SWAMP
Surface Water Ambient Monitoring Program

Final Quality Assurance Program Plan	2014

Study of Lakes and Reservoirs with Low Concentration of Contaminants in Sport Fish

May 2014

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

Study of Lakes and Reservoirs with Low Concentration of Contaminants in Sport Fish

The Bioaccumulation Oversight Group (BOG)

Surface Water Ambient Monitoring Program

May 2014

Program Title	SWAMP Bioaccumulation Oversight Group Wildlife BMF Study
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Primary Contact	Jay Davis, Lead Scientist San Francisco Estuary Institute 4911 Central Avenue Richmond, CA 94804 (510) 746-7368 Autumn Bonnema, Project Coordinator SJSURF Marine Pollution Studies Lab 7544 Sandholdt Road Moss Landing, CA 95039 (831) 771-4175
Effective Date	This Quality Assurance Project Plan (QAPP) is effective from April 2014 to May 2016 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 Wildlife Marine Pollution Studies Laboratory (MPSL-DFG), California Department of Fish and Wildlife Water 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 MPSL-DFG, DFG-WPCL, and SFEI also are contained within. The BOG selects the sampling sites, the types and size of tissue samples, and the number of analyses to be conducted.

This work is funded through the Surface Water Ambient Monitoring Program (SWAMP) fiscal year 13/14 Bioaccumulation funding.

Approvals

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

Rusty Fairey
Contract Manager

_____ Date_____

Jay Davis
Lead Scientist

_____ Date_____

Eric von der Geest
SWAMP Quality Assurance Liaison

_____ Date_____

Beverly can Buuren
Program Quality Assurance Officer

_____ Date_____

Autumn Bonnema
Project Manager/ MPSL-DFG Quality Assurance Officer

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Gail Cho
DFG-WPCL Quality Assurance Officer

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John DeWild
USGS-MRT 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	
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MOSS LANDING MARINE LABORATORIES **QUALITY ASSURANCE RESEARCH GROUP**	
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UNITED STATES GEOLOGICAL SURVEY, Water Resources Division	
Robin Stewart Amy Kleckner*	345 Middlefield Road MS496 Menlo Park, CA 94025 Phone: (650) 329-4550 Email: arstewar@usgs.gov
UNITED STATES GEOLOGICAL SURVEY, Mercury Research Team	
David Krabbenhoft John DeWild*	8505 Research Way Middleton, WI 53562 Phone: (608) 821-3843 Email: dpkrabbe@usgs.gov

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

Element 4. Project Organization

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

Table 2. Positions and duties

Position	Name	Responsibilities
Contract Manager	Rusty Fairey (MPSL-MLML)	Approve reports and invoices for payment.
Lead Scientist	Jay Davis (SFEI)	Advisory Roll; Data reporting
Project Manager/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	Autumn Bonnema (MPSL-DFG) Gail Cho (DFG-WPCL)	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	Wes Heim (MPSL-DFG) Pete Ode (DFG-WPCL) Collin Eagles-Smith (USGS FRESC) Robin Stewart (USGS WRD) David Krabbenhoft (USGS MRT)	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) Branden Johnson (USGS FRESC) Amy Kleckner (USGS WRD) John DeWild (USGS MRT) additional staff	Sample storage. Not responsible for any deliverables.
Technicians	Technical staff MPSL-DFG DFG-WPCL USGS	Conduct tissue dissection, digestion, and chemical analyses. Responsible for chemistry data submission

4.1. Involved parties and roles

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

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 will serve as the Project Manager (PM) and 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. As PM, she will 1) review, evaluate and document project reports, and 2) verify the completeness of all tasks.

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

Stephen Martenuk is responsible for sample storage and custody at MPSL. His duties will be to oversee compositing of tissue samples. Scot Harris will do the same for samples processed at DFG-WPCL; Robin Stewart, Collin Eagles-Smith, and Dave Krabbenhoft for USGS.

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.

Wes Heim will serve as the Laboratory Director (LD) for the MPSL-DFG component of this project. His specific duties will be to 1) review and approve the QAPP, 2) provide oversight for mercury analyses on fish tissues to be done for this project, and 3) ensure that all MPSL-DFG activities are completed within the proper timelines.

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

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, Stephen Louie, Carrie Austin and Patrick Morris (RWQCB5), Tom Suk (RWCQB6), Jeff Geraci (RWCB7), Chad Loflen (RWQCB9), Cassandra Lamerdin (MPSL-MLML), Jennifer Salisbury (State Water Resources Control Board (SWRCB)), and Jennifer Hunt (SFEI).

4.2. Quality Assurance Officer (QAO) Role

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

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

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

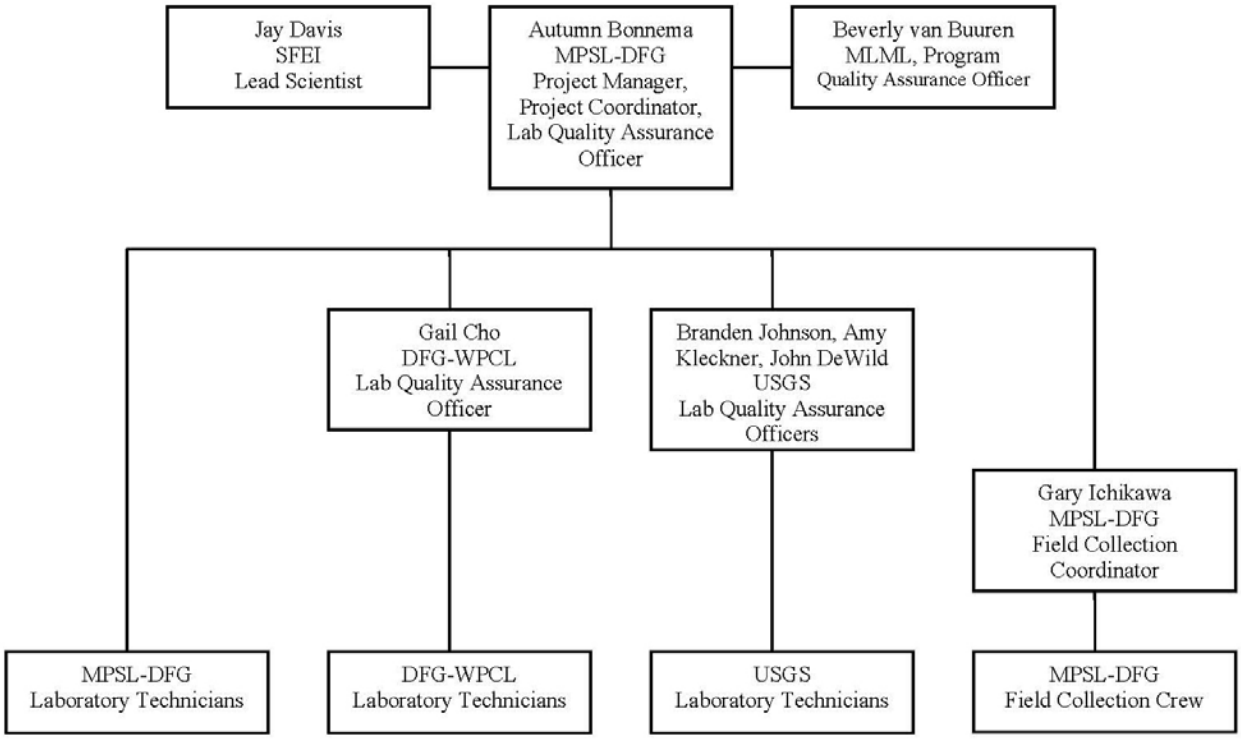
4.3. Persons responsible for QAPP update and maintenance

Revisions and updates to this QAPP will be carried out by Autumn Bonnema (PC), with technical input of the 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 Monitoring Objectives and Assessment Questions for 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. 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.

The current workplan describes an effort to refine the characterization of the status of lakes and reservoirs with regard to impairment due to bioaccumulation. SWAMP surveys to date have focused on identifying water bodies with elevated concentrations of bioaccumulative contaminants so that managers could develop strategies for addressing problem areas. In contrast, this survey will aim to provide information on another facet of status: identification of lakes and reservoirs with relatively low levels of contamination. This information will be useful to managers in their efforts to protect these relatively high quality ecosystems and to replicate these conditions in other water bodies. The information will also be valuable to the fishing public, drawing attention to water bodies where beneficial uses can be enjoyed with reduced exposure to bioaccumulative contaminants

5.2. Decisions or outcomes

Three management questions (one primary question, and two secondary questions) have been articulated to guide the design of this study. The primary question is the main driver of the sampling design. The secondary questions will be addressed to the extent possible with the resources available for the study, after assuring that the primary question is appropriately addressed.

5.2.1. Management Question 1 (MQ1)

Which popular lakes in California can be confirmed to have relatively low concentrations of contaminants in sport fish?

Answering this question will address the critical need of managers and the public to know which water bodies can be considered relatively clean. With this information, the fishing public can be directed to water bodies where they can enjoy the benefits of fishing and fish consumption and have reduced exposure to contaminants.

The data needed to answer this question are repeated observations of low concentrations of all contaminants of concern (including methylmercury, PCBs, legacy pesticides, and selenium) in the species with the greatest tendency to accumulate high concentrations. For methylmercury, top predators such as black bass tend to accumulate relatively high concentrations. High-lipid, bottom-feeding species such as catfish, carp, and sucker have the greatest tendency to accumulate relatively high concentrations of organic contaminants of concern (PCBs and legacy pesticides). 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. Measuring low concentrations of contaminants in both of these types of indicator species provides compelling evidence that a water body has a low overall degree of contamination. Given the variance associated with contaminant concentrations, the evidence becomes even more compelling if the low concentrations are observed on more than one occasion. This higher level of confidence obtained through repeated observation of low concentrations in both types of indicator species is desirable to be assured of providing reliable information to the public to guide their decisions on where to fish.

In some water bodies, it is not feasible to obtain both types of indicator species because they are not present in high enough abundance. Lakes at higher elevations with colder water where trout species predominate are a common example. For these lakes, repeated observation of the species that do occur there and are most likely to have high concentrations is the best basis that can be obtained for characterizing a lake as one with relatively low concentrations.

5.2.2. Management Question 2 (MQ2)

Why do some lakes have relatively low concentrations of methylmercury in sport fish?

Many of the lakes found to have low concentrations of contaminants in the 2007-8 survey were lakes where only rainbow trout were collected. Rainbow trout generally had low concentrations of methylmercury, with a statewide average of 0.05 ppm. Concentrations of organics in trout were also generally low. To some degree, this was due to lower concentrations of contaminants in these lakes, but other factors also likely played a role. Trout generally occupy a lower trophic position and accumulate lower concentrations of methylmercury and other pollutants than black bass. However, a factor that probably contributed to lower observed concentrations in trout is that, in many lakes, recently planted hatchery fish are part of the catch. A previous study found that hatchery trout consistently had very low concentrations of methylmercury (rainbow trout from four hatcheries all had less than 0.023 ppm – Grenier et al. 2007).

With the level of effort that could be expended in the statewide survey of 2007-8 it is possible that other resident species with a potential to have higher concentrations were missed, such as resident populations of trout or small populations of warmwater predators like black bass or bottom feeders like sucker. With the greater effort planned for the present study, it is anticipated that information will be obtained that will allow for some evaluation of the accuracy of the 2007-8 assessment for lakes where only one species was obtained..

5.2.3. Management Question 3 (MQ3)

Did the 2007-8 survey accurately characterize the status of lakes in which only rainbow trout were collected?

Many of the lakes found to have low concentrations of contaminants in the 2007-8 survey were lakes where only rainbow trout were collected. Rainbow trout generally had low concentrations of methylmercury, with a statewide average of 0.05 ppm. Concentrations of organics in trout were also generally low. To some degree, this was due to lower concentrations of contaminants in these lakes, but other factors also likely played a role. Trout generally occupy a lower trophic position and accumulate lower concentrations of methylmercury and other pollutants than black bass. However, a factor that probably contributed to lower observed concentrations in trout is that, in many lakes, recently planted hatchery fish are part of the catch. A previous study found that hatchery trout consistently had very low concentrations of methylmercury (rainbow trout from four hatcheries all had less than 0.023 ppm – Grenier et al. 2007).

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5.2.4. Overall Approach

The overall approach to be taken to answer these three questions is to re-sample a select subset of lakes that were identified as having relatively low concentrations of contaminants in the 2007-8 survey. The same basic design used in the 2007-8 survey will be repeated, as the goal is to obtain confirmation of the earlier results.

5.2.5. Coordination

The BOG is coordinating with other efforts to significantly leverage the funds for this survey and achieve a more thorough evaluation of California lakes with relatively low levels of contamination. These coordinated efforts are adding approximately $169,000 worth of work to the BOG funds available for sampling and analysis in this study ($240,000).

The Colorado River Basin Regional Water Quality Control Board (Region 7) will be conducting a survey of contaminants in sport fish in Region 7 lakes this summer. Region 7 has a relatively large proportion of lakes that meet the criteria for having low concentrations, including 10 of the 14 lakes that will be sampled in the Region. Resources for this statewide effort will be pooled with Region 7 resources to allow a more thorough and definitive assessment of the lakes in this region. The data from the Region 7 effort will be processed and reported along with the data from the statewide effort.

The Los Angeles Regional Water Quality Control Board (Region 4) will partner to expand this study in their region. Region 4 is covering the cost of all of the work in their region, including an extra lake (Castaic Lake) to complement sampling of Castaic Lagoon. In addition, they will pay for some of the costs of lakes outside their region.

The San Diego Regional Water Quality Control Board (Region 9) is planning a study of cyanotoxins in reservoirs for this summer. One of the lakes to be sampled in that effort (Lake Henshaw) is also a candidate for inclusion in this study. If Lake Henshaw is selected for this study, the work will be coordinated with the cyanotoxin study. Effort will be made to collect Lake Henshaw at a similar time the other lakes from the Region 9 study are being collected.

Several U.S. Geological Survey (USGS) research labs are partnering with this study as an opportunity to provide improved understanding of mercury cycling in the western US. The USGS Wisconsin Water Science Center's Mercury Research Team will partner with SWAMP on this study by performing chemical analysis of water and sediment samples for total mercury, methylmercury, and related parameters. The MRT operates one of the premier mercury labs in the country, and frequently contributes to mercury studies at regional and national scales.

The Corvallis Research Group of the USGS Forest and Rangeland Ecosystem Science Center in Corvallis OR will partner with SWAMP on this study by performing chemical analysis of small fish samples for total mercury.

The Water Resources Division of USGS in Menlo Park CA will partner with SWAMP on this study by performing chemical analysis of small fish samples for selenium. They will also be analyzing selenium in sport fish livers from select lakes

5.3. Tissue contamination criteria

The state is in the process of developing a statewide tissue objective for mercury that is anticipated to be 0.2 ppm wet weight (all concentrations mentioned in this document are presented on a wet weight basis). This threshold will be used for the next round of listing. Through BOG discussion, the 0.2 ppm objective and listing threshold was selected as the criterion for classifying lakes as having relatively low concentrations of mercury. To be confident that a lake truly has fish mercury concentrations below 0.2 ppm, it is desirable to have measured concentrations in species such as black bass that are known to accumulate high concentrations.

The California Office of Environmental Health Hazard Assessment (OEHHA) has established two sets of thresholds - fish contaminant goals and advisory tissue levels - that are relevant as selection criteria for lakes to be included in this study (Klasing and Brodberg [2008], Table 3). Fish contaminant goals (FCGs) are health protective values for lifetime exposure and consider only the toxicity of the contaminants. They were developed by OEHHA to assist other agencies to establish fish tissue-based criteria for cleanup. For the two main chemicals of concern in this study, the FCGs are 0.22 ppm for mercury and 3.6 ppb for PCBs. The FCG for mercury (0.22 ppm) is of the same magnitude as the statewide tissue objective of 0.2 ppm, based only on toxicity and one serving per week of consumption. FCGs are being used by the Water Boards in the latest round of 303(d) listing determinations.

Advisory Tissue Levels (ATLs) consider both the toxicity of contaminants and the health benefits of fish consumption. They are used to develop sport fish consumption advice for the public. OEHHA has developed ATL ranges for one to seven servings per week. A comparison of the same consumption frequency (one serving per week), shows that, for mercury, the low end of the ATL range (150 to 440 ppb) for the sensitive population (children and women of child-bearing age) encompasses the statewide tissue objective (200 ppb). For PCBs, the low end of the ATL range (21 ppb) for a 2 servings per week consumption rate was also considered as a lake selection criterion.

For organics, given their use in 303(d) listing determinations, the FCGs are a relevant benchmark to use in assessing the degree of contamination. To be confident that a lake truly has organics concentrations below FCGs, it is desirable to have measured concentrations in species such as catfish, carp, or sucker that are known to accumulate high concentrations

Water and sediment results will not be compared with any thresholds as they are not being assessed for human health concerns. These data are intended only to help inform what may be contributing to low contaminant concentrations in certain lakes and reservoirs.

Table 3. Sport fish assessment thresholds

Thresholds for concern based on an assessment of human health risk from these pollutants by OEHHA (Klasing and Brodberg, 2008). All values given in ng/g (ppb). The lowest available threshold for each pollutant is in bold font. One serving is defined as 8 ounces (227 g) prior to cooking. The FCG and 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

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 identifying lakes and reservoirs with relatively low levels of contamination. Chemistry and ancillary data will be collected from water, sediment and fish collected at water bodies suspected to not be contaminated based on the results from the 2007 and 2008 Lakes Studies, and a report of the findings will be made publicly available in 2015.

6.2. Constituents to be analyzed and measurement techniques.

A detailed Sampling and Analysis Plan (SAP) is in Appendix II. Chemistry analytical methods are summarized in Section E. Constituents to be analyzed are summarized in Tables 4-10. All sediment chemistry will be reported on a dry weight basis, and all tissue 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.

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 4. Constituents to be Analyzed – Field Measurements

Field measurements will be taken using a multi-probe YSI EXO2.

Field Measurements
Water Depth (m)
Temperature (ºC)
pH
Dissolved Oxygen (mg/L)
Redox
Specific Conductivity (µS/cm)
Chlorophyll *a*
Collection Location (UTMs)

Table 5. Constituents to be Analyzed – Conventionals

Analyte	Matrix Type	Analytical Method
Chlorophyll *a*	Subsurface and Near Bottom water	EPA 446.0 (USEPA 1997)
Sulfate	Unfiltered Subsurface and Near Bottom Water	EPA 300 (USPEA 1993, Appendix IV C)
Organic Carbon (Dissolved)	Filtered Subsurface and Near Bottom Water	METH011.00 (Appendix V B)
UV/EEMs	Filtered Subsurface and Near Bottom Water	Aqualog SOP_V1.3 (Appendix V F)
Loss on Ignition	Sediment	Volatile-on-Ignition (Appendix V D)

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)
Standard Length (mm; small fish only)
Weight (g)
Sex (sport fish only)
Moisture (%)
Lipid (%)
Collection Location (UTMs)

Table 7. Constituents to be Analyzed – Metals and Metalloids

Analyte	Matrix Type	Analytical Method
Total Mercury	Whole Body Small Fish and Sport Fish filet muscle	EPA 7473 (USEPA 1998)
Total Selenium	Whole Body Small Fish and Sport Fish Liver	ID HGICP-MS (Appendix V E)
Total Selenium	Sport Fish filet muscle	EPA 3052M (Appendix III E) EPA 200.8 (USEPA 1994a)
Total Mercury	Sediment	DFG SOP 103
Total Mercury	Unfiltered Water	EPA 1631E (USEPA 2002)
Methyl Mercury	Unfiltered Water	EPA 1630 (USEPA 2001) with Isotope Dilution (Appendix V C)

Table 8. Constituents to be Analyzed – Polychlorinated Biphenyls (PCB) in Tissue

Polychlorinated Biphenyl (PCB) Congeners (by USEPA Method 8082M, USEPA 1996e, Appendix IV B)		
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 9. Constituents to be Analyzed – Organochlorine (OC) Pesticides in Tissue

Organochlorine Pesticides (by EPA 8081BM using GC-ECD, USEPA 1996d, Appendix IV B)	
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 10. Constituents to be Analyzed – Algal Toxins

Microcystins and Biotoxins by LC/MS/MS (Appendix IV D)		
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.

Table 11. Project Schedule Timeline

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

6.4. Geographical setting and sample sites

Sampling will occur in freshwater lakes throughout California that have been shown to have low contaminant concentrations from the 2007 and 2008 Lake Studies. See the Sampling and Analysis Plan (Appendix II, Figure 1) for a map depicting targeted lakes.

6.5. Constraints

All sampling must be completed by the end of the current year's sampling season in order to meet analysis and reporting deadlines set forth in Table 11. Furthermore, all contract work must be completed prior to September 2015 to meet contract deadlines.

Element 7. Quality Indicators and Acceptability Criteria for Measurement Data

Data quality indicators for all laboratory analyses will include accuracy (bias), precision, recovery, completeness and sensitivity. Measurement Quality Indicators for analytical measurements in tissue are in Tables 12-15.

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

Table 12. Measurement acceptability criteria for field measurements in water.

Parameter	Units	Resolution	Instrument Accuracy Specs	Points per Calibration	Pre-Sampling Calibration Check Frequency	Post-Sampling Calibration Check Frequency	Allowable Drift
Dissolved oxygen	mg/L	0.01	±0.2	1	Before every monitoring day on-site (recalibrate if change is ≥500m or barometric pressure >2mmHg)	After every monitoring day (within 24 hours)	± 0.5 or 10%
pH	pH	0.01	±0.2	2	Per manufacturer	Per manufacturer	±0.2 units
Specific Conductivity	µS/cm	1	±0.5%	Per manufacturer	Per manufacturer	Per manufacturer	±10%
Temperature	ºC	0.1	±0.15	Per manufacturer	Per manufacturer	Per manufacturer	±0.5
Total Chlorophyll	µg/L	0.1	n/a	2	Per manufacturer	Per manufacturer	±10%

Table 13. Measurement quality indicators for laboratory measurements in water.

Parameter	Accuracy	Precision	Recovery	Completeness	Sensitivity
Mercury (Total)	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 27
Methylmercury (Total)	LCS 70% - 130%	Duplicate RPD <25%; n/a if concentration of either sample <RL Matrix Spike Duplicate RPD <25%	Matrix Spike 70% - 130%	90%	See Table 27
Chlorophyll *a*	CRM 80% - 120%	per method	Matrix Spike Not Applicable	90%	See Table 26
Organic Carbon (Dissolved)	CRM 80% - 120%	Duplicate RPD <25%; n/a if concentration of either sample <RL Matrix Spike Duplicate RPD <25%	Matrix spike 80% - 120%	90%	See Table 26
Sulfate	CRM 80% - 120%	Duplicate RPD <25%; n/a if concentration of either sample <RL Matrix Spike Duplicate RPD <25%	Matrix spike 80% - 120%	90%	See Tables 26

Table 14. Measurement quality indicators for laboratory measurements in sediment.

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 27
Loss on Ignition	CRM 80% - 120%	Duplicate RPD <25%; n/a if concentration of either sample <RL	Matrix spike Not Applicable	90%	See Tables 26

Table 15. Measurement quality indicators for laboratory measurements in tissue.

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 27
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 certified 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 28-29
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 30

7.1. Accuracy

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

$$\% \text{ recovery} = \frac{V_{analyzed}}{V_{certified}} x100$$

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 16-22.

Table 16. Measurement Quality Objectives – Conventional Analytes in Water

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	80-120% recovery
Matrix Spike	Per 20 samples or per batch, whichever is more frequent (n/a for Chlorophyll *a*)	80-120% recovery
Matrix Spike Duplicate	Per 20 samples or per batch, whichever is more frequent (n/a for Chlorophyll *a*)	80-120% recovery, RPD ≤25%
Laboratory Duplicate	Per 20 samples or per batch, whichever is more frequent (Chlorophyll *a*: per method)	RPD <25%; n/a if concentration of either sample <RL
Internal Standard	Accompanying every analytical run when method appropriate	60-125% recovery
Field Quality Control	**Frequency of Analysis**	**Measurement Quality Objective**
Field Duplicate	5% of total project sample count	RPD <25%; n/a if concentration of either sample <RL
Field Blank	per method	<RL for target analyte

*Unless method specifies more stringent requirements.
MDL = Method Detection Limit
RL = Reporting Limit
n/a = not applicable

Table 17. Measurement Quality Objectives – Inorganic Analytes in Water

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 (70-130% for methylmercury)
Matrix Spike	Per 20 samples or per batch, whichever is more frequent (n/a for Chlorophyll *a*)	75-125% recovery (70-130% for methylmercury)
Matrix Spike Duplicate	Per 20 samples or per batch, whichever is more frequent (n/a for Chlorophyll *a*)	75-125% recovery (70-130% for methylmercury), RPD ≤25%
Laboratory Duplicate	Per 20 samples or per batch, whichever is more frequent (Chlorophyll *a*: per method)	RPD <25%; n/a if concentration of either sample <RL
Internal Standard	Accompanying every analytical run when method appropriate	60-125% recovery
Field Quality Control	**Frequency of Analysis**	**Measurement Quality Objective**
Field Duplicate	5% of total project sample count	RPD <25%; n/a if concentration of either sample <RL
Field Blank	5% of total project sample count	<RL for target analyte

*Unless method specifies more stringent requirements.
MDL = Method Detection Limit
RL = Reporting Limit
n/a = not applicable

Table 18. Measurement Quality Objectives – Conventional Analytes in Sediment

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
Laboratory Blank	Total organic carbon only: one per analytical batch (n/a for other parameters)	<RL or <30% of lowest sample
Reference Material	Total organic carbon only: one per 20 samples or per analytical batch, whichever is more frequentn (n/a for other parameters)	80-120% recovery
Laboratory Duplicate	One per analytical batch	RPD <25%; n/a if concentration of either sample <RL
Field Quality Control	**Frequency of Analysis**	**Measurement Quality Objective**
Field Duplicate	5% of total project sample count	RPD <25%; n/a if concentration of either sample <RL
Field Blank	not applicable	

*Unless method specifies more stringent requirements.
MDL = Method Detection Limit
RL = Reporting Limit
n/a = not applicable

Table 19. Measurement Quality Objectives – Inorganic Analytes in Sediment

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 <RL
Internal Standard	Accompanying every analytical run when method appropriate	60-125% recovery
Field Quality Control	**Frequency of Analysis**	**Measurement Quality Objective**
Field Duplicate	5% of total project sample count	RPD <25%; n/a if concentration of either sample <RL
Field Blank	not applicable	

*Unless method specifies more stringent requirements.
MDL = Method Detection Limit
RL = Reporting Limit
n/a = not applicable

Table 20. Measurement Quality Objectives – Inorganic Analytes in Tissues

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 <RL
Internal Standard	Accompanying every analytical run when method appropriate	60-125% recovery

*Unless method specifies more stringent requirements.
MDL = Method Detection Limit
RL = Reporting Limit
n/a = not applicable

Table 21. 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 certified 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 <RL
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 22. Measurement Quality Objectives – Algal Toxins in tissues*

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

The acceptance criteria for laboratory duplicates are specified in Tables 16-22.

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.

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

Where:

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

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

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

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

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; detailed in Tables 16-22

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 (Appendix II, Tables 6-7) 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 may 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% (Tables 12-15). In the event laboratory documentation is incomplete, datasheets will be returned to the dissector for amendment.

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

Element 8. Special Training Requirements/Safety

8.1. Specialized training and safety requirements

Analysts are trained to conduct a wide variety of activities using standard protocols to ensure samples are analyzed in a consistent manner. Training of each analyst includes the use of analytical equipment and conducting analytical protocols, and other general laboratory processes including glassware cleaning, sampling preparation and processing, hazardous materials handling, storage, disposal. All laboratory staff must demonstrate proficiency in all the aforementioned and required laboratory activities that are conducted, as certified by the Laboratory QAO.

8.2. Training, safety and certification documentation

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

8.3. Training personnel

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

8.3.1. Laboratory Safety

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

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

8.3.2. Technical Training

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

Element 9. Documentation and Records

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

- Quality Assurance Project Plan (submitted to contract manager in paper and electronic formats)
- Sampling and Analysis Plan (submitted to contract manager in paper and electronic formats)
- Archived Sample Sheets (internal documentation available on request)
- Chain-of-Custody Forms (exchanged for signatures with chemistry lab, and kept on file)
- Lab Sample Disposition Logs (internal documentation available on request)

- Calibration Logs for measurements of water quality standards (internal documentation available on request)
- Refrigerator and Freezer Logs (internal documentation available on request)
- Quarterly Progress Reports (oral format to contract manager)
- Data Tables (submitted to contract manager in electronic formats)
- Draft Manuscript (produced in electronic format)
- Final Manuscript (in electronic format)
- Data Appendix (submitted to contract manager in paper and electronic spreadsheet formats)

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

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

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

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

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

Group B Elements. Data Generation and Acquisition

Element 10. Sample Process Design

The project design is described in the Sampling and Analysis Plan (SAP), Section E, pp. 10-15 (Appendix II). Twenty-four lakes and reservoirs identified as having relatively low contaiminatn concentrations will be sampled, where possible, water, sediment, small fish and sport fish.

Potential small fish and sport fish sampling equipment and methods can be found in MPSL-102a (Appendix III B). Once samples have been identified for composite creation, they will be processed according to the timeline in Table 11.

All measurements and analyses to be performed in tissue are critical to address the objectives laid out in Section III of the SAP (Appendix II), with the exception of fish weight, sex, age, and moisture content. These parameters may be used to support other data gathered. Water and sediment analyses are not as critical because they are not used to assess human health. These data may be used to inform MQ3.

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

Water samples will be collected after fish are collected, but before sediment is collected at the site. Samples will be collected according to MPSL Field SOP v1.1 and the clean-hands dirty-hands collection methods where appropriate. It is important to follow the clean-hands dirty-hands collection method when collecting total and methylmercury samples to avoid sample contamination. One sub-surface water grab will be collected each for unfiltered total and methyl mercury in a clear glass 250 mL bottle, demonstrated to be free of contaminants, at 0.1 m below the water surface. A sulfate sample will be collected at the same depth using a 125 mL HDPE bottle. Sample collection will occur in an area where the boat does not interfere with the sample, with the collector wearing clean polyethylene gloves. Containers will be opened and recapped under water to avoid surface water contamination of the sub-surface sample. Near-bottom water

will be collected utilizing a 2L capacity Kemmerer. Each analyte will be dispensed into the appropriate bottle for analysis.

A stainless steel Young-modified Van Veen grab will be deployed to collect bed sediments at the 3 locations where water is collected (MPSL Field SOP v1.1). The grab will be slowly lowered to the bottom of the lake with a minimum of substrate disturbance, and the closed grab will be retrieved at a moderate sped (< 2 ft s^{-1}). Upon retrieval the lids of the grab will be opened and the material examined to ensure it is undisturbed and of sufficient quality (recently deposited fine sediment) for use in chemical analyses. Specific rejection criteria are found in MPSL Field SOP v1.1, p59.

Only the top 2 cm of the collected material will be transferred to the sample bag using a pre-cleaned polyethylene scoop. All sediment will be stored in WhirlPac bags, and will be field frozen immediately on dry ice.

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

Details on targeted fish species, number of individuals and size ranges can be found in the SAP (Appendix II, Tables 6-7).

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

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

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

11.1. Corrective Action

In the event samples cannot be collected, the Sample Collection Coordinator will determine if corrective actions are appropriate. Table 23 describes action to take in the event of a collection failure.

Table 23. Field collection corrective actions

Collection Failure	Corrective Action
Field measurements	The instruments should be recalibrated following manufacturer cleaning and maintenance procedures. If measurements continue to fail MQOs, affected data should not be reported and the instrument should be returned to the manufacturer for maintenance. All troubleshooting and corrective actions should be recorded in calibration and field data logbooks.
Target Species not present	Collect secondary target; it is advisable to consult with OEHHA prior to choosing secondary target species; document the occurence
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.

All water and sediment samples will be preserved by the sample collection crew following Table 24. At the end of each collection event, samples will be delivered to MPSL and then subsequently shipped to each analytical laboratory according to an agreed upon schedule. A calendar of expected delivery dates and quantities can be found at http://swamp.mpsl.mlml.calstate.edu/calendar.

Samples for Dissolved Organic Carbon (DOC) will be preserved by keeping them cold (≤6 ◦C), and not be preserved with acid to pH<2 as recommended in the SWAMP Quality Control and Sample Handling tables. Dr. Dave Krabbenhoft (USGS) has indicated that DOC in water samples is stable for at least 30 days without addition of acid, therefore there is no impact on the integrity of the DOC results.

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

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 D) 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.

Holding times for each analyte can be found in Table 24.

Table 24. Sample handling and holding times for water, sediment and tissue samples.

Parameter	Container	Preservation	Holding Time
Chlorophyll *a* in water	Polyethylene	Filter as soon as possible after collection; if processing must be delayed, keep samples at ≤6°C; store in the dark	Samples must be frozen within 4 hours of collection; filters can be stored frozen for 28 days
Sulfate in water	Polyethylene	cool to ≤6°C	28 days
Organic Carbon (Dissolved) in water	Polyethylene	Filter within 15 minutes of collection, cool to ≤6°C	30 days
UV/EEMs in water	Polyethylene	Filter within 15 minutes of collection, cool to ≤6°C	30 days
Mercury (Total) in water	Glass	Preserve with 0.5% v:v pretested 12N HCl within 48 hours	90 days at room temperature following acidification
Methylmercury (total) in water	Glass	Immediately after collection, cool to ≤6°C in the dark; acidify to 0.5% v:v with pre-tested 12N HCl within 48 hours	6 moths at ≤6°C in the dark following acidification
Mercury (total) in sediment	WhirlPac	Freeze to ≤-20°C	1 year at ≤-20°C
Loss On Ignition in sediment	WhirlPac	Freeze to ≤-20°C	1 year at ≤-20°C
Mercury in Tissues	Wrapped in foil, zip top bag; Polyethylene	cool to ≤6°C within 24 hours, then freeze to ≤-20°C	1 year
Selenium in Sport Fish Tissues	Wrapped in foil, zip top bag; Polyethylene	cool to ≤6°C within 24 hours, then freeze to ≤-20°C	1 year
Selenium in Livers and Small Fish Tissues	Wrapped in foil, zip top bag; Polyethylene	cool to ≤6°C within 24 hours, then freeze to ≤-20°C	1 year
Organochlorine Pesticides	Wrapped in foil, zip top bag; Glass	cool to ≤6°C within 24 hours, then freeze to ≤-20°C	1 year; samples must be extracted within 14 days of thawing and analyzed within 40 days of extraction
Polychlorinated Biphenyls	Wrapped in foil, zip top bag; Glass	cool to ≤6°C within 24 hours, then freeze to ≤-20°C	1 year; samples must be extracted within 14 days of thawing and analyzed within 40 days of extraction
Algal Toxins	Wrapped in foil, zip top bag; Glass	cool to ≤6°C within 24 hours, then freeze to ≤-20°C	1 year; samples must be extracted within 14 days of thawing and analyzed within 40 days of extraction

Element 13. Analytical Methods

Methods and equipment for laboratory analyses are listed in Table 25. EPA methods can be downloaded from www.epa.gov/epahome/index/nameindx.htm. EPA method numbers followed by "M" indicate modifications have been made. Modifications and non-EPA SOPs can be found

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

Table 25. Methods for laboratory analyses

Parameter	Method	Instrument
Chlorophyll *a* in water	EPA 446.0 (USEPA 1997)	Genesis 10S
Sulfate in water	EPA 300.0 (USEPA 1993, Appendix IV C)	Dionex ICS 1000
Organic Carbon (Dissolved)	METH011.00 (Appendix V B)	Shimadzu TOC-L Series
UV/EEMs	Aqualog SOP_V1.3 (Appendix V F)	Aqualog Spectrofluorometer
Mercury (Total) in water	EPA 1631e (USEPA 2002)	Tekran 2600
Methylmercury (total) in water	EPA 1630 (USEPA 2001) with Isotope dilution (Appendix V C)	Brooks Rand MERX Automated methymercury Analytical System Perkin-Elmer Elan 9000 ICP-MS
Mercury (total) in sediment	EPA 7473 (USEPA 1998)	Nippon MA-2
Loss On Ignition in sediment	Solids, volatile-on-ignition, total-in-bottom-material, gravimetric (Appendix V D)	Not Applicable
Mercury in Tissues	EPA 7473 (USEPA 1998)	Milestone DMA 80 or Nippon MA-3000
Selenium in Sport Fish Tissues	EPA 3052M (USEPA 1996a, Appendix III E) EPA 200.8 (USEPA 1994a)	CEM MARSXpress Digester Perkin-Elmer Elan 9000 ICP-MS
Selenium in Livers and Small Fish Tissues	ID HGICP-MS (Appendix V E)	Perkin-Elmer SCIEX ELAN DRC II
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 Biotxins (Appendix IV D)	Agilent 1200 liquid chromatograph with Agilent 6410 Triple-Quad MS

Depth profiles for Dissolved Oxygen, pH, temperature, Specific Conductivity and chlorophyll a will be conducted using a YSI EXO2 multiparameter water quality sonde. Acceptibility criteria for field measurements can be found in Table 12.

