

Westside San Joaquin River Watershed Coalition

Quality Assurance Project Plan For Monitoring

**Designed for the Irrigated Lands Regulatory Program
Central Valley Regional Water Quality Control Board**

Prepared By:

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Revision 2

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Quality Assurance Project Plan For Monitoring for the Westside San Joaquin River Watershed Coalition

Revision 2

A. PROJECT MANAGEMENT

1. Approval Sheet

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3. Distribution List

Primary Distribution List for the Westside San Joaquin River Watershed Coalition Quality Assurance Project Plan

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4. Project Organization and Responsibility

The San Joaquin Valley Drainage Authority (SJVDA or Drainage Authority) is the umbrella organization for the Westside San Joaquin River Watershed Coalition (Westside Coalition or WSJRWC) for purposes of the Conditional Waiver of Waste Discharge Requirements for Discharges from Irrigated Lands Order No. R5-2006-0053 (Conditional Waiver). The Drainage Authority is a joint powers agency, and includes the Regional Water Quality Management Activity (RWQMA) subgroup. A Steering Committee, comprised of one representative per participating agency (defined below), provides budgeting and policy direction and makes recommendations for action to the Drainage Authority Board.

The Westside Coalition is comprised of the lands within the Del Puerto Water District, Patterson Irrigation District, the San Joaquin River Exchange Contractors Water Authority (which includes the Central California Irrigation District, San Luis Canal Company, Henry Miller Reclamation District, Firebaugh Canal Water District, and Columbia Canal Company), Tranquility Irrigation District/Fresno Slough Water District, Twin Oaks Irrigation District, West Stanislaus Irrigation District, Oak Flat Water District, El Solyo Water District, San Luis Water District, Stevenson Water District, White Lake Mutual Water Company, Lone Tree Mutual Water Company, Turner Island Water District, Grassland Water District/Grassland Resource Conservation District, State Refuges managed by the California Department of Fish and Game, and Federal Refuges managed by the US Fish & Wildlife Service. In addition, the Grassland Drainage Area is a cooperating participant in the Westside Coalition. Each of the above agencies is acting on behalf of the lands located within its boundaries and will engage its individual landowners and operators in the Westside Coalition's program for the monitoring requirements specified in the Monitoring and Reporting Program Order No. R5-2008-0831(MRP Order in further text) under the Conditional Waiver.

The RWQMA prepared budgets for the Westside Coalition to respond to the Conditional Waiver activities and developed a budget for implementing the monitoring program. Member agencies have committed to raise the required funds through their own budgets, which in turn are funded by collections from all of the landowners/operators within their boundaries. They have also agreed to continue to provide in-kind services such as data collection on existing best management practices and to distribute information on any needed changes in those practices to improve water quality.

The Westside Coalition monitoring program is managed by Summers Engineering, Inc. The Watershed Coordinator is Joseph McGahan of Summers Engineering. Mr. McGahan consults with the Westside Coalition and Central Valley Regional Water Quality Control Board (Central Valley Water Board) regarding the management of the program, submits reports to the Central Valley Water Board, and provides direction to the Technical Program Manager/QA Officer and Monitoring Program Coordinator regarding the implementation of the monitoring and reporting requirements. The project Technical Program Manager/Quality Assurance Officer is Chris Linneman of Summers Engineering. Mr. Linneman reviews all laboratory data for compliance with the QA/QC requirements specified in this QAPP, manages the electronic database for the program, and generates reports for submittal to the Central Valley Water Board. Stephen Clark of Pacific EcoRisk serves as the Monitoring Program Coordinator. Mr. Clark maintains the

official Quality Assurance Project Plan (QAPP), manages the generation of the field logs (and related documentation), coordinates sampling with the field crews, coordinates with analytical laboratories regarding sample analyses, submits reports and electronic data deliverables for toxicity testing, and manages the subcontracts with the analytical labs. Each laboratory selected to perform the analyses for this program has a QA Officer/Manager responsible for assuring that the testing is performed in accordance with the requirements specified in this QAPP.

This QAPP describes the quality assurance requirements for the Westside Coalition monitoring program; details on the program are available in the approved 2008 Westside San Joaquin River Watershed Coalition Monitoring and Reporting Plan (MRP in further text). APPL Inc., Caltest Laboratories, and Pacific EcoRisk have been contracted to analyze the Westside Coalition samples. Each laboratory selected to perform the analyses for this program has a QA Officer/Manager responsible for assuring that the testing is performed in accordance with the requirements specified in this QAPP. The organizations selected to perform the sampling and laboratory analyses have provided the precision, accuracy, detection and reporting limits in this QAPP, and meet the quality control criteria necessary to satisfy the data quality objectives of this program.

The organizational structure of the Westside Coalition monitoring program is illustrated in Figure A-1.

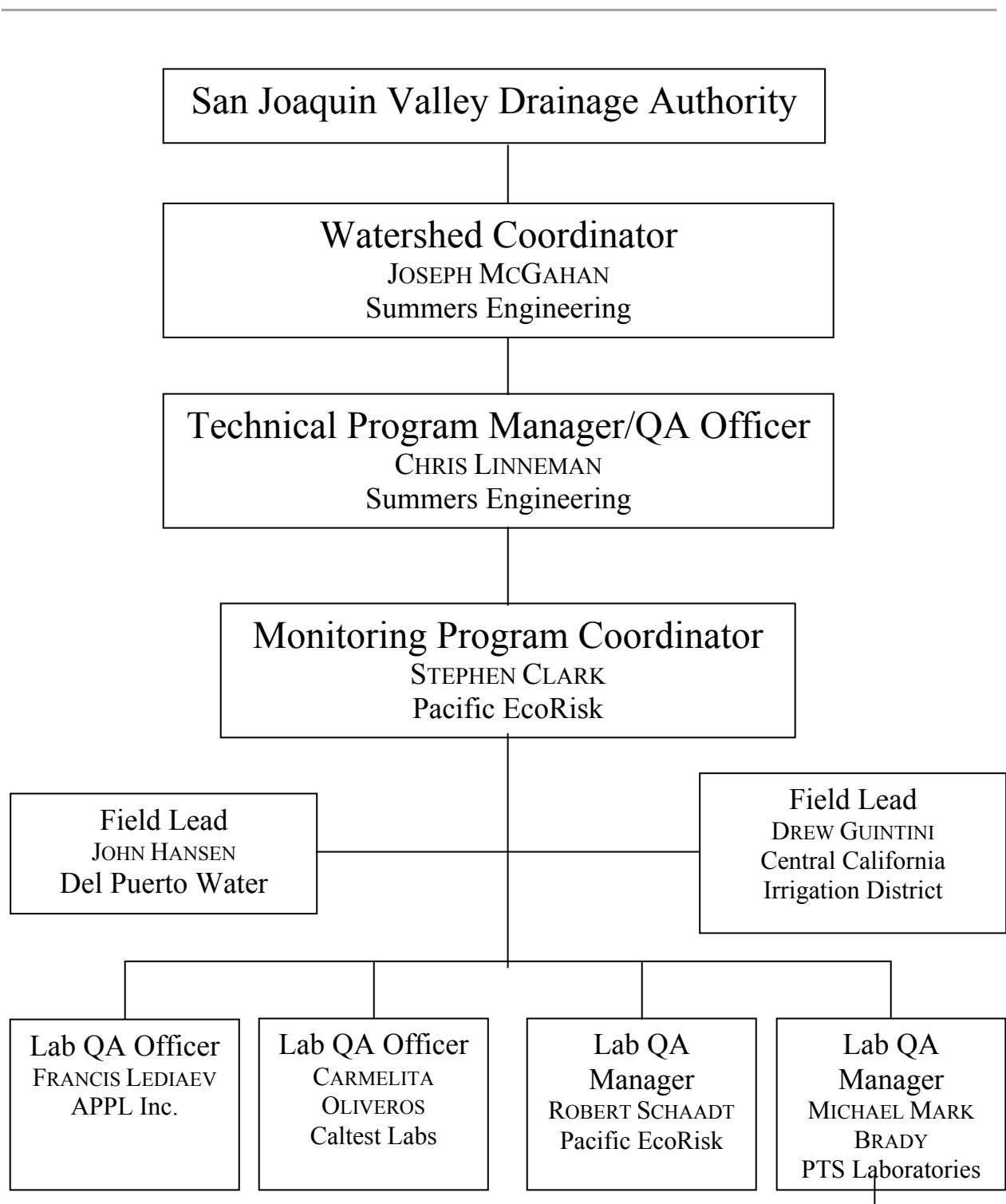


Figure A-1. Westside San Joaquin River Watershed Coalition Monitoring Program Management Structure

5. Problem Definition/Background

The Westside Coalition has reviewed historical data to determine problem areas, identify current water quality conditions, and determine future monitoring needs. The monitoring program is envisioned to be a multi-year effort that will provide information to promote the understanding of conditions in the watershed, improve water quality, and to assess the relative health of the watershed. The monitoring program will be a dynamic activity that will change over time as information is accumulated and new information needs are identified.

It is the objective of the Westside Coalition to coordinate with existing monitoring efforts so that the data generated by the various program are complementary and not duplicative. In addition to the work performed by the Westside Coalition, the region has a long history of water quality monitoring studies for a variety of constituents. Sampling has been conducted on water quality and chemistry, toxicity, and benthic macroinvertebrate communities by several agencies and academic institutions including:

- the Central Valley Regional Water Quality Control Board (CVRWQCB or Central Valley Water Board)
- the California Department of Pesticide Regulation (DPR)
- the California Department of Water Resources (DWR)
- the Bureau of Reclamation, and
- the U.S. Geological Survey (USGS)

Constituents monitored by these agencies include organophosphate (OP) pesticides, metals, drinking water constituents, nutrients, and dissolved oxygen (D.O.). An overwhelming majority of programs have monitored for OP pesticides. The Coalition watershed area will continue to be monitored as part of CVRWQCB programs such as the Total Maximum Daily Load (TMDL) monitoring program, and the Surface Water Ambient Monitoring Program (SWAMP). The aforementioned programs are not designed to address the requirements of the Conditional Waiver program, but may provide data useful for interpreting Westside San Joaquin River Watershed Coalition results. Other programs and locations are associated with monitoring storm water runoff from urban areas or transportation corridors and are not relevant when addressing runoff from irrigated agriculture.

For the purposes of the Conditional Waiver of Waste Discharge Requirements for Discharges from Irrigated Lands, the Westside Coalition is required to conduct water quality monitoring within the area defined in the Westside San Joaquin River Watershed Coalition Watershed Evaluation Report (submitted April 1, 2004). The Monitoring Program is structured to provide representative data on all of the sub-watersheds within the Westside Coalition while maintaining a cost effective and flexible program. The site selection strategy is based on three underlying objectives. The first objective is to achieve the characterization of discharge from irrigated agricultural lands as specified in the Conditional Waiver. The second objective is to maximize the geographic coverage of the characterization over the coalition region. The final objective is to be able to demonstrate improvement in water quality after implementation of BMPs in the Coalition region. During the development of the Monitoring Program, the previous three years of collected data were reviewed to determine where water quality issues existed, where resources should be expended, and how the Monitoring Program structure should be further refined.

The overall goal of the monitoring, as stated in the Westside Coalition's 2008 Monitoring and Reporting Plan, are to:

- build on the past three years of water quality monitoring generated through the first monitoring program of the Westside Coalition;
- continue characterizing the water quality conditions in the Westside San Joaquin River Watershed Coalition Watershed;
- track water quality as the Management Plan is implemented; and
- meet the requirements specified in the Central Valley Water Board Monitoring and Reporting Program (Westside Coalition Order No. R5-2008-0831) for the Conditional Waiver for Coalition Groups (Order No. R5-2006-0053).

These goals will be addressed through the evaluation of Westside Coalition data against the Basin Plan objectives for the San Joaquin River, applicable water quality criteria, and/or action limits listed in Appendix A. Exceedance reports will be submitted to the Central Valley Water Board if water quality objectives/criteria are exceeded at the monitoring sites. The Technical Program Manager will determine if follow-up monitoring or analyses are required or if other actions should be taken by the Westside Coalition to address an exceedance. Management Plans are required when more than one exceedance of the same constituent has occurred at a given site within a period of three years, and will be implemented per approved General Approach and Focused Watershed Management Plans;.

6. Project Description

The Monitoring Program is structured to account for the type of waterbody being monitored, amount of historical data, seasonal irrigation influence, and constituents to be analyzed.

Monitoring Site Groups

The monitoring sites are identified in Figure A-2 and Table A-1; latitude and longitude coordinates are provided in Table 1 of the MRP Order No. R5-2008-0831 (MRP Order, page 4, Table 1). Photoreconnaissance was performed at the beginning of the Westside Coalition site scoping in 2004, along with GPS coordinate recording. The sampling sites have been well documented, sampling staff has been consistent, and there have been no deviations from the established sampling sites.

Sites have been designated either "Source Water Sites" or "Discharge Sites" according to what type of water is conveyed through the site. Sites that characterize water that is primarily used for irrigation carry the "Source Water" designation. Sites that convey agricultural drainage water are designated as "Discharge Sites". The site designation will determine which constituent analyses will be performed at a given site (Tables A-3 through A-5).

Monitoring Season

The constituents analyzed at a given site are controlled, to a degree, by the time of year (or season) during which the sample is collected. Site conditions are documented on the field log data sheets. Table A-1 shows the type of monitoring performed at each monitoring site during each monitoring season. Monitoring is evaluated and strategy/sites are adjusted every three years.

Figure A- 2. Westside Coalition Monitoring Program Sampling Sites

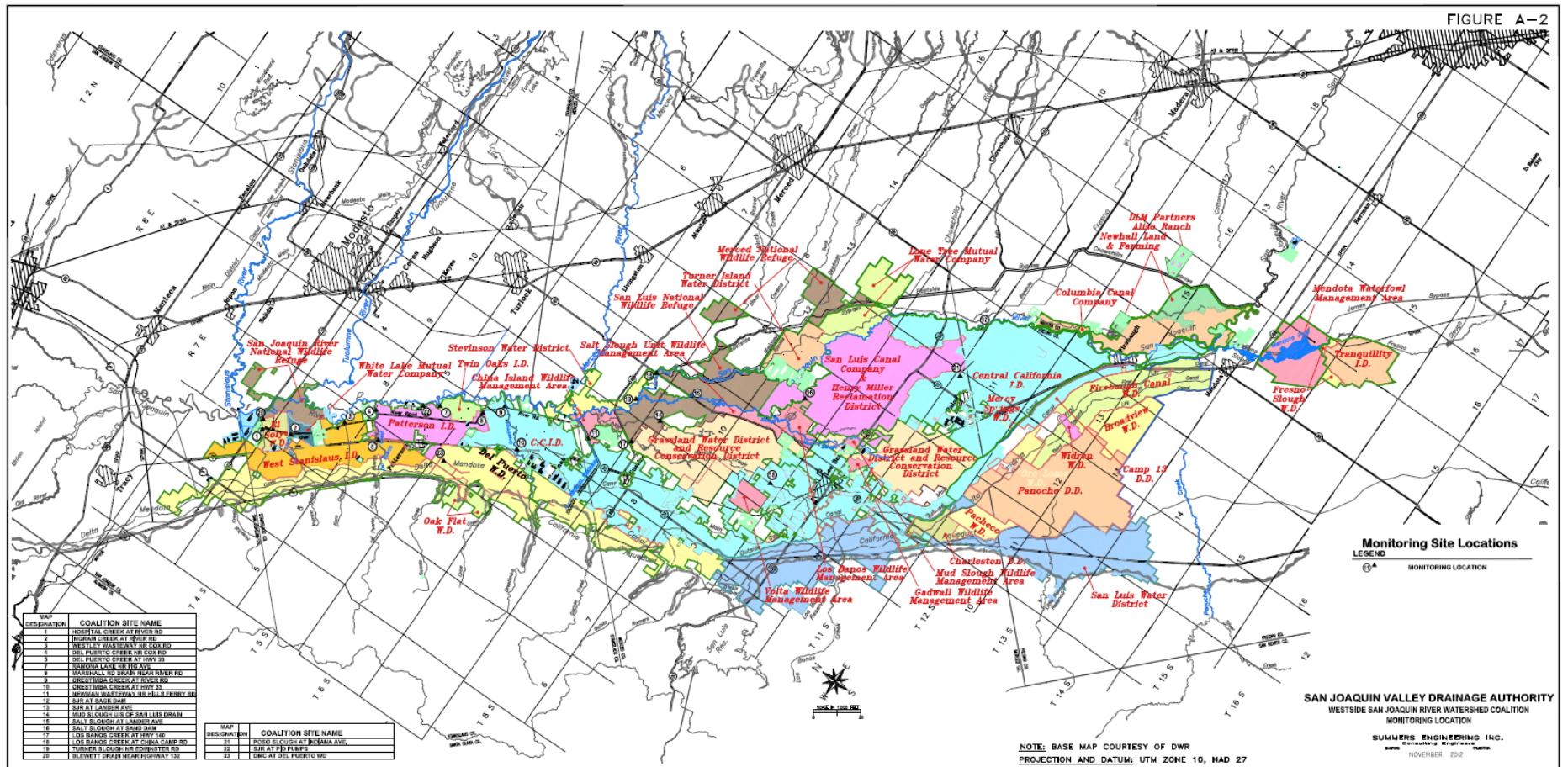


Table A- 1. Monitoring Sites and Special Study Constituents to be Analyzed for the Westside Coalition Monitoring Program
March 2012 through February 2014

Monitoring Site	Site Code	Season			Site-Specific Assessment Group Testing								
		Irrigation (Mar-Aug)*	Non-Irrigation (Sep-Feb)*	Rain Event 2x/year	Cerio Toxicity	Fathead Toxicity	Algae Toxicity	Sediment Toxicity	Metals	Pesticides			
										OP	OC	Carb	Herb
Discharge Sites													
Blewett Drain at Highway 132	VH132	Special	Core	Rain**	X			X		X	X		X
Poso Slough at Indiana Avenue	PSAIA	Special	Core	Rain**	X		X	X	X	X	X	X	X
Hospital Creek at River Road	HCARR	Special	-	Rain**	X		X	X	X	X		X	X
Ingram Creek at River Road	ICARR	Core + Special	Core	Rain**	X		X	X	X	X	X	X	X
Westley Wasteway near Cox Road	WWNCR	Core + Special	Core	Rain**	X		X	X	X	X	X		X
Del Puerto Creek near Cox Road	DPCCR	Core + Special	Core	Rain**	X			X		X	X	X	X
Del Puerto Creek at Highway 33	DPCHW	Special	-	Rain**	X			X		X	X	X	X
Ramona Lake near Fig Avenue	ROLFA	Core + Special	Core	Rain**	X			X		X	X		X
Marshall Road Drain near River Road	MRDRR	Core + Special	Core	Rain**	X					X	X		X
Orestimba Creek at River Road	OCARR	Core + Special	Core	Rain**	X		X	X	X	X	X	X	X
Orestimba Creek at Highway 33	OCAHW	Special	-	Rain**	X		X	X	X	X	X	X	X
Newman Wasteway near Hills Ferry Rd	NWHFR	Core + Special	Core	Rain**	X		X	X		X	X		X
San Joaquin River at Lander Avenue	SJRLA	Core + Special	Core + Special	Rain**	X					X			X
Mud Slough u/s San Luis Drain	MSUSL	Core + Special	Core + Special	Rain**	X					X	X	X	X
Salt Slough at Lander Avenue	SSALA	Core + Special	Core + Special	Rain**	X					X	X	X	X
Salt Slough at Sand Dam	SSASD	Special	-	Rain**	X			X		X		X	X
Los Banos Creek at Highway 140	LBCHW	Core + Special	Core+Special	Rain**	X		X		X	X			X
Los Banos Creek at China Camp Road	LBCCC	Core + Special	Core	Rain**	X	X	X	X	X	X			X
Turner Slough near Edminster Road	TSAER	Core + Special	Core	Rain**	X	X				X			X
Source Water Sites													
San Joaquin River at Sack Dam	SJRSD	Source	Source	Source						X			
Delta Mendota Canal at Del Puerto WD	DMCDP	Source	Source	Source						X			
San Joaquin River at PID Pumps	SJRPP	Source	Source	Source						X			

Note: Every third year, assessment monitoring is performed at all sites and sampled constituents may be adjusted. See MRP Order, page 5.

* Irrigation season is defined as March through August. Non-irrigation season is defined as September through February. The Westside Coalition, in collaboration with the Central Valley Water Board, may shift the seasons forward or backward 1 month to account for actual irrigation practices. Tabulated designations during irrigation and non-irrigation seasons are for periods from March 2009 through February 2011, and from March 2012 through February 2014. All sites are monitored following the assessment site schedule from March 2011 through February 2012, and from March 2014 through February 2015.

** During rain event sample collection, Discharge sites will be sampled for the constituents listed under the "Rain Event" category as described in Monitoring and Reporting Program Order No. R5-2008-0831.

Irrigation Season

The majority of the land within the Westside Coalition is irrigated agriculture that is actively farmed only in the spring and summer. This is the time of year during which most of the pesticides and other constituents of concern are applied. Irrigation season sampling will occur at all discharge sites from March through August, which has typically been the irrigation season run for this region. The Westside Coalition may shift Irrigation Season sampling up or back one month to account of seasonal variations. The Central Valley Water Board will be notified before this shift is enacted. Pesticide analyses and aquatic toxicity testing will be performed during irrigation season sampling. There are four sites that can carry agricultural runoff during the summer months and wetland irrigation runoff during the winter months (Los Banos Creek at Highway 140, Salt Slough at Lander Ave., the San Joaquin River at Lander Avenue, and Mud Slough upstream of the San Luis Drain). These four sites have the potential to carry pesticides all year and will be tested for toxicity and pesticides (MRP, page 8).

Non-Irrigation Season

The non-irrigation season is the period outside of the irrigation season, typically September through February. Discharge occurring during these months will be typically caused by activities other than irrigated agriculture. Physical and general chemical water quality monitoring will continue during the non-irrigation season, however most sites will not be tested for toxicity or pesticides.

Monitoring Strategy

The Monitoring Strategy consists of Core Monitoring for tracking of trends, Assessment Monitoring for characterizing water quality conditions including Rain, Special Study Monitoring components, and Source Monitoring (Tables A-2 through A-4).

Core Monitoring

Samples collected during the non-irrigation season for Core Monitoring will be analyzed for field measurements, general chemistry analyses, drinking water analyses, and nutrient analyses. These constituents will be consistent at all sites where this category is collected. Core monitoring does not include water column toxicity, pesticides or metals analyses.

Assessment Monitoring

Samples collected during Assessment Monitoring will include all of the analyses performed during the Core Monitoring, as well as toxicity testing for the three indicator species, pesticide analyses, and metal analyses. Assessment sample collection will occur at all sites every third year of monitoring (beginning March 2011, beginning March 2014, and so on) during the irrigation season.

Rain Monitoring

The Westside Coalition will attempt to collect runoff caused by storm events twice each year at all Core and Assessment monitoring sites. Rain event samples will be collected once enough rainfall has occurred to cause the majority of the flow at a monitoring site to consist of rain runoff; this will be determined by the field sampling crews on a site-by-site basis. Samples collected during the rain events will be analyzed for all of the constituents under the Core category, as well as aquatic toxicity testing for the three indicator species and pesticide analyses for the four pesticide categories for all discharge sites.

Special Study Monitoring

Special study monitoring sites and constituents are identified in the Monitoring and Reporting Program Order No. R5-2008-0831 (pages 11 through 14). Note that the sites and constituents undergoing special study monitoring may be updated periodically as the Management Plan evolves. Special Study sample collection will typically occur during the irrigation season for most sites.

Source Monitoring

Samples collected from source water sites will be used to evaluate potential contributions of a variety of field, general chemistry, drinking water, and metal constituents as well as for OP pesticides by the source irrigation water.

Table A-2, A-3, and A-4 show the chemistry, pesticide, and toxicity tests, respectively, performed during each type of monitoring.

So as to minimize resource/time (i.e., sampling staff) constraints, the sampling is targeted for the second Tuesday (and Wednesday, if necessary) of each month; this allows for the sampling staff to schedule activities around the targeted sampling dates. In an emergency, the sampling can be rescheduled later during the month.

Table A-2. Field Measurements, Drinking Water, General Physical, Metals, and Nutrients Analyzed for the Westside Coalition

Category	Monitoring Constituent	Matrix	Assessment	Core	Rain Event	Source
Field Measurements	Flow (cfs)	Water	X	X	X	X
	Photo Documentation ⁽¹⁾	Site	X	X	X	X
	Specific Conductivity (µS/cm)	Water	X	X	X	X
	Temperature (°C)	Water	X	X	X	X
	pH	Water	X	X	X	X
	Dissolved Oxygen (mg/L)	Water	X	X	X	X
Drinking Water	Bromide	Water	X	X	X	
	Dissolved Organic Carbon	Water	X	X	X	
	<i>E. coli</i>	Water	X	X	X	X
	Total Organic Carbon	Water	X	X	X	
General Physical	Hardness (as CaCO ₃)	Water	X	X	X	X
	Total Dissolved Solids	Water	X	X	X	X
	Total Suspended Solids	Water	X	X	X	X
	Turbidity	Water	X	X	X	X
Metals	Arsenic (total)	Water	X		X	
	Boron (total)	Water	X		X	X
	Cadmium (total and dissolved)	Water	X		X	
	Copper (total and dissolved)	Water	X		X	X
	Lead (total and dissolved)	Water	X		X	
	Nickel (total and dissolved)	Water	X		X	X
	Selenium (total)	Water	X		X	
	Zinc (total and dissolved)	Water	X		X	X
Nutrients	Ammonia (as N)	Water	X	X	X	
	Nitrogen, Nitrate-Nitrite	Water	X	X	X	
	Total Kjeldahl Nitrogen	Water	X	X	X	
	Total Phosphate as P	Water	X	X	X	
	Orthophosphate as P (soluble)	Water	X	X	X	
Toxicity	<i>Selenastrum capricornutum</i>	Water	X		X	
	<i>Ceriodaphnia dubia</i> (acute)	Water	X		X	
	<i>Pimephales promelas</i> (acute)	Water	X		X	

1 – One photo is taken per site (per event) and GPS coordinates are pre-printed in the field logs; field teams are not required to record GPS coordinates.

Table A- 3. Pesticides Analyzed for the Westside Coalition

Class	Monitoring Constituent	Matrix	Assessment	Core	Rain Event	Source
Organophosphate Pesticides	Azinphosmethyl	Water	X		X	X
	Chlorpyrifos	Water	X		X	X
	Demeton-S	Water	X		X	X
	Diazinon	Water	X		X	X
	Dichlorovos	Water	X		X	X
	Dimethoate	Water	X		X	X
	Disulfoton	Water	X		X	X
	Malathion	Water	X		X	X
	Methidathion	Water	X		X	X
	Methamidaphos	Water	X		X	X
	Parathion, ethyl	Water	X		X	X
	Parathion, methyl	Water	X		X	X
	Phorate	Water	X		X	X
	Phosmet	Water	X		X	X
	EPTC	Water	X		X	X
Herbicides	Atrazine	Water	X		X	
	Cyanazine	Water	X		X	
	Diuron	Water	X		X	
	Linuron	Water	X		X	
	Pendimethalin (Prowl)	Water	X		X	X
	Simazine	Water	X		X	
	Trifluralin	Water	X		X	X
Additional Group A Organochlorine Pesticides	Aldrin	Water	X		X	
	HCH, alpha (listed in the Order as a-BHC)	Water	X		X	
	HCH, beta (listed in the Order as b-BHC)	Water	X		X	
	HCH, delta (listed in the Order as d-BHC)	Water	X		X	
	HCH, gamma (listed in the Order as g-BHC)	Water	X		X	
	Chlordane, cis	Water	X		X	
	Chlordane, trans	Water	X		X	
	Endosulfan I	Water	X		X	
	Endosulfan II	Water	X		X	
	Endosulfan Sulfate	Water	X		X	
	Heptachlor	Water	X		X	
	Heptachlor epoxide	Water	X		X	
	Toxaphene	Water	X		X	

Table A- 3. Pesticides Analyzed for the Westside Coalition (continued)

Class	Monitoring Constituent	Matrix	Assessment	Core	Rain Event	Source
Organochlorine Pesticides (Base)	Dicofol	Water	X		X	
	DDD (p,p')	Water	X		X	
	DDE (p,p')	Water	X		X	
	DDT (p,p')	Water	X		X	
	Dieldrin	Water	X		X	
	Endrin	Water	X		X	
	Methoxychlor	Water	X		X	
Carbamate Pesticides	Aldicarb	Water	X		X	
	Carbaryl	Water	X		X	
	Carbofuran	Water	X		X	
	Methiocarb	Water	X		X	
	Methomyl	Water	X		X	
	Oxamyl	Water	X		X	

Table A- 4. Toxicity Analyses for the Westside Coalition

Class	Monitoring Constituent	Matrix	Assessment	Core	Rain Event	Source	Follow-up
Screen ¹	<i>Selenastrum capricornutum</i>	Water	X		X		
	<i>Ceriodaphnia dubia</i> (acute)	Water	X		X		
	<i>Pimephales promelas</i> (acute)	Water	X		X		
TIE ²	<i>Selenastrum capricornutum</i>	Water					X
	<i>Ceriodaphnia dubia</i> (acute)	Water					X
	<i>Pimephales promelas</i> (acute)	Water					X
Dil. Series ³	<i>Selenastrum capricornutum</i>	Water					X
	<i>Ceriodaphnia dubia</i> (acute)	Water					X
	<i>Pimephales promelas</i> (acute)	Water					X
Sed. Tox.	<i>Hyalella azteca</i> (survival only)	Sediment	X				
Supporting Chemical Analysis ⁴	Total Organic Carbon	Sediment	X				
	Grain Size	Sediment	X				
	Bifenthrin	Sediment					X
	Cypermethrin	Sediment					X
	Cyfluthrin	Sediment					X
	Esfenvalerate/Fenvalerate	Sediment					X
	Fenpropathrin	Sediment					X
	Lambda cyhalothrin	Sediment					X
	Permethrin	Sediment					X
	Chlorpyrifos	Sediment					X
	DDD (p,p')	Sediment					X
	DDE (p,p')	Sediment					X
	DDT (p,p')	Sediment					X

1 – Tested at 100% solution only.

2 – Phase I TIEs are performed immediately if $\geq 50\%$ reduction in the organism response when compared to the associated Lab Control is observed. TIE treatments are targeted for pesticide and metals-related toxicity.

3 – Dilution series testing is performed within 24 hours following the observation of 100% mortality during the initial screen test. Dilution tests are in addition to TIE.

4 – Sediment chemical analyses are performed on samples with statistically significant toxicity and a $\geq 20\%$ reduction compared the associated Lab Control.

7. Quality Objectives and Criteria

The objective of data collection for this monitoring program is to produce data that represent, as closely as possible, *in-situ* conditions of water bodies in the Westside San Joaquin River Watershed Coalition Watershed. This objective will be achieved by using accepted methods to collect and analyze surface water and sediment (Appendix B). Assessing the monitoring program's ability to meet this objective will be accomplished by evaluating the resulting laboratory measurements in terms of detection limits, precision, accuracy, representativeness, comparability, and completeness, as presented below.

Qualitative Objectives

Reporting Limits

Method detection limits (MDL) and reporting limits (RLs) must be distinguished for proper understanding and data use. The MDL is the minimum analyte concentration that can be measured and reported with a 99% confidence that the concentration is greater than zero. Laboratories generally establish limits that are reported with the analytical results based on the concentration of an analyte that can be routinely measured in the sampled matrix within stated limits and confidence in both identification and quantitation. These limits may be called quantitation limits, detection limits, reporting detection limits, or other terms. The limits that are reported with data are called laboratory reporting limits (RL) under this program. The laboratory RL must be less than or equal to the Project RLs included in this QAPP (Appendix A) and laboratories must have documentation to support quantitation at those levels, such as having the lowest non-zero calibration standard or calibration check sample concentration at or less than the RL.

For this program, Project RLs have been established based on the verifiable levels and general measurement capabilities demonstrated for each method to meet the data quality objectives (DQO). Project RLs should be considered as maximum allowable limits to be used for laboratory data reporting; data produced by different laboratories will be comparable at these levels. Laboratories must report analytical results between the MDL and Project RL. These results must be reported as the numerical values and qualified as estimates using appropriate data flags. Reporting as “trace” or “<RL” is not acceptable. The QC reviewers and data users must assess this information's usability. Note that samples diluted for analysis or corrected for percent moisture for sediment or tissue samples may have sample-specific RLs that exceed these RLs. This will be unavoidable in some cases.

When selecting an analytical method during the DQO process, data users must be sure to evaluate the laboratory RLs to verify that the method will meet the quantitation requirements for use in modeling, comparison with applicable water quality standards, or other planned uses. This approach ensures that the analytical method sensitivity has been considered and that the methods used can produce data that satisfy users' needs, making the most effective use of resources.

