Region 3 Conditional Waiver Cooperative Monitoring Program

Quality Assurance Project Plan (QAPP) for Monitoring for the Region 3 Conditional Ag Waiver Cooperative Monitoring Program

Designed for the Agricultural Waiver Monitoring Program in the Central Coast Region

Prepared For:

Central Coast Water Quality Preservation, Inc.
P.O. Box 1049
Watsonville, CA 95077

Prepared By:

Stephen L. Clark, Pacific EcoRisk
Dr. Scott Ogle, Pacific EcoRisk

Revision 6

PACIFIC ECORISK
ENVIRONMENTAL CONSULTING & TESTING

Quality Assurance Project Plan (QAPP) for Monitoring for the Region 3 Conditional Ag Waiver Cooperative Monitoring Program

A. PROJECT MANAGEMENT

1. Title Page and Approvals

Executive Director _____

Kirk Schmidt Date

Regional Board QA Officer _____

Karen Worcester Date

Technical Program Manager _____

Date

Project Manager _____

Claus Suverkropp Date

Monitoring Program Manager _____

Stephen Clark Date

Contract Manager _____

Date

Program QA Officer _____

Brian Laurenson Date

Field Sampling Coordinator _____

Stephen Clark Date

Field Sampling Coordinator	_____	
	Chris Minton	Date
PER Lab QA Officer	_____	
	Stephen Clark	Date
FGL Lab QA Officer	_____	
	David Terz	Date
CRG Lab QA Officer	_____	
	Misty Mercier	Date
EcoAnalysts QA Officer (sorting)	_____	
	John Pfieffer	Date
EcoAnalysts QA Officer (taxonomy)	_____	
	Shanda Cox	Date

2. DISTRIBUTION LIST

Primary Distribution List for the Region 3 Cooperative Monitoring Program Quality Assurance Project Plan

Name	*Agency*	*Contact Number*
Kirk Schmidt	Central California Water Quality Preservation, Inc.	(831) 761-7644
Amanda Bern	Central Coast Regional Water Quality Control Board	(805) 549-3147
Karen Worcester	Central Coast Regional Water Quality Control Board	(805) 549-3333
Claus Suverkropp	Larry Walker Associates – Davis Office	(530) 753-6400
Stephen Clark	Pacific EcoRisk, Inc.	(925) 313-8080
Brian Laurenson	Larry Walker Associates – Davis Office	(530) 753-6400
Chris Minton	Larry Walker Associates – Santa Monica Office	(310) 394-1036
Marc Los Huertos	University of California, Santa Cruz	(831) 459-4926
Edward Salinas	Pacific EcoRisk, Inc.	(925) 313-8080
John Pfeiffer	EcoAnalysts, Inc.	(208) 882-2588
Shanda Cox	EcoAnalysts, Inc.	(208) 882-2588
David Terz	Fruit Growers Laboratory	(805) 392-2024
Misty Mercier	CRG Marine Laboratories	(310) 533-5190

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4. Project Organization and Responsibility

The Central Coast Regional Board (Regional Board) and agricultural community representatives have worked cooperatively to develop the Central Coast Cooperative Monitoring Program to meet the requirements of the Conditional Waiver of Waste Discharge Requirements for Discharges from Irrigated Lands within the Central Coast Region (Resolution No. R3-2004-0117). During the initial planning, it was envisioned that non-profit organization(s) designated by the agricultural industry, and acceptable to the Regional Board, would oversee the implementation of the Cooperative Monitoring Program. As currently configured, the Central Coast Water Quality Preservation, Inc (CQWQPI) manages the overall program. However, as the Central Coast Region is rather large in scale and includes diverse agricultural interests, individuals from both the southern and northern growing regions are represented in the CCWQPI. The management structure initially included a northern and a southern management unit, each with a supporting non-profit organization: monitoring in the northern region was overseen by the Central Coast Water Quality Preservation Inc., and in the southern region by the Central Coast Wine Growers Association Foundation. Initially, each region had an Agriculture Committee (*i.e.,* a yet to be named committee in the north and the Southern San Luis Obispo/Santa Barbara Counties Ag Watershed Coalition in the south), Monitoring Subcommittee, and Cost Allocation Subcommittee. In addition, each region had a part-time Technical Program Manager whom supported the Ag Advisory Committees, and worked directly with an Executive Director who is responsible for oversight and management of the entire Cooperative Monitoring Program.

The CCWQPI has undergone changes in key staff and in the management structure since the monitoring program was initiated in January 2005. Briefly, there has been a change in the elected Board members, a new Executive Director has been appointed, and a new Technical Program Manager has been hired. The CCWQPI now has one Agricultural Committee and will have one Technical Program Manager, and is cooperating with Pajaro River monitoring program through the University of California, Santa Cruz (UC Santa Cruz). An additional sampling team from the UC Santa Cruz will be performing monthly sampling the Pajaro River sampling locations, and the UC Santa Cruz Project Manager will be submitting the monthly data directly to the CCWQPI.

The consulting team of Larry Walker Associates and Pacific EcoRisk have been contracted to fulfill the roles of the Monitoring Program Manager, QA Program Officer, Field Sampling Coordinators, and to select contract laboratories, which currently includes Fruit Growers Laboratory, Pacific EcoRisk, CRG Marine Laboratories, and EcoAnalysts. The Program QA Officer will review all laboratory data for compliance with the QA/QC requirements specified in this QAPP, and manage the electronic database for the program. Although the Program QA Officer is responsible for communicating with the Project Manager, the Program QA Officer will operate independently in regards to mandating that all parties meet the QA/QC requirements in this QAPP. The Program Management Structure, including individuals assigned for each of the roles described above, is provided in Figure A-1.

To initiate the monitoring program, the Cooperative Monitoring Program made use of available settlement funds in two areas (the lower Salinas River watershed and the Santa Maria watershed). Funds for the Cooperative Monitoring Program are collected and managed by the

non-profit organization(s), and may include the collection of dues from individual growers based on the number of irrigated acres and type of discharge. In addition, the non-profit organization(s) may seek grant funds through the Agricultural Water Quality Grant program. The Regional Board and the agricultural community have developed initial budgets for developing a monitoring program for the first year of monitoring and reporting designed to implement the requirements of the Conditional Waiver.

The contracting organization for the initial monitoring will be the Central Coast Water Quality Preservation, Inc. The Executive Director and Technical Program Manager are identified in Figure A-1.

This Quality Assurance Project Plan (QAPP) describes the quality assurance requirements for the Cooperative Monitoring Program. Contractors conducting sample collection and sample analyses under the Cooperative Monitoring Program were selected through a competitive bidding process. The organizations selected to perform the sampling and laboratory analyses must meet the precision, accuracy, detection and reporting limits in this QAPP, and meet the quality control criteria necessary to satisfy the method quality objectives of this program. This QAPP is based on the State's Surface Water Ambient Monitoring Program (SWAMP) Quality Assurance Management Plan.

Pacific EcoRisk will update this QAPP when necessary, and will be responsible for obtaining proper review and signatures for any revisions.

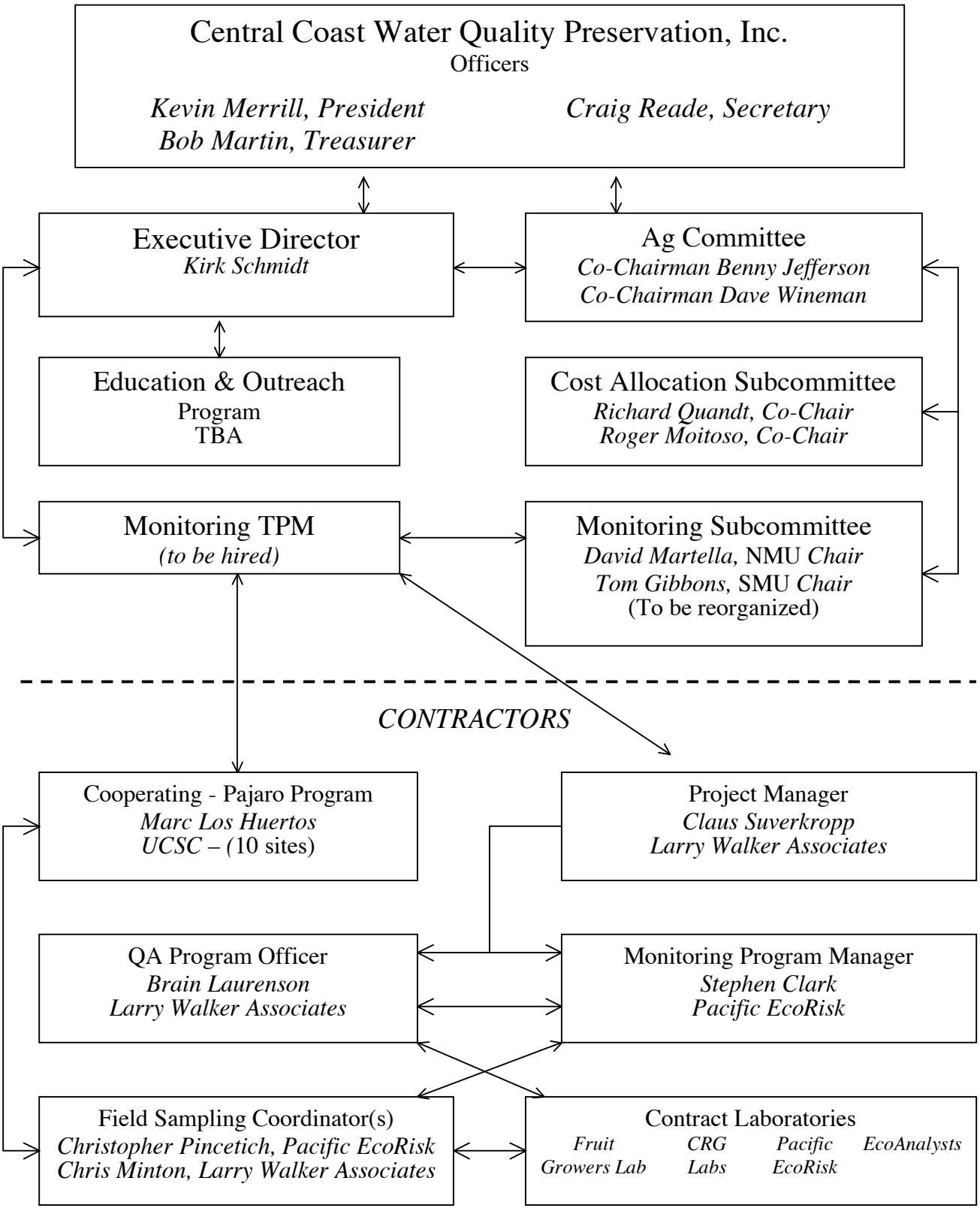

Figure A-1. Cooperative Monitoring Program Management Structure
(Figure provided courtesy of CCWQPI staff)

5. Problem Definition

For the purposes of the Conditional Waiver of Waste Discharge Requirements for Discharges from Irrigated Lands, the Cooperative Monitoring Program is required to conduct water quality monitoring within four primary watersheds: the Santa Ynez River and Santa Maria River (including Oso Flaco Creek) Watersheds in Santa Barbara County, and the Pajaro River and Salinas River Watersheds in Monterey County. The overall goal of the monitoring is to characterize the water quality conditions in these watersheds to understand long-tern water quality trends in agricultural areas, and to meet the requirements specified in the Monitoring and Reporting Program for the Conditional Waiver (MRP No. R3-2004-0117). A copy of the Monitoring and Reporting Program for the Conditional Waiver can be obtained at: http://www.swrcb.ca.gov/rwqcb3/AGWaivers/documents/MRP3-2004-0117AgWaiver.pdf.

6. Project Description

Project Objectives and Approach

The short-term objectives of the Cooperative Monitoring Program are to:

- Assess the status of water quality and associated beneficial uses in agricultural areas (through a comparison to Basin Plan objectives for the water bodies monitored in this study);
- Identify problems associated with agricultural activities;
- Conduct focused monitoring to further characterize problem areas and to better understand sources of impairment; and
- Provide feedback to growers in problem areas.

The long-term objective of the Cooperative Monitoring Program is to track changes in water quality and beneficial use support over time.

It is the intent of the Cooperative Monitoring Program to provide information to promote the understanding of conditions in the watersheds and to assess the relative health of the watersheds. The Cooperative Monitoring Program will be a dynamic program that will change over time as information is accumulated and new information needs are identified.

It is the objective of the Cooperative Monitoring Program to coordinate with existing monitoring efforts so that the data generated by the various program are complementary and not duplicative. The following current monitoring effort is not designed to address the requirements of the Ag Waiver program, but may provide data useful for interpreting CMP results:

- The Central Coast Ambient Monitoring Program (CCAMP) is the Regional Board's regionally scaled water quality monitoring and assessment program. This program is operated on a five-year rotation. Approximately 30 monitoring sites are placed in a rotation area, generally at the lower ends of tributaries, with conventional water quality

parameters monitored monthly. Sediment chemistry, toxicity, bioaccumulation, and benthic invertebrates are monitored once during the rotation year, as funding allows.

The results of this project may show a need for follow-up monitoring. Therefore, follow-up monitoring is part of the conceptual framework of the CMP. Once follow-up monitoring study designs are approved by the RWQCB QA Officer, they will incorporated (along with the appropriate QA/QC) into Section E and the appendices of this document.

The Cooperative Monitoring Program will track future developments to coordinate, whenever possible, with other monitoring efforts that may be planned for research-driven or regulatory programs.

Measurement

The following environmental monitoring elements are included in the Cooperative Monitoring Program:

- Conventional water quality constituents in water (*e.g.,* nutrients, temperature,) and flow;
- Organophosphate (OP) and possibly pyrethroid pesticides in water;
- Toxicity in water;
- Toxicity in sediment;
- Benthic invertebrate community assessment.

It is envisioned that the monitoring constituent list may be modified if changes to the MRP are agreed to by the Regional Water Quality Control Board.

Specific monitoring parameters measured for the Cooperative Monitoring Program are listed in Table A-1.

Project Schedule

This monitoring program will be implemented as specified in the Monitoring and Reporting Program. As specified in the MRP, monitoring is ongoing, and Phase I monitoring began in January 2005. Additional sites were added for Phase II monitoring, which began in January 2006. It is anticipated that the rainy season sampling (*i.e.,* "wet season") will be conducted during or shortly after river runoff events, possibly including the first event that results in significant flow increases. The team of Larry Walker Associates and Pacific EcoRisk will consult with the Central Coast Water Quality Preservation, Inc., the Technical Program Manager, and Regional Water Quality Control Board staff to resolve any constraints or limitations (e.g., resources and time) that may affect completion of any project schedules and/or goals.

Table A-1. Constituents to be Monitored

Constituent	Monitoring Frequency
Conventional Water Quality and Flow	
Flow (or at a minimum, stage data)	Monthly
pH	Monthly
Dissolved Oxygen	Monthly
Temperature	Monthly
Turbidity	Monthly
Total Dissolved Solids	Monthly
Nitrate + Nitrite	Monthly
Total Ammonia	Monthly
Orthophosphate as P	Monthly
Chlorophyll a	Monthly
Pesticides	
Organophosphates	Twice during wet season and twice during dry season
Pyrethroids	Twice during wet season and twice during dry season
Toxicity Test	
Chronic Water Column Toxicity	Twice during wet season and twice during dry season
Sediment Toxicity	Annually (Spring – March 1 – April 30)
Benthic Invertebrate Assessment	Annually (Spring – March 1 – April 30)

Sampling Schedule

The sample collection frequency will vary by the parameter tested, as summarized below:

- *Conventional Water Parameters* – monthly sampling will be conducted;
- *OP and possibly Pyrethroid Pesticides* – sampling will be four times annually, twice during the wet season (October 15 – March 15) and twice during the dry season (March 15 – October 15) at Phase I monitoring sites only;
- *Water Column Toxicity* – sampling will be four times annually, twice during the wet season (October 15 – March 15) and twice during the dry season (March 15 – October 15);
- *Sediment Toxicity* – sampling will be annually during spring (March 1 – April 30);
- *Benthic Invertebrate Assessment* – sampling will be annually during spring (March 1 – April 30).

The sample events will typically be conducted over a period of 1 to 3 days. A breakdown of sampling sites, sampling frequency, and general monitoring parameters to be analyzed are provided in Tables A-2a and A-2b.

Table A-2a. Summary of Phase I Sampling Sites, Frequency, and General Class of Parameters

Site Description	Conventional Water Quality & Flow	OP and Pyrethroid Pesticides[1]	Water Column Toxicity	Sediment Toxicity	Biological Assessment
Northern Sampling Region					
Moro Cojo Slough at Highway 1	12	4	4	1	1
Old Salinas River at Monterey Dunes Way	12	4	4	1	1
Tembladero Slough at Haro	12	4	4	1	1
Merritt Ditch u/s Highway 183	12	4	4	1	1
Espinosa Slough u/s Alisal Slough	12	4	4	1	1
Alisal Slough at White Barn	12	4	4	1	1
Blanco Drain Below Pump	12	4	4	1	1
Salinas Reclamation Canal at San Jon Road	12	4	4	1	1
Gabilan Creek at Boronda Road	12	4	4	1	1
Natividad Creek u/s Salinas Reclamation Canal	12	4	4	1	1
Salinas Reclamation Canal at La Guardia	12	4	4	1	1
Salinas River at Spreckels Gauge	12	4	4	1	1
Quail Creek at Highway 101	12	4	4	1	1
Salinas River at Chualar Bridge on River Road	12	4	4	1	1
Chualar Creek at Chualar River Road	12	4	4	1	1
Southern Sampling Region					
Santa Maria River at Estuary	12	4	4	1	1
Orcutt Solomon Creek u/s Santa Maria River	12	4	4	1	1
Little Oso Flaco Creek	12	4	4	1	1
Oso Flaco Creek at Oso Flaco Lake Road	12	4	4	1	1
Santa Maria River at Highway 1	12	4	4	1	1
Orcutt Solomon Creek at Highway 1	12	4	4	1	1
Main Street Canal u/s Ray Road at Highway 166	12	4	4	1	1
Green Valley at Simas	12	4	4	1	1
Bradley Canyon at Culvert	12	4	4	1	1
Bradley Channel at Jones Street	12	4	4	1	1
Number of Sites	25	25	25	25	25
Total Number of Samples	300	100	100	25	25
Additional Field QC Analyses*	17	7	7	2	2

* 1 in 20 samples requires a field split, and field blanks for conventional parameters are required during periodic field audits.
1 – Pesticide monitoring of Phase II sites will focus on organophosphate pesticides initially, and may be expanded to include pyrethroid pesticides.

Table A-2b. Summary of Phase II Sampling Sites, Frequency, and General Class of Parameters

Site Description	Conventional Water Quality & Flow	Water Column Toxicity	Sediment Toxicity	Biological Assessment
Northern Sampling Region				
Salinas River at Elm Road in Greenfield	12	4	1	1
Salinas River at Gonzalez River Road Bridge	12	4	1	1
Tequisquita Slough u/s Pajaro River at Shore Rd.	12	4	1	1
Carnadero Creek u/s Pajaro River	12	4	1	1
Salsipuedes Cr. d/s of Corralitos Cr u/s Hwy 129	12	4	1	1
Pajaro River @ Chittenton	12	4	1	1
Llagas Creek @ Southside	12	4	1	1
Millers Canal @ Frazier Lake Road	12	4	1	1
Watsonville Slough at San Andreas Road	12	4	1	1
Struve Slough at Lee Road	12	4	1	1
San Juan Creek @ Anzar Road	12	4	1	1
Pajaro River @ Main Street	12	4	1	1
Southern Sampling Sites				
Los Berros Creek at Century	12	4	1	1
Arroyo Grande Creek at old USGS gage	12	4	1	1
Warden Creek at Turri Road at the littering sige	12	4	1	1
Chorro Creek u/s from Chorro Flats	12	4	1	1
Prefumo Creek at Calle Joaquin	12	4	1	1
Davenport Creek at Broad	12	4	1	1
Santa Ynez River at River Park	12	4	1	1
Santa Ynez River at Flordale	12	4	1	1
Santa Ynez River at 13th	12	4	1	1
Bell Creek at Winchester Canyon Park	12	4	1	1
Glenn Annie	12	4	1	1
Franklin at Mountain View Lane	12	4	1	1
Arroyo Paredon at Via Real	12	4	1	1
Number of Sites	25	25	25	25
Total Number of Samples	300	100	25	25
Additional Field QC Analyses*	17	7	2	2

* 1 in 20 samples requires a field split, and field blanks for conventional parameters are required during periodic field audits.

7. Quality Objectives and Criteria for Data Measurement

The objective of data collection for this monitoring program is to produce data that represents, as closely as possible, *in-situ* conditions of water bodies in the Santa Ynez River, Santa Maria River (including Oso Flaco Creek), Pajaro River, and Salinas River Watersheds. This objective will be achieved by using accepted methods to collect and analyze surface water, sediment, and biota. 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, bias, and completeness, as presented in Section B of this document. Any previously collected information that may be used to bolster the data set for the CMP will be evaluated in accordance with the quality objectives noted in this QAPP. Previously collected data will only be used for trends analysis if the data do not meet the QC/QC criteria in this QAPP.

8. Training and Certification

All staff performing field sampling shall receive training to ensure that the work is conducted correctly and safely. All monitoring staff must receive training in "SWAMP compatible monitoring and data management" with RWQCB staff in San Luis Obispo, and an ongoing close working relationship is encouraged. At a minimum, all field staff shall have demonstrated experience with the field guidelines and sample collection standard operating procedures (SOP) included in this QAPP. All work shall be performed under the supervision of a senior field scientist experienced with the protocols described in this SOP, or under the supervision of a similarly experienced Field Sampling Coordinator. A copy of the safety training records is filed in the project file with the contractors. Annual training will also occur on a more informal basis during field audits that may be scheduled by the RWQCB. The Program QA Officer is responsible for overseeing all training.

Laboratories performing each analytical method must be either ELAP or NELAP certified, and their staff must have demonstrated experience with each method and be familiar with the laboratory standard operating procedures (SOP). All contractors and staff conducting fieldwork must receive field safety training. All work shall be performed under the supervision of experienced staff or a field coordinator. A copy of the safety training records must be filed in the project file with the contractors. Annual training will also occur on a more informal basis during field audits. The Program QA Officer is responsible for overseeing all training.

9. Documentation and Records

Data To Be Included In Data Reports

For each sample event, the field crew or monitoring agency shall provide the Quality Assurance Program Officer with copies of relevant pages of the field logs and copies of the chain-of-custody forms for all samples submitted for analysis. At a minimum, the following sample-specific information will be provided for each sample collected:

- Sample ID (unique for each sample and replicate);
- Monitoring location (*e.g.,* GPS coordinates);
- Sample type [*e.g.*, grab or composite type (cross-sectional, flow-proportional, etc.)];
- Number of sub-samples in composite (if appropriate);
- Quality Control (QC) sample type (if appropriate);
- Date and time(s) of collection (*i.e.,* military time); and
- Requested analyses (specific parameters or method references).

All field data should be recorded in hard copy on data forms (example provided on the CCAMP website - www.ccamp.org/Agriculture/Ag-Program_Demo.html). All data collected electronically using field probes with recording capability shall also be recorded simultaneously in the field in hard copy. All hard copy data must be maintained for the length of the Conditional Waiver in files at the offices of the Cooperative Monitoring Program, and should be available for review by the CCAMP Quality Assurance officer during performance reviews. Upon completion of the Conditional Waiver cycle, the records may be retained for an additional 5 years or delivered to the RWQCB QA Officer for storage.

For each sample analyzed, the analyzing laboratories shall provide the Quality Assurance Program Officer with the following information:

- Sample ID;
- Date of sample receipt;
- Dates of extraction and analysis (if appropriate);
- Analytical method(s);
- Method detection limit (if appropriate);
- Reporting limit (if appropriate); and
- Measured value of the analyte or parameter.

In addition, the analyzing laboratories shall provide results from all laboratory QC procedures (*e.g.,* blanks, duplicates, spikes, reference materials, etc.) and the sample IDs associated with each analytical sample batch.

Reporting Format

All results shall be reported on the laboratory's standard reporting format, which must include all data required to confirm compliance with the SWAMP method quality objectives. Data that do not meet SWAMP method quality objectives will be flagged either as qualified but acceptable, or as unacceptable. The final results shall include the results of all field and laboratory data, including all QC samples (*e.g.,* programmatic and internal laboratory QC samples). Laboratories will also be required to submit their data in an electronic data deliverable (EDD) format that is designed and/or accepted by the Regional Water Quality Control Board, and is consistent with the CCAMP EDD format. The required electronic reporting is provided at http://www.ccamp.org/Agriculture/html/HtmlDataDocumentation/TableList.htm. These EDDs

will be maintained at multiple locations (e.g., the laboratory, Program QA Officer, and RWQCB) to assure that backup records are available. Data deliverables will include the analytical results, method detection limits (MDL), practical quantification limits (PQL), and QA data including method blanks, method blank spikes or surrogates, matrix spikes, replicates and relative percent difference (RPD), and calibration verification data using certified reference materials.

Distribution of the Approved QA Project Plan

The Monitoring Program Manager at Pacific EcoRisk is responsible for distributing signed copies of the approved QAPP to all individuals listed on page *iv* of this document.

B. Data Generation and Acquisition

1. Sample Process Design

The CMP will include monitoring at 50 locations in the Pajaro River, and Salinas River, Santa Maria River (including Oso Flaco Creek), and Santa Ynez River Watersheds. The CMP sites are listed in Table B-1a and Table B-1b, and illustrated in Figures B-1 and B-2.

All general water quality samples (i.e., conventional water quality and flow) samples will be collected during scheduled monthly monitoring events. Water toxicity testing, and OP and pyrethroid pesticides, will be performed on grab samples collected during four sampling events, twice during the wet season (October 15-March 15) and twice during the dry season (May 15-October 15). Rainy season sampling shall be conducted during or shortly after river runoff events, preferably including the first event that results in significant flow increases. Sediment toxicity samples will be collected as composites annually during the spring (March 1 – April 30). Benthic invertebrates will also be collected annually during the spring (March 1 – April 30), concurrent with the sediment toxicity sampling.

Tables A-2a and A-2b in the previous section provides a summary of sampling frequency and parameters monitored at each site.

All information collected under this QAPP is to be reported for this program, including observations and notes recorded during sample collection. Although all data collected for the CMP is considered critical for addressing the program objectives, the completeness standard is the minimum benchmark that must be met for the program data (see page 33). Data that do not meet the QA/QC criteria in this document, or that do not meet the completeness standard may still be used for trends analyses.

Notes must be recorded in the field logbook; copies of the field log books must be submitted to the Monitoring Program Manager and Program QA Officer.

Table B-1a. Coordinated Monitoring Program Northern Monitoring Sites

Site Description	Site ID	Longitude	Latitude	Site Type
Moro Cojo Slough at Highway 1	306MOR	-121.78328	36.79646	Tributary Creek
Old Salinas River at Monterey Dunes Way	309OLD	-121.79008	36.77166	River
Tembladero Slough at Haro	309TEH	-121.75445	36.75952	Tributary Creek
Merritt Ditch u/s Highway 183	309MER	-121.74168	36.75158	Drain
Espinosa Slough u/s Alisal Slough	309ESP	-121.73372	36.73675	Tributary Creek
Alisal Slough at White Barn	309ASB	-121.72968	36.72482	Tributary Creek
Blanco Drain Below Pump	309BLA	-121.74393	36.71060	Drain
Salinas Reclamation Canal at San Jon Road	309JON	121.70496	36.70493	Canal
Gabilan Creek at Boronda Road	309GAB	-121.61641	36.71548	Tributary Creek
Natividad Creek u/s Salinas Reclamation Canal	309NAD	-121.60197	36.70254	Tributary Creek
Salinas Reclamation Canal at La Guardia	309ALG	-121.61297	36.65697	Canal
Salinas River at Spreckels Gauge	309SSP	-121.67339	36.62967	River
Quail Creek at Highway 101	309QUI	-121.56211	36.60943	Tributary Creek
Salinas River at Chualar Bridge on River Road	309SAC	-121.54951	36.55598	River
Chualar Creek at Chualar River Road	309CRR	-121.50995	36.56142	Tributary Creek
Salinas River at Elm Road in Greenfield	309GRN	-121.20429	36.33797	River
Salinas River at Gonzalez River Road Bridge	309SAG	-121.46854	36.48815	River
Tequisquita Slough u/s Pajaro River at Shore Road	305TSR	-121.44437	36.94279	Tributary Creek
Carnadero Creek u/s Pajaro River	305CAN	-121.53444	36.96002	Tributary Creek
Salsipuedes Cr. d/s of Corralitos Creek u/s from Hwy 129	305COR	-121.73183	36.92028	Tributary Creek
Pajaro River @ Chittenton	305CHI	-121.59770	36.90033	River
Llagas Creek @ Southside	305LCS	-121.53213	36.99053	Tributary Creek
Millers Canal @ Frazier Lake Road	305FRA	-121.49207	36.96344	Canal
Watsonville Slough at San Andreas Road	305WSA	-121.80430	36.88793	Tributary Creek
Struve Slough at Lee Road	305STL	-121.78249	36.90369	Tributary Creek
San Juan Creek @ Anzar Road	305SJA	-121.56144	36.87548	Tributary Creek
Pajaro River @ Main Street	305PJP	-121.75105	36.90533	River

Table B-1b. Coordinated Monitoring Program Southern Monitoring Sites

Site Description	Site ID	Longitude	Latitude	Site Type
Santa Maria River at Estuary	312SMA	-120.641796	34.963774	River
Orcutt Solomon Creek u/s Santa Maria River	312ORC	-120.631454	34.957554	Tributary Creek
Little Oso Flaco Creek	312OFN	-120.586157	35.022795	Tributary Creek
Oso Flaco Creek at Oso Flaco Lake Road	312OFC	-120.586259	35.016388	Tributary Creek
Santa Maria River at Highway 1	312SM1	-120.569832	34.977207	River
Orcutt Solomon Creek at Highway 1	312OR1	-120.572882	34.941374	Tributary Creek
Main Street Canal u/s Ray Road at Highway 166	312MSD	-120.486578	34.955227	Canal
Green Valley at Simas	312GVS	-120.556457	34.942280	Tributary Creek
Bradley Canyon at Culvert	312BCC	-120.35594	34.93526	Canal
Bradley Channel at Jones Street	312BCJ	-120.41711	34.94561	Canal
Los Berros Creek at Century	310LBC	-120.57837	35.10287	Tributary Creek
Arroyo Grande Creek at old USGS gage	310USG	-120.56907	35.12442	Tributary Creek
Warden Creek at Turri Road at the littering sige	310SYB	-120.81669	35.33106	Tributary Creek
Chorro Creek u/s from Chorro Flats	310CCC	-120.38124	35.35767	Tributary Creek
Prefumo Creek at Calle Joaquin	310PRE	-120.68168	35.24732	Tributary Creek
Davenport Creek at Broad	310SLD	-120.62369	35.22174	Tributary Creek
Santa Ynez River at River Park	314SYL	-120.43698	34.65180	River
Santa Ynez River at Flordale	314FYF	-120.49266	34.67192	River
Santa Ynez River at 13th	314SYN	-120.55442	34.67677	River
Bell Creek at Winchester Canyon Park	315BEF	-119.90579	34.43926	Tributary Creek
Glenn Annie	315GAN	-119.87635	34.44772	Tributary Creek
Franklin at Mountain View Lane	315FMV	-119.51766	34.40678	Canal
Arroyo Paredon at Via Real	315APF	-119.54445	34.41676	Canal

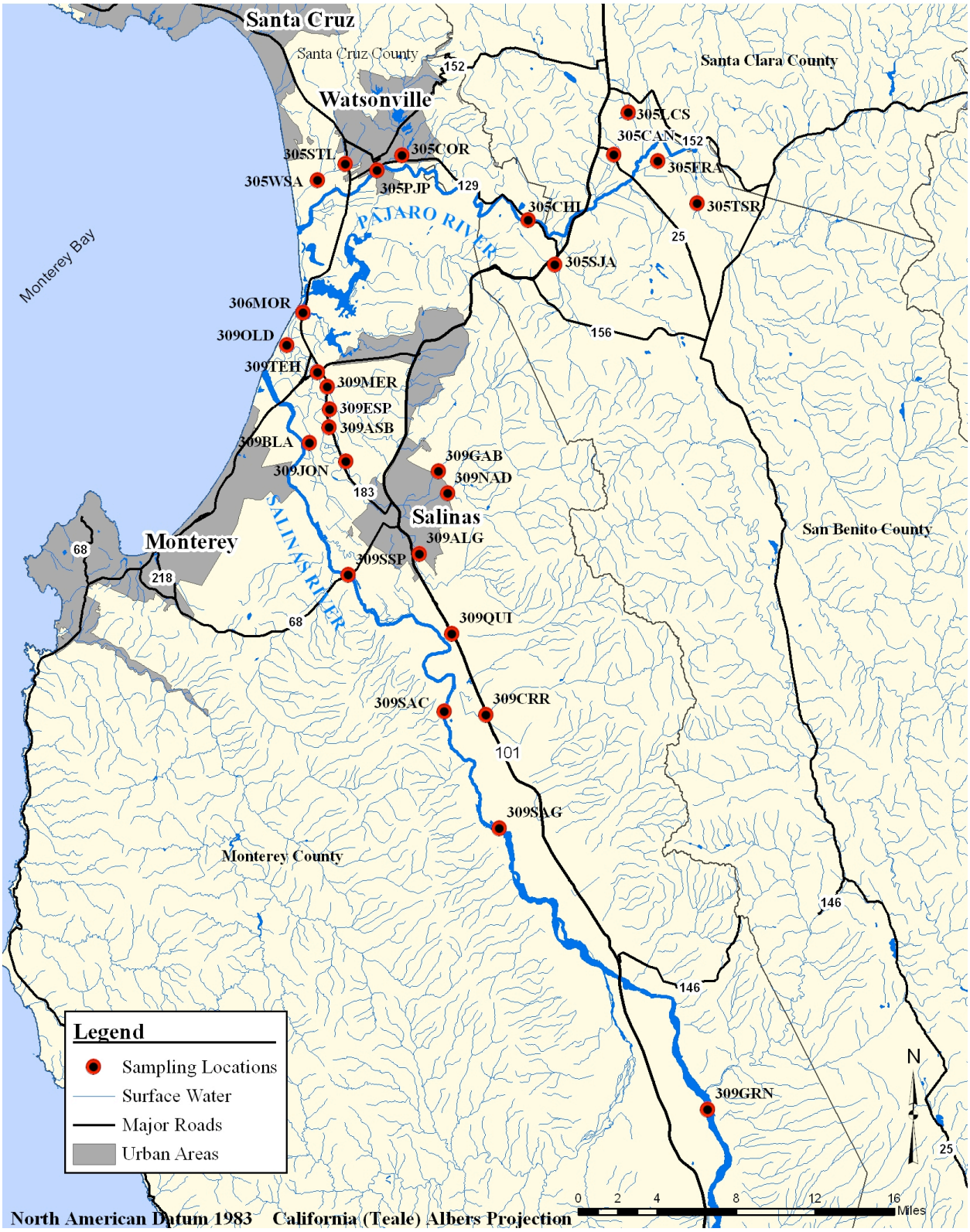

Figure B-1. Coordinated Monitoring Program Northern Sampling Sites

Figure B-2. Coordinated Monitoring Program Southern Sampling Sites

2. Rationale for the Design

The purpose of this study is to collect baseline water quality, aquatic toxicity, sediment toxicity, and benthic invertebrate community data within the Pajaro River, Salinas River, Santa Ynez River, and Santa Maria River Watersheds. Selection of waterbodies to be included in the monitoring program was based on several criteria, including:

- Waterbodies which are on the 303(d) list of impaired waterbodies for pollutants associated with agricultural practices;
- Waterbodies which have evidence of serious nitrate groundwater contamination in areas associated with intensive agricultural activity; and
- Waterbodies which have been documented with beneficial uses impairment from pollutants associated with agricultural activities and which are proposed for future listing on the 303(d) list.

Monitoring sites on these waterbodies are selected to best characterize agricultural inputs, and are generally located along the main stem and at the lower ends of tributaries in areas associated with agricultural activity. Sites may also in some cases be located to aide in distinguishing agricultural inputs from other sources. Most sites are selected from the suite of existing CCAMP monitoring sites, which already have at least one year of monitoring data available.

Procedure for Locating and Selecting Environmental Samples

Each sampling team will use road maps to reach each general sampling location. Upon arrival at the general location, Global positioning system (GPS) technology or specific site descriptions will be used to assure that samples are collected from the exact sampling site specified in this QAPP.

Validation of Any Non-Standard Methods

For non-standard sampling and analysis methods, sample matrices, or other unusual situations, appropriate method validation study information shall be documented to confirm the performance of the method for the particular need. The purpose of this validation method 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 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.

3. Sampling Methods

Samples will be collected from 3 environmental media: water, sediment, and biota. Two different sample collection methods will be used for the monitoring elements in water: (1) basic water quality sampling and (2) toxicity sampling. Sampling for biota will include methods for benthic macroinvertebrates. Sediment will be collected for an assessment of sediment toxicity. 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. Field crews are to notify the Monitoring Program Manager when sampling sites are inaccessible (e.g., due to unsafe conditions, removal of landowner access, or inclement weather conditions), and make appropriate notations in the field log. The Monitoring Program Manager will notify the Project Manager and Technical Program Manager, whom will identify appropriate replacement site(s) with the assistance of the Regional Board QA Officer should access be permanent prohibited.

Field Safety Procedures

All CMP field crews must receive field safety training prior to conducting fieldwork. Field crews conducting sampling are required to read the Field Manual prior to going to the field. The Field Sampling Manual consists of emergency information, field safety, site information, maps, and sampling SOPs. As field safety procedures must be followed at all times, there may be some cases where samples can not be collected due to safety concerns, or when a site is inaccessible.

Sources of Natural Variability

As the time of day that sample collection is performed may affect the outcome of the sample analyses (i.e., due to tides, temperature), the sequence of sample collection among sites should be maintained as consistent as possible among sampling events. In addition, the timing of sample collection at an individual site should be schedule so as to occur near the same time of previous sample collection efforts. As the location of sample collection at a given site may also affect the outcome of analytical results, every effort must be made to sample from a consistent location within a site; notes should be recorded in the field log should this not be possible. Such efforts will reduce bias and misrepresentation of a site, and should be a focus of field teams during each sampling event.

Conventional Water Quality Characteristics, and OP and Pyrethroid Pesticides

Conventional water quality monitoring will include sampling for nitrate + nitrite, total ammonia, orthophosphate as P, dissolved oxygen, temperature, total dissolved solids, pH, and turbidity. Temperature, dissolved oxygen, pH, turbidity, and chlorophyll a will also be measured using a Hydrolab DS4a field meter, as described in the "Field Measurements" section of this document. Note – no *in situ* monitoring is performed for the CMP, therefore equipment will not be deployed for this project.

All water quality samples will be collected using techniques that minimize sample contamination. Sampling methods will generally conform to the CCAMP Conventional Water

Quality Parameter Sampling SOP. Specific methods are documented in Appendix A[1]. Samples will generally be mid-channel, mid-depth grab samples and will be collected from a bridge or from shore either directly to the containers or using a stainless steel bucket; alternatively, and at the Technical Program Manager's discretion, samples may be collected using a peristaltic pump and acid-cleaned polyethylene or Teflon™ tubing, or may be collected directly into the sample containers. Sample container will be appropriate for the analyses specified in this document, and may include glass, polyethylene, fluorocarbon-lined polyethylene (FLPE), or Teflon™ sample containers (see Table B-2 for Phase I bottle requirements).

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.

Flow

Flow may be measured using a hand held flow meter, and must be measured at a sufficient number of minimum mid-depth locations at each site to sufficiently quantify the variability in flow; the CCAMP SOP method for measuring flow will be required for the CMP. If staff gage data is used as an estimation of flow, the gage height and stream cross-section width should be measured annually at a minimum so as to calibrate the measurements.

During certain times of the year, the lack discernable flowing water and/or the presence of only standing pools (e.g., puddles) of water can occur at some CMP sampling locations during sampling events. Such conditions may generate unrepresentative water quality data and reduces the value of collecting water samples. In addition, data from such samples present significant challenges when interpreting the data for impacts on water quality from agricultural practices. Standing pools of water and no-flow conditions can create stagnant conditions that are not attributable to irrigation return water quality.

To address the potential confounding interference that can occur under such conditions, sites sampled under the guidance of this QAPP should be assessed for the following conditions and sampled (or not sampled) accordingly:

- Ephemeral standing pools or puddles of water – site should not be sampled, but the field log should be completed for non-water quality data (including date and time of visit) and the site conditions should be photo documented;
- Semi-perennial or perennial standing water with zero flow and no visible connectivity to a larger surface water body site (i.e., based on visual observations, flow meter data, and a photo-documented assessment of conditions immediately upstream and downstream of the sampling site) – site should not be sampled but the field log should be completed for non-water quality data (including date, time of visit, and flow measurements);

[1] Water sampling chemical parameters collected by CMP field teams will also generally adhere to the CCAMP Conventional Water Quality Parameter Sampling Standard Operating Procedure (SOP), which is included in Appendix A.

- Flowing water, or semi-perennial or perennial standing water with zero flow and visible connectivity to a larger surface water body site (i.e., based on visual observations, flow meter data, and a photo-documented assessment of conditions immediately upstream and downstream of the sampling site) – site should be sampled.

Ambient Water Aquatic Toxicity Samples, and Chlorpyrifos and Diazinon 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 Chronic 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 five 1-gallon amber glass bottles (or other container approved by the CCAMP QA Officer), using the same equipment and procedures as for basic water quality samples (previously described above);
- Although follow-up testing (*e.g.,* dilution series testing, toxicity identification evaluation (TIE) procedures) is described in this QAPP, future monitoring may require the collection of sufficient water volume to support such follow-up options;
- Samples will be filtered in the laboratory as required for specific toxicity tests;
- After collection, samples will be chilled and maintained at 4°C until testing;
- Toxicity tests will be initiated within 36 hours of sampling;
- Should the CMP wish to obtain rapid analyses of toxicity samples for chlorpyrifos or diazinon, a small aliquot of the refrigerated aquatic toxicity sample would be used for ELISA analyses within 7 days of sample collection.

All observations of toxicity will be immediately communicated to the Monitoring Program Manager, who will communicate with the Technical Program Manager regarding any necessary follow-up measures (see below).

Ambient Sediment Aquatic 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). Specific methods are also documented in Appendix A[2]. In brief, the sampling requirements for sediment toxicity testing are as follows:

- Sediment will be collected in areas where fine-grained sediments have been deposited using a stainless steel petite ponar, Van Veen grab, or sediment scoop devices;

[2] Sediment samples collected by CMP field teams will also generally adhere to the SWAMP Bedded Sediment Sampling Standard Operating Procedure (SOP), which is included in Appendix A.

- Sediment from the top 2 cm of the grab will be transferred into a stainless steel homogenizing bowl using stainless steel spoons and thoroughly homogenized;
- Homogenized sediment will be transferred to glass sample containers;
- Sufficient volume will be collected to allow for any necessary re-testing;
- After collection, samples will be chilled and maintained at 4˚C until testing; and
- Toxicity tests will be initiated within 14 days of sampling.

Benthic Invertebrate Assessment

Bioassessment monitoring includes sampling of benthic invertebrates for bioassessment evaluations. The procedure for collecting samples of benthic invertebrates from wadeable streams is based on the method detailed in *California Stream Bioassessment Procedures (Habitat Assessment and Biological Sampling)* (Harrington and Born, 2000). Specific procedures for the collection of benthic invertebrates are provided in Appendix C (CSBP SOP, December 2003). In most cases, the low gradient stream protocol will be most appropriate. The sampling will require a 100 m reach from which three independent transects can be sampled for benthic invertebrates along the margins and thalweg. Alternatively, in streams where riffle/pool structure is present the standard stream protocol will be followed. The three independent samples will each be analyzed for 300 randomly selected invertebrates. Physical habitat methods in the CSBP SOP, as well as the CSBP Stream Habitat Characterization Form (see Appendix C), will be used to characterize habitat for the CMP.

Sample Storage, Preservation, Holding Times, and Disposal

Sample containers are pre-cleaned according to USEPA specification for the appropriate methods. Table B-2 lists the sample container, volume requirements, processing and storage requirements, and holding times 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 kept at approximately 4˚C. Samples will be shipped in insulated containers either via same-day courier or via overnight freight. All caps and lids will be checked for tightness prior to shipping.

All water, sediment, and biota samples will be handled, prepared, transported and stored in a manner so as to minimize bulk loss, analyte loss, 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 4˚C until analyzed.

Table B-2. Sampling Requirements

Parameter	Sample Container	Sample Volume[1]	Immediate Processing and Storage	Holding Time[2]
Conventional Parameters[3]				
Nitrate + Nitrite	Polyethylene	500 mL	Acidification and store at 4 ˚C	28 days
Total ammonia	Polyethylene	250 mL	Acidification and store at 4 ˚C	28 days
Orthophosphate as P	Polyethylene	250 mL	Store at 4 ˚C	48 hours
Turbidity	Probe analysis	N/A	Store on meter data recorder[4]	N/A
Total Dissolved Solids	Polyethylene	500 mL	Store at 4 ˚C	7 days
Chlorophyll a	Probe analysis	N/A	Store on meter data recorder[4]	N/A
Dissolved oxygen	Probe analysis	N/A	Store on meter data recorder[4]	N/A
Temperature	Probe analysis	N/A	Store on meter data recorder[4]	N/A
pH	Probe analysis	N/A	Store on meter data recorder[4]	N/A
Flow	Meter analysis	N/A	N/A	N/A
Pesticides				
Organophosphates	1-L amber glass	2-liters	Store at 4 ˚C	7 days extraction/ 40 days analyis
Pyrethroids	1-L amber glass			
Chlorpyrifos[6]	From tox bottle	50 mL	Store at 4 ˚C	7 days
Diazinon[6]				
Toxicity				
Aquatic bioassays	1-gallon amber glass[5]	5 gallons	Store at 4 ˚C	36 hours
Sediment bioassays	Polyethylene	1 gallon	Store at 4 ˚C	14 days
Biota				
Benthic Invertebrates	Polyethylene	NA	95% EtOH	5 years

1. Additional volumes may be required for QC analyses; NA = Not Applicable
2. Holding time after initial preservation or extraction.
3. Volume of water necessary to analyze the conventional parameters is typically combined in multiple 1-L polyethylene bottles, which provides sufficient volume for re-analyses and lab spike duplicates. This is only possible when the same laboratory provides the analyses for all of the physical parameters.
4. All probe data must also be stored on field data sheets in case of equipment download failure
5. FLPE jerricans may be used if published studies are provided to the RWQCB QA Officer demonstrating that their performance is comparable to glass for organophosphate and pyrethroid pesticides.
6. Optional follow-up monitoring using ELISA method, should CMP management request a need to rapid analytical results. Volume necessary for analyses would be collected from aquatic toxicity sample bottle.

Ice chests will be sealed with tape before shipping. 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. 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, proper preservation and holding times.

Contract laboratories will follow sample custody procedures outlined in their QA plans, and must be either certified by the California Department of Health Services to perform the analyses, or employ performance-based methodology as described in the SWAMP QAPP, Section A-7. 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.

The CMP QA Officer must approve the disposal of any sample.

Sample Identification Scheme

For monitoring performed during 2005, the four-part sample identification string used to identify samples. The four-part string includes a Project Description (CMP), the sampling event code (001), Station Code (*e.g.,* 306CAR– see Table B-1), and a unique identification number (001-999). For example, if the first sample collected for the program during event one is collected from Carneros Creek in Los Lomas at Blohm Road, the sample identification code would be: CMP-01-306CAR-001. This sample identification system did not allow for a rapid identification of a specific type of analyses (e.g., dissolved orthophosphate). So as to simplify the identification scheme and allow for a rapid assessment of the type of analysis performed on a sample by analyte type, a new sample identification scheme was used beginning in January 2006. The first three components of the sample identification string were maintained, however the forth component of the string was changed to reflect a specific analysis code rather than a unique sample identification number (Table B-3). The analysis codes also allow for a rapid differentiation between different quality control samples (e.g., field blanks and field splits) and event samples.

Field Measurements

Field-measured parameters (*e.g.,* temperature, dissolved oxygen, pH, turbidity, and chlorophyll a) will be measured at each site and event where basic water quality characteristic samples are collected. Field parameters will be measured using HydroLab DS4a multi-meter, or comparable instrument(s). Field meters must be calibrated the morning of the each day of sampling (i.e., prior to sample collection) and at the end of the last day of sampling. The data for each site may be logged into the meter data logger and downloaded upon completion of the sampling event. However, data must also be recorded in the field on the sample log sheets for each site.

Representative instantaneous flow measurements will be recorded at each sampling location. In addition, the stream stage will also be recorded, if available. These measurements will be used to determine discharge flow in cubic feet per second (cfs).

Table B-3. Coordinated Monitoring Program Sample Identification Scheme

Lab	Analysis	Sample Code	Analysis Code
Toxicity Analyses			
PER	Tox.Tests; Chronic larval fish, invertebrate, and algae	E	-T01
	Tox.Tests; Chronic larval fish, invertebrate, and algae	FD	-T02
	Tox.Tests: Chronic Hyalella	E	-T03
	Tox.Tests: Chronic Hyalella	FD	-T04
	Tox.Tests: Chronic Hyalella TIE	E	-T05
	Bioassessment	E	-T06
	Bioassessment	FD	-T07
Water Quality Analyses			
CRG	Orthophosphate (as P)	E	-Q01
	Orthophosphate (as P)	FD	-Q02
	Orthophosphate (as P)	FB	-Q03
	Pesticides (organophosphates, and possible pyrethroids)	E	-P01
	Pesticides (organophosphates, and possible pyrethroids)	FD	-P02
	Pesticides (organophosphates, and possible pyrethroids)	FB	-P03
FGL	TDS	E	-Q04
	TDS	FD	-Q05
	TDS	FB	-Q06
	Nitrate + Nitrite, Total Ammonia	E	-Q07
	Nitrate + Nitrite, Total Ammonia	FD	-Q08
	Nitrate + Nitrite, Total Ammonia	FB	-Q09
Sierra	Chlorophyll a	E	-Q10
	Chlorophyll a	FD	-Q11
	Chlorophyll a	FB	-Q12

Field Log

Field crews shall be required to keep a field log for each sampling event. The following items should be recorded in the field log for each sampling event:

- Time of sample collection;
- Sample ID numbers, including unique IDs for any replicate or blank samples;
- The results of any field measurements (*e.g.,* temperature, D.O., pH, and chlorophyll a) and the time that measurements were made;
    - When measuring D.O. with a field probe that reports measurements as both mg/L and % saturation, both values shall be reported in order to validate the proper performance of the prope.
- Qualitative descriptions of relevant water conditions (*e.g.,* color, flow level, clarity) or weather (*e.g.,* wind, rain) at the time of sample collection;

- A description of any unusual occurrences associated with the sampling event, particularly those that may affect sample or data quality.

The field crews shall have custody of samples during field sampling. Chain-of-custody forms will accompany all samples during shipment to contract laboratories. All water quality samples will be transported to the analytical laboratory directly by the field crew or by overnight courier.

Laboratory Custody Log

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.

QC Sample Collection

Field splits are collected at a frequency of about 1 per 20 normal samples. Additional sample containers will be collected for matrix spike analyses at frequency of about 1 per 20 normal samples. Field blanks will be collected during field audits. If contamination is detected, field blanks must be collected at a rate of 5% of samples until the next audit.

Decontamination Procedures

All field and sampling equipment that may contact samples must be decontaminated after each use in a designated area. The minimal decontamination procedures generally require washing with a detergent (*e.g.,* Alconox) followed by rinsing with de-ionized water. All waste materials must be collected during the sampling effort and properly disposed of upon return to the laboratory.

Field Documentation

All field activities must be adequately and consistently documented to support data interpretation and ensure defensibility of any data used for decision-making. Pertinent field information, including (as applicable), the width, depth, flow rate of the stream, the surface water condition and location of the tributaries are recorded on the field sheets. Sample control information is documented on the field sheet and chain-of-custody form.

The Monitoring Program Manager will provide project-specific field data sheets (Appendix F) that will be required by the field crews to complete field monitoring for each sampling event. Field personnel must record the following information:

- Name(s) of field personnel;
- Site/sampling location identification, including site tag ;
- Date and time of sample collection;
- Field calibration;
- All field measurements such as pH, temperature, dissolved oxygen, turbidity, chlorophyll a, and conductivity;
- Observation of weather and conditions that can influence sample results;

- Any problems encountered during sampling;
- Sample ID for each sample collected;
- Sample Custody and Documentation.

Sample possession during all sampling efforts must be traceable from the time of collection until results are reported and verified by the laboratory and samples are disposed. Sample custody procedures provide a mechanism for documenting information related to sample collection and handling.

The field sampling team(s) must immediately notify the Monitoring Program Manager when samples can not be collected, and the Monitoring Program Manager will communicate such cases to the Program QA Officer, Project Manager, and Technical Program Manager. Any deviation from a sampling protocol must be documented on the field logbook. The Monitoring Program Manager is responsible for reviewing the fieldlog books for compliance with this QAPP, and must notify the Program QA Officer of any deviations from acceptable protocols. The Program QA Officer is responsible for determining if documenting corrective actions, distributing corrective actions when necessary, and tracking the implementation of corrective actions over time.

4. Sample Handling and Custody

Documentation Procedures

The QA Program Officer 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
- 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 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;
- Signature blocks for release and acceptance of samples;
- Any comments to identify special conditions or requests.

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 refrigerated in a sample control area with temperature no greater than 4°C. An example of chain-of-custody form is shown in Figure B-3. An electronic chain-of-custody for is provided on the technical support website, and is necessary for completeness checking for the data management system. Both the laboratories hard copy chain-of-custody and the electronic copy on the technical support website must be completed for each sampling event.

Figure B-3. Chain-of-Custody Form

CHAIN-OF-CUSTODY RECORD

	Client Name: **Client Address:**						**REQUESTED ANALYSIS**																
	Sampled By:																						
	Phone:																						
	FAX:																						
	Project Manager:																						
	Project Name:																						
	PO Number:																						
	Client Sample ID	**Sample Date**	**Sample Time**	**Sample Matrix***	**Container Number**	**Container Type**																	
1																							
2																							
3																							
4																							
5																							
6																							
7																							
8																							
9																							
10																							
11																							

Correct Containers:	**Yes**	**No**			**RELIQUINSHED BY**	
Sample Temperature:	**Ambient**	**Cold**	**Warm**		**Signature:**	
Sample Preservative:	**Yes**	**No**			**Print:**	
Turnaround Time:	**STD**	**Specify:**			**Company:**	
Comments:					**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)

Sample Shipments and Handling

All sample shipments are accompanied by the chain-of-custody form, which identifies the content. The original form accompanies the shipment and a copy is retained in the project file.

All shipping containers are secured with chain-of-custody seals for transportation to the laboratory. Samples are shipped to the contract laboratories according to Department of Transportation standard. If the ice is packed with the samples, the ice must contact each sample and be approximately 2 inches deep at the top and bottom of the cooler. 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 contamination free shipping containers;
- The 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.

Laboratory Custody Procedures

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 such as temperature;
- Notify the project coordinator if any problems or discrepancies are identified;
- Ensure 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.

5. Analytical Methods

Data quality will be attained by maximizing and documenting the accuracy and precision of the methods used. Any changes in procedures due to equipment changes or to improved precision and accuracy will be documented. The SWAMP Program strongly encourages the use of "performance-based methodology" (PBM) for conducting analytical procedures and therefore

recognizes the use of modified standard procedures, as appropriately documented following CFR 40, Part 136.4. The use of PBM allows for approved procedures to be modified according to these guidelines, which provide results that are equal to or better than (more stringent than) the standard protocol that was modified. Any PBM selected for this study must be accepted by the Regional Board QA Officer and meet SWAMP requirements. The laboratory must maintain PBM method development records both electronically and in hard copy at their laboratory. SOPs presented in Appendices B-D describe the methods to be used for this program, as well as the instrumentation required to perform the required analyses.

Modified methods and techniques which have been determined to produce measurement data of a known and verifiable quality and which are of quality sufficient to meet the overall objectives of this QAPP are acceptable for use.

Conventional Water Quality Constituent, and OP and Pyrethroid Pesticide Analyses

Water quality samples may be analyzed for conventional water chemistry, and OP and pyrethroid pesticides, following approved analytical methods listed in this QAPP. Analytical methods are summarized in Table B-4.

Analyzing laboratories must demonstrate the ability to produce reporting limits approximately equal to or below the estimated reporting limits listed in Table B-4. Precision and replicate measurements in ambient waters should be less than 25% Relative Percent Difference for all constituents. Average recovery of appropriate reference materials should be between 80 and 120% for all constituents. Laboratory Standard Operating Procedures for these tests are documented in Appendix B of this QAPP, and include the laboratory equipment necessary to perform the analyses.

Chlorpyrifos and Diazinon Analyses

Should the CMP wish to obtain rapid analytical results of toxicity samples for chlorpyrifos and diazinon, an aliquot of the refrigerated aquatic toxicity samples could be analyzed using Enzyme-Linked ImmunoSorbent Assay (ELISA). The SOPs for the chlorpyrifos and diazinon analyses are provided as Attachment 6 and Attachment 7 in Appendix C.