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.

Sulfate in water samples will be determined by USEPA Method 300.0 “Determination of Inorganic Ions by Ion Chromatography” (USEPA 1993, Appendix IV C) using a Dionex ICS 1000 configured with a guard column, separator column, suppressor and detector. Samples for

sulfate analysis are collected in pre-cleaned plastic 250 mL containers. No chemical preservation is required after collection. Samples should be shipped and stored at <6°C, then analyzed within 28 days of collection. Standards, blanks, instrument checks, and batch quality control samples are prepared using ASTM Type II water. The instrument is calibrated with a 6-point calibration curve, then verified immediately after with a second-source reference standard. Instrument drift and sample carryover is monitored by the analysis of calibration verifications (CCV)and verification blanks after every 10 samples. The acceptance criteria for the CCV is $\pm$ 20% of the true value and $\pm$ reporting limit for the verification blank. A method blank, lab control spike, matrix spike, matrix spike duplicate, and sample duplicate is analyzed with every batch of 20 or fewer samples. If any instrument or batch quality control samples do not meet acceptance criteria, the corrective action is to investigate possible causes for the failure, correct the cause, and reanalyze the affected samples. Reporting Limits (RL) can be found in Table 26 and Measurement Quality Objectives (MQO) in Section 7, Table 16.

Chlorophyll a in water samples will be determined by USEPA Method 446, "In Vitro Determination of Chlorophylls *a, b,* c_1+c_2 Pheopigments in Marine and Freshwater Algae by Visible Spectrophotometry." (USEPA 1997) Periphyton are separated from water samples by filtering a measured volume of water through a glass fiber filter. The filter is wrapped to protect it from light then frozen for shipment to the laboratory. The filter is vortexed, sonicated, shaken, then steeped with a 90% acetone solution to extract the pigments from the periphyton. The UV spectrophotometer is zeroed using a blank, calibrated with standards, and the calibration verified with a certified reference standard. Absorbance of the blanks, standards, reference material, and sample extracts are recorded before and after acidification. Resultant readings are entered into "Lorenzen's Equation" as described in the method. A method blank, certified reference standard, and extract replicate are extracted with every batch of 20 or fewer samples. The mid-point calibration extract is reanalyzed after every 10 samples and end of analysis to monitor for drift (CCV). The acceptance criteria for the CCV is + 20% of the true value and + reporting limit for the method blank. If any instrument or batch quality control samples do not meet acceptance criteria, the corrective action is to investigate possible causes for the failure, correct the cause, and reanalyze the affected samples. Reporting Limits (RL) can be found in Table 26 and Measurement Quality Objectives (MQO) in Section 7, Table 16.

Loss On Ignition will be determined by Volatile on Ignition (Appendix V D). Sample is weighed into aluminum boats and heated to 550ºC for two hours. The percent of sample mass lost following heating is reported as Loss on Ignition (LOI). One sample per analysis is weighed in triplicate to assess method precision.

Table 26. Conventional analytical parameters, reporting units, and reporting limits (RL) for water and sediment samples.

Parameter	Method	RL
Chlorophyll *a*	EPA 446.0 (USEPA 1997)	5 µg/L
Sulfate	EPA 300.0 (USEPA,1993,Appendix IV C)	24 mg/L
Organic Carbon (Dissolved)	METH011.00 (Appendix V B)	Not Yet Determined
UV/EEMS	Aqualog SOP_V1.3	Not Applicable
Loss On Ignition in sediment	Volatile on Ignition (Appendix V D)	Not Applicable

Total mercury in water will be analyzed according to EPA 1631, revision E, "Mercury in water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry" (USEPA, 2002). 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 (NIST 1641d 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 27 and Measurement Quality Objectives (MQO) in Section 7, Table 17.

Total methylmercury in water will be analyzed according to EPA 1630, "Methylmercury in water by Distillation, Aqueous Ethylation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry" (USEPA, 2001) and Isotope Dilution (Appendix V C). 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 Laboratory Control Spike from a second source standard, 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 27 and Measurement Quality Objectives (MQO) in Section 7, Table 17.

Mercury in sediments will be analyzed according to EPA 7473, "Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry" (USEPA, 1998) using a Nippon MA-2 Mercury Analyzer. Solid sample is combusted at high temperature (850 deg C) in the presence of interference-reducing reagents, releasing mercury from the matrix as reduced gaseous mercury. In the resulting gas, matrix interference is further eliminated by catalytic treatment, adjusted to appropriate pH in a phosphate buffer, and then passed through a gold amalgam trap to quantitatively capture gaseous mercury. Lastly, the gold trap is heated, releasing the bound mercury into the sample stream, and detected by cold vapor atomic adsorption. 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 (NIST 1944 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 27 and Measurement Quality Objectives (MQO) in Section 7, Table 19.

Mercury in fish tissues will be analyzed according to EPA 7473, "Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry" (USEPA, 1998) using a Direct Mercury Analyzer (DMA 80). Samples, blanks, and standards will be prepared using clean techniques. ASTM Type II water and analytical grade chemicals will be used for all standard preparations. A continuing calibration verification (CCV) will be performed after every 10 samples. Initial and continuing calibration verification values must be within ±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 27 and Measurement Quality Objectives (MQO) in Section 7, Table 20.

Selenium sport fish composites will be digested according to EPA 3052M, "Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices" (USEPA, 1996a), modified (Appendix III E), 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 27 and Measurement Quality Objectives (MQO) in Section 7, Table 20.

Selenium in small fish composites and livers will be digested and analyzed according to ID HGICP-MS (Appendix V E). An ^{82}Se enriched isotope spike is used to measure isotope dilution. Calibration of the enriched ^{82}Se spike is achieved by reverse spike isotope dilution. The digestates are mixed with concentrated hydrochloric acid to reduce the selenium to the most favorable valence for hydride generation. The solutions are then analyzed by inductively coupled plasma mass spectrometry coupled with hydride generation (ID HGICP-MS). Polyatomic and isobaric interferences are removed through the use of hydride generation and background correction using ^{82}Se enriched isotope spike. 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 4-5 samples. Initial and continuing calibration verification values must be within ±20% of the true value, or the previous samples must be reanalyzed. Two blanks, two certified reference materials (NIST 2976, NRCC DORM-3 or similar), as well as two method duplicates and a matrix spike pair will be run with each set of samples. Reporting Limits (RL) can be found in Table 27 and Measurement Quality Objectives (MQO) in Section 7, Table 20.

Table 27. Trace metal analytical parameters, reporting units, and reporting limits (RL) for water, sediment and tissue samples.

Parameter	Method	RL
Mercury (total) in water	EPA 1631E (USEPA 2002)	0.04 ng/L
Methylmercury (total) in water	EPA 1630 (USEPA 2001) with Isotope dilution (Appendix V C)	0.04 ng/L
Mercury in Sediment	EPA 7473 (USEPA 1998)	1.38 ng/g dry wt
Mercury in Small Fish Tissues	EPA 7473 (USEPA 1998)	0.009 µg/g wet wt
Mercury in Sport FishTissues	EPA 7473 (USEPA 1998)	0.012 µg/g wet wt
Selenium in Small Fish Tissues	HGICP-MS (Appendix V E)	0.004 µg/g wet wt
Selenium in Sport Fish Tissues	EPA 3052M (USEPA 1996a, Appendix III E) EPA 200.8 (USEPA 1994a)	0.40 µg/g wet wt

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 B). PCBs will be analyzed according to EPA 8082M, "Polychlorinated Biphenyls (PCBs) by Gas Chromatography" (USEPA 1996e), modified (Appendix IV B). 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 28-29 and Measurement Quality Objectives (MQO) in Section 7, Table 21.

Table 28. Organochlorine Pesticide analytical parameters, reporting units, and reporting limits (RL) for tissue samples.

Organochlorine Pesticides (by USEPA 8081BM, USEPA 1996d, Appendix IV B)		
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 29. Polychlorinated Biphenyl analytical parameters, reporting units, and reporting limits (RL) for tissue samples.

Polychlorinated Biphenyl congeners (by USEPA Method 8082M, USEPA 1996e, Appendix IV B)			
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 D). 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, 22). Reporting Limits (RL) can be found in Table 30 and Measurement Quality Objectives (MQO) in Section 7, Table 22.

Table 30. Trace organic analytical parameters, reporting units, and reporting limits (RL) for tissue samples.

Microcystins and Biotoxins by LC/MS/MS (Appendix IV D)	
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 analyses must be completed within holding time specific to each analyte (Table 24). 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.

Each aspect of laboratory quality control is listed in Tables 12-22 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 25). These SOPs have been reviewed by each respective Laboratory QAO and found to be in compliance with SWAMP criteria. Analysts are responsible for equipment testing, inspection, and maintenance. Appendices III and IV list the referenced SOPs. DFG-WPCL SOPs are available upon request from the Laboratory Director by email: peter.ode@wildlife.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.

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 31) are calibrated, standardized and maintained according to procedures detailed in laboratory QAPs (Appendix I). Instrument manuals identify step-by-step calibration and maintenance procedures. 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 31. 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) (DFG-WPCL)	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 (DFG-WPCL)	As needed	At least once prior to each batch
Agilent 6410 Triple Quadropole LC/ESI/MS/MS in multiple reaction mode (DFG-WPCL)	As needed	At least once prior to each batch
Tekran 2600 (USGS)	As needed	At least once prior to each batch
Elan Inductively Coupled Plasma - Mass Spectrometer (USGS)	As needed	At least once prior to each batch
Nippon MA-2 (USGS)	As needed	At least once every 2 weeks
Nippon MA -3000 (USGS)	As needed	At least once every 2 weeks
Milestone DMA-80 Direct Mercury Analyzer (USGS and MPSL-DFG)	As needed	At least once every 2 weeks
Perkin-Elmer Elan 9000 Inductively Coupled Plasma - Mass Spectrometer (MPSL-DFG)	As needed	At least once prior to each batch
Perkin Elmer SCIEX ELAN DRC II (USGS)	As needed	At least once prior to each batch
Shimadzu	As needed	At least once prior to each batch
Aqualog Spectrofluorometer (USGS)	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 frequencies for this project are listed in Tables 16-22. All analyses are bracketed by an acceptable calibration verification; all samples not bracketed by an in control CCV should be reanalyzed. If the control limits for analysis of the calibration verification solution are not met, the initial calibration will have to be repeated. All samples analyzed before the calibration verification solution that failed the MQOs will be reanalyzed following the recalibration. Only the re-analysis results will be reported. If it is not possible or feasible to perform reanalysis of samples, all earlier data (i.e., since the last successful calibration control verification) are suspect. In this case, the laboratory QAO will contact the PM to determine proceedings, and will flag the data and note the issue in interim and final reports.

Element 17. Inspection/Acceptance of Supplies and Consumables

All supplies will be examined for damage as they are received. Laboratory ordering personnel will review all supplies as they arrive to ensure the shipment is complete and intact. All chemicals are logged in to the appropriate logbook and dated upon receipt. All supplies are stored appropriately and are discarded upon expiration date. Table 32 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 32. Inspection/acceptance testing requirements for consumables and supplies.

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

Element 18. Non-Direct Measures

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

Element 19. Data Management

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

All data generated by 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.

Furthermore, all USGS data will be generated and maintained according to the USGS Laboratory Quality Assurance Plan (Appendix I). The USGS QAO will be responsible for oversight of the collection of all dissection and metals analysis data and sending all QA-checked data to the PM.

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

All raw and statistical analysis data are subject to a 100% check for accuracy by the PM and Laboratory QAOs. Data are analyzed and proofread for accuracy, and then QA checked against the QAPP and SWAMP criteria before being entered into the SWAMP database. Original hard copies of the data are filed in a secure cabinet until requested by the PM and/or inclusion into the Final Report. Electronic copies are stored and backed up by each analyst and respective laboratory internal project manager.

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

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

Group C Elements: Assessment and Oversight

Element 20. Assessments and Response Actions

20.1. Audits

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

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

- Initial review of analytical and field data for complete and accurate documentation, chain of custody procedures, compliance with analytical holding times, and required frequency of laboratory QA samples.
- Comparison of all spike and duplicate results with the MQOs in Tables 16-22.
- 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, IV, and V), 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 LS and PM on the progress of all on-going chemical analyses in 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.

Data generated by project activities will be reviewed against the measurement quality objectives (MQOs) in Tables 16-22. 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 VI A).

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

Water and sediment chemistry data will not be validated for this project. These results aim to address why fish tissues may or may not show contamination, and are not intended to be used in any other way. Furthermore, there are currently no validation criteria developed for non-tissue chemistry results.

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

23.1. Blank Contamination Check

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

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

Element 24. Reconciliation with User Requirements

Data will be reported in the SWAMP Database version 2.5. Data that do not meet with the Measurement Quality Objectives in Table 16-22 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 will be summed for comparison with threshold values in Table 3. It is possible that some of the parameters that comprise each summation may be flagged as rejected through the Validation process (Appendix VI 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 3, 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 (Tables 13-15), to address the management questions laid out in the Sampling Plan (Appendix II). A failure to achieve the number of data points cited could mean an inability to answer these questions.

MQ1 will be assessed by comparing sport fish results from each location to the thresholds used for 303(d) listing determinations and to ATLs established by OEHHA (Klasing and Brodberg 2008) (Table 3). Data on water and sediment are being collected to address MQ2, and will not be compared to any assessment thresholds..

MQ2 will be assessed in collaboration with the Water Board staff working on the Reservoir TMDL. In addition to the parameters being measured in this study, other data that could help in addressing MQ2 include lake characteristics such as morphometry (surface area, shoreline length, bathymetry, volume), turnover, catchment area, water level fluctuation, fishing pressure, and landscape features such as wetlands (connected or adjoining), and agricultural land cover. If the budget allows, the influence of these parameters on concentrations of mercury in fish will be evaluated. If not covered by this study, it is likely that these factors will be evaluated by Water Board staff working on the statewide TMDL for reservoirs.

MQ3 will be assessed to the extent possible (depending on how many lakes are successfully sampled in a manner supporting this comparison) through a narrative summary of how the follow-up data compare to the previous results.

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Appendix I: List of Associated QAPs

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

CDFG WPCL Laboratory QAPP, Revision 9. August, 2006

Quality Assurance Plan for USGS Environmental Mercury Lab

USGS Mercury research Laboratory Quality Assurance Manual

Appendix II: Sampling and Analysis Plan

FINAL

Sampling and Analysis Plan for a Study of Lakes and Reservoirs with Low Concentrations of Contaminants in Sport Fish

The Bioaccumulation Oversight Group (BOG)

Surface Water Ambient Monitoring Program

May 2014

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I. INTRODUCTION

This document presents a plan for sampling and analysis of sport fish in a one-year effort to identify California lakes and reservoirs with low concentrations of contaminants in sport fish. This work will be performed as part of the State Water Resources Control Board's Surface Water Ambient Monitoring Program (SWAMP).

Oversight for this Project is being provided by the SWAMP Roundtable. The Roundtable is composed of State and Regional Water Board staff and representatives from other agencies and organizations including USEPA, the California Department of Fish and Wildlife, the California Office of Environmental Health Hazard Assessment, and the University of California. Interested parties, including members of other agencies, consultants, and other stakeholders are also welcome to participate.

The Roundtable has formed a subcommittee, the Bioaccumulation Oversight Group (BOG), which focuses on bioaccumulation monitoring. The BOG is composed of State and Regional Water Board staff and representatives from other agencies and organizations including USEPA, the Department of Fish and Wildlife, the Office of Environmental Health Hazard Assessment, 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), a two-year screening survey of the California coast (2009 and 2010), a one-year survey of California rivers and streams (2011), and a two-year study of mercury accumulation in grebes on California lakes and reservoirs (2012-2013). Final reports on the sport fish surveys are available (Davis et al. 2010; Davis et al. 2012; Davis et al. 2013; http://www.mywaterquality.ca.gov/monitoring_council/bioaccumulation_oversight_group/#mpr).

II. GENERAL ASPECTS OF THE SWAMP BIOACCUMULATION MONITORING PROGRAM

A. 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 (Table 1). This assessment framework is consistent with the 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 the most cost-effective tool for evaluating trends for many contaminants. Monitoring status and trends in bioaccumulation will provide 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.

This workplan describes an effort to refine the characterization of the status of lakes and reservoirs with regard to impairment due to bioaccumulation. SWAMP surveys to date have focused on identifying water bodies with elevated concentrations of bioaccumulative contaminants so that managers could develop strategies for addressing problem areas. In contrast, this survey will aim to provide information on another facet of status: identification of lakes and reservoirs with relatively low levels of contamination. This information will be useful to managers in their efforts to protect these relatively high quality ecosystems and to replicate these conditions in other water bodies. The information will also be valuable to the fishing public, drawing attention to water bodies where beneficial uses can be enjoyed with reduced exposure to bioaccumulative contaminants.

III. DESIGN OF THE CLEAN LAKES STUDY

A. Management Questions for this Study

Three management questions (one primary question, and two secondary questions) have been articulated to guide the design of this study. The primary question is the main driver of the sampling design. The secondary questions will be addressed to the extent possible with the resources available for the study, after assuring that the primary question is appropriately addressed.

Management Question 1 (MQ1)

Which popular lakes in California can be confirmed to have relatively low concentrations of contaminants in sport fish?

Answering this question will address the critical need of managers and the public to know which water bodies can be considered relatively clean. With this information, the fishing public can be directed to water bodies where they can enjoy the benefits of fishing and fish consumption and have reduced exposure to contaminants.

The data needed to answer this question are repeated observations of low concentrations of all contaminants of concern (including methylmercury, PCBs, legacy pesticides, and selenium) in the species with the greatest tendency to accumulate high concentrations. For methylmercury, top predators such as black bass tend to accumulate relatively high concentrations. High-lipid, bottom-feeding species such as catfish, carp, and sucker have the greatest tendency to accumulate relatively high concentrations of organic contaminants of concern (PCBs and legacy pesticides). 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. Measuring low concentrations of contaminants in both of these types of indicator species provides compelling evidence that a water body has a low overall degree of contamination. Given the variance associated with contaminant concentrations, the evidence becomes even more compelling if the low concentrations are observed on more than one occasion. This higher level of confidence obtained through repeated observation of low concentrations in both types of indicator species is desirable to be assured of providing reliable information to the public to guide their decisions on where to fish.

In some water bodies, it is not feasible to obtain both types of indicator species because they are not present in high enough abundance. Lakes at higher elevations with colder water where trout species predominate are a common example. For these lakes, repeated observation of the species that do occur there and are most likely to have high concentrations is the best basis that can be obtained for characterizing a lake as one with relatively low concentrations.

Management Question 2 (MQ2)

Why do some lakes have relatively low concentrations of methylmercury in sport fish?

A statewide control program for methylmercury is being developed by the State Water Resources Control Board: (http://www.waterboards.ca.gov/water_issues/programs/mercury/). Understanding the conditions associated with low concentrations of food web methylmercury is valuable to managers in their efforts to reduce concentrations in waters that are impaired. Supplemental measurements may provide valuable information on factors that drive methylmercury accumulation in lake food webs. Supplemental parameters that are expected to be very informative, based on data analysis conducted in support of the TMDL, include: mercury and selenium in prey fish; total mercury, methylmercury, sulfate, DOC, and chlorophyll in water; and total mercury and organic carbon in sediment.

Management Question 3 (MQ3)

Did the 2007-8 survey accurately characterize the status of lakes in which only rainbow trout were collected?

Many of the lakes found to have low concentrations of contaminants in the 2007-8 survey were lakes where only rainbow trout were collected. Rainbow trout generally had low concentrations of methylmercury, with a statewide average of 0.05 ppm. Concentrations of organics in trout were also generally low. To some degree, this was due to lower concentrations of contaminants in these lakes, but other factors also likely played a role. Trout generally occupy a lower trophic position and accumulate lower concentrations of methylmercury and other pollutants than black bass. However, a factor that probably contributed to lower observed concentrations in trout is that, in many lakes, recently planted hatchery fish are part of the catch. A previous study found that hatchery trout consistently had very low concentrations of methylmercury (rainbow trout from four hatcheries all had less than 0.023 ppm – Grenier et al. 2007).

With the level of effort that could be expended in the statewide survey of 2007-8 it is possible that other resident species with a potential to have higher concentrations were missed, such as resident populations of trout or small populations of warmwater predators like black bass or bottom feeders like sucker. With the greater effort planned for the present study, it is anticipated that information will be obtained that will allow for some evaluation of the accuracy of the 2007-8 assessment for lakes where only one species was obtained.

B. Overall Approach

The overall approach to be taken to answer these three questions is to re-sample a select subset of lakes that were identified as having relatively low concentrations of contaminants in the 2007-8 survey. The same basic design used in the 2007-8 survey will be repeated, as the goal is to obtain confirmation of the earlier results.

C. Coordination

The BOG is coordinating with other efforts to significantly leverage the funds for this survey and achieve a more thorough evaluation of California lakes with relatively low levels of contamination. These coordinated efforts are adding approximately $169,000 worth of work to the BOG funds available for sampling and analysis in this study ($240,000).

The Colorado River Basin Regional Water Quality Control Board (Region 7) will be conducting a survey of contaminants in sport fish in Region 7 lakes this summer. Region 7 has a relatively large proportion of lakes that meet the criteria for having low concentrations, including 10 of the 16 lakes that will be sampled in the Region. Resources for this statewide effort will be pooled with Region 7 resources to allow a more thorough and definitive assessment of the lakes in this region. The data from the Region 7 effort will be processed and reported along with the data from the statewide effort.

The Los Angeles Regional Water Quality Control Board (Region 4) will partner to expand this study in their region. Region 4 is covering the cost of all of the work in their region, including an extra lake (Castaic Lake) to complement sampling of Castaic Lagoon.

The San Diego Regional Water Quality Control Board (Region 9) is planning a study of cyanotoxins in reservoirs for this summer. One of the lakes to be sampled in that effort (Lake Henshaw) is also a candidate for inclusion in this study. If Lake Henshaw is selected for this study, the work will be coordinated with the cyanotoxin study. Effort will be made to collect Lake Henshaw at a similar time the other lakes from the Region 9 study are being collected.

Several U.S. Geological Survey (USGS) research labs are partnering with this study as an opportunity to provide improved understanding of mercury cycling in the western US. The USGS Wisconsin Water Science Center's Mercury Research Team will partner with SWAMP on this study by performing chemical analysis of water and sediment samples for total mercury, methylmercury, and related parameters. The MRT operates one of the premier mercury labs in the country, and frequently contributes to mercury studies at regional and national scales.

The Corvallis Research Group of the USGS Forest and Rangeland Ecosystem Science Center in Corvallis OR will partner with SWAMP on this study by performing chemical analysis of small fish samples for total mercury.

The Water Resources Division of USGS in Menlo Park CA will partner with SWAMP on this study by performing chemical analysis of small fish samples for selenium. They will also be analyzing mercury and selenium in sport fish livers from select lakes.

In addition, the Department of Fish and Wildlife will provide assistance in collecting fish from the Tahoe Keys.

D. Selection of Lakes to Be Sampled

The pool of lakes considered for sampling consisted primarily of those included in the 2007-8 SWAMP lakes survey, with the addition of a few others sampled from 2002-2012 for which data were placed in the California Environmental Data Exchange Network (CEDEN), a centralized repository of data on California's water bodies, including streams, lakes, rivers, and the coastal ocean.

Selection of the lakes to sample in this study was not straightforward because few lakes meet all of the standards that are under consideration for California use in assessing impairment for the purpose of 303(d) listing. Ideally, it would be good to avoid classifying a lake as having low concentrations and having that same lake appear on the 303(d) list of impaired waters. 303(d) listing determinations are based on the proportion of samples available that exceed the relevant threshold. When more than 10% of the samples exceed the threshold, the water body is classified as impaired.

The state is in the process of developing a statewide tissue objective for mercury that is anticipated to be 0.2 ppm wet weight (all concentrations mentioned in this document are presented on a wet weight basis). This threshold will be used for the next round of listing. Through BOG discussion, the 0.2 ppm objective and listing threshold was selected as the criterion for classifying lakes as having relatively low concentrations of mercury. To be confident that a lake truly has fish mercury concentrations below 0.2 ppm, it is desirable to have measured concentrations in species such as black bass that are known to accumulate high concentrations.

The California Office of Environmental Health Hazard Assessment (OEHHA) has established two sets of thresholds - fish contaminant goals and advisory tissue levels - that are relevant as selection criteria for lakes to be included in this study (Klasing and Brodberg [2008], Table 2). Fish contaminant goals (FCGs) are health protective values for lifetime exposure and consider only the toxicity of the contaminants. They were developed by OEHHA to assist other agencies to establish fish tissue-based criteria for cleanup. For the two main chemicals of concern in this study, the FCGs are 0.22 ppm for mercury and 3.6 ppb for PCBs. The FCG for mercury (0.22 ppm) is of the same

magnitude as the statewide tissue objective of 0.2 ppm, based only on toxicity and one serving per week of consumption. FCGs are being used by the Water Boards in the latest round of 303(d) listing determinations.

Advisory Tissue Levels (ATLs) consider both the toxicity of contaminants and the health benefits of fish consumption. They are used to develop sport fish consumption advice for the public. OEHHA has developed ATL ranges for one to seven servings per week. A comparison of the same consumption frequency (one serving per week), shows that, for mercury, the low end of the ATL range (150 to 440 ppb) for the sensitive population (children and women of child-bearing age) encompasses the statewide tissue objective (200 ppb). For PCBs, the low end of the ATL range (21 ppb) for a 2 servings per week consumption rate was also considered as a lake selection criterion.

For organics, given their use in 303(d) listing determinations, the FCGs are a relevant benchmark to use in assessing the degree of contamination. To be confident that a lake truly has organics concentrations below FCGs, it is desirable to have measured concentrations in species such as catfish, carp, or sucker that are known to accumulate high concentrations.

Only five lakes met these criteria for both mercury and organics, qualifying for Tier 1 of the list of candidate lakes for the study (Tables 3-5, Figure 1).

Given this outcome, slightly less stringent criteria were considered.

Since the FCGs for organics are much lower than the ATLs used to develop advisories, and OEHHA's advisories are the most important means of communicating information on fish contamination to the public, the use of the lowest ATLs for organics was considered. An additional six lakes had concentrations of mercury below the listing criterion and concentrations of organics below the lowest ATLs (Tier 2 in Table 5).

Another way in which the listing criteria are stringent is that they require 90% of the samples measured to be below the threshold. This leads to the fairly common occurrence that a lake has a mean mercury concentration below 0.2 ppm, but gets classified as impaired. The sampling approach employed in the SWAMP survey, which targets a wide range of sizes of black bass to provide a basis for ANCOVA that yields an accurate estimate of a size-standardized mean, has the unintended consequence of tending to trigger impairment listings because of the inclusion of large fish that tend to have relatively high mercury concentrations. Seven lakes had bass with size-standardized mean concentrations below 0.2 ppm and with organics means below the lowest ATLs (Tier 3 in Table 5). Many of the lakes included in Tier 3 are expected to be included on the next 303(d) list for mercury because while the means were below 0.2 ppm, 10% or more of the observations in individual fish were above this level due to the wide size ranges targeted for bass.

The last tier, Tier 4, is a more numerous category consisting of lakes where both indicator types were not sampled, but concentrations in the fish that were sampled were

below the 303(d) listing criteria for mercury and organics. This category is more numerous because it includes many lakes where only rainbow trout were sampled, and this species generally has low concentrations, in large part due to the origin of the fish at hatcheries.

Other criteria that were considered in selection of lakes for all tiers were having at least a moderate degree of fishing activity and a goal of having some lakes included from each of the Water Board regions.

E. Sampling Design At Each Lake

The general goal of this study is to replicate and expand upon the observations of low concentrations observed in some lakes in the 2007-8 survey. Given this, the sampling design for sport fish at each location will generally match that of the prior survey (BOG 2007). Another aspect of this goal is to generate information that can be communicated to the public to raise awareness of locations with relatively low concentrations and promote exposure reduction. In accordance with this, OEHHA has provided detailed input on the data needed to support development of consumption advice for each lake targeted in this study. OEHHA's guidance will be followed to the extent possible. In some cases, additional fish will be collected beyond OEHHA's specifications, most notably to support estimation of average mercury in largemouth bass at a standard size of 350 mm.

This study will also aim to understand factors that may contribute to the low concentrations of mercury in the food webs of these lakes (MQ2). Detailed statistical evaluation of available data on sport fish mercury and related parameters has been conducted in development of the forthcoming TMDL for mercury in California reservoirs. Key parameters identified in the TMDL analysis will also be measured in this study.

1. Sport Fish

a. Sport Fish Species Targeted

Given the focus of the study on the fishing beneficial use, the species to be sampled, as in prior sampling, 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 and temporal patterns in contamination. For example, largemouth bass are 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 study is to identify lakes and reservoirs with relatively low 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 - if these species have low concentrations, then it is likely that the food web in general has a low degree of contamination. 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, although the organic contaminants of concern biomagnify, they do so primarily through accumulation in lipid. Concentrations of organics are therefore 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.

Consequently, this study will target, where possible, two indicator species at each location: 1) a top predator (e.g., largemouth bass) as a mercury indicator, and 2) a high-lipid, bottom-feeding species (e.g., channel catfish, common carp) as an organics indicator.

Some lakes, particularly high elevation lakes, may have only one abundant top trophic level species (e.g., rainbow trout, and frequently these are stocked fish). In these cases in the 2007-8 survey, the one species present was often sampled as an indicator of all the target analytes. In contrast, in this study a greater effort (more hours spent fishing per lake) will be made to collect both mercury and organics indicator species.

In addition to the indicator species, species that are popular and accumulate lower concentrations have been identified for each lake by OEHHA and the Water Boards will be targeted for sampling (Table 6). Sampling of these species will allow for more comprehensive advice for each lake.

If the species recommended by OEHHA are not available, other potential targets will be considered (Table 7). 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. To cope with this, the sampling crew will have a prioritized menu of several potential target species. 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 electroshocking. 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 additional follow-up studies are needed at any of the sampled lakes.

b. Sport Fish Sampling Locations Within Each Lake

Lakes and reservoirs in California vary tremendously in size, from hundreds of small ponds less than 10 ha to Lake Tahoe at 50,000 ha. As lakes increase in size it becomes necessary to sample more than one location to obtain a representative

characterization of the water body. As much as possible, the same sampling locations visited in 2007-8 will be visited again for this survey.

In sport fish sampling using an electroshocking boat, it is frequently necessary to sample over a linear course of 0.5 – 1 mi to obtain an adequate number of fish. A sampling location in this study can therefore be thought of as a circle with a diameter of 1 mile. For small lakes less than 500 ha in size, one sampling location covers a significant fraction of the surface area of the lake. Therefore, for lakes less than 500 ha, one location will be sampled. For lakes of medium size (500 – 1000 ha), two locations will generally be sampled. For lakes in the large (1000 – 5000 ha) and very large categories (>5000 ha), two to four locations will be sampled. Since the goal of the study is to characterize human exposure, the existing locations have been established near centers of fishing activity.

Decisions regarding the number and placement of any new locations will be made in consultation with Regional Board staff with local knowledge of the lakes. Criteria to be considered in determining the placement of sampling locations will include the existence of discrete centers of fishing activity, known patterns of spatial variation in contamination or other factors influencing bioaccumulation, road or boat ramp access, and possibly other factors.

c. Sport Fish Compositing and Size Ranges for Each Species

Chemical analysis of trace organics is relatively expensive, and the management questions established for the 2007-8 survey and this study can be addressed with good information on average concentrations. Therefore the compositing strategy employed in the 2007-8 survey will again be employed for these chemicals (Figures 2 and 3).

Chemical analysis of mercury is much less expensive, and SWAMP partners would like to be able to answer additional questions related to trends over time and differences among lakes. Consequently, the sampling design for the mercury indicator species includes analysis of mercury in individual fish. For the mercury indicator species, an analysis of covariance approach will be employed where possible, in which the size:mercury relationship will be established for each location and an ANCOVA will be performed. The ANCOVA will allow evaluation of differences in slope among the locations and comparison of mean concentrations and confidence intervals at a standardized total length, following the approach of Tremblay (1998). Experience applying this approach in the Central Valley indicates that to provide robust regressions, 11 fish spanning a broad range in size are needed (Davis et al. 2003, Melwani et al. 2007).

Specific size ranges to be targeted for each species are listed in Table 8. Black bass (including largemouth, smallmouth, and spotted bass), Sacramento pikeminnow (included in Group 1) and brown trout are the key mercury indicators. These species have a high trophic position and a strong size:mercury relationship. These species will be analyzed for mercury only (unless a bottom-feeding species is not present), and will be

analyzed individually. 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 standardized total 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). In past sampling, brown trout have been observed to accumulate high concentrations in some lakes, due to the existence in some cases of resident, self-sustaining populations and a switch to piscivory for larger fish. Brown trout will therefore have a similar target as black bass - 11 fish analyzed as individuals with the data analyzed through ANCOVA.

In many high elevation lakes, trout species predominate, especially rainbow trout. Trout will be sampled again in this study, though a greater effort will be made to obtain resident predators and bottom-feeders in trout lakes. Past sampling of rainbow trout in the Bay-Delta watershed has found low concentrations and a weak size:mercury relationship. Therefore, for rainbow trout the ANCOVA approach will not be used. Mercury will be analyzed in individuals, but a specified size range will be targeted to control for size rather than a wide span to support a regression-based analysis. Trout species 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.

Catfish, carp, bullhead, and sucker are the primary targets for high lipid bottom-feeders. These species will be analyzed for organics and mercury. 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). Mercury is expected to be highest in the pelagic predators, but concentrations may also be above thresholds for concern in the bottom-feeders, so mercury will be analyzed in these samples as well. Samples for these species will be analyzed as composites.

Secondary targets have been identified (Table 8) that will be collected if the primary targets are not available. These species would be processed for potential analysis of mercury and organics. The samples would be analyzed as composites. The size ranges established for bottom-feeders 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 complications.

d. Sport Fish Compositing and Archiving Strategies

Strategies for compositing and archiving will vary somewhat for lakes of different size. The overall strategy will be described first for small lakes, followed by a discussion of the differences for larger lakes.

Small Lakes

Figure 2 illustrates the approach to be taken for the predator and bottom-feeding species in small lakes (<500 ha). As described above, the predator species will be analyzed for mercury only and as individual fish. All samples of the predator species will be analyzed. Small lakes will be treated as one sampling location, so fish from anywhere in the lake will be counted toward meeting the targets for each size range listed in Table 8. For ANCOVA, one common regression line will be developed to describe the size:mercury relationship for the lake as a whole. Aliquots from these samples will also be archived after they are analyzed in case of any problems or other circumstances calling for reanalysis at a later time.

The bottom-feeding species will be analyzed as composites for organics and mercury (Figure 2). These composite samples will be analyzed and processed in a stepwise fashion. One representative composite sample will be analyzed first. Another composite sample will also be collected but analyzed only in the unanticipated event that the first composite sample has problematic concentrations. Aliquots from all composites will be archived, whether they are analyzed or not, in case of any problems or other circumstances calling for analysis or reanalysis at a later time.

Larger Lakes

For lakes in the medium, large, and very large categories the basic approach will be similar, with a couple of modifications. Figure 3 illustrates the approach using a medium lake as the example. The first difference from the small lake approach is that sampling locations will be treated discretely. For the predator species, this means that 11 fish spanning a wide range of sizes will be targeted for each location to support the development of a size:mercury regression and an estimated mean concentration at standardized total length for each location. From these location means a lakewide mean will be calculated.

For the bottom-feeder species, discrete composites will be prepared for each location. These composites will be homogenized and archived. Aliquots of homogenate from each location composite will be pooled to form a lakewide composite. The lakewide composite will be analyzed first. If the lakewide composite concentrations of any of the organics are problematic, then all the discrete location composites can be analyzed if that is desired by the Regional Board responsible for that lake. Since the goal of this study is to identify relatively clean lakes, these additional composites will not be automatically analyzed as part of this study. Aliquots from all composites will also be

archived, whether they are analyzed or not, in case of any problems or other circumstances calling for analysis or reanalysis at a later time.

2. Prey Fish

Prey fish (25-100 mm) will be sampled using traps, seines, and dip nets from shoreline areas adjacent to the locations where sport fish are collected. Ten individuals each from three different prey fish species will be sampled from each lake. We will target the following primary prey fish target species at all lakes: Inland silversides, young-of-the-year largemouth bass, young-of-the-year bluegill, and threadfin shad. Other species that are within the target size range may be collected if the primary targets are not available. Efforts will be made to sample the same species across all lakes, and when not possible fish that overlap in trophic guild will be sampled. Extra species of fish in the correct size ranges will be retained, and decisions on species to analyze for mercury will be made after all fish are collected each year.

Prey fish will be composited by species in each lake and analyzed for mercury and selenium.