Reporting limits established for the analytical methods used for this program are provided in Tables A-5 through A-7.

Table A-5. Physical Parameters, Drinking Water Constituents, and Nutrients: Analytical Methods, analyzing laboratory, Laboratory Reporting Limits (RL), and Method Detection Limits (MD).

Constituent	Method # ⁽¹⁾	Laboratory	RL	MDL
Field Parameters				
Discharge	Site Dependant ⁽²⁾	Field team	1 cfs	n/a
pH	EPA 150.1	Field team	0.1 pH unit	n/a
Specific Conductivity	EPA 120.1	Field team	100 µS/cm	n/a
Dissolved Oxygen	SM 4500-O	Field team	0.1 mg/L	n/a
Temperature	SM2550	Field team	0.1 °C	n/a
Physical Parameters				
Turbidity, Total	EPA 180.1	Caltest	0.05 NTU	0.03 NTU
Suspended Solids, Total	SM 2540 D	Caltest	3.0 mg/L	2.0 mg/L
Dissolved Solids, Total	SM 2540 C	Caltest	10 mg/L	4.0 mg/L
Hardness as CaCO ₃ , Total	SM 2340 C	Caltest	5 mg/L	1.7 mg/L
Drinking Water Parameters				
Bromide, Total	EPA 300.0	Caltest	1 mg/L	0.01 mg/L
<i>Escherichia coli</i> (<i>E. coli</i>)	SM 9223	Caltest	1 MPN/100 mL	1 MPN
Organic Carbon, Dissolved	SM 5310 B	Caltest	0.5 mg/L	0.3 mg/L
Organic Carbon, Total	SM 5310 B	Caltest	0.5 mg/L	0.3 mg/L
Nutrients				
Nitrogen ,Total Kjeldahl	SM 4500-NH3 v20	Caltest	0.1 mg/L	0.07 mg/L
Nitrate +Nitrite as N, Total	EPA 353.2	Caltest	0.05 mg/L	0.02 mg/L
Ammonia as N, Total	SM 4500-NH3 v20	Caltest	0.1 mg/L	0.04 mg/L
Phosphate as P, Total	SM 4500-P E	Caltest	0.01 mg/L	0.007 mg/L
Orthophosphate as P, Dissolved	SM 4500-P E	Caltest	0.01 mg/L	0.006 mg/L

(1) Standard Methods (SM) or EPA Method number.

(2) The “float method” is used for most sites. Alternatively, data will be obtained online for sites with gauging stations or rated weirs.

Table A-6. Trace Elements: Laboratory Performance Requirements for Analysis of Water Quality Samples for Total and Dissolved Trace Metals

Analyte	Method ⁽¹⁾	Laboratory	RL (µg/L)	MDL (µg/L)
Arsenic (total)	EPA 200.8	Caltest	0.5	0.06
Boron (total)	EPA 200.8	Caltest	10	2
Cadmium (total and dissolved)	EPA 200.8	Caltest	0.1	0.05
Copper (total and dissolved)	EPA 200.8	Caltest	0.5	0.07
Lead (total and dissolved)	EPA 200.8	Caltest	0.25	0.03
Nickel (total and dissolved)	EPA 200.8	Caltest	0.5	0.06
Selenium (total)	EPA 200.8	Caltest	1	0.06
Zinc (total and dissolved)	EPA 200.8	Caltest	1	0.5

(1) SOP or EPA Method number

Table A-7. Supporting Sediment Analyses: Laboratory Performance Requirements for Analysis of Sediment Quality Samples for TOC, Grain Size, and Pesticides

Analyte	Method ⁽¹⁾	Laboratory	RL (µg/kg) ⁽²⁾	MDL (µg/kg) ⁽²⁾
Physical Parameters				
Total Organic Carbon	Walkley-Black	PTS Labs	200 mg/kg	100
Grain Size	ASTM D4464M	PTS Labs	0.01%	0.01
Pesticides				
Bifenthrin	EPA 8270 NCI	Caltest	0.43	0.11
Chlorpyrifos	EPA 8270 NCI	Caltest	0.43	0.12
Cyfluthrin	EPA 8270 NCI	Caltest	0.43	0.11
Lambda-cyhalothrin	EPA 8270 NCI	Caltest	0.43	0.06
Cypermethrin	EPA 8270 NCI	Caltest	0.43	0.11
Esfenvalerate	EPA 8270 NCI	Caltest	0.43	0.13
Permethrin	EPA 8270 NCI	Caltest	0.43	0.11
DDD (p,p')	EPA 8081A	Caltest	0.0097	0.0039
DDE (p,p')	EPA 8081A	Caltest	0.0097	0.0058
DDT (p,p')	EPA 8081A	Caltest	0.0097	0.0029

(1) SOP or EPA Method number

(2) RL and MDL are µg/kg, unless otherwise noted.

Table A-8. Pesticides: Analytical Methods, Laboratory Reporting Limits (RL), and Method Detection Limits (MDL). Analyzing laboratory is APPL, Inc.

Analyte	RL (µg/L)	MDL (µg/L)	Analyte	RL (µg/L)	MDL (µg/L)
<i>Carbamate pesticides and herbicides analyzed by EPA 8321A</i>					
Aldicarb	0.4	0.2	Methiocarb	0.4	0.2
Carbaryl	0.07	0.05	Methomyl	0.07	0.05
Carbofuran	0.07	0.05	Oxnamyl	0.4	0.2
Diuron	0.4	0.2	Linuron	0.4	0.2
<i>Base Organochlorine pesticides by EPA 8081A/8082</i>					
Dicofol	0.1	0.01	Dieldrin	0.01	0.005
DDD	0.01	0.003	Endrin	0.01	0.007
DDE	0.01	0.004	Methoxychlor	0.01	0.008
DDT	0.01	0.007	-	-	
<i>Group A Organochlorine pesticides by EPA 8081A/8082</i>					
Aldrin	0.01	0.009	Endosulfan I	0.01	0.005
HCH, alpha	0.01	0.005	Endosulfan II	0.01	0.004
HCH, beta	0.01	0.008	Endosulfan sulfate	0.01	0.005
HCH, delta	0.01	0.005	Heptachlor	0.01	0.008
HCH, gamma	0.01	0.005	Heptachlor epoxide	0.01	0.007
Chlordane, cis-	0.01		Toxaphene	0.5	0.38
Chlordane, trans-	0.01		-	-	
<i>Organophosphorus pesticides and Select Herbicides by EPA 8141A</i>					
Azinphos methyl	0.1	0.02	Methamidophos	0.2	0.1
Chlorpyrifos	0.015	0.0026	Methidathion	0.1	0.04
Demeton-s	0.1	0.08	Parathion, ethyl	0.1	0.02
Diazinon	0.02	0.004	Parathion, methyl	0.1	0.075
Dichlorvos	0.1	0.02	Phorate	0.1	0.072
Dimethoate	0.1	0.08	Phosmet	0.2	0.06
Disulfoton	0.05	0.02	Pendimethalin ¹	0.1	0.04
EPTC	0.1	0.03	Trifluralin ¹	0.05	0.04
Malathion	0.1	0.05	Cyanazine	0.5	0.09
Atrazine	0.5	0.07	Simazine	0.5	0.08

(1) Herbicides analyzed using the same assay as OP.

Representativeness

Representativeness can be defined as the degree to which the environmental data generated by the monitoring program accurately and precisely represent actual environmental conditions. For the Westside Coalition, this objective is addressed by the overall design of the monitoring program, adherence with sampling SOPs (including decontamination SOPs) and meeting holding times. Assuring that the data is representative of the program objectives is addressed primarily by selecting appropriate locations, methods, times, and frequencies of sampling for each environmental parameter, and by maintaining the integrity of the sample after collection. Each of these elements of the quality assurance program is addressed elsewhere in this document.

Comparability

Comparability of the data can be defined as the similarity in content and quality of data generated by different monitoring programs. For the purpose of the Westside Coalition, meeting the statewide consistency goals outlined by the SWAMP program and California Data Exchange Network (CEDEN) is addressed by using standard sampling and analytical procedure and adherence to data reporting guidelines. Additionally, comparability of analytical data is addressed by analysis of standard reference materials (addressed in Section B.5 this document).

Completeness

Data completeness is a measure of the amount of successfully collected and validated data obtained relative to the amount of data planned to be collected for the project. Completeness is usually expressed as a percentage value. Factors that affect data completeness include sample breakage during transport or handling, insufficient sample volume, laboratory error, QC failure and equipment failure. A project objective of 90% completeness has been selected for this program and is based on the percentage of the data needed for the program or study to reach valid conclusions. The 90% data completeness objectives are based on the planned sampling frequency and a subjective determination of the relative importance of the monitoring element within the Monitoring Program. Because the Westside Coalition is intended to be a long term monitoring program, data that are not successfully collected for a specific sample event or site can be recollected at a later sampling event.

The completeness goals apply to all aspects within the field collection, transport, and laboratory analysis to meet the 90% total requirement for the project completeness. Field and transport completeness is assessed by confirming that each site was successfully visited, conditions were documented in the field log, in-field measurements were made, samples were collected in the correct bottle (e.g., preserved, when necessary) and that a sufficient volume was collected, in-field QA/QC samples were properly collected, COCs were properly completed, and that samples were successfully transported to the labs. Proper documentation of dry or inaccessible sites is required in the field log and via photos, and meets the completeness goal for that site and event. Laboratory completeness is assessed by confirming that the samples were properly received (and completion of the COCs), stored and preserved, extracted (i.e., when appropriate), analyzed, and that lab QA/QC measures were met. The Project will provide a narrative describing this assessment for each area as well as outline goals for improvement or maintenance of the 90% completeness as a part of the semi-annual reporting requirement. The program QA Officer will confirm that total program completeness is assessed for each monitoring event.

Precision and Accuracy

The evaluation of precision and accuracy takes place at the analytical measurement level for both field and laboratory measurements. These are defined in Section B-5 of this document and in the pertinent field and laboratory SOPs.

8. Special Training Needs/Certification

The Technical Program Manager is responsible for overseeing training of all project personnel, and the Monitoring Program Manager assures that appropriate certifications are current.

All Field Leads have received training on field sampling methods and field safety during a workshop provided by the Monitoring Program Coordinator and Pacific EcoRisk Senior Field Scientists on July 10, 2009. During the training, Field Leads were provided a Field Sampling Manual, which consists of emergency information, field safety, site information, maps, and sampling SOPs. Hands-on training in the field followed the workshop. This training was performed to ensure that the monitoring work is conducted following standard health and safety protocols, and per the methods required in the field sampling and field equipment decontamination SOPs. At a minimum, all field staff are familiar with the field guidelines and sample collection standard operating procedures (SOPs) included in this QAPP. The training records are documented via the attendance list, and are maintained at the office of the Technical Program Manager. Additional training of field teams will be coordinated by the Monitoring Program Manager (e.g., when new field crew members are needed) as request by the Technical Program Manager.

Laboratories performing each sample analysis must be either ELAP or NELAP certified, and their staff must have demonstrated experience with each method and be certified by the laboratory to perform the analyses; analytical labs must follow their internal training program specified in their individual Lab QA Plan and by their laboratory certification. Laboratories are required to renew their laboratory certifications via the biennial renewals with the California Department of Public Health. All work shall be performed under the supervision of experienced staff or a Monitoring Program Coordinator. The program QA Officer is responsible for coordinating all training. Figure A-1 identifies the staff involved with this monitoring program.

9. Documentation and Records

Project Documents

For each sample event, each field crew shall provide the program QA Officer with copies of relevant pages of the Westside Coalition field logs and copies of the chain-of-custody forms for all samples submitted for analysis. The Technical Program Manager maintains electronic copies of the field logs; copies are submitted to ILRP staff. All data recorded by the field teams (e.g., sample date, time, etc.) must be consistently recorded on the chain of custody record and the field data sheets, and be consistent with the data reported in the laboratory reports (see below).

For each sample analyzed, the analyzing laboratories shall provide the program QA Officer with the sample custody and integrity forms, and analytical results for field and QC samples. The analyzing laboratories shall provide results from all laboratory QC procedures (e.g., calibrations,

blanks, duplicates, spikes, reference materials, etc.) and the sample IDs associated with each analytical sample batch. The laboratories must also report any results that exceed standards (e.g., lab QC, method QC) for the methods.

Reporting Format

All results meeting data quality objectives, and results having satisfactory explanations for deviations from objectives shall be reported on the laboratory's standard reporting format. The final results shall include the results of all field and laboratory QC samples. Laboratories will also be required to submit their data in a ILRP specific CEDEN-comparable electronic data deliverable (EDD) format for the Westside Coalition. The Technical Program Manager will submit semi-annual reports in both electronic format and hard copy to the Central Valley Water Board as required in the ILRP MRP. Semi-annual report data package contains copies of field logs, chain-of-custody forms, laboratory sheets, site photos for each sampling event, and summaries of QC and sample analyses. All hard copy reports, associated raw data, and backup electronic copies for this program are maintained for at least 5 years either by the laboratories (i.e., lab reports and EDDs) or the Technical Program Manager (e.g., exceedance reports). Each participating organization is responsible for maintaining monitoring program records. Copies of the semi-annual reports are kept in hard and electronic copy by the Westside Coalition and by the Regional Board.

Distribution of the QAPP

Pacific EcoRisk prepares the Westside Coalition QAPP. For the original distribution of this document, or for any necessary revision, copies of the QAPP are sent to Program Managers or Quality Assurance Managers, as appropriate, for each consulting team, sampling organization, and laboratory involved in this study (see page *vii* for a distribution list). These parties also receive a signature page (see page *i*) that they must sign and return to Pacific EcoRisk. Following the receipt of all necessary signatures, a completed signature page is distributed to all participants such that they will have a completed and sign copy of the QAPP from Pacific EcoRisk. Each organization is responsible for distributing to appropriate parties within their organization to assure that the QAPP is followed by all of their staff participating in the project.

B. DATA GENERATION AND ACQUISITION

1. Sampling Process Design

The Westside San Joaquin River Watershed Coalition monitoring program includes monitoring at 26 monitoring locations within the Coalition area (Table A-1 and Figure A-2). The monitoring schedule is described in Section A-6 of this QAPP. Samples will be collected during irrigation season, non-irrigation season, and rain event based on the type of water that is conveyed through the site (i.e., source water or discharge sites). The varying sampling regime is also divided into five categories of monitoring: core, assessment, rain, source, and special study (see Section A-6), therefore total number of samples for the program will vary by event type, monitoring category, site, and constituent.

The Watershed Coordinator will communicate to the Central Valley Water Board every exceedance of water quality standards detected during this program. The exceedance follow-up plan is identified in the MRP (page 22). The location and type/magnitude of the exceedance will be considered as part of the adaptive management process of using such information within the categorized monitoring framework (e.g., core, assessment, rain, source, and special study) described in section A-6.

Rationale for the Design

The purpose of this study is to collect baseline water quality, aquatic toxicity, and sediment toxicity data within the Westside San Joaquin River Watershed. The monitoring stations were selected to provide representative stations among the drainages throughout the watershed. The sampling design is based on a judgmental data collection method (i.e., based on historical data from the watershed), with adjustments to the sampling design as water quality issues are identified, as per the requirements in the Monitoring and Reporting Program.

Sample Identification Scheme

Each sample collected under this program will be assigned a three-part sample identification string. The three-part string includes a sampling event code (e.g., 01), Station Code (ICARR –the 5 letters reflect an acronym for the site - see Table A-1), and a two letter code for the type of sample (e.g., PE = pesticide, PB = pesticide blank, PD = pesticide duplicate, PM = pesticide matrix spike, QE = non-pesticide analytical sample, TE = toxicity). For example, if the pesticide samples collected for the program during event 46 were collected from Ingram Creek at River Road, the sample identification code would be: 46-ICARR-PE.

Procedure for Locating and Selecting Environmental Samples

Each sampling site location is well defined and the sampling teams are thoroughly familiar with the sampling sites and how to access them. GPS locations for each site have been tabulated (MRP Order, page 4, Table 1) and each site is mapped in Figure A-2. Should the sampling site become inaccessible, the field crew must notify the Technical Program Manager, whom will open a dialogue with the Central Valley Water Board regarding how to proceed.

Classification of Measurements as Critical

Because the Westside Coalition is intended to be a long term monitoring program, samples that are not successfully collected for a specific sample event or site can typically be recollected at a later sampling event. However, all measurements made for the program are important. Therefore, no one specific measurement made is deemed critical (e.g., required to achieve project objectives). The expected number of samples, specific analytical methods and procedures, and defined acceptance criteria for QC samples (as described other section of this QAPP) shall be included as part of the data review process.

Sources of Natural Variability and Potential Sources of Bias

Ambient water and sediment monitoring can be dramatically affected by naturally variability and bias. Sources of natural variability may include, but are not limited to, time of day, season, meteorological conditions (e.g., rainfall), and hydrological conditions (e.g., flow regimes). As this program is intended to be a long-term monitoring study, it is expected that the effect of such sources of natural variability will have a minimal effect on the data over time (i.e., a sufficient number of monitoring events will occur such that no one source of natural variability will negatively affect the data). Sources of bias are primarily driven by the people involved in any given program, and may occur in the field (e.g., differences between individuals in the time used to measure a dissolved oxygen reading) or the lab (e.g., how mortality is recorded in a toxicity testing lab). Bias is being minimized in this program by applying a standardized training program for both field crews and laboratory staff (i.e., intra-laboratory training).

2. Sample Collection Methods

Field Procedures

High quality fieldwork is dependent on the laboratories providing pre-cleaned sample containers that are certified free of contamination for each method (i.e., to be addressed in each lab's QA Plan). Only field crews instructed in the collection of environmental water and sediment samples are permitted to collect samples for this program. Pacific EcoRisk provides training to the field crews, that includes training on the proper sample collection methods, how to recognize and avoid sources of contamination, how to distinguish acceptable versus non-acceptable water (e.g., leaking bottle) and sediment samples (e.g., dry sediment), how to clean and decontaminate field equipment.

Pacific EcoRisk generates the Field Logs for each sampling team. Data to be recorded by the field crews on the field log sheets for each site must include the site name and GPS coordinates, a single site photo, flow measurements, calculated discharge, and field measurements (see Appendix E). Flow will be estimated using the float method at most sites (i.e., a measurement tape is placed along the bank and the flow rate is estimated based on timing the distance that a piece of floating debris moves over a measured distance); the field crew will also measure the stream width and depth so that discharge can be calculated. All samples must have the sample identification/numbering as described above in section B-1. Field crews are also required to record comments for any samples or data that should be flagged or qualified (e.g., water quality parameters were collected from puddled water). Any changes to the sample site must be

approved by the Technical Program Manager, and must be accompanied by both new GPS coordinates and site photos.

The Technical Program Manager oversees the field sampling teams, assures that each field team adheres to proper custody and documentation procedures, assures that flagged field data are properly flagged in a comments column for electronic data submittals, and maintains the final logbooks for each sampling event.

Sample Type

Samples will be collected from 2 environmental media: water and sediment. Three different sample collection methods will be used for the monitoring elements in water: (1) basic water quality sampling, (2) pathogen sampling, and (3) toxicity sampling. For each of these methods described or referenced, it is the combined responsibility of all members of the sampling crew to determine if the performance requirements of the specific sampling method have been met, and to collect an additional sample if required. Sampling personnel should carry copies of the QAPP and any relevant SOPs with them in the field for reference during sampling. Descriptions of specific sampling methods and requirements are provided below.

Decontamination Procedures

All field and sampling equipment that may contact samples must be decontaminated after each use in a designated area. A detailed description of cleaning procedures for water sampling and sediment sampling equipment is included in Appendix B of this QAPP.

Sample Storage, Preservation, and Holding Times

Sample containers are pre-cleaned according to USEPA specification for the appropriate methods. Table B-1 lists the sample container, volume requirements, processing and storage requirements for this QAPP.

In the field, all water and sediment samples will be packed in wet ice or frozen ice packs during shipment, so that they will be shipped at approximately 0-6°C. Samples will be shipped in insulated containers. All caps and lids will be checked for tightness prior to shipping.

All water and sediment samples will be handled, prepared, transported and stored in a manner so as to minimize bulk loss, analyte loss, and contamination or biological degradation. Sample containers will be clearly labeled with an indelible marker. Where appropriate, samples may be frozen to prevent biological degradation. Samples will be kept cool at a temperature of 0-6°C until analyzed.

Samples will be placed in the ice chest with enough ice (in ziplock bags or tied off trash bags) to completely fill the ice chest. Chain-of-custody forms will be placed in a plastic zip lock bag and taped to the inside of the ice chest lid. Ice chests will be sealed with tape before shipping. It is assumed that samples in tape-sealed ice chests are secure whether being transported by staff vehicle, by common carrier, or by commercial package delivery. The receiving laboratory will have a sample custodian(s) who examines the samples for correct documentation and proper preservation.

Table B-1. Sampling Requirements

Parameter	Sample Container	Sample Volume ⁽¹⁾	Immediate Processing and Storage	Holding Time ⁽²⁾
Physical Parameters				
Turbidity, TDS, TSS, Bromide, and soluble orthophosphate	Polyethylene	2 liter	Store at 0-6°C	48 hours
Drinking Water				
E. coli (pathogens)	Polyethylene (sterile)	125 mL	Store at 0-6°C	24 hours ⁽³⁾
Total Organic Carbon	Amber Glass VOA, PTFE-lined cap	3 x 40 mL	Preserve w/HCl; Store at 0-6°C;	28 days
Toxicity				
Aquatic bioassays	Amber glass	5 gallons	Store at 0-6°C	36 hours
Sediment bioassays	Glass	2-Liter	Store at 0-6°C	14 days
Sediment Analyses				
Total Organic Carbon	Glass	500 mL	Store at 0-6°C	28 days
Sediment Grain Size				
Pesticides ⁽⁴⁾		500 mL	Frozen	1 year
Pesticides				
Carbamates	Amber Glass	1 Liter	Store at 0-6°C; Extract within 7 days	Extract within 7 days, analyze within 40 days
Organochlorines	Amber Glass	1 Liter		
Organophosphates	Amber Glass	1 Liter		
Herbicides	Amber Glass	1 Liter		
Nutrients				
TKN, Ammonia, Nitrate-Nitrite, and Total Phosphorus	Polyethylene	500 mL	Preserve to ≤pH 2 with H ₂ SO ₄ ; Store at 0-6°C.	28 days
Orthophosphate	Sample collected in bottle with physical parameters			
Metals/Trace Elements				
Dissolved trace elements ⁽⁵⁾	Polyethylene	500 mL	Filter as necessary; Preserve to ≤pH 2 with HNO ₃	40 days

1. Additional volumes may be required for QC analyses; NA = Not Applicable

2. Holding time after initial preservation or extraction.

3. Samples for bacteria analyses should be set up as soon as possible.

4. Pyrethroid insecticide and chlorpyrifos.

5. To include arsenic, boron, cadmium, copper, lead, nickel, selenium, and zinc.

Contract laboratories will follow sample custody procedures outlined in their QA plans.

Contract laboratory QA plans will be on file with the respective laboratory.

All samples remaining after successful completion of analyses will be disposed of properly. It is the responsibility of the personnel of each analytical laboratory to ensure that all applicable regulations are followed in the disposal of samples or related chemicals.

Chain-of-custody procedures require that possession of samples be traceable from the time the samples are collected until completion and submittal of analytical results. A complete chain-of-custody form is to accompany the transfer of samples to the analyzing laboratory.

Samples that do not meet the preservation and/or holding times are required to be re-sampled. Since there may be circumstances when this is not possible (e.g., rain events), the labs must

immediately notify the Technical Program manager for any such sample so that the Central Valley Water Board can be contacted before proceeding.

Basic Water Quality Characteristics

Basic water quality monitoring will include sampling for metals, pesticides, total dissolved solids, turbidity, nitrogen and phosphorus compounds, and TOC.

All water quality samples will be collected using techniques that minimize sample contamination. Sampling methods will generally conform to those described in Appendix B. Samples will generally be mid-depth grab samples and will be collected from shore using a stainless-steel bucket or mid-channel directly into the sample container; Alternatively, and at the Technical Program Manager's discretion, samples may be collected using a depth-integrated sampling device, peristaltic pump and acid-cleaned polyethylene or Teflon™ tubing. Sample container(s) will be appropriate for the analyses specified in this document, and may include glass, polyethylene, or Teflon™ sample containers.

Should a peristaltic pump be used to collect samples, this sample collection method requires that the sample collection tubing, and the sample bottle and lid come into contact only with surfaces known to be clean, or with the water sample. If the performance requirements for specific samples are not met, the sample will be re-collected. If contamination of the sample container is suspected, a fresh sample container will be used.

Pathogens

Pathogen monitoring will include sampling for a pathogen indicator organism (*E. coli* bacteria). *Note:* Samplers must wear gloves when collecting any pathogen samples. Samples analyzed for bacteria will be collected as near-surface grab samples. Sampling for bacteria will be performed according to the sampling procedures detailed for Standard Methods 9221B and 9221E (APHA *et al.* 1998). In brief, the sampling procedures are summarized as follows:

- Sample containers should be cleaned and sterilized using procedures described in Standard Methods 9030 and 9040
- For waters suspected to contain a chlorine residual, sample bottles should contain a small amount of sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) sufficient to neutralize bactericidal activity. For water containing high concentrations of copper or zinc, sample bottles should contain sufficient EDTA solution to reduce metal toxicity. *Note:* These conditions are rare in surface waters
- Sample bottles may be glass or plastic (e.g. polypropylene) with a capacity of at least 120 mL. After sterilization, sample bottles should be kept closed until they are to be filled
- When removing caps from sample bottles, be careful to avoid contaminating inner surface of caps or bottles
- Using aseptic techniques fill sample bottles leaving sufficient air space to facilitate mixing by shaking. Do not rinse bottles
- Recap bottles tightly

If at any time the sampling crew suspects that the sample or sampling container has been contaminated, the sample should be re-collected into a new sample container. After collection, store samples at 0-6°C until evaluation. Bacteriological tests must be set up within 24 hours of collection. The 20th edition of Standard Methods (APHA et al. 1998) recommends analysis of samples as soon as possible, but specifies that non-drinking water samples analyzed for non-compliance purposes may be held for up to 24 hours (below 10°C) until time of analysis.

Aquatic Toxicity Samples

Collection of water samples for analysis of ambient water column toxicity will be performed in accordance with guidance for sampling and sample handling documented in *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms* (USEPA 2002). In brief, the sampling requirements for toxicity testing are as follows:

- Water collected for toxicity tests will consist of grab samples
- Samples will be collected directly into 1-gallon amber glass bottles, using the same equipment and procedures as for basic water quality samples (described above)
- Sufficient volume will be collected to allow for any necessary re-testing (*i.e.*, persistence testing), dilution series testing, or to conduct the characterization and identification phases (Phase I and II) of acute or chronic toxicity identification evaluation (TIE) procedures
- Samples will be filtered in the laboratory as required for specific toxicity tests
- After collection, samples will be chilled and maintained at 0-6°C until testing
- Toxicity tests will be initiated within 36 hours of sampling

Sediment Toxicity Samples

Collection of sediment samples for analysis of ambient sediment toxicity will be performed in accordance with guidance for sampling and sample handling documented in *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates* (USEPA 2000). Sediment samples shall be collected and analyzed for toxicity twice per year (one sample collected between August 15 and October 15, and one sample collected between March 1 and April 30); exceptions would be when there is insufficient sediment accumulation or when there is absence of overlying water. Sites that do not include overlying water should be flagged in the field log as potential outlying data points for sediment toxicity. Specific methods are also documented in Appendix B. In brief, the sampling requirements for sediment toxicity testing are as follows:

- Sediment will be collected using either stainless steel petite ponar or Van Veen grab devices, or alternatively using a stainless steel scoop;
- Sediment from the top 2 cm of the grab will be transferred into glass sample containers using stainless steel spoons;
- Sufficient volume will be collected to allow for recommended chemical and physical analyses as well as for any necessary re-testing (Table B-1);
- After collection, samples will be chilled and maintained at 0-6°C until testing; and

-
- Toxicity tests will be initiated within 14 days of sampling.

In some cases where significant toxicity is observed during sediment toxicity testing, samples may be analyzed for any of the chemical parameters included in this QAPP. The specific analyses to be performed will depend on the pattern of toxicity observed. Every effort will be made to be consistent with the sample requirements documented herein for the specific analytes. Because requirements for sample holding times may not be strictly met, the results of the analyses will be used primarily for determining or confirming causes of toxicity, and will be qualified for any other use. Laboratories selected to perform these analyses must meet the same QA performance criteria used to select other laboratories for this monitoring program.

3. Sample Handling and Custody

Sample Holding Times

The maximum holding times for all analyses are identified in Table B-1. The analytical laboratories must report to the program QA Officer (QAPP, page 3) any sample that does not meet the program holding time limit or preservation requirements, and must implement internal corrective actions to eliminate reoccurrences. Corrective actions may include follow-up communications with the labs about assuring that staff receive appropriate training to assure that holding time limits are not exceeded.

Documentation Procedures

The program QA Officer (QAPP, page 3) is responsible for ensuring that the field sampling team adheres to proper custody and documentation procedures. Field datasheets are completed for all samples collected during each sampling activity. Field personnel have the following responsibilities:

- Keep an accurate written record of sample collection activities on the field forms;
- Ensure that all entries are legible, written in waterproof ink and contain accurate and inclusive documentation of the field activities;
- Date and initial daily entries;
- Note errors or changes using a single line to cross out the entry and date and initial the change;
- Complete the chain of custody forms accurately and legibly; and
- A label is affixed to each sample collected. Sample labels uniquely identify samples with an identification number, date and time of sample collection, and the initials of the sampling crew.

Chain-of-Custody Form

A chain-of-custody (COC) form is completed after sample collection, and prior to sample shipment or release. The chain-of-custody form, sample labels, and field documentation are crossed-checked to verify sample identification, type of analyses, and number of containers, sample volume, preservatives and type of containers.

Information to be included in the chain of custody forms includes:

-
- Sample identification;
 - Date and time of collection;
 - Sampler(s) names;
 - Analytical method(s) requested;
 - Sample matrix ;
 - Any comments to identify special conditions or requests; and
 - Ice chest temperature at log-in.

Sample transfer between field staff and laboratory is documented by signing and dating “relinquished by” and “received by” blocks whenever sample possession changes. If samples are not shipped on the collection day, they are to be refrigerated in a sample control area with a temperature range of 0-6 °C. An example of chain-of-custody form is shown in Figure B-2.

Sample Shipments and Handling

All sample shipments are accompanied by the COC form, which identifies the content. The original COC accompanies the shipment and the field crew retains a copy. Samples are shipped to the contract laboratories according to Department of Transportation standard. A sufficient amount of ice must be packed around the sample to maintain the shipping conditions at 0-6 °C. The ice may be contained in re-closeable bags, but must contact the samples to maintain temperature. The method(s) of shipments, courier name, and other pertinent information is entered in the “Received By” or “Remark” section of the chain of custody form.

The following procedures are used to prevent bottle breakage and cross-contamination:

- Prior to packaging, outside of the bottles need to be rinsed off with DI water
- Bubble wrap or foam pouches are used to keep glass bottles from contacting one another to prevent breakage
- All samples are transported inside hard plastic coolers or other contaminated free shipping containers
- Coolers are taped shut and sealed with chain-of-custody seals to prevent accidental opening
- If pre-arrangements are not made, prior to shipment of the samples field staff must notify laboratory sample control

Figure B- 1. Chain-of-Custody Form



Pacific EcoRisk

ENVIRONMENTAL CONSULTING & TESTING

835 Arnold Drive, Suite 104, Martinez, CA 94553

(925) 313-8080 FAX (925) 313-8089

CHAIN-OF-CUSTODY RECORD

Client Name:						REQUESTED ANALYSIS													
Client Address:																			
Sampled By:																			
Phone:																			
FAX:																			
Project Manager:																			
Project Name:																			
PO Number:																			
Client Sample ID	Sample Date	Sample Time	Sample Matrix*	Container															
				Number	Type														
1																			
2																			
3																			
4																			
5																			
6																			
7																			
8																			
9																			
10																			
11																			
Correct Containers:		Yes	No			RELIQUINSHED BY													
Sample Temperature:		Ambient	Cold	Warm															
Sample Preservative:		Yes	No																
Turnaround Time:		STD	Specify:																
Comments:						Signature:													
						Print:													
						Company:													
						DATE:												TIME:	
						RECEIVED BY													
						Signature:													
Print:																			
Company:																			
DATE:												TIME:							

*MATRIX CODES: (SED = Sediment); (TISS = Tissue); (SW = Seawater, Saltwater); (FW = Freshwater); (WW = Wastewater); (STRMW = Stormwater); (AW = Ambient Water)

Laboratory Custody Procedures

Laboratories shall maintain custody logs sufficient to track each sample submitted and to verify that samples are preserved, extracted and analyzed within specified holding times. The following sample control activities must be conducted in the laboratory:

- Initial log-in and verification of samples received with the chain of custody form;
- Document any discrepancies noted during log-in on the chain of custody;
- Verify sample preservation and temperature at log-in must be recorded;
- Notify the project coordinator if any problems or discrepancies are identified;
- Proper sample storage, including daily refrigerator temperature monitoring and sample security;
- Distribute samples or notify the laboratory of sample arrival; and
- Return shipment of coolers.