Ambient Water Aquatic Toxicity Analyses

Water samples will be analyzed for chronic toxicity to *Selenastrum capricornutum, Ceriodaphnia dubia*, and *Pimephales promelas*. Determination of chronic toxicity shall be performed generally as described in SWAMP Standard Operating Procedures and *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* (USEPA 2002b). As some of the sampling stations designated in this QAPP may at times exceed the conductivity (*e.g.,* >3000 μS/cm) for testing with freshwater species, alternative test species will be tested. *Skeletonema or Thalassiosira* will replace *Selenastrum*, *Hyalella azteca* will replace *Ceriodaphnia* using water-only exposures for waters <15 ppt, *Americamysis bahia* will replace *Ceriodaphnia* for waters ≥15 ppt, and either topsmelt or sheepshead minnow will replace the fathead minnow (based on organism availability). Laboratory Standard Operating Procedures for these tests are documented in Appendix B.

Table B-4. Conventional Water Quality and Pesticides: Analytical Methods and Project Reporting Limits

Constituent	Fractions	Method #[1]	Range	RL[2]
Conventional Parameters				
Chlorophyll a	N/A	Probe – optical fluorometric sensor	0.02 - 150 µg/L	2 µg/L
Dissolved oxygen	N/A	Probe – membrane	0 - 50 mg/L	0 mg/L
Turbidity	N/A	Probe – nephelometric	0 - 1000 NTU	0.5 NTU
Temperature	N/A	Probe – high stability thermistor	-5 – 50 °C	0 ºC
pH	N/A	Probe – glass membrane	0 – 14 standard units	0 standard units
Nitrate + Nitrite	Total	SM 4500-P	-	0.1 mg/L
Ammonia	Total	EPA 350.3	-	0.1 mg/L
Orthophosphate as P	Dissolved	EPA 300.0	-	0.01 mg/L
Dissolved Solids, Total	Total	SM 2540C	-	10.0 mg/L
Pesticides				
Organophosphates	Total	EPA 625	-	2 – 16 ng/L
Pyrethroids	Total	EPA 625	-	10 – 25 ng/L
Chlorpyrifos	Total	ELISA	-	50 ng/L
Diazinon	Total	ELISA	-	30 ng/L

(1) Standard Methods (SM) or EPA Method number.
(2) Reporting Limit for project, based on detection limits achievable by analyzing laboratory

Ambient Sediment Aquatic Toxicity Analyses

Sediment samples will be analyzed for toxicity to the amphipod *Hyalella azteca*. 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). Laboratory Standard Operating Procedures for this test are documented in Appendix B.

Biota

Analysis of benthic invertebrates for community abundance and diversity parameters will adhere to the protocols described in *California Stream Bioassessment Procedures (Macroinvertebrate Laboratory and Data Analyses)* (Harrington and Born, 2000). This document describes sorting and identification procedures used to identify and quantify benthic invertebrate samples, and various community metrics calculated for each sample; benthic invertebrates will be identified using the CSBP Level 1 taxonomic identification protocol. SWAMP Standard Operating Procedures for this protocol are documented in Appendix C.

Detection and Quantitation Limits

Method detection limits (MDL) and quantitation limits (QLs) 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. The QL represents the concentration of an analyte that can be routinely measured in the sampled matrix within stated limits and confidence in both identification and quantitation. For this

program, QLs must be verifiable by having the lowest non-zero calibration standard or calibration check sample concentration at or less than the QL.

For this program, QLs have been established based on the verifiable levels and general measurement capabilities demonstrated for each method to meet the method quality objectives (DQO) and are compatible with SWAMP QLs. These QLs 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. Note that samples diluted for analysis or corrected for percent moisture for sediment may have sample-specific QLs that exceed these QLs. This will be unavoidable in some cases.

When selecting an analytical method during the DQO process, data users must be sure to evaluate the QLs 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.

Project Quantitation Limits

Laboratories generally establish limits that are reported with the analytical results; these may be called reporting limits, detection limits, reporting detection limits, or other terms. The limits that are reported with data are called quantitation limits under this program. These laboratory limits must be less than or equal to the project QLs included in this QAPP and the laboratories must have documentation to support quantitation at those levels.

Laboratories must report analytical results between the MDL and project QL. These results must be reported as the numerical values and qualified as estimates. Reporting as “trace” or “<QL” is not acceptable. The QC reviewers and data users must assess this information's usability.

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.

6. Quality Control Requirements

The types of quality control assessments used in the CMP are discussed below. Quality control requirements and schedules are summarized in Tables B-5 through B-10. Detailed SOPs are provided in Appendix B for toxicity testing and Appendix C for analytical chemistry analyses.

SWAMP Laboratory Quality Control Requirements are attached in Appendix E. All laboratories must be substantially compatible with SWAMP requirements. The Monitoring Program Manager is responsible for reviewing field data for QC compliance, whereas each laboratory QA Officer is responsible for maintaining compliance with the QC requirements described in the following sections. The Monitoring Program Manager will notify the Program QA Officer of any violation of QC protocol by the field crews, and the Program QA Officer will generate a corrective action and document future compliance. As required by their ELAP or NELAP certification, each laboratory QA Officer is responsible for generating corrective actions for violations of QC protocols. The laboratory QA Officers must also notify the Program QA Officer of deviations from QC protocols that are related to samples collected for this program.

Qualitative Objectives

Comparability

Comparability of the data can be defined as the similarity of data generated by different monitoring programs. For the purpose of the CMP, this objective is addressed primarily by using standard sampling and analytical procedures where possible. Additionally, comparability of analytical data is addressed by analysis of certified reference materials (discussed subsequently in this document).

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 CMP, this objective is addressed by the overall design of the monitoring program. Specifically, assuring the representativeness of the data 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 are addressed elsewhere in this document.

Completeness

Data completeness is a measure of the amount of successfully collected and validated data relative to the amount of data planned to be collected for the project. Completeness is usually expressed as a percentage value. A project objective for percent completeness is typically based on the percentage of the data needed for the program or study to reach valid conclusions. Because the CMP is intended to be a long-term monitoring program, data that are not successfully collected for a specific sample event or site can typically be recollected at a later sampling event. For this reason, most of the data planned for collection cannot be considered absolutely critical, and it is difficult to set a meaningful objective for data completeness. However, some reasonable objectives for data are desirable, if only to measure the effectiveness of the CMP. The following program goals for data completeness are based on the planned sampling frequency and a subjective determination of the relative importance of the monitoring element within the CMP:

Monitoring Element	Completeness Objective
Conventional Parameters	90%
Pesticides	90%
Aquatic Toxicity – Water Column	90%
Aquatic Toxicity – Sediment	90%
Benthic Invertebrates	90%

Field Procedures

For basic water quality analyses, quality control samples to be prepared in the field will consist of field blanks and field splits, which will be collected at different stations for each event.

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 analytes of interest during periodic field audits (held by the CCAMP QA Officer at least every 6 months and more frequently at the beginning of the program) 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. If contamination is detected then field blanks must be collected at a rate of 5% of samples until the next audit.

Field splits

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

Each measured value is for the event sample is compared against the results for the split sample, and accuracy is expressed as the relative percent difference.

$$RPD = \frac{[Vm - Vk]}{Vk} \times 100\%$$

Where: RPD = the relative percent difference
Vm = the measured value
Vk = the known value (event sample).

If the relative Percent Difference (RPD) of field split results is greater than 25% and the absolute difference is greater than the RL, both samples should be reanalyzed. If an RPD greater than 25% is confirmed by reanalysis, environmental results will be qualified as *estimated*. 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.

Laboratory Analyses

For basic water quality analyses, quality control samples prepared in the contract laboratory(s) will typically consist of equipment blanks, method blanks, certified reference materials, laboratory duplicates, matrix spikes, and matrix spike duplicates. Laboratory analyses for bacteria will include negative and positive quality control samples, as specified in the method documents.

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. If reanalysis is not possible, the associated sample results should be qualified as an *upper limit* (UL) at the reported value.

Laboratory Control Samples

The purpose of analyzing laboratory control samples is to demonstrate the accuracy of the analytical method. 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:

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

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. Bias is the systematic or persistent distortion of a measurement process that causes errors in one direction. If reanalysis is not possible, the associated sample results should be qualified as *low or high biased*.

Laboratory 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, or one in 20 samples, whichever is more frequent; 10% of bioassessment samples must be reexamined (*e.g.,* sorting, counting, and identification) for QA purposes. Laboratory duplicates will consist of duplicate laboratory fortified method blanks. If the Relative Percent Difference (RPD) for any analyte is greater than the precision criterion (25% for SWAMP conventional constituents) *and* the absolute difference between duplicates is greater than the RL, the analytical process is not being performed adequately for that analyte. In this case, the sample batch should be prepared

again, and laboratory duplicates should be reanalyzed. If reanalysis is not possible, 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, or one in 20 samples, whichever is more frequent. 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.

If matrix spike recovery of any analyte is outside the acceptable range, 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. 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, the results for that analyte have failed the acceptance criteria. If the RPD for laboratory 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 include monthly reference toxicant testing. Test acceptability requirements are documented in the method documents for each bioassay method and are included in Appendix B.

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 splits, for field blank analyses are generally not required for toxicity testing studies.

Benthic Invertebrates Processing and Analysis

Accuracy of identifications and precision of enumeration of benthic invertebrate collections are assessed by re-analysis of samples at the rate of one for every ten samples analyzed. This consists of complete re-examination of the organisms in the archived original sample, including remnants from the sorting process. If any additional organisms are identified in the "remnant" fraction of the archived sample, the numbers of taxa and organisms are recorded. The total number of organisms and enumeration of individual taxa for the re-examined sample should be within 5% of the original total. Discrepancies in taxonomic identification or enumeration should be resolved as soon as possible.

Sample Disposal

Samples must be disposed of in accordance with any pertinent local, State, of federal guidelines and/or regulations. The Program QA Officer must approve sample disposal.

Turnaround Times

All analytical, toxicological, and taxonomic data must be reported to the Program QA Officer within 28 days of sample receipt, unless otherwise approved by the Project Manager. Electronic data deliverables (EDDs) are due within 45 days of sample receipt.

Table B-5. Project Quality Control Requirements for Analysis of Water Quality Samples: Frequency[1] and Numbers of Field Quality Assurance Samples

Parameter(s)	Field splits	Field Blanks	Total QA Samples
Conventional Parameters	5% of all samples	5% of all samples if problems are found in field audits	34
Pesticides	5% of all samples	5% of all samples if problems are found in field audits	7
Toxicity – Water Column	5% of all samples	N/A	14
Toxicity – Sediment	5% of all samples	N/A	4
Bioassessment	5% of all samples	N/A	4

(1) External QA samples are rotated among sites to provide field splits for 5% of all samples collected (as appropriate for specific analyses).

Table B-6. Project Quality Control Requirements for Analysis of Water Quality Samples: Conventional Water Quality Parameters.

QA Procedure	QA Parameter	Frequency[1]	Criterion	Corrective Action
Field Blanks	Contamination	Various, see Table B-5	< MDL *or* < sample ÷ 5	Examine field log. Identify contamination source. Qualify data as needed.
Field split	Precision	Various, see Table B-5	RPD ≤ 25% if |Difference| ≥ RL	Reanalyze both samples. Identify variability source. Qualify data as needed.
Method Blank	Contamination	≥1 per batch or per 20 samples, (trace metals and OC)	< MDL *or*, if n≥3, avg ± 2 s.d. < RL	Identify contamination source. Reanalyze method blank and all samples in batch.
LCS or CRM	Accuracy	1 per batch	80-120% REC	Recalibrate and reanalyze LCS or CRM and samples
Lab Duplicate	Precision	1 per batch or 1 in 20, whichever is more frequent	RPD ≤ 25% if |Difference| ≥ RL	Recalibrate and reanalyze.
Matrix Spike	Accuracy	1 per batch or 1 in 20, whichever is more frequent	80-120% REC	Check CRM recovery. Attempt to correct matrix problem and reanalyze sample. Qualify data as needed.
Matrix Spike Duplicate	Precision	1 per batch or 1 in 20, whichever is more frequent	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	100%	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; CRM = Certified Reference Material (=Certified Reference Material)

(1) The term "lot" refers to a set of bottles or reagents identifiable by a common production lot number, or to sampling equipment subjected to the same cleaning procedures as a set. The term "batch", as used in this document, refers to an uninterrupted series of analyses.

Table B-7. Project Quality Control Requirements for Analysis of Water Quality Samples: Organophosphate Pesticides in Water.

QA Procedure	QA Parameter	Frequency[1]	Criterion	Corrective Action
Field Blanks	Contamination	Various, see Table B-5	< MDL *or* < sample ÷ 5	Examine field log. Identify contamination source. Qualify data as needed.
Field split	Precision	Various, see Table B-5	RPD ≤ 25% if |Difference| ≥ RL	Reanalyze both samples. Identify variability source. Qualify data as needed.
Method Blank	Contamination	≥1 per batch or per 20 samples, (trace metals and OC)	< MDL *or*, if n≥3, avg ± 2 s.d. < RL	Identify contamination source. Reanalyze method blank and all samples in batch.
LCS[2] or CRM Surrogates Pesticides	Accuracy	1 per batch	 40-130% REC 45-125% REC	Recalibrate and reanalyze LCS or CRM and samples
Lab Duplicate	Precision	1 per batch or 1 in 20, whichever is more frequent	RPD ≤ 25% if |Difference| ≥ RL	Recalibrate and reanalyze.
Matrix Spike[2] Surrogates Pesticides	Accuracy	1 per batch or 1 in 20, whichever is more frequent	 40-130% REC 45-125% REC	Check CRM recovery. Attempt to correct matrix problem and reanalyze sample. Qualify data as needed.
Matrix Spike Duplicate	Precision	1 per batch or 1 in 20, whichever is more frequent	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	100%	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; CRM = Certified Reference Material (=Certified Reference Material)

(1) The term "lot" refers to a set of bottles or reagents identifiable by a common production lot number, or to sampling equipment subjected to the same cleaning procedures as a set. The term "batch", as used in this document, refers to an uninterrupted series of analyses.

(2) See table at the end of Attachment 5 of Appendix C for list of analytes and analyte-specific accuracy requirements.

Table B-8. Project Quality Control Requirements for Analysis of Water Quality Samples: Pyrethroid Pesticides in Water.

QA Procedure	QA Parameter	Frequency[1]	Criterion	Corrective Action
Field Blanks	Contamination	Various, see Table B-5	< MDL *or* < sample ÷ 5	Examine field log. Identify contamination source. Qualify data as needed.
Field split	Precision	Various, see Table B-5	RPD ≤ 25% if |Difference| ≥ RL	Reanalyze both samples. Identify variability source. Qualify data as needed.
Method Blank	Contamination	≥1 per batch or per 20 samples, (trace metals and OC)	< MDL *or*, if n≥3, avg ± 2 s.d. < RL	Identify contamination source. Reanalyze method blank and all samples in batch.
LCS[2] or CRM Surrogates Pesticides	Accuracy	1 per batch	 40-130% REC 65-125% REC	Recalibrate and reanalyze LCS or CRM and samples
Lab Duplicate	Precision	1 per batch or 1 in 20, whichever is more frequent	RPD ≤ 25% if |Difference| ≥ RL	Recalibrate and reanalyze.
Matrix Spike[2] Surrogates Pesticides	Accuracy	1 per batch or 1 in 20, whichever is more frequent	 40-130% REC 65-125% REC	Check CRM recovery. Attempt to correct matrix problem and reanalyze sample. Qualify data as needed.
Matrix Spike Duplicate	Precision	1 per batch or 1 in 20, whichever is more frequent	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	100%	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; CRM = Certified Reference Material (=Certified Reference Material)

(1) The term "lot" refers to a set of bottles or reagents identifiable by a common production lot number, or to sampling equipment subjected to the same cleaning procedures as a set. The term "batch", as used in this document, refers to an uninterrupted series of analyses.

(2) See table at the end of Attachment 5 of Appendix C for list of analytes and analyte-specific accuracy requirements.

Table B-9. Project Quality Control Requirements for Analysis of Water Quality Samples: Chlorpyrifos and Diazinon Analyses using ELISA Methods.

QA Procedure	QA Parameter	Frequency[1]	Criterion	Corrective Action
Field Blanks	Contamination	Various, see Table B-5	< MDL *or* < sample ÷ 5	Examine field log. Identify contamination source. Qualify data as needed.
Field split	Precision	Various, see Table B-5	RPD ≤ 25% if |Difference| ≥ RL	Reanalyze both samples. Identify variability source. Qualify data as needed.
Method Blank	Contamination	≥1 per batch or per 20 samples, (trace metals and OC)	< MDL *or*, if n≥3, avg ± 2 s.d. < RL	Identify contamination source. Reanalyze method blank and all samples in batch.
LCS or CRM	Accuracy	1 per batch	65-125% REC	Recalibrate and reanalyze LCS or CRM and samples
Lab Duplicate	Precision	1 per batch or 1 in 20, whichever is more frequent	RPD ≤ 25% if |Difference| ≥ RL	Recalibrate and reanalyze.
Matrix Spike	Accuracy	1 per batch or 1 in 20, whichever is more frequent	65-125% REC	Check CRM recovery. Attempt to correct matrix problem and reanalyze sample. Qualify data as needed.
Matrix Spike Duplicate	Precision	1 per batch or 1 in 20, whichever is more frequent	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	100%	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; CRM = Certified Reference Material (=Certified Reference Material)

(1) The term "lot" refers to a set of bottles or reagents identifiable by a common production lot number, or to sampling equipment subjected to the same cleaning procedures as a set. The term "batch", as used in this document, refers to an uninterrupted series of analyses.

Table B-10. Project Quality Control Requirements for Analysis of Benthic Invertebrates.

QA Procedure	Parameter	Frequency	Criterion	Corrective Action
Re-examination of sample	Accuracy Precision	1 per 10 benthic invertebrate samples	≤5% difference ≤5% difference	Resolve differences in identification and enumeration.
Assess percent of data successfully collected	Data Completeness	1 per planned sample event	100%	Reschedule sample events as necessary or appropriate.

7. Instrument/Equipment Testing, Inspection, and Maintenance

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 samples are specific for each analytical approach. Standard conventional parameters proposed for the CMP will require cleaning of the equipment with Alconox, followed by copious rinsing with de-ionized water, and a triple rinse with site water for each site sampled. As the CMP expands to include additional analytical constituents in the future, this QAPP will be updated to include any additional analyte-specific cleaning procedures.

For all analytes where contamination is considered a significant concern, field blanks will be collected and analyzed as directed in Section B-6 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.

Laboratories must have sufficient spare parts for all testing equipment so as to meet holding time limits and other QC measures specified in this QAPP. Only qualified laboratory scientist and/or technicians should clean, provide preventative maintenance, test and repair equipment in accordance with the manufacturers guidelines. Any deficiencies in equipment performance should be managed in accordance with the individual laboratories QA Plan. Any equipment performance issues that affect the quality of the data generated for samples collected under this QAPP must be reported to the Program QA Officer.

Water Quality Field Probe Maintenance

The Hydrolab DS4a multimeter should be maintained by the Field Sampling Coordinators or lead field sampling scientists according to the manufacturer instructions so as to assure that the meter and probes are properly functioning during each sampling event. This will include routine replacement of the batteries (and carrying back-up batteries in the field), inspection of the probe, meter, and cable for damage, and properly cleaning and storing the probes in between uses. Sampling teams are to carry sufficient spare parts (e.g., turbidity wiper kit, o-rings, DO membranes, and DO electrolyte) and solutions (e.g., calibration standards) to perform the required analyses and any necessary maintenance.

Analytical Instrument and Equipment Testing Procedures and Corrective Actions

Testing, inspection, maintenance of analytical equipment used by the contract laboratory, and corrective actions are documented in the Quality Assurance manuals for each analyzing laboratory. Laboratory QA Manuals are made available for review at the analyzing laboratory.

8. Instrument/Equipment Calibration and Frequency

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. Any deficiencies in equipment calibration performance should be managed in accordance with the individual laboratories QA Plan. Any equipment calibration issues that affect the quality of the data generated for samples collected under this QAPP must be reported to the Program QA Officer.

Field Instruments

Routine calibration must be performed prior to and during use to ensure instruments are operating properly and produce accurate and reliable data. Calibration of the Hydrolab DS4a multimeter used for measurement of field parameters (*e.g.,* temperature, pH, dissolved oxygen, and chlorophyll a) are performed as described in the owner's manual and the CCAMP SOP (Appendix A). The multimeter should be calibrated the morning of each day of sampling (i.e., prior to sample collection) and at the end of the last day of sampling, which will permit an assessment of the "drift" of the meter during the course of a day's worth of sampling. Turbidity, chlorophyll a, and pH, and conductivity all require two point calibrations, using laboratory certified standards that bracket the expected values to be measured. 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 dissolved oxygen measurements is accomplished using an oxygen-saturated water sample;
- Chlorophyll a calibration will follow the manufacturer's specifications (a two-point calibration using de-ionized water (true zero) and a solids standard calibrated against the RWQCB Hydrolab meter);
- Turbidity calibration will follow manufacturer's specifications.

Chlorophyll a will also require (at a minimum) a single sample collection for submittal to an analytical laboratory to provide for a comparison to the data generated by the field meter.

Should the calibration result in errors that do not meet the instruments specifications, the field sampling teams must recalibrate the instrument. Should errors still occur, a calibration should be performed with new calibration solutions and/or maintenance should be performed (e.g., replace DO membrane, check batteries, etc.). If field calibration with new standards reveals that the instrument is outside established accuracy limits, the instrument should be serviced in the field with the service kit provided by the manufacturer. Back-up instruments must also be available for each of the critical real-time instruments used in the field. In the event that a field instrument cannot be properly calibrated and the in-field servicing does not mitigate the calibration problems, then the back up instrument should be used. If the errors in the calibration can not be rectified, the field sampling team must report the problem to the Monitoring Program Manager

and Program QA Officer and record the appropriate information in the field log calibration sheets. The meter should be immediately returned to Hydrolab for maintenance and an identical loaner meter should be requested. All calibration data must be recorded in the electronic format provided at: http://www.ccamp.org/Agriculture/html/HtmlDataDocumentation/TableList.htm.

9. Inspection/Acceptance for Supplies and Consumables

The procurement of supplies, equipment, and services must be controlled to ensure that specifications are met for the high quality and reliability required for each field and laboratory function. All equipment and material specifications used by contract laboratories or CMP contracted field personnel for surface water quality monitoring are outlined in the respective laboratories operating procedures and policies. Equipment and materials are purchased independently by each contract laboratory, or by contracted field sampling teams. It is the responsibility of each staff person doing the ordering to inspect the equipment and materials for quality.

Upon receipt of materials or equipment, a designated employee receives and signs for the materials. The items are reviewed to ensure the shipment is complete and they are then delivered to the proper storage location. All chemicals are dated upon receipt. All supplies are stored appropriately and are discarded upon expiration date.

10. Non-Direct Measurements

Water quality data collected by this monitoring program is intended to complement data collected by CCAMP (see Section A-6). CCAMP has stringent quality assurance and quality control elements comparable to those described in this document. Any data to be used by the CMP, including data from other sources (i.e., non-direct measurements) must be substantially compatible (i.e., must meet the QA/QC requirements in this QAPP) with the SWAMP Method quality objectives. If non-direct measurements do not meet the QA/QC requirements, the data may be used for trends analyses only.

As the data generated for this program are submitted in an electronic format (as an electronic data deliverable), all data must be generated using software compatible with the CCAMP EDD provided by the RWQCB and independently reviewed within each laboratory generating the EDD, preferably by the laboratory QA or Data Management Officer. As the EDDs are to be submitted to the Regional Water Quality Control Board for eventual public record, they should be 100% accurate with the data generated in the field and laboratory. Each laboratory and the Program QA Officer should maintain backup files for all EDDs and computer-generated reports related to this program.

11. Data Management

Copies of all program data must be submitted to the Quality Assurance Program Manager. The following parties are responsible for the deliverables noted:

- Field Sampling Coordinator: copies of field logs (see Appendix F) and Hydrolab sonde file downloads;
- Laboratory QA Officers: copies of chain-of-custody forms, original preliminary and final lab reports, and electronic media reports.

Each type of report will be stored separately and ordered chronologically by the Quality Assurance Program Manager. Original field logs will be retained by the field crew. Original chain-of-custody forms will be retained by the contract laboratory. Copies of the preliminary and final data reports will be retained by the contract laboratory(s). All original electronic laboratory data files, as well as the sonde files from the Hydrolab multimeter, must be submitted to the Regional Board by the Quality Assurance Program Manager and archived in their original format.

Concentrations of chemicals and toxicity endpoints, and all numerical biological parameters will be calculated as described in the laboratory Standard Operating Procedures or referenced method document for each analyte or parameter.

Each laboratory has flexibility for selecting the software and hardware that will best serve their needs as long as the software and hardware are capable of producing the electronic data deliverables in the CCAMP format.

The various data and information generated from the CMP will be stored and maintained at the Monitoring Program Manager's offices. The data generated from the monitoring program must be transmitted to the Quality Assurance Program Manager within 45 days of sample collection in the format required for the CMP (*i.e.,* CCAMP electronic data deliverable) for agriculture waiver monitoring. After data entry or data transfer procedures are completed for each sample event, data will be inspected for data transcription errors, and corrected as appropriate. Following this review, the data will be made available to the Technical Program Manager. After the final QA checks for errors are completed, the data are added to the final database. The production of data tables can be generated from this database.

The CMP differs from many existing regulatory monitoring programs in that it emphasizes the delivery of data as opposed to reports. In this respect it shares common properties with the Department of Health Services Drinking water quality data management approach, the Department of Pesticide Regulation Pesticide Use Reporting System, and the Underground Tanks data management system (GEOTRACKER). Accordingly, the approach to monitoring data management stresses electronic exchange of data. The data exchange and reporting system is not a database management system. Data collected through the exchange system will reside in the Ambient Monitoring Module of the State Board's CIWQS system and/or the USEPA STORET system.

A number of internet based data management tools are being provided with the intention of serving four types of data provider:

1) Field Samplers
2) Water Quality Laboratories
3) Bioassay (Toxicity Testing) Laboratories
4) Benthic Invertebrate Rapid Bio-assessment Laboratories

While several forms of electronic delivery are possible, the initial system is being set up to enable the use of delimited text files for delivery of all monitoring data in order to address potential requirements of the State Water Resources Control Board to comply with previously established precedents for methods of electronic data delivery.

Documentation and examples of the proposed electronic deliverable formats are available at www.ccamp.org/Agriculture/Ag-Program_Demo.html. Central Coast Regional Board staff will work with laboratories involved in the program to adapt the examples for ease of use with existing Laboratory Information Systems (LIMS).

In cases where environmental results are less than the reporting limit but greater than the Method Detection Limit for a parameter, the results will be reported and "J flagged"; e.g. an analytical result of 4 μg/L for an analyte with a reporting limit of 5 μg/L will be reported as 4 μg/L with a J-flag. In cases where field blank results exceed the acceptance criteria listed in Table B-6, a flag can be attached to the data to indicate that the field blank was over acceptable criteria. Any data qualifications resulting from QC analyses must be reported with the environmental data.

C. ASSESSMENT AND OVERSIGHT

1. Assessments and Response Actions

Assessments of compliance with quality control procedures will be undertaken for each sampling event during the data collection phase of the project:

- The field sampling crews will conduct a performance assessments of the sampling procedures each day of sampling. Corrective actions shall be carried out by the field sampling crew, recorded on the field log, and reported to the Quality Assurance Program Manager.
- The Quality Assurance Program Manager will review the Field Logs and associated COCs within 14 days of sample collection and develop corrective actions should errors be observed. Periodic field audits will be conducted by the RWQCB QA/QC Officer.
- Assessment of laboratory QC results and implementation of corrective actions will be the responsibility of the QA officer at each laboratory and shall be reported to the Quality Assurance Program Manager as part of any data reports.
- The Quality Assurance Program Manager will review of the laboratory hard copy and electronic reports within 14 days of receipt (or at ~45 days post-collection) and develop corrective actions should errors be observed.

Routine procedures to assess precision and accuracy, criteria for success, and corrective actions have been discussed previously (Section B) and are summarized in Table B-7. The Quality Assurance Program Manager will maintain an open dialogue with each contract laboratory and field coordinator regarding the QC review performed following each sampling event, and will maintain a QA Log of all communications and any specified corrective actions. The Quality Assurance Program Manager will make the QA Log available to the Technical Program Manager upon request. The Quality Assurance Program Manager is authorized to submit stop work orders to the Project Manager should a laboratory or field sampling team prove incapable of meeting the project QA/QC requirements. The Project Manager must communicate the reasoning for the stop work order to the Technical Program Manager and the Executive Director.

2. Reports to Management

A quality assurance report will be prepared by the Quality Assurance Program Manager following each year of monitoring, as part of the annual data report produced for the CMP. 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 annual report will be distributed to the project managers, as well as to all other program participants and interested parties.

3. Corrective Actions

During the course of sample collection and analysis in this study, the laboratory supervisors and analysts, and field supervisors and team members will make sure that all measurements and procedures are followed as specified in this QAPP, and measurements meet the prescribed acceptance criteria. If a problem arises, prompt action to correct the immediate problem and to identify its root causes is imperative. Any related systematic problems must also be identified. Problems regarding analytical data quality that require corrective action are documented in the laboratories' QA/QC Guidance. Problems regarding field data quality that may require corrective action are documented in the field data sheets. The Monitoring Program Manager is responsible for reviewing field data for QC compliance, whereas each laboratory QA Officer is responsible for maintaining compliance with the QC requirements described in the following sections. The Monitoring Program Manager will notify the Program QA Officer of any violation of QC protocol by the field crews, and the Program QA Officer will generate a corrective action and document future compliance. As required by their ELAP or NELAP certification, each laboratory QA Officer is responsible for generating corrective actions for violations of QC protocols. The laboratory QA Officers must also notify the Program QA Officer of deviations from QC protocols that are related to samples collected for this program. Corrective actions will be documented and included in the Analytical Data and Quality Assurance Report for each event.

4. Analytical Data and Quality Assurance Report

The Contractors, as stated in each contract, will prepare a report after conducting data validation.

All laboratory quality assurance data shall be reported electronically as well as in hard copy.

The elements described below will be addressed and included in the report:

- Description of the project including the number of samples, analyses, completeness and any significant problems or occurrences that influence data use;
- The QA/QC activities performed during this project;
- QC sample results, type and number of samples including the results that did not meet the projective objectives, and the impact on usability;
- Tables of analytical results for usable and unusable data.

5. Site Management

The responsible field sampling teams will observe field activities to ensure tasks are conducted according to the project specifications. The field coordinator is equipped with a cellular telephone for improved communication among the team members. Decontamination of field equipment will occur at a designated area assigned by the field manager. Access for sites is coordinated through CMP partners.

D. DATA VALIDATION AND USABILITY

1. Data Review, Validation, and Verification

In addition to the method quality objectives presented in Tables B-5 through B-6, 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 will be responsible for validating data generated by the laboratory. The CMP 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 and the SWAMP QAPP. The Program QA Officer will contact the Laboratory QA Officers should QC issues be identified and work collectively to resolve any data and or procedures that are not consistent with the QC measures described in this document.

The CMP will generally follow the informal guidance provided by the EPA regarding data verification and data validation [see "*Guidance on Environmental Data Verification and Data Validation (EPA QA/G-8)"*].

Data Verification will be confirmed by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled. Data verification is the process of evaluating the completeness, correctness, and conformance/compliance of a specific data set against the method, procedural, or contractual requirements.

Data Validation will be confirmed by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled. Data validation is an analyte- and sample-specific process that extends the evaluation of data beyond method, procedural, or contractual compliance (*i.e.,* data verification) to determine the analytical quality of a specific set of data.

All data reported for the CMP will be assessed for errors in transcription, calculation, and computer input. Field data will be entered electronically and verified against the field data log sheets. Laboratory data will be reviewed by the Laboratory QA Officers to assure that the QC program QC requirements in this QAPP have been met. Finally, the QA Program Officer will provide an additional data verification and validation assessment using Microsoft Access. This software will have all QC requirements entered so as to allow for an automated assessment of data verification. Should an outlier or other questions arise with the data, the QA Officer from the appropriate laboratory will be contacted and the data will be verified. When the QA Program Officer is satisfied with the accuracy of the laboratory data in question, the data will then be submitted electronically.

2. Data Reporting

Laboratory personnel will verify that the measurement process was "in control" (*i.e.,* all specified method 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.

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. Data that do not meet the method quality objectives following any necessary reanalysis must be flagged as not acceptable or qualified if they clearly do not meet SWAMP criteria. All CMP data must be reported.

As the period between the first sample collection, data review, distribution to the Technical Program Manager, and final submittal to the CCAMP database is expected to take approximately 90 days, the first quarterly data set will be submitted on the CCAMP website at the end of the 2nd quarter of 2005, and quarterly thereafter.

3. Data Validation

Data validation is a data quality audit and is conducted to verify whether an analytical method has been performed according to the method and project specifications, and the results have been correctly calculated and reported. The Quality Assurance Program Manager, or contractor, will conduct the data validation prior to submitting the data to Technical Program Manager. 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).

4. Reconciliation with User Requirements

The goals of the CMP is to characterize the water quality conditions in the monitored watersheds, to understand long-term water quality trends in agricultural areas, and to meet the requirements specified in the Monitoring and Reporting Program for the Conditional Waiver. The CMP has been designed to have a sufficient number of sampling locations and sampling events to generate the necessary water quality data necessary to address these goals. The project will need a sufficient number of data points, as represented by the completeness data quality objective, in order to perform the necessary trend analyses. A failure to achieve a sufficient

number of data points could result in the inability of the CMP to perform these analyses. Any uncertainty of the data will be addressed during the data validation procedures. Limitations on data use (i.e., data that do not meet the CMP QAPP QA/QC requirements) will be flagged accordingly in the database so that the data user can clearly identify data that should only be used for trends analyses.

The CMP has been designed to complement the CCAMP data, and in fact CCAMP data will be used to understand agricultural impacts in outlying areas. Sampling sites have maintained the same CCAMP site tags and will be accessed in the SWAMP database in a way that will be compatible with the CCAMP data collected at the same location. All data is collected in a way that is SWAMP compatible. As all electronic data submitted for the CMP is required to be SWAMP compatible, the data will be uploaded as noted previously in this document. The RWQCB QA Officer will then upload the data, as submitted to the RWQCB, to the SWAMP database.

5. Field Technical Audits

The Field Sample Coordinators will routinely observe field operations to ensure consistency and compliance with sampling specifications presented in the QAPP. Audit checklists document field observations and activities. At least once every 6 months field audits will include collection of field blanks.

E. REVISIONS TO THE QUALITY ASSURANCE PROJECT PLAN

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.

1. REVISIONS FOR MONITORING PERFORMED IN 2006

The QAPP was updated for monitoring performed in 2006. Revisions to the QAPP were required for several reasons:

- Additional monitoring locations have been added to the program as required in the Monitoring and Reporting Program;
- Changes to the sample identification scheme so as to simplify the identification codes by analyte type;
- The collection of field duplicates has been eliminated and replaced with the collection of field splits;
- Clarification regarding flow conditions that would not require water sample collection;
- Changes in the method for the analysis of orthophosphate and nitrate;
- Changes to the chronic fathead minnow test protocol to address pathogen interference.

Additional monitoring locations

As is required in the Monitoring and Reporting Program for the Conditional Waiver (MRP No. R3-2004-0117), monitoring was to commence in January 2005, with an agreement with the Central Coast Regional Water Quality Control Board (CCRWQCB) to perform monitoring at 25 sites from Northern Monterey County and Santa Maria River Watershed during 2005. Monitoring at an additional 25 sites for Phase II monitoring in other watersheds listed in Table 1 of the MRP was initiated in January 2006. Again, the additional sites were selected following consultation between the CCWQPI and the CCRWQCB. A list of Phase II sampling locations is provided in Table A-2b.

Changes to the sample identification scheme

So as to simplify the identification scheme and allow for a rapid assessment of the type of analysis performed on a sample by analyte type, a new sample identification scheme was used beginning in January 2006. Section B-3 of this document has been updated to reflect the new identification scheme.

The collection of field splits instead of field duplicates

Field duplicate sample collection required in the 2005 CCWQPI QAPP requires the collection of "back-to-back" samples in the field. Approximately 10 minutes can pass between the filling of the first 1-gallon aquatic toxicity event sample bottle and the last 1-gallon aquatic toxicity field duplicate sample bottle, since a total of 5 gallons of water must be collected for both the event and the field duplicate samples. This difference in sample collection time can account for significant differences in water quality parameters that may affect the outcome of the toxicity tests. Although shorter periods of time would pass between the collection of event and duplicate

samples for other constituents (e.g., nutrients), there is still a distinct possibility of varying in-stream water quality affecting the variability between the event and duplicate sample analyses. So as to eliminate this difference of sample collection time, the CCRWQCB has approved the collection of "field splits", which requires the collection of a composite sample in a large bucket and then splitting the sample volume into the appropriate containers. This approach will allow for a more realistic assessment of laboratory variability for the analysis of field samples. All notations of field duplicates in the 2005 CCWQPI QAPP have been changed to field splits in this revision.

Clarification of flow conditions that would not require sample collection

During certain times of the year, the lack discernable flowing water and/or the presence of only standing pools (e.g., puddles) of water can occur at some CMP sampling locations during sampling events. Such conditions may generate unrepresentative water quality data and reduces the value of collecting water samples. In addition, data from such samples present significant challenges when interpreting the data for impacts on water quality from agricultural practices. Standing pools of water and no-flow conditions can create stagnant conditions that are not attributable to irrigation return water quality.

To address the potential confounding interference that can occur under such conditions, sites sampled under the guidance of this QAPP should be assessed for the following conditions and sampled (or not sampled) accordingly:

- Ephemeral standing pools or puddles of water – site should not be sampled, but the field log should be completed for non-water quality data (including date and time of visit) and the site conditions should be photo documented;
- Semi-perennial or perennial standing water with zero flow and no visible connectivity to a larger surface water body site (i.e., based on visual observations, flow meter data, and a photo-documented assessment of conditions immediately upstream and downstream of the sampling site) – site should not be sampled but the field log should be completed for non-water quality data (including date, time of visit, and flow measurements);
- Flowing water, or semi-perennial or perennial standing water with zero flow and visible connectivity to a larger surface water body site (i.e., based on visual observations, flow meter data, and a photo-documented assessment of conditions immediately upstream and downstream of the sampling site) – site should be sampled.

The "Sample Collection Methods" section on of this QAPP has been updated to address this issue.

Changes in analytical protocols

Two analytical protocols have been modified in this QAPP:

- Standard Method 4500-P (SM 4500-P) was originally cited as the method that would be used for the analysis of orthophosphate as P. However, SM 4500-P is an indirect method for the analysis for orthophosphate as P since a spectrophotometer is used to obtain the date. The EPA 300.0 method is a direct method for the analysis of orthophosphate as P that involves the use of ion chromatography has been used instead of SM 4500-P. So as to assure that a direct analysis of the sample is performed, the method in Table B-4 of the

QAPP has been updated to reflect the use of EPA 300.0 for the analysis of orthophosphate as P, and the SOP has been replaced in Attachment 3 of Appendix C.

- Nitrate was analyzed following EPA 300.0, since the method has the advantage of distinguishing between nitrate and nitrite species. Nitrite typically makes up less than 1% of the total nitrate plus nitrite nitrogen species in ambient surface waters (based on a review of EPA 300 sample results by David Terz of FGL). The EPA 300 method also has a disadvantage in having a 48-hour hold time. In general, the hold time limit for this analysis has not been a problem for the CMP, since the labs receive samples the day after sample collection. However, in many of the CMP samples, nitrate concentrations are very high and exceed the standard calibration curve limit for the method. In these cases, the laboratory is required to dilute and re-analyze the sample to achieve a result in the acceptable range for the analytical instrument. Because of the time interval between sample receipt and re-analysis, these samples will exceed the allowable hold time for the EPA 300 method. To address this issue, the specified method for nitrate has been changed in Table B-4 of the QAPP to the SM4500 method, which provides for a 28-day holding time limit. The SOP has been replaced in Attachment 1 of Appendix C.

Changes in toxicological protocols

One toxicological protocol has been modified in this QAPP. The chronic fathead minnow test is susceptible to Pathogen Related Mortality (PRM), a phenomenon that is not uncommon in toxicity tests of ambient and ponded waters. PRM is characterized by high inter-replicate variability in mortality and pathogenic "coronas" around the fish, resulting in fish mortality related to a pathogen infestation and not due to toxicant exposure. The US EPA recognized this test interference in the 2002 edition of the freshwater chronic toxicity testing manual (EPA-821-R-02-013), and suggests the use of a modified exposure method that increases the number of replicates while decreasing the number of fish in each replicate as an approach to reduce pathogen infestation among the fish in a replicate. The testing laboratory involved with the CMP has demonstrated success in the application of this modified protocol. The chronic fathead minnow SOP in Attachment 6 of Appendix B has been updated to include this PRM exposure protocol.

2. REVISIONS IN MONITORING - FOLLOW-UP MONITORING TOOLS

The CCWQP Inc., and RWQCB staff have worked together to develop potential follow-up monitoring tools that could be applied to sites where toxicity was observed during 2005. Revisions to the QAPP include the following potential follow-up tools:

- A method for the analysis of organophosphate (OP) and pyrethroid pesticides, which are two classes of pesticides used for irrigated agriculture in the Central Coast region;
- An analytical screening method for the analysis of two specific OP pesticides, chlorpyrifos and diazinon, which have been detected in water samples collected by the CCRWQB for the Central Coast Ambient Monitoring Program, which has some overlapping sites with the CMP;
- A method for the performance of Toxicity Identification Evaluations (TIE), should TIEs be included as a follow-up tool for identifying the contaminant responsible for toxicity observed during future monitoring.

Organophosphate and pyrethroid analysis method

Organophosphate and pyrethroid pesticides may be analyzed by a variety of standardized analytical methods. Often these methods require the use of specific equipment (e.g., detectors) to obtain results for each class of pesticides, resulting in the need to perform multiple analytical methods to obtain results for both OP and pyrethroid pesticides. EPA 625 is a gas chromatography/mass spectrometry (GC/MS) analytical method that will provide results for both OP and pyrethroid pesticides. Briefly, the pesticides are recovered from the ambient water sample using liquid-liquid extraction. The GC is temperature programmed to separate the compounds and confirmation is achieved for the single component peaks using ions specific to each target compound. Compounds eluting from the GC are identified by matching the retention times of the unknown peaks with those from a known calibration standard and the concentration of each identified component is measured by comparison of the responses to those generated for reagent-grade pesticide standards stored in a mass spectral library.

So as to begin to quantify OP and pyrethroid pesticides in CMP ambient water samples, ambient water samples will be collected and analyzed via EPA 625 at the 2005 CMP monitoring sites listed in Table A-2a; samples will be collected four times annually coinciding with the timing of the aquatic toxicity sampling. A total of 2-L of ambient water will be collected into amber glass bottles for each site; an additional 6-L of sample will be required to perform necessary QA procedures (e.g., lab duplicate, matrix spike, matrix spike duplicate). Tables A-1, B-2, B-3, and B-4 in this QAPP have been updated to include information relevant to the EPA 625 analysis; narrative text has been added to Sections B-3 and B-5 relative to the pesticide sample collection and analytical method requirements. The Quality Control requirements for the OP and pyrethroid pesticide analyses have been added as Tables B-7 and B-8, respectively. The OP and pyrethroid pesticide analysis SOP is provided as Attachment 5 of Appendix C.

Diazinon and chlorpyrifos analysis method

Both diazinon and chlorpyrifos have been detected in water samples collected by the CCRWQB for the Central Coast Ambient Monitoring Program, which has some overlapping sites with the CMP. Although diazinon and chlorpyrifos are two organophosphate pesticides which are detected using EPA 625, the standard laboratory turn around time (TAT) for the EPA 625 can be as long as 30 days. Enzyme-Linked ImmunoSorbent Assay (ELISA) methods can be used as a screening tool that can provide rapid analyses of ambient water samples, with results available within 24 hours of sample collection. In performing ELISA analyses, an aliquot of each sample is pipetted into replicate wells containing polyclonal antibodies specific to diazinon or chlorpyrifos. An enzyme-linked substrate that binds to available antibodies is then added to the wells. After a reaction period of 1 hr, the wells are rinsed with RO/DI water and a color formation solution is added to the wells. Upon completion of color development, the diazinon and chlorpyrifos concentrations are determined by spectrophotometric analysis. Although not a required component of the CMP monitoring program, the ELISA method may be used should the CMP management desire rapid analytical results for diazinon and chlorpyrifos for designing any desired follow-up work.

Table B-2 in this QAPP has been updated to include information relevant to the ELISA analysis; narrative text has been added to Sections B-3 and B-5 relative to the pesticide sample collection and analytical method requirements. The Quality Control requirements for the chlorpyrifos and diazinon analyses by ELISA are provide in Table B-9. The SOPs for the chlorpyrifos and diazinon analyses are provided as Attachment 6 and Attachment 7 in Appendix C.

Toxicity Identification Evaluations

If Toxicity Identification Evaluations (TIEs) be included as a follow-up tool for identifying the contaminant responsible for toxicity observed during future monitoring, Phase I TIEs would be "targeted" toward contaminant classes likely to be present in irrigation return waters, including pesticides and metals. The methods for the targeted Phase I TIEs would be as follows:

- Centrifugation – to remove particulate-associated contaminants (and a pre-treatment step for the carbon column treatment);
- Solid Phase Extraction (SPE) using C-8 columns – to remove non-polar organic contaminants, such as pesticides;
- CHELEX cation exchange columns – to remove divalent cations, such as copper;
- Piperonyl butoxide (PBO) – inhibits the cytochrome P450 enzyme system, which is responsible for making organophosphate pesticides toxic to invertebrates and vertebrates.

F. REFERENCES

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Harrington and Born. 2000. Measuring the Health of California Streams and Rivers. – A Methods Manual for Water Resources Professionals, Citizen Monitors, and Natural Resources Students. Sustainable Lands Stewardship International institute, Sacramento, CA.

USEPA 1995. Method 1669: Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels. U.S. Environmental Protection Agency (USEPA), Office of Water. EPA 821-R-95-034.

USEPA 2000. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates. U.S. Environmental Protection Agency (USEPA), Office of Research and Development. EPA 600/R-99/064.

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

SUPPORTING DOCUMENTS FOR CHEMICAL WATER QUALITY MONITORING

ATTACHMENT 1: Pacific EcoRisk 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. Garman® 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 Garman® 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: Pacific EcoRisk 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 retrevial line (efter 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 Garman® 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 an 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 Garman® 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: SWAMP Bed Sediment Sampling SOP

Field Collection Procedures for Bed-Sediment Samples

Bed sediment (hereafter termed "sediment") samples are collected after any water samples have been collected. Care must be taken not to sample sediments that have been walked on or disturbed in any manner by field personnel collecting water samples. Sediment samples are collected into a composite jar, where they are thoroughly homogenized in the field, and then aliquoted into separate jars for chemical or biological analysis. Sediment samples for metals and organics are submitted to the respective analytical laboratories in separate glass jars, which have been pre-cleaned according to laboratory protocol

Sediment chemistry samples give information regarding both trends in contaminant loading and the potential for adverse effects on sediment and aquatic biota. In order to compare samples over time and from site to site, they must be collected in a consistent manner. If a suitable site for collecting sediments cannot be found at a station, sampling personnel should not collect the sediment sample, and should instead attempt to reschedule the sample collection. If this is not possible, make a note so that the missing sample is accounted for in the reconciliation of monitoring events during preparation of sample collection "cruise reports". Sites that are routinely difficult to collect should be considered for elimination from the sample schedule, if appropriate.

Characteristics of Ideal Sediment Material to be Collected

Many of the chemical constituents of concern are adsorbed onto fine particles. One of the major objectives in selecting a sample site, and in actually collecting the sample while on site, is to obtain recently deposited fine sediment, to the extent possible. Avoid hard clay, bank deposits, gravel, and disturbed and/or filled areas. Any sediment that resists being scooped by a dredge is probably not recently deposited fine sediment material. In following this guidance, the collection of sediment is purposefully being biased for fine materials, which must be discussed thoroughly in any subsequent interpretive reporting of the data, in regards to representativeness of the collected sample to the environment from which it was collected.

Characteristics of an Ideal Site

Quiescent areas are conducive to the settling of finer materials (EPA/USACOE, 1981).

Choose a sampling site with lower hydrologic energy, such as the inner (depositional) side of bends or eddies where the water movement may be slower. Reservoirs and estuaries are generally depositional environments, also.

Field Collection Procedures for Bed-Sediment Samples

Selecting the Appropriate Sediment Type for Analysis

See filename: "FieldSiteEvaluation_and_Recon_guidelines_1202.doc", within Appendix D of this QAMP for more specific guidance.

Sediment will vary from site to site and can vary between sample events at a particular site.

Streams and Rivers: Sediment collection in flowing streams is often a challenge. In areas of frequent scouring there may not be sufficient sediment for collection during or following periods of high flow. Sediment collection during these times may prove unsuccessful and may have to be rescheduled.

When the suspended load in rivers and streams precipitates due to reduction of velocity, most of the resulting sediment will be fine-grained. More often than not, a dredge or mechanical grab device does not function well for collection of sediment in smaller streams. In many cases, sediment will have to be collected using a pre-cleaned Teflon scoop. Collect the top two (2) cm for analysis. Five or more (depending on the volume of sediment needed for conducting analyses) fine-sediment sub-sites within a 100-meter reach are sampled into the composite jar.

Reservoirs and Estuaries: Collect the top two (2) cm for analysis. Five or more grabs are composited for the sediment sample, depending on the volume of sediment needed for conducting analyses.

GENERAL PROCEDURE FOR COLLECTION OF BED SEDIMENT

After choosing an appropriate site, and identifying appropriate fine-grained sediment areas within the general reach, collect the sample using one or more of the following procedures, depending on the setting:

A. Sediment Scoop Method—Primary Method for Wadeable, Shallow Streams

The goal is to collect the top 2cm of recently-deposited fine sediment only .

- Wear gloves and protective gear, per appropriate protocol (make sure gloves are long enough to prevent water from overflowing gloves while submerging scoop).

Field Collection Procedures for Bed-Sediment Samples

- Survey the sampling area for appropriate fine-sediment depositional areas before stepping into the stream, to avoid disturbing possible sediment collection sub-sites.
- Carefully enter the stream and start sampling at the closest appropriate spot, after rinsing the homogenizing jar and lid with ambient water. Then continue sampling UPSTREAM. Never advance downstream, as this could lead to sampling disturbed sediment.
- Use a clean polyethylene scoop for each site (pre-cleaned beforehand in the laboratory, with Micro™ detergent and acid, rinsed, dried and double-bagged). Scoop can be transported from vehicle to site inside the homogenizing jar. Gently lead the scoop under water and towards the sediment. Run scoop slowly underneath sediment at about 2cm depth till about ½ to ¾ filled. Then carefully lift the scoop out of the water and slowly poor off most of the overlying water over one of the BACK corners of the scoop. Make sure that the top layer of fine sediment is not discarded. Fill homogenizing jar as far as necessary to fill all required sample volumes. I-CHEM 4000mL tall clear 300 Series glass jars are used to collect and homogenize sediment samples.
- Cap homogenizing jar, put on ice, and transport to site where sample containers are to be filled.
- Make sure all containers are capped tightly.
- Write date and time on each container label (container bag label for TM [trace metals] and Hg [mercury] prior to aliquoting.
- Single bag all containers (except TM [trace metals] and Hg [mercury] containers – are double bagged already) in zip lock bags.
- Store in cooler on cube ice at 4°C.
- Check cooler temperature and record in log book every 8-12 hours or whenever sampler suspects that the temperature has not been maintained at 4 C.

B. Sediment Grab Method—Primarily for Lake, River, Bridge, and Estuarine Settings (or deeper streams)

Description of sediment grab equipment

- A mechanical sediment grab is used for the SWAMP bed sediment collection field effort for lake, river, bridge, and estuarine/coastal settings (or deeper, non-wadeable streams).
- The mechanical grab is a stainless steel “Young-modified Van Veen Grab", and is 1 square meter in size.
- The mechanical grab is deployed primarily from a boat, and is used in deeper, non-wadeable waters, such as lakes, rivers, estuaries, and coastal areas.
- It is also deployed by field personnel from land in settings which allow its use: primarily from bridges; from smaller vessels in streams or drainage channels too deep or steep to wade into, but too shallow for a larger boat.

Field Collection Procedures for Bed-Sediment Samples

Field Collection Procedures for Bed-Sediment Samples

Deploying and retrieving the grab

- Slowly lower the grab to the bottom with a minimum of substrate disturbance.
- Retrieve the closed dredge at a moderate speed (less than two feet per second).
- Upon retrieval, examine the grab to ensure that the sediment surface is undisturbed and that the grab sample should not be rejected.

Rejection Criteria—reject the sample if the following are not met:
Mud surface must not be pressing out of the top of the sampler. If it is, lower the grab more slowly.

- Overlying water must not be leaking out along the sides of the sediment in the grab. This ensures the surficial sediment is not washed out.
- Sediment surface is flat and level in the sampler. If it is not level, the grab has tilted over before closing.

Processing the sediment sample from the grab equipment:
The water overlying the sediment in the grab is very gently decanted by slightly tipping the grab with the lid closed until the water runs out the top.

- The decanting process should remove all of the overlying water but not remove the surficial sediments. The laboratory reports percent water for the sample, so overlying water is not included in the sample container.
- The sediment is examined for depth of penetration, color and thickness of top aerobic zone, and texture. These observations are recorded in the logbook.
- Collect the top 2 cm from at least five sub samples, and otherwise, exclude the bottom-most layer and composite.
- In streams or other settings with excessive bottom debris (rocks, sticks, leaves) where the use of a grab is determined to be ineffective (dredge does not close, causing loss of sediment), samples may be collected by hand using a clean plastic scoop, or by a variety of coring methods, if appropriate for the situation.
- Sediment is handled as described below in the metals and organic sections.

Cleaning the Grab Equipment and Protection from Potential Contaminating Sources:

- The sediment sampler will be cleaned prior to sampling EACH site by: rinsing all surfaces with ambient water, scrubbing all sediment sample contact surfaces with Micro™ or equivalent detergent, rinsing all surfaces with ambient water, rinsing sediment sample contact surfaces with 5% HCl, and rinsing all sediment sample contact surfaces with methanol.
- The sediment grab will be scrubbed with ambient water between successive deployments at ONE site, in order to remove adhering sediments from contact surfaces possibly originating below the sampled layer, thus preventing contamination from areas beyond target sampling area.
- Sampling procedures will attempt to avoid exhaust from any engine aboard any vessel involved in sample collection. An engine will be turned off when possible during portions of the sampling process where contamination from engine exhaust

Field Collection Procedures for Bed-Sediment Samples

may occur. It is critical that sample contamination be avoided during sample collection. All sampling equipment (i.e., siphon hoses, scoops, containers) will be made of non-contaminating material and will be appropriately cleaned before use. Samples will not be touched with un-gloved fingers. In addition, potential airborne contamination (e.g., from engine exhaust, cigarette smoke) will be avoided.

Sediment Sub-sample Collection Procedure (Removal from Grab): Before sub-samples from the grab sampler are taken, the overlying water will be removed. One method of removing this water is by slowly siphoning it off to pipette out overlying water. Other methods, such as decanting the water or slightly opening the sampler to allow the water to escape, will be done slowly and with care to minimize disturbance or loss of fine-grained surficial sediment. Once the overlying water has been removed, the top 2 cm of surficial sediment can be sub-sampled from the grab. Sub-samples are taken using a pre-cleaned flat bottom scoop. This device allows a relatively large sub-sample to be taken accurately. Because accurate and consistent sub sampling requires practice, a trained and experienced person performs this task. When sub sampling surficial sediments, unrepresentative material (e.g., large stones or vegetative material) will be removed from the sample in the field. The smaller rocks and other small foreign material remain in the sample.

C. Core Method--alternative for fast-moving, wadeable streams

The core method is used in soft sediments when it is difficult to use the other methodologies. The cores can be used in depths of water from 0 to 10 feet by using a pole deployment device or in deeper water using SCUBA divers. The pole deployment device consists of a pole that attaches to the top of the core. The top of the core is fitted with a one-way valve, which allows the core to be filled with sediment, but when pulled from the sediment catches the sediment within the core. The core is then brought to the surface and the sediments within the core are extruded out the top of the core so that 2 cm. of sediment is above the top of the plastic core. The 2 cm of sediment is then sliced off and placed in the homogenizing jar. The core, homogenizing jar, and device used to slice off the top two cm. are all cleaned according to field protocols herein in Appendix D.

GENERAL PROCEDURE FOR PROCESSING OF BED SEDIMENT SAMPLES, ONCE THEY ARE COLLECTED

Sediment Homogenization, Aliquoting and Transport

For the collection of bed sediment samples, the top 2-cm is removed from the scoop, or the grab, or the core, and placed in the 4-liter glass compositing/homogenizing container.

Field Collection Procedures for Bed-Sediment Samples

The composited sediment in the container is homogenized and aliquoted on-site in the field. The sample is stirred with a polycarbonate stirring rod for at least 5 minutes, but longer if necessary, until sediment/mud appears homogeneous. All sample identification information (station numbers, etc.) will be recorded prior to homogenizing and aliquoting.

All pre-labeled jars will be filled using a clean plastic scoop. The sediment sample is then aliquoted into appropriate containers for trace metal chemistry, organic chemistry, and bioassay testing. Four-liter sample containers will be packed surrounded by enough ice to keep them cool for 48 hours. Each container will be sealed in one large plastic bag to prevent contact with other samples or ice or water.