3. Water

Sampling locations for water will be selected with the aim of obtaining information that is representative of the lake and where the sport fish are accumulating their mercury. Three locations will be sampled in each lake.

1. Near dam at deepest part of lake (deepest part of lake could be in middle, if a natural lake), top and bottom (less than 1 m above the bottom) sample.
2. In a location where the water is <100 ft deep (likely off-thalweg), top and bottom sample.
3. Various, top and bottom sample.

If the lake has major tributary arms, then the arms will be sampled, if possible. If the lake has coves where the majority of the fish reside, these will be sampled. At each location, subsurface (0.1 m depth) and near-bottom grab samples will be collected and analyzed for unfiltered total mercury, unfiltered methylmercury, DOC, sulfate, and chlorophyll a. Depth profiles for Dissolved Oxygen, pH, temperature, Specific Conductivity and chlorophyll a will be conducted using a YSI EXO2 multiparameter water quality sonde.

4. Sediment

Three sediment grab samples (top 2 cm) will be collected where waters are collected in each lake using a Van Veen grab sampler (0.5 m^2). Sediment samples will be analyzed for total mercury and total organic carbon loss on ignition (LOI).

F. Sample Processing and Analysis

1. Sport Fish

Fish will be collected in accordance with MPSL-102a, Section 7.4. Whenever possible an electro-fishing boat will be used; however, it may be necessary to employ another method also described in Section 7.4.

The following adaptation to MPSL-102a, Section 7.4.5 (Appendix II) has been made for this study: at the dock, all fish collected will be placed on a measuring board covered with a clean plastic bag; fork and total length will be recorded. Weight will be recorded with a digital spring scale. Large fish will be partially dissected in the field using the following protocol: fish will be placed on a cutting board covered with a clean plastic bag where the head, tail, and guts are removed using a clean (laboratory detergent, DI) cleaver. The cleaver and cutting board are re-cleaned between fish species, per site if multiple stations are sampled.

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., carp, which can be greater than 40 lb) there will be times when it is necessary to process fish in the field.

Whole fish or field-processed fish will be wrapped in aluminum foil and placed in a clean labeled zipper-style bag. All samples will be kept cold on ice until frozen in a freezer or on dry ice within 24 hours of collection. Samples will be stored at -20°C at the laboratory until dissection and homogenization. Homogenates will also be frozen until analysis is performed. Frozen tissue samples have a 12-month hold time from the date of collection (USEPA 2000); however, the scientific advisory board has stated that samples kept frozen, with minimal thaw-freeze cycles, for several years have no appreciable degradation of organic contaminants.

All sport fish will be dissected "skin off". 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 mercury. 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).

Fish are filleted to expose the flesh. It is important to maintain the cleanliness of the tissue for analysis; therefore any flesh that has been in direct contact with the skin, with instruments in contact with skin, or with any potential contaminant surface such as foil or a plastic bag, must be eliminated from the analyzed sample. The exposed edges of the fillet should be trimmed by 1/4 inch with a clean scalpel or fillet knife to remove this contaminated tissue.

How a sample is dissected is greatly dependent on the types of analyses being conducted. Tissue from individual fish for mercury analysis only will be dissected from the fillet above the lateral line. When composites must be created, equal tissue weights are taken from 5 individual fish following the 75% size rule recommended by USEPA (2000) and homogenized into a Location Composite with a target weight of 200g or greater. Tissue for composites will be taken from the fillet of each fish above the lateral line and from the belly to include areas of higher lipid content. A subsequent lakewide composite will be created from equal portions of each contributing Location Composite within each lake. Figures 2 and 3 diagram compositing strategies and target weights for predator and bottom species. Post-homogenization aliquots will be taken from the lakewide composite for mercury, selenium and organics analyses.

Livers from selected fish will be preserved and processed for analysis of total mercury and selenium by USGS. Methods for these analyses are described in the "Prey Fish" section below.

Mercury will be analyzed by the Moss Landing Marine Laboratory Marine Pollution Studies Lab 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 analyzed by the Moss Landing Marine Laboratory Marine Pollution Studies Lab. 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 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 and PCBs 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 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.

2. Prey Fish

Inserting tags into small fish is not always possible, therefore upon collection, each prey fish will be individually bagged in well labeled zipper-style bag and placed in a larger zipper bag clearly labeled with the lake and species. Each fish will be linked to the latitude/longitude or UTM where it was collected. Several parameters will be measured in the field for each fish, including total length (longest length from tip of tail fin to tip of nose/mouth), fork length (longest length from fork to tip of nose/mouth), standard length, and weight. Once measurements have been recorded, prey fish will be frozen on dry ice in the field.

Prey fish will be analyzed as composites of whole fish for total mercury and total selenium only.

Analysis of total mercury in prey fish will be conducted by the Corvallis Research Group of the USGS Forest and Rangeland Ecosystem Science Center in Corvallis OR using the same analytical method as for the sport fish (EPA 7473).

Analysis of selenium in prey fish will be conducted by the Water Resources Division of USGS in Menlo Park CA. Selenium digested and analyzed by Isotope Dilution Hydride Generation Inductively Coupled mass Spectrometry. An ^{82}Se enriched isotope spike is used to measure isotope dilution. Calibration of the enriched ^{82}Se spike

is achieved by reverse spike isotope dilution. The digestates are mixed with concentrated hydrochloric acid to reduce the selenium to the most favorable valence for hydride generation. The solutions are then analyzed by inductively coupled plasma mass spectrometry coupled with hydride generation (ID HGICP-MS). Polyatomic and isobaric interferences are removed through the use of hydride generation and background correction using ^{82}Se enriched isotope spike. 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 4-5 samples. Initial and continuing calibration verification values must be within ±20% of the true value, or the previous samples must be reanalyzed. Two blanks, two certified reference materials (NIST 2976, NRCC DORM-3 or similar), as well as two method duplicates and a matrix spike pair will be run with each set of samples.

3. Water

Water samples will be collected after fish are collected, but before sediment is collected at the site. Samples will be collected according to MPSL Field SOP v1.1 and the clean-hands dirty-hands collection methods where appropriate. It is important to follow the clean-hands dirty-hands collection method when collecting total and methylmercury samples to avoid sample contamination. One sub-surface water grab will be collected each for unfiltered total and methyl mercury in a clear glass 250 mL bottle, demonstrated to be free of contaminants, at 0.1 m below the water surface. Mercury and methylmercury samples will be preserved in the field with 2.5 ml 50 percent HCl. A sulfate sample will be collected at the same depth using a 125 mL HDPE bottle. Sample collection will occur in an area where the boat does not interfere with the sample, with the collector wearing clean polyethylene gloves. Containers will be opened and recapped under water to avoid surface water contamination of the sub-surface sample. Near-bottom water will be collected utilizing a 2L capacity Kemmerer. Each analyte will be dispensed into the appropriate bottle for analysis. Chlorophyll A up to 1000 ml may be filtered depending upon the turbidity of the water.

Total and methylmercury samples will be stored in the dark, on ice or refrigeration (4±2 ºC) until transfer to the laboratory within 48 hr of collection. When necessary, samples may be shipped on ice via freight carrier in a well-sealed ice chest. Ice will be double bagged to prevent water leakage into the samples. Glass bottles will be wrapped in bubble wrap to prevent breakage during shipment. Appropriate chain of custody records (COCs) will accompany each shipment. Samples collected will have the salinity (in parts per thousand) or specific conductivity (µS/cm), depth of collection, and date/time collected for each station on every COC.

Total mercury and methylmercury analysis will be performed by the U.S. Geological Survey Wisconsin Water Science Center's Mercury Research Team (MRT). Total mercury will be analyzed by oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry following EPA Method 1631, Rev. E. Bromine Monochloride (BrCl) is added to the sample container to oxidize all forms of Hg to the HgII oxidation

state. After 5 days at 50 deg C, the BrCl is neutralized by the addition of Hydroxylamine Hydrochloride (NH2OH*HCl). Following neutralization, Stannous Chloride (SnCl2) is added to the sample to reduce the Hg from HgII to Hg0. The Hg0 is purged onto gold-coated glass bead traps (sample). The mercury vapor is thermally desorbed to a second gold trap (analytical) and from that detected by cold vapor atomic fluorescence spectrometry (CVAFS). Samples high in organic matter may require initial pretreatment in an ultra violet (UV) digester to remove the organic color from the sample.

Methymercury analysis will be performed by aqueous phase ethylation, followed by gas chromatography separation with speciated isotope dilution mass spectrometry (USGS 2002: Open-File Report 01-445). Water samples are spiked with isotopically enriched standard and distilled to remove potential interferences. The pH of the distillate is adjusted to 4.9 using acetate buffer. The distillate is then ethylated using sodium tetraethyl borate (NaTEB4) and allowed to react for 15 minutes. Following reaction with NaTEB the distillate is purged with grade 5 Argon gas (Ar) for 20 minutes and the ethylated mercury species are collected on a Carbotrap. The ethylated mercury species are thermally desorbed from the Carbotrap, separated using a gas chromatography (GC) column, reduced and ionized using the ICP-MS, and detected using Speciated Isotope Dilution Mass Spectrometry (SIDMS).

Sulfate and chlorophyll *a* analyses will be performed by the WPCL. Sulfate samples will be kept cold (<6 ºC) until transfer to and analysis at WPCL within 28 days of collection. Sulfate will be analyzed according to WPCL-AA-041: Inorganic Anions by Ion Chromatography, EPA Method 300. 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 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. A blank, laboratory control sample, certified reference material (NIST 1641d or similar), as well as a method duplicate and matrix spike pair will be run with each set of samples.

Chlorophyll a in water samples will be determined by USEPA Method 446, "In Vitro Determination of Chlorophylls *a, b,* c_1+c_2 Pheopigments in Marine and Freshwater Algae by Visible Spectrophotometry." Periphyton are separated from water samples by filtering a measured volume of water through a glass fiber filter. The filter is wrapped to protect it from light then frozen for shipment to the laboratory. The filter is vortexed, sonicated, shaken, then steeped with a 90% acetone solution to extract the pigments from the periphyton. The UV spectrophotometer is zeroed using a blank, calibrated with standards, and the calibration verified with a certified reference standard. Absorbance of the blanks, standards, reference material, and sample extracts are recorded before and after acidification. Resultant readings are entered into "Lorenzen's Equation" as described in the method. A method blank, certified reference standard, and extract replicate are extracted with every batch of 20 or fewer samples. The mid-point calibration extract is reanalyzed after every 10 samples and end of analysis to monitor for drift (CCV). The acceptance criteria for the CCV is + 20% of the true value and + reporting limit for the method blank. If any instrument or batch quality control samples

do not meet acceptance criteria, the corrective action is to investigate possible causes for the failure, correct the cause, and reanalyze the affected samples. No certified reference material is available for chlorophyll a, therefore one will not be analyzed, and neither will a matrix spike/ duplicate pair.

DOC analysis will be performed by the USGS MRT using a catalytically-aided platinum 680°C combustion technique (http://wi.water.usgs.gov/mercury-lab/analysis-methods.html).

4. Sediment

A stainless steel Young-modified Van Veen grab will be deployed to collect bed sediments at the 3 locations where water is collected (MPSL Field SOP v1.1). The grab will be slowly lowered to the bottom of the lake with a minimum of substrate disturbance, and the closed grab will be retrieved at a moderate sped (< 2 ft s^{-1}). Upon retrieval the lids of the grab will be opened and the material examined to ensure it is undisturbed and of sufficient quality (recently deposited fine sediment) for use in chemical analyses. Specific rejection criteria are found in MPSL Field SOP v1.1, p59.

Only the top 2 cm of the collected material will be transferred to a whirl pac bag, frozen in the field and shipped on dry ice.

Total mercury and LOI analysis will be performed by the USGS MRT. Total mercury will be analyzed by atomic adsorption following direct combustion with a Nippon MA-2 Mercury Analyzer (EPA Method 7473 [SW-846] Rev. 0). Solid sample is combusted at high temperature (850 deg C) in the presence of interference-reducing reagents, releasing mercury from the matrix as reduced gaseous mercury. In the resulting gas, matrix interference is further eliminated by catalytic treatment, adjusted to appropriate pH in a phosphate buffer, and then passed through a gold amalgam trap to quantitatively capture gaseous mercury. Lastly, the gold trap is heated, releasing the bound mercury into the sample stream, and detected by cold vapor atomic adsorption.

LOI will be analyzed following USGS Techniques of Water-Resources Investigations 5-A1, 3rd ed. Sample is weighed into aluminum boats and heated to 550°C for two hours. The percent of sample mass lost following heating is reported as LOI. One sample per analysis is weighed in triplicate to assess method precision.

G. Analytes in Sport Fish

Table 9 provides a summary list of sport fish 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. A detailed list of analytes is provided in Table 10.

Additional discussion of the analytes is provided below.

Ancillary Parameters

Ancillary parameters to be measured in the lab include moisture and lipid (Table 10). 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.

Scales will be collected from black bass species and analyzed for age.

Methylmercury

Methylmercury is the contaminant of greatest concern with respect to bioaccumulation on a statewide basis (Davis et al. 2010). 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 it is possible that samples of each species will exceed thresholds of concern.

PCBs

PCBs are the contaminant of second greatest concern with respect to bioaccumulation on a statewide basis (Davis et al. 2010). PCBs will be analyzed using a congener-specific method. A total of 55 congeners will be analyzed (Table 10). PCBs will be analyzed in one composite sample from each lake. The species with the greatest expected concentrations (i.e., the organics indicator species where they are present) will be included.

Legacy Pesticides

Legacy pesticides may exceed FCGs in some locations. Individual compounds recommended by USEPA (2000) will be analyzed (Table 10). Legacy pesticides will be analyzed in one composite sample from selected lakes. The species with the greatest expected concentrations (i.e., the organics indicator species where they are present) will be analyzed.

Selenium

Past monitoring (Davis et al. 2010) indicates that selenium concentrations are generally not likely to be above thresholds in this study. Selenium analysis will be included for a select few lakes where selenium may approach thresholds.

Microcystins

Sampling of Lake Henshaw will be conducted in coordination with Region 9, which is conducting a regional study of cyanotoxins. Cyanotoxins will be analyzed in the fish samples collected from Lake Henshaw.

Other Contaminants

Assessment thresholds are essential in this study, and are not available for the other contaminant categories.

H. Quality Assurance

This effort will adhere to quality assurance requirements established for the SWAMP. A QAPP specific to this effort is in preparation (Bonnema 2014).

I. Archiving

1. Sport Fish

Samples will be stored in short-term archives. Samples in the short-term archive are stored at -20 °C and are intended for use in the identification of short-term time trends (i.e. < 5-10 years), the investigation of yet-unidentified chemical contaminants, and addressing quality assurance issues that may arise during the routine analyses of samples. These samples are intended for the analysis of chemicals that 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 equipped with a backup generator in the event of a power outage.

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 each sampling location, up to three 40-50 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 or polypropylene for fluorinated chemical (i.e. PFCs) or trace metals analysis. Two of the three 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.

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.

J. 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 follow-up studies are needed in any of the sampled locations.

K. Timing

Sampling will be conducted from May 2014 through October 2014. Seasonal variation in body condition and reproductive physiology, as well as limnological characteristics, are recognized as factors that could affect contaminant concentrations. To the extent practical, the seasonal timing of sampling will replicate the timing of the previous round of sampling.

L. Data Assessment

MQ1 will be assessed by comparing sport fish results from each location to the thresholds used for 303(d) listing determinations and to ATLs established by OEHHA (Klasing and Brodberg 2008) (Table 2). Data on water and sediment are being collected to address MQ2, and will not be compared to any assessment thresholds.

MQ2 will be assessed in collaboration with the Water Board staff working on the Reservoir TMDL. In addition to the parameters being measured in this study, other data that could help in addressing MQ2 include lake characteristics such as morphometry (surface area, shoreline length, bathymetry, volume), turnover, catchment area, water level fluctuation, fishing pressure, and landscape features such as wetlands (connected or adjoining), and agricultural land cover. If the budget allows, the influence of these parameters on concentrations of mercury in fish will be evaluated. If not covered by this study, it is likely that these factors will be evaluated by Water Board staff working on the statewide TMDL for reservoirs.

MQ3 will be assessed to the extent possible (depending on how many lakes are successfully sampled in a manner supporting this comparison) through a narrative summary of how the follow-up data compare to the previous results.

M. Products and Timeline

A report on this 2014 sampling will be drafted by June 2015. The final report, incorporating revisions in response to reviewer comments, will be completed and released in September 2015.

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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 2a. OEHHA Fish Contaminant Goals. From Klasing and Brodberg (2008).

Table 1. Fish Contaminant Goals (FCGs) for Selected Fish Contaminants Based on Cancer and Non-Cancer Risk* Using an 8-Ounce/Week (prior to cooking) Consumption Rate (32 g/day)**	
	FCGs (ppb, wet weight)
Contaminant Cancer Slope Factor $(mg/kg/day)^{-1}$	
Chlordane (1.3)	**5.6**
DDTs (0.34)	**21**
Dieldrin (16)	**0.46**
PCBs (2)	**3.6**
Toxaphene (1.2)	**6.1**
Contaminant Reference Dose (mg/kg-day)	
Chlordane ($3.3x10^{-5}$)	100
DDTs ($5x10^{-4}$)	1600
Dieldrin ($5x10^{-5}$)	160
Methylmercury ($1x10^{-4}$)[S]	**220**
PCBs ($2x10^{-5}$)	63
Selenium ($5x10^{-3}$)	**7400**
Toxaphene ($3.5x10^{-4}$)	1100

*The most health protective Fish Contaminant Goal for each chemical (cancer slope factor- versus reference dose-derived) for each meal category is bolded.
**g/day represents the average amount of fish consumed daily, distributed over a 7-day period, using an 8-ounce serving size, prior to cooking.
[S]Fish Contaminant Goal for sensitive populations (i.e., women aged 18 to 45 years and children aged 1 to 17 years.)

Tabled values are rounded based on laboratory reporting of three significant digits in results, where the third reported digit is uncertain (estimated). Tabled values are rounded to the second digit, which is certain. When data are compared to this table they should also first be rounded to the second significant digit as in this table.

Table 2b. OEHHA advisory tissue levels. From Klasing and Brodberg (2008).

Table 2. Advisory Tissue Levels (ATLs) for Selected Fish Contaminants Based on Cancer or Non-Cancer Risk Using an 8-Ounce Serving Size (Prior to Cooking) (ppb, wet weight)

Contaminant	Three 8-ounce Servings* a Week	Two 8-ounce Servings* a Week	One 8-ounce Servings* a Week	No Consumption
Chlordane[c]	≤190	>190-280	>280-560	>560
DDTs[nc**]	≤520	>520-1,000	>1,000-2,100	>2,100
Dieldrin[c]	≤15	>15-23	>23-46	>46
Methylmercury (Women aged 18-45 years and children aged 1-17 years)[nc]	≤70	>70-150	>150-440	>440
Methylmercury (Women over 45 years and men)[nc]	≤220	>220-440	>440-1,310	>1,310
PCBs[nc]	≤21	>21-42	>42-120	>120
Selenium[nc]	≤2500	>2500-4,900	>4,900-15,000	>15,000
Toxaphene[c]	≤200	>200-300	>300-610	>610

[c]ATLs are based on cancer risk
[nc]ATLs are based on non-cancer risk
*Serving sizes are based on an average 160 pound person. Individuals weighing less than 160 pounds should eat proportionately smaller amounts (for example, individuals weighing 80 pounds should eat one 4-ounce serving a week when the table recommends eating one 8-ounce serving a week).
**ATLS for DDTs are based on non-cancer risk for two and three servings per week and cancer risk for one serving per week.

Tabled values are rounded based on laboratory reporting of three significant digits in results, where the third reported digit is uncertain (estimated). Tabled values are rounded to the second digit, which is certain. When data are compared to this table they should also first be rounded to the second significant digit as in this table.

Table 3. Criteria for assigning candidate lakes to tiers. Colors refer to shading in Table 4.

Tier 1 (blue)
Both indicator types sampled
Hg: Below 303(d) listing criterion (90% of samples below 0.2 ppm)
Organics: Below 303(d) listing criteria (90% of samples below FCGs)
At least some fishing activity

Tier 2 (green)
Both indicator types sampled
Hg: Below 303(d) listing criterion (90% of samples below 0.2 ppm)
Organics: means in the ATL range for three servings per week
At least some fishing activity

Tier 3 (purple)
Both indicator types sampled
Hg: mean below 0.2
Organics: means in the ATL range for three servings per week
At least some fishing activity

Tier 4 (yellow)
Both indicator types not sampled
Hg: Below 303(d) listing criterion (90% of samples below 0.2 ppm)
Organics: Below 303(d) listing criteria (90% of samples below FCGs)
The more fishing the better

Table 4. Candidates for inclusion in the Clean Lakes Study.

Candidates for the Clean Lakes Study: Region 1. See Table 3 for tier and color scheme. Letters indicate priority of the Tier 4 lakes.

Region	Lake	Species	Tier	Comments
1	Kangaroo Lake	RBT		Remote back country lake.
1	Reservoir F	LMB		Remote back country lake.
1	Lewiston Lake	RBT	4a	Popular. More heavily fished than Cleone.
1	Trinity Lake	RBT		Listed for Hg. Do not eat LMB advisory.
1	Howard Lake	RBT		Remote back country lake.
1	Plaskett Lake	Hardhead		Remote back country lake.
1	Cleone Lake	RBT	4b	Popular.

Candidates for the Clean Lakes Study: Region 2. See Table 3 for tier and color scheme. Letters indicate priority of the Tier 4 lakes.

Region	Lake	Species	Tier	Comments
2	Horseshoe Lake, Quarry Lakes	CCAT		Not impaired for Hg. One of three PCB samples above FCG. Difficult to get bass.
2	Lago Los Osos	CCAT		No fishing allowed.
2	Lake Cunningham	CARP		Not popular. PCBs, DDTs, dieldrin, chlordanes above FCGs.
2	Lake Elizabeth	CARP		Not impaired for Hg. PCBs, DDTs, dieldrin above FCGs
2	Briones Reservoir	LMB		Fishing not allowed.
2	Lake Madigan	Bluegill		Only got bluegill.

Table 4. Candidates for inclusion in the Clean Lakes Study (continued).

Candidates for the Clean Lakes Study: Region 3. See Table 3 for tier and color scheme. Letters indicate priority of the Tier 4 lakes.

Region	Lake	Species	Tier	Comments
3	Loch Lomond Reservoir	LMB, Bluegill		Impaired. Hg 0.11 at 350 mm.
3	Lopez Lake	LMB, Sucker	2	Not impaired. PCBs, dieldrin above FCGs

Table 4. Candidates for inclusion in the Clean Lakes Study (continued).

Candidates for the Clean Lakes Study: Region 4. See Table 3 for tier and color scheme. Letters indicate priority of the Tier 4 lakes.

Region	Lake	Species	Tier	Comments
4	Castaic Lagoon	LMB, CARP, RBT, Redear	3	Need repeat of low Hg, PCBs above FCG. New 303(d) Listing
4	Elizabeth Lake	Crappie, Bullhead, RBT		Hg 0.21 in 2007, under in 2010 - repeat? (Tier 4 based on 2010)
4	Lake Lindero	Carp		Not impaired. Hg low in 2007 and 2010. Organics above FCGs.
4	Malibou Lake	LMB, Carp, Bluegill	2	Not impaired. Hg low in 2007 & 2010. PCBs, dieldrin, chlordanes above FCGs.
4	Westlake Lake	LMB		Not impaired. PCBs, dieldrin above FCGs.
4	Cerritos Park Lake	LMB, Carp, RBT		Impaired. PCBs above ATL, DDTs, dieldrin above FCGs. New 303(d) Listing
4	Wilderness Park Lake	CCAT, Carp		Dieldrin above FCG.
4	Harbor Lake (Lake Machado)	Carp		Not impaired. PCBs above FCG.
4	Balboa Lake	Carp		DDTs, dieldrin above FCG.
4	Belvedere Park Lake	Carp	*	PCBs at 22 ppb (above ATL). Strong Region 4 interest in sampling this lake.
4	Lake Calabasas	LMB		Not impaired. PCBs above FCG.
4	Legg Lake	LMB, Carp, Redear, CCAT	3	Impaired. PCBs very high in 2005. Hg low in 2007 and 2010. PCBs above FCG in 2010. New 303(d) Listing.
4	Lincoln Park Lake	LMB, Carp	2	Not impaired. Hg low in 2007 & 2010. PCBs above FCG.
4	Sepulveda Lake	Carp		PCBs, DDTs, dieldrin above FCGs.
4	Toluca Lake	LMB		Not impaired. PCBs above FCG.
4	Echo Lake	LMB, Carp	*	PCBs above ATLs in past sampling, but cleanup and restocking have occurred. Expected to be clean now.

Table 4. Candidates for inclusion in the Clean Lakes Study (continued).

Candidates for the Clean Lakes Study: Region 5. See Table 3 for tier and color scheme. Letters indicate priority of the Tier 4 lakes.

Region	Lake	Species	Tier	Comments
5	McCumber Reservoir	RBT		
5	North Battle Creek Reservoir	Brown Trout		
5	Blue Lakes	LMB		Tribes interested. Impaired.
5	Big Reservoir	RBT		
5	Caples Lake	Brown Trout	4b	Not impaired.
5	French Meadows Reservoir	RBT		Impaired.
5	Hell Hole Reservoir	Brown Trout		Impaired.
5	Ice House Reservoir	RBT	4e	
5	Union Valley Reservoir	RBT		Also SMB, 1 sample = 0.419
5	Lake of the Pines	LMB	4a	Not impaired.
5	Bowman Lake	Brown Trout		PCBs above FCG
5	Faucherie Lake	RBT		Impaired.
5	Jackson Meadow Reservoir	RBT		Impaired.
5	Lake Spaulding	RBT		Brown Tr(n=5) = 1.1, Chinook(n=3) = 0.58
5	Scotts Flat Reservoir	RBT		Impaired, BG, LMB, Brown Tr, Green Sunfish
5	Fuller Lake	Brown Trout		Not impaired.
5	Feeley Lake	Bullhead		
5	Kidd Lake	Bullhead		
5	Antelope Lake	LMB, Bullhead	1	Not impaired.
5	Bucks Lake	RBT		Not impaired. Brown Tr(n=10) = 0.069, Lake Tr(n=5) = 0.024
5	Butt Valley Reservoir	SMB		Impaired.
5	Frenchman Lake	RBT		
5	Gold Lake	RBT	4c	
5	Little Grass Valley Reservoir	RBT		
5	Lake Almanor	SMB		Impaired.
5	Lake Davis	RBT, Bullhead		

5	Lower Bucks Lake	Kokanee		
5	Paradise Lake	LMB		Impaired.
5	Whiskeytown Lake	LMB		Impaired.
5	Castle Lake	RBT		
5	Gumboot Lake	RBT		
5	Big Lake	RBT, Sucker		
5	Reservoir C	RBT		
5	Duncan Reservoir	RBT, Bullhead		
5	Iron Canyon Reservoir	RBT		
5	Lake Britton	SMB, Carp	3	Impaired. 303(d) Listed Sucker up to 0.5 in 2006. SMB 350 mean above 0.2 in 2008.
5	Medicine Lake	Brook Trout		
5	Cave Lake	Brook Trout		
5	Lily Lake	RBT		
5	Lower Bear River Reservoir	RBT		
5	Lower Blue Lake - Alpine County	RBT		Impaired. Dieldrin above FCG
5	Upper Blue Lake	RBT		
5	White Pines Lake	RBT		
5	Lake Alpine	RBT	4d	Nearby Spicer Meadow also possible, but Alpine had lower Hg.
5	Beardsley	RBT		
5	Pinecrest	RBT		
5	Spicer Meadow Reservoir	RBT		
5	La Grange Reservoir	RBT		
5	Bass Lake	LMB, Bullhead	1	Not impaired.
5	Florence Lake	Brown Trout		Not impaired.
5	Huntington Lake	RBT	4f	Kokanee(n=1) = 0.10
5	Mammoth Pool Reservoir	RBT		
5	Contra Loma Reservoir	LMB		Impaired.

5	545TU0164	LMB, Carp		Impaired. Would be Tier 3, but not popular for fishing. PCBs, DDTs, dieldrin above FCG, New 303(d) Listing
5	Marsh in Fresno Slough	LMB, Bullhead		Impaired. Would be Tier 3, but not popular for fishing. DDTs, dieldrin above FCG. New 303(d) Listing
5	Courtright Reservoir	RBT		Brown Tr(n=1) = 0.06
5	Hume Lake	RBT		
5	Wishon Reservoir	RBT		Brown Tr(n=1) = 0.29

Table 4. Candidates for inclusion in the Clean Lakes Study (continued).

Candidates for the Clean Lakes Study: Region 6. See Table 3 for tier and color scheme. Letters indicate priority of the Tier 4 lakes.

Region	Lake	Species	Tier	Comments
6	Ellery Lake	RBT		
6	Grant Lake	RBT		
6	Gull Lake	RBT		
6	June Lake	RBT		
6	Lundy Lake	RBT		Dieldrin above FCG.
6	Saddlebag Lake	RBT		Dieldrin above FCG.
6	Tioga Lake	RBT		
6	Convict Lake	RBT		
6	Lake Crowley	RBT		
6	Lake George	RBT		
6	Lake Mary	RBT		
6	Lake Mamie	RBT		
6	Pleasant Valley Reservoir	RBT		
6	Rock Creek Lake	RBT		
6	Lake Sabrina	RBT		
6	Twin Lakes	RBT		Dieldrin above FCG.
6	Apollo Lake	RBT		
6	Palmdale Lake	LMB, CCAT	3	PCBS, dieldrin above FCG. New 303(d) Listing
6	Lake Gregory	LMB, Carp	3	Dieldrin above FCG. New 303(d) Listing
6	Spring Valley Lake	RBT		PCBs above FCG.
6	Bridgeport Reservoir	RBT		
6	Virginia Lakes	RBT		
6	Topaz Lake	Sucker, RBT	4b	Very popular.
6	Indian Creek Reservoir	RBT	4d	Dieldrin above FCG. Wastewater was discharged into this reservoir for decades, Nutrient TMDL was done, and they are actively oxygenating the bottom to reduce nutrient mobilization. Due to all the waste discharged over the decades, i'm curious what any non-trout species may show. This reservoir is rather warm, and may not support trout

				long-term without continual physical manipulations, Probably will shift to warm-water species as climate warms).
6	Fallen Leaf Lake	Lake Trout	4c	Not impaired. PCBs, DDTs, dieldrin, chlordane above FCG. Suspect pesticides from numerous homes around the lake; however may not be able to capture any "bottom" species in this oligotrophic (cold) lake; if design requires multiple species, may kick this out of clean lakes study)
6	Lake Tahoe	RBT		
6	Lake Tahoe - Tahoe Keys		4a	Little/no data for non-trout species, which are caught & eaten by local people "of color" [potential EJ issue]; also, JRowan of DFW is actively shocking in the Keys Lagoons so costs could be modest compared to mobilizing a whole crew
6	Prosser Creek Reservoir	RBT		
6	Boca Reservoir	Sucker, RBT		
6	Stampede Reservoir	RBT		
6	Eagle Lake	Eagle Lk Trout		
6	Crater Lake	RBT		
6	Dodge Reservoir	RBT		

Table 4. Candidates for inclusion in the Clean Lakes Study (continued).

Candidates for the Clean Lakes Study: Region 7. See Table 3 for tier and color scheme. Letters indicate priority of the Tier 4 lakes.

Region	Lake	Species	Tier	Comments
7	Lake Havasu	Carp		RB7 plans to sample in 2014
7	Gene Wash Reservoir	LMB, Carp	1	Not impaired. RB7 plans to sample in 2014
7	Ferguson Lake	LMB, Carp	1	Not impaired. RB7 plans to sample in 2014
7	Senator Wash Reservoir	LMB, Carp	1	Not impaired. RB7 plans to sample in 2014
7	Lake Cahuila	Carp		DDTs above FCG
7	Fig Lake	Tilapia, Carp		
7	Ramer Lake	Crappie, Carp		Not impaired. RB7 plans to sample in 2014
7	Wiest Lake	CCAT, Carp		Not impaired. RB7 plans to sample in 2014. PCBs, DDTs, dieldrin above FCG.
7	Sunbeam Lake	LMB, CCAT	2	Not impaired. RB7 plans to sample in 2014. PCBs, DDTs, dieldrin above FCG. Data from 2004 only.
7	Salton Sea	Tilapia		RB7 plans to sample in 2014

Table 4. Candidates for inclusion in the Clean Lakes Study (continued).

Candidates for the Clean Lakes Study: Region 8. See Table 3 for tier and color scheme. Letters indicate priority of the Tier 4 lakes.

Region	Lake	Species	Tier	Comments
8	Lee Lake/Corona Lake	LMB		Impaired. PCBs above FCG.
8	Lake Evans	LMB, Carp	2	Not impaired. PCBs abo e FCG.
8	Prado Lake	LMB, Carp	2	Not impaired. PCBs above FCG.
8	Lake Hemet	RBT, Carp		Not impaired.
8	Perris Reservoir	LMB		Not impaired. PCBs and DDTs above FCG.

Table 4. Candidates for inclusion in the Clean Lakes Study (continued).

Candidates for the Clean Lakes Study: Region 9. See Table 3 for tier and color scheme. Letters indicate priority of the Tier 4 lakes.

Region	Lake	Species	Tier	Comments
9	Lake Henshaw	LMB, Carp	3	Impaired. Popular. Sampled lots of large bass. Organics below FCGs. R9 Sampling in late summer for cyanotoxins in tissues. New 303(d) Listing
9	Dixon Lake	LMB	4a	Not impaired. In/close to urbanized areas. Lots of fishing for stocked species (catfish in summer and trout in winter). Popular for fishing. Newer Reservoir, dam built in 1960s? Storage for imported water treatment.
9	Lake Wohlford	LMB		Not impaired. Moderately popular. Private boats prohibited. Downstream from Henshaw...receives Henshaw water via canal
9	Lake Poway	LMB	4b	Not impaired. In/close to urbanized areas. Lots of fishing for stocked species (catfish in summer and trout in winter). Popular for fishing. Newer Reservoir, dam built in 1971. Storage for imported water treatment.
9	Lake Jennings	LMB, CCAT	3	Impaired. Dieldrin above FCG. New 303(d) Listing

Table 5. Tier assignments for candidate lakes.

Tier 1 (blue)

1. Antelope Lake (R5)
2. Bass Lake (R5)
3. Gene Wash Reservoir (R7)
4. Senator Wash Reservoir (R7)

Tier 2 (green)

5. Lopez Lake (R3)
6. Lincoln Park Lake (R4)
7. Malibou Lake (R4)
8. Sunbeam Lake (R7)
9. Lake Evans (R8)
10. Prado Lake (R8)

Tier 3 (purple)

11. Castaic Lagoon (R4)
12. Legg Lake (R4)
13. Palmdale Lake (R6)
14. Lake Gregory (R6)
15. Lake Henshaw (R9)
16. Lake Jennings (R9)

Tier 4 (yellow - top choices for each region shown, in priority order)

17. Lewiston Lake (R1)
18. Lake Merced (R2)
19. Lake of the Pines (R5)
20. Caples Lake (R5)
21. Gold Lake (R5)
22. Lake Tahoe (Tahoe Keys) (R6)
23. Dixon Lake (R9)
24. Loch Lomond (R3) (not included due to budget limitations)
25. Huntington Lake (R5) (not included due to budget limitations)
26. Lake Alpine (R5) (not included due to budget limitations)
27. Ice House Reservoir (R5) (not included due to budget limitations)
28. Topaz Lake (R6) (not included due to budget limitations)
29. Fallen Leaf Lake (R6) (not included due to budget limitations)
30. Indian Creek Reservoir (R6) (not included due to budget limitations)

Table 6a. OEHHA recommendations for sampling at each of the lakes to be included in the study. Species in **bold** are especially important target species.