4. Analytical Methods and Field Measurements

Field Measurements

Field-measured parameters (*e.g.*, temperature, D.O., specific conductivity, and pH) will be measured at each site and event where basic water quality characteristic samples are collected. Field parameters will be measured using a YSI 556 multi-meter. The multi-meter includes a glass membrane pH probe with a measurement range from 0-14 standard pH units, a membrane D.O. probe with a range of 0-20 mg/L, and a conductivity probe with a range of 0 – 3,000 $\mu\text{S}/\text{cm}$. Representative instantaneous flow measurements will be recorded at each sampling location. These measurements will be used to estimate flow in cubic feet per second (cfs).

Laboratory Standard Operating Procedures and Reporting Limits

Water quality samples may be analyzed for aquatic toxicity, sediment toxicity, physical parameters, drinking water constituents, nutrients, metals/trace elements, and pesticides. Analytical methods and reporting limits are summarized in Tables A-5 through A-8.

Laboratory Certification Requirements

The analysis of any material required for this program must be performed by a laboratory that has accreditation or certification pursuant to Environmental Laboratory Accreditation Program (California Health and Safety Code § 100825-100920).

Aquatic Toxicity Analyses

Water samples will be analyzed for acute toxicity to *Ceriodaphnia dubia* (water flea) and *Pimephales promelas* (fathead minnow). Determination of acute toxicity shall be performed generally as described in *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms* (USEPA 2002a). Water samples will also be analyzed for chronic toxicity to the green algae *Selenastrum capricornutum*. Determination of chronic toxicity shall be performed generally as described in *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* (USEPA 2002b). Laboratory SOPs for these tests are documented in Appendix C. *Selenastrum* testing is to be performed without EDTA.

Samples that exhibit a statistically significant reduction in organism response when compared to the laboratory control must be reported to the Central Valley Water Board as an exceedance of the narrative water quality objective for toxicity. The Westside Coalition is establishing clearly defined triggers for follow-up testing for the initial toxicity screening tests. The follow-up triggers are:

- Dilution series testing (*i.e.*, 6.25%, 12.5%, 25%, 50%, and 100%) will be conducted within 24 hours of reaching 100% mortality during testing for any sample, which will provide for a determination of the magnitude of the toxic response.
- Since it may be difficult to conduct a successful Toxicity Identification Evaluation (TIE) with samples that exhibit marginal toxicity, and as per EPA guidance (EPA 1996), a TIE (USEPA 1991, 1992, 1993a-b) will be performed immediately when an acceptable initial toxicity test exhibits ≥ 2 Toxic Units (*i.e.*, $\geq 50\%$ reduction in organism response when compared to the associated Laboratory Control). Phase I TIE procedures will at minimum determine the general class of the chemicals causing toxicity (metals, non-polar organics, polar organics), and may be targeted toward a specific contaminant class should existing information (*e.g.*, analytical or historical data) indicate that a specific contaminant class may be responsible for the toxicity. TIEs will be initially targeted toward classes of contaminants (*e.g.*, pesticides) that are most likely to be involved with toxicity in agricultural return water or storm water. For example, the targeted TIE for *Ceriodaphnia* and the fathead minnow will include the following treatments:
 - Centrifugation – to remove particulate associated contaminants (*e.g.*, pyrethroids) and a pre-treatment step for solid phase extraction (SPE and Chelex);
 - C-8 SPE – carbon column to remove non-polar organic compounds (*e.g.*, many pesticides);
 - Chelex – an ion exchange column that removes divalent cations (*e.g.*, metals); and
 - Piperonyl butoxide – inhibits the toxicity of pesticides that are metabolize to toxic forms in the cytochrome P450 enzyme system.

As the growth of *Selenastrum* can be inhibited or promoted by many TIE treatments, the *Selenastrum* TIE will be limited to the following treatments:

- C-8 SPE – carbon column to remove non-polar organic compounds (*e.g.*, many pesticides) – note that there is no centrifugation pre-treatment step since the *Selenastrum* test protocol requires sample filtration to remove resident algae from the sample prior to testing; and
- CHELEX SPE – ion exchange column treatment that removes divalent cations (*e.g.*, many metals).

All observations of toxicity will be immediately communicated to the Technical Program Manager, whom will make decisions regarding follow-up testing and/or sampling. In some cases where significant toxicity is observed during aquatic toxicity testing, samples may be analyzed for any of the chemical parameters included in this QAPP. The specific analyses to be performed will depend on the pattern of toxicity observed, including any decision to filter samples for chemical analysis. Every effort will be made to be consistent with the sample requirements documented herein for the specific analyte. Because requirements for sample and preservation holding times, filtration, and original sample containers may not be strictly met, the results of the

analyses will be used primarily for determining or confirming causes of toxicity, and will be qualified for any other use. Laboratories selected to perform these analyses must meet the same QA performance criteria used to select other laboratories for this monitoring program.

Sediment Toxicity Analyses

Sediment samples will be analyzed for toxicity to the amphipod *Hyalella azteca*, and will be analyzed only for survival. Determination of toxicity shall be performed generally as described in *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Organisms* (USEPA 2000). The laboratory SOP for this test is documented in Appendix C. Data critical for the evaluation of sediment toxicity test interpretation (e.g., storm conditions, flow rates, etc.) will be incorporated into program reports by the Technical Program Manager since they are not available to the laboratory.

Sediment samples that show statistically significant toxicity to *Hyalella azteca* at the end of an acceptable test and that exhibit a $\geq 20\%$ reduction in survival compared to the Laboratory Control require pesticide analysis of the same sample within 5 business days when the toxicity criterion described above is exceeded (i.e., an archived aliquot of the same sample used for the toxicity test that is held frozen until the toxicity test results have been analyzed) in an effort to determine the possible cause of toxicity.

Laboratory Calibration Curves

Any changes to laboratory calibration curves that occur during the implementation of this project need to be communicated to the Technical Program Manager, whom will share the information with the Central Valley Water Board staff. This is done to assure that any changes will comply with the program requirements. Only linear regression is acceptable for organic analyses since non-linear calibrations create a potential for poor quantification at low and high concentrations. In order to conduct linear regression analyses for calibrations, the lab must prepare a 5-point calibration curve where the low level standard concentration is less than or equal to the analyte reporting limit.

Alternative Analytical Methods

Only the methods specified in this document may be used to analyze samples for this program. Should a laboratory determine that an alternative method should be considered, then the lab must submit a performance-based evaluation of their SOP, performance-based validation package, and initial demonstration of capability to the Technical Program Manager for review. If the Technical Program Manager finds the method to be acceptable, it will then be submitted to the Central Valley Water Board's Executive Officer for review.

Physical Parameters, Drinking Water Constituents, and Nutrients

Analyzing laboratories must demonstrate the ability to produce reporting limits equal to or below the reporting limits (RLs) listed in Table A-5. Laboratory SOPs for these analyses are documented in Appendix D.

Metals/Trace Elements

Prior to analysis of any environmental samples for metals/trace elements, the laboratory must have demonstrated the ability to meet the minimum RLs for each analytical method. Initial

demonstration of laboratory capability includes the ability to produce a RL equal to or less than those listed in Table A-6. Procedures for demonstrating analytical performance requirements, extraction procedures, and waste disposal and pollution prevention requirements are detailed in the SOPs or EPA Method documents for each analytical method.

Pesticides

Pesticide analyses are performed on whole (unfiltered) samples. Prior to analysis of any environmental samples for pesticides, the laboratory must have demonstrated the ability to meet the RLs for each analytical method. Initial demonstration of laboratory capability includes the following:

- the ability to produce a reporting limit equal to or less than the RL listed in Table A-7 for sediments and A-8 for water samples; and
- the ability to generate acceptable precision and recovery, as defined by the specified method.

Procedures for demonstrating analytical performance requirements, extraction procedures, and waste disposal and pollution prevention requirements are detailed in the EPA Method documents for each analytical method. EPA's recommended minimum performance requirements are summarized in the method documents. Laboratory SOPs for pesticide analyses are documented in Appendix D.

All analytes detected between the MDL and the RL will be reported with a with a "DNQ" identifier. The laboratory will continue to provide acceptable detection limits with as much accuracy as possible in order to meet the water quality objectives and other State and Federal Water Quality Criteria in the future. This QAPP will be amended in the future should any lower RLs be developed for constituents of concern.

Sample Disposal

Laboratories are responsible for the proper disposal of the Westside Coalition sample. The laboratory Project Manager must seek approval from the Technical Program Manager prior to the disposal of all samples.

Corrective Actions

Program corrective actions are presented in Tables B-2a through B-2c. Laboratories must document any corrective actions that are related to Westside Coalition samples and report them to the program QA Officer. When a situation that is not "in control" occurs, analyses or work must be stopped until the problem has been identified and resolved. The analyst responsible must document the problem and a solution, and all analyses since the last control point must be repeated or discarded. The nature and disposition of the problem must be documented in the data report to the Central Valley Water Board.

Laboratory Turn Around Times

The laboratory turn around time is 30 days from the date of sample collection for hard copy reports and 45 days for EDDs.

Validation of Any Non-Standard Methods

For non-standard sampling and analysis methods, sample matrices, or other unusual situations, method validation study shall be documented to evaluate the appropriateness and performance of the method for the particular need. The purpose of this validation is to assess the potential impact on the representativeness of the data generated. For example, if a non-standard method is used, rigorous validation of the method may be necessary. Such validation studies may include round-robin studies performed by USEPA or other organizations. If previous validation studies are not available, some level of single-user validation study (i.e., a performance-based method validation study) should be performed during the project and included as part of the project's final report. Approval of non-standard methods ultimately is the responsibility of the Technical Program Manager, and must be approved by the Executive Officer of the Central Valley Water Board.

5. Quality Control

The types of quality control assessments used in the Westside Coalition monitoring program are discussed below. Quality control requirements and schedules are summarized in Tables B-2a through B-2c, as are the corrective actions required should control limits be exceeded.

Field Procedures

For basic water quality analyses, quality control samples to be prepared in the field will consist of field blanks and field duplicates. The QC site for a given event is selected from a limited number of sites that consistently have flow in all months.

Field Blanks

The purpose of analyzing field blanks is to demonstrate that sampling procedures do not result in contamination of the environmental samples. Field blanks will be prepared and analyzed for all chemical and microbiological analyses at the rate of one per event and at least 5% of all samples, along with the associated environmental samples. Field blanks will consist of laboratory-prepared blank water processed through the sampling equipment using the same procedures used for environmental samples. If any analytes of interest are detected at levels greater than the Reporting Limit (RL) for the parameter, the sampling crew should be notified so that the source of contamination can be identified (if possible) and corrective measures taken prior to the next sampling event. If the concentration in the associated samples is less than five times the value in the field blank, the results for the environmental samples may be unacceptably affected by contamination and should be qualified as an *upper limit* (UL) at the reported value.

Field Duplicates

The purpose of analyzing field duplicates is to demonstrate the precision of sampling. Field duplicates will be prepared at the rate of one per event and at least 5% of all samples, and analyzed along with the associated environmental samples. Field duplicates will consist of two aliquots from the same composite sample, or of two grab samples collected in rapid succession.

Field duplicates will be used to evaluate the Relative Percent Difference (RPD) between regularly collected field samples and the corresponding duplicate samples. The measured value for each field sample will be compared to the measured value for the corresponding field duplicate, and is the RPD expressed as:

$$RPD = \frac{V_m - V_k}{V_k} \times 100\%$$

Where: RPD = the relative percent difference
Vm = the measured value in the duplicate sample
Vk = the known value of the primary field sample.

If the relative Percent Difference (RPD) of field duplicate results is greater than 25% and the absolute difference is greater than the RL, both samples should be re-analyzed. If an RPD greater than 25% is confirmed by reanalysis, re-sampling is not required, but the environmental results of field duplicate will be qualified as *estimated* with the SWAMP code “FDP” (field duplicate replicate above QC limit). The sampling crew should be notified so that the source of sampling variability can be identified (if possible) and corrective measures taken prior to the next sampling event. It is important to note the collection of true field duplicates will also capture natural variability in the field, so if no errors were made during the sampling of a field duplicate that exceeds an RPD of 25%, no corrective actions will be taken. Results for field duplicates must be reported independently and not be averaged for determining an exceedance.

Laboratory Analyses

For basic water quality analyses, quality control samples prepared in the contract laboratory(s) will typically consist of equipment blanks, method blanks, standard reference materials, laboratory duplicates, matrix spikes, and matrix spike duplicates. It is important to note that laboratory control spikes and matrix spikes can't be performed on several basic water quality analyses (e.g., turbidity, TDS, and TSS). Laboratory analyses for bacteria will include method blanks, lab duplicates, and negative and positive quality control samples, as specified in the method documents. Laboratory analyses for pesticide analysis of sediment samples will include method blanks, laboratory duplicates, laboratory control spikes, laboratory control spike duplicates, matrix spikes, and matrix spike duplicates. Laboratory analyses for TOC analysis of sediment samples will include a method blank and laboratory spike.

Method Blanks

The purpose of analyzing method blanks is to demonstrate that the analytical procedures do not result in sample contamination. Method blanks will be prepared and analyzed by the contract laboratory at a rate of at least one for each analytical batch. Method blanks will consist of laboratory-prepared blank water processed along with the batch of environmental samples. The method blank should be prepared and analyzed before analysis of the associated environmental samples. If the result for a single method blank is greater than the MDL, or if the average blank concentration plus two standard deviations of three or more blanks is greater than the RL, the source(s) of contamination should be corrected, and the associated samples should be reanalyzed.

Some analytes may be detected even in the cleanest laboratory blanks. In these circumstances, the magnitude of a contaminant found in the blanks should be compared to the concentrations found in the samples. Subtracting method blanks from sample results is not permitted. For a blank metal analysis that is above the RL and the lowest concentration of the samples is 10x the method blank, the lab should report the data and the blank as above the RL. If the samples are <

10x the method blank concentration, the sample must be re-digested and re-analyzed for that analyte. For organics with a method blank above the RL, all samples must be re-extracted and re-analyzed for that analyte. The exception is for common laboratory contaminants (e.g., solvents and phthalates) where all samples associated with the blank may be < 10x the method blank and above the RL must be re-digested and re-analyzed for that analyte. Any blank contamination should be discussed with the Technical Program Manager and must be reported in the monitoring reports that are submitted to the Central Valley Water Board.

Laboratory Control Spike Samples

The purpose of analyzing laboratory control samples is to demonstrate the accuracy of the analytical method, and instrument bias. Laboratory control samples will be analyzed at the rate of one per sample batch. Laboratory control samples will consist of laboratory fortified method blanks. Recovery is the accuracy of an analytical test measured against a known analyte addition to a sample. Recovery is calculated as follows:

$$\text{Recovery} = \frac{\text{Measured Concentration}}{\text{Spiked Concentration}} \times 100$$

The acceptable recovery range varies by analyte and is specified in the appropriate method. If recovery of any analyte is outside the acceptable range for accuracy, the analytical process is not being performed adequately for that analyte. In this case, the sample batch should be prepared again, and the laboratory control sample should be reanalyzed. If reanalysis is not possible, the associated sample results should be qualified as *low or high biased*.

Laboratory Control Duplicates

The purpose of analyzing laboratory duplicates is to demonstrate the precision of the analytical method. Laboratory duplicates will be analyzed at the rate of one pair per sample batch. Specific duplicate analyses requirements for *E. coli* are specified in Table B-c. Laboratory duplicates will consist of a comparison of the laboratory control spike and laboratory control spike duplicate samples. The measured value for each sample will be compared to the measured value for the corresponding lab duplicate, and is the RPD expressed as:

$$\text{RPD} = \frac{V_m - V_k}{V_k} \times 100\%$$

Where: RPD = the relative percent difference
 V_m = the measured value in the duplicate sample
 V_k = the known value of the primary field sample.

If the Relative Percent Difference (RPD) for any analyte is greater than the precision criterion *and* the absolute difference between duplicates is greater than the RL, the analytical process is not being performed adequately for that analyte; note that the acceptable RPD will change with annual method detection limit studies performed by the laboratories. In this case, the sample batch should be prepared again, and laboratory duplicates should be reanalyzed. If reanalysis is not possible, or the reanalysis is greater than the acceptable RPD, the associated sample results should be qualified as *not reproducible* due to analytical variability.

Matrix Spikes and Matrix Spike Duplicates

The purpose of analyzing matrix spikes and matrix spike duplicates is to demonstrate the performance of the analytical method in a particular sample matrix. Matrix spikes and matrix spike duplicates will be analyzed at the rate of one pair per sample batch. Each matrix spike and matrix spike duplicate will consist of an aliquot of laboratory-fortified environmental sample. Spike concentrations should be added at between 2 to 10 times the expected sample value. Recovery is the accuracy of an analytical test measured against a known analyte addition to a sample. Recovery is calculated as follows:

$$\text{Recovery} = \frac{(\text{Matrix plus spike result} - \text{Matrix result})}{\text{Expected matrix plus spike result}} \times 100$$

If matrix spike recovery of any analyte is outside the acceptable range specified in the appropriate method, the results for that analyte have failed the acceptance criteria. If recovery of laboratory control samples is acceptable, the analytical process is being performed adequately for that analyte, and the problem is attributable to the sample matrix interference. Attempt to correct the problem (by dilution, concentration, etc.) and re-analyze the samples and the matrix spikes. If the matrix problem can't be corrected, qualify the results for that analyte as appropriate (*low or high biased*) due to matrix interference.

If matrix spike duplicate RPD for any analyte is greater than the precision criterion for the appropriate method, the results for that analyte have failed the acceptance criteria; note that the acceptable RPD will change with annual method detection limit studies performed by the laboratories. If the RPD for laboratory control spike and laboratory control spike duplicates is acceptable, the analytical process is being performed adequately for that analyte, and the problem is attributable to the sample matrix. Attempt to correct the problem (by dilution, concentration, etc.) and re-analyze the samples and the matrix spike duplicates. If the matrix problem can't be corrected, qualify the results for that analyte as *not reproducible*, due to matrix interference.

Aquatic Toxicity Quality Control

For aquatic toxicity tests, the acceptability of test results is determined primarily by performance-based criteria for test organisms, culture and test conditions, and the results of control bioassays. Control bioassays may include monthly reference toxicant testing, and negative and solvent controls (for TIEs). In addition to the QA requirements for the toxicity testing methods, a minimum of 5% of the samples collected for aquatic toxicity testing will be reserved for other QC analyses. These analyses will consist of field duplicates, as field blank analyses are generally not required for toxicity testing studies. Test acceptability requirements are documented in the method documents for each bioassay method and are included in Appendix C. In addition to the method test acceptability criteria, the Central Valley Water Board has developed specific follow-up requirements should a test not meet the test acceptability criteria (MRP Order, Attachment C, pages 22-23). The laboratory performing the toxicity testing must comply with these requirements. The laboratory is required to report what may have caused

the test control performance issue, the corrective measures that the laboratory took to prevent future control failures, and compare the data against the ILRP completeness criteria.

Table B-2a. Project Quality Control Requirements for Analysis of Water Quality Samples: Physical Parameters, Drinking Water Constituents, Nutrients, and Metals/Trace Elements.

QA Procedure	QA Parameter	Frequency ¹	Criterion	Corrective Action
Field Blanks	Contamination	1 per event and at least 5% of total samples	< RL or < sample ÷ 5	Examine field log. Identify contamination source. Qualify data as needed.
Field Duplicate	Precision	1 per event and at least 5% of total samples	RPD ≤ 25% if Difference ≥ RL	Reanalyze both samples. Identify variability source. Qualify data as needed.
Method Blank	Contamination	≥ 1 per batch	< MDL or, if n ≥ 3, avg ± 2 s.d. < RL	Identify contamination source. Reanalyze method blank and all samples in batch.
LCS or SRM ²	Accuracy	1 per batch	80-120% REC/ 75-125% REC (metals)	Recalibrate and reanalyze LCS or SRM and samples
Lab Control Spike Duplicate ²	Precision	1 per batch	RPD ≤ 25% if Difference ≥ RL	Recalibrate and reanalyze.
Matrix Spike ²	Accuracy	1 per batch	80-120% REC/ 75-125% REC (metals)	Check SRM recovery. Attempt to correct matrix problem and reanalyze sample. Qualify data as needed.
Matrix Spike Duplicate ²	Precision	1 per batch	RPD ≤ 25%	Check lab dup RPD. Attempt to correct matrix problem and reanalyze samples. Qualify data as needed.
Assess percent of data successfully collected	Data Completeness	1 per event	90%	Reschedule sample events as necessary or appropriate.

Notes: MDL = Method Detection Limit; RL = Reporting Limit; RPD = Relative Percent Difference; RSD = Relative Standard Deviation; REC = Recovery; LCS = Laboratory Control Sample; SRM = Standard Reference Material (=Certified Reference Material)

- (1) The term “batch”, as used in this document, refers to an uninterrupted series of analyses.
- (2) LCS, LCSD, MS, and MSD can’t be performed on turbidity, total dissolved solids, or total suspended solids. MSD may be used as the laboratory measure of precision in place of LCSD if the laboratory opts to not perform a LCSD.

Table B-2b. Project Quality Control Requirements for Analysis of Water Quality Samples: Requirements for Carbamate Pesticide and Urea Herbicide Analyses by EPA Method 8321, Organochlorine Pesticide Analyses by EPA Method 8081A, Organophosphorus Pesticide and Select Herbicide Analyses by EPA Method 8141A, EPA 547, and EPA 549.2, and for Sediment Quality Samples: Requirements for Pyrethroid Pesticide Analyses by EPA Method 8270.

QA Procedure	QA Parameter	Frequency ¹	Criterion	Corrective Action
Field Blanks ²	Contamination	1 per event and at least 5% of total samples	< RL or < (sample ÷ 5)	Examine field log. Identify contamination source. Qualify data as needed.
Field Duplicate	Precision	1 per event and at least 5% of total samples	RPD ≤ 25% if Difference ≥ RL	Reanalyze both samples. Identify variability source. Qualify data as needed.
Method Blank	Contamination	≥1 per batch	< MDL or, if n≥3, avg ± 2 s.d. < RL	Identify contamination source. Reanalyze method blank and all samples in batch.
Matrix Spike & LCS	Accuracy	1 per batch	50-150% REC or control limits at ± 3 S.D. of actual lab data	Check SRM recovery. Attempt to correct matrix problem and reanalyze sample. Qualify data as needed.
Matrix Spike & LCS Duplicates ³ :	Precision	1 per batch	RPD ≤ 25% if Difference ≥ RL	Check lab dup RPD. Attempt to correct matrix problem and reanalyze samples. Qualify data as needed.
Assess percent of data successfully collected	Data Completeness	1 per event	90%	Reschedule sample events as necessary or appropriate.

Notes: MDL = Method Detection Limit; RL = Reporting Limit; RPD = Relative Percent Difference; RSD = Relative Standard Deviation; REC = Recovery; LCS = Laboratory Control Sample; SRM = Standard Reference Material (=Certified Reference Material)

(1) The term “batch”, as used in this document, refers to an uninterrupted series of analyses.

(2) Field blank for sediment quality samples not collected.

(3) MSD may be used as the laboratory measure of precision in place of LCSD if the laboratory opts to not perform a LCSD.

Table B-2c. Project Quality Control Requirements for Analysis of Water Quality Samples for Pathogen Indicators by SM 9223.

<i>QA Procedure</i>	<i>Parameter</i>	<i>Frequency¹</i>	<i>Criterion</i>	<i>Corrective Action</i>
<i>E. coli Analyses</i>				
Field Blanks	Contamination	1 per event and at least 5% of total samples	< RL or < sample ÷ 5	Examine field log. Identify contamination source. Qualify data as needed.
Field Duplicate	Precision	1 per event and at least 5% of total samples	$RPD \leq 25\%$ if $ Difference \geq RL$	Reanalyze both samples. Identify variability source. Qualify data as needed.
Method Blanks (Sterility Checks)	Contamination	1 per batch	< RL	Identify contamination source. Clean equipment and slides. Check reagents. Re-analyze blank.
Lab Duplicate	Precision ²	1 per 10 samples, and at least 1 per batch	$R_{log} \leq 3.27 \cdot \text{mean } R_{Log}$	Recalibrate and reanalyze.
Negative Control Samples	Contamination	1 per culture medium or reagent lot	< RL	Identify source. Clean equipment and prepare new media. Re-examine negative control
Positive Control Samples	Assay function	1 per culture medium or reagent lot	$\geq RL$	Identify and correct problem. Re-examine positive control.
Assess percent of data successfully collected	Data Completeness	1 per planned sample event	90%	Reschedule sample events as necessary or appropriate.

Notes: MDL = Method Detection Limit; RL = Reporting Limit; RPD = Relative Percent Difference; RSD = Relative Standard Deviation; REC = Recovery; LCS = Laboratory Control Sample; SRM = Standard Reference Material (=Certified Reference Material)

(1) The method documentation defines an analytical batch as an “uninterrupted series of analyses”.

(2) R_{log} is the absolute difference between logarithms of coliform counts for duplicate analyses. The mean R_{log} is determined by performing duplicate analyses on the first 15 positive samples analyzed for each matrix type.

6. Instrument/Equipment Testing, Inspection, and Maintenance

All field and laboratory equipment is maintained based on manufacturer's specifications, and by qualified staff. Instrument-specific maintenance schedule, testing procedures and criteria, troubleshooting and any corrective actions are included in the appropriate SOPs.

Sample Equipment Cleaning Procedures

Equipment used for sample collection (*e.g.*, peristaltic pump tubing, carboys and carboy caps, and sample bottles) will be cleaned according to the specific procedures documented for each analytical method. Clean sample containers will be provided by the laboratories performing the analyses. Note that the same pump tubing and carboys may also be used to collect samples for analysis of other parameters. The cleaning procedures for equipment used to collect water quality and sediment quality samples are documented in Appendix B.

At least one equipment blank will be generated and analyzed for metals/trace elements prior to initiating monitoring for the program, and additional equipment blanks will be analyzed for new lots of critical cleaning reagents. In addition, for all analytes where contamination is considered a significant concern, field blanks will be collected and analyzed as directed in Section B-5 of this document. If the results of these analyses indicate any contamination, the source will be identified and corrected, and the equipment will be re-cleaned and re-tested. The combined regimen of equipment blanks and field blanks is considered to provide adequate control against potential systematic equipment contamination problems.

Analytical Instrument and Equipment Testing Procedures and Corrective Actions

Each laboratory has a QA program and a QA Manual, as per their certification by the Department of Public Health. Each laboratory must maintain a list of the individuals responsible for the testing, inspection, maintenance of analytical equipment used by the contract laboratory are specified by each laboratory and make this available upon request of the Technical Program Manager. As each laboratory has latitude within their accreditation to develop their own corrective actions protocols, such corrective actions are documented in the Quality Assurance manuals for each analyzing laboratory.

7. Instrument/Equipment Calibration and Frequency

Field Instruments

Calibration of all instruments used for measurement of field parameters (*e.g.*, temperature, pH, D.O., and specific conductivity) are performed as described in the owner's manual for YSI 556 multi-meter. Typical field instrument calibration procedures are as follows:

- Temperature calibration is factory-set and requires no subsequent calibration;
- Calibration for pH measurement is accomplished using standard buffer solutions;
- Calibration for D.O. measurements is accomplished using air oxygen saturation; and
- Calibration for specific conductivity measurements is generally accomplished using potassium chloride standard solutions.

Calibration of the field meter is performed at least twice daily, typically prior to the use of the instrument for sample analyses each day and at end of sample collection. However, the calibration frequency may be greater if the manufacturer requires increased calibration (e.g., for instrument failures). Calibration records are recorded in the field log.

Laboratory Analytical Equipment

Frequency and procedures for calibration of analytical equipment used by each contract laboratory is documented in the Quality Assurance Manual for each contract laboratory. Laboratory QA Manuals are made available for review at the analyzing laboratory.

Corrective Actions

Laboratory or field equipment that does not meet the manufacturer's requirements for calibration must be re-calibrated. Should re-calibration not address the problem, calibration standards should be replaced and/or the owner's manual maintenance requirements must be followed until the issue is resolved. Should the problem continue to occur, the equipment must be either repaired or replaced and a corrective action measures must be documented and submitted to the Technical Program Manager if the quality of the samples is affected.

8. Inspection/Acceptance of Supplies and Consumables

A list of critical supplies and consumables for field and laboratory analyses are identified in the attached field and laboratory SOPs. Each laboratory's QA Officer/Manager (QAPP, page 3) is responsible for overseeing inspection and acceptance of supplies and consumables in respective laboratories.

Field Consumables and Supplies

Gloves, sample containers, standard calibration solutions for the field multi-meter and any other consumable equipment used for sampling will be inspected by the field leads on receipt and will be rejected/returned if any obvious signs of contamination (e.g., torn packages, etc.) are observed.

Laboratory Standards and Reagents

With the exception of common laboratory solvents, all stock standards and reagents must be tracked through the laboratory. Standards must comply with method-specified holding time requirements. The preparation and use of all working standards must be recorded in bound laboratory notebooks that document standard traceability to U.S. EPA, A2LA or National Institute for Standards and Technology (NIST) criteria. Records must provide sufficient detail to allow determination of the identity, concentration, and viability of the standards including any dilutions performed to obtain the working standard. Date of preparation, analyte or mixture, concentration, name of preparer, lot or cylinder number, and expiration date, if applicable, is recorded on each working standard. All laboratory reagents used for the analyses described in this QAPP are noted in the SOPs in Appendix C and Appendix D. Inspection protocols and acceptance criteria for laboratory analytical reagents and other consumables are documented in the Quality Assurance Manuals for individual laboratories. Laboratory QA Manuals are made available for review at the analyzing laboratory.

9. Non-Direct Measurements

Water quality data collected by this monitoring program are intended to complement data collected by several other programs (see Section A-5). Although it is unlikely that data reported by these programs (e.g., data from CDEC rated flow tables, which could be used to calculate discharge, PUR data to determine the time and place of application of pesticides) will be used, the program's internal QA/QC should allow for use of the data without limitation for the purposes of the Westside Coalition monitoring program.

10. Data Management

Copies of field logs, copies of chain of custody forms, original preliminary and final lab reports, and electronic media reports will be sent to the program QA Officer. Each type of report will be stored separately and ordered chronologically. Field crews will retain the original field logs for a minimum of five years (electronic copies will be retained). Contract laboratory will retain the original chain of custody forms and copies of the preliminary and final data reports.

The laboratories have data management requirements specified in their QA Manuals. After data entry or data transfer procedures are completed for each sample event by the contract labs, data must be inspected for data transcription errors by approved staff, and corrected as appropriate. After the final QA checks for errors through the CEDEN data online data checker are completed, the data are sent to the Technical Program Manager in the form of hard copy reports and ILRP specific CEDEN-compatible EDDs. EDDs must meet the ILRP formatting and business rule requirements at the time of submission.

The various data and information generated from the Westside Coalition monitoring program will be stored and maintained at the Technical Program Manager's office. The data are transferred to a common database format maintained on personal computers in the Technical Program Manager's offices. The production of data tables for reporting to the Central Valley Water Board is generated from this database.

For the Semi-Annual Monitoring Reports, there may be cases where analytical results are less than the reporting limit for a parameter. In such cases, the results detected above the method detection limit (MDL) are reported numerically and flagged as DNQ ("j" flagged). The "<" flag can potentially be reported as a flag for *E. coli* or other analyses as appropriate. In cases where field blank results exceed the acceptance criteria listed in Tables B-2a – B-2c, data collected during the associated sample run will be qualified and reported as follows:

- Measured environmental sample concentrations greater than or equal to 5 times the field blank level will be reported with no qualification.
- Measured environmental sample concentrations less than 5 times the field blank level will be qualified as "less than" the measured value, e.g. if a field blank is equal to 1.5 µg/L, a measured environmental concentration of 4.0 µg/L will be reported as <4.0 µg/L.

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- Any data qualifications resulting from QC analyses will be reported with the environmental data as appropriate.