Metals and Semi-volatile Organics in Sediment	For trace metals and semi-volatile organics, a minimum of three grabs is distributed to the composite bottle and/or sample containers. Mixing is generally done with a polycarbonate stirring rod. <u>Collecting Metal, Semi-Volatile, or Pesticide Samples:</u> Make sure the sample volume is adequate, but the containers do not need to be filled to the top. Seal the jars with the Teflon liner in the lid.
Sediment Conventionals	Sediment conventionals are sometimes requested when sediment organics, sediment metals, and/or sediment toxicity tests are requested for analysis of samples. The collection method is the same as that for metals, semi-volatile organics, and pesticides. Sediment conventionals include: grain size analysis and total organic carbon. These are used in the interpretation of metals and organics in sediment data.
Sample Containers	See "Sediment Sample Handling Requirements" Table at end of this document, as well as in Appendix C of this QAMP.
Sediment Sample Size	Must collect sufficient volume of sediment to allow for proper analysis, including possible repeats, as well as any requested archiving of samples for possible later analysis. See "Sediment Sample Handling Requirements" Table at end of this document, as well as in Appendix C of this QAMP.
Labeling	Label the jars with the station ID, sample code, matrix type, project ID, and date of collection, as well as the type of analysis requested (i.e., metals, conventionals, organics, or archives).
Short-term Field Preservation	Immediately place the labeled jar on ice, cool to 4°C, and keep in the dark at 4°C until delivery to the laboratory.
Field Notes	Record the depth at the location where the sample was taken in the field logbook. Record a gross description of the sample, i.e., color, texture, number of grabs, and thickness of grab sample that was composited. This information can be reported as comments with the sediment analytical results. Fill out SWAMP Station Occupation Data Sheet and the Sediment Data Sheet.

Field Collection Procedures for Bed-Sediment Samples

Field Collection Procedures for Bed-Sediment Samples

SUMMARY OF SEDIMENT SAMPLE COLLECTION METHODS, PRESERVATION, STORAGE, AND HANDLING REQUIREMENTS

Parameters for Analysis	Recommended Containers	Typical Sample Volume (ml)	Initial Field Preservation	Maximum Holding Time
Bed Sediment Samples				
Trace Metals, including Hg and As (except for Se--see below)	60 ml I-Chem 300-series clear glass jar with Teflon lid-liner; Pre-cleaned	60 ml (one jar)	Cool to 4°C, dark, up to 14 days	12 months(1) (-20°C)
Selenium (separate container required)	60 ml I-Chem 300-series clear glass jar with Teflon lid-liner; Pre-cleaned	60 ml (one jar)	Cool to 4°C, dark, up to 14 days	12 months(1) (-20°C)
Synthetic Organic Compounds	250 ml I-Chem 300-series amber glass jar with Teflon lid-liner; Pre-cleaned	500 ml (two jars)	Cool to 4°C, dark, up to 14 days	12 months(1) (-20°C)
Sediment TOC	125 ml(3) clear glass jar; Pre-cleaned	125 ml (one jar)	Cool to 4°C, dark, up to 28 days	12 months(2) (-20°C)
Sediment Grain Size	125 ml(3) clear glass jar; Pre-cleaned	125 ml (one jar)	Cool to 4°C, dark, up to 28 days	28 days (4°C) ***Do not freeze***
Sediment Toxicity Testing	1-Liter I-Chem wide-mouth polyethylene jar with Teflon lid-liner; Pre-cleaned	2-Liters (two jars filled completely)	Cool to 4°C, dark, up to 14 days	14 days (4°C) ***Do not freeze***

(1) Sediment samples for parameters noted with one asterisk (*) may be refrigerated at 4°C for up to 14-days maximum, but analysis must start within the 14-day period, or the sediment sample must be stored frozen at minus (-) 20°C for up to 12 months maximum.

(2) Sediment samples for sediment TOC analysis can be held at 4°C for up to 28 days, and should be analyzed within this 28 day period, but can be frozen at any time during the initial 28 days, for up to 12 months maximum at minus (-) 20°C.

(3) Sediment samples for TOC AND grain size analysis can be combined in one 250 ml clear glass jar, and sub-sampled at the laboratory in order to utilize holding time differences for the two analyses. If this is done, the 250 ml combined sediment sample must be refrigerated only (not frozen) at 4°C for up to 28 days, during which time the sub-samples must be aliquoted in order to comply with separate storage requirements (as shown above).

ATTACHMENT 4: CCAMP Sampling SOP

CCAMP Conventional Water Quality Monitoring Standard Operating Procedure

Introduction

The CCAMP Conventional Water Quality (CWQ) Standard Operating Procedures (SOPs) document describes techniques used by CCAMP staff in the collection of surface water quality data. This document also describes site selection criteria, all field measurement procedures, collection of water samples for laboratory analysis, sample handling, quality assurance and data management

Sampling Preparations

1) Lab Coordination (1 week in advance of sampling)
   a) BC labs (661) 327-4911
      i) Arrange for bottles to be delivered (2, 1L/site, 1 coliform/site)
      ii) Arrange for sample pickup
   b) Creek (805) 545-9838
      i) Notify of sample drop off (dates and number of samples per day)
2) Sign Out a Vehicle (1 week in advance of sampling)
3) Sign out a digital camera (1 week in advance of sampling)
4) Sign out a cell phone, from Karen (1 week in advance of sampling)
5) Prepare & print (ex. S: CCAMP/Santa Barbara/Monitoring…)
   a) Labels for bottles (edit/replace-change dates, etc.) (s:CCAMP/CCAMPers/in personal folders)
   b) Data sheets (s:CCAMP/CCAMPers/in personal folders)
   c) Chain of Custody forms (COCs) (s:CCAMP /CCAMP monitoring/BC chain or Creek chain)
6) Calibrate the Hydrolab DS4a (See appendix A for Calibration procedures)

Field Equipment and Supplies

Field data sheet (see Appendix)
Clipboard
2 Chain of custody sheets (see Appendix)
Pencil and pen
Thermometer
20 Coliform bottles
1 chlorophyll bottles
41 cwq bottles
Bottle labels and permanent marker
Disposable Gloves
Digital camera / Disks
Cell Phone
Hydrolab Datasonde and surveyor 4a
1 gallon of water
Squirt bottle
First aid kit and a change of clothes
Cooler with ice
5 gallon bucket
100m rope
Bottle dipping device
Hand wipes
Leather work gloves

Safety equipment and considerations

Tall rubber boots, felt soled if necessary
Elbow length rubber gloves
Cones
Orange vests

Personal safety equipment:
For your own protection from pathogens and other organic pollutants each field personnel will have long sleeve rubber gloves and watertight tall rubber boots available for collecting samples. See your supervisor to have personal safety equipment issued to you.

Considerations for sampling from bridges:
Park the vehicle in the shoulder of the lane, at the beginning of the bridge
Turn on hazard lights
Wear orange vest with reflective tape
Place cones along the shoulder preceding the location where personnel is collecting the sample
Move the vehicle a safe distance from the road's edge before processing samples in the back.

Sampling Procedures

Surveyor 4a File Storage Procedures

Create a file in the hand held surveyor for storage of all data collected in the field. Procedures for file set up can be found in Appendix A, Surveyor file set up procedures. A new file should be used every day and named by the date of sampling, i.e. {01/22/02}.

Pre-Sampling QAQC Sample (Field equipment verification sample)

A QAQC sample of tap water is measured prior to sampling. The data is stored in the hand held Surveyor 4a and the sample is kept in a clean sample bottle labeled "QAQC" with the day's date. The sample is measured again after all sampling is concluded. These measurements are recorded in the pre-blank and post-blank rows on the field data sheet. They are used to ensure that pH, conductivity and turbidity have not significantly drifted during the sampling day. This sample is subsequently sent to BC lab to verify accuracy of Hydrolab measurements. It's recorded on the COC form as QAQC.

Instream Sampling Location

For all wadeable streams and rivers, samples are collected from the center of the stream flow or the thalweg. Stream profiles have been established at several sites. In this case samples should be collected at this location and accompanied by a stage and flow measurement. Sampling from the shore is the least acceptable location. In streams or rivers that are not wadeable samples are collected from an overpass or bridge. Specific procedures for field techniques are discussed in the following text.

Site Photographs

Take a photograph each time a site is visited. Site photographs are taken from the location the sample is collected looking up stream. Take any additional photographs you find interesting, such as aquatic fauna, unusual structures or flow patterns. Record picture numbers for all photographs taken at a site on the data sheet.

Field Measurements and Sample Collection

1) Air Temperature: Place thermometer in an area not directly in the sun, but where the air temperature for that area will be obtained (above ground, in the shade, avoid influence from vehicle).

2) In Stream Procedures
   a) Affix labels to bottles (two qt bottles and 1 coliform bottle). Labels contain the following information: Site name, date, time, project ID, and samplers name.

b) Take the Hydrolab and labeled bottles along with the data sheet to the site. Select an area in the water that has flow and is deep enough to submerge the Hydrolab probes, preferably in the thalweg. Record Hydrolab results on data sheet and store the measurements in the hand held Surveyor 4a.

c) Collecting a Grab Sample
   i) Wear clean disposable gloves
   ii) Collect a depth-integrated sample from the thalweg facing up stream. Submerge the bottles slowly, obtaining a sample representing the entire water column.
   iii) Collect the coliform sample last, open and close the bottle underwater to avoid sampling surface scum. Be sure to leave an air space and avoid touching the bottle rim or the inside of the lid.

3) Bridge and Pole samples
if river, tributary or creek is not wadeable samples are collected either from a bridge using a 5-gallon bucket, the bottle dipper and the 100m rope or from the shore using a pole and bottle dipping attachment. Use your best professional judgment to evaluate the conditions and choose the safest sampling method.
   a) Bridge Sample collection
      i) Hydrolab Sample Collection: Lower and rinse the bucket three times. Fill the bucket, and submerge Hydrolab probes into bucket to obtain measurements.
      ii) Grab Sample Collection: Place properly labeled bottles into the bottle dipper, remove lids (coliform last) using a glove. Hold the lids face down while the second field person lowers the bottles into Waterbody to fill. Replace lids immediately following collection of samples, coliform lid first.
   b) Pole sampling
      i) Hydrolab Sample Collection: Lower and rinse the bucket three times. Fill the bucket, and submerge Hydrolab probes into bucket to obtain measurements.
      ii) Grab Sample Collection: Place properly labeled bottles into the bottle dipper, remove lids using a glove. Hold the lids face down while the second field person lowers the bottles into the waterbody facing upstream to fill. Replace lids immediately following collection of samples, coliform lid first.

4) Field Observations
   a) Record information relating to the shading of the stream, algal cover in stream as well as riparian vegetation and aquatic vegetation.
      i) Shading: Record the percent of the stream's surface (water surface), up stream from your sample location, that you estimate would be shaded if the sun was directly over the creek.
      ii) Algae:
         (1) Filamentous (Fill): Record the percent of the flowing water surface, up stream from your sample location, that you estimate is occupied by filamentous algae.
         (2) Other Periphyton (Peri): Record the percent of substrate in the active channel, up stream from your sample location, that you estimate is covered in periphyton. Other periphyton is defined here as the living community attached to the substrate, including algae that is not the green filamentous type, aquatic mosses, fungi, diatoms and sessile invertebrates. To make this estimate feel the surface of the rocks and other substrate materials and estimate the percent of the substrate that is covered with a slimy organic community.

iii) Plants:
(1) Bank: Record the percent of the surface of both banks, up stream from your sample location that you estimate to be covered by vegetation. This estimate refers only to plants and roots at the waters edge.
(2) In Stream: Record the percent of the flowing waters surface, up stream from your sample location that you estimate to be occupied by aquatic vegetation. This is a percent of the total waters surface that is occupied by aquatic vegetation.

iv) Dominant Substrate:
Record the dominant substrate in the upstream reach of the sample location using one of the following categories: Boulder (B), cobble (C), gravel (G), sand (S), fines (F).

b) Visually assess the stream corridor and comment on anything that you fell may directly affect or contribute to changes in water quality. Some standard comments and categories of observations follow.

i) Biological Activity: Count the number of fish, birds or invertebrates observed and record one of the following categories: True count up to 25, 25-50, 51-75, 76-100, >100. For fish estimate the size of the fish that are present.
ii) Color: Record any unusual watercolor, suspended matter, foam or other debris.
iii) Odors: Record any odors such as H_2S, animal waste or any identifiable odor, else record unidentified odor.
iv) Instream activities: Record any construction, major erosion events or other instream activities that may impact water quality
v) Weather: Record any recent weather events such as heavy rains, cold front or heat spells, and comments that reflect changes in flow such as recent scour.
vi) Trash and Debris: Record the extent of trash and discarded debris on the banks and in the stream. Certain types of trash should be specifically listed such as human wastes, homeless encampments, aerosol cans, batteries or any other debris that directly affect water quality. Other categories of trash that should be noted include dumping of furniture or appliances, and the presence of paper and recyclable trash. Record the true number of paper and recyclable trash items up to 10 items (count 1-10 items of trash), greater than 10 items should be recorded as >10 items of trash.
vii) Tidal Influences: Record evidence of recent tidal surge (i.e. kelp or driftwood) or record possible salt-water influence.

5) Upon returning to the vehicle:
a) Record the air temperature for that site
b) Place the bottles in cold ice chests (4°C)
c) Rinse the Hydrolab cup and probes and keep some clean water in the cup between sites, keeping the probes moist.

Sample Handling

All samples are to be stored in ice chests at 4°C until transferred to the contract laboratory. Ensure that the number of samples in the ice chest does not compromise the sample temperature. The maximum number of bottles per ice chest is determined by ensuring that each bottle is in contact with at least one blue ice cube.

Holding Time is defined as the amount of time between collection of the sample and the initiation of its analysis. The holding time for coliform samples is 6 hours and for all other CWQ parameters the holding time is 24 hours. All samples must reach the lab within this time allotment.

Post sampling QAQC sample

1. Turbidity, pH and conductivity drift and accuracy verification
   The procedure is as follows: after the last site is sampled, rinse the Hydrolab cup and fill cup with the blank. Record data in the section labeled "Post Measurements" and store measurements in the surveyor 4a. Pour sample back into the blank bottle, labeled with a unique name (QAQC), date and time and place in the ice chest. This sample is to be analyzed by the contract laboratory for *pH, conductivity* and *turbidity* only. Dissolved oxygen is not verified using this procedure.
2. Lab verification of Dissolved Oxygen
   DO accuracy is measured at Creek Labs. Upon arrival at Creek Labs you can quality check the Hydrolab's DO measurement using a different blank in the Hydrolab and comparing the DO readings to those obtained using the lab's equipment. Use the Hydrolab cup to take measurements with the Hydrolab and record these measurements on the data sheet under post Blank. Use the same Hydrolab cup to have the lab measure DO. This ensures that the sample has not been stirred or agitated between measurements. Record their measurement under post Lab.

Quality Assurance

a) Duplicate Samples: In order to assure quality and consistency of lab results, duplicate samples are collected at 10% of the sites. These samples are collected at the same site every time and each replicate sample has a unique site name to disguise duplicate samples from the lab. The duplicate sample is collected to measure the variability in the environment as well as variation that is introduced by collection, handling and processing of samples. Duplicate samples are collected by submerging two bottles side by side, collecting 2 depth integrated samples simultaneously.

b) Pre / Post Sampling field equipment verification sample:
   i) Turbidity, pH and conductivity drift and accuracy verification
      Samples of tap water are measured by the field equipment pre and post sampling and subsequently are sent to the lab to verify that the Hydrolab's readings are accurate. This pre and post QAQC measurement is used to detect any drift in probe measurements (for ph, turbidity and conductivity) over the course of the sampling event.
   ii) Lab verification of Dissolved Oxygen
      Dissolved Oxygen is measured by the Hydrolab and subsequently by Creek labs following each sampling day. This sample is used to evaluate the accuracy of the Hydrolab measurements of DO taken on a given field day.

c) Chlorophyll calibration QA sample: A chlorophyll QAQC sample is collected from a site at which chlorophyll is measurable. At this site, a single chlorophyll sample is collected in a 100 ml amber bottle and processed by Creek Labs.

d) Chain of custody: A chain of custody form documents the change in possession of the samples between the time it is collected and analyzed (see attached). Retain copies of all chains with the field data sheet. The following information is to be included on the chain of custody:

Project name	Sample ID names
Sample date and time	Name of collector
Type of sample	EPA or SM method and Reporting format
Billing information	Receiving signature
Relinquishing signature	

Analysis used to assess conventional water quality

Analyte	Units	MDLs	Sampling Approach
PH	pH units	n/a	DataSonde 4a
Conductivity	US/cm	1.0	DataSonde 4a
Turbidity	NTU	0.1	DataSonde 4a
Dissolved Oxygen	Ppm	0.01	DataSonde 4a
Oxygen Saturation	% Saturation	n/a	DataSonde 4a
Water Temperature	Celsius	n/a	DataSonde 4a
Air Temperature	Celsius	n/a	Thermometer (°C)
Total Coliform Bacteria	MPN/100 ml	2	25-tube dilution
Fecal Coliform Bacteria	MPN/100 ml	2	25-tube dilution
Nitrate-N	mg/l	0.02	EPA 300.0
Nitrite-N	mg/l	0.01	
Total Kjeldahl Nitrogen	mg/l	0.1	EPA 351.2
Ammonia-NH_3	mg/l	0.02	EPA 350.1
Ortho Phosphate	mg/l	0.01	EPA 365.1
Total Phosphate	mg/l	0.06	EPA 365.4
Chlorophyll a	ug/l	0.1	DataSonde 4a
Total Suspended Solids	mg/l	0.5	EPA 160.2
Fixed Suspended Solids	mg/l	0.5	EPA 160.2
Total Dissolved Solids	mg/l	4.0	EPA 160.1
Fixed Dissolved Solids	mg/l	4.0	EPA 160.4
Volatile Dissolved Solids	mg/l	5.0	EPA 160.4
Volatile Suspended Solids	mg/l	0.5	EPA 160.4
SO_4	mg/l	5	SM-2340B
Chloride	mg/l	0.06	EPA 300.0
Boron, Dissolved	mg/l	0.05	EPA 6010
Calcium	mg/l	0.01	EPA 6010
Magnesium	mg/l	0.02	EPA 6010
Sodium	mg/l	0.06	EPA 6010
Corridor Shading	%	n/a	Visual estimate
Algal Cover	%	n/a	Visual estimate
Instream Plant Cover	%	n/a	Visual estimate

Post Sampling Procedures

1) Equipment Checklist
   a) Prepare equipment for next use
      i) Hydrolab
         (1) Ensure there is at least 1inch of water in the Hydrolab cup to keep the probes moist
         (2) Rinse and inspect the Hydrolab probes.
         (3) Rinse the glass surfaces of the chlorophyll probe with deionized water and wipe with lens paper or a q-tip
      ii) Ice chests
         (1) Empty all ice chests and return blue ice to freezer. Stack ice chests with lids open so that residual water will evaporate
      iii) Camera
         (1) Return camera to the regional board cabinet, place battery on charger
      iv) Vehicle
         (1) Make sure vehicle has a full tank of gas, windshield clean and any obvious dirt inside the vehicle has been vacuumed out!

2) Electronic Chain of Custody

   This file documents the field data collected and the analysis requested from the lab. The purpose is to identify all data that we should expect to receive, including lab, meter, subjective estimates and notes.
   a) Open file in s:/ Master Data Files & backups/ COCentry.
   b) Use the COC to enter each site tag, date, time and all analysis requested from the lab, blue columns in the COCentry file refer to lab data.
      i) The lab tag column contains all site tags listed on the COC, including the unique tags given to duplicate sites. The Site Tag column should reflect the actual site tag used by the project.
   c) Use the Field data sheet to enter all analyses to be downloaded from the Hydrolab, including pre, post and lab analyzed blanks. Green columns refer to data collected in the field Blue to data to be received from the lab.
   d) Make sure that the **duplicates and blanks** are **designated in the purpose column** using the following: FD, BLANK-PRE, BLANK_POST, AND BLANK-LAB. Every single sampling day should have these 4 rows associated with sites entered into the electronic COC.

3) Slurp in the COC
   a) Open Admin Code (s:/Master Data Files& backups/AdminCode)
   b) Hit the [perform COC] button on the control page
   c) If no error message comes up on the report worksheet continue to step 4) if an error message appears fix all errors and hit the [perform COC] button again
   d) Check the Big 8 columns (2-9) in Mastermon and hand enter any information that was not slurped in.

4) Surveyor 4a download
   a) Download surveyor 4a file following the procedures in Appendix A.
   b) Name file the Date you were in the field (i.e. 01/16/02)
   c) Download file into the folder created for data (s: /data processing/ slurp/Hydrolab).
      This is the file where all original incoming data is stored.
   d) Make a copy of this file and paste it in to s: /master data files/Hydrolab
      This is the file where we store all Hydrolab data to be slurped into MasterMon.

5) Slurp in the Hydrolab data
   a) Open admin code (s:/Master Data Files& backups / AdminCode)
   b) Hit the [scan hydrolab files] button on the control page. If no error message comes up on the report worksheet continue to step c). If an error message appears fix all errors and hit the [scan hydrolab files] button again. **YOU MUST SCAN BEFORE YOU READ FILES !!!!!!!!**
   c) Following scan hit the [read hydrolab files] button
   d) Check the Big 8 columns (2-9) in Mastermon and hand enter any information that was not slurped in.

6) Enter Field Data and QA Hydrolab slurp
   a) Use field data sheets to ensure that all field data is present in the database and to hand enter the following parameters into MasterMon
   b) Find the row of data with the hydrolab measurements (Indicated in the ID column, by the letter H and the date time (for example Jan 16th 2001 at noon looks like H011602120000)
   c) If Hydrolab data for a given site is not present hand enter data for that date and time.
   d) Compare data in Mastermon to field data sheet (time, DO, pH etc.)
   e) Hand enter the following parameters on the same row: Air temp, Cshade, Algae_fill, Algae_peri, Cover_Bank, Cover_In, Flow_Q and notes.

7) Site Photographs
   All photographs taken in the field must be labeled in a dated folder.
8) Download all site photographs into a dated folder in the project's file found in s/CCAMP/CCAMP monitoring/ Site Pics/ Dated folder.
   a) Name each picture with the site tag and date (month. year), for example Jalama in January of 2002 picture is named 315JAL.01.02

Appendix A

Hydrolab DS4a procedures

Calibration Procedures (Hydrolab DS4a)

Prior to each sampling event, the Hydrolab DS4a must be calibrated using the following procedure.

Calibration Supplies:

Calibration log binder	1L Mason jar
Deionized water	PH standards 7.0 and 10.0
DS4a	Conductivity standard ~1500us
Surveyor 4a	Turbidity standard 100 NTU
Calibration cord	Chlorophyll calibration donut

1) **Pre Calibration QAQC Sample**
   Fill a 1L Mason jar filled with tap water and submerge the Hydrolab probes, this is the pre calibration **blank.** Record the "pre cal blank" parameters on the Calibration log, and set jar aside until calibration is completed.
2) **Two-point Calibrations**
   For each parameter except oxygen conduct a two-point calibration; this is to bracket the highest and the lowest values you expect to encounter in the field.
   a) **PH**
      - Rinse the Hydrolab cup with the pH solution 7.0
      - Fill the cup with the 7.0 solution and submerge the probes
      - Let stabilize and record "pre cal" values in the calibration log
      - Calibrate to 7.0 using the Surveyor 4a:
        select "Calibration"
        select "sonde"
        select "pH"
        select "7.0"
        press "done"
        press any key
        select "go back"
        record the "post cal" value on the calibration log.
      - Discard the pH 7.0 solution, rinse the Hydrolab cup with the pH solution 10.0, rinse the Hydrolab probes with deionized water
      - Fill the cup with the 10.0 solution and submerge the probes
      - Let stabilize and record the pre cal values in the calibration log
      - Calibrate to "10.0" following above procedures for surveyor 4a.

   b) **Conductivity**
      - Rinse the cup and probes with deionized water; fill the cup with deionized water, submerge the probes and record the "pre cal" value following the procedures above; calibrate to 0.0 us and record the "post cal" value.

- Rinse the cup with the calibration standard, fill the cup with the calibration standard (1413us), submerge the probes, let stabilize and record the "pre cal" value; calibrate to 1413us following the above procedures and record the "post cal" value.

c) **Turbidity**
- Rinse the cup and probes with deionized water; fill the cup with deionized water and submerge the probes; record the "pre cal" value; following above procedures calibrate to 0.0 NTU and record the "post cal" value.
- Rinse the cup with the calibration standard (100 NTU); submerge the probes and record the "pre cal" value; calibrate to 100 NTU following the above procedures and record the "post cal" value.

d) **Dissolved Oxygen mg/L**
- Calibrate D.O. "mg/L"; Record the "pre cal" measurement on the calibration log. Using standard methods print out on front of "Meter Calibration Log" binder, find temperature measured by Hydrolab on the print out and calculate appropriate DO value at "0" chlorinity. For example, if temperature is 20 degrees, calibrate to 9.092 mg/L; select 9.920 and record "post cal" measurement.

e) Dissolved Oxygen, %Saturation *Note: automatically done when part "d" is finished.
- Set up for oxygen: fill cup with tap water so that probes are <u>just above</u> the water line; wipe off probe tops using lens tissue to remove all water droplets; leave Hydrolab running with the cup sealed for at least 20 mins.
- While the Dissolved Oxygen is stabilizing calibrate the chlorophyll probe without splashing the DO probe.
- After at least 20 mins calibrate % saturation. Record "pre cal" in "D.O. sat" section; select "done" when barometer reading says "760;" record "post cal."

f) Chlorophyll
- Submerge chlorophyll probe in DI by holding a full cup up to the probe and record "pre cal;" calibrate to "0" following the above procedures for the surveyor 4a and record "post cal."
- Place the calibration donut on the chlorophyll probe and record "pre cal"; calibrate to "50" following the above procedures for the surveyor 4a and record "post cal."

3) Post Calibration QAQC sample
- Re-measure water used as the pre cal blank (1L Mason jar filled with tap water). Record the "post cal blank" parameters on the Calibration log.

Surveyor 4a File Set Up Procedures

1. Create a File on the Surveyor 4a for each sampling event
    - Select "Files", "svr 4a", "create", "manual", Give the file a name
      File names are the date you were in the field (i.e. 01.18.02)
2. Storing data in the Surveyor 4a
    - Select "Files" and select the file name (i.e. 01.18.02)

Surveyor 4a Downloading Procedures

1) Connect the surveyor to the PC (use the COM 1 connection), **and then** turn both machines on.
2) On your PC, go to **Start > Programs > Accessories > Communications >Hyperterminal**.
3) Select the icon titled Hypertrm or Hypertrm.exe.
4) Select a name and an icon, the same name as the file in the surveyor is a good bet.
5) A window will open titled 'Phone Number'. In the field titled 'Connect Using:' Use the pull-down menu to select 'Direct to Com 1' and Click OK.
6) Another window will open titled 'Com 1 Properties'. In this window, you will select
    a) the baud rate at 19200
    b) data bits should be 8
    c) parity should be none
    d) stop bits should be 1
    e) The flow control should be set to 'Xon/Xoff'
7) The screen should clear. At this time select **File > Properties**. A window will open with two tabs at the top of it. One tab will say 'Phone Number', the other will say 'Settings'. Click on the tab entitled 'Settings'. Your terminal emulation should be ANSI. Also make sure that the button next to terminal keys is highlighted. Click OK.
8) In the top, left-hand corner of your screen there should be two phone icons. Click on the one with the receiver off the hook. This will disconnect the session. Next, click on the icon with the receiver on the hook. This will re-connect you. This is done to allow the program to remember the changes that you have just made.
9) On the PC, go to Transfer/Receive and set "use receiving protocol" to X-modem and set the location you would like the file to be saved in as S:\Data processing\Slurp Originals\Hydrolab slurp. Click on 'Receive' and enter the file name (Use the same file name that you created in the Hydrolab.).
10) You are now ready to transmit from your sonde. On the Sonde, select files > transmit.
    a) Select the appropriate file on the surveyor.
    b) At the prompt, select file type "SS importable" and data should flow.

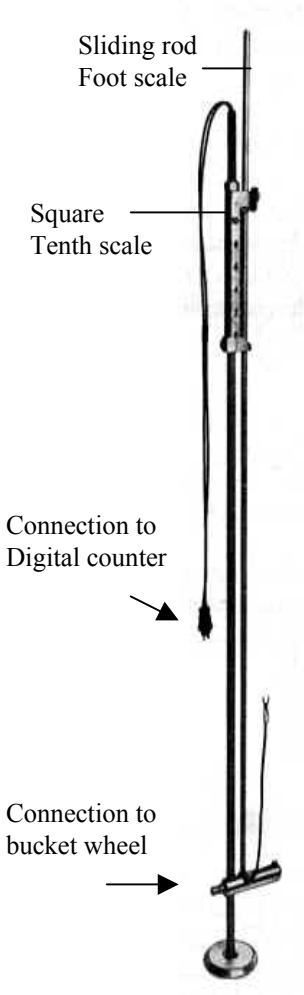
Flow Measurement Procedures

Preparation for collection Flow Measurements

- Remove the brass travel pin from the bucket wheel and replace it with the silver pin.
- Attach bucket wheel to the Top Setting Rod
- Attach the digital counter to the connector at the top of the rod

Collection of Flow Data

- Visually inspect the stream channel to identify the location of the cross section. The ideal cross section will have a flat uniform substrate, the least desirable substrate is uneven with large obstructions and immeasurable sections of flow.
- Measure the stream width, wetted channel and record on the data sheet. Stretch a measurement tape (in 10^{th} of feet) across the flowing stream bed and record stream width
- Determine the number of flow measurements to be taken. Five measurements are a minimum for all streams. If it is possible, collect 7-10 measurements at each site. Use your best professional judgment to determine the spacing and location of flow measurement sections. Evaluate the shape of the streambed, dimensions of the substrate and volume of water to determine measurement locations, which will provide data representing the many different flow regimes present. Flow measurements sections do not have to be of equal width, however no section should have greater than 50% of the flow when 5 measurements are taken and no more than 10% of the flow when 10 measurements are taken.
- Use the top setting rod to set the bucket wheel at 60% depth of the water column.
    - The sliding rod measurements refer to whole foot depths The rod has single marks representing .10 of a foot, double marks representing .5 foot and triple marks representing 1.0 foot.
    - The square, which the rod slides through, is marked in tenths of feet. If the creek is 1.2 meters deep position the 1 foot (triple mark) on the rod next to the .2 mark on the square. The bucket wheel is now set to 60% of the total depth of the water.
- Set the digital counter to 40 seconds and hit start.
- Ensure the wading rod is kept vertical and the bucket wheel kept perpendicular to the flow.
- Record the number of rotations of the wheel counted on the digital display.



Appendix B

CCAMP Monitoring Forms

1. BC Laboratories, Chain of Custody Form
2. Creek Environmental Laboratories Chain of Custody Form
3. Field Data Sheet
4. Calibration Log
5. Maintenance log

BC LABORATORIES, INC.	4100 Atlas Court • Bakersfield, CA 93308 (661) 327-4911 • FAX (661) 327-1918	CHAIN OF CUSTODY

Report to/Contact person:

Mary S Adams

Central Coast RWQCB
81 Higuera St. Suite 200
San Luis Obispo, CA 93401
(805) 542-4768
Fax 788-3502

Project: **CCAMP**

Sampler:

Analysis Requested

Lab #	SiteTag	Date and Time sampled	MATRIX = Water (w), Sediment (s)	NUTRIENT SERIES																			
				EPA 300.0 NO3 as N	**EPA 353.2** NO2 as N	**EPA 350.1** NH3 as N	**EPA 351.2** TKN	**EPA365.4** Total PO4 as P	**EPA 365.1** Ortho PO4 as P	**EPA 160.2** TSS	**EPA 160.4** Fixed & dissolved SS	**EPA160.1** TDS	**EPA 6010** Calcium	**EPA 6010** Sodium	**EPA 6010** Dissolved Boron	**EPA 6010** Magnesium	**EPA 300.0** Chloride	**SM 2340B** Hardness (CaCO3)	**EPA 300.0** Sulfate	**25 tube dilution** Total and Fecal Coliform		PH, SPC (us), and Turbidity

Comments:

Billing Info

Name: Regional Water
Address: 81 Higuera St. Suite 200
San Luis Obispo, CA 93401-5427

Attention: **Cyndee Jones**

Relinquished By:

Date and Time:

Received By:

Date and Time:

2. Creek Labs Chain of Custody Form

Please Print in Pen

Client Name	Contact	Phone	Due Date:
Regional Water Quality Control Board	Mary S. Adams	542-4768	24Hr 48Hr Other Normal TAT

Address	City	State	Zip	Fax	Cell	Beeper
81 Higuera, Suite 200	San Luis Obispo	CA	93402	543-0397		

Project Name/Number	PO#	Copies To:
CCAMP		

Bill to: (if different from above) BC Laboratories

Sampler Name (Print)	Comments:	Matrix Key: DW = Drinking Water AQ = Aqueous SL = Soil/Solid

Sample Description	Date/Time Sampled	Analysis		Matrix	# of Bottles	Preservative / Type Bottles	Lab Sample #
		25 tube Total and Fecal coliform	**SM 10200H** Chlorophyll a	**Aq**			

RELINQUISHED BY (Sign)	(Print)	(Organization)	DATE/TIME	RECEIVED BY (Sign)	(Print)	(Organization)
		RWQCB				**Creek Environmental Laboratories, Inc.**

FOR LAB USE ONLY: Shipping Method: Client/ Lab/ Courier**:** Sample Conditions: Intact: Y/ N Cold: Y/ N Custody Sealed: Y/ N

REMARKS

3. Field Data Sheet for Conventional Water Quality Monitoring

Site	Time	pH	Cond(uS)	Turbidity	DO(ppm)	DO%sat	H_2OTemp	Salinity	Chlorophyll	Air Temp	shading	algae	plants	flow	**Substrate**
Cal date												Fill / Peri	Bank / in		**Dates of calibration**
Blank															**Pre Measurements**
Blank															**Post Measurements**
DS4a/ Lab															**Post Meas, Lab**
313SAB															
314SYP															
314SYC															
314SYI															
314SYL															
314SAL															
314MIG															
314SYF															
314SYN															
314SAC															
314SAR															
314SAI															
314SAE															

3. Page 2 Field Data Sheet for Conventional Water Quality Monitoring

Site	Picture #	Picture Notes	Stage	Depth/Flow	Depth/Flow	Depth/Flow	Depth/Flow	Depth/Flow	General Notes
308BSU									
305BSR									
308SUR									
308GAR									
308LSU									
308SJC									
307CML									
307CMD									
307CMU									
307TUL									
307CMN									

4. Calibration Log For DS4a

Analyte	pH	pH	Cond	Cond	Turb	Turb	DO %	Chlorophyll	Chlorophyll	
Units			us/cm	us/cm	NTU	NTU		mg/l	mg/l	
Date										
Pre Cal Blank										
Post Cal Blank										
Pre Cal										
Post Cal										
Method										
Your Initials										
Date										
Pre Cal Blank										
Post Cal Blank										
Pre Cal										
Post Cal										
Method										
Your Initials										
Date										
Pre Cal Blank										
Post Cal Blank										
Pre Cal										
Post Cal										
Method										
Your Initials										
Date										
Pre Cal Blank										
Post Cal Blank										
Pre Cal										
Post Cal										
Method										
Your Initials										

5. Maintance Log for DS4a

Maintenance Log

Hydrolab unit ID#	Date Repaired	Part Repaired	Repaired By	Notes

APPENDIX B

SUPPORTING DOCUMENTS FOR AQUATIC TOXICITY MONITORING

ATTACHMENT 1: Chronic *Selenastrum capricornutum* Toxicity Testing SOP

Revision #2 (Date last modified: 1/14/05 8:05 PM by Pacific EcoRisk)
Effective Date: December 8, 2002
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).

1.0 INTRODUCTION

This test is based on a 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 $ZnCl_2$ (Zinc 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

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 4°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). <u>For each test, a minimum of 4 Liters of sample will be needed each day.</u>

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 <u>prior to test initiation</u> and read the attached "**Summary of Test Conditions for *Selenastrum capricornutum***".

3.1 Water Preparation

<u>3.1.1 Diluent</u>

1. Figure out how much water you need. Remember to have extra for rinsing the flasks.
2. Deionized water raised to moderate hardness with EPA chemicals (EPAMH) is used as control and diluent for freshwater algal tests unless specified otherwise.
3. Prepare sterile filtered water (0.45 μm). If performing an effluent test or testing a volatile material positive pressure filtration must be used.
4. Add nutrients. These are stored in the refrigerator. 1 mL/L of each of the five nutrient solutions (A-E) should be added to the filtered water. See **Table 1** for more information on the nutrient solutions.
5. Adjust the diluent pH to 7.5 ± 0.2 with HCl or NaOH.

<u>3.1.2 Test Sample Water</u>

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

1. Prepare sample
2. Positive pressure sterile filter sample
3. Add nutrients

3.2 Test Setup

1. Measure and record light intensity using a light meter throughout the water bath. Intensity should be 86 ± 8.6 μE/m2/s (400+40 foot candles). Replace bulbs if necessary to maintain light intensity within the acceptable range.
2. Prepare the test flasks for each concentration. You will need 5 containers per treatment. Label the flasks with their treatment and replicate I.D. Rinse each flask with control water.
3. Prepare dilutions of the test material.
4. 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.
5. 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 in the dark, cold storage to prevent any algal growth.
6. 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 x 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 culture. Add 10 μL of the culture to each side of the hemacytometer and allow algae to settle for five to ten minutes before counting. Count 3-5 subsamples on the hemacytometer. To determine algal density in the culture, simply multiply the average hemacytometer count by 10^4.
4. The formula for determining inoculation is as follows:

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

3.3.2 Test Inoculation

1. Inoculate each control, test and reference toxicant dilution container with the appropriate volume of algae as calculated in section 3.3.1.
2. 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.
3. Randomly place the test containers within the temperature-controlled water bath. Make sure that all of the necessary data are recorded upon the data sheets.

4.0 MAINTAINING THE TEST

1. Swirl flasks twice daily and randomize the flasks positions in the water bath.
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 water bath temperature once daily.

5.0 TEST TERMINATION

1. Take down the test after 96 hours. Keep flasks cold and in the dark to restrict any further growth. Remove the blanks that were collected for each concentration at test initiation from the cold storage.
2. Tests are taken down using the turbidity method on the Hach spectrophotometer DR 2010. After turning on spectrophotometer, enter program number 951 when prompted by the meter. Set wavelength at 750 nm.
3. Starting with the Control, 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 can be taken without having to re-zero.
4. Swirl the first Control flask so that the contents are thoroughly mixed. Pour 25 mL into a cuvette (that is paired with the cuvette used for zeroing), place the cuvette into the spectrophotomer, and press read. Record reading. Repeat the procedure twice more for the replicate. Read three measurements on each of the remaining Control replicates.
5. Use the blank for the next concentration up to zero the meter. Take three turbidity readings on each of the four replicates of that concentration. Repeat the procedure up through the concentration series.
6. Spectrophotometer method #951 automatically converts turbidity readings into cells Selenastrum/mL. If the normal turbidity method is used (#750), a calibration curve must be constructed to convert turbidity values into cells/mL.
7. Measure and record final water quality (pH, D.O., conductivity, and temperature) for each control, sample or reference toxicant concentration.

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 "***Selenastrum capricornutum* Growth Reference Toxicity Test SOP**".

The toxicant generally used is Zinc chloride at concentrations of 1, 2.5, 5, 10 and 20 μg Zn/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™ v1.023 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 x 10^6 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 (Deionized water raised to moderate hardness with EPA chemicals) with nutrients added, is used.
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 *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 appropriately stored for hazardous waste disposal.

Table 1. Nutrient Stock Solutions for Maintaining Algal Stock Cultures and Test Control Cultures.

Nutrient Stock Solution	Compound	Amount dissolved in 500 mL
		Distilled Water
MACRONUTRIENTS:		
SOLUTION A	$MgCl_2•6H_2O$	6.08 g
	$CaCl_2•2H_2O$	2.20 g
	$NaNO_3$	12.750 g
SOLUTION B	$MgSO_4•7H_2O$	7.350 g
SOLUTION C	K_2HPO_4	0.522 g
SOLUTION D	$NaHCO_3$	7.50 g
MICRONUTRIENTS:		
SOLUTION E	H_3BO_3	92.8 mg
	$MnCl_2•4H_2O$	208.0 mg
	$ZnCl_2$	1.64 mg[a]
	$FeCl_3•6H_2O$	79.9 mg
	$CoCl_2•6H_2O$	0.714 mg[b]
	$Na_2MoO_4•2H_2O$	3.63 mg[c]
	$CuCl_2•2H_2O$	0.006 mg[d]
	$Na_2EDTA•2H_2O$	150.0 mg[e]
	Na_2SeO_4	1.196 mg[f]

a $ZnCl_2$ - Weigh out 164 mg and dilute to 100 mL. Add 1 mL of this solution to Stock E.

b $CoCl_2•6H_2O$ - Weigh out 71.4 mg and dilute to 100 mL. Add 1 mL of this solution to Stock #1.

c $Na_2MoO_4•2H_2O$ - Weigh out 36.6 mg and dilute to 10 mL. Add 1 mL of this solution to Stock E.

d $CuCl_2•2H_2O$ - Weigh out 60.0 mg and dilute to 1000 mL. Take 1 mL of this solution and dilute to 10 mL. Take 1 mL of the second dilution and add to Stock E.

e $Na_2EDTA•2H_2O$ - Use this chemical only for culturing and when specifically required by the project. EDTA will chelate metals and can alter the toxicity of the sample.

f Na_2SeO_4 – Weigh out 119.6 mg and dilute to 100 mL. Add 1 mL of this solution to Stock E.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR ALGAL (***SELENASTRUM CAPRICORNUTUM***) GROWTH TEST (TEST METHOD 1003.0)	
1. Test type	Static
2. Test duration	96 hours
3. Temperature	25 ± 1ºC
4. Light quality	"Cool white" flourescent lighting
5. Light intensity	400 ± 40 ft-c (86 ± 8.6 $\mu E/m^2/s$)
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%)

ATTACHMENT 2: Chronic *Skeletonema* and *Thalassiosira* Toxicity Testing SOP

Revision #2 (Date last modified: 1/14/05 3:24 PM by Pacific EcoRisk)
Effective Date: December 8, 2002
Accepted:_____

Skeletonema and *Thalassiosira* Algal Growth Bioassay

Standard Operating Procedures

This S.O.P. is based upon the guidelines described in ASTM Standards on Aquatic Toxicology and Hazard Evaluation (E 1218-97a; PCN #03-547093-16).

1.0 INTRODUCTION

This test is based on a 96 hour static exposure of the marine diatoms, *Skeletonema costatum* or *Thalassiosira pseudonana* 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. Reference Weights, for calibration of balance.
4. pH, D.O., and conductivity/salinity meter, needed to document test water quality.
5. ASTM certified Thermometer, for documenting test water temperature.
6. Test Containers, 250 mL Erlenmeyer flasks with cotton or foil stoppers. Flasks must be appropriately cleaned, rinsed and autoclaved.
7. De-Ionized water, for rinsing of probes, etc.
8. Wash Bottles, for rinsing of probes, etc.
9. Volumetric and Graduated Flasks and Pipettes, for making up dilution series and reference toxicant test solutions.
10. Transfer Pipettes, for transfer of mysids to and from test containers.
11. NITEX mesh sieves (150 μm), for removing debris or predatory zooplankton.
12. Cubitainers may be necessary for the client's collection of effluent.
13. ACS Reagent $CuSO_4$ Copper Sulfate), for use as reference toxicant.
14. Filter system for water.

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. Ideally, algae should be cultured at the desired test salinity for two weeks prior to the test's initiation.
2. Order Algal cultures from: Botany Dept. of the University of Texas, Austin (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 4°C. The sample should be shipped or transported to the testing laboratory ASAP. Upon receipt of the sample(s) in the laboratory, sample login water quality measurements should be taken. For instruction on the login 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). <u>For each test, a minimum of 4 Liters of sample will be needed.</u>

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 <u>prior to test initiation</u> and read the attached "**Summary of Test Conditions for *Skeletonema and Thalassiosira***."

3.1 Water Preparation

<u>3.1.1 Diluent</u>

1. Figure out how much water you need. Remember to have extra for rinsing the flasks.
2. Distilled water, brought to the appropriate salinity with Crystal Sea®, is used for the salt-water algal tests unless specified otherwise.
3. Prepare 0.45 μm sterile filtered water when preparing culture media or running a ref-tox. When performing an effluent test or testing a volatile material, positive pressure filtration must be used.
4. Add nutrients to both the diluent and test waters in the following concentrations: 15mL/liter of the metal mix solution, 1 mL/liter of the minor salt solution, and 0.5 mL/liter of the vitamin stock solution (1.0 mL/liter for *Thalassiosira*). See **Table 1** for more information on the nutrient solutions.
5. Adjust the diluent pH to 8.0± 0.2 with HCl of NaOH.

<u>3.1.2 Test Sample Water</u>

Follow same procedure as in 3.1.1.

1. Prepare sample
2. Add nutrients

3. Adjust pH
4. Positive pressure sterile filter sample

3.2 Test Setup

1. Prepare the test flasks for each concentration. You will need 4 flasks per treatment. Label the flasks with their treatment and replicate I.D.
2. Prepare dilutions of the test material.
3. 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.
4. Beginning with the Control treatment and working up through the concentration series, pour 100 mL of sample water into each of the 4 containers.

3.3 Test Inoculation

3.3.1 Determination of Inoculation Volumes:

1. Inoculation should be 2 x 10^4 cells/mL
2. Use 4-7 day old cultures in log growth
3. Count 1 mL of culture to find the inoculation stock algal density. Use a Sedgewick-Rafter chamber slide and count 3 or more fields on the Whipple grid. Allow algae to settle for 25 minutes on slide before counting. Use the appropriate formula calculation to determine if stock culture needs to be diluted or concentrated. Preferably, the inoculation volume should be between 0.8-1.3 mL/flask so as not to unduly dilute the test sample.
    a. Formula: cells/mL = N(1000) / # fields (A)
    b. N = total number of cells counted
    c. A = area of the grid (labeled on the microscope)
    d. # fields = the number of fields (grids) counted
4. The formula for determining inoculation is as follows: (algal density)(x) = 2 x 10^4
    a. x = mL of algal stock needed (x should be approximately 1 mL + 0.3 mL

3.3.2 Test Inoculation

1. Randomize the flasks containing test solutions and inoculate with the calculated inoculation volume.
2. Separate out 3 flasks and pull 1 mL of each for counting, to check that the inoculation was correct. Inoculation numbers should be within 80% of each other.
3. Randomly place the test containers within the temperature-controlled water bath. Make sure that all of the necessary data are recorded upon the data sheets.

4.0 MAINTAINING THE TEST

Swirl flasks twice daily.

5.0 TEST TERMINATION

1. Take down the test after 96 hours. Keep flasks cold and in the dark to restrict any further growth. Record pH, DO and salinity in the high, low and control concentrations.
2. Count cell densities on a microscope (5 fields minimum or 400 cells in more than five fields, up to 20 fields).
3. Collect and prepare data for statistical analysis.

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 "***Skeletonema & Thalassiosira* Growth Reference Toxicity Test SOP**".

7.0 DATA ANALYSIS

The cell density data for each replicate, which are recorded on the appropriate data sheets, are entered into a CETIS™ v1.023 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

Test acceptability criteria for both the *Skeletonema costatum* or *Thalassiosira pseudonana* test are as follows: there must be an average of at least $1x10^5$ cells/mL in the Control treatment.

9.0 QUALITY CONTROL

1. Control water (Dionized water adjusted to the appropriate salinity with Crystal Sea®) with nutrients added, is used.
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 *Skeletonema & Thalassiosira* chronic toxicity tests poses little risk to those performing them. Effluents can contain pathogens and appropriate safety precautions should be observed when handling these materials. 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 appropriately stored for hazardous waste disposal.

Table 1. Nutrient Stock Solutions for Maintaining Saltwater Algal Stock Cultures and Test Control Cultures.

Nutrient Stock Solution	Compound	Amount dissolved in 1 L Distilled Water[A]
METAL MIX SOLUTION (A):		
	$FeCl_3 \cdot H_2O$	0.048 g
	$MnCl_2 \cdot 4H_2O$	0.144 g
	$ZnSO_4$	0.045 g
	$CuSO_4 \cdot 5H_2O$	0.000157 g (0.157 mg) [B]
	$CoCl_2 \cdot 6H_2O$	0.000404 g (0.404 mg) [C]
	H_3BO_3	1.140 g
	$Na_2EDTA \cdot 2H_2O$	1.0 g [D]
MINOR SALT MIX (B)		
	K_2HPO_4	0.3 g
	$NaNO_3$	5.0 g
	$NaSiO_3 \cdot 9H_2O$	2.0 g
VITAMIN STOCK SOLUTION (C)		
	Thiamine HCl	0.5 g
	Biotin	0.001 g (1 mg)[E]
	B_{12}	0.001 g (1 mg) [E]

A Add salts to 900 mL of distilled water and dilute to a final volume of 1 L when all salts are dissolved.

B $CuSO_4 \cdot 5H_2O$ - Weigh out 0.0157 g and dilute to 100 mL. Add 1 mL of this solution to Stock A.

C $CoCl_2 \cdot 6H_2O$ - Weigh out 0.0404 g and dilute to 100 mL. Add 1 mL of this solution to Stock A.

D $Na_2EDTA \cdot 2H_2O$ - Use this chemical only for culturing and when specifically required by the project. EDTA will chelate metals and can alter the toxicity of the sample.

E Biotin & B12 - Weigh out 0.1 g of each and dilute to 100 mL. Add 1 mL of this solution to Stock C.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR ALGAL (***SKELETONEMA COSTATUM***) GROWTH TEST	
1. Test type	Static
2. Test duration	96 hours
3. Temperature	20 ± 2ºC
4. Light quality	"Cool white" flourescent lighting
5. Light intensity	60 ± 9 $\mu E/m^2/s$
6. Photoperiod	16 hours light: 8 hours darkness
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	20,000 cells/mL
12. # of replicates chambers per concentration	Four
13. Shaking rate	Twice daily by hand
14. Test chamber cleaning	Rinsed with dilution water
15. Dilution water	0.45μm filtered seawater or deionized water and sea salts. Salinity: 24–35ppt
16. Dilution factor	According to NPDES permit. Effluents: ≥ 0.5 dilution series Receiving Waters: None, or ≥ 0.5 dilution series
17. Test endpoint	Growth
18. 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 10^5 cells/mL in controls

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR ALGAL (***THALASSIOSIRA PSEUDONANA***) GROWTH TEST	
1. Test type	Static
2. Test duration	96 hours
3. Temperature	20 ± 2ºC
4. Light quality	"Cool white" flourescent lighting
5. Light intensity	82-90 μE/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	20,000 cells/mL
12. # of replicates chambers per concentration	Four
13. Shaking rate	Once daily by hand
14. Test chamber cleaning	Rinsed with dilution water. None during test.
15. Dilution water	0.45μm filtered seawater or deionized water and sea salts
16. Dilution factor	According to NPDES permit. Effluents: ≥ 0.5 dilution series Receiving Waters: None, or ≥ 0.5 dilution series
17. Test endpoint	Growth
18. 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 10^5 cells/mL in controls

ATTACHMENT 3: Chronic *Ceriodaphnia dubia* Toxicity Testing SOP

Revision #2 (Date last modified: 1/14/05 8:06 PM by Pacific EcoRisk)
Effective Date: December 8, 2002
Accepted:_____

Ceriodaphnia dubia (Cerio)
Chronic Survival and Reproduction 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)

1.0 INTRODUCTION

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

2.0 TEST PREPARATION

2.1 Equipment and Supplies Needed

1. Food: Selenastrum, YCT and the vitamins Thiamin, Biotin and B_{12}
2. Control/dilution (80:20) Water: Calistoga 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. Beakers: (2) 1-L beakers and (4) 250-mL beakers.
6. "Cerio" 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.
7. Cerio board: Foam Board containing pre-cut holes to support replicate cups in waterbath.
8. Water quality test cups: 100-mL plastic cups for water quality analysis.
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. Transfer pipettes, wide-bore: for transfer of organisms to and from test containers.
13. Cubitainers may be necessary for the client's collection of effluent.
14. Temperature controlled water bath under cool white fluorescent lighting.
15. ACS reagent $CuS0_4$ (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 < 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 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 S.O.P**."
3. Alternatively, <24 hour old neonates can be obtained from in-house Stock Cultures (See ***Ceriodaphnia dubia* Culture Maintenance SOP** for methods).

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 4°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). <u>For each test, a minimum of 1 Liter of sample will be needed each day</u>.

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 <u>prior to test initiation</u> and read the attached "**Summary of Test Conditions for *Ceriodaphnia dubia***".

1. Label (1) 1-L beaker for the Control treatment and (1) 1-L beaker for the 100% sample treatment.
2. Fill the Control beaker with 800 mL of Control water and fill the 100% sample beaker with 600 mL of the 100% sample; volume requirements may need to be adjusted for each client. Check the D.O. level of the Control water and the 100% sample and make sure that there is enough oxygen; if not, you must aerate the sample until the D.O. reaches adequate levels.
3. Add food into the 800 mL of Control and 600 mL of sample water:
   a. *Selenastrum* (algae) (13 mL/L): 10.4 mL in Control; 7.8 mL in 100% sample
   b. YCT (7 mL/L): 5.6 mL in Control; 4.2 mL in 100% sample
4. Label (4) 250-mL beakers with the appropriate intermediate test dilutions. See client NPDES permit for specific dilution requirements.
5. Prepare 10 replicate "cerio" test cups for each treatment dilution according to client requirements (e.g.: Control, 6.25%, 12.5%, 25%, 50%, 100%). Label the cups with their treatment and replicate I.D. (i.e. as A-J) using a Sharpie pen.
6. Label (1) water quality test cup (~100-mL size) for each treatment using a Sharpie pen.
7. Using the Control water and the 100% effluent that have been spiked with food, prepare

200-mL volumes of test solution at each of the intermediate test treatment concentrations.

8. For each treatment, dispense 25-50 mL into a water quality test cup and 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.
9. Place the replicate "cerio" test cups onto a foam support board. Then, beginning with the Control treatment and working up through the concentration series, aliquot 15 mL of test media into each of the 10 replicate cups at each treatment **using a clean 60-cc syringe**.
10. Use the Sharpie pen and trace the water level line onto each container. Place the foam support board with the dilution containers into the water bath to allow test waters to acclimate to test temperature.
11. Identify 10 adult cerio females from the brood board cultures that have had 8 or more offspring within the past 24 hours. There must not be more than an 8-hour deviation in the ages of the neonates.
12. Using a wide-bore transfer pipette, *carefully* capture and randomly allocate 1 neonate cerio into each of the test containers. Each of the neonates from one adult will be used to load one replicate (e.g. all A replicates) from each treatment concentration (this is done so that any anomalies, such as a high proportion of males in a particular replicate, can be addressed by omitting that particular replicate from the statistical analysis).
    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 stock water used to transfer the organisms. Take care to avoid excessive dilution of the test treatments.
13. Place the foam support board, now containing the test replicate cups, into a temperature controlled water bath at 25ºC under cool-white fluorescent lighting on a 16L:8D photoperiod.
14. Place transparent plastic cover sheet over the top of the test replicate cups on the foam support board.
15. Record the water bath temperature onto the test data sheet.

4.0 MAINTAINING THE TEST

4.1 Each day

1. Check the D.O. level of the Control water and the highest concentration of treatment media and make sure that there is enough oxygen (at least 4.0 mg/mL); if not, you must aerate the sample until the D.O. reaches adequate levels.
2. Prepare the appropriate beakers, test cups, and test solutions with the appropriate amounts of food, as above in section 3.0 Test Initiation (steps 1-7).
3. Record the "new" water quality measurements (temperature, pH, D.O. and conductivity) before pouring into new cerio test cups (section 3.0, step 8).
4. Fill each new "cerio" test cup with 15 mL of new test solution using **a clean 60-cc syringe**

(section 3.0, step 9) and trace the water level onto each container (section 3.0, step 10).

5. Pull the foam support board containing the test replicate cups out of the water bath, and examine each replicate cup. Record observations of dead organisms onto the test data sheets.
6. Using a pipette, carefully transfer each original test organism into its designated new test replicate cup (see section 3.0, step 12).
7. Examine each old test replicate cup to determine the number of neonates (if any) and record the count onto the test data sheet. Discard neonates after counting. Save ~25-50 mL of old test solution for each treatment and measure the "old" pH and D.O., recording the data onto the test data sheets.
8. Place the test organisms within their new test replicate containers onto a foam support board, and return the test replicates to the water bath. Record the water bath temperature onto the test data sheets.

5.0 TEST TERMINATION

Test are terminated when 60% or more of the surviving females in the controls have produced their third brood or at the end of 8 days, whichever occurs first. All observations on organisms' survival and number of offspring should be completed within two hours of test termination.

1. Pull the foam support board containing the test replicate cups out of the water bath, and examine each replicate cup. Record observations of dead organisms onto the test data sheets.
2. Examine each old test replicate cup to determine the number of neonates (if any) and record the count onto the test data sheet. Save ~25-50 mL of old test solution for each treatment and measure the "old" pH and D.O., recording the data onto the test data sheets.
3. Count the number of total offspring produced by each individual test organism and record onto the test data sheets. Any animal not producing young should be examined to determine if it is male.

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 *Ceriodaphnia* reference toxicity test is presented in the "**Chronic *Ceriodaphnia dubia* Reference Toxicity Test SOP**".