Water Body	BOG list #	County	Region	OEHHA recommendations-Hg	OEHHA PCBs	Comments
Antelope Lake	1	Plumas	5	bass (6 legal, indiv) resident brown trout (10 fish, 2 comps) Eagle Lake or rainbow trout (10 fish or 5 fish each species, 2 comps) catfish (10 fish, 2 comps)	brown trout catfish Eagle Lake trout or rainbow trout	
Bass Lake	2	Madera	5	bass (6 legal, indiv) **kokanee** (10 fish, 2 comps) bluegill (10 fish, 2 comps) crappie (10 fish, 2 comps) catfish (10 fish, 2 comps) rainbow trout (10 fish, 2 comps)	catfish kokanee Rainbow trout	
Gene Wash Reservoir	3	San Bernardino	7	bass (6-9 legal, indiv) channel catfish (10 fish, 2 comps) bluegill (10 fish, 2 comps) crappie (10 fish, 2 comps)	catfish	Also Se (all species) and OCs (catfish)
Senator Wash Reservoir	4	Imperial	7	bass (6-9 legal, indiv) channel catfish (10 fish, 2 comps) bluegill (10 fish, 2 comps) crappie (10 fish, 2 comps)		Also Se (all species) and OCs (catfish)
Lopez Lake	5	San Luis Obispo	3	crappie (10 fish, 2 comps) bluegill (10 fish, 2 comps) catfish (10 fish, 2 comps)	catfish	
Lincoln Park Lake	6	Los Angeles	4	bass (6 legal, indiv) catfish (10 fish, 2 comps) bluegill (10 fish, 2 comps) crappie (10 fish, 2 comps) rainbow trout (10 fish, 2 comps)	Catfish Rainbow trout	
Malibou Lake	7	Los Angeles	4	bass (6 legal, indiv.) catfish (10 fish, 2 comps) crappie (10 fish, 2 comps)	catfish	
Sunbeam Lake	8	Imperial	7	bass (9 legal, indiv.) rainbow trout (10 fish, 2 comps) catfish (15 fish, 3 comps) carp (15 fish, 3 comps) bluegill (15 fish, 3 comps)	catfish rainbow trout carp	Also Se (all species) and OCs (catfish, trout, carp)

1

Water Body	BOG list #	County	Region	OEHHA recommendations-Hg	OEHHA PCBs	Comments
Lake Evans	9	Riverside	8	bass (6 legal, indiv) catfish (10 fish, 2 comps) rainbow trout (10 fish, 2 comps) bluegill (10 fish, 2 comps)	Catfish Rainbow trout	
Prado Lake	10	Riverside, San Bernardino	8	bass (6 legal, indiv) catfish (10 fish, 2 comps) rainbow trout (10 fish, 2 comps)	Catfish Rainbow trout	
Castaic Lagoon	11	Los Angeles	4	bass (6 legal, indiv) catfish (10 fish, 2 comps) crappie (10 fish, 2 comps) rainbow trout (5 fish, 1 comp) carp (5 fish, 1 comp)	catfish (if no catfish or just one composite of catfish, do carp	Sampling the Lagoon without sampling Castaic Lake will cause communication problems
Castaic Lake	See comment	Los Angeles	4	Striped bass (10 indiv, 2 comps) catfish (10 fish, 2 comps) crappie (10 fish, 2 comps) rainbow trout (10 fish, 2 comps)	Striped bass and catfish and rainbow trout	Not sampled as part of the BOG study - to be sampled concurrently but separately for Region 4
Legg Lake	12	Los Angeles	4	bass (6-9 legal, indiv) crappie (10 fish, 2 comps) bluegill (10 fish, 2 comps) rainbow trout (10 fish, 2 comps)	Rainbow trout	high Hg mean, may not be good candidate for clean lake study
Palmdale Lake	13	Los Angeles	6	bass (6 legal, indiv) catfish (5 fish, 1 comp) rainbow trout (10 fish, 2 comps) bluegill (10 fish, 2 comps) crappie (10 fish, 2 comps)	Catfish Rainbow trout	private lake
Lake Gregory	14	San Bernardino	6	bass (6 legal, indiv) catfish (10 fish, 2 comps) brown trout if present, otherwise rainbow(10 fish, 2 comps) bullhead (5 fish, 1 comp)	catfish carp Rainbow trout carp (5 fish, 1 comp)	high Hg mean, may not be good candidate for clean lake study)
Lake Henshaw	15	San Diego	6	bass (6 legal, indiv) catfish (10 fish, 2 comps) **crappie** (10 fish, 2 comps) bluegill (10 fish, 2 comps) bullhead (10 fish, 2 comps)	catfish	high Hg mean, may not be good candidate for clean lake study)

2

Water Body	BOG list #	County	Region	OEHHA recommendations-Hg	OEHHA PCBs	Comments
Lake Jennings	16	San Diego	9	bass (6 legal, indiv, good size range) channel (large) or blue catfish (10 fish, 2 comps) bluegill (10 fish, 2 comps) rainbow trout (10 fish, 2 comps) "whipers" (9-10, indiv)	Catfish Rainbow trout "Whipers" (comp)	
Lewiston Lake	17	Trinity	1	brook trout (10 fish, 2 comps) brown trout (10 fish, 2 comps)	brown trout (or brook if don't get browns)	Do not expect warm water spp. But if there (bass, catfish), collect
Lake Merced	18	San Francisco	2	9 largemouth bass (individuals) Trout (2 comps) Carp (2 comps)—unless they catch catfish, which is better for PCBs Bluegill (2 comps)	Trout Carp or catfish	Not previously sampled, but included to have a lake in Region 2.
Lake of the Pines	19	Nevada	5	Bass (largemouth and smallmouth, 6 legal each, indiv) Sunfish (10 fish, 2 comps) Crappie (10 fish, 2 comps) Catfish (10 fish, 2 comps)	catfish	
Caples Lake	20	Alpine	6	rainbow trout (10 fish, 2 comps) brook trout (10 fish, 2 comps) lake trout (10 fish, 2 comps)	lake trout	
Gold Lake	21	Sierra	5	Brown trout (10 fish, 2 comps) Lake trout (10 fish, 2 comps)	Brown or lake	
Tahoe Keys	22	Placer, El Dorado	6	bass (6 legal, indiv); crappie (10 fish, 2 comps) bluegill (10 fish, 2 comps) bullhead (10 fish, 2 comps)	Bullhead	
Dixon Lake	23	San Diego	9	bass (6 legal, indiv) **crappie** (10 fish, 2 comps) rainbow trout (10 fish, 2 comps) channel (large) or blue catfish (10 fish, 2 comps)	Catfish Rainbow trout	

Table 6b. OEHHA recommendations for sampling at other lakes that were considered for inclusion in the study. Species in **bold** are especially important target species.

Water Body	BOG list #	County	Region	OEHHA recommendations-Hg	OEHHA PCBs	Comments
Lake Alpine	21	Alpine	6	Rainbow trout (large, 6-10 1-2 comps	none needed unless other species present	
Ice House Reservoir	22	El Dorado	5	Brown trout (large) (10 fish, 2 comps)	Brown trout	
Huntington Lake	23	Fresno	5	kokanee (10 fish, 2 comps) rainbow trout (large) (10 fish, 2 comps) brown trout (10 fish, 2 comps)	brown trout or kokanee and Rainbow trout	
Topaz Lake	26	Mono	6	Rainbow trout (10 fish, 2 comps)	Rainbow trout Sucker (10 fish, 1 comp)	
Fallen Leaf Lake	27	El Dorado	6	Lake trout (at least 16") (10 fish, 2 comps) Kokanee (10 fish, 2 comps)	Lake trout or kokanee	
Indian Creek Reservoir	28	Alpine	6	Eagle Lake trout (10 fish, 2 comps) Rainbow trout (5 fish, 1 comp, or if less than 2 comps of Eagle lake, then 10 fish, 2 comps Rainbow trout)	Eagle Lake (or rainbow trout if don't have Eagle Lake)	
Lake Tahoe	See comment	Placer, El Dorado	6	Lake trout (10 fish, 2 comps) Brown trout (10 fish, 2 comps) Kokanee (10 fish, 2 comps)	Brown, Lake, or kokanee and rainbow trout	Not included in this BOG study.

Table 7. Target species and their characteristics.

	Foraging Type		Trophic Level	Distribution			
Species	Water column	Bottom feeder		Low Eleva-tion	Foothills	High Elevation	Priority for Collection
Largemouth bass	X		4	**X**	**X**		**A**
Smallmouth bass	X		4	x	**X**		**A**
Spotted bass	X		4	x	**X**		**A**
Sacramento pikeminnow	X		4	x	x		B
White catfish		X	4	x	x		**A**
Brown bullhead		X	3	x			B
Channel catfish		X	4	**X**	**X**		**A**
Carp		X	3	**X**	**X**		**A**
Sacramento sucker		X	3	x	x		B
Tilapia		X	3				B
Bluegill	X		3	**X**	**X**		B
Green sunfish	X		3	**X**	**X**		B
Crappie	X		3/4	x	x		B
Redear sunfish	X		3	**X**	**X**		B
Rainbow trout	X		3/4	x	x	**X**	**A**
Brown trout	X		3/4		x	x	**A**
Brook trout	X		3			x	**A**
Kokanee	X		3	?	x	x	B

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 8. Target species, size ranges, and processing instructions. I - process as individuals. C - process as composites.

	Process for Mercury	Process for Organics and Selenium	Numbers and Size Ranges (mm)
Primary Targets: stay on location until one of these targets from both Group 1 and 2 is obtained, or collect secondary targets if primary targets are not available			
Group 1) Predator			
Black bass	I		2X(200-249), 2X(250-304), 6X(305-407), 2X(>407)
Sacramento pikeminnow	I		3X(200-300), 5X(300-400), 3X(400-500)
Brown trout	I	C	3X(200-300), 5X(300-400), 3X(400-500)
Rainbow trout	C	C	5X(300-400)
Brook trout	C	C	5X(300-400)
Group 2) Bottom feeder			
White catfish	C	C	5X(229-305)
Channel catfish	C	C	5X(375-500)
Common carp	C	C	5X(450-600)
Brown bullhead	C		5X(262-350)
Sacramento sucker	C	C	5X(375-500)
Secondary Targets: collect these if primary targets are not available			
Bluegill	C	C	5X(127-170)
Redear sunfish	C	C	5X(165-220)
Black crappie	C	C	5X(187-250)
Tilapia	C	C	5X(235-314)
Green sunfish	C	C	Xx
Kokanee	C	C	5X(300-400)

Table 9. Summary of sport fish analytes included in the study.

Analyte	Included in Study?
Methylmercury[1]	Some individuals, all composites
PCBs	Selected composites
DDTs	Selected composites
Dieldrin	Selected composites
Aldrin	Selected composites
Chlordanes	Selected composites
Selenium	Selected composites
Microcystins	Not included (except for work funded by Region 9)
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 10. Parameters to be measured in sport fish.

FISH ATTRIBUTES

1. Total length
2. Fork length
3. Standard length (small fish only)
4. Weight
5. Sex
6. Moisture
7. Lipid content
8. Age (for black bass)

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')

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
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
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. MCY-RR
2. MCY-LR
3. MCY-YR
4. MCY-LA

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

Cyanotoxins
1. anatoxin a

Figure 1. Map of sampling locations. Lake names are indicated in Table 5.

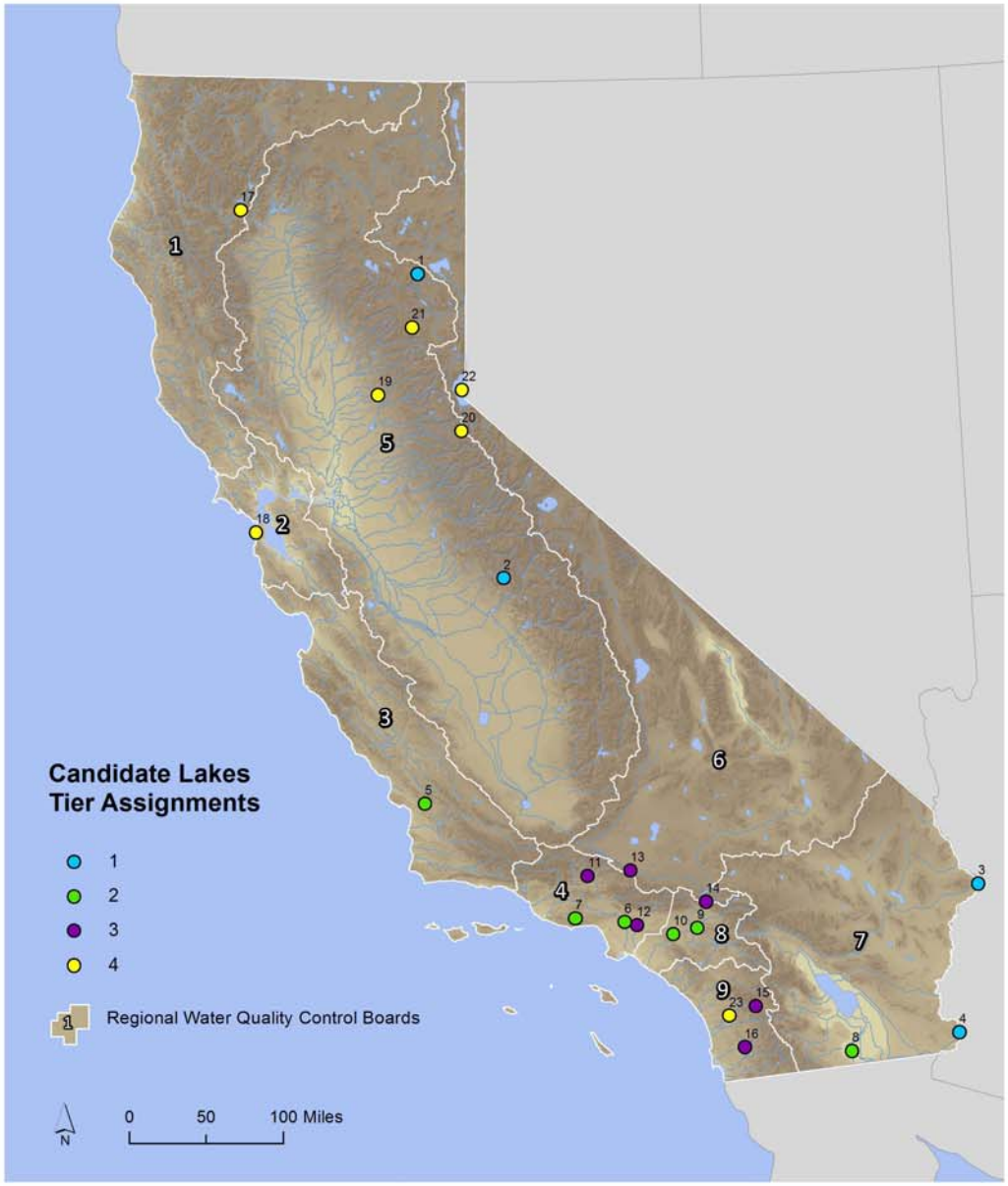


Figure 2. Sampling design for a small lake.

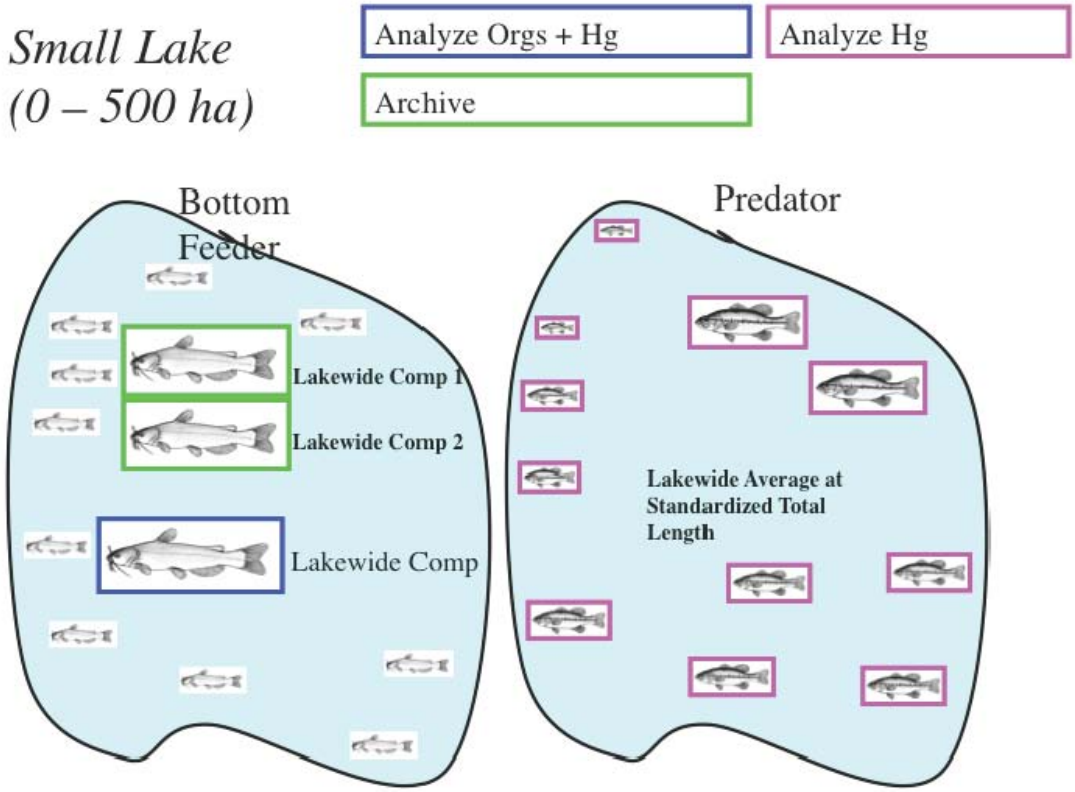
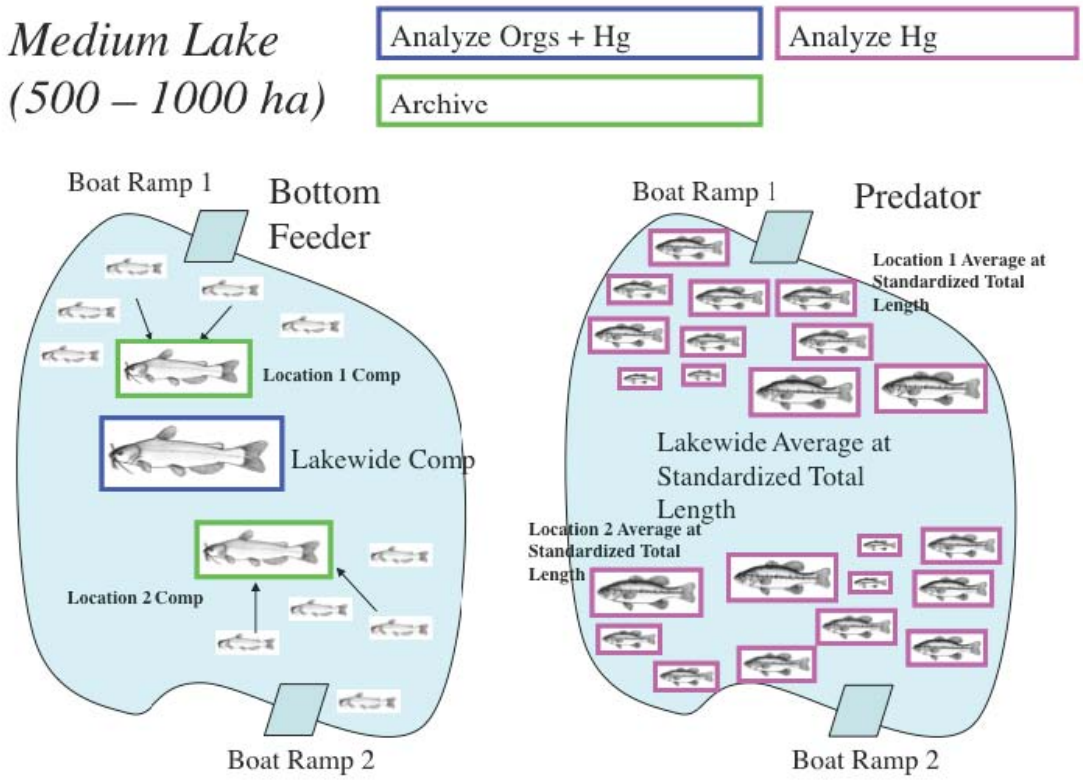


Figure 3. Sampling design for a medium-sized lake.



Appendix III: MPSL-DFG SOPs

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

Appendix III A: 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

7.3.2.1 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 B: 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.

10.2 Goldberg, E.D., ed. 1980. The International Mussel Watch. National Academy of Sciences Publ., Washington, D.C.

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

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

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

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

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 D: 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.

11.2 Goldberg, E.D., ed. 1980. The International Mussel Watch. National Academy of Sciences Publ., Washington, D.C.

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

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

Appendix III E: 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 IV: WPCL SOPs

WPCL Standard Operating Procedures			
Page	Procedure/Equipment	SOP number/Reference	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	March 2005
C	Inorganic Anions by ion Chromatography, EPA Method 300	WPCL-AA-041	March 2012
D	Method: Microcystins and Biotoxins by LC/MS.MS	WPCL-LC-065	
E	Procedures for Disposal of Waste	HAZMAT_ Rev4_SOP	March 2009
F	Protocol for Corrective Action Procedures	WPCL-QA-050	December 2009

Appendix IV A: Sample Custody, Receipt 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

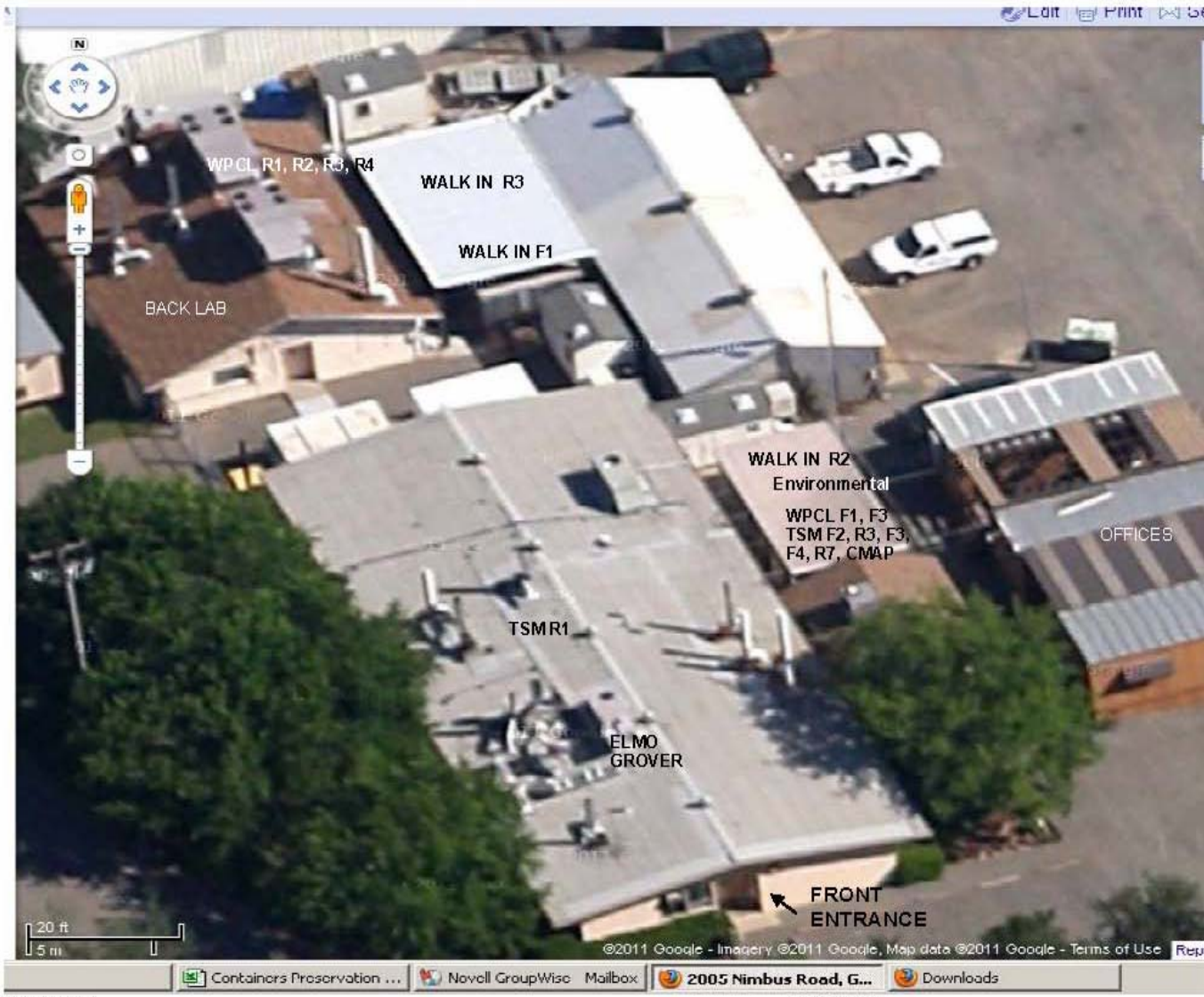
	EPA	Standard	LabWorks Code	Container	Preservation		Holding Time		Minimum Volume		Sa
Analyses	**Method**	**Methods**			**Water (includes dissolved)**	**Solids, Tissues**	**Water**	**Solids**	**Water**	**Solids**	
Aluminum by GFAAS		SM 3113B	AL_GF_*				6 months	6 months	250 mL		
Aluminum, Total monomeric		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*	P,FP,G	HNO3 to pH<= 2	<= -20°c	6 months	6 months	250 mL	100 g	
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% Mositure					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 = JAOAC65

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.


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
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Appendix IV B: Determination of OC and PCB in Sediment and Tissue – Modifications to EPA 8081B and 8082

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:

<u>PCB Internal Standards</u>
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

<u>OC Internal Standards</u>
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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SOP Section Approval: _____ Date: _____

SOP Final Approval: _____ Date: _____

SOP QA Officer Approval: _____ Date: _____

Appendix IV C: Inorganic Anions by Ion Chromatography, EPA Method 300

STANDARD OPERATING PROCEDURE
TITLE: Inorganic Anions by Ion Chromatography, EPA Method 300.

REVISION HISTORY		
Revision #	**Summary of Changes**	**Date**
4	Changed lab director. Updated format. Added iodide, Tables 1 to 4, Figure 1, eluent preparation.	03/01/12
3	Unknown.	08/10/09
2	Unknown.	Unknown.
1	Unknown.	Unknown.
0	Initial release.	Unknown.

Author:	*Mallory Pirie*		Date: 03/01/12
Approved:	Section Leader *Kendall Penney*		Date:
Approved:	Laboratory Director *Pete Ode*		Date:
Approved:	Quality Assurance *Gail Cho*		Date:
Approved:	Health and Safety *Thomas Lew*		Date:

STANDARD OPERATING PROCEDURE

TITLE: Inorganic Anions by Ion Chromatography (IC)

1.0 Scope and Application

1.1 This method is applicable to the determination of the following inorganic anions in water.

- 1.1.1 Bromide (Br-)
- 1.1.2 Chloride (Cl-)
- 1.1.3 Fluoride (F-)
- 1.1.4 Nitrate-N (NO3-N)
- 1.1.5 Nitrite-N (NO2-N)
- 1.1.6 Ortho-Phosphate-P (OPO4-)
- 1.1.7 Sulfate (SO4=)
- 1.1.8 Iodide (I-)

1.2 The matrices applicable to this method include: surface water, mixed domestic and industrial wastewaters, groundwater, reagent waters, solids (after extraction 9.1.2), and leachates (when no acetic acid is used).

1.3 The WPCL Method Detection Limit (MDL) for the above analytes is listed in Table 1. The MDL for a specific matrix may differ from those listed, depending upon the nature of the sample. MDLs and reporting limits are adjusted when samples are diluted.

1.4 Some of the reporting limits are based on water quality objectives specified in National Pollution Discharge Elimination System (NPDES) permits by Regional Water Quality Control Boards. Water quality objectives in mg/L for the Hatchery Monitoring Program (based on the Hot Creek Fish Hatchery requirements) are as follows:

Analyte	Limit, mg/L
NO3-N	0.21
OPO4=	0.65
SO4=	24.0
F-	0.10

.

1.5 The current reporting limits for the ICS 1000 are listed in Table 1.

1.6 This method is recommended for use only by or under the supervision of analysts experienced in the use of ion chromatography and in the interpretation of the resulting ion chromatogram. Each analyst must demonstrate the ability to generate acceptable results with this method. Refer to WPCL-QA-003 Training.

1.7 When this method is used to analyze unfamiliar samples for any of the above anions, anion identification should be supported by the use of fortified sample matrix covering the anions of interest. The fortification procedure is described in Section 9.2.5.

2.0 Summary of Method.

2.1 A small volume of sample, typically 2 to 3 mL, is introduced into an ion chromatograph. The anions of interest are separated and measured, using a system comprised of a guard column, separator column, suppressor device, and conductivity detector.

2.2 In order to use this method for solids an extraction procedure must be performed as described in section 9.1.2.

3.0 Interferences

3.1 Interferences can be caused by substances with retention times that are similar to and overlap those of the anion of interest. Large amounts of an anion can interfere with the peak resolution of an adjacent anion. Sample dilution and/or fortification can be used to solve most interference problems.

3.2 The water dip or negative peak that elutes near and can interfere with the fluoride peak can usually be eliminated by the addition of the equivalent of 1 mL of concentrated eluent (section 6.3, 100X) to 100 mL of each standard and sample.

3.3 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baseline in ion chromatograms.

3.4 Any anion that is not retained by the column or only slightly retained will elute in the area of fluoride and interfere. Known coelution is caused by carbonate and other small organic anions. At concentrations of fluoride above 1.5 mg/L this interference may not be significant; however, it is the responsibility of the user to generate precision and accuracy information in each sample matrix.

3.5 The acetate anion elutes early during the chromatographic run. The retention times of the anions also seem to differ when large amounts of acetate are present.

Therefore, this method is not recommended for leachates of solid when acetic acid is used for pH adjustment.

3.6 The quantitation of unretained peaks should be avoided, such as low molecular weight organic acids (formate, acetate, propionate, etc.) which are conductive and coelute with of near fluoride and would bias the fluoride quantitation in some drinking and most waste waters.

4.0 Safety

4.1 Wear gloves, lab coats, and safety glasses when handling samples and reagents.

4.2 Dispose of samples according to WPCL-EH-049 Disposal of Hazardous Wastes.

5.0 Equipment and Supplies

5.1 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.

5.2 Ion chromatograph - Analytical system complete with ion chromatograph and all required accessories including auto-sampler, analytical column, guard columns, compressed helium gas, detectors and data system. The current system is the Dionex ICS-1000, S/n 04090303, purchased In October 2004.

5.2.1 Anion guard column: A protector of the separator or analytical column. If omitted from the system the retention times will be shorter. Usually packed with a substrate the same as that in the analytical column. See Table 2 for guard column information.

5.2.2 Anion separator (analytical) column: See Table 2 for analytical column information.

5.2.3 Anion suppressor device: The Dionex ASRS-Ultra Anion Suppressor is used for this system (P/N 064555).

5.2.4 Detector – 35°C Heated Conductivity cell: See Table 2 for sample loop sizes.

5.2.5 Dionex Chromeleon DC36R051 Data Chromatography Software used to generate the data.

5.2.6 The Dionex AS-40 Autosampler.

6.0 Reagents and Standards

6.1 Sample bottles: Glass or polyethylene of sufficient volume to allow replicate analyses of anions of interest.

6.2 Reagent water: Distilled or deionized water, free of the anions of interest. Water should contain particles no larger than 0.20 microns.

6.3 Eluent solution, Sodium Bicarbonate

6.3.1 Add 10 mL of 4.5 mM sodium carbonate/1.4 mM sodium bicarbonate solution to 1000 mL of Milli-Q water. Invert to mix well. Prepare fresh daily.

6.4 Stock standard solutions, 1000 mg/L (1 mg/mL): Stock standard solutions may be purchased as certified solutions from ERA or may be prepared from ACS reagent grade materials (dried at 105°C for 30 min.) as listed below.

6.4.1 Bromide (Br^-) 1000 mg/L: Dissolve 1.2876 g sodium bromide (NaBr) in reagent water and dilute to 1 L or use purchased ERA certified stock standard.

6.4.2 Chloride (Cl^-) 1000 mg/L: Dissolve 1.6485 g sodium chloride (NaCl) in reagent water and dilute to 1 L or use purchased ERA certified stock standard.

6.4.3 Fluoride (F^-) 1000 mg/L: Dissolve 2.2100 g fluoride (NaF) in reagent water and dilute to 1 L or use purchased ERA certified stock standard.

6.4.4 Nitrate (NO_3^--N) 1000 mg/L: Dissolve 6.0679 g sodium nitrate ($NaNO_3$) in reagent water and dilute to 1 L or use purchased ERA certified stock standard.

6.4.5 Nitrite (NO_2^--N) 1000 mg/L: Dissolve 4.9257 g sodium nitrite ($NaN0_2$) in reagent water and dilute to 1 L or use purchased ERA certified stock standard.

6.4.6 Phosphate (HPO_4^{2-}-P) 1000 mg/L: Dissolve 4.3937 g potassium phosphate, monobasic (KH_2PO_4) in reagent water and dilute to 1 L or use purchased ERA certified stock standard.

6.4.7 Sulfate (SO_4^{2-}) 1000 mg/L: Dissolve 1.8141 g potassium sulfate (K_2SO_4) in reagent water and dilute to 1 L or use purchased ERA certified stock standard.

6.4.8 Iodide (I^-) 1000 mg/L: Use purchased ERA certified stock standard.

6.5 Stability of standards: Stock standards are stable for at least one month when stored at room temperature.

6.6 Dilute working standards should be prepared at 28 day intervals, except those that contain **nitrite, nitrate and phosphate which should be prepared fresh daily.**

7.0 Preservation and Holding Times

7.1 Samples should be collected in scrupulously clean glass or polyethylene bottles.

7.2 Sample preservation and holding times for the anions that can be determined by this method: See Table 2.

7.3 The method of preservation and the holding time for samples analyzed by this method are determined by the anions of interest. In a given sample, the anion that requires the most preservation treatment and the shortest holding time will determine the preservation treatment.

7.4 All samples must be cooled to <6°C. Analysis of preserved analytes must be completed within 28 days of collection.

8.0 Calibration and Standardization/Instrument Set Up.

8.1 Instrument Operating Conditions: Instrument operating conditions are controlled by the Data System and a copy of the instrument operating parameters is presented in the Dionex Operator's Manual.

8.2 Calibration

8.2.1 Ion chromatographic operating parameters should be established which are equivalent to parameters in the Manual.

8.2.2 For each analyte of interest, prepare calibration standards at six concentration levels consisting of a blank, and five standards ranging from the reporting limit to the established upper limit. Each attenuation range of the instrument used to analyze a sample must be calibrated individually.

8.2.3 Tabulate peak height or area responses against the concentration. The results are used to prepare a calibration curve for each analyte. During this procedure retention times must also be recorded. These steps are done by the Data System which displays the peak height and area, the calibration curves and the linearity of the calibrations at the different concentrations.

8.2.4 The calibration curve must be verified on each working day, or whenever the anion eluent is changed, and after every 10 samples. A certified reference standard at a concentration near the mid-range of the curve must be used to verify the calibration curve. The standard must be purchased from a different vendor than the working standard. If the response or retention time for any analyte varies from the expected values by more than ±20% (10% if used for compliance monitoring), the test must be repeated, using fresh calibration

standards. If the results are still more than ±20%, a new calibration curve must be prepared for that analyte.

8.2.5 Non-linear response can result when the separator column capacity is exceeded (overloading). The response of the detector to the sample when diluted 1:1, and when undiluted, should be compared. If the calculated responses are the same, samples of this total anionic concentration need not be diluted.

9.0 Procedure

9.1 Sample Preparation.

9.1.1 **Water**: If "dissolved" anions are requested, filter water samples through a 0.45 um filter prior to analysis. The samples are poured into the autosampler vials and the plug/filter is placed on the top of the vial and is forced into the vial using the tool supplied by Dionex. The plug filter is optional.

9.1.2 **Solid materials**: The following extraction should be used for solid materials.

9.1.2.1 Add an amount of reagent water equal to ten times the weight of dry solid material taken as a sample. This slurry is mixed together for ten minutes using a magnetic stirring device. Filter the resulting slurry using a 0.45 µm membrane type filter before injecting. This filter can be the type that attaches directly to the end of the syringe. Care should be taken to show that good recovery and identification of peaks is obtained. A matrix spike should be performed. Apply a dilution factor of 10 to final results for solid samples extracted using this procedure.

9.2 Analysis sequence.

9.2.1 Table 2 summarizes the operating conditions stored under the file name AS14A in the data system Program section. Included in the Analysis table are the estimated retention times of the anions of interest established under the conditions of this method.

9.2.2 Check system calibration daily, if required, recalibrate as described in Tables 3 and 4.

9.2.3 Load standards, blanks, and samples by filling polyethylene autosampler vials and inserting cap/filter into the vial with the tool supplied by Dionex. Load autosampler holders with the ridges facing the front of the autosampler.

9.2.4 Begin analysis.

9.2.4.1 Turn gas on. Adjust helium to 20 psi.

9.2.4.2 Switch eluent degas module on and set pressure to 50 psi.

9.2.4.3 If the eluent is freshly prepared, prime the pump by opening the priming valve one-quarter to one-half turn counterclockwise. On the Chromeleon Control Panel click Pump Settings and then click **Eluent Flow Valve Open**. Place a 10 ml syringe in the hole of the priming valve and draw back about 3-4 syringes of eluent to eliminate air bubbles from the system.

9.2.4.4 After priming the lines thoroughly, close the priming valve. Close the eluent valve by clicking **Eluent Flow Valve Closed** on the control panel. Open the waste valve in the secondary pump head by turning the knob one-quarter to one–half turn counterclockwise. Click **Prime on the Control Panel** and continue until no air bubbles are exiting the waste pump line. Press **Pump Off** and close the waste valve.