C. ASSESSMENT AND OVERSIGHT

1. Assessments and Response Actions

Assessments of compliance with quality control procedures will be undertaken on a routine basis during the data collection phase of the project:

- Performance of sampling procedures will be assessed by the lead field scientist during each sampling event and any quality control problems will be reported to the Monitoring Program Manager. Upon consultation with the program QA Officer, the Monitoring Program Manager will propose corrective actions to be carried out by the field sampling crew.
- Assessment of laboratory QC results and implementation of corrective actions will be the responsibility of the QA Officer/Manager at each laboratory and shall be reported to the program QA Officer as part of each data report. A similar review is performed twice annually when the semi-annual reports are prepared.
- Assessment of field QC results and implementation of corrective actions shall be the responsibility of the program QA Officer.

Routine procedures to assess precision and accuracy, criteria for success, and corrective actions have been discussed previously (Section B-3 and B-4 of this QAPP). The program QA Officer will maintain an open dialogue with each contract laboratory and Monitoring Program Coordinator regarding the QC review performed following each sampling event, and will maintain a QA Log of all communications and any specified corrective actions.

2. Reports to Management

A quality assurance report will be prepared by the program QA Officer, as part of the semi-annual monitoring report produced by Westside Coalition (MRP Order, page 17, Table 7). The quality assurance report will summarize the results of QA/QC assessments and evaluations, including precision, accuracy, comparability, representativeness, and completeness of the monitoring data. The semi-annual report will be distributed to the Regional Board, project managers and the Watershed Coordinator, as well as to all other program participants and interested parties.

D. DATA VALIDATION AND USABILITY

1. Data Review, Validation, and Verification

In addition to the data quality objectives presented in Tables B-2a through B-2c, the standard data validation procedures documented in the contract laboratory's Quality Assurance Manuals will be used to accept, reject, or qualify the data generated by the laboratory. Each laboratory's QA Officer/Manager (QAPP, page 3) will be responsible for validating data generated by the laboratory. The program QA Officer will be responsible for verification of data submitted by analyzing labs, including electronic data reports, and for qualifying all data based on the evaluation of field and laboratory quality control samples and based on the QA guidelines in this QAPP.

2. Verification and Validation Methods

Laboratory Procedures

Laboratory personnel will verify that the measurement process was "in control" (*i.e.*, all specified data quality objectives were met or acceptable deviations explained) for each batch of samples before proceeding with the analysis of a subsequent batch. In addition, each laboratory will establish a system for detecting and reducing transcription and/or calculation errors prior to reporting data.

Only data that have met data quality objectives, or data that have acceptable deviations explained, will be submitted by the laboratory. When QA requirements have not been met, the samples will be reanalyzed when possible and only the results of the reanalysis will be submitted, provided they are acceptable.

Validation of Field Data

The Technical Program Manager reviews field operations to ensure consistency and compliance with sampling specifications presented in the QAPP. At the request of the Technical Program Manager/QA Officer, the Monitoring Program Coordinator may perform field audits and use an audit checklists to document field observations and activities. The Technical Program Manager will work with the Field Leads to address any data quality issues that arise during data validation. The Technical Program Manager will be the ultimate arbiter for issues that can't be resolved between the Monitoring Program Coordinator and the Field Leads.

Analytical Data Validation

Prior to submitting the data to Technical Program Manager/QA Officer, the lab QA Officers/Managers will verify whether an analytical method has been performed according to the method and project specifications, and the results have been correctly calculated and reported. Specific items that are reviewed during data validation are:

- Chain of custody records
- Documentation of the laboratory procedures (e.g., standard preparation records, run logs, data reduction and verification)

-
- Accuracy of data reduction, transcription, and reporting
 - Adherence to method-specific calibration procedures and quality control parameters
 - Precision and accuracy of recorded results (with an objective of 100% accuracy).

Issues Resolution

The Technical Program Manager/ QA Officer (QAPP, page 3) will communicate with the individual lab QA Officers/Managers should data quality issues arise during data validation. The lab QA Officers/Managers must address the data validation issues in writing and propose appropriate corrective actions. The Technical Program Manager/QA Officer will be the ultimate arbiter for issues that can't be resolved with the laboratory QA Officers/Managers.

3. Reconciliation with User Requirements

The primary user requirement for the generated data is to determine potential exceedance of water quality objectives within the Westside San Joaquin River Watershed. Other questions asked within the monitoring objectives are to assess the baseline water quality, aquatic toxicity, and sediment toxicity. The data quality and completeness will be used to evaluate how well the user requirements have been addressed. Possible anomalies or any limitations on data use will be reported in the SAMR. Limitations on the use of data are also qualified on the database level, using SWAMP-defined codes and comments

The Westside Coalition needs a sufficient number of data points, as represented by the completeness data quality objective, in order to do trend analyses to determine if water quality conditions are improving in the Westside San Joaquin River Watershed. A failure to achieve the numbers of data points cited could mean an inability to provide this assessment. However, the monitoring program is expected to be a long-term study of the conditions in the watershed, which should allow for evaluations of water quality conditions, trends, and improvements to be evaluated.

E. REVISIONS TO THE QAPP

The purpose of this section is to document significant additions, deletions and revisions to the approved QAPP for this project, and to provide the rationale for these changes. Because this is the first version of this QAPP, no further information is presented in this section.

F. REFERENCES

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USEPA 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. U.S. Environmental Protection Agency (USEPA), Office of Water. EPA-821-R-02-012.

USEPA 2002. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. U.S. Environmental Protection Agency (USEPA), Office of Water. EPA-821-R-02-013.

APPENDIX A

**APPLICABLE WATER QUALITY CRITERIA,
ACTION LIMITS, TMDLs AND
BASIN PLAN OBJECTIVES**

	Reporting Unit	WQTL	Project RL
<i>Field, drinking and general physical</i>			
Electrical Conductivity	µS/cm	700	100
Temperature	° Celsius	variable	0.1
pH	pH units	6.5-8.5	0.1
Dissolved Oxygen	mg/L	7	0.1
Bromide			
E. coli	MPN/100ml	235	2
Total Organic Carbon	mg/L	NA	0.5
Hardness (as CaCO ₃)	mg/L		10
Total Dissolved Solids	mg/L	450	10
Total Suspended Solids	mg/L	NA	10
Turbidity	NTU	variable	1
<i>Metals</i>			
Arsenic	µg/L	10	1
Boron	µg/L	700	10
Cadmium (total and dissolved)	µg/L	variable	0.1
Copper (total and dissolved)	µg/L	variable	0.5
Lead (total and dissolved)	µg/L	variable	0.5
Nickel (total and dissolved)	µg/L	variable	1
Selenium	µg/L	5	1
Zinc (total and dissolved)	µg/L	variable	1
<i>Nutrients</i>			
Total Ammonia (as N)	mg/L	1.5	0.1
Nitrate + Nitrite (as N)	mg/L	10	0.05
Nitrogen, Total Kjeldahl	mg/L	NA	0.1
Total Phosphorous (as P)	mg/L	NA	0.01
Soluble Orthophosphate	mg/L	NA	0.01
<i>Organophosphorus Pesticides</i>			
Azinphos methyl	µg/L	0.01	0.1
Chlorpyrifos	µg/L	0.015	0.015
Demeton-s	µg/L	NA	0.1
Diazinon	µg/L	0.1	0.02
Dichlorvos	µg/L	0.085	0.1
Dimethoate	µg/L	1	0.1
Disulfoton	µg/L	0.05	0.05
Malathion	µg/L	ND	0.1
Methidathion	µg/L	0.7	0.1
Methamidophos	µg/L	0.35	0.2
Parathion, Ethyl			
Parathion, Methyl	µg/L	ND	0.1
Phorate	µg/L	0.7	0.2
Phosmet	µg/L	140	0.2
EPTC			
<i>Herbicides</i>			

Atrazine	µg/L	1	0.5
Cyanazine	µg/L	1	0.5
Diuron	µg/L	2	0.5
Linuron	µg/L	1.4	0.5
Prowl (Pendimethalin)	µg/L		
Simazine	µg/L	4	0.5
Trifluralin	µg/L	5	0.05
<i>Carbamate Pesticides</i>			
Aldicarb	µg/L	3	0.5
Carbaryl	µg/L	2.53	0.5
Carbofuran	µg/L	ND	0.5
Methiocarb	µg/L	0.5	0.5
Methomyl	µg/L	0.52	0.5
Oxamyl	µg/L	50	0.5
<i>Group A Pesticides</i>			
Aldrin	µg/L	0.00013	0.01
Chlordane, Total	µg/L	0.00057	0.01
Endosulfan (I & II)	µg/L	0.056	0.005
Heptachlor	µg/L	0.00021	0.005
Heptachlor epoxide	µg/L	0.0001	0.005
HCH, Total (in. Lindane)	µg/L	0.0039	0.005
Toxaphene	µg/L	0.00073	0.5 / 0.05
<i>Organochlorines Pesticides</i>			
Dicofol	µg/L	NA	0.1
DDD	µg/L	0.00083	0.02
DDE	µg/L	0.00059	0.01
DDT	µg/L	0.00059	0.01
Dieldrin	µg/L	0.00014	0.01
Endrin	µg/L	0.036	0.01
Methoxychlor	µg/L	0.03	0.01
<i>Sediment analyses</i>			
Total Organic Carbon	mg/kg dw	NA	200
Grain Size	%	NA	1
Bifenthrin	µg/kg dw	NA	1
Cyfluthrin	µg/kg dw	NA	1
Cypermethrin	µg/kg dw	NA	1
Esfenvalerate	µg/kg dw	NA	1
Fenpropathrin	µg/kg dw	NA	1
Cyhalothrin, lambda	µg/kg dw	NA	1
Permethrin	µg/kg dw	NA	1
Chlorpyrifos	µg/kg dw	NA	3
DDD (p,p')	µg/kg dw	NA	0.0061
DDE (p,p')	µg/kg dw	NA	0.0061
DDT (p,p')	µg/kg dw	NA	0.0061

APPENDIX B

**SUPPORTING DOCUMENTS FOR WATER QUALITY
SAMPLING**

ATTACHMENT 1: AMBIENT WATER SAMPLING SOP

Ambient Water Sampling Standard Operating Procedures

This S.O.P. is based, in part, upon methods described in EPA Method 1669: Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels (EPA 821-R-95-034).

1.0 Introduction

This sampling protocol is designed to support water quality monitoring programs authorized under the Clean Water Act, and permit the analysis of samples at or near the water quality criteria levels, while precluding sample contamination. Although this protocol was specifically designed for the collection of trace metals, the methods described below can also be applied toward the collection of ambient water samples for other analyses (*i.e.*, pesticides, general water quality characteristics, and aquatic toxicity).

2.0 Equipment And Supplies Needed

2.1 Collection by Boat

1. Battery operated peristaltic pump and connectors.
2. Peristaltic pump head.
3. Polyethylene tubing.
4. Teflon/Tygon® tubing.
5. Tubing connectors.
6. 3/4" PVC cut into 2-foot sections and 3/4" PVC elbows and couplers.
7. Rechargeable marine gel cell battery and back-up power source (Prestone Jump It®).
8. Battery charger.
9. Pre-cleaned sample bottles from analytical laboratory - appropriate for parameter sampled.
10. Sampling gloves - powder free (supplied by analytical laboratory) for metals and standard laboratory gloves for other parameters.
11. Ziplock bags, either provided by analytical laboratory or in-house, for bagging individual samples after collection and for bagging ice for Federal Express shipments.
12. Sample labels (may be project specific).
13. Bubble-wrap, or related packing material, to prevent bottle breakage during shipment.
14. Ice chests for shipping samples.

15. 30-gallon garbage bags for double bagging multiple sample bottles prior to shipping (specific to FedEx shipping - “no loose wet ice” is now a requirement for Federal Express).
16. “Field blank” water (consists of reverse-osmosis, de-ionized water from lab; split sample = “Lab Blank”).
17. Sharpie and pens.
18. Federal Express forms.
19. Address list for analytical laboratories that are to receive samples.
20. Project-specific QAPP.
21. Water proof field log book.
22. Sample log sheets (may be project specific).
23. Chain-of-custody forms.
24. Pacific EcoRisk research vessel (**and keys**).
25. Garmin® dash-mounted GPS and manual.
26. Motor and gear oil for boat.
27. D.O. meter.
28. pH meter & calibration standards.
29. Conductivity meter.
30. NIST calibrated thermometer or digital thermometer calibrated against NIST thermometer.
31. De-ionized water for and wash bottles for rinsing water quality meter probes.
32. Cell phone(s).
33. Cash for boat ramp fees.
34. Portable saw horses (for portable table top).
35. Plexiglass sheet (for portable table top).
36. Clip boards.
37. Acid-cleaned plastic sheets (from analytical lab).
38. Acid-cleaned tubing and bottle caps (from analytical lab).
39. 0.45 μm cartridge filters.

40. Expense record book.
41. Batteries for all field equipment.
42. 30-gallon trash bags.
43. Paper towels.
44. Tool box with tools.
45. First aid kit.
46. Tape gun and extra roll of tape.

2.2 Bridge or Bank Sampling (in addition to list above)

1. Bucket grab.
2. Bottle grab.
3. Rope.
4. Traffic cones.
5. Traffic vests.
6. Waders.
7. Rubber boots.
8. Hand-held Garman® GPS.

3.0 Sample Collection

****Never endanger yourself, your crew, or your equipment for samples - SAFETY FIRST****

3.1 Boat preparation at ramp/parking lot

1. Pay boat ramp fee, record expense in expense log book, and place receipt into envelope.
2. Load all necessary gear and coolers into boat.
3. Turn on vent to remove fuel vapors from engine compartment and turn on GPS.
4. Insert plug below the motor.
5. Remove stern boat straps from trailer and place into boat.
6. Release crank strap and safety strap from the bow of the boat.

7. Place vehicle into 4-WD Low to optimize traction on boat ramp.
8. Connect bow and stern lines and gently release boat into the water while other scientist holds boat near dock or shore.

3.2 Arrival on site and collection of basic water quality parameters

1. Navigate to exact sampling location using Garmin® GPS. Point the boat so the bow is heading upstream. Set the anchor, or alternatively tie off to a snag, and turn off the motor.
2. Record date, time, sample crew information, general weather information, and list of samples to be collected into field log book and field log sheets (if required for project).
3. Calibrate pH meter at first site of the day using the 7.0 and 10.0 pH buffers. Record the slope in field logbook.
4. Turn on dissolved oxygen (D.O.) meter and calibrate after 15 minutes (warm-up period). Record calibration information in field logbook.
5. Place pH, D.O., and salinity probes into water. Be careful - place meters on a stable surface inside the boat so they do not fall into the water. Record readings in field log book.
6. Place thermometer into the water and record ambient temperature in field log book.

3.3 Collection of samples using peristaltic pump (regardless of site)

1. Put on a pair of gloves (powder free if sampling trace metals). If “clean hands” sampling, do not touch anything (or anyone) other than what is described below . If your gloves are contaminated by touching anything, you must replace them.
2. Hook up peristaltic pump to marine gel cell battery and test for operation.
3. Remove polyethylene tubing from clean bag (do not touch “free ends”). After checking depth finder, feed enough polyethylene tubing through an appropriate number of PVC pipe sections to reach mid-channel depth. Cover the “free ends” of the tubing so as to minimize contamination.
4. Remove teflon/Tygon tubing from clean bag (do not touch “free ends”). Cover one free end with a powder free glove. Using a sharp utility knife, cut a 3-foot section and connect it to the polyethylene tubing.
5. Feed tubing through the pump head and turn pump on. Using PVC extensions, place end of tubing to mid-channel depth, upstream of the boat. Adjust pump head clamp pressure to a point where water begins to flow through. Pump blank water through the pump (if collected) **first** and collect into appropriately labeled bottle. Next pump site water through for five minutes to purge the lines.
6. Label bottles with time, date, and field crew. Cross reference sample number with sample log sheet to assure sample identification number is correct for sample to be collected.
7. If collecting sample by “clean hands/dirty hands method”, put label on outside of outer bag containing sample bottle. Both crew members collecting sample must put on

powder-free gloves. “Dirty hands” scientist will only operate pump and open only the outer bag that contain the sample bottle (do not touch anything or anyone (including yourself or you must replace your gloves)). “Dirty hands” scientist must not touch the neck of the bottle with tubing while pumping sample into the bottle. “Clean hands” scientist will open only the inner bag and remove the bottle cap (do not touch anything or anyone (including yourself or you must replace you gloves)).

8. Fill bottle with approximately 50-100 mL of site water, rinse, and discard; larger volume bottles (*e.g.*, 1-gallon amber bottles) should be rinsed with a greater volume of site water to so as to easily rinse all interior surfaces). Repeat 3 times then collect full bottle of sample while minimizing water spillage into ziplock bags (trace metals).
9. If appropriate, place sample into bubble wrap sleeves or ziplock bag. If sampling following “cleans hands technique”, “clean hands” scientist should seal only the inner ziplock bag. “Dirty hands” scientist should seal the outer ziplock bag. Immediately place sample into cooler with wet ice.
10. Record sample collection date, time, and sample identification number into field logbook, on field log sheets, and on chain-of-custody (COC) forms (COC can be done in transit between stations).

3.4 Collection of samples from shore or within a shallow stream

1. Follow general methods described above (connect pump, label bottles, etc.).
2. Attempt to eliminate contamination from dirt on the banks.
3. Put on waders or rubber boots, as the stream conditions require. Enter the stream carefully to minimize stirring up in-stream sediments.
4. Do not walk upstream of sample location or you risk contaminating the sample.
5. Collect sample as described above, remembering to collect upstream side of where you are standing.
6. Place sample bottles into cooler with wet ice.
7. Record sampling information into sample logbook and on sample log sheets and COC forms.

3.5 Collection of samples from a bridge

1. Put on traffic safety vest.
2. Place traffic cones on the upstream side of the bridge so as to warn on-coming traffic of your location.
3. Place 1-gallon amber glass bottle into bucket grab and remove cap. Drop bottle down below the surface for approximately 20 seconds. Pull bucket grab back up to bridge and pour out water. Repeat 3 times.
4. Drop bucket grab into water until bubbles are no longer seen. Pull bucket grab back up to bridge and decant ambient water sample into appropriately labeled sample containers.

5. Record sampling information into sample logbook and on sample log sheets and COC forms.
6. Place sample bottles into cooler with wet ice.

3.6 Collection of Samples for Specific Analyses

3.6.1 Collection of *E. coli* samples

1. Put on clean gloves.
2. Remove the cap from the pre-cleaned 100-mL sterile bottle; be careful to not loose the STS pellet in the bottle, which is necessary for the analysis.
3. *E. coli* bottles **must be collected as a surface hand grab**. Simply dip the bottle into the water and fill to the 100-mL mark on the bottle and do not rinse the bottle with site water.
4. Cap the bottle and loop the attached plastic tie (if present) through the hole to prevent the cap from coming loose.
5. Place the *E. coli* bottle into a ziplock bag (usually provided by the lab) and seal the bag.
6. Place the bottle/bag into a cooler with ice; be sure that the ice doesn't melt and enter the ziplock bags.
7. **Field duplicate** samples must be collected simultaneously with the event sample by filling appropriately labeled bottles with both bottles dipped at the same time using one hand; otherwise the duplicate variability may increase.
8. **Field blank** analyses are collected by directly filling a sterile bottle with de-ionized or distilled water.

3.6.2 Collection of pesticide samples

1. Put on clean gloves.
2. Remove the cap from the pre-cleaned 1000-mL amber glass bottle.
3. Collect the sample in accordance with the approved sampling method for the study; Sections 3.3 – 3.5 of this SOP are possible options.
4. Triple-rinse the bottle with site water, then completely fill and cap the bottle. Place the bottle into a bubble-wrap bag to minimize the possibility of breakage.
5. Place the bottle into a cooler with ice.
6. **Field duplicate** samples must be collected simultaneously with the event sample by filling appropriately labeled bottles either from the same bucket grab, dipped simultaneously for direct-to-container sampling, or using a "t-splitter" for peristaltic pump tubing.
7. **Field blank** analyses are collected by directly filling a sample bottle with de-ionized or distilled water.

3.6.3 Collection of toxicity samples

1. Put on clean gloves.
2. Remove the cap from the pre-cleaned sampling containers (e.g., 1-gallon amber glass bottles).
3. Collect the sample in accordance with the approved sampling method for the study; Sections 3.3 – 3.5 of this SOP are possible options.
4. Triple-rinse the bottle with site water, then completely fill and cap the bottle.
5. Place the bottle into a cooler with plenty of ice; it may take a considerable amount of ice to chill the large volumes collected for toxicity testing.
6. **Field duplicate** samples must be collected simultaneously with the event sample by filling appropriately labeled bottles either from the same bucket grab, dipped simultaneously for direct-to-container sampling, or using a “t-splitter” for peristaltic pump tubing. Collection of a true field duplicate will be difficult should bucket grabs be used due to the large volume of water collected and the relatively small volume of water captured by the bucket grab; collect the field duplicate by splitting the water from each bucket grab between the event sample and field duplicate sample bottles.

3.6.4 Collection of TOC samples

1. Put on clean gloves.
2. Remove the cap from the pre-cleaned 250 to 500-mL amber glass bottle; use care as the TOC bottles contain acid.
3. Collect the sample in accordance with the approved sampling method for the study; Sections 3.3 – 3.5 of this SOP are possible options.
4. Do not triple-rinse the bottle with site water, otherwise you will rinse away the acid that is necessary to preserve the sample. Completely fill the bottle, being careful to not overfill, and cap the bottle. Place the bottle into a bubble-wrap bag (if provided) to minimize the possibility of breakage.
5. Place the bottle into a cooler with ice.
6. **Field duplicate** samples must be collected simultaneously with the event sample by filling appropriately labeled bottles either from the same bucket grab, dipped simultaneously for direct-to-container sampling, or using a “t-splitter” for peristaltic pump tubing.
7. **Field blank** analyses are collected by directly filling a sample bottle with de-ionized or distilled water.

3.6.5 Collection of color, turbidity, and TDS samples

1. Put on clean gloves.

2. Color, turbidity, and TDS are collected using the same sample bottle. Remove the cap from the pre-cleaned 500 to 1000-mL amber glass bottle; acceptable alternative sample bottles are 500 to 1000-mL HDPE bottles.
3. Collect the sample in accordance with the approved sampling method for the study; Sections 3.3 – 3.5 of this SOP are possible options.
4. Triple-rinse the bottle with site water and completely fill and cap the bottle. Place the bottle into a bubble-wrap bag (if provided) to minimize the possibility of breakage.
5. Place the bottle into a cooler with ice.
6. **Field duplicate** samples must be collected simultaneously with the event sample by filling appropriately labeled bottles either from the same bucket grab, dipped simultaneously for direct-to-container sampling, or using a “t-splitter” for peristaltic pump tubing.
7. **Field blank** analyses are collected by directly filling a sample bottle with de-ionized or distilled water.

3.7 Field filtration of samples (as required)

1. Set up saw horses. Place plexiglass sheet on top. With powder free gloves on, place clean plastic sheet on top of plexiglass and clamp down with clipboards. Scientist without gloves on should place pump and battery on top of sheet and connect for pumping.
2. “Dirty hands” scientist should remove appropriate empty sample bottles from the analytical lab coolers and the 1-gallon bottle with the ambient water sample and place on them on top of the clean plastic sheet. “Dirty hands” scientist should place completed sample labels onto outer bag containing empty sample bottles and open the outer ziplock bag.
3. Using “clean hands/dirty hands” technique as described above, open outer and inner bag of 1-gallon sample bottle.
4. “Dirty hands” scientist will open outer bag containing tubing apparatus. “Clean hands” scientist will then remove tubing from inner bag. “Dirty hands” scientist will then carefully feed tubing into pump head without touching anything except the tubing. “Clean hands” scientist will open the 1-gallon sample bottle, place the lid top-side up in the inner sample bag, and feed tubing into one-gallon amber bottle. Vigorously shake the bottle containing the sample.
5. “Dirty hands” scientist will hold end of tubing and operating the pump. Pump approximately 100 mL through the tubing and discard. Flush the end of the tubing by pointing the tubing straight up while dispensing water from the pump. “Clean hands” scientist will open the inner bag and remove the lid from the bottle. Hold lid top-side up so as to minimize settling of dust into lid. “Clean hands” scientist should hold the sample bottle by the neck while the “dirty hands” scientist pumps water into bottle (**do not touch tubing to bottle!**). Rinse the sample bottle three times with approximately 25-50 mL of sample and discard the rinse water.

6. For the “unfiltered” fraction of any given trace metal, after thoroughly shaking the bottle containing the sample, fill the empty sample bottle to the top and “clean hands” scientist will cap the bottle and seal the inner ziplock bag. “Dirty hands” scientist will seal the outer bag.
7. After the “unfiltered” sample(s) have been collected, “dirty hands” scientist will remove a 0.45 μm cartridge filter from the analytical lab cooler and open the bag. “Clean hands” scientist will connect the tubing the appropriate end of the filter. The “dirty hands” scientist will purge the filter by running the pump for approximately 20 seconds while the “clean hands” scientist holds the filter. Rinse and fill the bottles as described above.
8. Place samples into cooler with wet ice.

4.0 Shipping Samples

1. Place sample bottles into 30-gallon garbage bag and seal bag.
2. Place this bag into another 30-gallon garbage bag containing sufficient ice so as to maintain sample at 4°C during shipping.
3. Enclose completed COC(s) in a zip lock bag and tape to inside lid of cooler along with appropriate samples. Tape outside of cooler and place “this side up” labels on front and back.
4. Complete Federal Express form and either drop off at Federal Express or retain for pick up by courier or Pacific EcoRisk staff.

5.0 Quality Assurance/Quality Control

The sampling team will follow the study-specific Quality Assurance Project Plan (QAPP) at all times.

6.0 Safety

Collection of ambient water samples can be done with minimal risk as long safety is considered first above all other factors involved with sampling. Be sure all staff members and other parties are aware of where safety equipment is on the boat. During inclement conditions, all parties on the boat must wear a life vest. Life vests should also be worn when collecting samples in more than a few feet of water or when sampling in swift currents. Care should also be taken to avoid hypothermia when collecting “in-stream” samples, and dehydration and heat stroke during hot conditions. In the event someone falls overboard, immediately throw a float cushion and maintain visual contact. Move the boat downstream and position the boat so as to “receive” the person in the water. Use the extendable pole to assist the person into the boat. Never retrieve a person from the water on the stern of the boat with the motor running. In the event difficulties are encountered retrieving the person in the water, immediately use cell phone to call 911 and describe your location and situation. Make sure first aid kit is stocked and all field crew members are familiar with the location.

ATTACHMENT 2: SEDIMENT SAMPLING SOP

SEDIMENT CORE/SAMPLE COLLECTION USING A ECKMAN GRAB AND/OR A PUSH-CORER Standard Operating Procedures

1.0 INTRODUCTION

Sediment samples will be collected with a Eckman or a push-core apparatus, depending upon the study needs and sediment conditions (*e.g.*, grain size). A Eckman is dropped through the water and allowed, via gravity, to “bite” into the surficial sediments. A “messenger” is then sent down the line to close the Eckman, which is facilitated by a quick upward pull on the retrieval line (after the messenger has hit the Eckman).

The push-corer, on the other hand, is lowered through the water column under manual control, and penetrates the sediment by means of its weight and manual pushing.

The following steps outline the procedure for collection of sediment samples using a petite ponar and push-corer.

2.0 EQUIPMENT AND SUPPLIES NEEDED

2.1 Collection by Boat

1. Eckman – The Eckman, messenger, and associated line.
2. Coring Device –The push core device has a 2-inch diameter barrel and is adjustable in length up to 15 feet; however most cores obtained using this method will not be greater than one foot, since only the top two centimeters of sediment are typically retained.
3. Sampling Containers – 2-L polyethylene containers.
4. Navigation and Positioning Methods – Project-specific sites/stations will be identified based on study needs, and GPS coordinates will be determined using a hand held Garmin® GPS.
5. Pond Master Boat – The 10’ Master is used in narrow channels and or creeks access that would prohibit the use of the 21’ Tracker®.
6. Boat Oars
7. Boat Dolly – clamps onto boat transom and allow boat to be rolled easily.
8. River anchor (18 lb.) and associated line
9. Min-Kota Endura® trolling motor, and battery
10. Hummingbird depth-finder
11. Pre-cleaned sample bottles from analytical laboratory - appropriate for specific analyses.
12. Sampling gloves - powder free (supplied by analytical laboratory) for metals and standard laboratory gloves for other parameters.

13. Ziplock® bags, either provided by analytical laboratory or in-house, for bagging individual samples after collection, and for bagging ice for Federal Express shipments.
14. Sample labels (may be project specific).
15. Bubble-wrap, or related packing material, to prevent bottle breakage during transportation.
16. Ice chests for shipping/storing samples.
17. 30-gallon polyethylene bags for double bagging multiple sample bottles prior to shipping (specific to FedEx shipping - “no loose wet ice” is now a requirement for Federal Express).
18. Sharpie pens.
19. Chain-of-custody forms.
20. Address list for analytical laboratories that are to receive samples.
21. Project-specific QAPP, if required.
22. Water-proof Field Log Book.
23. Sample log sheets (may be project specific).
24. Multi-meter.
25. NIST calibrated thermometer or digital thermometer calibrated against NIST thermometer.
26. De-ionized water (3-gallon jerrican full) for and wash bottles for rinsing water quality meter probes.
27. Cell phone(s).
28. Alconox/liquinox for decontamination of equipment
29. Scrub brush for decontamination.
30. Bucket for alconox waste
31. Expense record book (as needed).
32. Batteries for all field equipment, including water quality meters.
33. Paper towels.
34. Tool box with tools.
35. First aid kit.
36. Tape gun and extra roll of tape.
37. Foul weather gear and boots.
38. Stainless steel spoon and bowl.

2.2 Sampling while wading (in addition to list in Section 2.1)

1. Waders.

2.3 Sampling for Benthic Macroinvertebrates (in addition to list in Section 2.1)

1. BMI sampling manual
2. Project Labels (specific for invertebrate sample containers.
3. Measuring tape
4. D-shaped kick net (0.5 mm mesh)
5. Standard size 35 stainless steel sieve (0.5 mm mesh)
6. Pre-cleaned wide-mouth 500 mL plastic jars
7. Forceps
8. 95% ethanol
9. California Bioassessment Worksheet (for riffle sampling)
10. Physical/Habitat quality form (as needed)
11. Random number tables (for riffles)
12. Clinometer and stadia rod (for riffles)
13. Densiometer
14. White enamel pans

3.0 SEDIMENT SAMPLE COLLECTION

****Never endanger yourself, your crew, or your equipment for samples - SAFETY FIRST****

3.1 Tracker Boat preparation at ramp/parking lot

1. Pay boat ramp fee, record expense in expense log book, and place receipt into envelope.
2. Load all necessary gear and coolers into boat.
3. Turn on vent to remove fuel vapors from engine compartment and turn on GPS.
4. Insert plug below the motor.
5. Remove stern boat straps from trailer and place into boat.

6. Release crank strap and safety strap from the bow of the boat.
7. Place vehicle into 4-WD Low to optimize traction on boat ramp.
8. Connect bow and stern lines and gently release boat into the water while other scientist holds boat near dock or shore.

3.2 Pond Master Boat preparation

1. Carefully remove boat from vehicle.
2. Attach dolly if carrying boat long distances.
3. Once on site, load necessary coolers and gear into boat.
4. Once on site, attach trolling motor, battery, and depth finder.

3.3 Arrival on site and measurement of basic water quality parameters and habitat characteristics

1. Navigate to exact sampling location using Garmin® GPS or station markers. Orient the boat so that the bow is heading upstream; in lentic water, orient the boat upwind. Set the anchor, or alternatively tie off to a snag, and turn off the motor. If sampling multiple stations longitudinally along one localized stretch of water, sample the most downstream station first and work towards upstream stations.
2. Record date, time, sample crew information, general weather information, and list of samples to be collected into Field Log Book and Field Log Sheets (if required for project).
3. Calibrate pH meter at first site of the day, using the 7.0 and 10.0 pH buffers. Record calibration slope in field log book.
4. Turn on dissolved oxygen (D.O.) meter, and calibrate after 15 minutes (warm-up period). Record calibration information in field log book. In lentic water, the pH and D.O. probes must be kept in motion in order to obtain accurate measurements.
5. Place pH, D.O., and salinity probes into water. Be careful - place meters on a stable surface inside the boat so they do not fall into the water. Record readings in field log book.
6. Place thermometer into the water and record ambient temperature in field log book.
7. Perform habitat assessment for station following California Rapid Bioassessment protocol. Sites without riffles will require description of bank stability, canopy cover, channel width/depth and flow rate.

3.3 Collection of samples using the Eckman

1. Put on a pair of gloves (powder free if sampling trace metals). If “clean hands” sampling, do not touch anything (or anyone) other than what is described below . If your glove is contaminated by touching anything, you must replace them with new gloves.