7.0 DATA ANALYSIS

The two endpoint data for each replicate, which are recorded on the appropriate data sheets, are entered into a CETIS™ v1.023 data file labeled for identification of the specific test. Statistical analysis are performed in accordance with EPA guidelines for statistical analysis.

8.0 TEST ACCEPTABILITY CRITERIA

Test acceptability criteria for the *Ceriodaphnia dubia* chronic test includes:

1. 80% or greater control survival
2. Production of three broods of offspring by >60% of surviving control females
3. An average of ≥15 offspring per surviving control female.
4. Identified males must be excluded for analysis of the reproduction endpoint, but may be used for the survival endpoint. If 50% or more of the surviving organisms in a replicate block are males, the entire replicate block is excluded from analysis of the reproduction endpoint.
5. The entire test is invalid if fewer than 8 control replicates remain after excluding males.

9.0 QUALITY CONTROL

1. Control water, consisting of a mixture of commercial spring waters for tests and cultures.

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 *Ceriodaphnia dubia* 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 appropriately stored for hazardous waste disposal.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR CHRONIC ***CERIODAPHNIA DUBIA*** REPRODUCTION AND GROWTH TEST (TEST METHOD 1002.0)	
1. Test type	Static renewal
2. Temperature	25 ± 1ºC
3. Light quality	Ambient laboratory illumination
4. Light intensity	50-100 ft-c (10-20 $\mu E/m^2/s$)
5. Photoperiod	16 hours light: 8 hours darkness
6. Test chamber size	30 mL
7. Test solution volume	15 mL
8. Renewal of test solutions	Daily
9. Age of test organisms	Less than 24 hour old neonates (8 hour release)
10. No. of organisms per test chamber	One
11. No. of replicate chambers per concentration	Ten
12. No. of organisms per concentration	Ten
13. Feeding regime	Algae(13mL/L) & YCT (7mL/L) 0.1 mL daily
14. Test chamber cleaning	New cups daily. Rinse with deionized water.
15. Test chamber aeration	None
16. Dilution water	According to NPDES permit.
17. Test Concentrations	According to NPDES permit. Effluents: 5 and a control Receiving Waters: 100% and a control.
18. Test duration	7 days or until 60% of the surviving females in the control have 3 broods
19. Test endpoint	% survival and reproduction
20. 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)
21. Test acceptability	80% control survival, 60% of surviving females in control have three broods, and an average of 15 neonates per surviving control female. Exclude males for repro.
22. Sample volume required	1 Liter per day

ATTACHMENT 4: 10-day *Hyalella azteca* Water-Only Toxicity Testing SOP

***Hyalella azteca* 10-Day Water Toxicity Test**

1.0 OBJECTIVE

In laboratory tests designed to determine the toxicity of low-salinity water samples, *Hyalella azteca* are exposed to test solutions for 10 days, after which the percentage mortality is determined. Observed effects may be related to the presence of contaminants or to naturally occurring factors. In order to correctly interpret toxicity results, concentrations of chemical contaminants should be analyzed, as well as other water quality parameters, such as dissolved oxygen, pH, conductivity, ammonia, hardness, alkalinity, and temperature.

In this procedure, water samples collected from field stations are divided into randomly numbered replicate test containers in the laboratory. Ten *H. azteca* are placed into each replicate container. Each beaker is monitored daily for mortality, and is renewed at 5 days. After a 10-day exposure, survival is counted and recorded to give an estimate of sample toxicity. Because the test measures effects on an early life-stage of an ecologically important species possessing relatively stringent water quality requirements, the results constitute a good basis for decisions concerning either hazard evaluation or the suitability of estuarine waters for aquatic life (EPA 1993).

2.0 EQUIPMENT

The following equipment is necessary to conduct the toxicity test at the Marine Pollution Studies Laboratory at Granite Canyon (MPSL). The word "clean" here and throughout this procedure means that the item has been cleaned according to the MPSL glassware cleaning procedures outlined in a separate standard operating procedure (MPSL SOP 1.3).

2.1 Culture

- Pipettes, tubing, and clean air system
- Short buckets or trays
- Granite Canyon well water (23 ± 1°C)
- YCT for feeding, purchased from Aquatic Biosystems (Fort Collins, CO)
- *Hyalella azteca* (9-14 days old, supplied by Chesapeake Cultures (Gloucester, VA)

2.2 Test Initiation/Termination

- Environmental chamber (23 ± 1°C, ambient laboratory illumination for 16 hours/day)
- 300-mL clean glass beakers (5 per sample)
- 250-mL clean plastic tripour beakers (3 per reference toxicant concentration)
- Clear plastic sheets to cover beakers
- 1000-mL volumetric flask for reference toxicant concentrations
- 10-mL and micropipettors and pipettes for reference toxicant concentrations

• Cadmium chloride stock solution (100,000 µg/L Cd)
• Randomization sheet to arrange and identify test containers
• Data sheets
• Gloves and appropriate safety gear (see MPSL lab safety manual)
• Sample vials for reference toxicant analysis (new polyethylene 30 mL, acid washed)
• Dissecting microscope for counting neonates
• Disposable plastic pipettes with cut-off tips (for handling animals)

2.3 Water Quality

• Meters, probes, spectrophotometer, digital titrator and standards for measuring pH, dissolved oxygen, hardness, alkalinity, ammonia, and conductivity
• Thermometers (glass spirit thermometer and continuously recording thermometer)
• Graduated pipettes (10 mL) and hand pipette pump for water quality sampling
• Water quality vials (30 mL glass)
• Gloves and appropriate safety gear (see MPSL lab safety manual)

2.4 Dilution Water

In every step of this procedure, use Granite Canyon well water.

3.0 EXPERIMENTAL DESIGN

Aquatic toxicity tests can be used as screening tools or as part of more comprehensive studies to assess water quality. Careful consideration must be given to site characteristics, reference site selection, field replication, choice of synoptic measures, seasonal factors, and comprehensive planning and peer review to determine that study designs are adequate to meet program objectives.

This laboratory toxicity test consists of five replicate test beakers for each sample concentration. Beakers are arranged randomly, and each receives ten *H. azteca*. The quality of test animals and testing conditions is determined through concurrent testing of reference toxicants (positive controls) and control water (negative controls). Testing of reference sites or receiving water is recommended to demonstrate the suitability of test sites in the absence of toxic contaminant concentrations. Test conditions of temperature and photoperiod are controlled as indicated below, and dissolved oxygen, pH, conductivity, and ammonia are measured at the beginning and end of the exposure. Temperature is measured continuously, and hardness and alkalinity are measured at the beginning of the test.

4.0 PREPARATION OF SAMPLES FOR TESTING

One day before test initiation, the volume necessary for test initiation should be placed in the constant temperature room (23°C) to allow oxygen concentrations to equilibrate below super-saturated levels; the minimum time should be 12 hours. Prepare five replicate 300-mL beakers for each sample to be tested. Consult the random number sheet to ensure proper randomization. Each container receives 100 mL of test solution.

5.0 CONTROLS

5.1 Dilution Control

The dilution control should consist of Granite Canyon well water.

5.2 Reference Toxicant Tests

For cultured organisms, conduct a concurrent reference toxicant at least monthly. The reference toxicant test indicates the sensitivity of the organisms and the suitability of the test methodology.

Reagent grade cupric chloride ($CdCl_2$) should be used as the reference toxicant for *H. azteca* tests, unless another toxicant is specified. Prepare a 100,000 μg/L Cd stock solution by adding 0.1630 g of reagent grade $CdCl_2$ to a final volume of one liter of distilled water in a plastic volumetric flask. Cap tightly and mix thoroughly. Sample and log the reference toxicant stock solution at the beginning of the test for chemical verification of the copper concentration. Acidify samples for analysis in clean sample vials with 1% by volume 14N-reagent grade nitric acid..

Reference toxicant solutions should be three to five replicates of 0 (control), 5.6, 10, 18, 32, and 56 μg Cd/L. Other concentrations may be added between these if greater precision is desired for quality control chart purposes. Prepare 1000 mL of each concentration by adding stock solution (see dilution schedule) to a 1000-ml plastic volumetric flask and fill with culture water. Aliquot each concentration to randomly numbered test containers as indicated on the random number sheet, and into water quality vials. Start with the control solutions and progress to the highest concentration to minimize contamination. Place the reference toxicant test containers in the constant temperature room, cover, and equilibrate.

All tests (sample and reference toxicant) must use *H. azteca* from the same culture. They must be handled in the same way and delivered to the test containers at the same time.

6.0 TEST ORGANISMS

6.1 Laboratory Acclimation

Order amphipods to arrive between two and seven days before test initiation. Amphipods must be between 7 and 14 days old at test initiation. Place the amphipods in a culture tray containing well water at a temperature that varies by no more than 3°C from transport conditions. Acclimate the amphipods to test temperature and conductivity. Hold amphipods at test temperature for 48 hours prior to initiating sediment testing. Remove any dead or moribund animals. Make sure water in the tray is constantly aerated. Check the amphipods daily, and monitor the health of amphipods by observing appearance. If more than 5% of the amphipods appear unhealthy during the 48 hours prior to the test, reschedule the test and immediately arrange for another amphipod shipment. Renew the culture daily before the test with dilution water, and feed 10 mL YCT daily.

6.2 Amphipod Loading

Using a clean transfer pipette with the tip removed, transfer the amphipods from the culture tray into the test containers. Only transfer animals that are healthy and moving. Replace injured or stressed amphipods. Continue until each container has 10 animals. Maintain water temperature (23 ± 1°C) by sorting animals in the constant temperature room where the test is being held.

7.0 MONITORING THE TOXICITY TEST

7.1 Counting *Hyalella* Mortality

Test duration is 10 days. Check all test containers daily, and record the number of live animals. Also attempt to count the number dead to ensure that the total number of animals in the container at the start of the test was 10; if not, record this on the data sheet. Immobile *H. azteca* that do not respond to a stimulus are considered dead. The stimulus should be a gentle stream of water from a disposable pipette. *H. azteca* that exhibits a visible response is considered living. Remove dead animals.

7.2 Measuring Water Quality in Test Containers

Measure temperature, dissolved oxygen, pH, ammonia, and conductivity at the beginning and end of the test. Hardness and alkalinity should be measured at the beginning of the test. Sample the initial test solutions at the time of dilution preparation. Water quality should also be measured on old and new dilutions at the time of renewal. Renewal water quality parameters include dissolved oxygen, pH, conductivity, and temperature. Water quality should be measured only initially on reference toxicant tests.

7.3 Feeding

Test containers are fed 1.5 mL YCT every other day.

8.0 TEST SOLUTION RENEWAL

The test duration is 10 days. Because toxicity may change over short periods in test containers, the test solutions must be renewed after 5 days. Prepare new test solutions as in section 4. One day before solution renewal, the volume necessary for renewal should be placed in the constant temperature room (23°C) to allow oxygen concentrations to equilibrate below super-saturated levels. These samples must be aerated if dissolved oxygen concentrations exceed maximum values allowed.

9.0 TERMINATING THE TOXICITY TEST

After 10 days of exposure, final mortality counts are made.

Final water quality must be sampled at the termination of the test. Deliver a sample from each site into pre-labeled water quality containers. Measure and record temperature, dissolved oxygen, pH and conductivity of each sample. Only measure temperature for the reference toxicant test.

Take the completed data sheet to the office for data entry and analysis. Notify the data analyst that the data has arrived. Make sure the data sheets are placed in the proper location and that the person keeping track of the data knows where it is.

10.0 DATA HANDLING AND TEST ACCEPTABILITY

Immediately after test termination, check the data sheet to determine whether dilution water and conductivity controls have acceptable survival (≥90%). If not, notify the project officer without delay. Tests with temperature, salinity, or dissolved oxygen measurements outside the specified ranges, may be considered conditionally acceptable based on the project officer's best professional judgment. Acceptable temperatures are 23 ± 1°C; acceptable dissolved oxygen concentration is 60-100% saturation.

11.0 REFERENCES

US EPA. 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. U.S. Environmental Protection Agency, Office of Research and Development. EPA/600/4-90/027F. August 1993.

12.0 TEST SUMMARY

Species:	*Hyalella azteca*
Test Duration:	10 Days
Endpoint:	Survival
Renewals:	At day 5
Organism Source	Chesapeake Cultures (Gloucester, VA)
Age of Test Organisms:	7-14 Days
Test Salinity Range:	0-15 ‰
Test Temperature:	23 ± 1°C
Dilution Water:	Granite Canyon well water
Light intensity:	Ambient laboratory illumination (10-20 µE/m^2/s)
Photoperiod:	16 hour light: 8 hour dark
Replication:	5 (samples), 3 (reference toxicant)
Test Containers:	300-mL glass beakers
Test Solution Volume:	100 mL
Loading:	10 animals per beaker
Feeding:	In culture prior to test initiation and 1.5 mL YCT every other day
Water Quality:	pH, dissolved oxygen, temperature, conductivity, hardness, alkalinity, ammonia
Reference Toxicant:	cadmium chloride ($CdCl_2$)
Daily Monitoring:	count alive and remove dead
Acceptability Criteria:	mean survival in dilution water controls ≥90%

ATTACHMENT 5: Chronic *Americamysis bahia* Toxicity Testing SOP

Revision #2 (Date last modified: 1/14/05 3:23 PM by Pacific EcoRisk)
Effective Date: December 8, 2002
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Americamysis bahia
Chronic (7-Day) Survival Growth and Fecundity 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 Marine and Estuarine Organisms, Third Edition (EPA-821-R-02-014). See Addendum, for tests that require the guidelines set forth in previous editions (EPA/600/4-87/028 and EPA-600-4-91-003).

1.0 INTRODUCTION

This test is based on a seven-day static-renewal exposure of 7 day old *Americamysis bahia* to different concentrations of effluents and/or receiving waters during the life period when eggs are produced by the females. The test endpoints are survival, growth (measured as dry weight) and fecundity (measured as the number of mature females with eggs in the oviduct and brood sac).

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 (*Artemia*) nauplii at least twice per day. These nauplii should be produced with in-house cultures that will require brine shrimp eggs, seawater (natural or artificial), and egg incubation containers. Incubation of the eggs should begin long enough prior to receiving the test organisms so as to assure a ready supply of newly-hatched nauplii as needed.
2. Aeration System: needed for aeration when D.O. drops below acceptable levels.
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: 400-mL glass beakers or 200-mL disposable 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. 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. Wide-bore transfer pipettes or small handheld dip nets: for transfer of organisms to and from test containers.
10. NITEX mesh sieves (150 μm & 500 μm); for concentrating organisms.
11. Dissecting microscope, for examination of organisms and enumeration of females with brood sacs.

12. Depression slide, for microscopic examination of live mysids.
13. Balance: capable of weighing to 0.01 mg.
14. Reference Weights: for calibration of balance.
15. Drying Oven: for drying fish at 105°C at test termination.
16. Desiccator: for holding dried fish.
17. Forceps: for transfer of organisms to weighing pans.
18. Aluminum Foil Weighing Pans: for drying and weighing of fish.
19. Cubitainers: may be necessary for the client's collection of effluent.
20. Artificial Sea Salt (Crystal Sea®): for salting up of effluent to acceptable test salinity.
21. ACS Reagent K_2CrO_4 (Potassium Dichromate), 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 7 day old animals by the day of test set-up. Approximately 25-33% more animals should be ordered than are actually needed for generation of larvae, so as to allow for some attrition of organisms that are stressed from the shipping, etc.
   Order mysids from: (1) - Aquatic Indicators: (904) 829-2780
   (2) - Aquatic Biosystems Inc.: (303) 223-2938
   (3) – Aquatox (501)767-9120
2. Order the juvenile mysids to be pre-adapted to the test salinity. This is important as the supplier may be culturing the mysids at a different salinity than the desired test salinity. If the mysids come in at a "non-test" salinity, they must begin acclimation ASAP. Place them in control water at the receiving salinity and immediately begin the adjust the holding salinity towards the test salinity.
3. 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 4°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 3 Liters of sample will be needed each day.

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 *Americamysis bahia***".

1. Mysids should be fed artemia at least two hours prior to the start of the test.
2. Label an appropriate number of 400-mL beakers with the appropriate test treatments for your dilution series (e.g.: control, 5%, 10%, 25%, 50%, and 100%). You will need **8 replicate containers** per treatment. Label the beakers with their treatment and replicate I.D. using 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 filtered seawater or appropriately salted receiving water according to client NPDES permit. Always work from low to high concentration and rinse out any glassware 3X with dH_2O and one final with seawater prior to use.
5. For each treatment, record the initial water quality (pH, D.O., salinity, conductivity and temperature) onto the data sheets; check to make sure that all parameters, especially the D.O. are within acceptable levels.
6. Then, beginning with the Control treatment and working up through the concentration series, pour 150 mL of sample water into each of the 8 containers.
7. Use the Sharpie pen and trace the water level line onto each container (if using cups).
8. Clean the tank holding the test organisms as much as possible (i.e., remove uneaten food and any dead mysids) minimizing any disturbance of the live animals.
9. Using a net or sieve, concentrate some of the animals into a corner of the holding tank or into a smaller container of control water. Using a wide-bore transfer pipette, capture and randomly allocate **5 mysids** into each of the test containers following the randomization template.
    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. Note any excess dilution of the test solution.
10. Randomly place the test containers within the temperature-controlled water bath (26 ± 1°C) 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.

4.0 MAINTAINING THE TEST

1. The test organisms should be fed newly-hatched brine shrimp nauplii first thing in the morning.
2. At the time of the media renewal, pull the test containers from the water bath and arrange, in replicate # order, and by treatment. From one randomly selected container at each treatment, measure the "old" temperature, pH, D.O. and salinity/conductivity. Check these measurements to make sure that the water quality is within acceptable limits.
3. Remove the uneaten food and any dead animals. This can be accomplished either of two

methods: (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; (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.

4. Count the number of live animals in each replicate and record the number on the data sheet.
5. 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. Carefully (and slowly) pour fresh media back into the replicate containers until the water level is at the 150-mL 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.
6. From one randomly selected replicate container at each treatment (beginning with the Control, and working upward through the concentration series), measure the "new" pH, D.O., and salinity/conductivity. Record these data on the data sheet and randomly place the replicate beakers back in the water bath. Sometime between 3 p.m. and 5 p.m., feed each replicate container with newly-hatched brine shrimp nauplii.

5.0. TEST TERMINATION

1. After 7 days, pull the test containers from the water bath. Measure and record the "old" pH, D.O., and salinity/conductivity. Count and record the number of live animals in each replicate container and remove the dead ones. Siphon or pour out approximately 90% of the old media and replace with control water.
2. Examine the **live** animals within 12 hours of termination. Using a microscope (240X), determine the number of immature animals, sex of the mature animals, and the presence or absence of eggs in the oviduct or brood sacs for surviving females. Record data on the data sheet.
3. Pour the remaining test solution containing the surviving mysids into a <300 μm mesh screen submerged in deionized water so as to wash away debris that may alter final weights.
4. Euthanize the organisms from each replicate in an ice-bath with filtered seawater, rinse in de-ionized water and transfer the organisms onto a pre-dried and pre-weighed aluminum drying pan (the pans should be weighed as per the **Weighing of Test Organisms S.O.P.**). When all of the replicates have been transferred into their respective drying pans, place the pans into the drying oven, and dry at 105°C for at least 6 hrs.
5. After drying, place the aluminum pans into the dessicator and seal. Allow to cool at least 4 hrs, after which each pan must be weighed and the weight data recorded. The weight recorded for the empty pans <u>minus</u> the weight of the pans + dried animals = the pooled dry weight of the organisms for that replicate. Divide this number by the number of organisms in the replicate to obtain the mean dry weight for individual mysids in that replicate. For the control treatment, calculate the mean weight per surviving fish for each replicate to determine if the weights met test acceptability criteria.

6. At this point, there should be two endpoint data for each replicate: percentage survival and mean dry weight per individual.
7. 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 data base for that reference toxicant to make this determination. Once the various reference toxicant concentrations are prepared, test set-up, maintenence, and termination are identical to those above. Information regarding the *Americamysis bahia* reference toxicity test is presented in the "**Chronic (7-day) *Americamysis bahia* Reference Toxicity Test SOP**".

7.0 DATA ANALYSIS

The three endpoint data for each replicate, which are recorded on the appropriate data sheets, are entered into a CETIS™ v1.023 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

Test acceptability criteria for the *Americamysis bahia* chronic test are as follows: (1) 80% or greater survival in the control; (2) average dry weight of 0.20 mg/individual in controls; and (3) fecundity can be used if >50% of females in controls produce eggs.

9.0 QUALITY CONTROL

1. Control water, consisting of deionized water salted up to test salinity with Crystal Sea® sea salt, is used.
2. The test mysids shall be maintained in the laboratory 4-7 days prior to use in tests in order to monitor and examine them for health and quality.
3. All equipment is calibrated and operated as described in each applicable equipment SOP.
4. 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 *Americamysis bahia* 7-day 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 appropriately stored for hazardous waste disposal.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR MYSID, ***AMERICAMYSIS BAHIA***, LARVAL SURVIVAL, GROWTH, AND FECUNDITY TEST (TEST METOD 1007.0)	
1. Test type	Static renewal
2. Test duration	7 days
3. Salinity	20 to 30 ppt ± 2 ppt
4. Temperature	26 ± 1ºC
5. Light quality	Ambient laboratory illumination
6. Light intensity	50-100 ft-c (10-20 $\mu E/m^2/s$)
7. Photoperiod	16 hours light: 8 hours darkness
8. Test chamber size	400-mL
9. Test solution volume	150 mL
10. Renewal of test solutions	Daily
11. Age of test organisms	7 days old
12. No. of organisms per test chamber	Five (5)
13. No. of rep. chambers per concentration	Eight (8)
14. No. of organisms per concentration	Forty (40)
15. Feeding regime	Artemia nauplii twice daily
16. Test chamber cleaning	Siphon daily, immediately before test solution renewal
17. Test chamber aeration	None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min
18. Dilution water	According to NPDES permit. Filtered (1 μm) seawater, dH_2O salted up with Crystal Sea® sea salt.
19. Test concentration	According to NPDES permit. Effluents: 5 and a control Receiving Waters: 100% and a control.
20. Dilution factor	According to NPDES permit. None, or ≥ 0.5 dilution series
21. Test endpoint	% survival and growth
22. 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)
23. Sample volume required	3 Liters per day
24. Test acceptability	80% or greater average survival in controls; average weight of control animals ≥ 0.2 mg; fecundity may be used if > 50% of control females produce eggs.

ATTACHMENT 6: Chronic Fathead Minnow Toxicity Testing SOP

Revision #4
Effective Date: 4/27/06 12:13 PM
Accepted:_____

Pimephales promelas (Fathead Minnow) Chronic (7-Day) Survival and 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). Pathogen control modifications are based on Geis S.W. *et al.* 2003 (Modifications to the fathead minnow (*Pimephales promelas*) chronic test method to remove mortality due to pathogenic organisms. Environ Toxicol Chem, 22 (10), 2400-2404) as approved by the EPA.

1.0 INTRODUCTION

This test is based on a seven-day static-renewal exposure of < 24 hr old ***Pimephales promelas*** to different concentrations of effluents and/or receiving waters during the larval life period. The test endpoints are survival and growth (measured as dry weight).

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 (*Artemia*) nauplii at least twice per day. These nauplii should be produced with in-house cultures that will require brine shrimp eggs, seawater (natural or artificial), and egg incubation containers. Incubation of the eggs should begin long enough prior to receiving the test organisms so as to assure a ready supply of newly-hatched nauplii as needed.
2. Balance: capable of weighing to 0.01 mg.
3. Reference Weights: for calibration of balance.
4. Drying Oven: for drying fish at 105°C at test termination.
5. Desiccator: for holding dried fish.
6. Aeration System: needed for aeration when D.O. drops below acceptable levels.
7. Meters: D.O., pH and conductivity/salinity, needed to document test water quality.
8. Thermometer: ASTM certified, for documenting test water temperature.
9. Test Containers: 600 mL glass beaker. Beakers must be appropriately cleaned and rinsed, and then pre-soaked for 24 hrs (overnight) in control water, before use in test.
10. De-Ionized water: for rinsing of probes, etc.
11. Wash Bottles: for rinsing of probes, etc.
12. Volumetric and Graduated Flasks and Pipettes: for making up dilution series and reference toxicant test solutions

13. Wide-bore transfer pipettes or small handheld dip nets: for transfer of organisms to and from test containers.
14. NITEX mesh sieves (150 μm); for concentrating organisms.
15. Forceps: for transfer of organisms to weighing pans.
16. Aluminum Foil Weighing Pans: for drying and weighing of fish.
17. Cubitainers: may be necessary for the client's collection of effluent.
18. ACS Reagent $CuS0_4$ (Copper Sulfate), for use as reference toxicant.

2.2 Alternative Equipment and Supplies Needed for Pathogen Control Modified Testing

1. "Cerio" 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.
2. Cerio board: Foam Board containing pre-cut holes to support replicate cups in waterbath.

2.3 Ordering and Holding of Test Organisms

1. Test organisms should be ordered far enough in advance so as to ensure arrival of < 24 hrs old animals by the day of test setup. 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 Inc. (303)223-2938
   (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 S.O.P.**"

2.4 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 4°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). <u>For each test, a minimum of 4 Liters of sample will be needed each day</u>.

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 *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 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.

   a. For Pathogen Control Modifications: Prepare replicate "cerio" test cups for each treatment dilution according to client requirements (e.g.: Control, 6.25%, 12.5%, 25%, 50%, 100%). Use 20 replicates for effluents (e.g. NPDES testing) and 10 replicates for ambient waters (e.g. Ag Waiver testing). Label the cups with their treatment and replicate I.D. (e.g. as A-J) using a Sharpie pen.

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.
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 water is made and for effluent when sample(s) arrive at the laboratory.
6. 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.

   a. For Pathogen Control Modifications: Place the replicate "cerio" test cups onto a foam support board. Then, beginning with the Control treatment and working up through the concentration series, aliquot 25 mL of test media into each of the 10 replicate cups at each treatment **using a clean 60-cc syringe**.

7. 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.
8. Using wide-bore pipette (or dipnet 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. For Pathogen Control Modifications: allocate **2 fish** per treatment replicate.
   b. The organisms are delicate. When transferring, release organisms under the surface of the water. Make sure that each individual is uninjured. Replace injured individuals.
   c. Be consistent with the volume of water used to transfer the organisms. Take care to avoid excessive dilution of the test treatments. Note any excess dilution of the test solution.

9. Randomly place the test containers within the temperature-controlled water bath (25 ± 1ºC), 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.

10. Feed each replicate with newly-hatched brine shrimp nauplii. 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 THE TEST

1. The test organisms should be fed newly-hatched brine shrimp nauplii first thing in the morning.
2. At the time of the media renewal, pull the test containers from the water bath and arrange, in replicate # order, and by treatment. From one randomly selected container at each treatment, measure the "old" temperature, pH, D.O. and salinity/conductivity. Check these measurements to make sure that the water quality is within acceptable limits.
3. Remove the uneaten food and any dead animals. This can be accomplished either of two methods: (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; (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.

    a. For Pathogen Control Modifications: Fish are transferred daily into new replicate "cerio" test cups with new 25 mL of test media.

4. Count the number of live animals in each replicate and record the number on the data sheet.
5. 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. Carefully (and slowly) pour fresh media back into the replicate containers until the water level is at the 400 mL 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.
6. From one randomly selected replicate container at each treatment (beginning with the Control, and working upward through the concentration series), measure the "new" pH, D.O., and conductivity. Record these data on the data sheet and randomly place the replicate beakers back in the water bath. Sometime between 3 p.m. and 5 p.m., feed each replicate container with newly-hatched brine shrimp nauplii.

5.0 TEST TERMINATION

1. After 7 days, pull the test containers from the water bath. Measure and record the "old" temperature, pH and D.O.. Siphon or pour out approximately 80% of the old media. Count and record the number of live animals in each replicate container. Pour the remaining test solution containing the surviving fish into a 500 μm mesh screen submerged in deionized water so as to wash away debris that may alter final weights.
2. Carefully transfer the animals from each replicate onto a pre-dried and pre-weighed aluminum drying pan (the pans should be weighed as per the **Weighing of Test Organisms S.O.P.**). When all of the replicates have been transferred into their respective

drying pans, place the pans into the drying oven, and dry at 105°C for at least 6 hours.

a. For Pathogen Control Modifications: Fish from adjacent replicates are pooled to obtain 5 composite replicates for the growth endpoint (e.g. A+B, C+D, E+F, etc…).

3. 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. The weight recorded for the weight of the pans + dried animals minus the empty pans = the pooled dry weight of the organisms for that replicate. Divide this number by the number of organisms originally loaded in the replicate to obtain the mean dry weight for individual fish in that replicate. For the control treatment, calculate the mean weight per surviving fish for each replicate to determine if the weights met test acceptability criteria.
4. At this point, there should be two endpoint data for each replicate: percentage survival and mean dry weight per individual.
5. 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 set-up, maintenance, and termination are identical to those above. Information regarding the fathead minnow reference toxicity test is presented in the "**Chronic *Pimephales promelas* Reference Toxicity Test SOP**".

7.0 DATA ANALYSIS

The two endpoint data for each replicate, which are recorded on the appropriate data sheets, are entered into a CETIS™ v1.023 data file labeled for identification of the specific test. Statistical analysis 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 80%. The average dry weight of surviving control fish must be at least 0.25 mg.

9.0 QUALITY CONTROL

1. Control water, consisting of deionized water adjusted to moderate hardness with EPA salts, is used.

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 fathead minnow 7-day chronic toxicity test poses little risk to those performing it. Effluent and/or receiving waters may contain pathogens so appropriate safety precautions should be used when handling these solutions including gloves, eye protection and lab coat. 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 appropriately stored for hazardous waste disposal.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR FATHEAD MINNOW, ***PIMEPHALES PROMELAS***, LARVAL SURVIVAL AND GROWTH TEST (TEST METHOD 1000.0)	
1. Test type	Static renewal
2. Test duration	7 days
3. Temperature	25 ± 1ºC
4. Light quality	Ambient laboratory illumination
5. Light intensity	50-100 ft-c (10-20 $\mu E/m^2/s$)
6. Photoperiod	16 hours light: 8 hours darkness
7. Test chamber size	600 mL
8. Test solution volume	400 mL
9. Renewal of test solutions	Daily
10. Age of test organisms	Newly hatched larvae should be less than 24 h old, but no more than 48 hours old (all hatched within 24 h)
11. # of organisms per test chamber	Ten
12. # of replicate chambers per concentration	Four
13. # of organisms per concentration	Forty
14. Feeding regime	Artemia nauplii twice daily
15. Test chamber cleaning	Siphon daily, immediately before test solution renewal
16. Test chamber aeration	None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min
17. Dilution water	Moderately hard water (deionized water with reagent grade chemicals added to make EPA moderately hard synthetic water)
18. Dilution factor	≥0.5
19. Test endpoint	% survival and growth
20. 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)
21. Sample volume required	4 Liters per day
22. Test acceptability	80% or greater average survival in controls; average weight of control fish ≥ 0.25 mg

Supplemental SOP Language

Definitions:

ACS:	American Chemical Society
ASAP :	As soon as possible
ASTM :	American Society for Testing Materials
°C :	degrees Celsius
dH_2O :	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 generates 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 7: Chronic Sheepshead Minnow Toxicity Testing SOP

Revision #2 (Date last modified: 1/14/05 3:23 PM by Pacific EcoRisk)
Effective Date: December 8, 2002
Accepted:_____

Cyprinodon variegatus (Sheepshead Minnow) Chronic (7-Day) Survival and 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 Marine and Estuarine Organisms, Third Edition (EPA-821-R-02-014). See Addendum, for tests that require the guidelines set forth in previous editions (EPA/600/4-87/028 and EPA-600-4-91-003).

1.0 INTRODUCTION

This test is based on a seven-day static-renewal exposure of < 24 hr old ***Cypriniodon variegatus*** to different concentrations of effluents and/or receiving waters during the larval life period. The test endpoints are survival and growth (measured as dry weight).

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 (*Artemia*) nauplii at least twice per day. These nauplii should be produced with in-house cultures that will require brine shrimp eggs, seawater (natural or artificial), and egg incubation containers. Incubation of the eggs should begin long enough prior to receiving the test organisms so as to assure a ready supply of newly-hatched nauplii as needed.
2. Aeration System: needed for aeration when D.O. drops below acceptable levels.
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: 600 mL glass beaker. Beakers must be appropriately cleaned and rinsed, and then pre-soaked for 24 hrs (overnight) in control water, before use in test.
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. Wide-bore transfer pipettes or small handheld dip nets: for transfer of organisms to and from test containers.
10. NITEX mesh sieves (150 μm & 500 μm); for concentrating organisms.
11. Balance: capable of weighing to 0.01 mg.
12. Reference Weights: for calibration of balance.

13. Drying Oven: for drying fish at 105°C at test termination.
14. Desiccator: for holding dried fish.
15. Forceps: for transfer of organisms to weighing pans.
16. Aluminum Foil Weighing Pans: for drying and weighing of fish.
17. Cubitainers: may be necessary for the client's collection of effluent.
18. Artificial Sea Salt (Crystal Sea®): for salting up of effluent to acceptable test salinity.
19. ACS Reagent $CuS0_4$ (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 < 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 fish from: (1) - Aquatic Indicators (904)829-2780
   (2) - Aquatic Biosystems Inc. (303)223-2938
   (3) - Aquatic Research Organisms (603)926-1650

2. Order the juvenile fish to be pre-adapted to the test salinity. This is important as the supplier may be culturing the fish at a different salinity than the desired test salinity. If the fish come in at a "non-test" salinity, they must begin acclimation ASAP. Place them in control water at the receiving salinity and immediately begin to adjust the holding salinity towards the test salinity.
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 4°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 6 Liters of sample will be needed each day.

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 *Cyprinodon variegatus***"

1. Feed fish *Artemia* nauplii at least two hours prior to the start of the test.
2. Label an appropriate number of 600-mL or 1-L beakers with the appropriate test treatments for your dilution series (e.g.: control, 10%, 25%, 50%, and 100%). You will need 2-4

containers per treatment (test dependent). 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 filtered seawater or appropriately salted receiving water according to client NPDES permit. Adjust salinity of control and sample waters, if necessary, by adding Crystal Sea® Sea Salt into solution while stirring on a stir plate. Always work from low to high concentration and rinse out any glassware 3X with dH_2O and one final with control salinity water prior to use.
5. For each treatment, record the initial water quality (**pH**, **D.O.**, **salinity**, **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.
6. Beginning with the Control treatment and working up through the concentration series, pour 500-750 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.
7. Try to arrange the work schedule so that loading of organisms into the test containers will take place around 2-3 p.m. Prior to loading fish, clean the tank holding the test organisms as much as possible (i.e. remove uneaten food and any dead organisms) minimizing any disturbance of the live animals.
8. Using a net and pipette, *carefully* capture and randomly allocate 10 larval 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. Note any excess dilution of the test solution.
9. 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.
10. Randomly place the test containers within the temperature-controlled (25°C ±1°C) water bath 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.

4.0 MAINTAINING A STATIC RENEWAL TEST

1. The test organisms should be fed newly-hatched brine shrimp nauplii first thing in the

morning.

2. At the time of the media renewal, pull the test containers from the water bath and arrange, in replicate # order, and by treatment. From one randomly selected container at each treatment, measure the "old" temperature, pH, D.O. and salinity/conductivity. Check these measurements to make sure that the water quality is within acceptable limits.
3. Remove the uneaten food and any dead animals. This can be accomplished either of two methods: (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; (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.
4. Count the number of live animals in each replicate and record the number on the data sheet.
5. 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. Carefully (and slowly) pour fresh media back into the replicate containers until the water level is at the fill line (500-750 mL). 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.
6. From one randomly selected replicate container at each treatment (beginning with the Control, and working upward through the concentration series), measure the "new" pH, D.O., and salinity/conductivity. Record these data on the data sheet and randomly place the replicate beakers back in the water bath. Sometime between 3 p.m. and 5 p.m., feed each replicate container with newly-hatched brine shrimp nauplii.

5.0 TEST TERMINATION

1. After 7 days, pull the test containers from the water bath. Measure and record the "old" pH, D.O., and salinity/conductivity. Siphon or pour out approximately 80% of the old media. Count and record the number of live animals in each replicate container.
2. Pour the remaining test solution containing the surviving fish into a 500 μm mesh screen submerged in deionized water so as to wash away debris that may alter final weights.
3. Euthanize the organisms from each replicate in an ice-bath with filtered seawater, rinse in de-ionized water and transfer the organisms onto a pre-dried and pre-weighed aluminum drying pan (the pans should be weighed as per the **Weighing of Test Organisms S.O.P.**). When all of the replicates have been transferred into their respective drying pans, place the pans into the drying oven, and dry at 105°C for at least 6 hrs.
4. 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. The weight recorded for the weight of the pans + dried animals <u>minus</u> the empty pans = the pooled dry weight of the organisms for that replicate. Divide this number by the number of organisms in the replicate to obtain the mean dry weight for individual fish in that replicate. For the control treatment, calculate the mean weight per surviving fish for each replicate to

determine if the weights met test acceptability criteria.

5. At this point, there should be two endpoint data for each replicate: percentage survival and mean dry weight per individual.
6. 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 may be 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 sheephead reference toxicity test is presented in the "**Chronic *Cyprinodon variegatus* Reference Toxicity Test SOP"**.

7.0 DATA ANALYSIS

The two endpoint data for each replicate, which are recorded on the appropriate data sheets, are entered into a CETIS™ v1.023 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

Test acceptability criteria for the sheephead minnow chronic test are as follows:
80% or greater survival in the controls and an average dry weight of the control larvae, when dried immediately after test termination, equal to or greater than 0.60 mg per individual. If the larvae have been preserved in fixative, for no more than 7 days, the average dry weight per control individual should be equal to or greater than 0.50 mg.

9.0 QUALITY CONTROL

1. Control water, consisting of deionized water salted up to test salinity with Crystal Sea® sea salt, is used.
2. Test fish arriving as embryos shall be maintained in the laboratory 4-7 days prior to use in tests in order to monitor and examine them for health and quality.
3. All equipment is calibrated and operated as described in each applicable equipment SOP.
4. 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 sheepshead minnow 7-day chronic toxicity test poses little risk to those performing it. Effluent and/or receiving waters may contain pathogens so appropriate safety precautions should be used when handling these solutions. 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 appropriately stored for hazardous waste disposal.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR SHEEPSHEAD MINNOW, ***CYPRINIDON VARIEGATUS***, LARVAL SURVIVAL AND GROWTH TEST (TEST METHOD 1004.0)	
1. Test type	Static renewal
2. Test duration	7 days
3. Salinity	20 to 32 ppt ± 2 ppt
4. Temperature	25 ± 1ºC
5. Light quality	Ambient laboratory illumination
6. Light intensity	50-100 ft-c (10-20 $\mu E/m^2/s$)
7. Photoperiod	16 hours light: 8 hours darkness
8. Test chamber size	600-mL to 1-L
9. Test solution volume	500 mL to 750 mL
10. Renewal of test solutions	Daily
11. Age of test organisms	<24 hours old
12. No. of organisms per test chamber	Ten (10)
13. No. of rep. chambers per concentration	Four (4)
14. No. of organisms per concentration	Forty (40)
15. Feeding regime	*Artemia* nauplii twice daily
16. Test chamber cleaning	Siphon daily, immediately before test solution renewal
17. Test chamber aeration	None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min
18. Dilution water	According to NPDES permit. Filtered (1 μm) seawater, dH_2O salted up with Crystal Sea® sea salt.
19. Test concentration	According to NPDES permit. Effluents: 5 and a control Receiving Waters: 100% and a control.
20. Dilution factor	According to NPDES permit. None, or ≥ 0.5 dilution series
21. Test endpoint	% survival and growth
22. 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)
23. Sample volume required	6 Liters per day
24. Test acceptability	80% or greater average survival in controls; average weight of control fish ≥ 0.6mg

ATTACHMENT 8: Chronic Topsmelt Toxicity Testing SOP

Revision #2 (Date last modified: 1/14/05 3:24 PM by Pacific EcoRisk)
Effective Date: December 8, 2002
Accepted:_____

ATHERINOPS AFFINIS (TOPSMELT)
Chronic (7-Day) Survival and 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 West Coast Marine and Estuarine Organisms (EPA/600/R-95/136).

1.0 INTRODUCTION

This test is based on a seven-day static-renewal exposure of 9 -15 day old ***Atherinops affinis*** to different concentrations of effluents and/or receiving waters during the juvenile life period. The test endpoints are % survival and growth (measured as dry weight).

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 (*Artemia*) nauplii at least twice per day. These nauplii should be produced with in-house cultures that will require brine shrimp eggs, seawater (natural or artificial), and egg incubation containers. Incubation of the eggs should begin long enough prior to receiving the test organisms so as to assure a ready supply of newly-hatched nauplii as needed.
2. Aeration System: needed for aeration when D.O. drops below acceptable levels.
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: 600 mL glass beaker. Beakers must be appropriately cleaned and rinsed, and then pre-soaked for 24 hrs (overnight) in control water, before use in test.
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. Wide-bore transfer pipettes or small handheld dip nets: for transfer of organisms to and from test containers.
10. NITEX mesh sieves (150 μm); for concentrating organisms.
11. Balance: capable of weighing to 0.01 mg.
12. Reference Weights: for calibration of balance.
13. Drying Oven: for drying fish at 105°C at test termination.

14. Desiccator: for holding dried fish.
15. Forceps: for transfer of organisms to weighing pans.
16. Aluminum Foil Weighing Pans: for drying and weighing of fish.
17. Cubitainers: may be necessary for the client's collection of effluent.
18. Artificial Sea Salt (Crystal Sea®): for salting up of effluent to acceptable test salinity.
19. ACS Reagent $CuS0_4$ (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 9 to 15-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) - Aquatic Biosystems Inc. (303)223-2938

2. Order the juvenile fish to be pre-adapted to the test salinity. This is important as the supplier may be culturing the fish at a different salinity than the desired test salinity. If the fish come in at a "non-test" salinity, they must begin acclimation ASAP. Place them in control water at the receiving salinity and immediately begin the adjust the holding salinity towards the test salinity.

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 4°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 4 Liters of sample will be needed each day.

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 *Atherinops affinis***".

1. Feed fish *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, 10%, 25%, 50%, and 100%). You will need 2-4 containers per treatment (test dependent). 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 filtered seawater or appropriately salted receiving water according to client NPDES permit. Adjust salinity of control and sample waters, if necessary, by adding Crystal Sea® Sea Salt into solution while stirring on a stir plate. Always work from low to high concentration and rinse out any glassware 3X with dH_2O and one final with control salinity water prior to use.
5. For each treatment, record the initial water quality (**pH**, **D.O.**, **salinity**, **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.
6. Beginning with the Control treatment and working up through the concentration series, pour 400 mL (200 mL minimum) 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.
7. Try to arrange the work schedule so that loading of organisms into the test containers will take place around 2-3 p.m. Prior to loading fish, clean the tank holding the test organisms as much as possible (i.e. remove uneaten food and any dead organisms) minimizing any disturbance of the live animals.
8. Using a net and pipette, *carefully* capture and randomly allocate 5 fish into each of the test containers.
    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. Note any excess dilution of the test solution.
9. Randomly place the test containers within the temperature-controlled (**20°C ±1°C**) water bath 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.
10. Feed each replicate with newly-hatched brine shrimp nauplii. 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 THE TEST

1. The test organisms should be fed newly-hatched brine shrimp nauplii first thing in the morning.
2. At the time of the media renewal, pull the test containers from the water bath and arrange, in replicate # order, and by treatment. From one randomly selected container at each

treatment, measure the "old" temperature, pH, D.O. and salinity/conductivity. Check these measurements to make sure that the water quality is within acceptable limits.

3. Remove the uneaten food and any dead animals. This can be accomplished either of two methods: (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; (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.
4. Count the number of live animals in each replicate and record the number on the data sheet.
5. 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. Carefully (and slowly) pour fresh media back into the replicate containers until the water level is at the 400 mL 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.
6. From one randomly selected replicate container at each treatment (beginning with the Control, and working upward through the concentration series), measure the "new" pH, D.O., and salinity/conductivity. Record these data on the data sheet and randomly place the replicate beakers back in the water bath. Sometime between 3 p.m. and 5 p.m., feed each replicate container with newly-hatched brine shrimp nauplii.

5.0 TEST TERMINATION

1. After 7 days, pull the test containers from the water bath. Measure and record the "old" pH, D.O., and salinity/conductivity. Siphon or pour out approximately 80% of the old media. Count and record the number of live animals in each replicate container.
2. Pour the remaining test solution containing the surviving fish into a 500 μm mesh screen submerged in deionized water so as to wash away debris that may alter final weights.
3. Euthanize the organisms from each replicate in an ice-bath with filtered seawater, rinse in de-ionized water and transfer the organisms onto a pre-dried and pre-weighed aluminum drying pan (the pans should be weighed as per the **Weighing of Test Organisms S.O.P.**). When all of the replicates have been transferred into their respective drying pans, place the pans into the drying oven, and dry at 105°C for at least 6 hrs.
4. 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. The weight recorded for the weight of the pans + dried animals <u>minus</u> the empty pans = the pooled dry weight of the organisms for that replicate. Divide this number by the number of organisms in the replicate to obtain the mean dry weight for individual topsmelt in that replicate. For the control treatment, calculate the mean weight per surviving fish for each replicate to determine if the weights met test acceptability criteria.
5. At this point, there should be two endpoint data for each replicate: percentage survival and mean dry weight per individual.

6. 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 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 *Atherinops affinis* reference toxicity test is presented in the "**Chronic *Atherinops affinis* Reference Toxicity Test SOP**.

7.0 DATA ANALYSIS

The survival and growth data for each replicate, which are recorded on the appropriate data sheets, are entered into a CETIS™ v1.023 data file labeled for identification of the specific test. Statistical analysis are performed in accordance with EPA guidelines for statistical analysis.

8.0 TEST ACCEPTABILITY CRITERIA

Test acceptability criteria for the *Atherinops affinis* chronic test are as follows: 80% or greater survival in the controls and an average dry weight of the control larvae, when dried immediately after test termination, is equal to or greater than 0.85 mg per individual. The survival LC_{50} for copper in the reference toxicant test must be <205 μg/L. There must be <25% and <50% MSD for survival and growth, respectively, relative to controls in the reference toxicant test.

9.0 QUALITY CONTROL

1. Control water, consisting of deionized water salted up to test salinity with Crystal Sea® sea salt, is used.
2. The test fish shall be maintained in the laboratory 4-7 days prior to use in tests in order to monitor and examine them for health and quality.
3. All equipment is calibrated and operated as described in each applicable equipment SOP.
4. 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 *Atherinops affinis* 7 Day 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 appropriately stored for hazardous waste disposal.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR TOPSMELT, ***ATHERINOPS AFFINIS***, LARVAL SURVIVAL AND GROWTH TEST	
1. Test type	Static renewal
2. Test duration	7 days
3. Salinity	5 to 34 ppt ± 2 ppt
4. Temperature	20 ± 1ºC
5. Light quality	Ambient laboratory illumination
6. Light intensity	50-100 ft-c (10-20 $\mu E/m^2/s$)
7. Photoperiod	16 hours light: 8 hours darkness
8. Test chamber size	600-mL
9. Test solution volume	400 mL (200 mL minimum)
10. Renewal of test solutions	Daily
11. Age of test organisms	9-15 days old
12. No. of organisms per test chamber	Five
13. No, of rep. chambers per conc'n	Five
14. No. of organisms per conc'n	Twenty-five
15. Feeding regime	40 *Artemia* nauplii per larvae twice daily
16. Test chamber cleaning	Siphon daily, immediately before test solution renewal
17. Test chamber aeration	None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min
18. Dilution water	According to NPDES permit. Filtered (1 μm) seawater, dH_2O salted up with Crystal Sea® sea salt.
19. Test concentration	According to NPDES permit. Effluents: 5 and a control Receiving Waters: 100% and a control.
20. Dilution factor	According to NPDES permit. None, or ≥ 0.5 dilution series
21. Test endpoint	% survival and growth
22. 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)
23. Sample volume required	4 Liters per day
24. Test acceptability	80% or greater average survival in controls; average weight of control fish ≥ 0.85 mg; Cu LC_{50} ≤205 μg/L; <25% & <50% MSD for survival and growth.

ATTACHMENT 9: 10-day *Hyalella azteca* Sediment Toxicity Testing SOP

Revision #2 (Date last modified: 1/14/05 8:05 PM by Pacific EcoRisk)
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Hyalella azteca
Acute (10-day) Survival & Growth Sediment Toxicity Test
Standard Operating Procedures

This SOP is based upon the U.S. EPA 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 120-μm 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 *Hyalella* 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 *Hyalella 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 4°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 *Hyalella 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 substratate 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 TERMINANTION

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 *Hyalella* 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 ***HYALELLA AZTECA*** 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 *outside* 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.

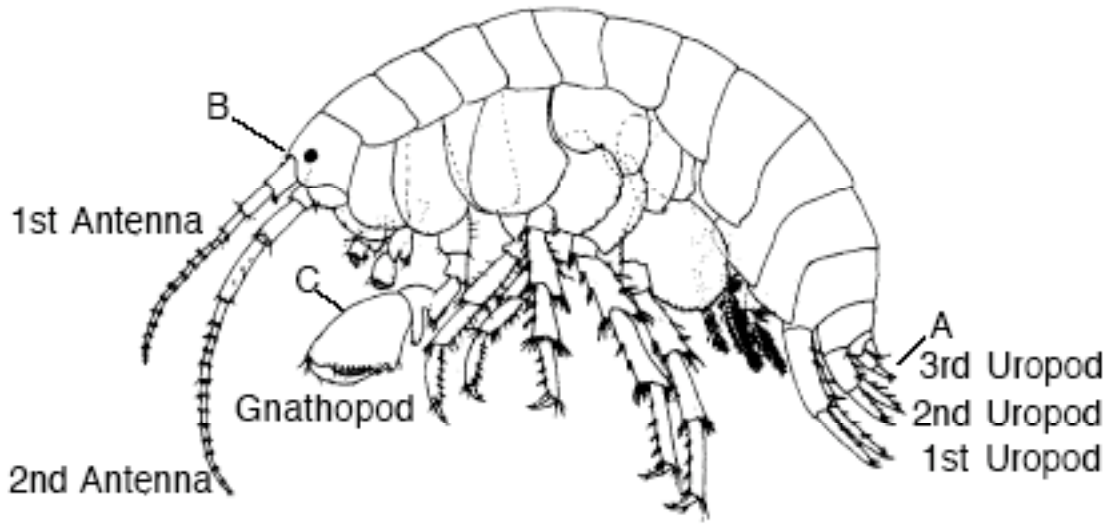

Figure 11.1 ***Hyalella azteca.*** **(A) denotes the uropods; (B) denotes the base of the first antennae; (C) denotes the gnathopod used for grasping females. Meaurement of length is made from base of the 3rd uropod (A) to (B). Females are recognized by the presence of egg cases or the absence of an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

ATTACHMENT 10: Chronic *Selenastrum capricornutum* Ref Tox SOP

Revision #2
Effective Date: December 8, 2002
Accepted:_____

96 hour *Selenastrum capricornutum* Reference Toxicant Test
Standard Operating Procedure

The reference toxicant for Selenastrum is: Zinc (as ZnCl2)

MW = 249.68

The Standard Range of Zn concentrations is: control, 1.25, 2.5, 5, 10, and 20 ug/L

Reference Toxicant Test Procedures:

(Note: Recommended Test Conditions are summarized on the following page)

1. Prepare control/dilution water (0.45 um filtered distilled water amended with 1 ml/L of each of the five nutrient solutions). See Table 1 in the Selenastrum Toxicant Test SOP for more information regarding preparation of nutrient solutions. Adjust the diluent pH to 7.5 ± 0.2 with Hcl or NaOH.

2. Prepare a 20 mg/L stock solution of Zn (0.02085 g of ZnCl2 diluted to 500 mL w/ DI water).

3. Prepare 1000 mL of 20 μg/L Cu. (Add 1.0 mL of stock solution, dilute to 1000 mL with 80:20 water).

4. Prepare test media:
- control - 500 mL of control water.
- 1.25 ug/L - 31.25 mL of 20 g/L Zn stock diluted to 500 mL w/ dilution water.
- 2.5 ug/L - 62.5 mL of 20 g/L Zn stock diluted to 500 mL w/ dilution water.
- 5 ug/L - 125 mL of 20 g/L Zn stock diluted to 500 mL w/ dilution water.
- 10 ug/L - 250 mL of 20 g/L Zn stock diluted to 500 mL w/ dilution water.
- 20 ug/L - 500 mL of 20 g/L Zn stock solution (from step 3 above).

5. Measure and record initial water chemistries (pH, D.O., conductivity, and temp.) onto the Test Data Sheet.

6. Dispense 25 ml of each treatment in clearly labeled vials for use as blanks at test termination. Store vials in the dark, cold storage to prevent algal growth.

7. An inoculation density of 1 x 104 cells/mL for each flask can be obtained through the following procedure:

(a). Obtain a 4 to 7 day old culture which should be in the log phase of growth.

(b). Load 10 L of the well mixed culture into each side of a hemacytometer, allow the algae to settle for 5-10 minutes, and count the algae. Repeat 3-5 times and record data on Algal Inoculation Worksheet.

(c). Determine the algal density in the culture by multiplying the average hemacytometer count by 104.

(d). The volume of algae used for inoculation is calculated by:

(10,000 cells/mL) x [volume of test solution (=500 mL)]/algal density

(e). Inoculate each 500 mL of test media with the appropriate inoculation volume and mix well.

8. For each test media, transfer 100 ml into 4 clean, pre-rinsed, and labeled 250 ml glass Erlenmeyer flasks.

9. Place loaded flasks into temperature-controlled water bath set at 25 C.

10. Follow procedures outlined in the Selenastrum test SOP.

Preparation of Stock Solution:

mg of ZnCl2 need: _____

mg of ZnCl2 measured: _____

Date prepared: _____

ATTACHMENT 11: Chronic *Skeletonema* and *Thalassiosira* Ref Tox SOP

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96-hour *Thalassiosira pseudonana* Reference Toxicant Test
Standard Operating Procedure

The reference toxicant for Ceriodaphnia is: Copper (as CuSO4•5H2O) MW = 249.68

The standard range of Cu concentrations is: control, 5, 10, 25, 50, 100, and 250 ug/L

Reference Toxicant Test Procedures:

1. Prepare control/dilution water (0.45 μm filtered distilled water adjusted to 30 ppt using Crystal Sea® bioassay-grade sea salts) and amended with nutrients as follows:
   a. 15 ml/L of each of the metals solution;
   b. 1 mL/L of the minor salts solution;
   c. 1.0 ml/L of the vitamin stock solution.

2. Adjust the control/dilution water pH to 8.0 ± 0.2 with HCl or NaOH.

3. Prepare 1000 mL of 250 μg/L Cu. (Add 1.25 mL of 200 mg/L stock solution, dilute to 1000 mL with control/dilution water).

4. Prepare test media:

control -	500 mL of control water.
5 μg/L -	10 mL of 250 μg/L Cu stock diluted to 500 mL w/ dilution water.
10 μg/L -	20 mL of 250 μg/L Cu stock diluted to 500 mL w/ dilution water.
25 μg/L -	50 mL of 250 μg/L Cu stock diluted to 500 mL w/ dilution water.
50 μg/L -	100 mL of 250 μg/L Cu stock diluted to 500 mL w/ dilution water.
100 μg/L -	200 mL of 250 μg/L Cu stock diluted to 500 mL w/ dilution water.
250 μg/L -	500 mL of 250 μg/L Cu stock solution (from step 3 above).

5. Measure and record initial water chemistries (pH, D.O., salinity, and temp.) onto the Test Data Sheet.

6. Dispense 25 ml of each treatment in clearly labeled vials for use as blanks at test termination. Store vials in the dark, cold storage to prevent algal growth.

7. An inoculation density of 2 x 10^4 cells/mL for each flask can be obtained through the procedure noted in the *Skeletonema* and *Thalassiosira* Algal Groth Bioassay SOP.

8. For each test media, transfer 100 ml into 4 clean, pre-rinsed, and labeled 250 ml glass Erlenmeyer flasks.

9. Place loaded flasks into temperature-controlled water bath or incubator set at 20º C.

10. Follow procedures outlined in the *Thalassiosira* test SOP.

Preparation of Stock Solution:

mg of $CuCl_2$ need: _____

mg of $CuCl_2$ measured: _____

Date prepared: _____

ATTACHMENT 12: Chronic *Ceriodaphnia dubia* Ref Tox SOP

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7 day Chronic *Ceriodaphnia dubia* Reference Toxicant Test

Standard Operating Procedure

The reference toxicant for *Ceriodaphnia dubia* is: **Cu (as $CuSO_4 \cdot 5H_2O$)** MW = 249.68

Reference Toxicant Test Procedures:

1. Prepare control/dilution water (80% Arrowhead spring water + 20% Evian).
2. Prepare a **64 mg/L** stock solution of Cu (0.02515 g of $CuSO_4 \cdot 5H_2O$ diluted to 100 mL w/ DI water).
3. Prepare 400 mL of 128 µg/L Cu. (Add 0.8 mL of stock solution, dilute to 400 mL with 80:20 water).
4. Prepare test media:

Control -	400 mL of control water.
8 µg/L -	12.5 mL of 128 µg/L Cu stock diluted to 200 mL w/ dilution water.
16 µg/L -	25 mL of 128 µg/L Cu stock diluted to 200 mL w/ dilution water.
32 µg/L -	50 mL of 128 µg/L Cu stock diluted to 200 mL w/ dilution water.
64 µg/L -	100 mL of 128 µg/L Cu stock diluted to 200 mL w/ dilution water.
128 µg/L -	200 mL of 128 µg/L Cu stock solution (from step 3 above).