9.2.4.5 Press **startup** to begin the pump and **control acquisition on** to view the baseline. If the instrument does not begin, make sure the connected boxes are checked in the ICS-1000_Panel_1.pan.

9.2.4.6 The pump setting and eluent flow valve icons should be green.

9.2.4.7 Create a schedule or call up an existing one and modify it in the sequence folder. The calibration curve may be saved as raw data to use it in the current schedule.

9.2.4.8 If the response for a peak exceeds the working range of the system, dilute the sample with an appropriate amount of reagent water and reanalyze.

9.2.5 If the resulting chromatogram fails to produce adequate resolution, or if identification of specific anions is questionable, fortify the sample with an appropriate amount of standard and reanalyze.

9.2.6 **Note:** Retention time is inversely proportional to concentration. Nitrate and sulfate exhibit the greatest amount of change, although all anions are affected to some degree. In some cases this peak migration may produce poor resolution or identification.

10.0 Data Analysis and Calculations

10.1 After the standards have been analyzed review the calibration curves using the data system. The calibration curves for each anion should be reviewed for response and linearity ($r \geq 0.995$). If a problem is observed the run should be stopped and corrective action should be taken prior to restarting the run. The standards may need to be prepared and analyzed again.

10.2 For the blank, a response less than the desired reporting limit (or MDL for compliance testing) should be observed for all analytes. If there is a positive response for the blank the run should be stopped and corrective action should be taken prior to restarting the run. The blank should be prepared again and reanalyzed. Any affected samples must be reanalyzed.

10.3 All anions except for Iodide should be reported in mg/L. Iodide should be reported in ug/L.

10.4 Report:

10.4.1 NO_2^- as N

10.4.2 NO_3^- as N

10.4.3 HPO_4^{2-} as P.

10.5 Concentration from linear calibration curves are calculated:

10.5.1 Concentration, mg/L = (y-b)/m

Where : m = slope of the curve.
y = area of analyte in sample.
B = y-intercept.

10.6 All results will be reported using three significant figures. If a target analyte is detected between the MDL and the RL, the result will be reported as detected not quantified (DNQ) and if the result is detected below the MDL the result will be reported as ND (not detected) and be followed by the MDL value.

11.0 Quality Control

11.1 A batch is defined as 20 or fewer samples of the same matrix processed concurrently using the same reagents, standards, and instrument. Each batch will include quality control samples: a blank, a certified reference material, a laboratory

control sample, a duplicate, matrix spike, and matrix spike duplicate. The corrective actions for quality control samples are described in Tables 3 and 4 Corrective Actions.

11.2 A method blank (MBLK) is an aliquot of reagent water processed as if it were a sample, including filtration. The method blank will demonstrate that all glassware and reagent interferences are under control. A new method blank must be processed whenever there is a change in reagents.

11.3 A certified reference material (CRM) is a commercially available solution accompanied by a vendor certificate of analysis, analyte true values, and sometimes acceptance control limits. The CRM is used to document an acceptable analysis and to check standards preparation by comparing recovered concentrations to certified values. The CRM is from a source different from the standards used to prepare the calibration curve and may also be used as the initial calibration verification (ICV).

11.4 Laboratory control sample (LCS) is an aliquot of lab reagent water spike with known concentrations of known analytes. Typically, the LCS concentration selected is near the midpoint of the calibration curve. If a CRM is unavailable, a LCS must be processed with the batch of samples. Calculate percent recovery and compare to acceptance limits. The LCS is used to assess analyte recovery in the absence of matrix effects.

11.5 A duplicate (DUP) is a second aliquot of a selected sample within the batch. The DUP is analyzed to assess analytical reproducibility. Calculate relative percent difference and compare to acceptance limits.

11.6 Matrix spike/spike duplicate (MS/MSD). A MS is an aliquot of a selected sample fortified with known concentrations of known analytes. Typically, the concentrations selected for spiking are near the midpoint of the curve or 3 to 5 times the native concentration. Adjust the recovered concentration for any contribution from the native sample, then calculate percent recovery. Used in conjunction with the LCS, the matrix spike is used to assess the bias that may be imparted from the matrix on analyte recovery. The matrix spike duplicate provides an indicator of sample homogeneity and also serves to confirm the presence of any matrix effects.

11.7 When doubt exists over the identification of a peak in the chromatogram, confirmatory techniques such as sample dilution and fortification, must be used.

12.0 References

12.1 U.S. Environmental Protection Agency, *The Determination of Inorganic Anions in Water by Ion Chromatography*, Method 300.0. August 1991. Environmental Monitoring and Systems Laboratory, Cincinnati, OH 45268.

12.2 Dionex Corporation, Document No. 031879, ICS -1000, *Ion Chromatography System Operator's Manual*.

12.3 WPCL-EH-049, Disposal of Hazardous Wastes.

13.0 Attachments

13.1 Table 1: Analyte lists (with reporting limits).

13.2 Table 2: IC Summary Sheet

13.3 Figure 1: Sodium Hydroxide Eluent Preparation

13.4 Table 3: Corrective Action, Enforcement and Compliance Samples

13.5 Table 4: Corrective Action, Other Clients

Table 1: Analytes, Reporting Limits (RL), and Method Detection Limits (MDLs)

ANION	MDL, mg/L	RL mg/L
Bromide	0.125/0.50	0.150/1.0
Chloride	0.04	0.20
Nitrate-N	0.10	0.20
Fluoride	0.018	0.080
Ortho-PO4	0.10	0.25
Sulfate	0.08	0.40
Iodide	25.0 ug/L	25.0 ug/L

Table 2: IC Summary Sheet

Analyte	Analytical/ Guard Column	Sample Loop	Current	Eluent	Preparation/ Preservation	Holding Times	Comments
Chloride, Sulfate	AS 22(P/N 064137) AG 22 (P/N 064135)	10uL (042949)	11 mA	4.5mM Sodium Carbonate 1.4mM Sodium Bicarbonate	Filter/Cool to <6°C	28 days	
Fluoride	AS 22(P/N 064137) AG 22 (P/N 064135)	10uL (042949)	11 mA	4.5mM Sodium Carbonate 1.4mM Sodium Bicarbonate	Filter/Cool to <6°C	28 days	Add 1mL of eluent to 100mL of each standard and sample
Iodide	AS 20(P/N 063065) AG 20 (P/N 063066)	10uL (042949)	100 mA	35mM KOH (see Figure 1)	Don't Filter. Let settle if there is particles in it.	28 days	Prime for 30 min when column has just been put on
Perchlorate	AS 20(P/N 063065) AG 20 (P/N 063066)	250uL (042953)	100 mA	35mM KOH	Filter/Cool to <6°C	28 days	Prime for 30 min when column has just been put on
Nitrite-N, Nitrate-N, Ortho-Phosphate-P	AS 22(P/N 064137) AG 22 (P/N 064135)	10uL (042949)	11 mA	4.5mM Sodium Carbonate 1.4mM Sodium Bicarbonate	Filter/Cool to <6°C	***48 hours***	
Bromide	AS 22(P/N 064137) AG 22 (P/N 064135)	10uL (042949)	11 mA	4.5mM Sodium Carbonate 1.4mM Sodium Bicarbonate	Filter/Cool to <6°C	28 days	

Figure 1: Sodium Hydroxide Eluent Preparation

5.2 Eluent Preparation

Sodium Hydroxide Eluent Concentration

Weight Method

When formulating eluents from 50% sodium hydroxide, DIONEX recommends weighing out the required amount of 50% sodium hydroxide.

Example: To make 1 L of 38 mM NaOH use 3.04 g of 50% sodium hydroxide:
(as used in Section 5.3, "Production Test Chromatogram")

$$\text{For 38 mM:} \quad \frac{0.038 \text{ mole/L} \times 40.01 \text{ g/mole}}{50\%} = 3.04 \text{ g diluted to 1 L}$$

Volume Method

Although it is more difficult to make precise carbonate-free eluents for gradient analysis volumetrically, you may choose to use the following formula to determine the correct volume of 50% sodium hydroxide to be diluted.

$$g = dvr$$

Where: g = weight of sodium hydroxide required (g)
d = density of the concentrated solution (g/mL)
v = volume of the 50% sodium hydroxide required (mL)
r = % purity of the concentrated solution

Example: To make 1 L of 38 mM NaOH, use 1.99 mL of 50% sodium hydroxide:
(as used in Section 5.3, "Production Test Chromatogram")

$$\text{For 38 mM:} \quad \frac{0.038 \text{ mole/L} \times 40.01 \text{ g/mole}}{50\% \times 1.53 \text{ g/mL}} = 1.99 \text{ mL diluted to 1 L}$$

* This density applies to 50% NaOH. If the concentration of the NaOH solution is significantly different from 50%, the upper (weight method) calculation should be used instead.

Sodium Hydroxide Eluents

Dilute the amount of 50% (w/w) NaOH (in water) specified in Table 6, "Dilution of 50% (w/w) NaOH to Make Standard AS15 Eluents" with degassed, deionized water having a specific resistance of 18.2 megohm-cm to a final volume of 1,000 mL using a volumetric flask. Avoid the introduction of carbon dioxide from the air into the aliquot of 50% (w/w) NaOH or the deionized water being used to make the eluent. Do not shake the 50% (w/w) NaOH or pipette the required aliquot from the top of the solution where sodium carbonate may have formed.

Table 6
Dilution of 50% (w/w) NaOH to Make Standard AS15 Eluents

50% (w/w) NaOH g (mL)	Concentration of NaOH Eluent (mM)
0.80 (0.52)	10
2.72 (1.78)	34
3.04 (1.99)	38
3.20 (2.09)	40
8.00 (5.25)	100
16.00 (10.5)	200

Table 3: Calibration and QC Summary for Compliance/Enforcement Testing (Hatcheries, DFG enforcement cases)

QC Type	Frequency	Criteria	Corrective Actions	Comments
Method Blank	Per batch per matrix, up to 20 samples.	≤ MDL	• Reanalyze to verify. • Assess impact on samples. • Re-prepare affected samples and QC.	If solid samples are extracted, use as the preparation blank.
Lab control Sample (LCS)	Per batch per matrix, up to 20 samples.	90-110%	• Reanalyze to verify. • Re-analyze associated samples and QC.	
MS/MSD	Per batch, up to 20 samples.	80-120% RPD ±25%	• Reanalyze to verify. • Compare to LCS to assess matrix effects.	
Sample Duplicate	Per batch, up to 20 samples	0-25%	• Reanalyze to verify.	
Initial calibration	Daily or system failure of ICV, CCV, initial calibration.	Minimum 6 points (including blank). $R^2 \geq 0.995$	• Review curve. • Reprepare standards and recalibrate.	
SRM/CRM=ICV (conc. between stds. bracketing mid-point)	Immediately after calibration.	90-110%	• Reanalyze. • Re-calibrate.	
ICB	Immediately after ICV.	≤ MDL	• Reanalyze. • Reanalyze if carryover suspected.	.
RL check	After calibration and prior to sample analysis.	± 30% of expected value.	• Reanalyze. • Review curve.	
CCV	After every 10 injections and end of run. Use midpoint concentration standard.	90-110%	• Reanalyze samples back to the last acceptable CCV.	
CCB	Immediately after CCV.	≤ MDL	• Reanalyze samples back to the last acceptable CCB.	
Sample concentration higher than highest standard.	Each sample	Exceeds highest standard.	• Dilute and reanalyze. • Apply dilution to MDL and RL.	

Attachment 2: **Calibration and QC Summary for Non-Compliance/Non-Enforcement Testing SWAMP, PSA, RCMP, Index**

QC Type	Frequency	Criteria	Corrective Actions	Comments
Method Blank	Per batch per matrix, up to 20 samples.	≤ Reporting Limit (RL)	• Reanalyze to verify. • Assess impact on samples. • Re-prepare affected samples and QC.	Report DNQ (MDL to RL). If solid samples are extracted, use as the preparation blank.
Lab control Sample (LCS)	Per batch, up to 20 samples.	80-120%	• Reanalyze to verify. • Re-prep associated samples and QC.	Spike preparation check
MS/MSD	Per batch, up to 20 samples.	80-120% RPD ±25%	• Reanalyze to verify. • Compare to LCS to assess matrix effects.	
Sample Duplicate	Per batch, up to 20 samples	0-25%	• Reanalyze to verify.	
Initial calibration	Daily or changes in system failure of ICV, CCV, initial calibration.	Minimum 6 points (including blank). $R^2 \geq 0.995$	• Review curve. • Reprepare standards and recalibrate.	
SRM/CRM=ICV (conc. between stds. bracketing mid-point)	Prepare one per batch up to 20 samples. Analyze immediately after multipoint calibration	80-120%	• Reanalyze. • Recalibrate.	.
ICB	Immediately after ICV.	≤ RL	• Reanalyze. • Recalibrate if drift suspected.	.
RL check	After calibration and prior to sample analysis.	± 30% of expected value.	• Reanalyze. • Review curve.	
CCV	After every 10 injections and end of run. Use midpoint standard.	80-120%	• Reanalyze samples back to the last acceptable CCV.	
CCB	Immediately after CCV.	≤ RL	• Reanalyze samples back to the last acceptable CCB.	
Sample concentration higher than highest standard.	Each sample	Exceeds highest standard.	• Dilute and reanalyze. • Apply dilution to MDL and RL.	

Appendix IV D: 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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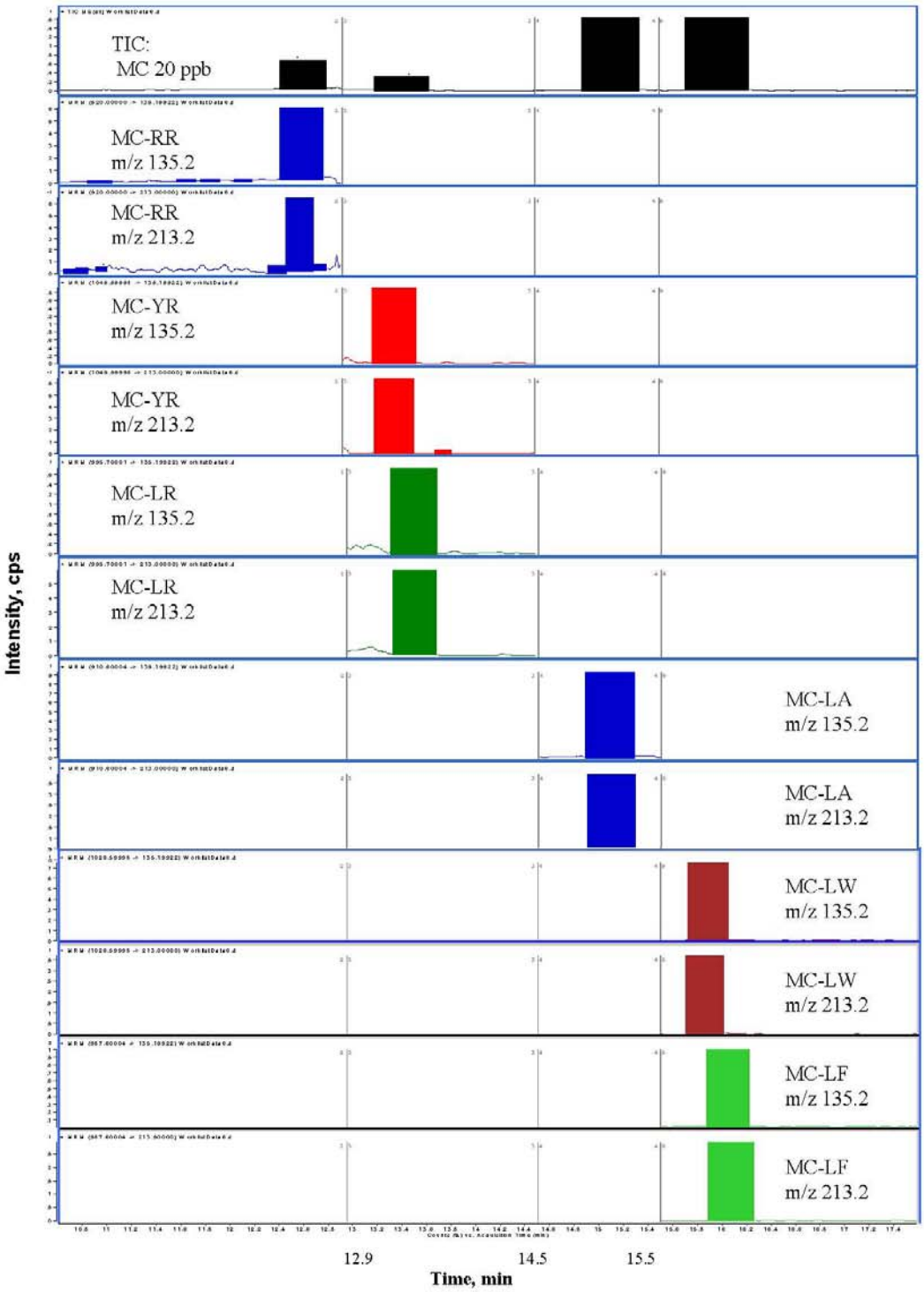
Fig. 1. MRM constructed ion chromatogram for microcystin standard.



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.

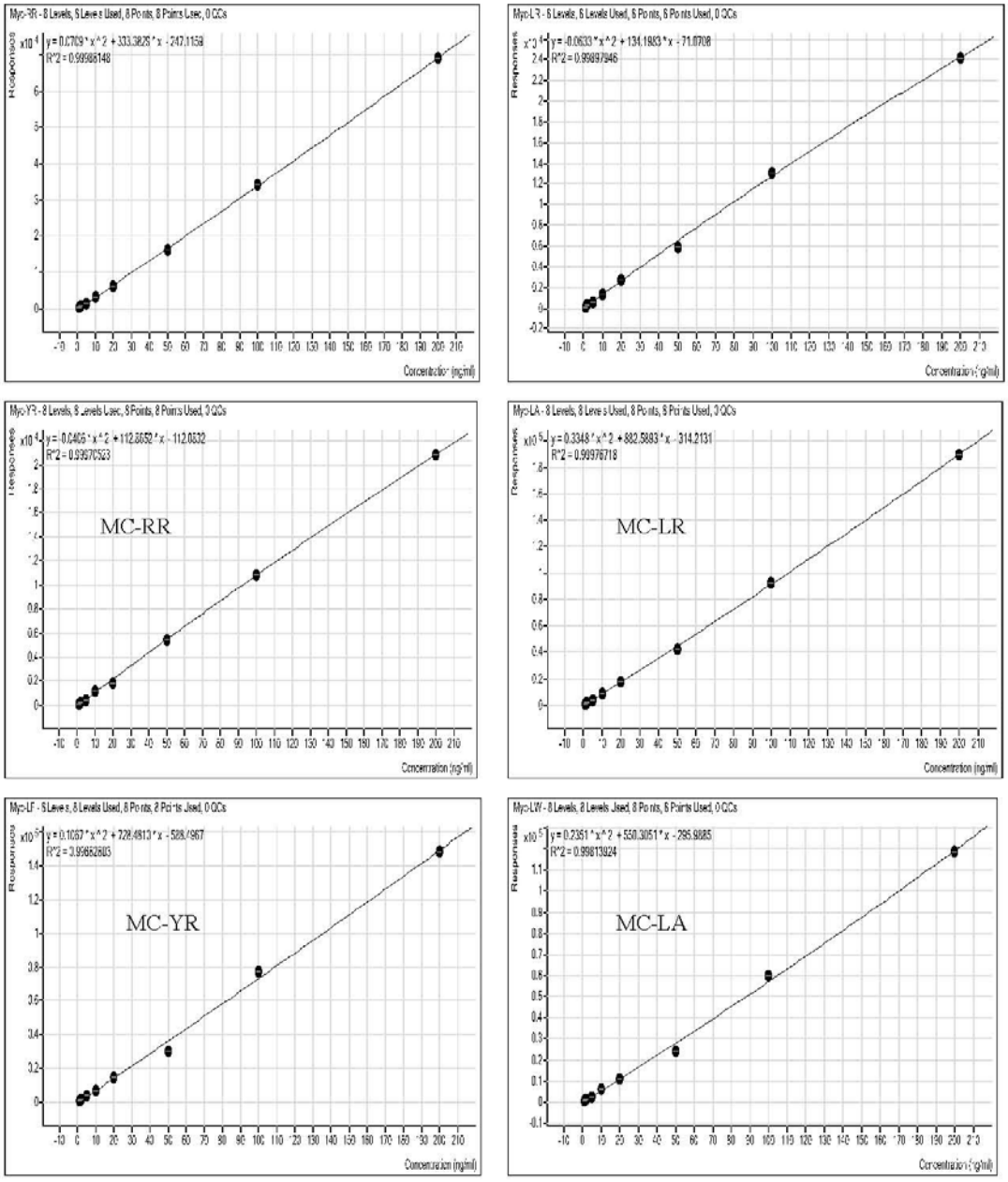


Fig. 3. Total ion chromatogram of a microcystin standard at 1 pg on column.

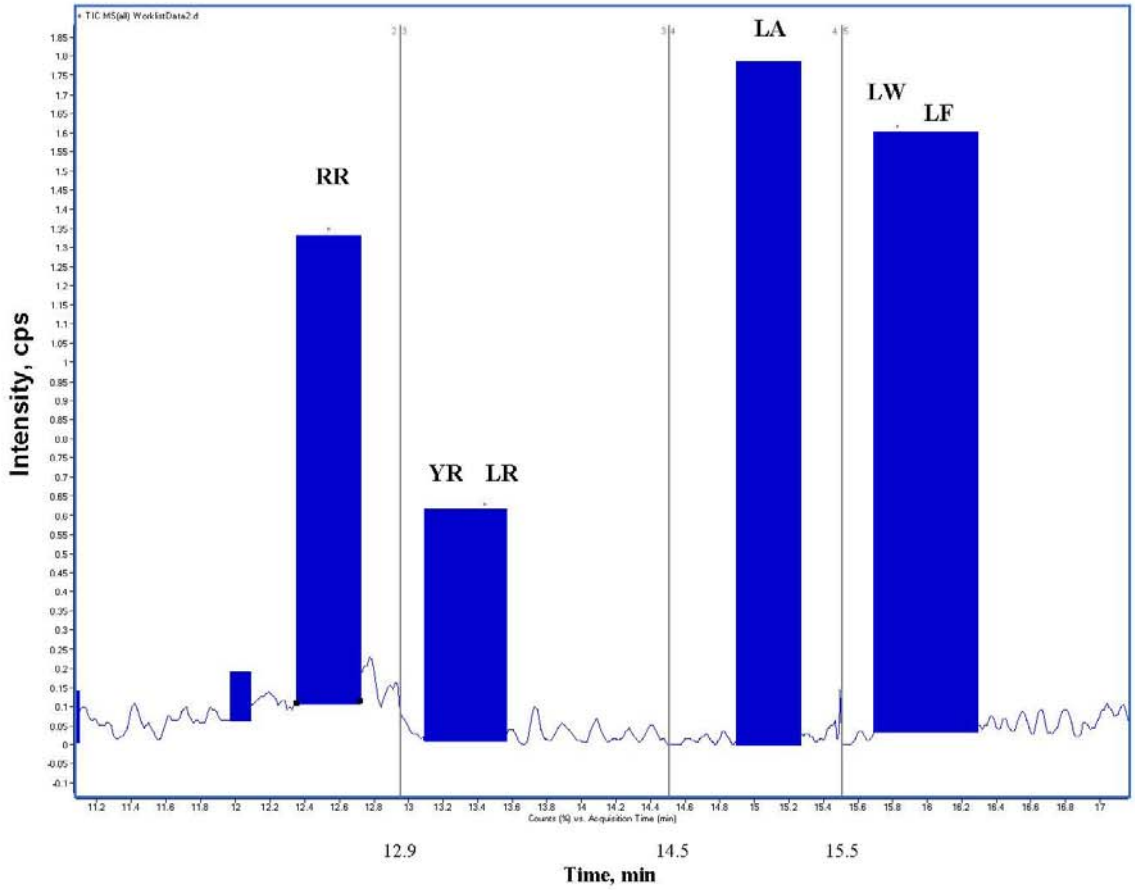
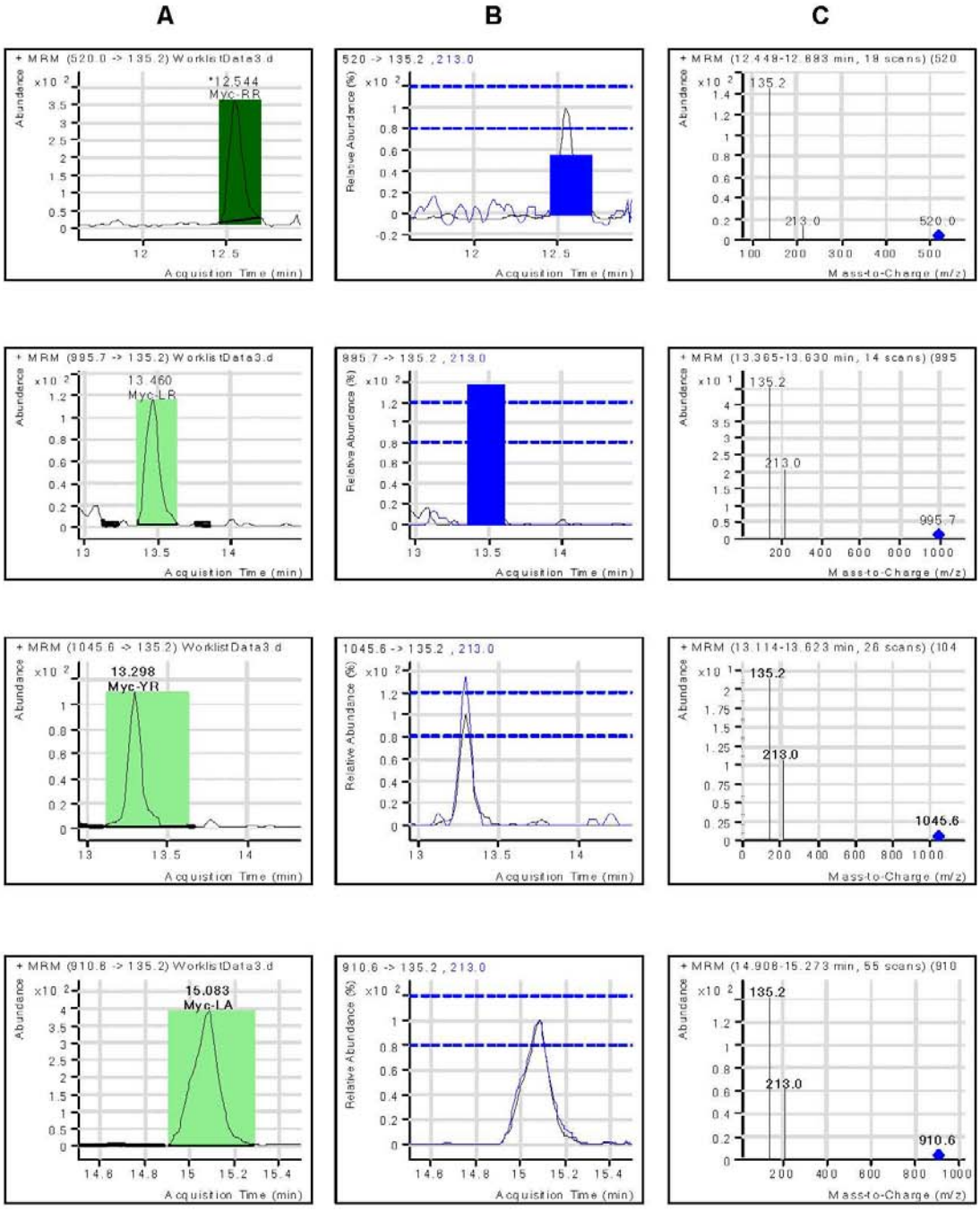
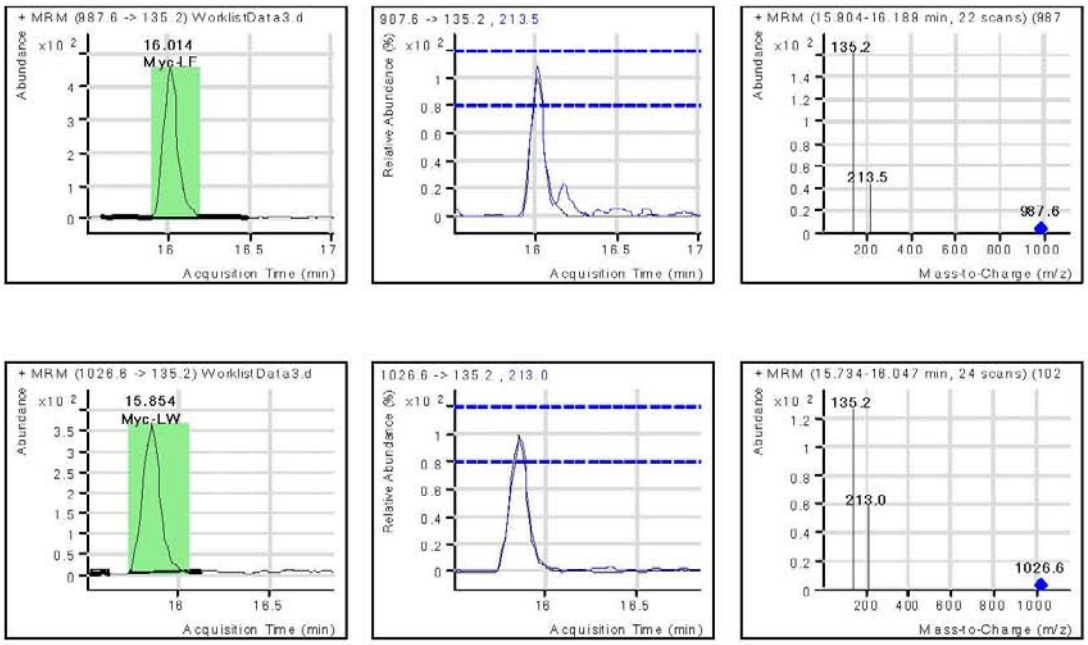


Fig. 4. Microcystin chromatograms in MRM mode: precursor ion (A), product ion (B) and spectra (C).





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 IV E: Procedures for Disposal of Waste

File: HAZMAT_Rev4_SOP
Date: 3/23/09
Revision #: 4
Prepared By: DBC
Page 1 of 8

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 60ºC (140ºF);
- 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 F: 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:** _____

Page 1 of 2

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:** _____

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Appendix V: USGS SOPs

USGS Standard Operating Procedures			
Page	Procedure/Equipment	SOP number/Reference	Revision Date
A	Sample Processing (USGS-FRESC)		December 2011
B	Water TOC Analysis Using the Shimadzu TOC-VCSN and ASI-V Autosampler	METH011.00 (Appendix V B)	July 2013
C	Analysis of Methylmercury in Water by Distillation, Gas Chromatography Separation, and Speciated Isotope Dilution Mass Spectrometry		
D	Solids, volatile-on-ignition, total-in-bottom-material, gravimetric	Techniques of water-Resrouces Investigations of the United States Geological Survey, Third Edition, Book 5 Laboratory Analysis, p 451	1989
E	Digestion of Sediments and Biological Tissues for Analysis by Isotope Dilution Hydride Generation Inductively Coupled Mass Spectrometry (ID HGICP-MS)		May 2014
F	Measurement of Dissolved Organic Matter Fluorescence and Absorbance	Aqualog SOP_V1.3	DRAFT

Appendix V A: Sample Processing (USGS-FRESC)

Sample Processing Standard Operating Procedures

Forest and Rangeland Ecosystem Science Center

CONTAMINANT ECOLOGY RESEARCH PROGRAM

Collin Eagles-Smith

Revised 12/27/2011

USGS
science for a changing world

Quick View Processing SOP

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

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

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

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

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

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

2.6. Prepare tough tags with appropriate ***CERP ID Code*** (refer to the ***Master Sample ID codes_ToughTAGs*** (*S:\Projects\Eagles-SmithLab\Sample ID Codes_ToughTags*) Excel sheet and the Access database to identify a list of unique codes. Note: all tissue samples have different codes (refer to the '***tbl TissueCode***' in the database (*S:\Projects\Eagles-SmithLab\Databases*). See ***ID Code SOP*** for more detail.

2.7. Store all data sheets in the respective binder until all samples have been processed.

3. **Sample cleaning and wet weight** – ***This step may seem redundant with the weighing involved in the cataloging step. However, recording the exact wet weight of a sample just prior to drying is critical for data integrity. We often assess these values in comparison to the catalog weights to evaluate desiccation in the freezer due to sublimation.***

3.1. Wearing powderless nitrile gloves remove sample from container and rinse the surface of each thawed sample with DI water and pat dry with clean KimWipe.

3.2. Obtain one drying vessel (e.g. aluminum or plastic weigh boat, sample vial, etc.) for each sample and write the appropriate***CERP ID Code*** on each weigh boat.

3.3. With the balance empty, press the tare button to zero the balance. Place each weigh boat on the balance and record the weight in the "**Drying vessel weight**" cell on the hard copy data sheet.

3.4. With a clean, dry KimWipe, pat the surface of the sample dry and place in weigh boat. Obtain a sample wet weight (be sure that the weight of the drying vessel is included in this – **i.e. DON'T TARE THE WEIGHT BOAT!**) and hand record in the "**Lab wet weight + drying vessel**" cell on the hard copy data sheet.

4. **Dissection** – ***The dissection process varies by project. The following are general procedures outlining the most basic dissection steps. In most cases either 1) the whole organism will be processed and thus no dissection process is warranted, or 2) tissue samples will be extracted and processed for later analyses.***

4.1. With the balance empty, press the tare button to zero the balance.

4.2. Label each drying weigh boat with ***CERP ID Code***.

4.3. Place each weigh boat on the balance and record the weight in the "Drying vessel weight" cell on the data sheet.

4.4. Dissect out appropriate tissue(s). Record ***CERP ID*** with respective tissue code in appropriate column(s).

4.5. For samples in which a muscle tissue is extracted for processing instead of the whole organism, dissect the dorsal portion of muscle between the head and dorsal fin, along the side of the spine (see Figure 1). Remove at least **1 gram** of muscle (both sides of the fish can be pooled if necessary; when applicable make note on data sheet).

4.6. Obtain a sample wet weight for each tissue (be sure that the weight of the drying vessel is included in this – ***i.e. DON'T TARE THE WEIGHT BOAT!***) in the "**tissue wet weight + drying vessel**" cell on the data sheet. Be sure to indicate the tissue type on the data sheet or that the appropriate column exists for the tissue (e.g. IDMuscleAxial, IDKidney, etc.).

4.7. Place tissue in drying oven.

5. Sample drying

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

6. Sample dry weight

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

7. Sample grinding

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

8. Post processing

8.1. Enter all data from hard copy datasheet into the appropriate electronic file and save as "***ProjectID_Proc_MATRIX_data_ddmmyy.xls***" (Where **MATRIX** is the type of sample being analyzed fish/eggs/inverts/etc…). Use the ***DataEntryTemplate_Easy AccessUpload*** excel file as a template for data entry. This file contains the correct column headings and additional information used in the database but not recorded in the laboratory during dissecting/processing samples. Note: column headings used on laboratory data sheets are identified in row 2 (under the headings used in access). Appropriate column headings may be found on different worksheets for database purposes (i.e., data collected during the "cataloging" process in the lab may actually be found in the "morphology/dissection" worksheet in the DateEntryTemplate excel file). Add and delete columns as necessary for each particular project. All data can be entered on one excel worksheet. For further description of column headings see the access database design view for the respective table

8.2. Proof (verify) all entered data.

8.3. Initial the data sheets following data entry and proofing data.

8.4. Scan hard copy datasheets and save the file as "***ProjectID_Proc_MATRIX_scandata_ddmmyy.xls***" (Where **MATRIX** is the type of sample being analyzed fish/eggs/inverts/etc…) Place PDF of datasheets into the Scanned datasheets folder on the share drive.

8.5. File the hard copy of the original datasheets in the current year's data folder in the data repository filing cabinet.

8.6. Data are ready for uploading to the database, so move excel files to data folder on the share drive (*S:\Projects\Eagles-SmithLab\Data*).

