SWAMP
Surface Water Ambient Monitoring Program

Final Quality Assurance Program Plan	2011

Quality Assurance Program Plan for a Screening Study of Bioaccumulation in California Rivers and Streams

July 2011

California Water Boards

http://www.waterboards.ca.gov/water_issues/programs/swamp

Group A Elements: Project Management

Element 1. Title and Approval Sheets

QUALITY ASSURANCE PROJECT PLAN

SCREENING STUDY OF BIOACCUMULATION IN CALIFORNIA RIVERS AND STREAMS

The Bioaccumulation Oversight Group (BOG)

Surface Water Ambient Monitoring Program

July 2011

Program Title	SWAMP Bioaccumulation Oversight Group Rivers Study
Lead Organization	Marine Pollution Studies Lab California Department of Fish and Game 7544 Sandholdt Road Moss Landing, CA 95039 Mark Stephenson, Project Manager Autumn Bonnema, Project Coordinator
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Effective Date	This Quality Assurance Project Plan (QAPP) is effective from April 2011 to March 2012 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 California Department of Fish and Game Marine Pollution Studies Laboratory (MPSL-DFG), California Dept. of Fish and Game Fish Wildlife Pollution Control Laboratory (DFG-WPCL), 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 SFEI, MPSL-DFG, and DFG-WPCL also are contained within. The BOG selects the sampling sites, the types and size of fish, and the number of analyses to be conducted.

This work is funded through the Surface Water Ambient Monitoring Program (SWAMP) fiscal year 10/11 Bioaccumulation funding.

Approvals

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

Mark Stephenson
Project Manager/MPSL-DFG Laboratory Director

_____ Date_____

Rusty Fairey
Contract Manager

_____ Date_____

Jay Davis
Lead Scientist

_____ Date_____

Beverly van Buuren
SWAMP Quality Assurance Officer

_____ Date_____

Autumn Bonnema
Project Coordinator/ MPSL-DFG Quality Assurance Officer

_____ Date_____

Pete Ode
DFG-WPCL Laboratory Director

_____ Date_____

Gail Cho
DFG-WPCL 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
SAN FRANCISCO ESTUARY INSTITUTE	
Jay Davis*	SFEI 7770 Pardee Lane Oakland, CA 94621-1424 Phone: (415) 746-7368 Email: jay@sfei.org
CALIFORNIA DEPARTMENT OF FISH AND GAME **FISH AND WILDLIFE WATER POLLUTION CONTROL LABORATORY**	
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MARINE POLLUTION STUDIES LAB **CALIFORNIA DEPARTMENT OF FISH AND GAME**	
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MOSS LANDING MARINE LABORATORIES **QUALITY ASSURANCE RESEARCH GROUP**	
Beverly van Buuren* Eric von der Geest	QA Research Group, MLML c/o: 4320 Baker AVE NW Seattle, WA 98107 Phone: (206) 297-1378 Email: bvanbuuren@mlml.calstate.edu

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.
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	Gail Cho DFG-WPCL 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	Gary Ichikawa MPSL-DFG	Sampling coordination, operations, and implementing field-sampling procedures.
Laboratory Director	Pete Ode DFG-WPCL Mark Stephenson MPSL-DFG	Organizing, coordinating, planning and designing research projects and supervising laboratory staff; Data validation, management and reporting
Sample Custodian	Stephen Martenuk MPSL-DFG Scot Harris DFG-WPCL additional staff	Sample storage. Not responsible for any deliverables.
Technicians	Technical staff MPSL-DFG DFG-WPCL	Conduct fish tissue dissection, digestion, and chemical analyses. Not responsible for any deliverables.

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.

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.

Gary Ichikawa 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.

Stephen Martenuk is responsible for sample storage and custody at MPSL. His duties will be to oversee compositing of tissue samples. Laurie Smith will do the same for samples processed at DFG-WPCL.

Pete Ode will serve as the Laboratory Director (LD) for the DFG-WPCL component of this project. His specific duties will be to 1) review and approve the QAPP, 2) provide oversight for all organic chemical analyses to be done for this project, and 3) ensure that all DFG-WPCL activities are completed within the proper timelines.

Mark Stephenson 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 all trace metal analyses 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)), Billy Jakl (MPSL-DFG), Dylan Service (MPSL-DFG), and Aroon Melwani (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 and Gail Cho is the DFG-WPCL 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 (Puckett, 2002).

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.

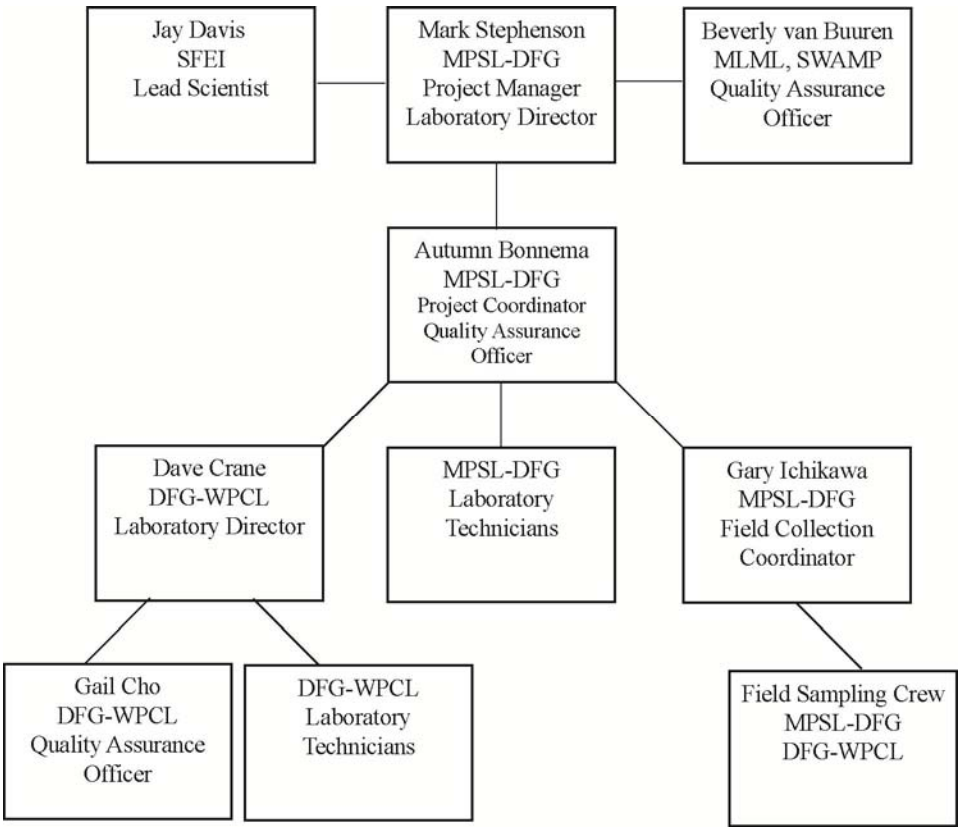
4.3. Persons responsible for QAPP update and maintenance

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, DFG-WPCL 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 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 has decided to focus on sampling that addresses the issue of bioaccumulation in sport fish and impacts on the fishing beneficial use. This approach is intended to provide the information that is the highest priority for the state government and the public. Monitoring focused on evaluating the aquatic life beneficial use should be included in the Project in the future.

5.1.2. Addressing Multiple Monitoring Objectives and Assessment Questions for the Fishing Beneficial Use

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 (Table 3). 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.

Table 3. Bioaccumulation monitoring assessment framework for the fishing beneficial use.

D.1. ***Determine the status of the fishing beneficial use throughout the State with respect to bioaccumulation of toxic pollutants***

D.1.1 What are the extent and location of water bodies with sufficient evidence to indicate that the fishing beneficial use is at risk due to pollutant bioaccumulation?
D.1.2 What are the extent and location of water bodies with some evidence indicating the fishing beneficial use is at risk due to pollutant bioaccumulation?
D.1.3 What are the extent and location of water bodies with no evidence indicating the fishing beneficial use is at risk due to pollutant bioaccumulation?
D.1.4 What are the proportions of water bodies in the State and each region falling within the three categories defined in questions D.1.1, D.1.2, and D.1.3?

D.2. ***Assess trends in the impact of bioaccumulation on the fishing beneficial use throughout the State***
D.2.1 Are water bodies improving or deteriorating with respect to the impact of bioaccumulation on the fishing beneficial use?
D.2.1.1 Have water bodies fully supporting the fishing beneficial use become impaired?
D.2.1.2 Has full support of the fishing beneficial use been restored for previously impaired water bodies?
D.2.2 What are the trends in proportions of water bodies falling within the three categories defined in questions D.1.1, D.1.2, and D.1.3 regionally and statewide?

D.3. ***Evaluate sources and pathways of bioaccumulative pollutants impacting the fishing beneficial use***
D.3.1 What are the magnitude and relative importance of pollutants that bioaccumulate and indirect causes of bioaccumulation throughout each Region and the state as a whole?
D.3.2 How is the relative importance of different sources and pathways of bioaccumulative pollutants that impact the fishing beneficial use changing over time on a regional and statewide basis?

D.4. ***Provide the monitoring information needed to evaluate the effectiveness of management actions in reducing the impact of bioaccumulation on the fishing beneficial use***
D.4.1 What are the management actions that are being employed to reduce the impact of bioaccumulation on the fishing beneficial use regionally and statewide?
D.4.2 How has the impact of bioaccumulation on the fishing beneficial use been affected by management actions regionally and statewide?

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 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 will be the last in the series of water body types to be covered with a statewide screening study. The Roundtable has decided that the rivers and streams survey will be a one-year study, given available resources and that it is possible to provide reasonable coverage of popular fishing locations in a one-year effort. Wetlands will not be covered due to the low fishing pressure in those habitats. Another cycle of statewide surveys of lakes and reservoirs, the coast, and rivers and streams will occur, but the timing of the next round of surveys has not yet been established.

In summary, focusing on two closely associated habitat types (rivers and streams), one objective (status), and one beneficial use (fishing) will allow us to provide reasonable coverage and a thorough assessment of bioaccumulation in these habitats in a one-year study.

5.2. Decisions or outcomes

In response to information needs articulated by the state and regional Water Boards, two management questions have been articulated to guide the 2011 screening survey of the status of bioaccumulation in sport fish on the California coast. 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): Status of the Fishing Beneficial Use

For popular fish species, what percentage of popular fishing areas have low enough concentrations of contaminants that fish can be safely consumed?

Answering this question is critical to determining the degree of impairment of the fishing beneficial use across the state due to bioaccumulation. This question places emphasis on characterizing the status of the fishing beneficial use through monitoring of the predominant pathways of exposure – the popular fish species and fish areas. This focus is also anticipated to enhance public and political support of the program by assessing the resources that people care most about. The determination of percentages captures the need to perform a statewide assessment of the entire California coast. While a significant amount of monitoring in rivers and streams has been conducted (reviewed in Davis et al. [2007]), a systematic statewide survey has

never been performed. The emphasis on safe consumption calls for: a positive message on the status of the fishing beneficial use; evaluation of the data using thresholds for safe consumption; and performing a risk-based assessment of the data.

The data needed to answer this question are average concentrations in popular fish species from popular fishing locations. Inclusion of as many popular species as possible is important to understanding the nature of impairment in any areas with concentrations above thresholds. In some areas, some fish may be safe for consumption while others are not, and this is valuable information for anglers. 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): Need for Further Sampling

Should additional sampling of bioaccumulation in sport fish (e.g., more species or larger sample size) in an area be conducted for the purpose of developing consumption guidelines?

This screening survey of California rivers and streams will provide a preliminary indication as to whether some areas that have not been sampled thoroughly to date may require consumption guidelines. Consumption guidelines provide a mechanism for reducing human exposure in the short-term. The California Office of Environmental Health Hazard Assessment (OEHHA), the agency responsible for issuing consumption guidelines, considers a sample of 9 or more fish from a variety of species abundant in a water body to be the minimum needed in order to issue guidance. It is valuable to have information not only on the species with high concentrations, but also the species with low concentrations so anglers can be encouraged to target the low species. Answering this question is essential as a first step in determining the need for more thorough sampling in support of developing consumption guidelines. Large stretches of rivers in the Central Valley that are popular for fishing are already under advisories.

5.2.3. Overall Approach

The overall approach to be taken to answer these two questions is to perform a statewide screening study of bioaccumulation in sport fish in California rivers and streams. Answering these questions, as has been done for lakes and reservoirs and the coast, will provide a basis for decision-makers to understand the scope of the bioaccumulation problem both in rivers and streams and across all of these water body types, and will provide regulators with information needed to establish priorities for both cleanup actions and development of consumption guidelines.

It is anticipated that the screening study may lead to more detailed followup investigations of areas where consumption guidelines and cleanup actions are needed. Funding for these followup studies will come from other local or regional programs rather than the SWAMP statewide monitoring budget.

The approach in this study is consistent with the approaches taken in the previous statewide surveys of bioaccumulation in California lakes and reservoirs (Davis et al. 2010) and on the California coast (BOG 2009). Adding information on bioaccumulation in rivers and streams to

that already obtained for the other water body types will complete a comprehensive statewide assessment of the impact of contaminants on the fishing beneficial use in California.

5.2.4. 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 rivers and streams.

One significant collaboration will be with the Central Valley Regional Water Quality Control Board (CVRWQCB). The CVRWQCB is providing $16K for supplemental sampling at 13 sites to support development of a mercury TMDL for the Sierra Nevada foothill region. The Water Board will fund analysis of sediment (total mercury: sieved for fines [<63 microns], 2 samples per site), water (total mercury, total methylmercury, SSC; 1 sample per site), and additional fish (total mercury; whatever large species is most abundant at the time of sampling other than rainbow or brown trout; at least 7 inches in total length; 3 samples of the same species per site). It is highly likely that the additional fish species collected will coincide with the secondary target list for this study (Sacramento pikeminnow, Sacramento sucker, etc. – see Table 3).

The study will also be coordinated with a study conducted by USGS and funded by the State Board to develop assessment tools for evaluating mercury cleanups and for making 303(d) listing decisions. The $700,000 project will be designed to validate the use of sediment mercury concentration data for listing. The project will begin in 2011 with a review of existing data, followed by sampling to fill data gaps in 2012. The project will attempt to establish a consistent relationship between mercury bioaccumulation in fish tissue and sediment total mercury. The study will conduct sampling at 20 stream reaches and 13 lakes and reservoirs in gold mining regions of the Sierra Nevada foothills. Sediment analyses will include total mercury, methylmercury, reactive mercury, and iron and sulfur species. Fish tissue analyses will also be conducted where they are needed. Water analyses will also be conducted. Coordination with the SWAMP survey will allow the USGS study to establish a more extensive empirical dataset to support the development of the assessment tools.

Coordination on a small-scale will occur with the Water Board from Region 6 to obtain information on microcystin in fish fillets. Microcystin is a toxin produced by cyanobacteria that can undergo blooms in eutrophic water bodies. Cyanobacteria blooms are known to occur in Bridgeport Reservoir in Region 6. In coordination with Region 6, microcystin in fish fillets will be analyzed in fish collected from the station on the East Walker River below Bridgeport Reservoir.

5.3. Fish tissue contamination criteria

Threshold levels for determining impairment of a body of water based on pollutants in fish tissue are listed in Table 4. 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 $1x10^{-6}$ 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 $1x10^{-4}$ 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 the ATL table 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."

Thresholds for Total PCBs, DDTs, and Chlordanes are based on the summation of concentrations from the compounds listed in Table 5. The summations will be compared with the threshold values in Table 4, and may lead to the identification of species which meet the beneficial uses of MQ1.

Table 4. 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 ATLs 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

Table 5. Compounds summed for comparison with FCGs and ATLs levels.

Pollutant	Components	Reference
Total PCBs	Sum of all congeners analyzed	
Total Chlordanes	Chlordane, cis- Chlordane, trans- Nonachlor, cis- Nonachlor, trans- Oxychlordane	USEPA 2000
Total DDTs	DDD(o,p') DDD(p,p') DDE(o,p') DDE(p,p') DDT(o,p') DDT(p,p')	USEPA 2000

Element 6. Project Description

6.1. Work statement and produced products

This study will be completed in one year of sampling. Sampling will focus on the popular fishing sites identified along the rivers and streams in California. 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 6-10. All chemistry data will be reported on a wet weight basis. Analytical methods are listed in each table as appropriate.

Past studies have calculated PCB as Aroclors for comparison with older data sets and health thresholds. OEHHA no longer intends to use these data, and they will not be reported in SWAMP reports. The BOG agrees that these calculations are not as valuable as individual congener data, and will therefore cease reporting these calculated values. If necessary, these values can be calculated at a later time by the data management team using the provided congener data.

Algal toxins will only be analyzed in fish collected from East Walker River below Bridgeport Reservoir. Some compounds will be reported as screening level data only due to the unavailability of a reliable standard source material (Table 10).

In the SWAMP Lakes Study (conducted in 2007 and 2008), PBDE data were provided at a screening level only as a free service from the analytical lab. These compounds are important emerging contaminants however they are cost prohibitive and not part of our current analyte list. Archives of each sample will be retained for potential future analysis.

Also, Tedion has been removed from the analyte list. This compound was discontinued from use in 1985 and has a very short residence time. Furthermore, it is a compound that is not bioaccumulated.

Table 6. 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
Moisture (%)
Lipid Content (%)
Collection Location (lat./long.)

Table 7. Constituents to be Analyzed – Metals and Metalloids

Analyte	Analytical Method
Total Mercury	EPA 7473 (USEPA 1998)
Total Selenium	EPA 200.8 (USEPA 1994a)

Table 8. Constituents to be Analyzed – Organochlorine (OC) Pesticides

Organochlorine Pesticides (by EPA 8081BM using GC-ECD, USEPA 1996d)	
Group	**Parameter**
Chlordanes	Chlordane, cis-
	Chlordane, trans-
	Heptachlor
	Heptachlor epoxide
	Nonachlor, cis-
	Nonachlor, trans-
	Oxychlordane
DDTs	DDD(o,p')
	DDD(p,p')
	DDE(o,p')
	DDE(p,p')
	DDMU(p,p')
	DDT(o,p')
	DDT(p,p')
Cyclodienes	Aldrin
	Dieldrin
	Endrin
HCHs	HCH, alpha
	HCH, beta
Others	Dacthal
	Endosulfan I
	Hexachlorobenzene
	Methoxychlor
	Mirex
	Oxadiazon

Table 9. Constituents to be Analyzed – Polychlorinated Biphenyls (PCB)

Polychlorinated Biphenyl (PCB) Congeners (by USEPA Method 8082M, USEPA 1996e)		
PCB 008	PCB 095	PCB 157
PCB 018	PCB 097	PCB 158
PCB 027	PCB 099	PCB 169
PCB 028	PCB 101	PCB 170
PCB 029	PCB 105	PCB 174
PCB 031	PCB 110	PCB 177
PCB 033	PCB 114	PCB 180
PCB 044	PCB 118	PCB 183
PCB 049	PCB 126	PCB 187
PCB 052	PCB 128	PCB 189
PCB 056	PCB 137	PCB 194
PCB 060	PCB 138	PCB 195
PCB 064	PCB 141	PCB 198/199
PCB 066	PCB 146	PCB 200
PCB 070	PCB 149	PCB 201
PCB 074	PCB 151	PCB 203
PCB 077	PCB 153	PCB 206
PCB 087	PCB 156	PCB 209

Table 10. Constituents to be Analyzed – Algal Toxins

Microcystins and Biotoxins by LC/MS/MS (Appendix IV E)		
Group	**Parameter**	**CAS #**
Microcystins	MCY-RR	111755-37-4
	MCY-LR	101043-37-2
	MCY-YR	101064-48-6
	MCY-LA	101043-37-2
	MCY-LW*	157622-02-1
	MCY-LF*	154037-70-4
	MCY-LY*	123304-10-9
Microcystin Metabolites	Desmethyl-LR*	NA
	Desmethyl-RR*	NA
Cyanotoxins	Anatoxin A	64285-06-9

* These compounds will be reported at a screening level only

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 11.

One to two species will be collected from each of 59 river, stream and hatchery sites, resulting in an estimated 69 composites analyzed for the constituents found in Tables 6-9. The compounds in Table 10 will only be analyzed from East Walker River below Bridgeport Reservoir.

Table 11. Project Schedule Timeline

Item	Activity and/or Deliverable	Deliverable Due Date
1	**Contracts**	
	Subcontract Development	February 2011
2	**Quality Assurance Project Plan & Monitoring Plan**	
2.1	Draft Monitoring Plan	February 2011
2.2	Final Monitoring Plan	June 2011
2.3	Draft Quality Assurance Project Plan	May 2011
2.4	Final Quality Assurance Project Plan	June 2011
3	**Sample Collection**	March-September 2011
4	**Sample Selection and Chemical Analysis**	
4.1	Selection of Tissue for Analysis	June-October 2011
4.2	Creation of Sample Composites	June-November 2011
4.3	Chemical Analysis	July 2011-February 2012
4.4	Data Reported to SWAMP	March 2012
5	**Data Quality Assessement and Narrative**	May 2012
6	**Interpretive Report**	
6.1	Draft Report	December 2012
6.2	Final Report	March 2013

6.4. Geographical setting and sample sites

California has over 211,000 miles of rivers and streams (Davis et al. 2007) that span a diversity of habitats and fish populations, and dense human population centers with a multitude of popular fishing locations. Conducting a statewide survey with a limited budget is a challenge. The approach being employed to sample this vast area is to conduct a complete sampling (or census) of the entire population of the most popular river and stream fishing locations in the state. Popular fishing locations were identified from Stienstra (2004) and discussions with stakeholders. Stienstra (2004) rated fishing spots on a scale of 1 to 10 based on three elements:

number of fish, size of fish, and scenic beauty. With the budget available for this survey we are able to sample all of the river and stream locations with a Stienstra rating of 6 or higher. The locations selected for inclusion are listed in Table 2 and shown in Figures 1a-e of the Sampling and Analays Plan (Appendix II).

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 11.

Ultimately, additional zones may be sampled pending time remaining in the sampling season and available funding within the project once cost savings from analysis has been determined.

Element 7. Quality Indicators and Acceptability Criteria for Measurement Data

Data quality indicators for the analysis of fish tissue concentrations of analytes will include accuracy (bias), precision, recovery, completeness and sensitivity. Measurement Quality Indicators for analytical measurements of organics and metals in tissue are in Table 12.

Field duplicates and blanks will not be collected for this study, and are consequently not included in Table 12. These QA elements are not appropriate for discrete tissue collections, and are not valuable for data interpretation.

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

Table 12. 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 18
Synthetic Organics (including PCBs, and pesticides)	Certified Reference Materials (CRM, PT) within 70-130% of the certified 95% CI stated by provider of material. If not available then within 50-150% of reference value.	Duplicate RPD <25%; n/a if concentration of either sample <RL Matrix Spike Duplicate RPD <25%	Matrix spike 50% - 150% or control limits control limits based on 3x the standard deviation of laboratory's actual method recoveries	90%	See Tables 19-20
Algal Toxins*	50-150% recovery for selected spiked target analytes	Duplicate RPD <25%; n/a if concentration of either sample <RL Matrix Spike Duplicate RPD <25%	50-150% recovery, or based on 3x the standard deviation of laboratory's actual method recoveries	90%	See Table 21

* Some compounds will be reported at a screening level only and are not subject to the MQIs.

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.

$$\% \text{ recovery} = \frac{\mathbf{V}_{\text{analyzed}}}{\mathbf{V}_{\text{certified}}} \text{x}100$$

Where:

$v_{analyzed}$: the analyzed concentration of the reference material
$v_{certified}$: the certified concentration of the reference material

The acceptance criteria for reference materials are listed in Tables 13-15.

While reference materials are not available for all analytes, a way of assessing the accuracy of an analytical method is still required. Laboratory control samples (LCSs) provide an alternate method of assessing accuracy. 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. If an LCS needs to be substituted for a reference material, the acceptance criteria are the same as those for the analysis of reference materials. These are detailed in Tables 13-15.

Table 13. 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

Table 14. Measurement Quality Objectives – Synthetic Organic Compounds 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	<RL 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% of the certified 95% confidence interval stated by provider of material. If not available then within 50-150% of reference value.
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 <25%
Laboratory Duplicate	Per 20 samples or per batch, whichever is more frequent	RPD <25%; n/a if concentration of either sample <MDL
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)
RL = Reporting Limit
n/a = not applicable

Table 15. Measurement Quality Objectives – Algal Toxins*

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	85-115% recovery
Laboratory Blank	Per 20 samples or per batch, whichever is more frequent	<RL 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)	CRM is not available for microcystins. 50-150% recovery for selected spiked target analytes.
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 <25%
Laboratory Duplicate	As specified in method	RPD <25%; n/a if concentration of either sample <RL
Surrogate or Internal Standard	As specified in method	Per method. Surrogate is unavailable for this method.

* Some compounds will be reported at a screening level only and are not subject to the MQIs.

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).

$$RPD = \left| \frac{(V_{sample} - V_{duplicate})}{mean} \right| x100$$

Where:

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

Specific requirements pertaining to the analysis of laboratory duplicates vary depending on the type of analysis. The acceptance criteria for laboratory duplicates are specified in Tables 13-15.

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:

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

Where:

Stdev(v_1,v_2,…,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.

$$\% \text{ recovery} = \frac{(V_{MS} - V_{ambient})}{V_{spike}} x100$$

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.

$$RPD = \left| \frac{(V_{MS} - V_{MSD})}{mean} \right| x100$$

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 ($recovery_{MS}$ + $recovery_{MSD}$)

The MQO for the RPD between the MS and MSD is the same regardless of the method of calculation. These are detailed in Tables 13-15.

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 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.

7.5. Routine monitoring of method performance for organic analysis – surrogates

Surrogates are compounds chosen to simulate the analytes of interest in organic analyses. Surrogates are used to estimate analyte losses during the extraction and clean-up process, and must be added to each sample, including QC samples, prior to extraction. The reported concentration of each analyte is adjusted to correct for the recovery of the surrogate compound. The surrogate recovery data will be carefully monitored. If possible, isotopically-labeled analogs of the analytes will be used as surrogates. Surrogate recoveries for each sample are reported with the target analyte data. Surrogate is considered acceptable if the percent recovery is within 50-150%.

7.6. Internal standards

For Gas Chromatography Mass Spectrometry (GC-MS) analysis, internal standards (i.e., injection internal standards) are added to each sample extract just prior to injection to enable optimal quantification, particularly of complex extracts subject to retention time shifts relative to the analysis of standards. Internal standards are essential if the actual recovery of the surrogates added prior to extraction is to be calculated. The internal standards can also be used to detect and correct for problems in the GC injection port or other parts of the instrument. The compounds used as internal standards will be different from those already used as surrogates. The analyst(s) will monitor internal standard retention times and recoveries to determine if instrument maintenance or repair, or changes in analytical procedures, are indicated. Corrective action will be initiated based on the judgment of the analyst(s). Instrument problems that may have affected the data or resulted in the reanalysis of the sample will be documented properly in logbooks and internal data reports and used by the laboratory personnel to take appropriate corrective action.

7.7. Dual-column confirmation

Dual-column chromatography is required for analyses using Gas Chromatography Electron Capture Detector (GC-ECD) due to the high probability of false positives arising from single-column analyses.

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 3, 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 fish cannot be collected from a particular location, another location will be chosen to replace it. Additional locations will be chosen by the PI with input from Regional Board staff.

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 12). In the event laboratory documentation is incomplete, datasheets will be returned to the dissector for amendment.

Occasionaly digestates or extracts are rendered unusable for various reasons in the preparation process. If this occurs, the sample(s) affected will be re-processed. In rare occasions, the laboratory may need to request additional material to complete the analysis. Archived material will be made available.

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 DFG-WPCL 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 DFG-WPCL 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 DFG-OSPR Industrial Hygiene 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)

- Monitoring 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 III, pp. 6-19 (Appendix II). Fifty-four locations along California's rivers and streams will be sampled, where possible, for two indicator species – a top predator (e.g., largemouth bass) as a mercury indicator and a high lipid, bottom-feeding species (e.g., channel catfish, common carp) as an organics and selenium indicator. Specific details on locations selected and target species are found in Sections III D and E, pp. 9-14 of the SAP.

Potential sampling equipment and methods can be found in MPSL-102a (Appendix III). Once samples have been identified for composite creation, they will be shipped to the dissection laboratory for processing and analysis according to the timeline in Table 11.

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 fish weight, sex, moisture, and lipid content. These parameters may be used to support other data gathered.

10.1. Variability

Due to potential variability of contaminant loads in individual tissue samples, samples will be analyzed in composites as outlined in the SAP (Appendix II) and MPSL-DFG SOPs (Appendix III).

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

Fish will be collected in accordance with MPSL-102a, Section 7.4 (Appendix III) except where noted here. Because river and stream habitats 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 3-4).

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 sharks will is 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 organism 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, Section III, E, pp. 12-14 (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 16 describes action to take in the event of a collection failure.

Table 16. Field collection corrective actions

Collection Failure	Corrective Action
No Bottom Feeder Present	Collect one species of predator and analyze for all constituents; document the occurrence
No Predator Present	Collect one species of bottom feeder and analyze for all constituents; document the occurrence
No Fish present	Inform PC and move on to another location – another location may be substituted; document the occurrence

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.

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). Samples delivered to DFG-WPCL will undergo a similar handling procedure (SAMPMAN_REV_Aug08, Appendix IV).

Authorization forms will be provided to each dissecting laboratory detailing the dissection and analysis to be performed (Attachment 3). Samples will be dissected according to MPSL-105 (Appendix III) and data retained on the lab data sheets in Attachment 4.

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.

Organic compounds frequently have 40 day hold times between extraction and analysis. Please refer to the appropriate method for specific holding time requirements. Violations will be flagged appropriately in the final results. This type of hold time is not applicable to metals and metalloids

Element 13. Analytical Methods

Methods and equipment for laboratory analyses are listed in Table 17. 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.)

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.

Table 17. Methods for laboratory analyses

Parameter	Method	Instrument
Mercury	EPA 7473 (USEPA 1998)	Milestone DMA 80
Selenium	EPA 3052M (USEPA 1996a) EPA 200.8 (USEPA 1994a)	CEM MARSXpress Digester Perkin-Elmer Elan 9000 ICP-MS
Organochlorine Pesticides	EPA 8081BM (USEPA 1996d)	Agilent 6890 GC-ECD Varian 3800 GC with Varian 1200 Triple-Quad MS
Polychlorinated Biphenyls	EPA 8082M (USEPA 1996e)	Varian 3800 GC with Varian 1200 Triple-Quad MS
Algal Toxins	WPCL Microcystins and Biotoxins (Appendix IV E)	Agilent 1200 liquid chromatograph with Agilent 6410 Triple Quad Mass Spectrometer

Mercury 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 ±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 18 and Measurement Quality Objectives (MQO) in Section 7, Table 13.

Selenium composites will be digested according to EPA 3052M, "Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices" (USEPA, 1996a), modified (Appendix III), and will be analyzed according to EPA 200.8, "Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Mass Spectrometry" (USEPA, 1994a). 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 ±20% of the true value, or the previous 10 samples must be reanalyzed. Two blanks, a certified reference material (NIST 2976, NRCC DORM-3 or similar), as well as a method duplicate and a matrix spike pair will be run with each set of samples. Reporting Limits (RL) can be found in Table 18 and Measurement Quality Objectives (MQO) in Section 7, Table 13.

Table 18. Trace metal analytical parameters, reporting units, and reporting limits (RL) for tissue samples.

Parameter	Method	RL (µg/g wet wt)
Mercury	EPA 7473 (USEPA 1998)	0.02
Selenium	EPA 3052M (USEPA 1996a) EPA 200.8 (USEPA 1994a)	0.40

Organochlorine and PCB compounds will be extracted following EPA Methods 3545, 3640A, and 3620B. (USEPA 1994b, 1996b,c) Organochlorine pesticides will be analyzed according to EPA 8081BM, "Organochlorine Pesticides by Gas Chromatography" (USEPA 1996d), modified (Appendix IV). PCBs will be analyzed according to EPA 8082M, "Polychlorinated Biphenyls (PCBs) by Gas Chromatography" (USEPA 1996e), modified (Appendix IV). 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 ±25% of the true value, or the previous 10 samples must be reanalyzed. One blank, a laboratory control spike (LCS), as well as a method duplicate and a matrix spike pair will be run with each set of samples. Reporting Limits (RL) can be found in Tables 19-20 and Measurement Quality Objectives (MQO) in Section 7, Table 14.

Table 19. Trace organic analytical parameters, reporting units, and reporting limits (RL) for tissue samples.

Organochlorine Pesticides (by USEPA 8081BM, USEPA 1996d)		
Group	**Parameter**	**RL (ng/g wet wt)**
Chlordanes	Chlordane, cis-	1
	Chlordane, trans-	1
	Heptachlor	1
	Heptachlor epoxide	0.5
	Nonachlor, cis-	1
	Nonachlor, trans-	1
	Oxychlordane	1
DDTs	DDD(o,p')	0.5
	DDD(p,p')	0.5
	DDE(o,p')	0.5
	DDE(p,p')	1
	DDMU(p,p')	1
	DDT(o,p')	1
	DDT(p,p')	1
Cyclodienes	Aldrin	1
	Dieldrin	0.5
	Endrin	1
HCHs	HCH, alpha	0.5
	HCH, beta	1
	HCH, gamma	0.5
Others	Dacthal	0.5
	Endosulfan I	1
	Hexachlorobenzene	0.7
	Methoxychlor	1
	Mirex	1
	Oxadiazon	1

Table 20. Trace organic analytical parameters, reporting units, and reporting limits (RL) for tissue samples.