5. Measure and record initial water chemistries (pH, D.O., conductivity, and temp.) onto the Test Data Sheet. For the control and 128 µg/L treatments, determine alkalinity and hardness.
6. To each 200 mL of media add: 1.3 mL of YCT and 2.6 mL of *Selenastrum.*
7. For each treatment media, transfer 15 ml into 10 clean, pre-rinsed, and labeled 30 mL plastic cups.
8. Identify 10 adult *Ceriodaphnia* females from the brood board cultures that have had 8 or more offspring. Each of the neonates from one adult will be used to load one replicate from each treatment concentration.
9. Place loaded cups in temperature-controlled water bath set at 25°C.
10. Follow procedures outlined in the chronic *Ceriodaphnia* test SOP.

Preparation of Stock Solution:

mg of $CuSO_4$ need: _____

mg of $CuSO_4$ measured: _____

Date prepared: _____

ATTACHMENT 13: Chronic *Americamysis bahia* Ref Tox SOP

Revision #2 (Date last modified: 6/25/04 11:07 AM by Stephen Clark)
Effective Date: December 8, 2002
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7 day Chronic *Americamysis bahia* Reference Toxicant Test

Standard Operating Procedure

The reference toxicant for *Americamysis bahia* is: Cr (as $K_2Cr_2O_7$) MW = 294.19
There are 2 Cr per $K_2Cr_2O_7$; for 2 Cr, the MW = 103.992

Reference Toxicant Test Procedures:

1. Prepare control/dilution water (DI water salted up to 25 ppt w/ 40 Fathoms Salt).

2. Prepare 4.0 L of 14 mg/L Cr (0.15841 g of $K_2Cr_2O_7$ diluted to 4.0 L w/ dilution water).

3. Prepare test media:

Control - 1.6 L of control water
0.88 mg/L - 100 mL of 16 mg/L Cr stock diluted to 1.6 L w/ dilution water.
1.75 mg/L - 200 mL of 16 mg/L Cr stock diluted to 1.6 L w/ dilution water.
3.5 mg/L - 400 mL of 16 mg/L Cr stock diluted to 1.6 L w/ dilution water.
7 mg/L - 800 mL of 16 mg/L Cr stock diluted to 1.6 L w/ dilution water.
14 mg/L - 1600 ml of 16 mg/L Cr stock solution (from step 2 above).

4. Measure and record initial water chemistries (pH, D.O., salinity, and temp.) onto the Test Data Sheet.

5. For each of the treatment media, transfer 200 mL into 8 clean, pre-rinsed, and labeled 400-mL plastic beakers.

6. Make sure that mysids are well-fed prior to loading animals into the test beakers. From a set of 7 -day old animals, randomly allocate 5 mysids into each of the replicate containers. Make sure that each individual is undamaged.

7. Place loaded beakers in temperature-controlled water bath set at 26°C.

8. Follow procedures outlined in the chronic *M. bahia* toxicity test SOP.

Preparation of Stock Solution:

mg of $K_2Cr_2O_7$ need: _____

mg of $K_2Cr_2O_7$ measured: _____

Date prepared: _____

ATTACHMENT14: Chronic Fathead Minnow Ref Tox SOP

Revision #2 (Date last modified: 6/24/04 9:35 AM by Stephen Clark)
Effective Date: December 8, 2002
Accepted:_____

Pimephales promelas (Fathead Minnow) Chronic (7-day) Reference Toxicant Test

Standard Operating Procedure

1.0 INTRODUCTION

To ensure that the organisms being used in a bioassay are responding to chemical stress in a "typical" manner, a reference toxicant test is performed. With the exception of the preparation of the working stock, the performance of this test is identical as described in the **"*Pimephales promelas* Chronic (7-Day) Survival and Growth Bioassay SOP"**. Read this SOP prior to starting this test.

2.0 REFERENCE TOXICANT TREATMENT PREPARATION

The reference toxicant for *Pimephales promelas* is copper (Cu, as $CuSO_4$). A certified standard solution of 40 mg/mL copper specific to this test is available in the chemistry refrigerator. Record the following information from the stock solution bottle:

Manufacturer	**Inorganic Ventures Inc.**		
Certified Concentration	**Lot Number**	**Expiration Date**	**Today's Date**
Signature			

1. Pour 10-15 mL of Certified Cu stock into a 25-mL beaker. Do not pipet directly from the stock solution bottle.

2. Volumetrically pipette 10 mL of stock into a 2-L volumetric flask and dilute to 2-L mark with control water; pour into 6-L HDPE beaker dilute volumetrically to 4 L with control water. This will be your "working" solution of 100 μg/L Cu. Do not pour any excess stock solution back into the stock bottle.

3. Prepare treatment dilutions according to the following table. Always work from low to high concentration.

Treatment	**Vol. of Working Solution (100 μg/mL)**	**Vol. Dilution Water (80:20)**	**Final Vol.**	**Initials**
control	0	1600 mL	1600 mL	
6.25 μg/L	100 mL	1500 mL	1600 mL	
12.5 μg/L	200 mL	1400 mL	1600 mL	
25 μg/L	400 mL	1200 mL	1600 mL	
50 μg/L	800 mL	800 mL	1600 mL	
100 μg/L	1600 mL	0	1600 mL	

3.0 DATA ANALYSIS

The survival data for each replicate, which are recorded on the appropriate data sheets, are entered into a CETIS™ v1.023 data file labeled for identification of the specific test. Statistical analyses are performed in accordance with EPA guidelines for statistical analysis. The reference toxicant results are then compared with 20 most recent tests from in-house database for that reference toxicant.

4.0 SAFETY

The fathead minnow chronic reference toxicant 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 copper stock solution. After the reference toxicant spiking solution has been used, any remaining solution should be appropriately stored for hazardous waste disposal.

ATTACHMENT 15: Chronic Sheepshead Minnow Ref Tox SOP

Revision #2 (Date last modified: 2/11/05 6:31 PM by Stephen Clark)
Effective Date: December 8, 2002
Accepted:_____

7 day Chronic *Cyprinodon variegatus* Reference Toxicant Test

Standard Operating Procedure

The reference toxicant for *Cyprinodon variegatus* is: **KCl**
MW = 74.55
The Standard Range of Cu concentrations is: control, 0.25, 0.5, 1, 1.5, and 2 mg/L

Reference Toxicant Test Procedures:

(Note: Recommended Test Conditions are summarized on the following page)

1. Prepare control/dilution water (Arrowhead spring water salted up to 25 ppt w/ 40 Fathoms Salt).

2. Prepare a 200 mg/L stock solution of KCl (0.040 g of KCl diluted to 200 mL w/ DI water).

3. Prepare 4.0 L of 2 mg/L KCl (dilute 40 mL of stock solution to 4.0 L with dilution water).

4. Prepare test media: Control - 1.6 L of control water
   - 0.25 mg/L- 125 mL of 2 mg/L KCl stock diluted to 1.6 L w/ dilution water.
   - 0.5 mg/L - 250 mL of 2 mg/L KCl stock diluted to 1.6 L w/ dilution water.
   - 1 mg/L - 500 mL of 2 mg/L KCl stock diluted to 1.6 L w/ dilution water.
   - 1.5 mg/L - 1000 mL of 2 mg/L KCl stock diluted to 1.6 L w/ dilution water.
   - 2 mg/L - 1.6 L of 2 mg/L KCl stock (from step 3 above).

5. Measure and record Initial water chemistries (pH, D.O., salinity, and temp.) onto the Test Data Sheet.

6. For each of the treatment media, transfer 400 mL into 4 clean, pre-rinsed, and labeled 600-mL glass beakers.

7. Make sure that fish are well fed prior to loading animals into the test beakers. From a set of fish <24 hours post-hatch, randomly allocate 10 fish into each of the replicate containers. Make sure that each individual is undamaged.

8. Place loaded beakers in temperature-controlled water bath set at 25°C.

9. Follow procedures outlined in the chronic *Cyprinodon* test SOP.

Revision #2 (Date last modified: 2/11/05 6:31 PM by Stephen Clark)
Effective Date: December 8, 2002
Accepted:_____

Preparation of Stock Solution:

mg of KCl need: _____

mg of KCl measured: _____
Date prepared: _____

ATTACHMENT 16: Chronic Topsmelt Ref Tox SOP

Revision #2 (Date last modified: 2/11/05 8:28 PM by Stephen Clark)
Effective Date: December 8, 2002
Accepted:_____

7 day Chronic Topsmelt Reference Toxicant Test

Standard Operating Procedure

The reference toxicant for *Atherinops affinis* is: **Cu (as $CuSO_4 \bullet 5H_2O$)** MW = 249.68

The Standard Range of Cu concentrations is: control, 56, 100, 180, 320, 560, & 1000 μg/L

Reference Toxicant Test Procedures:

(Note: Recommended Test Conditions are summarized on the following page)

1. Prepare control/dilution water (De-ionize water salted up to 25 ppt w/ 40 Fathoms Salt).

2. Prepare a 2.8 g/L Cu stock solution (11.02 g of $CuSO_4 \bullet 5H_2O$ diluted to 1000 mL w/ DI water).

3. Prepare 5.0 L of 1000 μg/L Cu (dilute 1.79 mL of stock solution to 5.0 L with dilution water).

4. Prepare test media:

Control -	2 L of control water
56 μg/L-	1 mL of 1000 μg/L Cu stock diluted to 2 L w/ dilution water.
100 μg/L -	200 mL of 1000 μg/L Cu stock diluted to 2 L w/ dilution water.
180 μg/L -	360 mL of 1000 μg/L Cu stock diluted to 2 L w/ dilution water.
320 μg/L -	640 mL of 1000 μg/L Cu stock diluted to 2 L w/ dilution water.
560 μg/L -	1120 mL of 1000 μg/L Cu stock diluted to 2 L w/ dilution water.
1000 μg/L-	2 L of 1000 μg/L Cu stock (from step 3 above).

5. Measure and record Initial water chemistries (pH, D.O., salinity, and temp.) onto the Test Data Sheet.

6. For each of the treatment media, transfer 400 mL into 5 clean, pre-rinsed, and labeled 600-mL glass beakers.

7. Make sure that fish are well fed prior to loading animals into the test beakers. From a set of 9-15 day old fish, randomly allocate 5 fish into each of the replicate containers. Make sure that each individual is undamaged.

8. Place loaded beakers in temperature-controlled water bath set at 20°C.

9. Follow procedures outlined in the chronic Topsmelt test SOP.

Revision #2 (Date last modified: 2/11/05 8:28 PM by Stephen Clark)
Effective Date: December 8, 2002
Accepted:_____

Preparation of Stock Solution:

g of $CuSO_4 \bullet 5H_2O$ need: _____

g of $CuSO_4 \bullet 5H_2O$ measured: _____
Date prepared: _____

ATTACHMENT 17: 4-day *Hyalella azteca* Ref Tox SOP

Revision #2
Effective Date: December 8, 2002
Accepted:_____

96 hour *Hyalella azteca* Reference Toxicant

The reference toxicant for Hyalella azteca is: Potassium Chloride
KCl
MW = 74.56

The Standard Range of KCl concentrations is: Control, 0.025, 0.05, 0.10, 0.20, 0.40 g/L

Reference Toxicant Test Procedures:

(Note: Recommended Test Conditions are summarized on the following page)

1. Prepare 6.0 L of 70:30 (Control/Dilution) water.

2. Prepare the test media:
Control - 1.0 L of 70:30 in a one liter volumetric flask
0.025 g/L - 0.025 g of KCl diluted to 1.0 Liter with 70:30
0.05 g/L - 0.05 g of KCl diluted to 1.0 Liter with 70:30
0.10 g/L - 0.10 g of KCl diluted to 1.0 Liter with 70:30
0.20 g/L - 0.20 g of KCl diluted to 1.0 Liter with 70:30
0.40 g/L - 0.40 g of KCl diluted to 1.0 Liter with 70:30

3. Measure and record Initial Water Chemistries onto the Test Water Quality Data Sheet

4. For each of the treatment media, transfer 20 mL into 10 pre-rinsed, labeled 30-mL plastic cups (i.e. Cerio cups) placed in a cup-holding board (i.e. Cerio board).

5. Add 0.1 mL of YCT food into each of the 30 mL cups (= replicate containers).

6. Place a one cm2 piece of 120 um NITEX into each of the replicate containers to provide clinging substrate for the test organisms.

7. From a neonate set of 12-14 day old animals, randomly allocate one individual amphipod into each of the replicate containers. Make sure that each individual is submerged and undamaged.

8. Place test board in temperature-controlled water bath.

9. Each day: (a) examine the replicate cups, recording live and dead animals;
(b) then feed each replicate container 0.1 mL of the YCT food;
(c) measure and record the water temperature of the water bath.

10. After 96 hours, examine the replicate cups, and record the number alive and dead.

11. Pool the water from the replicate cups for each treatment into labeled beakers and then measure and record Final Water Chemistries onto the Test Water Quality Data Sheet.

Recommended Test Conditions for Conducting Hyalella azteca Ref-Tox Test

Parameter	**Condition**
Test Type	Static 96 hour exposure
Toxicant	KCl (Potassium Chloride)
Treatment Levels	Control, 0.025, 0.05, 0.10, 0.20, 0.40 g/L
Control/Dilution Water	70:30 (mix of 70% Arrowhead and 23% Evian spring waters).
Temperature	23 + 1o C
Photoperiod	16L:8D
Age of Organisms	12-14 days old
Test Containers	30 mL plastic cups (Cerio cups)
Volume of Test Water	20 mL in each cup
# of Animals per Cup	One
# of Cups per Treatment	10
Feeding	0.1 mL of YCT into each cup daily.
Substrate	One cm2 of 120 um NITEX in each cup.
Aeration	None
Water Qualities	pH, D.O., conductivity, alkalinity, and hardness at Ti and Tf Temperature recorded daily
Test Duration	96 hours
Test Endpoint	Survival (LC50)
Test Acceptability	90% control survival.

96 hour Hyalella azteca Reference Toxicant Test Data

Date _____Client _____ Project _____ Time _____

Treatment	Temp.	Day	Survival										Results Summary
Control		Time 0											96 hr
		24 hrs											% Survival
		48 hrs											
		72 hrs											=_____
		96 hrs											
0.025 g/L		24 hrs											96 hr
		48 hrs											% Survival
		72 hrs											
		96 hrs											=_____
0.05 g/L		24 hrs											96 hr
		48 hrs											% Survival
		72 hrs											
		96 hrs											=_____
0.10 g/L		24 hrs											96 hr
		48 hrs											% Survival
		72 hrs											
		96 hrs											=_____
0.20 g/L		24 hrs											96 hr
		48 hrs											% Survival
		72 hrs											
		96 hrs											=_____
0.40 g/L		24 hrs											96 hr
		48 hrs											% Survival
		72 hrs											
		96 hrs											=_____

Treatment	Initial Water Chemistries				Final Water Chemistries			
	pH	DO	Salinity	Conduct.	pH	DO	Salinity	Conduct.
Control								
0.025 g/L								
0.05 g/L								
0.10 g/L								
0.20 g/L								
0.40 g/L								

APPENDIX C

SUPPORTING DOCUMENTS FOR CHEMICAL ANALYSES

ATTACHMENT 1: Nitrate + Nitrite Analysis SOP

Standard Operating Procedure
Standard Method 4500-NO_3 F
Nitrate, Nitrate as N, NO_3+NO_2 as N in Drinking Water and Wastewater

APPROVAL: The undersigned has verified that this SOP is complete and meets requirements specified in document S0QA010.XXX:

Wet Chemistry Manager:_____ Date:_____

Quality Assurance Director:_____ Date:_____

1.0 Scope and Application:

1.1 This is a flow injection analysis (FIA) method applicable to the determination of Nitrate, Nitrate as N, and Nitrate+Nitrite as N in drinking water and wastewater.

1.2 The specific Practical Quantitation Limits (PQL) for each matrix is maintained in the FGL LIMS.

1.3 Prior to the use of this procedure FGL employees must perform and document an initial demonstration of proficiency for the method and LIMS. For continued use, annual method proficiency must be documented. Refer to S0QA185.XXX for employee training.

2.0 Summary of Method:

2.1 Summary:

2.1.1 The nitrate is paristaltically introduced to the flow injection analysis system. The nitrate is reduced to nitrite using cadmium reduction. The nitrite then reacts with the ammonium chloride/EDTA and sulfanilimide to form a purple dye.

2.1.2 The concentration is quantified using a five point standard curve read colorimetrically with two filters ranging from 540 - 720 nm.

2.2 Definitions:

2.2.1 Definitions are listed in the FGL Quality Assurance Plan, Section 17.0.

2.3 Method Modifications:

2.3.1 The cleaning solution, reagent section 8.5, is not listed in the Standard Methods reference but is specified by the manufacturer.

3.0 Interferences:

3.1 All glassware must be properly cleaned before use. Refer to S0QA135.XXX for glassware cleaning.

3.2 Preservation with sulfuric acid will give nitrate+nitrite results by this method because nitrite is converted to nitrate. For wastewaters when the client specifically wants nitrate or nitrate-N then nitrite has to be run on the un-preserved sample and subtracted from the nitrate+nitrite result. For drinking waters it is assumed there is no nitrite present. Samples preserved with mercuric chloride cannot be run by this method. Turbidity may be removed by filtration.

3.3 Oil and grease will coat cadmium surfaces. Filter as necessary.

4.0 Safety:

4.1 A lab coat and gloves should be worn while performing analyses. Protective eyewear must be worn at all times. Refer to the individual MSDS's for safe handling of chemicals.

5.0 Sample Containers, Preservation and Handling:

5.1 Samples should be collected in 1 pint plastic containers.

5.2 Sample preservation is 2 mL 1+1 H_2SO_4 and refrigeration at 4˚ C. If the sample is received unpreserved then 100 mL of sample is preserved with 0.4 mL of conc. H_2SO_4 and refrigeration at 4˚ C.

5.3 The holding time for Nitrate is 28 days for preserved samples and 48 hours for un-preserved samples.

5.6 Procedures for sampling are maintained in the FGL field services department.

5.7 Procedures for sample shipment are maintained in the FGL shipping department.

6.0 Equipment and Supplies:

6.1 Technicon autoanalyzer using 90 sec. cam/ FIASTAR 5000 w/a 120 tray autosampler

6.2 Autosampler tray with 2 mL vials/ 10 ml vials

6.3 IBM compatible computer as specified in SOP S0LIM055.XXX

7.0 Quality Control:

7.1 **Preparation Quality Controls:** the batch size for extraction is 20 samples.

7.1.1 Matrix Spike (MS): a set of duplicate MS's are run per batch.

7.2 **Analysis Quality Controls:** the batch size for analysis is 10 samples.

7.2.1 Initial/Continuing Calibration Blank (ICB/CCB): the ICB is run after the calibration and the CCB is run after every analytical batch and at the end of the analytical run.

7.2.2 Initial/Continuing Calibration Verification (ICV/CCV): the ICV is run after the calibration and the CCV is run after every analytical batch and at the end of the analytical run.

7.3 **Detection Limit Quality Controls:**

7.3.1 Method Detection Limit (MDL) determination: MDL's are analyzed as part of the initial demonstration and whenever major changes are made to the method (i.e. sample volume changed, analyte added). For MDL guidance please see SOP S0QA060.XXX.

8.0 Reagent Preparation:

8.1 All reagents received and prepared must be properly labeled and recorded in the LIMS electronic reagent/standard preparation log. Login the reagent using FGL LIMS, Laboratory, Inorganics, Standards. In order to minimize reagent waste, an appropriate quantity of reagent should be created or purchased which will be completely used during the lifetime of the reagent. Refer to S0QA030.XXX for proper reagent preparation and documentation.

8.2 Record the reagent code for all reagents on the prep sheet.

8.3 NO3 Reagents_Analytical:
Ammonium Chloride Buffer: Dissolve 85g NH_4OH (under a fume hood) to solution and bring final volume up to l L with DI H2O.

8.4 Sulfanilamide Reagent: Dissolve 10g of Sulfanilamide in 500 ml DI H2O. Add 100 ml of Phosphoric Acid, slowly, and bring to a final volume of 1 L with DI H2O. This is stable for several months.

8.5 NED Reagent: Dissolve 0.5g N-(1-naphtyl)-Ethylene diamine Dihydrochloride in approximately 300 ml DI

H2O. Bring up to a final volume of 500 ml with DI H2O. This solution should be prepared fresh weekly.

9.0 Standard Preparation:

9.1 All standards received and prepared must be properly labeled and recorded in the LIMS electronic reagent/standard preparation log. The LIMS then maintains the specific concentrations for each standard mix and subsequent dilutions. Login the standard using FGL LIMS, Laboratory, Wet Chem, Standards. In order to minimize standard waste, an appropriate quantity of standard should be created or purchased which will be completely used during the lifetime of the standard. Refer to S0QA030.XXX for proper standard preparation and documentation.

9.2 Record the standard code for all standards on the prep sheet. Also record the volumes of standards used and their concentrations.

9.3 Primary Calibration Standard:

9.3.1 Stock: Nitrate-N, 1000 mg/L: Purchase from current vendor.

9.3.2 Working: Nitrate-N, 8, 4, 2, 1, 0.5 and 0.1 mg/L: dilute 0.8, 0.4, 0.2, 0.1 and 0.05 mL (respectively) of Nitrate-N 9.3.1 stock standard to 100 mL.

9.4 Secondary Calibration Verification Standard: **this standard must be from either a different lot or supplier than the Primary calibration standard.**

9.4.1 Stock: Nitrate-N, 1000 mg/L: Purchase from current vendor.

9.4.2 Working: Nitrate-N, 4 mg/L: dilute 0.4 mL of 9.4.1 stock standard to 100 mL.

10.0 Sample Preparation:

10.1 **LIMS Batching:**

10.1.1 Batch the samples for preparation using FGL LIMS, Laboratory, Inorganics, Preparation. Completely fill in all information requested on the prep sheet. All sample observations are noted in the comments column.

10.2 **QC Sample Preparation:**

10.2.1 MS/MSD: to a 1 mL aliquot of sample add 1.0 mL of working standard (9.4.2).

10.2.2 Continue the QC samples with step 12.2.

10.3 **Sample Preparation:**

10.3.1 Refer to S0QA165.XXX for guidance on obtaining a representative sample.

10.3.2 UV screen for Nitrate dilution estimates:

10.3.2.1 Set spectrophotometer to 275 nm (UV/VIS). Zero the instrument (at 275) with deionized water in a quartz cuvette. Switch the wavelength to 220 nm and take a reading of the deionized water. This is the blank reading. Do a 1:10 dilution on all samples, and a 1:100 if the 1:10 dilution reads over 0.999 at 220 nm. Record samples readings at 220 nm and 275 nm. Multiply all the absorbances by 1000. Calculate the Nitrate-N using the following calculation:

$$\text{Nitrate-N mg/l} = \frac{(220_{abs} - 275_{abs} - \text{blank}_{abs}) \times \text{dilution}}{254}$$

10.3.2.2 Interpret the appropriate dilution according to the following table:

mg/L Nitrate as N	Dilution
0-5	None
5-10	1:2
10-15	1:4
15-20	1:8

11.0 Calibration:

11.1 **Frequency:**

11.1.1 The calibration is performed at the beginning of each analytical run or when continuing calibration verifications indicate a new calibration is required.

11.2 **Procedure and Calculation:**

11.2.1 Prepare standards as outlined in section 9.3.2.

11.2.2 Analyze standards as outlined in sections 12.2.

11.2.3 The calibration calculation is performed by the instrument and must be reviewed by the analyst.

11.3 **Acceptance Criteria:**

11.3.1 The criteria for passing this calibration is the correlation coefficient has to be greater than 0.995.

11.4 **Failure Resolution:**

11.4.1 If the failure can be corrected by performing instrument maintenance and/or recalibration then initiate the correction. Log the instrument maintenance performed.

11.4.2 If you are unsure of how to handle the failure please contact your supervisor or the QA director.

11.4.3 Refer to S0QA035.XXX if it is determined that a nonconformance and/or corrective action are required.

12.0 Analytical Procedure:

12.1 **LIMS Batching:** batch the samples for analysis using FGL LIMS, Laboratory, Wet Chem, Analysis. Check that samples have not already been run on the IC for nitrate, or have not been logged in under the EPA 353.2 method.

13.0 Calculations:

13.1 The analysis results including analysis dilutions are automatically calculated and expressed as mg/L values by the instrument. Preserved wastewaters should be reported as nitrate+nitrite as N unless nitrite can be subtracted. Drinking water is assumed to have no nitrite.

13.2 All solids prep dilutions are entered into the LIMS and calculated by the LIMS. The LIMS also corrects for the unit changes to mg/Kg.

13.3 Calculation for NO_3 mg/L = NO_3-N mg/L x 4.43
This is normally performed automatically by the LIMS.

14.0 Data Assessment and Failure Resolution:

14.1 All current Data Quality Objectives (DQO's) for quality controls listed below are maintained in the FGL LIMS and automatically checked during data assessment. Qualify the data using FGL LIMS, Laboratory, Wet Chem, Analysis or Prep.

14.2 **Preparation Quality Controls:**

14.2.1 MS/BS: the percent recovery and the relative percent difference of the duplicate spikes should be within the FGL acceptance range based on the control chart.

14.3 **Analysis Quality Controls:**

14.3.1 ICB/CCB: the ICB/CCB must be less than the instrument reporting limit (IRL).

14.3.2 ICV/CCV: the ICV/CCV must be within the FGL acceptance range of 90-110%.

14.4 **Detection Limit Quality Controls:**

14.4.1 MDL: the MDL must be lower than the PQL.

14.5 **Failure Resolution:**

14.5.1 If the failure can be corrected by repreparation and/or reanalysis then initiate the correction.

14.5.2 If the failure can't be corrected by repreparation and/or reanalysis or if you are unsure of how to handle the failure please contact your supervisor or the QA director. If the data must be accepted with a failure, an explanation must accompany the failure. Refer to S0QA095.XXX for guidance on proper handling of failures.

14.5.3 Refer to S0QA035.XXX if it is determined that a nonconformance and/or corrective action are required.

15.0 Method Performance:

15.1 MDL studies are used to determine precision, accuracy and sensitivity for evaluation of performance at the reporting level. MDL's are electronically stored in the LIMS and a hard copy is maintained in the QA department.

15.2 Control charts are used for analysis and preparation quality controls to determine precision and/or accuracy on an ongoing basis for evaluating the performance of the method. Control charts are electronically maintained in the LIMS. Where applicable the control charts are used to generate the data quality objectives.

15.3 Performance Testing (PT) through interlaboratory studies is performed semi-annually (where applicable) to independently verify performance. PT Study results are maintained in the QA department.

15.4 Refer to the referenced test method(s) for published interlaboratory method performance.

16.0 Review and Reporting:

16.1 **Preparation Review and Reporting:**

16.1.1 Preparation Data Packages are compiled, reviewed, signed and dated by the analyst. They are then turned in to the supervisor or peer for review. Refer to S0QA105.XXX for data package review.

16.1.2 Samples are reported in mg/L units. They are printed on the Prep Summary Report of the Preparation Data Package and electronically maintained in the FGL LIMS. Refer to S0QA090.XXX for data reduction and recording.

16.2 **Analysis Review and Reporting:**

16.2.1 Analysis Data Packages are compiled, reviewed, signed and dated by the analyst. They are then turned in to the supervisor or peer for review. Refer to S0QA105.XXX for data package review.

16.2.2 Samples are analyzed in mg/L units. They are printed on the Raw Data for instrumental methods or the Analysis Summary Report for manual methods and electronically maintained in the FGL LIMS. Refer to S0QA090.XXX for data reduction and recording.

17.0 Record Storage and Archiving:

17.1 **Preparation Records:**

17.1.1 The records generated during preparation are Preparation Package Narrative, Prep Confirmation Report, Quality Control Reports, Prep Summary Report and Prep Worksheet. These all form the Prep/Extraction Data Package.

17.1.2 After the Preparation Data Package has been reviewed, it is filed in the QA department filing boxes. Periodically the files are removed for archival. Refer to S0QA215.XXX for archiving records.

17.2 **Analysis Records:**

17.2.1 The records generated during analysis are Analysis Package Narrative, Analysis Confirmation Report, Quality Control Reports, Analysis Runlog and Raw Data or Analysis Summary Report. These all form the Analysis Data Package.

17.2.2 After the Analysis Data Package has been reviewed, it is filed in the QA department filing boxes. Periodically the files are removed for archival. Refer to S0QA215.XXX for archiving records.

17.3 The data generated by the analytical instruments, Lims Raw Data (LRD), is archived on a monthly basis. Refer to S0LIM045.XXX for archiving LRD.

18.0 Maintenance:

18.1 A maintenance log book is utilized to document repair on the instrument. It is imperative that these logs are kept up to date. Log books will be checked for completeness during the annual QA audit. Refer to SOP S0QA140.XXX for instrument maintenance.

18.2 Specific instrument maintenance information is located in the manufacturers instructions.

18.3 The instrument identification information is maintained in the FGL Quality Assurance Plan.

18.4 Tubing is replaced approximately every month but varies depending on sample load.

19.0 Pollution Prevention and Waste Disposal:

19.1 Where feasible, techniques have been used to reduce the amount of standards and reagents in order to minimize the amount of waste generated by this procedure. This is primarily through the proper management of reagents and standards (see sections 8.1 and 9.1) and the reduction of sample and reagents (in the proper ratios) used to perform the procedure.

19.2 Waste from this analysis meets standard sewage discharge requirements. Waste from the analysis may be disposed of down the sink while rinsing with tap water. Samples are kept for one month prior to disposal.

19.3 Used Cadmium is given to the Hazardous Waste Officer for proper segregation and disposal.

20.0 References:

20.1 *"Standard Methods for the Examination of Water and Wastewater,"* AWWA, 18th ed, 1992, Method No. 4500-NO_3 F.

20.2 *"Addendum to the Nitrate and Nitrite in Water and Wastewater Method,"* Westco Scientific Instruments, 189-B001-02.

21.0 Associated Documents:

21.1 S0QA030.XXX - SOP for Standard/Reagent Preparation and Documentation

21.2 S0QA035.XXX - SOP for Non-conformance/Corrective Action Program

21.3 S0QA060.XXX - SOP for Performing MDL/IDL Studies

21.4 S0QA090.XXX - SOP for Data Reduction and Recording

21.5 S0QA095.XXX - SOP for Qualifying Data

21.6 S0QA105.XXX - SOP for Review of Data Packages by Analysts and Manager

21.7 S0QA135.XXX - SOP for Glassware and Plasticware Cleaning

21.8 S0QA140.XXX - SOP for Instrument Maintenance

21.9 S0QA165.XXX - SOP for Spiking, Diluting and Homogenizing

21.10 S0QA185.XXX - SOP for Analyst Demonstration of Proficiency

21.11 S0QA215.XXX - SOP for Records Archiving, Retrieving and Disposal
21.12 S0LIM045.XXX-SOP for LRD Data Archiving
21.13 S0LIM055.XXX-SOP for PC Workstation Program

ATTACHMENT 2: Ammonia Analysis SOP

Standard Operating Procedure
Standard Method 4500-NH_3 BG
Ammonia as N in Drinking Water, Wastewater and Solids
by Distillation and Technicon AA2 - Flow Injection Analysis

APPROVAL: The undersigned has verified that this SOP is complete and meets requirements specified in document S0QA010.XXX:

Wet Chemistry Manager:_____ Date:_____

Quality Assurance Director:_____ Date:_____

1.0 Scope and Application:

1.1 This is a distillation and flow injection analysis (FIA) method applicable to the determination of Ammonia as N in drinking waters, wastewaters, and solids.

1.2 The specific list of Practical Quantitation Limits (PQL) for each matrix is maintained in the FGL LIMS.

1.3 Prior to the use of this procedure FGL employees must perform and document an initial demonstration of proficiency for the method and LIMS. For continued use, annual method proficiency must be documented. Refer to S0QA185.XXX for employee training.

2.0 Summary of Method:

2.1 Summary:

2.1.1 The ammonia, as ammonium hydroxide, is released from samples by means of a distillation operation under basic conditions and absorbed in a scrubber containing sulfuric acid. The ammonia in the absorbing solution is then determined colorimetrically by FIA.

2.1.2 The ammonia is paristaltically introduced to the flow injection analysis system. The ammonia then reacts with the sodium potassium tartrate, bleach and phenate to form a blue color.

2.1.2 The concentration is quantified using a five point standard curve read colorimetrically at XXX nm.

2.2 Definitions:

2.2.1 Definitions are listed in the FGL Quality Assurance Plan, Section 17.0.

2.3 Method Modifications:

2.3.1 This SOP has used a reduction of sample and reagents (in the correct ratios) from the published test method.

3.0 Interferences:

3.1 All glassware must be properly cleaned before use. Refer to S0QA135.XXX for glassware cleaning.

3.2 The distillation is performed to remove interferences.

4.0 Safety:

4.1 A lab coat and gloves should be worn while performing analyses. Protective eyewear must be worn at all times. Refer to the individual MSDS's for safe handling of chemicals.

5.0 Sample Containers, Preservation and Handling:

5.1 Samples should be collected in 1 pint plastic containers.
5.2 Sample preservation is 2 mL 1+1 H_2SO_4 and refrigeration at 4˚ C.
5.3 The holding time is 28 days.
5.4 Procedures for sampling are maintained in the FGL field services department.
5.5 Procedures for sample shipment are maintained in the FGL shipping department.

6.0 Equipment and Supplies:

6.1 Technicon autoanalyzer using 90 sec. cam with IBM compatible PC
Rapid distillation apparatus
Autosampler tray with 2 mL vials

7.0 Quality Control:

7.1 **Preparation Quality Controls:** the batch size for extraction is 20 samples.
7.1.1 Method Blank: one method blank is run per batch.
7.1.2 Laboratory Control Sample (LCS): one LCS is run per batch.
7.1.3 Matrix Spike (MS): a set of duplicate MS's are run per batch.
7.2 **Analysis Quality Controls:** the batch size for analysis is 10 samples.
7.2.1 Initial/Continuing Calibration Blank (ICB/CCB): the ICB is run after the calibration and the CCB is run after every analytical batch and at the end of the analytical run.
7.2.2 Initial/Continuing Calibration Verification (ICV/CCV): the ICV is run after the calibration and the CCV is run after every analytical batch and at the end of the analytical run.
7.3 **Detection Limit Quality Controls:**
7.3.1 Method Detection Limit (MDL) determination: MDL's are run on an annual basis for both aqueous and solid matrices. For MDL guidance please see SOP S0QA060.XXX.

8.0 Reagent Preparation:

8.1 All reagents received and prepared must be properly labeled and recorded in the LIMS electronic reagent/standard preparation log. Login the reagent using FGL LIMS, Laboratory, Wet Chem, Standards. In order to minimize reagent waste, an appropriate quantity of reagent should be created or purchased which will be completely used during the lifetime of the reagent. Refer to S0QA030.XXX for proper reagent preparation and documentation.
8.2 Record the reagent code for all reagents on the prep sheet.
8.3 Distillation:
8.3.1 0.04 N H_2SO_4: dilute 1.2 mL of conc. H_2SO_4 to 1000 mL in a volumetric flask with deionized water.
8.3.2 50% NaOH: dissolve 500 g of NaOH pellets and add 1000 mL of deionized water. This is an extremely exothermic reaction. This procedure should be carefully performed under a hood. Allow ample time for solution to cool; preferably, prepare the reagent a day or two in advance.
8.3.3 Borate Buffer: 88 mL of 0.01 in NaOH + 9.5 g $Na_2B_4O_7$ x 10 H_2O to 1000 mL of deionized water.
8.4 Analysis:
8.4.1 25% Bleach: dilute 250 mL of bleach to 1000 mL with deionized water.
8.4.2 Phenol/NaOH color reagent: dissolve 100 g NaOH pellets in 400 mL of deionized water. Add 278 mL

of liquid phenol very slowly. Fill up to 1000 mL with deionized water and store in amber bottle.

8.4.3 EDTA/NaOH reagent: dissolve 6 pellets of NaOH in 400 mL deionized water. Add 50g of EDTA and let it dissolve completely. Fill up to 1000 mL of deionized water. Store in amber bottle.

8.4.4 Sodium Nitroprusside: dissolve 0.5 g of Sodium Nitroprusside in 1000 mL of deionized water. Store in amber bottle.

9.0 Standard Preparation:

9.1 All standards received and prepared must be properly labeled and recorded in the LIMS electronic reagent/standard preparation log. The LIMS then maintains the specific concentrations for each standard mix and subsequent dilutions. Login the standard using FGL LIMS, Laboratory, Wet Chem, Standards. In order to minimize standard waste, an appropriate quantity of standard should be created or purchased which will be completely used during the lifetime of the standard. Refer to S0QA030.XXX for proper standard preparation and documentation.

9.2 Record the standard code for all standards on the prep sheet. Also record the volumes of standards used and their concentrations.

9.3 Primary Calibration Standard:

9.3.1 Stock: (Ammonia-N 1000 mg/L): purchase from current vendor. Store in refrigerator.

9.3.2 Intermediate: (Ammonia-N 50.0 mg/L): dilute 5 mL of stock standard 9.3.1 to 100 mL of 0.04 N H_2SO_4.

9.3.3 Working: undistilled standards for standard curve: independently dilute 0, 0.25, 0.5, 1.0, and 2.5 mL of stock standard 9.3.1 to 500 mL with 0.04 N H_2SO_4 to make corresponding concentrations of 0, 0.5, 1.0, 2.0 and 5.0 mg/L.

9.4 Secondary Calibration Verification Standard: **this standard must be from either a different lot or supplier than the Primary calibration standard.**

9.4.1 Stock: (Ammonia-N 1000 mg/L): purchase from current vendor. Store in refrigerator.

9.4.2 Working: (Ammonia-N 2.0 mg/L): dilute 1.0 mL of stock standard (9.4.1) to 500 mL of 0.04 N H_2SO_4.

10.0 Sample Preparation:

10.1 **LIMS Batching:**

10.1.1 Batch the samples for preparation using FGL LIMS, Laboratory, Wet Chem, Preparation. Completely fill in all information requested on the prep sheet. All sample observations are noted in the comments column.

10.2 **Liquid QC Sample Preparation:**

10.2.1 Method Blank: to the distillation chamber add 25 mL amount of 0.04 N H_2SO_4.

10.2.2 LCS: to the distillation chamber add 25 mL of working standard (9.4.2).

10.2.3 MS/MSD: to the distillation chamber add a 24 mL aliquot of sample then add 1.0 mL of intermediate standard (9.3.2). This is performed in duplicate.

10.2.4 Continue the QC samples with step 10.3.2.9.

10.3 **Solids QC Sample Preparation:**

10.3.1 Method Blank: to a 4 oz plastic container add 40 mL of KCl.

10.3.2 LCS: to a 4 oz plastic container add 2 mL of working standard (9.4.2) and dilute to 40 mL with KCl.

10.3.3 MS/MSD: to duplicate a 4 oz plastic container add 10 g aliquots of sample then add 2 mL of working

standard (9.4.2) and dilute to 40 mL with KCl.

10.3.4 Continue the QC samples with step 10.3.2.9.

10.4 **Sample Preparation:**

10.4.1 Refer to S0QA165.XXX for guidance on obtaining a representative sample.

10.4.2 **Distillation procedure:**

10.4.2.1 Turn on cooling water and adjust to normal rate.

10.4.2.2 Check water level in boiling flask (steam reservoir). It needs to be 3/4 full.

10.4.2.3 Turn on heater (highest setting) and allow steam reservoir to come to a boil.

10.4.2.4 Place a waste cup under the outlet of the condenser.

10.4.2.5 Allow still to reach thermal equilibrium (4-5 mL/min).

10.4.2.6 Open wasteline stopcock and let sample chamber drain completely. (Don't forget to close wasteline after draining, otherwise you will lose your sample.)

10.4.2.7 Add 25 mL deionized water through the addition funnel into the sample chamber. Then add 10 mL of 50% NaOH. Let it come to a boil and continue for about 10 minutes to clean out the still out. After the still is cleaned, open the wasteline stopcock and let the sample chamber drain. Rinse the inside of the still and the outlet of the condenser about three times with deionized water.

10.4.2.8 Label and add 5 mL of 0.04 N H_2SO_4 to 50 mL centrifuge tubes. These are your sample receivers.

10.4.2.9 Neutralized 25 mL of sample to approximately pH 7 by adding sodium hydroxide. After neutralizing add 1.2 mL borate buffer then adjust the pH to 9.5. After adjustment transfer the sample to the addition funnel and let drain into sample chamber. pH Adjustment is not required for TKN digests - add the 25 mL digest directly to the sample chamber. Place your sample receiver under the condenser outlet so that the tip of the outlet is submerged into the 5 mL 0.04 N H_2SO_4. Rinse addition funnel about three times with a small amount of deionized water. Add 0.35 mL of 50% NaOH and rinse again three times with deionized water. 0.35 mL NaOH should be enough for ammonia samples to turn alkaline which is necessary for the ammonia distillation. Check samples before distilling for the amount of NaOH needed by using 25 mL of sample. Add NaOH until pH paper turns alkaline ($pH \geq 10$). Close the stopcock and fill additional funnel up with deionized water. Open stopcock for a short time so that deionized water stands in the line to the sample chamber. Distill for about 4 minutes. Lower sample receiver so that the condenser tip is now above the sample level. Let distill to 25 mL total volume. If you go over 25 mL note final volume on batch sheet.

10.4.2.10 Drain sample chamber completely and rinse three times with deionized water. Rinse condenser outlet with deionized water after every sample.

10.4.2.11 Fill steam reservoir up to 3/4 with deionized water if necessary and wait until still reaches thermal equilibrium. The still is then ready for next sample.

10.4.3 **Soil Extraction:**

10.4.3.1 If volatile analysis is also required for the sample, it must be returned to the volatiles refrigerator ASAP and if in a brass tube, mark an X on the end that was opened.

10.4.3.2 If the sample is free of any solid (e.g. rocks or glass) particles >2 mm, simply break up any clumps, mix well, and weigh into an acid cleaned beaker. Any semi-solid particles should be crushed.

10.4.3.3 Thoroughly mix the sample.

10.4.3.4 Weigh 10 g of soil into a 4 oz plastic container and add 40 mL 2 N KCl.
10.4.3.5 Put on shaker for one hour.
10.4.3.6 The extract is then distilled as a listed in section 10.3.2.

11.0 Calibration:

11.1 **Frequency:**

11.1.1 The calibration is performed at the beginning of each analytical run or when continuing calibration verifications indicate a new calibration is required.

11.2 **Procedure and Calculation:**

11.2.1 Prepare standards as outlined in section 9.3.3. The ammonia-N standards do not require distillation.
11.2.2 Analyze standards as outlined in sections 12.2.
11.2.3 The calibration calculation is performed by the instrument and must be reviewed by the analyst.

11.3 **Acceptance Criteria:**

11.3.1 The criteria for passing this calibration is the correlation coefficient has to be greater than 0.995.

11.4 **Failure Resolution:**

11.4.1 If the failure can be corrected by performing instrument maintenance and/or recalibration then initiate the correction. Log the instrument maintenance performed.
11.4.2 If you are unsure of how to handle the failure please contact your supervisor or the QA director.
11.4.3 Refer to S0QA035.XXX if it is determined that a nonconformance and/or corrective action are required.

12.0 Analytical Procedure:

12.1 **LIMS Batching:** batch the samples for analysis using FGL LIMS, Laboratory, Wet Chem, Analysis.

12.2 **Technicon Start-up:**

12.2.1 Turn lamps on and let warm up for at least 1/2 hour.
12.2.2 Check 5 gal. waste container and inform lab safety manager if it needs emptying.
12.2.3 Check reagent and water containers, refill if necessary.
12.2.4 Let reagents run though tubes at least 10 min. before using instrument . To do this, unclip water lines at water container, put tubes marked with the reagent in the corresponding reagent bottle.

12.3 **Sample Analysis:**

12.3.2 Fill out prep sheet with vial positions and dilutions. Record all codes for any standards spikes, or external reference checks used in this analysis. The outside circle is Channel 4. Therefore, even out the batches so that the channel runs are even in length. The 5 mg/l standard is placed in positions 1, 2, and 3 in the tray. The 2, 1 and 0.5 mg/l standards are placed into positions 4 5 and 6 respectively. Spaces 7 and 8 are for two blanks (the second blank is the zero standard). Next the ICB is in position 9, the ICV is position 10, then the Distillation blank and LCS in position 11 and 12. The MS/MSD's are then put in 13 and 14. Then continue with samples and CCB's and CCV's every 10. Blanks may be inserted between very high and low samples to eliminate carryover. After samples are poured and placed on the tray, 2 more positions are used for a the final CCB and CCV.

Position	Sample	Position	Sample
1,2,3	Standard 5 ppm	9, 10	ICV
4	Standard 2 ppm	11	Method Blank
5	Standard 1 ppm	12	LCS
6	Standard 0.5 ppm	13	MS
7	0 for curve	14	MSD
8	ICB	15+	samples with QC as needed

12.3.3 At the Technicon computer, use ESC to change screens, ALT F4 to change channels, and ALT 4 to turn the channels ON/OFF. (Be careful not to confuse these). F4 will bring up the sample table. Use ALT F to call up a file, and ALT L to load it. A basic AMMONIA file outline is under C:\DP4\DATA\WLNH4. The standards should be labeled as such: S15, S3, S2, S1 and S7 for standards 5, 2, 1, 0.5 and 0. Note: only the third 5 mg/L standard is to be labeled S15. To type in the sample schedule (example: for January 2, 1999) the naming convention is as such: P010200A BLK (for the method blank), P0102OOA LCS (for the LCS). The matrix spike ID's for a sample that has a Santa Paula lab number of SP50012301, on batch A, would be typed in as"P012301A MS" and “P012301A MSD”. Stockton samples use T, and Visalia use V. Also enter the correct dilutions in the dilution column.

12.3.4 Use ALT 4 to start the channels. F5 and F3 will allow you to view the graph. Adjust the gain by running the standards through first. The highest standard should be between 85-100% of the screen . Use the gain to adjust this while the high standard is running. Make sure the base line is between 6.0 and 7.0 and is straight and stable. Make the necessary adjustments and then turn the channels off to clear the baseline.

12.3.5 Turn the channels back on after several seconds, and then press the red button on the autosampler to turn it on. The cam should be turning. Wait to see that the first sample is picked up.

12.3.6 After the run is completed, turn the channels off and put the reagent tubes in the D.I. rinse water, allowing it to rinse for 10-15 minutes before disconnecting the pump and reclipping the tubes.

12.3.7 To calculate the data use F8. Then F8 again and calculate data from raw data file, press enter, and enter file name. It should bring up the graph of your AMMONIA samples. F2 is used to print the graph. Use F9 to continue to the standard graph. Make sure the correlation coefficient is at least 0.995. Use F9 again to view the schedule file and calculations. F2 will print to LPT1, and use F2 again to print to the LIMS system with W:\LABTRONC\T-0102-1.NH3. (for January 2, 1999 and first NH3 run). Use F9 to Save and Exit, do not overwrite existing file, but save as C:\ DP4\DATA\T-0102-1.DAT and then save to the calibration file.

12.3.8 Repeat step 8 for channel 4. Switch to channel 4 by using ALT F4. Save the data to the LIMS by printing the channel to the LIMS system. Use the following naming convention: for the third file for January 2, use W:\LABTRONC\T-0102-3.NH3 and save the data as C:\DP4\DATA\T-0102-3.DAT.

12.3.9 Use extension .TKN for electronic TKN files.
12.3.10 Process the data using the LIMS.

13.0 Calculations:

13.1 The analysis results including analysis dilutions are automatically calculated and expressed as mg/L values by the instrument.
13.2 All other dilutions are entered into the LIMS and calculated by the LIMS. The LIMS also corrects for the unit changes to mg/L or mg/Kg.

14.0 Data Assessment and Failure Resolution:

14.1 All current Data Quality Objectives (DQO's) for quality controls listed below are maintained in the FGL LIMS and automatically checked during data assessment. Qualify the data using FGL LIMS, Laboratory, Wet Chem, Analysis or Prep.

14.2 **Preparation Quality Controls:**

14.2.1 Method Blank: the method blank must be less than the Practical Quantitation Limit used for reporting (PQL).
14.2.2 LCS: the percent recovery for the LCS must be within the FGL acceptance range based on the control chart.
14.2.3 MS/BS: the percent recovery and the relative percent difference of the duplicate spikes should be within the FGL acceptance range based on the control chart.

14.3 **Analysis Quality Controls:**

14.3.1 ICB/CCB: the ICB/CCB must be less than the instrument reporting limit (IRL).
14.3.2 ICV/CCV: the ICV/CCV must be within the FGL acceptance range of 90-110%.

14.4 **Detection Limit Quality Controls:**

14.4.1 MDL: the MDL must be lower than the PQL.

14.5 **Failure Resolution:**

14.5.1 If the failure can be corrected by repreparation and/or reanalysis then initiate the correction.
14.5.2 If the failure can't be corrected by repreparation and/or reanalysis or if you are unsure of how to handle the failure please contact your supervisor or the QA director. If the data must be accepted with a failure, an explanation must accompany the failure. Refer to S0QA095.XXX for guidance on proper handling of failures.
14.5.3 Refer to S0QA035.XXX if it is determined that a nonconformance and/or corrective action are required.

15.0 Method Performance:

15.1 MDL studies are used to determine precision, accuracy and sensitivity for evaluation of performance at the reporting level. MDL's are electronically stored in the LIMS and a hard copy is maintained in the QA department.
15.2 Controlcharts are used for analysis and preparation quality controls to determine precision and/or accuracy on an ongoing basis for evaluating the performance of the method. Control charts are electronically maintained in the LIMS. Where applicable the control charts are used to generate the data quality objectives.

15.3 Performance Testing (PT) through interlaboratory studies is performed semi-annually (where applicable) to independently verify performance. PT Study results are maintained in the QA department.

15.4 Refer to the referenced test method(s) for published interlaboratory method performance.

16.0 Review and Reporting:

16.1 Preparation Review and Reporting:

16.1.1 Preparation Data Packages are compiled, reviewed, signed and dated by the analyst. They are then turned in to the supervisor or peer for review. Refer to S0QA105.XXX for data package review.

16.1.2 Samples are reported in mg/L or mg/Kg units. They are printed on the Prep Summary Report of the Preparation Data Package and electronically maintained in the FGL LIMS. Refer to S0QA090.XXX for data reduction and recording.

16.2 Analysis Review and Reporting:

16.2.1 Analysis Data Packages are compiled, reviewed, signed and dated by the analyst. They are then turned in to the supervisor or peer for review. Refer to S0QA105.XXX for data package review.

16.2.2 Samples are analyzed in mg/L units. They are printed on the Raw Data for instrumental methods or the Analysis Summary Report for manual methods and electronically maintained in the FGL LIMS. Refer to S0QA090.XXX for data reduction and recording.

17.0 Record Storage and Archiving:

17.1 Preparation Records:

17.1.1 The records generated during preparation are Preparation Package Narrative, Prep Confirmation Report, Quality Control Reports, Prep Summary Report and Prep Worksheet. These all form the Prep/Extraction Data Package.

17.1.2 After the Preparation Data Package has been reviewed, it is filed in the Wet Chemistry department filing cabinet. Periodically the files are removed for archival. Refer to S0QA215.XXX for archiving records.

17.2 Analysis Records:

17.2.1 The records generated during analysis are Analysis Package Narrative, Analysis Confirmation Report, Quality Control Reports, Analysis Runlog and Raw Data or Analysis Summary Report. These all form the Analysis Data Package.

17.2.2 After the Analysis Data Package has been reviewed, it is filed in the Wet Chemistry department filing cabinet. Periodically the files are removed for archival. Refer to S0QA215.XXX for archiving records.

18.0 Maintenance:

18.1 A maintenance log book is utilized to document repair on the instrument. It is imperative that these logs are kept up to date. Log books will be checked for completeness during the annual QA audit. Refer to SOP S0QA140.XXX for instrument maintenance.

18.2 Specific instrument maintenance information is located in the manufacturers instructions.

18.3 The instrument identification information is maintained in the FGL Quality Assurance Plan.

19.0 Pollution Prevention and Waste Disposal:

19.1 Where feasible, techniques have been used to reduce the amount of standards and reagents in order to minimize the amount of waste generated by this procedure. This is primarily through the proper management of reagents and standards (see sections 8.1 and 9.1) and the reduction of sample and reagents (in the proper ratios) used to perform the procedure.

19.2 Waste from this analysis meets standard sewage discharge requirements. Waste from the analysis may be disposed of down the sink while rinsing with tap water. Samples are kept for one month prior to disposal.

20.0 References:

20.1 *"Standard Methods for the Examination of Water and Wastewater,"* AWWA, 18th ed, 1992, Method No. 4500-NH_3 BG.

21.0 Associated Documents:

21.1 S0QA030.XXX - SOP for Standard/Reagent Preparation and Documentation
21.2 S0QA035.XXX - SOP for Non-conformance/Corrective Action Program
21.3 S0QA060.XXX - SOP for Performing MDL/IDL Studies
21.4 S0QA090.XXX - SOP for Data Reduction and Recording
21.5 S0QA095.XXX - SOP for Qualifying Data
21.6 S0QA105.XXX - SOP for Review of Data Packages by Analysts and Manager
21.7 S0QA135.XXX - SOP for Glassware and Plasticware Cleaning
21.8 S0QA140.XXX - SOP for Instrument Maintenance
21.9 S0QA165.XXX - SOP for Spiking, Diluting and Homogenizing
21.10 S0QA185.XXX - SOP for Analyst Demonstration of Proficiency
21.11 S0QA215.XXX - SOP for Archiving Records.

ATTACHMENT 3: Orthophosphate Analysis SOP

CRG MARINE LABORATORIES
2020 Del Amo Blvd, Suite 200, Torrance, Ca 90501 (310) 533-5190

DETERMINATION OF INORGANIC ANIONS BY ION CHROMATOGRAPHY

Approved by:

_____ _____

Richard Gossett, Laboratory Manager Date

METHOD 300.0

DETERMINATION OF INORGANIC IONS BY ION CHROMATOGRAPHY

REFERENCE: [US EPA Method 300.1 and Standard Methods 20th Edition-SM4110 C]

0.0 SCOPE AND APPLICATION

0.1 This method is used to determine the concentration of inorganic ions in water, seawater or sediments.

1.O SUMMARY OF METHOD

1.1 A drinking water, groundwater or wastewater sample is filtered and introduced to an ion Chromatograph (IC) instrument (Metrohm 761) containing a chromatography Column that separates the anions, and a conductivity cell that detects the ions. The detector output is recorded and integrated, and the integrated signals are compared to those from standard solutions of known anion concentrations to calculate the anion concentrations in the sample. Anions are identified by their characteristic retention times, also known from standard solutions. The following anions are calibrated for: Bromide, Chloride, Fluoride, Nitrate-Nitrogen, Nitrite-Nitrogen, Phosphate as PO4 and Sulfate as SO4. The Metrohm-peak operating manual must be read and understood prior to performing this method.

2.O SAFETY

2.1 Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section *3*.

2.2 It is mandatory to wear a laboratory coat, closed toe shoes, and safety glasses in the Laboratory. Gloves shall be worn while working with solvents.

2.3 All steps involving the use of large volumes of solvents shall be performed in a fume hood.

2.4 Material Safety Data Sheets (MSDS) are on file and available at all times to personnel using hazardous materials. It is the

responsibility of everyone using these materials to be familiar with the potential hazards of the chemicals in their work area. If the analyst is uncertain of the potential hazards of specific chemicals, contact the supervisor prior to using the chemicals.

2.5 Extreme caution, awareness and knowledge of the location and safe use of fire extinguishers, eye wash fountains, and safety showers are required.

3.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

3.1 Samples should be collected in plastic containers. There is no chemical preservative added.

3.2 Samples must be kept at 4 degrees C during storage prior to analysis.

3.3 The holding time varies by analyte, as follows: Nitrate 48 hrs, Nitrite 48 hrs, Chloride 28 days, Sulfate 28 days, Bromide 28 days and Fluoride 28 days.

4.0 STANDARDS

4.1 Stock standards are stable for at least one month at 4 °C (refrigerator temperature).

4.2 Prepare dilute working standards daily, as nitrite is relatively unstable. The working standards are used to run calibrations, prepare spikes, etc., as detailed below.

4.3 STOCK STANDARD: These are bought ready made from vendors (Fischer).

Fluoride as F	100 ppm
Chloride as Cl	1000 ppm
Nitrite as N	1000 ppm
Bromide as Br	1000 ppm
Nitrate as N	1000 ppm
Phosphate as PO4	1000 ppm
Sulfate as SO4	1000 ppm

4.4 WORKING STANDARDS: Pipette an appropriate volume of each of the six stock standards into a single volumetric flask and dilute to the mark with reagent water. This yields a solution containing all six anions. The concentrations typically used are listed in the last column of the table above. Prepare four dilutions of this concentrated solution to yield a total of five working standards. The dilutions typically yield anion concentrations that will be 0.01, 0.05, 0.1, 0.5,2 and 10mg/L. These concentrations span two orders of

magnitude, giving a sufficiently large calibration range. The calibration is performed at least once per quarter.