8.7. Open the Google Docs file entitled "***Lab_project tracking***" and update the processing fields with your initials and necessary information. The following fields **must** be completed on the Google Docs project tracking datasheet before moving on to processing:

- 8.7.1. Samples dried (N) N= number of samples
- 8.7.2. Samples ground (N) N=number of samples
- 8.7.3. Drying and grinding data entered
- 8.7.4. Drying and grinding data sheet file names and locations
- 8.7.5. Drying and grinding data sheet page numbers
- 8.7.6. Drying and grinding data sheet file names and locations
- 8.7.7. Processing data proofed
- 8.7.8. Drying data sheets scanned and filed

Sample Data sheet

Project ID#: CERP Project ID
Datasheet ID: ProjectID_Proc_12/21/2011_1

Year "Project Name"
Processing Data

Date (mm/dd/yyyy):_______________

Name(s):

Tissue ID (YY____####_)	CERP ID Code (YY____####) (Note if different)	Sample or Tissue Type (e.g. whole fish)	Drying Vessel (DV) Weight (g)	Tissue Wet Weight +DV (g)	Dry start date	Dry temp (C)	Dry time (hrs)	Tissue Dry Weight + DV (g)	Grind Method	Grind date	Notes

Data Entered by:________ Date:______

Data Verified by:_____________ Date:_______

Appendix V B: Water TOC Analysis Using the Shimadzu TOC-VCSN and ASI-V Autosampler

California Department of Pesticide Regulation
Environmental Monitoring Branch
1001 I Street, Sacramento CA 95814-2828
P.O. Box 4015, Sacramento CA 95812-4015

SOP Number: METH011.00
Previous SOP: None

STANDARD OPERATING PROCEDURE
Water TOC Analysis Using the Shimadzu TOC-VCSN and ASI-V Autosampler

KEY WORDS

TOC-V, total carbon, inorganic carbon, oxygen, NPOC, HCl, calibration curve

APPROVALS

APPROVED BY: original signed by _____ DATE: July 26, 2013
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APPROVED BY: original signed by _____ DATE: July 29, 2013
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Staff Environmental Scientist

APPROVED BY: original signed by _____ DATE: July 30, 2013
Chang Sook Lee Peoples
Environmental Monitoring Branch Quality Assurance Officer

PREPARED BY: original signed by _____ DATE: July 26, 2013
Michael Ensminger, Ph.D.
Staff Environmental Scientist

Environmental Monitoring Branch organization and personnel, such as management, senior scientist, quality assurance officer, project leader, etc., are defined and discussed in SOP ADMN002.

California Department of Pesticide Regulation
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STANDARD OPERATING PROCEDURE
Water TOC Analysis Using the Shimadzu TOC-VCSN and ASI-V Autosampler

1.0 INTRODUCTION

1.1 Purpose

The Shimadzu Total Organic Carbon (TOC) Analyzer in combination with the ASI-V automated sampler is used to measure TOC in water samples. The amount of TOC in a water sample can be an indicator of the amount of pollution in a sample.

2.0 MATERIALS

2.1 TOC-V CSH/CSN component (herein after referred to as TOC-V)

2.2 ASI-V autosampler

2.3 2M HCl

2.4 DI water

2.5 Low TOC water (certified < 100 ppb TOC)

2.6 40 ml vials

2.7 Parafilm®M (cut into 1 inch squares)

2.8 Calibration standard(s)

2.9 Alconox® or Liquinox®

2.10 Potassium hydrogen phthalate (KHP [$KHC_8H_4O_4$])

3.0 PROCEDURES

3.1 Equipment Checks

3.1.1 Open the front door of the TOC-V to check the DRAIN VESSEL volume (back of machine). If needed, fill with DI water to the level indicated by the drain discharge tube on the side of the container. Use DI wash bottle to fill the container through the slits in the top of the black cap *in situ*. Do not remove the cap or unclip the bottle from its current location.

3.1.2 With the door open, check the HUMIDIFIER water container level in the front of the TOC-V; fill if needed. Add low TOC water using a wash bottle to fill the container to the 'Hi' level mark. Squirt water through the water supply port opening in the top of the container. Close the door of the TOC-V.

3.1.3 Fill the RINSE BOTTLE with DI water to the 2000 ml mark (outside the ASI-V).

3.1.4 Fill the DILUTION WATER BOTTLE (located between the ASI-V and TOC-V) with low TOC water. Verify that the intake tubing nearly reaches to the bottom.

3.1.5 Check the ACID BOTTLE located between ASI-V and TOC-V. Ensure acid tube is near the bottom of the acid bottle and that the bottle is at least 50% full. If not, fill with 2M HCl. Recap bottle at the end of the day (or seal with Parafilm®M). If making up 2M HCl, always wear gloves and safety glasses with side shields (or goggles) and prepare under the hood. Always add concentrated HCl to the low TOC water (acid to water). If you spill some acid on your skin, IMMEDIATELY wash it off with copious amounts of running cold water and seek medical attention.

3.1.6 Monitor the PRINTER PAPER by removing the cover to the paper roll. Replace the paper roll if necessary (to run a full tray of 68 vials will take approximately 10 feet of printer paper).

Figure 1. TOC-V machine.

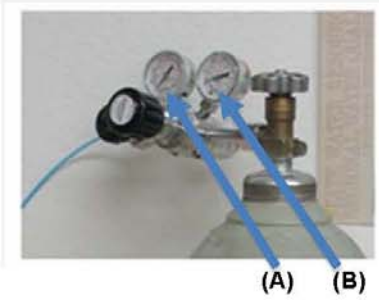

Figure 2. Oxygen tank; gas pressure (A) and tank volume (B).

3.2 Turning on the TOC-V

3.2.1 Press the "Power" button located in the lower right hand corner to power on the TOC-V (Figure 1). It will take 20 to 30 min to reach the operating temperature. The TOC-V is at the correct operating temperature when the green light below the READY signage on the keypad is steady green (not flashing).

3.2.2 Immediately after turning the machine on, supply oxygen by turning the knob on the oxygen tank counter-clockwise. Use the two gauges on the top of the tank to regulate the amount of oxygen available to the TOC-V. The right gauge measures the amount of O_2 gas in the tank whereas the left gauge regulates the pressure of the gas entering the TOC-V (Figure 2). The needle of the left gauge should be between 44-87 psi; 60 psi is the optimum operating pressure. If the needle of the right gauge is below 400 psi, there may not be enough O_2 to properly conduct an analysis and the oxygen tank should be replaced.

3.2.3 Open the front door of the machine and adjust the two carrier gas regulators (Figure 3). The round CARRIER GAS PRESSURE REGULATOR is the located on the left hand side of the TOC-V. Use the knob below the gauge to adjust the carrier gas pressure to 200 KPa. The long rectangular CARRIER GAS FLOW METER is located on the right hand side. Adjust the carrier flow using the carrier gas knob to the left of the gauge. The bottom of the ball should be level to the 150 mL/min increment in the scale. Close door.

3.3 Preparing Samples for Analysis

3.3.1 As the TOC-V comes up to operating temperature, prepare the water samples. Using a Sharpie®, label the vials according to your protocol. Fill the glass vials with approximately 35 mL of water with the meniscus just below the shoulders of the vial and cap or cover with a 1-inch square of parafilm®M. Do not let the parafilm®M hang below the threads on the vial.

Figure 3. Setting the carrier gas pressure and flow.

3.3.2 Set vials into the sampling rack. To ensure the correct QA/QC for the analysis it is recommended that for every 20 vials analyzed, include one sample of each: 1) low TOC water; 2) lab duplicate; and 3) concentration standard. Repeat this pattern for every 20 samples. Check with project lead to confirm. Set rack into the ASI-V Autosampler.

3.3.3 No visible sediment or organic matter should be included in the water samples as this could clog the uptake needle.

3.4. Programming Conditions

3.4.1 In the INITIAL DISPLAY screen, press the F4 key on the keypad to access the CONDITIONS screen. Set the parameters for conducting a water TOC analysis (Figure 4). To change any of the parameters use the arrow keys to scroll down and highlight the selected measurement. Press the SELECT key and scroll through the options until the desired setting is highlighted. Press the ENTER key to change the parameter.

3.4.2 Next, press the F4 key again to access the MEASUREMENT CONDITIONS screen. Ensure that the parameters are set correctly

(Figure 5). Use the arrow keys to change any parameters in the same manner used in 3.4.1.

3.4.3 Press the F1 (RETURN) key to return to the Initial Display screen.

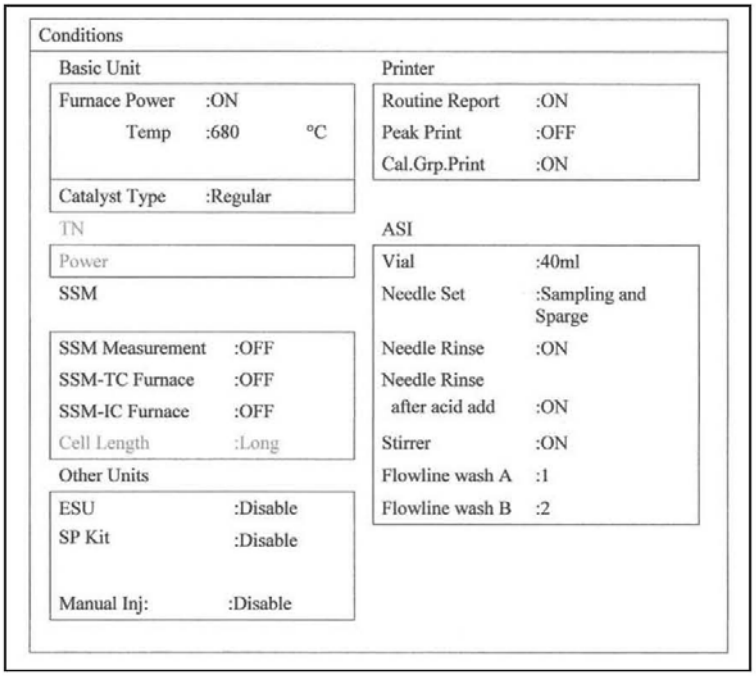

Figure 4. Parameters on the Conditions screen (F4) for conducting a TOC water analysis.

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STANDARD OPERATING PROCEDURE
Water TOC Analysis Using the Shimadzu TOC-VCSN and ASI-V Autosampler

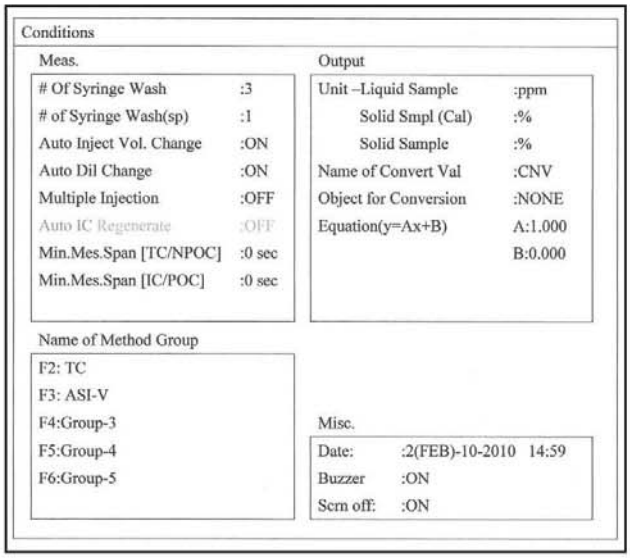


Figure 5. Default parameters on the Measurement Conditions screen (F4, then F4 again) for conducting a TOC water analysis.

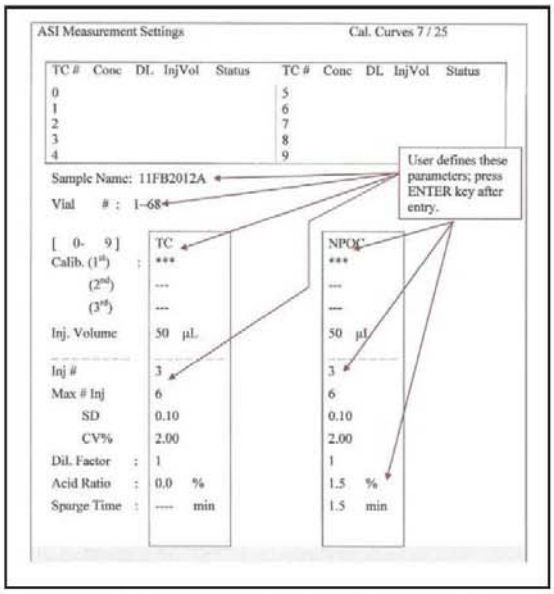


Figure 6. Recommended settings on the ASI Measuring Setting screen. Most of the parameters are user defined.

3.5 Conducting a TOC water analysis using the ASI-V autosampler

3.5.1 In the Initial Display screen, press the ASI key to select the ASI SETTING screen.

3.5.2 Move the cursor to an empty row in the ASI Schedule Setting screen. Press the MEASURE SAMPLE key and the ASI Measurement Setting screen will appear.

3.5.3 In the ASI Measurement Setting screen, enter a unique sample name and the total number vials that will be processed (1 ~ n; n = total number of vials). Select the SHIFT key to toggle between numbers/alphabet (Figure 6).

3.5.4 Press the F5 key to select NPOC. Most analyses will be acidified with 2M HCl to convert inorganic carbon into CO_2, then purged with oxygen to remove the CO_2. Hence most analyses will be processed using Non Purgeable Organic Carbon (NPOC) method. Using the Total Carbon (TC) method instead of the NPOC method will analyze CO_2 as part of the TC.

3.5.5 Set other parameters in ASI Measurement Setting screen, including calibration curve and number of injections (Figure 6). More than one calibration curve may be selected. Only select acid ratio and sparge time if samples have not already been acidified with HCl.

3.5.6 Press the NEXT key to complete the setup. The schedule has been stored and the display will return to the ASI Schedule Settings screen. The sample name and analysis mode of the schedule will be displayed with the message “Not Measured”.

3.5.7 Using arrow keys highlight the desired row for analysis.

3.5.8 Press the NEXT key

3.5.9 Select which option is to be performed when analysis is completed: 1) POWER OFF [F3], powers off 30 minutes after completion and carrier gas flow (oxygen) is halted; 2) SLEEP [F4], temporarily stopped, and will start again at a specified time and date; or 3) WAITING [F5], will remain on, ready to run another analysis. For

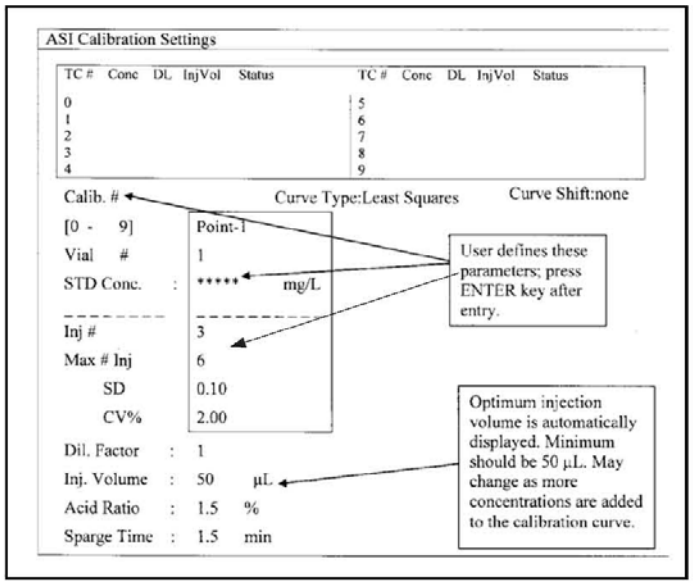


Figure 7. ASI Calibration Setting screen.

3.6.6 Additional concentration points may be added in the same manner once the parameters box for the first calibration point has been completed. With "Point-1" highlighted, press the right facing arrow key to display the parameters box for the second concentration point.

3.6.7 If more than five concentration points are required use the arrow keys to toggle between parameter boxes. To delete a point, highlight the parameters box for that point and press the F6 [DELETE] key.

3.6.8 When all desired points are added to the curve, press the NEXT key. This returns the user to the ASI Schedule Setting.

3.6.9 Check that all of the vials are in their correct position in the sample rack. Highlight the row for analysis. Once verified, press the NEXT key to run the analysis.

most analyses, including ones to be conducted over night, select POWER OFF [F3].

3.5.10 Ensure that the vials are in the sample rack in the correct order.

3.5.11 Press the flashing START key to begin the analysis.

3.5.12 If POWER OFF is not selected, return to ASI Settings Screen. Highlight your analysis (message should display "Measure End") and select Delete (F4), Edit (F5), or Reset Schedule (F6). Press F6 (Yes) to complete.

3.5.13 Deselect the ASI key to return to the Initial Display screen.

3.6 Creating a Calibration Curve using the ASI-V

3.6.1 While in the Initial Display screen press the ASI key.

3.6.2 Move the cursor to an empty row in the ASI Schedule Setting screen.

3.6.3 Press the CAL key; this will display the ASI Calibration Settings screen (Figure 7). A list of previously generated calibration curves will appear at the top of the screen.

3.6.4 Press the F2 (TC) key to create a new calibration curve.

3.6.5 Enter the user defined parameters (press ENTER after each entry):

3.6.5.1 calibration curve number not previously used

3.6.5.2 vial number

3.6.5.3 calibration standard concentration(s)

3.6.5.4 number of injections

3.6.5.5 maximum number of injections

3.6.5.6 injection volume.

the F6 [YES] key to shut down the machine. You will get the message "It is a termination".

3.7.3.3 It will take the TOC-V about 30–40 minutes to cool. DO NOT turn off the oxygen until the TOC-V has completely cooled and the furnace has shut down (the green light on the Power Button is off).

3.7.4 Calibration standards and stock solutions. Standard solutions can be purchased or prepared from potassium hydrogen phthalate (KHP [$KHC_8H_4O_4$]). Directions to make a 1000 mg carbon/L (1000 ppm carbon) stock solution:

3.7.4.1 Dry KHP at 105-120°C for 1 hr, then cool to room temperature in a desiccator.

3.7.4.2 Weigh out 1.0625 g KHP and add to a 500 ml volumetric flask.

3.7.4.3 Bring volumetric flask up to volume with low TOC water; ensure that the KHP is completely dissolved.

3.7.4.4 To make up 100 mL of a 10 ppm standard, add 1 ml of the 1000 ppm stock solution to a 100 mL volumetric flask. Add 99 ml of low TOC water to bring up to volume.

3.7.4.5 Confirm concentration with known standard.

3.7.4.6 Store solution in refrigerator; discard after 2-3 weeks.

4.0 CALCULATIONS

4.1 TOC units can be expressed in %, mg/L, µ/L, and ppm. For TOC water, ppm units are recommended. The TOC machine will print out the result of TC or NPOC, the mean (MN), the standard deviation (SD) and the coefficient of variation (CV). No calculations are necessary by the user.

4.2 Accuracy and precision calculations. Accuracy can be measured against the results of a known TOC standard included in the analysis. Precision can be measured by comparing the results of a lab duplicate to the

original sample. Both accuracy and precision can be quantified by calculating the relative percent differences (RPD):

$$RPD = \frac{|C_1 - C_2| \times 100\%}{(C_1 + C_2)/2}$$

For Accuracy calculations: C_1 = the results of the standard, and C_2 = the know value of the standard.
For Precision calculations: C_1 = the result of the original sample; C_2 = the result of the lab duplicate

5.0 REMEDIAL ACTION IN CASE OF FAILURE AND USER HELP

5.1 See DPR video presentations of this SOP: http://www.youtube.com/watch?v=bx7eky_bABc&feature=related (Part I), http://www.youtube.com/watch?v=o9Ln8nCCNfY&feature=related (Part II), and http://www.youtube.com/watch?v=AGW3kTqrDKU&list=UUqC2ZGkVe7XKFBRrKL_3J7g&index=22&feature=plcp (Part III).

5.2 For minor problems, visit Shimadzu's TOC Advisor at https://tocvva.ssi.shimadzu.com/.

5.3 For additional help contact a Shimadzu sales rep at http://www.ssi.shimadzu.com/about/aboutssi_id8.cfm.

6.0 SAFETY

6.1 Wear gloves for personal protection and to prevent sample contamination.

6.2 Wear closed toe shoes when conducting TOC analysis.

6.3 Wear safety glasses with side shields or goggles for eye protection, especially when using HCl.

6.4 Ensure the oxygen tanks are secured to the wall.

6.5 Do not touch hot components or open up housing when unit is in use.

6.6 Keep hands away from moving components (i.e., syringe injector). Never remove the sample cover to the automated sampler (ASI-V) when an analysis is in process.

6.7 Ensure that the external drain tubing does not touch the surface of the liquid in the waste container.

3.6.10 When instructed, press the flashing START key. A calibration curve will be generated at the completion of the analysis. The curve will be stored and may be used in future TOC sample analyses.

3.6.11 When the calibration curve is completed, return to the ASI Schedule Settings Screen. Highlight your analysis (message should display "Measure End"). Select Delete (F4), Edit (F5), or Reset Schedule (F6).

3.6.12 Press F6 (Yes) to complete selection.

3.6.13 Deselect the ASI key to return to the Initial Display screen.

3.7 Housekeeping

3.7.1 Cleanup: remove all vials from sample rack, erase labeling, and wash vials with Alconox® or Liquinox® and triple rinse with DI water.

3.7.2 Deleting data. Data is stored internally in the TOC machine. Delete your data after confirming the printout of the data is complete.

3.7.2.1 In the Initial Display, press the F6 [Data Report] key.

3.7.2.2 The Data Report – Measurement Log screen is displayed.

3.7.2.3 Press the F4 [Clear All] key.

3.7.2.4 Press the F6 [Yes] key to delete data.

3.7.2.5 Press F1 key to return to the Initial Display.

3.7.3 Manual shutdown. Prior to turning off the TOC-V manually, follow the sequence below to ensure the furnace cools without damage.

3.7.3.1 Press the Press F1 [STANDBY OPTION] key in the Initial Display screen.

3.7.3.2 Press F5 [POWER OFF], then F6 [Execute]. After the message "Do you want to stop this process?" press

Appendix V C: Analysis of Methylmercury in Water by Distillation, Gas Chromatography Separation, and Speciated Isotope Dilution Mass Spectrometry

Analysis of Methylmercury in Water by Distillation, Gas Chromatography Separation, and Speciated Isotope Dilution Mass Spectrometry

Ogorek, J.

Scope and Application

The following standard operating procedure (SOP) describes the (1) preparation of water samples by distillation, and (2) subsequent analysis for methylmercury (MeHg). Analysis is facilitated by coupling the Brooks-Rand "MERX" Automated Methylmercury Analytical System with the Elan Inductively Coupled Plasma-Mass Spectrometer (ICPMS). Quantification of MeHg is by isotope dilution. This method is used by the Wisconsin Mercury Research Laboratory (WMRL) to determine MeHg concentrations in filtered or unfiltered water samples. Quality assurance and control protocols are employed throughout sample distillation and analysis, including: laboratory practices to prevent sample contamination, method blanks, and matrix spikes.

Distillation is required to remove components in natural waters that interfere with methylmercury (MeHg) analysis. In Teflon distillation vials, approximately 50 ml of acidified environmental samples (0.5% HCl) are spiked with isotopically enriched MeHg, amended with copper sulfate, heated in an aluminum block (121° C), and purged with argon gas. The resulting vapor is carried to and condensed in chilled Teflon receiving vials. Distillation is stopped when approximately 25% of the water remains in the distillation vessels.

The MERX consists of three interconnected modules (autosampler, purge and trap module, GC/Pyrolytic module), and is coupled to the ICPMS for detection. In 42 ml glass vials, distillate is buffered to a pH of 4.5 – 5.0 and treated with Sodium Tetraethylborate (NaTEB), resulting in ethylation of oxidized mercury species. These volatile ethylated species (as well as elemental mercury) are stripped from the liquid phase with Argon gas, retained on Tenex traps, desorbed back into the sample stream, and separated with a gas chromatography column. Each ethylated mercury species is released from the column *en masse* into the sample stream, thermally oxidized to elemental mercury, and introduced to the ICPMS. Elemental mercury in the sample stream is ionized by a radio-frequency generated plasma field, and detected using Speciated Isotope Dilution Mass Spectrometry (SIDMS).

This document is intended as an SOP designed to guide the user through MeHg analysis specific to the WMRL. Additional details helpful to the analyst can also be found following the SOP (Appendices 1-3), and is intended as a quick reference bench guide. However, the analyst is required to be familiar with the detailed SOP as well as the original user's manuals provided by Brooks-Rand and Elan which will be referred to when appropriate.

Safety Concerns

Multiple safety concerns are present in the conduct of this method. Persons involved must have read, understood, and signed the Chemical Hygiene Plan for the WMRL prior to potential exposure to any chemicals. Although MeHg is an extremely toxic organic metal, concentrations encountered in samples and

working standards on this instrument are generally low. However, caution should still be exercised to limit chronic exposure during daily operations. Concentrated stock solutions containing elevated MeHg levels are occasionally encountered, and should only be handled by experienced lab personnel. Reagents used in this method include strong acid and an organometallic ethylating compound. The analyst must have a thorough understanding of these chemicals, including their required safety protocols, prior to their use. More detailed information is included for each reagent later in this SOP, and additional information can be found in the attached material data safety sheet. During analysis the automated sample introduction system may begin moving without warning and presents a mechanical hazard. Finally, equipment and sample in excess of 100° C will be encountered and presents a burn hazard.

Distillation Procedure

A typical distillation contains 32 samples, four blanks, and two duplicate spikes. In small sample sets (< 16), four method blanks, and one duplicate spike should be included in each run.

1. Throughout the distillation process, it is important for the analyst to develop and maintain a structured and organized system. Each distillation and receiving vessel has a unique identification code etched onto the wall. The corresponding vials must be appropriately linked to each other, as well as back to the original sample. Good record keeping must occur not only throughout the distillation process, but must be similarly well documented in the Excel data sheet (See Appendix 1 for more details). A template of the Excel data sheet can be found in the "ID TRACER TEMPLATE" folder (HG4→ hg4data→Isotope methyl data).

2. Before beginning sample setup, turn on the aluminum block heater so that it can reach temperature while samples are being prepared.

3. Arrange an adequate number of clean Teflon distillation and receiving vials into four wire racks.

4. Weigh approximately 50 grams of sample (or reagent water for method blanks) into each of the distillation vials. Be sure to homogenize the sample by inverting the sample bottle several times before dispensing to the distillation vial. Setup two samples in triplicate for the duplicate spike analysis. Be sure that you record the sample ID, distillation vial ID, distillation vial tare weight, and the sample mass into the appropriate places in the Excel data sheet.

5. To each of the distillation vials, add 1 ml of 25% $CuSO_4$ solution. The method blanks should be acidified with 500 µl of concentrated HCl.

6. Add 100 µl of the working standard to each of the duplicate spike samples.

7. Add 50 µl of the isotopically enriched ($Me^{199}Hg$) working solution. If working with isotopically enriched samples, be sure that the spiked isotope fraction has not been amended in the sample.

8. Fit each distillation vial with a combined distillation cap/transfer tube assembly corresponding to the block position to be occupied by that vial (each cap has been engraved with a number between 1 and 40).

9. To each of the receiving vials, add 10 ml of reagent water. Be sure that you record the receiving vial ID and receiving vial tare weight.

10. Fit each receiving vial with a distillation cap corresponding to the rack position to be occupied by that vial (each cap has been engraved with a number between 1 and 40).

11. Place the distillation vials in the bore holes of the preheated (approximately 120° C) aluminum block. Attach an argon gas line to each of the distillation caps and ensure that gas flows through the sample.

12. In an ordered manner, thread the transfer tubes through the Teflon ports in the side of the cooler.

13. In the cooler, attach each transfer tube to the corresponding receiving vial. Ensure that gas flows in the reagent water of the receiving vial.

14. Throughout the distillation, check the temperature of the heating block often. Adjust to maintain a temperature of 121° C (± 5).

15. Check the distillation vials regularly. Samples are finished distilling when approximately 25% of the original sample is left.

16. Once a sample is finished, disconnect the transfer tube from the receiving vial and the gas line from the distillation cap. Cover the gas inlet port of the distillation cap quickly with a gloved finger to keep the sample in the vial from discharging.

17. Thoroughly rinse the combined distillation cap/transfer tube assembly with copious amounts of reagent water. Place the caps in the laminar flow hood to dry.

18. Once the entire distillation is finished, remove the receiving vials from the cooler and remove the distillation caps. Record the mass of the receiving vial in the Excel data sheet and attach a standard cap.

19. Distillates should be analyzed within 48 hours of distillation.

Brooks-Rand Operation

Start Up

1. Check that all modules of the instrument have power and the Argon gas supply is turned on. Empty the waste receptacle located on the floor.

2. If necessary, open the Mercury Guru4 software with the shortcut on the desktop.

3. Open the analytical file BR549.brd (found on HG4) and save the file as data (from the "File" dropdown menu).

4. From the "Instrument" dropdown menu, select "Connect", prompting a popup window displaying three communication ports. Select the appropriate ports (CVAFS = ICPMS, Purge and Trap = COM5, and Autosampler = COM4) and click "Accept". The communication status at the top of the screen will turn green indicating connection with each module.

Preparation of Vials for Analysis

The MERX instrument is designed to operate on a specific mixture of reagents that are prepared in sealed 42 ml amber glass vials. The autosampler holds three removable 24 vial sample racks, each consisting of 3 rows of 8 vials. Vial number one is the upper right position, with vial position descending from right to left-then top to bottom. Once prepared, the vials are sealed to the atmosphere and remain viable for analysis up to 48 hours. A typical analytical run is shown in Appendix 2.

1. Place the clean vials in the sample rack and add approximately 40 ml of sample distillate or reagent water (for instrument blanks and MeHg standard additions) to each vial.

2. Record the identity of the sample, analytical vial number, date, and standard information on a bench sheet (Appendix 3).

3. Adjust the pH of the mixture to 4.5 – 5.0 by adding 200 μL of the sodium acetate buffer reagent to every vial.

4. Add 50 μL of 1% NaTEB to every vial.

    a. NaTEB is an unstable reagent and must always remain at freezing temperatures to slow degradation. Begin thawing several minutes

before use but always make sure that some frozen NaTEB remains in the vial. Promptly cap and return the vial of NaTEB to the freezer after use.

b. NaTEB is toxic and spontaneously combustible in air. Only open vials and dispense NaTEB under a fume hood. Add NaTEB directly to the sample mixture (not to the glass surface inside the vial) to reduce volatilization.

5. Fill the vials with reagent water using a squirt bottle until a reverse meniscus forms (convex water surface). Seal the vial carefully (without headspace or spilling) with a new clean cap and septa assembly. Vigorously shake the vial, check for any air bubbles in the vial, and refill if necessary.

6. Place the full rack on the autosampler tray, making sure that the rack is properly positioned and oriented.

Elan ICPMS Operation

Start up (see figures below for reference)

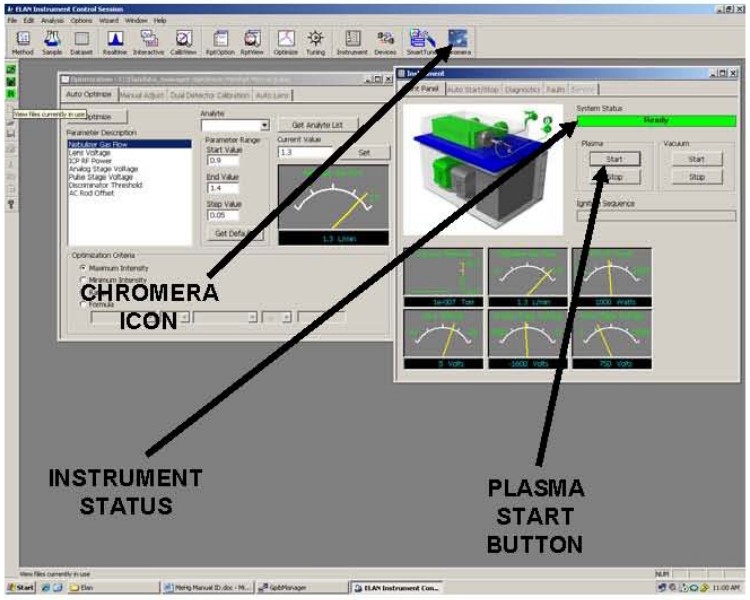
1. Start the Elan software from the desktop shortcut.

2. Turn on the coolant pump with the switch near the door and check that the external mass flow controller displays 30.0 ml/minute.

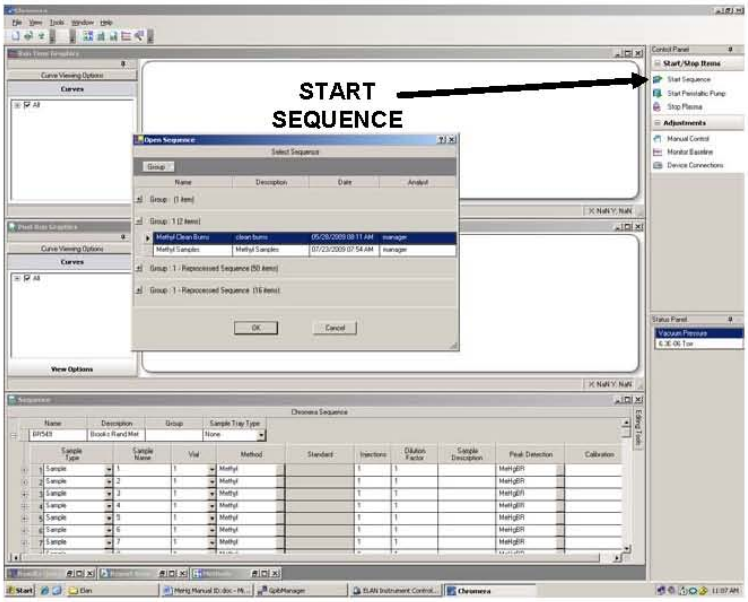
3. Click the "**R**" button in the upper left corner of the workspace and verify that the Methyl Mercury.dac file has been loaded.

4. Click the "instrument" icon to display the ICPMS status.

5. When the status of the instrument displays "Ready" click the "Start" button to initiate the plasma field.



6. Launch the Chromera software by clicking the icon at the far right of the toolbar.

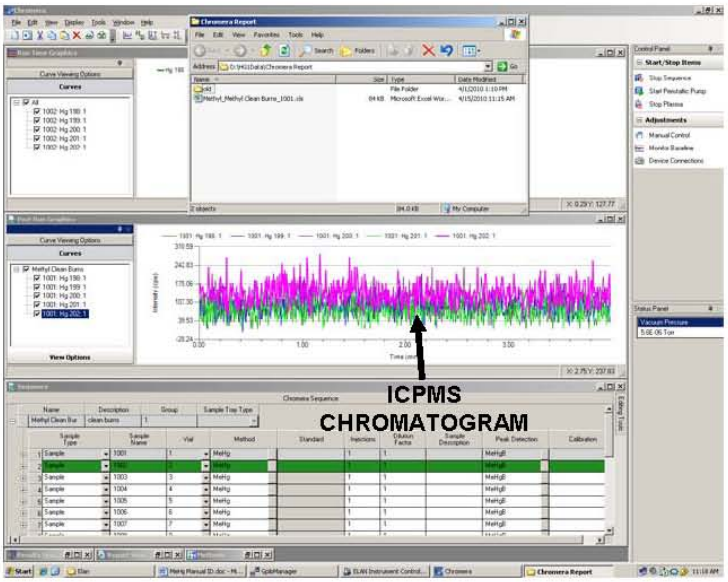


7. Click on the sequence window to make it the active application and choose "Open Sequence" from the File menu. The group "BR549" should have 75 preprogrammed analytical sequences.

8. In the Control Panel, select "Start Sequence". The first line of the sequence should turn green. It is necessary to start the analytical sequence in the Chromera software (to "prepare" the ICPMS detector to detect the sample) prior to initiating the Brooks Rand for sample analysis.

9. Activate the Brooks Rand software. Under the Automation tab, select the number of vials to be analyzed and the starting position. Click the start button to activate the Brooks Rand modules and subsequent detection by the ICPMS.

10. After approximately 15 minutes, chromatograms will start appearing in the Chromera software. The initial three chromatograms will be Tenex trap desorption blanks, and the following are the chromatograms from the analytical vials.



11. The report files for the integration of the chromatograms will be sent to the "Chromera Report" folder. Record the file number of the report file for each analytical event in the bench sheet.

12. Copy the peak areas out of the Chromera report files and past them into the Excel data sheet. Orientate the data horizontally by selecting Paste Special-Transpose and paste into the appropriate conversion section located in the "peak area-curve" workbook. The data will be converted to non-comma delimited. Copy and Paste these non-comma data into the appropriate field of the workbook.

Reagents and Standards

Reagents

All reagents and/or dry chemicals used to make reagents must be of the highest purity available from the vendor and shown to be low in mercury. Upon receipt at the laboratory, containers will be marked with the date of receipt and stored in the appropriate areas. When reagents are mixed for use in this method, the person who mixes them will initial and date the reagent container. Reagents and manufacture instructions follow below.

Reagent Water: Ultra pure reagent grade water containing less than 0.1 ng/L Hg with a resistance greater than 18 MΩ-cm. The water is delivered through a 0.2 µm filter, as obtained from a Millipore Academic water-purification system or equivalent.

Argon: Ultra high purity grade 5.0 Argon is used as the carrier gas in the analytical system. The Argon is first passed through a gold bead trap to remove any Hg.