2. Drop Eckman to the bottom, allowing the drop velocity to allow the Eckman to take a “bite” into the sediment. Send messenger down the line and then pull Eckman line swiftly upward to set the Eckman jaws into the closed position.
3. Pull Eckman up to boat, and rest on clean work surface.
4. Carefully open Eckman doors, gently allow the sediment to slide out, keeping the “grab” intact. Using a stainless steel spatula, remove top two cm of sediment. It is important to track the number of samples you remove from the Eckman so as to characterize the total volume of sediment collected.
5. Place sediment into sample container(s) if compositing for sediment chemistry or toxicity testing.
6. Place sediment into sieve if collecting and sorting benthic macroinvertebrates (BMI) identification. Sieve samples using site water and place BMI into pre-labeled 500 mL containers with ethanol.
7. If collecting samples for BMI tissue analyses, place BMI into pre-labeled laboratory sample jars only after rinsing with DI water.
8. Record sample collection date, time, and sample identification number into field log book, on field log sheets, and on chain-of-custody (C.O.C.) forms (C.O.C. can be done in transit between stations). Make sure that sample ID information is consistent with the sample container labels.

3.4 Collection of samples using push-core

1. Follow general methods described above.
2. Check to ensure that the polycarbonate core barrel is securely fastened to the extension pole and place it over the bow of the boat.
3. Determine the depth of the corer in the water column and track its’ subsequent penetration into the sediment with the reference being 0 ft at the tip of the core. Push the core in approximately one foot and turn the valve to “lock in” the sample.
4. Pull the corer up onto the boat and allow all but the top 2 cm to be extruded from the core.
5. Process the top 2 cm as described above.

4.0 SHIPPING OF SAMPLES

4.1 Shipping samples to analytical laboratory

1. Place sample bottles into 30-gallon garbage bag and seal bag.
2. Place this bag into another 30-gallon garbage bag containing sufficient ice so as to maintain sample at 4°C during shipping.
3. Enclose completed C.O.C.(s) in a zip lock bag and tape to inside lid of cooler along with appropriate samples. Close cooler and seal by wrapping tape 2-4 times around each end of the

cooler. Place “this side up” labels on front and back. If required by the project, place a custody seal on the container, and record your name and the date.

4. Complete Federal Express form and either drop off at Federal Express or retain for pick up by courier or Pacific EcoRisk staff.

4.2 Shipping of BMI samples for tissue analyses

1. Place samples into ice chest as described above.
2. Upon receipt at Pacific EcoRisk laboratory, place samples in freezer.
3. Once frozen, samples can be placed into an ice chest with dry ice and shipped to the analytical laboratory, along with a C.O.C.

4.3 Shipping of BMI samples for identification

1. Place preserved samples into an ice chest without ice.
2. Transport to the laboratory and store in a cool, dry place.

5.0 Quality Assurance/Quality Control

The sampling team will follow the Quality Assurance Project Plan (QAPP) set forth for each project at all times.

ATTACHMENT 3: FIELD EQUIPMENT DECONTAMINATION SOP

Revision #2 (Date last modified: 7/31/06 5:13 PM by Stephen Clark)

Effective Date: December 8, 2002

Accepted: _____

Field Equipment Decontamination STANDARD OPERATING PROCEDURE

References: SJR sample collection protocol, USGS Surface-Water Cleaning Procedures 1998.

1.0 PURPOSE

This Standard Operating Procedure (SOP) describes procedures for field equipment cleaning during sampling events. In addition, this SOP describes the quality control (QC) procedures for each type of instrument cleaning.

2.0 APPLICABILITY

The procedures described in this SOP are applicable to standard equipment cleaning procedures. Cleaning methods are specified in this SOP.

3.0 TERMS AND DEFINITIONS

QC-	Quality Control
SOP-	Standard Operating Procedure
MeOH-	Methanol
DI-	De-ionized Water

4.0 EQUIPMENT AND PROCEDURES

4.1 Equipment and Supplies

- Latex gloves for cleaning when using potentially hazardous chemicals
- Clean zip-lock bags for cleaned containers
- Liqui-Nox soap for pre-rinse
- MeOH for final rinse
- Container for MeOH waste
- Container for 100% MeOH
- Container for DI water for rinsing

4.2 Procedures

1. Follow the general procedures described below for all equipment that will be re-used for sampling; a Teflon sampler is provided as an example.
2. Disassemble the used sampler into component parts (bottle, cap, nozzle) so that all of the pieces can be thoroughly wetted with various rinses. Discard the previously used holding bag (do not attempt to clean it for reuse).
3. Wearing appropriate disposable gloves, thoroughly rinse the sampler components with DI. Use a stream of DI from the wash bottle, if required.

4. If a 3-L Teflon sampler will be used for collecting samples for analysis of organic compounds only, change gloves. Follow the procedures listed below:
 - Use Liqui-Nox to wash out all applicable equipment. Use only 2 to 3 squirts from a squirt bottle since the Liqui-Nox is in high concentration.
 - Rinse the cap and bottle of the 3-L Teflon sampler thoroughly with DI water until agitated rinse water produces no more suds.
 - Change to solvent resistant gloves.
 - Rinse the cap and bottle of the 3-L Teflon sampler three times with pesticide-grade methanol thoroughly.
 - Collect the used methanol into a container labeled “waste container” for safe storage and delivery for appropriate disposal.
 - Rinse the cap and bottle of the 3-L Teflon sampler thoroughly three times with DI water.
5. Reassemble sampler once all the components are dried. If the sampler is dedicated to sampling for organic compounds, then double wrap sampler in plastic bags for storage and transport.

APPENDIX C

**SUPPORTING DOCUMENTS FOR AQUATIC
TOXICITY TESTING**

**ATTACHMENT 1: CHRONIC *Selenastrum capricornutum* TOXICITY
TESTING SOP**

Revision #6

Effective Date: November 17, 2008

Accepted: _____

Selenastrum capricornutum
Algal Growth Bioassay
Standard Operating Procedures

This S.O.P. is based upon the U.S. EPA Guidelines described in Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms (EPA-821-R-02-013 and EPA-600-4-91-002). This SOP also meets the requirements of ASTM Method E 1218-04.

1.0 INTRODUCTION

This test is based on a 72-96-hour static exposure of the freshwater alga, *Selenastrum capricornutum*, to different concentrations of effluents and/or receiving waters. The test endpoint is growth (measured as cell density).

2.0 TEST PREPARATION

2.1 *Equipment and Supplies Needed*

1. Autoclave
2. Balance: capable of weighing to 0.01 mg.
3. Meters: D.O., pH and conductivity/salinity, needed to document test water quality.
4. Thermometer: ASTM certified, for documenting test water temperature.
5. Test Containers: 250-mL Erlenmeyer flasks with aluminum foil for covering. Flasks must be appropriately cleaned and rinsed
6. De-Ionized water: for rinsing of probes, etc.
7. Wash Bottles: for rinsing of probes, etc.
8. Volumetric and Graduated Flasks and Pipettes: for making up dilution series and reference toxicant test solutions
9. Pipettor: Eppendorf micro-pipettor with tips capable of pipetting 10-1000 μL .
10. Cubitainers: may be necessary for the client's collection of effluent.
11. ACS Reagent NaCl (Sodium Chloride), for use as reference toxicant.
12. Filter apparatus and 0.45 μm filters.
13. Chemicals necessary for making algal nutrient solutions.
14. Light meter
15. Hemocytometer

2.2 Ordering and Holding of Test Organisms

1. Test cultures should be ordered far enough in advance so as to ensure algal cultures are in log growth prior to the test set-up.

Order Algal cultures from:

Aquatic Research Organisms (603)926-1650 or
Botany Department, Univ. Of Texas (512)471-4019

3. For additional instruction on the receipt and handling of algal test cultures, see the “**Algal Test Culture Receipt and Handling S.O.P.**”

2.3 Collection and Holding of Water Samples

Grab or composite samples should be collected into appropriately-cleaned glass or plastic container(s) (e.g. cubitainers), and immediately be placed on ice (or “blue ice” type product) to bring the temperature to $\leq 6^{\circ}\text{C}$. The sample should be shipped or transported to the testing laboratory ASAP. Upon receipt of the sample(s) in the laboratory, sample log-in water quality measurements should be taken. For instruction on the log-in of incoming samples, see the “**Test Sample(s) Log-In Procedures**”. The test sample(s) used to start the test must be <36 hrs old (i.e. 36 hrs after collection). No sample > 72 hrs old should be used in any test(s). For each test, a minimum of 2 Liters of sample will be needed.

3.0 TEST INITIATION

These guidelines may be modified according to specific test conditions required by the NPDES permit of the individual client. Identify these specific requirements prior to test initiation and read the attached “**Summary of Test Conditions for *Selenastrum capricornutum***”.

3.1 Water Preparation

3.1.1 Diluent

1. Figure out how much water you need and determine which type of diluent (e.g., lab water or receiving water) is needed. Account for the extra volume that will be necessary for rinsing the flasks.
2. De-ionized water is used as Control and diluent for NPDES freshwater algal tests, including reference toxicant tests. Toxicity tests on ambient waters that require micronutrients without EDTA require Arrowhead drinking water as the Control and diluent, unless specified otherwise.
3. Filter water (0.45 μm). If performing an effluent test or testing a volatile material positive pressure filtration must be used.
4. Add nutrients. Have a second scientist spot the addition of the nutrients. These are stored in the refrigerator. 1 mL/L of each of the five nutrient solutions (A-D and micronutrients) should be added to the filtered water. For instruction on the preparation of the nutrient solutions, see the “***Selenastrum capricornutum* Nutrient Preparation Worksheet**” Adjust the Control pH to 7.5 ± 0.1 with HCl or NaOH.

3.1.2 Test Sample Water

Follow same procedure as in 3.1.1, except test samples should **not** be pH adjusted.

1. Prepare sample.
2. Filter sample using positive pressure.
3. Add nutrients.

3.2 Test Setup

1. Measure and record light intensity using a light meter throughout the test area. Follow procedures outlined in the “*Selenastrum capricornutum* Light Mapping SOP” Light intensity should be 4300+/-430 lux (400+/-40 foot candles). Record the mean light intensity for the test area on the test data sheet.

ASTM Method Modifications: Light intensity should be 4300+/- 645 lux (400+/-60 foot candles).

2. Prepare the test flasks for each concentration. You will need 5 containers per treatment; four test replicates and one water quality replicate. Label the flasks with their treatment and replicate I.D. Be sure to identify the client and test date at the minimum on the A replicates and the water quality flasks.

ASTM Method Modifications: You will need 4 containers per treatment; three test replicates and one water quality replicate.

3. Prepare dilutions of the test material. Prepare and adequate volume of each treatment for rinsing of the treatment flasks. Pour the volume of solution to be used at test initiation into the inoculation chamber.
4. Rinse all labeled test flasks vigorously with DI water, followed by a rinse with water from appropriate test treatment.
5. For each treatment, record the initial water quality (pH, D.O., conductivity, and temperature) onto the data sheets; check to make sure that all parameters are within acceptable levels.
6. Set aside at least 25 mL of solution for each control and test concentration in clearly labeled vials. These will be used as blanks at test termination. Store these subsamples at 4 +/- 1° C in the dark.
7. From the control and low, middle and high test concentrations, collect subsamples of approximately 100 mL for alkalinity and hardness measurements to be performed later.

3.3 Test Inoculation

3.3.1 Determination of Inoculation Volumes:

1. Inoculation density of *Selenastrum* should be 1×10^4 cells/mL
2. A 4 to 7 day old culture should be used so that the culture is in log growth phase.
3. Use a hemacytometer to count the density of the culture. Swirl the culture flask vigorously so that the contents are thoroughly mixed. Using a sterile pipet or pipet tip, transfer approximately 20 μ L of the culture onto the loading “V” of the hemocytometer and allow the chamber to fill by capillary action. Do not overfill or underfill the chamber. Let the algae to settle for five to ten minutes before counting..

4. Count 3-5 subsamples of the culture on the hemacytometer using the 20X objective on the compound or inverted microscope. To determine algal density in the culture, simply multiply the average hemacytometer count by 10^4 . Record microscopic and macroscopic observations of culture quality on the “**Algal Growth Test Inoculation Worksheet**”. Do not initiate tests with contaminated cultures.
5. The formula for determining inoculation is as follows:

$$\text{Inoculum volume} = (\text{volume to be inoculated}) \times \frac{(1 \times 10^4)}{(\text{algal density of culture})}$$

ASTM Method Modifications: Multiply inoculation volume by a factor of 1.2 in order to provide a surplus of inoculum.

3.3.2 Test Inoculation

1. Verify that the pipette is delivering an appropriate and consistent volume, set the pipet to deliver the inoculation volume and weigh a sample of DI on the analytical balance. Repeat this process. Verify that the % error is within the manufacturer’s acceptable range. Record this information on the “**Algal Growth Test Inoculation Worksheet**”.
2. Inoculate each control, test and reference toxicant treatment with the appropriate volume of algae as calculated in section 3.3.1. Have a second scientist observe the inoculation and sign off as doing so on the “**Algal Growth Test Inoculation Worksheet**”.
3. Beginning with the Control treatment and working up through the concentration series, pour 100 mL of the inoculated solution into each of the 5 containers associated with each concentration. Each concentration will have four test replicates and one water chemistry replicate for daily pH measurements. Make sure the algae are not allowed to settle when pouring out each 100 mL aliquot.
4. Randomly place the test containers within the temperature-controlled test area. Make sure that all of the necessary data are recorded upon the data sheets.

4.0 MAINTAINING THE TEST

1. Swirl flasks three times daily and randomize the flasks positions in the test area each time the flasks are swirled. Follow procedures outlined in the “***Selenastrum capricornutum* Daily Test Maintenance SOP**”
2. Once daily, measure and record pH of each concentration. The pH readings should be taken from the flasks designated for water chemistry measurements so that the test flasks do not get contaminated.
3. Measure the test area temperature once daily from a designated flask within that test area. Do not measure water quality parameters in test chambers when axenic conditions are to be maintained.

ASTM Method Modifications: For dilution series tests using the ASTM method, it is recommended that biomass measurements are performed daily in each test vessel to allow evaluation of test material effects during the test.

5.0 TEST TERMINATION

5.1 Turbidity (Absorbance) Method

1. Terminate the test after 96 +/-1 hours. Keep flasks cold and in the dark to restrict any further growth while test is being terminated. Remove the blanks that were collected for each concentration at test initiation from the cold storage, and allow to warm to room temperature prior to use.

ASTM Method Modifications: Test duration for *Selenastrum capricornutum* is 72 to 96 hours and is project dependant. See study plan for project-specific requirements.

2. Cell density is scored using the *in-vitro* turbidity (absorbance) method on the Hach DR 4000 spectrophotometer. To use the spectrophotometer, follow the procedures outlined in the “**HACH Spectrophotometer SOP**”. After turning on spectrophotometer, select user program number 101. This program measures *Selenastrum* cell density at 750 nm. This user program was generated following the methods described in the “**Selenastrum capricornutum: Calibration of Spectrophotometer for Determination of Algal Growth SOP**”.
3. Starting with the Control treatment, pour 25 mL of the appropriate blank solution into a cuvette. Place the filled cuvette into the machine and press zero. After the blank has been zeroed, all readings for the corresponding replicates of that treatment can be taken without having to re-zero. Save the blank solutions until the data has been reviewed and approval has been given to dispose of the test and blanks.
4. Swirl the first Control flask vigorously so that the contents are thoroughly mixed. Pour 25 mL into a cuvette (the same cuvette used for zeroing, or one that is paired with the cuvette used for zeroing) and place the cuvette into the spectrophotometer. The spectrophotometer should begin reading automatically. Record reading. Repeat for the remaining Control replicates. Once it has been determined that the Control treatment has a mean cell density of 1.0×10^6 cells/mL (2.0×10^5 for test without EDTA), and a CV of $\leq 20\%$, proceed with the termination of the remainder of the test. If these test acceptability criteria are not met, follow the procedures outlined on the “**Improving Selenastrum capricornutum Testing: Scientists’ Responsibilities**” worksheet.

ASTM Method Modifications: For tests using the ASTM method, two samples should be taken from each flask and two counts made of each sample. Record all four measurements on each of the remaining Control replicates. If only the turbidity method is used, representative samples of cells should be examined microscopically for morphological abnormalities at test termination.
5. Use the blank for the next highest concentration to zero the meter. Take readings on each of the four replicates of that concentration. Repeat the procedure up through the concentration series.
6. Measure and record final water quality (pH, D.O., conductivity, and temperature) for each control, sample or reference toxicant concentration.

5.2 Microscopic Method

1. Terminate the test after 96 +/-1 hours. Keep flasks cold and in the dark to restrict any further growth while test is being terminated. Rinse the hemocytometer and cover slip

thoroughly with DI water and dry with a Kimwipe.

ASTM Method Modifications: Test duration for *Selenastrum capricornutum* is 72 to 96 hours and is project dependant. See study plan for project-specific requirements.

2. Starting with the Control treatment, swirl the first flask vigorously so that the contents are thoroughly mixed.
3. Using a sterile pipet or pipet tip, transfer approximately 20 μl onto the loading “V” of the hemocytometer and allow the chamber to fill by capillary action. Do not overfill or underfill the chamber. Allow the cells to settle 5 to 10 minutes before counting the total number of cells in the 25 square grid.
4. Count 2 subsamples of each flask on the hemacytometer using the 20X objective on the compound or inverted microscope. To determine algal density, simply multiply the each hemacytometer count on the 25 square grid by 10^4 and record on the data sheet.

ASTM Method Modifications: Whenever feasible, at least 400 cells per flask should be counted.

6.0 REFERENCE TOXICANT TESTING

To ensure that the organisms being used in the test are responding to chemical stress in a “typical” manner, a reference toxicant test is run side-by-side with the effluent test. The reference toxicant results are then compared with an in-house data base for that reference toxicant to make this determination. Once the various reference toxicant concentrations are prepared, test set-up, maintenance, and termination are identical to those above. Information regarding the algal growth reference toxicity test is presented in the “**96 hour *Selenastrum capricornutum* Reference Toxicant Test SOP**”.

The toxicant generally used is Sodium chloride at concentrations of 0.5, 1, 2, 4 and 8 g NaCl/L.

7.0 DATA ANALYSIS

The cell density data for each replicate, which are recorded on the appropriate data sheets, are entered into a CETIS™ data file labeled for identification of the specific test. Statistical analyses are performed in accordance with EPA guidelines for statistical analysis.

8.0 TEST ACCEPTABILITY CRITERIA

1. The average algal cell density in the control replicates must be greater than or equal to 1×10^6 at the end of the test. For test using micronutrients without EDTA the average algal cell density in the control replicates must be greater than or equal to 2×10^5 at the end of the test.
2. The algal cell density in the control replicates must not vary more than 20% among replicates (CV%).

9.0 QUALITY CONTROL

1. Control water with nutrients added, is used.
2. All equipment is calibrated, maintained and operated as described in each applicable equipment SOP.
3. All staff working independently on any test shall have previously demonstrated familiarity and competency with the test, analytical equipment used, and the corresponding SOPs.

10.0 SAFETY

The *Selenastrum* chronic toxicity test poses little risk to those performing it. Care should be taken in the preparation of the reference toxicant spiking solution. Review the MSDS for the reference toxicant. After the ref-tox spiking solution has been used, any remaining solution should be disposed appropriately according to the “**Sample and Chemical Disposal SOP**”.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR ALGAL (<i>SELENASTRUM CAPRICORNUTUM</i>) GROWTH TEST (TEST METHOD 1003.0)	
1. Test type	Static
2. Test duration	96 hours
3. Temperature	25 ± 1°C
4. Light quality	“Cool white” fluorescent lighting
5. Light intensity	400 ± 40 ft-c (86 ± 8.6 μ E/m ² /s)
6. Photoperiod	Continuous illumination
7. Test chamber size	250 mL
8. Test solution volume	100 mL
9. Renewal of test solutions	None
10. Age of test organisms	4-7 days old
11. Initial cell density	10,000 cells/mL
12. No. of rep. chambers per concentration	Four (4)
13. Shaking rate	Twice daily by hand
14. Test chamber cleaning	Rinsed in algal culture media. None during test.
15. Dilution water	Algal culture media
16. Test Concentrations	According to NPDES permit. Effluents: 5 and a control Receiving Waters: 100% and a control.
17. Dilution factor	According to NPDES permit. Effluents: ≥ 0.5 dilution series Receiving Waters: None, or ≥ 0.5 dilution series
18. Test endpoint	Growth
19. Sampling and holding requirements	Grab or composite samples must be used to start test within 36 hours (no sample > 72 hours is used in test)
19. Sample volume required	2 Liters
20. Test acceptability	1 x 10 ⁶ cells/mL in controls; control variability ≤ 20% (CV%)

Supplemental SOP Language

Definitions:

ACS:	American Chemical Society
ASAP :	As soon as possible
ASTM :	American Society for Testing Materials
°C :	degrees Celsius
dH ₂ O :	distilled water
D.O.:	dissolved oxygen
ECx:	Effective concentration in X% of the population.
hrs :	hours
ICx:	Inhibitory concentration in X% of the population.
LCx:	Lethal concentration in X% of the population.
LOEC:	Lowest Observed Effect Concentration
mg :	milligram
mg/L :	milligram per liter
mL :	milliliter
NOEC:	No Observed Effect Concentration
NPDES :	National Pollutant Discharge Elimination System
S.O.P.:	Standard Operation Procedure
TIE:	Toxicity Identification Evaluation
U.S. EPA :	United States Environmental Protection Agency

Interferences:

In an effort to eliminate interferences, SOPs have been established for every procedure involved in conducting a successful bioassay test. Additionally, a rigorous daily QA/QC inspection is designed to identify potential sources of interference. Prior to the initiation of toxicity tests every effort is made to identify and eliminate potential sources of interference that could compromise test results. These can include but are not limited to the following: clean and functional facilities, equipment and test chambers; sample storage and handling; test organism and food quality; laboratory water quality.

Pollution Prevention

As a pollution prevention measure, wastes generated during toxicity testing must be properly handled and disposed of in an appropriate manner. Care should be taken not to generate excessive wastes when preparing solutions for testing. All materials identified as hazardous should be labeled and appropriately stored for hazardous waste disposal.

Data Assessment

Bioassay and water quality data are assessed each day during the course of testing for accuracy and compliance with established criteria. At test termination, the data for each replicate, which are recorded on the appropriate data sheets, are entered into a CETIS™ data file labeled for identification of the specific test. Statistical analyses are performed in accordance with EPA guidelines for statistical analysis. Control data for all endpoints are evaluated for compliance with established test acceptability criteria. Water Quality data are assessed for compliance with specifications outlined in the appropriate USEPA testing manuals.

Corrective Actions and Contingencies for Out-of-Control Data

If control performance is not met, a project manager should be notified immediately and, upon approval, the test is to be repeated. The potential cause(s) of poor control performance will be documented by scientific staff and evaluated and assessed by a project manager. Corrective actions will be determined on a case-by-case basis. The results of all tests will be summarized in reports for the regulatory authorities with an explanation of the results.

ATTACHMENT 2: ACUTE *Ceriodaphnia dubia* TOXICITY TESTING SOP

Revision #5

Effective Date: December 1, 2008

Accepted: _____

***Ceriodaphnia dubia* (“Cerio”)**

Acute Bioassay

Standard Operating Procedures

This S.O.P. is based upon the U.S. EPA guidelines described in Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition (EPA-821-R-02-012). See Addendum, for tests that require the guidelines set forth in the Fourth Edition (EPA/600/4-90/027F) or Third Edition (EPA/600/4-85/013) of this manual.

1.0 INTRODUCTION

This test is based on a 24, 48 or 96-hour static or static-renewal exposure of < 24 hr old (neonate, first instar) *Ceriodaphnia dubia* to different concentrations of effluents and/or receiving waters. The test endpoint is survival.

2.0 TEST PREPARATION

2.1 Equipment and Supplies Needed

1. Food: *Selenastrum*, YCT and the vitamins Thiamin, Biotin and B₁₂
2. Control/dilution (80:20) Water: Arrowhead® spring water and Evian® spring water, mixed at a ratio of 80:20, respectively.
3. Meters: D.O., pH and conductivity/salinity, needed to document test water quality.
4. Thermometer: ASTM certified, for documenting test water temperature.
5. Test containers: 30-mL plastic cups. Cups must be appropriately-cleaned and rinsed, and then pre-soaked for 24 hrs (overnight) in control water, before use in test.
6. Cerio board: Foam Board containing pre-cut holes to support replicate cups in waterbath.
7. De-ionized water: for rinsing of probes, etc.
8. Wash bottles: for rinsing of probes, etc.
9. Graduated flasks and pipettes: for making up dilution series and reference toxicant test solutions.
10. Transfer pipettes, wide-bore: for transfer of organisms to and from test containers.
11. Cubitainers may be necessary for the client's collection of effluent.
12. Temperature controlled water bath under cool white fluorescent lighting.

2.2 Ordering and Holding of Test Organisms

1. Neonate (<24 hr old) cerios can be obtained from in-house Stock Cultures (see *Ceriodaphnia dubia* Culture Maintenance SOP for methods.)

2. Alternatively, test organisms are only ordered from a supplier when in-house Stock cultures are not healthy enough to provide neonates. Test organisms should be ordered far enough in advance so as to ensure arrival of <24 hrs old animals on test set-up day. Approximately 25-33% more animals should be ordered than are actually needed for the test, so as to allow for some attrition of organisms that are stressed from the shipping, etc.

Order cerios from: - Aquatic BioSystems (800) 331-5916

3. For additional instruction on the receipt and handling of the test organisms, see the “Test Organism Receipt and Handling S.O.P.”

2.3 Collection and Holding of Water Samples

Grab or composite samples should be collected into appropriately-cleaned glass or plastic container(s) (e.g. cubitainers), and immediately be placed on ice (or “blue ice” type product) to bring the temperature to $\leq 6^{\circ}\text{C}$. The sample should be shipped or transported to the testing laboratory ASAP. Upon receipt of the sample(s) in the laboratory, sample log-in measurements should be taken. For instruction on the log-in of incoming samples, see the “Test Sample(s) Log-In SOP”. The test sample(s) used to start the test must be <36 hrs old (i.e. 36 hrs after collection). No sample > 72 hrs old should be used in any test(s).

3.0 TEST INITIATION

These guidelines may be modified according to specific test conditions required by the NPDES permit of the individual client. Identify these specific requirements prior to test initiation and read the attached “**Summary of Test Conditions for *Ceriodaphnia dubia***”.

1. At least two hours prior to loading the test organisms, collect neonate (<24 hr old) *C. dubia* into a volume of 1 L of culture water and feed them 7 mL of YCT and 13 mL of algae (*Selenastrum*).
2. Label an appropriate number of beakers and test cups with the appropriate test treatments for the test dilution series (e.g.: control, 5%, 10%, 25%, 50%, and 100%). You will need 4 containers per treatment. Label the cups with their treatment and replicate I.D. using colored tape and a Sharpie pen. See client NPDES permit for specific requirements regarding sample dilutions, number or replicates per test treatment, and number of organisms per replicate.
3. Label test cups for water quality measurements with treatment and replicate I.D. using a Sharpie pen.
4. Prepare dilutions (as needed) with lab water or receiving water according to client NPDES permit. Always work from low to high concentration and rinse out any glassware 3X with dH_2O prior to use. Place the beakers containing the test solutions into a temperature-controlled water bath at 20°C to allow test solutions to come to test temperature.
5. For each treatment, record the initial water quality (**pH, D.O., conductivity, and temperature**) onto the data sheets; check to make sure that all parameters, especially the D.O. are within acceptable levels. **Total residual chlorine** analysis is required for the highest concentration of municipal effluents. **Alkalinity** and **hardness** are measured for the control

when the water is made and for effluents/ambient waters when sample(s) arrive at the lab.

6. Place the replicate “cerio” test cups onto a foam support board. Beginning with the Control treatment and working up through the concentration series, aliquot 15 mL of test media into each of the containers **using a clean 60-cc syringe**. Place the replicate containers into the water bath.
7. Using a wide-bore transfer pipette, *carefully* capture and allocate 5 neonate *C. dubia* (from the fed stock) into each of the test containers, following the random template selected for the test. (Note – some NPDES permits may require a different number of organisms per replicate).
 - a. The organisms are delicate. When transferring, gently “release” organisms under the surface of the water. Make sure that each individual is uninjured. Replace injured individuals.
 - b. Be consistent with the volume of stock water used to transfer the organisms. Take care to avoid excessive dilution of the test treatments.
8. Place the test containers into the temperature-controlled room. The acute *Ceriodaphnia* test is either performed at $20 \pm 1^\circ\text{C}$ or $25 \pm 1^\circ\text{C}$, as specified by project guidelines. The test should be performed under a 16-h light, 8-h dark photoperiod where the light intensity is 50-100 foot-candles.

4.0 MAINTAINING A STATIC RENEWAL TEST

4.1 Each day

1. Monitor water quality parameters (temperature, pH and D.O.) in separate test cups and make sure the water quality is in acceptable limits. If dissolved oxygen falls below 4.0 mg/L, aerate the entire test.
2. Record the # of live animals on the appropriate data sheet. Remove any dead (immobile, white/opaque) cerios; it may be necessary to examine the organisms under a dissecting scope to confirm absence of heartbeat.

4.2 For renewals (on a 96-hour test only)

1. Approximately 2 hours before the renewal at 48 hours, feed each cerio test chamber 0.1 mL each of YCT and algae.
2. Check the D.O. level of the control water and the highest concentration of treatment media and make sure that there is enough oxygen; if not, you must aerate the sample until the D.O. reaches adequate levels.
3. Prepare the appropriate test solutions. Record pH, D.O., and conductivity before pouring into new test cups.
4. Transfer each organism into its new cup of media, saving the old media that will be pooled for each treatment to measure the “old” pH, D.O., and conductivity. Check these measurements to make sure that the water quality is within acceptable limits.
5. Place transferred organisms and test media back into the trough.

5.0 TEST TERMINATION

1. At time of test termination (24, 48, or 96 hrs from the test initiation \pm 2 hrs), pull the test containers from the water bath. Examine each cup and record the number of live animals. Pool the media for each treatment and measure final water chemistry parameters (temperature, pH, D.O. and conductivity) on the composite.
2. At this point, there should be percent survival data for each replicate for use in statistical analyses and report preparation.
3. Notify client immediately if toxicity is observed.

6.0 REFERENCE TOXICANT TESTING

To ensure that the organisms being used in the test are responding to chemical stress in a “typical” manner, a reference toxicant test is run side-by-side with the effluent test. The reference toxicant results are then compared with an in-house database for that reference toxicant to make this determination. Once the various reference toxicant concentrations are prepared, test initiation, maintenance, and termination are identical to those above. Information regarding the *Ceriodaphnia* reference toxicity test is presented in the “Acute *Ceriodaphnia dubia* Reference Toxicity Test SOP”.

7.0 DATA ANALYSIS

The survival data for each replicate, which are recorded on the appropriate data sheets, are entered into a CETIS™ data file labeled for identification of the specific test. Statistical analyses are performed in accordance with EPA guidelines for statistical analysis.

8.0 TEST ACCEPTABILITY CRITERIA

Tests are acceptable if the mean survival in control treatments is at least 90%.

9.0 QUALITY CONTROL

1. Control water, consisting commercial spring waters mixed together to create EPA moderately hard mineral waters is used for tests and cultures.
2. All equipment is calibrated and operated according to specific equipment SOP guidelines.
3. All staff working independently on any test shall have previously demonstrated familiarity and competency with the test, analytical equipment used, and the corresponding SOPs.