4.5 Calibration Verification Solution: A second source of the chemicals in the table above (purchased from a different manufacturer) are prepared as above and used to verify the calibration standards

5.0 REAGENTS

5.1 It is important that all solutions for ion chromatography be prepared using deionized water, free of the anions of interest. Use deionized water of Resistivity of at least 16.67 mS/cm, as per manufacturer specifications. All reagents must be of high quality (>99% pure), dried at 105 °C for 60 min prior to use, and stored in a desiccator.

5.2 Carbonate-Bicarbonate (2.5 mM sodium carbonate, Na2CO3/ 2.4 mM sodium bicarbonate, NaHCO3)

5.3 To prepare eluent, use an analytical balance to weigh out 0.339g Na2CO3 and 0.084g NaHCO3. Next transfer the weighed salts analytically to a 1 L volumetric flask and dilute to the mark, and then transfer the solution to the eluent reservoir. Carbonate and bicarbonate solutions are sensitive to atmospheric carbon dioxide and will change composition over time, store the eluent away from air and use the solution promptly. This is a minor concern and, considering the projected daily consumption of eluent, should not affect the analyses.

6.0 APPARATUS

6.1 Syringe with filter disks, 0.45 µm pore size

6.2 Analytical balance

6.3 Metrohm-peak ion chromatogram, whose components include:

- 6.3.1 Eluent reservoir bottle
- 6.3.2 Regenerant reservoir bottle
- 6.3.3 Delivery pump, to deliver a constant flow of eluent at moderate pressure
- 6.3.4 Auto sampler, to introduce the sample to the instrument
- 6.3.5 Sample loop (100 µL), to conduct the sample to the columns
- 6.3.6 Guard column, to protect the separator column from particulates and organics
- 6.3.7 Separator (analytical) column (6.1006.020 metrosep anion dual 1), to separate the anions by selective adsorption
- 6.3.8 Suppressor (chemical suppression), to convert the eluent and analyte anions to their acid forms
- 6.3.9 Conductivity detector (digital output), to quantify the analytes
- 6.3.10 Waste bottle, to collect the effluent
- 6.3.11 Recorder (software), to note the output of the detector for further processing

6.3.12 Integrator (software), to process the recorded signals to yield meaningful data
6.3.13 Volumetric flasks, pipettes
6.3.14 All glassware used should be class A

7.0 PROCEDURE

7.1 To set up instrument, verify that the eluent is flowing, and that the baseline detector output is steady (13-16 µS for eluent).

7.2 Using the software, prepare a sample schedule (sequence) to be run. For each sample choose the appropriate method. This method file contains the system parameter settings established for the particular system. Analyze the initial calibration standards described above. The correlation coefficient should be 0.995 or better for each analyte. If not, correct the problem and re-analyze the initial calibration. If the continuing calibration standards do not meet the criteria found in the QC Section of this SOP, the initial cal must be repeated.

7.3 To prepare the samples, first filter about 5 mL of each using the syringe + filter disk. This prolongs column life and keeps the sample path clean by removing sizeable particles. Next fill each sample vial with about 5 mL of sample and load the vials in the auto sampler according to the schedule already defined. To verify the operation of the system, we include calibration standards, blanks and spikes as described in the Quality Control section below.

7.4 Before starting the run, make sure that the eluent flow and column pressure readings are appropriate (13-16 µS and 4.5-6.5 psi respectively).

7.5 Initiate the run. As defined in the selected method, the system parameters are controlled by the software. In the ICnet 2.3 main menu, click on the systems icon.

7.5.1 From the file dropdown menu, click on open and choose method.

7.5.2 From the systems window, click on control and choose startup hardware (measure baseline). This allows the instrument to warm up. Let it warm up for at least 20 minutes or until the baseline is stable.

7.5.3 From the systems window, click on system and choose a sample queue. Make sure that all samples have the correct method assigned.

7.5.4 Type sample IDs in the sample column. When calibration standard is chosen, Level 1,2,3,4, 5or 6 must be selected under the level column. Save the changes on the queue and finally check the box that says, “Shut down after queue finishes”. When baseline is stable, click on “start” on the sample queue window.

7.5.5 When the run is complete, the system settings are set automatically to inactive status as defined in the method.

8. CALCULATIONS

8.1 The software is highly automated and does all the calculations. After reviewing the output (chromatograms and tabular integration and quantification results), reprocess the raw data using the IC-net 2.3 software.

8.2 The signals for each of the five calibration standards are integrated. Baseline Subtraction is automatic. Standards are designated as such, with their analyte Concentrations, in the schedule.

8.3 A linear calibration is performed using the 5 data points for each anion. If the Correlation coefficient R <0.995, the calibration data are rejected and the process is repeated.

8.4 The slope and intercept of each line are used to interpret the signals for a given analyte in the samples of that run. In this manner area counts (in μS·min) are converted to concentrations (in mg/L). Analytes are identified by retention time; the software allows some flexibility in these times, as they vary slightly from sample to sample. The separations are quite good, so generally there is no difficulty in associating a signal with the proper anion.

9. QUALITY CONTROL

9.1 Analyze a laboratory reagent blank sample after every 10 samples, to verify the baseline reading. The blank sample is the same deionized water used to prepare the eluent and all solutions. All blank samples must contain less than ½ the reporting limit of all analytes. If this is not the case, all samples associated with the blank must be re-analyzed.

9.2 The initial calibration verification standard, prepared from a source different from the calibration, is analyzed after each calibration. All standards must meet 90-110 % recovery limits. If this is not the case, the calibration must be repeated.

9.3 Analyze an instrument performance check (CCV, “continuing cal check”) sample immediately after calibration, after every 10 samples and at the end of the run. The ICV and CCV results must be 90–110% of the true value. If they are not passing, they must be repeated. If a second result is still outside of the limits, identify the cause, recalibrate and rerun the samples that have been analyzed since the last passable CCV check.

9.4 Analyze a matrix spike sample per batch of 20 samples. The recommended spike recovery is in the 80-120% range. The MS/MSD sample is prepared similar to the LCS/LCSD sample, except that a suitable sample is used as the diluent, instead of deionized water. Using the same equation as in **2)** above, except that the background concentration B is the result obtained from the

prior analysis of the unspiked sample. No specific corrective action is taken if the MS recovery is not 80-120 %; however the analyst must make certain that systematic spike failures are not occurring due to changes in spiking solutions, etc.

9.5 Analyze a sample duplicate every batch of 20 samples. The duplicate RPD should be +/- 20 %. No specific corrective action is taken if the RPD criterion is not met, however the analyst must make certain that systematic failures are not occurring. This may indicate that the IC instrument is not functioning properly and that maintenance is needed.

9.6 Annually (or if a significant system change occurs, *e.g.* moving the entire setup) perform an MDL study by analyzing 7 replicate samples of 0.01 ppm standard over 3 days.

10.O DATA ASSESSMENT AND CORRECTIVE ACTIONS

10.1 All sample results must be evaluated for adherence with the QC requirements discussed in the preceding section.

10.2 No samples should be analyzed without demonstrating that the blank is interference free (concentration is below 1/2 the reporting limit). A blank is analyzed every 10 samples. Those samples that are associated with a contaminated blank must be reanalyzed.

10.3 Evaluation of continuing calibration standard (CCV) data: evaluation of the performance of this analytical method is primarily controlled by the recovery that is obtained in the CCV sample. A CCV is analyzed every 10 samples and the standard is prepared from a source different from the one used for calibration. Since there are no matrix interferences present in the CCV, the recoveries should always fall within the 90-110 % limits. Samples that are associated with a CCV having lower than expected recoveries must be re-analyzed. If the CCV recovery exceeds criteria and no analyte is detected in the sample, then the data is acceptable without re-analysis since the bias was high and the sample was non-detect. Corrective action for CCV recoveries outside of criteria most often would be re-calibration. If this does not correct the problem, prepare the CCV solution afresh.

10.4 Initial Calibration: Each analyte must first have an acceptable initial calibration. The calibration must contain the appropriate number of standards for the method and should cover the expected linear concentration range of the method with the lowest standard close to or at the reporting limit and the highest standard near the linear range.

10.5 Samples that have analyte concentrations exceeding the highest standard must be diluted to bring the concentration near the middle of the calibration. There are several calibration scenarios: linear calibration (preferred), quadratic (used for non-linear calibrations)

and average response factor. A correlation coefficient of 0.995 to be considered acceptable. Corrective actions for calibrations which do not meet this requirement would be to drop the low or high level standard and add levels which cover a narrower range. It also may be necessary to prepare all standards afresh and repeat the calibration. This method always uses a linear calibration.

ATTACHMENT 4: TDS Analysis SOP

Standard Operating Procedure
Standard Method 2540 C
Total, Fixed and Volatile Dissolved Solids Dried at 180°C
in Drinking Water and Wastewater
by Gravimetric Analysis

APPROVAL: The undersigned has verified that this SOP is complete and meets requirements specified in document S0QA010.XXX:

Wet Chemistry Manager:_____ Date:_____

Quality Assurance Director:_____ Date:_____

1.0 Scope and Application:

1.1 This is a gravimetric method applicable to the determination of Total Dissolved Solids (TDS, total filterable residue) Fixed Dissolved Solids (FDS, Inorganic Dissolved Solids) or Volatile Dissolved Solids (VDS, Organic Dissolved Solids) in drinking water and wastewaters.

1.2 The specific list of analytes and their associated Practical Quantitation Limits (PQL) for each matrix is maintained in the FGL LIMS.

1.3 Prior to the use of this procedure FGL employees must perform and document an initial demonstration of proficiency for the method and LIMS. For continued use, annual method proficiency must be documented. Refer to S0QA185.XXX for employee training.

2.0 Summary of Method:

2.1 Summary:

2.1.1 Drinking waters and wastewaters are filtered through a glass fiber filter, Whatman Grade 934AH or equivalent. The filtrate is evaporated and the weight of residue is determined. The result is calculated from the volume of sample used and weight of residue found.

2.1.2 For fixed or volatile dissolved solids the TDS residue is heated at 500˚C and the remaining residue (FDS) or loss (VDS) is calculated.

2.2 Definitions:

2.2.1 Definitions are listed in the FGL Quality Assurance Plan, Section 17.0.

2.3 Method Modifications:

2.3.1 This SOP has used a reduction of sample and reagents (in the correct ratios) from the published test method.

3.0 Interferences:

3.1 All glassware must be properly cleaned before use. Refer to S0QA135.XXX for glassware cleaning.

3.2 Samples with high amounts of calcium, magnesium, chloride and sulfate may be hygroscopic and may require longer drying times. For these samples use a smaller aliquot of sample for analysis. Samples high

in undissolved solids should be filtered by vacuum for better efficiency.

3.3 Samples high in organics can not be compared with the TDS to EC ratio.

4.0 Safety:

4.1 A lab coat and gloves should be worn while performing analyses. Protective eyewear must be worn at all times. Refer to the individual MSDS's for safe handling of chemicals.

5.0 Sample Containers, Preservation and Handling:

5.1 Samples should be collected in 1 pint containers.
5.2 Sample preservation is refrigeration at 4˚ C.
5.3 The holding time is 7 days.
5.4 Procedures for sampling are maintained in the FGL field services department.
5.5 Procedures for sample shipment are maintained in the FGL shipping department.

6.0 Equipment and Supplies:

6.1 Filtration manifold using 60 mL disposable syringes
6.2 Whatman 934AH syringe filters, 1.5 u, 13cm (or equivalent)
6.3 Pretared glass 100 mL beakers
6.4 Pretared crucibles (for FDS or VDS only)
6.5 Oven, set at 90ºC for initial evaporation
6.6 Oven, set at 180ºC for final drying
6.7 Muffle furnace set at 500˚C (for FDS or VDS only)
6.8 Desiccator
6.9 Analytical balance

7.0 Quality Control:

7.1 **Preparation Quality Controls:** the batch size for extraction is 20 samples.
7.1.1 Method Blank: one method blank is run per batch.
7.1.2 Laboratory Control Sample/Continuing Calibration Verification (LCS/CCV): one LCS and one CCV are run per batch. The CCV is grouped with the prep information and essentially used as a duplicate LCS.
7.1.3 Duplicate: One duplicate is analyzed per batch.
7.2 **Detection Limit Quality Controls:**
7.2.1 Method Detection Limit (MDL) determination: The TDS MDL is run on an annual basis. Because of method similarities between TDS, FDS and VDS analyses the MDL from the TDS procedure is applied to FDS and VDS. For MDL guidance please see SOP S0QA060.XXX.

8.0 Reagent Preparation: N/A

9.0 Standard Preparation:

9.1 All standards received and prepared must be properly labeled and recorded in the LIMS electronic reagent/standard preparation log. The LIMS then maintains the specific concentrations for each standard mix and subsequent dilutions. Login the standard using FGL LIMS, Laboratory, Wet Chem, Standards.

In order to minimize standard waste, an appropriate quantity of standard should be created or purchased which will be completely used during the lifetime of the standard. Refer to S0QA030.XXX for proper standard preparation and documentation.

9.2 Record the standard code for all standards on the prep sheet. Also record the volumes of standards used and their concentrations.

9.3 Primary Calibration Standard:

9.3.1 Stock: reagent grade sodium chloride. Purchase from current vendor.

9.3.2 Working Standard (approx. 1000 mg/L): Weigh 1g of NaCl, record exact weight, and add to 1L volumetric partially filled with deionized water. Dissolve and adjust volume to 1 L with deionized water. The final concentration in mg/L is the weight recorded x 1000 x purity of .

10.0 Sample Preparation:

10.1 **LIMS Batching:**

10.1.1 Batch the samples for preparation using FGL LIMS, Laboratory, Wet Chemistry, Preparation. Completely fill in all information requested on the prep sheet. All sample observations are noted in the comments column.

10.2 **QC Sample Preparation:**

10.2.1 Method Blank: to the 60 syringe add 50 mL of deionized water.

10.2.2 LCS and CCV: to the 60 mL syringe add 50.0 mL of working standard (9.3.2).

10.2.3 Duplicates: to duplicate 60 mL syringes add 50.0 mL aliquots of sample.

10.2.4 Continue the QC samples with step 10.3.2.5.

10.3 **Sample Preparation:**

10.3.1 Refer to S0QA165.XXX for guidance on obtaining a representative sample.

10.3.2 **Sample filtering:**

10.3.2.1 For obtaining pretared beakers refer to SOP S2WCI180.XXX sections 12.1,12.2 and 12.3.

10.3.2.2 Place 6 beakers inside the vacuum manifold below each syringe mount.

10.3.2.3 Put the 934AH filter on a clean dry syringe, twist to lock and mount onto the vacuum manifold.

10.3.2.4 Pour at least 50 mL of sample into the syringe and tap out any air bubbles. Adjust the volume to the 50 mL mark with a pipet. This equates to 51 mL.

10.3.2.5 Initiate vacuum to pass the sample through the filter and into the beaker.

10.3.2.6 When filtering is complete, rinse the syringe 2 times with approximately 10 mL of deionized water each time.

10.3.2.7 When a filter clogs perform the following steps:

10.3.2.7.1 Disconnect the filter and syringe from the manifold. Dispense the remaining sample into a new filter and syringe. Keep the clogged filter and syringe.

10.3.2.7.2 The new filter and syringe is then mounted on the manifold and the vacuum initiated. When the filtering is complete the new filter and syringe are rinsed with deionized water. After rinsing the vacuum is turned off and the new filter and syringe are remove from the manifold.

10.3.2.7.3 The clogged filter is then reattached to the manifold and the vacuum initiated. The filter is then rinsed with deionized water to rinse remaining sample which was left in the clogged filter.

10.3.2.8 Carefully transfer the samples to oven set at 90°C for initial evaporation. When dry, set oven to 180 ± 2°C for at least one hour.

10.3.2.9 After drying, immediately place pans with filters in desiccator until cool. This is approximately one hour.
10.3.2.10 For postweighing procedures refer to SOP S2WCI180.XXX section 12.4.
10.3.2.11 Repeat steps 10.3.2.8 through 10.3.2.10.
10.3.2.12 Reweigh the dishes to verify the weights are within 0.0005g or 4% of each other (after subtracting the tare weight).
10.3.2.13 Repeat this drying and weighing cycle until the criteria in 10.3.2.12 are met.
10.3.2.14 For fixed or volatile solids place the sample in muffle furnace set at 500°C overnight, then cool sample in desiccator.
10.3.2.15 After ashing, immediately place pans with filters in desiccator until cool. This is approximately one hour.
10.3.2.16 For postweighing procedures refer to SOP S2WCI180.XXX section 12.4.
10.3.2.17 Repeat steps 10.3.2.14 through 10.3.2.16.
10.3.2.18 Reweigh the dishes to verify the weights are within 0.0005g or 4% of each other (after subtracting the tare weight). Record the second weight.
10.3.2.19 Repeat this drying and weighing cycle until the criteria in 10.3.2.18 are met.

11.0 Calibration:
11.1 Refer to SOP S0QA155.XXX

12.0 Analytical Procedure:
12.1 Refer to S2WCI180.XXX

13.0 Calculations:
13.1 The LIMS System calculates the concentration after the final weight, tare weight (already entered from **PREWT**), and sample volume are entered.
13.2 Total Dissolved Solids mg/L = $\frac{\text{(final beaker wt g - tare wt g) x 1000000}}{\text{mL sample}}$
13.3 Fixed Dissolved Solids mg/L = $\frac{\text{(final beaker wt g @ 500˚C - tare wt g) x 1000000}}{\text{mL sample}}$
13.4 Volatile Dissolved Solids mg/L = $\frac{\text{(final beaker wt g @ 105˚C - final beaker wt g @ 500˚C) x 1000000}}{\text{mL sample}}$

14.0 Data Assessment and Failure Resolution:
14.1 All current Data Quality Objectives (DQO's) for quality controls listed below are maintained in the FGL LIMS and automatically checked during data assessment. Qualify the data using FGL LIMS, Laboratory, Wet Chem, Analysis or Prep.
14.2 **Preparation Quality Controls:**
14.2.1 Method Blank: the method blank must be less than the Practical Quantitation Limit used for reporting (PQL).
14.2.2 LCS/CCV: the percent recoveries for the LCS and CCV must be within the FGL acceptance range

based on the control chart.

14.2.3 Duplicates: the relative percent difference (RPD) of the duplicates should be within the FGL acceptance range based on the control chart.

14.2.4 TDS to EC Check: the TDS to EC ratio should be within the 0.55 to 0.7. If it is not, the result is suspect and should be verified unless sample site historically has a different ratio or the sample is known to contain organics.

14.3 **Detection Limit Quality Controls:**

14.3.1 MDL: the MDL must be lower than the PQL.

14.4 **Failure Resolution:**

14.4.1 If the failure can be corrected by repreparation and/or reanalysis then initiate the correction.

14.4.2 If the failure can't be corrected by repreparation and/or reanalysis or if you are unsure of how to handle the failure please contact your supervisor or the QA director. If the data must be accepted with a failure, an explanation must accompany the failure. Refer to S0QA095.XXX for guidance on proper handling of failures.

14.4.3 Refer to S0QA035.XXX if it is determined that a nonconformance and/or corrective action are required.

15.0 Method Performance:

15.1 MDL studies are used to determine precision, accuracy and sensitivity for evaluation of performance at the reporting level. MDL's are electronically stored in the LIMS and a hard copy is maintained in the QA department.

15.2 Control charts are used for analysis and preparation quality controls to determine precision and/or accuracy on an ongoing basis for evaluating the performance of the method. Control charts are electronically maintained in the LIMS. Where applicable the control charts are used to generate the data quality objectives.

15.3 Performance Testing (PT) through interlaboratory studies is performed semi-annually (where applicable) to independently verify performance. PT Study results are maintained in the QA department.

15.4 Refer to the referenced test method(s) for published interlaboratory method performance.

16.0 Review and Reporting:

16.1 **Preparation Review and Reporting:**

16.1.1 Preparation Data Packages are compiled, reviewed, signed and dated by the analyst. They are then turned in to the supervisor or peer for review. Refer to S0QA105.XXX for data package review.

16.1.2 Samples are reported in mg/L units. They are printed on the Prep Summary Report of the Preparation Data Package and electronically maintained in the FGL LIMS. Refer to S0QA090.XXX for data reduction and recording.

16.2 **Analysis Review and Reporting:**

16.2.1 Analysis Data Packages are compiled, reviewed, signed and dated by the analyst. They are then turned in to the supervisor or peer for review. Refer to S0QA105.XXX for data package review.

16.2.2 Samples are analyzed in g units. They are printed on the Raw Data for instrumental methods or the Analysis Summary Report for manual methods and electronically maintained in the FGL LIMS. Refer to S0QA090.XXX for data reduction and recording.

17.0 Record Storage and Archiving:

17.1 **Preparation Records:**

17.1.1 The records generated during preparation are Preparation Package Narrative, Prep Confirmation Report, Quality Control Reports, Prep Result Summary and Prep Worksheet. These all form the Prep/Extraction Data Package.

17.1.2 After the Preparation Data Package has been reviewed, it is filed in the QA department filing boxes. Periodically the files are removed for archival. Refer to S0QA215.XXX for archiving records.

17.2 **Analysis Records:**

17.2.1 The records generated during analysis are Analysis Package Narrative, Analysis Confirmation Report, Quality Control Reports, Analysis Runlog and Raw Data or Result Summary Report. These all form the Analysis Data Package.

17.2.2 After the Analysis Data Package has been reviewed, it is filed in the QA department filing boxes. Periodically the files are removed for archival. Refer to S0QA215.XXX for archiving records.

18.0 Maintenance: N/A

19.0 Pollution Prevention and Waste Disposal:

19.1 Where feasible, techniques have been used to reduce the amount of standards and reagents in order to minimize the amount of waste generated by this procedure. This is primarily through the proper management of reagents and standards (see sections 8.1 and 9.1) and the reduction of sample and reagents (in the proper ratios) used to perform the procedure.

19.2 Waste from this analysis meets standard sewage discharge requirements. Waste from the analysis may be disposed of down the sink while rinsing with tap water. Samples are kept for one month prior to disposal.

20.0 References:

20.1 *"Standard Methods for the Examination of Water and Wastewater,"* AWWA, 18th ed, 1992, Method No. 2540 C.

21.0 Associated Documents:

21.1 S0QA030.XXX - SOP for Standard/Reagent Preparation and Documentation
21.2 S0QA035.XXX - SOP for Non-conformance/Corrective Action Program
21.3 S0QA060.XXX - SOP for Performing MDL/IDL Studies
21.4 S0QA090.XXX - SOP for Data Reduction and Recording
21.5 S0QA095.XXX - SOP for Qualifying Data
21.6 S0QA105.XXX - SOP for Review of Data Packages by Analysts and Manager
21.7 S0QA135.XXX - SOP for Glassware and Plasticware Cleaning
21.8 S0QA140.XXX - SOP for Instrument Maintenance
21.9 S0QA165.XXX - SOP for Spiking, Diluting and Homogenizing
21.10 S0QA185.XXX - SOP for Analyst Demonstration of Proficiency
21.11 S0QA215.XXX - SOP for Records Archiving, Retrieving and Disposal

ATTACHMENT 5: Organophosphate and Pyrethroid Pesticide Analysis SOP

CRG MARINE LABORATORIES
2020 Del Amo Blvd., Suite 200, Torrance, CA 90501 (310) 533-5190

SEPARATORY FUNNEL LIQUID-LIQUID EXTRACTION AND ANALYSIS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY

Approved by:

_____ _____

Richard Gossett, Laboratory Manager Date

METHOD 625:

SEPARATORY FUNNEL LIQUID-LIQUID EXTRACTION AND ANALYSIS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY

REFERENCES: U.S. EPA 40CFR Part 136

1.0 SCOPE AND APPLICATION

1.1 This method covers the extraction and concentration procedures required for the determination of chlorinated pesticides and PCBs and semi-volatile base/neutral and acid-extractable compounds in Laboratory Operating Procedure Methods (LOPM) 625. It is applicable to liquid samples.

1.2 The glassware cleaning procedure for extraction glassware is listed in this method.

Table 1. Target Compound List

COMPOUND	RETENTION TIME DB-5 COLUMN	METHOD DETECTION LIMIT (µg/kg)
D5-Phenol (Recovery Surrogate)	8.00	*
Naphthalene-d8 (Recovery Surrogate)	13.34	*
2-Fluorophenol (Recovery Surrogate)	15.00	*
Acenaphthene-d10 (Recovery Surrogate)	21.49	*
2,4,5,6-Tetrachloro-m-xylene (Recovery Surrogate)	26.45	*
2,4,6-Tribromophenol (Recovery Surrogate)	27.17	*
PCB030 (Recovery Surrogate)	31.66	*
Phenanthrene-d10 (Recovery Surrogate)	32.29	*
Anthracene-d10 (Internal Standard)	32.84	*
PCB112 (Recovery Surrogate)	46.29	*
2,2'-5,5'-Tetrabromobiphenyl (Internal Standard)	52.07	*

Chrysene-d12 (Recovery Surrogate)	56.25	*
PCB198 (Recovery Surrogate)	60.57	*
Perylene-d12 (Recovery Surrogate)	68.49	*
Phenol	8.21	100
2-Chlorophenol	10.56	50
Aniline	11.37	100
2,4-Dimethylphenol	11.11	100
bis(2-Chloroethoxy) methane	11.44	50
1,3-Dichlorobenzene	11.98	10
1,4-Dichlorobenzene	12.13	10
1,2-Dichlorobenzene	12.39	10
Benzyl Alcohol	12.23	100
2-Nitrophenol	12.41	100
bis(2-Chloroisopropyl) ethane	12.61	50
2,4-Dichlorophenol	12.83	50
N-nitrosodi-n-propylamine	12.95	50
N-nitrosodimethylamine	12.97	50
Hexachloroethane	13.15	50
Nitrobenzene	13.32	50
Naphthalene	13.39	1.0
Isophorone	13.99	50
Dichlorvos	14.52	10
bis(2-Chloroethyl) ether	14.59	50
1,2,4-Trichlorobenzene	14.81	10
Benzidine	15.31	50
4-Chloro-3-methylphenol	15.59	100
4-Chloroaniline	15.74	50
2-Methylnaphthalene	15.98	1.0
Hexachlorobutadiene	16.24	50
1-Methylnaphthalene	16.39	1.0
2,4,6-Trichlorophenol	17.42	50
Benzoic Acid	17.70	100
Biphenyl	18.30	1.0
2,6-Dimethylnaphthalene	19.05	1.0
Mevinphos	19.55	10
Hexachlorocyclopentadiene	19.83	50
Acenaphthylene	20.47	1.0
Dimethyl Phthalate	20.56	5
Acenaphthene	21.74	1.0
4-Nitroaniline	21.82	50

2,4-Dinitrophenol	22.27	100
4-Nitrophenol	23.30	100
2,3,5-Trimethylnaphthalene	24.42	1.0
3-Nitroaniline	24.57	50
Fluorene	25.18	1.0
Diethyl Phthalate	25.45	5
2,6-Dinitrotoluene	25.89	50
2-Methyl-4,6-dinitrophenol	26.04	100
Dibenzofuran	26.12	50
Demeton	26.21	10
2,4-Dinitrotoluene	26.55	10
Ethoprop	26.83	10
4-Chlorophenyl phenyl ether	28.97	50
2-Nitroaniline	29.13	50
Phorate	29.17	10
N-nitrosodiphenylamine	30.04	50
Azobenzene	30.21	50
Dimethoate	30.60	5
Pentachlorophenol	31.70	50
Phenanthrene	32.67	1.0
4-Bromophenyl phenyl ether	32.96	50
Anthracene	32.96	1.0
alpha-BHC	33.26	1.0
Diazinon	33.48	5
Disulfoton	33.65	10
Hexachlorobenzene	33.90	1.0
beta-BHC	35.29	1.0
gamma-BHC	35.79	1.0
Methyl Parathion	36.69	10
delta-BHC	37.56	1.0
Fenchlorophos	37.91	10
1-Methylphenanthrene	38.01	1.0
Dibutyl Phthalate	39.46	5
Malathion	39.91	5
Fenthion	40.38	10
Chlorpyrifos	40.53	5
Heptachlor	41.36	1.0
Trichloronate	41.44	10
Fluoranthene	42.98	1.0
Aldrin	43.99	1.0
Pyrene	44.85	1.0
Tetrachlorvinphos	45.61	10
Tokuthion	46.91	10
Heptachlor Epoxide	47.00	1.0

Bolstar	47.40	10
gamma-Chlordane	48.77	1.0
2,4'-DDE	49.18	1.0
Endosulfan I	49.69	1.0
alpha-Chlordane	49.91	1.0
Fensulfothion	50.10	10
trans-Nonachlor	50.29	1.0
4,4'-DDE	51.50	1.0
Merphos	51.55	10
Dieldrin	51.61	1.0
2,4'-DDD	52.11	1.0
Butylbenzyl Phthalate	53.13	5
Endrin	53.19	1.0
Endosulfan II	53.84	1.0
4,4'-DDD	54.53	1.0
2,4'-DDT	54.79	1.0
Endrin Aldehyde	55.20	1.0
Benz[a]anthracene	56.25	1.0
Chrysene	56.52	1.0
Endosulfan Sulfate	56.93	1.0
4,4'-DDT	57.24	1.0
bis-(2-ethylhexyl) Phthalate	59.24	5
Endrin Ketone	60.23	1.0
Guthion	60.47	10
Coumaphos	60.52	10
Methoxychlor	61.36	1.0
Mirex	64.27	1.0
Di-n-octyl Phthalate	64.71	5
Benzo[b]fluoranthene	65.47	1.0
Benzo[k]fluoranthene	65.87	1.0
Benzo[e]pyrene	67.71	1.0
Benzo[a]pyrene	68.07	1.0
Perylene	68.77	1.0
3,3'-Dichlorobenzidine	72.45	50
Indeno[1,2,3-c,d]pyrene	76.29	1.0
Dibenz[a,h]anthracene	76.68	1.0
Benzo[g,h,i]perylene	77.87	1.0
PCBs By Congener		1
PCBs By Aroclor		10

2.0 SUMMARY OF METHOD

A measured volume of sample, usually 2 liters, is serially extracted with methylene chloride at pH >11 and again at pH <2 using a separatory funnel. The methylene chloride extract is concentrated in preparation for instrumental analysis. Samples are to be stored at 4 °C, extracted within 7 days of collection, and analyzed within 40 days of extraction.

A 1-3 µL sample is injected into a gas chromatograph (GC) equipped with a mass selective detector. The GC is temperature programmed to separate the compounds and confirmation is achieved for the single component peaks using ions specific to each target compound. Compounds eluting from the GC are identified by matching the retention times of the unknown peaks with those from a known calibration standard and the concentration of each identified component is measured by comparison of the responses.

3.0 PREVENTION OF INTERFERENCES

3.1 Solvents, glassware, and other processing apparatus are to be free of any interferences. A procedural blank is be analyzed with each sample batch to demonstrate the absence of any method interferences.

3.2 High purity solvents are to be used to minimize interferences.

3.3 Phthalate esters and PCBs are contaminants found in many types of products commonly used in the laboratory. Care should be taken to avoid or eliminate the use of plastic products during sample processing and handling.

3.4 Impurities in the carrier and makeup gases may be avoided by using Ultra-High purity gases and/or gas purifying cartridges. See the instrument manufacturer for guidelines.

3.5 Contamination by carryover may occur whenever high level and low-level samples are sequentially analyzed. To reduce carryover, the syringe used for sample injection shall be rinsed a minimum of 5 times between samples using n-hexane. Whenever possible, samples shall be analyzed from low to high concentrations.

3.6 A procedural blank shall be analyzed with each batch of 20 or less samples to check for contamination during sample processing.

4.0 SAFETY

4.1 It is mandatory to wear a laboratory coat, closed-toe shoes and safety glasses in the laboratory. Gloves are to be worn when working with samples.

4.2 All glassware cleaning and extraction procedures involving any solvent exposure shall take place in a fume hood. Use of a respirator and appropriate safety gloves are recommended for working with solvents.

4.3 Material Safety Data Sheets (MSDS) are on file in the laboratory and are available to all personnel involved in the use of hazardous materials during any procedure.

4.4 Extreme caution and the proper use of safety equipment are required during the handling of any hazardous material. If the analyst has any questions regarding safety, he or she should contact a supervisor or the laboratory director prior to the start of this procedure.

5.0 APPARATUS AND MATERIALS

5.1 Glassware

A. Separatory funnel: 2 L, with Teflon stopcock

B. Round-bottom flasks: 250 mL

C. Pear-shaped flasks: 25 mL

D. Graduated cylinder: 100 mL

E. Erlenmeyer flask: 1 L

F. Glass filter funnel

G. Pasteur pipettes

H. Gastight volumetric syringes: 100, 500 μL

I. Autosampler vials with Teflon-lined screw caps: 2 mL

5.2 Glass wool

5.3 pH indicator paper: pH 0-6

5.4 Graduated cylinder: 2 L

5.5 Heavy duty aluminum foil

5.6 Roto-evaporator system with aspirator pump and water bath set at 30 ± 5 °C

5.7 Chiller unit set at 10 ± 5 °C or cool tap water

5.8 High-temperature oven set at 1000 ± 50 °F

5.9 Non-ionic detergent

5.10 Shimadzu GC2010 GC equipped with a Mass Selective Detector, an AOC 20i Low-volume Autosampler and split/splitless injector or an Agilent 6890 GC equipped with a Mass Selective Detector (5973 or 5975), a 7683/7683b Low-volume Autosampler and a split/splitless injector.

5.11 J&W Scientific DB5 Column (or equivalent), 30 meters in length, 0.25 µm film thickness, and 0.25 mm I.D

5.12 10 µL syringe for the AOC20i/7683/7683b Autosamplers

5.13 Ultra-high purity helium

5.14 Fused Silica Retention Gap, 5 meters in length, 0.53 mm I.D.

6.0 REAGENTS

6.1 Deionized water

6.2 Pesticide grade hexane and methylene chloride

6.3 Method 625 spike solutions prepared from stock solutions obtained from an accredited supplier. The solution used is dependent on the clients target analyte list.

- Chlorinated Pesticides
- PCB Congeners
- Base/Neutral Extractables
- Acid Extractables

6.4 Anhydrous granular sodium sulfate

6.5 Concentrated sulfuric acid

6.6 Stock solutions. All stock solutions are purchased from NIST traceable commercial suppliers. Store at or below 4 °C and protect from light. Stock standards shall be replaced after one year or sooner if comparison with check standards indicates a problem.

6.7 Calibration Standards. Prepare a minimum of five concentration levels for each parameter of interest. One of the concentration levels shall be near the method detection limit. The remaining concentration levels shall bracket the expected concentrations found in the samples. Calibration solutions shall be replaced after 6 months or sooner if a problem is indicated.

6.8 Internal Standards. Select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst shall demonstrate that the selected compound(s) is not affected by the method or matrix interference.

6.8.1 Just prior to analysis, add a known constant amount of internal standard to all calibration solutions, blanks, and samples.

6.9 Recovery Surrogates. Select one or more internal standards that are similar in analytical behavior to the compounds of interest.

6.9.1 Prior to the extraction of the samples, add a known amount of recovery surrogate to all blanks and samples. See Methods 3510 and 3545 for additional information about this procedure.

6.10 Sodium Hydroxide

7.0 CALIBRATION AND MAINTENANCE

7.1 Shimadzu QP2010 or Agilent 6890 GC/MS

7.1.1 GC Oven Operating Conditions:
Initial Oven Temperature = 45 °C
Initial Hold = 5 min
Ramp 1 = 20 °C/min to 125 °C
Hold Time = 0 min
Ramp 2 = 2.5 °C/min to 285 °C
Hold Time = 17 min

7.1.2 Injector Operating Conditions:
Injector = Splitless or On-Column
Mode = Track Oven Temperature (On-Column Only)

Nominal Initial Pressure = 23.5 psi (on)

7.1.3 Column Operating Conditions:
Max Temp = 325 °C
Mode = Constant Flow
Initial Flow = 1.5 mL/min
Average Carrier Velocity = 40 cm/sec
Carrier Gas = Helium

7.1.4 Detector Operating Conditions:
Transfer Line Temperature = 285 °C
Ionization Voltage = 70 ev
Gain = +100 to +300 volts over standard sensitivity target tune

7.1.5 Autosampler Operating Conditions (Back Injector):
Sample Washes = 2
Sample Pumps = 2
Injection Volume = 2.0 µL
Syringe Size = 10 µL
Post Injection Washes Solvent A = 3
Post Injection Washes Solvent B = 3
Plunger Speed = Fast

7.1.6 System Maintenance

Prior to each set of samples, remove ca. 30 cm of the retention gap or column, replace injector septum if needed, and refill the solvent wash bottles.

Clean glass inlet liners or replace as needed.

Replace the gas cartridges every 6 months.

Replace the GC columns as needed.

Enter all maintenance actions into the instrument maintenance logbook.

8.0 QUALITY CONTROL

8.1 With each batch of samples (maximum 20 samples per batch), a procedural blank is extracted and analyzed to demonstrate that procedural interferences are under control. Deionized water is used as the blank matrix.

8.2 With each batch of samples, a duplicate sample and/or matrix spike/matrix spike duplicate (MS/MSD) set of samples is analyzed with the appropriate extraction procedure to measure the precision of the extraction procedure. A non-spiked sample of an MS/MSD set is analyzed to determine background concentrations for each parameter of interest. The MS/MSD samples are spiked with specific parameters at a concentration greater than ten times the method detection limit and analyzed to determine the percent recovery of the spiked compounds. For concentrations at ten times the method detection limit, a precision factor between the duplicate samples or MS/MSD samples is calculated and compared to the corresponding QC acceptance criteria.

8.3 Every sample, spike set, and blank is spiked with an appropriate surrogate spike solution consisting of 1 to 6 surrogate compounds. The surrogate spike is used to demonstrate the efficiency of the extraction and analytical procedure by allowing calculation of the percent recovery of each surrogate compound.

Control charts and control limits are generated by measuring the mean and standard deviation of the surrogate percent recovery for the previous 20 samples. Upper and lower *warning limits* are calculated at two times the standard deviation from the mean. Upper and lower *control limits* are calculated at three times the standard deviation from the mean. Surrogate control limits and results are presented with the analytical results. When surrogate results indicate atypical method performance, a quality control check sample is analyzed and an evaluation of the procedure and instrumentation is made.

8.4 If any individual parameter falls outside of the designated range for percent recovery, that parameter has failed the acceptance criteria. An evaluation of the method procedure and instrumentation shall be made to uncover evidence of any atypical performance. If there is atypical performance of the method procedure and/or instrumentation, the problem shall be immediately identified and corrected prior to the analysis of any further samples. A re-spike and/or quality control check sample shall be analyzed and evaluated. If possible, all samples from the suspect batch shall be re-analyzed under corrected method conditions. If samples can not be re-analyzed, the analytical results for the non-spiked samples are suspect and shall be reported with the result flagged and followed by an explanation of the problem.

8.5 QA/QC records are maintained to document the quality of data generated. If any constituent falls outside the designated range, that compound has failed the acceptance criteria. Failure to meet the stated requirement shall require that corrective action be taken to eliminate the problem prior to the analysis of any samples. Samples from the batch being analyzed at the time the failure is detected shall be reanalyzed after the corrective action has been taken. A batch is defined as 20 or less samples. If any sample cannot be reanalyzed, the result for that element shall be flagged and a detailed report is included with the result.

8.5.1 **Initial Calibration Check-** Prior to analyzing any samples, using a second-source calibration standard an initial calibration of the instrument is performed with each batch of samples. This calibration shall be within 15% of the initial calibration curve.

8.5.2 **Calibration Check-** Using a second-source calibration standard, a calibration check will be performed every 12 hours and at the end of every batch of samples. The calibration check shall be within 15% of the initial calibration curve.

8.5.3 **Matrix Spikes-** Matrix spike and matrix spike duplicates as well as duplicate samples shall be analyzed with each batch of samples to determine the precision for each compound. A control chart is generated to document the precision. Control limits are established by using the mean and standard deviation from 20 results. Upper and lower warning limits are two times the standard deviation and upper and lower “out of control” limits are three times the standard deviation for those compounds that are greater than 10 times the method detection limit.

8.5.4 **CRM/LCM-** Certified reference materials and/or lab control materials shall be analyzed with each batch of samples. The reported value shall be within the limits set forth by the agency providing the material.

8.5.5 **Blanks-** Lab reagent blanks shall be analyzed with each batch of samples. No compound shall be detected at greater than 3 times the method detection limit.

8.5.6 **QCS-** A method standard is extracted along with each batch of samples. Prepare the QC check standard to 1L of reagent water.

8.5.6 **Internal Standards-** Internal standards shall be added in known amounts to blanks, calibration standards, continuing calibration check solutions, and samples to compensate for instrumental drift.

8.5.7 **Recovery Surrogates-** Recovery surrogates shall be added in known amounts to all blanks and samples to indicate sample processing efficiency. Sample results shall not be adjusted for surrogate recovery efficiency unless specifically requested by the client.

8.5.8 Daily GCMS Performance Test- At the beginning of each batch of samples, the GCMS system must be checked to see of acceptable performance criteria are achieved for DFTPP. The criteria are presented in the following table.

Mass	m/z Abundance Criteria
69	0-100 percent of Mass 198
70	< 2 percent of Mass 69
127	40-60 percent of Mass 198
198	Base peak, 100 percent relative abundance
199	5-9 percent of Mass 198
275	10-30 percent of Mass 198
365	> 1 percent of Mass 198
441	Present but < Mass 443
442	> 40 percent of Mass 198
443	17-23 percent of Mass 442

9.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

9.1 All samples are collected in amber glass jars with Teflon-lined screw caps. All samples are kept at 4 ± 2 °C from the time of collection until extraction.

9.2 If Residual Chlorine is present, add 80mg of sodium thiosulfate per liter of sample and mix well. Please refer to the SOP for Residual Chlorine determination.

10.0 PROCEDURE

10.1 Glassware cleaning procedure: High-temperature oven option

Wash glassware with non-ionic detergent and water. Rinse glassware thoroughly with tap water, then rinse once with deionized

water. Place glassware in high temperature oven and bake at a minimum of 1000 ± 50 °F for 2 hours according to the following conditions:

Set initial temperature ramp to 536°C over 1 hours then hold for 3 hours. Once the oven program shuts off, the oven begins to cool back down to 30 ºF. Consecutive oven runs can be done once the oven has cooled to less than 150 °F.

CAUTION: Do not open oven door or turn off blower until over temperature is below 570 °F.

Once the glassware has cooled, cover all exposed areas that will touch the sample with aluminum foil or place it upside down onto foil until use.

10.2 Glassware cleaning procedure: Solvent rinse option

Wash glassware with non-ionic detergent and water. Rinse glassware thoroughly with tap water, then rinse once with deionized water. Let dry, then use Teflon squeeze bottles to rinse three times with methylene chloride and three times with hexane.

10.3 Sodium sulfate cleaning procedure

Clean sodium sulfate either by heating in the high-temperature oven using the same program as the glassware cleaning procedure or by rinsing with several mLs of methylene chloride.

10.4 Glass wool cleaning procedure

Clean glass wool either by heating in the high-temperature oven using the same program as the glassware cleaning procedure or by rinsing with several mLs of methylene chloride.

10.5 Sample extraction

10.5.1 Remove sample from the refrigerator and bring to room temperature.

10.5.2 Decant some of the sample into the sink, if necessary, to allow for the addition of solvent.

10.5.3 Use a gas-tight volumetric syringe to pipette the appropriate QC surrogates into the sample. The surrogate solution(s) should be at room temperature prior to use. Record the sample ID, name and volume of surrogate used, standard solutions logbook page number containing details of solution preparation, and analyst initials in the laboratory notebook.

10.5.4 For matrix spike/matrix spike duplicate samples, use a gas-tight volumetric syringe to pipette the appropriate QA/QC spikes into the sample. The spike solution(s) should be at room temperature prior to use. Record the sample ID, name and volume of spike used, standard solutions logbook page number containing details of solution preparation, and analyst initials in the laboratory notebook.

10.5.5 Adjust the pH to >11 using NaOH solution. Then use the 100 mL graduated cylinder to measure 100mL of methylene chloride and add it directly to the sample bottle. Recap the bottle tightly and shake it continuously and vigorously for at least 2 minutes.

10.5.6 Allow the sample bottle to sit untouched for 5 minutes so that the organic solvent and aqueous layers can separate.

10.5.7 Decant approximately half of the sample into the 2 L Erlenmeyer flask and the remainder, including the solvent layer, into the separatory funnel. Allow the organic and aqueous layers in the separatory funnel to separate for 5 minutes.

10.5.8 Prepare the collection flask as follows:

Place a small amount of glass wool into the bottom of a glass filter funnel, then add approximately 50 g of anhydrous sodium sulfate. Place the funnel into the neck of a 250 mL round bottom flask.

10.5.9 Filter the solvent extract through the sodium sulfate and collect it in the 250 mL round bottom flask.

10.5.10 Add 75 mL methylene chloride to the empty sample bottle and swirl it around thoroughly to wash down the walls of the bottle. Pour the sample portions from the Erlenmeyer flask and the separatory funnel back into the sample bottle and repeat the shaking step in 10.4.5. Allow for layer separation, decant, and collect the extract in the same 250 mL flask.

10.5.11 For samples being analyzed only for Base/Nuetral compounds only, the third extraction is identical to the second. For samples being analyzed for acid extractable compounds, adjust the sample pH to less than 2 by adding a small amount of concentrated sulfuric acid prior to the shaking step of the third extraction. The third extraction is otherwise identical to the second.

10.5.12 After the third extraction is complete, measure the total volume of the sample using the 2 L graduated cylinder and record it in the laboratory notebook.

10.6 Sample concentration

10.6.1 Prepare the roto-evaporator for use according to the following parameters:

water bath temperature at 30 ± 5 °C
chiller temperature at 10 ± 5 °C or cool tap water

10.6.2 Attach the 250 mL round bottom flask to the distillation trap and secure it with a plastic spring clip.

10.6.3 Close the stopcock and lower the flask into the water bath. Turn on the roto-evaporator motor and adjust the rotation to a medium speed. Adjust the vacuum so that no solvent flashes up into the distillation trap.

10.6.4 Concentrate the sample to approximately 10 mL. Break the internal vacuum by opening the stopcock. Stop the motor, raise the arm, and remove the flask from the trap.

10.6.5 Use a Pasteur pipette to transfer the sample to a 25 mL pear-shaped flask. Rinse the 250 mL flask three times with approximately 1 mL methylene chloride and transfer each rinse to the 25 mL flask.

10.6.6 Attach the 25 mL pear-shaped flask to the distillation trap using the adaptor and concentrate the sample to approximately 500 μL. Take care not to let the sample go to dryness.

10.6.7 Transfer the sample to an autosampler vial using a Pasteur pipette. Rinse the 25 mL flask three times with approximately

250 μL methylene chloride, transfering each rinse to the autosampler vial.

10.6.8 The sample extract is now ready for instrumental analysis.

10.7 Using the Shimadzu/Agilent data system, load the appropriate method for the parameters of choice.

10.8 Using the Shimadzu/Agilent data system, set up a sequence table for the analysis of the samples. The sequence table should include all calibrations necessary for five calibration levels of each parameter of interest, the recovery surrogate solution, a calibration check solution for every 12 hours of operation, and all blanks and samples.

10.9 Place the vials in the autosampler tray insuring that they are in the same order as the sequence table.

10.10 Load and run the sequence file and insure that the autosampler operates correctly.

10.11 From the results of the calibrations, build a calibration table.

10.12 Once the calibration table is completed, load the result file for each sample and print the appropriate report.

11.0 CALCULATIONS

11.1 The qualitative identification of compounds determined by this method is based on retention time matching. Results are confirmed by quantification using a specific mass for each compound and comparison wiith the retention times.

11.2 An internal standard calibration procedure is used by calculating the relative response factor (RRF) for each analyte using the following formula:

$$RRF = \frac{(A_x)(C_{IS})}{(A_{IS})(C_X)}$$

Where:
A_x = Area of the Target Analyte Peak
C_{IS} = Mass of the Internal Standard
A_{IS} = Area of the Internal Standard Peak
C_X = Concentration of the Target Analyte

11.3 The quantitation of each analyte of interest shall be based on the area of the peak of each ion at the retention corresponding to the calibration standard. The concentration is calculated using the following formula:

$$\text{Concentration} = \frac{(A_{UNK})(C_{IS})}{(A_{IS})(RRF_{TA})(SW)}$$

Where:

A_{UNK} =	Peak area of the sample
C_{IS} =	Mass of the Internal Standard
A_{IS} =	Area of the Internal Standard Peak
RRF_{TA} =	Relative Response Factor for the Target Analyte
SW =	Weight of sample extracted

11.4 The Method Detection Limit (MDL) is defined as the minimum concentration of a compound that can be measured and reported with 99% confidence that the value is greater than zero. The MDLs listed in Table 1 were determined using a clean marine sediment sample following US EPA guidelines in 40CFR.

Parameter	Group	MDL	MDL_Units	AccRange	RL	Method
(PCB030)	Organophosphorus Pesticides		ng/L	40 - 130%		EPA 625m
(PCB112)	Organophosphorus Pesticides		ng/L	60 - 120%		EPA 625m
(PCB198)	Organophosphorus Pesticides		ng/L	60 - 120%		EPA 625m
(TCMX)	Organophosphorus Pesticides		ng/L	40 - 130%		EPA 625m
Bolstar (Sulprofos)	Organophosphorus Pesticides	2	ng/L	65 - 125%	4	EPA 625m
Chlorpyrifos	Organophosphorus Pesticides	1	ng/L	65 - 125%	2	EPA 625m
Demeton	Organophosphorus Pesticides	1	ng/L	45 - 105%	2	EPA 625m
Diazinon	Organophosphorus Pesticides	2	ng/L	65 - 125%	4	EPA 625m
Dichlorvos	Organophosphorus Pesticides	3	ng/L	65 - 125%	6	EPA 625m
Dimethoate	Organophosphorus Pesticides	3	ng/L	65 - 125%	6	EPA 625m
Disulfoton	Organophosphorus Pesticides	1	ng/L	45 - 105%	2	EPA 625m
Ethoprop (Ethoprofos)	Organophosphorus Pesticides	1	ng/L	65 - 125%	2	EPA 625m
Fenchlorphos (Ronnel)	Organophosphorus Pesticides	2	ng/L	65 - 125%	4	EPA 625m
Fensulfothion	Organophosphorus Pesticides	1	ng/L	65 - 125%	2	EPA 625m
Fenthion	Organophosphorus Pesticides	2	ng/L	65 - 125%	4	EPA 625m
Malathion	Organophosphorus Pesticides	3	ng/L	65 - 125%	6	EPA 625m
Merphos	Organophosphorus Pesticides	1	ng/L	65 - 125%	2	EPA 625m
Methyl Parathion	Organophosphorus Pesticides	1	ng/L	60 - 120%	2	EPA 625m
Mevinphos (Phosdrin)	Organophosphorus Pesticides	8	ng/L	65 - 125%	16	EPA 625m
Phorate	Organophosphorus Pesticides	6	ng/L	45 - 105%	12	EPA 625m
Tetrachlorvinphos (Stirofos)	Organophosphorus Pesticides	2	ng/L	65 - 125%	4	EPA 625m
Tokuthion	Organophosphorus Pesticides	3	ng/L	65 - 125%	6	EPA 625m
Trichloronate	Organophosphorus Pesticides	1	ng/L	65 - 125%	2	EPA 625m
Allethrin	Pyrethroids	5	ng/L	65 - 125%	10	EPA 625m
Bifenthrin	Pyrethroids	5	ng/L	65 - 125%	25	EPA 625m
Cyfluthrin	Pyrethroids	5	ng/L	65 - 125%	25	EPA 625m
Cypermethrin	Pyrethroids	5	ng/L	65 - 125%	25	EPA 625m
Danitol	Pyrethroids	5	ng/L	65 - 125%	25	EPA 625m
Deltamethrin	Pyrethroids	5	ng/L	65 - 125%	25	EPA 625m
L-Cyhalothrin	Pyrethroids	5	ng/L	65 - 125%	25	EPA 625m
Permethrin	Pyrethroids	5	ng/L	65 - 125%	25	EPA 625m
Prallethrin	Pyrethroids	5	ng/L	65 - 125%	25	EPA 625m

ATTACHMENT 6: Chlorpyrifos Analysis SOP

Revision #2
Effective Date: December 8, 2002
Accepted:_____

Chlorpyrifos ELISA
Standard Operating Procedures

This S.O.P. is based upon the instructions provided by Ensys, the company that manufactures the chlorpyrifos EnviroGard Plate test kit.

1.0 INTRODUCTION

This test uses polyclonal antibodies, which bind either chlorpyrifos or a chlorpyrifos-enzyme conjugate. Chlorpyrifos antibodies are immobilized by the factory into the walls of the plate's test wells. The manufacture's chlorpyrifos-enzyme conjugate is added to each test well. Any chlorpyrifos in a water sample competes with the manufacture's chlorpyrifos-enzyme conjugate for the antibody binding sites on the wall. The addition of the manufacture's substrate solution causes the enzyme-conjugate to change color. Color formation is inversely proportional to the chlorpyrifos concentration.

Dark color=lower concentration of chlorpyrifos (more manufacture's enzyme-conjugate bound to antibodies)

Lighter color=higher concentration of chlorpyrifos (less manufacture's enzyme-conjugate bound to antibodies).

Chlorpyrifos concentration is read by using the ThermoElectric 96-well plate reader.

2.0 TEST PREPARATION

2.1 Pre-testing procedure

1. Remove test kit from refrigerator. Let kit components warm up to room temperature for 1.5-2 hours. (Never let kit components sit at room temperature for more than 8 hours).

2.2 Equipment and Supplies Needed

1. EnviroGard Chlorpyrifos Plate Kit (Catalog #72500).

2. 2 glass beakers with Optima Methanol.

3. Waste beaker.

4. 100 mL glass beaker with Milli-Q water.

5. Positive displacement pipettes to deliver 100μLs.
6. Repeator Eppendorf pipette with 3, clean, 5.0 mL combtips.

7. 25 μL Hamiliton syringe.

8. Milli-Q water or equivalent.

9. 1-100 mL glass beaker, 4-50 mL glass beakers.

10. 1-25 mL clean, glass volumetric flasks. 4, clean glass 10 mL volumetric flasks. Caution: do not use plastics, chlorpyrifos binds to plastic.

11. Timer capable of timing 1 hour.

12. A readily available source of tap or distilled water to rinse test wells.

13. ThermoElectric 96-well plate reader

14. Orbital Shaker.

15. 5-5 mL, clean volumetric pipettes

2.3 Preparation of Chlorpyrifos Standards

1. Always use the 1 ppm chlorpyrifos-ethyl standard to create your standard curve. Unless you are analyzing grain samples, never use the 1 ppm chlorpyrifos-methyl standard. All standards should be made with Milli-Q or equivalent water. These calibrators are unstable and should be prepared immediately before use.

2. Remove independent 1. ppb chlorpyrifos calibrator from refrigerator and allow to warm up to room temperature.

3. Prepare 0.05, 0.3, 1.0 ppb chlorpyrifos standards:

1.0 ppb standard: Gently swirl manufacture's 1.0 ppm stock solution. Using a cleaned Hamiliton syringe, (see below), dispense 10 μLs of 1 ppm stock into a clean, glass, 10 mL volumetric flask, containing Milli-Q water. Bring up to 10mL with Milli-Q water. Stopper the flask and invert 5X to mix. Remember, prior to this step, you should coat the inside barrel of the syringe with the stock solution by first drawing up 25 μLs of the 1 ppm stock solution and dispensing this volume back into the stock solution vial.

Note: Always clean the Hamiliton syringe before and after each use. Clean the syringe by drawing up a full syringe volume of Optima MeOH. Repeat this step 3X, discarding the MEOH into a waste beaker. After the third rinse, pour MeOH down the syringe, then repeat the syringe rinsing procedure 3X using a fresh batch of MeOH. Following this final rinsing stage, work the plunger up and down to expel any excess MeOH. Wipe the outside of the syringe with a clean chem-wipe and return the syringe to storage.

4.0 Using the freshly made 1.0 ppb standard, prepare 0.05 and 0.3 ppb standards.

0.3 ppb standard: Using a clean volumetric pipette, pipette 3.0 mLs of 1.0 ppb stock into a clean, glass, 10 mL volumetric flask, containing Milli-Q water. Bring up to 10.0 mL with Milli-Q water. Stopper the flask and invert 5X to mix.

0.05 ppb standard: Using a clean volumetric pipette, pipette 0.5mLs of 1.0 ppb stock into a clean, glass, 10 mL volumetric flask, containing Milli-Q water. Bring up to 10.0 mL with Milli-Q water. Stopper the flask and invert 5X to mix.

3.0 TEST INITIATION

Unless otherwise instructed, always run two replicates for each treatment

1. Leave the first two wells of your first ELISA strip empty. Beginning with the 0.05 standard, pipette 100 μLs of the appropriate standard solution into each test well. Calibrator solutions should be pipeted from lowest chlorpyrifos concentration to highest. Pipette tips should be changed for each new standard. Like the standards, run 2 replicates/sample and 100 μLs of sample per test well. .

2. Use the repeator Eppendorf with its dial set to 1. Place a clean 5.0 mL combtip onto the pipettor. Use this combtip to add 100 μL of enzyme conjugate to each well. Caution: the first and second repeat pipette volumes may not be accurate. Discard these first two volumes back into the conjugate vial.

Note: This step should be conducted fairly quickly. Do not leave to answer the phone, etc., until the next step (step 3.3) is completed. Try to minimize hand shaking when pipetting the conjugate. It is very important to be as accurate as possible.

3. Cover the wells with a clean piece of Parafilm. Place on orbital shaker and shake @ 200 rpm for 1 hour.

4. After 1 hour, remove strips from shaker, rinse all wells 5X with running tap water. Invert the wells onto a clean paper towel and shake dry.

5. Use the repeator Eppendorf with its dial set to 1. Place a clean 5.0 mL combtip onto the pipettor. Use this combtip to add 100 μL of substrate to each well. Caution: the first and second repeat pipette volumes may not be accurate. Discard these first two volumes back into the substrate vial. Caution: do not expose substrate to direct sunlight!!!!!

6. Cover the wells with a clean piece of Parafilm. Place on orbital shaker and shake @ 200 rpm for 30 minutes.

7. After 30 minutes, remove strips from shaker. Use the repeat Eppendorf with its dial set to 1. Place a clean 5.0 mL combtip onto the pipettor. Use this combtip to add 100 μL of stop solution to each well. Caution: the first and second repeat pipette volumes may not be accurate. Discard these first two volumes back into the stop solution vial.

8. Place on orbital shaker and shake @ 200 rpm for 5 minutes.

9. Read results using the ThermoElectric 96-well plate reader. Enter Test #4 in the ThermoElectric 96-well plate reader. Strips must be read within 30 minutes of adding the stop solution.

4.0 Helpful Hints

1. Always store all plate kit components at 4-8º C when not in use.

2. Do not store test components for more than 8 hours at room temperature.

3. Do not freeze plate kit components or expose them to temperatures greater than 37º C.

4. Do not use plate kit components after the expiration date.

5. Do not use reagents or test well strips from one plate kit with reagents or test well strips from a different plate kit.

6. Never store chlorpyrifos samples in plastic. Always store in glass. Always mix all calibrators in glass.

7. Chlorpyrifos can be destroyed under alkaline conditions. Collect all samples and prepare all calibrators in glassware that has been rinsed free of all alkaline detergent residues.

8. Do not expose substrate to direct sunlight

ATTACHMENT 7: Diazinon Analysis SOP

Revision #2
Effective Date: December 8, 2002
Accepted:_____

Diazinon ELISA
Standard Operating Procedures

This S.O.P. is based upon the instructions provided by Strategic Diagnostics, the company that manufactures the diazinon EnviroGard Diazinon Plate test kit.

1.0 INTRODUCTION

This test use polyclonal antibodies which bind either diazinon or a diazinon-enzyme conjugate. Diazinon antibodies are immobilized by the factory into the walls of the plate's test wells. The manufacture's diazinon-enzyme conjugate is added to each test well. Any diazinon in a water sample competes with the manufacture's diazinon-enzyme conjugate for the antibody binding sites on the wall. The addition of the manufacture's substrate solution causes the enzyme-conjugate to change color. Color formation is inversely proportional to the diazinon concentration.

Dark color=lower concentration of diazinon (more manufacture's enzyme-conjugate bound to antibodies)

Lighter color=higher concentration of diazinon (less manufacture's enzyme-conjugate bound to antibodies).

Diazinon concentration is read by using the Thermo Electric 96-well plate reader.

2.0 TEST PREPARATION

2.1 Pre-testing procedure

1. Remove test kit from refrigerator. Let kit components warm up to room temperature for 1.5-2 hours. (Never let kit components sit at room temperature for more than 8 hours).

2.2 Equipment and Supplies Needed

1. EnviroGard Diazinon Plate Kit (Catalog #72700).
2. 2 glass beakers with Optima Methanol.
3. Waste beaker.
4. 100 mL glass beaker with Milli-Q water.
5. Positive displacement pipette to deliver 100μLs.
6. Repeator Eppendorf pipette with 3, clean, 5.0 mL combtips.

7. 100 μL Hamiliton syringe
8. Milli-Q water or equivalent.
9. 5-20 mL Glass beakers for calibrator solutions. Caution: do not use plastics, diazinon binds to plastic.
10. Timer capable of timing 1 hour and 30 minutes.
11. A readily available source of tap or distilled water to rinse test wells.
12. Thermo Electric 96-well plate reader.
13. Orbital Shaker.
14. 5, clean, glass 10 mL volumetric flasks
15. 5-5 mL, clean volumetric pipets

2.3 Preparation of Diazinon Standards

1. Always use the manufacture's 100 ppb diazinon standard to create your standard curve. All standards should be made with Milli-Q or equivalent water. These standards used for the calibration curve are unstable and should be prepared immediately before use.

2. Remove independent 100 ppb diazinon calibrator from refrigerator and allow to equilibrate to room temperature.

3. First prepare a 500 ppt stock solution:

500 ppt standard: Gently swirl the manufacture's 100 ppb stock solution. Using a cleaned Hamiliton syringe, (see below), dispense 50 μLs of 100 ppb stock into a clean, glass, 10 mL volumetric flask, containing Milli-Q water. Bring up to volume with Milli-Q water. Stopper the flask and invert 5X to mix. Remember, prior to this step, you should coat the inside barrel of the syringe with the stock solution by first drawing up 100 μLs of the 100 ppb stock solution and dispensing this volume back into the stock solution vial.

Note: Always clean the Hamiliton syringe before and after each use. Clean the syringe by drawing up a full syringe volume of Optima MeOH. Repeat this step 3X, discarding the MeOH into a waste beaker. After the third rinse, pour MeOH down the syringe, then repeat the syringe rinsing procedure 3X using a fresh batch of MeOH. Following this final rinsing stage, work the plunger up and down to expel any excess MeOH. Wipe the outside of the syringe with a clean chem-wipe and return the syringe to storage.

4. Using the freshly made 500 ppt standard, prepare 100 and 30 ppt standards.

100 ppt standard: Using a clean volumetric pipette, pipette 1.0 mLs of 500 ppt stock into a clean, glass, 10 mL volumetric flask, containing Milli-Q water. Bring up to 5.0 mL with Milli-Q water. Stopper the flask and invert 5X to mix.

30 ppt standard: Using a clean volumetric pipet, pipet 0.3 mLs of 500 ppt stock into a clean, glass, 10 mL volumetric flask, containing Milli-Q water. Bring up to 5.0 mL with Milli-Q water. Stopper the flask and invert 5X to mix.

Note: These aqueous standards may be unstable and should be prepared just prior to use.

3.0 TEST INITIATION

Unless otherwise instructed, always run two replicates for each treatment

1. Leave the first two wells of your first ELISA strip empty. Beginning with the 30 ppt standard, pipette 100 μLs of the appropriate standard solution into each test well. Calibrator solutions should be delivered by pipette from lowest diazinon concentration to highest. Pipette tips should be changed for each new standard or sample. After loading the standards, proceed with your sample waters. Like the standards, run 2 replicates/sample and 100 μLs of sample per test well.

2. Use the repeator Eppendorf with its dial set to 1. Place a clean 5.0 mL combtip onto the pipette. Use this combtip to add 100 μL of enzyme conjugate to each well. Caution: the first and second repeat pipette volumes may not be accurate. Discard these first two volumes back into the conjugate vial.

Note: This step should be conducted fairly quickly. Do not leave to answer the phone, etc., until the next step (step 3.3) is completed. Try to minimize hand shaking when pipetting the conjugate. It is very important to be as accurate as possible.

3. Cover the wells with a clean piece of Parafilm. Place on orbital shaker and shake @ 200 rpm for 1 hour.

4. After 1 hour, remove strips from shaker, rinse all wells 5X with running tap water. Invert the wells onto a clean paper towel and shake dry.

5. Use the repeator Eppendorf with its dial set to 1. Place a clean 5.0 mL combitip onto the pipettor. Use this combtip to add 100 μL of substrate to each well. Caution: the first and second repeat pipette volumes may not be accurate. Discard these first two volumes back into the substrate vial. Caution: do not expose substrate to direct sunlight!!!!!

6. Cover the wells with a clean piece of Parafilm. Place on orbital shaker and shake @ 200 rpm for 30 minutes.

7. After 30 minutes, remove strips from shaker. Use the repeator Eppendorf with its dial set to 1. Place a clean 5.0 mL combitip onto the pipettor. Use this commbtip to add 100 μL of stop solution to each well. Caution: the first and second repeat pipette volumes may not be accurate. Discard these first two volumes back into the stop solution vial. Warning: stop solution is 1 N hydrochloric acid

8. Place on orbital shaker and shake @ 200 rpm for 5 minutes.

9. Read results using the ThermoElectric 96-well plate reader. Enter Test #7 in the

ThermoElectric 96-well plate reader. Strips must be read within 30 minutes of adding the stop solution.

4.0 Helpful Hints

1. Always store all plate kit components at 4-8∞ C when not in use.

2. Do not store test components for more than 8 hours at room temperature.

3. Do not freeze plate kit components or expose them to temperatures greater than 37∞ C.

4. Do not use plate kit components after the expiration date.

5. Do not use reagents or test well strips from one plate kit with reagents or test well strips from a different plate kit.

6. Never store diazinon samples in plastic. Always store in glass. Always mix all calibrators in glass.

7. Diazinon can be destroyed under acidic conditions. Collect all samples and prepare all calibrators in glassware that has been rinsed free of all acidic detergent residues.

8. Do not expose substrate to direct sunlight.

APPENDIX D

SUPPORTING DOCUMENTS FOR BIOASSESSMENT MONITORING

ATTACHMENT 1: California Stream Bioassessment Protocol SOP (December 2003)

CALIFORNIA STREAM BIOASSESSMENT PROCEDURE

(Protocol Brief for Biological and Physical/Habitat Assessment in Wadeable Streams)

The California Stream Bioassessment Procedure (CSBP) is a standardized protocol for assessing biological and physical/habitat conditions of wadeable streams in California. The CSBP is a regional adaptation of the national Rapid Bioassessment Protocols outlined by the U.S. Environmental Protection Agency in "Rapid Bioassessment Protocols for use in Streams and Rivers" (**EPA/841-B-99-002**). The CSBP is a cost-effective tool that utilizes measures of the stream's benthic macroinvertebrate (BMI) community and its physical/habitat characteristics to determine the stream's biological and physical integrity. The purpose of this Protocol Brief is to introduce the techniques of bioassessment to aquatic resource professionals and help standardize data for statewide bioassessment efforts. The Protocol Brief is only a summary and does not contain all the necessary information that may be required to understand the concepts of bioassessment and to implement a successful monitoring program. Additional information and updates on bioassessment can be obtained by visiting the **DFG Aquatic Bioassessment Laboratory website at www.dfg.ca.gov/cabw/cabwhome.html**.

History of the CSBP

The CSBP was originally developed in 1993 to measure biological response from point-source discharges of chemical contaminants, inorganic sediment and elements of organic enrichment. The method was based on sampling the single richest habitat in a stream reach; this was the most common technique at the time (Rosenberg and Resh 1993, Loeb and Spacie 1994, Lenat and Barbour 1994) and consistent with the U.S. EPA's Rapid Bioassessment Protocols (RBP) (Plafkin et al. 1989). In 1995, the CSBP was adapted for use in ambient and non-point source pollution monitoring programs and this version was reviewed by a Technical Advisory Committee assembled by DFG and the U.S. EPA. The 1996 edition of the CSBP was widely distributed in California and accepted as the state's standardized RBP protocol (Davis et al. 1996 U.S. EPA 2002). A 1999 revision added quality assurance and control (QA/QC) techniques to ensure high quality field collections, laboratory analysis and taxonomic consistency.

As of 2003, the CSBP is the most often used RBP protocol in California (Barbour and Hill, 2003). This unique protocol allows the user to produce biological and physical/habitat data that can be used to measure differences between sites, compare to a regional Index of Biological Integrity (IBI) (Ode *et al.* 2003) and help diagnose response to individual stressors. In addition to the high gradient riffle based procedure, the 2003 edition of the CSBP describes techniques for use in unique channels and a technique for low gradient channels that blends elements of the CSBP with those of a multi-habitat technique recommended by the U.S. EPA (Barbour et al. 1999).

The CSBP 2003 has four notable changes to the existing protocol; 1) the stream reach for the assessment is no longer defined by a set of five pool-riffle sequences, but rather by a discreet length of 100 m (300 ft); 2) the area of benthos sampled has been reduced from 1.6 m^2 (18 ft^2) to 0.8 m^2 (9 ft^2); 3) although 3 independent samples will be collected at each reach, there is now an option to composite the 3 samples in the laboratory and reduce the total number of BMIs identified at each reach from 900 to 500; and 4) there is a new QA/QC procedure to collect a set of duplicate samples

at 10% of the reaches for projects with more than 20 sites. These changes were based on experiences gained from several years of field testing, changes in the national RBP (Barbour et al. 1999), recommendations from Barbour and Hill (2003) and methods comparison studies conducted by DFG. **Data collected with these modifications can easily be made compatible with previous CSBP data and these changes make the CSBP more consistent with other BMI protocols used in the western US**.

OVERVIEW OF THE CSBP

The CSBP can be used to measure biological and physical/habitat condition in all freshwater lotic environments (streams and rivers) shallow enough to allow safe wading (≤1.5 m). The CSBP samples benthic macroinvertebrates with a 0.5mm mesh net from the richest habitat along 3 randomly selected transects within a 100 m (300 ft) reach of stream or river. The 3 transects are placed within shallow-fast water habitat (usually riffle) for high gradient channels and throughout the entire reach for low gradient channels. At each transect, three 0.09 m^2 (1 ft^2) areas of stream benthos are sampled and composited into a single sample. In low gradient channels, the 3 collections along the transect are selected to represent the relative proportions of the different richest habitat categories present (submerged vegetation, hard substrate of natural rock or concrete, soft substrate of sand or mud, stream bank vegetation and woody debris). Physical/habitat is measured using a qualitative U.S. EPA procedure throughout the entire reach and additional quantitative measures within the vicinity of the BMI samples. Taxonomic identification of the BMI samples is performed on a fixed count of 300 organisms from the 3 samples (total of 900 for the entire reach) or 500 from the composite of the 3 samples. There are two standard levels of taxonomic identification: one standardized for the state by the California Bioassessment Laboratory Network (CAMLnet; www.dfg.ca.gov/cabw/camlnetste.pdf) and a more precise level based on the U.S. EPA's Environmental Monitoring and Assessment Program (EMAP).

CALIFORNIA DEPARTMENT OF FISH AND GAME SCIENTIFIC COLLECTING PERMIT

Anyone who collects fish, amphibians, or invertebrates from the waters of the state must have a DFG Scientific Collecting Permit in their possession. The permit can be obtained from the DFG License and Revenue Branch in Sacramento (916-227-2225). Those conducting bioassessment in California should specify on the permit application that they will take freshwater invertebrates (authorization 5), incidental fish (authorization 6) and amphibians (authorization 8). It is also advisable to contact the local Game Warden and District Fisheries Biologist at the closest Regional Office prior to collecting.

FIELD PROCEDURES FOR COLLECTING BMI SAMPLES

The CSBP can be used to sample BMIs from all streams and rivers where the access and depth (≤1.5 m) do not require the use of a boat. The step-by-step procedures described in this document have been divided into three sections: high gradient channels, low gradient channels and considerations for unusual channel conditions. **Contact DFG or visit the DFG Aquatic Bioassessment Laboratory website for more information on Rapid Bioassessment procedures for boatable streams and rivers and lentic or still water environments.**

CSBP for High Gradient Channels

High gradient channels usually have greater than a 1% slope and will always contain pool-riffle sequences with a ratio high enough to contain at least 3 riffles per 100 m (300 ft) reach. Riffle substrate could be rock, sand or mud, but must be at least 1 m (3 ft) wide with flow velocities greater than 0.3 m/sec (1 ft/sec).

Biological and Physical/Habitat Equipment List

Measuring tape (300 ft or 100 m)
D-shaped kick net (0.5 mm mesh)
Standard size 35 sieve (0.5 mm)
Wide-mouth 500 ml plastic jars
White enameled pan and forceps
95% ethanol
California Bioassessment Worksheet (CBW)
Physical/Habitat Quality Form
Chain of Custody Form (COC)
Random Number Table
pH, temp, DO and conductivity meter
Stadia rod and hand level or clinometer
Densiometer

Step 1. Measure a 100 m (300 ft) reach of channel and count the number of riffles greater than 1 m (3 ft) wide and 1 m (3 ft) long. Randomly choose 3 of the riffles within the stream reach.

Step 2. Starting with the downstream riffle, place the measuring tape along the bank of the entire riffle while being careful not to walk in the stream. Select one transect from all possible 1/3 m (1 ft) marks using a random number table. For riffles longer than 10 m (30 ft), randomly place the transect within the top third of the riffle.

Step 3. Inspect the transect before collecting BMIs by imagining a line going from one bank to the other, perpendicular to the flow. Choose 3 locations along that line where you will place your net to collect BMIs. If the substrate is fairly similar and there is no structure along the transect, the 3 locations will be on the side margins and the center of the stream. If the substrate is structurally complex along the transect, then place the 3 collections to reflect it.

Step 4. Collect BMIs at the 3 locations along the transect by placing the D-shaped net on the substrate and disturbing an area as wide as the net and 1 ft upstream. Excavate the 0.09 m^2 ($1ft^2$) area to an approximate depth of 10-15 cm (4-6 in) by kicking or by using a tool to loosen the substrate. Pick-up and scrub large rocks by hand under water in front of the net. If the substrate is sand or mud, a hand rake can be used to prevent substrate from filling the net. Maintain a consistent sampling effort (approximately 1-3 minutes) at each area. Combine the 3 collections within the net to make one "composite" sample.

Step 5. Place the contents of the net in a standard size 35 sieve (0.5 mm mesh) or white enameled tray. Remove the larger twigs, leaves and rocks by hand after carefully inspecting for clinging organisms. If the pan is used, place the material through the sieve to remove excess water before placing the material in the jar. Place the sampled material in a jar and completely fill with 95% ethanol. Never fill a jar more than 2/3 full with course sampled material or 1/2 full with sand or mud. **Gently** agitate jars that contain primarily mud or sand to help mix the alcohol, taking care to not damage any organisms present.

Step 6. Place a label containing descriptive information about the sites (see box) in each jar. An additional label can be taped to the outside of the jar to help with the sample log-in process at the laboratory. A Chain of Custody (COC) should accompany the samples during transportation to the laboratory.

Bioassessment Sample Label
Project Name:
Site Name/Code:
County:
Riffle/Reach Number:
Transect Number:
Date/Time:
Sampled by:

Step 7. Proceeding upstream, Repeat Steps 2 through 5 for the next two riffles within the stream reach.

Step 8. **QA/QC Repeat Sampling Procedure. For projects with 20 or more sites, duplicate samples must be collected at 10% of the reaches.** For reaches containing more than six riffles, randomly choose 3 riffles for the primary set of samples and randomly choose 3 more riffles for the duplicate set of samples. For reaches that contain 6 or less riffles, measure the entire length of all riffle habitat and randomly select 3 transects from the total length for the primary samples and randomly select 3 for the duplicate samples. For both methods, start at the downstream riffle or transect, proceeding upstream collecting the 6 samples designating them as primary or duplicate.

CSBP for Low Gradient Channels

Low gradient channels usually have less than a 1% grade and will never have more than two riffles. These channels can be as deep as 1.5 m, but with low enough water velocity to allow safe wading. **Channels greater than 1.5 m deep, with swift water velocities and/or which can not be accessed on at least one bank will require a boat**.

Step 1. Measure a 100 m (300 ft) section of channel trying to avoid large human-made structures such as bridges or dams. The stream reach can be less than 100 m (300 ft) if access or obstacles are a problem, especially if the channel is morphologically homogeneous.

Step 2. Without entering the water, survey the entire reach for approximate percentages of 5 generalized habitat categories: a. submerged vegetation, b. hard substrate of natural rock or concrete, c. soft substrate of sand or mud, d. stream bank vegetation and e. woody debris. Record the proportions and make note if it was difficult to determine depth and habitat type (e.g. water was highly turbid).

Step 3. Determine how many 2 m (6 ft) intervals can be established along the entire length of the reach. Randomly select 3 of the intervals and using a range finder or measuring tape, locate the three points on the bank of the reach.

Step 4. Starting with the downstream point, establish a transect across the channel perpendicular to the flow. Sample BMIs at 3 locations along that transect, choosing areas representing the generalized habitats identified in Step 2. Collect BMIs by placing the D-shaped kick-net on the substrate or vegetation and disturb a 0.09 m^2 (1 ft^2) portion of habitat upstream of the kick-net. Maintain a consistent sampling effort (approximately 1-3 minutes) at each site. Combine the 3 collections within the kick-net to make one "composite" sample. Note the 3 generalized habitats that were sampled along the transect on the field form.

Step 5. Place the contents of the kick-net in a standard size 35 sieve (0.5 mm mesh) or white enameled tray. Remove the larger twigs, leaves and rocks by hand after carefully inspecting for clinging organisms. If the pan is used, place the material through the sieve to remove excess water before placing the material in the jar. Place the sampled material and label (see box) in a jar and completely fill with 95% ethanol. Never fill a jar more than 2/3 full with course sampled material or 1/2 full with sand or mud. **Gently** agitate jars that contain primarily mud or sand to help mix the alcohol, taking care to not damage any organisms present.

Step 6. Place a label containing descriptive information about the sites (see page 4 box) in each jar. An additional label can be taped to the outside of the jar to help with the sample log-in process at the laboratory. A Chain of Custody (COC) should accompany the samples during transportation to the laboratory.

Step 7. Proceeding upstream, Repeat Steps 4 and 5 for the next two transects within the reach. Try to choose generalized habitats for the 9 collections (3 areas along 3 transects) in proportion to what was determined in Step 2.

Step 8. **QA/QC Repeat Sampling Procedure. For projects with 20 or more sites, duplicate samples must be collected at 10% of the reaches.** After determining how many 2 m (6 ft) intervals can be established along the entire length of the reach, randomly select 3 of the intervals for collecting the primary samples and randomly select 3 more intervals for the duplicate samples. Starting with the downstream transect, proceed upstream collecting the 6 samples and designating them as primary or duplicate.

PROTOCOL CONSIDERATIONS FOR UNUSUAL CHANNEL CONDITIONS

CSBP for Intermittent or Ephemeral Channels: Intermittent or ephemeral channels will have flowing water during the rainy season and be dry during mid to late summer. These channels can be sampled using the CSBP for high or low gradient streams, but must be sampled in a spring (March through May) index period or at the end of the wet period.

CSBP for No Flow Conditions in High and Low Gradient Channels: Although this is very problematic for sampling BMIs, sometimes sampling areas in high gradient streams have pocket water with little or no flow. In this case, put the net at the downstream portion of the sampling area, disturb the substrate and push the water into the net with vigorous hand motions. Strained water from the surface of a nearby pool with a bucket can be used to move organisms into the net by pouring the water into the pocket area in front of the net. In low gradient channels, low flow or no flow conditions can be quite common. In this case, put the net downstream of the sampling area, get in front of the net and agitate the substrate with a twisting foot motion for 30 seconds. At 5-10 second intervals throughout the agitation, step aside and swiftly move the net in a "figure eight" motion through the cloud of suspended substrate.

CSBP for Bifurcated or Braided Channels: Low gradient channels can have two or more channels flowing through a typically wide riparian corridor. There is no need to extend the transect through islands or sand bars separating these bifurcated or braided high gradient channels. Use the

standard procedure for sampling the dominant channel or randomly selected one channel if there are more than 2 similar channels >1 m (>3 ft) wide.

CSBP for Channels <1 M (3 ft) Wide (the "Spot-Sampling" modification): High gradient channels <1 m (<3 ft) wide can not be sampled using the 1/3 m (1 ft) wide D-frame net at three places along the transect. In this case, divide the channel into an upper, middle and lower section, relative to the flow. Each section should be approximately 30 m long, but could be divided by natural breaks in the morphology of the channel. Survey each section, without stepping into the channel for all 0.09 m^2 (1 ft^2) areas where the substrate and flow resemble a riffle. Randomly select 3 of these "sampleable areas" in the lower section and composite them into one sample. Proceed upstream and repeat for each section.

CSBP for Large Boulder Channels: High gradient channels that are dominated by boulder substrates too large to move, but with enough gravel substrate in patches between the boulder can be sampled similarly to the previous modification. After dividing the channel into three sections, count the patches of substrate small enough to sample and randomly select three patches. Composite the three samples and proceed upstream to sample the next two sections.

CSBP for Channels Immediately Below Water Impoundments: High gradient channels immediately below a water impoundment structure that prevents gravels and fines from moving downstream will often not contain shallow-fast water habitats with gravel or cobble substrates. These channels can be sampled either using the modification for large boulder channels or by using the low gradient procedure where 3 transects are chosen randomly from the entire reach.

CSBP for Cement Channels: Cement channels in urban areas will typically have uniform shape and depth with no natural habitat. These channels should be sampled using the low gradient protocol of 3 randomly selected transects along 100 m (300 ft) of channel. The 3 collections can be simply taken from the left margin, center and right margin of the channel. Try to avoid human made habitats such as shopping carts and other transient debris.

CSBP for Channels with Gradient Controls: Some low gradient urban streams will have low level dams to control the gradient. The channel will be transformed into small impoundments separated by extremely high gradient sections of large boulders to dissipate the energy. Do not sample the high gradient sections. Sample the impounded areas using the low gradient protocol or if the impoundments are too deep to wade, sample along the littoral zone of one bank. Divide the bank into upper, middle and lower sections, randomly pick three points at 1 m (3 ft) intervals and at each point, take a 0.09 m^2 (1ft^2) sweep through the vegetation trying to disturb the sediment if present. Composite the 3 collections and repeat for each section.

CSBP for Channels with Three or Fewer Riffles: High gradient channels that are wider than 1 m (3 ft), but have 3 or fewer riffles within the 100 m (300 ft) reach will not allow for an independent sample from several riffles. In these cases, measure the entire length of all riffle habitat and select the 3 transects randomly from the total length.

CSBP for Channels with Continuous Riffle Habitat: Stream reaches (usually very high gradient) that have continuous riffle habitat should be sampled using the low gradient procedure where 3 transects are chosen randomly from the entire reach.

CSBP for Channels with Transitional Gradient: Large watersheds can have wide channels where the gradient transitions from high to low. Riffle pool sequences can be present, but further apart than in higher gradient channels. In these cases, expand the reach length to 40 times the average width to allow for an adequate number of riffles to sample. If riffle habitat is limited to one or two riffles in a greater than 100 m (300 ft) transitional gradient reach, then consider the riffle to be hard substrate and use the low gradient procedures.

FIELD PROCEDURES FOR MEASURING CHEMICAL AND PHYSICAL/HABITAT QUALITY

The EPA's physical/habitat scoring criteria is a nationally standardized method (Barbour et al. 1999). It is used to measure the physical integrity of a stream and can provide a stand alone evaluation or used in conjunction with a bioassessment sampling event. DFG recommends that this procedure be conducted on every 100 m (300 ft) reach as part of a bioassessment program. A detailed description of the scoring criteria is available through the DFG Aquatic Bioassessment Laboratory website. **This procedure is an effective measure of a stream's physical/habitat quality, but can produce inconsistent measures if QA/QC measures are not regularly implemented. This procedure requires field training prior to its use and field audits throughout the program.**

The following list of quantitative measures of chemical and physical/habitat characteristics are considered minimal and should be measured when rapid bioassessments are not part of an existing chemical or fisheries habitat program where a more extensive list of parameters are measured. The information produced from measuring chemical and physical/habitat characteristics can be used to classify stream reaches and to help explain data anomalies.

Reach-Wide Parameters:

- GPS coordinates at the top and bottom of the reach
- Water temperature, specific conductance, pH, alkalinity and dissolved oxygen at the center of the reach using approved standardized procedures and instruments
- Reach length, average width and gradient
- Visually estimated substrate composition using the following categories: fines (<0.25 cm) (<0.1in.), gravel (0.25-0.8 cm) (0.1-2 in.), cobble (0.8-25 cm) (2-10 in.), boulder (>25 cm) (>10 in.) and bedrock (solid)

Sample Site Specific Parameters:

- Average length, width and depth for each of the 3 randomly chosen riffles (for unmodified high gradient protocol only)

- Water velocity immediately upstream of the three composite samples along each of the 3 transects

- Percent cover upstream of the three composite samples along each of the 3 transects. Measure this parameter using a densiometer 1/3 m (1 ft) above the water surface and averaged for each transect

- Substrate consolidation at the three sample excavations along the 3 transects. Estimates are obtained while collecting the BMI sample by noting whether the substrate is loosely, moderately or tightly cemented

- Pebble count and percent embeddedness immediately upstream of the 3 transects where BMI samples were collected. Measure this parameter by establishing a transect approximately 1/3 m (1 ft) upstream of the sample transect, randomly choosing 10 points along the transect, reaching down to the point at the end of a wooden dowel or tip of the boot and measure the width of the particle. For every third particle (3 on each transect), estimate percent embeddedness by noting how much of the particle was surrounded by fine substrate.

LABORATORY PROCEDURES FOR ANALYZING BMI SAMPLES

DFG recommends that taxonomic identification of BMI samples collected using the CSBP is performed by a professional or permanent university laboratory with extensive experience with California taxa. **These bioassessment laboratories should participate in the California Bioassessment Laboratories Network (CAMLnet) to ensure that they are aware of the standardized level of taxonomy and QA/QC procedures recommended for bioassessments conducted in California.** To ensure a high quality product, all contracts to a bioassessment laboratory should require:

1. A Laboratory Standard Operation Procedure (SOP) document and Quality Assurance Protection Plan (QAPP)

2. A list of all taxonomists that will work on the samples including their education, years of experience and any specialized training they have received.

3. Internal QA/QC documentation for sub-sampling and taxonomic validation (can be specified to provide this information upon request);

4. Be able and willing to perform taxonomy consistent with the CAMLnet Taxonomic Effort Standards (www.dfg.ca.gov/cabw/camlnetste.pdf).

Project managers are encouraged to subject all laboratory data to an external review by an independent laboratory at the rate of 10% to 20% (depending on experience and nature of the project) of the project samples. The DFG Aquatic Bioassessment Laboratory performs this QC procedure and can be contacted about information on the procedure requirements and costs.

Taxonomic Level of BMI Identification

There are two levels of taxonomic identification for samples collected using the CSBP. It is the ultimate responsibly of the contractor or project manager to guarantee that the level of taxonomy reported is consistent with the CSBP standards.

CSBP Level 1 is used for most state-wide rapid bioassessment projects and it is imperative when comparing data to the Southern California IBI. In general, Level 1 taxonomic effort is to genera where possible for most taxonomic groups, order for oligochaetes and family for chironomids.

CSBP Level 2 is based on the taxonomic effort levels established by the U.S. EPA for the Western Pilot EMAP. In general, Level 2 taxonomic effort identifies insects to species level where possible and the Dipteran Family: Chironomidae to genus.

Compositing Samples or Data

There will always be 3 samples collected at each sampling reach when using the CSBP. Depending on the objectives of the project, the samples can be processed as individual samples and subsampled for 300 organisms/sample (900 organisms total per site) or **composited at the laboratory** and subsampled for 500 organisms.

Subsampling

The CSBP requires fixed count subsampling with a +/- 10% accuracy. The total count of BMIs must come from at least 3 randomly selected grids within a subsampling tray. The last grid must be fully counted to get an estimate of relative abundance. The debris from processed grids should be put in a clean "remnant" jar and the remaining contents of the tray should be placed back into the original sample jar. If a "large and rare" survey is preformed on the sample, it should be conducted after the subsampling procedure and counted separately.

Data Production, Storage and Analysis

DFG has developed a Microsoft Access® database based loosely on the U.S. EPA's Environmental Data Analysis System (EDAS). The structure of the CalEDAS database is available through the DFG Aquatic Bioassessment Laboratory website, but it does not currently come with end-user support. Whether using the DFG database or other software, the laboratory analysis should produce a BMI taxa list that is consistent with CAMLnet (see above) for all samples and a list of common or project specific biological metrics. Many common biological metrics are listed in the U.S. EPA's RBP document (Barbour et al 1999) and several other sources of bioassessment literature. When BMI samples are processed independently, there are two options for calculating metrics depending on the needs of the project:

1. Calculate metrics for all three samples independently and calculate metric averages at each site

2. The three samples can be composited in the analysis stage, and a 500 count subsample of the 900 organisms can be used to generate one set of **cumulative** metrics for each site.

QA/QC CONSIDERATIONS FOR USING THE CSBP

All private and public entities conducting bioassessment using the CSBP should have a Standard Operating Procedures document (SOP) and a Quality Assurance Protection Plan (QAPP). Large programs and laboratories can have a quality assurance officer and some smaller operations may only have a field or laboratory supervisor. In either case, those individuals responsible for assuring the quality of samples collected in the field and processed in the laboratory should be trained on all aspects of the CSBP. Two 3-day courses on bioassessment concepts and the use of the CSBP are available through the American Fisheries Society (CalNeva AFS) and the Society of Environmental Toxicology and Chemistry (NorCal and SoCal SETAC). Information on these courses can be found at www.slsii.org

The details of a QAPP should be tailored for particular bioassessment operations. Depending on the nature of the project, appropriate boiler plate for QAPPs may be available through Regional Water Quality Control Boards or the State Water Resources Control Board. These agencies should be contacted before developing a QAPP and initiating a bioassessment program.

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ATTACHMENT 2: CSBP Stream Habitat Characterization Forms

PHYSICAL HABITAT QUALITY
(California Stream Bioassessment Procedure)

WATERSHED/ STREAM: _____ DATE/ TIME: _____

COMPANY/ AGENCY: _____ SAMPLE ID NUMBER: _____

SITE DESCRIPTION: _____

Circle the appropriate score for all 20 habitat parameters. Record the total score on the front page of the CBW.

Parameters to be evaluated within the sampling reach

HABITAT PARAMETER	CONDITION CATEGORY			
	OPTIMAL	**SUBOPTIMAL**	**MARGINAL**	**POOR**
1. Epifaunal Substrate/ Available Cover	Greater than 70% (50% for low gradient streams) of substrate favorable for epifaunal colonization and fish cover; most favorable is a mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are <u>not</u> new fall and <u>not</u> transient).	40-70% (30-50% for low gradient streams) mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale).	20-40% (10-30% for low gradient streams) mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 20% (10% for low gradient streams) stable habitat; lack of habitat is obvious; substrate unstable or lacking.
	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
2. Embeddedness	Gravel, cobble, and boulder particles are 0-25% surrounded by fine sediment. Layering of cobble provides diversity of niche space.	Gravel, cobble, and boulder particles are 25-50% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 50-75% surrounded by fine sediment.	Gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment.
	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
3. Velocity/ Depth Regimes *(deep<0.5 m, slow<0.3 m/s)*	All four velocity/depth regimes present (slow-deep, slow-shallow, fast-deep, fast-shallow).	Only 3 of the 4 regimes present (if fast-shallow is missing, score lower than if missing other regimes).	Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score low).	Dominated by 1 velocity/ depth regime (usually slow-deep).
	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
4. Sediment Deposition	Little or no enlargement of islands or point bars and less than 5% (<20% for low-gradient streams) of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5-30% (20-50% for low-gradient) of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% (50-80% for low-gradient) of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 50% (80% for low-gradient) of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
5. Channel Flow Status	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

Parameters to be evaluated in an area longer than the sampling reach

HABITAT PARAMETER	CONDITION CATEGORY			
	OPTIMAL	**SUBOPTIMAL**	**MARGINAL**	**POOR**
6. Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely.
	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
7. Frequency of Riffles (or bends)	Occurrence of riffles relatively frequent; ratio of distance between riffles divided by width of the stream <7:1 (generally 5 to 7); variety of habitat is key. In streams where riffles are continuous, placement of boulders or other large, natural obstruction is important.	Occurrence of riffles infrequent; distance between riffles divided by the width of the stream is between 7 to 15.	Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 to 25.	Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is a ratio of >25.
	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
8. Bank Stability (score each bank) Note: determine left of right side by facing downstream	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
	Left Bank 10 9	8 7 6	5 4 3	2 1 0
	Right Bank 10 9	8 7 6	5 4 3	2 1 0
9. Vegetative Protection (score each bank) Note: determine left or right side by facing downstream.	More than 90% of the streambank surfaces and immediate riparian zones covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
	Left Bank 10 9	8 7 6	5 4 3	2 1 0
	Right Bank 10 9	8 7 6	5 4 3	2 1 0
10. Riparian Vegetative Zone Width (score each bank riparian zone)	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities haveimpacted zone a great deal.	Width of riparian zone <6 meters: little or no riparian vegetation due to human activities.
	Left Bank 10 9	8 7 6	5 4 3	2 1 0
	Right Bank 10 9	8 7 6	5 4 3	2 1 0

ATTACHMENT 3: BMI Sorting SOP

ECOANALYSTS, INC.

STANDARD OPERATING PROCEDURES

January 2004

Sorting

105 East 2nd Street, Suite 1
Moscow, Idaho 83843

Contents

Introduction

Sorting, the removal of benthic macroinvertebrates (insects, mollusks, worms, etc.) from the surrounding organic and inorganic substrate is the first step in the laboratory processing of a macroinvertebrate sample. The quality of the final product depends as much on sorting as it does on taxonomy and data management. It is vital that each sample be sorted accurately and consistently. A great deal of time and money are invested in these samples, and the clients whom requested it will use the data extracted from them for making important decisions. Ask questions and take your time to do it right.

Equipment required

At each workstation have the following items within reach:

- ✓ One or two pairs of forceps
- ✓ One tally counter
- ✓ Glass or plastic petri dish
- ✓ Spatula, pipette, pencil, micron pen, Sharpie®, scissors
- ✓ Squirt bottle containing 70% ETOH
- ✓ Vials-general taxa, large/rare, worm, and midge vials
- ✓ Squares of foam for vials

Preservatives

There are several preservatives possible to encounter in the lab. By far the most common (90+%) is ethyl alcohol (ETOH). It comes from the factory at 95% and is diluted to 70% to be used as a preservative. It will burn, and when burning the flame is difficult to see, so be careful. Formalin is the second most common preservative. It preserves better than alcohol, but is rather unpleasant to work with. Avoid inhaling the vapors and getting it on the skin or in the eyes. Any more than a 10% solution formalin should not be encountered in the lab. Samples preserved in formalin need to be rinsed very thoroughly. Isopropyl alcohol (rubbing alcohol) and acetic acid (vinegar) are used as preservatives from time to time. They smell funny but are more or less harmless. Rinse as normal.

Standard Procedures

1. Sorting Protocols

a. Set Up, Rinsing, and Elutriation

The first step when starting any project is reading and clearly understanding the *sorting protocol* located in that *project's folder*. Check off, date and initial the next available sample on the *sorting check sheet.* Cut out all labels provided for that sample and keep them with your *sorting log sheet.* At this point, remove the sample from the shelf and begin recording *sort time.* Make sure to pick up the entire sample, as there may be more than one jar per sample. Fill out the first two sections of the sort sheet (see Appendix A-2: *Sorting Sheet Instructions*). Estimate the sample's total volume of matrix (in liters) and record in the "Pre E" section on the sort sheet.

Dump the sample into the sieve of the appropriate mesh size indicated on the specific project sorting protocols. If the sample substrate is mostly inorganic (sand, rocks, etc.), then E*lutriation* may be possible before continuing. Rinse thoroughly with water to remove any ultra fine sediment and the preservative.

Elutriation:
Elutriation is essentially panning for bugs (as in panning for gold). Samples in which majority of the substrate is inorganic- sand, rocks, precious metals, rusty nails- can be reduced dramatically in volume and scope time by using this simple technique.

Dump the sample into a tray and fill the tray with water. Swish around the sample material until the organic material is floating and suspended in the water. Pour off the water and anything in it through the appropriate sieve. Add more water and repeat the process until no more organic material remains in the tray. A fairly well separated sample, the heavier inorganics in the tray, light organics in the sieve should remain. Once the sample is separated, use a magnifying lamp to check the inorganics for snails, clams, stone cased trichopterans, or any other critter that was too heavy to have floated off. Have a fellow technician take a second look.

Take any critters found within the inorganic material and toss them in with the organic material. It is important to put the critters back in the sample so they can be sub sampled appropriately. If it is the case that there are so many things that did not float off that it makes the elutriation process worthless, recombine everything and sort as per usual.

After rinsing, choose an appropriate size caton and tray in which to place the sample material.

Generally, choose:
4-grid, 6-grid or 12-grid caton for up to 0.50L of matrix,
24 or 30-grid caton for 1L to 2L of matrix,
and a 42-grid caton for 2L or more of matrix in a sample.

Fill the tray with enough water so that all of the material is floating inside of the gridded caton. Agitate and separate the floated material in an effort to distribute any bugs equally throughout the entire caton. Slowly pull up the screen until the water drains off and the material settles evenly to the mesh. Dump the water, and return the screen containing the sample matrix and bugs to the tray. Be very sure to distribute the sample material **thinly** (up to _ inch) and **evenly,** completely covering the mesh bottom of the caton.

There are instances where the volume of a sample may be too small or too large to use a caton and get the desired thin and even distribution. For sample volumes less than 75 to 100 ml, using a petri dish (marked into 8 even grids) may allow for an even distribution and easier handling of the matrix. For extremely large sample volumes (samples that when distributed in the largest caton prove to "overload" the caton resulting in the sample material distribution thicker than _ inch), using two or more catons and keeping subsampling random will allow for a more even distribution and easier handling of the matrix.

Grab the jar(s) and any internal jar label(s), and head for the scope. Fill the vials _ way with alcohol. Use a random number generator to find a coordinate that corresponds to a numbered grid in the caton. The grid chosen will be the first square of material to be sorted through. Each square can be subdivided into quarters and placed into a petri dish one quarter at a time. Keep track of rinse, elutriation and all preparation time and record it as part of the "overall time" on the sort sheet.

b. Sorting Procedure

Using the microscope, remove all the invertebrates in the petri dish according to project specific protocols concerning target count (section 1-b-i), standard rejections (section 1-b-ii) and Large/Rare picks (section 1-d). Record all identifiable taxa on a tally counter being sure to place worms and midges in two separate vials (small glass ones) from the general taxa vial. Once all of the bugs contained in the matrix in the entire petri dish have been removed, place the resulting "sorted residue" into a specific jar marked as "Sorted" and labeled with the PIN and SIN.

Repeat this process, being sure to use a random grid generator throughout, until the subsample target count is reached. Any sample matrix that is left over or left unsorted will be replaced into its original sample jar that is already labeled with the PIN and SIN and then marked as "Unsorted". All time spent looking through the scope is recorded as "scope time" for that sample on the sort sheet.

i. Subsampling and Target counts

The specific methods for subsampling is a controversial subject among benthic ecologists, and will probably remain so for some time. The reasons for subsampling, however, are more or less universal, to save time and money. In productive areas, invertebrates can achieve densities of 100,000 individuals per square meter or more. It would be impossible from a standpoint of both time and money to routinely sort and identify that many critters, hence subsampling. The method used is a fixed count, known area subsampling protocol. This protocol allows for a target number of individuals to be set at a manageable level without sacrificing the ability to get an estimate of the density of invertebrates in the sample. The target count will vary with project, and although similar methods will be employed for each, there are subtle differences. Each method will employ a gridded caton or petri dish and a random number generator.

Full sorts: (AKA complete sorts, 100% sorts)
Some projects demand we remove all individuals, regardless of density. This is straightforward; just pick every identifiable aquatic invertebrate from the sample and keep count on a tally counter. Once the sample has been rinsed, put it in a caton tray. Be sure to label sorted material jar with "FULL SORT" so the QC tech does not hunt for an "unsorted" jar.

500 count subsample:
This is the most common target value we use. The intent of this subsample target value is to capture as many of the available taxa as possible with a minimum of effort. Many studies have shown that if you remove 500 individuals you will collect 90% or more of the total available taxa, missing only those that are very rare. What is important here as with all subsample target counts, is consistency. The closer a sample is sorted to the target count, the better are the comparisons between samples.
Remove a *minimum* of 500 individuals. As a rule of thumb, the target value may be exceeded by up to 50 bugs (10% over the target count). Make liberal use of subdividing squares to try to keep the counts within the 50-bug margin. If you do exceed the 550 individuals, you may need to distribute the picked bugs in a gridded petri dish and randomly select 500 bugs (see section 1-c). Alternatively, it is probably best to exceed 500 by 20 or 30 bugs, in case the taxonomist is forced for some reason to reject one or two specimens.

300-count subsample:
This is the second most common target count. The same general explanations apply here as they did for the 500 count. However, the smaller number of individuals increases the importance of consistently hitting the target count. Again, remove a *minimum* of 300 individuals. The target count may be exceeded by 30 bugs (10% over the target count).

200 counts and fewer:
200 and 100 counts are less common but will occur. The intent of these subsample counts is to minimize effort and still produce a reliable and comparable representation of the benthic community. They do not attempt to exhaustively remove the available taxa in the sample. It is of the utmost importance to pick as close to the target value as possible. Some clients require we remove and identify *exactly* the target number. Again, make sure to get *at least* the target count. It is OK to exceed the count by 10% over the target count.

NOTE You may find you are dealing with a sample where the density of invertebrates is so high that even sorting a quarter square will cause you to exceed the 10% margin. In this case, it may be necessary to randomly subsample the entire collection of picked macros after the sample has passed QC (section 1-c) or via the computer, after they have been identified. Discuss which approach to take with Laboratory Coordinator in every case.

When a 500, 300, 200, 100 count becomes a Full Sort
It is possible to determine if a sample that does not have enough bugs to reach the minimum target count or will exceed the target count using estimation.
For example: The project calls for 500 counts. You have sorted 6 out of 30 squares and only found 50 bugs. Divide the total number of squares (30) by the number of squares you have picked (6) then multiply that number by the number of invertebrates you have removed, (30/6)*50=250 bugs in the entire sample. You need to sort a minimum of 20% (e.g. 6 of 30 squares) of the sample before you make the above calculation. Once you are convinced you will fully sort the sample, you can go ahead and switch to the Full Sort protocol explained above.

Subdivide, or not to Subdivide:
Make it a habit to take a mental estimate of how long the sample will take to prepare, sort and QC while rinsing it. Whether or not it will be necessary to subdivide a square into quarters or smaller portions will depend on the subsample target count, the density of the invertebrates in the sample, and our QC, which requires that bugs are selected from at least three different squares. This can be explained best through example:

> Your target count is 300. The sample is distributed and you have removed the first quarter square and found 10 bugs. You then calculate 10 bugs X 4 quarters X 30 squares in the tray and estimate there are 1200 individuals total. Since each square has about 40 bugs, you know you will have to sort about 7.5 squares. This means you can probably sort whole squares pretty safely, switching back to quarter squares as you approach the target count. Change your target count to 100, and you would have to alter your approach. After sorting the same first quarter square and doing the calculation, you find that you will get 100 bugs in only 2.5 squares, which does not pass the QC requirement of three separate squares. It would probably be best to sort quarter squares for the duration, drawing a new random grid between each. In that case you would sort from at least 3 different squares and will have more control over the count.

This is all dependent on if the sample is distributed evenly in the sample caton. If the sample is carelessly distributed, the density estimates could skew if a less dense or very dense patch is chosen to begin with. Many of the conclusions drawn from these data depend on the assumption that each bug had an equal chance of being included in the subsample.

Minimizing sampling error is the watchword from the moment the investigator steps into the stream to collect the sample, until we hand the data to the client. Also, it is always better to start small and move up than vice versa. If all bugs are picked in the first square the sample would not meet the QC requirement of 3 squares and everything would have to be tossed back and redistribute the sample. Keep in mind as small of an area as needed can be sorted to hit the target count, (e.g. a _ of a _ of a _) as long as recorded correctly.

ii. Standard Rejections

Most of the samples sorted at EcoAnalysts, Inc. are benthic samples. This means they are collected from the benthos, or bottom of streams, rivers and lakes. Therefore the general interest is of those critters that actually live in the bottom substrata, not ones that were accidentally captured. This is important, especially in the case of low target values, to make sure to get the correct number of truly benthic critters, and not a bunch of terrestrials not used in a benthic study. It will take time to recognize these, so if there is any doubt what any critter is, ask. Occasionally other types of samples (drift, multiplate, etc.) will be sorted in the lab. Each could require a different set of exclusions or none at all.

Standard rejections are:

- Terrestrial invertebrates: grasshoppers, ants, leafhoppers, aphids, etc.
- Adult aquatic insects, *except* aquatic beetles, and some Hemiptera (Notonectidae, Corixidae, Belostomatidae).
- Sub-aquatic invertebrates: Collembola, surface dwelling hemipterans (Gerridae, Vellidae), etc.
- Zooplankton not associated with the benthos: daphnia, bosmina, Copepoda
- Exuviae.
- Insects that are missing their head.
- Worms that are missing their head
- Any vertebrates (fish, salmanders, tailed frogs, etc.)
- Empty snail or clam shells (dead before sampling).

When in doubt, ask.

c. Subsampling Macros

Due to some project protocols that may require sorting any square of material chosen to completion or when a QA/QC check results in picking a large number of "extra" bugs, a target count may be largely exceeded.

In the case where the final number of bugs picked significantly exceeds the target count (>10% of the target count) it may be necessary to subsample the macros before they are sent to taxonomy.

The procedure for subsampling macros in the sorting lab is as follows:

1. Recombine all midges, worms, and generals into a gridded petri dish that is marked into 8 even grids.
2. Distribute all bugs evenly throughout the grids.
3. Choose a random grid and count all bugs whose heads are within that grid.
4. Continue picking from random grids of the dish until the true target count is reached, all the while placing midges, worms, and generals back into their respective vials.
5. Record the number of picked grids out of 8 on that sample's sort sheet along with the amount of time it took to subsample.
6. Make amendments to any vial labels by crossing out the original bug count and recording the new actual bug count at the bottom of the general-vial label.
7. Make any amendments and detailed notes in the "comments" section of the data base for that sample explaining original and actual count, initial sort and subsampling times.

d. Procedure For Picking the Large/Rare Component

A Large/Rare sort is intended to account for rare organisms present in the sample that were not picked when subsampling. This is a qualitative procedure that is used in some bioassessment protocols and is the very last step after picking the target count.

After picking the fixed-count portion of the sample, perform a timed scan of five minutes duration on the unsorted material to remove any invertebrates that may not be represented in the fixed-count component. Some rules for the Large/Rare sort:

- Take a full 5 minutes to scan the unsorted material.
- Use your unaided eye—do not use any kind of magnification.
- Rare organisms do not necessarily need to be large—however, do not pick chironomids (midges).
- Use size, color, and other morphological characters to determine whether an organism differs from those encountered in the fixed-count portion—if in doubt, pick it.
- Wash any large specimens in a separate dish containing a small amount of alcohol to remove any detritus and clinging invertebrates that are not intended for the Large/Rare component.
- If possible, pick three representatives of each organism that you encounter that you believe is different.
- Pick no more than 50 total specimens.

Put the Large/Rare specimens into a separate vial with the pre-printed "Large/Rare label. Use a rubber band to bind the fixed-count component to the Large/Rare component. Return the material in the wash dish to the subsampler.

e. Labeling and Data Entry

The label that came in the original sample jar goes back in with the "unsorted" portion. Use only pencil or the provided alcohol proof pens (micron pens) and label paper when filling out all sample labels. Regular ink and marker ink will dissolve in alcohol.

Make sure the back your pre-printed labels and worm and midge labels contain the following information and format:

General Vial and Sorted Jar

___# ___**Bugs**

Sorter Initials

Sort Date

Large/Rare Vial

Sorter Initials

Sort Date

Worms: Back

Sorter Initials & Date

Midges: Back

Sorter Initials & Date

Note: If there are no L/R present in the sample or The sample is a full sort, write "NONE" on the front of the L/R label and place in front the general vial label.

Take the time to write neatly and make sure all numerals are unambiguous. Taking that simple step will save great frustration. The sort information on the labels, sort sheet, and data base will match exactly for each sample. Be sure to orient labels into each vial so that pre-printed information is facing out.

Using pencil, write the SIN on the top of the general vial lid. When left with separate jars of "sorted" and "unsorted" residues, write "Sorted" on the outer label of the sorted jar and "Unsorted" on the outer label of the unsorted jar. When left with one jar containing a "fully sorted" residue, write "Full Sort" on the outer jar label. Place all jars and vials together in the staging area to await QC.

Enter all data recorded on the sort sheet for each sample into the data base (see Appendix A-3) immediately upon completion of the sorting process of each sample. Don't forget to include the amount of time it takes to complete paperwork, labels, and data entry as part of the sample's "Overall" sort time.

f. Other things to remember

- <u>The last step after labeling your macros is to scan each of your own vials for misplaced bugs.</u>
- We process many samples from many different projects and specific protocols vary. Make sure you know what project you are working on.
- Always clean both the caton, caton tray and sieve thoroughly after each use. Cross-contamination is absolutely the worst possible error, especially if it occurs between projects.
- Make sure the preservative you are using is actually alcohol, not water in disguise. It takes very little time for the invertebrates to rot if not properly preserved.
- When in doubt, ask.

2. Internal Sorting Quality Assurance / Quality Control Protocols

a. Set up and preliminary checks

Before beginning a QC sort, all labels will be double checked for accuracy by the tech performing the QC for the sorted sample. Any labels that are incorrectly filled out or mistakenly placed in the wrong vials must be brought to the attention of the original sorter and fixed immediately. Briefly look over the picked invertebrates to see if there are any obvious reject taxa and retain any invertebrates you remove to show to the original sorter for further reference. Most importantly, scan each of the vials for "misplaced" bugs. Have the original sorter return appropriate bugs to the correct vials. This begins the "QC time" to be recorded on the QA/QC log sheet (Appendix B-1) by the QC'er.

b. QA/QC procedure

The QC tech will redistribute only the *sorted* portion of the sample in the appropriately sized caton or gridded petri dish. Remove randomly selected squares and inspect until 20% of the material has been checked (i.e.,6 of 30 squares) for any bugs that should've been picked during the original sort.

c. Calculations

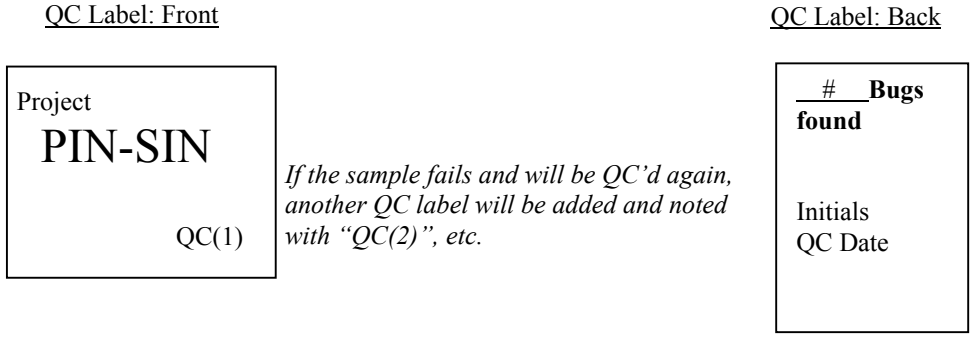
Calculate an estimated percent efficacy according to the directions provided on the QA/QC log sheet (see Appendix B). If the estimated % Efficacy is 90 percent or greater the sample passes QC. Move onto labeling (section 2-d) and the final steps of a QC (section 2-e).

If the estimate is less than 90 percent, the sorted portion must be completely resorted (section 3). If this occurs, the QC sorter returns the sample to the original sorter. The QC sorter should discuss what types of invertebrates and where these were found (i.e. moss, inside tricop cases, etc.) with the original sorter. The sample will have to undergo the sort and QC process again until it does exceed 90% efficacy. Record re-QC and resort information on the provided forms and data base for each step.

There is a case in which further investigation is warranted. If the actual % efficacy (obtained after a full resort) is more than 10 percentage points away from the estimated % efficacy, the QC tech, original sorter and a supervisor need to get together and discuss why that occurred.

d. Labeling and data entry

Complete a hand-written QC label for each QC as follows and place in general taxa vial:



Enter all data recorded on the QA/QC sheet for each sample into the data base (see Appendix B-3) immediately upon completion of the QCing process of each sample. Don't forget to include the amount of time it takes to complete paperwork, labels, and data entry as part of the sample's QC time.

e. Final Steps

The QC tech's final responsibility and the most important aspect of the QC process is completing the feedback loop. Make sure to discuss the results of the QC with the original sorter especially if the sorted sample failed the QC.

3. Resort and Re-QA/QC Protocols

a. Definition of a resort and re-QC

A "resort" is the term for the procedure applied to a sorted sample that has failed the QC. When a QC'er finds too many missed bugs in the sorted portion of a sample, thereby resulting in less than a 90% efficacy, the sorted portion and QC sheet is returned to the original sorter for resorting.

b. Resorting and re-QCing effort and procedure

A sorter who needs to resort a failed sample should place as much effort into picking through the sorted portion again as the first time. The same effort applies to the subsequent re-QC.

Redistribute the sorted portion into a caton. Sort through the entire caton picking any missed bugs and placing them into the midge, worm, and general vials for that sample keeping a new tally for the newly picked number of bugs. Place the resorted material back into the "sorted" jar. Keep track of the resort data on the same sort sheet as the original sort data for that sample, but on a new line and starring or indicating in some way that line's data is for a resort. Fill out the resort portion of the QA/QC sheet (Appendix B) and enter the appropriate information into the data base (see section 3-c).

As always, double check vials for any misplaced bugs or "stragglers" and complete labeling. The resorter will place all sample jars, vials, and QC sheet for the resorted sample in the QC staging area for re-QC.

The tech performing a second or third QC on a resorted sample will follow the same procedures outlined in section 2. There will be a few differences in labeling and record keeping (see Appendix B for how to record a re-QC on the QA/QC sheet).

c. Labeling and data entry for a resort and a re-QC

Create your own labels using cardstock and a pencil. Both labels will go into the general vial behind all other labels.