Sodium Acetate Buffer: To make a stock solution of sodium acetate buffer, measure approximately 50 ml reagent grade water, 47.2 ml glacial acetic acid, and 108.8 g sodium acetate into a 500 ml Teflon bottle. Bring up to 400 ml volume, shake until all solids dissolve, and expose to ultraviolet light for 1 week (acetate buffer may be contaminated with MeHg). Transfer to a 125 ml Teflon bottle for use as a working solution.

Sodium Tetraethylborate (NaTEB): Sodium tetraethyborate is a toxic organometallic compound that is spontaneously combustible in the presence of oxygen and other oxidizing chemicals (such as strong acids), and volatilizes toxic gases (triethyl boron). Sodium tetraethylborate has a distinctive "sweet" smell, and should be considered an indication of analyst exposure. Although the long-term health affects of NaTEB exposure is unknown, it should be assumed that repeated exposure may have adverse health affects. All use of NaTEB should take place inside a high-volume fume hood, and special consideration for equipment exposed to NaTEB in the fume hood (i.e. gloves, wipes, pipette tips, containers, etc...) must be made.

Pure solid NaTEB is purchased in 1 gram sealed glass vials (stored under N_2 gas) and kept in the freezer until use. To dilute NaTEB to a 1% working solution, dissolve 2 g of KOH in 100 mL of reagent water in a 125 ml Teflon vial and chill to sub-freezing temperatures. Check the condition of the solution often. As soon as the KOH solution begins freezing, remove the vial of $NaBEt_4$ from the freezer and score the neck of the bottle with a glass cutter or the back of a ceramic knife. Wrap the vial in a lab wipe and break the neck of the vial. It is best to work quickly at this point as to keep the pure

NaTEB cold and to limit its exposure to oxygen to reduce the risk of combustion. Immediately dump the pure NaTEB into the 2% KOH solution and gently swirl to dissolve. Rinse the glass vial with the solution if any significant amount of NaTEB remains in the vial. When the NaTEB solution is almost entirely melted, homogenize, and pour equally into 20 clean chilled 5 mL Teflon vials. Cap the vials, store in a sealed bag, and record the date prepared. This solution should be kept frozen and made fresh every 2 weeks. Never use $NaBEt_4$ solid or solutions that are yellow in color. Following use, NaTEB should be stored in an appropriately labeled and sealed bag in the freezer until the solution can be disposed of properly.

To dispose of old or unused portions of the 1% NaTEB solutions, thaw the vials and pour into a beaker under a fume hood. Fill the beaker with an equivalent volume of 6M HCl (50% concentrated solution), place on a hotplate, boil down to half-volume, and then discard the remaining solution as an acid waste. Never dispose of concentrated NaTEB in this fashion, as that it will combust, but rather dilute to a 1% concentration with water and then process as previously described.

1M KOH rinse solution: In a 500 ml Teflon bottle, add 28 g of KOH to 250 ml of reagent water and bring up to 500 ml.

Aqua Regia rinse solution: In a 1000 ml Teflon bottle, add 25 and 75 ml of concentrated HNO_3 and HCl (respectively) to approximately 100 ml of reagent water and bring up to 1000 ml.

25% $CuSO_4$ solution: In a 500 ml Teflon bottle, add 125 g $CuSO_4$ and bring up to 500 ml volume with reagent water. Shake well until all solids dissolve.

Standards

Upon receipt at the laboratory or on the day of preparation, standards should be labeled with the date received or made and the initials of the person preparing them. Highly concentrated stock solutions should be stored away from the main working areas to prevent contamination of the clean lab. Working standards and (if necessary) subsequent sub-stock dilutions should be made in a class A volumetric flask in a matrix of reagent grade water at a 2% and 0.2% concentration of glacial acetic acid and hydrochloric acid, respectively. This solution should be transferred to a Teflon bottle designated specifically for mercury standards, stored in an amber bag at 4° C, and remade every 6 months. All standards must be assigned a unique letter-number-letter identification code and be entered into the laboratory database system. Isotopcially enriched MeHg standard concentrations are determined by ICPMS analysis. For working solutions of native MeHg, allow the solution to equilibrate for at least 24 hours and then determine the concentration by analysis via cold vapor atomic fluorescence spectrometry as follows:

1. Mass of mercury in the MeHg standard: To four 15 ml Teflon vials, add 8.0 ml of reagent grade water, 1.000 ml of the MeHg working standard, and 1.0 ml of BrCl.

2. Blank contribution of mercury: To four 15 ml Teflon vials, add 9.0 ml of reagent grade water and 1.0 ml of BrCl.

3. Store the vials in a rack, seal in a bag, and heat in an oven to 50°C for eight hours.

4. Analyze the contents of the eight Teflon vials by EPA method 1631.

5. Analyze four 1.000 ml additions of the MeHg working standard to determine the $SnCl_2$ reducible fraction of Hg^{II}

6. Subtract the average blank mercury mass and the $SnCl_2$ reducible fraction of Hg^{II} from the total mercury mass determined MeHg working standard to determine the actual MeHg mass in the vials and subsequent concentration.

Quality Assurance and Control Objectives

During the analytical run, the analyst must evaluate the calibration data, instrument blank values, duplicate spike recoveries, and check standard recoveries to ensure acceptance criteria are being met. The "summary" workbook in the Excel data sheet is where this information is displayed.

Matrix Interference

A sample should be run as a duplicate spike twice in every distillation/analysis, with a recovery within 75 – 125% of the known addition and the relative percent difference between the recoveries of less than 25%. A duplicate spike is set up similar to a triplicate analysis, except that two of the three samples are spiked with MeHg prior to the distillation. In the case of failure, repeat the duplicate spike (if possible) and bring to the attention of the quality assurance officer.

Instrumental Carryover

Instrumental carryover is assessed by the instrument blanks, which is the analysis of reagent water (with buffer and NaTEB), and should be analyzed throughout the run. Excessive instrument carryover indicates that the sample train has been contaminated with MeHg and requires cleaning (see below).

Method Blank

A method blank should be analyzed at least once every ten samples. Method blanks are part of the distillation set up and consist of a distillation vial with reagent water, 1 ml of 25% $CuSO_4$, isotopic MeHg spike, and 500 µl of HCl. Elevated method blanks indicate contamination in the distillation vials or reagents and are used to calculate the DDL (daily detection limit) for the extraction batch. The Absolute Detection Limit (three times the standard deviation of the method blank masses) must be less than or equal to 0.0024.

Instrument Calibration

Instrument calibration requires the measurement of mass bias (measured isotopic fractionation of native MeHg compared to published values) and the analysis of five isotopically spiked native MeHg standards (reverse ID). The native MeHg mass in the reverse ID calibration and check standards should be near that expected in the samples.

Reverse ID calibration: Add 25 – 100 µl of native MeHg standard to 5 analytical vials. Add 25 – 100 µl of isotopically enriched MeHg standard to these same vials. Continue with the setup of analytical vials as previously described. Enter the volume of native and isotopically enriched standards added to the appropriate fields of the "peak area-curve" workbook.

Reverse ID check standard: A reverse ID check standard is prepared similar to the reverse ID calibration vials. Vary the volumes of native MeHg standard throughout the check standards so that the mass brackets the native MeHg mass of the samples-with emphasis near the most common levels within the samples. At the minimum, a reverse ID check standard should be analyzed to verify instrument calibration in every eighth position, and have a measured mass within 80 – 120% of its true value. Enter the volumes of native and isotopically enriched standards added to the appropriate fields of the "peak area-curve" workbook. The failure of subsequent check standards is an indication of instrumental drift which may require recalibration of the instrument.

Mass Bias: The typical calibration curve (created with native MeHg) is used to determine the mass bias correction. Create a five point calibration curve with volumes of native MeHg standard spanning 10 – 200 µl. Mass bias is determined by dividing the measured ratio of the isotopes of interest (amended isotope/non-amended isotope) by published values (IUPAC) and must be within 5% of the expected value. The standard deviation for the ratio among the measured isotopes must be less than 10%.

Isotopic Fractionation of Enriched Standard: The enriched isotopes used to create the reverse ID calibration/check standards and to amend environmental samples is contaminated with small amounts of other

isotopes. Due to the sensitive nature of isotopic analysis, the instrument must be calibrated to account for these contaminants. The isotopic fractionation of the enriched isotopes can be found in the "isotope info" workbook. See the quality control officer for further direction as to the appropriate source for which to reference.

Additional Instructions

Instrument Maintenance

The MERX system requires some short- and long-term maintenance. Empty the waste receptacle daily to prevent the overflow of spent sample medium. The purge vessel and sample lines should be cleaned monthly or sooner if necessary. The three analytical Tenex traps will last for approximately 2000 desorption's each before they need replacing, and the detector lamp life is approximately 4-6 months. See pages 29-30 of the MERX user's guide for detailed instructions for lamp and trap replacement.

Sample Line/Purge Vessel Cleaning

The purge vessel and sample line will require monthly cleaning under regular use or sooner as evidenced by elevated instrument blanks. Clean this equipment in the following order: 1 M KOH (8 hrs), reagent water rinse, 10% aqua regia (8 hrs), and reagent water rinse. Dry the purge vessel with Argon gas and double bag prior to storage.

Equipment Cleaning

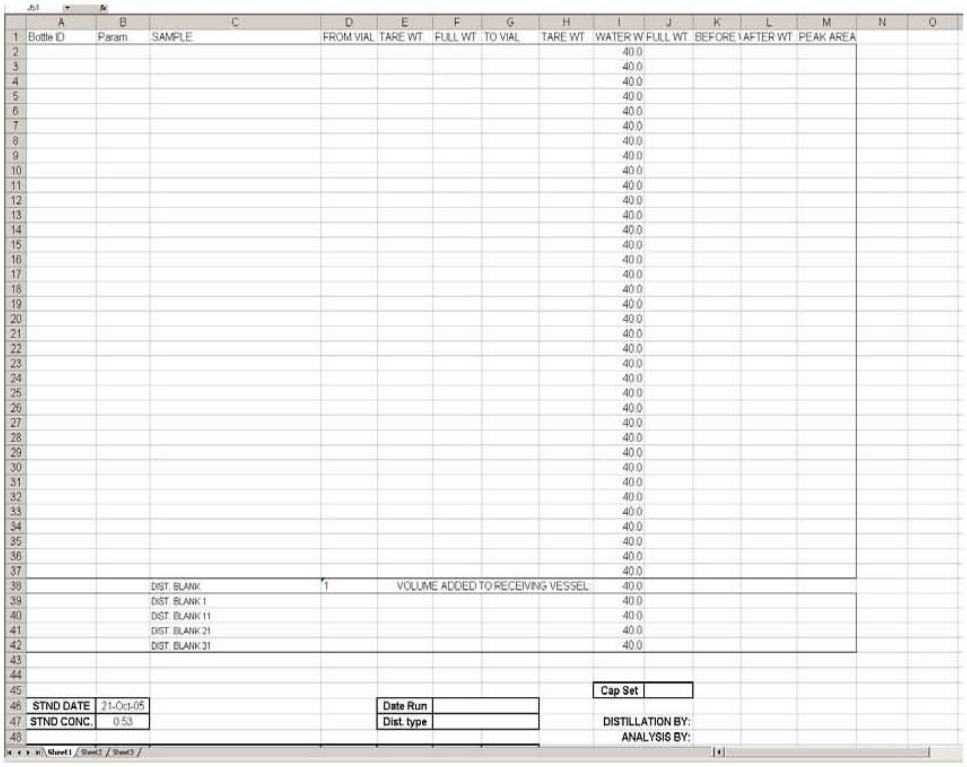
Trace level mercury analyses of samples at parts per billion concentrations are susceptible to contamination. Equipment that comes into contact with samples or reagents should be free of residual mercury and can consist of (but not be limited to) Teflon, glass, and polypropylene containers. Brand new and previously used Teflon equipment should be washed in acid before use. The equipment is first rinsed with tap water, and then cleaned by immersing in 4 N HCl heated to 65°C for at least 12 hours (48 hours for new Teflon equipment). Immediately following removal from the bath, equipment is completely immersed in reagent-grade water and then additionally triple-rinsed in reagent-grade water. After rinsing, each container is air dried under a mercury-free class 100 laminar flow hood. Dry equipment is stored double bagged in zip-type bags.

Analytical Vial Cleaning

Clean the amber glass vials with the following method. Wash the vials with lab detergent and rinse with reagent water. Once dry, wrap the vials in aluminum foil and heat at 550 ° C for 2 hrs. Inspect the vials prior to use for chips or cracks.

Shutdown: Turn off the plasma and exit Elan. Do not save changes to the method file when prompted. Turn the coolant pump off. Create a new folder in the "old" folder in the Chromera Report folder. The naming convention is Mxxxxxx, where xxxxxx represents the six digit date MMDDYY. Cut all of the reports from the Chromera Report folder (except the "old" folder) and paste them into the new folder within the old folder.

Appendix 1. Additional instructions for distillation data entry.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Bottle ID	Param	SAMPLE	FROM VIAL	TARE WT	FULL WT	TO VIAL	TARE WT	WATER W	FULL WT	BEFORE \	AFTER WT	PEAK AREA		
2									40.0						
3									40.0						
4									40.0						
5									40.0						
6									40.0						
7									40.0						
8									40.0						
9									40.0						
10									40.0						
11									40.0						
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32									40.0						
33									40.0						
34									40.0						
35									40.0						
36									40.0						
37									40.0						
38			DIST. BLANK	1	VOLUME ADDED TO RECEIVING VESSEL				40.0						
39			DIST. BLANK:1						40.0						
40			DIST. BLANK:11						40.0						
41			DIST. BLANK:21						40.0						
42			DIST. BLANK:31						40.0						
43															
44															
45									Cap Set						
46	STND DATE	21-Oct-05			Date Run										
47	STND CONC.	0.53			Dist. type				DISTILLATION BY:						
48									ANALYSIS BY:						

Enter the following information into the appropriate fields: STND DATE, STND CONC., Date Run, DISTILLATION BY, and ANALYSIS BY.

Enter the 3 digit numeric codes (etched onto the wall of the vial) for the corresponding distillation and receiving vials into the columns titled "FROM VIAL" and "TO VIAL" (respectively). Be sure to enter the blanks into the appropriate area.

Enter the corresponding sample ID's into the column titled "BOTTLE ID". Duplicate spikes should be chosen randomly from the sample group.

Enter the full weight of the distillation vials after the sample has been added to the column "FULL WT".

Enter the tare weights for the distillation and receiving vials and the sample information using the database query tool found in *query_TAre_FieldSampleInfo.xls*. Follow the directions in each tab to query for the needed information and enter it into the spreadsheet.

Following the distillation, enter the final mass of the receiving vial into the column titled “FULL WT.”

Following the analysis, enter the peak area of the analyzed samples into the column titled “PEAK AREA”.

APPENDIX 2. Example of a typical analytical run.

Rack 1

8 Check Std.	7 Instrument Blank	6 Sample 6	5 Sample 5	4 Sample 4	3 Sample 3	2 Sample 2	1 Sample 1
16 Check Std.	15 Instrument Blank	14 Sample 12	13 Sample 11	12 Sample 10	11 Sample 9	10 Sample 8	9 Sample 7
24 Check Std.	23 Sample 19	22 Sample 18	21 Sample 17	20 Sample 16	19 Sample 15	18 Sample 14	17 Sample 13

Rack 2

32 Check Std.	31 Instrument Blank	30 Sample 25	29 Sample 24	28 Sample 23	27 Sample 22	26 Sample 21	25 Sample 20
40 Check Std.	39 Instrument Blank	38 Sample 31	37 Sample 30	36 Sample 29	35 Sample 28	34 Sample 27	33 Sample 26
48 Check Std.	47 Sample 38	46 Sample 37	45 Sample 36	44 Sample 35	43 Sample 34	42 Sample 33	41 Sample 32

Rack 3

56	55	54	53	52 Check Std.	51 Instrument Blank	50 Sample 40	49 Sample 39
64	63	62	61	60	59	58	57
72	70	70	69	68	67	66	65

Appendix 3. Analytical bench sheet for the ICPMS.

Date: _____ ID Std: _____ Native Std: _____ Initials: _____

ID	Sample ID	Vial ID	Chromera File #
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			
29			
30			
31			
32			

ID	Sample ID	Vial ID	Chromera File #
33			
34			
35			
36			
37			
38			
39			
40			
41			
42			
43			
44			
45			
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61			
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63			
64			

Appendix V D: Solids, volatile-on-ignition, total-in-bottom-material, gravimetric

USGS
science for a changing world

Techniques of Water-Resources Investigations
of the United States Geological Survey

Chapter A1

METHODS FOR DETERMINATION OF INORGANIC SUBSTANCES IN WATER AND FLUVIAL SEDIMENTS

By Marvin J. Fishman and Linda C. Friedman, Editors

First Edition 1970
Second Edition 1979
Third Edition 1989

Book 5
LABORATORY ANALYSIS

Solids, volatile-on-ignition, total-in-bottom-material, gravimetric

Parameter and Code:

Solids, volatile-on-ignition, total-in-bottom-material, dry wt, I-5753-85 (mg/kg): 00496

1. Application

This method may be used to analyze samples of bottom material. Usually, only the material that will pass a 2-mm sieve is taken for analysis.

2. Summary of method

A portion of well-mixed sample is dried at 105 °C. A portion of dry, well-mixed sample is carefully weighed and then ignited at 550 °C. The loss of weight on ignition represents the amount of volatile solids in the sample.

3. Interferences

None.

4. Apparatus

4.1 *Desiccator*, charged with indicating silica gel or other efficient desiccant.

4.2 *Muffle furnace*, 550 °C.

4.3 *Oven*, 105 °C, uniform temperature throughout.

4.4 *Platinum evaporating dishes*, 75- to 125-mL capacity, weighing less than 50 g. Platinum is recommended because the change in weight of glass or porcelain dishes may introduce appreciable error into the determination.

5. Reagents

None required.

6. Procedure

6.1 Spread approx 1 g of sample in the bottom of a platinum evaporating dish. Place in an oven at 105 °C and heat overnight.

6.2 Place the dish containing the dry sample in a desiccator, cool, and weigh to the nearest 0.1 mg.

6.3 Ignite the weighed dry residue at 550 °C for 1 h, cool in a desiccator, and weigh to the nearest 0.1 mg.

7. Calculations

Solids, volatile-on-ignition (mg/kg) =

$$\frac{(DR - IR) \times 10^6}{DR}$$

where

IR = weight of ignited residue, milligrams, and

DR = weight of dry residue, milligrams.

8. Report

Report solids, volatile-on-ignition, total-in-bottom-material (00496), concentrations as follows: less than 1,000 mg/kg, whole numbers; 1,000 mg/kg and above, three significant figures.

9. Precision

Precision data are not available for this method.

Appendix V E: Digestion of Sediments and Biological Tissues for Analysis by Isotope Dilution Hydride Generation Inductively Coupled Mass Spectrometry (ID HGICP-MS)

Standard Operating Procedure (SOP) for Digestion of Sediments and Biological Tissues for Analysis by Isotope Dilution Hydride Generation Inductively Coupled Plasma Mass Spectrometry (ID HGICP-MS)

May 7, 2014

Originated by: Amy Kleckner, Evangelos Kakouros, Robin Stewart, & Kent Elrick
Revised by: Amy Kleckner, Evangelos Kakouros, & Robin Stewart

I. Introduction:

This method outlines digestion and analysis of sediments and biological tissues for selenium concentrations using a multi-step wet digestion with a mixture of nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). An ^{82}Se enriched isotope spike is used to measure isotope dilution. Calibration of the enriched ^{82}Se spike is achieved by reverse spike isotope dilution. The digestates are mixed with concentrated hydrochloric acid to reduce the selenium to the most favorable valence for hydride generation. The solutions are then analyzed by inductively coupled plasma mass spectrometry coupled with hydride generation (ID HGICP-MS). Polyatomic and isobaric interferences are removed through the use of hydride generation and background correction using ^{82}Se enriched isotope spike.

II. Safety Issues:

Before beginning any of the procedures involved in this method, individuals must read and sign the Chemical Hygiene Plan developed for the lab. Specific safety concerns for each chemical can be found in the Material Safety Data Sheets (MSDS) for that chemical – all of which are located in the laboratory.

Strong acid solutions are employed in the cleaning of equipment, preparation of reagents and in sample preservation. Proper acid handling techniques should be employed whenever acids are being used. These techniques include the use of acid resistant clothing and the utilization of high volume fume hoods.

III. Materials

1. Freeze dried/ground or otherwise homogenized samples and certified reference materials.
2. Acid washed 6 mL screw cap Teflon vials. Vials are soaked for 24 hours in dilute micro solution, rinsed 3x with Milli-Q water, soaked for 3 days in a 15% HNO3 bath, rinsed 3x with Milli-Q water, dried in a Laminar flow hood and stored in a zip lock bag until use.
3. 50 ppb enriched ^{82}Se standard solution.
4. Nitric Acid (HNO_3) (Trace metal grade).
5. 30% Hydrogen Peroxide (H_2O_2) (Ultrex II grade).

6. Hydrochloric acid (HCl) (Trace metal grade).
7. Hot plates.
8. Pressure steam sterilizer.
9. Milli-Q Water – 18 megohm ultrapure (<0.2 ng/L total Hg)
10. Selenium standard solution.
11. 2% hydrochloric acid solution.
12. Water bath heated to ~90 °C
13. KOH pellets
14. Sodium borohydride powder

IV. Selenium Standard and Reagent Preparations

Enriched ^{82}Se spike solution in 2% HCl:
Prepare enriched isotope stock by accurately weighing approximately 1.25 mL secondary ^{82}Se stock (4-Feb-2013) into a tared, empty, 50 mL Falcon tube or pre-cleaned PE plastic bottle. The solution is brought to 50 mL with 2% HCl. The resulting concentration should be near 50 ppb.

Selenium Standard solutions in 2% HCl:
Prepare standards according to the nominal concentrations listed for each standard. Selenium standard solutions are prepared by accurately weighing a suitable amount of Stock or Intermediate Stock solution (density approximately 1.011 g/mL) into a tared, empty, 50 mL Falcon tube or pre-cleaned PE plastic bottle. The solutions are brought to final volume with 2% HCl (density approximately 1.007 g/mL). RECORD the exact weight of each addition. Mix well and allow standards to equilibrate before using. Final concentrations are calculated by weight. Note: Secondary and tertiary stock solutions are made up in 2% HNO_3

~Conc. (µg/L, ppb)	Stock Se: ~1,000 mg/L, 1000 ppm (mL)	Secondary Stock Se: 10000 µg/L, 10000 ppb (mL)	Tertiary Stock Se: 100 µg/L, 100 ppb (mL)	2% HCl (mL) *2% HNO_3	Final Volume (mL)
10000	0.5	--	--	49.5*	50
100	--	0.5	--	49.5*	50
0.2	--	--	0.1	49.9	50
1	--	--	0.5	49.5	50
2	--	--	1	49	50
4	--	--	2	48	50
10	--	0.05	--	49.95	50
20	--	0.1	--	49.9	50
40	--	0.2	--	49.8	50
60	--	0.3	--	49.7	50

Standard solutions can be stored at room temperature for several months but must be reduced prior to HGICP-MS analysis. Note that concentrations of reduced standards used for creating calibration curves for the analysis will be ½ their original concentrations.

RSID in 2% HCl:

Prepare the RSID by accurately weighing approximately 1 mL of the enriched ^{82}Se spike solution, and 2.5 mL of ambient 100 ppb Se stock into a tared, empty, 50 mL Falcon tube or pre-cleaned PE plastic bottle. The solution is brought to 50 mL with 2% HCl.

One day prior to ID HG-ICP-MS analysis three replicate (RSID) solutions are prepared by transferring an aliquot of the RSID in 2%HCl solution to a 15mL Falcon tube and adding an equal amount of 12M HCl. (minimum total volume of 8 mL). The RSID is reduced along with all the ambient standards and samples.

*For ~45 lines/samples you will need approximately 1L $NaBH_4$ solution and 2.5L **50% HCl**.

$NaBH_4$:

1. Fill a 1L volumetric flask with approximately 800mL Milli-Q water.
2. Add 18 g KOH to the volumetric flask and dissolve.
3. Add 6 g $NaBH_4$ to the volumetric flask and dissolve.
4. Bring to volume with Milli-Q water.
5. Store solution in an airtight container in the fridge. Will keep for ~ 1 month.

Calibration/Cleaning Blank:

Prepare a 1:1 2% HCl : 12M HCl solution to be sampled as a calibration blank and as a cleaning blank periodically during the run.

V. Digest Procedure

1. Weigh and transfer 10-20 mg of freeze dried/ homogenized sample to a labeled 6ml Teflon vial. Store dried samples in desiccator. For every ten samples digested one sample should be run in duplicate.
2. Weigh out appropriate reference standard materials, and use empty vials for blanks. (ex. TORT ~ 10 mg, PACS ~ 50 mg)
3. Two reagent blank samples will be run with each sample set. This is prepared with all the reagents and digested exactly like the actual samples.
4. Add 100uL of a solution of 50ppb ^{82}Se enriched isotope to each vial. Record weight.
5. Add 0.6 mL of concentrated HNO_3 (Trace metal grade) to each vial and cap tightly.
6. Place samples in pressure steam sterilizer and incubate for 3 hours at ~ 20psi and ~ 125C.
7. After 3 hours turn off pressure steam sterilizer and carefully remove samples. Allow the samples to come back to room temperature overnight.

8. Place a hotplate in a fume hood, place aluminum heating block on top of the hotplate, and adjust the hot plate to heat the block to a temperature between 170 & 190C.
9. Remove the lids and place them on a clean kimwipe with the label up.
10. Add 200µL H_2O_2 to each vial and allow it to react for ~15 minutes at room temperature.
11. Place all samples in the heating block.
12. Heat until the residue is nearly dry (<~100 µL or "lentil-sized" drop). They will need to be monitored continuously when they are almost evaporated to prevent "charring." This step can take anywhere from 20-40 minutes.
13. Remove samples from the heating block and allow them to come to room temperature.
14. Bring samples up to volume with 5 mL 2% HCl solution. Samples can now be stored at room temperature until ready for analysis.

VI. Sample Reduction Procedure

1. One day prior to HG-ICP-MS analysis, transfer an aliquot (min. 4 mL) of each sample, 3 RSID, and each nominal standard solution to a 15mL Falcon tube and add an equal amount of 12M HCl. (minimum total volume of 8 mL).
2. Samples and standards need to be reduced one day prior to analysis. To do this place Falcon tubes in a water bath (temp ~90 °C) in a fume hood, and heat to "near boiling" for 30 minutes.
3. Allow samples to equilibrate and come to room temperature over night.

*For PACS-2 samples, pipette off supernatant. Do not allow for undigested sediments (sample) to go into ICP-MS.

Samples are now ready for analysis by hydride generation ICP-MS.

VII. Analysis procedure – HGICP-MS (Perkin Elmer SCIEX Elan DRC II)

1. Check that there is sufficient Argon gas and that the gauge reads ~ 80 psi.
2. Check the chiller/recirculator gauge is at ~70 psi and that water level is about 4/5ths full.
3. Check the gas gauge on the back of the ICP-MS is at ~55 psi.
4. Replace waste container.
5. Assemble tubing on FIAS.
6. Open gas valve to FIAS.
7. Turn on FIAS. Gas pressure on the front of the FIAS should be ~130 psi.
8. Make sure the ICP-MS "ready light" is ON.
9. Log onto computer: username: pki, password is found in the log book.
10. Start ELAN software.
11. Go into "Instrument" and "start plasma." May come back as "failed", just try again.
12. In log book, record start time etc.

13. If peristaltic pump on ICP-MS is running go to "Devices", stop peristaltic pump.
14. Allow ICP-MS to "warm up" for about 30-60 minutes.
15. Under "Method", check that you are using the correct files.
"File", "Open"
Method: C:\elandata_pki\Method\Se-total-FIASHGstdmode3.mth
Optimization: C:\elandata_pki\Optimize\Default.dac
Tuning: C:\elandata_pki\Tuning\Default.tun
16. In "Method", "Report", Report Filename, "Browse" create new file with today's date.
17. Send to printer? Check or don't check box for print outs of every sample. Check that there is sufficient paper in the printer.
18. Under "Report", Options template: seextract1.rop.
19. Under "Sample", create "Batch", with MQ in autosampler position 1. Always start with a MQ sample and MQ in place of acid and $NaBH_4$ to check for leaks and to make sure everything is running in the proper directions. If OK, then replace with acid and $NaBH_4$ solutions.
20. Fill autosampler probe cup with 10% HCl solution for rinsing the autosampler probe between samples.
21. Load autosampler.
22. Now create a sample "Batch" according to the run list starting with a couple of cleaning blanks and then the standard curve. After the highest standard and again after the RSID samples, insert a couple cleaning blanks. Every 4-5 samples insert a cleaning blank, standard to check for instrument drift, and another cleaning blank.
23. Highlight all lines then hit "Analyze Batch". ("Save changes?"- No)
24. At some point during the run, go under "Instrument" and record Int. temp., Box temp., Pressure, etc. in the log book.
25. Before shutting down the instrument, run a MQ sample and then air to clean out the FIAS tubing.
26. Turn off FIAS, close FIAS gas valve, release tubing, stop plasma, remove waste, in the log book record end time, and log off computer.

Samples and reverse ID samples are run on a same day to obtain ratios (mass bias corrected) which are then used to obtain final analyte concentration using Eq1.

RSID: $$C_y = C_z \cdot \frac{m_z}{m'_y} \cdot \frac{B_z \cdot R'_n - A_z}{A_y - B_y \cdot R'_n} \cdot \frac{AW_y}{AW_z} \qquad (1)$$

where:

C_z is the concentration of analyte in the primary standard (mg/kg);
m_z is the mass of primary standard used (g);
m'_y is the mass of spike used to prepare the mixture of spike and primary standard (g);
A_y is the abundance of the reference isotope in the spike;
B_y is the abundance of the spike isotope in the spike;
A_z is the abundance of the reference isotope in the primary standard;

B_z is the abundance of the spike isotope in the primary standard;
R'_n is the measured reference/spike isotope ratio (mass bias corrected) in the mixture solution of spike and primary standard;
AW_y is the atomic weight of the analyte element in the spike (g/mol);
AW_z is the atomic weight of the analyte element in primary standard(g/mol);

$$\text{ID: } C_x = C_y \cdot \frac{m_y}{w \cdot m_x} \cdot \frac{A_y - B_y \cdot R_n}{B_x \cdot R_n - A_x} \cdot \frac{AW_x}{AW_y} \qquad (2)$$

where:

C_x is the concentration of analyte in the sample (mg/kg);
C_y is the concentration of analyte in the enriched spike (mg/kg);
m_y is the mass of spike solution used to prepare the mixture of sample and spike (g);
m_x is the mass of sample used (g);
w is the dry weight correction factor;
A_y is the abundance of the reference isotope in the spike;
B_y is the abundance of the spike isotope in the spike;
A_x is the abundance of the reference isotope in the sample;
B_x is the abundance of the spike isotope in the sample;
R_n is the measured reference/spike isotope ratio (mass bias corrected) in the mixture solution of sample and spike;
AW_x is the atomic weight of the analyte element in the sample (g/mol).

VIII. Quality Assurance

1. Sample Batch Size: Approximately 25 samples can be analyzed over a 5-day period (digested and run).
2. Certified Reference Material: Use an appropriate matrix and prepare at least 2 per batch. Digest and analyze with the same method as the samples. Recovery Limit = 80-120%.
3. Reagent Blanks: Prepare 2 sample blanks per batch. Digest and analyze with the same method as the samples.
4. Laboratory duplicate: At least 2 samples per batch should be run in duplicate. RPD limit = 15%
5. For a 10mg sample MDL = 0.026 ug/g MRL = 0.1 ug/g. (as of 4/15/14)

IX. References

Liber, K. 2011. Cold Digestion of Invertebrates for the Selenium Project. Method developed C.W. and E.F. 2007. Revised Mar 2011 MK. Water Quality Laboratory, Toxicology Center, University of Saskatchewan.

Elrick, K.A., and Horowitz, A.J., 1985. Analysis of rocks and sediments for arsenic, antimony, and selenium, by wet digestion and hydride generation atomic absorption: U.S. Geological Survey Open-File Report 85-497, p. 14.

X. Appendix

Appendix V F: Measurement of Dissolved Organic Matter Fluorescence and Absorbance

Updated: March 17, 2014
Version 1.3
Pete L. Lenaker

Procedure for the Measurement of Dissolved Organic Matter Fluorescence and Absorbance using the Aqualog Spectrofluorometer at the Wisconsin Water Science Center, Middleton, WI

Standard Operating Procedure

1. **Initial preparations**
   - **a.** Always were gloves (nitrile or polypropylene) when handling cuvettes and any samples
   - **b.** Computer login information
     - i. Username: plenaker
     - ii. Password: USGS1234
   - **c.** Plug USB cable from Aqualog into USB port in back left corner of Dell laptop
   - **d.** Plug USB dongle into USB port located on left side of Dell laptop
   - **e.** Plug Passport external hard drive into remaining USB port on right side of Dell laptop
   - **f.** Turn on the Aqualog and let it warm up (at least 30 minutes)
   - **g.** Remove cuvettes from 2 M HNO_3^- solutions and soak in 3 consecutive 18.2 MΩ MilliQ baths for 10 minutes at a minimum. Further rinse 10 times with MilliQ to insure they are clean of any remaining acid.
   - **h.** Remove samples to be run from refrigerator and let equilibrate to room temperature prior to analysis
   - **i.** Open Aqualog on desktop

2. **Reagents and Standard Solutions**
   - **a.** 1% Lipton Unsweetened Tea Standard - ***Daily***
     - i. Add 2.5 ml of Lipton unsweetened concentrate tea to a clean 250 ml volumetric flask containing 175 ml 18.2 MΩ Milli Q water. Bring up to 250 ml volume
     - ii. Pour 10 ml into a 250 ml baked amber bottle to rinse, then transfer remaining solution to the 250 ml baked amber bottle.
   - **b.** 2 M HNO_3^- cuvette bath solution - ***Monthly***
     - i. Add 12.7 ml HNO_3^- to a clean 100 ml volumetric flask containing 25 ml 18.2 MΩ Milli Q water. Bring up to 100 ml volume
     - ii. Transfer solution to Teflon container and mark with date prepared

3. **Record lamp hours**

Updated: March 17, 2014
Version 1.3
Pete L. Lenaker

a. Click on the Windows start menu 
    i. Then select All Programs
    ii. Jobin Yvon
    iii. Utilities
    iv. Lamp reset
        1. Record Lamp On time in hours in the Aqualog lab book (do NOT reset the lamp or filter, just close window when finished)

4. Create Project File for the Day

a. Click on File and select Save Project As

b. Create a folder (YYYYMMDD) in the following directory to save the project file in
    i. Computer/OS(C:)/Users/Public/Public Documents/Jobin Yvon/Data/Projects/YYYYMMDD
    ii. Save project file in above directory as YYYYMMDD (file will be given a .opj extension)

5. Create Data Folder for the Day

a. Create a folder (YYYYMMDD) in the following directory to save the Aqualog data files in
    i. Computer/OS(C:)/ Users/Public/Public Documents/Jobin Yvon/Data/Output Files/YYYYMMDD

b. Create an additional folder called "output" in the above data folder (YYYYMMDD). This will be used to export the absorbance and EEM python script data

6. Create Sample Log file

a. Open previous day/sample run Microsoft excel sample log file and save in
    i. Computer/OS(C:)/ Users/Public/Public Documents/Jobin Yvon/Data/Output Files/YYYYMMDD/Sample Log_YYYYMMDD.xlsx
    ii. Follow the format provided in Table 1. If more than 10 samples are to be run, continue "Group 2" format by making a "Group 3". You do NOT need to run "Group 1" again
    iii. Dilution factor should stay 1, otherwise enter the dilution factor used
    iv. "Project ID" should represent samples you are running, i.e. GLRI, MMSD, GMIA, Lake MI….

Updated: March 17, 2014
Version 1.3
Pete L. Lenaker

Sample Type	Field ID	Aqualog ID	Dilution	Aqualog Sample #	Aqualog Experiment #
Initial Baseline	Baseline	Group001Baseline	1		1
Blank	blank	Group001"ProjectID"001	1	1	2
Blank	blank	Group001"ProjectID"002	1	2	3
Blank	blank	Group001"ProjectID"003	1	3	4
1% Tea Std.	1% Tea Std.	Group001"ProjectID"004	1	4	5
1% Tea Std.	1% Tea Std.	Group001"ProjectID"005	1	5	6
Initial Baseline	Baseline	Group002Baseline	1		1
Sample 1	BK ###	Group002 "ProjectID"001	1	1	2
Sample 2	CG ###	Group002 "ProjectID"002	1	2	3
Sample 3	MC ###	Group002 "ProjectID"003	1	3	4
Sample 4	UW ###	Group002 "ProjectID"004	1	4	5
Sample 5	MW ###	Group002 "ProjectID"005	1	5	6
Sample 6	RK ###	Group002 "ProjectID"006	1	6	7
Sample 7	DG ###	Group002 "ProjectID"007	1	7	8
Sample 8	TR ###	Group002 "ProjectID"008	1	8	9
Sample 9	PO ###	Group002 "ProjectID"009	1	9	10
Sample 10	MA ###	Group002 "ProjectID"010	1	10	11
1% Tea Std.	1% Tea Std.	Group002 "ProjectID"011	1	11	12
1% Tea Std.	1% Tea Std.	Group002 "ProjectID"012	1	12	13
Sample Replicate	MC ###-R	Group002 "ProjectID"013	1	13	14
Blank	blank	Group002 "ProjectID"014	1	14	15
Blank	blank	Group002 "ProjectID"015	1	15	16

Table 1. An example of your Microsoft excel sample log (YYYYMMDD_Sample Log.xls) and a typical days sample run using Sample Q. Group 001 is only run once per day, so additional samples would following the Group 002 sequence.