10.0 SAFETY

The *Ceriodaphnia dubia* 96-hr acute toxicity test poses little risk to those performing it.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR <i>Ceriodaphnia dubia</i> , ACUTE TOXICITY TESTS WITH EFFLUENTS AND RECEIVING WATERS (EPA-821-R-02-012 and EPA/600/4-90/027F)	
1. Test type	Static non-renewal, static-renewal
2. Test duration	24, 48, or 96 h
3. Temperature	20°C ±1°C; or 25°C ±1°C
4. Light quality	Ambient laboratory illumination
5. Light intensity	50-100 ft-c (10-20 µE/m ² /s)
6. Photoperiod	16 h light: 8 h darkness
7. Test chamber size	30 mL
8. Test solution volume	15 mL
9. Renewal of test solutions	After 48 h (on 96-h acute test <i>only</i>)
10. Age of test organisms	Neonate, less than 24-h old
11. No. organisms per test chamber	5
12. No. of replicate chambers per concentration	4
13. No. organisms per concentration	20
14. Feeding regime	2 hours prior to test initiation and 48-h renewal. YCT (7 mL/L) and algae (13 mL/L)
15. Test chamber cleaning	Cleaning not required
16. Test solution aeration	None
17. Dilution water	According to NPDES permit.
18. Test concentrations	According to NPDES permit. Effluents: 5 and a control Receiving Waters: 100% and a control.
19. Dilution series	According to NPDES permit. Effluents: ≥ 0.5 dilution series Receiving Waters: None, or ≥ 0.5 dilution series
20. Endpoint	Percent survival (mortality)
21. Sample and sample holding requirements	Grab or composite sample first used within 36 h of completion of the sampling period (no sample > 72 hours is used in a test)
22. Sample volume required	1 L
23. Test acceptability criteria	90% or greater survival in controls

Supplemental SOP Language

Definitions:

ACS:	American Chemical Society
ASAP :	As soon as possible
ASTM :	American Society for Testing Materials
°C :	degrees Celsius
dH ₂ O :	distilled water
D.O.:	dissolved oxygen
ECx:	Effective concentration in X% of the population.
hrs :	hours
ICx:	Inhibitory concentration in X% of the population.
LCx:	Lethal concentration in X% of the population.
LOEC:	Lowest Observed Effect Concentration
mg :	milligram
mg/L :	milligram per liter
mL :	milliliter
NOEC:	No Observed Effect Concentration
NPDES :	National Pollutant Discharge Elimination System
S.O.P.:	Standard Operation Procedure
TIE:	Toxicity Identification Evaluation
U.S. EPA :	United States Environmental Protection Agency

Interferences:

In an effort to eliminate interferences, SOPs have been established for every procedure involved in conducting a successful bioassay test. Additionally, a rigorous daily QA/QC inspection is designed to identify potential sources of interference. Prior to the initiation of toxicity tests every effort is made to identify and eliminate potential sources of interference that could compromise test results. These can include but are not limited to the following: clean and functional facilities, equipment and test chambers; sample storage and handling; test organism and food quality; laboratory water quality.

Pollution Prevention

As a pollution prevention measure, wastes generated during toxicity testing must be properly handled and disposed of in an appropriate manner. Care should be taken not to generate excessive wastes when preparing solutions for testing. All materials identified as hazardous should be labeled and appropriately stored for hazardous waste disposal.

Data Assessment

Bioassay and water quality data are assessed each day during the course of testing for accuracy and compliance with established criteria. At test termination, the data for each replicate, which are recorded on the appropriate data sheets, are entered into a CETIS™ data file labeled for identification of the specific test. Statistical analyses are performed in accordance with EPA guidelines for statistical analysis. Control data for all endpoints are evaluated for compliance with

established test acceptability criteria. Water Quality data are assessed for compliance with specifications outlined in the appropriate USEPA testing manuals.

Corrective Actions and Contingencies for Out-of-Control Data

If control performance is not met, a project manager should be notified immediately and, upon approval, the test is to be repeated. The potential cause(s) of poor control performance will be documented by scientific staff and evaluated and assessed by a project manager. Corrective actions will be determined on a case-by-case basis. The results of all tests will be summarized in reports for the regulatory authorities with an explanation of the results.

**ATTACHMENT 3: ACUTE FATHEAD MINNOW TOXICITY TESTING
SOP**

Revision #4

Effective Date: November 17, 2008

Accepted: _____

***Pimephales promelas* (Fathead Minnow)**

Acute Bioassay

Standard Operating Procedures

This S.O.P. is based upon the U.S. EPA guidelines described in Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition (EPA-821-R-02-012). See Addendum, for tests that require the guidelines set forth in the Fourth Edition (EPA/600/4-90/027F) or Third Edition (EPA/600/4-85/013) of this manual.

1.0 INTRODUCTION

This test is based on a 24, 48 or 96-hour static, static-renewal, or flow-through exposure of larval *Pimephales promelas* to different concentrations of effluents and/or receiving waters. The test endpoint is survival.

2.0 TEST PREPARATION

2.1 Equipment and Supplies Needed

1. Food: Brine shrimp nauplii - The test organisms will need to be fed brine shrimp nauplii (*Artemia*) daily until test initiation; depending upon the discharger's permit, feeding may be required immediately prior to the 48-hour renewal. Incubation of brine shrimp eggs should begin early enough so as to provide newly hatched nauplii when the fish arrive in the lab.
2. Balance: capable of weighing to 0.01 mg.
3. Reference weights: for calibration of balance.
4. Aeration system: needed for aeration when D.O. drops below acceptable levels.
5. Meters: D.O., pH and conductivity/salinity, needed to document test water quality.
6. Thermometer: ASTM certified, for documenting test water temperature.
7. Test Containers: 600-mL beakers appropriately cleaned and rinsed, and then pre-soaked for 24 hrs (overnight) in control water, before use in test (plumbed for flow-through if needed).
8. Peristaltic pump and tubing: to deliver test solutions (flow-through only)
9. De-Ionized water: for rinsing of probes, etc.
10. Wash Bottles: for rinsing of probes, etc.
11. Volumetric and Graduated Flasks and Pipettes: for making up dilution series and reference toxicant test solutions
12. Wide-bore transfer pipettes: for transfer of organisms to and from test containers.
13. Cubitainers: may be necessary for the client's collection of effluent.

14. ACS Reagent Cu (as Copper Sulfate), for use as reference toxicant.

2.2 Ordering and Holding of Test Organisms

1. Test organisms should be ordered far enough in advance so as to ensure arrival of 1-14 day old animals by the day of test set-up. Approximately 25-33% more animals should be ordered than are actually needed for the test, so as to allow for some attrition of organisms that are stressed from the shipping, etc.

Order fish from:

- (1) – Aquatox (501)767-9120
- (2) - Aquatic Biosystems (800)331-5916
- (3) - Aquatic Research Organisms (603)926-1650

2. For additional instruction on the receipt and handling of the test organisms, see the “**Test Organism Receipt and Handling SOP**”.

2.3 Collection and Holding of Water Samples

Grab or composite samples should be collected into appropriately cleaned glass or plastic container(s) (e.g. cubitainers), and immediately be placed on ice (or “blue ice” type product) to bring the temperature to $\leq 6^{\circ}\text{C}$. The sample should be shipped or transported to the testing laboratory ASAP. Upon receipt of the sample(s) in the laboratory, sample log-in water quality measurements should be taken. For instruction on the log-in of incoming samples, see the “**Test Sample(s) Log-In SOP**”. The test sample(s) used to start the test must be <36 hrs old (i.e. 36 hrs after collection). No sample > 72 hrs old should be used in any test(s). For each test, a minimum of 2 liters of sample will be needed each day.

2.4 Description of Flow-Through System

If this test will be performed as a flow-through exposure, test media will be delivered to test chambers using a peristaltic pump apparatus equipped with multi-channel pump heads. Test solution flow-rates to the test replicate chambers will provide a minimum of five (5) 90% replacements of water volume in each test chamber every 24 hours with test media delivered from test treatment solution head tanks via pre-cleaned (e.g., acid- and solvent-rinsed) silicone tubing. Flow rates will be checked and recorded for each treatment on a daily basis and appropriate adjustments performed. See “**Peristaltic Pump SOP**” for further guidance. Test treatment solutions will be prepared as described in Section 3.0.

3.0 TEST INITIATION

These guidelines may be modified according to specific test conditions required by the NPDES permit of the individual client. Identify these specific requirements prior to test initiation and read the attached “**Summary of Test Conditions for *Pimephales promelas***”

1. Fish should be fed with *Artemia* nauplii at least two hours prior to the start of the test.
2. Label an appropriate number of 600-mL beakers with the appropriate test treatments for your dilution series (e.g.: control, 5%, 10%, 25%, 50%, and 100%). You will need 2-4 containers per treatment. Label the beakers with their treatment and replicate I.D. using colored tape and a Sharpie pen. See client NPDES permit for specific dilution

requirements.

3. Label test cups for water quality measurements with treatment and replicate I.D. using a Sharpie pen.
4. Prepare dilutions (as needed) with lab water or receiving water according to client NPDES permit. Always work from low to high concentration and rinse out any glassware 3X with dH₂O prior to use
5. If flow through testing is used, adjust peristaltic pump to deliver appropriate volume of test solution per day. Make sure that the gravity outflow is functioning properly.
6. For each treatment, record the initial water quality (**pH, D.O., conductivity, and temperature**) onto the data sheets; check to make sure that all parameters, especially the D.O. are within acceptable levels. **Total residual chlorine** analysis is required for the highest concentration of municipal effluents. **Alkalinity** and **hardness** are measured for the control when water is made and for effluent when sample(s) arrive at the laboratory see the “**Test Sample(s) Log-In SOP**”.
7. Beginning with the Control treatment and working up through the concentration series, pour 400 mL of test media into each of the containers. Use the Sharpie pen and trace the water level line onto each container. Place dilution containers into the water bath to allow test waters to acclimate to test temperature.
8. Prior to loading any fish, clean the tank holding the test organisms as much as possible (i.e. remove uneaten food) minimizing any disturbance of the live animals.
9. Using wide-bore pipette (or dip net for older fish), *carefully* capture and randomly allocate 10 fish into each of the test containers. This is best accomplished by gradually placing allocations of 2-4 animals into each container until 10 animals are in each.
 - a. The organisms are delicate. When transferring, release organisms under the surface of the water. Make sure that each individual is uninjured. Replace injured individuals.
 - b. Be consistent with the volume of water used to transfer the organisms. Take care to avoid excessive dilution of the test treatments.
10. Randomly place the test containers within the temperature-controlled water bath. The water bath should be either at $20 \pm 1^{\circ}\text{C}$ or $25 \pm 1^{\circ}\text{C}$ (depending on project specifications), under a 16 hour light, 8 hour dark photoperiod at a light intensity of 50-100 foot-candles. Make sure that all of the necessary data are recorded upon the data sheets.
11. Let the replicate containers (with the animals within) sit for one hour and then re-examine each replicate, removing any organism(s) that appear to have been unduly stressed or injured from the loading procedure, and replace with a freshly caught animal.

4.0 MAINTAINING A STATIC RENEWAL TEST

4.1 *Each day*

1. For each test treatment, collect a sub-sample of test solution from a random replicate and determine the pH, D.O., and conductivity. Evaluate the water quality data to ensure that all water quality characteristics are within acceptable limits. If dissolved oxygen falls below

4.0 mg/L, aerate the entire test.

2. In flow-through tests, temperature, pH, DO, conductivity, and flow rate are measured at the beginning of the test, daily thereafter in the control and all test concentrations, and at test termination. In addition, total alkalinity, total hardness, and total residual chlorine and ammonia are measured daily in the highest effluent concentration.
3. Record the # of live animals on the appropriate data sheet. Remove any dead fish.

4.2 For renewals (on a 96-hour test only)

1. Approximately 2 hours prior to water renewals, feed each replicate newly hatched brine shrimp nauplii.
2. Record bath temperature. Check the D.O. level of the Control water and the highest concentration of treatment media and make sure that there is enough oxygen; if not, you must aerate the sample until the D.O. reaches adequate levels.
3. Prepare dilutions. Measure and record the “new” pH, D.O., conductivity and total residual chlorine (if required). If D.O. levels are not acceptable, you must aerate the samples until the D.O. reaches adequate levels. Place dilution containers into the bath for test waters to acclimate to test temperature.
4. Record the # of live animals on the appropriate data sheet. Remove any dead organisms.
5. Pull containers from the water bath and arrange in replicate number order and by treatment. Remove the uneaten food and any dead animals. This can be accomplished either of two methods:
 - a. (1) siphon out the debris and approximately 80% of the “old” test media from each replicate container, being careful not to accidentally siphon any of the live animals;
 - b. (2) use a transfer pipette to squirt water across the bottom of the replicate container, stirring up debris from the bottom in the process, and then quickly pour approximately 80% of the “old” media out, being careful not to pour out any live animals.
6. For each test treatment, collect a sub-sample of test solution from a random replicate and measure the “old” temperature, pH, D.O. and conductivity.
7. Carefully (and slowly) pour fresh media back into the replicate containers until the water level is at the original line. In order not to stress the animals while pouring in this media, tilt the containers and slowly pour in the new media down the side of the tilted container.
8. Verify the number of live fish in each replicate. Place the replicate beakers back in the water bath.

5.0 TEST TERMINATION

1. At time of test termination (24, 48, or 96 hours from the test initiation \pm 0.5h/24h of test duration), pull the test containers from the water bath. Measure and record the final water chemistry parameters (temperature, pH, D.O., and conductivity). Count and record the number of live animals in each replicate container.

2. At this point, there should be percent survival data for each replicate for use in statistical analyses and report preparation.
3. Notify client immediately if toxicity is observed.

6.0 REFERENCE TOXICANT TESTING

To ensure that the organisms being used in the test are responding to chemical stress in a “typical” manner, a reference toxicant test is run side-by-side with the effluent test. The reference toxicant results are then compared with an in-house database for that reference toxicant to make this determination. Once the various reference toxicant concentrations are prepared, test initiation, maintenance, and termination are identical to those above. Information regarding the fathead minnow reference toxicity test is presented in the “**Acute *Pimephales promelas* Reference Toxicity Test SOP**”.

7.0 DATA ANALYSIS

The survival data for each replicate, which are recorded on the appropriate data sheets, are entered into a CETIS™ data file labeled for identification of the specific test. Statistical analyses are performed in accordance with EPA guidelines for statistical analysis.

8.0 TEST ACCEPTABILITY CRITERIA

Tests are acceptable if the mean survival in control treatments is at least 90%.

9.0 QUALITY CONTROL

1. Control water, consisting of de-ionized water adjusted to moderate hardness with EPA salts, is used.
2. All equipment is calibrated and operated according to specific equipment SOP guidelines.
3. All staff working independently on any test shall have previously demonstrated familiarity and competency with the test, analytical equipment used, and the corresponding SOPs.

10.0 SAFETY

The fathead minnow acute toxicity test poses little risk to those performing it. Care should be taken in the preparation of the reference toxicant spiking solution. After the reference toxicant spiking solution has been used, any remaining solution should be appropriately stored for hazardous waste disposal.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR FATHEAD MINNOW, <i>PIMEPHALES PROMELAS</i> , ACUTE TOXICITY TESTS WITH EFFLUENTS AND RECEIVING WATERS (EPA-821-R-02-012 and EPA/600/4-90/027F).		
1.	Test type	Static non-renewal, static-renewal, flow-through
2.	Test duration	24, 48, or 96 h
3.	Temperature	20°C ±1°C; or 25°C ±1°C
4.	Light quality	Ambient laboratory illumination
5.	Light intensity	50-100 ft-c (10-20 µE/m ² /s)
6.	Photoperiod	16 h light: 8 h darkness
7.	Test chamber size	600-mL (250-mL minimum)
8.	Test solution volume	400 mL (200 mL minimum)
9.	Renewal of test solutions	After 48 h (on 96-h acute test <i>only</i>)
10.	Age of test organisms	1-14 days old with ≤ 24-h range in ages
11.	No. organisms per test chamber	10 (≤ 0.65 g/L static@20°C; ≤ 0.40 g/L static@25°C; or ≤ 2.5 g/L flow through@20°C)
12.	No. of replicate chambers per concentration	2-4
13.	No. organisms per concentration	20-40
14.	Feeding regime	2 hours prior to test initiation and 48-h renewal. 0.2 mL <i>Artemia</i> nauplii concentrate
15.	Test chamber cleaning	Cleaning not required
16.	Test solution aeration	None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min
17.	Dilution water	According to NPDES permit.
18.	Test concentrations	According to NPDES permit. Effluents: 5 and a control Receiving Waters: 100% and a control.
19.	Dilution series	According to NPDES permit. Effluents: ≥ 0.5 dilution series Receiving Waters: None, or ≥ 0.5 dilution series
20.	Endpoint	Percent survival (mortality)
21.	Sample and sample holding requirements	Grab or composite sample first used within 36 h of completion of the sampling period (no sample > 72 hours is used in a test)
22.	Sample volume required	2 L
23.	Test acceptability criteria	90% or greater survival in controls

Supplemental SOP Language

Definitions:

ACS:	American Chemical Society
ASAP :	As soon as possible
ASTM :	American Society for Testing Materials
°C :	degrees Celsius
dH ₂ O :	distilled water
D.O.:	dissolved oxygen
ECx:	Effective concentration in X% of the population.
hrs :	hours
ICx:	Inhibitory concentration in X% of the population.
LCx:	Lethal concentration in X% of the population.
LOEC:	Lowest Observed Effect Concentration
mg :	milligram
mg/L :	milligram per liter
mL :	milliliter
NOEC:	No Observed Effect Concentration
NPDES :	National Pollutant Discharge Elimination System
S.O.P.:	Standard Operation Procedure
TIE:	Toxicity Identification Evaluation
U.S. EPA :	United States Environmental Protection Agency

Interferences:

In an effort to eliminate interferences, SOPs have been established for every procedure involved in conducting a successful bioassay test. Additionally, a rigorous daily QA/QC inspection is designed to identify potential sources of interference. Prior to the initiation of toxicity tests every effort is made to identify and eliminate potential sources of interference that could compromise test results. These can include but are not limited to the following: clean and functional facilities, equipment and test chambers; sample storage and handling; test organism and food quality; laboratory water quality.

Pollution Prevention

As a pollution prevention measure, wastes generated during toxicity testing must be properly handled and disposed of in an appropriate manner. Care should be taken not to generate excessive wastes when preparing solutions for testing. All materials identified as hazardous should be labeled and appropriately stored for hazardous waste disposal.

Data Assessment

Bioassay and water quality data are assessed each day during the course of testing for accuracy and compliance with established criteria. At test termination, the data for each replicate, which are recorded on the appropriate data sheets, are entered into a CETIS™ data file labeled for identification of the specific test. Statistical analyses are performed in accordance with EPA guidelines for statistical analysis. Control data for all endpoints are evaluated for compliance with

established test acceptability criteria. Water Quality data are assessed for compliance with specifications outlined in the appropriate USEPA testing manuals.

Corrective Actions and Contingencies for Out-of-Control Data

If control performance is not met, a project manager should be notified immediately and, upon approval, the test is to be repeated. The potential cause(s) of poor control performance will be documented by scientific staff and evaluated and assessed by a project manager. Corrective actions will be determined on a case-by-case basis. The results of all tests will be summarized in reports for the regulatory authorities with an explanation of the results.

ATTACHMENT 4: 10-day *Hyaella azteca* TOXICITY TESTING SOP

Revision #4

Effective Date: November 17, 2008

Accepted: _____

Hyalella azteca
Acute (10-day) Survival & Growth Sediment Toxicity Test
Standard Operating Procedures

This SOP is based upon the U.S. EPA Method 100.1 guidelines described in “Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates, Second Edition (EPA/600/R-99/064)”. It is also in general accordance with ASTM Standard E1706-95b, Test methods for measuring the toxicity of sediment-associated contaminants with freshwater invertebrates.

1. INTRODUCTION

This test is based on a 10 day static-renewal exposure of 7-14 day old *Hyalella azteca* to sediments. The test endpoints are survival (and an optional endpoint of growth (measured as mean dry weight)).

2. TEST PREPARATION

2.1 Equipment and Supplies Needed

1. **Sample containers** may be necessary for the client’s collection of sediment. Containers must be pre-cleaned consistent with EPA guidelines. A minimum volume of 1-L of sediment is necessary (2-L is preferred) to provide sediment for the bioassay and for the accompanying sediment porewater characterization. Additional volume will be necessary for further characterization of sediment (e.g., grain size characteristics, contaminant concentrations).
2. **Stainless steel bowls and spatulas (or spoons)** to homogenize sediments prior to placement in replicate containers.
3. **Test containers**, consisting of 300-mL tall-form glass beakers, modified as follows:
 - a. The flared lip of the beakers should be cut off, and the upper rim flame-polished. This service can be provided by Orca Glassworks in Benicia. The prepared beakers must be appropriately cleaned before further use.
 - b. Cut a 2.5 cm-wide band of 425- μ m Nitex[®], approximately 25 cm in length. Using aquarium-safe silicon sealant, attach the band of Nitex around the upper lip of the beaker, such that ~two-thirds of the width of the Nitex band is above the glass. Make sure to completely seal the Nitex such that there are no openings or seams into which the test organisms might become entrapped. Allow the silicon sealant to cure for a minimum of 24 hrs. The resulting test containers must be appropriately cleaned and rinsed, and then pre-soaked for 48 hrs in reverse-osmosis, de-ionized (RO/DI) water, before use in testing.
4. **Modified Zumwalt-type water delivery system**, consisting of lower plastic tub to hold replicate containers in position, and upper plastic tub, plumbed with 75 mL syringes for delivery of water to replicate containers.

5. **Synthetic Test Water**, consisting of synthetic freshwater, prepared as per EPA guidelines (see Section 7.1.3.4 of guidelines):
 - a. Transfer ~75 L of reverse-osmosis, de-ionized (RO/DI) water into an appropriately-cleaned 120-L HDPE tank.
 - b. Add 5-gm of CaSO_4 and 5 gm of CaCl_2 to a 2-L aliquot of RO/DI water and mix on magnetic stir plate for 30 min or until the salts completely dissolve.
 - c. Add 3 gm of MgSO_4 , 9.6 gm of NaHCO_3 , and 0.4 gm of KCl to a second 2-L aliquot of RO/DI water, and mix on a magnetic stir plate for 30 min.
 - d. While vigorously stirring, pour each of the 2-L aliquots of salt solutions into the 75-L of RO/DI water, and fill to a total volume of 100-L with RO/DI water.
 - e. Vigorously aerate the water for at least 24 hrs prior to use.
 - f. The water quality should be:
 - i. Hardness, 90-100 mg/L as CaCO_3
 - ii. Alkalinity, 50-70 mg/L as CaCO_3
 - iii. Conductivity, 330-360 mS/cm
 - iv. pH, 7.8-8.2
6. **Water quality (pH, DO, and conductivity/salinity) meters**, calibrated and used as per the appropriate SOPs.
7. **Glass or electronic thermometer**, calibrated and used as per the appropriate SOP.
8. **Pipets**, disposable plastic Pasteur pipets, for the collection and transfer of test organisms.
9. **Fine-tip Forceps**, for use in collecting individual organisms from culture material at test initiation.
10. **Glass dishes**, for the sorting and collection of test organisms at test initiation and at test termination.
11. **Light boxes**, for the sorting and collection of test organisms at test initiation and at test termination.
12. **Aeration System**, in case needed to aerate should D.O. drops below acceptable levels.
13. **Test Food**, consisting of YCT.
14. **Sieves**, #25, #40, and #50, for collection of organisms at test termination.
15. **Aluminum Foil Weighing Pans**, for drying and weighing of *Hyaella* at end of test.
16. **Drying Oven**, at 105°C for drying larval amphipods at test termination.
17. **Desiccators**, for holding dried organisms.
18. **Balance**, capable of weighing to 0.01 mg. Calibrate and use as per the appropriate SOP

2.2 **Ordering and Holding of Test Organisms**

2.2.1 **Ordering and Holding of Test Organisms from Commercial Supplier**

1. Test organisms should be ordered far enough in advance so as to ensure arrival of 13-day old animals 24 hrs prior to Day 0. Approximately 25-33% more animals should be

ordered than are actually needed for the test, so as to allow for some attrition of organisms that are stressed from the shipping, etc.

2. Order *Hyaella azteca* from:
 - a. Aquatic Biosystems Inc.
 - b. Aquatic Research Organisms
3. Upon receipt, the test organism culture should be transferred into 4-L HDPE tanks containing test water at 23°C; the culture should be gently aerated, and should be fed slurried ground flake fish food and YCT.

2.2.2 Organisms from In-Lab Culture

1. Test organisms must be isolated from the In-Lab culture at least 13 days before the test is to begin in order to have 14 day-old animals on Day 0. Adults from each of the culture tanks should be collected and transferred to the top of the sieve bowls in the three neonate collection bowls. Add a few conditioned leaves to each of the sieve bowls as well, and provide gentle aeration. Allow to sit undisturbed overnight.
2. The following day, carefully remove the leaves, shaking to dislodge any clinging adults. Gently shake the top sieve bowl and lift out of the bowl assembly, carefully transferring the retained adults into a temporary holding container (make sure the transferred adults are not trapped at the water surface!). The remaining control water in the bowl assembly contains all of the neonates released. These should be transferred into a new culture tank containing a few conditioned leaves. During this transfer, the neonates should be counted. There should be at least 150% of the number needed for the test. If not, repeat this process with the adults and collect a second day's batch of neonates, which will be combined with the first days. After enough neonates are collected, the adults can be returned to their culture tanks.
3. The collected neonates should be fed a suspension of ground Tetra-Min, YCT, and powdered Spirulina. Change the water at 7 days and at 11 days, inspecting the animals to ensure adequate abundance, health and quality.

2.3 Collection and Holding of Sediment Samples

Grab or composite samples should be collected into appropriately-cleaned glass or plastic container(s), and immediately be placed on ice (or "blue ice" type product) to bring the temperature to $\leq 6^{\circ}\text{C}$. The sample should be shipped or transported to the testing laboratory ASAP. Upon receipt of the sample(s) in the laboratory, each sample should be logged in, and then placed in the sample refrigerator at 4°C. For instruction on the log-in of incoming samples, see the "**Test Sample(s) Log-In Procedures**". The test sample(s) used to start the test should be < 2 weeks old. For each site tested, a minimum of 16 L of sample will be needed for the bioaccumulation testing. Chemistry analyses will require additional samples. The total organic carbon content of each sediment type should be determined before starting test in order to validate the organism loading rate.

3.0 TEST INITIATION

Before test initiation begins be aware of any client-specific testing requirements and read the attached "**Summary of Test Conditions for *Hyaella azteca*.**"

3.1 On the Day Before Test Initiation (Day -1):

1. Remove the test replicate containers from soaking in the tank of R/DI water and shake excess water off. Each test treatment, including each Control, will require 8 test replicate containers. Label the test containers with their treatment and replicate ID code (Replicates “A” through “H”) using an indelible black ink (Sharpie®) pen.
2. Remove the sediment from the sample storage refrigerator and allow to come to room temperature. Using a stainless steel spoon and bowl, re-homogenize the sediment along with any overlying water that has developed.
3. For each sediment sample, use a stainless steel spoon or spatula to transfer approximately 100 mL of homogenized sediment into each of the 8 replicates, carefully “tamping” down the sediments. Carefully pour approximately 175 mL of control water into each beaker, taking care to minimize disturbance of the sediment.
4. Place the test replicates into the water bath, with the temperature controlled at 23°C, under cool-white fluorescent lighting on a 16L:8D photoperiod.

3.2 Pre-Test Sediment Porewater Characterization (Day -1, or before):

1. Place approximately 500 mL of each homogenized sediment into a 750-mL centrifuge bottle, and centrifuge at 2500 g for 30 min.
2. Decant supernatant (= sediment porewater), and measure routine water quality characteristics of the porewater (pH, DO, conductivity, and total ammonia). Record the water quality data into the Sediment Porewater Data Log Book.

3.3 Immediately Prior to Test Initiation (Day 0):

1. Using the Zumwalt water delivery system, renew the overlying water in each of the replicate containers.
2. After the water is renewed, collect ~25 mL of test water from 1-2 cm above the sediment in each test replicate using a disposable 25 ml glass pipet; composite the replicate water samples for each test treatment to provide a total volume of ~200 mL.
3. Measure the initial water quality conditions (temperature, pH, DO, conductivity, hardness, alkalinity, and total ammonia). Record the water quality data onto the Sediment Toxicity Test Water Quality Data Sheet.
4. If the DO levels fall below 2.5 mg/L, implement gentle aeration of each test replicate.
5. Isolation and Collection of Individual Test Organisms:
 - a. Immediately prior to test initiation, transfer small portion of test organism culture and test water into shallow glass dish placed on top of light box.
 - b. Using plastic pipet, agitate the culture material. This disturbance will cause the *Hyalella* to disengage from the substratum and swim around in the water, facilitating their capture.

3.4 Initiate the Test (Day 0):

1. Gently draw individual *Hyalella* into the pipet and transfer organisms directly into test replicate containers, gently expelling organism from pipet below the water surface.

Alternatively, transfer organisms into a small transfer dish (e.g., plastic weigh boats) containing small aliquot of test water, continuing process until there are 10 organisms in the transfer dish, that can subsequently be poured into the test replicates, again making sure that organisms are below the water surface. Note – this process must take place quickly, as extended period in the transfer dish will stress the organisms.

2. Allocate 10 randomly-selected 7-14 day old *Hyalella azteca* into each replicate beaker. Load test replicates following a randomized block approach. Load all “A” replicate containers first, with the order of test treatments being randomized. Repeat process for the “B” replicates, with the order of test treatments being re-randomized. Continue until all test replicates are loaded.
3. Immediately re-examine the replicates, replacing any dead or injured animals. Due to surface tension, some organisms may be “trapped” on the water surface. Examine each replicate to ensure that all test organisms are below the water surface. Using a plastic pipet, organisms that are at the water surface should be moved into the water by gently squirting the organisms with test water.
4. Randomly place the replicate containers into the temperature-controlled waterbath at 23°C, under cool-white fluorescent lighting on a 16L:8D photoperiod.
5. Feed each replicate 1.0 mL of YCT.

4.0 TEST MAINTENANCE (DAYS 1-9)

1. Examine each replicate container. Any dead organisms should be removed via pipet, and the number of mortalities recorded onto the test data sheet.
2. Each day, measure the temperature in the test water in one randomly-selected replicate for each treatment and record data onto test data sheet.
3. Using a disposable 25 mL pipet, collect “old” test water from 1-2 cm above the sediment for each replicate, compositing the replicate water samples for each test treatment to provide a total volume of ~200 mL. Measure the “old” DO and record data onto test data sheet. If the DO levels fall below 2.5 mg/L, implement gentle aeration of each test replicate.
4. Renew the overlying water using the Zumwalt water delivery system to deliver 2 replicate water volumes to each replicate container.
5. Collect ~25 mL of “new” test water from from each replicate, compositing the replicate water samples for each test treatment to provide a total volume of ~200 mL. Measure the “new” DO and record data onto test data sheet.
6. Return the test replicates to the test waterbath, and feed each replicate 1.0 mL of YCT.

5.0 TEST TERMINATION

1. Measure the temperature in the test water in one randomly-selected replicate for each treatment and record data onto test data sheet.

2. Collect ~25 mL of test water from 1-2 cm above the sediment in each test replicate using a disposable 25-mL glass pipet; composite the replicate water samples for each test treatment to provide a total volume of ~200 mL.
3. Measure the remaining final water quality conditions (pH, DO, conductivity, hardness, alkalinity, and total ammonia). Record the water quality data onto the Sediment Toxicity Test Water Quality Data Sheet.
4. Working one treatment and one replicate at a time, examine each replicate, noting and recording the number of any pupae, pupal exuvia, and/or adults, and recording this data onto the test weight data sheet.
5. Using a pipet or a squirt bottle containing clean test water, vigorously squirt water onto the top of the sediment so as to disturb the surficial layer – this will often result in the emergence of many of the *Hyalella*, facilitating their collection. Using a pipet and/or forceps, collect and transfer any emerging larvae into a glass sorting dish atop a light box. Using a squirt bottle, rinse the organisms with clean test water to remove any sediment or other clinging material. Using the forceps, transfer the individual larvae into a pre-labeled, -dried, and -weighed aluminum foil drying pan.
6. Carefully wash the sediment from the same replicate container through a #40 stainless steel sieve, washing the retained materials into the glass sorting dish. Using a pipet and/or forceps, collect and transfer any emerging larvae into a glass sorting dish. Using a squirt bottle, rinse the organisms with clean test water to remove any sediment or other clinging material. Using the forceps, transfer the individual larvae into the same pre-labeled, -dried (via muffle furnace), and -weighed aluminum foil drying pan that was used for the organisms collected in the earlier step (Step 6.5, above).
7. Record the number of live larvae collected from that replicate onto the test weight data sheet.
8. Repeat steps 6.4 through 6.7 for each test replicate.
9. When all of the replicate organisms have been transferred into their respective drying pans, place the pans into the drying oven, and dry at 105°C for 48 hrs.
10. After drying, place the aluminum pans into the desiccator and seal. Allow to cool at least 4 hrs, after which each pan must be weighed and the weight data recorded onto the test weight data sheet.

6.0 REFERENCE TOXICANT TESTING (OPTIONAL)

To ensure that the organisms being used in the test are responding to chemical stress in a “typical” manner, a reference toxicant test is run side-by-side with the effluent test. The reference toxicant results are then compared with an in-house database to make this determination. Information regarding the reference toxicity test is presented in the “*Hyalella* Reference Toxicity Test SOP”.

7.0 DATA ANALYSIS

1. For each sediment, sum up the total number of live organisms that were counted at test termination and record total number of live organisms at test termination onto the toxicity test data sheet.
2. On the test weight data sheet, subtract the weight of the pans + dried animals from the tare weight (the weight recorded for the empty pans) to determine the pooled dry weight of the larval organisms for that replicate. Divide this number by the number of larval organisms for that replicate to obtain the mean dry weight for individual organisms in that replicate.
3. Using the CETIS[®] statistical software, input the survival and relevant weight data for the Control treatment and for a given test sediment into a linked-file specific for that test sediment.
4. Analyze the test data, as per the EPA guidelines statistical flowchart procedures, comparing the test responses of the test sediment against the Control treatment to determine whether the test sediment exposure resulted in statistically significant reductions in survival or growth (as dry-ash weight) of the larval amphipods.