Resort Labels

Front

PIN-SIN

Resort(1)

Back

__#__**Bugs**
(for resort)

Sorter Initials

Sort Date

Re-QC Labels

Front

PIN-SIN

QC(2)

Back

__#__**Bugs**
(for re-QC)

QC'er Initials

Re-QC Date

The resorter will need to enter the resort bug count, which is called the "remainder count", and resort time into the QC portion of the data base for that sample (see Appendix B-3) immediately when the resort is completed.

The re-QC'er will need to enter the data for the re-QC into the QC portion of the data base for that sample (see Appendix B-3) immediately when the re-QC is completed.

If after the second QC, the sample fails to meet the 90% efficacy requirements, the sample has failed again and will need to go through the resort and re-QC process another time. This process is repeated until at least 90% of all bugs in the sorted portion of the sample have been removed from the matrix.

Contact your lab supervisor when a sample has failed a QA/QC check more than once before proceeding to discuss causation and possible tactics for a more efficient sort on that sample.

4. Short Counts

From time to time it will be necessary to remove additional bugs from a sample that has already been sorted due to an insufficient number of identifiable bugs picked during the

original sort. All techs should make the largest effort possible to prevent this from happening in the sorting labs.

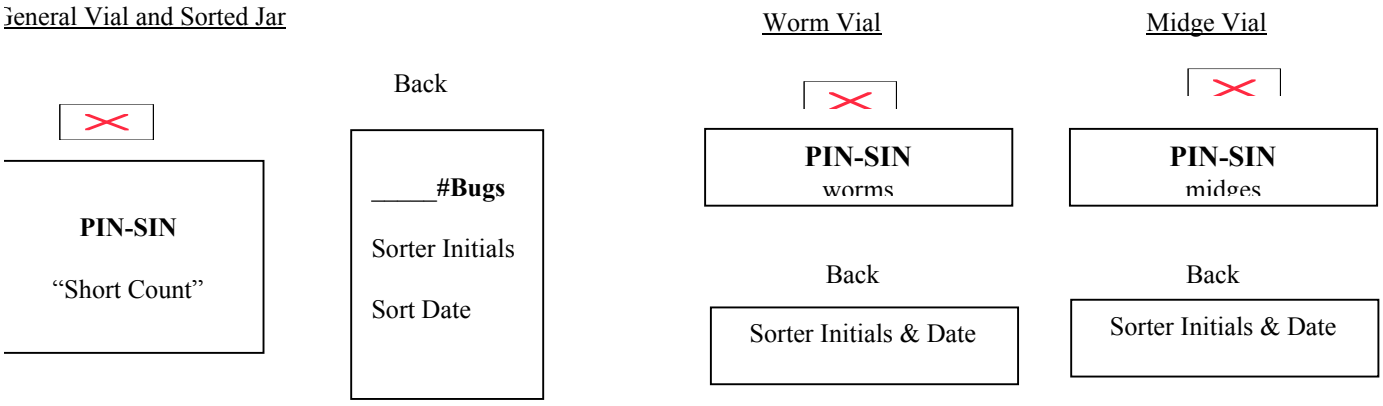
Some ways to prevent "short counts" in the sorting lab are to be very sure to pick and count at least the number of bugs to reach the target count of identifiable bugs or bugs that contain all of the head and at least some of the body and bugs that are not rejects - i.e. terrestrials or exuviae.

a. Setting up a "short count" sample for sorting

Redistribute the unsorted portion and sort as outlined in section 1.

b. Labeling and record keeping for "short counts"

Record the number of grids sorted and total grids as you would on a regular sort as well as all necessary label information. When possible, pre-printed vial and sorted jar labels will be available. Fill those out as outlined in section 1-e and add "Short Count" on the back of the labels. If pre-printed labels are not available make labels out of cardstock as outlined below.



Keep a sort sheet for short count samples as required for a standard sort. There is a section in the data base for select projects to enter "short count" sorting information. See Appendix C for an example of what information needs to be entered.

5. Project Wrap-up Protocols

At the end of each project, all sample residues, vials of macros, data base entry and paperwork needs to be placed in the appropriate location. All techs are responsible for making sure their own data is correct and immediately put into the database and all paperwork for that project is placed into the project folder. The tech who sorted the last sample for a project is responsible for making sure the data base integrity is upheld for that project, all paperwork is in the folder, and all vials are labeled and accounted for. The same tech is responsible for handing off the project to the Laboratory Coordinator the day the project is completed.

Appendix A-1

Macroinvertebrate Sorting Sheet

PIN-
Project:________________________________Sorter:______________________

N	Volume (liters) PreE / PostE	Matrix Type	Sort Date	Count	S/T Grids	L/R?	Time (hrs) Overall/Scope	Data Entered?
9	0.70 / 0.20	Inorganic	5/24/00	510	8/30	no	1.5/1.0	√
	/				/		/	
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	/				/		/	
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Appendix A-2

Sorting Sheet Instructions

1) Write in the **PIN**, **Project Name**, and **Sorter Name** at the top of each sort sheet.

2) **SIN:** Sample identification number, EcoAnalysts lab identification number.

3) **Volume (liters):**
   PreElutriation: Before dumping out any material for a sample to be rinsed, estimate the amount of material the entire sample contains in liters.
   PostElutriation: After rinsing the sample and tossing any inorganic material, estimate how much of the sample material remains.

4) **Matrix Type:** Material from which bugs are removed, background substrate. Classify each sample by the dominant matrix type using the following categories:
   Inorganic: Substrate consisting mainly of sand, gravel, silt, any mineral substrate.
   Fine Organic: Also known as FPOM (fine particulate organic matter). Substrate characterized by fine (approx. less than 4mm) generally homogenous organic (wood fragments, shredded leaves, etc.) particles. Often the most common substrate found in large river and lake samples.
   Coarse Organic: Also know as CPOM (coarse particulate organic matter). Characterized by coarse (greater than 4mm) often heterogeneous organic material. Most often semi decomposed leaves, twigs, sticks, and/or larger chunks of woody debris. Common substrate type for smaller, forested streams.
   Vegetation: Substrate composed mainly of recently dead (still green) aquatic vegetation. Usually mosses and macrophytes. Can be found in samples from almost anywhere.
   Filamentous algae: Substrate composed of dense, tangled, stringy aquatic algae. Often requires the use of two sets of forceps to remove invertebrates. Most common in samples from warmer, open canopy streams.

5) **Sort Date:** self

6) **Count:** self

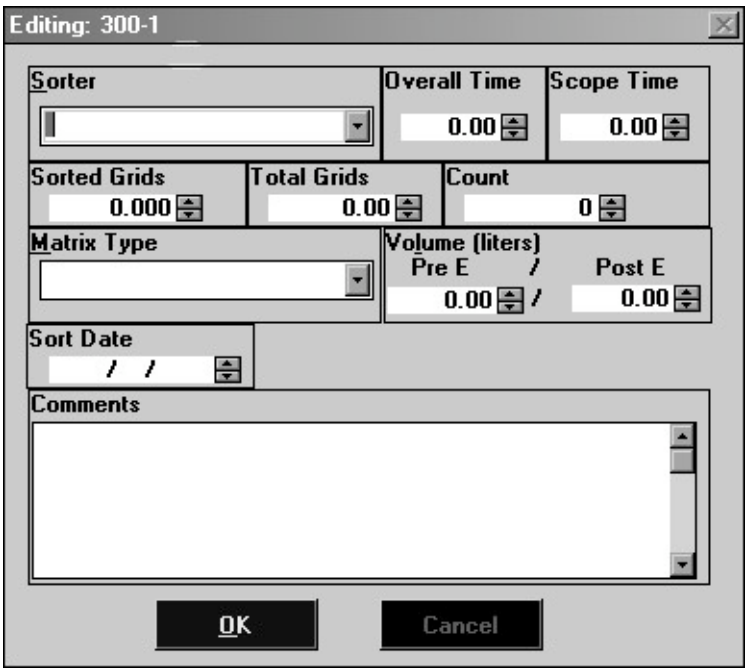
7) **S/T Grids:** Grids Sorted / Total Grids

8) **Large/Rare:** Were L/R specimens picked – YES or NO?

9) **Sort Time (Overall and Scope):** Record in hours, and quarter hours. Overall is from the time you check the sample off from the sort check list to when you put sort information into the data base. Scope time is time actually spent sitting at the scope sorting, i.e. not including elutriation and other set up time such as paperwork and data entry.

10) **Data Entered:** Check when data entry is executed for each sample.

Appendix A-3


Editing: 300-1
Sorter
Overall Time
0.00
Scope Time
0.00
Sorted Grids
0.000
Total Grids
0.00
Count
0
Matrix Type
Volume (liters)
Pre E / Post E
0.00 / 0.00
Sort Date
/ /
Comments
OK
Cancel

Appendix B-1

QA/QC Form: Sorting Efficacy/Label Quality, PIN: _____ **Project:** _____

A		B		C	D	E	F	G	H	I	J	K	L
mple	OS **by**	**Original Count**	**QC by**	**20% QC Count**	**QC'd Grids**	**Total Grids**	**Est. Total Missed**	**Est. Total Count**	**Est. % Effective**	**Remainder count**	**Remainder count + 20% QC count**	**Overall Total Count**	**Actual % Effective**
		510		10	2	16	(C/D)*E= 80	B + F = 590	(B/G)*100 = 86	70	I + C = 80	B + J = 590	(B/K)*100 = 86
		590		1	2	16	(C/D)* E = 8	B + F = 598	(B/G)*100 = 99				
										Comments:			
										Comments:			
Hrs:				QC2 Hrs:			QC3 Hrs:						
Date:				Date:			Date:						
										Comments:			
Hrs:				**QC2 Hrs:**			**QC3 Hrs:**						
Date:				**Date:**			**Date:**						
										Comments:			
Hrs:				QC2 Hrs:			QC3 Hrs:						
Date:				Date:			Date:						
										Comments:			
Hrs:				**QC2 Hrs:**			**QC3 Hrs:**						
Date:				**Date:**			**Date:**						
										Comments:			
Hrs:				QC2 Hrs:			QC3 Hrs:						
Date:				Date:			Date:						

Appendix B-2

QA/QC Log Sheet Instructions

1) Write the **PIN** and **Project Name** at the top of each QC sheet.

2) **SIN:** Sample identification number, EcoAnalysts lab identification number.

3) **OS by:** The Original Sorter's initials

4) **Original Count:** Record the original sorter macro count taken from the general vial label.

5) **QC by:** The QC tech's initials

6) **20% QC Count:** The number of identifiable bugs found in 20% of the QC'd portion of the sample.

7) **QC'd Grids:** The number of grids QC'd from the caton that the sorted portion of the sample is redistributed into. (Should equal 20% of the total grids)

8) **Total Grids:** The number of total caton grids the sorted portion is redistributed into.

9) **Estimated Total Missed:** The number of bugs estimated to have been missed during the previous sort. This number is calculated by dividing the number of bugs found during the QC of 20% of the sorted portion of the sample by the number of QC'd grids then multiplying the quotient by the total number of grids the sorted portion was redistributed in.

10) **Estimated Total Count:** The estimated total number of bugs that existed in the sorted portion of the sample. This number is calculated by adding the original (or previous) sort count to the estimated number of bugs missed.

11) **Estimated % Effective:** This is the estimated percentage of bugs picked during the original (or previous) sort. This number is calculated by dividing the original sort count by the estimated total count and multiplying the quotient by 100.

12) **QC1 Hrs:** The amount of time in hours and quarter hours from the set up of the QC to when data entry for that sample is complete and includes all paperwork time for that sample.

13) **Date:** Self

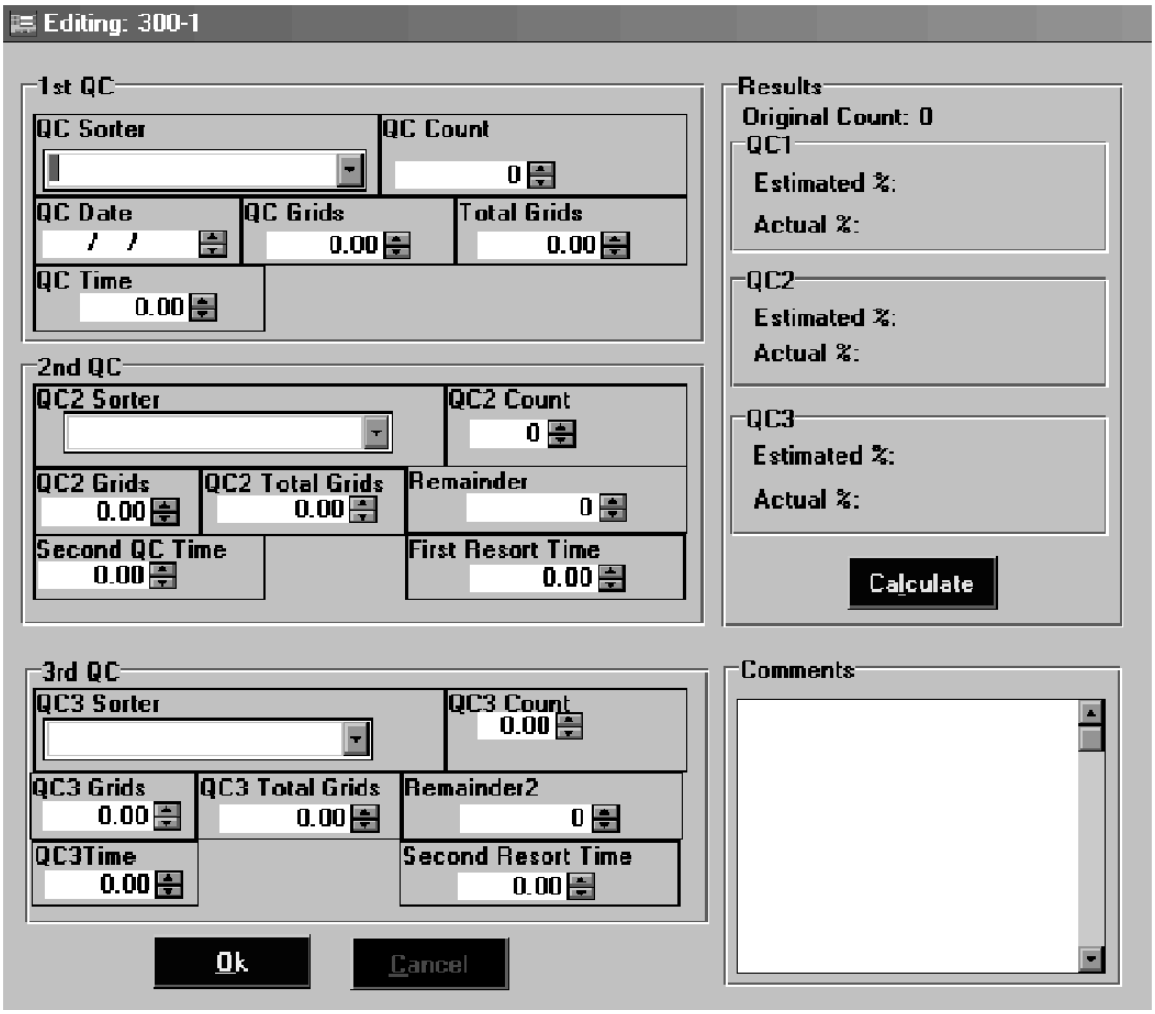
14) **Remainder Count:** If the sample is resorted due to a failed QC, this is where the number of bugs found during the resort is recorded.

15) **Remainder count + 20% QC count:** Record the appropriate calculation.

16) **Overall Total Count:** The total number of bugs contained in the vials for that sample which were found during the previous sorts (and resorts), previous QC's and the present resort.

17) **Actual % Effective:** Instead of an estimated percentage, this number is the actual percentage of bugs that were picked during the previous sort.

Appendix B-3

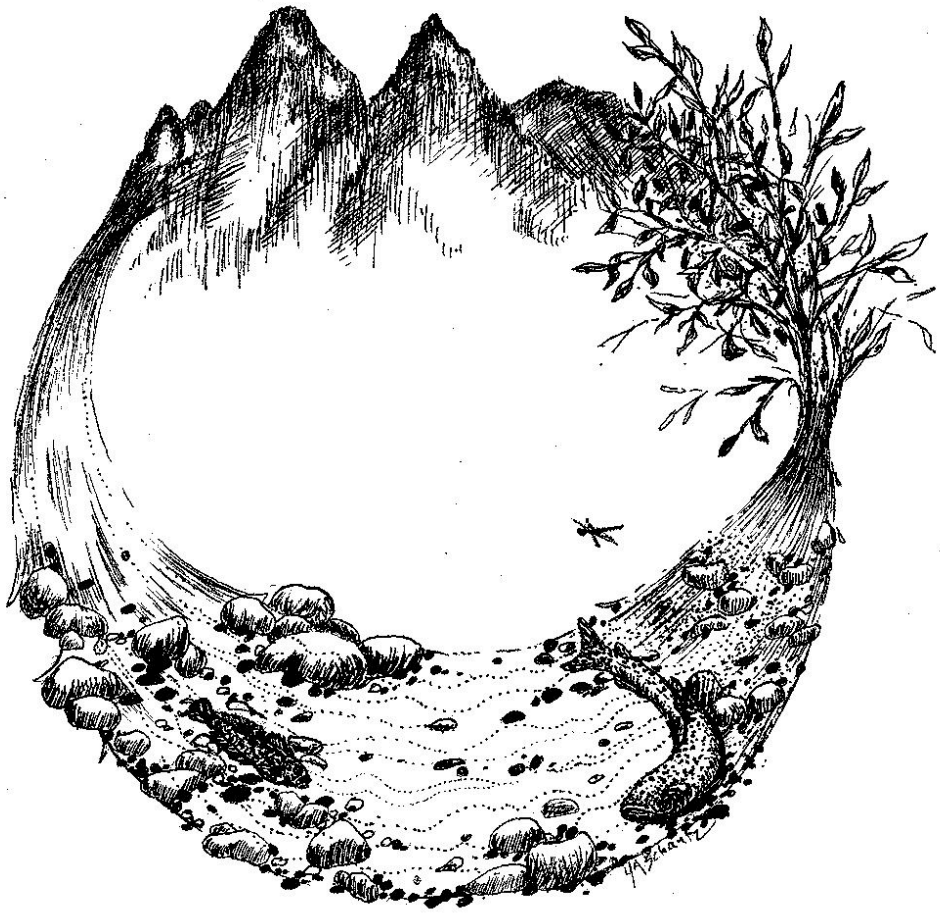

Editing: 300-1
1st QC
QC Sorter
QC Count
0
QC Date
/ /
QC Grids
0.00
Total Grids
0.00
QC Time
0.00
2nd QC
QC2 Sorter
QC2 Count
0
QC2 Grids
0.00
QC2 Total Grids
0.00
Remainder
0
Second QC Time
0.00
First Resort Time
0.00
3rd QC
QC3 Sorter
QC3 Count
0.00
QC3 Grids
0.00
QC3 Total Grids
0.00
Remainder2
0
QC3Time
0.00
Second Resort Time
0.00
Ok
Cancel
Results
Original Count: 0
QC1
Estimated %:
Actual %:
QC2
Estimated %:
Actual %:
QC3
Estimated %:
Actual %:
Calculate
Comments

ATTACHMENT 4: BMI Taxonomic Identification SOP

ECOANALYSTS, INC.

STANDARD OPERATING PROCEDURES

Taxonomy



105 East 2nd Street, Suite 1
Moscow, Idaho 83843

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Sample Identification

EcoAnalysts, Inc. taxonomists emphasize morphological characters as the primary method of identifying macroinvertebrates. Identifications are made to the level specified by the client using dissecting and compound microscopes. An extensive library of taxonomic literature, as well as a reference collection of specimens verified by nationally known taxonomists is used to determine the identity of invertebrates. Species limits are often defined by specialists using the adult male that has distinct morphological and genitalia characters. Reliable species-level identification of immature stages is often impossible as larvae of several species within a genus cannot be distinguished using morphological characters. Therefore generic-level determinations are common in datasets. Some taxonomists use distributional data in order to put a species name on a specimen. However, we discourage this practice as many historical distributions are now outdated and reliance upon distributional data can ultimately be a considerable source of error. Where possible, and if indicated by the client, identifications are made to the genus/species level (including Chironomidae). This taxonomic effort level corresponds to USEPA RBP Level III biological assessment protocols (USEPA 2000). The EcoAnalysts, Inc. process of identifying non-oligochaete benthic macroinvertebrates to the lowest practical level follows these steps:

- A sorted sample vial is selected for identification and the contents are emptied into a petri dish.
- Under a dissecting microscope, the invertebrates are separated into higher-level taxonomic groups (e.g. order or family for insects)
- The specimens within each taxonomic group are then identified to the lowest practical level, unless otherwise specified by the client.
- The number of individuals of each taxon is counted and entered into our macroinvertebrate data entry program.
- At least one specimen (preferably 3-5) of each taxon encountered is placed into a 1-dram vial containing 70% ethanol. A label with taxonomic identification and sample number is inserted into the vial. These specimens will comprise the project synoptic reference collection.
- Once an entire sample is completed the non-reference collection organisms are returned to the original vial and a taxonomic bench sheet for the sample is printed.
- This process is repeated with each sample until the entire project is completed.

Quality Assurance of Taxonomic Identification

EcoAnalysts, Inc. uses one of two methods of quality control to ensure taxonomic accuracy and consistency. As a comprehensive, qualitative QC we retain a synoptic voucher collection consisting of at least one good specimen (preferably 3-5 specimens) of each taxon encountered within a project. If multiple taxonomists are involved, they each maintain their own collections. Upon completion, this collection is reviewed by a second taxonomist or by an outside laboratory. On occasion, we will encounter an unfamiliar specimen that we must send to an outside specialist for verification. We will then retain these verified specimens in our laboratory reference collection.

At an additional cost, a quantitative QC can also be performed. The quantitative QC is obtained by choosing 10% of the samples for re-identification. The original taxonomists do not know in advance which samples will be re-identified. After all samples have been identified, the taxonomic coordinator uses a computer program to generate a list of random QC sample numbers. Projects with more than approximately one hundred samples or projects that need to be QC'd by region may have random sample numbers selected from subsets within the project. After the QC taxonomist re-identifies the sample the data manager produces a taxonomic QC report which lists taxa, abundance by taxonomist, and a similarity index for the each sample. Typically, at genus/species level, our taxonomists using a common standard taxonomic effort can attain uncorrected similarities of about 90%. The two taxonomists review the QC report, discuss any differences and determine how best to reconcile those differences. Any errors in identification, enumeration, or data entry are discussed and corrected. If it is determined that misidentifications were pervasive the original taxonomist will revisit any sample with the taxon in question and ascertain the accuracy of the determination. If enumeration is determined to be an important source of error the original taxonomist will reexamine the samples and attempt to reconcile the count. Exact similarity (100%) is virtually unattainable due to subtle and complex factors, e.g. destructive methods that must be applied to identify some taxa or removal of type specimens for the reference collection.

The EcoAnalysts, Inc. process of non-Oligochaete taxonomic quality assurance follows these steps:

- As a qualitative quality control check, the original taxonomist gives the synoptic reference collection to a QC taxonomist to verify the accuracy of all taxa identified in the project.
- In addition to the qualitative QC, and at an additional cost, a quantitative QC can be performed:
    - Ten percent of the samples are randomly selected for re-identification by the QC taxonomist.
    - A percent similarity is calculated for the original and QC sets of data.
    - Discrepancies are discussed and/or re-examined by the original and QC taxonomist.
    - The final data is adjusted according to mutual agreement by both taxonomists.

Mounting and Identifying Aquatic Oligochaeta

All genus/species identification of Oligochaeta are performed by an expert subcontractor. The resume for our specialist is available upon request. The process entails several steps: separating the oligochaetes from other sorted sample organisms, mounting and shipping oligochaetes to the specialist, identification of the specimens by the specialist, and importing the electronic data from the specialist into the project data set.

The sorters first separate Oligochaeta from the sorted portion of each sample into individual vials. Each vial has an internal label with the project and sample numbers and the word “Oligochaeta”. These vials along with the Chironomidae and general vials for the project are given to the EcoAnalysts taxonomists. As the taxonomists identify each sample they remove any Oligochaeta found in the general or Chironomidae vials and put them in the Oligochaeta vial for that sample. Once the project has been completed by the general and Chironomidae taxonomists the Oligochaeta vials are returned to the lab coordinator.

A specially trained lab technician mounts the oligochaetes on slides and completes an Oligochaeta log sheet indicating the number of oligochaetes per slide and the number of slides per sample. When all oligochaetes are mounted and the slides are dried the technician prepares a UPS shipping label, inserts one copy into the lab shipping log book and labels the package with the other. The package is shipped via UPS air to the specialist along with a copy of the log book page enumerating abundance per slide and slide per sample. The package is tracked until received by the subcontractor.

The Oligochaeta subcontractor completes identifications for the project according to guidelines set forth in the Oligochaeta Taxonomist Instruction Sheet. The subcontractor also uses the EcoAnalysts, Inc. taxonomic code list and a data entry template to ensure accurate species identification, data entry, and electronic data transfer into the project data set. The specialist reconciles his counts with the lab counts of specimens per slide and sample and explains reasons for any discrepancies there may be. The data is sent electronically to the EcoAnalysts data manager and the project slides are retained by the Oligochaeta taxonomist for thirty days unless otherwise specified by the client. Electronic data received from the Oligochaeta taxonomist is reconciled a second time by the EcoAnalysts data manager and conflicts are resolved before the data is imported into the general and Chironomidae data set. Throughout the year, a random selection of samples identified by our specialist is shipped to a second specialist for quality control and verification. The following steps will be followed by EcoAnalysts to prepare Oligochaeta for taxonomic identification:

- A log sheet is filled out with project and sample numbers.
- A vial of oligochaetes is selected for slide mounting.
- The vial is emptied into a petri dish.
- Under a fume hood, a slide is labeled with the unique sample number and slide number (e.g. Slide 1 of _) and a few drops of mounting CMC-10 media are placed onto the left and right halves of the slide.
- Using fine-tipped jeweler’s forceps individuals are removed from the petri dish and lined up in a head-down position in the mounting media. The number of organisms under each cover slip will vary according to their size.

- A cover slip is gently put over the slide and any air bubbles are removed by applying gentle pressure.
- When both cover slips are in place the slide is placed on a slide dryer over low heat to dry.
- The number of individuals on the slide is recorded on the log sheet.
- Once all slides for the sample are made the numbering sequence on each is completed (e.g. Slide 1 of 7).
- The project slides are boxed and shipped to the Oligochaeta taxonomist for identification.
- The specialist records all identifications to an electronic data entry form, reconciles sample and slide counts with those of EcoAnalysts, and emails the data to the EcoAnalysts data manager.
- The data manager checks the accuracy of the counts per sample in the Oligochaeta data set and appends it to the project data set when complete.

Quality Assurance of Non-Oligochaeta Taxonomic Data Entry

The taxonomists enter data into a database using a custom data entry program. For each project, each taxonomist maintains a data file on a network drive that is backed up daily. The identification data is also backed up to a network drive at the end of each session. As each taxon is identified, it is entered into the database using a unique code number. Taxa that are new to the system are assigned a unique code. A complete taxonomic reference including higher classification and known characteristics is entered for each new taxon. At the end of each sample the taxonomist examines the on-screen data and makes any necessary corrections before moving on to the next sample. The program generates a printed identification bench sheet upon completion of each sample. The data entry program has several built-in QC loops that eliminate most of the common data entry errors, e.g. typographical errors, duplications, omissions, and misspellings. When a taxonomist completes a project he gives the printed bench sheets to the taxonomic coordinator who then puts them in a project file folder. When an entire project is complete the taxonomic coordinator compiles all the data from the project taxonomists into a new database table and reviews the data set for completeness, accuracy and target organism counts. A synoptic taxa list is printed by the coordinator and is then routed to each of the project taxonomists for review, correction if necessary, and signature. The signed synoptic taxa list is put in the project folder with all printed bench sheets. After all reviews and corrections are made the taxonomic coordinator delivers the project folder to the data manager.

The data manager appends Oligochaeta data to the project data set if necessary, runs a series of quality control checks on the data and then compiles the data, graphs, and/or reports into the format specified by the client. All deliverables are reviewed by the project leader before being sent to the client. If necessary, corrections are made and the data is delivered to the client. On the date of data delivery, all project samples are placed in a bin and labeled with a date thirty days in the future. These samples are retained by EcoAnalysts for this period of time in the event they need to be reevaluated. After thirty days the samples are returned to the client.

APPENDIX E

SWAMP LABORATORY QUALITY CONTROL REQUIREMENTS

SWAMP LABORATORY QUALITY CONTROL REQUIREMENTS

SWAMP will require all participating laboratories to demonstrate capability continuously through:

1. Strict adherence to common QA/QC procedures.
2. Routine analysis of certified reference materials (CRMs).
3. Regular participation in an on-going series of interlaboratory comparison exercises.

Because SWAMP is specifically designed to provide information on "ambient" conditions in the state's surface waters, the ability to provide low-level contaminant analysis is critical. This is a "performance-based" approach for measurements of low-level contaminant analyses, involving continuous laboratory evaluation through the use of accuracy-based materials (e.g., CRMs), laboratory matrix spikes, laboratory method blanks, calibration standards, laboratory- and field-duplicated samples, and others as appropriate. The definition and use of each of these types of quality control samples are explained further below.

Quality control operates to make sure that data produced are satisfactory, consistent, and dependable. Under SWAMP's performance-based chemistry QA program, laboratories are not required to use a single, standard analytical method for each type of analysis, but rather are free to choose the best or most feasible method within the constraints of cost and equipment that is suitable for meeting the method quality objectives (DQO's), as outlined in **Appendix C** of the SWAMP QAPP (Data Acceptability Criteria tables). SWAMP has developed specific guidelines for measurement precision, accuracy, and levels of detection that are reflected in sampling, handling, and analysis requirements to satisfy a large spectrum of potential management questions. Each laboratory will continuously demonstrate proficiency and data comparability through routine analysis of accuracy-based performance evaluation samples, split samples, and reference materials representing actual sample matrices. No single analytical method has been officially approved for low-level analysis of organic and inorganic contaminants in water or sediments. Recommended methods are available and are provided in **Appendix C's** Target Reporting Limits section (listing of recommended methods) in the SWAMP QAPP.

All laboratories providing analytical support for chemical or biological analyses will have the appropriate facilities to store, prepare, and process samples, and appropriate instrumentation and staff to provide data of the required quality within the time period dictated by the project. Laboratories are expected to conduct operations in a way that includes:

1. A program of scheduled maintenance of analytical balances, microscopes, and other laboratory equipment and instrumentation.
2. Routine checking of analytical balances using a set of standard reference weights (American Society of Testing and Materials (ASTM) Class 3, NIST Class S-1, or equivalents).
3. Checking and recording the composition of fresh calibration standards against the previous lot. Acceptable comparisons are <5 percent difference from previous value.

4. Recording all analytical data in bound (where possible) logbooks, with all entries in ink, or electronic format.
5. Monitoring and documenting the temperatures of cold storage areas and freezer units once per week.
6. Verifying the efficiency of fume hoods.
7. Having a source of reagent water meeting ASTM Type I specifications (ASTM, 1984) available in sufficient quantity to support analytical operations. The resistivity of the reagent water will not exceed 18 megaohm at 25°C. Alternately, the conductivity of the reagent water will exceed 10 □mhos/cm.
8. Labeling all containers used in the laboratory with date prepared, contents, initials of the individual who prepared the contents, and other information as appropriate.
9. Dating and safely storing all chemicals upon receipt. Proper disposal of chemicals when the expiration date has passed.
10. Having QAPP, SOPs, analytical methods manuals, and safety plans readily available to staff.
11. Having raw analytical data, such as chromatograms, accessible so that they are available upon request.

Laboratories will be able to provide information documenting their ability to conduct the analyses with the required level of data quality. Such information might include results from interlaboratory calibration studies, control charts and summary data of internal QA/QC checks, and results from certified reference material analyses.

QA/QC Documentation

All laboratories will have the latest revision of the SWAMP QAMP. In addition, the following documents and information will be current, and they will be available to all laboratory personnel participating in the processing of SWAMP samples, as well as to SWAMP project officials:

1. Laboratory QA Plan: Clearly defined policies and protocols specific to a particular laboratory, including personnel responsibilities, laboratory acceptance criteria and corrective actions to be applied to the affected analytical batches, qualification of data, and procedures for determining the acceptability of results.
2. Laboratory Standard Operating Procedures (SOPs): Containing instructions for performing routine laboratory procedures.
3. Laboratory Analytical Methods Manual: Step-by-step instructions describing exactly how a method is implemented in the laboratory for a particular analytical procedure. Contains all analytical methods utilized in the particular laboratory for SWAMP.
4. Instrument Performance Information: Information on instrument baseline noise, calibration standard response, analytical precision and bias data, detection limits, etc. This information is usually recorded in logbooks or laboratory notebooks.

5. Control Charts: Control charts are useful in evaluating internal laboratory procedures and are helpful in identifying and correcting systematic error sources. Contract laboratories are encouraged to develop and maintain control charts whenever they may serve in determining sources of analytical problems.

Recommended Typical Laboratory Performance Measurements

Typical laboratory performance measurements included in the analysis stream and designed to check if data quality criteria are met are recommended and briefly defined below. SWAMP **Data Acceptability Criteria are provided for all analytical groups for all media in Appendix C of the SWAMP QAPP. Note that not all media may have all of these performance measurements (See App C).**

1. Method Blanks (also called extraction blanks or preparation blanks): These account for contaminants present in the preservative and analytical solutions and equipment used during the preparation and quantification of the parameter.
2. Injection Internal Standards and/or Surrogates: These account for error introduced by the analytical instrument or extraction process.
3. Matrix Spike Samples: These are field samples to which a known amount of contaminant is added and used to measure potential analytical interferences present in the field sample.
4. Replicate Samples: These are replicates of extracted material that measure the instrumental precision.

   a. Laboratory Replicate Samples: These are replicates of the raw material that are extracted and analyzed to measure laboratory precision.

   b. Matrix Spike Replicate Samples: These are used to assess both laboratory precision and accuracy. They are particularly useful when the field samples analyzed do not contain many of the target compounds (measuring non-detects in replicate does not allow the data reviewer to measure the precision or the accuracy of the data in an analytical batch).
5. Certified Reference Materials (CRM): Analysis of CRMs is another way of determining accuracy of the analysis by comparing a certified value of material with similar concentrations as those expected in the samples to be analyzed.

These types of samples serve to check if errors were introduced during the analysis process and if so, at what step(s) and at what magnitude. The remainder of this document will provide RMP guidance for general laboratory requirements and protocols for checking and tracking possible sources of errors (outlined above) in the analytical process.

Laboratory Quality Control Procedures

The performance-based protocols utilized in SWAMP for analytical chemistry laboratories consist of two basic elements: initial demonstration of laboratory capability for new laboratories (e.g., documentation that the analyses of samples are within the data quality criteria), and

ongoing demonstration of capability. Prior to the initial analysis of samples, each new laboratory will demonstrate capability and proficiency.

INITIAL DEMONSTRATION OF CAPABILITY

Instrument Calibration

Upon initiation of an analytical run, after each major equipment disruption, and whenever on-going calibration checks do not meet recommended DQOs (see **Appendix C of the SWAMP QAPP**), the system will be calibrated with a full range of analytical standards. Immediately after this procedure, the initial calibration must be verified through the analysis of a standard obtained from a different source than the standards used to calibrate the instrumentation, prepared in an independent manner, and ideally having certified concentrations of target analytes of a certified reference material (CRM) or certified solution. Frequently, calibration standards are included as part of an analytical run, interspersed with actual samples. However, this practice does not document the stability of the calibration and is incapable of detecting degradation of individual components, particularly pesticides, in standard solutions used to calibrate the instrument. The calibration curve is acceptable if it has a r^2 of 0.990 or greater for all analytes present in the calibration mixtures. If not, the calibration standards, as well as all the samples in the batch must be re-analyzed. All calibration standards will be traceable to a recognized organization for the preparation and certification of QA/QC materials (e.g., NIST, National Research Council Canada (NRCC), US EPA, etc.).

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

Initial Documentation of Method Detection Limits

Analytical chemists have coined a variety of terms to define "limits" of detectability; definitions for some of the more commonly used terms are provided in Keith *et al.* (1983) and in Keith (1991). In SWAMP, the method detection limit (MDL) is used to define the analytical limit of detectability. The MDL represents a quantitative estimate of low-level response detected at the maximum sensitivity of a method. The Code of Federal Regulations (40 CFR Part 136) gives the following rigorous definition:

> "The MDL is the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte."

The American Society of Testing and Materials (ASTM) defines the limit of detection as:

"A concentration of twice the criterion of detection...when it has been decided that the risk of making a Type II error is to be equal to a Type I error."

In order to compare MDLs in quantitative terms by different laboratories participating in SWAMP analyses, MDLs will initially be determined according to 40 CFR 136.2 (f) and Appendix B of 40 CFR 136. Determining the MDL with this procedure is elaborate and need not be determined annually provided that:

1. No process or method changes have been made.
2. Check samples containing an analyte spike at about 2x MDL indicate that the sample is detected. The required frequency of check samples is quarterly.

The matrix and the amount of sample (i.e., dry weight of sediment or tissue) used in calculating the MDL will match as closely as possible the matrix of the actual field samples and the amount of sample typically used.

Limits of Quantification

In order to ensure comparability of results among different laboratories, recommended Reporting Limit (quantification) values have been established for SWAMP, termed **Target Reporting Limits, or TRL's (see Appendix C of the SWAMP QAPP)**. These TRL's have been derived empirically. In most cases, they are 2-5 times the MDL as determined by the above process. Most are considerably lower than water quality objectives or sediment and tissue quality guidelines and provide the foundation for having a high level of certainty in the data.

The laboratory shall confirm the ability to analyze low-level samples with each batch. This shall be accomplished by analyzing a method blank spiked at 3 to 5 times the method detection limit or a reference material in the appropriate range. Recoveries for organic analyses shall be between 50 and 150% for at least 90% of the target analytes.

Taylor (1987) states that "a measured value becomes believable when it is larger than the uncertainty associated with it". The uncertainty associated with a measurement is calculated from the standard deviation of replicate measurements (s_0) of a low concentration standard or a blank. Normally, the MDL is set at three times the standard deviation of replicate measurements, as it is at this point that the uncertainty of a measurement is approximately ±100% at the 95% level of confidence. The limit of quantification (LOQ, which SWAMP is referring to as the Reporting Limit, or RL), as established by the American Chemical Society, is normally ten times the standard deviation of replicate measurements, which corresponds to a measurement uncertainty of ±30% (see Taylor, 1987). By these standard definitions, measurements below the MDL are not believable, measurements between the LOQ (RL) and the MDL are only semi-quantitative, and confidence in measurements above the LOQ is high.

Initial Blind Analysis of Representative Samples

As appropriate, representative sample matrices which are uncompromised, homogeneous, and contain the analytes of interest at concentrations of interest will be used to evaluate performance of analytical laboratories new to SWAMP prior to the analysis of field samples. The samples used for this initial demonstration of laboratory capability typically will be distributed blind (i.e.,

the laboratory will not know the concentrations of the analytes of interest) as part of the SWAMP interlaboratory comparison exercises, with the SWAMP QA Program staff conducting and evaluating the exercise. Based on results that have typically been attained by experienced laboratories, a new laboratory's performance generally will be considered acceptable if its submitted values are within the DQO's **(outlined in Appendix C of the SWAMP QAPP)** of the known concentration, or the consensus value, of each analyte of interest in the samples. These criteria apply only for analyte concentrations equal to or greater than three times the RL. If the results for the initial analysis fail to meet these criteria, the laboratory will be required to repeat the analysis until the performance criteria are met, prior to the analysis of SWAMP field samples.

Record of Certified Reference Material

As CRMs are routinely included in analysis of batches of reputable laboratories, the historical record of results may also serve as a suitable performance indicator.

ONGOING DEMONSTRATION OF CAPABILITY

Participation in Interlaboratory Comparison Exercises

Through an interagency agreement, NOAA's NIST Program and EPA's EMAP program jointly sponsor an on-going series of interlaboratory comparison exercises (round-robins). All SWAMP analytical laboratories are at this point encouraged to participate in these intercomparison exercises, which are conducted jointly by NIST and NRCC. In the near future, this most likely will become a mandatory participation, with approval from NOAA/NIST. SWAMP would then be conducting its own annual interlaboratory calibration exercise for media types not covered within the NOAA/NIST intercalibration (primarily water media), and it will be mandatory for all participating SWAMP labs. These exercises provide a tool for continuous improvement of laboratory measurements by helping analysts identify and resolve problems in methodology and/or QA/QC. The results of these exercises are also used to evaluate both the individual and collective performance of the participating analytical laboratories on a continuing basis and to insure that ongoing measurements are meeting Data Acceptability Criteria. The SWAMP laboratories will be required to initiate corrective actions if their performance in these comparison exercises falls below pre-determined minimal standards.

It is planned for there to be one exercise conducted over the course of a year. In a typical exercise as planned for SWAMP, the 3rd party (referee) contractor will distribute performance evaluation samples of an "unknown" and a certified reference material (CRM) to each laboratory, along with detailed instructions for analysis. A variety of performance evaluation samples could be utilized, including accuracy-based solutions, sample extracts, and representative matrices (e.g., sediment or tissue samples). Laboratories are required to analyze the sample(s) "blind" and will submit their results in a timely manner to the SWAMP interlaboratory calibration study coordinator (as instructed). Laboratories which fail to maintain acceptable performance may be required to provide an explanation and/or undertake appropriate corrective actions. At the end of each calendar year, coordinating personnel at the 3rd party (referee) contract QA Program will participate in a QA workshop to present and discuss the comparison exercise results. Additionally, a written summary of the evaluation will be provided.

Routine Analysis of Certified Reference Materials or Laboratory Control Materials

Certified reference materials generally are considered the most useful QC samples for assessing the accuracy of a given analysis (i.e., the closeness of a measurement to the "true" value). CRMs can be used to assess accuracy because they have "certified" concentrations of the analytes of interest, as determined through replicate analyses by a reputable certifying agency using two independent measurement techniques for verification. In addition, the certifying agency may provide "non-certified or "informational" values for other analytes of interest. Such values are determined using a single measurement technique, which may introduce unrecognized bias. Therefore, non-certified values must be used with caution in evaluating the performance of a laboratory using a method which differs from the one used by the certifying agency.

A laboratory control material (LCM) is similar to a certified reference material in that it is a homogeneous matrix that closely matches the samples being analyzed. A "true" LCM is one that is prepared (i.e., collected, homogenized, and stored in a stable condition) strictly for use in-house by a single laboratory. Alternately, the material may be prepared by a central laboratory and distributed to others (so-called regional or program control materials). Unlike CRMs, concentrations of the analytes of interest in LCMs are not certified but are based upon a statistically valid number of replicate analyses by one or several laboratories. In practice, this material can be used to assess the precision (i.e., consistency) of a single laboratory, as well as to determine the degree of comparability among different laboratories. If available, LCMs may be preferred for routine (i.e., day to day) analysis because CRMs are relatively expensive.

Routine analysis of CRMs or, when available, LCMs represents a particularly vital aspect of the "performance-based" SWAMP QA philosophy. At least one CRM or LCM must be analyzed along with each batch of 20 or fewer samples (i.e., QA samples should comprise a minimum of 5% of each set of field samples). For CRMs, both the certified and non-certified concentrations of the target analytes will be known to the analyst(s) and will be used to provide an immediate check on performance before proceeding with a subsequent sample batch. Performance criteria for both precision and accuracy have been established for analysis of CRMs or LCMs **(Appendix C of the SWAMP QAPP)**; these criteria are discussed in detail in the following paragraphs. If the laboratory fails to meet either the precision or accuracy control limit criteria for a given analysis of the CRM or LCM, the data for the entire batch of samples is suspect. Calculations and instruments will be checked; the CRM or LCM may have to be reanalyzed (i.e., reinjected) to confirm the results. If the values are still outside the control limits in the repeat analysis, the laboratory is required to find and eliminate the source(s) of the problem and repeat the analysis of that batch of samples until control limits are met, before final data are reported. The results of the CRM or LCM analysis will never be used by the laboratory to "correct" the data for a given sample batch.

Precision criteria: Precision is the reproducibility of an analytical method. Each laboratory is expected to maintain control charts for use by analysts in monitoring the overall precision of the CRM or LCM. Upper and lower control chart limits (e.g., warning limits and control limits) will be continually updated; control limits based on 99% confidence intervals around the mean are recommended. The relative standard deviation (RSD) will be calculated for each analyte of interest in the CRM based on the last 7 CRM analyses. Acceptable precision targets for various analyses are listed in **Appendix C of the SWAMP QAPP**.

Laboratory Replicates for Precision

A minimum of one field sample per batch of SWAMP samples submitted to the laboratory will be processed and analyzed in duplicate or more for precision. The relative percent difference among replicate samples (RPD expressed as percent) will be less than the DQO's listed in **Appendix C of the SWAMP QAPP** for each analyte of interest. Following are the calculations:

Each measured value is compared against the known value of the standard, and accuracy is expressed as the relative percent difference.

$$RPD = \text{ x } 100\%$$

Where: RPD = the relative percent difference
Vm = the measured value,
Vk = the known value.

If results for any analytes do not meet the DQO's for the RPD, calculations and instruments will be checked. A repeat analysis may be required to confirm the results. Results which repeatedly fail to meet the objectives indicate sample in-homogeneity, unusually high concentrations of analytes or poor laboratory precision. In this case, the laboratory is obligated to halt the analysis of samples and eliminate the source of the imprecision before proceeding.

Accuracy criteria: The "absolute" accuracy of an analytical method can be assessed using CRMs only when certified values are provided for the analytes of interest. However, the concentrations of many analytes of interest to SWAMP are provided only as non-certified values in some of the more commonly used CRMs. Therefore, control limit criteria are based on "relative accuracy", which is evaluated for each analysis of the CRM or LCM by comparison of a given laboratory's values relative to the "true" or "accepted" values in the LCM or CRM. In the case of CRMs, this includes both certified and noncertified values. The "true" values are defined as the 95% confidence intervals of the mean.

Based on typical results attained by experienced analysts in the past, accuracy control limits have been established both for groups of compounds (**Appendix C of the SWAMP QAPP**).

Continuing Calibration Checks (CCC's)

Calibration check solutions traceable to a recognized organization must be inserted as part of the sample stream. The source of the calibration check solution shall be independent from the standards used for the calibration. Calibration check solutions used for the continuing calibration checks will contain all the analytes of interest. The frequency of these checks is dependent on the type of instrumentation used and, therefore, requires considerable professional judgment. All organic analyses shall be bracketed by an acceptable calibration check. **Appendix C of the SWAMP QAPP** provides specific frequencies and other criteria for CCC's. If the control limits for analysis of the calibration check solution (set by the laboratories) are not met, the initial calibration will have to be repeated. If possible, the samples analyzed before the calibration check solution that failed the DQO's in **Appendix C of the SWAMP QAPP** will be reanalyzed following recalibration. The laboratory will begin by reanalyzing the last sample analyzed before the calibration check solution which failed. If the RPD between the results of this

reanalysis and the original analysis exceeds precision DQO's (**Appendix C of the SWAMP QAPP**), the instrument is assumed to have been out of control during the original analysis. If possible, reanalysis of samples will progress in reverse order until it is determined that the RPD between initial and reanalysis results are within DQO's (**Appendix C of the SWAMP QAPP**). Only the re-analysis results will be reported by the laboratory. If it is not possible or feasible to perform reanalysis of samples, all earlier data (i.e., since the last successful calibration control check) are suspect. In this case, the laboratory will flag the data and prepare a narrative explanation to accompany the submitted data.

Laboratory Method Blank

Laboratory method blanks (also called extraction blanks, procedural blanks, or preparation blanks) are used to assess laboratory contamination during all stages of sample preparation and analysis. For both organic and inorganic analyses, one laboratory method blank will be run in every sample batch. The method blank will be processed through the entire analytical procedure in a manner identical to the samples. Method blank criteria are provided in **Appendix C of the SWAMP QAPP**. If eliminating the blank contamination is not possible, all impacted analytes in the analytical batch shall be flagged. In addition, a detailed description of the contamination source and the steps taken to eliminate/minimize the contaminants shall be included in the transmittal letter. Subtracting method blank results from sample results is not permitted.

Completeness

Completeness is defined as "a measure of the amount of data collected from a measurement process compared to the amount that was expected to be obtained under the conditions of measurement" (Stanley and Verner, 1985). Field personnel will always strive to achieve or exceed the SWAMP completeness goals of 90% (85% for fish, clam, and mussel tissues) for water, sediment, or biota (biological assessment) samples.

Surrogates

The usage of the terms "surrogate", "injection internal standard", and "internal standard" varies considerably among laboratories and is clarified here.

Surrogates are compounds chosen to simulate the analytes of interest in organic analyses. Surrogates are used to estimate analyte losses during the extraction and clean-up process and must be added to each sample, including QA/QC samples, prior to extraction. The reported concentration of each analyte is adjusted to correct for the recovery of the surrogate compound, as done in the NOAA NS&T Program. The surrogate recovery data will be carefully monitored; each laboratory must report the percent recovery of the surrogate(s) along with the target analyte data for each sample. If possible, isotopically-labeled analogs of the analytes will be used as surrogates.

Each laboratory will set its own warning limit criteria based on the experience and best professional judgment of the analyst(s). It is the responsibility of the analyst(s) to demonstrate that the analytical process is always "in control" (i.e., highly variable surrogate recoveries are not

acceptable for repeat analyses of the same certified reference material and for the matrix spike/matrix spike duplicate). The warning limit criteria used by the laboratory will be provided in the standard operating procedures submitted to SWAMP.

Data will be reported as surrogate-corrected values.

Internal Standards (if they are used)

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

Dual-Column Confirmation

Dual-column chromatography is required for analyses using GC-ECD due to the high probability of false positives arising from single-column analyses.

Matrix Spike and Matrix Spike Duplicate

A laboratory fortified sample matrix (commonly called a matrix spike, or MS) and a laboratory fortified sample matrix duplicate (commonly called a matrix spike duplicate, or MSD) will be used both to evaluate the effect of the sample matrix on the recovery of the compound(s) of interest and to provide an estimate of analytical precision. Frequencies and specifications for MS and MSD's are provided in **Appendix C of the SWAMP QAPP**. A field sample is first homogenized and then split into three subsamples. Two of these subsamples are fortified with the matrix spike solution and the third subsample is analyzed to provide a background concentration for each analyte of interest. The matrix spike solution should contain as many representative analytes from the SWAMP analyte list as feasible. The final spiked concentration of each analyte in the sample will be at least 5 times the MDL for that analyte, as previously calculated by the laboratory. Additionally, the total number of spikes should cover the range of expected concentrations. Recovery is the accuracy of an analytical test measured against a known analyte addition to a sample. Recovery is calculated as follows:

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

Recovery data for the fortified compounds ultimately will provide a basis for determining the prevalence of matrix effects in the samples analyzed during the project. If the percent recovery for any analyte in the MS or MSD is less than the recommended warning limit, the chromatograms (in the case of trace organic analyses) and raw data quantitation reports will be reviewed. If an explanation for a low percent recovery value is not discovered, the instrument response may be checked using a calibration standard. Low matrix spike recoveries may be a result of matrix interferences and further instrument response checks may not be warranted, especially if the low recovery occurs in both the MS and MSD, and the other QC samples in the batch indicate that the analysis was "in control". An explanation for low percent recovery values for MS/MSD results will be discussed in a cover letter accompanying the data package. Corrective actions taken and verification of acceptable instrument response will be included. Analysis of the MS/MSD is also useful for assessing laboratory precision. The RPD between the MS and MSD results should be less than the target criterion listed in **Appendix C of the SWAMP QAPP** for each analyte of interest.

APPENDIX F

CMP FIELD LOG SHEETS

Central Coast Cooperative Monitoring Program: SAMPLE EVENT 01

Field Meter Calibration Log **Meter ID:** _____ **Date:** _____

Time	Analyte	Pre-Cal Measurement	Post-Cal Measurement	CalMethod	Initials
_____AM	D.O.	_____	_____	_____mmHg	_____
_____AM	Chlorophyl a	_____	_____	0.0 Std	_____
_____AM	Chlorophyl a	_____	_____	10.0 Std	_____
_____AM	Turbidity	_____	_____	0 NTU	_____
_____AM	Turbidity	_____	_____	100 NTU	_____
_____AM	Turbidity	_____	_____	1000 NTU	_____
_____AM	Turbidity	_____	_____	3000 NTU	_____
_____AM	E.C.	_____	_____	500 μS/cm Std	_____
_____AM	E.C.	_____	_____	10,000 μS/cm Std	_____
_____AM	pH	_____	_____	7.0 Std	_____
_____AM	pH	_____	_____	10.0 Std	_____
_____PM	D.O.	_____	_____	_____mmHg	_____
_____PM	Chlorophyl a	_____	_____	0.0 Std	_____
_____PM	Chlorophyl a	_____	_____	10.0 Std	_____
_____PM	Turbidity	_____	_____	0 NTU	_____
_____PM	Turbidity	_____	_____	100 NTU	_____
_____PM	Turbidity	_____	_____	1000 NTU	_____
_____PM	Turbidity	_____	_____	3000 NTU	_____
_____PM	E.C.	_____	_____	500 μS/cm Std	_____
_____PM	E.C.	_____	_____	10,000 μS/cm Std	_____
_____PM	pH	_____	_____	7.0 Std	_____
_____PM	pH	_____	_____	10.0 Std	_____

HydroLab DS4a multi-meter must be calibrated at the beginning and end of each day.

CMP FIELD SAMPLING DATA LOG SHEET: EVENT 01
Ag Waiver Program

Station: Old Salinas River at Monterey Dunes Way

Latitude: *36.772291*
Longitude: *-121.787855*

GPS Reading .....

Personnel: .....

Date: .....

Field Meter Data

Time	Temp (°C)	pH

D.O. (mg/L) / %sat	Turbidity (NTU)	Chlorophyll a (µg/L)

E.C. (µS/cm)	Salinity (ppt)	Midchannel Depth (m)

Site Discharge Characterization

Wet Channel Width: ..... **Stage:** .....

Flow and depth recordings

.....
.....
.....
.....
.....
.....
.....
.....

Samples Collected

	Sample ID	Analyte	Time	Sample Depth	Notes*
	CMP-01-309OLD-010	*Nitrate (as N); TDS*			
	CMP-01-309OLD-011	*Total Ammonia*			
	CMP-01-309OLD-012	*Orthophosphate (as P)*			

*Field Observations***

Photo ID: 309OLD_2005_01 **Photo #:**

Dominant Substrate**	Algae % Filamentous	Algae % Periphyton	% Shading	Bank Vegitation Left Bank	Bank Vegitation Right Bank	Air Temp

Additional Notes or Comments:

Weather:
Water Color:
Odor:
In stream Activity:
Other Foreign Matter:

* SG = Surface Grab, direct to container; BG = Bucket Grab
** See attached "Field Observations" sheet for standard comments and further guidance.

Central Coast Cooperative Monitoring Program

Field Observation Guidance and Standard Comments

Qualitative Measures

1) Dominant Substrate:
   Record the dominant substrate in the upstream reach of the sample location using one of the following categories: Boulder (B), cobble (C), gravel (G), sand (S), fines (F) or cement (K).
2) Algae:
   a) Filamentous: Record the percent of the flowing water surface, up-stream from your sample location, that you estimate is occupied by filamentous algae.
   b) Other Periphyton: Record the percent of substrate in the wetted channel, looking up stream from your sample location, that you estimate is covered in periphyton. Other periphyton is defined here as the living community attached to the substrate, including algae that is not the green filamentous type, aquatic mosses, fungi, diatoms and sessile invertebrates. To make this estimate feel the surface of the rocks and other substrate materials and estimate the percent of the substrate that is covered with a slimy organic community.
3) Shading: Record the percent of the stream's surface (water surface), up-stream from your sample location, that you estimate would be shaded if the sun was directly over the creek.
4) Plants:
   a) Bank: Record the percent of the surface of both banks, up-stream from your sample location that you estimate to be covered by vegetation. This estimate refers only to plants and roots at the water's edge.
   b) In-Stream: Record the percent of the flowing water's surface, up-stream from your sample location that you estimate to be occupied by aquatic vegetation. This is a percent of the total water surface that is occupied by aquatic vegetation.

Other Notes

Visually assess the stream corridor and comment on anything that you feel may directly affect or contribute to changes in water quality. Some standard comments and categories of observations follow.

a) Recent/Current Weather Events: heavy rains, cold front or heat spells
b) Water Color: black, brown, yellow, white, green, etc.
c) Site Odors: sulfides, sewage, petroleum, unidentifiable odor or none.
d) In stream Activities: construction, major erosion events, recent scour or other
e) Other Foreign Matter: suspended matter, oily sheen, foam or other debris.
f) Biological Activity: Note the presence of fish, birds, mammals or invertebrates observed and record one of the following categories: True count up to 25, then estimate > 25, > 50 or >100.
g) Trash : Bank and in stream debris such as fertilizer bags, aerosol cans, batteries human wastes, homeless encampments, dumping of furniture or appliances. Record the true number of paper and recyclable trash items up to 10 items (count 1-10 items of trash), greater than 10 items should be recorded as >10 items of trash.
h) Tidal Influences: evidence of recent tidal surge (i.e. kelp or driftwood) or of possible salt-water influence.