Water Raman Test

b. Use the certified Raman Reference cell from Starna Cells for the water Raman test.
   i. Remove sealed cuvette from Starna container and wipe clean w/ lens paper ensuring the cuvette is clear of all fine debris
   ii. Place standard in position 1 of sample chamber and make sure the etched side of the cuvette faces the center of the sample carousel

c. Click on Raman Scattering Area Unit Tool icon 

d. Data Description
   i. Change the data identifier to RSYYYYMMDD (e.g. RS20131217)

e. Aqualog Experiment Options
   i. Integration time = 10
   ii. Accumulation = 1
   iii. Excitation and emission do not change
   iv. Increment or "bin" (nm) = 2.33 nm (4 pixel)
   v. CCD gain = Medium

NOTE: These settings, except the integration time, should match the settings you plan to use. The normalization factor can be adjusted for different integration times used when analyzing samples.

f. Blank/Sample Setup

i. "Sample only" should be selected

g. Sample Selection

i. "Position 1" should be selected

h. Click **RUN** when ready

i. When instrument is finished, click on the "Raman Area Graph" tab at bottom to see the intensity vs. wavelength graph

i. Visually inspect the water raman graph, should be relatively stable and provide a nice peak around 398

ii. Make sure the baseline is flat

iii. Record the Peak, Height and Area in the Aqualog lab book

iv. Height and Area, at a minimum, should be within the 3% range in Table 2

Settings: Integration time: 10 bin: 2 gain: medium			Settings: Integration time: 10 bin: 4 gain: medium			Settings: Integration time: 10 bin: 8 gain: medium		
%	Variable	Range	%	Variable	Range	%	Variable	Range
1	Height	971.42 – 991.05	1	Height	1910.17 – 1948.76	1	Height	3718.36 – 3793.47
1	Area	11581.58 – 11815.55	1	Area	23088.56 – 23555.00	1	Area	46492.57 – 47431.82
3	Height	951.80 – 1010.67	3	Height	1871.58 – 1987.34	3	Height	3643.24 – 3868.59
3	Area	11347.61 – 12049.52	3	Area	22622.12 – 24021.43	3	Area	45553.33 – 48371.06

Table 2. Area and Height ranges depending on which bin setting is chosen for the sealed Water Raman Standard.

j. Click on the "RSU_Adjust" tab at bottom of workbook

i. Integration time = 1 (column C(Y), row 1)

1. Integration time can be modified to reflect integration time used for running samples

ii. Right click on the Area value (column D(Y), row 2) and copy it. Also, record value in Aqualog lab notebook

1. This will be used later when setting up your sample Q to normalize the EEMs data to Raman Standard units (RSU)

7. Quinine Sulfate Unit (QSU) Normalization Factor Test

a. Use the certified Quinine Sulfate Reference Standard from Starna Cells to collect and calculate the QSU normalization factor
   i. Remove Quinine Sulfate Blank cuvette from container, wipe clean with lens paper
      1. Place blank cuvette in position 1 and make sure the etched side of the cuvette faces the center of the sample carousel
   ii. Remove Quinine Sulfate cuvette from container, wipe clean with lens paper
      1. Place QS cuvette in position 2 and make sure the etched side of the cuvette faces the center of the sample carousel

b. Click on Quinine Sulfate Normalization Factor Tool icon 

c. Data Description
   i. change the data identifier to QSYYYYMMDD (e.g. QS20131217)

d. Aqualog Experiment Options
   i. Integration time = .05
   ii. Accumulation = 1
   iii. Excitation and emission do not change
   iv. Increment or "bin" (nm) = 2.33 nm (4 pixel)
   v. CCD gain = Medium

      NOTE: These settings, except the integration time, should match the settings you plan to use. The normalization factor can be adjusted for different integration times used when analyzing samples.

e. Blank/Sample Setup
   i. "Sample and Blank" should be selected along with "Collect Blank"

f. Sample Selection
   i. The QS Blank should be "Position 1" and the QS Sample should be "Position 2"

g. Click **RUN** when ready

h. When instrument is finished, click on the "Emission Spectrum Graph" tab at bottom to see the intensity vs. wavelength graph
   i. Visually inspect the QS emission graph, should be relatively stable and provide a nice peak around 450 nm
   ii. Record the Excitation wavelength (EX WL), Absorbance (AU) and Percent Transmittance (%T) in the Aqualog lab book

i. Click on the "QSU Calculation Worksheet" tab

i. Record the "QSU Adjust" value (column G(Y), row 1) in the Aqualog lab notebook.
    1. If planning to normalize the EEMs data to Quinine Sulfate units (QSU), this will be used later when setting up your sample Q

8. Run "Group 1" a Baseline, Set of Three Blanks and Two 1% Tea Standards

a. Click on Aqualog SampleQ Tool icon  to set up initial baseline and blank evaluation

b. Sample Q File
- i. Click "Save As"
- ii. Open Projects folder and select Project folder (YYYYMMDD) created for today's run
- iii. Change file name to "AqualogSampleQ_YYYYMMDD.aqu"
- iv. Make sure the proper folder was selected to save the file in: C:\Users\Public\Documents\JobinYvon\Data\Projects\YYYYMMDD\AqualogSampleQ_YYYYMMDD.aqu

c. Experimental File click "Create"
- i. Change "Experiment File" name to DfltAqualogSampleQ_YYYYMMDD.xml.
    1. Click "Save As" to save the experiment file in the Project File established for today's run.
- ii. Set up **Aqualog Experiment Options**
    1. Integration time = 1
    2. Excitation wavelength 800 – 240 with increment (nm) = 3
    3. Emission does not change
    4. Increment or "bin" (nm) = 2.33 nm (4 pixel)
    5. CCD gain = Medium.
- iii. Click "Save" to save your experimental file settings
- iv. Click OK when ready

d. Output File Names
- i. Leave the Blank Group Prefix as "Group"
- ii. Change the Blank Base to "Baseline"
- iii. Change Sample Prefix from "Sample" to "Project ID" representing the samples
- iv. Set Output Folder location by clicking "Browse"

1. Scroll down to output folder created in section 5, part a C:\Users\Public\Documents\Jobin Yvon\Data\Output Files\YYYYMMDD
2. Select the New Days Folder (YYYYMMDD) and click ok
3. Verify the Output Folder is correct. This is where all the data you collect for the day will be exported to

e. Sample Setup

i. Number of Blank Groups should be 1
ii. Blank Group Start # should be 1
iii. Samples per Blank should be 5 (we plan to run 3 blanks and 2 tea stds). You should notice that the Total Samples should equal 6 (initial baseline followed by 3 blanks and 2 teas)

f. Post Processing Options

i. Check IFE
ii. Check Normalize and Normalization Factor
iii. Enter the RSU or QSU normalization factor
iv. Check Rayleigh Masking and select 1st and 2nd order
v. Change the Sum of slit widths from 10 to 12.

g. Export Options

i. Check "Save Raw Data in Workbook Format (*.ogw)"
ii. Check "Save worksheets as ASCII (*.dat)
iii. Select all data files you would like to save (Most common below)
    1. ABS
    2. Blank XYY
    3. Sample-Blank Raw (XYY) and (XYZ)
    4. Sample-Blank Processed (XYY) and (XYZ)

h. Sample Q File

i. Click "Save" to make sure all settings above are saved

i. Click **Run** when ready

i. A pop up box will appear telling you to insert the samples
ii. Fill your 4 cuvette's with MilliQ, wipe cuvette's clean with lens paper, check for air bubbles (tap cuvette if air bubbles are present to get air bubbles to move to top of cuvette) and load them into Sample Changer
iii. Click "Continue" when samples are loaded and ready

j. When Sample run is complete open a second Aqualog window and open the "baseline/initial Blank" and the 3 BLANK workbooks to visually evaluate baseline/Blank and BLANKS.

i. Click "folder" icon at top to open baseline and blanks workbooks. Navigate to the day's output folder you created earlier to find the four workbooks.
ii. Visually inspect the initial baseline/Blank, all you should see are Rayleigh lines near the bottom left corner of the EEM
iii. Visually inspect the 3 BLANK EEMs. They should be free of any high intensity peaks and have only signal noise
iv. Visually inspect the Absorbance of the sample by clicking on the "Abs Spectra Graphs" to view Absorbance and percent transmittance. Should have very low absorbance readings if any.
v. Run the raw blank data through the python/R script to evaluate the baseline and blanks
vi. If baseline and blank samples pass the evaluation, then begin running samples

9. QA/QC Check the Baseline and Blank

a. Open Windows command window
i. Change working directory by typing the following:
cd C:\Users\Public\Documents\EEM_scripts
ii. Type cd and hit enter to confirm change in working directory

b. Open Aqualog_input.py file using Notepad or wordpad
i. Change the #date of analysis, #working directory, #output directory, #sample log file, #Raman normalization factor. This change will be updating the dates in all the above categories and entering the day's normalization factor. .
ii. Save the Aqualog_input.py changes you just made

c. Execute absorbance_01.py and eem_03.py scripts in Windows command window
i. Type: python absorbance_01.py and hit enter
ii. Type: python eem_03.py
iii. Open output file within the days run data folder and check to see that the script ran. You should see an absorbance and EEM plot for each of your blanks and tea standards. You should also see a absorbance and EEM summary text documents
iv. Inspect absorbance .png files and insure there are no trends in absorbance blank data. Should be a flat, but noisy line
1. If trends do appear, do additional MilliQ rinses on cuvettes showing the trend and re-run them with new baseline.

v. Inspect EEM .png files and look for indications of contamination in the EEM
vi. Evaluate the absorbance summary text file. Look for high values in any of the absorbance parameters, evaluate DDL for various absorbance parameters
vii. Evaluate the EEM summary text file. Look at blanks for high values in EEM parameters (Peak_B and Peak_T tend to have high values, relative to other peaks), evaluate DDL for various EEM parameters

10. Running Samples and setting up Sample Queue

a. Click on Aqualog SampleQ Tool icon  to set up sample Q for samples, blanks and standards
   i. Refer to Table 1 for an idealized run of samples
b. Check the **post processing** and **export** options
   i. Confirm normalization factor is correct
   ii. Confirm all data files desired are checked for export
c. Sample Setup
   i. Number of Blank Groups should be 1 (this will be the baseline for this group of samples)
   ii. Blank Group Start # should change to 2
   iii. Samples per Blank should be equal to the number of samples you plan to run (Table 1).
      NOTE: This does not include the initial blank. You should notice that the Total Samples should equal 1 more than the samples per blank
   iv. Sample start number should be 1
d. Output File Names
   i. Leave the Blank Group Prefix ID
   ii. Leave the Blank Base as "Baseline"
   iii. Leave the Sample Prefix "Project ID" representing the samples
   iv. Verify the Output Folder is referring to the correct Output folder.
      1. C:\Users\Public\Documents\Jobin Yvon\Data\Output Files\YYYYMMDD
e. Experimental File
   i. click "Open"

ii. Confirm your experimental settings have not changed and they are set how you want them.
iii. If changes are made to experimental file, click "Save," then click OK

f. Sample Q File
i. Click "Save"

g. Click **Run** when ready

h. A pop up box will show up telling you to insert the samples

i. Prepare cuvettes for running a sample
i. Rinse cuvette 3x with MilliQ water
ii. Gently shake sample bottle to mix
iii. Rinse cuvette with sample water 3x
iv. Pour sample slowly into cuvette
v. Dry cuvette and clean with lens paper to ensure cuvette is free of spots, streaks, lint, etc.
vi. Check cuvettes prior to hitting "Continue" to make sure no air bubbles are present on cuvette, if so, tap with finger until they rise out of solution.

j. Upon completion of first four samples a pop up box will tell you to insert the next four samples prior to hitting continue.

k. When Sample run is complete open a second Aqualog window and open the workbooks to visually evaluate your initial baseline/blank, samples, standards, replicate and blanks.
i. Click "folder" icon at top to open workbooks. Navigate to the day's output folder you created earlier to find the four workbooks.
ii. Visually inspect the initial baseline, all you should see are Rayleigh lines near the bottom left corner of the EEM
iii. Visually inspect the 2 Tea standard samples EEMs. They should have a very noticeable signal at EX 248-275 and EM 315-355
iv. Visually compare the replicate samples EEM and Absorbance plots
v. Visually inspect the 2 blanks by looking at EEM and Absorbance plots
vi. Visually inspect the EEM and Absorbance plots of the sample
vii. Run the blank data through the python/R script to evaluate them compared to the DDL
viii. If blank samples, replicates and tea standards pass the evaluation, then sample data is good and you can continue running more samples

Appendix VI: 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 VI 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 VI B: BOG Data Validation SOP

Please note that BOG QAPP table references do not refer to this document but rather previous BOG QAPPs. Please use the information in the tables in the above sections.

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 VII: Signatures of Approval

Attachment 1: Chain of Custody Forms

Fiscal Year: 1314 **Project ID:** SWB_FishLk_LC_2014 **Contact Person:** Autumn Bonnema
Region: **Season:** **Phone:** 831-771-4175
Field Crew: **Date:** **email:** bonnema@mlml.calstate.edu
Mailing Address: 7544 Sandholdt Rd.
Moss Landing, CA 95039

LabSampleID	StationCode	Station Name	Species	BagID	MPSL_ID	Sample Date	Tissue THg	Tissue Se	Tissue PCBs	Tissue Ocs	Tissue MYCs	# of Containers Foil wrap in Plastic	Preservation Frozen
						TOTAL	0	0	0	0	0	0	0

Comments: Please see attached AA form for explicit instructions and data reporting format.

Samples Relinquished by:		**Samples Received by:**	
Name (Print and Sign)	**Date**	**Name (Print and Sign)**	

Attachment 2: Field Data Sheets

SWAMP Field Data Sheet (Water Chemistry & Discrete Probe) - EventType=WQ				Entered in d-base (initial/date)	Pg of Pgs
*StationID: _ _ _ _ _ _ _ _ _ _	*Date (mm/dd/yyyy): / /			*Group:	*Agency:
*Project: SWB_FishLk_LC_2014	ArrivalTime:	DepartureTime:		*SampleTime (1st sample): see below	*Protocol:
*FundingCode: 1 3 S W B G 0 1	*Personnel:		*Purpose (all applic): WaterChem Habitat Continuous		*PurposeFailure:

		*GPS/DGPS	Lat (dd.ddddd)	Long (ddd.ddddd)	OCCUPATION METHOD: Walk-in Bridge R/V _____ Other
GPS Device:		Target:		-	STARTING BANK (facing downstream): LB / RB / NA
Datum: NAD83	Accuracy (ft / m):	*Actual:		-	Point of Sample (if Integrated, then -88 in dbase)

Habitat Observations (CollectionMethod = Habitat_generic)		WADEABILITY: Y / N / Unk	BEAUFORT SCALE (see attachment):	
SITE ODOR:	None,Sulfides,Sewage,Petroleum,Smoke,Other_____			
SKY CODE:	Clear, Partly Cloudy, Overcast, Fog, Smoky, Hazy	WIND DIRECTION (from):		HYDROMODIFICATION: None, Bridge, Pipes, ConcreteChannel, GradeControl, Culvert, AerialZipline Other LOCATION (to sample): US / DS / WI / NA
OTHER PRESENCE:	Vascular,Nonvascular,OilySheen,Foam,Trash,Other___			**PHOTOS** (RB & LB assigned when facing downstream; RENAME to StationCode_yyyy_mm_dd_uniquecode) 1: (RB / LB / BB / US / DS / ##)
DOMINANT SUBSTRATE:	Bedrock, Concrete, Cobble, Gravel, Sand, Mud, Unk, Other_____			
WATERCLARITY:	Clear (see bottom), Cloudy (>4" vis), Murky (<4" vis)	PRECIPITATION:	None, Fog, Drizzle, Rain, Snow	2: (RB / LB / BB / US / DS / ##)
WATERODOR:	None, Sulfides, Sewage, Petroleum, Mixed, Other___	PRECIPITATION (last 24 hrs):	Unknown, <1", >1", None	
WATERCOLOR:	Colorless, Green, Yellow, Brown	EVIDENCE OF FIRES:	No, <1 year, <5 years	3: (RB / LB / BB / US / DS / ##)
OVERLAND RUNOFF (Last 24 hrs):	none, light, moderate / heavy, unknown			

Field Measurements (SampleType = FieldMeasure; Method = Field)

Location (circle)	Bottom Depth (m)	StartTime	EndTime	Calibration date:	latitude (both probe and grab)	longitude (both probe and grab)				
Bank OpenWater ___										
Bank OpenWater ___										
Bank OpenWater ___										
Collection Device:										

Samples Taken (# of containers filled) - Method=Water_Grab — **Field Dup YES / NO:** (SampleType = Grab / Integrated; LABEL_ID = FieldQA; create collection record upon data entry

SAMPLE TYPE: Grab / Integrated — COLLECTION DEVICE: Indiv bottle (by hand, by pole, by bucket); Teflon tubing; Kemmer; Pole & Beaker;

Location: (circle)	position	Time first sample	Collect Depth (m)	Device	Clarity	Odor	Color	ChlA (ml)	THg (250ml)	TMMHg (250ml)	DOC	Sulfate (125 ml)	
Bank OpenWater ___	Subsurface			by-hand									
Bank OpenWater ___	NearBottom			kemmerer									
Bank OpenWater ___	Subsurface			by-hand									
Bank OpenWater ___	NearBottom			kemmerer									
Bank OpenWater ___	Subsurface			by-hand									
Bank OpenWater ___	NearBottom			kemmerer									

COMMENTS:

SWAMP Field Data Sheet (Sediment Chemistry) - EventType=WQ				Entered in d-base (initial/date)	Pg of Pgs
***StationID:** ___ ___ ___ ___ ___ ___ ___ ___ ___ ___	***Date (mm/dd/yyyy):** / /			***Group:**	***Agency:**
***Project: SWB_FishLk_LC_2014**	ArrivalTime:	DepartureTime:		***SampleTime (1st sample):**	***Protocol:**
***FundingCode: 13SWBG01**	***Personnel:**		***Purpose (circle applicable):** SedChem Habitat Benthic		***PurposeFailure:**

***Location:** Bank Thalweg Midchannel OpenWater	***GPS**	**Lat (dd.ddddd)**	**Long (ddd.ddddd)**	OCCUPATION METHOD: Walk-in Bridge R/V _____ Other
GPS Device:	**Target:**		-	STARTING BANK (facing downstream): LB / RB / NA
	***Actual:**		-	Point of Sample (if Integrated, then -88 in dbase)
Datum: NAD83 Accuracy (ft / m):	Same as Water/Probe Collection? YES NO			

Habitat Observations (CollectionMethod = Habitat_generic) ****Only complete Sed Observations (bolded) if WQ Observations are already recorded**		WADEABILITY: Y / N / Unk	BEAUFORT SCALE see Attachtment
SITE ODOR:	None,Sulfides,Sewage,Petroleum,Smoke,Other_____		
SKY CODE:	Clear, Partly Cloudy, Overcast, Fog, Smoky, Hazy	WIND DIRECTION (from):	
OTHERPRESENCE:	Vascular,Nonvascular,OilySheen,Foam,Trash,Other___		
DOMINANTSUBSTRATE:	Bedrock, Concrete, Cobble, Gravel, Sand, Mud, Unk, Other_____		

HYDROMODIFICATION: None, Bridge, Pipes, ConcreteChannel, GradeControl, Culvert, AerialZipline, Other LOCATION (to sample): US / DS / WI / NA

PHOTOS (RB & LB assigned when facing downstream; RENAME to StationCode_yyyy_mm_dd_uniquecode
1: (RB / LB / BB / US / DS / ##)
2: (RB / LB / BB / US / DS / ##)
3: (RB / LB / BB / US / DS / ##)

SEDODOR:	None, Sulfides, Sewage, Petroleum, Mixed, Other____	PRECIPITATION:	None, Fog, Drizzle, Rain, Snow
SEDCOLOR:	Colorless, Green, Yellow, Brown	PRECIPITATION (last 24 hrs):	Unknown, <1", >1", None
SEDCOMPOSITION:	Silt/Clay, FineSand, CoarseSand, Gravel, Cobble, Mixed, HardPanClaEVIDENCE OF FIRES:		No, <1 years, <5 years

Samples Taken (# of containers filled) - Method=Sed_Grab

Field Dup YES / NO: (SampleType = Grab / Integrated; LABEL_ID = FieldQA; create collection record upon data entry

COLLECTION DEVICE: Scoop (SS / PC / PE, Core (SS / PC / PE), Grab (Van Veen / Eckman / Petite Ponar)

COLLECTION DEVICE AREA (m2): _____

Location: (circle)	Sample Type:	DepthCollec (cm)	CollectionTime	Equipment Used	Water Depth (m)	THg (60ml)	TOC included in THg	odor	color	composition	lat long: same as water?	Latitude	Longitude
Bank OpenWater ___	Integrated Grab										yes / no		
Bank OpenWater ___	Integrated Grab										yes / no		
Bank OpenWater ___	Integrated Grab										yes / no		

COMMENTS:

SWAMP Tissue Sampling - Non-Trawl (Event Type = TI) **SWB_FishLk_LC_2014**				Entered in d-base (initial/date)		Pg of Pgs
***StationCode:** __ __ __ __ __ __ __ __ __ __	***StationName:**				***Purpose Failure Code:**	**Agency**
***FundingCode: 1 3 S W B G 0 1**	***Date (mm/dd/yyyy):** / /					
***Sampling Crew:**	**ArrivalTime:**		**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)
	DepartureTime:					1: (RB / LB / BB / US / DS / ##)
DOMINANTSUBSTRATE: Concrete,Cobble,Gravel,Sand,Mud,Other_____,unk			WATERCOLOR: Colorless, Green, Yellow, Brown			
WATERCLARITY: Clear (see bottom), Cloudy (>4" vis), Murky (<4" vis)			OTHER PRESENCE: Vascular,Nonvascular,OilySheen,Foam,Trash,O			2: (RB / LB / BB / US / DS / ##)
Comments:						3: (RB / LB / BB / US / DS / ##)

Tissue Collection

COLLECTION DEVICE: RV _____Masta-Blasta, Big E, Sparky , Backpack Model _____, Net (length & mesh) _____

Target:	**Lat (dd.ddddd)**		**Long (dd.ddddd)**	-		
GPS Model:		Datum: **NAD83** WGS84 Other _____			*GPS / DGPS	Elevation (ft):

Location	*Depth (m):		Distance from Bank (m):		Accuracy (ft / m)	Latitude (dd.ddddd)	Longitude (-ddd.ddddd)	Depth (m)
COLLECTION METHOD:	E-boat, Backpack shocker, Fyke net, gill net, seine, hook & line		Start Time	Coord. 1				
SAMPLE LOCATION:	Bank, Thalweg, Midchannel, Open Water, NA			Coord. 2				
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Grade Control, Culvert,		End Time	Coord. 3				
HYDROMODLOC(to sample):	US / DS / NA/ WI	Other _____ Geoshape: Line Poly Point		Coord. 4				
Location	*Depth (m):		Distance from Bank (m):			Latitude (dd.ddddd)	Longitude (-ddd.ddddd)	Depth (m)
COLLECTION METHOD:	E-boat, Backpack shocker, Fyke net, gill net, seine, hook & line		Start Time	Coord. 1				
SAMPLE LOCATION:	Bank, Thalweg, Midchannel, Open Water, NA			Coord. 2				
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Grade Control, Culvert,		End Time	Coord. 3				
HYDROMODLOC(to sample):	US / DS / NA/ WI	Other _____ Geoshape: Line Poly Point		Coord. 4				
Location	*Depth (m):		Distance from Bank (m):			Latitude (dd.ddddd)	Longitude (-ddd.ddddd)	Depth (m)
COLLECTION METHOD:	E-boat, Backpack shocker, Fyke net, gill net, seine, hook & line		Start Time	Coord. 1				
SAMPLE LOCATION:	Bank, Thalweg, Midchannel, Open Water, NA			Coord. 2				
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Grade Control, Culvert,		End Time	Coord. 3				
HYDROMODLOC(to sample):	US / DS / NA/ WI	Other _____ Geoshape: Line Poly Point		Coord. 4				

Failure Codes: Dry (no water), Instrument Failure, No Access, Non-sampleable, Pre-abandoned, Other

Comments:

Modified 06/08/07

SWAMP Tissue Sampling - Non-Trawl (Event Type = TI) **SWB_FishLk_LC_2014**		Entered in d-base (initial/date)	Pg of Pgs
***StationCode:** ___ ___ ___ ___ ___ ___ ___ ___ ___	*StationName:	*Purpose Failure Code:	**Agency**
***FundingCode:** _1_ _3_ _S_ _W_ _B_ _G_ _0_ _1_	*Date (mm/dd/yyyy): / /		

Tissue Collection

Location	*Depth (m):		Distance from Bank (m):			Accuracy (ft / m)	Latitude (dd.ddddd)	Longitude (-ddd.ddddd)	Depth (m)
COLLECTION METHOD:	E-boat, Backpack shocker, Fyke net, gill net, seine, hook & line			Start Time	Coord. 1				
SAMPLE LOCATION:	Bank, Thalweg, Midchannel, Open Water, NA				Coord. 2				
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Grade Control, Culvert,			End Time	Coord. 3				
HYDROMODLOC(to sample):	US / DS / NA / WI	Other ________	Geoshape: Line Poly Point		Coord. 4				
Location	*Depth (m):		Distance from Bank (m):				Latitude (dd.ddddd)	Longitude (-ddd.ddddd)	Depth (m)
COLLECTION METHOD:	E-boat, Backpack shocker, Fyke net, gill net, seine, hook & line			Start Time	Coord. 1				
SAMPLE LOCATION:	Bank, Thalweg, Midchannel, Open Water, NA				Coord. 2				
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Grade Control, Culvert,			End Time	Coord. 3				
HYDROMODLOC(to sample):	US / DS / NA / WI	Other ________	Geoshape: Line Poly Point		Coord. 4				
Location	*Depth (m):		Distance from Bank (m):				Latitude (dd.ddddd)	Longitude (-ddd.ddddd)	Depth (m)
COLLECTION METHOD:	E-boat, Backpack shocker, Fyke net, gill net, seine, hook & line			Start Time	Coord. 1				
SAMPLE LOCATION:	Bank, Thalweg, Midchannel, Open Water, NA				Coord. 2				
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Grade Control, Culvert,			End Time	Coord. 3				
HYDROMODLOC(to sample):	US / DS / NA / WI	Other ________	Geoshape: Line Poly Point		Coord. 4				
Location	*Depth (m):		Distance from Bank (m):				Latitude (dd.ddddd)	Longitude (-ddd.ddddd)	Depth (m)
COLLECTION METHOD:	E-boat, Backpack shocker, Fyke net, gill net, seine, hook & line			Start Time	Coord. 1				
SAMPLE LOCATION:	Bank, Thalweg, Midchannel, Open Water, NA				Coord. 2				
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Grade Control, Culvert,			End Time	Coord. 3				
HYDROMODLOC(to sample):	US / DS / NA / WI	Other ________	Geoshape: Line Poly Point		Coord. 4				
Location	*Depth (m):		Distance from Bank (m):				Latitude (dd.ddddd)	Longitude (-ddd.ddddd)	Depth (m)
COLLECTION METHOD:	E-boat, Backpack shocker, Fyke net, gill net, seine, hook & line			Start Time	Coord. 1				
SAMPLE LOCATION:	Bank, Thalweg, Midchannel, Open Water, NA				Coord. 2				
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Grade Control, Culvert,			End Time	Coord. 3				
HYDROMODLOC(to sample):	US / DS / NA / WI	Other ________	Geoshape: Line Poly Point		Coord. 4				
Location	*Depth (m):		Distance from Bank (m):				Latitude (dd.ddddd)	Longitude (-ddd.ddddd)	Depth (m)
COLLECTION METHOD:	E-boat, Backpack shocker, Fyke net, gill net, seine, hook & line			Start Time	Coord. 1				
SAMPLE LOCATION:	Bank, Thalweg, Midchannel, Open Water, NA				Coord. 2				
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Grade Control, Culvert,			End Time	Coord. 3				
HYDROMODLOC(to sample):	US / DS / NA / WI	Other ________	Geoshape: Line Poly Point		Coord. 4				

Failure Codes: Dry (no water), Instrument Failure, No Access, Non-sampleable, Pre-abandoned, Other

Comments:

Modified 06/08/07

SWAMP Tissue Sampling - Non-Trawl (Event Type = TI) **SWB_FishLk_LC_2014**

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)	StdL (mm)	Weight (g)	Count	Count Est.	Sex	Anomaly	Condition
										M F U L		
										M F U L		
										M F U L		
										M F U L		
										M F U L		
										M F U L		
										M F U L		
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										M F U L		
										M F U L		
										M F U L		
										M F U L		

Location #: Match fish with Location # from Tissue Collection sheet

Organism ID: Combine composite # and fish # (e.g., fish 1 of composite WC01 is WC01-01) to be uni

Tag #: Use if applicable

Species Code: Largemouth Bass (**LMB**), Smallmouth Bass (**SMB**), Spotted Bass **(SPB),**Sacramento Pike Minnow **(SPM)**, Rainbow Trout **(RT),** Brown Trout **(BT),** Brook Trout **(BKT)**, White Catfish **(WC),** Carp **(CAR),** Channel Catfish **(CC)**, Brown Bullhead **(BRB)**, Sacramento Sucker **(SS)**, Redear **(RES)**, Black Crappie (**CRP**), Bluegill (**BG**), Tilapia **(TIL)**, Green Sunfish **(GRS)**, Kokanee **(KOK)**

Stage: Adult (A), Juvenile (J), Subadult (SA), Not Recorded (NR)

Count Est: If appropriate, add < or > if count is e

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), Popeye (PE), Tumor (T), Ulceration (U), White Spots (W), and any combination

Sex:unk(U),taken at Lab(L)

BodyLocation: Branchial Chamber(BRC), Buccal Cavity(BC), Eyes(E), Musculoskeleton(M), Skin/Fins(SF)

Comments: Mark fish requiring further ID; **SEPARATE FISH BY LOCATION AND INDICATE LOCATION # ON LABEL**

Modified 06/19/07

Attachment 3: Analysis Authorization Forms

Analysis Authorization
Fiscal Year: 1314
Region:

Project ID: SWB_FishLk_LC_2014
Season:
Date:

Contact Person: Autumn Bonnema
Phone: 831-771-4175
email: bonnema@mlml.calstate.edu
Mailing Address: 7544 Sandholdt Rd.
Moss Landing, CA 95039

				Analyze Tissue Flesh							
Station	StationName	OrganismName	CompositeIDText	Ind Hg	Comp Hg	Comp Se	Ind Se	PCBs	OCs	MYCs	Age
Total				0	0	0	0	0	0	0	0

Attachment 4: Laboratory Data Sheets

SWAMP Lab Data Sheet - FISH	ProjectID: SWB_FishLk_LC_2014	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: Rainbow Trout		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												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												
24												
25												

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

SWAMP Lab Data Sheet - FISH	ProjectID: **SWB_FishLk_LC_2014**	PrepPres: Skin OFF	LabID:	Pg: 2 of 2 Pgs
StationCode:	Tissue: fillet		Entered d-base (initial/date)	
StationName:	Homog. Method: BUCCHI POLYTRON OTHER_____		Staff: Diss. Homog.	
Species Name: Rainbow Trout	Date Diss. (mm/dd/yyyy): / /		Date Homog. (mm/dd/yyyy): / /	

CHEMISTRY JARS

CompositeID:	**CompositeID**:	**CompositeID**:
Analysis: WPCL Organics	**Analysis:** Mercury and Selenium	**Analysis:** Short Term Archive 1
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):
		Glass 30g
CompositeID:	**CompositeID**:	
Analysis: Short Term Archive 2	**Analysis:** Short Term Archive 3 (PFAs)	
Jar Weight Full (g):	Jar Weight Full (g):	
Jar Weight Empty (g):	Jar Weight Empty (g):	
Comp Tissue Wt (Jar Full - Empty; g):	Comp Tissue Wt (Jar Full - Empty; g):	
Glass 30g	**Plastic 30g**	

Comments: Keep archive tissue if possible; If a duplicate is made, use Dup ID as identification for analysis

SWAMP Lab Data Sheet - FISH	ProjectID: **SWB_FishLk_LC_2014**	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: Largemouth Bass		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												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												
24												
25												

OrganismID: xxxxxxxxLLXX##YYYzz-ZZ; unique code - StationCode (xxxxxxxx), 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_FishLk_LC_2014**	PrepPres: Skin OFF	LabID:	Pg: 2 of 2 Pgs
StationCode:	Tissue: fillet		Entered d-base (initial/date)	
StationName:	Homog. Method: BUCCHI POLYTRON OTHER_____		Staff: Diss.	Homog.
Species Name: Largemouth Bass	Date Diss. (mm/dd/yyyy): / /		Date Homog. (mm/dd/yyyy): / /	

CHEMISTRY JARS

CompositeID:	**CompositeID**:	**CompositeID**:
Analysis: WPCL Organics	**Analysis:** Selenium	**Analysis:** Short Term Archive 1
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):
		Glass 30g
CompositeID:	**CompositeID**:	
Analysis: Short Term Archive 2	**Analysis:** Short Term Archive 3 (PFAs)	
Jar Weight Full (g):	Jar Weight Full (g):	
Jar Weight Empty (g):	Jar Weight Empty (g):	
Comp Tissue Wt (Jar Full - Empty; g):	Comp Tissue Wt (Jar Full - Empty; g):	
Glass 30g	**Plastic 30g**	
Individual ID:	**Individual ID**:	**Individual ID**:
Analysis: Mercury	**Analysis:** Mercury	**Analysis:** Mercury
Jar Weight Full (g):	Jar Weight Full (g):	Jar Weight Full (g):
Jar Weight Empty (g):	Jar Weight Empty (g):	Jar Weight Empty (g):
Comp Tissue Wt (Jar Full - Empty; g):	Comp Tissue Wt (Jar Full - Empty; g):	Comp Tissue Wt (Jar Full - Empty; g):
Individual ID:	**Individual ID**:	
Analysis: Mercury	**Analysis:** Mercury	
Jar Weight Full (g):	Jar Weight Full (g):	
Jar Weight Empty (g):	Jar Weight Empty (g):	
Comp Tissue Wt (Jar Full - Empty; g):	Comp Tissue Wt (Jar Full - Empty; g):	

Comments: Keep archive tissue if possible; If a duplicate is made, use Dup ID as identification for analysis

Modified 06/08/07

SWAMP Lab Data Sheet - FISH	ProjectID: **SWB_FishLk_LC_2014**	PrepPres: Skin ON	LabID:	Pg: 1 of 2 Pgs
StationCode:		Tissue: whole	Entered d-base (initial/date)	
StationName:		Homog. Method: BUCCHI POLYTRON OTHER_____	Staff: Diss. Homog.	
Species Name: Threadfin Shad		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												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												
24												
25												

OrganismID: xxxxxxxxLLXX##YYYzz-ZZ; unique code - StationCode (xxxxxxxx), 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

SWAMP Lab Data Sheet - FISH	ProjectID: **SWB_FishLk_LC_2014**	PrepPres: Skin ON	LabID:	Pg: 2 of 2 Pgs
StationCode:	Tissue: whole		Entered d-base (initial/date)	
StationName:	Homog. Method: BUCCHI POLYTRON OTHER_____		Staff: Diss. Homog.	
Species Name: Threadfin Shad	Date Diss. (mm/dd/yyyy): / /		Date Homog. (mm/dd/yyyy): / /	

CHEMISTRY JARS

CompositeID:	**CompositeID**:	**CompositeID**:
Analysis: USGS-WRD Selenium	**Analysis:** USGS-FRESC Mercury	**Analysis:** Short Term Archive 1
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):
		Glass 30g
CompositeID:	**CompositeID**:	
Analysis: Short Term Archive 2	**Analysis:** Short Term Archive 3 (PFAs)	
Jar Weight Full (g):	Jar Weight Full (g):	
Jar Weight Empty (g):	Jar Weight Empty (g):	
Comp Tissue Wt (Jar Full - Empty; g):	Comp Tissue Wt (Jar Full - Empty; g):	
Glass 30g	**Plastic 30g**	

Comments: Keep archive tissue if possible; If a duplicate is made, use Dup ID as identification for analysis