8.0 TEST ACCEPTABILITY CRITERIA

1. Age of *H. azteca* at the start of the test must be between 7- to 14-d old. The 10-d test should start with a narrow range in size or age of *H. azteca* (i.e., 1- to 2-d range in age) to reduce potential variability in growth at the end of a 10-d test.
2. Average survival of *H. azteca* in the control sediment must be greater than or equal to 80% at the end of the test. Growth of test organisms should be measurable in the control sediment at the end of the 10-d test (i.e., relative to organisms at the start of the test).
3. Hardness, alkalinity, and ammonia in the overlying water typically should not vary by more than 50% during the test, and dissolved oxygen should be maintained above 2.5 mg/L in the overlying water.

9.0 QUALITY CONTROL

1. All measured water quality should be within the limits established by the US EPA guidelines; any deviations must be noted in lab notebook and explained.
2. All equipment is calibrated and operated as described in each applicable equipment SOP.
3. All staff working independently on any test shall have previously demonstrated familiarity and competency with the test, analytical equipment used, and the corresponding SOPs.

10.0 SAFETY

The *Hyaella* survival and growth toxicity test poses little risk to those performing it. Sediments can contain pathogenic organisms and appropriate precautions should be observed when handling this material. After the test is complete, the sediments should be disposed of in an appropriate fashion.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR CONDUCTING THE 10-DAY <i>HYALELLA AZTECA</i> SURVIVAL AND GROWTH SEDIMENT TOXICITY TEST (TEST METHOD 100.1)	
1. Test type	Whole-sediment toxicity test with renewal of overlying water
2. Test duration	10 days
3. Temperature	23 ± 1°C
4. Light quality	Wide-spectrum fluorescent lights
5. Light intensity	About 100 to 1000 lux
6. Photoperiod	16L:8D
7. Test chamber size	300-mL high-form lipless beaker
8. Test sediment volume	100 mL
9. Overlying water	USEPA MH Culture water, well water, surface water, site water, or reconstituted water
10. Overlying water volume	175 mL
11. Overlying water quality	Temperature and D.O. daily. Hardness, alkalinity, conductivity, pH, and ammonia at beginning and end of test.
12. Overlying water renewal	2 volume additions/d @ one volume addition every 12 h
13. Age of test organisms	7- to 14-d old at the start of the test (1- to 2-d range in age)
14. No. of organisms per test chamber	10
15. No. of rep. chambers/concentration	8 but depends on the objective of the test.
16. Feeding regime	YCT food, fed 1.0 mL daily (1800 mg/L stock) to each test chamber.
17. Test chamber cleaning	If screens become clogged during the test, gently brush the <i>outside</i> of the screen
18. Test solution aeration	None, unless DO in overlying water drops below 2.5 mg/L
19. Endpoints	Survival and growth
20. Sample and sample holding requirements	Grab or composite samples should be stored at 4°C.
21. Sample volume required	16 Liter
22. Test acceptability criteria	Minimum mean control survival of 80% and measurable growth of test organisms in the control sediment.

Supplemental SOP Language

Definitions:

ACS:	American Chemical Society
ASAP :	As soon as possible
ASTM :	American Society for Testing Materials
°C :	degrees Celsius
dH ₂ O :	distilled water
D.O.:	dissolved oxygen
ECx:	Effective concentration in X% of the population.
hrs :	hours
ICx:	Inhibitory concentration in X% of the population.
LCx:	Lethal concentration in X% of the population.
LOEC:	Lowest Observed Effect Concentration
mg :	milligram
mg/L :	milligram per liter
mL :	milliliter
NOEC:	No Observed Effect Concentration
NPDES :	National Pollutant Discharge Elimination System
S.O.P.:	Standard Operation Procedure
TIE:	Toxicity Identification Evaluation
U.S. EPA :	United States Environmental Protection Agency

Interferences:

In an effort to eliminate interferences, SOPs have been established for every procedure involved in conducting a successful bioassay test. Additionally, a rigorous daily QA/QC inspection is designed to identify potential sources of interference. Prior to the initiation of toxicity tests every effort is made to identify and eliminate potential sources of interference that could compromise test results. These can include but are not limited to the following: clean and functional facilities, equipment and test chambers; sample storage and handling; test organism and food quality; laboratory water quality.

Pollution Prevention

As a pollution prevention measure, wastes generated during toxicity testing must be properly handled and disposed of in an appropriate manner. Care should be taken not to generate excessive wastes when preparing solutions for testing. All materials identified as hazardous should be labeled and appropriately stored for hazardous waste disposal.

Data Assessment

Bioassay and water quality data are assessed each day during the course of testing for accuracy and compliance with established criteria. At test termination, the data for each replicate, which are recorded on the appropriate data sheets, are entered into a CETIS™ data file labeled for identification of the specific test. Statistical analyses are performed in accordance with EPA guidelines for statistical analysis. Control data for all endpoints are evaluated for compliance

with established test acceptability criteria. Water Quality data are assessed for compliance with specifications outlined in the appropriate USEPA testing manuals.

Corrective Actions and Contingencies for Out-of-Control Data

If control performance is not met, a project manager should be notified immediately and, upon approval, the test is to be repeated. The potential cause(s) of poor control performance will be documented by scientific staff and evaluated and assessed by a project manager. Corrective actions will be determined on a case-by-case basis. The results of all tests will be summarized in reports for the regulatory authorities with an explanation of the results.

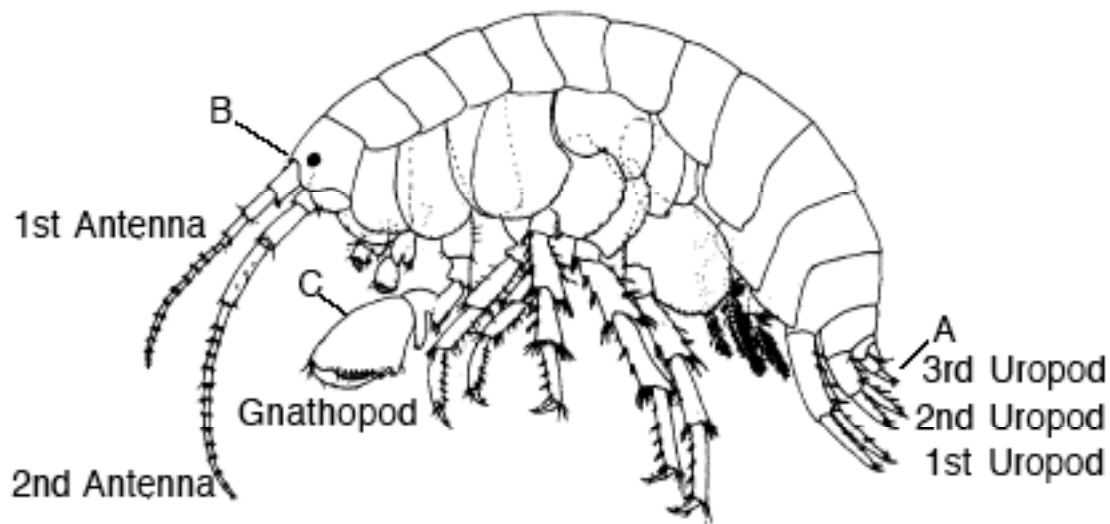


Figure 11.1 *Hyalella azteca*. (A) denotes the uropods; (B) denotes the base of the first antennae; (C) denotes the gnathopod used for grasping females. Measurement of length is made from base of the 3rd uropod (A) to (B). Females are recognized by the presence of egg cases or the absence of an enlarged gnathopod. (Reprinted from Cole and Watkins, 1997 with kind permission from Kluwer Academic Publishers.)

ATTACHMENT 5: TOXICITY IDENTIFICATION EVALUATION SOP

Revision #2

Effective Date: July 7, 2008

Accepted: _____

Targeted TIE Procedures for the Central Valley Irrigated Lands Regulatory Program Standard Operating Procedures

This S.O.P. is based upon the guidelines described in the following U.S. EPA documents:

- Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition (EPA-821-R-02-012)
- Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition (EPA-821-R-02-013)
- Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures, Second Edition (EPA-600/6-91/003)
- Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity (EPA-600/6-92/080)

1.0 INTRODUCTION

The US Environmental Protection Agency (US EPA) acute and chronic freshwater bioassays are used to characterize water quality in ambient monitoring programs. The species used in these bioassays include a green alga, *Selenastrum capricornutum*, an invertebrate, *Ceriodaphnia dubia*, and a minnow, *Pimephales promelas*. When toxicity is detected in a sample, Toxicity Identification Evaluation (TIE) procedures can be conducted to aid in the classification of chemical(s) causing toxicity to the bioassay organisms. For the Central Valley Regional Water Quality Control Board (CVRWQCB) Irrigated Lands Regulatory Program (ILRP), A TIE is initiated when there is a $\geq 50\%$ reduction in organism response when compared to the associated Lab Control. This information can be found in the Quality Assurance Project Plan, or QAPP.

TIEs involve chemical and physical manipulations to either remove or inactivate specific classes of chemicals. The toxicity of a manipulated sample is compared to that of an un-manipulated sample in a bioassay to ascertain whether toxicity is reduced by the manipulation. If organism performance improves in a manipulated sample, then a specific class of chemical is implicated as responsible for the toxicity.

The Regional Board has approved an abbreviated list of manipulations for the Irrigated Lands Monitoring Program that include the following:

- Centrifugation, see the “**Centrifugation SOP**”
- C-8 Solid Phase Extraction (SPE), see the “**C-8 Solid Phase Extraction SOP**”
- Chelex anion exchange resin, see the “**Chelex 100 Cationic Exchange Columns SOP**”
- Piperonyl Butoxide (PBO), see the “**PBO SOP**”

2.0 SAMPLE COLLECTION AND HANDLING

Grab samples should be collected into appropriately-cleaned 1 gallon amber glass bottles, and immediately be placed on ice (or “blue ice type product) to bring the temperature to 0-6°C. The sample should be shipped or transported to the testing laboratory ASAP so as to assure that the holding time limit is met. Upon receipt of the sample(s) in the laboratory, sample login water quality measurements should be taken. For instructions on the login of incoming samples, see the “**Sample_Receipt_Handling_SOP**”. The test sample(s) used to start the original toxicity test must be <36 hrs old (i.e., 36 hrs after collection), but the TIE will often be initiated outside this hold-time, as approved by the EPA. TIEs for the CVRWQCB ILRP must be initiated within 24 hrs of the observation of a $\geq 50\%$ reduction in organism response when compared to the associated Lab Control.

3.0 ACUTE AND CHRONIC TOXICITY TEST PROCEDURES

3.1 Algal Growth TIE with *Selenastrum capricornutum*

The performance of this test is based on the procedures outlined in the “*Selenastrum capricornutum* Algal Growth Bioassay SOP”. The procedures for sample manipulation are described in the appropriate TIE treatment SOP (see section 1.0). The test conditions that differ from the above mentioned SOPs are described in the TIE experimental design listed below.

Treatments:

Sample Treatment	Control/Blank Treatment
Baseline	Control
C-8 SPE	C-8 SPE Blank
CHELEX	CHELEX Blank

Dilution Series:	100% Sample treatment
Test Duration:	96 hours
Replicates:	4 reps/treatment + WQ rep
Test Volumes:	50 mL
Test Chambers:	250-mL Erlenmeyer flasks
Additional WQ:	Standard algae WQ (pH, DO, EC, and temperature at To and Tf, pH and temperature only at 24, 48, 72 hrs)
Manual Edition:	4 th edition protocols, <u>without</u> EDTA addition
Test acceptability:	2 x 10 ⁵ cells/mL in Control (w/o EDTA); Control variability < 20% (CV%)

3.1.1 Test Initiation

These guidelines may be modified according to specific test conditions required by the individual client. Identify these specific requirements prior to test initiation and read the “**TIE Study Design**” provided by the Project Lead.

3.1.1.1 Water Preparation

3.1.1.1.1 Control Medium

1. Determine the volume of Control water that will be needed. Commercially available Arrowhead[®] drinking water is used as the Control and treatment blanks for freshwater algal TIEs, unless otherwise specified. The minimum volume required for the Control is 1 L, which provides enough volume to rinse the flasks prior to test initiation and to set up the Lab Control treatment. Additional volume of Arrowhead[®] drinking water is required for rinsing the C-8 SPE and CHELEX columns. The **minimum volume for the C-8 SPE and CHELEX treatment blanks is 1 L.**

Control = 1 L

C-8 SPE Blank= 1 L
CHELEX Blank = 1 L
Minimum Volume = 3 L

2. Perform TIE treatment manipulations. See TIE treatment SOPs for procedures (section 1.0). The C-8 SPE treatment utilizes a **1g** column. A total of 1 L of Arrowhead® drinking water should be passed over the both the C8 and the CHELEX columns as a rinse; dispose of this rinsate. Then pass 1 L of Arrowhead® drinking water over the columns and collect as the treatment blank. Discard the first 100 ml, and then **collect the remaining volume**. The Ca/Mg add-back (see “**Ca/Mg Add-back SOP**”) must be completed on the CHELEX blank prior to nutrient addition.
3. Remove the five nutrient solutions (A-D and Micronutrients) from the refrigerator; be sure that the micronutrients without EDTA are used. Add one (1) mL/L of each of the five nutrient solutions (A-D and Micronutrients) to the Control and treatment blanks while thoroughly mixing the water as each nutrient spike is added.
4. Adjust the Control and treatment blanks to pH to 7.5 ± 0.1 with HCl or NaOH. ***Be sure to pH adjust the CHELEX treatment blank.***
5. There is 200 ml of Control water available for rinsing inoculation flasks.

3.1.1.1.2 Sample Water

1. Determine the volume of sample that will be needed. The **minimum volume for the C-8 SPE and CHELEX treatments is 1 L**.
Baseline = 1L
C-8 SPE = 1L
CHELEX = 1L
Volume lost during filtration = 500 mL
Minimum volume = 3.5 L
2. Filter the sample (0.45 µm), preferably using a high capacity groundwater filter. Filter with positive pressure using a peristaltic pump and an in-line filter. Rinse the filter with the first 500 mL of sample; dispose of this rinsate. Collect the volume of filtered sample you will need for the test.
3. Perform TIE treatment manipulations. See TIE treatment SOPs (section 1.0). The C-8 SPE treatment utilizes a **1g** column. 1 L of Arrowhead® drinking water should be passed over the C-8 and CHELEX columns as a rinse; dispose of this rinsate. Pass 1 L of the ambient water sample over the columns, discard the first 100 ml, then **collect the remaining volume**. The Ca/Mg add-back (see “**Ca/Mg Add-back SOP**”) must be completed on the CHELEX treatment prior to nutrient addition.
4. Add nutrients, mixing the water thoroughly as the nutrient spikes are being added.
5. ***Be sure to pH adjust the CHELEX treatment back to the pH of the baseline sample***

+/- 0.1pH unit prior to nutrient addition.

3.1.1.2 Test Solution Preparation

1. Pour 500 ml of the Control, treatment blanks, and sample treatments into inoculation chambers. **500 ml is the inoculation volume for the TIE.** If volume is limited, the minimum inoculation volume is 250 mL.
2. Set aside at least 40 mL of solution from each control, TIE blank and sample treatment (not from the inoculation chambers) in clearly labeled vials. These will be used as blanks at test termination as spectrophotometer blanks.
3. Collect 100 mL of test solution from the each treatment (not from the inoculation chamber) for alkalinity and hardness measurements to be performed later.
4. Measure pH, dissolved oxygen (DO), and conductivity from the spectrophotometer blanks set aside for test termination. Record values on the test data sheet. Store these spectrophotometer blanks in the refrigerator.

3.1.2 Test Maintenance

The maintenance of this test is the same as the procedures outlined in the “*Selenastrum capricornutum* Algal Growth Bioassay SOP”.

3.1.3 Test Termination

The termination of this test is the same as the procedures outlined in the “*Selenastrum capricornutum* Algal Growth Bioassay SOP”.

3.2 Acute Toxicity TIE with *Ceriodaphnia dubia*

The performance of this test is based on the procedures outlined in the “*Ceriodaphnia dubia* (Cerio) Acute Bioassay SOP”. The procedures for sample manipulation are described in the appropriate TIE treatment SOP (see section 1.0). The test conditions that differ from the above mentioned SOPs are described below.

Treatments:

Sample Treatment	Control/Blank Treatment
Baseline	Conditioned 80:20 Control
Centrifugation	Centrifugation Blank
Centrifugation + C8 SPE	Centrifugation + C8 SPE Blank
Centrifugation + CHELEX	Centrifugation + CHELEX
PBO (spiked at 100 µg/L)	PBO Blank (spiked at 100 µg/L)

Dilution Series: 100% Sample treatment
 Test Duration: 96 hours
 Replicates: 4 reps/treatment + WQ replicate
 Test Volumes: 15 mL/replicate
 # Organisms: 5/replicate
 Organism Age: <24 h old
 Test Chambers: 30-mL *Cerio* cups
 Additional WQ: Standard WQ parameters for an acute test
 Solution Renewal: 48 hours
 Feeding: 48 hours
 Manual Edition: 5th edition protocols
 Test acceptability: \geq 90% survival in Control treatment

3.2.1 Test Initiation

These guidelines may be modified according to specific test conditions required by the individual client. Identify these specific requirements prior to test initiation and read the “**TIE Study Design**” provided by the Project Lead.

3.2.1.1 Water Preparation

3.2.1.1.1 Control Medium

1. Determine the volume of Control water that will be needed. Conditioned 80:20 is used for

the Lab Control and treatment blanks for acute Cerio TIEs, unless otherwise specified. Additional volume of the Control water is required for rinsing the C-8 SPE and CHELEX columns. The minimum volume required for the Centrifuge and PBO treatment blanks is 300 mL, but **1 L is preferred**. The **minimum volume for the C-8 SPE and CHELEX treatment blanks is 1 L**.

Control = 1 L
Centrifuge Blank = 1 L
C-8 SPE Blank = 1 L
CHELEX Blank = 1 L
PBO = 1 L
Volume lost during centrifugation ~ 500 mL
Preferred Volume = 5.5 L, Minimum Volume = 4.1 L

2. Pass the Conditioned 80:20 through a 60 µm “Cerio” screen.
3. Perform TIE treatment manipulations. The C-8 SPE treatment utilizes a **1g** column. See TIE treatment SOPs for procedures (section 1.0). Centrifuge sufficient volume of conditioned 80:20 for the following treatments:
Centrifugation = 1 L
C-8 SPE = 1 L
CHELEX = 1 L
Ambient water column rinse water = 2 L
Volume lost during centrifugation ~ 500 mL
Preferred Volume = 5.5 L, Minimum Volume = 4.8 L
4. 1 L of centrifuged conditioned 80:20 water should be passed over the C-8 and CHELEX columns as a rinse; dispose of this rinsate. Pass 1 L of centrifuged conditioned 80:20 over the columns to be used as the treatment blank. Discard the first 100 ml, then **collect the remaining volume**. The Ca/Mg add-back (see “**Ca/Mg Add-back SOP**”) must be completed on the CHELEX blank prior to use.
5. ***Be sure to pH adjust the CHELEX treatment blank back to the pH of the Control water +/- 0.1.***

3.2.1.1.2 Sample Water

1. Determine the volume of sample that will be needed. The minimum volume required for the Centrifuge and PBO treatments is 300 mL, but **1 L is preferred**. The **minimum volume for the C-8 SPE and CHELEX treatments is 1 L**.
Baseline = 1 L
Centrifugation = 1 L
C-8 SPE = 1 L
CHELEX = 1 L
PBO = 1 L
Volume lost during centrifugation = 500 mL
Preferred Volume = 5.5 L, Minimum Volume = 4.1 L

2. Pass the sample through a 60 µm “Cerio” screen.
3. Perform TIE treatment manipulations. The C-8 SPE treatment utilizes a **1g** column. See TIE treatment SOPs for procedures (section 1.0). Centrifuge sufficient volume of the ambient water sample for the following treatments:
 - Centrifugation = 1L
 - C-8 SPE = 1L
 - CHELEX = 1L
 - Volume lost during centrifugation ~ 500 mL
 - Preferred Volume = 3.5 L, Minimum Volume = 2.8 L**
4. 1 L of centrifuged conditioned 80:20 water should be passed over the C8 and CHELEX columns as a rinse; dispose of this rinsate. Pass 1 L of sample over the columns, discard the first 100 ml, then **collect the remaining volume**. The Ca/Mg add-back (see “**Ca/Mg Add-back SOP**”) must be completed on the CHELEX treated sample prior to use.
5. ***Be sure to pH adjust the CHELEX treatment back to the pH of the baseline sample +/- 0.1.***
6. **PBO treatment:** Before adding the PBO to the solutions, place the 5 ppm PBO stock on the stir plate and allow warm up to room temperature. Place the test volumes for the PBO treatment blank and the PBO sample treatment (150 mL – see section 3.2.1.2) in the 20 °C water bath. When the solutions have warmed up, add the 5 ppm PBO stock to the blank and sample at a ratio of 2 ml of the 5 ppm PBO stock per 100 ml of solution. Stir the solutions. New solutions for the PBO treatment and treatment blank should be prepared at T48 as well.

3.2.1.2 Test Solution Preparation

1. Solution Prep: Pour off 150 mL of each TIE treatment and treatment blank for use at T0 .
2. From this volume, pour off 30 mL for characterization of initial water quality parameters.
3. Initial water quality parameters include pH, dissolved oxygen (DO), and conductivity.
4. Dispense the solution to the test replicate chambers.
5. Repeat at T48.

3.2.2 Test Maintenance

The maintenance of this test is the same as the procedures outlined in the “*Ceriodaphnia dubia* (Cerio) Acute Bioassay SOP”.

3.2.3 Test Termination

The termination of this test is the same as the procedures outlined in the “*Ceriodaphnia dubia* (Cerio) Acute Bioassay SOP”.

3.3 Acute Toxicity TIE with Larval Fathead Minnows

The performance of this test is based on the procedures outlined in the “*Pimephales promelas* (Fathead Minnow) Acute Bioassay SOP”. The procedures for sample manipulation are described in the appropriate TIE treatment SOP (see section 1.0). The test conditions that differ from the above mentioned SOPs are described below.

Treatments:

Sample Treatment	Control/Blank Treatment
Baseline	EPAMH Control
Centrifugation	Centrifugation Blank
Centrifugation + C8 SPE	Centrifugation + C8 SPE Blank
Centrifugation + CHELEX	Centrifugation + CHELEX
PBO (spiked at 100µg/L)	PBO Blank (spiked at 100µg/L)

Dilution Series: 100% Sample treatment
 Test Duration: 96 hours
 Replicates: 2 reps/treatment + WQ replicate
 Test Volumes: 75 mL/replicate
 # Organisms: 5 /replicate
 Organism Age: <24 h old (or youngest available fish)
 Test Chambers: 100 ml beakers
 Additional WQ: Standard WQ parameters for an acute test
 Solution Renewal: 48 hours
 Feeding: 48 hours
 Manual Edition: 5th edition protocols
 Test acceptability: \geq 90% survival in Control treatment

3.3.1 Test Initiation

These guidelines may be modified according to specific test conditions required by the individual client. Identify these specific requirements prior to test initiation and read the “**TIE Study Design**” provided by the Project Lead.

3.3.1.1 Water Preparation

3.3.1.1.1 Control Medium

1. Determine the volume of Control water that will be needed. EPAMH is used as the Lab Control and treatment blanks for acute fathead monnow TIEs, unless otherwise specified. Additional volume of the Control water is required for rinsing the C-8 SPE and CHELEX

columns. The minimum volume required for the Centrifuge and PBO treatment blanks is 600 mL, but **1 L is preferred**. The **minimum volume for the C-8 SPE and CHELEX treatment blanks is 1 L**.

Control = 1 L

Centrifuge Blank = 1 L

C-8 SPE Blank = 1 L

CHELEX Blank = 1 L

PBO = 1 L

Volume lost during centrifugation ~ 500 mL

Preferred Volume = 5.5 L, Minimum Volume = 4.7 L

2. Perform TIE treatment manipulations. The C-8 SPE treatment utilizes a **1g** column. See TIE treatment SOPs for procedures (section 1.0). Centrifuge sufficient volume of EPAMH for the following treatment blanks and rinsing the columns.

Centrifugation = 1 L

C-8 SPE = 1 L

CHELEX = 1 L

Ambient water column rinse water = 2 L

Volume lost during centrifugation ~ 500 mL

Preferred Volume = 5.5 L, Minimum Volume = 4.7 L

3. 1 L of centrifuged EPAMH should be passed over the C-8 and CHELEX columns as a rinse; dispose of this rinsate. Pass 1 L of EPAMH over the columns to be used as the treatment blank. Discard the first 100 ml, then **collect the remaining volume**. The Ca/Mg add-back (see “**Ca/Mg Add-back SOP**”) must be completed on the CHELEX blank prior to use.
4. ***Be sure to pH adjust the CHELEX treatment blank back to the pH of the Control water +/- 0.1.***

3.3.1.1.2 Sample Water

1. Determine the volume of sample that will be needed. The minimum volume required for the Centrifuge and PBO treatments is 600 mL, but **1 L is preferred**. The **minimum volume for the C-8 SPE and CHELEX treatments is 1 L**.

Baseline = 1 L

Centrifugation = 1 L

C-8 SPE = 1 L

CHELEX = 1 L

PBO = 1 L

Volume lost during centrifugation = 500 mL

Preferred Volume = 5.5 L, Minimum Volume = 4.7 L

2. Perform TIE treatment manipulations. The C-8 SPE treatment utilizes a **1g** column. See TIE treatment SOPs for procedures (section 1.0). Centrifuge sufficient volume of conditioned 80:20 for the following treatments.

Centrifugation = 1 L
C-8 SPE = 1 L

CHELEX = 1 L

Volume lost during centrifugation ~ 500 mL

Preferred Volume = 3.5 L, Minimum Volume = 2.8 L

3. 1 L of centrifuged EPAMH should be passed over the C8 and CHELEX columns as a rinse; dispose of this rinsate. Then pass 1 L of sample over the columns, discard the first 100 ml, then **collect the remaining volume**. The Ca/Mg add-back (see “**Ca/Mg Add-back SOP**”) must be completed on the CHELEX treated sample prior to use.
4. ***Be sure to pH adjust the CHELEX treatment back to the pH of the baseline sample +/- 0.1.***
4. **PBO treatment:** Before adding the PBO to the solutions, place the 5 ppm PBO stock on the stir plate and allow warm up to room temperature. Place the volume for the PBO treatment blank and the PBO sample treatment (300 mL – see section 3.3.1.2) in the 20 °C water bath. When the solutions have warmed up, add the 5 ppm PBO stock to the sample at a ratio of 2 ml of the 5 ppm PBO per 100 ml of solution. Stir the solutions. New solutions for the PBO treatment and treatment blank should be prepared at T48 as well.

3.3.1.2 Test Solution Characterization

1. Solution Prep: Pour off 150 mL of each TIE treatments and treatment blanks for use at T0.
2. From this volume, pour off 30 mL for characterization of initial water quality parameters. Initial water quality parameters include pH, dissolved oxygen (DO), and conductivity.
3. Dispense the solution to the test replicate chambers.
4. Repeat at T48.

3.2.2 Test Maintenance

The maintenance of this test is the same as the procedures outlined in the “***Pimephales promelas* (Fathead Minnow) Acute Bioassay SOP**”

3.2.3 Test Termination

The termination of this test is the same as the procedures outlined in the “***Pimephales promelas* (Fathead Minnow) Acute Bioassay SOP**”

Supplemental SOP Language

Definitions:

ACS:	American Chemical Society
ASAP :	As soon as possible
ASTM :	American Society for Testing Materials
°C :	degrees Celsius
dH ₂ O :	distilled water
D.O.:	dissolved oxygen
ECx:	Effective concentration in X% of the population.
hrs :	hours
ICx:	Inhibitory concentration in X% of the population.
LCx:	Lethal concentration in X% of the population.
LOEC:	Lowest Observed Effect Concentration
mg :	milligram
mg/L :	milligram per liter
mL :	milliliter
NOEC:	No Observed Effect Concentration
NPDES :	National Pollutant Discharge Elimination System
S.O.P.:	Standard Operation Procedure
TIE:	Toxicity Identification Evaluation
U.S. EPA :	United States Environmental Protection Agency

Interferences:

In an effort to eliminate interferences, SOPs have been established for every procedure involved in conducting a successful bioassay test. Additionally, a rigorous daily QA/QC inspection is designed to identify potential sources of interference. Prior to the initiation of toxicity tests every effort is made to identify and eliminate potential sources of interference that could compromise test results. These can include but are not limited to the following: clean and functional facilities, equipment and test chambers; sample storage and handling; test organism and food quality; laboratory water quality.

Pollution Prevention

As a pollution prevention measure, wastes generated during toxicity testing must be properly handled and disposed of in an appropriate manner. Care should be taken not to generate excessive wastes when preparing solutions for testing. All materials identified as hazardous should be labeled and appropriately stored for hazardous waste disposal.

Data Assessment

Bioassay and water quality data are assessed each day during the course of testing for accuracy and compliance with established criteria. At test termination, the data for each replicate, which are recorded on the appropriate data sheets, are entered into a CETIS™ data file labeled for identification of the specific test. Statistical analyses are performed in accordance with EPA guidelines for statistical analysis. Control data for all endpoints are evaluated for compliance with

established test acceptability criteria. Water Quality data are assessed for compliance with specifications outlined in the appropriate USEPA testing manuals.

Corrective Actions and Contingencies for Out-of-Control Data

If control performance is not met, a project manager should be notified immediately and, upon approval, the test is to be repeated. The potential cause(s) of poor control performance will be documented by scientific staff and evaluated and assessed by a project manager. Corrective actions will be determined on a case-by-case basis. The results of all tests will be summarized in reports for the regulatory authorities with an explanation of the results.

**ATTACHMENT 6: SAMPLE TIE TREATMENT –
CENTRIFUGATION TREATMENT SOP**

Revision #2

Effective Date: July 17, 2008

Accepted: _____

Centrifugation

Standard Operating Procedures

This S.O.P. outlines the centrifugation procedures for the ThermoForma® General Purpose Centrifuge, Model 5682. This centrifuge has a 5800218 4-Place Swinging Bucket Rotor with a windshield cover.

1.0 PROCEDURES

1.1 Equipment and Supplies Needed

1. ThermoForma® General Purpose Centrifuge, Model 5682
2. Centrifuge bottles
3. Top-Loading Balance: capable of weighing up to 2 kg with an accuracy of 0.01 g.
4. Spoon or Transfer Pipet
5. Labeling Tape

1.2 Weighing Out Samples

Always make sure that the rotor assembly is loaded symmetrically in order to prevent damage to the unit. Each centrifuge bottle containing sample needs to weigh within 1 grams of it's opposing container.

Opposing containers must not only be alike in mass, but must also have the same center of gravity. The containers should be alike in shape, thickness, and material. The type of samples in the opposing containers must also have the same density.

1. Turn on the balance and make sure it is level. Refer to the “**Balance Calibration and Use SOP**” for instructions on the use of the balance. Tare the balance.
2. Put a piece of labeling tape on each of the centrifuge bottles.
3. Place the centrifuge bottle and cap on the balance.
4. Place the sample in the bottle. Do not fill the bottle more that 3/4 full.
5. Record the weight of the container on the label
6. Repeat steps 1-5 for the opposing centrifuge bottle, ensuring that it weighs within 1 g of the first bottle. Repeat as necessary for the sample volume that requires centrifugation.

1.2 Centrifuging Samples

8. Press the On/Off switch on the lower right-hand corner of the front panel.
9. Press the OPEN key to open the centrifuge lid.
10. Remove the windshield cover by pressing on the two metal latches in the center of the lid,

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and lifting it off.

11. Place either 2 or 4 of the centrifuge bottles in the rotor assembly making sure the bottles that are within 1 g of each other are in opposing positions.
12. Adjust the run parameters using the touch switches on the front panel. Adjust the parameters to the following settings. Press the arrow keys twice to change each parameter:

Run Parameters	Sample Matrix	
	Water	Sediment
Temp	4 °C	4 °C
Speed	3500 RPM*(~2500 RCF*)	3500 RPM* (~2500 RCF*)
Time	15 minutes	30 minutes
Accelerate	Maximum	Maximum
Brake	Maximum	Maximum
Rotor	58000218	58000218

* RCF = relative centrifugal force, RPM = revolutions per minute

To convert between the RCF and the RPM for a particular rotor, use the following equation:

$$\text{RCF} = (1.118 \times 10^{-5}) \times R \times S^2$$

Where RCF = relative centrifugal force

R = radius of the rotor (cm)

S = speed in RPM

The diameter of the 5800218 4-Place Swinging Bucket Rotor is 19.2 cm.