Polychlorinated Biphenyl congeners (by USEPA Method 8082M, USEPA 1996e)			
PCB	RL ppb (ng/g wet wt)	PCB	RL ppb (ng/g wet wt)
PCB 008	0.6	PCB 128	0.6
PCB 018	0.6	PCB 137	0.6
PCB 027	0.6	PCB 138	0.6
PCB 028	0.6	PCB 141	0.6
PCB 029	0.6	PCB 146	0.6
PCB 031	0.6	PCB 149	0.6
PCB 033	0.6	PCB 151	0.6
PCB 044	0.6	PCB 153	0.6
PCB 049	0.6	PCB 156	0.6
PCB 052	0.6	PCB 157	0.6
PCB 056	0.6	PCB 158	0.6
PCB 060	0.6	PCB 169	0.6
PCB 064	0.6	PCB 170	0.6
PCB 066	0.6	PCB 174	0.6
PCB 070	0.9	PCB 177	0.6
PCB 074	0.6	PCB 180	0.6
PCB 077	0.6	PCB 183	0.6
PCB 087	0.9	PCB 187	0.6
PCB 095	0.9	PCB 189	0.6
PCB 097	0.6	PCB 194	0.6
PCB 099	0.6	PCB 195	0.6
PCB 101	0.9	PCB 198/199	0.6
PCB 105	0.6	PCB 200	0.6
PCB 110	0.9	PCB 201	0.6
PCB 114	0.6	PCB 203	0.6
PCB 118	0.9	PCB 206	0.6
PCB 126	0.6	PCB 209	0.6

Algal toxins will be analyzed following WPCL Method: Microcystins and Biotoxins by LC/MS/MS (Appendix IV E). Samples are subjected to a volume of acidified methanol/water solution and sonicated. The supernatant is poured through solid phase extraction cartridges and eluted. The resulting eluate is analyzed by LC/MS/MS using acidified HPLC-grade water (1% formic acid) and acetonitrile in the mobile phase. 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 ±15% of the true value, or the previous 10 samples must be reanalyzed. One blank, a laboratory control spike (LCS), as well as a method duplicate and a matrix spike pair will be run with each

set of samples. Some compounds will be reported at a screening level only and matrix spikes will not be performed (Tables 10, 21). Reporting Limits (RL) can be found in Table 21 and Measurement Quality Objectives (MQO) in Section 7, Table 15.

Table 21. Trace organic analytical parameters, reporting units, and reporting limits (RL) for tissue samples.

Microcystins and Biotoxins by LC/MS/MS (Appendix IV E)	
Analyte	**RL ppb (ng/g wet wt)**
MCY-RR	1.00
MCY-LR	1.00
MCY-YR	1.00
MCY-LA	1.00
Anatoxin a	10.0
MC-LW*	1.00
MC-LF*	1.00
MC-LY*	1.00
Desmethyl-LR*	1.00
Desmethyl-RR*	1.00

* These compounds will be reported at a screening level only

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 11.

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. A written report containing all corrective actions will be submitted to the SWAMP QAO on a quarterly basis.

Each aspect of laboratory quality control is listed in Tables 13-15 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 17). These SOPs have been reviewed by each respective Laboratory QAO and found to be in compliance with SWAMP criteria. DFG-WPCL and MPSL-DFG analysts are responsible for equipment testing, inspection, and maintenance. Appendices III and IV list the referenced SOPs. DFG-WPCL SOPs are available upon request from the Laboratory Director by email: dcrane@ospr.dfg.ca.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. In some cases, the cost of instruments (i.e., GC-MS, EFD, etc) prohibits the procurement of additional spare parts. However, those instruments are typically maintained and repaired by the manufacturer.

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 22) are calibrated, standardized and maintained according to procedures detailed in laboratory SOPs (Appendices III and IV). Instrument

manuals identify step-by-step calibration and maintenance procedures. Instruments and types of calibration required are listed in Table 22. 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 22. Equipment maintenance and calibration frequency.

Instrument	Inspection/Maintenance Frequency	Calibration Frequency
Agilent 6890 Gas Chromatograph equipped with micro-ECD detectors and autosamplers using Enviroquant Software (Agilent)	As needed	At least once prior to each batch
Varian 3800 Gas Chromatograph with Varian 1200 Triple Quadrupole Mass Spectrometer equipped with Combi-Pal autosampler	As needed	At least once prior to each batch
Perkin-Elmer Elan 9000 Inductively Coupled Plasma - Mass Spectrometer	As needed	At least once prior to each batch
Milestone DMA-80 Direct Mercury Analyzer	As needed	At least once every 2 weeks
Agilent 6410 Triple Quadropole LC/ESI/MS/MS in multiple reaction mode	As needed	At least once prior to each batch

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 Tables 13-15. 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, DFG-WPCL 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 23 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 23. Inspection/acceptance testing requirements for consumables and supplies.

Project-Related Supplies (source)	Inspection / Testing Specifications	Acceptance Criteria	Frequency	Responsible Individual
Certified pre-cleaned glass (I-Chem/Fisher Scientific or similar)	Carton custody seal is inspected	Carton custody seal intact	At receipt date of shipment	MSPL-DFG or DFG-WPCL 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	MSPL-DFG or DFG-WPCL 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	MSPL-DFG or DFG-WPCL 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	MSPL-DFG or DFG-WPCL 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 DFG-WPCL will be maintained as described in DFG-WPCL SOPs (Appendix IV) and the DFG-WPCL Quality Assurance Manual (Appendix I). The DFG-WPCL 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 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 Tables 13-15.
- 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 11.

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 11.

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. DFG-WPCL checklists and forms are in Attachment 5. MPSL-DFG does not have specific forms; comments are made on original data sheets and reports.

Data generated by project activities will be reviewed against the measurement quality objectives (MQOs) in Tables 13-15. Furthermore, the final dataset as a whole will scrutinized for usability to answer the three Management Questions.

Element 23. Verification and Validation Methods

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).

Data will be validated by Stacey Swenson of the DMT 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.

A QA narrative will be produced to be incorporated in the BOG Rivers Report. This narrative will summarize the data set from a QA standpoint. Validated data will be made available to users via the SWAMP Database 2.5 provided by the DMT on 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 Tables 13-15 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.

As stated earlier, PCBs, DDTs, and Chlordanes will be summed for comparison with threshold values in Table 4. It is possible that some of the parameters that comprise each summation may be flagged as rejected through the Validation process (Appendix V B). When this occurs, the censored results will not be included in the summation used for comparison. However, the difference between summations with and without rejected values will be compared to each other. If the rejected values comprise more than 30% of the total sum for a sample, and the concentration prior to censoring was above the threshold level in Table 4, then the sample

will be designated for reanalysis. Samples with censoring of more than 30% but with uncensored sums below the threshold level will not be designated for reanalysis.

The project needs sufficient data, as represented by the completeness objective (Table 12, Section 7), to address the management questions laid out in Section 5; specifically MQ1 and MQ2. A failure to achieve the number of data points cited could mean an inability to answer these questions.

To address MQ1, the concentrations from all composites will be compared with the BOG adopted thresholds presented in Table 4.

In order to answer MQ2 the analytical results will be compared to the BOG adopted thresholds as described in the previous paragraph. For each analyte the percent of locations that have fish that exceeded the threshold will be calculated.

Those locations with analyte results greater than the OEHHA FCGs or ATLs in Table 4 will be called to the attention of the California Regional Water Quality Control Boards in the technical report. It will be up to each Region to compare the measured chemistry results of this study with the appropriate regional 303(d) list requirements and to determine if further sampling is needed (MQ3).

Since this study is a screening study with primarily the two management questions as objectives, complex statistical analysis is not anticipated except as mentioned above. The data collected by this study is not intended to be used with traditional statistics.

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Appendix I: List of Associated QAPPs

CDFG MPSL MLML Laboratory QAP, Revision 5. February, 2006

CDFG WPCL Laboratory QAPP, Revision 9. August, 2006

Appendix II: Sampling and Analysis Plan

FINAL

Sampling and Analysis Plan for a Screening Study of Bioaccumulation in California Rivers and Streams

The Bioaccumulation Oversight Group (BOG)

Surface Water Ambient Monitoring Program

July 2011

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I. INTRODUCTION

This document presents a plan for sampling and analysis of sport fish in a one-year screening survey of bioaccumulation in California rivers and streams. This work will be performed as part of the State Water Resources Control Board's Surface Water Ambient Monitoring Program (SWAMP). This effort is part of a new long-term Bioaccumulation Monitoring Project that is providing comprehensive monitoring of bioaccumulation in California water bodies.

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, the California Office of Environmental Health Hazard Assessment, and the University of California. Interested parties, including members of other agencies, consultants, or other stakeholders are also welcome to participate.

The Roundtable has formed a subcommittee, the Bioaccumulation Oversight Group (BOG), which 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 (2007 and 2008) and another two-year screening survey of the California coast in 2009 and 2010. A final report on the lakes survey is available (Davis et al. 2010; http://www.swrcb.ca.gov/water_issues/programs/swamp/lakes_study.shtml). A report presenting results from the first year of the coast survey is available (Davis et al. 2011; http://www.waterboards.ca.gov/water_issues/programs/swamp/coast_study.shtml).

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 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 has decided to focus on sampling that addresses the issue of bioaccumulation in sport fish and impacts on the fishing beneficial use. This approach is intended to provide the information that is the highest priority for the state government and the public. Monitoring focused on evaluating the aquatic life beneficial use should be included in the Project in the future.

B. Addressing Multiple Monitoring Objectives and Assessment Questions for the Fishing Beneficial Use

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 (Table 1). 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 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 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.

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 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 will be the last in the series of water body types to be covered with a statewide screening study. The Roundtable has decided that the rivers and streams survey will be a one-year study, given available resources and that it is possible to provide reasonable coverage of popular fishing locations in a one-year effort. Wetlands will not be covered due to the low fishing pressure in those habitats. Another cycle of statewide surveys of lakes and reservoirs, the coast, and rivers and streams will occur, but the timing of the next round of surveys has not yet been established.

In summary, focusing on two closely associated habitat types (rivers and streams), one objective (status), and one beneficial use (fishing) will allow us to provide reasonable coverage and a thorough assessment of bioaccumulation in these habitats in a one-year study.

III. DESIGN OF THE RIVERS AND STREAMS SURVEY

A. Management Questions for this Survey

In response to information needs articulated by the state and regional Water Boards, two management questions have been articulated to guide the 2011 screening survey of the status of bioaccumulation in sport fish on the California coast. 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.

Management Question 1 (MQ1)
Status of the Fishing Beneficial Use
For popular fish species, what percentage of popular fishing areas have low enough concentrations of contaminants that fish can be safely consumed?

Answering this question is critical to determining the degree of impairment of the fishing beneficial use across the state due to bioaccumulation. This question places emphasis on characterizing the status of the fishing beneficial use through monitoring of the predominant pathways of exposure – the popular fish species and fish areas. This focus is also anticipated to enhance public and political support of the program by assessing the resources that people care most about. The determination of percentages captures the need to perform a statewide assessment of the entire California coast. While a significant amount of monitoring in rivers and streams has been conducted (reviewed in Davis et al. [2007]), a systematic statewide survey has never been performed. The emphasis on safe consumption calls for: a positive message on the status of the fishing beneficial use; evaluation of the data using thresholds for safe consumption; and performing a risk-based assessment of the data.

The data needed to answer this question are average concentrations in popular fish species from popular fishing locations. Inclusion of as many popular species as possible is important to understanding the nature of impairment in any areas with concentrations above thresholds. In some areas, some fish may be safe for consumption while others are not, and this is valuable information for anglers. 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.

Management Question 2 (MQ2)
Need for Further Sampling
Should additional sampling of bioaccumulation in sport fish (e.g., more species or larger sample size) in an area be conducted for the purpose of developing comprehensive consumption guidelines?

This screening survey of California rivers and streams will provide a preliminary indication as to whether some areas that have not been sampled thoroughly to date may require consumption guidelines. Consumption guidelines provide a mechanism for

reducing human exposure in the short-term. The California Office of Environmental Health Hazard Assessment (OEHHA), the agency responsible for issuing consumption guidelines, considers a sample of 9 or more fish from a variety of species abundant in a water body to be the minimum needed in order to issue guidance. It is valuable to have information not only on the species with high concentrations, but also the species with low concentrations so anglers can be encouraged to target the low species. Answering this question is essential as a first step in determining the need for more thorough sampling in support of developing consumption guidelines. Large stretches of rivers in the Central Valley that are popular for fishing are already under advisories.

Overall Approach

The overall approach to be taken to answer these two questions is to perform a statewide screening study of bioaccumulation in sport fish in California rivers and streams. Answering these questions, as has been done for lakes and reservoirs and the coast, will provide a basis for decision-makers to understand the scope of the bioaccumulation problem both in rivers and streams and across all of these water body types, and will provide regulators with information needed to establish priorities for both cleanup actions and development of consumption guidelines.

It is anticipated that the screening study may lead to more detailed followup investigations of areas where consumption guidelines and cleanup actions are needed. Funding for these followup studies will come from other local or regional programs rather than the SWAMP statewide monitoring budget.

The approach in this study is consistent with the approaches taken in the previous statewide surveys of bioaccumulation in California lakes and reservoirs (Davis et al. 2010) and on the California coast (BOG 2009). Adding information on bioaccumulation in rivers and streams to that already obtained for the other water body types will complete a comprehensive statewide assessment of the impact of contaminants on the fishing beneficial use in California.

B. 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 rivers and streams.

One significant collaboration will be with the Central Valley Regional Water Quality Control Board (CVRWQCB). The CVRWQCB is providing $16K for supplemental sampling at 13 sites to support development of a mercury TMDL for the Sierra Nevada foothill region. The Water Board will fund analysis of sediment (total mercury: sieved for fines [<63 microns], 2 samples per site), water (total mercury, total methylmercury, SSC; 1 sample per site), and additional fish (total mercury; whatever large species is most abundant at the time of sampling other than rainbow or brown trout; at least 7 inches in total length; 3 samples of the same species per site). It is highly likely

that the additional fish species collected will coincide with the secondary target list for this study (Sacramento pikeminnow, Sacramento sucker, etc. – see Table 3).

The study will also be coordinated with a study conducted by USGS and funded by the State Board to develop assessment tools for evaluating mercury cleanups and for making 303(d) listing decisions. The $700,000 project will be designed to validate the use of sediment mercury concentration data for listing. The project will begin in 2011 with a review of existing data, followed by sampling to fill data gaps in 2012. The project will attempt to establish a consistent relationship between mercury bioaccumulation in fish tissue and sediment total mercury. The study will conduct sampling at 20 stream reaches and 13 lakes and reservoirs in gold mining regions of the Sierra Nevada foothills. Sediment analyses will include total mercury, methylmercury, reactive mercury, and iron and sulfur species. Fish tissue analyses will also be conducted where they are needed. Water analyses will also be conducted. Coordination with the SWAMP survey will allow the USGS study to establish a more extensive empirical dataset to support the development of the assessment tools.

Coordination on a small-scale will occur with the Water Boards from Regions 1 and 6 to obtain information on microcystin in fish fillets. Microcystin is a toxin produced by cyanobacteria that can undergo blooms in eutrophic water bodies. Cyanobacteria blooms are known to occur in the Klamath River in Region 1. In coordination with Region 1, microcystin in fish fillets will be analyzed in fish collected from the Klamath River station and in salmon collected from the Iron Gate Fish Hatchery on the Klamath River. Cyanobacteria blooms also occur in Bridgeport Reservoir in Region 6. In coordination with Region 6, microcystin in fish fillets will be analyzed in fish collected from the station on the East Walker River below Bridgeport Reservoir.

C. Sampling Locations

California has over 211,000 miles of rivers and streams (Davis et al. 2007) that span a diversity of habitats and fish populations, and dense human population centers with a multitude of popular fishing locations. Conducting a statewide survey with a limited budget is a challenge. The approach being employed to sample this vast area is to conduct a complete sampling (or census) of the entire population of the most popular river and stream fishing locations in the state. Popular fishing locations were identified from Stienstra (2004) and discussions with stakeholders. Stienstra (2004) rated fishing spots on a scale of 1 to 10 based on three elements: number of fish, size of fish, and scenic beauty. With the budget available for this survey we are able to sample all of the river and stream locations with a Stienstra rating of 6 or higher. The locations selected for inclusion are listed in Table 2. Table 2 also includes the Stienstra rating and other information regarding the rationale and specifications of each sampling location.

Consideration was also given to information obtained from and priorities expressed by staff from the Regional Water Boards. In some instances, Water Board staff were aware of popular locations not rated or not given a high rating by Stienstra

(2004). In other instances Water Board information needs were a factor that drove inclusion of particular locations.

In all, the available budget can accommodate sampling of 56 river and stream locations. In addition, the budget covers collection and analysis of anadromous species (salmon and steelhead) upon their return migration to six hatcheries (three of each). This was considered to be the most efficient and appropriate approach to collecting these species that range throughout the river systems and are not closely connected with any particular location.

A list of alternate locations was also developed in case problems are encountered at any of the 56 primary candidate locations or additional funds are identified to allow coverage of more locations.

D. Sampling Design At Each Location

1. Species Targeted

Given the focus of the screening study on the fishing beneficial use, the species to be sampled will be those that are commonly caught and consumed by anglers. Other factors considered include abundance, geographic distribution, and value as indicators for the contaminants of concern. The abundance and geographic distribution of species are factors that facilitate sample collection and assessment of spatial patterns in contamination. For example, largemouth bass is very common and widely distributed, and these factors contribute to making this an appropriate indicator species even though it is less popular for consumption than some other species.

The goal of this screening study is to determine whether or not popular fishing locations in California rivers and streams have unacceptably high concentrations of contaminants. Given this goal, the study is focusing on indicator species that tend to accumulate the highest concentrations of the contaminants of concern. Different contaminants tend to reach their highest concentrations in different species. Methylmercury biomagnifies primarily through its accumulation in muscle tissue, so top predators such as largemouth bass tend to have the highest concentrations. In contrast, the organic contaminants of concern biomagnify, but primarily through accumulation in lipid. Concentrations of organics are therefore are also influenced by the lipid content of the species, with species that are higher in lipid having higher concentrations. Bottom-feeding species such as channel catfish and common carp tend to have the highest lipid concentrations in their muscle tissue, and therefore usually have the highest concentrations of organics. Selenium also biomagnifies primarily through accumulation in muscle, but past monitoring in the San Joaquin Valley (Beckon et al. 2010) suggests that bottom-feeders accumulate slightly higher concentrations, perhaps an indication of a stronger association with the benthic food web.

Consequently, this study will target, where possible, two indicator species at each location – a top predator (e.g., largemouth bass) as a mercury indicator and a high lipid,

bottom-feeding species (e.g., channel catfish, common carp) as an organics and selenium indicator. Another advantage of this approach is that it provides a characterization of both the pelagic and benthic food chains. These considerations led USEPA (2000) to recommend this two-species approach in their guidance document for monitoring in support of development of consumption advisories. Most of the river and stream sampling locations selected are expected to have only one abundant group of species: trout. In these cases, one trout species will be sampled as an indicator for all the target analytes. This approach is practical, as it is not common to find multiple trout species in abundance at a single location, and cost-effective. If both rainbow and brown trout are present, brown trout will be collected as they have the potential to have a higher trophic position and accumulate more methylmercury than rainbow trout.

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 3). Primary target species will be given the highest priority. If primary targets are not available in sufficient numbers, secondary targets have been identified. 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 followup studies are needed at any of the sampled locations.

2. Locations

In sport fish sampling it is frequently necessary to sample over a linear course of 0.5 – 1 miles to obtain an adequate number of fish. A sampling location in this study can therefore be thought of as a reach of river or stream channel with an length of 1 mile. An example of the target boundaries for one sampling location is shown in Figure 1.

Since the goal of the study is to characterize human exposure, the locations will be established near centers of most intensive fishing activity for a given river or stream site. For the locations mentioned in Stienstra (2004), an attempt will be made to sample those locations as precisely as possible.

3. Size Ranges and Compositing

Chemical analysis of trace organics is relatively expensive ($544 per sample for PCB congeners and $584 per sample for organochlorine pesticides), and the management questions established for this survey can be addressed with good information on average concentrations, so a compositing strategy will be employed for these chemicals. This is consistent with the approach taken for the previous surveys of lakes and the coast.

Chemical analysis of total mercury is much less expensive ($60 per sample), and, consistent with the previous surveys, SWAMP stakeholders would like to obtain information pertaining to management questions in addition to the ones listed on page 6.

The additional questions relate to evaluation of spatial variation among locations and of trends over time. Consequently, the sampling design for the mercury indicator species (black bass, pikeminnow, and striped bass) includes analysis of mercury in individual fish. For the mercury indicator species, 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 4. The key mercury indicators include largemouth bass, striped bass, and any other black bass species that may be collected. These species have a high trophic position and a strong size:mercury relationship. These species will be analyzed as individuals for mercury. 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). Similarly, the size range for striped bass takes the legal limit for these species (457 mm, or 18 inches) into account, and would provide the range of sizes needed to establish the length:mercury relationship within locations.

In many rivers and streams only trout species will be available. Past sampling of rainbow trout in the Bay-Delta watershed has found low concentrations and a weak size:mercury relationship. Therefore, for these species the ANCOVA approach will not be used. Mercury will generally be analyzed in composites, with a specified size range targeted to control for size rather than a wide span to support a regression-based analysis. These trout will also be analyzed as composites for organics. The size ranges established for trout are based on a combination of sizes prevalent in past sampling (Melwani et al. 2007) and the 75% rule recommended by USEPA (2000) for composite samples. In some cases larger trout may be available. If this occurs (except for rainbow trout larger than 16 inches in anadromous waters because they are considered steelhead and are protected by CDFG), the larger fish will be retained and all of the trout from that location will be analyzed as individuals. This will help in determining whether there are differences between resident or older hatchery transplants and newer hatchery transplants.

Catfish, carp, bullhead, and sucker are the primary targets for high lipid bottom-feeders. These species will be the primary targets for organics and selenium. Organics are expected to be highest in these species based on past monitoring in the Toxic Substances Monitoring Program and other studies (Davis et al. 2007). Selenium is expected to be highest in these species, although the difference is not as distinct as for the organics, based on data from the Grassland Bypass Project (Beckon et al. 2010).

Methylmercury is expected to be highest in the pelagic predators, but concentrations are also expected to be above thresholds for concern in the bottom-feeders, so mercury will be analyzed in the bottom-feeder composites as well. Samples for these species will be analyzed as composites. The size ranges established for trout are based on a combination of sizes prevalent in past sampling (Melwani et al. 2007) and the 75% rule recommended by USEPA (2000) for composite samples.

Secondary targets have been identified that will be collected if the primary targets are not available. These species would be processed for potential analysis of mercury, selenium, and organics. The samples would be analyzed as composites. The size ranges established are based on a combination of sizes prevalent in past sampling (Melwani et al. 2007) and the 75% rule recommended by USEPA (2000) for composite samples.

The sampling crew will be reporting their catch back to the BOG on a weekly basis to make sure that the appropriate samples are collected and to address any unanticipated departures from sampling protocols.

E. Sample Processing and Analysis

Upon collection each fish collected will be tagged with a unique ID. Each fish collected will be linked to the latitude/longitude 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), and weight. Total length changes with freezing and thawing and is best noted in the field for greatest accuracy and because it is the measure used by fishers and wardens to determine whether a fish is legal size. Determining fork length at the same time simplifies matters, and might help with IDs later to sort out freezer mishaps. For large fish (e.g., salmon, carp, and steelhead which can be greater than 40 lb) there will be times that it is necessary to process fish in the field.

Whole fish will be wrapped in aluminum foil and frozen on dry ice for transport 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. Dissection and compositing of muscle tissue samples will be performed following USEPA guidance (USEPA 2000). At the time of dissection, fish will be placed in a clean lab to thaw. After thawing, fish will cleaned by rinsing with de-ionized (DI) and ASTM Type II water, and handled only by personnel wearing polyethylene or powder-free nitrile gloves (glove type is analyte dependent). All dissection materials will be cleaned by scrubbing with Micro® detergent, rinsed with tap water, DI water, and finally ASTM Type II water.

Composites will be created based on the 75% rule recommended by USEPA (2000). In general, fish will have the skin dissected off, and only the fillet muscle tissue will be used for analysis. This is inconsistent with the guidance of USEPA (2000) that recommends that fish with scales have the scales removed and be processed with skin on, and skin is only removed from scaleless fish (e.g. catfish). The BOG is aware of this difference, but favors skin removal. Skin removal has been repeatedly used in past

California monitoring. All fish (with limited exceptions) in Toxic Substances Monitoring Program, the Coastal Fish Contamination Program, and the Fish Mercury Project have also been analyzed skin-off. Processing fish with the skin on is very tedious and results in lower precision because the skin is virtually impossible to homogenize thoroughly and achieving a homogenous sample is difficult. Also, skin-on preparation actually dilutes the measured concentration of mercury because there is less mercury in skin than in muscle tissue. The most ubiquitous contaminant in fish in California that leads to most of our advisories is methylmercury. By doing all preparation skin-off we will be getting more homogeneous samples, better precision for all chemicals, and definitely a better measure of mercury concentrations, which are our largest concern. The analysis of axial fillets without skin was also advised by a bi-national workgroup concerning the monitoring and analysis of mercury in fish (Wiener et al. 2007).

Mercury will be analyzed according to EPA 7473, "Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry" using a Direct Mercury Analyzer. 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 ±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.

Selenium will be digested according to EPA 3052M, "Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices", modified, and analyzed according to EPA 200.8, "Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Mass Spectrometry". 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 ±20% of the true value, or the previous 10 samples must be reanalyzed. Two blanks, a standard reference material (2976 or NRCC DORM-3), as well as a method duplicate and a matrix spike pair will be run with each set of samples.

Organics analyses will be performed by the California Department of Fish and Game Water Pollution Control Lab in Rancho Cordova, CA. Organochlorine pesticides, PCBs, and PBDEs will be analyzed according to WPCL-GC-006 "Analysis of Extractable Synthetic Organic Compounds in Tissues and Sediment (including Organochlorine Pesticides, Polychlorinated Biphenyls (PCBs) and PBDEs) by GC/ECD or Gas Chromatography with detection and quantitation by tandem mass spectrometry (MSMS). Microcystins and microcystin metabolites will be analyzed according to WPCL-LC-065, "Determination of Microcystins and Microcystin Metabolites in Water and Tissue by Enhanced LC/MS/MS." 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 ±25% of the true value, or the previous 10 samples must be reanalyzed. One blank, a laboratory control spike (LCS), a CRM (if available), and a method duplicate and a matrix spike pair will be run with each set of samples.

F. Analytes

Table 5 provides a summary of list of analytes for the study. Since the study is focused on assessing the impacts of bioaccumulation on the fishing beneficial use, the list is driven by concerns over human exposure. Contaminants were included if they were considered likely to provide information that is needed to answer the management questions for the study (see pages 6-7). A detailed list of analytes is provided in Table 6.

Additional discussion of the analytes is provided below.

Ancillary Parameters

Ancillary parameters to be measured in the lab include moisture and lipid (Table 6). Fish sex will also be determined for all samples as it comes at no extra cost and can be valuable in interpreting the data. Each fish collected will be linked to the latitude/longitude where it was collected.

Methylmercury

Methylmercury is the contaminant of greatest concern with respect to bioaccumulation on a statewide basis. Based on past monitoring (reviewed by Davis et al. 2007), methylmercury is expected to exceed thresholds of concern at many locations. Methylmercury will be measured as total mercury. Nearly all of the mercury present in edible fish muscle is methylmercury, and analysis of fish tissue for total mercury provides a valid, cost-effective estimate of methylmercury concentration (Wiener et al. 2007). Mercury will be analyzed in all samples because a substantial proportion of samples of each species are expected to exceed thresholds of concern.

PCBs

PCBs are the contaminant of second greatest concern with respect to bioaccumulation on a statewide basis (Davis et al. 2007). PCBs will be analyzed using a congener specific method. A total of 55 congeners will be analyzed (Table 6). PCBs will be analyzed in one composite sample from each location. The species with the greatest expected concentrations (i.e., the organics indicator species where they are present) will be analyzed.

Legacy pesticides

Based on past monitoring (Davis et al. 2007), legacy pesticides are generally expected to exceed thresholds of concern in a very small percentage of California river and stream locations. Individual compounds recommended by USEPA (2000) will be analyzed (Table 6). Legacy pesticides will be analyzed in one composite sample from each location. The species with the greatest expected concentrations (i.e., the organics indicator species where they are present) will be analyzed.

Selenium

Past monitoring (e.g., Beckon et al. 2010) indicates that selenium concentrations are not likely to be above thresholds in this study. However, selenium analysis of one composite from each location was included primarily to support a national effort by USEPA to develop a selenium criterion for fish tissue.

PBDEs

Few data are currently available on PBDEs in California sport fish, and a threshold of concern has not yet been established. However, a rapid increase in concentrations in the 1990s observed in San Francisco Bay and other parts of the country raised concern about these chemicals, and led to a ban on the production and sale of the penta and octa mixtures in 2006 (Oros et al. 2005). The deca mixture is still produced commercially. A threshold of concern is anticipated to be established soon by USEPA. The most important PBDE congeners with respect to bioaccumulation are PBDEs 47, 99, and 100. Coverage of a larger number of locations was considered a higher priority than inclusion of PBDE analysis, which is relatively expensive ($584 per sample). PBDEs are presently a low priority due to the lack of accepted assessment thresholds. In addition, since PBDEs were not included in the lakes or coast surveys, there are no data to place river data in context. Archived samples will be available for analysis if PBDE analysis is desired in the future. The archiving plan will include selection of a subset of locations that are particularly valuable for trend analysis, and long-term storage of samples from these locations.

Dioxins and Dibenzofurans

Few data are available on dioxins and dibenzofurans in California sport fish. Perhaps the best dataset exists for San Francisco Bay, where samples from 1994, 1997, 2000, 2003, and 2006 indicated that concentrations in high lipid species exceeded a published screening value of 0.3 TEQs (for dioxins and furans only) by five fold (Greenfield et al. 2003). However, there are no known major point sources of dioxins in the Bay Area and the concentrations measured in the Bay are comparable to those in rural areas of the U.S. OEHHA did not include dioxins in their recent evaluation of guidance tissue levels for priority contaminants due to the lack of data for dioxins in fish throughout the state (Klasing and Brodberg 2008). Given the relatively high cost of

dioxin analysis and these other considerations, OEHHA recommended that dioxins not be included in this screening study (Table 7).

Organophophates, PAHs, TBT, and Cadmium

Past monitoring (e.g., San Francisco Bay work – SFBRWQCB 1995) indicates that concentrations of these chemicals in sport fish are generally far below thresholds of concern for human exposure. Therefore, they will not be included in the present study.

Other Emerging Contaminants

Other emerging contaminants are likely to be present in California sport fish. Examples include perfluorinated chemicals, other brominated flame retardants in addition to PBDEs, and others. Thresholds do not exist for these chemicals, so advisories or 303(d) listing are not likely in the near future. However, early detection of increasing concentrations of emerging contaminants can be very valuable for managers, as evidenced by the PBDE example. Measuring emerging contaminants would not directly address the management questions guiding this study, so analysis of these chemicals is not included in the design. Archives of each composite will be retained and made available for analysis of emerging contaminants in the future (see Section G). The archiving plan will include selection of a subset of locations that are particularly valuable for trend analysis, and long-term storage of samples from these locations with particular consideration given to evaluating trends in emerging contaminants.

Microcystin

Concerns regarding microcystin were described in Section III.B.

Omega-3 Fatty Acids

Klasing and Brodberg (2008) concluded that there is a significant body of evidence and general scientific consensus that eating fish at dietary levels that are easily achievable, but well above national average consumption rates, appears to promote significant health benefits, including decreased mortality, and that because of the unique health benefits associated with fish consumption, the advisory process should be expanded beyond a simple risk paradigm in order to best promote the overall health of the fish consumer. Much of the health benefits of fish consumption are derived from their relatively high content of key omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). When these data are available, OEHHA can take them into consideration in developing safe eating guidelines. Few data are available on the omega-3 content of wild fish. Due to the limited funding available, omega-3 fatty acids were not included on the analyte list.

F. Quality Assurance

This effort will adhere to quality assurance requirements established for the SWAMP. A QAPP specific to this effort is in preparation (Bonnema 2011).

G. Archiving

Samples will be stored in both short-term and long-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. These samples are intended for the analysis of chemicals which are not expected to degrade in five years of storage at -20 °C. 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.

Through a partnership with the Regional Monitoring Program for Water Quality in the San Francisco Estuary, selected samples can also be stored in a state-of-the-art long-term storage facility operated by NIST (Klosterhaus 2010). Samples in this long-term archive will be stored at -150 °C in liquid nitrogen (LN2) vapor freezers and are primarily intended for use in the identification of time trends occurring over decadal time frames (i.e. > 10 years). Samples stored in LN2 vapor freezers are not expected to degrade over time and are thus reliable for chemical contaminant studies occurring well into the future. The long-term archive was established in 2010 and is located in the Marine Environmental Specimen Bank (Marine ESB), operated by NIST at the Hollings Marine Laboratory in Charleston, SC. The Marine ESB is characterized by having well-developed banking protocols and standard operating procedures (SOPs), computerized sample tracking (chain-of-custody) systems, maintenance of many forms of data associated with original specimens, and large investments in state-of-the-art facilities and equipment required to store specimens over long periods of time. The Marine ESB emphasizes cryogenic storage using LN2 vapor storage freezers, security systems, and electronic monitoring of storage conditions 24 hours a day, 365 days a year. The Marine ESB also maintains high efficiency particulate air (HEPA)-filtered clean air laboratories for cleaning storage containers, preparing banked specimens for analysis, and processing and storing samples. Additional details about the Marine ESB facility are described in Pugh et al. (2007).

A number of small volume sub-samples, rather than one or two large volume samples, are prepared for archiving to avoid subjecting the samples to several freeze-thaw cycles. Each sub-sample contains a sufficient amount of material for most chemical analysis, and when needed, can be removed from the freezer and sent to the appropriate laboratory without the need to sub-sample.

For routine sampling locations, up to five 50 g aliquots of each composite analyzed for organics will be archived. This will provide an integrative, representative sample for each location that can be reanalyzed in later years to confirm earlier analyses, look for new chemicals of concern, provide material for application of new analytical methods, provide material for other ecological research, and other purposes. Samples for the short-term archive will be stored in either glass jars with Teflon-lined lids for non-fluorinated organic chemical and trace metal analysis or in polyethylene (PE) or polypropylene (PP) for fluorinated chemical (i.e. PFCs) or trace metals analysis. Four of the five archive jars will be glass with a Teflon lined lid (e.g., I-Chem 200 series glass jars). One separate aliquot will be kept in a polypropylene jar for potential analysis of perfluorinated compounds. These archived samples will be stored at -20°C.

At sites considered a high priority for trend analysis of emerging contaminants (Table 2), five aliquots will be archived. Three of the five archived aliquots will be stored in the long-term archive at NIST. Two 15-20 g aliquots for the long-term archive will be stored in two 22 ml Teflon jars for non-fluorinated organic chemical and trace metal analysis and one 15-20 g aliquot in two 10 ml PP cryovials for fluorinated chemical analysis (in order to obtain sufficient mass for future analysis, two cryovials will replace one standard archive jar). Glass and PE/PP containers are the least expensive containers and thus are used when possible; however, only Teflon and PP cryovials are able to withstand liquid nitrogen temperatures for long periods without shattering and are therefore used for storing samples in the long-term archive. The other two of the five aliquots will be stored in 50 g glass jars with Teflon lids and archived at -20C.

Teflon and cryo-containers used for the storage of samples in the long-term archive are pre-cleaned by NIST Marine ESB personnel using established protocols (Pugh et al. 2007) and shipped to SFEI contract laboratories or designated field personnel for use. For storage of samples in the short-term archive, glass and plastic containers are pre-cleaned using appropriate acids or solvents by MPSL-DFG or purchased pre-cleaned commercially (e.g. from Fisher or ESS Vial). For containers purchased 'pre-cleaned' from ESS Vial or other companies, a minimum of two per shipment will not be opened and kept in storage with the other samples in case container contamination issues arise.

H. Ancillary Data

In addition to the primary and secondary target species, other species will also be observed in the process of sample 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 followup studies are needed in any of the sampled locations.

I. Timing

Sampling will be conducted from February 2011 through November 2011. Seasonal variation in body condition and reproductive physiology are recognized as factors that could affect contaminant concentrations. However, sampling as many

locations as possible is essential to a statewide assessment, and it will take this many months to sample the locations targeted.

J. Data Assessment

MQ1 will be assessed by comparing results from each location to thresholds established by OEHHA in Klasing and Brodberg (2008) (Table 7). Maps, histograms, and frequency distributions will be prepared to summarize these comparisons.

MQ2 will be assessed in consultation with OEHHA.

K. Products and Timeline

A report on the 2011 sampling will be drafted by January 2013. The final report, incorporating revisions in response to reviewer comments, will be completed and released in May 2013.

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Table 1. Bioaccumulation monitoring assessment framework for the fishing beneficial use.

D.1. Determine the status of the fishing beneficial use throughout the State with respect to bioaccumulation of toxic pollutants

D.1.1 What are the extent and location of water bodies with sufficient evidence to indicate that the fishing beneficial use is at risk due to pollutant bioaccumulation?

D.1.2 What are the extent and location of water bodies with some evidence indicating the fishing beneficial use is at risk due to pollutant bioaccumulation?

D.1.3 What are the extent and location of water bodies with no evidence indicating the fishing beneficial use is at risk due to pollutant bioaccumulation?

D.1.4 What are the proportions of water bodies in the State and each region falling within the three categories defined in questions D.1.1, D.1.2, and D.1.3?

D.2. Assess trends in the impact of bioaccumulation on the fishing beneficial use throughout the State

D.2.1 Are water bodies improving or deteriorating with respect to the impact of bioaccumulation on the fishing beneficial use?

D.2.1.1 Have water bodies fully supporting the fishing beneficial use become impaired?

D.2.1.2 Has full support of the fishing beneficial use been restored for previously impaired water bodies?

D.2.2 What are the trends in proportions of water bodies falling within the three categories defined in questions D.1.1, D.1.2, and D.1.3 regionally and statewide?

D.3. Evaluate sources and pathways of bioaccumulative pollutants impacting the fishing beneficial use

D.3.1 What are the magnitude and relative importance of pollutants that bioaccumulate and indirect causes of bioaccumulation throughout each Region and the state as a whole?

D.3.2 How is the relative importance of different sources and pathways of bioaccumulative pollutants that impact the fishing beneficial use changing over time on a regional and statewide basis?

D.4. Provide the monitoring information needed to evaluate the effectiveness of management actions in reducing the impact of bioaccumulation on the fishing beneficial use

D.4.1 What are the management actions that are being employed to reduce the impact of bioaccumulation on the fishing beneficial use regionally and statewide?

D.4.2 How has the impact of bioaccumulation on the fishing beneficial use been affected by management actions regionally and statewide?

Table 2. Sampling locations.

Region	Station Code	Station Name	Rationale	Trend Station	SPoT Station	Target Specie	Target Latitude	Target Longitude
1	111VD6485	Van Duzen River near Dinsmore	Replaced Smith River @ Crescent City	X		trout	40.48892	-123.62577
1	103SFSRGC	South Fork Smith River near Goose Creek	ranked high by Stienstra			trout	41.68481	-123.91932
1	109MADHAT	Mad River (Mad River Fish Hatchery)	Requested by RB			steelhead	40.85413	-123.99074
1	111EELFCS	Eel River (Van Arsdale Fishing Counting Stati	Requested by RB			salmon	39.38569	-123.11677
1	113GUASRP	Gualala River, South Fork near Rockpile Creek	ranked high by Stienstra			trout	38.75050	-123.47024
1	114CCPOTV	Cold Creek at Potter Valley	ranked high by Stienstra			trout	39.24405	-123.12179
1	114RURHAT	Russian River (Coyote Valley Dam Egg Collection Site)	Large amount industry/agriculture			steelhead	39.19679	-123.18668
1	114LDSROR	Laguna de Santa Rosa at Occidental Rd	Large immigrant population catching	X		blackfish?	38.42381	-122.82803
1	105KLMHAT	Klamath River (Iron Gate FH)	Microcystin, Huge Tribal fishery			salmon	41.92956	-122.44210
1	105KLAMOR	Klamath River at Orleans	ranked high by Stienstra	X		trout	41.30162	-123.53607
1	106TRWILC	Trinity River at Willow Creek	ranked high by Stienstra	X		trout	40.93784	-123.61863
5	541MER522	San Joaquin River at Lander Avenue	ranked high by Stienstra	X	X	trout, largemouth bass	37.29528	-120.85028
5	506SHA950	Pit River at Big Bend	ranked high by Stienstra			trout	41.02071	-121.91032
5	526FRIRMA	Fall River at Island Road near McArthur	ranked high by Stienstra			trout	41.08887	-121.49308
5	526HCRDOS	Hat Creek downstream Old Station	Se data would be helpful for comparison to the Hg data.			trout	40.73061	-121.43757
5	505MRLFFC	McCloud River at Lower Falls below Fowlers Camp	ranked high by Stienstra			trout	41.24317	-122.02470
5	525SRCCSP	Sacramento River near Castle Crags State Park	ranked high by Stienstra			trout	41.14893	-122.31209
5	508ADVSBB	Sacramento River at Bend Bridge Near Red Bluf	ranked high by Stienstra	X		trout, pike minnow, sucker	40.25283	-122.22667
5	520SACLSA	Sacramento River at Colusa near Bridge Street	already 303(d) listed for PCBs	X	X	largemouth bass, trout, sunfish,striped bass	39.21415	-122.00031
5	515FRUPYC	Feather River upstream Yuba City	OCs TMDL underway; important site for future Hg control program compliance	X		largemouth bass, trout, striped bass	39.33486	-121.63230
5	510INDM44	Sacramento River at RM44	already 303(d) listed for PCBs. However, this is a good long-term monitoring location. Delta Hg Control Program compliance location.	X	X (3.5 mi upstream of SPoT)	largemouth bass	38.43520	-121.51960
5	510ST1492	San Joaquin River off Pt Antioch near fishing	OCs TMDL underway. Very near Delta Hg Control Program compliance location.	X		striped bass, largemouth bass, catfish	38.03233	-121.76566

Table 2. Sampling locations.

Region	Station Code	Station Name	Rationale	Trend Station	SPoT Station	Target Specie	Target Latitude	Target Longitude
5	519SWPDCP	American River near Discovery Park	more PCBs data and Se data might be useful	X	X	largemouth bass, catfish, sunfish	38.59970	-121.50550
5	518SPCOCR	Spanish Creek at Oakland Camp Road crossing	Could be important to Tribes.			trout	39.97902	-120.90526
5	518PLU901	Feather River Middle Fork at Sloat	ranked high by Stienstra			trout	39.86085	-120.72789
5	518SED030	Warner Creek 30	Could be important to Tribes.			trout	40.36374	-121.30668
5	518SED082	Jamison Creek 82	Could be important to Tribes. Also, a mine cleanup is planned for 2011 that could affect fish Hg levels.			trout	39.74051	-120.70642
5	518MFFRUC	Feather River, Middle Fork upstream Clio	ranked high by Stienstra	X		trout	39.74776	-120.56605
5	518NFFRBB	Feather River, North Fork above Beldon Bridge	close to site ranked high by Stienstra (below Bridge site catch and release only)			trout	40.01370	-121.22616
5	517YRSFNW	Yuba River, South Fork near Washington	ranked high by Stienstra			trout	39.36081	-120.78331
5	517YRSFLS	Yuba River, South Fork upstream Lake Spaulding	ranked high by Stienstra			trout	39.30588	-120.53559
5	514RRRUBS	Rubicon River downstream Rubicon Springs	ranked high by Stienstra			trout	39.02538	-120.25095
5	514ARSFCL	American River, South Fork at Coloma	ranked high by Stienstra			trout	38.80123	-120.88978
5	541INDVRN	San Joaquin River at Vernalis (FMP)	OCs TMDL underway. However, this is a good long-term monitoring location. Delta Hg Control Program compliance location. Ranked high by Stienstra.	X	X	largemouth bass, catfish	37.67130	-121.25920
5	544MOKNH5	Mokelumne River near I-5	OC TMDL underway. Delta Hg Control Program compliance location.	X	X (5 mi away)	largemouth bass	38.25593	-121.44257
5	544LSAC12	San Joaquin R at Louis Park	ranked high by Stienstra	X		largemouth bass, catfish	37.95558	-121.34626
5	544MREMPC	Middle River near Empire Cut	OC TMDL underway. Very near Delta Hg Control Program compliance location.	X		largemouth bass	37.96942	-121.53339
5	531ADVMOK	Mokelumne River (Mokelumne River FH)	Representative of steelhead/salmon in rivers.	X		steelhead/salm	38.2254	-121.02562
5	540SJRMFA	San Joaquin River, Middle Fork near Agnew Mea	ranked high by Stienstra			trout	37.67504	-119.09097
5	537MCRABB	Merced River at Briceburg	ranked high by Stienstra			trout	37.60495	-119.96703
5	536TRCHEC	Tuolumne River at Cherry Creek	ranked high by Stienstra			trout	37.88902	-119.97229
5	532MFCRPP	Cosumnes River, Middle Fork at Pi Pi	Only public fishing site in the entire Cosumnes Watershed mentioned in the DFG Online Fishing Guide.			trout	38.56680	-120.44250
5	554KRKRNV	Kern River at Kernville	ranked high by Stienstra			trout	35.75578	-118.42219
6	633WCR004	West Fork Carson River, at HWY 89 (Hope Valley)	ranked high by Stienstra			trout	38.77819	-119.91694

Table 2. Sampling locations.

Region	Station Code	Station Name	Rationale	Trend Station	SPoT Station	Target Specie	Target Latitude	Target Longitude
6	635MTR002	Middle Truckee River, Below Bronco Cr	ranked high by Stienstra	X		trout	39.38455	-120.02211
6	632ECR009	Carson River, East Fork upstream of Hangman's	ranked high by Stienstra			trout	38.65837	-119.72553
6	630EWK002	East Walker River below Bridgeport Reservoir	Microcystin	X		trout	38.34209	-119.20743
6	630VIR002	Virginia Creek S of Bridgeport	ranked high by Stienstra			trout	38.15060	-119.18927
6	631WWK011	West Walker River, near Chris Flat Campground	ranked high by Stienstra			trout	38.39542	-119.45165
6	637CE0143	Susan River 0.6mi above Jensen Slough	requested by RB6	X		trout	40.41203	-120.64571
6	630BUC003	Buckeye Cr, above Eagle Cr (abv campground)	ranked high by Stienstra			trout	38.23491	-119.35887
6	601LVC001	Lee Vining Cr, at Moraine Camp	ranked high by Stienstra			trout	37.92998	-119.16364
6	603LOW009	Owens River at Hwy 6	ranked high by Stienstra	X		trout	37.39752	-118.35485
6	603BSP009	Bishop Creek near USFS boundary	ranked high by Stienstra			trout	37.33046	-118.49630
6	603BIG003	Big Pine Creek, near USFS boundary	ranked high by Stienstra			trout	37.14488	-118.31767
6	603IND002	Independence Creek above Independence	ranked high by Stienstra			trout	36.79825	-118.20801
6	603LPC001	Lone Pine Creek, at USGS gage	ranked high by Stienstra			trout	36.60118	-118.08231
7	715CRBLYT	Colorado River at Blythe	ranked high by Stienstra	X		largemouth bass, bluegill, catfish	33.76634	-114.50677
8	801SARERL	Santa Ana River E of Redlands	ranked high by Stienstra	X		trout	34.18105	-116.92853

Table 3. Target species and their characteristics.

	Foraging Type		Trophic Level	Distribution			
Species	Water column	Bottom feeder		Low Eleva-tion	Foothi lls	High Elevati on	Priority for Collection
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
Striped bass	X		4	x			2
White catfish		X	4	x	x		2
Brown bullhead		X	3	x			2
Channel catfish		X	4	**X**	**X**		**1**
Common carp		X	3	**X**	**X**		**1**
Sacramento sucker		X	3	x	x		2
Tilapia		X	3				2
Bluegill	X		3	**X**	**X**		2
Green sunfish	X		3	**X**	**X**		2
Black crappie	X		3/4	x	x		2
Redear sunfish	X		3	**X**	**X**		2
Rainbow trout	X		3	x	x	**X**	**1**
Brown trout	X		3/4		x	x	**1**
Brook 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 "A" primary target for collection "B" secondary target for collection

Table 4. Target species, size ranges, and processing instructions.

	Process as Individuals and/or Composites	Process for Organics: 1=first choice, 2=second choice	Numbers and Size Ranges (mm)
Primary Targets			
Group 1: Pelagic Predators			
Black bass (largemouth, smallmouth, spotted)	I*	2	2X(200-249), 2X(250-304), 5X(305-407), 2X(>407)
Striped bass	I*	2	2X(<250), 2X(250-457), 6X(>457)
Sacramento pikeminnow	I*	2	3X(200-300), 3X(300-400), 3X(400-500)
Rainbow trout	C*	2	5X(300-400)
Brown trout	C*	2	5X(300-400), and keep up to five fish > 400 if present
Brook trout	C*	2	5X(300-400), and keep up to five fish > 400 if present
Group 2: Bottom feeder			
White catfish	C	1	5X(229-305)
Channel catfish	C	1	5X(375-500)
Common carp	C	1	5X(450-600)
Brown bullhead	C	1	5X(262-350)
Sacramento sucker	C	1	5X(375-500)
Secondary Targets: collect these if primary targets are not available			
Bluegill	C	2	5X(127-170)
Redear sunfish	C	2	5X(165-220)
Black crappie	C	2	5X(187-250)
Tilapia	C	2	??
Green sunfish	C	2	??
Kokanee		2	??

I* - process as individuals for mercury, also prepare a composite using middle of size range for selenium and if other species are not available for organics;
C* - process as composites, but as individuals for mercury if fish > 400 mm are collected

Table 5. Summary of analytes included in the study.

Analyte	Included in Screening Study?
Methylmercury[1]	Some individuals, all composites
Selenium	All composites
PCBs	One composite per location
DDTs	One composite per location
Dieldrin	One composite per location
Aldrin	One composite per location
Chlordanes	One composite per location
Microcystins	Included at two locations and a hatchery
PBDEs	Not included
Dioxins	Not included
Perfluorinated chemicals	Not included
Omega-3 fatty acids	Not included

[1] Measured as total mercury, which provides a direct estimate of methylmercury in fish muscle.

Table 6. Parameters to be measured.

FISH ATTRIBUTES

1. Total length
2. Fork length
3. Weight
4. Sex
5. Moisture
6. Lipid content

METALS AND METALLOIDS

1. Total mercury
2. Selenium

PESTICIDES

Chlordanes

1. Chlordane, cis-
2. Chlordane, trans-
3. Heptachlor
4. Heptachlor epoxide
5. Nonachlor, cis-
6. Nonachlor, trans-
7. Oxychlordane

DDTs

1. DDD(o,p')
2. DDD(p,p')
3. DDE(o,p')
4. DDE(p,p')
5. DDMU(p,p')
6. DDT(o,p')
7. DDT(p,p')

Table 6. Parameters to be measured (continued).

Cyclodienes

1. Aldrin
2. Dieldrin
3. Endrin

HCHs

1. HCH, alpha
2. HCH, beta

Others

1. Dacthal
2. Endosulfan I
3. Hexachlorobenzene
4. Methoxychlor
5. Mirex
6. Oxadiazon

PCBs

1. PCB 008
2. PCB 011
3. PCB 018
4. PCB 027
5. PCB 028
6. PCB 029
7. PCB 031
8. PCB 033
9. PCB 044
10. PCB 049
11. PCB 052

Table 6. Parameters to be measured (continued).

12. PCB 056
13. PCB 060
14. PCB 064
15. PCB 066
16. PCB 070
17. PCB 074
18. PCB 077
19. PCB 087
20. PCB 095
21. PCB 097
22. PCB 099
23. PCB 101
24. PCB 105
25. PCB 110
26. PCB 114
27. PCB 118
28. PCB 126
29. PCB 128
30. PCB 137
31. PCB 138
32. PCB 141
33. PCB 146
34. PCB 149
35. PCB 151
36. PCB 153
37. PCB 156
38. PCB 157
39. PCB 158
40. PCB 169
41. PCB 170

Table 6. Parameters to be measured (continued).

42. PCB 174
43. PCB 177
44. PCB 180
45. PCB 183
46. PCB 187
47. PCB 189
48. PCB 194
49. PCB 195
50. PCB 198/199
51. PCB 200
52. PCB 201
53. PCB 203
54. PCB 206
55. PCB 209

Algal Toxins

Microcystins
1. MC-RR
2. MC-LR
3. MC-YR
4. MC-LA

MC metabolites
1. Desmethyl-LR
2. Desmethyl-RR

Cyanotoxins
1. anatoxin a

Table 7. Assessment thresholds (ng/g wet weight).

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 ATLs 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

Figure 1. Example of the 0.5 mi sampling radius surrounding each sampling location.

Appendix III: MPSL-DFG SOPs

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

Appendix III A: Modifications to EPA 3052

Modification of EPA Method 3052

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

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

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

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

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

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

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

Method # MPSL-101

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

1.0 Scope and Application

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

2.0 Summary of Method

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

3.0 Interferences

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

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

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

4.0 Apparatus and Materials

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

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

4.3 Garbage Bag, clear 30 gallon

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

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

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

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

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

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

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

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

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

4.13 Heavy Duty Aluminum Foil

4.14 Homogenization Jar: Büchi Analytical Part # 26441

4.15 Immersion Heater: VWR Part # 33897-208

4.16 Lab Coats

4.17 Non-metal Scrub Brush

4.18 Non-metal Bottle Brush

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

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

4.21 Plastic Knife

4.22 Polyethylene Bin, 63 L

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

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

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

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

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

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

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

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

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

4.32 Polyethylene Scoop: VWR Part # 56920-400

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

4.34 Polypropylene Cutter Tool: Büchi Analytical Part #24225

4.35 Polypropylene Diaphragm Seal: Büchi Analytical Part # 26900

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

4.37 Polypropylene Spacer: Büchi Analytical Part # 26909

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

4.39 Sapphire Thermowell: CEM Part # 326280

4.40 Shoe covers: Cellucap Franklin Part # 28033

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

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

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

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

4.45 Teflon HP500+ Control Cover: CEM Part # 431255

4.46 Teflon HP500+ Cover: CEM Part # 431250

4.47 Teflon HP500+ Liner: CEM Part # 431110

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

4.49 Teflon Tape (plumbing tape)

4.50 Teflon Thermowell Nut: CEM Part #325028

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

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

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

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

4.55 Teflon Wash Bottle, 500 mL

4.56 Teflon Vent Nut: CEM Part # 431313

4.57 Titanium Cutter Screw: Büchi Analytical Part # 34376

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

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

4.60 Titanium Displacement Disc: Büchi Analytical Part # 26471

4.61 Ventilation Hood

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

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

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

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

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

5.0 Reagents

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

5.1 Tap water (Tap)

5.2 Deionized water (DI)

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

5.4 All-purpose Cleaner, 409™

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

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

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

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

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

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

5.11 Methanol: VWR Part # JT9263-3

5.12 Micro Detergent: ColeParmer Part # 18100-20

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

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

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

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

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

5.18 Petroleum Ether: VWR Part # JT9265-3

6.0 Sample Collection, Preservation and Handling

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

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

7.0 Procedures

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

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

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

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

7.1.1 Carboy

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

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

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

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

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

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

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

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

7.1.2 Carboy Spigots and Tubing

7.1.2.1 Soak in dilute Micro/Tap solution overnight.

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

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

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

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

7.1.3 Syringes for Field Filtration (not for Hg use)

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

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

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

7.1.3.4 Rinse three to five times with MilliQ.

7.1.3.5 Allow to completely dry on clean Crew Wipers.

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

7.1.4 Polyethylene Water Containers (not for Hg use)

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

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

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

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

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

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

7.1.5 Polyethylene Tissue Dissection Containers

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

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

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

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

7.1.5.5 Fill with MilliQ and soak for three days.

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

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

7.1.6 Polyethylene Scoops

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

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

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

7.1.6.4 Rinse three to five times with MilliQ.

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

7.1.7 Polypropylene Knives for Aliquoting

7.1.7.1 Scrub knives in dilute Mirco/Tap solution.

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

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

7.1.8 Teflon Digestion Vessel and Lids

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

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

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

7.1.8.4 Rinse three to five times in MilliQ.

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

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

7.1.9 Teflon and Sapphire Digestion Nuts and Thermowells

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

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

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

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

7.1.9.5 Rinse three to five times in MilliQ.

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

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

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

7.1.10 Polyethylene Digestate Bottles

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

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

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

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

7.1.10.5 Fill with MilliQ and soak for three days.

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

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

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

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

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

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

7.1.11.4 Rinse each tube and cap three times with MilliQ.

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

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

7.2 Mercury Only Sample Containers

7.2.1 Water Composite Bottles, 4L

7.2.1.1 Caps do not get micro cleaned.

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

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

7.2.1.4 Rinse with Tap until no more suds appear.

7.2.1.5 Rinse three times with DI.

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

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

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

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

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

7.2.2 Tubing Sets

7.2.2.1 Cable Ties

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

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

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

7.2.2.2 Polyethylene Caps with Holes

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

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

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

7.2.2.2.4 Soak in 4N HCl for 3 days.

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

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

7.2.2.3 Teflon Tubing

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

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

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

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

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

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

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

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

7.2.2.4 C-Flex Tubing

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

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

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

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

7.2.2.4.5 Rinse three to four times in MilliQ.

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

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

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

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

7.2.2.5 Tubing Set Assembly (using cleaned parts described above)

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

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

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

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

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

7.2.2.6 In-Lab Mercury Filters

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

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

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

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

7.2.2.6.5 Discard MilliQ after each rinse.

7.2.3 Water Sample Bottles, 250 mL

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

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

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

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

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

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

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

7.2.4.1 Rinse new tubes in dilute Micro/Tap.

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

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

7.2.4.4 Rinse cooled tubes three to four times in MilliQ.

7.2.4.5 Let dry completely on clean Crew Wipers.

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

7.3 Methylmercury Only Sample Containers

7.3.1 Teflon Digestion or Distillation Vials

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

7.3.1.2 Rinse three times in DI.

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

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

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

7.3.2 Teflon Distillation Caps and Tubing

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

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

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

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

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

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

7.2.4.1 Rinse new tubes in dilute Micro/Tap.

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

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

7.2.4.4 Rinse cooled tubes three to four times in MilliQ.

7.2.4.5 Let dry completely on clean Crew Wipers.

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

7.3 Methylmercury Only Sample Containers

7.3.1 Teflon Digestion or Distillation Vials

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

7.3.1.2 Rinse three times in DI.

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

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

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

7.3.2 Teflon Distillation Caps and Tubing

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

7.3.2.2 Rinse three times in DI.

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

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

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

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

7.4 Organic Sample Containers

7.4.1 Aluminum Foil Sheets

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

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

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

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

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

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

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

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

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

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

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

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

7.4.2.4 Set jars aside in the hood to dry.

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

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

7.5.1 Teflon sheets

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

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

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

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

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

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

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

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

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

7.5.2 Teflon Squares for Dissection Jars

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

7.5.2.2 Soak in 6% Seastar HNO_3 coldbath overnight.

7.5.2.3 Rinse three times with MilliQ.

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

7.5.2.5 Lay on clean crew wipers to dry.

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

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

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

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

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

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

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

7.5.3.4 Rinse each jar three times in MilliQ.

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

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

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

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

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

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

7.5.4.3 Rinse 3 times with MilliQ.

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

7.5.4.5 Allow parts to dry completely before assembly and homogenization.

8.0 Analytical Procedure

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

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

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

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

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

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

9.0 Quality Control

9.1 See individual methods.

10.0 Method Performance

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

10.2 Carboys are tested for all metals after cleaning.

11.0 References

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

Method # MPSL-102a

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

1.0 Scope and Application

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

2.0 Summary of Method

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

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

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

3.0 Interferences

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

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

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

4.0 Apparatus and Materials

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

4.1 Anchor Chains

4.2 Backpack Shocker (electro-fishing)

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

4.4 Bone Saw

4.5 Camera, digital

4.6 Cast Nets (10' and 12')

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

4.8 Daypacks

4.9 Depth Finder

4.10 Dip Nets

4.11 Dry Ice or Ice

4.12 Gill Nets (various sizes)

4.13 GPS

4.14 Heavy Duty Aluminum Foil, prepared

4.15 Heavy Duty plastic bags, Clear 30 gallon

4.16 Inflatable Buoy

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

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

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

4.20 Otter Trawl (various widths as appropriate)

4.21 Permanent Marking Pen

4.22 Plastic bucket, 30 gallon

4.23 Plastic Ice Chests

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

4.25 Polypropylene Mesh, 76mm wide with 13mm mesh

4.26 Polypropylene Mesh, 50mm wide with 7mm mesh

4.27 Polypropylene Line, 16mm

4.28 Rods and Reels

4.29 Screw in Earth Anchor, 4-6" diameter

4.30 Scuba Gear

4.31 Seines (various size mesh and lengths as appropriate)

4.32 Stainless Steel Dive Knives

4.33 Trap Nets (hoop or fyke nets)

4.34 Teflon Forceps

4.35 Teflon Sheet, prepared

4.36 Teflon Wash Bottle, 500 mL

4.37 Wading Gear

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

5.0 Reagents

5.1 Tap water (Tap)

5.2 Deionized water (DI)

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

5.4 Micro Detergent: ColeParmer Part # 18100-20

5.5 Methanol: VWR Part # JT9263-3

5.6 Petroleum Ether: VWR Part # JT9265-3

6.0 Sample Collection, Preservation and Handling

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

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

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

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

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

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

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

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

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

7.0 Procedure

7.1 Sample collection - mussels and clams

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

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

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

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

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

7.2 Transplanted sample deployment

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

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

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

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

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

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

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

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

7.3 Sample Retrieval

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

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

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

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

7.4 Sample Collection – Fish

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

7.5 Sample Collection- Crabs

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

8.0 Analytical Procedure

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

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

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

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

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

9.0 Quality Control

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

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

10.0 References

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

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

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

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1.0 SCOPE AND APPLICATION

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

2.0 SUMMARY OF METHOD

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

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

4.0 SAFETY

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

5.0 EQUIPMENT

Perkin Elmer FIMS system with autosampler

6.0 REAGENTS

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

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

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

6.4. Stannous Chloride (1.1%) Baker Reagent Grade

7.0 PROCEDURES

7.1. Preparation of Mercury Standard Solutions

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

7.2. Mercury Analyzer Calibration Sequence

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

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

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

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

7.3. ANALYZING SAMPLES

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

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

8.0 QUALITY ASSURANCE

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

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

9.0 CORRECTIVE ACTION

Key to Corrective Actions in Table 8.1:

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

12. Clean Instrument
13. Check reagent sources

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

10.0 EQUATIONS

10.1. Mercury in sediment and tissue

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

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

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

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

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

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

Method # MPSL-104

SAMPLE RECEIPT AND CHECK-IN

1.0 Scope and Application

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

2.0 Summary of Method

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

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

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

3.0 Interferences

3.1 Not Applicable

4.0 Apparatus and Materials

4.1 Bound logbook with numbered pages

4.2 Permanent Pen

4.3 Permanent Marker (i.e. Sharpie)

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

4.5 3-Ring Binder

4.6 Copy Machine

5.0 Reagents

5.1 Not Applicable

6.0 Sample Collection

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

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

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

7.0 Procedure

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

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

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

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

7.4.1 Date of entry.

7.4.2 Project Name and Number

7.4.3 Unique 9-digit Lab Number

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

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

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

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

7.4.5 Sample Identification; station information taken directly from the COC

7.4.6 Analyte of suite of analytes requested for each sample.

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

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

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

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

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

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

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

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

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

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

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

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

8.0 Analytical Procedure

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

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

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

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

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

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

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

9.0 Quality Control

9.1 MS Access database does not allow duplicate Lab Numbers

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

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

Method # MPSL-105

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

1.0 Scope and Application

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

2.0 Summary of Method

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

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

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

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

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

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

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

3.0 Interferences

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

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

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

4.0 Apparatus and Materials

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

4.1 Brinkmann Polytron model PT 10-35

4.2 Büchi Mixer B-400

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

4.4 Ear Protection

4.5 Fillet knives

4.6 Glass Jar Class 100, 500 mL, prepared

4.7 Glass Jar Class 200, 500 mL, prepared

4.8 Glass Jar Class 300, 500 mL, prepared

4.9 Glass Jar Class 100, 125 mL, prepared

4.10 Glass Jar Class 200, 125 mL, prepared

4.11 Glass Jar Class 300, 125 mL, prepared

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

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

4.14 Heavy Duty Beakers, 1000 mL

4.15 Heavy Duty Beakers, 400 mL

4.16 Garbage Bags, Clear 30 gallon

4.17 Lab Coats

4.18 Plastic Knives, prepared

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

4.20 Polyethylene (HDPE) jar, 30 mL, prepared

4.21 Polyethylene (HDPE) jar, 125 mL, prepared

4.22 Shoe Covers: Cellucap Franklin Part # 28033

4.23 Teflon Forceps, prepared

4.24 Titanium Bars

4.25 Titanium Generator: Brinkmann Part # PTA 20

5.0 Reagents

5.1 Tap water (Tap)

5.2 Deionized water (DI)

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

5.4 Micro Detergent: ColeParmer Part # 18100-20

5.5 Methanol: VWR Part # JT9263-3

5.6 Petroleum Ether: VWR Part # JT9265-3

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

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

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

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

6.0 Sample Collection, Preservation and Handling

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

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

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

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

7.0 Procedure

7.1 Dissection

7.1.1 Bivalve Dissection

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

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

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

7.1.1.4 TM Bivalve Dissection

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

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

Note: Gonads are not removed from clams.

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

7.1.1.5 SO Bivalve Dissection

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

7.1.1.6 "Split" Bivalve Dissection

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

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

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

7.1.2 Fish Dissection

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

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

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

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

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

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

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

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

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

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

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

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

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

7.1.2.13 Total length, fork length and weight are recorded.

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

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

7.2 Homogenization

7.2.1 TM Bivalve Homogenization

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

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

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

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

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

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

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

7.2.2 SO Bivalve Homogenization

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

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

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

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

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

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

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

7.2.3 "Split" Bivalve (TM and SO) Homogenization

7.2.3.1 Samples are homogenized as TM with the following exceptions:

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

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

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

7.2.4 Fish

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

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

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

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

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

7.2.4.6 Assemble the homogenizer according to manufacturer specifications.

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

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

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

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

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

8.0 Analytical Procedure

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

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

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

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

9.0 Quality Control

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

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

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

10.0 Method Performance

10.1 See individual analytical methods.

11.0 References

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

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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: DFG-WPCL SOPs

DFG-WPCL EPA Modifications and Laboratory Procedures			
Page	Procedure/Equipment	SOP number	Revision Date
A	Sample Custody, Receipt, and Storage	WPCL-AB-001	June 2011
B	Determination of OC and PCB in Sediment and Tissue – Modifications to EPA 8081B and 8082	WPCL-GC-006	Mar 2005
C	Procedures for Disposal of Waste	HAZMAT_Rev4_SOP	Mar 2009
D	Protocol for Corrective Action Procedures	WPCL-QA-050	Dec 2009
E	Method: Microcystins and Biotoxins by LC/MS/MS	WPCL-LC-065	June 2008

Appendix IV A: Sample Custody, Receiving and Storage

STANDARD OPERATING PROCEDURE
TITLE: Sample Custody, Receiving and Storage

REVISION HISTORY		
Revision #	**Summary of Changes**	**Date**
6	Updated format. Added sample receiving checklist. Move sections referring to LabWorks. Added holding time tables and maps. Added sample disposal periods.	06/10/2011
5	Unknown.	08/06/2007
4	Unknown.	
3	Unknown.	
2	Unknown.	
1	Unknown.	
0	Initial release.	

Author:	*Scot Harris*		Date:
Approved:	Section Lead *Patricia Bucknell*		Date:
Approved:	Laboratory Director *David B. Crane*		Date:
Approved:	Quality Assurance *Gail Cho*		Date:
Approved:	Health and Safety *Thomas Lew*		Date:

STANDARD OPERATING PROCEDURE
TITLE: SAMPLE RECEIPT AND STORAGE

1.0 Scope and Application

1.1 This procedure describes chain-of-custody and the procedures for receiving, handling, scheduling, storing, and disposing of samples received by the DFG-OSPR/WPCL laboratory located at 2005 Nimbus Road, Rancho Cordova, California.

1.2 Samples submitted to WPCL fulfill data needs for routine monitoring, research, compliance, investigations, or enforcement actions. The procedures in this SOP help ensure sample traceability, chain-of-custody, integrity, timeliness, completeness, and proper sample disposal.

1.3 If an individual receives samples for testing at WPCL, this procedure must be followed.

2.0 Summary

2.1 The WPCL sample receiving area is located in the sample storage room at the back of the main laboratory. All samples are immediately unpacked, logged, checked for temperature, inventoried, preserved; reviewed for holding time, volume limitations, and clarity of instructions. Chain-of-custody (COC) records (Form FG1000 Rev. 9/01) or chains of custody submitted with samples are completed. The Sample Receipt Checklist is also completed. After the COC information is verified, samples are labeled, then stored (refrigerated or frozen) in designated units. If samples are delivered frozen, they are immediately transferred to the freezer after they are logged-in.

2.2 Clients are notified of discrepancies or anomalies as soon as possible after discovery. If samples are delivered in-person, ask the deliverer to remain until after sample inventory is complete so that any changes can be made real-time.

2.3 Copies of the chain-of-custody are provided to all departments and quality assurance for scheduling and secondary review. Copies must be circulated on the same day of sample receipt.

2.4 Samples are stored until data review and reporting have been completed.

2.5 Samples are assigned a unique laboratory identifier known as the "L-number" during the sample logging process. The identifier is labeled on each sample, tracked in a manual log as well as in the laboratory information management system (LIMS).

See WPCL-AB-002 (TBD), Sample Logging for LIMS entry instructions. The L-number and sub-numbers are used to track samples through the laboratory.

2.6 Samples will not be stored in offices, desks, or other non-designated sample storage units. Samples will not be transferred from the Sample Custodian to laboratory departments until samples have been inspected, inventoried, assigned an L-number, and labeled. Any exceptions (i.e. holding time) must be approved by the Sample Custodian or his designee.

2.7 Enforcement samples are stored in designated areas or units.

2.8 Highly contaminated samples or pure product will be stored separately from other samples in areas designated by the Sample Custodian.

2.9 Hours and location.

2.9.1 Routine sample receiving hours are Monday through Friday, 8:00 AM to 4:30 PM. Shipment receipt for extended hours, holidays and weekends may be prearranged with the project manager, or sample custodian or his designee.

2.9.2 Samples received after hours will be stored in WPCL R2. Leave a note on the Sample Custodian's desk or write a note on the dry erase board of samples stored in WPCL R2.

2.9.3 All other samples will be delivered to the WPCL sample receiving area located at the back of the main laboratory building at 2005 Nimbus Road, Rancho Cordova, California.

2.9.4 Samples will be logged as soon as possible and copies of the COC distributed on the date of receipt.

2.10 Designated Personnel.

2.10.1 Primary Sample Custodian: Scot Harris.

2.10.2 Secondary contact: Patty Bucknell.

3.0 Responsibilities

3.1 The Sample Custodian is the primary individual responsible for the receipt and inspection of sample delivery groups, storage of unprocessed samples, chain-of-custody distribution, notification to the laboratory, sample disposal, and LIMS log in.

3.2 The Project Manager or designee has the responsibility to contact clients of any discrepancies or anomalies. All discrepancies and communications will be documented on the sample receiving documents or in Labworks.

3.3 All personnel receiving samples are responsible for following this procedure and for reviewing distributed COC copies for scheduled analyses.

3.4 All entries will be written in blue or black ink. Error correction protocols defined in WPCL-QA-002 Documentation Practices will be followed by all personnel.

3.5 Any corrections to original COCs or sample labels should be made by the sample deliverer.

3.6 Any changes will be made on the original COC located in the QA office.

3.7 Copies of corrections to COCs after receipt must be distributed to all departments.

4.0 Definitions

4.1 Traceability: Ability to recreate the sample progression through the laboratory from receipt to disposal.

4.2 Chain-of-custody: Sample possession from collection to disposal. A sample is under custody if:

4.2.1 It is in your physical possession.

4.2.2 It is in your view.

4.2.3 It is in a secure area.

4.2.4 It was in your possession, but the sample was stored while processing.

4.3 Sample Integrity: The character of a sample/analyte of interest is unaltered by collection, shipping, preservation, storage and handling activities.

4.4 Timeliness: Samples are preserved, processed, and reported within regulatory or contractual requirements.

4.5 Completeness: All requested analyses are performed, reported, and traceable.

5.0 Safety

5.1 Assume that samples are potentially hazardous and exercise caution when opening packages containing samples. Wear nitrile gloves, lab coats, closed-toe shoes, and safety glasses when handling all incoming samples and shipping containers.

5.2 Unpack samples in a well-ventilated area or in a fume hood. If a shipping container is leaking, use spill pillows to absorb spills.

5.3 If containers are broken upon receipt, notify the Project Manager who will contact the client to determine the next course of action. Document discussions on the chain-of-custody or on the Sample Receipt Checklist. Dispose of container contents, broken containers, and shipping container rinsates as hazardous wastes. Use caution when handling and disposing of broken glass containers- do not use your bare hands. Dispose of broken glass in broken glass containers.

5.3.1 Use spill pillows to mop up any liquids. Dispose of used pillows as hazardous waste.

5.4 Follow disposal requirements specified in WPCL-EH-049 Disposal of Hazardous Wastes.

6.0 Equipment and Supplies

6.1 Chain of Custody (COC) form FG 1000 (Rev. 09/01).

6.2 Sample Receipt Checklist FM 006.

6.3 Calibrated infrared temperature gun.

6.4 Calibrated thermometers.

6.5 Spill kits as needed.

6.6 Sample Receiving Log Book.

7.0 Procedure

7.1 Receive and unpack sample shipping containers.

7.1.1 Shipping containers may be delivered by commercial courier, mail, or in person. Sign delivery manifests (either paper or electronic). If delivered in-person, ask the deliverer to remain until sample inventory is complete.

7.1.2 As soon as possible after receipt, inspect the shipping container for leaks or damage.

7.1.2.1 If leaking, refer to WPCL-EH-049 and the Safety section of this SOP.

7.1.2.2 If damaged, open the container cautiously. Be prepared to move the damaged container to a hood if odors are detected.

7.1.2.3 Note any leakage or damage on the COC or Sample Receiving Checklist.

7.1.3 Remove any paperwork, shipping manifests that are attached to the outside of the shipping container. Cut packaging tape to open the package or ice chest.

7.1.4 Inspect the contents for broken, leaking, or damaged containers. If intact, continue.

7.1.5 Remove any COCs or other paperwork.

7.1.5.1 If no COC is provided, initiate a COC (FG 1000). Fill out the header information as completely as possible.

7.2 Assign a unique sample delivery group tracking number.

7.2.1 Locate the hardbound Sample Receipt Log. Locate the most recent last entry. The assigned internal tracking number will be L-number-YY where "number" is a sequential number and YY indicates the current year. Record this number in the far left margin of the logbook page. Date and initial the entry. Example: L-345-11 indicates the 345th delivery group received in 2011. "Number" resets to -001- at the beginning of each calendar year. Record the following in the logbook next to the L-number:

7.2.1.1 Client name.

7.2.1.2 Number of samples.

7.2.1.3 Matrix of sample.

7.2.1.4 Project location. Include Index-PCA code if provided.

7.2.1.5 General description of analyses.

7.2.2 Pull a FG 1000 Chain of Custody Record and FM 006 Sample Receiving Checklist for completion. Write the L-number on client-provided documentation and in the Lab Number space indicated on the forms.

7.3 Measure and record sample temperature on the COC.

7.3.1 Turn on the infrared temperature gun. Allow to equilibrate.

7.3.1.1 If the IR gun is unavailable, place a thermometer in the cooler, close, allow to equilibrate for 15 minutes prior to reading.

7.3.2 Aim the IR gun on sample containers. Do not aim the gun at ice or packing material or at other people.

7.3.3 Record the temperature on the COC under "Water Temp:" and on the Sample Receipt Checklist, "cooler temperature upon arrival." Read in Fahrenheit.

7.3.4 Acceptable temperature ranges:

7.3.4.1 <= 43°F

7.3.4.2 <= 6° C.

7.3.5 Note any exceedances on the Sample Receipt Checklist and notify the client.

7.4 Inventory and inspect samples.

7.4.1 Note the presence or absence of custody seals on shipping containers on the Sample Receipt Checklist.

7.4.2 Remove samples from the shipping container and arrange them on the counter in client identifier order (if possible). If multiple containers are

provided, group containers according to client identifiers. Count sample containers and compare to the COC.

7.4.3 On the COC, record the number of containers for each bottle type under "# of Containers."

7.4.4 Check each sample for cracks, leaks, or breakage. Note any problems on the COC.

7.4.5 Compare client ID labels to the provided COC. Note any discrepancies on the COC for immediate client notification by the Project Manager or designee.

7.4.5.1 COC information and sample labels must match exactly.

7.4.5.2 If provided, the date and time of Collection indicated on the COC must match sample labels.

7.5 Check holding times and preservation.

7.5.1 Using the table in Attachment 1, calculate the remaining holding times. Compare the remaining days and/or hours against the table.

7.5.2 If 50% or more of the holding time has elapsed, contact the Project Manager and the affected department immediately and make a note on the COC.

7.5.3 If the COC does not indicate that the sample is preserved and the table indicates that the sample should be preserved, notify the affected department immediately. Note any preservation problems on the COC.

7.6 Check volumes and containers.

7.6.1 Confirm with affected departments if you are unsure that there is sufficient volume to perform the requested analyses. Contact the Project Manager or the Sample Custodian if there is a shortage of volume.

7.7 Verify that requested analyses are present and that instructions are clear.

7.7.1 Assign and write sub-numbers on the COC.

7.7.1.1 For organics analyses: One line = one sample = sub-number.

Example: L-number = L-100-11.

Sample A is listed on line 1 of the COC.

All containers of Sample A will be labeled as L-100-11-001.

7.7.1.2 For inorganics and biologicals tests: Each sample container = unique number.

7.7.1.3 For combined organics and inorganics:

7.7.1.3.1 Label organics as in 7.7.1.1.

7.7.1.3.2 After all lines are assigned, the next sequential sub-numbers are assigned to each bottle received for inorganics testing..

7.8 Complete the COC and Sample Receipt Checklist. Fill out all spaces.

7.8.1 Indicate the storage location of samples o the COC in the upper right hand box under "Lab Storage."

7.8.2 Sign and print your name in the box Received By.

7.8.3 Record the date and time of receipt.

7.9 Distribute copies of the COC.

7.9.1 Yellow or make a copy = Client.

7.9.2 Pink copy, FM 006, and original paperwork = Sample Receiving Binder.

7.9.3 Xerox copies for all departments: Back Lab, Petroleum, TSM, Inorganics, Dissection.

7.9.4 The original = Quality Assurance to be included in the client report.

7.10 Samples are ready for Labworks log in by the Sample Custodian or designee.

8.0 Designated Storage Locations for unprocessed samples. See Figure 1.

8.1 All temperatures will be monitored daily by the Sample Custodian. Exception: Saturday and Sunday.

8.2 Refrigerators will be monitored and maintained at <6°C.

8.3 Freezers will be monitored and maintained at <= -20°C.

8.4 Sample storage locations for unprocessed samples.

8.4.1 Volatiles/VOAs/Method 8260 : ELMO.

8.4.2 Petroleum samples: GROVER.

8.4.3 Inorganics samples.

8.4.3.1 Walk-in R2 (primary)

8.4.3.2 WPCL R7 (Enforcement; must be locked).

8.4.3.3 CMAP (frozen AFDM, chlorophyll a)

8.4.3.4 Environmental Walk-In (9 month archive pre-disposal).

8.4.4 Tissue Samples.

8.4.4.1 TSM F1.

8.4.4.2 Walk-In F1

8.4.5 Pesticides.

8.4.5.1 WPCL R1, R2, R3, R4 (extracts, back lab).

8.4.5.2 Walk-in R3 (waters).

8.4.5.3 Walk-in F1.

8.4.5.4 TSM F2 (tissues).

8.4.5.5 TSM F1 (tissues).

8.4.6 Sediments.

8.4.6.1 WPCL F1

9.0 Sample Archive and Disposal (minimum periods)

9.1 All samples and extracts will be disposed according to WPCL-EH-049 Disposal of Hazardous Wastes.

9.2 Non-enforcement water samples, SWAMP water and algae samples may be disposed 9 months after reported to the client or 20 months after receipt.

9.3 Tissues and sediments will be stored at <= (-20°C) until directed to dispose.

9.4 Enforcement samples, California Department of Fish and Game samples, samples from the Pesticides Investigation Unit will be held in proper storage until directed to dispose.

9.5 Solvent extracts may be disposed 18 months after results are reported to the client. Extracts are stored in the dark at <= (-20°C).

10.0 References

10.1 WPCL-EH-049, "Disposal of Hazardous Wastes"

10.2 WPCL-QA-002, "Documentation Practices"

10.3 USEPA, Table II, "Required Containers, Preservation Techniques, and Holding Times," Federal Register 40 CFR Part 136, March 26, 2007.

10.4 USEPA, Office of Solid Waste, Chapter 3 and Chapter 4, SW-846.

11.0 Attachments

11.1 Attachment 1: Analyses and Holding Times-Metals

11.2 Attachment 2: Analysis and Holding Times-Inorganics

11.3 Attachment 3: Analysis and Holding Times-Organics

11.4 Figure 1: Storage Locations Map.

11.5 Figure 2: FM006 Sample Receipt Checklist

METALS CONTAINERS, PRESERVATION, HOLD TIMES

Updated 2/17/10

	EPA	Standard	LabWorks Code	Container	Preservation		Holding Time		Minimum Volume		Same Container
Analyses	**Method**	**Methods**			**Water (includes dissolved)**	**Solids, Tissues**	**Water**	**Solids**	**Water**	**Solids**	
Aluminum by GFAAS		SM 3113B	AL_GF_*	P,FP,G	HNO3 to pH<= 2	<= -20°c	6 months	6 months	250 mL	100 g	1
Aluminum, Total monomeric		SM 3500 Al-E	ALIM_FIA				6 months	6 months	250 mL		
Aluminum, Organic monomeric		SM 3500 Al-E	ALOM_FIA				6 months	6 months	250 mL		
Arsenic by GFAAS	EPA 204.2		AS*				6 months	6 months	250 mL		
Arsenic by Hydride			AS_HYDR*				6 months	6 months	250 mL		
Cadmium by GFAAS		SM 3113B	CD_GF*				6 months	6 months	250 mL		
Cadmium by FLAAS		SM 3111B	CD*				6 months	6 months	250 mL		
Calcium by FLAAS		SM 3111B	CA*				6 months	6 months	250 mL		
Copper by FLAAS		SM 3111B	CU*				6 months	6 months	250 mL		
Total Copper by GFAAS		SM 3113B	CU_GF				6 months	6 months	250 mL		
Dissolved Copper by GFAAS		SM 3113B	CU_GF_DIS				6 months	6 months	250 mL		
Iron by FLAAS		SM 3111B	FE				6 months	6 months	250 mL		
Iron by GFAAS		SM 3113B	FE_GF*				6 months	6 months	250 mL		
Total and Dissolved Lead by FLAAS		SM 3111B	PB*				6 months	6 months	250 mL		
Total and Dissolved Lead by GFAAS		SM 3113B	PB_GF*				6 months	6 months	250 mL		
Magnesium by FLAAS (0.05-2.00ppm)		SM 3111B	MG*				6 months	6 months	250 mL		
Magnesium by FAAS (1.00-20.0ppm)		SM 3113B	MG*				6 months	6 months	250 mL		
Manganese by GFAAS		SM 3113B	MN_GF*				6 months	6 months	250 mL		
Mercury in Water	EPA 245.5		HG_COLD*				**28 days**	6 months	500 mL		
Molybdenum by GFAAS		SM 3113B	MO_GF				6 months	6 months	250 mL		
Nickel by FLAAS		SM 3111B	NI*				6 months	6 months	250 mL		
Nickel by GFAAS		SM 3113B	NI_GF*				6 months	6 months	250 mL		
Potassium Permanganate (KMnO4)	Calculation from Mn						6 months	6 months	250 mL		
Potassium by FLAAS (0.50-20.0ppm)		SM 3111B	K*				6 months	6 months	250 mL		
Se in Tissue by Hydride on PE300, in soln	EPA 7742M		SE_HY*				6 months	6 months	250 mL		
Se in Tissue by Hydride on PE300, wet wt.	EPA 7742M						6 months	6 months	250 mL		
Se in Tissue by Hydride on PE300, dry wt.	EPA 7742M						6 months	6 months	250 mL		
Se in Sediment by Hydride on PE300, in soln	EPA 7742M						6 months	6 months	250 mL		
Se in Sediment by Hydride on PE300, wet wt.	EPA 7742M	5% Moisture					6 months	6 months	250 mL		
Se in Sediment by Hydride on PE300, dry wt.	EPA 7742M						6 months	6 months	250 mL		
Sodium by FLAAS		SM 3111B	NA*				6 months	6 months	250 mL		
Silver		SM 3111B	AG*				6 months	6 months	250 mL		
Zinc by GFAAS		SM 3113B	ZN_GF*				6 months	6 months	250 mL		
Zinc by FLAAS		SM 3111B	ZN				6 months	6 months	250 mL		

NA = not applicable

Selenium Digestion = JAOAC85

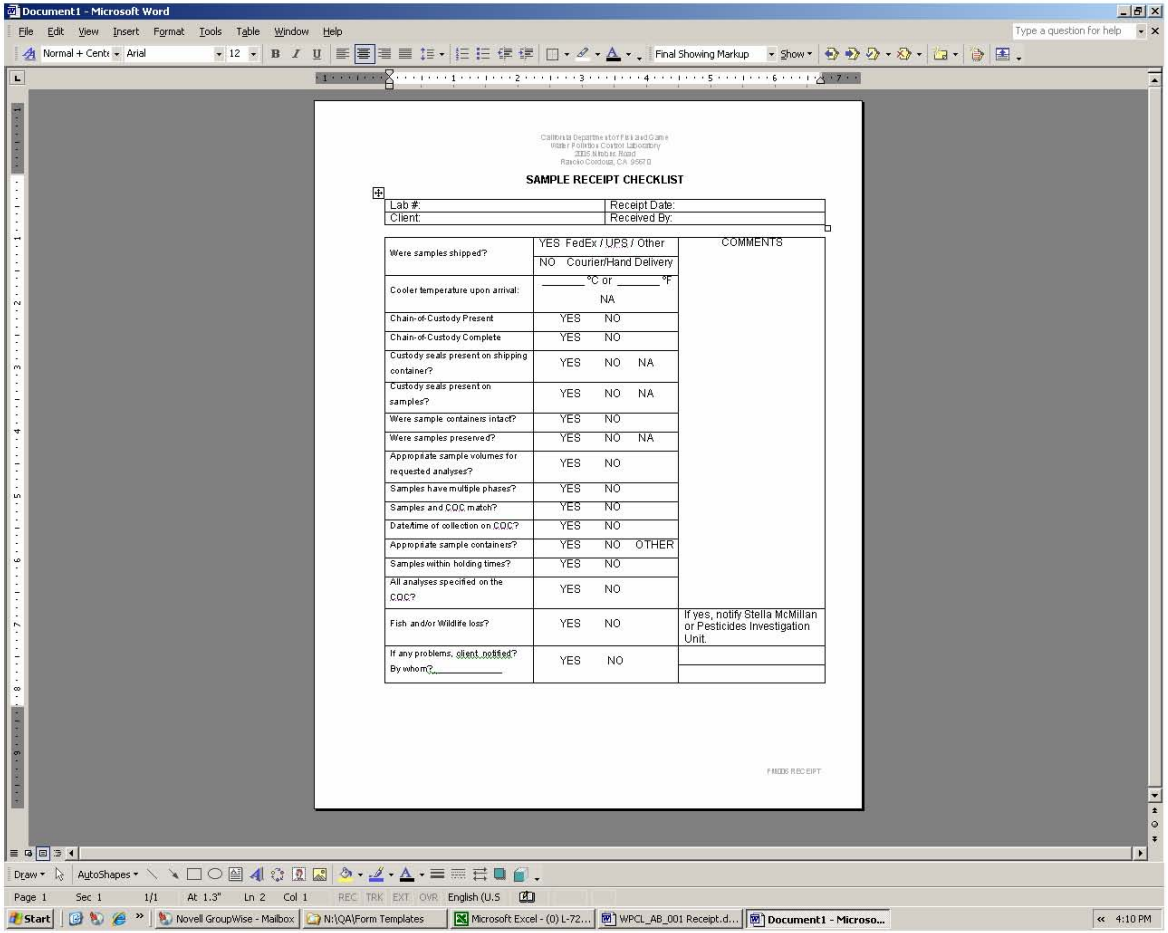
Methods for Chemical Analysis of Water and Wastewater, EPA-600/4-79-020, March 1983.

Standard Methods for the Examination of Water and Wastewater, 18th edition, 1992, American Public Health Association, American Water Works Association, WPCF.

Organics: TBD

ORGANICS CONTAINERS, PRESERVATION, HOLD TIMES (INCOMPLETE)

Analyses	EPA Method	Other Method	LabWorks Code	Container		Preservation		Holding Time		Minimum Volume		Same Container		Additional for QC
				Water	Solid	Water	Solid	Water	Solid	Water	Solid	Water	Solid	
Anatoxin a				G amber	G jar	<=6°C	<= -20°C	7/40	1 yr; 14/40	1000 mL	50 g	2	2	
Anticoagulants	HPLC			G amber	G jar	<=6°C	<= -20°C	7/40	1 yr; 14/40	1000 mL	50 g			
Carbamates	8321	LCMSMS		G amber	G jar	<=6°C	<= -20°C	7/40	1 yr; 14/40	1000 mL	50 g	1		
Glyphosate	547	HPLC		G amber	G jar	<=6°C	<= -20°C	14/18 months	NA	30 mL	NA			
Herbicides (fipronil*, metalochlor, trifluralin)	8270M	GCMSMS		G amber	G jar	<=6°C	<= -20°C	7/40	1 yr; 14/40	1000 mL	50 g			
Microcystins				G amber	G jar	<=6°C	<= -20°C	7/40	1 yr; 14/40	1000 mL	50 g	2	2	
Organochlorine Pesticides (OCH)	8081			G amber	G jar	<=6°C	<= -20°C	7/40	1 yr; 14/40	1000 mL	50 g			
Organophosphorus (organophosphate) Pesticides (OP, OPP)	8141	8270		G amber	G jar	<=6°C	<= -20°C	7/40	1 yr; 14/40	1000 mL	50 g			
Organotin	8323	GC-FPD		G amber	G jar	<=6°C	<= -20°C	7/40	6 months	1000 mL	50 g			
PCBs	8081			G amber	G jar	<=6°C	<= -20°C	7/40	1 yr; 14/40	1000 mL	50 g			
Pharmaceuticals and Personal Care Products				G amber	G jar	<=6°C	<= -20°C	48 hours/40	7/40	1000 mL	50 g			
Phenols (PCP, TCP)	8321			G amber	G jar	<=6°C	<= -20°C	7/40	1 yr; 14/40	1000 mL	50 g			
Piperonyl Butoxide (PBO)				G amber	G jar	<=6°C	<= -20°C	7/40	1 yr; 14/40	1000 mL	50 g	1		
Polybrominated Diphenyl Ethers (PBDE)	8270M	GCMSMS		G amber	G jar	<=6°C	<= -20°C	7/40	1 yr; 14/40	1000 mL	50 g			
Polynuclear Aromatic Hydrocarbons (PAH)	8270 SIM			G amber	G jar	<=6°C	<= -20°C	7/40	1 yr; 14/40	1000 mL	50 g			
Pyrethrins	LCMSMS			G amber	G jar	<=6°C	<= -20°C	7/40	1 yr; 14/40	1000 mL	50 g			
Pyrethroids	8081M	GCMSMS		G amber	G jar	<=6°C	<= -20°C	72 hours; 7/40	1 yr; 14/40	1000 mL	50 g			
Semi-Volatiles by GCMS	8270	PAH, PAH-SIM		G amber	G jar	<=6°C	<= -20°C	7/40	1 yr; 14/40	1000 mL	50 g			
Surfactants (Nonylphenol, nonylphenol ethoxylates)	HPLC			G amber	G jar	<=6°C	<= -20°C	7/40	1 yr; 14/40	1000 mL	50 g			
Triazines	GC-TSD			G amber	G jar	<=6°C	<= -20°C	7/40	1 yr; 14/40	1000 mL	50 g			
Volatiles	8260	BTEX, MTBE		G vial	G	<=6°C	<=6°C	14 days	14 days	40 mL	50 g			



California Department of Fish and Game
Water Pollution Control Laboratory
2005 Nimbus Road
Rancho Cordova, CA 95670

SAMPLE RECEIPT CHECKLIST

Lab #:	Receipt Date:
Client:	Received By:

Were samples shipped?	YES FedEx / UPS / Other NO Courier/Hand Delivery	COMMENTS
Cooler temperature upon arrival:	_____ °C or _____ °F NA	
Chain-of-Custody Present	YES NO	
Chain-of-Custody Complete	YES NO	
Custody seals present on shipping container?	YES NO NA	
Custody seals present on samples?	YES NO NA	
Were sample containers intact?	YES NO	
Were samples preserved?	YES NO NA	
Appropriate sample volumes for requested analyses?	YES NO	
Samples have multiple phases?	YES NO	
Samples and COC match?	YES NO	
Date/time of collection on COC?	YES NO	
Appropriate sample containers?	YES NO OTHER	
Samples within holding times?	YES NO	
All analyses specified on the COC?	YES NO	
Fish and/or Wildlife loss?	YES NO	If yes, notify Stella McMillan or Pesticides Investigation Unit.
If any problems, client notified? By whom?_____	YES NO	

FM005 RECEIPT


Edit
Print
Send
Link
Map
Traffic
WPCL R1, R2, R3, R4
WALK IN R3
WALK IN F1
BACK LAB
WALK IN R2
Environmental
WPCL F1, F3
TSM F2, R3, F3,
F4, R7, CMAP
OFFICES
TSM R1
ELMO
GROVER
FRONT
ENTRANCE
20 ft
5 m
©2011 Google - Imagery ©2011 Google, Map data ©2011 Google - Terms of Use
Report a problem
Containers Preservation ...
Novell GroupWise Mailbox
2005 Nimbus Road, G...
Downloads
2:23 PM

Appendix IV B: Determination of OC and PCB in Sediment and Tissue (Modifications to EPA 8081B and 8082)

WPCL Method: OCH, PCBs
SOP# WPCL-MS-006
Revision #10
Prepared by: DBC/GJB
Date: 10/15/08
Page 1 of 25

ANALYSIS OF EXTRACTABLE SYNTHETIC ORGANIC COMPOUNDS IN TISSUE AND SEDIMENT
(Organochlorine Pesticides, Polychlorinated Biphenyls and Polybrominated Diphenyl Ethers)

1.0 Scope and Application

1.1 This method describes the sample preparation using an automated extraction system for the determination of trace residue levels of a selected list of organochlorine (OCs) pesticides, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in fish and shellfish tissues and sediments. Dual column gas chromatography with dual electron capture detectors (GC-ECD) and/or gas chromatography with triple quadrupole mass spectrometry (GC-MSMS) are used to analyze OC pesticides, PCBs and PBDEs. Table 1 lists the target OC pesticide compounds currently analyzed with their method detection limits and reporting limits. Table 2 lists the PCB congeners and Aroclor mixtures analyzed with their reporting limits. Table 3 lists the PBDE congeners analyzed with their method detection limits and reporting limits.

1.2 These procedures are applicable when low parts per billion analyses are required to monitor differences between burdens in organisms and sediment concentrations from relatively uncontaminated reference areas and contaminated areas. In addition, the procedures are applicable when low detection limits are required for the estimation of potential health effects of bioaccumulated substances.

Table 1. Organochlorine Compounds Analyzed and their Minimum Detection Limits (MDL) and Reporting Limits (RL) in Tissue (ng/g, wet wt.) and Sediment (ng/g dry weight), based on 50 % moisture.

	Tissue		Sediment	
	MDL, ng/g wet wt.	RL, ng/g wet wt.	MDL, ng/g dry wt.	RL, ng/g dry wt.
aldrin	0.414	1.00	0.800	2.00
chlordane, cis	0.400	1.00	0.800	2.00
chlordane, trans	0.450	1.00	0.900	2.00
chlorpyrifos	0.204	1.00	0.400	2.00
dacthal	0.096	1.00	0.200	2.00
DDD, o,p'	0.096	1.00	0.200	2.00
DDD, p,p'	0.124	1.00	0.250	2.00
DDE, o,p'	0.178	2.00	0.400	4.00
DDE, p,p'	0.480	2.00	1.00	4.00
DDMU, p,p'	0.108	3.00	0.200	6.00
DDT, o,p'	0.216	3.00	0.400	6.00
DDT, p,p'	0.156	5.00	0.300	10.0
diazinon	4.80	20.0	10.0	40.0
dieldrin	0.432	0.500	1.00	1.00
endosulfan I	0.560	2.00	1.00	4.00
endosulfan II	0.682	5.00	1.40	10.0
endosulfan sulfate	0.546	5.00	1.00	10.0
endrin	0.180	2.00	0.400	4.00
HCH, alpha	0.262	0.500	0.500	1.00
HCH, beta	0.210	1.00	0.400	2.00
HCH, gamma	0.144	0.500	0.300	1.00
heptachlor	0.356	1.00	0.700	2.00
heptachlor epoxide	0.246	1.00	0.500	2.00
hexachlorobenzene	0.346	0.692	0.700	1.40
methoxychlor	0.146	3.00	0.300	6.00
mirex	0.300	1.50	0.600	3.00
nonachlor, cis	0.308	1.00	0.600	2.00
nonachlor, trans	0.194	1.00	0.400	2.00
oxadiazon	0.544	1.00	1.00	2.00
oxychlordane	0.474	1.00	1.00	2.00
parathion, ethyl	0.524	2.00	1.00	4.00
parathion, methyl	0.756	4.00	1.50	8.00
tedion	1.07	2.00	2.00	4.00
DBOB(surrogate)	NA	NA	NA	NA
DBCE(surrogate)	NA	NA	NA	NA
DDD*deuterated (surrogate)	NA	NA	NA	NA

Table 2. PCB Congeners and Aroclor mixtures Analyzed and their Reporting Limits (RL) in Tissue (ng/g, wet weight) and Sediment (ng/g, dry weight).

NIST PCB Congeners:	
PCB Congener 8	PCB Congener 128
PCB Congener 18	PCB Congener 138
PCB Congener 28	PCB Congener 153
PCB Congener 44	PCB Congener 170
PCB Congener 52	PCB Congener 180
PCB Congener 66	PCB Congener 187
PCB Congener 87	PCB Congener 195
PCB Congener 101	PCB Congener 206
PCB Congener 105	PCB Congener 209
PCB Congener 118	PCB Congener 209 C^{13}(surrogate)

Additional PCB Congeners:	
PCB Congener 27	PCB Congener 141
PCB Congener 29	PCB Congener 146
PCB Congener 31	PCB Congener 149
PCB Congener 33	PCB Congener 151
PCB Congener 49	PCB Congener 156
PCB Congener 56	PCB Congener 157
PCB Congener 60	PCB Congener 158
PCB Congener 64	PCB Congener 169
PCB Congener 70	PCB Congener 174
PCB Congener 74	PCB Congener 177
PCB Congener 77	PCB Congener 183
PCB Congener 95	PCB Congener 189
PCB Congener 97	PCB Congener 194
PCB Congener 99	PCB Congener 198_199
PCB Congener 110	PCB Congener 200
PCB Congener 114	PCB Congener 201
PCB Congener 126	PCB Congener 203
	PCB Congener 137

All individual PCB Congener reporting limits (RL) are 0.2 ng/g (wet weight) or 0.4 ng/g (dry weight, based on 50 % moisture). Estimated Aroclor concentrations calculated from the congener concentrations have the following RLs:

Aroclors:	RL ng/g (wet wt.)	RL ng/g (dry wt.)
Aroclor 1248	25	50
Aroclor 1254	10	20
Aroclor 1260	10	20

Table 3. Polybrominated Diphenyl Ethers (PBDEs) and their Minimum Detection Limits (MDL) and Reporting Limits (RL) in Tissue (ng/g, wet wt.) and Sediment (ng/g, dry wt., based on 50 % moisture.)

	Tissue		Sediment	
	MDL, ng/g wet wt.	RL, ng/g wet wt.	MDL, ng/g dry wt.	RL, ng/g dry wt.
BDE 17	0.139	0.600	0.278	1.20
BDE 28	0.148	0.600	0.296	1.20
BDE 47	0.196	0.800	0.391	1.60
BDE 66	0.135	0.600	0.269	1.20
BDE 100	0.157	0.600	0.314	1.20
BDE 99	0.197	0.800	0.394	1.60
BDE 85	0.177	0.800	0.354	1.60
BDE 154	0.165	0.600	0.329	1.20
BDE 153	0.185	0.800	0.370	1.60
BDE 138	0.200	0.800	0.400	1.60
BDE 183	0.297	1.20	0.594	2.40
BDE 190	0.437	1.80	0.874	3.60
BDE 209	1.00	10.0	2.00	20.0

2.0 Summary of Method

2.1 Sets of 10-18 homogenized tissue or sediment samples are scheduled for extraction by the project lead chemist. Extraction method employed was developed and validated by the Water Pollution Control Laboratory (WPCL) and is a modification of EPA Method 3545A Pressurized Fluid Extraction (PFE). Extract cleanup and partitioning methods are modifications of EPA Methods 3640A Gel Permeation Cleanup and 3620C Florisil Cleanup and the multi-residue methods for fatty and non-fatty foods described in the U.S. Food and Drug Administration, Pesticide Analytical Manual, Vol. 1, 3rd Edition 1994, Chapter 3, Multi-residue Methods, Section 303-C1.

Homogenized tissue or sediment samples are removed from the freezer and allowed to thaw. A separate extraction bench sheet is initiated for each set of samples which are distinguished by project, sample matrix type and analysis type.

2.2 A 3-4 g (tissue or sediment homogenate) sample is weighed into a pre-weighed aluminum planchet and placed in a 70°C oven for 48 hours to determine moisture content. A 10 g sample is mixed using a clean glass stirring rod with approximately
7 g of pre-extracted Hydromatrix® in a 250 mL Trace Clean Wide Mouth Jar until the mixture is free flowing. The mixture is then poured into a 33 mL stainless steel Dionex Accelerated Solvent Extractor (ASE 200) extractor cell

and packed by tamping the mixture. A solution containing pesticide, PCB and PBDE surrogate compounds is added to the cell and the cap is screwed onto the cell. The extractor cells (maximum of 24) are placed on the ASE 200 autosampler rack and the samples are extracted twice with a 50/50 mixture of acetone/dichloromethane (DCM) using heat and pressure. The extracts are automatically collected in two 60 mL VOA vials.

2.3 The combined extracts (~100 mL) are dried using sodium sulfate, evaporated to approximately 1.0 mL using Kuderna-Danish (K-D) glassware equipped with 3-ball Snyder columns and micro-Snyder apparatus and diluted to 10 mL using DCM. The extracts are then filtered through a 0.45 µm syringe filter into J_2 Scientific AccuPrep 170 (GPC) autosampler tubes. If the lipid content needs to be determined, two milliliters each of the filtered extracts are removed and placed in a pre-weighed aluminum planchet.

2.4 The GPC autosampler tubes are then placed on the GPC autosampler for initial sample cleanup by gel permeation (size exclusion) chromatography.

2.5 The cleaned-up extracts are evaporated using K-D apparatus and solvent exchanged into petroleum ether. The extracts are then fractionated using 5 grams of Florisil® in a 11 mm x 300 mm column with a 250 mL reservoir. The Florisil® columns prepared for tissue samples are eluted with 6% diethyl ether/PE (Fraction 1), 15% diethyl ether/PE (Fraction 2), and. Florisil® columns prepared for sediment samples are eluted with 6% diethyl ether/PE (Fraction 1) and 50% diethyl ether/PE (Fraction 2).The fractions are concentrated to an appropriate volume using K-D/micro K-D apparatus prior to analysis by dual column high resolution gas chromatography and/or GC-MSMS. The distribution of synthetic organic compounds in the fractions is listed in Table 4.

Table 4. Distribution of Synthetic Organic Compounds Among the Three Fractions of a Standard Florisil® Column.

6% Fraction 1/	15% Fraction 2/
aldrin	dacthal
chlordane (cis-)	DBCE*
chlordane (trans-)	dieldrin
DBOB*	endosulfan I 4/
DDE, o,p'	endosulfan II 5/
DDE, p,p'	endrin
DDD, o,p'	oxadiazon
DDD, p,p'/DDD-d10*,p,p'	tetradifon
DDMU, p,p'	
DDT, o,p'	
DDT, p,p'	
endosulfan I 4/	
heptachlor	
heptachlor epoxide	
hexachlorobenzene	
HCH-alpha	
HCH-beta	
HCH-gamma	
methoxychlor	
nonachlor (cis-)	
nonachlor (trans-)	
oxychlordane	
polybrominated diphenyl ethers (PBDEs)	
polychlorinated biphenyls (PCBs)/PCB 209*(C^{13})	
toxaphene	

* surrogate
1/ 6% ethyl ether in petroleum ether (analysis by GC-MSMS)
2/ 15% ethyl ether in petroleum ether (analysis by GC-ECD)
3/ 50% ethyl ether in petroleum ether (analysis by GC-ECD).
4/ In both 6% and 15% fractions.
5/ In both 15% and 50% fractions.

3.0 Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may cause GC artifacts and/or elevated baselines, resulting in the misinterpretation of chromatograms. 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. Specific selection of reagents and purification of solvents by distillation in all-glass systems are required. High-purity, distilled-in-glass solvents are commercially available.

An effective way of cleaning laboratory glassware is by rinsing with polar and non-polar solvents before use. The cleaning procedure used must be tested by analyzing procedural blanks prior to analyzing samples.

3.2 Phthalates are common laboratory contaminants that are used widely as plasticizers. Sources of phthalate contamination include plastic lab-ware, plastic tubing, plastic gloves, plastic coated glassware clamps, and have been found as a contaminant in Na_2SO_4. Polytetrafluoroethylene (PTFE) can be used instead of polypropylene or polyethylene to minimize this potential source of contamination. However, use of PTFE lab-ware will not necessarily preclude all phthalate contamination.

3.3 Interferences co-extracted from tissue and sediment samples limit the method detection and quantitation limits. For this reason, sample extract cleanup is necessary to yield reproducible and reliable analyses of low level contaminants.

4.0 Apparatus and Materials

4.1 Wide mouth, borosilicate glass, pre-cleaned and certified, 250 mL, Qorpak or equivalent.

4.2 Chromatographic Column - (300 mm x 11 mm) borosilicate glass chromatography column with 250 mL reservoir and Teflon stopcock.

4.3 Glass wool, Pyrex - solvent washed prior to use.

4.4 Kuderna-Danish (K-D) Apparatus

4.4.1 Concentrator tube - 10 mL, graduate (Kontes K0570050-1025, or equivalent). A ground stopper, 19/22 joint, is used to prevent evaporation of extracts.

4.4.2 Evaporation flask - 500 mL (Kontes K-570050-0500, or equivalent), attached to concentrator tube with blue clamp (Kontes K-662750-0012).

4.4.3 Snyder column - three ball (Kontes K-503000-0121, or equivalent).

4.4.4 Micro-Snyder column - (Kontes VWR KT569261-0319 or equivalent).

4.4.5 Boiling stones, Chemware® Ultra-Pure PTFE, extracted with acetone and petroleum ether. Note that boiling chips can be a significant source of contamination if not properly cleaned.

4.5 Water bath, Organomation Assoc. Inc.(OA-SYS/S-EVAP-KD), 115 V, thermostatically controlled with stainless steel cover to fit 5 K-D apparatus, installed in a fume hood. Water bath is equipped with solvent recovery system.

4.6 Extractor, automated, Dionex Accelerated Solvent Extractor (ASE 200), Dionex P/N 047046.

4.6.1 Extraction Cells, 33 mL, Dionex P/N 049562

4.6.2 Filters, cellulose for ASE extraction cells, Dionex P/N 049458.

4.6.3 VOA Vials, 60 mL, pre-cleaned and certified.

4.7 Sample vials - glass, 2.5 mL with PTFE-lined screw cap.

4.8 Analytical balance - capable of weighing 0.1 mg.

4.9 Drying oven.

4.10 Balance - capable of 100 g to the nearest 0.01 g.

4.11 Disposable Pasteur Pipettes - (rinsed with solvents before use).

4.12 Aluminum dishes for moisture and lipid determination.

4.13 Desiccator with indicating desiccant.

4.14 Glass funnel, 75 mm.

4.15 Graduated cylinder, 250 mL and 100 mL.

4.17 Culture tubes, 13 x 100mm and 16 x 100 mm, with PTFE lined cap.

4.18 Centrifuge tubes, 15 mL, graduated to 0.1 mL and calibrated to 1.0 mL.

4.19 Gas chromatographs (GC) (3): Hewlett-Packard HP 6890 plus, equipped with dual micro-ECD. All are equipped with split-splitless injector with EPC and autosampler.

4.20 GC Capillary columns, 60 meter DB5 and 60 meter DB17MS (J&W Scientific) (0.25 mm I.D. and 25 µm film thickness) connected to a single injection port using a "Y" press fit connector.

4.21 GC Data System, Hewlett-Packard, to collect and record GC data, generate reports, and compute and record response factors for multi-level calibrations. Data system should be capable of calibrating a method using a minimum of 5 concentrations of analytical standards.

4.22 Gas chromatograph-mass spectrometer (triple quadrupole), Varian Model 1200L with Varian Model 3800 gas chromatograph, split-splitless injector with EPC and Combi-Pal autosampler.

4.23 Homogenizer, Bucchi Model B-400 (Brinkman P/N 16-07-200-1) or equivalent equipped with titanium knife assembly (Brinkman P/N 16-07-222-2) and glass sample vessel (Brinkman P/N 16-07-245-1).

4.24 Homogenizer, Brinkman Polytron or equivalent equipped Teflon and titanium generator assembly (for homogenization of small sample amounts).

4.25 Gel Permeation (size exclusion) Chromatograph, automated, J2 Scientific AccuPrep 170, equipped with 70 g S-X3 BioBeads J_2 Scientific P/N C0070G (100% DCM).

5.0 Reagents

5.1 Petroleum ether (PE), Burdick and Jackson, distilled in glass and pesticide residue or HRGC grade or equivalent.

5.2 Acetone. (Same as above).

5.3 Iso-Octane. (Same as above).

5.4 Diethyl ether preserved with 2% ethanol.(Same as above).

5.5 Dichloromethane (DCM). (Same as above).

5.6 Chem Elut-Hydromatrix®, Varian P/N 0019-8003. Pre-extracted on ASE-200 with acetone/DCM prior to use.

5.7 Sodium sulfate. Anhydrous granular reagent grade, rinsed with PE prior to use.

5.8 Florisil®, 60/100 mesh, PR grade, U.S. Silica.

5.9 Nitrogen, pre-purified grade (99.9999%) or better (used for ASE and GPC).

5.10 Nitrogen, ultra-pure (99.99999%) for ECD makeup.

5.11 Helium, ultra-pure (99.99999%) for GC carrier gas.

5.12 Air, compressed, breathing quality, for ASE pneumatics.

5.13 OC/PCB/PBDE Surrogate Mix containing: 40 ppb of deuterated p,p'-DDD-d10, PCB 209(C^{13}), and dibutylchlorendate (DBCE).

5.14 Standard Reference Material (SRM), National Institute of Standards and Technology (NIST): SRM 1588b (Organics in Cod Liver Oil) and SRM 1944 (New York/New Jersey Waterway sediment).

CAUTION

The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of data handling Material Safety Data Sheets should also be made available to all personnel involved in these analyses.

6.0 Sample Collection, Preparation, and Storage

6.1 In the field, sources of contamination include sampling gear, grease from ship winches or cables, ship and/or motor vehicle engine exhaust, dust, and ice used for cooling. Efforts should be made to minimize handling and to avoid sources of contamination. This will usually require that resection (i.e., surgical removal) of tissue be performed in a controlled environment (e.g., a laboratory). The samples should be double wrapped in aluminum foil and immediately frozen with dry ice in a covered ice chest. Ice should be in water tight plastic bags for transporting live shellfish.

6.2 To avoid cross-contamination, all equipment used in sample handling should be thoroughly cleaned before each sample is processed. All instruments must be 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 tap water, rinsed with a high-purity acetone, and finally rinsed with Type II water.

6.3 Resection should be carried out by or under the supervision of a competent biologist. Each organism should be handled with clean high carbon steel, titanium, quartz, or Teflon instruments (except for external surfaces). The

specimens should come into contact with pre-cleaned glass surfaces only. Polypropylene and polyethylene surfaces are a potential source of contamination and should not be used. To control contamination when resecting tissue, separate sets of utensils should be used for removing outer tissue and for resecting tissue for analysis. For fish samples, special care must be taken to avoid contaminating target tissue (especially muscle) with slime and/or adhering sediment from the fish interior (skin) during resection. The incision "troughs" are subject to such contamination; thus, they should not be included in the sample. In case of muscle, a "core" of tissue is taken from within the area bordered by the incision troughs, without contacting them. Unless specifically sought as a sample, the dark muscle tissue that may exist in the vicinity of the lateral line should not be mixed with the light muscle tissue that constitutes the rest of the muscle tissue mass.

6.4 The resected tissue sample should be placed in a clean glass or PTFE container which has been washed with detergent, rinsed twice with tap water, rinsed once with distilled water, rinsed with acetone, and, finally, rinsed with high-purity petroleum ether.

6.5 The U.S. EPA has published a guidance document containing specific recommendations regarding holding times and temperatures for tissue samples to be analyzed for semi-volatile organic compounds. The following holding conditions should be observed. Tissue samples should be maintained at $\leq$-20° C and analyzed as soon as possible, but within 12 months of sample receipt.

6.6 Sediment samples may be refrigerated at 4°C for up to 14-days maximum or must be stored frozen at minus (-) 20°C for up to 12 months maximum.

7.0 Sample Extraction

7.1 Remove homogenized tissue or sediment samples from freezer and allow to thaw. Prior to extraction, the tissue samples are homogenized using a Bucchi B-400 mixer equipped with a titanium knife assembly or for small samples a Brinkman Polytron® equipped with a titanium and Teflon generator. Decant any excess water from the sediment samples prior to thoroughly mixing by hand using a clean glass rod or may be homogenized using a Polytron homogenizer equipped with stainless steel generator equipped with Teflon bearings. Sample sets of 10-18 should be extracted when possible. The ASE-200 extractor will extract 24 cells. Be sure to reserve enough cells for method blanks, matrix spikes, and laboratory control spikes.

7.2 A separate extraction bench sheet is initiated for each project, sample matrix type, and analysis type. Several bench sheets may be used for an extraction set.

7.3 Prepare a glass rod or Teflon spatula for each sample to be weighed by rinsing 3 times with petroleum ether using a Teflon wash bottle.

7.4 Label 60 mL VOA vials for the collection of the sample extract. The labels must be placed between 1.5" and 3" from the top of the VOA cap; if they are placed outside of this area, they will interfere with the ASE optical sensor. Use two VOA vials for each sample. Label the first VOA vial with the ASE position number, bench sheet number and the sample name. Label the second VOA vial the same but add "RE" to distinguish between the two vials. Label and weigh aluminum planchets for lipid and moisture determinations (write sample ID on the bottom of planchets using a ball point pen).

7.5 Tare a 250 mL glass jar. Using a clean (solvent rinsed) glass rod, stir the tissue or sediment so that the mixture is homogeneous. Weigh 10 g of sample into the jar, record the weight on the bench sheet, and add the twice-extracted Hydromatrix® from one ASE cell. Stir the mixture thoroughly and go on to the next sample. After approximately 15 minutes stir the sample again. Repeat this at 15 minute intervals two more times or until the sample mixture is free flowing.

7.6 Weigh 3-4 g of additional sample into a pre-weighed and tared aluminum planchet for % moisture analysis. Place planchets in 70°C oven for 48 hours and re-weigh dry weight.

7.7 Place a pre-rinsed powder funnel on top of a 33 mL ASE cell containing a pre-extracted cellulose filter *(the filter is the one that was used to pre-extract the Hydromatrix®)*.

7.8 Pour the tissue or sediment/Hydromatrix® mixture through the powder funnel back into the extraction cell that the Hydromatrix® was poured from. Tap the cell against the counter top to settle the contents. The mixture will fill the cell and it may be necessary to pack it slightly using the glass rod and the end of the powder funnel. The cells used for the method blank and laboratory control spike and its duplicate *(if used)* will contain only Hydromatrix®.

7.9 All of the extraction cells are spiked with the OC/PCB/PBDE pesticide surrogate standard. Spike each cell with exactly 0.5 mL of the appropriate surrogate solution. Surrogate spikes must be witnessed, recorded and dated on the extraction bench sheet.

7.10 The extraction cells used for the matrix spike (MS) and duplicate matrix spike (MSD) and laboratory control spike (LCS) and its duplicate (LCSD) (if used) are spiked with exactly 0.5 mL of the OC/PCB/PBDE matrix spike solution (40 ng/mL). A separate MS/MSD and LCS/LCSD (if used) is required for each

class of compounds being analyzed. Matrix spikes must be witnessed, recorded and dated on the extraction bench sheet.

7.11 The extraction cells are capped *(Firmly tightened but do not overtighten)* and placed on the ASE 200 carrousel. The first set of labeled VOA collection vials are placed on the ASE 200 collection carrousel with the position numbers corresponding to the position numbers of the extraction cells. Make sure that the solvent reservoir contains enough solvent for the extraction.

7.12 Samples are extracted with acetone/methylene chloride (DCM) 50:50 using the following conditions:

Pre-heat	0 min.
Heat	5 min.
Static	5 min.
Flush	60%
Purge	300 sec.
Cycles	1
Pressure	1500 psi
Temp	100 °C
Sol A Other	100%

7.13 After the initial extraction is complete, remove full VOA vials and place in a Wheaton rack. Place the second set of collection VOA vials labeled "RE" on the ASE carrousel. Check each of the extraction cells to make sure that the caps are *(firmly tightened)* as they tend to loosen with the first extraction. Make sure that the replacement vials are in the correct order. Make sure that the solvent reservoir contains enough solvent for the re-extraction. Re-start the ASE-200.

7.14 When extraction is completed, place VOA vials in a Wheaton rack with the "RE" vials next to the vials from the first extraction. The extracts should be re-capped with solid green caps (Qorpak) and placed in a refrigerator for storage until they are removed for the GPC cleanup procedure.

8.0 Gel Permeation Chromatography

IMPORTANT: ***All glassware, glass wool, and sodium sulfate must be triple-rinsed with petroleum ether before they are used for this procedure.***

8.1 Remove VOA vials containing the sample extracts from the refrigerator. Make sure the vials are capped with the green Qorpak caps. Allow them to sit out until they are at room temperature.

8.2 Set up and label pre-cleaned K-D flasks (4-6) with concentrator tubes attached on ring stands in the fume hood. Place a funnel containing a plug of pre-cleaned glass wool in the bottom of the funnel and place the funnel in the top of the K-D flask. Add about two inches of pre-rinsed sodium sulfate to the funnel. Make sure that the level of the sodium sulfate is uniform across the funnel to prevent any possible splashing out.

8.3 Pour sample extracts from the VOA vials through sodium sulfate into the K-D flask. Add about 10 mL of DCM to the VOA vial, cap and shake and add this rinse to the sodium sulfate. Repeat with another 10 mL DCM rinse. Rinse the sodium sulfate with an additional portion of DCM (~50 mL) by pouring from a clean and rinsed 400 mL beaker. After the solvent has completely drained through the sodium sulfate add one more additional rinse of DCM (~50 mL) from the beaker of clean DCM. Allow the DCM to completely drain through the sodium sulfate (~3-5 minutes).

8.4 Add 0.5 mL Iso-Octane using a macro-pipetter and a solvent rinsed boiling chip to each K-D flask. Place a Snyder column on the K-D flask, clamp with a green clamp and place the flask on the hot water bath set at 80-82°C. Drop down the inverted Hopkins condenser from the solvent recovery system and attach it to the top of the Snyder column. Turn the water supply on to the solvent recovery system until the water flow is between 1500-2000 cc/min. Evaporate the solvent until the apparent volume is 2-5 mL. Remove the inverted Hopkins condenser and secure using the set clamps so that it is out of the way. At this point there should be between 2-5 mL visible in the concentrator tube while the K-D apparatus is still on the hot water bath and 10 mL or less of the solvent remaining after the K-D flask is removed from the hot water bath and the solvent drains from the Snyder column. Dry off the water using a WyPall X60 towel to remove any water from around the ground glass union of the concentrator tube and the K-D flask to prevent any of it from entering the concentrator tube upon removal.

8.5 After the K-D apparatus has cooled and all of the solvent has drained from the Snyder column, remove the Snyder column, label the concentrator tube and then remove the concentrator tube from the flask and place the tube in a test tube rack and cover with pre-rinsed aluminum foil. Rinse the Snyder column with dichloromethane and place back in the column rack for storage. After all of the flasks have been removed from the hot water bath, repeat steps 2-5 for the remaining samples extracted with this set.

8.6 Add a new micro-boiling stone and place a clean micro-Snyder column on the concentrator tube with a blue clamp and place in a 400 mL beaker containing hot water heated to approximately 75°C on a hot plate. If the solvent does not begin to boil, remove the tube from the bath immediately, allow it to cool slightly, add a new micro boiling stone to prevent it from bumping and place it

back in the bath. Evaporate the solvent until only 1.0 mL remains in the concentrator tube. Four or five tubes can be evaporated at one time.

8.7 When the solvent has been evaporated to 1.0 mL remove the tube from the bath and allow it to cool in a test tube rack. Remove the micro-Snyder column and add DCM to the concentrator tube to reach a final volume of 10.0 mL.

8.8 Whatman filter (0.45 µm) the sample into a 12 mL culture tube. Using a volumetric pipette remove 2.0 mL of the filtered sample and place it in a pre-weighed aluminum planchet if lipid determination is needed. Cap the culture tube with the Teflon-insert style caps. Mark the bottom of the meniscus with a pen in case of evaporation before clean-up on GPC.

8.9 All samples are cleaned using a J_2 Scientific GPC (Autoinject 110, AccuPrep 170, DFW-20 Fixed Wavelength Detector, 1" ID glass column with 70g Bio-Beads SX-3 in 100% DCM)

8.9.1 From the desktop double click on the AccuPrep.exe shortcut to open the program. Click on the Use Injector button and allow the instrument time to initialize. Activate the pump by using the top left hand button. A solvent Control Pump window will open up. Click on the Apply Defaults button and then OK on the Selected Pressure Limit 30 psi. The pump should audibly be heard coming on and the green light should show that the system is on line and status flowing. Make sure that the bottle of clean DCM is full and the waste bottle is empty. Allow the system to pump for about 5 minutes before switching the column in-line (gray button next to Column that has 'Put in line' on it). The pressure will be observed to normally go up to the 12-16 psi range. Turn the power on to the detector to allow it at least 30 minutes of time to warm up before use. Because the scale is auto-adjusted in the software now it is no longer necessary to manually adjust the range on the unit itself.

8.9.2 While the system is equilibrating, the sequence can be entered. Click on the Seq button next to the Pump button. An 'Editing new sequence' window will pop up. This gives a view of the instrument which clearly shows the sample tray locations and the corresponding sample collection locations. By clicking on the sample tray position, a new window 'Adding sample at tray position #' will pop up. This allows information to be included about each specific sample. Sample position 1 will always be a calibration standard (CLP-340) which is run prior to any sequence of runs to verify instrument integrity. In the Sample ID field just type in 'CLP-340'. In the Descrip (optional), information pertaining to the project, laboratory control number, bench sheet number and date are typically added. The Method File needs to be changed to 'ZGPC Calib' for only this sample and in the Sample Type field the 'Calibration' type can be chosen. After this information is completed click on the OK to continue. This returns you back to the main sequence window but

now the first position will be highlighted in green. Continue by adding the next sample information to tray position 2, again following the same steps as before. By default the Method File will be on the program SOPAH which is used for both pesticides (SO) and petroleum (PAH) clean-up. Also by default, the Sample Type field will already be set at 'Sample'. This will not need to be changed until a duplicate sample (Duplicate), matrix spike (Matrix Spike), matrix spike duplicate (Spike Duplicate), laboratory control spike (Spiked Blank), and the SRM (Lab Control Std) are encountered. After all the samples have been added to the sequence, save it as the bench sheet number (BS###). From the Editing sequence window print out the sample list. Compare the information to your original bench sheet to insure there are no mistakes. Make sure the ZGPC method is being used for the calibration standard and the SOPAH method is being used for the samples. Next verify that the samples are still at the marked line on the culture tubes (add DCM to the marked line if they are not). Place a tube with the GPC Calibration Standard Solution (CLP-340) in sample tray position 1 and then follow as the sequence was made in the remaining positions.

8.9.3 Get two boxes of the 125 mL Trace Clean amber bottles for sample collection. A bottle does not need to be placed in collection position #1 because that is the GPC Calibration Std (all goes to waste). Remove the white caps from the bottles and place them on top of the detector (so that Teflon side is not exposed to possible contamination). Label the boxes with bench sheet and laboratory control numbers and keep them for the post-GPC samples to be stored in. Now that the pump as had plenty of time to equilibrate the system and the detector has had plenty of time to warm up, in the Signal field click to adjust the setting to 'Absorbance Units' and click on the 'Zero Signal' button to set the baseline.

8.9.4 If the pressure seems to be pretty stable between the 12-16 psi range and all the sample positions and collection positions have been loaded, then click on the large button with the stop watch to begin the program. A window will pop up asking if the correct column method is loaded (100%DCM). Click on 'yes' to engage the syringe pump to begin priming. The sample probe will move over to sample position #1 and aspirate the sample. After the samples have all been processed (~1 hour per sample), remove the label from the sample position and place it on the bottle in corresponding collection position. Cap the bottle and place it back in the box that was retained for their storage. At the end of the sequence there will be a window that pops up saying that the 'Sequence has been successfully completed'. The column will switch offline and the pump will automatically shut down. The only thing that has to manually be turned off is the power to the detector. Empty the waste container into a 4L waste bottle labeled with a hazardous waste label.

8.10 Pour the GPC eluate into a rinsed K-D flask. Rinse the bottle with some DCM

and add that to the K-D flask. Add 0.5 mL Iso-Octane and a micro boiling chip to each K-D flask. Attach a Snyder column to the flask and place in the hot water bath. Attach the inverted Hopkins condenser to the top of the Snyder column and turn to water on to the solvent recovery system (~1500-2000 cc/min). When the volume of the solvent in the concentrator tube is level with the base of the K-D flask, remove the inverted Hopkins condenser and secure out of the way. Lift the K-D apparatus up enough to be able to angle it slightly and add 40-50 mL Petroleum Ether through the top of the Snyder column. By holding the K-D apparatus at an angle, it allows the solvent to more easily drain back into the flask. Return to the K-D apparatus back into the hot water bath. Repeat this step 2 more times to successfully solvent exchange the sample from DCM to Petroleum Ether. When the apparent volume in the concentrator tube is 5-10 mL remove it from the hot water bath. Wipe down the K-D apparatus with a WyPall X60 towel especially around the ground glass junction. Remove the Snyder column from the K-D apparatus and allow to completely drain into the concentrator tube. Add a new micro boiling chip to the aliquot and place it in a 400 mL beaker containing water heated to approximately 75°C on a hot plate (4-5 tubes can be evaporated at one time). Evaporate the solvent down to 1-2 mL. Remove it from the water bath and allow it to cool.

8.11 Transfer the solution to a 13 x 100 culture tube with a Pasteur pipette, rinse the concentrator tube with 0.5 ml of Petroleum Ether, vortex, and transfer the rinse to the culture tube. Repeat the rinse step two more times, and add each rinse to the culture tube. Cap the culture tube with a Teflon faced cap. Mark the volume on the tube with a permanent marker.

8.12 SEDIMENT SAMPLES ONLY: Add acid rinsed copper to the culture tubes to remove any residual sulfur from the extract. Allow copper to stay in contact with extract overnight.

9.0 Florisil® Column Fractionation

IMPORTANT: ***All glassware, glass wool, and sodium sulfate must be triple-rinsed with petroleum ether (PE) before they are used for this procedure. Florisil® must be activated in an oven at 130°C for at least 24 hours prior to use.***

9.1 This procedure is performed after the GPC cleanup procedure for all tissue and sediment samples analyzed for pesticides and PCBs.

9.2 **PCB ONLY:** When the samples are to be analyzed for only PCBs prepare only the 6% ethyl ether in petroleum ether Florisil column eluant. Make an amount slightly in excess of what is actually needed to allow for any loss which may occur during solvent transfer. The required volume is 50 mL per sample for the 6% eluant.

9.3 **TISSUE:** Prepare the reagents to be used for Florisil® cleanup for tissue: 6% ethyl ether in petroleum ether, 15% ethyl ether in PE Make an amount slightly in excess of what is actually needed to allow for any loss which may occur during solvent transfer. The required volume is 50 mL per sample for the 6%, 50 ml per sample for the 15% (F2).

9.4 **SEDIMENT:** Prepare the reagents to be used for Florisil® cleanup for sediment: 6% ethyl ether in petroleum ether and 50% ethyl ether in PE. Make an amount slightly in excess of what is actually needed to allow for any loss which may occur during solvent transfer. The required volume is 50 mL per sample for the 6% and 50 ml per sample for the 50% fraction.

9.5 Prepare the chromatography columns. Place a small piece of PE rinsed glass wool in the bottom of the column and tap into place with a PE rinsed glass rod. Cover with a small portion (0.5 inch) of sodium sulfate. Fill the column with 5 grams of Florisil® that has been measured using a dedicated pre-calibrated culture tube. Tap column with rubber "mallet" to firmly settle the Florisil®. Top the column with 3/4-1 inch of sodium sulfate. This will prevent the column from being disrupted when solvent is added and will remove any residual water.

9.6 Place a 600 mL beaker under the column and pre-wet the column with about 25 mL of petroleum ether.

IMPORTANT: ***From this point and through the elution process, the solvent level should never be allowed to go below the top of the sodium sulfate layer.***

9.7 When approximately 1 inch of PE remains above the surface of the column, add 0.5 mL of iso-octane to a K-D flask and place it under the column making sure that the stopcock is in the full open position. This will allow for a flow rate of about 2 to 3 mL/min. When the meniscus of the PE rinse reaches the column bed surface, decant the sample onto the column. Immediately add approximately 0.5 mL of PE to the tube, vortex, and add the rinse to the sample extract on the column. Add another 0.5 ml of PE to the tube, vortex, and add this final rinse to the sample extract on the column. Start the columns in a sequential fashion, and the lag time will be adequate to perform the necessary tasks for up to six columns.

9.8 When the combined sample and rinses reach the sodium sulfate layer, add 50 mL of 6% diethyl ether/petroleum ether that has been carefully measured out using a graduated cylinder to the column reservoir. Make sure that the stopcock is fully open in order to achieve the desired flow rate of 2 to 3 mL per minute. Place a 50 mL clean, dry, petroleum ether rinsed beaker over the top

of the reservoir to prevent evaporation during the elution process. If only **PCB** analyses are requested, allow the column to completely drain and stop here.

TISSUE SAMPLES

9.9 Just as the last of the 6% diethyl ether/PE solvent reaches the top of the sodium sulfate layer, add 0.5 mL of iso-octane to a new K-D flask and exchange it for the K-D containing the 6% elution, add 50 mL of the 15% diethyl ether/PE mixture to the column reservoir, replace the 50mL beaker, and elute as before. Add a micro boiling chip and attach a Snyder column with a blue clamp to the K-D flask containing the 6% diethyl ether/PE fraction and place vessel in the hot water bath with the temperature set at 80-82 °C and reduce volume to an apparent volume of 1 mL. Tap the Snyder column to make sure solvent is not trapped between the balls then remove the vessel from the bath and place in the vessel stand to cool.

9.10 Repeat the above adding 0.5 mL of iso-octane to a new K-D flask and exchange it for the K-D flask containing the 15% eluant. Allow all of the eluant to drain into the K-D flask.

SEDIMENT SAMPLES

9.11 Just as the last of the 6% diethyl ether/PE solvent reaches the top of the sodium sulfate layer, add 0.5 mL of iso-octane to a new K-D flask and exchange it for the K-D flask containing the 6% eluant, add 40 mL of the 50% diethyl ether/PE mixture to the column reservoir, replace the 50mL beaker, and elute as before. Add a micro boiling stone and attach a Snyder column with a blue clamp to the K-D flask containing the 6% diethyl ether/PE fraction and place vessel in the hot water bath with the temperature set at 80-82 °C and reduce volume to an apparent volume of 1 mL. Tap the Snyder column to make sure solvent is not trapped between the balls then remove the vessel from the bath and place in the vessel stand to cool.

9.12 When the vessels are cool, remove the concentrator tube from the K-D flask add a new micro boiling stone and attach a clean micro-Snyder column to the concentrator tube with a blue clamp and place in a 400 mL beaker containing hot water heated to approximately 75°C on a hot plate. Evaporate the solvent until only 0.5-1 mL remains in the concentrator tube. Four or five tubes can be evaporated at one time.

9.13 When the solvent has been evaporated to 0.5-1 mL remove the tube from the bath and allow it to cool in a test tube rack. Remove the micro-Snyder column and transfer the contents to a calibrated centrifuge tube rinsing the concentrator tube with a small amount of PE and adding the rinsate to the centrifuge tube. If the volume in the centrifuge tube is greater than 1 mL,

evaporate to 1 mL using nitrogen. Mix the tube contents by tapping the bottom of the tube causing a vortex which will rinse the sides of the tube. A Vortex Genie mixer may be used for this step. Transfer the extract to a clean labeled culture tube and cap.

9.14 Repeat for 15% (tissue only) and 50% extracts (sediments only). The extracts are ready for analysis by GC-ECD and GC-MSMS.

10.0 Analytical Procedure

10.1 Before the sample extracts can be analyzed, a sequence listing the order of calibration standards, second source check standards, initial and continuing calibration blanks, initial and continuing calibration verification standards and sample extracts is written using Agilent Chemstation (GC) or Varian (GC-MSMS) Software.

10.2 Each sequence includes a minimum of seven calibration standards. The calibration curve concentration for chlorinated hydrocarbons differs for different analytes, but in general the range is 0.5 ppb to 500 ppb. The calibration curve concentration range for polychlorinated biphenyl congeners (PCBs) is 0.5 ppb to 100 ppb. Higher concentrations of PCB standards (50 ppb to 1000 ppb) are analyzed with samples containing higher concentrations of PCBs.

10.3 To verify the calibration standards, second source pesticide check standards (Radian Corp., Pesticide Check Standard Mix A, ERP-009L; Pesticide Check Standard Mix B, ERP-011L) and PCB congener check standard (Ultra Scientific, RPC-EPA) are analyzed. The second source analytes and their concentrations are listed in Table 5 (pesticides) and Table 6 (PCB congeners).

Table 5. Radian Pesticide Calibration Check Standards (Mix A and B)

Mix A	Certified Concentration (ng/µL)
Aldrin	10.0
Gamma-HCH	5.00
DDT, p,p'	20.0
Dieldrin	10.0
Endosulfan I	10.0
Endosulfan II	20.0
Heptachlor	10.0
Heptachlor epoxide	10.0
Methoxychlor	80.0
Mix B	
Alpha-HCH	5.00
Beta-HCH	20.0

Delta-HCH		10.0
Cis-chlordane		10.0
Trans-chlordane		10.0
DDD, p,p'		20.0
DDE, p,p'		10.0
Endosulfan sulfate	20.0	
Endrin		20.0

Table 6. Ultra Scientific PCB Congener Check Standard

RPC-EPA	Certified Concentration (ng/µL)*
PCB 8	4.0
PCB 18	4.0
PCB 28	4.0
PCB 52	4.0
PCB 44	4.0
PCB 66	4.0
PCB 101	4.0
PCB 118	4.0
PCB 153	4.0
PCB 105	4.0
PCB 138	4.0
PCB 187	4.0
PCB 128	4.0
PCB 180	4.0
PCB 170	4.0
PCB 195	4.0
PCB 206	4.0
PCB 209	4.0

* Initial concentration of RPC-EPA is 0.2 µg/mL in iso-octane. This solution is diluted 2:100 in iso-octane

10.4 An initial calibration blank and initial calibration verification standard is analyzed after the calibration standards and prior to the first sample extract. For the 6% Fraction and 15% Fraction runs, continuing calibration blanks (CCBs) and calibration verification standards (CCVs) are analyzed after ten sample extracts have been analyzed. The 50% Fraction extracts contain more lipid material and can cause the CCVs to fail to meet the % recovery criteria, therefore the CCBs and CCVs are analyzed after every five sample extracts. If a CCV fails, the five samples prior to the failed CCV and the five samples after the failed CCV are re-analyzed after a new calibration curve is analyzed.

10.5 The CCV analyte concentrations are mid-range of the calibration curve (5 – 10 ppb).

10.6 As the run proceeds, sample extracts are monitored for analyte concentrations that are greater than the calibration curve and need dilution.

10.7 Instrumentation

Gas Chromatographs with Electron Capture Detectors:

10.7.1 Agilent 6890*plus* gas chromatograph equipped with two ^{63}Ni micro-electron capture detectors with EPC and autosampler. Two 60 meter, 0.25 mm ID, 0.25 µm (film thickness) fused silica columns (J&W) are used. A 5 meter length of DB-5 column is connected to a press fit "Y" union which splits the column effluent into two 60 m columns, a DB-5 and a DB-17MS. The injector is a split-splitless injector with EPC.

10.7.2 Chromatograph conditions:
The injector is operated isothermal at 240°C. The oven has an initial temperature of 80°C which is held for 1 minute and then temperature programmed to 210°C at a rate of 15°C/min and held for 10 min. It is then programmed to 280°C at a rate of 2°C/min and is held for 51 min (for PBDE analysis the oven is held at 280°C for 110 min). Helium is used as the carrier gas at a linear velocity of 35 cm/sec. Nitrogen is used for the detector makeup at 30 mL/min.

10.7.3 Sample volume:
Three microliters of samples and standards are injected and split approximately 50/50 onto the 60 m DB-5 and the 60 m DB-17MS.

10.7.3 Instrument calibration:
External standard calibration is used.

10.7.4 Data acquisition and processing:
Detector signals are acquired and processed with a Agilent 3365 Series II Chemstation. Data processing may also be done using Enviroquant Software.

Gas Chromatograph-Triple Quadrupole Mass Spectrometer:

10.7.5 Varian Model 3800/1200L gas chromatograph/triple quadrupole mass spectrometer equipped with a Model 1177 split-splitless injector with EPC and CombiPal autosampler. A J&W 60 meter, 0.25 mm ID, 0.25 µm (film thickness) XLB fused silica columns (J&W) is used. The injector is a split-splitless injector with EPC.

10.7.6 Chromatograph Conditions:
The injector is operated isothermal at 280°C in splitless mode with pressure pulse (45 psi for 1.05 min). The oven has an initial

temperature of 80°C which is held for 1 minute and then temperature programmed to 210°C at a rate of 15°C/min and held for 10 min. It is then programmed to 280°C at a rate of 2°C/min and is held for 8 min. Helium is used as the carrier gas at a constant column flow of 1 mL/min.

10.7.7 Mass Spectrometer Conditions:
The mass spectrometer is operated in electron impact (EI) ionization and MSMS mode using argon as the CID gas. A collision energy of 10 to 30 volts is used depending on the analyte. Q1 and Q3 mass fragments were selected to optimize selectivity and sensitivity. See Table 7.

Table 7. Varian 1200 MS collision energies and mass fragments (Q1 and Q3) for targeted analytes.

	Segment	Q1	Q3	Collision Energy	Internal Standard
DBOB	1	296	246	-20	HCH, alphaC^{13}
HCH, alpha	2	219	183	-10	HCH, alphaC^{13}
HCH, alphaC^{13}	2	223	187	-10	Internal Std
HCB	3	284	214	-30	HCB C^{13}
HCBC^{13}	3	290	220	-30	Internal Std
HCH, gamma	4	219	183	-15	HCH, alphaC^{13}
HCH, beta	4	219	183	-15	HCH, alphaC^{13}
Heptachlor	5	272	237	-15	HeptachlorC^{13}
HeptachlorC^{13}	5	277	242	-15	Internal Std
Chlorpyrifos	6	314	258	-10	ChlorpyrifosC^{13}
ChlorpyrifosC^{13}	6	325	260	-15	Internal Std
Aldrin	6	293	258	-10	ChlorpyrifosC^{13}
Oxychlordane	7	387	263	-10	Nonachlor, transC^{13}
Heptachlor epoxide	7	387	353	-10	HeptachlorC^{13}
DDE, o,p'	8	318	246	-10	DDE, p,p'C^{13}
DDMU, p,p'	9	284	212	-15	DDE, p,p'C^{13}
Chlordane, trans	9	373	266	-15	Nonachlor, transC^{13}
Chlordane, cis	9	373	266	-15	Nonachlor, transC^{13}
Nonachlor, trans	10	409	310	-15	Nonachlor, transC^{13}

Nonachlor, transC^{13}	10	418	310	-15	Internal Std
DDE, p,p'	12	318	246	-15	DDE, p,p'C^{13}
DDE, p,p'C^{13}	12	329	258	-15	Internal Std
DDD, o,p'	13	235	165	-20	DDE, p,p'C^{13}
DDT, o,p'	15	235	165	-15	DDT, p,p'C^{13}
Nonachlor, cis	16	409	275	-15	Nonachlor, transC^{13}
DDD, p,p-deuterated	16	243	173	-20	DDT, p,p'C^{13}
DDD, p,p'	16	235	165	-15	DDT, p,p'C^{13}
DDT, p,p'	17	235	165	-25	DDT, p,p'C^{13}
DDT, p,p'C^{13}	17	248	177	-20	Internal Std
Methoxychlor	18	227	169	-20	DDT, p,p'C^{13}
Mirex	20	272	237	-15	DDE, p,p'C^{13}

10.7.8 Instrument Calibration:

Internal standard calibration is used. Internal standards are added to the standards and sample extracts just prior to analysis. The following internal standards are used at 1.0 ng/µL:

PCB Internal Standards
PCB 52 (C^{13}) – 4Cl congeners
PCB 97 (C^{13}) – 5Cl congeners
PCB 128 (C^{13}) – 6Cl and 7Cl congeners
PCB 194 (C^{13}) – 8Cl congeners
PCB 206 (C^{13}) – 9Cl congeners
PCB 209 (C^{13}) – 10Cl congeners

OC Internal Standards
HCH, alpha (C^{13})
HCB, (C^{13})
Heptachlor, (C^{13})
Chlorpyrifos, (C^{13})
Nonachlor, trans (C^{13})
DDE, p,p' (C^{13})
DDT, p,p' (C^{13})

Nine target analyte calibration levels are used (0.25, 0.50, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100 ng/µL).

10.7.9 Sample volume:

Two microliters of samples and standards are injected.

10.7.10 Data processing:
Mass spectrometer signals are acquired and processed using Varian 1200L software .

11.0 References

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Appendix IV C: Procedure for the Handling, Storage and Disposal of Hazardous and General Laboratory Waste

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CDFG Fish and Wildlife Water Pollution Control Laboratory (WPCL) and Petroleum Chemistry Laboratory (PCL) Standard Operating Procedure for the Handling, Storage, and Disposal of Hazardous and General Laboratory Waste

1. Scope and Application

1.1 Federal and state regulatory control over hazardous waste has become extraordinarily stringent in recent years. These changes have dramatically increased the complexity of handling the hazardous waste produced by laboratories.

1.2 These procedures are provided to ensure safe, efficient, and legally compliant handling and disposal of hazardous waste.

2. Summary of Hazardous Waste Disposal

2.1 **NEVER DISPOSE OF LIQUIDS, SOLID CHEMICALS, LABORATORY SAMPLES, HAZARDOUS WASTE OR HAZARDOUS MATERIALS IN THE LABORATORY TRASH RECEPTICALS OR DUMPSTERS. NEVER DISCHARGE LIQUID HAZARDOUS WASTE TO THE SANITARY SEWER (bathroom drains) OR EVAPORATION POND (laboratory sinks, fume hood drains, floor drains).** Non-hazardous aqueous laboratory waste can be disposed of by discharging to the evaporation pond.

2.2 Characteristics of Hazardous Waste (these definitions apply to waste potentially generated by WPCL, for complete definitions see Title 22 Article 2 section 66261.10)

Ignitability – Hazardous Waste Number **D001**

- is liquid, other than an aqueous solution containing less than 24 percent alcohol by volume, with flash point less than 60°C (140°F);
- is not a liquid and is capable of causing fire through friction, absorption of moisture or spontaneous chemical changes and, when ignited burns so vigorously and persistently that it creates a hazard;
- is an ignitable compressed gas;
- is an oxidizer defined in 49 CFR section 173.151.

Corrosivity – Hazardous Waste Number **D002**

- is aqueous and has a pH less than or equal to 2 or greater than or equal to 12.5;
- is not aqueous and, when mixed with an equivalent weight of water, produces a solution having a pH less than or equal to 2 or greater than or equal to 12.5.

Reactivity – Hazardous Waste Number **D003**

- is normally unstable and readily undergoes violent change without detonating;
- reacts violently with water;
- forms potentially explosive mixtures with water;
- when mixed with water, generates toxic gases, vapors or fumes in a quantity sufficient to present a danger to human health or the environment;
- is a cyanide or sulfide bearing waste which, when exposed to pH conditions between 2 and 12.5, can generate toxic gases, vapors or fumes in a quantity sufficient to present a danger to human health or the environment;
- is capable of detonation or explosive reaction if it is subjected to a strong initiating source or if heated under confinement;
- is readily capable of detonation or explosive decomposition at STP;
- is a Class A explosive.

Toxicity – Hazardous Waste Number **D004-D043**

See attached definitions of toxicity.

2.3 All chemical hazardous waste must be properly identified, labeled, segregated, and stored prior to removal by a qualified and licensed hazardous waste contractor.

2.4 Maximum Storage Times

- The maximum length of time that hazardous waste may be stored by the laboratory is 270 days from the initial date of accumulation.
- On the date that 55 gallons of waste have accumulated, the laboratory has 90 days to have the waste removed.
- Hazardous waste should be transferred from the laboratory to the hazardous material storage building within 6 months of the initial date of accumulation. The date that the waste is transferred to the hazardous material storage building, that date must be entered on the hazardous waste label under "Accumulation Start Date". Waste must be removed within 90 days of the Accumulation Start Date.
- Any hazardous waste container stored over 270 days is a violation.

2.5 Labeling Hazardous Waste Containers

- All hazardous waste containers must be labeled properly.
- Hazardous waste labels must be completely filled out.
- The first date of accumulation (WPCL waste log-in code) must always be entered on the upper right corner of the label with the individual's initials. **The date that the waste is transferred from the lab to the hazardous waste storage building must be entered on the label in the "Accumulation Start Date" section and that starts the 90 day removal requirement.**

2.6 Neutralization of Aqueous Acids and Bases

Small volumes of aqueous corrosive hazardous waste may be neutralized and discharged to the evaporation pond if the following procedures are followed. A hazardous waste facilities permit or other grant of authorization is not required for treatment of laboratory hazardous waste (neutralization of aqueous acid and base waste) generated onsite, if all of the following requirements are met:

- The hazardous waste is treated in containers using recommended procedures and quantities for treatment of laboratory wastes published by the National Research Council (NRC) or procedures for treatment of laboratory wastes published in peer-reviewed scientific journals.
- The waste is treated at a location that is as close as practical to the location where the laboratory hazardous waste is generated, **and the treatment is conducted within 10 calendar days after the date that the waste is generated.**
- The amount of laboratory hazardous waste treated in a single batch does not exceed the quantity limitation specified in subparagraph (A) or (B), whichever is the smaller quantity:
  - (A) **Five gallons** or 18 kilograms, whichever is greater.
  - (B) (i) Except as otherwise provided in clause (ii), the quantity limit recommended in the procedures published by the NRC or in other peer reviewed scientific journals for the treatment procedure being used. (ii) a qualified chemist has demonstrated that a larger quantity can be safely treated and documentation to that effect is maintained onsite.
- The laboratory hazardous waste treated is from a single procedure, or set of procedures that are part of the same laboratory process.
- The person performing the treatment has knowledge of the laboratory hazardous waste being treated, including knowledge of the procedure that generated the laboratory waste, and has received hazardous waste training, including how to conduct the treatment, manage treatment residuals, and respond effectively to emergency situations.
- Training records for all persons performing treatment of laboratory hazardous wastes pursuant to this subdivision are maintained for a minimum of three years.
- All records maintained by the laboratory pertaining to treatment conducted pursuant to this subdivision are made available for inspection upon request by a representative of the department or the CUPA or other authorized agency.

3. Source Reduction and Waste Minimization

3.1 Whenever possible, experiment protocols should include provisions to both reduce the volume of the source, and minimize the generation of hazardous waste.

3.2 Waste such as motor oil, paper, corrugated boxes, toner cartridges etc. that is eligible for recycling should be recycled.

4. Storage of Waste in the Laboratory

4.1 Each laboratory should have a designated location in which to store hazardous materials to be discarded. This location should be out of the way of the normal lab activities, but should be easily accessible and recognizable. This space should be properly labeled. Fume hoods may be used temporarily to store small quantities of materials being generated but hazardous materials should not be allowed to accumulate in hoods because it could block air flow. Cabinets under fume hoods are appropriate storage locations for small quantities of hazardous waste.

4.2 All waste materials must be kept in secondary containers and segregated by hazard class (i.e., oxidizing agents such as potassium permanganate or hydrogen peroxide should be separated from organics or corrosives, acids should be separated from bases, etc.). Secondary containers can be lab trays, or any such device that will contain 110% of the largest container.

5. Labeling

5.1 Containers must be labeled prior to being used as receptacles for hazardous waste. Printed Hazardous Waste Labels must be used and filled out completely with all mandatory information including (see attached examples and summary of hazardous waste labeling codes commonly used at WPCL):

- the words "Hazardous Waste"
- starting date of accumulation in upper right corner and initials of person labeling waste container (waste identification number)
- CDFG Fish and Wildlife Water Pollution Control Lab
  2005 Nimbus Road (916) 358-2858
  Rancho Cordova, CA 95670
- WPCL's EPA ID Number (**CAD980815401**)
- the "Accumulation Start Date" or the date the waste is transferred to the hazardous materials storage building which starts the 90 day storage period (satellite storage rule)
- the composition (name of the waste) and physical state (gas, liquid, solid, sludge)
- a description of the hazardous properties of the waste (i.e. flammable, reactive, toxic, corrosive)
- EPA waste code and California waste code
- Approved D.O.T. Shipping Name and "UN" number (proper shipping names must be written exactly as listed in the D.O.T. regulations)

5.2 Handwritten labels made from tape or unprinted labels are **not** acceptable .

5.3 Chemical names must be specific. Nonspecific labels such as "organic waste", "waste solvents", "acid waste", etc., are not sufficient.

5.4 Chemical formulas or abbreviated chemical names are **not** acceptable.

5.5 Specific waste identification labels may be used **in addition to** the Hazardous Waste Label, such as PCB waste labels.

5.6 Hazardous Waste Labels are available in the cabinet in the photocopier room in the main laboratory.

6. Documentation (Hazardous Waste Inventory Logs)

6.1 When a waste container is started in the laboratory or moved to the hazardous materials storage building, it must be logged-in on laboratory's (lab room or hazmat storage building) Hazardous Waste Inventory Log. Each laboratory room where hazardous waste is generated and the hazardous materials storage building must have a logbook for recording the information described in 6.2.

6.2 The log must include the following information:
Container Log # (Identification No. – month/day/year, initials)
- -Date In (date that waste accumulation started)
- -Date Out (date that waste is transferred to the hazardous material storage building)
- -Waste Description
- -Waste Amount (size of container)
- -Hazard (Toxic, Flammable, etc)
- -First initial and surname of the person starting the waste

6.3 When the hazardous waste container is moved to the hazardous materials storage building, the waste must be logged out of the laboratory and logged into the hazardous materials storage building. The date that the waste is transferred must be entered on the Hazardous Waste label in the "Accumulation Start Date" section.

7. Containers

7.1 Containers must be properly labeled and in good condition (i.e., structurally sound and leak-proof) and **kept closed** unless you are adding or removing wastes. Liquids must be in a screw-capped container that will not leak if tipped over. Corks, parafilm, lab beakers, or other open containers are **not** acceptable. If waste is not in a proper container, transfer the waste.

7.2 The size of the container should correspond with the quantity of materials discarded. For example, it is not cost effective to ship 50 mL of material in a 4 L container, etc.

7.3 Contaminated lab ware such as glassware, gloves, paper towels, etc., must not have liquid in them. They must be placed in clear, double plastic bags and properly labeled with a Hazardous Waste Label. For disposal of broken glassware and sharps see Section 11.

7.4 Glass, paper, or plastic must not be placed in liquid waste containers.

7.5 The material must be compatible with the container - acids or bases cannot be stored in metal containers or solvents in plastic.

7.6 Containers must be inspected weekly for leaks and deterioration (**this must be documented with any deficiencies and corrective action**).

7.7 Hazardous waste storage building must be inspected weekly by the WPCL safety officer or his designate. **This inspection must be documented with any deficiencies and corrective action.**

8. Waste Segregation

8.1 Proper segregation of waste chemicals in the laboratory helps facilitate waste disposition options such as recycling. This can also result in cost savings for disposal. Any questions about waste segregation should be directed to the WPCL safety officer.

8.2 Examples of responsible and cost-effective segregation include:

- Separating halogenated from non-halogenated solvents
- Isolating metals from other wastes
- Keeping waste acetone separate from other solvents

9. Empty Containers

9.1 Empty (nothing can be poured out if the container is inverted) chemical containers of **five gallons or less** that have had the caps removed and labels defaced or removed may be disposed of as regular refuse. **Full or partially full containers should never be thrown in the regular trash.**

9.2 Empty containers that held extremely hazardous materials (waste) must be triple rinsed prior to disposal. **All rinsate** must be handled as hazardous waste.

10. Unknowns

10.1 Unknowns must be characterized prior to disposal. If the Laboratory can

not characterize the material it will have to be characterized by the hazardous waste contractor or another lab specializing in the characterization of hazardous waste prior to disposal.

11. Sharps

11.1 Broken glass, pipets, and any other sharp material that is not contaminated with hazardous material must be disposed of in a rigid sharps or broken glassware container.

11.2 Sharps that have been contaminated with hazardous materials should either be triple rinsed and discarded with non-contaminated sharps or should be discarded in a separate rigid sharps container designated and labeled as hazardous waste.

12. Tissue and Sediment Waste

12.1 Tissue waste resulting from dissection of fish should remain frozen until it is to be discarded. The frozen fish tissue should be bagged and transported to the sanitary landfill where it is discarded in a designated area used for that purpose. The county landfill should be contacted ahead of time to find out dates that these types of refuse are accepted.

12.2 Waste sediment and soils that meet the definition of hazardous waste must be labeled and treated accordingly. Waste sediment and soils that are not hazardous waste must be disposed of as non-hazardous laboratory waste and removed by the laboratory's hazardous waste contractor.

13. Hazardous Materials Storage Building Weekly Inspections

13.1 The hazardous materials storage building must be inspected weekly. A permanent record of the inspections, inspection log and deficiency and corrective action reports must be kept. Examples of the checklist, inspection log and deficiency report are attached.

14. References

California Environmental Protection Agency, Department of Toxic Substances Control (DTSC) Fact Sheets January 2002 and December 2006.

CCR Title 22, sections 66261.10, 66262.20-24, 66262.34

California Health and Safety Code, section25200.3.1

Safety Officer	Signature	Date
Laboratory Director	Signature	Date

Appendix IV D: Protocol for Corrective Action Procedures

STANDARD OPERATING PROCEDURE
TITLE: Nonconformance, Corrective Action and Preventative Action

REVISION HISTORY		
Revision #	**Summary of Changes**	**Date**
1	Reformatted.	02/01/10
0	Initial release.	09/18/06

Author:	*David Crane*		Date:
Approved:	Laboratory Director *David B. Crane*		Date:
Approved:	Quality Assurance *Gail Cho*		Date:
Approved:	Health and Safety *Thomas Lew*		Date:

STANDARD OPERATING PROCEDURE

TITLE: Nonconformance, Corrective Action and Preventative Action

1.0 Scope and Application

1.1 The purpose of this document is to describe the procedure used by the DFG Water Pollution Control Laboratory (WPCL) for the identification and documentation of nonconforming events, items, or procedures and the assessment of their impact on the quality of data generated by the laboratory.

1.2 This procedure also describes the laboratory's corrective action and preventive action procedures and monitoring.

1.3 This SOP is applicable to all laboratory systems involved in the quality system and analytical processes in the laboratory, including but not limited to, sample receiving and logging, storage, preparation, analysis, reporting, auditing, and proficiency testing.

1.4 This SOP also addresses instances of nonconformance for which no corrective action is possible or appropriate, but documentation of the nonconformance is still required.

1.5 Documentation of all nonconformances is required by WPCL and is maintained on file by the QA Officer.

1.6 This procedure also requires documentation of nonconformances resulting from errors made by persons submitting samples to the laboratory. The documentation required is maintained and archived with the appropriate data set.

1.6.1 Errors made by persons submitting samples to the laboratory (i.e. errors on chain of custody documents, sample labeling, etc.) are documented and resolved by immediately contacting the sampler or person responsible for the samples.

1.6.2 Errors that are identified following receipt of analytical results that do not result from any laboratory mistake (i.e. incorrect sample identifier, test method requested, etc.) are resolved using directions taken from consultation with the data user.

2.0 Definitions

2.1 Nonconformance – An item, event, or procedure which does not comply or agree with the governing documents, procedures, policies or requirements (e.g. QAPP, QAMP, WPCL QA Manual, etc.).

2.2 Corrective action – A twofold measure that is taken to correct a nonconforming event and to eliminate or severely restrict the reoccurrence of the same type of nonconformance.

2.3 Preventive action – A measure taken to address needed improvements and potential sources of nonconformances.

3.0 Responsibilities

3.1 Any individual within the laboratory can stop an analysis when nonconformance occurs (i.e. nonconformance not caused by sample matrix or similar unpreventable condition, etc.). Problems will be identified, documented and resolved prior to continuing the analysis.

3.1.1 The quality assurance manager also possesses the authority and responsibility to stop any work that does not meet quality standards of the laboratory and to take all necessary steps to return the system in question to a state of control.

3.1.2 The Laboratory Director is responsible for the determination of "official" work stoppages and for notifying all parties of concern regarding work stoppages, redistribution, subcontracting, if necessary, and subsequent work resumption.

3.1.2.1 The Laboratory Director, in conjunction with the Section Lead Chemist and QA officer is responsible for redistributing workload during work stoppages to ensure that requirements are met with respect to hold and turnaround times.

3.1.2.2 The Section Lead Chemist, QA officer, or Laboratory Director are responsible for notifying the data user of significant problems requiring work stoppages.

3.2 Each employee who detects a deficiency is responsible for initiating documentation of the nonconformance and forwarding the documentation to the appropriate Section Lead Chemist and /or QA Officer for review and assessment.

3.3 The Section Lead Chemist, in conjunction with the QA Officer and Laboratory Director, is responsible for analyzing the source of the nonconforming item, determining the impact of the nonconformance on the quality of the data and /or operations and implementing corrective actions to correct and/or restrict the noted deficiency according to the requirements detailed in the project QAPP or laboratory QA manual.

3.4 The QA officer is responsible for maintaining nonconformance/corrective action records and aiding personnel in the identification of nonconforming items, determining the extent of the nonconformance, and planning corrective action.

3.5 Laboratory personnel are responsible for participating in cause analysis and implementing corrective actions in response to nonconformances and for timely written response(s).

3.5.1 Generally, corrective actions, taken in response to nonconformances and cause analysis, are to be initiated immediately upon the identification of the event.

3.5.2 Some nonconformances, cause analyses and appropriate corrective actions may require additional time due to external factors, including purchasing new materials, obtaining additional training etc.

3.5.3 The QA Officer is responsible for participating in and monitoring nonconformance identification, cause analysis and corrective actions to ensure that each nonconformance is addressed quickly and effectively.

3.6 The Laboratory Director is ultimately responsible for assuring that laboratory procedures are performed in accordance to written instructions.

4.0 Procedure

4.1 Each performance event is documented by the individual who identifies the event. Documentation is effected using the Nonconformance/Corrective Action Record (NC/CAR). The NC/CAR is completed following the instructions on the form and is forwarded to the Section Lead Chemist followed by the QA Officer for review.

4.1.1 All laboratory staff have access to electronic NC/CAR forms.

4.1.2 The person(s) identifying the nonconformance will complete the Set ID, Sample Matrix, Analysis, Date Documented, and Date of Occurrence along with a brief description of the nonconformance.

4.1.3 If a specific data set is not appropriate to identify the nonconformance, complete a descriptive title in the space marked Set ID to allow for clear and concise identification of the nonconformance addressed.

4.1.4 The identifier will also sign the initiated form and forward it to the Section Lead Chemist who will pass it on to the QA Officer.

4.1.5 The Section Lead Chemist and/or QA Officer will assess the impact of the nonconformance on the data generated and will formulate a cause analysis study, if necessary.

4.1.6 Nonconformances may also be generated by the QA Officer in response to specific Measurement Quality Objectives and Method Quality Objectives. These reports do not require the Section Lead Chemist's review.

4.1.7 One NCR is completed for each nonconformance identified; however multiple sample sets may be documented on one NCR form if the deficiency is the same for each set listed.

4.1.8 The Section Lead Chemist and QA Officer review the NCR for assignment of cause analysis investigation and potential corrective actions. If following the

determination of cause, a corrective action is deemed appropriate; procedures are followed as described in Section 4.3.

4.2 Root Cause Analysis

4.2.1 Root cause analysis may involve any number of people, from 1 or 2 to the entire laboratory staff, and may involve informal conversations to lengthy reports to various individuals but must include a sufficient number of people to effectively and efficiently identify what happened and more importantly the cause of the nonconforming event (why it happened) or item and all related factors that contribute to the nonconformance.

4.2.1.1 The root cause of the nonconformance may not always be the obvious source of the problem.

4.2.1.2 Root causes that result in nonconformances can include: staff skills and training, client requirements, sample composition, methods requested, equipment, calibration, supplies, etc.

4.2.1.3 Investigations include historical sample performance (i.e. samples from the same site submitted by the same client), method performance, analyst training, and any other factors relating to system performance that could make an impact on the nonconformance identified.

4.2.1.4 Additionally upon the identification of cause of the nonconformances, internal audits may be performed where appropriate areas of activity are audited as soon as possible if the identification of the nonconformance casts doubts on the laboratory's compliance with its own policies and procedures or project QAMP or QAPP.

4.2.2 Root cause analysis studies must be appropriate to the scope and severity of the nonconformance identified.

4.2.3 Root cause analysis is generally undertaken by the QA Officer, in conjunction with the specific Section Lead Chemist and staff, and is monitored by the QA Officer for effectiveness in addressing the original nonconformance identified.

4.2.4 Upon identification of the root cause, the QA Officer and/or Section Lead Chemist will complete the Cause of Nonconformance section on the NC/CAR report and will then decide if a corrective action is needed, what steps should be performed to implement that corrective action to remedy and restrict the reoccurrence of the nonconformance and will designate the laboratory personnel who will be assigned to implement the steps required.

4.3 Corrective Action

4.3.1 Upon identification of the root cause, the QA Officer and Section Lead Chemist then decide if a corrective action is needed, what steps should be performed to correct the nonconformance and to severely restrict reoccurrence and will also determine the personnel who will be assigned to implement the steps selected.

4.3.2 Common corrective actions include: recalibration, instrument maintenance, sample repreparation, analysis of spiking solutions for degradation, etc. but must be appropriate to the scope and the magnitude of the nonconformance identified.

4.3.3 The corrective action portion of the NC/CAR form is then completed and retained by the QA Officer for further review and a copy is placed with the documentation for the project affected by the nonconformance/corrective action.

4.4 Corrective Action and Follow-Up

4.4.1 Allowing an appropriate period of reasonable time to fully implement the corrective action, the QA Officer will then perform a review of the subsequent implementation and effectiveness of all corrective actions.

4.4.2 This follow-up is usually performed within 3-5 days, but the time frame may vary depending on the complexity of the corrective action required.

4.4.3 Following this review, the NC/CAR form is then completed by the QA Officer.

4.4.4 If corrective action has been successful, the NC/CAR form is copied and the copy placed in the appropriate sample set for archive and the original is retained by the QA Officer in the NC/CAR file.

4.4.5 If corrective actions have not been successful, the Section Lead Chemist and QA Officer will conduct another review to determine other possible courses of action and repeat procedures in Sections 4.2 – 4.3.

4.4.6 If no corrective action has been taken by the individual assigned to implement the corrective action, the issue will be reported to the Laboratory Director for further action.

4.5 Preventive Actions Procedure.

4.5.1 Preventive action are a pro-active process to determine the areas where potential improvements can be made to reduce the likelihood of problems or complaints.

4.5.2 Preventive actions may originate with any member of the laboratory, from analyst to Laboratory Director, and should be brought to the attention of the Section Lead Chemist and/or QA Officer for consideration.

4.5.3 It may be necessary for the originator to prepare a short report regarding the type of improvement needed and potential improvements to be made to provide ample information for a thorough discussion among the laboratory's lead chemists and director.

4.5.4 Preventive actions generally result from the Section Lead Chemists or the Laboratory Director as a result of conversations with laboratory staff or daily activities.

4.5.5 Preventive actions can result from needed changes as instrumentation or procedures become outdated, newer technology is created to improve the laboratory's throughput and data quality, or as a result of trends identified during control charting or data analysis/review, etc.

4.5.6 Once issues are identified for possible preventive actions and the QA Officer is informed, the issues are discussed with the Section Lead Chemist and analysts.

4.5.7 The issue will be discussed with the laboratory staff affected by the proposed preventive actions, including possible benefits and costs, for formulation of an action plan.

4.5.8 If subsequent investigations are necessary, they will be assigned to specific personnel and will be monitored by the QA Officer for resolution by the date assigned for completion of the investigation.

4.5.9 Following the reception of all required supporting information, the Laboratory Director is responsible for determining the need for the proposed preventive action, for assigning personnel to perform the preventive action duties, and for determining the time frame in which the duties will be completed.

4.5.10 If the situation becomes an actual nonconformance or the result of nonconformances prior to the resolution of the preventive action; the preventive actions taken will be assistive, but the issue is then addressed using the procedure for nonconformance/cause analysis/corrective action and that procedure will take precedence over the preventive action activities.

5.0 Appendices

5.1 NC/CAR Report (2 pages)

6.0 References

6.1 "General Requirements for the Competence of Testing and Calibration Laboratories," ISO/IEC 17025:1999(E).

Appendix 5.1 NC/CAR Report

CDFG WATER POLLUTION CONTROL LABORATORY
NONCONFORMANCE/CORRECTIVE ACTION REPORT

NC/CAR# _____
(assigned by QA Officer)

Directions: Fill in all information in the top box and briefly explain the nonconformance. Forward this document to the QA Officer for review/follow-up and archive in the project folder.

SUBMIT THIS REPORT TO THE QA OFFICER WITHIN ONE WORKING DAY AFTER NONCONFORMANCE HAS BEEN DOCUMENTED

Set ID#(s): _____

Sample Matrix: _____ **Analysis:** _____

Date Documented: _____ **Date of Occurrence:** _____

Briefly describe nonconformance: *(Check all that apply)*

1. _____ LCS compounds outside warning/control limits.
2. _____ Contamination in blank outside warning/control limits
3. _____ MS/MSD compounds outside warning/control limits.
4. _____ Surrogate outside warning/control limits.
5. _____ Calibration curve/check standard outside warning/control limits.
6. _____ SRM outside warning/control limits.
7. _____ Other: (describe) _____

_____

Was client contacted? _____ Yes (If yes, complete the following information) _____ No

Client contact: _____ **Organization:** _____

Date: _____ **Time:** _____

Signature: _____ **Date:** _____

Supervisor's Signature: _____ **Date:** _____

QUALITY ASSURANCE USE ONLY

Date NCR received by QA: _____

Signature: _____ **Date:** _____

CDFG WATER POLLUTION CONTROL LABORATORY
NONCONFORMANCE/CORRECTIVE ACTION REPORT

NC/CAR# _____
(assigned by QA Officer)

Directions: Cause and corrective action are to be completed by the Section Lead Chemist with the aid of any responsible parties. A two-week (or appropriate) follow-up to the corrective action will be given to resolve the issue by the personnel assigned to address the nonconformance. If corrective action is not addressed, the report will be forwarded to the Lab Director for resolution.

Section Lead Chemist

Cause of Nonconformance:

_____ Matrix Effect.
_____ Spiking solution/Standard mix degradation.
_____ Instrument malfunction.
_____ Preparation error.
_____ Other (describe): _____

Section Lead Chemist

Corrective Action: (Check all that apply)

_____ Sample was re-prepared and reanalyzed.
_____ Standards were re-prepared and reanalyzed.
_____ Instrument maintenance was performed.
_____ Spiking solution/standard solution was analyzed for degradation.
_____ Other (describe): _____
_____ No action necessary/possible. Why? _____

Person assigned to correct nonconformance: _____

Date corrective action is to be initiated: _____

Assigned by: _____

QA USE ONLY (Follow Up Comments)

Was corrective action initiated? _____ Yes _____ No* _____ Not Required

Did corrective action correct nonconformance? _____ Yes _____ No*

** (if no to either, forward to Laboratory Director for further action)*

Comments:

Signature: _____ **Date:** _____

Laboratory Director (if applicable)

Comments:

Signature: _____ **Date:** _____

Page 2 of 2

Appendix IV E: Method: Microcystins and Biotoxins by LC/MS/MS

Determination of Microcystins and Microcystin Metabolites in Water and Tissue by Enhanced Liquid Chromatography Tandem Mass Spectrometry

1.0 Scope and Application

A liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) method has been developed and thoroughly validated to identify and quantify trace levels of cyanotoxins or microcystins (MC) in water, bivalves and fish tissue with enhanced sensitivity and specificity. The method enables confirmation and quantification of six MCs (MC-LA, LF, LR, LW, RR and YR) with a single chromatographic run. The applied chromatography also allows determination of certain MC metabolites (Desmethyl-LR and -RR). By using LC-ESI-MS/MS in Multiple Reaction Monitoring (MRM) mode, the limit of detection and quantitation for the microcystins studied, were determined to be between 0.2 pg and 1 pg on column (5:1 S/N ratio).

2.0 Summary of Method

An aliquot of water sample is mixed with 10% acidified methanol and extracted using sonication techniques. Each batch of samples (20 or less) contains a blank, laboratory control sample (LCS), matrix spike and duplicate (MS/MSD) and field sample duplicate, when provided (100 mL total). Identification and quantification of MCs is performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in MRM mode. All quantitation is performed using certified standards, except the demethylated (dm) congeners which are quantified as the parent non-methylated analog since no certified standard is commercially available. All extracts are analyzed using a five level calibration curve and second source standards are obtained when available. The microcystins currently analyzed are MC-RR, -dmRR, -LR, -dmLR, -YR, -LA, -LF, and -LW. Nodularin is used for internal standard. The reporting limit for all microcystins is 1 µg/L (ppb). The average method recovery range for MCs in water is 65-120%.

3.0 Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may cause LC artifacts and/or elevated baselines, resulting in the misinterpretation of chromatograms. 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. Specific selection of reagents and purification of solvents by distillation in all-glass systems are required. High-purity distilled-in-glass solvents are commercially available.

An effective way of cleaning laboratory glassware is by rinsing with polar and non-polar solvents before use. The cleaning procedure used must be tested by analyzing procedural blanks prior to analyzing samples.

3.2 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source. Solid phase extraction (SPE) can be used to overcome many of these interferences.

3.3 SPE Clean Up Procedure

Pre-filtered water samples (100 mL) were extracted with J.T.Baker™ C18, 6 mL, 500 mg solid phase cartridges (Milford, MA) mounted on a Resprep™ vacuum manifold, (Restek Corp., Bellefonte, PA). The cartridges were first pre-conditioned with 10 mL methanol followed by 10 mL water. The samples were loaded through the cartridges at a rate of 5 mL/min, not to exceed 20 psi. The cartridges were then dried for 5 minutes with vacuum and finally, eluded with 2 mL methanol, vortexed and filtered through 0.45 µm filters.

4.0 Material and methods

4.1 Chemicals and reagents

Certified MC standards (LR, RR, LF, LW and NOD-R) were purchased from Calbiochem (EMD Chemicals, La Jolla, CA) and LR, RR, YR, LA were purchased from Sigma-Aldrich (Allentown, PA). Burdick and Jackson HPLC grade solvents (acetonitrile, methanol, water), glass fiber filters (Type A/E, 90mm, 1 µm) and Gelman Acrodisc® CR PTFE syringe filters (13 mm, 0.45 µm) were obtained from Pall Corp., Ann Arbor MI, USA. Mobile phase additives, ACS grade formic acid (98%) and trifluoroacetic acid (99%) were purchased from Sigma Aldrich, Milwaukee, WI, USA). For method validation purposes, Sacramento River water and Rainbow Trout tissue and livers were obtained from the Nimbus Fish hatchery, Rancho Cordova, CA. Mussels were purchased from a local fish market. A combined intermediate working solution of MCs was made in methanol from the purchased standards.

4.2 Sample storage

Tissue samples are kept frozen until time of extraction. Water samples for cyanotoxin analysis should be refrigerated in the dark to prevent toxin degradation but it is essential that storage be kept to a minimum (preferably less than 72 hours). Where prolonged storage is required, samples can be frozen, although this will release toxins from the cells and only the total amount of toxin in the sample can then be determined.

4.3 Sample preparation

4.3.1. Liquid sample extraction

MCs in water bodies at the time of a bloom will be present in both the water (free, dissolved or extra cellular toxins) and the cyanobacterial cells (intracellular toxins). In order to determine total MC in the water the cell wall must be ruptured or lysed by repeated freeze-thawing and sonication. An aliquot of sample (100 mL) was filtered under vacuum through a glass fiber filter (1 µm). The water and filters were extracted separately, as follows: 1) Pre-filtered water samples were acidified with 0.1 % FA and 0.05 % TFA to obtain pH~2 and extracted by SPE using JT BakerBond C18, 6 cc, 500 mg solid phase cartridges (Mallinckrodt Baker, Phillipsburg, NJ) mounted on a Resprep™ vacuum manifold (Restek Corp., Bellefonte, PA). The cartridges were first pre-conditioned with 10 mL methanol followed by 10 mL acidified water. The samples were loaded through the cartridges at a rate of 5 mL/min, not to exceed 20 psi. The cartridges were then dried for 5 minutes with vacuum and finally, eluded with 2 x 1 mL mixture methanol:water (90:10) acidified with 0.1% TFA, vortexed and filtered through 0.45 µm filters. Extracts are now ready for analysis. 2) Filters with planktonic material or lyophilized biomass shellfish were extracted twice with 15 mL of methanol:acidified water (90:10, v/v) by homogenizing for 1-2 minutes using a Polytron, followed by 10 minute sonication in ultrasonic bath. The extracts were centrifuged and the supernatant was evaporated at 35°C to 5 mL with rotary-evaporator. The concentrated extract was diluted to 100 mL in order to decrease the methanol concentration, acidified and followed by the SPE procedure.

4.3.2 Bivalve sample extraction

Tissue (mussel, liver, fish tissue) samples were homogenized using a Bucchi B-400 mixer equipped with a titanium knife assembly. A 2-5 g sample was transferred to conical centrifuge tubes with 10 mL methanol:acidified water (90:10, v/v) and finely-ground with an Arrow 850 tissue grinder (Arrow Engineering Co., Inc., Hillside, NJ) equipped with a glass pestle for five minutes, followed by sonication with a Branson® 3510 Ultrasonic for one hour. The extracts were then centrifuged at 3500 rpm for 30 minutes using a HN-S centrifuge (Damon-IEC Division, Needham Heights, MA). The extract is reduced to minimum volume and diluted with water (not to exceed 5 % methanol), acidified and finally, cleaned-up using SPE, as described above. For extremely dirty samples, an extra step is recommended by washing the HLB cartridge with 10-20% methanol:water solution before eluting the target analytes.

4.4 Analysis parameters and set up

The LC-MSD single quadrupole used was an Agilent 1100 liquid chromatograph connected to Hewlett Packard MSD-SL and the LC-MS/MS used was an Agilent 1200 liquid chromatograph connected to a 6410 triplequadrupole (QqQ). Both LCs were equipped with a vacuum degasser, a binary pump, an autosampler and a thermostatted column compartment kept at 40°C. These instruments were purchased from Agilent Technologies, Santa Clara, CA. Agilent Chemstation

software was used to collect and process data from LC-MS, while Agilent Mass Hunter software was used for LC triplequadrupole.

4.4.1. Chromatography

The mobile phase consisted of HPLC water (A) and acetonitrile (B) (both contained 0.1 % formic acid (v/v)). The gradient elution program started with 5 % B and held for 2 min. The first linear gradient from 5 % B to 50 % B over 8 min, a second linear gradient from 50 % B to 75 % B over 3 min and held at this gradient for 5 min before returning to initial mobile phase ratio at 19 min and held for 1 min. The run time was 20 minutes. The flow rate was set at 0.25 mL/min. The injection volume was 10 µL. After each run, the column was equilibrated for 5 min at the initial conditions before the next injection. A Guard column C18, 3.5 µm, 2.1 x 30 mm was used to protect the analytical column; dC18, 3 µm and 2.1 X 100mm (Waters Atlantis). The effluent from the LC column was directed from the waste to the mass spectrometer source after the first 5 minutes of the run.

4.4.2. MS-SIM parameters

Nitrogen (less than 1 ppm oxygen, Praxair, Rancho Cordova, CA) was used as the nebulizing and drying gas. The MSD was run using electrospray ionization (ESI) interface operated in positive mode as follows: 350°C drying gas temp, 13.0 L/min drying gas flow, 40 psi nebulizer pressure, 110 fragment voltage and 4.0 kV electrospray capillary voltage. MS detection was performed in Selected Ion Monitoring (SIM). The following MC ions (m/z) were monitored: 519.8 RR and 512.8 desmethyl-RR are both $[M+2H]^{2+}$; 1045.6 YR, 995.7 LR, 981.7 demethyl-LR, 910.6 LA, 1026.6 LW, 987.6 LF and 825.5 NOD-R were monitored using $[M+H]^{+}$. Full scan was also collected over the range 100-1100 Da. The UV-Diode Array Detector (DAD) was set at 238 nm , Agilent ChemStation was used to collect data.

First, experiments were carried out by direct injection of high concentrations of individual toxin standards into the mass spectrometer using the Flow Injection Automated (FIA) program. The obtained full scan spectra showed the exclusive presence of protonated molecular ions $[M+H]^{+}$ for all microcystins (MC) except MC-RR, which had a doubly charged $[M+2H]^{2+}$ ion. This correlates well with the presence of two arginine residues in MC-RR whose side chains are capable of retaining external protons and producing stabilized $[M+2H]^{2+}$. Those ions were then chosen for SIM mode. Table 1 shows the toxin fragments monitored and their respective m/z values. Nodularin could be used as surrogate (Sur) or internal standard (IS) since it is a pentacyclicpeptide and found primarily in marine water.

Table 1. MC toxin fragments monitored by LC-MS.

MC toxin fragment	m/z $[M+H]^{+}$	m/z $[M+2H]^{2+}$
Adda fragment	135.1	
MC-RR		519.8

dm-MC-RR		512.8
MC-LR	995.5	
dm-MC-LR	981.5	
MC-YR	1045.5	
MC-LA	910.6	
MC-LF	987.5	
MC-LW	1026.5	
NOD-(IS)	825.5	

The fragment voltage was optimized by re-analyzing the individual microcystin standards in SIM mode using FIA programming and changing the fragment voltage over the range from 10-130 volts. The fragment voltage corresponding to the most intense peak was chosen (110V). Instrument default settings were used for drying gas, capillary voltage and the remaining MS parameters. Finally, the LC system was attached to the MS and MCs standard mixture was analyzed. All analytes were well separated under the LC conditions listed above. It was possible to increase analyte response in the SIM mode by using multiple acquisitions and time programming modes, where five segments were used.

4.4.3. MS/MS-MRM parameters

The triplequadrupole was operated using the same conditions as the single quadrupole, except the detection was in multiple reaction monitoring (MRM) mode. The parameters for running MRM are as follows: ultra-pure nitrogen gas was used for collision induced dissociation (CID). The protonated fragment ions used for SIM mode served as the precursor ions for MRM mode, therefore, only the transition from the precursor to the product ion needed to be optimized by varying the voltage of collision induced dissociation (CID) gas from 0 to 50 eV. As a result, the most intense product ions obtained by these voltage settings were selected. The collision energy (CE) was set at 50V for MC-RR/dm-RR and 70V for the remaining MCs. The MRM windows were established for MCs using the daughter ions, which are the Adda fragments of m/z 135.2 and m/z 213 produced by the transition of the protonated precursor ions (SIM). Fragment at m/z 135 corresponding to the O-methylphenylacetaldehyde $[Ph\text{-}CH2\text{-}CHOMe]^+$ structure from the Adda moiety and the fragment at m/z 213 corresponding to $[Glu\text{-}Mdha+H]^+$ resulted from the MRM transition were the predominate product ions for all MC analytes. The fragment ions, m/z 135.2 and m/z 213 were chosen as quantifier and qualifier ions, respectively. Fragment of Adda at m/z 135.2 and fragment ion at m/z 227.1 correspond to $[Glu\text{-}Mdhb+H]^+$ were obtained for the internal standard, NDLN. Table 2 shows the optimum CE setting for all MCs and time segments of the MRM method.

Table 2. Time segments and MRM parameters.

Time Segment #	Time (min)	Target Compound	Scan Type	Precursor Ion	Product Ions	Fragment (V)	Collision Energy (V)
1	0	na*	MS2 Scan (to waste)				
2	5	MC-RR	MRM	520	213, 135.2	110	50
		dm-MC-RR	MRM	512.8	213, 135.2	110	50
3	12.9	MC-LR	MRM	995.7	213, 135.2	110	70
		dm-MC-LR	MRM	981.7	213, 135.2	110	70
		MC-YR	MRM	1045.6	213, 135.2	110	70
4	14.5	MC-LA	MRM	910.6	213, 135.2	110	70
5	15.5	MC-LF	MRM	987.6	213, 135.2	110	70
		MC-LW	MRM	1026.6	213, 135.2	110	70
* na: not applicable							

Under these LC-MS/MS conditions a 0.2 µg/L microcystins standard mixture was analyzed and easily identified with S/N greater than 5.0 for most toxins. Typical MRM and reconstructed ion chromatograms are shown in Fig. 1.

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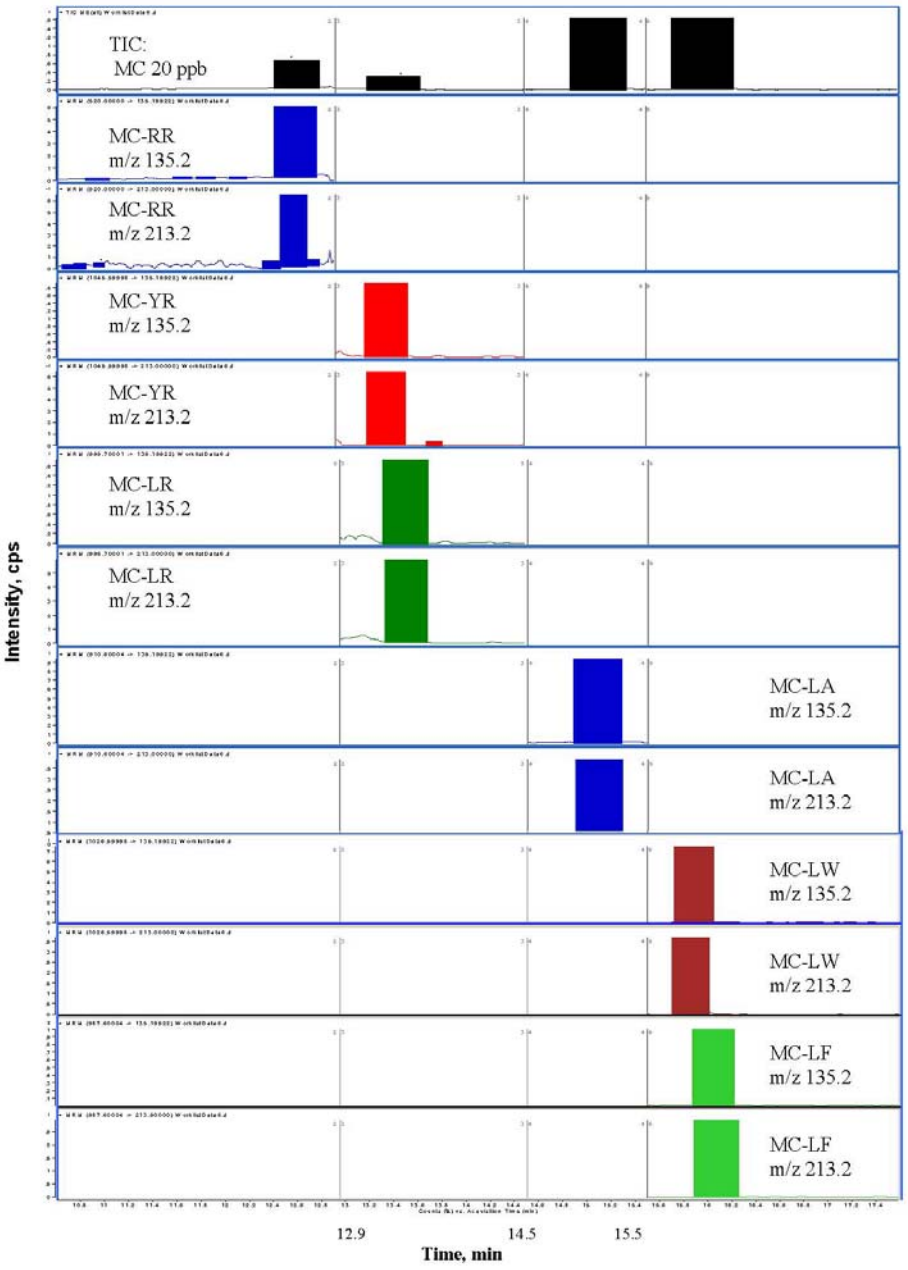
Fig. 1. MRM constructed ion chromatogram for microcystin standard.



4.5 Calibration curve

To evaluate the linearity of the system various concentrations of certified MC mixture standard solution were prepared in methanol-water (90:10) (v/v) to obtain a seven level calibration curve ranging from 0.2 µg/L to 200 µg/L. An individual calibration curve was drawn for all the toxins except the demethylated variants. A linear response was found between concentration and area for MCs. As shown in Fig. 2, the linearity was very good for all MCs with correlation coefficient (r^2) greater than 0.998. The limit of detection in MRM mode was calculated using USEPA procedures found in Title 40 Code of Federal Regulations Part 136 (40CFR 136, Appendix B, revision 1.11) and were below 0.1 µg/L or lower for all the MCs.

Using the above listed MRM parameters, 1 µg/L microcystin standard mixture was easily detected and separated (Fig. 3). The constructed ion chromatogram (1 µg/L) showing the transition from the individual microcystin precursor ion to its corresponding product ions chromatogram and spectra are shown in Fig. 4. By using LC triplequadrupole MS with ESI in Multiple Reaction Monitoring (MRM) mode, the limit of detection and quantitation for all microcystins were determine to be between 0.2 and 1 pg on column, with 5:1 S/N ratio.

Fig. 2. Calibration curves of individual MCs ranging from 1-200 µg/L.

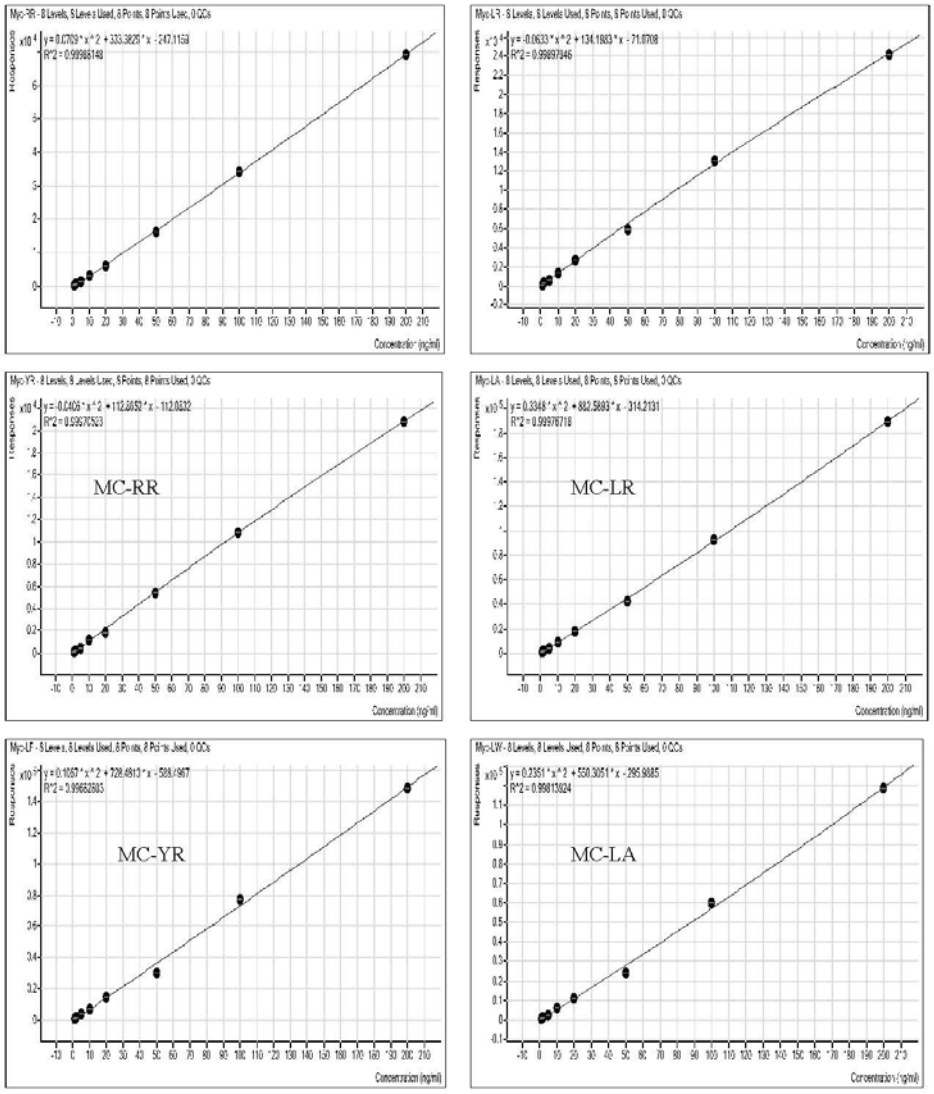


Fig. 3. Total ion chromatogram of a microcystin standard at 1 pg on column.

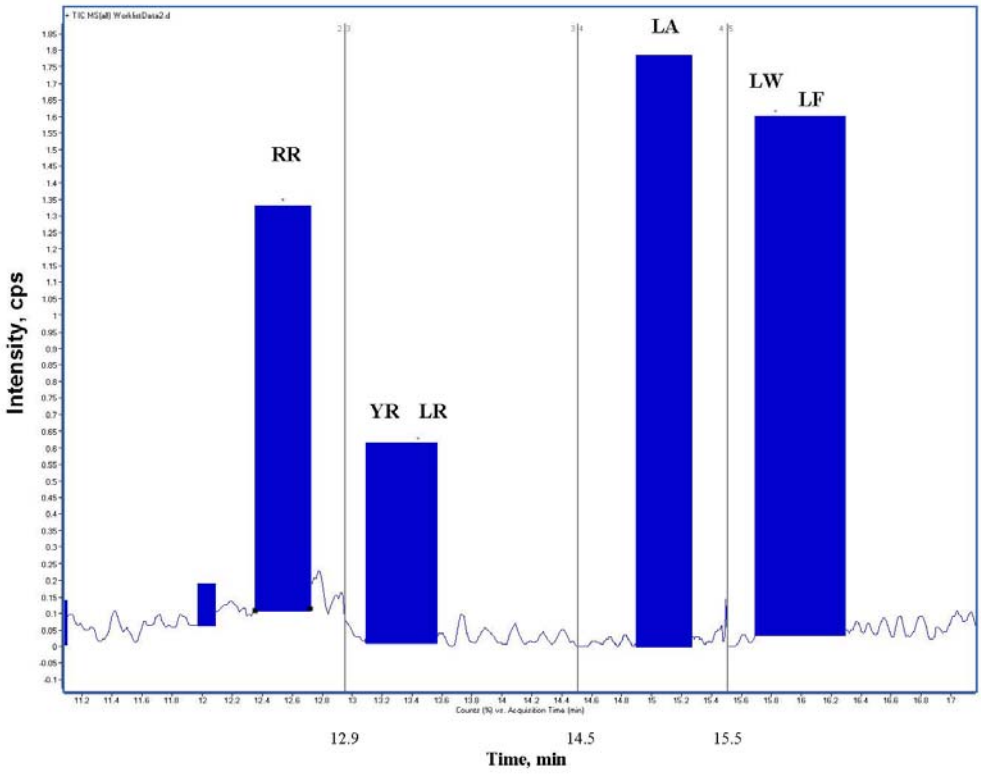
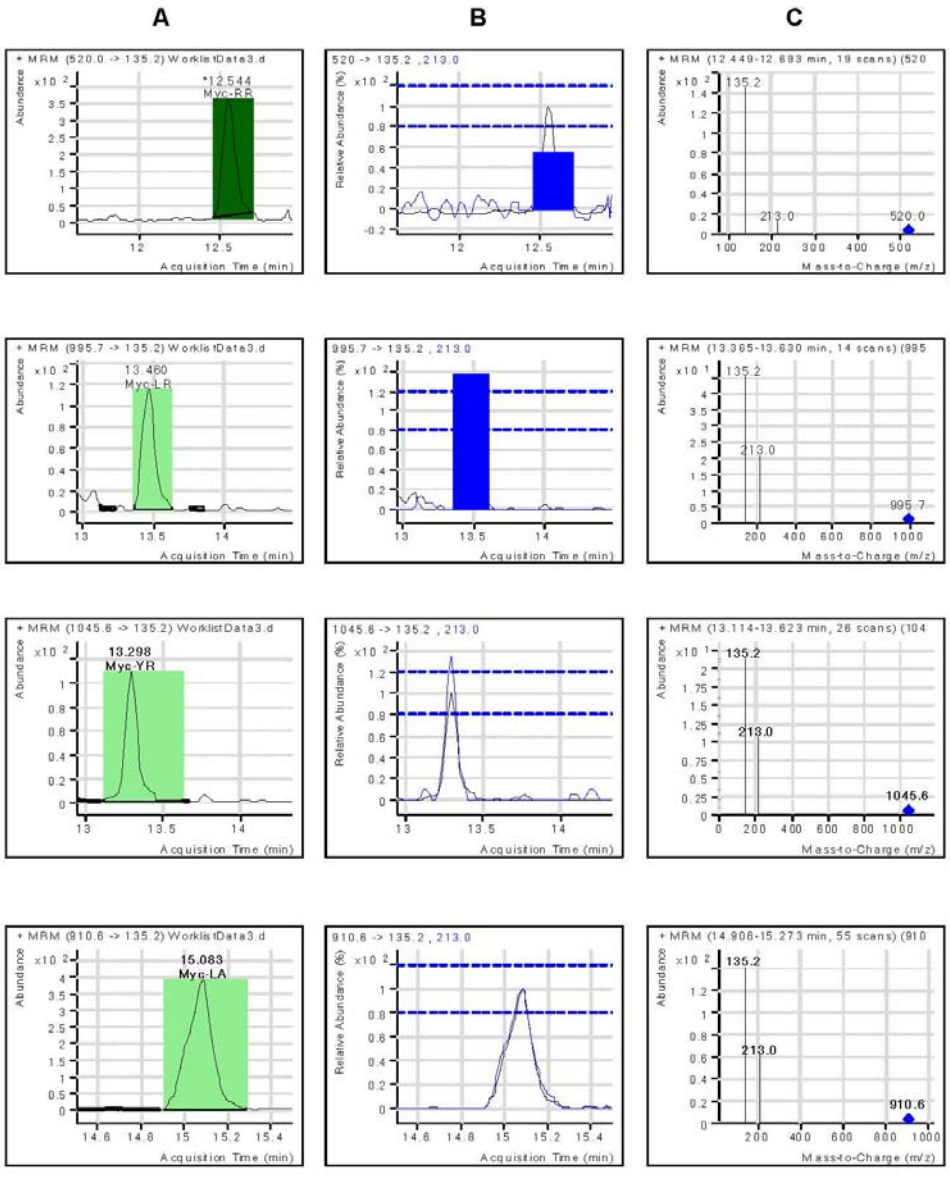
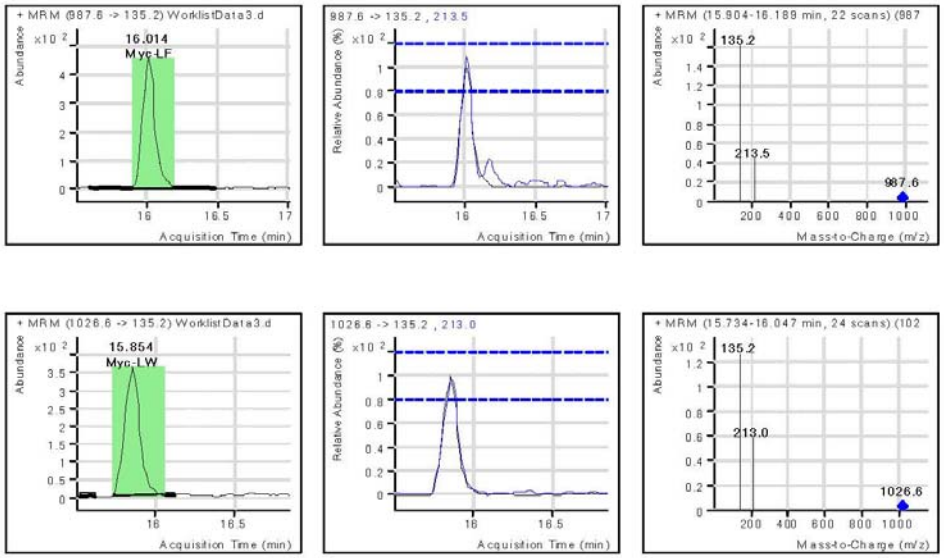


Fig. 4. Microcystin chromatograms in MRM mode: precursor ion (A), product ion (B) and spectra (C).





5.0 Method validation

5.1 Liquid samples method validation

5.1.1. Fresh water solid phase extraction (SPE)

The method was first tested with several types of SPE cartridges (Supelco C8, Oasis HLB, C18 J.T Baker Strata X). Acidified water samples (100 mL), fortified with MCs mixture at 5 µg/L, went thru the SPE procedure detailed earlier in Section 3.3.3. The result from this study shows that J.T Baker C18 cartridges extract all the tested microcystins and Nodularin from the water with acceptable recovery as shown in Table 3.

Table 3. Microcystin Solid Phase Extraction Results.

Biotoxin Compounds	C8-Supelco	C18-JTBaker	C18-HLB Oasis	Strata X
	% Recovery	% Recovery	% Recovery	% Recovery
Myc-YR	114	118	60.5	60.0
Noduralin	78.8	82.8	56.3	50.0
Myc-LW	74.1	139	10.8	8.08
Myc-LF	86.1	157	13.2	ND
Myc-LR	60.8	109	58.8	60.3
Myc-LA	4.99	48.1	18.6	13.2
Myc-RR	151	143	95.4	114

The selected SPE C18 cartridge (J.T.Baker) was used to validate the method. Triplicate river water samples fortified with MCs and NDLN at 5µg/L level (LCS) and triplicate glass fiber filters spiked (FS) with MCs and NDLN at 0.2u/g were extracted following the procedure listed in Section 4.3. Recoveries obtained for all tested MCs were ranging from 74.0-125 % and from 73.8-110% for water and filter extracts respectively. NDLN recoveries were ranging from 89.7-113 for both. MC-LW showed lower recoveries in the filter extracts. This loss could be contributed to the sorption of this MC to the wall of the C18 cartridges. Recoveries for all individual MC and the estimated method detection limit (MDL) for MCs calculated from students *t* times standard deviation for water samples (200mL) using this procedure are listed in Table 4.

Table 4. C18 SPE water (LCS) and filters (FS) method validation.

Microcystin Congeners	LCS1	LCS2	LCS3	Ave% Rec	StDev	%RSD	MDL=t*stdev µg/L
MCY-RR	102	99.6	102	101.3	1.45	1.43	0.009
MCY-LR	104	103	111	106.0	4.51	4.25	0.005
MCY-YR	114	115	125	118	6.08	5.15	0.015
MCY-LA	92.0	89.9	101	94.3	5.90	6.25	0.013
MCY-LF	85.6	74.0	84.8	81.5	6.48	7.95	0.020
MCY-LW	66.7	72.3	81.9	73.6	7.69	10.4	0.024
Nodularin	100	101	113	104.7	7.51	7.18	0.009

Microcystin Congeners	FS1	FS2	FS3	Ave% Rec	StDev	%RSD	MDL=t*stdev µg/L
MCY-RR	73.8	79.2	76.0	76.3	2.72	3.56	0.005
MCY-LR	87.2	89.6	86.8	87.9	1.51	1.72	0.016
MCY-YR	103	111	110	108	4.36	4.04	0.021
MCY-LA	82.0	77.0	84.0	81.0	3.61	4.45	0.021
MCY-LF	92.0	84.8	80.8	85.9	5.68	6.61	0.023
MCY-LW	38.8	44.8	52.7	45.4	6.97	15.3	0.027
Nodularin	94.7	89.7	92.0	92.1	2.50	2.72	0.026

5.2 Fresh water direct injection

LC-MS/MS triplequadrupole operated in MRM has shown the ability to achieve extremely low detection of MCs (2 pg on column). For this reason a direct injection method was validated on the most common microcystins (MC-RR, MC-LR and MC-YR). A set of nine fortified river water samples (0.5 µg/L) were diluted with methanol to obtain (9:1) water-methanol (v/v). A portion of the sample was filtered through 0.45 µm Gelman filters then directly injected into LC-MS/MS (QqQ).The MDL for water using direct injection was determined to be 0.1 µg/L based on signal-to-noise equivalent to 7:2. The MRM results obtained in Table 5 shows the mean recoveries were 104, 97.0 and 95.4 % for MC RR, -LR and -YR, respectively, with RSD< 11%.

Table 5. Direct injection results from 0.2 µg/L fortified water samples.

Toxins	LCS1	LCS2	LCS3	LCS4	LCS5	LCS6	LCS7	LCS8	LCS9	Avg	StDev
MC-RR	106	100	102	98.8	104	110	110	110	96.8	104	5.09
MC-LR	88.0	85.1	90.2	87.2	93.9	107	104	101	116	97.0	10.6
MC-YR	100	88.4	96.5	94.0	103	106	95.0	83.5	92.4	95.4	7.00

This validated method was tested by analyzing split contaminated water samples received as part of a Round Robin study organized by Florida Department of Environmental Protection (EPA). Twelve laboratories throughout the United States participated in this exercise. Three types of water samples were received:

1) water from natural bloom (2007) occurred in Lake Munson (M) which was caused by cyanobacteria, *Microcystis aeruginosa*, 2) water sample containing toxin produced by cultures from University of Texas laboratory (T) and 3) a microcystin standard (S) diluted in water. Each laboratory was provided with 10 blind water samples. Either three or four replicates were provided to each laboratory for each sample type. Laboratories were required to hold the samples in the dark at 4°C for no more than one week before analysis. Two different extractions were performed on the water samples for comparison:

a) An aliquot of round robin water sample (200mL) was first filtered thru 0.45 µm glass fibers filters then extracted with SPE cartridge. The filters were sonicated and both SPE and filters were extracted according to the procedure outlined in Section 4.3.
b) The second extraction consisted of 1 mL of methanol added to 9 mL of the round robin water sample sonicated for 45 min, centrifuged for 30 min and a portion of the methalonic solution was filtered thru Gelman filters and directly injected into LC-MS/MS. Results from both type of extraction are listed in Tables 6-8.

Table 6. Comparison of direct injection (sonication) vs. SPE and filters from Round Robin standard (S).

Sonication	S-1	S-2	S-3	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR						
Myc-LR	2.80	2.96	2.96	2.91	0.09	3.18
Myc-YR						
Total MCs	2.80	2.96	2.96	2.91	0.09	3.18

SPE	S-1	S-2	S-3	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR						
Myc-LR	0.37	0.49	0.55	0.47	0.09	19.4
Myc-YR						
Total MCs	0.37	0.49	0.55	0.47	0.09	19.4

Filters	S-1	S-2	S-3	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR						
Myc-LR	1.28	1.27	1.17	1.24	0.06	5.12
Myc-YR						
Total MCs	1.28	1.27	1.17	1.24	0.06	5.12

Total MC	S-1	S-2	S-3
Sonication	2.80	2.96	2.96
SPE+filters	1.65	1.76	1.72

Table 6 contains the results obtained from the analysis of round robin sample (S) showing the presence of low levels of MC-LR (~2 ppb). The combined results obtained from SPE and filters correlate well with the direct injection results. The average MC-LR value obtained from sonication was 2.90 ppb compared to 1.71 ppb obtained with SPE and filters, the difference could be contributed to losses during sample preparation.

Table 7. Comparison of direct injection vs. SPE and filters from University of Texas culture (T).

Sonication	T-1	T-2	T-3	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR						
Myc-LR	65.0	60.6	63.1	62.9	2.21	3.51
Myc-YR						
Total MCs	65.0	60.6	63.1	62.9	2.21	3.51

SPE	T-1	T-2	T-3	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR	0.51					
Myc-LR	37.5	40.0	39.6	39.0	1.37	3.51
Myc-YR						
Total MCs	38.0	40.0	39.6	39.2	1.1	2.7

Filters	T-1	T-2	T-3	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR						
Myc-LR	11.69	7.54	6.27	8.50	2.84	33.4
Myc-YR						
Total MCs	11.69	7.54	6.27	8.5	2.84	33.4

Total MC	T-1	T-2	T-3
Sonication	65.0	60.6	63.1
SPE+filters	49.7	47.5	45.9

Microcystins	T-1	T-2	T-3
Demethyl-RR	6.20	5.65	7.61
Demethyl-LR	16.9	16.5	18.0

Table 7 shows the presence of mainly MC-LR in the Texas culture samples. With an average of 62.9 ppb for the direct injection compare to 48.1 ppb obtained by SPE extraction. The RSD values were below 5% for all replicates. Desmethylated

microcystin (demethyl-MC) RR and LR were also found in these samples with an average value of 5.82 and 17.13 ppb, respectively.

Table 8. Comparison of direct injection vs. SPE and filters from Lake Munson (M) natural bloom.

Sonication	M-1	M-2	M-3	M-4	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR	57.3	71.9	72.7	70.7	68.2	7.28	10.7
Myc-LR	63.6	84.2	79.4	78.8	76.5	8.93	11.7
Myc-YR	1.80	1.50	1.50	1.90	1.68	0.21	12.3
Total MCs	123	158	154	151	147	15.9	10.9

SPE	M-1	M-2	M-3	M-4	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR	60.3	67.3	60.3	72.1	65.0	5.77	8.88
Myc-LR	58.5	81.5	70.0	80.4	72.6	10.8	14.8
Myc-YR	0.19	0.79	1.25	0.65	0.72	0.44	60.6
Total MCs	119	150	132	153	138	16.0	11.6

Filters	M-1	M-2	M-3	M-4	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR	9.88	11.4	16.7	10.1	12.0	3.21	26.7
Myc-LR	8.73	7.08	8.96	9.08	8.46	0.93	11.0
Myc-YR	0.35	0.54	0.74	0.54	0.54	0.16	29.4
Total MCs	19.0	19.0	26.4	19.7	21.0	3.62	17.2

Total MC	M-1	M-2	M-3	M-4
Sonication	123	158	154	151
SPE+filters	138	169	158	173

Microcystins	M-1	M-2	M-3	M-4
Demethyl-RR	60.0	74.2	74.2	72.6
Demethyl-LR	56.5	72.5	68.1	68.8

MC-RR, MC-LR and MC YR were found in Munson Lake samples with an average of 68.2, 76.5 and 1.68 ppb, respectively. The total microcystins obtained from direct

injection of the four replicates were practically the same as the one obtained from SPE and filters combined and were 151 and 173 ppb, respectively. Desmethyl-MC-RR and desmethyl-MC-LR were also found in the samples with an average of 70.2 and 66.5 ppb, respectively (Table 8).

In summary, the total concentration of microcystins obtained from direct injection was slightly better compared to the combined MCs obtained from SPE and filters extractions. The method showed excellent precision by comparing replicate results. Only this LC-MS/MS technique was able to detect and report the presence of desmethylated variants compared to other participating laboratory methods. Since desmethylated-MC standards were not available at the time, the desmethyl-MCs values were estimated using the methylated congeners response factor.

5.3 Biota samples (fish and mussels)

California coastal mussels (M), oysters (O), Rainbow Trout fillets and livers were used for method validation. Samples (2-5 g fresh weight) were polytronned, homogenized, fortified with 5 ng/g microcystins mixture standard and extracted with methanol-water (90:10) using the sonication procedure listed in Section 4.3. Recovery experiments were performed using replicate samples. The results showed that all tested MCs were extracted with high degree of efficiency using sonication technique (Table 9). Recoveries obtained from mussels ranged from 79.9-104 % with percent RSD<15 (n=8). The average microcystin recovery for oysters was 102 % with average standard deviation of ±14.9. The mean recoveries were 106 % for fish fillet (n=4) and 85.7 % for fish liver (n=3). The % RSD was below 11 % for both.

Table 9. Recoveries of microcystins by sonication in various matrices: mussel (M), oyster (O), fish fillet (FF) and fish liver (FL).

	M-1	M-2	M-3	M-4	M-5	M-6	M-7	M-8
MC-RR	112	115	125	118	85.4	92.5	94.0	89.2
MC-LR	82.9	77.5	81.8	114	114	107	115	114
MC-YR	72.0	87.3	97.9	116	109	117	121	115
MC-LA	73.5	72.9	73.6	80.3	82.5	74.5	75.6	106
MC-LW	75.4	74.3	83.8	92.0	81.9	74.3	79.0	85.6
MC-LF	82.8	80.7	89.1	96.1	85.9	71.5	78.1	89.2

The method detection limit (MDL) calculated from student's *t* times standard deviation for mussels (n=8) determine to be ≤ 1 ng/g using MRM (Table 10).

Table 10. Determination of microcystin MDL in tissue (n=8).

Microcystins	Avg (%Rec)	StDev	RSD (%)	MDL=t* StDev
MCY-RR	104	15.2	14.6	0.91
MCY-LR	101	16.8	16.7	1.01
MCY-YR	104	17.3	16.5	1.04
MCY-LA	79.9	11.1	13.9	0.67
MCY-LF	80.8	6.27	7.77	0.38
MCY-LW	84.2	7.62	9.05	0.46

	O-1	O-2	O-3	O-4	FF-1	FF-2	FF-3	FF-4	FL-1	FL-2	FL-3
MC-RR	106	103	105	93.3	114	106	99.1	101	81.6	77.0	83.8
MC-LR	79.1	74.8	107	117	120	122	108	103	78.4	84.4	82.4
MC-YR	80.1	79.0	115	118	123	119	109	108	89.0	83.2	87.0
MC-LA	102	101	103	110	108	107	110	107	88.6	76.4	84.8
MC-LW	118	116	101	88.8	125	111	112	103	79.8	68.8	85.4
MC-LF	120	118	103	90.3	110	106	105	104	78.4	84.4	82.4

6. QUALITY CONTROL

Quality control checks are routinely performed in the WPCL operations. These checks may be increased or modified to meet the needs of a particular analysis or project.

6.1 QA Samples

Internal quality assurance samples (fortified samples and duplicates, appropriate reference materials, duplicate samples, and method or procedural blanks) will be analyzed with each set or every twenty analyses being performed. These internal quality assurance analyses are conducted for the parameters being monitored by that analytical procedure. In addition, the compounds contained in the quality assurance sample will be representative of those compounds being monitored.

Accuracy is measured by calculating percent recovery for laboratory control spikes (fortified reagent sample), matrix spikes (fortified samples) and when available, certified reference materials (CRMs or SRMs). Accuracy is also determined for CRMs by comparing the analysis results with the certified (consensus) or reference (non-certified) values. CRM results are acceptable if they are within 65-135% of the 95^{th} percentile confidence interval of the consensus values for certified materials.

Precision is measured by calculating the relative percent difference (RPD) for analytes from duplicate analysis of samples, fortified samples and fortified blanks.

The results of all QA analyses and the percent recoveries for fortified samples and reference materials will be calculated and documented.

6.2 Duplicate Samples

One duplicate sample and/or a matrix spike duplicate or laboratory control spike duplicate will be analyzed for each set of twenty samples analyzed. The relative percent difference for each constituent is calculated as follows:

$$RPD = \{(D_1-D_2)/[(D_1+D_2)/2]\}x100$$

Where, RPD = Relative Percent Difference
D_1 = First Sample Value
D_2 = Second Sample Value (duplicate)

The results of all duplicate determinations and the calculated relative percent difference will be reported with the data sets. For RPD, use a control limit of 25 percent unless otherwise specified by a project specific QAPP.

If either sample value is less than the MDL, the notation of "ND" (not detected) will be reported. If the precision falls outside the control limits, the analysis results will be reported with the appropriate data qualifier.

6.3 Fortified Matrix (MS/MSD) Sample Analyses

When required, matrix spike and matrix spike duplicate analyses will be conducted at a rate of five percent. The spike will be added prior to any digestion, extraction, or distillation steps as a check on the sample preparation and analysis. An amount of analyte will be added to the sample that is five to ten times the reporting limit for the analyte of interest. Recovery values are calculated as follows:

$$\text{Recovery} = [(D_a - D)/D_s] \times 100$$

Where, Recovery = Percent Recovery

D_a = Analysis value of fortified sample
D = Analysis value of sample without spike
D_s = Amount of spike added

Recovery values for fortified samples must be greater than 50 percent except where a specific method (SOP) or project specific QAPP require a different acceptable range. Exceptions shall be noted in the project specific data quality objectives. When a specific method and analyte require a different acceptable recovery range, as determined by actual spike recovery runs, the acceptable range shall be noted in the Standard Operating Procedure for that method. If the recovery falls outside of the acceptable recovery range, the analysis results will be qualified or rejected. If the results are rejected, the batch of samples associated with the rejected results may need to be re-analyzed. When sample concentrations are less than the MDL, the value of "0" will be used as the sample result concentration for purposes of calculating spike recoveries. All fortified sample results will be reported with the data package.

If the percent recovery for matrix spike is unacceptable, there might be an interference due to the matrix. The sample will be diluted to lower the interference and re-analyzed. If matrix interference is determined to be the cause of unacceptable recoveries, the data will be qualified.

6.4 Method Blanks

Method blanks will be analyzed at a minimum of once for every batch of samples. Blank concentrations should not exceed the reporting limit for the analyte. If blank values exceed the reporting limit, the source of the contamination should be investigated and corrected, and the results associated with the contaminated blank re-analyzed or qualified. All blank analysis results will be reported with the data package.

6.5 Laboratory Control Samples

While reference materials are not available for all analytes, a way of assessing the accuracy of an analytical method is still required. Laboratory control samples (LCSs) provide an alternate method of assessing accuracy. 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.

SOP Section Approval: _____ Date: _____

SOP Final Approval: _____ Date: _____

SOP QA Officer Approval: _____ Date: _____

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 ≤25%
Laboratory Duplicate	Per 20 samples or per batch, whichever is more frequent	RPD ≤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 ≤25%
Laboratory Duplicate	Per 20 samples or per batch, whichever is more frequent	RPD ≤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 Duplicate[1]

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-lower limit of the range))
   - Upper Rejection Limit = 100+(2*(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 (≤25%), or marginally fail (25%<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

Approvals

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

Mark Stephenson
Project Manager/MPSL-DFG Laboratory Director

_____ Date_____

Rusty Fairey
Contract Manager

_____ Date_____

Jay Davis
Lead Scientist

_____ Date_____

Beverly van Buuren
SWAMP Quality Assurance Officer

_____ Date 07/14/11

Autumn Bonnema
Project Coordinator/ MPSL-DFG Quality Assurance Officer

_____ Date_____

David Crane
DFG-WPCL Laboratory Director

_____ Date_____

Gail Cho
DFG-WPCL Quality Assurance Officer

_____ Date_____

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Mark Stephenson
Project Manager/MPSL-DFG Laboratory Director

_____ Date_____

Rusty Fairey
Contract Manager

_____ Date_____

Jay Davis
Lead Scientist

_____ Date 8/3/11

Beverly van Buuren
SWAMP Quality Assurance Officer

_____ Date 07/14/11

Autumn Bonnema
Project Coordinator/ MPSL-DFG Quality Assurance Officer

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Rusty Fairey
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_____ Date 8/3/11

Jay Davis
Lead Scientist

_____ Date _____

Beverly van Buuren
SWAMP Quality Assurance Officer

_____ Date _____

Autumn Bonnema
Project Coordinator/ MPSL-DFG Quality Assurance Officer

_____ Date 14 July 2011

David Crane
DFG-WPCL Laboratory Director

_____ Date _____

Gail Cho
DFG-WPCL Quality Assurance Officer

_____ Date _____

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Lead Scientist

_____ Date_____

Beverly van Buuren
SWAMP Quality Assurance Officer

_____ Date_____

Autumn Bonnema
Project Coordinator/ MPSL-DFG Quality Assurance Officer

_____ Date_____

~~David Crane~~ PETER ODE 8/4/2011
DFG-WPCL Laboratory Director

Peter R. Ode Date 8/4/2011

Gail Cho
DFG-WPCL Quality Assurance Officer

Gail Cho Date 08/02/11

Attachment 1: Chain of Custody Forms

DFG REQUEST FOR ANALYSIS AND CHAIN OF CUSTODY RECORD Page ____ of ____ Page 1 of 1

Fiscal Year: 1011	**Project ID:** 10SWSBG1	SWB_FishRiv_2011		**Contact Person:**				**Phone:**		
Field Crew				Autumn Bonnema				831-771-4175		
Mailing Address				**Address**						
7544 Sandholdt Road				7544 Sandholdt Road						
City	**State**	**Zip**		**City**		**State**	**Zip**			
Moss Landing	CA	95039		Moss Landing		CA	95039			
				email: bonnema@mlml.calstate.edu						
			Analysis Requested			**BOG Analyte List**	**Sample Type**	**# of Containers**	**Preservation**	
							Fish	Foil Wrap in plastic	Temp	
Sample Identification/Location										
StationCode	**StationName**	**BagID**	**Collection Date**							
Comments/Special Instructions										
Please see AA form for compositing and analysis instructions										
Samples Reliquished By (Signature)			**Print Name and Date**		**Received By** (Signature)			**Print Name and Date**		

SWAMP REQUEST FOR ANALYSIS AND CHAIN OF CUSTODY (COC) RECORD page 1 of 1

Analysis Authorization
Fiscal Year: 1011
Region:

Project ID: SWB_FishRiv_2010
Season:
Date:

Contact Person: Autumn Bonnema
Phone: 831-771-4175
email: bonnema@mlml.calstate.edu

LabSampleID	Comp ID	Station Name	Species	BagID	Sample Date	Tissue Organics	# of Containers 60ml Glass	Preservation Frozen
					TOTAL	0	0	

Comments:

Please see AA form for explicit instructions and data reporting format.

Earliest sample collected 04/19/210

Samples Relinquished by:		Samples Received by:	
Name (Print and Sign)	Date	Name (Print and Sign)	Date

Attachment 2: Field Data Sheets

SWAMP Tissue Sampling - Non-Trawl (Event Type = TI)				Entered in d-base (initial/date)			Pg of Pgs	
*StationCode: __ __ __ __ __ __ __ __ __ __		*StationName:			*Trip:		*Sampling Crew:	**Agency:**
*FundingCode1: 10SWBG01		*Date (mm/dd/yyyy):	/ /					
*FundingCode2: __ __ __ __ __ __ __ __ __ __		ArrivalTime:		*Purpose (circle all appl.): Tissue Habitat			*Purpose Failure:	
*ProjectCode: SWB_FishRiv_2011		DepartureTime:		BEAUFORT SCALE (see attachment):		WIND DIRECTION (from):	N W E S	**PHOTOS** (RB & LB assigned when facing downstream; RENAME to StationCode_yyyy_mm_dd_uniquecode):
HabitatObs (CollectionMethod= Not App.) associated w Location1								1: (RB / LB / BB / US / DS / ##)
DOMINANTSUBSTRATE:	Concrete,Cobble,Gravel,Sand,Mud,Other_____,unk							
WATERCLARITY:	Clear (see bottom), Cloudy (>4" vis), Murky (<4" vis)	WATERCOLOR:	Colorless, Green, Yellow, Brown					2: (RB / LB / BB / US / DS / ##)
OTHER PRESENCE:	Foam, OilySheen, None, Trash, MacroAlgae, Other_____							3: (RB / LB / BB / US / DS / ##)

Comments:

OCCUPATIONMETHOD: Walk-in / Boat (RV_____)				GPS Model:		accur (ft/m)	Datum: **NAD83** Other _____	
Location:	Bank/MidChan #___	*StationDepth(m):	*StationWidth(m):	DistanceFromBank(m):	Coordinate		Lat (dd.ddddd)	Long (-ddd.ddddd)
COLLECTION METHOD:	Hook, Net, Seine, Trap, Shock			Start Time	1			
COLLECTIONDEVICE:	Hook/Line, Gill Net (mesh size)_____Backpack, Seine, Other_____				2			
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Pier, Rip-rap, Hatchery			End Time	3			
HYDROMODLOC(to sample):	US / DS / NA/ WI	Other _____	GEOSHAPE: Line Poly Point		4			
Location:	Bank/MidChan #___	*StationDepth(m):	*StationWidth(m):	DistanceFromBank(m):	Cordinate	(ft/m)	Lat (dd.ddddd)	Long(-ddd.ddddd)
COLLECTION METHOD:	Hook, Net, Seine, Trap, Shock			Start Time	1			
COLLECTIONDEVICE:	Hook/Line, Gill Net (mesh size)_____Backpack, Seine, Other_____				2			
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Pier, Rip-rap, Hatchery			End Time	3			
HYDROMODLOC(to sample):	US / DS / NA/ WI	Other _____	GEOSHAPE: Line Poly Point		4			
Location:	Bank/MidChan #___	*StationDepth(m):	*StationWidth(m):	DistanceFromBank(m):	Cordinate	(ft/m)	Lat (dd.ddddd)	Long (-ddd.ddddd)
COLLECTION METHOD:	Hook, Net, Seine, Trap, Shock			Start Time	1			
COLLECTIONDEVICE:	Hook/Line, Gill Net (mesh size)_____Backpack, Seine, Other_____				2			
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Pier, Rip-rap, Hatchery			End Time	3			
HYDROMODLOC(to sample):	US / DS / NA/ WI	Other _____	GEOSHAPE: Line Poly Point		4			
Location:	Bank/MidChan #___	*StationDepth(m):	*StationWidth(m):	DistanceFromBank(m):	Cordinate	(ft/m)	Lat (dd.ddddd)	Long (-ddd.ddddd)
COLLECTION METHOD:	Hook, Net, Seine, Trap, Shock			Start Time	1			
COLLECTIONDEVICE:	Hook/Line, Gill Net (mesh size)_____Backpack, Seine, Other_____				2			
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Pier, Rip-rap, Hatchery			End Time	3			
HYDROMODLOC(to sample):	US / DS / NA/ WI	Other _____	GEOSHAPE: Line Poly Point		4			

Failure Codes: Dry (no water), Instrument Failure, No Access, Non-sampleable, Pre-abandoned, Other

Collection Comments:

Modified6/14/2011

SWAMP Tissue Sampling - Fish Abundance

Entered in d-base (initial/date) | Pg: of Pgs

*StationCode: ___ ___ ___ ___ ___ ___ ___ ___ ___ | StationName: | Date (mm/dd/yyyy): / /

Location #	Organism ID	Tag #	Species Name/Code	FL (mm)	TL (mm)	Weight (g)	Count	Sex	Range (mm)	Count Est.	Anomaly	Condition	Stage
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR
								M F Unk/ LAB					A J SA NR

Location #: Match fish with LocationName and # from Tissue Collection sheet

Organism ID: Combine BAG # and FISH # (e.g., fish 1 of composite WC01 is WC01-1) to be unique

Tag #: Use if applicable; must be unique

Species Code: see attached list for codes

Stage: Adult (A), Juvenile (J), Subadult (SA), Not Recorded (NR)

Count Est: If appropriate, add < or > if count is est

Anomalies: Fin Erosion (**FinEro**), Gill Erosion (**GillEro**), Lesion (**Les**), Parasite (**Par**), Popeye (**PE**), Tumor (**Tum**), Hemmorage (**Hem**), Skeletal Deformity (**SkDef**)

Comments: 1) Bag Numbers must be consecutive by previous trips per StationCode, 2) Smaller fish that are bagged by species but not tagged must be bagged by location.

Modified 6/14/2011

Fish Species	Species Code
American shad	AMS
black crappie	BCR
bluegill	BGL
black bullhead	BLB
blue catfish	BLC
brown trout	BNT
brown bullhead	BRB
brook trout	BRT
carp, common	CAR
channel catfish	CHC
chinook salmon	CHS
coho salmon	COH
California roach	CRH
delta smelt	DTS
flathead catfish	FHC
fathead minnows	FHM
goldfish	GLF
golden trout	GLT
green sunfish	GRS
hitch	HIT
hardhead	HRH
inland silverside	ISS
killifish	KIL
kokanee salmon	KOK
lamprey	LAM
longfin smelt	LFS
lake trout	LKT
largemouth bass	LMB
mosquitofish	MQF
pumpkinseed sunfish	PKS

Fish Species	Species Code
rainbow trout	RBT
redeye bass	REB
redear sunfish	RES
red shiner	RSR
Sacramento sucker	SAS
Sacramento blackfish	SBF
sculpin ssp	SCP
shiner perch	SHP
smallmouth bass	SMB
spotted bass	SPB
Sacramento perch	SPH
Sacramento pike minnow	SPM
Sacramento splittail	SST
striped bass	STB
steelhead	STH
striped mullet	STM
sturgeon, white	WST
threadfin shad	TFS
tilapia ssp	TIL
topsmelt	TPS
threespine stickleback	TSS
tui chub	TUC
tule perch	TUP
warmouth	WAR
white crappie	WCR
white catfish	WHC

Notes to Standardize SWAMP Field Data Sheets (For in the field use)

Key Reminders to identify samples:

1. **Sample Time** is the SAME for all samples (Water, Sediment, & Probe) taken at the sampling event. Use time of FIRST sample; important for COC.
2. **Group;** many diffrent ways to do a group, one suggestion is to create groups which assign trips to assess frequency of field QA

Collection Details

1. **Personnel**: S. Mundell, G Ichikawa (first person listed is crew leader)
2. **Location:** Use "openwater" in bay/estuary/harbor only if no distinguishable channel exists
3. **GRAB vs INTEGRATED**: GRAB samples are when bottles are filled from a single depth; INTEGRATED sample are taken from MULTIPLE depths and combined.
   a. GRAB: use 0.1 for subsurface samples; if too shallow to submerge bottle; depth =0
   b. INTEGRATED: -88 in depth sampled, record depths combined in sample comments
4. **TARGET LAT/LONG**: Refers to the existing station location that the sampling crew is trying to achieve; can be filled out prior to sampling
5. **ACTUAL LAT/ LONG**: is the location of the current sample event.
6. **HYDROMODIFICATION:** Describe existing hydromodifications such as a grade control, drainage pipes, bridge, culvert
7. **HYDROMOD LOC**: if there is an IMMEDIATE (with in range potentially effecting sample) hydromodification; Is the hydromodification upstream/downstream/within area of sample; if there is no hydromodification, NA is appropriate
8. **STREAM WIDTH and DEPTH**: describe in meters at point of sample.

FIELD OBSERVATIONS: (each one of these observations has a comment field in the database so use comment space on data sheet to add information about an observation if necessary)

1. **PICTURES:** use space to record picture numbers given by camera; be sure to rename accordingly back in the office. (StationCode_yyyy_mm_dd_uniquecode)
2. **WADEABILITY**: in general, is waterbody being sampled wadeable to the average person AT the POINT of SAMPLE
3. **DOMINANT SUBSTRATE**: if possible; describe DOMINANT substrate type; use UNK if you cannot see the dominant substrate type
4. **BEAUFORT SCALE**: use scale 0-12; refer to scales listed below.
5. **WIND DIRECTION**: records the direction from which the wind is blowing
6. **OTHER PRESENCE**: VASCULAR refers to terrestrial plants or submerged aquatic vegetation (SAV) and NONVASCULAR refers to plankton, periphyton etc. These definitions apply to vegetation IN the water at the immediate sampling area.
7. **OBSERVED FLOW**: Visual estimates in cubic feet/ second.
8. **WATER COLOR**: This is the color of the water from standing creek side
9. **WATER CLARITY**: this describes the clarity of the water while standing creek side; clear represents water that is clear to the bottom, cloudy may not be clear to bottom but greater than 4" can be seen through the water column.
10. **SedimentComp**: generally described sediments used for chemistry sample

Note: these reminders do not give all details needed to maintain equivalent SWAMP sampling protocols, they are strictly for "infield" use to help insure comparability of field observations.

BEAUFORT SCALE: Specifications and equivalent speeds for use at sea

FORCE	EQUIVALENT SPEED 10 m above ground		DESCRIPTION	SPECIFICATIONS FOR USE AT SEA
	miles/hour	knots		
0	0-1	0-1	Calm	Sea like a mirror
1	1-3	1-3	Light air	Ripples with the appearance of scales are formed, but without foam crests.
2	4-7	4-6	Light breeze	Small wavelets, still short, but more pronounced. Crests have a glassy appearance and do not break.
3	8-12	7-10	Gentle breeze	Large wavelets. Crests begin to break. Foam of glassy appearance. Perhaps scattered white horses.
4	13-18	11-16	Moderate breeze	Small waves, becoming larger; fairly frequent white horses.
5	19-24	17-21	Fresh breeze	Moderate waves, taking a more pronounced long form; many white horses are formed. Chance of some spray.
6	25-31	22-27	Strong breeze	Large waves begin to form; the white foam crests are more extensive everywhere. Probably some spray.
7	32-38	28-33	Near gale	Sea heaps up and white foam from breaking waves begins to be blown in streaks along the direction of the wind.
8	39-46	34-40	Gale	Moderately high waves of greater length; edges of crests begin to breakinto spindrift. The foam is blown in well-marked streaks along the direction of the wind.
9	47-54	41-47	Severe gale	High waves. Dense streaks of foam along the direction of the wind. Crests of waves begin to topple, tumble and roll over. Spray may affect visibility.
10	55-63	48-55	Storm	Very high waves with long over-hanging crests. The resulting foam, in great patches, is blown in dense white streaks along the direction of the wind. On the whole the surface of the sea takes on a white appearance. The 'tumbling' of the sea

Source:

Last edited on 09 January, 1999 Dave Wheeler weatherman@zetnet.co.uk
Web Space kindly provided by Zetnet Services Ltd, Lerwick, Shetland.

heavy and shock-like. Visibility affected.

BEAUFORT SCALE: Specifications and equivalent speeds for use on land

FORCE	EQUIVALENT SPEED 10 m above ground		DESCRIPTION	SPECIFICATIONS FOR USE ON LAND
	miles/hour	knots		
0	0-1	0-1	Calm	Calm; smoke rises verticall.
1	1-3	1-3	Light air	Direction of wind shown by smoke drift, but not by wind vanes
2	4-7	4-6	Light Breeze	Wind felt on face; leaves rustle; ordinary vanes moved by wind
3	8-12	7-10	Gentle Breeze	Leaves and small twigs in constant motion; wind extends light flag
4	13-18	11-16	Moderate Breeze	Raises dust and loose paper; small branches are moved.
5	19-24	17-12	Fresh Breeze	Small trees in leaf begin to sway crested wavelets form on inland waters
6	25-31	22-27	Strong Breeze	Large branches in motion; whistling heard in telegraph wires umbrellas used with difficulty
7	32-38	28-33	Neargale	Whole trees in motion; inconvenience felt when walking against the wind
8	39-46	34-40	Gale	Breaks Twigs and generally impedes progress

Source:

Last edited on 09 January, 1999 Dave Wheeler weatherman@zetnet.co.uk
Web Space kindly provided by Zetnet Services Ltd, Lerwick, Shetland.

Attachment 3: Analysis Authorization Forms

Analysis Authorization
Fiscal Year: 1011
Region:

Project ID: SWB_FishRiv_2011
Season:
Date:

Contact Person: Autumn Bonnema
Phone: 831-771-4175
email: bonnema@mlml.calstate.edu
Mailing Address:

				Dissect and Analyze						Dissect and Send to MPSL				
				Tissue Flesh						Tissue Flesh			Archive	Archive
StationCode	StationName	OrganismName	CompositeIDText	OC	PCB	%Moist	%Lipid	Sex	Wt/Length	Ind Hg	Comp Hg	Comp Se	Ind	Comp
Total				0	0	0	0	0	0	0	0	0	0	0

Analysis Authorization
Fiscal Year: 1011
Region:

Project ID: SWB_FishRiv_2011
Season:
Date:

Contact Person: Autumn Bonnema
Phone: 831-771-4175
email: bonnema@mlml.calstate.edu
Mailing Address:

				Dissect and Analyze Tissue Flesh							
StationCode	StationName	OrganismName	CompositeIDText	Ind Hg	Comp Hg	Comp Se	%Moisture	Sex	Wt/Length	Archive Ind	Archive Comp
Total				**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Dissect and Send to WPCL Tissue Flesh			
OC	PCB	%Moisture	%Lipid
0	**0**	**0**	**0**

Attachment 4: Laboratory Data Sheets

SWAMP Lab Data Sheet - FISH	ProjectID: SWB_FishRiv_2011	PrepPres:	LabID:	Pg: 1 of 2 Pgs
StationCode:	Tissue: Fillet		Entered d-base (initial/date)	
StationName:	Homog. Method: BUCCHI POLYTRON OTHER_____		Staff: Diss. Homog.	
Species Name:	Date Diss. (mm/dd/yyyy): / /		Date Homog. (mm/dd/yyyy): / /	

#	Tissue/Bag ID	Fish #	Organism ID	Composite / Individual ID	FL (mm)	TL (mm)	Whole Fish Wt (g)	Part Wt (g)	Sex	Part	Anomaly	Body Location
1									M / F / Unk	T / L / O		
2									M / F / Unk	T / L / O		
3									M / F / Unk	T / L / O		
4									M / F / Unk	T / L / O		
5									M / F / Unk	T / L / O		
6									M / F / Unk	T / L / O		
7									M / F / Unk	T / L / O		
8									M / F / Unk	T / L / O		
9									M / F / Unk	T / L / O		
10									M / F / Unk	T / L / O		
11									M / F / Unk	T / L / O		
12									M / F / Unk	T / L / O		
13									M / F / Unk	T / L / O		
14									M / F / Unk	T / L / O		
15									M / F / Unk	T / L / O		
16									M / F / Unk	T / L / O		
17									M / F / Unk	T / L / O		
18									M / F / Unk	T / L / O		
19									M / F / Unk	T / L / O		
20									M / F / Unk	T / L / O		
21									M / F / Unk	T / L / O		
22									M / F / Unk	T / L / O		
23									M / F / Unk	T / L / O		
24									M / F / Unk	T / L / O		
25									M / F / Unk	T / L / O		

OrganismID: xxxxxxxxLLXX##YYYzz-ZZ; unique code - StationCode (xxxxxxxxx), Location (LL), Project (XX), ProjectYear (##), OrganismCode (YYY), Bag # (zz), Fish # (ZZ); ex. 203SRF101L1SW04CAR01-01

TissueID: Differentiates different parts from same fish or differentiates composited vs. individual fish | **Part**: Tissue (T), Liver (L), Other (O) - list in Comments

Comp/IndID: Unique code; include Agency code in the ID; e.g., 2003-1823-MLML or C031501-MLML

Anomalies: Ambicoloration (A), Albinism (B), Cloudiness (CL), Deformity-skeletal (D), Discoloration (DC), Depression (DS), Fin Erosion (F), Gill Erosion (T), Hemorrhage (H), Lesion (L), Parasite (P),

Body Locations: Branchial Chamber (BRC), Buccal Cavity (BC), Eyes (E), Musculoskeleton (M), Skin/Fins (SF) | Popeye (PE), Tumor (T), Ulceration (U), White Spots (W), and any combination

Comments: Measure length to nearest 1 mm; Measure weight to nearest 0.01 g; Keep archive tissue if possible; If a duplicate is made, use DupID as identification for analysis

Modified 06/08/07

SWAMP Lab Data Sheet - FISH	ProjectID: SWB_FishRiv_2011	PrepPres: Skin OFF	LabID:	Pg: 1 of 2 Pgs
StationCode:	Tissue: Fillet		Entered d-base (initial/date)	
StationName:	Homog. Method: BUCCHI POLYTRON OTHER__________		Staff: Diss.	Homog.
Species Name:	Date Diss. (mm/dd/yyyy): / /		Date Homog. (mm/dd/yyyy): / /	

CHEMISTRY JARS

Composite/Individual ID:	**Composite/Individual ID**:	**Composite/Individual ID**:
Analysis: Mercury Organics Archive	**Analysis:** Mercury Organics Archive	**Analysis:** Mercury Organics Archive
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):
Duplicate: Yes / No **DUP ID:**	**Dup**: Yes / No **DUP ID:**	**Duplicate**: Yes / No **DUP ID:**
Composite/Individual ID:	**Composite/Individual ID**:	**Composite/Individual ID**:
Analysis: Mercury Organics Archive	**Analysis:** Mercury Organics Archive	**Analysis:** Mercury Organics Archive
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):
Duplicate: Yes / No **DUP ID:**	**Dup**: Yes / No **DUP ID:**	**Duplicate**: Yes / No **DUP ID:**
Composite/Individual ID:	**Composite/Individual ID**:	**Composite/Individual ID**:
Analysis: Mercury Organics Archive	**Analysis:** Mercury Organics Archive	**Analysis:** Mercury Organics Archive
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):
Duplicate: Yes / No **DUP ID:**	**Dup**: Yes / No **DUP ID:**	**Duplicate**: Yes / No **DUP ID:**
Composite/Individual ID:	**Composite/Individual ID**:	**Composite/Individual ID**:
Analysis: Mercury Organics Archive	**Analysis:** Mercury Organics Archive	**Analysis:** Mercury Organics Archive
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):
Duplicate: Yes / No **DUP ID:**	**Dup**: Yes / No **DUP ID:**	**Duplicate**: Yes / No **DUP ID:**
Composite/Individual ID:	**Composite/Individual ID**:	**Composite/Individual ID**:
Analysis: Mercury Organics Archive	**Analysis:** Mercury Organics Archive	**Analysis:** Mercury Organics Archive
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):
Duplicate: Yes / No **DUP ID:**	**Dup**: Yes / No **DUP ID:**	**Duplicate**: Yes / No **DUP ID:**

Comments: Keep archive tissue if possible; If a duplicate is made, use Dup ID as identification for analysis

Modified 06/08/07