13. Replace the windshield cover. Pull in the center once it is in place to make sure it is secure.
14. Close the cover by lowering the centrifuge lid so that the cover rests on the chamber gasket. Place hands on both sides of the cover and press down firmly.
15. Press START.

**ATTACHMENT 7: SAMPLE TIE TREATMENT –
C-8 SOLID PHASE EXTRACTION TREATMENT SOP**

Revision #3

Effective Date: July 17, 2008

Accepted: _____

C8 Solid Phase Extraction

1.0 Background

- 1.1 In Phase I and II Toxicity Identification Evaluations (TIEs), C8 solid phase extraction (SPE) is used to determine whether a toxicant in a water sample is a non-polar organic compound. C8 SPE columns bind non-polar organic compounds.

2.0 Equipment Needed

- 2.1 Column adapter
- 2.2 5/32" outside diameter 1" long piece of rigid Teflon tubing (one for each column)
- 2.3 Teflon tape
- 2.4 C8SPE columns (Varian brand)
- 2.5 2.5N HNO₃
- 2.6 Three 50-ml beakers (one labeled glass distilled water, one labeled HNO₃, and one labeled MeOH) (one set for each column)
- 2.7 Glass distilled water
- 2.8 Methanol
- 2.9 Propanol (if running columns for an algae TIE)
- 2.10 500-ml glass transfer containers (one for each column)
- 2.11 Pump (Cole Parmer Model 7553-80) with pump heads (Masterflex Model 7518-10)
- 2.12 Silicone tubing (about 5' long) for each column (Masterflex flexible tubing)
- 2.13 Ring/clamp stand (enough to fit all of the columns)
- 2.14 Clamps (one for each column)
- 2.15 Stop cock (one for each column)
- 2.16 Large Erlenmeyer flask (for blank water)
- 2.17 10 ml graduated cylinder (for timing rate of pump)
- 2.18 Amber bottles labeled and marked for blanks and solid phase extraction waters

- 2.19 Long rigid Teflon tubing (one for each column)
- 2.20 Parafilm
- 2.21 Aluminum foil
- 2.22 Luer tip syringe (for drying columns)
- 2.23 Ziploc bag labeled with mount of sample and pump rate (for storing columns after extraction)
- 2.24 Timer

3.0 Equipment Preparation

- 3.1 Label a clean 2.5-L and a 1-L amber bottle for each sample being extracted. Be sure to use a different color for each sample being run. The 1-L amber bottle will be used to catch the column blank water and the 2.5-L amber bottle will be used to catch the solid phase extracted water.
- 3.2 Mark the 2.5-L amber bottle at the 1600 ml mark for *Ceriodaphnia* columns or at the 1000 ml mark for algae columns. Mark the 1-L amber bottle at the 550 ml mark.
- 3.3 Rinse all amber bottles (with caps) three times with glass distilled water. Place them upside down on a clean paper towel for five minutes.
- 3.4 Place a colored piece of tape on each of the columns. Use a different color for each. Make sure the color coding matches that used to label the amber bottles.
- 3.5 Wrap the end of the column adapter and the blunt end of the 1" piece of Teflon tubing with the Teflon tape.
- 3.6 Secure the wrapped end of the 1" piece of Teflon tubing in the top of the column adapter. Attach the silicone column tubing to the pointed end of the 1" piece of Teflon tubing.
- 3.7 For algae columns, before activation with methanol, fill the column with propanol and allow that to drip into a transfer container until the column runs dry.

4.0 Tubing Cleaning

- 4.1 Run 50ml of 2.5N HNO₃ through the tubing. Allow the solution to drip into a transfer container. Dump acid waste into the acid waste carboy.
- 4.2 Rinse tubing end and column adapter with glass distilled water
- 4.3 Run 25 ml of glass distilled water through the tubing. Repeat this step once (i.e., a total of 50 ml glass distilled water will be run through each column). Allow the solution to drip into a waste beaker. Dump glass distilled waste.

- 4.4 Shut off pump after tubing runs dry.

5.0 Column Activation

- 5.1 Attach clamps to the ring stands. Attach columns to clamps.
- 5.2 Set the column adapter on top of the column. DO NOT completely plug the adapter into the column yet.
- 5.3 Place a waste beaker under each of the columns.
- 5.4 Plug a stop cock into the bottom of the column and turn it to the off position.
- 5.5 Pump 50 ml of methanol (for *Ceriodaphnia* columns) at a rate of 5 ml/min (about 2.5 on the speed control knob). Allow the solvent to run into the column until the column is approximately 2/3 full. From this point on DO NOT allow the column to run dry.
- 5.6 Stop the pump.
- 5.7 Plug the column adapter into the column until it is flush with the lip of the column.
- 5.8 Open the stop cock on the column and turn the pump back on. Make sure that the liquid level in the column remains about the same.
- 5.9 When the 50 ml of solvent in the beaker is gone, immediately move the tubing to the beaker containing 50 ml of glass distilled water. Dump the solvent into the solvent waste carboy and put the transfer container back under the column.

6.0 Column Blank Extraction

- 6.1 Fill the large Erlenmeyer flask with moderately hard water. Cover the top with Parafilm and set this aside until needed.
- 6.2 When the 50 ml of glass distilled water in the beaker is gone, move the tubing into the flask of moderately hard water. All of the column tubing can be placed into the same flask. Dump the glass distilled water and put the transfer container back under the column.
- 6.3 Moderately hard column blank water should now be pumping through the column.
- 6.4 Time the rate of the pump and adjust it to about 10 ml/min.
- 6.5 Allow 200 ml of moderately hard water to run through the column into the waste beaker. Dump the moderately hard water.
- 6.6 Put a 1-L amber bottle labeled for the C8 blank under the column. Parafilm the top of the bottle and put aluminum foil around the column (to be consistent with the sample extraction procedure).

- 6.7 Allow 550 ml of moderately hard water to run through the column into the 1-L amber bottle (the level of the liquid inside the bottle should match the 550 ml mark made on the bottle).
- 6.8 Once you have collected 550 ml of moderately hard column blank water, put the waste beaker back under the column and cap the 1-L amber bottle.

7.0 Sample Extraction

- 7.1 Transfer the tubing into the sample water. If your sample is an ambient water collected in the field, do not shake it and make sure you do not suck up and sediment from the bottom of the bottle when you put the tubing inside.
- 7.2 Allow 200 ml of your sample to collect in the waste beaker. This is a good time to re-check the flow rate. Dump this into an ambient sample transfer container.
- 7.3 Once you have collected the first 200 ml of sample, put the 2.5-L amber bottle labeled for the solid phase extracted water under the column. Make sure to place the bottle in ice while collecting the solid phase extracted water.
- 7.4 Adjust the column so that the sample drips down the side of the bottle (to reduce volatilization). To do this, you may need to attach one of the long rigid Teflon tubes to the bottom of the stop cock on the bottom of the column so that it touches the side of the bottle.
- 7.5 Parafilm the top of the bottle and put aluminum foil around the column (to prevent photo degradation).
- 7.6 Allow 1600 ml (for *Ceriodaphnia* columns) or 1000 ml (for algae columns) to be collected in the 2.5-L amber bottle (the level of the liquid in the bottle should match the mark made previously).
- 7.7 Once the water reaches the 1600 or 1000 ml mark, remove the tubing from your sample and allow the column to run dry for 5 minutes.

8.0 Drying the Column

- 8.1 Detach the stop cock from the bottom of the column.
- 8.2 Leave the column adapter in the top of the column. Remove the tubing from inside the column adapter.
- 8.3 Insert a clean Luer tip 60 cc syringe in the hole in the column adapter and force air through the column ten times. You must detach the syringe each time you draw air into it otherwise you will draw up the frit inside the column.
- 8.4 Dry the inside and outside of the column with a Kimwipe.

- 8.5 Label the column with the following:
- Sample name
 - Sample collection date
 - Extraction date
- 8.6 Label a Ziploc bag with the following:
- Sample name(s)
 - Sample collection date(s)
 - Amount of sample pumped through the column(s).
 - Rate at which the sample was pumped through the column(s).
 - Date the sample(s) was extracted.
 - Initials of person who extracted the column(s).
- 8.7 Put the column(s) into the Ziploc bag and place the bag into the freezer for storage.
- 8.8 Be sure to log the column(s) extracted into the C8 Column Logbook.

9.0 Troubleshooting

- 9.1 Occasionally leaks will occur at the column adapter connection to the tubing or to the column. When this happens, pressure is released through the leak and the column fills with liquid and stops dripping. If leakage occurs, immediately turn off the pump and turn the column stop cocks to the off position (this will keep the column from running dry). Remove the column adapter from the column and the tubing from the column adapter. Remove the Teflon tape from column adapter and the Teflon tubing and re-tape the connections to ensure a secure seal between the tubing and the column. Re-attach the column adapter and the tubing, turn the column stop cock back to the on position, and turn the pump back on.
- 9.2 When a sample contains a lot of suspended sediment, columns may become plugged. When the column plugs, the column adapter will pop off the column. When this occurs, immediately turn off the pump. Record the actual amount of water extracted prior to the column plugging (in the C8 Column Logbook and on the column label).
- 9.3 Occasionally, the tubing sticks to the pump head rollers and causes the tubing to "walk". When this occurs, the tubing may actually get pulled out of the sample. Turn off the pump and re-seat the tubing and the tubing clamps inside the pump head.

**ATTACHMENT 8: SAMPLE TIE TREATMENT –
CATION EXCHANGE TREATMENT SOP**

Revision #2

Effective Date: July 17, 2008

Accepted: _____

Sample Application to Ion Exchange Resin Columns

1.0 BACKGROUND

- 1.1 Ion exchange columns are common laboratory tools that are useful in the quantification and evaluation of the impact of metal levels on aquatic organisms in ambient waters.
- 1.2 This laboratory has adapted ion-exchange techniques to determine both the biotoxicity and concentration of trace metals.
- 1.3 Sample waters should be run through resin columns as soon as possible. No more than 2 gallons of sample is needed, however the samples must be settled or filtered through a 0.45 µm glass fiber filter prior to use. Ask the laboratory manager for the volume needed and whether the sample should be filtered or settled.
- 1.4 Please read all of the MSDSs for the chemicals used in this process before you start.

2.0 EQUIPMENT NEEDED

- 2.1 Column adapter (custom made)
- 2.2 5/32" outside diameter 1" long piece of rigid Teflon tubing (one for each column)
- 2.3 Chelex 100 or Ag2-X8 resin columns
- 2.4 2.5N HNO₃
- 2.5 Two 50-ml beakers (one labeled glass distilled water, one labeled HNO₃; one set for each column)
- 2.6 Glass distilled water
- 2.7 500-ml glass transfer containers (one for each column)
- 2.8 Pump (Cole Parmer Model 7553-80) with pump heads (Masterflex Model 7518-10)
- 2.9 Silicone tubing for each column (about 5" in length of Masterflex flexible tubing)
- 2.10 Ring/clamp stand (enough to fit all of the columns)
- 2.11 Clamps (one for each column)
- 2.12 Stop cock (one for each column)

- 2.13 Large Erlenmeyer flask (for blank water)
- 2.14 10 ml graduated cylinder (for timing rate of pump)
- 2.15 Amber bottles labeled and marked for blanks and metal extraction waters
- 2.16 Long rigid Teflon tubing (one for each column)
- 2.17 Parafilm
- 2.18 Ziploc bag labeled with amount of sample and pump rate (for storing columns after extraction)
- 2.19 Timer

3.0 EQUIPMENT PREPARATION

- 3.1 Label a clean amber bottle for the control blank and each sample being extracted. Bottle sizes depend on how much rinsate is needed to run the biotoxicity test. This volume varies with the type of test (acute or chronic) and with the species being tested. Additionally, the rinsate may be collected in plastic containers, as opposed to the amber bottles, depending on the project. Be sure to use a different color for each sample being run.
- 3.2 Mark the amber bottles for the appropriate sample volumes (see step 3.1) that are needed.
- 3.3 Rinse all amber bottles (with caps) three times with glass distilled water. Place them upside down on a clean paper towel for five minutes.
- 3.4 Place a colored piece of tape on each of the columns. Use a different color for each. Make sure the color coding matches that used to label the amber bottles.
- 3.5 Attach the silicone column tubing to the pointed end of the column adapter.

4.0 TUBING CLEANING

- 4.1 Run 50 ml of 2.5N HNO₃ through the tubing. Allow the solution to drip into a transfer container. Dump the acid waste into the hazardous waste carboy.
- 4.2 Rinse the tubing end and column adapter with glass distilled water.
- 4.3 Run 25 ml of glass distilled water through the tubing - Repeat this step once (i.e., a total of 50 ml glass distilled water will be run through each tube). Allow the solution to drip into a waste beaker. Dump the glass distilled waste down the drain.
- 4.4 Shut off the pump after the tubing runs dry.

5.0 COLUMN BLANK EXTRACTION

- 5.1 **Note: The column blank extraction is run simultaneously with the ambient sample extraction.**
- 5.2 There should be one column for each treatment and then one for the blank. The volumes of the control blank and the samples that are run through the columns must be the same.
- 5.3 Fill the large Erlenmeyer flask with moderately hard water. Move the tubing for the column blank into the flask of moderately hard water.
- 5.4 Laboratory reconstituted column blank water should now be pumping through the column.
- 5.5 Time the rate of the pump and adjust it to 3 ml/min.
- 5.6 Allow 200 ml of the laboratory reconstituted water to run through the column into the waste beaker. Dump the rinsate into the holding tank
- 5.7 Place the appropriately labeled amber bottle under the column designated for the control blank. Parafilm the top of the bottle.
- 5.8 Allow the required volume of laboratory water to run through the column into the amber bottle (the level of the liquid inside the bottle should match the mark made on the bottle; see step 3.2).
- 5.9 Once the rinsate reaches the pre-designated mark on the amber bottle, put the waste beaker back under the column and cap the amber bottle. **Do not let the columns run dry** because these columns need to be stored wet.

6.0 SAMPLE EXTRACTION

- 6.1 Place the tubing into the sample water. If your sample is an ambient water collected in the field, do not shake it and make sure you do not suck up any sediment from the bottom of the bottle when you put the tubing inside. If the sample is to be gravity fed, the sample must be filtered prior to use.
- 6.2 Allow 200 ml of your sample to collect in the waste beaker. The flow rate should be 3 ml/minute, which is the same rate as the concurrently running control blank. Dump this into an ambient sample transfer container.
- 6.3 Once you have discarded the first 200 ml of sample, place the appropriately labeled amber bottle under the column designated for that sample. Make sure to pack the bottle in ice while collecting the water.
- 6.4 Adjust the column so that the sample drips down the side of the bottle (to reduce volatilization). To do this, you may need to attach one of the long rigid Teflon tubes to the bottom of the stop cock on the bottom of the column so that it touches the side of the bottle. Parafilm the top of the bottle.

- 6.5 Once the water reaches the pre-designated mark, on the amber bottle, remove the amber bottle and place the tubing in glass distilled water. Run glass distilled water through the columns for wet storage of the columns. For proper storage of the Chelex 100/Ag2-X8 columns refer to SOP 11-6 or 11-7

7.0 TROUBLESHOOTING

- 7.1 Occasionally leaks will occur at the column adapter connection to the tubing or to the column. When this happens, pressure is released through the leak and the column fills with liquid and stops dripping. If leakage occurs, immediately turn off the pump and turn the column stop cocks to the off position (this will keep the column from running dry). Remove the column adapter from the column and the tubing from the column adapter. Re-attach the column adapter and the tubing, turn the column stop cock back to the on position, and turn the pump back on.
- 7.2 When a sample contains a lot of suspended sediment, columns may become plugged. When the column plugs, the column adapter will pop off the column. When this occurs, immediately turn off the pump. Record the actual amount of water extracted prior to the column plugging (in the Column Logbook and on the column label).
- 7.3 Occasionally, the tubing sticks to the pump head rollers and causes the tubing to "walk". When this occurs, the tubing may actually get pulled out of the sample. Turn off the pump and re-seat the tubing and the tubing clamps inside the pump head.

Literature Cited

Conner V., Deanovic L., Masaki P., 1991. A laboratory manual for the preparation and use of ion exchange resins to determine the concentration and biotoxicity of dissolved trace metals in freshwater samples

**ATTACHMENT 9: SAMPLE TIE TREATMENT -
CALCIUM/MAGNESIUM ADD-BACK SOP**

Revision #2

Effective Date: July 17, 2008 through

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Calcium / Magnesium Add-Back

Standard Operating Procedures

This S.O.P. is based upon the U.S. EPA guidelines described in Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity (EPA-600/6-92/080).

1.0 INTRODUCTION

Ion exchange resins are common laboratory tools that are useful in the quantification and evaluation of the impact of anionic or cationic ions on aquatic organisms in ambient waters. Cation exchange resins are effective in the removal of multivalent cations such as Ammonium, Cadmium, Copper, Zinc, Calcium and Magnesium. Removal of calcium and magnesium from the sample will result in a lowered hardness value. When the sample is being processed for use in toxicity testing, the amount of calcium and magnesium present in the original sample needs to be replaced following treatment with a cationic exchange resin. Lowering the hardness can have a negative effect on species exposed to the sample and is not representative the original sample. The following SOP describes this process, which is referred to as “Ca/Mg add-back”.

2.0 TEST PREPARATION

2.1 Equipment and Supplies Needed

1. HACH® Company Digital Titrator Model 16900
2. 0.0800 M EDTA titration cartridge
3. 0.800 M EDTA titration cartridge
4. Delivery tubes
5. 100 ml graduated cylinder
6. Hardness 1 Buffer Solution
7. ManVer® 2 Hardness Indicator
8. Spatula
9. De-ionized water
10. 100 ml class A volumetric flask
11. 10 ml class A volumetric pipet
12. 100 ml beaker
13. Stir bar
14. Stir plate

15. Kimwipe®
16. Hardness Logbook
17. Balance: capable of weighing to 0.01 mg
18. ACS Reagent Grade $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (calcium chloride dihydrate)
19. ACS Reagent Grade $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (magnesium chloride hexahydrate)
20. HACH® CalVer® 2 Calcium Indicator
21. 8 N Potassium Hydroxide Solution

3.0 PROCEDURE

1. Measure the total hardness and the calcium hardness of the sample and treatment blank PRIOR TO manipulation. Refer to the “**Hardness SOP**”.
2. Record the values on the attached “**Ca/Mg Add-back worksheet**”.
3. Measure the total hardness and the calcium hardness of the sample AFTER manipulation.
4. Record the values on the attached “**Ca/Mg Add-back worksheet**”.
5. Determine the volume of solution that the add-back will be performed on.
6. Use the “Ca/Mg Add-back worksheet” to calculate the amount of calcium chloride and magnesium chloride necessary for the sample volume.
7. Using the analytical balance, measure out the appropriate amounts of calcium chloride and magnesium chloride.
8. Add the calcium chloride and magnesium chloride to the sample
9. Measure the total hardness to conform that it is the same as the original sample.

3.1 Addback Formulas

	Total Hardness	-	Calcium	=	Magnesium
Pre – treatment	A_1	-	B_1	=	C_1
Post – treatment	A_2	-	B_2	=	C_2
<hr/>					
Add Back	$A_1 - A_2$		$B_1 - B_2$		$C_1 - C_2$

Titration digit conversion factors:

Digits = mg/l CaCO_3 in 100 ml

Digits x 0.001 = mmole or 1mole = Digits x 10^{-6}

$1 \times 10^{-6} = 0.000001$

$$\frac{\text{g}}{\text{L}} \text{CaCl}_2 \cdot 2\text{H}_2\text{O} = \frac{(B_1 - B_2)(1 \times 10^{-6}) \text{ moles Ca}}{0.1 \text{ L solution}} \times \frac{147.01 \text{ g CaCl}_2 \cdot 2\text{H}_2\text{O}}{1 \text{ mole CaCl}_2 \cdot 2\text{H}_2\text{O}} \times \frac{1 \text{ mole CaCl}_2 \cdot 2\text{H}_2\text{O}}{1 \text{ mole Ca}}$$

$$\frac{\text{g}}{\text{L}} \text{MgCl}_2 \cdot 6\text{H}_2\text{O} = \frac{(C_1 - C_2)(1 \times 10^{-6}) \text{ moles Mg}}{0.1 \text{ L solution}} \times \frac{203.3 \text{ g MgCl}_2 \cdot 6\text{H}_2\text{O}}{1 \text{ mole MgCl}_2 \cdot 6\text{H}_2\text{O}} \times \frac{1 \text{ mole MgCl}_2 \cdot 6\text{H}_2\text{O}}{1 \text{ mole Mg}}$$

Final amount to add-back depends on volume of treated sample. Multiply volume to be used by the calculated g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$.

$$\frac{\text{g}}{\text{L}} \text{CaCl}_2 \cdot 2\text{H}_2\text{O} \times \# \text{ Liters to AddBack} = \text{grams of } \text{CaCl}_2 \cdot 2\text{H}_2\text{O}$$

$$\frac{\text{g}}{\text{L}} \text{MgCl}_2 \cdot 6\text{H}_2\text{O} \times \# \text{ Liters to AddBack} = \text{grams of } \text{MgCl}_2 \cdot 6\text{H}_2\text{O}$$

Revision #2

Effective Date: July 17, 2008 through

Accepted: _____

Calcium / Magnesium Add-Back Worksheet

Date: _____

Sample ID: _____

Client: _____

Sample Date: _____

CALCULATIONS

	Total Hardness	Calcium	Magnesium
<i>Pre – treatment</i>	-	=	
<i>Post – treatment</i>	-	=	
<i>Result</i>			

$$\left(\frac{\quad}{0.1 \text{ L solution}} \right) (1 \times 10^{-6}) \text{ moles Ca} \times \frac{147.01 \text{ g CaCl}_2 \cdot 2\text{H}_2\text{O}}{1 \text{ mole CaCl}_2 \cdot 2\text{H}_2\text{O}} \times \frac{1 \text{ mole CaCl}_2 \cdot 2\text{H}_2\text{O}}{1 \text{ mole Ca}} = \boxed{\frac{\text{g}}{\text{L}} \text{ CaCl}_2 \cdot 2\text{H}_2\text{O}}$$

$$\left(\frac{\quad}{0.1 \text{ L solution}} \right) (1 \times 10^{-6}) \text{ moles Mg} \times \frac{203.3 \text{ g MgCl}_2 \cdot 6\text{H}_2\text{O}}{1 \text{ mole MgCl}_2 \cdot 6\text{H}_2\text{O}} \times \frac{1 \text{ mole MgCl}_2 \cdot 6\text{H}_2\text{O}}{1 \text{ mole Mg}} = \boxed{\frac{\text{g}}{\text{L}} \text{ MgCl}_2 \cdot 6\text{H}_2\text{O}}$$

$$\frac{\text{g}}{\text{L}} \text{ CaCl}_2 \cdot 2\text{H}_2\text{O} \times \quad \text{L} = \boxed{\quad \text{g CaCl}_2 \cdot 2\text{H}_2\text{O}}$$

$$\frac{\text{g}}{\text{L}} \text{ MgCl}_2 \cdot 6\text{H}_2\text{O} \times \quad \text{L} = \boxed{\quad \text{g MgCl}_2 \cdot 6\text{H}_2\text{O}}$$

AMOUNT WEIGHTED OUT

_____ g CaCl₂•2H₂O _____ g MgCl₂•6H₂O

HARDNESS CONFIRMATION

Total Hardness = _____ mg/L CaCO₃

INITIALS: _____

**ATTACHMENT 10: SAMPLE TIE TREATMENT –
PIPERONYL BUTOXIDE (PBO) SOP**

Revision #2

Effective Date: July 17, 2008

Accepted: _____

Protocol For Making a 5 ppm Solution of PBO and Spiking it into Sample Waters

1.0 BACKGROUND

- 1.1 Piperonyl Butoxide (PBO) is used in TIE work to determine whether or not toxicity is due at least in part to the presence of a metabolically activated OP pesticide. If toxicity is due to a metabolically activated OP pesticide, then PBO will block the toxicity.
- 1.2 NOTE: Be sure to read the Material Safety Data Sheet for PBO before attempting to make a PBO stock solution.

2.0 EQUIPMENT NEEDED

- 2.1 26,600 ppm PBO in methanol (pesticide refrigerator)
 - This solution is made by dispensing 1.4 ml 90% pure PBO into a 50 ml volumetric flask and filling the flask to the 50 ml mark with methanol.
- 2.2 200 or 500 ml volumetric flask (depending on how much stock solution you are making)
- 2.3 50 ml beaker
- 2.4 Yellow adjustable Eppendorf pipette
- 2.5 Yellow Eppendorf tips (in box to keep dust out)
- 2.6 Nitrile gloves
- 2.7 Scissors
- 2.8 Parafilm
- 2.9 Ziploc bag

3.0 PREPARATION OF 5 ppm PBO SOLUTION

- 3.1 This 5 ppm solution must be made in the hood.
- 3.2 Be sure to wear Nitrile gloves at all times during preparation procedures.
- 3.3 Rinse the 200 or 500 ml volumetric 3 times with control water. Then fill with about 195 or 495 ml of control water.
- 3.4 In the hood, lay down a few paper towels. Then pour a very small amount of 26,600 ppm PBO into the 50 ml beaker. After dispensing 37.6 µl of the 26,600 ppm PBO into the 200 ml volumetric flask (or 94.0 µl of the 26,600 ppm into the 500 ml volumetric flask) pour the remaining unused

PBO in the 50 ml beaker into the PBO waste bottle. Leave the 50 ml beaker in the hood.

- 3.5 Cover the 26,600 ppm with Parafilm. Discard the yellow tip in the plastic PBO bag. Discard the paper towels and gloves in a regular trashcan unless you have spilled. For minor spills (a few milliliters), discard paper towels in trash dumpster in front of the Institute of Ecology building. For larger spills, follow the hazardous waste disposal procedures.
- 3.6 Top off the 200 or 500 ml volumetric with glass distilled water. Cover the volumetric flask with Parafilm and gently shake it to mix the solution.
- 3.7 Label the flask with the concentration of PBO made, the date it was made, and the initials of the person who made it. Return the stock solution and the newly made solution to the pesticide refrigerator.
- 3.8 Be sure to log the PBO stock solution into the stock solution logbook.

4.0 Adding PBO to a Water Sample

- 4.1 You can add PBO to a water sample after the sample has been warmed to 25°C.
- 4.2 Add 20 µl of the 5 ppm PBO stock solution (made in advance) to 1 ml of sample (i.e., add 4 ml to 200 ml of sample, etc.).
- 4.3 Be sure to stir the sample well prior to using it in the TIE.
- 4.4 Dump all PBO waste (including waters spiked with PBO) into the hazardous waste carboy.

APPENDIX D

**SUPPORTING DOCUMENTS FOR CHEMICAL
TESTING**

ATTACHMENT 1: Turbidity Analysis SOP

PROPRIETARY SOP:

**SUBMITTED UNDER SEPARATE COVER TO
REGIONAL WATER QUALITY CONTROL BOARD**

ATTACHMENT 2: Total Dissolved Solids Analysis SOP

PROPRIETARY SOP:

**SUBMITTED UNDER SEPARATE COVER TO
REGIONAL WATER QUALITY CONTROL BOARD**

ATTACHMENT 3: Total Suspended Solids Analysis SOP

PROPRIETARY SOP:

**SUBMITTED UNDER SEPARATE COVER TO
REGIONAL WATER QUALITY CONTROL BOARD**

ATTACHMENT 4: Total and Dissolved Organic Carbon Analysis SOP

PROPRIETARY SOP:

**SUBMITTED UNDER SEPARATE COVER TO
REGIONAL WATER QUALITY CONTROL BOARD**

ATTACHMENT 5: Coliform and *E. coli* Analysis SOP

PROPRIETARY SOP:

**SUBMITTED UNDER SEPARATE COVER TO
REGIONAL WATER QUALITY CONTROL BOARD**

ATTACHMENT 6: Carbamate Pesticide Analysis SOP

PROPRIETARY SOP:

**SUBMITTED UNDER SEPARATE COVER TO
REGIONAL WATER QUALITY CONTROL BOARD**

ATTACHMENT 7: Organochlorine Pesticide Analysis SOP

PROPRIETARY SOP:

**SUBMITTED UNDER SEPARATE COVER TO
REGIONAL WATER QUALITY CONTROL BOARD**

ATTACHMENT 8: Organophosphorus and Triazine Pesticide Analysis SOP

PROPRIETARY SOP:

**SUBMITTED UNDER SEPARATE COVER TO
REGIONAL WATER QUALITY CONTROL BOARD**

ATTACHMENT 9: Bromide Analysis SOP

PROPRIETARY SOP:

**SUBMITTED UNDER SEPARATE COVER TO
REGIONAL WATER QUALITY CONTROL BOARD**

ATTACHMENT 10: Metals Analysis SOP

PROPRIETARY SOP:

**SUBMITTED UNDER SEPARATE COVER TO
REGIONAL WATER QUALITY CONTROL BOARD**

ATTACHMENT 11: Hardness Analysis SOP

PROPRIETARY SOP:

**SUBMITTED UNDER SEPARATE COVER TO
REGIONAL WATER QUALITY CONTROL BOARD**

ATTACHMENT 12: Total Kjeldahl Nitrogen and Ammonia Analysis SOP

PROPRIETARY SOP:

**SUBMITTED UNDER SEPARATE COVER TO
REGIONAL WATER QUALITY CONTROL BOARD**

ATTACHMENT 13: Nitrogen, Nitrate-Nitrite Analysis SOP

PROPRIETARY SOP:

**SUBMITTED UNDER SEPARATE COVER TO
REGIONAL WATER QUALITY CONTROL BOARD**

ATTACHMENT 14: Total and Ortho Phosphorous Analysis SOP

PROPRIETARY SOP:

**SUBMITTED UNDER SEPARATE COVER TO
REGIONAL WATER QUALITY CONTROL BOARD**

ATTACHMENT 15: Sediment Organochlorine Pesticide Analysis SOP

PROPRIETARY SOP:

**SUBMITTED UNDER SEPARATE COVER TO
REGIONAL WATER QUALITY CONTROL BOARD**

ATTACHMENT 16: Sediment Pyrethroid Analysis SOP

PROPRIETARY SOP:

**SUBMITTED UNDER SEPARATE COVER TO
REGIONAL WATER QUALITY CONTROL BOARD**

ATTACHMENT 17: Sediment Total Organic Carbon Analysis SOP

PROPRIETARY SOP:

**SUBMITTED UNDER SEPARATE COVER TO
REGIONAL WATER QUALITY CONTROL BOARD**

ATTACHMENT 18: Sediment Grain Size Analysis SOP

APPENDIX E

WSJRWQC FIELD LOG SHEETS

WSJRWC FIELD SAMPLING DATA LOG SHEET: EVENT 101

Ag Waiver Program

Station: Blewett Drain at Highway 132

Date: 4/9/2013

Latitude: GPS Reading Longitude: GPS Reading
37° 38.397' -121° 13.797'
 Datum: _____ Accuracy (ft/m): _____

Personnel: _____

Photo Numbers	
U/S	D/S

Field Observations:

Circle your observations

Wadeable: Yes No

Sky code:..... clear hazy overcast partly cloudy fog other
Site odor:..... none sulfides sewage petroleum mixed other
Water odor:..... none sulfides sewage petroleum mixed other
Dominant substrate:.. sand cobble gravel concrete mud other
Other presences:..... vascular nonvascular oily sheen foam trash none other
Observed flow:..... NA dry waterbody bed no observed flow isolated pool 0.1-1 cfs 1-5 cfs 5-20 cfs 20-50 cfs 50-200 cfs >200 cfs

Field Meter Data:

Wet Channel Width: _____

Est. Velocity (fps): _____

Staff Gage: _____

H₂O Temp (°C) _____ pH _____ D.O. (mg/L) / %sat _____ / _____
 E.C. (µS/cm) _____ Midchannel Depth (ft) _____

Samples Collected:

Circle location: bank thalweg mid-channel

Sample
Depth (ft)

Notes*

Sample ID	Analyte	Time	Sample Depth (ft)	Notes*
101-VH132-QE	DOC			
101-VH132-QE	E. coli			
101-VH132-QE	Hardness			
101-VH132-PE	Herbicides			
101-VH132-PE	OC Pesticides (Base)			
101-VH132-PE	OC Pesticides (Group A)			
101-VH132-PE	OP Pesticides			
101-VH132-QE	TKN, Total NH3, Nitrate+Nitrite as N (NO3+NO2-N), Total			
101-VH132-QE	TOC			
101-VH132-TE	Tox. Tests: Acute Ceriodaphnia			
101-VH132-QE	Turbidity, TDS, TSS, Bromide (Total), Solub. Orthophos (as P)			

Additional Notes or Comments: