Marina Copper Monitoring Study

State Water Resources Control Board Agreement No. 04-236-190-0

Quality Assurance Project Plan

Southern California Coastal Water Research Project
7171 Fenwick Lane
Westminster, CA 92683

June 2005

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PROJECT: Marina Copper Monitoring Study
State Water Resources Control Board Agreement No. 04-236-190-0

PREPARED BY: Southern California Coastal Water Research Project
7171 Fenwick Lane
Westminster, CA 92683

1. APPROVED BY:

Grant Organization

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Ken Schiff, Project Director Date
Southern California Coastal Water Research Project

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Jeff Brown, QA Officer Date
Southern California Coastal Water Research Project

______________________________________ ____________________
Gary Ichikawa, Lab Manager Date
Moss Landing Marine Laboratory

Funding Organization (RWQCB)

______________________________________ ____________________
Pete Michael, Contract Manager Date
San Diego Regional Water Quality Control Board

______________________________________ ____________________
First Last, QA Officer Date
San Diego Regional Water Quality Control Board

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3. DISTRIBUTION LIST

The final QAPP will be kept on file at SCCWRP. The following individuals will receive copies of the approved QAPP and any subsequent revisions:

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4. PROJECT/TASK ORGANIZATION

4.1 Involved Parties and Roles.

Southern California Coastal Water Research Project (SCCWRP) is a joint powers agency that was formed by several government agencies with a common mission to gather the necessary scientific information to effectively, and cost-efficiently, protect the Southern California aquatic environment. As the lead agency in this project, SCCWRP will organize the sample collection, field and in-house analysis of samples and data, the maintenance of contracts with California Department of Fish & Game, and all report preparation.

Ken Schiff will be the SCCWRP coordinator for this study and will establish a project team for planning and conducting the study (Table 1, Figure 1).

The Moss Landing Marine Laboratory (MLML) analytical chemistry laboratory located in Santa Cruz will perform the majority of chemical analyses of the water samples. Gary Ichikawa will oversee these analyses. SCCWRP will conduct the toxicity analyses. Steve Bay will oversee the toxicity analyses.

4.2 Quality Assurance Officer Role

Jeff Brown is SCCWRP's Quality Assurance Officer. Jeff's role is to establish the quality assurance and quality control procedures found in this QAPP as part of the sampling, field analysis, and in-house analysis procedures. Jeff will also work with the Laboratory Managers from MLML analytical laboratory and SCCWRP by communicating all quality assurance and quality control issues contained in this QAPP.

Jeff will also review and assess all procedures during the life of the contract against QAPP requirements. Jeff will report all findings to Ken Schiff, including all requests for corrective action. Jeff may stop all actions, including those conducted by the MLML if there are significant deviations from required practices or if there is evidence of a systematic failure.

4.3 Persons Responsible for QAPP Update and Maintenance.

Changes and updates to this QAPP may be made after a review of the evidence for change by SCCWRP's Project Director and Quality Assurance Officer, and with the concurrence of the both Regional Board's Contract Manager and Quality Assurance Officer. The Project Director will be responsible for making the changes, submitting drafts for review, preparing a final copy, and submitting the final for signature.

Table 1. (Element 4) Personnel responsibilities.

Name	Organizational Affiliation	Title	Contact Information (Telephone number, fax number, email address)
Ken Schiff	SCCWRP	Project Director	Tel: (714) 372-9202 Fax: (714) 894-9699 kens@sccwrp.org
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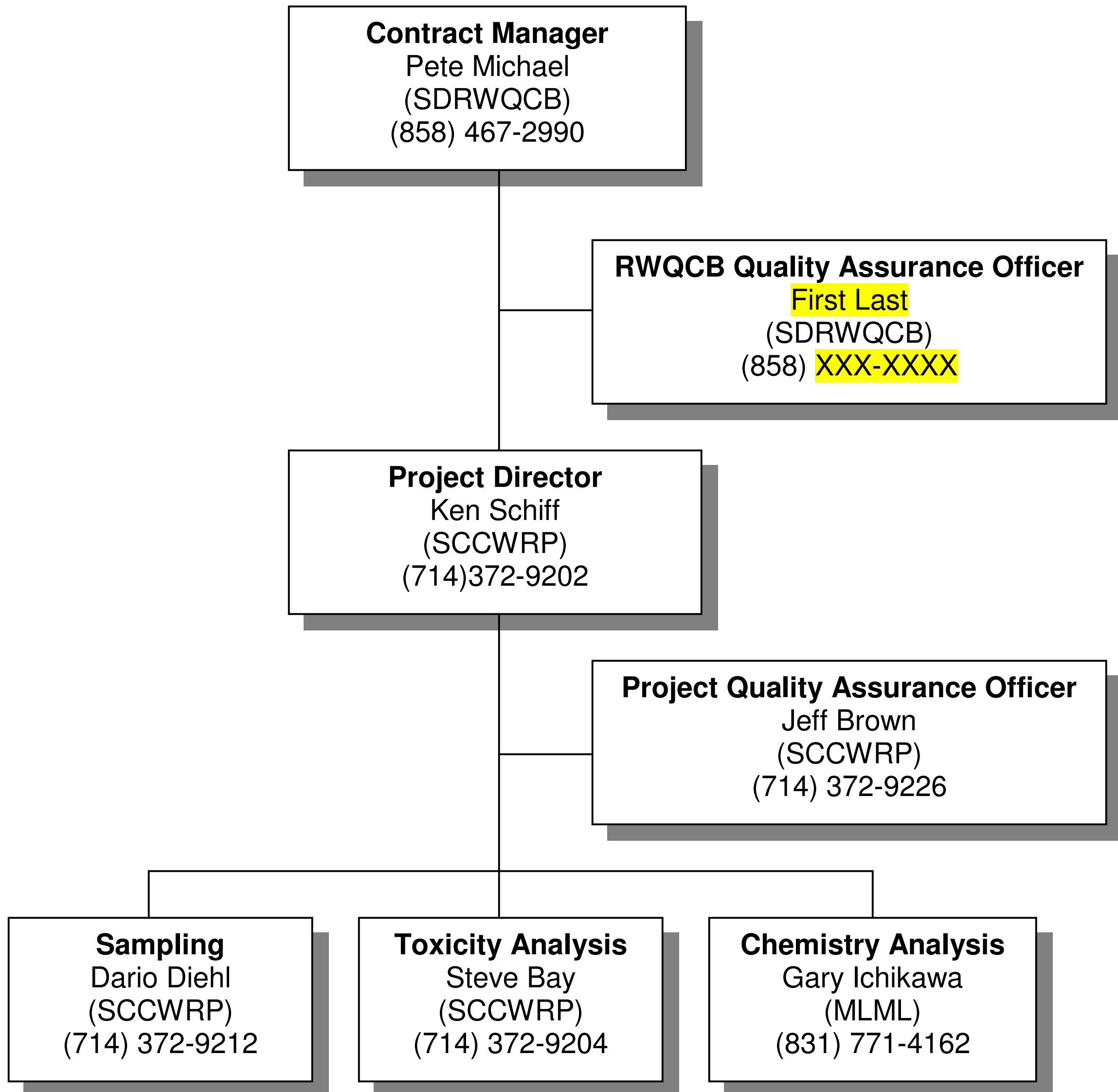
Gary Ichikawa	MLML	Laboratory Manager	Tel (831) 771-4162 Fax: (831) 633-0805 email: gichikawa@mlml.calstate.edu
Pete Michael	SDRWQCB	RWQCB Contract Manager	Tel (858) 467-2990 Fax: (858) pmichael@waterboards.ca.gov
First Last	SDRWQCB	RWQCB QA officer	Tel: Fax: email

4.4 Organizational Chart and Responsibilities

Figure 1. Organization chart



5. PROBLEM DEFINITION / BACKGROUND

5.1 Problem Statement

Marinas, especially in southern California, serve multiple beneficial uses. First, they are important waterbodies because they serve as protected anchorage and storage areas for recreational vessels in the region. Second, they are important ecological areas because there are so few shallow water embayments in the region. As such, many organisms use marinas for habitat.

Despite their relative importance, marinas also receive numerous discharges of potential pollutants. In specific, copper has been identified as a pollutant of concern. For example, measurements of copper in the Shelter Island Marina in San Diego Bay demonstrated that concentrations exceeded water quality thresholds of concern and resulted in aquatic toxicity to larval mussels (SDRWQCB reference). Sources of copper to Shelter Island include vessel antifouling coatings, shipyard or boatyard discharges, runoff from urban and industrial areas, atmospheric deposition, and resuspension of contaminated sediments. The contamination and toxicity found in Shelter Island Marina has resulted in its listing on the State's list of impaired waterbodies and has been subjected to a total maximum daily load (TMDL) for copper.

The extent of copper contamination in other marinas of the San Diego Region is unknown. No waterbody assessment in this region has been conducted to determine if the conditions in Shelter Island are unique or are more widespread. There are four harbors in the San Diego region, from Dana Point to San Diego Bay, that encompass 12 marinas. Almost all of the marinas have the same sources of copper as Shelter Island Marina, but differ in size, mixing, and circulation patterns.

5.2 Decisions or Outcomes

The contamination in Shelter Island Marina, and the lack of information from other marinas in the region, has led to two primary questions asked by environmental managers. The first question is "what is the extent and magnitude of copper contamination in marinas of the San Diego region?" Answering this question will help to determine if the copper problems in the Shelter Island Marina are an isolated occurrence or a more widespread water quality problem. The extent and magnitude of copper contamination will be assessed by comparing measured copper concentrations in marina waters to water quality thresholds. The second question is "Does the copper contamination in marinas result in biological impacts?" If the extent and/or magnitude of copper contamination is large, then the next logical step is to determine if this contamination is resulting in important effects on the ecosystem. We will assess the potential for ecological impacts by conducting toxicity tests on a subset of the water samples collected for copper concentrations. Ultimately, if toxicity is measured, toxicity identification evaluation can be used to determine if copper is the contaminant responsible for the observed toxicity.

5.3 Water Quality Regulatory Criteria

The main focus in this study is on the acute and chronic water quality criterion for dissolved copper in saltwater. These values are established by the US EPA (2002) and the State of California (2000):

Criterion maximum concentration (acute) = 4.8 µg/L

Criterion continuous concentration (chronic) = 3.1 µg/L

6. PROJECT/TASK DESCRIPTION

6.1 Work Statement and Produced Products

This element of the project will consist of three primary tasks including sampling, analysis, and reporting.

Sampling will be focused on the water column for chemistry and toxicity. In total, there will be thirty sites randomly distributed in marinas throughout the San Diego region. Water column samples will be collected at three depths (surface, mid-depth, and bottom) for chemical measurements of copper. An additional surface water sample at each site will be collected for toxicity analysis using larvae of the bivalve *Mytilus galloprovincialis*. The product for this task will be a sampling summary memo indicating sampling success and field observations at each site visited during the field program.

The second task will involve laboratory analysis. Laboratory analysis includes chemical measurements of copper in seawater. Laboratory analysis also includes toxicity testing using larvae of the bivalve *Mytilus galloprovincialis*. The product for this task will be a laboratory analysis summary memo indicating analytical success for all samples delivered to laboratory.

The final task will be reporting. This task involves information management, data analysis, and a final report. Information management will ensure consistency with the State's Surface Water Ambient Monitoring Program (SWAMP). Report writing will provide a description of all methods, tabulations of raw data, and interpretation of results. The product for this task will include a SWAMP compliant relational database for study results (including metadata) and a written final report.

6.2 Constituents to be Monitored and Measurement Techniques

For this element of the study, we will analyze copper using US EPA method 1638, Determination of Trace Elements in Ambient Waters by Inductively Coupled Plasma — Mass Spectrometry. In addition, we will measure toxicity using the larvae of the bivalve *Mytilus galloprovincialis* following US EPA/600/R-95/136. Toxicity Identification Evaluation (TIE) will be used to characterize the cause(s) of toxicity, where appropriate, using EPA/600/R-96/054.

Table 2. (Element 6) Analytical constituents and method requirements.

Analyte	Method
Metal	
Dissolved Cu	EPA 1638
Toxicity	
Mussel embryo development test	EPA/600/R-95/136, EPA/600/R-96/054 (TIE)

6.3 Project Schedule

Table 3. (Element 6) Project schedule.

Activity	**Anticipated date of completion**	**Deliverable**	**Deliverable due date**
QAPP Production	7/31/05	QAPP	7/31/05
Workplan	7/31/05	Workplan	7/31/05
Sampling & Analysis	8/31/05	Sample event table	8/31/05
Draft Report	12/31/05	Draft Report	12/31/05
Final Report	3/1/06	Final report	3/1/06

6.4 Geographic Setting

The San Diego Regional Harbor Monitoring Program (RHMP) addresses four harbors in southern Orange and San Diego Counties. These include Dana Point Harbor, Oceanside Harbor, Mission Bay and San Diego Bay (Figure 2). Dana Point Harbor is located in the City of Dana Point in southern Orange County. It has berths for up to 2500 pleasure craft in two separate marinas. The harbor is protected by a single jetty that parallels the coast. There are no significant freshwater inputs to Dana Point Harbor other than storm drains servicing the local area. A variety of land uses occur around the harbor, including commercial (retail and restaurants), marina-related industry (fueling and dry-dock) and recreation.

Oceanside Harbor is located in the City of Oceanside in northern San Diego County. The Oceanside Harbor has berths for 950 pleasure craft and additional anchorage for U.S. Coast Guard vessels, commercial and sport fishing vessels. There are no significant freshwater inputs to Oceanside Harbor other than storm drains servicing the local area. Retail shops and restaurants are located on the piers around the harbor and there is one fuel station. There are also residential units and recreational opportunities adjacent to the harbor. Separated from Oceanside Harbor, but protected by the same jetties, another harbor approximately ¼ mile north supports U.S. Navy vessels operating at Camp Pendleton.

Mission Bay is located in the City of San Diego in central San Diego County. Mission Bay is one of the largest man-made recreation aquatic parks in the world, encompassing 4,235 acres that used to be predominantly marshland until the mid-twentieth century. Tecolote and Rose Creeks discharge into the eastern side of the Bay. Storm drains and groundwater discharge enter the Bay. There are numerous marinas and anchorages in Mission Bay, located primarily in the southwest corner,

near the entrance to the Bay. Sandy beaches surround most of the bay, with the majority of adjacent land uses parks and residential areas. Mission Bay and the surrounding parks are used year-round for walking, jogging, picnicking, and a variety of water contact sports, including swimming, sailing, water-skiing and fishing.

San Diego Bay is located in the City of San Diego. The entrance to San Diego Bay is between Point Loma on the west and North Island to the east. The bay curves around North Island and extends to the south, bound by the Silver Strand on the west and the Cities of San Diego, National City and Chula Vista to the east. Otay River, Sweetwater River and Chollas Creek discharge to the Bay. Other drainages discharge through storm drains into San Diego Bay, including Switzer Creek and the Downtown Anchorage Drainage. San Diego Bay is a deep water harbor, with the majority of shipping traffic related to military operations, tourist industry and fishing. Small boat marinas are located throughout the Bay. Land uses adjacent to San Diego Bay include commercial, industrial, military, residential and parks and recreation.

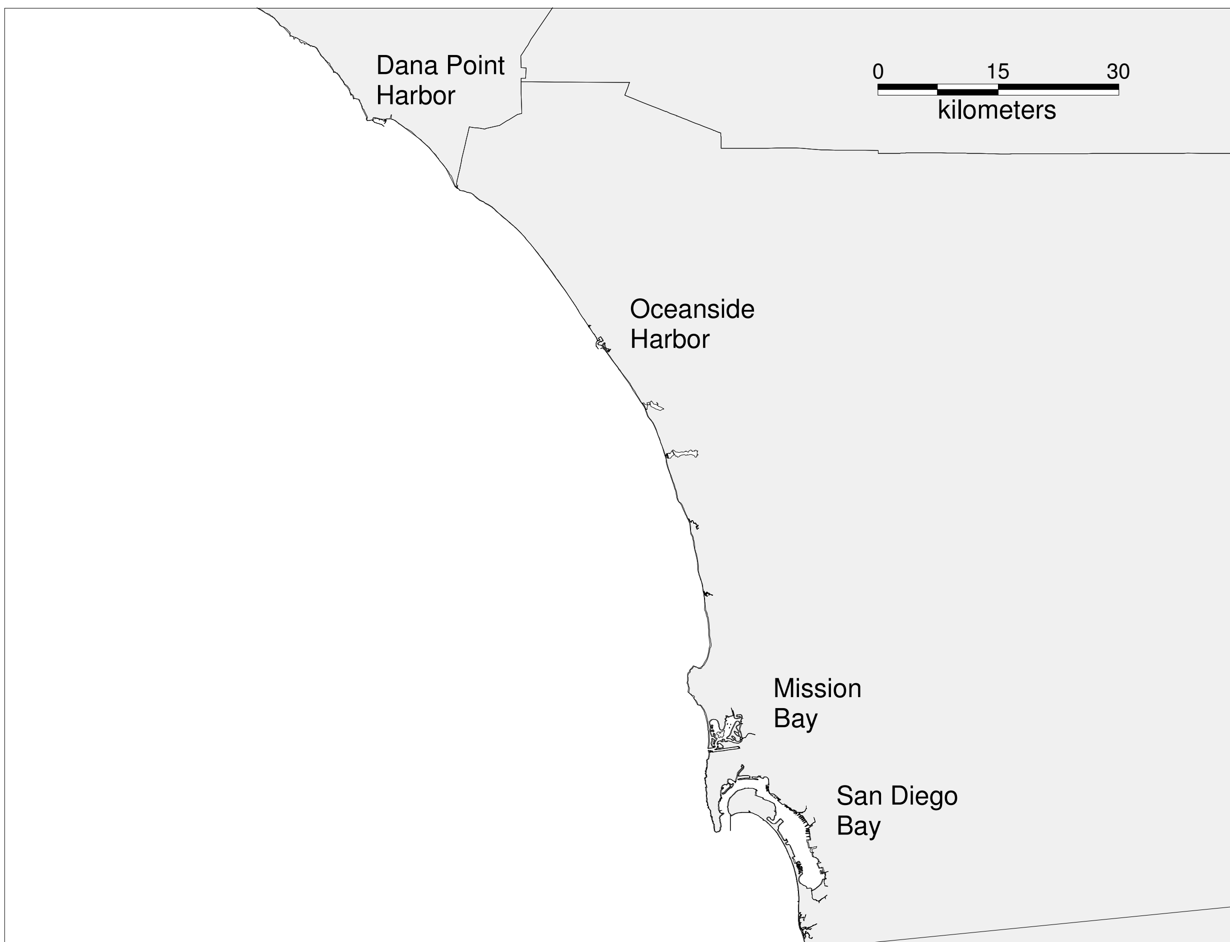


Figure 2. Study locations in Orange and San Diego Counties.

6.5 Constraints

This study is designed to assess the extent and magnitude of copper contamination and toxicity in the water column of marinas in the San Diego Region. However, there are several constraints that may affect this assessment. Because this study will be conducted over a relatively short time period, this project represents only a snapshot of water quality and potential biological effects in marinas. Factors such as water circulation and boating activity can alter the concentrations of dissolved copper in the harbors. However, the time period we have selected likely represents the critical condition in marinas because summertime has the greatest potential input of copper as a result of: 1) the most boating activity of the year including occupation of guest berths and addition of trailered vessels; 2) an increase in boatyard vessel maintenance and reapplication of copper-based paints; 2) an increase in frequency of in-water hull cleaning activities; 3) water column stratification due to temperature driven thermoclines; and 4) increase in summer spawning of many fish and invertebrate species that inhabit local marinas. A second constraint is that this study will not be representative of conditions following wet weather events. Stormwater inputs to these harbors can be potentially significant sources of additional copper. An assessment of water quality and potential biological effects during or following wet weather events would require substantial study design revisions and resource allocation.

7. QUALITY OBJECTIVES AND CRITERIA

Data Quality Objectives (DQOs) are quantitative and qualitative statements that specify the tolerable levels of potential errors in the data (U. S. EPA, 2000) and ensure that the data generated meet the standards for published data in the peer-reviewed literature. As defined in this plan, DQOs specify the quantity and quality of data required to support the study objectives. Each data quality category is described below. Numerical DQOs for the constituents being sampled are listed in Table 4.

7.1 Precision

Precision describes how well repeated measurements agree. The precision objectives in this study apply to laboratory duplicate samples and matrix spike samples for chemical measurements (see Section 14). Precision for chemical measurements is quantified using relative percent difference (RPD) between duplicate samples (Table 4). Precision objectives for toxicity measurements focus on reference toxicant survival or larval development. Precision for toxicity measurements is quantified relative to the mean and standard deviation of previous reference toxicant exposures (Table 4).

7.2 Accuracy

Accuracy describes how close the measurement is to its true value. The accuracy of chemical measurements in this study applies to laboratory control standards (LCS) and matrix spike (MS) samples (See section 14). The accuracy of chemical measurements is quantified as percent recovery (Table 4). Accuracy objectives for toxicity measurements focus on reference toxicant survival or larval development. Accuracy for toxicity measurements is quantified relative to the mean and standard deviation of previous reference toxicant exposures (Table 4).

7.3 Completeness

Completeness describes the success of sample collection and laboratory analysis, which should be sufficient to fulfill the statistical criteria of the project (Table 4). Completeness is measured as the fraction of samples sampled and/or analyzed relative to the quantity targeted in the study design (See Section 10). While no specific statistical criteria have been established for this study, it is expected that 90% of all measurements could be taken when anticipated. This DQO accounts for adverse weather conditions, safety concerns, and equipment problems. A loss of 10% of the samples in this study would represent a minimal loss in statistical power to address the study objectives.

7.4 Representativeness

Representativeness describes how characteristic the sample is of the actual condition attempting to be assessed. Representativeness in this study is addressed at three scales: 1) randomized sampling design avoids bias associated with known or assumed hot spots; 2) multiple sampling depths that will integrate any bias associated with water stratification; and 3) use of an index period to dissassociate any bias associated with seasonality.

Table 4. (Element 7) Measurement quality objectives.

Analyte	Accuracy	Precision (RPD)	Target Reporting Level	Completeness
Metal				
Cu (dissolved)	75-125% recovery for MS or LCS	±30% for Lab Dup or MSD	0.01 µg/L	90%
Toxicity				
Mussel development test	± 2 SD[1]	± 2 SD[1]	30%[2]	90%

[1] Within 2 standard deviations of recent reference toxicant tests.
[2] Minimum significant difference.

8. SPECIAL TRAINING NEEDS/CERTIFICATION

8.1 Specialized Training or Certifications

The MLML analytical lab holds certification for analysis of dissolved Cu. No other specialized training is required for this study.

8.2 Training and Certification Documentation

Both SCCWRP and the MLML maintain records of their training. Those records can be obtained, if needed, through the Laboratory Managers. The Contractor's QA Officer is responsible for overseeing training.

8.3 Training Personnel

SCCWRP and CFF&G maintain rigorous field and laboratory training programs based on written, oral and performance-based guidelines. Training and performance are also evaluated on an ongoing basis based, in part, on the QA parameters defined in this plan. Standard Operating Procedures (SOPs) for field, laboratory, and data management tasks have been developed and will be updated on a regular basis in order to maintain procedural consistency (see Appendices). The maintenance of an SOP Manual will provide project personnel with a reference guide for training new personnel as well as a standardized information source that personnel can access.

9. DOCUMENTS AND RECORDS

All documents generated by this project will be stored at SCCWRP (Table 5). Sampling records and toxicity testing laboratory records will be stored and maintained at SCCWRP. Chemical testing records pertinent to this study will be maintained at the MLML laboratory. Copies of all records held by MLML or SCCWRP will be provided to the Project QA Officer or Project Director upon request.

Persons responsible for maintaining records for this project are as follows. Jeff Brown will maintain all sample collection, sample transport, chain of custody, field analyses forms, all records associated with the receipt and analysis of samples analyzed for all parameters, and all records submitted by MLML. Gary Ichikawa will maintain MLML's records. Steve Bay will maintain SCCWRP's toxicity testing records. Ken Schiff will oversee the actions of these persons and will arbitrate any issues relative to records retention and any decisions to discard records.

All data will be entered into an electronic database using a set of standardized data protocols for data entry and sharing. Database tables will include information on the location and character of each sampling site, physical and biological features, and results of toxicity and chemistry analyses, including QA Data.

All field results will be recorded at the time of completion, using standardized field data sheets. Data sheets will be reviewed for outliers and omissions before leaving the sample site. Chain of custody forms will be completed for all water samples before leaving each sampling site. Data sheets and chains of custody will be stored by SCCWRP in hard copy form for five years from the time the study is completed. The directory where electronic files are stored will be backed up nightly on a second hard drive, and backed up monthly off-site.

Copies of this QAPP will be distributed to all parties involved with the project, including field collectors and laboratory analysts. Copies will be sent to MLML for distribution within this lab. Any future amended QAPPs will be distributed in the same fashion. All originals of this and subsequent amended QAPPs will be held at SCCWRP. Copies of versions, other than the most current, will be discarded so as not to create confusion.

All data from this project will be made publicly available. Release of data will include comprehensive documentation. This documentation will include database table structures (including table relationships) and lookup tables used to populate specific fields in specific tables. Release to the public will also include quality assurance classifications of the data (i.e. flags, as appropriate) and documentation of the methods by which the data were collected (metadata). Data will be released to the general public once a final report documenting the study has been prepared. Final deposition of databases and reports will be passed to the Regional Board Contract Manger on CD.

Table 5. (Element 9) Document and record retention, archival, and disposition information.

	Identify Type Needed	Retention	Archival	Disposition
Station Occupation Log	Notebook	Paper	Notebook	5 years
	Field data sheet	Paper	Notebook	5 years
Sample Collection Records	Chain of Custody	Paper	Notebook	5 yeas
Analytical Records	Lab notebooks	Paper	Notebook	3 years
	Lab Results QA/QC	Paper and electronic	Notebook/Excel	3 years
	Electronic data file	Electronic	Database	3 years
Data Records	Data Entry	Electronic	Database	Indefinite
Assessment Records	QA/QC assessment	Paper and electronic	Document	Indefinite
	Final Report	Paper and electronic	Document	Indefinite

GROUP B DATA GENERATION AND ACQUISITION

10. SAMPLING PROCESS DESIGN

A total of 30 sites randomly allocated using a probabilistic design dispersed throughout Dana Point Harbor, Oceanside Harbor, Mission Bay, and San Diego Bay will be assessed for concentrations of dissolved Cu and toxicity (Table 6, Figure 3). Each site will be sampled once during the index period of July 1 to August 31, 2005. Samples will be collected from three depths at each site; one meter below the surface, at mid-depth, and one meter above the bottom. Dissolved Cu will be measured in each sample, while toxicity to mussel development will be measured in the surface samples only. Samples from up to six sites, preferably distributed across multiple marinas, which exhibit sufficient toxicity ($\geq$25% reduction in normal development) will also be characterized with a Toxicity Identification Evaluation (TIE). The randomized design will incorporate both natural variability and variability associated with anthropogenic contributions.

Table 6. (Element 10). Number and frequency of water samples.

Sample Location	SiteID	Total Number of Samples	Frequency of Sampling
Dana Point Harbor	D1	3 for dissolved Cu, 1 for toxicity	Once
Dana Point Harbor	D2	3 for dissolved Cu, 1 for toxicity	Once
Dana Point Harbor	D3	3 for dissolved Cu, 1 for toxicity	Once
Dana Point Harbor	D4	3 for dissolved Cu, 1 for toxicity	Once
Dana Point Harbor	D5	3 for dissolved Cu, 1 for toxicity	Once
Mission Bay	M1	3 for dissolved Cu, 1 for toxicity	Once
Mission Bay	M2	3 for dissolved Cu, 1 for toxicity	Once
Mission Bay	M3	3 for dissolved Cu, 1 for toxicity	Once
Oceanside Harbor	O1	3 for dissolved Cu, 1 for toxicity	Once
Oceanside Harbor	O2	3 for dissolved Cu, 1 for toxicity	Once
San Diego Bay	S1	3 for dissolved Cu, 1 for toxicity	Once
San Diego Bay	S2	3 for dissolved Cu, 1 for toxicity	Once
San Diego Bay	S3	3 for dissolved Cu, 1 for toxicity	Once
San Diego Bay	S4	3 for dissolved Cu, 1 for toxicity	Once
San Diego Bay	S5	3 for dissolved Cu, 1 for toxicity	Once
San Diego Bay	S6	3 for dissolved Cu, 1 for toxicity	Once
San Diego Bay	S7	3 for dissolved Cu, 1 for toxicity	Once
San Diego Bay	S8	3 for dissolved Cu, 1 for toxicity	Once
San Diego Bay	S9	3 for dissolved Cu, 1 for toxicity	Once
San Diego Bay	S10	3 for dissolved Cu, 1 for toxicity	Once
San Diego Bay	S11	3 for dissolved Cu, 1 for toxicity	Once
San Diego Bay	S12	3 for dissolved Cu, 1 for toxicity	Once
San Diego Bay	S13	3 for dissolved Cu, 1 for toxicity	Once
San Diego Bay	S14	3 for dissolved Cu, 1 for toxicity	Once
San Diego Bay	S15	3 for dissolved Cu, 1 for toxicity	Once
San Diego Bay	S16	3 for dissolved Cu, 1 for toxicity	Once
San Diego Bay	S17	3 for dissolved Cu, 1 for toxicity	Once
San Diego Bay	S18	3 for dissolved Cu, 1 for toxicity	Once
San Diego Bay	S19	3 for dissolved Cu, 1 for toxicity	Once
San Diego Bay	S20	3 for dissolved Cu, 1 for toxicity	Once

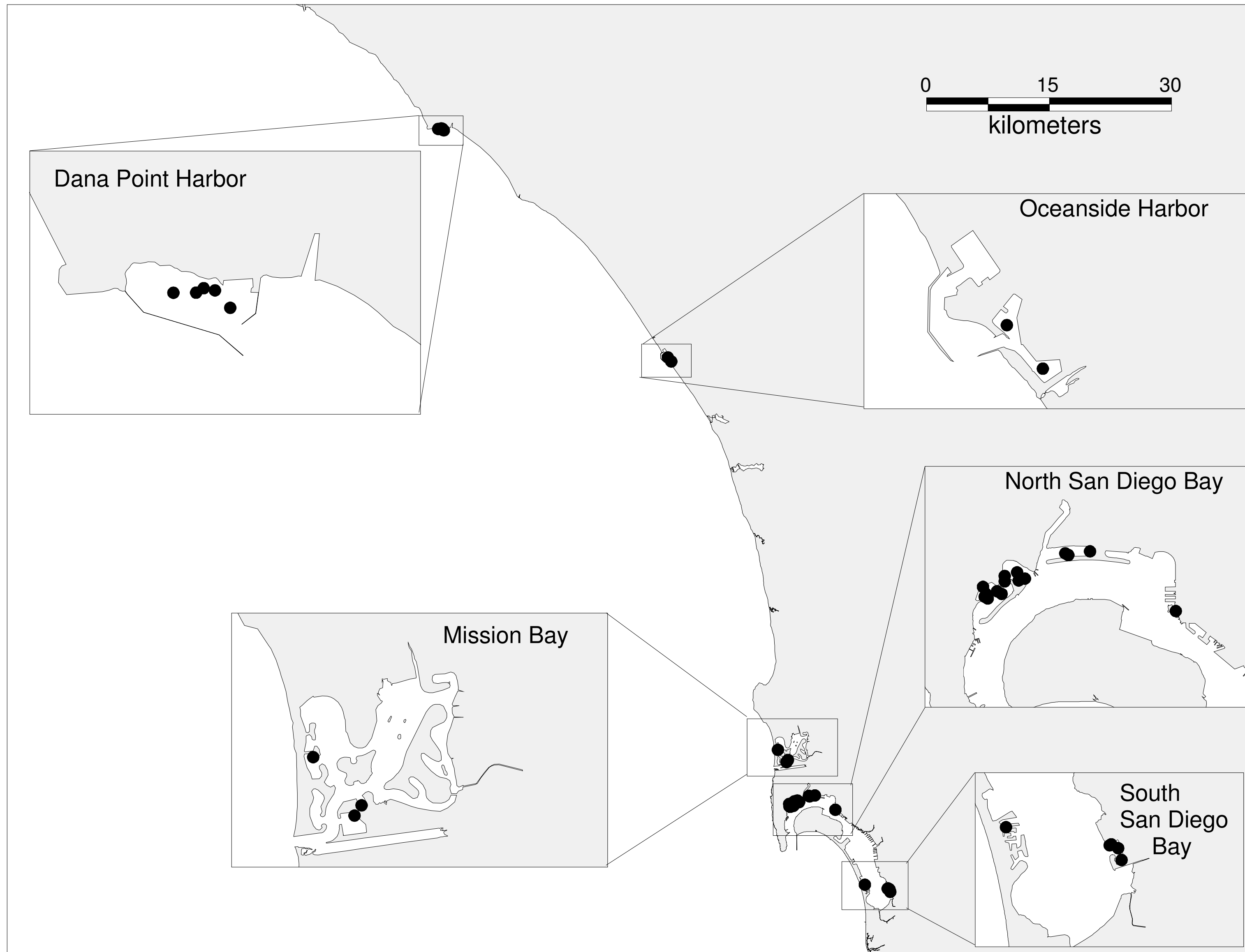


Figure 3. Sampling stations distributed among marinas throughout the San Diego Region.

11. SAMPLING METHODS

Sampling requires the manual collection of grab samples using a Niskin Bottle at each of the monitoring locations. The complete sampling SOP appears in Appendix A.

Sample containers and preservatives are identified in Table 7. Appropriate pre-cleaned sample containers will be used. Sample bottles and bottle caps will be protected from contact with solvents, dust, or other contaminants. Sample bottles for this project will not be reused. A field blank will be used to determine possible contamination during the sampling process.

The sampling coordinator has responsibility for assessing the safety of sampling teams. A two-person team will conduct all sampling, and the sampling team will have access to a cellular phone in order to alert rescue agencies should an accident occur. Sampling will be postponed if the sampling team determines that the conditions are unsafe.

Failure to collect a sample due to safety concerns or technical issues will be promptly reported to the Project Director, who will determine if any corrective action is needed and make arrangements to collect a replacement sample (if possible). The Quality Assurance Officer will document sampling failures and the effectiveness of corrective actions.

Table 7. (Element 11) Sample handling.

Analyte	Bottle Type/Size	Preservative	Maximum Holding Time
Chemistry			
Cu (dissolved)	125 mL HDPE	Cool at <4°C Acidify <2 pH	4 h filtration and acidification, 6 months analysis
Toxicity			
Mussel development test	1 L Polyethylene	Cool at <4°C	36 h preferred, 48 h max

12. SAMPLE HANDLING AND CUSTODY

Samples will be kept properly chilled and will be transferred to the analytical laboratories within the holding times specified in Table 7. To provide for proper tracking and handling of the samples, documentation will accompany the samples from the initial collection to the final extractions and analysis.

All bottles will be pre-labeled. Once sample containers are filled, they will be placed on ice, in a cooler, in the dark and transported to the laboratory for processing.

Field data sheets and chains of custody will accompany the collection of water samples. Sampled water will be kept properly chilled and transferred to an analytical laboratory within holding times.

All samples will be marked with a unique number to track their analysis. These identification labels will also be entered directly on to field and laboratory data sheets. All observations recorded in the field as well as information recorded in processing all field samples in the laboratory will be tracked using these identification labels.

Chain-of-Custody Forms for the samples will be completed and transport of the samples to the analytical laboratory will be coordinated to ensure that all samples are handled and analyzed within the proper holding time. An example of the Chain-of-Custody form is shown in Appendix E.

13. ANALYTICAL METHODS

13.1 Analysis Methods

The samples will be analyzed for chemistry and toxicity as indicated below.

13.1.1 Chemistry

Inductively coupled plasma-mass spectrometry (ICP-MS, EPA 1638) will be used in order to analyze concentrations of dissolved copper in water samples.

13.1.2 Toxicity

The mussel embryo development test (EPA/600/R-95/136) will be used to assess the toxicity of the surface water samples. Toxicity Identification Evaluation (TIE) will be used to characterize the cause(s) of toxicity, where appropriate, using EPA/600/R-96/054.

Table 8. (Element 13). Analytical methods. NA = not applicable.

Analyte	Method	Modifications to Method	Method Detection Limit
Chemistry			
Cu (dissolved)	EPA 1638	None	0.01 µg/L
Toxicity			
Mussel development test	EPA/600/R-95/136 EPA/600/R-96/054 (TIE)	None	NA

13.2 Sample Disposal

After analysis, including QA/QC procedures, any excess sample will be disposed of by the analytical laboratories. All samples will be disposed of in a manner consistent with the SOP (Appendix B)

13.3 Corrective Action

Corrective action is taken when an analysis is deemed suspect for some reason. These reasons include exceeding RPD ranges and/or problems with spike recoveries or blanks. The corrective action will vary on a case-by-case basis, but at a minimum involves the following:

- A check of procedures.

- A review of documents and calculations to identify possible errors.
- Correction of errors.
- A re-analysis of the sample digest, if sufficient volume is available, to determine if results can be improved.
- A complete reprocessing and re-analysis of additional sample material, if sufficient volume is available and if the holding time has not been exceeded.

The Lab Managers at SCCWRP and the MLML analytical lab each have systems in place to document problems and make corrective actions. All corrective actions will be documented to the Project Manager.

14. QUALITY CONTROL

Samples for QA/QC will be collected both in the field and in the lab. Field QA/QC samples are used to evaluate potential contamination and sampling error occurring prior to sample delivery to the analytical laboratory. Field QA/QC samples include field blanks. Lab QA/QC samples are used to evaluate the analytical process for contamination, accuracy, and reproducibility. Internal laboratory quality control checks will include method blanks, matrix spike/matrix spike duplicate (MS/MSDs), and duplicates (See Section 7). These QA/QC activities are discussed below.

14.1 Blanks

Blanks help verify that the equipment, sample containers, and reagents are not a source of contamination, and that the sampling techniques used are non-contaminating. Both field and laboratory blanks are included in the program.

Field blanks will be used to determine if field sampling activities are a potential source for contamination. These blanks will be collected by sampling "blank water" (contaminant-free deionized water) in the field during a sampling event. The same equipment used for collection of the grab samples will be used to transfer the blank water into the blank sample containers.

Method blanks will be run by the analytical laboratory to determine the level of contamination associated with laboratory reagents and equipment. A method blank is a clean sample in a known matrix that has been subjected to the same complete analytical procedure as the submitted samples to determine if contamination has been introduced into the samples during processing. Results of method blank analysis should be less than the reporting limits for each analyte, or less than 5% of the native sample concentration.

For toxicity tests, blanks are represented by negative control samples. In this study, filtered seawater from an uncontaminated location will be used in the mussel development test.

14.2 Spikes and Duplicates

Matrix spike/matrix spike duplicates (MS/MSD) will be used to assess precision and accuracy of the laboratory analytical method. A MS is created when the laboratory adds a known quantity of analyte to an aliquot of field sample. After accounting for native concentrations, the percent recovery is calculated as the proportion of the known compound in the sample. The acceptable recovery limits are shown in Table 4. Percent recovery is calculated as:

Percent Recover = ((spike concentration – sample concentration)*100)/spike concentration

A MSD will be the reanalysis of the MS. The MSD results are compared to the MS results to assess the precision of the laboratory analytical method. MS/MSD results are evaluated by calculating the relative percent difference (RPD) between the two sets of results. The acceptable RPD limits are shown in Table 4. The RPD is calculated as:

Relative Percent Difference = (100 * (MS – MSD/2))/(MS + MSD)/2)

14.3 Reference Toxicants

Organism health can be impacted by how the animals were collected, handled or shipped, and exposure parameters. To increase precision as a result of test exposure variability, environmental parameters are kept to a strict range of temperature, pH, salinity, light intensity, photoperiod, and dissolved oxygen (See Appendix C). To ensure that a particular batch of organisms are not overly sensitive or tolerant, concurrent toxicity tests are conducted using spiked reference toxicants in laboratory dilution water. Copper will be the reference toxicant in this study. The results of these reference toxicity tests are compared with the mean response for the same organism from previous tests conducted in the SCCWRP laboratory. Acceptable reference toxicants limits are achieved if the results are within 2 standard deviations of the grand mean for the laboratory's control chart (Table 4).

15. INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE

15.1 Sampling Equipment

SCCWRP staff has established standard operating procedures for each piece of field equipment in use. Sampling equipment receive regular maintenance based on a combination of manufacturer requirements and the actual amount of equipment use in the field. A second Niskin sampler will be taken into the field in case the first sampler fails for any reason.

15.2 Analytical Instruments

The MLML analytical lab maintains its equipment in accordance with its SOPs, which include those specified by the manufacturer and those specified by the method. Problems with the ICP/MS during analysis will require repair, recalibration, and re-analysis of the sample.

Table 9. (Element 15). Testing, inspection and maintenance of sampling equipment and analytical instruments.

Equipment / Instrument	Responsible Person	Frequency	SOP Reference
Niskin sampler	Dario Diehl	Once per sampling	Appendix A
ICP-MS	Gary Ichikawa	Refer to SOP	Appendix B

16. INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY

All laboratory equipment is calibrated based on manufacturer recommendations and accepted laboratory protocol. The MLML analytical laboratory maintains calibration practices as part of the method SOPs. The instrument will be recalibrated if the calibration curve does not meet acceptable limits. Problems with the instrument calibration will be documented by the analyst if the problem is persistent, or if the resulting data are questionable.

17. INSPECTION/ACCEPTANCE FOR SUPPLIES AND CONSUMABLES

Glassware, sample bottles, and collection equipment will all be inspected prior to their use for chips, cracks, leaks, contamination, and other deformities that can affect the outcome of the study results. Sampling bottles will be purchased from VWR (vwr.com, 800-932-2500). Supplies will be examined for damage as they are received. Precleaned 125 mL high density polyethylene containers will be used for metals analysis. Precleaned 1L cubitainers will be used for toxicity analysis. Mussels for toxicity testing will be purchased from Carlsbad Aquafarms (Carlsbad, CA 760-438-2444). Mussels will be checked for viability and reproductive status upon receipt. The field manager will be responsible for acquisition and inspection of sampling containers. The toxicity manager will be responsible for acquisition and inspection of test organisms. The chemistry manager will be responsible for acquisition and inspection of chemical supplies including standards.

18. NON-DIRECT MEASUREMENTS

Previous studies that have performed metals and toxicity measurements in the study areas will be referred to in the study report, but this study will not incorporate existing data or other non-direct measurements.

19. DATA MANAGEMENT

The management of water quality and toxicological data will be initiated with the use of field and laboratory data sheets. Analysis results will be electronically sent to the Project Director following the completion of quality control checks by each of the laboratories. Data will be screened for the following major items:

- A 100 percent check between electronic data provided by the laboratory and the hard copy reports
- Conformity check between the Chain-of-Custody Forms and laboratory reports
- A check for laboratory data report completeness
- A check for typographical errors on the laboratory reports
- A check for suspect values

The MLML and SCCWRP laboratories will provide data in both hard copy and electronic format. The required form of electronic submittals will be provided to the laboratories to ensure the files can be imported into the project database with a minimum of editing. The data will be managed in SCCWRP's project database, which has a relational structure and is compatible for incorporation into the SWAMP database. This database has been inspected and validated through the use on other programs including Southern California Regional Monitoring Efforts (2003 Southern California Regional Marine Monitoring Information Management Plan ftp://ftp.sccwrp.org/pub/download/PDFs/BIGHT03/bight03_infoplan.pdf). The Project Director will be responsible for ensuring that data are entered into the database.

Following the initial screening, a more complete QA/QC review process will be performed, which will include an evaluation of holding times, method and equipment blank contamination, and analytical accuracy and precision. Accuracy will be evaluated by reviewing MS/MSD and LCS recoveries; precision will be evaluated by reviewing MSD and laboratory sample duplicate RPDs.

GROUP C ASSESSMENT AND OVERSIGHT

20. ASSESSMENTS AND RESPONSE ACTIONS

The Project Director will be responsible for the day-to-day oversight of the project. The Project QA Officer will conduct systematic reviews of the data for the specified DQOs every time data packets are delivered and entered into the SCCWRP database. Any problems will be relayed to the Project Director. The SCCWRP QA Officer has the power to halt all sampling and analytical work by the MLML lab or SCCWRP if the deviation(s) noted are considered detrimental to data quality. Problems that cannot be corrected, will be documented by the QA Officer, flagged in the database, and acknowledged in the final report.

21. REPORTS TO MANAGEMENT

The status of data collection during this project will be reported by the Project Director to the Contract Manager on a quarterly basis beginning July 15, 2005 and continuing until the completion of the project in March 2006. A draft final project report will be filed no later than December 31, 2005. The Project QA Officer has complete access to the Project Director on an ongoing basis. Any QA deviations will be detailed in the sample event summary report and draft/final report.

Table 10. (Element 21) QA management report

Report	Due by
Quarterly progress reports	October 15, 2005 and quarterly thereafter
Sample event summary	August 31, 2005
Draft final report for review	December 31, 2005
Final Report	March 1, 2006

GROUP D DATA VALIDATION AND USABLILITY

22. DATA REVIEW, VERIFICATION, AND VALIDATION

Laboratory validation and verification of the data generated is the responsibility of the laboratory. The laboratory manager will maintain analytical reports in a database format as well as all QA/QC documentation for the laboratory.

SCCWRP will review all data packages received for adherence to guidelines set forth in this QAPP. COC forms will be reviewed to ensure adherence to collection, transport, and receipt requirements, including test initiation within the required holding time. Toxicity data will be evaluated for completeness, adherence to test methodology, passing acceptability criteria, choice of appropriate statistical methods, and proper reporting.

Laboratories will conduct a 100 percent raw data versus electronic data audit before delivering results to SCCWRP.

23. VERIFICATION AND VALIDATION METHODS

Data collected in the field will be validated and verified by the Project QA Officer. Reconciliation and correction will be the responsibility of the Project Director.

Laboratory validation and verification of the data generated is the responsibility of each laboratory. Each laboratory supervisor maintains analytical reports in a database format as well as all QA/QC documentation for the laboratory.

The Project Director is responsible for oversight of data collection and the initial analysis of the raw data obtained from the field and the contracted laboratory. The Project Director responsibilities also include the generation of rough drafts of quarterly and final reports. The Project Director has final oversight on the submission of quarterly and final reports.

24. RECONCILIATION WITH USER REQUIREMENTS

These data will be used to define the extent and magnitude of copper contamination in marinas throughout the San Diego Region. These data can be used directly by SWAMP in their assessment of California's waterbodies by inclusion in the State's 305(b) report. Data analysis will address study uncertainty (see section 6.5).

For data that do not meet DQOs, management has two options:

1. Retain the data for analytical purposes, but flag these data for QA deviations.
2. Do not retain the data and exclude them from all calculations and interpretations.

The choice of option is the decision of the Project Manager. If qualified data are to be used, then it must be made clear in the final report that these deviations do not alter the conclusions of the study.

APPENDIX A
STANDARD OPERATING PROCEDURE FOR NISKIN BOTTLE SAMPLER

A) PROCEDURES

1.0 SCOPE AND APPLICATION

The Niskin bottle is used to collect a water sample from a discrete depth. Being able to collect samples from discrete depths is useful for profiling the vertical distribution of water column parameters. The plastic-bodied Niskin sample apparatus is better suited for collecting samples for metals analysis than a metal-bodied sampling apparatus.

2.0 SUMMARY OF METHOD

The Niskin bottle has stoppers on both ends, which are held in place by springs. The bottle is prepared by cocking open both ends of the bottle. The Niskin bottle is then lowered by a nylon rope to the desired depth. A small weighted "messenger" is then loosely attached to the line, and released. When the messenger reaches the Niskin bottle, the cocking mechanism releases the two stoppers, and a sample of water from that depth is captured in the bottle. The rope is raised to retrieve the Niskin bottle, and the sample is released into a precleaned container by opening the valve on the lower side of the sampler.

3.0 DEFINITIONS

No special acronyms, abbreviations, or terms have been identified.

4.0 HEALTH AND SAFETY WARNING

General safety considerations regarding working in the field should be followed. However, misuse of the sampling device will probably not result in serious injury or death. If the sample procedure is not followed or is not followed correctly, the sample will not be collected at the discrete depth.

5.0 CAUTIONS

The Niskin bottle must be properly secured to the nylon rope in order to prevent loss of the sampler. The valve on the side of the sampler must be closed before deploying the sampler in order to prevent sample loss.

6.0 INTERFERENCES

If the sampler is prematurely tripped, the sample will not represent the desired depth. The O rings in the end closures should be checked periodically for seal integrity. Cracked seals could result in sample loss. In addition, the Niskin bottle should be rinsed with deionized water before the sampler is stored.

7.0 PERSONNEL QUALIFICATIONS

The user should understand how the sampling apparatus works, and be able to demonstrate competence before actual samples are collected.

8.0 EQUIPMENT AND SUPPLIES

- Niskin bottle
- Messenger
- Nylon rope or wire. The line should have knots or tape to indicate depth
- Precleaned sample container
- 2 lb weight to hold sampler in place if a current is suspected (optional)
- Rubber tube to attach to the valve on the apparatus (optional)

9.0 PROCEDURE

Field operation

- Tie the end of the rope to the boat to secure the Niskin Sampler.
- Place top-plug lanyard loop over bottle cap release pin (the longer pin).
- Release pushrod cap and snap bottom plug lanyard between top plug lanyard ball and pin. Do not put into loop.
- Simultaneously (with one hand) depress thumb block, and hold pushrod in place (at base of lower mounting block).
- Lower Niskin Sampler to the desired depth.
- Attach messenger to the line, then trigger the sampler to close by sending down the weighted messenger.
- Retrieve the sampler.
- Attach tubing to the valve.
- Open the valve and draw water to the precleaned container(s).
- Open the tops and dump out the leftover water.

Maintenance

- Wash bottle with fresh water before storage.
- After extended storage, especially before every deployment, make sure that all moving parts and seals are free to perform.
- Replace spring when necessary
- Inspect O rings for damage.

10.0 DATA AND RECORDS MANAGEMENT

No data forms or records identified.

B) QUALITY CONTROL AND QUALITY ASSURANCE

No additional QA/QC procedures than those in the “Interference” and “Maintenance” sections above.

C) REFERENCES

No references.

APPENDIX B
STANDARD OPERATING PROCEDURE FOR ICP/MS ANALYSIS OF SEAWATER SAMPLES

APPENDIX B
STANDARD OPERATING PROCEDURE FOR ICP/MS ANALYSIS OF SEAWATER SAMPLES

This technique is a modification of EPA Method 1638, Determination of Trace Elements in Ambient Waters by Inductively Coupled Plasma — Mass Spectrometry. This procedure outlines the methods specifically used in this laboratory and is intended as a supplement to the material in the protocol.

Overview

Seawater is a very complex matrix for trace element analysis by ICPMS with numerous isobaric, polyatomic and physical interferences. These interferences are overcome through the use of proper instrumentation and matrix matched standards. The method involves the use of a double focusing, high resolution ICPMS, the Element 2, coupled to an Aridus desolvation system and a low flow, Teflon self-aspirating nebulizer on the inlet side. The desolvation system greatly reduces the formation of oxide and other polyatomic interferences in the plasma. With this method the sample is in contact only with acid cleaned polyethylene and Teflon prior to entering the ICP. Quantitative analysis of seawater samples is achieved through the use of matrix matched standards which are made using clean, open ocean seawater with extremely low trace element concentrations. Seawater samples are diluted 1:10 to reduce total dissolved solids to acceptable levels.

Instrumentation

Element 2 High resolution Inductively Coupled Plasma Mass Spectrometer
100 µL/min. µflow Teflon nebulizer
Aridus desolvation system
ASX-100 autosampler with Teflon sipper probe

Supplies

Eppendorf pipettes (200µL-1mL and 20µL-100µL)
Polyethylene 2mL autosampler cups
Quartz distilled nitric acid made from Fisher Trace Metal Grade acid ($QHNO_3$)
>18 megohm cm MilliQ water or equivalent
1000 µg/L Spex Certiprep or equivalent primary standard
Open ocean seawater with a extremely low concentration of the elements of interest
Rhodium internal standard

Sample, standard and blank preparation

The standards used to quantitate the sample are made using Spex Certiprep or equivalent primary standards using low density polyethylene bottles. Appropriate standard concentrations are made using $QHNO_3$, MQ, and the open ocean seawater. The acid concentration is matched to that contained in the samples. The final seawater content in the standards is 10%. Standards are dispensed into autosampler cups with the appropriate amount of internal standard prior to analysis. Standard blanks are prepared in the same manner without the addition of primary standard.

Seawater samples are prepared directly in the autosampler cups. They are diluted 1:10 using $QHNO_3$ and MQ to a final acid concentration of 3%. An appropriate amount of internal standard is then added. Sample blank solutions are prepared in the same manner without the addition of sample material.

Analytical procedure

After plasma ignition the Element 2 ICP-MS is allowed to warm up 15-20 minutes. The instrument is then tuned using a 1 ng/mL tuning solution containing the appropriate elements made up in 10% seawater. The instrument is tuned for maximum sensitivity and signal stability while keeping cerium oxide levels below 0.05%.

Once the instrument is tuned and stable, a blank solution is aspirated. The blank is repeatedly aspirated until the signal is stable. The instrument is ready for quantitation. Blank solutions are analyzed first, followed by standard analysis. The calibration is monitored for outliers and standards are reanalyzed if necessary. If there are still outliers, fresh standards are prepared. Samples are then analyzed. A calibration standard is analyzed as a sample every 10 analyses to check that instrument performance is stable. If the standard deviates more than 10% from the true value, the instrument is recalibrated and samples are reanalyzed from the last good standard check. QA/QC samples (spikes, spike duplicates, and sample duplicates) are interspersed among the samples as appropriate.

Hardware Maintenance

Seawater samples contain very high levels of total dissolved solids that can create many problems with ICPMS instrumentation. Sample and skimmer cones must be cleaned or replaced daily at a minimum. The membrane in the Aridus is subject to clogging, which requires regular cleaning, usually daily, for optimal performance. The desolvation system should be cleaned according to manufacturers specifications with hot acids followed by membrane conditioning with isopropyl alcohol.

APPENDIX C
STANDARD OPERATING PROCEDURE FOR MUSSEL EMBRYO DEVELOPMENT TEST

Southern California Coastal Water Research Project Toxicology Laboratory

Electronic Index

If you are using a hard copy version of this SOP, this index will be of little use. However, if you are using an electronic version, click on any of the descriptions below to hyperlink to the pertinent section of the document.

Animal Collection and Culturing
Brine
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Test Procedure
    Urchin Spawning
    Gamete Collection
    Egg Counting
    Sperm Counting
    Trial Fertilization
    Test Initiation
Water quality analysis
Microscopic Evaluation
Forms

I. Overview

This method estimates the toxicity in aqueous samples by a 48 hour exposure of *Mytilus galloprovincialis* embryos. The test endpoint is normal embryo development and survival. The test is based on methods in the EPA's 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). The purpose of this SOP is to detail the test procedure as specifically applied in our laboratory. The SOP is intended to supplement the material in the protocol, not replace it.

II. Supply Checklist

Deep trays for use as water baths (2)
Glass bread pan (2)
Seawater and DIW squirt bottles
pH, DO and conductivity meter/probes
Graduated cylinders 50-1000 ml for making gamete and solution dilutions
Automatic pipets 0.1 ml up to 10 ml
Water pump
Tubing with Y-joint
Thermometer

250 ml, 400 ml and 1 L beakers (several)
Inverted microscope
Counter, 2 unit
Sedgwick-Rafter counting chamber
Perforated plunger to fit 250 ml, 400 ml and 1 L beakers
Nitex screening 100 µm or smaller openings
Razor blades
Eppendorf Pipet tips (100 µl, 1 ml and 10 ml)
Shell vials with translucent caps, 5 dram
Formalin, 30% borax buffered (see recipe below)
Dispenser for formalin to repeatedly deliver 1 ml
Pasteur pipets and bulbs (both 5 ¾ and 9 in)
Scintillation vial racks (plastic for exposure, cardboard for storage)
Spawning and gamete calculation data sheet
Glass or Fiberglass aquaria tanks (3)
Air pump
Pairing knife.
Air stones

III. Animals Collection and Culturing

Adult Bivalves (*Mytilus galloprovincialis*) are obtained from (Carlsbad Aquafarms, John Davis ph# 760-438-2444, FAX# 760-438-3568) a commercial supplier.
Set up glass aquaria tanks in the cold room. To each tank add about six inches of seawater the day before the mussels arrive. Temperature shock may cause the animals to spawn; therefore once you have received the mussels, the animals should be acclimated to the cold room by opening the travel cooler. After acclimation, transfer the mussels equally among the tanks, and add air stones.

The seawater should be changed everyday. Mussels can remain in holding under optimal conditions up to eight weeks from receiving date. No food is given to the mussels while in holding.

Water quality measurements (pH, ammonia, DO and salinity) should be made on the system on a weekly basis.

IV. Test Design

<u>Summary of test conditions</u>

Type: Static non-renewal
Salinity: 32 ± 2 g/kg
Temperature: 15 ± 1 °C
Duration: 48 hours
Endpoint: normality of development and survival
Exposure volume: 10 ml
Test containers: 29.35 x 55 mm (5 dram) glass shell vial with snap cap.
Lighting: Ambient laboratory

Photoperiod: 16 hours Light and 8 hours Dark
Salinity adjustment: Hypersaline brine
Dilution water: natural seawater (activated carbon and 0.45 µm filtered)
Water Quality: DO, pH, salinity and ammonia (optional)
Reference toxicant: concurrent with each experimental batch, copper chloride

Exposures should be conducted in 5 dram glass shell vials. The vials should be vigorously rinsed with DIW and allowed to dry before use. Vials should be labeled and randomly distributed in vials racks (based on our experiment set-up randomization program).

The sample volume is 10 ml per replicate, with 5 replicates per concentration. Include an additional 5 vials of 32 ‰ seawater to determine the actual embryo density. After the samples are in the vials, the vials should be placed in the 15 ºC room for at least ½ hr before starting the exposure. The vials should kept covered with parafilm whenever possible from the time of labeling through the end of the exposure to prevent cross contamination and evaporation.

V. Sample Handling

Care should be taken during sample preparation and dilution that cross contamination of glassware used for the samples and for the gametes does not occur. The exposure vials should be covered at all times to prevent contamination.

Samples having a salinity of less than 30 ‰ should be adjusted using hypersaline brine. To make the brine, first place a glass container (usually a 1 L beaker or 1 Gal jar) of seawater in a freezer for at least 18 hr. Remove the container from the freezer and allow the ice to thaw at room temperature. During the thawing process, occasionally pour off the thawed brine to a clean beaker. Check the salinity of the brine by taking a 6 ml sample and dilute it with 6 ml DIW. Multiply the salinity by 2 (Note: the conductivity to salinity algorithm is not accurate above 40 ‰, which necessitates making the dilution just described). When the salinity of the brine is close to the desired level, or the volume needed is achieved, final dilution of the brine to the desired level should be made using seawater. The salinity of the brine used for sample adjustment should never exceed 80 ppt, as higher levels have been known to cause toxicity. When testing samples that have no saline content (stormwater, sewage effluent, etc) it is usually desirable to make the brine at 64 ‰ so that a 50:50 mixture of sample and brine has a final salinity of 32 ‰. We have found that brine may be stored in the refrigerator for up to a week.

Water quality measurements are made at the beginning and end of the testing time. Separate sub-samples for water quality analysis of each test sample or dilution should be taken at the time the samples are prepared. Samples should be measured for pH, DO and salinity. Ammonia analysis should be considered optional.

VI. Reference Toxicant

Each test of field or laboratory samples should include a concurrent reference toxicant exposure to copper. The reference toxicant exposure should include a control (0 µg/L) and five concentrations of copper.

The copper concentrations are prepared by first making a stock solution of 10,000 µg/L copper. This stock solution consists of 0.0268 g $CuCl_2{\cdot}2{\cdot}H_20$ in 1 L DIW. A working stock is prepared by diluting 10 ml of stock solution into 90 ml of seawater to produce a concentration of 1,000 µg/L. The concentrations tested should be 0, 4.5, 6.5, 9.5, 13.9,20.4, and 30.0µg/L. These concentrations are achieved by adding 0.45, 0.65, 0.95, 1.39, 2.04, and 3.00 mL of working stock to seawater to make 100 ml of each concentration. An approximately 40 ml sample of the highest concentration should be saved in a plastic container for copper concentration verification. This sample should be preserved by adding two drops of concentrated, redistilled nitric acid then storing it in the refrigerator.

VII. Test Procedure

A. Before Spawning Mussels

Fill about half full with 32‰ seawater two deep trays and heat to 20 ºC. Place both bread pans and the pump with tubing in one of the trays. With seawater, rinse about ten 250 ml beakers and fill with 75 mL of seawater at 15 ºC.

Gently scrap off the barnacles and other encrusting organisms with a pairing knife from twenty mussels. Then rinse animals with 32 ‰ seawater.

B. Mussel Spawning

Place the animals into bread pans in the 20 ºC seawater bath. Turn on the water pump so that there is flow in each pan. Note initial time of mussel addition, look for spawning mussels, after 30 min. stop the pump. Wait 15 min. If no spawning occurs place the mussels in a 15 ºC, 32 ‰ seawater bath for 15 min. then start the process again. At least two animals of each sex with good gamete quantity and quality are necessary.

C. Gamete Collection

When individual animals are observed shedding gametes, remove them from the pan. Rinse each animal individually with 32 ‰ seawater and place in their own 250 mL beaker that has enough seawater to cover the animal at 15 ºC.

Early in the spawning process, using a clean Pasteur pipet mix up the eggs in the beaker from one female and transfer about 0.5 ml of egg solution to the rafter cell. Check the eggs on the microscope at 100X power. Greater than 90% of the eggs should be round, of average size, not clumped, and not containing germinal vesicles. If the eggs appear to be of good quality, add a very small amount of sperm to the eggs in the Rafter cell. Watch for motility of the sperm and the

ability to fertilize. Continue checking so that all of the males and females are tested in this manner.

D. Egg Counting

Allow the eggs of the females that were deemed to be in good condition to settle to the bottom of their collection beakers. Pour off most of the water from each beaker, then pour the remaining water with the eggs through the 100 um nitex screen into a 1 L beaker. After adding the eggs from all the "good" females, bring the water level in the beaker up to about 600 ml. Allow the eggs to resettle (about ½ hr. After the eggs have settled, again pour off most of the water, then again pour the eggs through the nitex into a clean 1 L beaker. Again bring the water up to about 600 ml.

Put 9 ml of seawater into each of two scintillation vials, labeled A and B. Using the perforated plunger mix the egg solution well and take a 1 ml sample and place it into vial A. Mix vial A well and take 1 ml sample from it and place in vial B. Mix vial B well and place a 1 ml sample onto the Rafter cell. Count all of the eggs on the Rafter cell on a microscope a 100X. If total count is less than 30, then use vial A for counting. Record the count in the appropriate place on the egg and sperm count form. Take a second sample from vial B and count. Record the second count. If the two counts are within 20% calculate the mean. If the counts are not within 20%, count one more sample before calculating the mean. The egg density target should be about 5000-8000 eggs/mL. This is a stock solution so if the egg density is higher or lower it is ok just use the actual value when calculating the embryo density. Density must not be less than 1500. If the density of the eggs is less than 1500, let the eggs settle and decant excess water.

E. Sperm suspension

Filter high quality sperm through a 100 um nitex screen into one beaker and make a note as to which animals were used on the mussel spawning data sheet.

F. Trial fertilization test

A trial fertilization must be performed with each spawning event. A series of sperm dilutions will be performed to achieve final sperm to egg ratio. Use a 10 mL pipet with the tip cut off and place10 mL of egg suspension into three scintillation vials. Add 0.1, 0.3, and 1.0 mL of sperm suspension using pipets. Let these solutions sit for 1.5 –2.5 hours in the lab. Transfer about 0.5 ml of egg solution to the rafter cell. Check the eggs on the microscope at 100X power. Fertilized eggs will have a single polar body, a very small clear circle attached to an egg, or they will have multiple cells that look like Micky Mouse ears. Use the ratio of egg to sperm that uses the lowest amount of sperm to achieve >90% fertilization.

While the eggs are being fertilized, finish the egg counts and determine the eggs/mL concentration. (See Mussel Spawning Datasheet)

To calculate the sperm suspension volume necessary to add to the egg solution, take the volume of the egg suspension prepared in section D and multiply by the sperm to egg ratio determined in the trial fertilization.

G. Test Initiation

Add sperm to eggs(embryo suspension), and use the perforated plunger to mix the suspension. Adjust the embryo suspension density to 1500 – 3000/ mL. Our target density and volume for the embryo suspension is 2500 embryos/mL in 300mL of 32 ‰ seawater. (See Mussel Spawning Datasheet) Achieve this by measuring out the needed amount of embryo stock solution and add 15 °C seawater to 300mL. Use the perforated plunger to mix the suspension. Cover the beaker with parafilm and set aside until ready to use (do not let stand for more than one hour).

On the mussel spawning record form record the time that you will add the embryo solution to the first vial. Using the perforated plunger, continually agitate the embryo solution while adding 0.1 ml to each exposure container. Be careful to insure that the embryo solution is added to the liquid in the exposure containers and does not contact the side of the vials first. Record the time that you finish the embryo addition. Recover the vials with the parafilm. Record the temperature at which the exposure is being performed.

The 5 additional vials of seawater will serve as the initial embryo density sub-samples. One mL of 30% borax buffered formalin will be added to each vial within minutes of the embryo solution addition. These will be used to determine the survival in the controls and the other treatments. Record the counts embryo count form. Calculate the actual embryo density by averaging the 5 sub-samples.

48 hours after the start of the addition of embryos, transfer the racks of exposure vials to the Biology Lab. Terminate the test by adding 1 ml of 30% borax buffered formalin to each vial. This should be done inside a fume hood. The formalin should be dispensed from the re-pipettor. Secure a snap cap on each vial and give the vial a quick swirl to ensure that the formalin is evenly distributed. This task is made easier with two people; one adding the formalin and the other capping and swirling the vials.

VIII. Microscopic Evaluation

The samples can be evaluated whenever convenient. There is not a known maximum holding time for preserved samples.

The samples are evaluated by placing the entire vial in a small petri dish and placing this over the objective port in the stage of the inverted microscope. The embryos are easily viewed at 100 X. Start at the top of the vial and move across

to the opposite side, scoring all “D” shape embryos as normal and those without the “D” shape as abnormal. Move the stage down one field of view and make another complete pass of the vial, continue this process until the entire vial has been counted. Record the results on the mussel embryo development examination data sheet and put a colored dot on the cap to designate it as counted.

IX. Data Analysis

There are three endpoints that can be analyzed. One endpoint is the percent normal. In this case the number of normal embryos is divided by the total number of normal and abnormal embryo present in a vial then multiplied by 100. a second endpoint is percent normal alive data, which is the number of normal embryos present in the vial divided by the mean of the initial count of embryos in the 5 sub-samples then multiplied by 100. The third endpoint is percent alive. In figuring the percent alive one assumes that if embryos are present, no matter what condition, then they are alive. You compare the sum of both the normal and the abnormal embryos against the mean of the initial count of embryos in the 5 sub-samples then multiply by 100.

Enter the endpoint data into the Excel spreadsheet by container number. The means and standard deviations are calculated automatically by the spreadsheet. For each experiment, run an ANOVA and Dunnett’s test using toxstat. Use a point estimation program (such as Toxstat) to calculate the EC50 using the probit method.

The reference toxicant data are similarly entered in the appropriate Excel spreadsheet. Calculate the EC50 as above and plot this value on the running laboratory control chart for this bioassay.

X. Quality Assurance

Test Acceptability Criteria

Mean normal development in the controls must be at least 90%. Mean survival in the controls must be > 50%. The percent minimum significant difference (MSD) must be less than 25%.

Reference toxicant results

The reference toxicant EC50 should fall within two standard deviations of the mean on the control chart. If the EC50 falls outside this range, results of concurrent tests should be examined carefully. The investigator should include a discussion of the significance of the exceedance in any report of the data.

Deviations from test conditions

Deviations from acceptable test conditions must be recorded (i.e. temperature out of range). Best professional judgment will be applied to determine whether the deviation was significant enough to render the results of the test questionable. The investigator should include a discussion of the significance of the deviation in any report of the data.

XI. Cleaning procedures

The exposure vials are used as shipped except that they should be vigorously rinsed with DIW and allowed to dry before use. All glassware and plasticware used in handling the gametes or samples should be processed under the normal toxicology lab cleaning procedure to remove metals and organics.

After it is decided that the embryo samples can be discarded, the vials should be emptied into the sink under a fume hood with running water. The vials should then be rinsed once with tap water and then discarded in the trash. To prevent injuries from broken glass, it best to accumulate the discarded vials in a separate trash bag and then discard directly to the dumpster.

XII. References

USEPA, 1995. "Short-term methods of estimating the chronic toxicity of effluents and receiving water to west coast marine and estuarine organisms. National Exposure Research Laboratory, Office of Research and Development. Cincinnati, Ohio.

Mussel Spawning Data Sheet

Experiment No. ___________ Animal Source __________________
Date ____________________ Time in Culture _________________
Temperature of Water Bath __________________

Mussel No.	Induction	Spawn	Sex	Comments
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				

Pooled eggs from mussels _____________________
Pooled sperm from mussels ____________________

EGG COUNTS

Sample	Dilution	Count	Eggs/mL

For 300 mL of embryo suspension at 2500 embryos/mL use:
300 x 2500 / (counted eggs/mL) = mL of egg stock

750000 eggs / ___________ eggs/mL = _____________ mL of egg stock

Time of embryo addition _________________

Mussel Bioassay Number____________

EMBRYO EXAMINATION

Initials	Count Date	Vial Number	Sample	Normal	Abnormal	% Normal Alive

APPENDIX D
STANDARD OPERATING PROCEDURE FOR CONDUCTING A PHASE I TOXICITY IDENTIFICATION EVALUATION (TIE) USING THE MUSSEL DEVELOPMENT TEST

Southern California Coastal Water Research Project Toxicology Laboratory

OVERVIEW

A phase I TIE uses physical or chemical manipulation of an aqueous sample to selectively remove or render non-toxic substances found in the sample. Through these means, the class (i.e. metals, non-polar organics) of compound causing the toxicity in the original sample may be determined. The methods used in this SOP are based on those in Marine Toxicity Identification Evaluation (TIE): Phase I Guidance Document (EPA/600/R-96/054). The methods for the mussel development test can be found in SOP #T12.0 and EPA's Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to West Coast Marine and Estuarine Organisms (EPA/600/R-95/136).

SUPPLY CHECKLIST

Sodium Thiosulfate, 5-hydrate (STS)
(Ethylenedinitrilo) Tetraacetic Acid, Disodium salt (EDTA)
0.1 N NaOH
500 ml polycarbonate centrifuge tubes
Centrifuge capable of 3000 X G and holding 500 ml centrifuge tubes
pH, DO and conductivity meter/probes
Automatic pipets 0.025 ml up to 10 ml
C-18 cartridges (1 gram, 2 gram and/or 10 gram)
Adapters for cartridges
Masterflex pump with at least 2 pump heads
60 ml syringe bodies for reservoirs
Cation exchange columns, 0.5 gram (optional)
50 ml, 125 ml, 250 ml, 500 ml and 1000 ml Erlenmeyer (many)
Teflon tubing
Pasteur pipets
Parafilm
Aluminum foil

PREPARATIONS

Samples

Before any further manipulation of the samples, salinity must be measured and the appropriate amount of brine added to achieve a salinity of 34 $\pm$ 2 g/kg. For details of the brining procedure, see the mussel development test SOP.

Stock Solutions

Sodium thiosulfate (STS)

STS is not stable and must be made fresh on the day it is to be used. The stock solution concentration is 15 g/L and is made by dissolving 2.35 g of Sodium Thiosulfate, 5-hydrate in 100 ml of DIW. Measure out the crystals in 100 ml beaker and add about 75 ml of DIW. Add a stir bar and mix until dissolved. Transfer to a 100 ml volumetric flask. Rinse the beaker twice with DIW and add to the flask. Bring the volume up to the line with DIW and mix by inversion. Transfer to a 125 ml Erlenmeyer flask and cover with Parafilm.

(Ethylenedinitrilo) Tetraacetic Acid, Disodium salt (EDTA)

EDTA is stable can be stored in the refrigerator for up to one month. The stock solution concentration is 25 g/L and is made by dissolving 2.78 g of EDTA in 100 ml of DIW. EDTA is difficult to dissolve, so plan on allowing about 1 hr between making and using the stock solution. Weigh out the EDTA in a 100 ml beaker, then rinse the compound with DIW into a 100 ml volumetric flask. Bring the level up to the line and add a stir bar. Mix until completely dissolved. Transfer to a 125 ml Erlenmeyer flask and cover with Parafilm.

TEST DESIGN

Bioassay Method: Mussel embryo development
Replicates per treatment concentration: 3
Concentrations of Sample per Treatment: 2 or 3
Salinity adjustment: Hypersaline brine
Dilution water: natural seawater (activated carbon and 0.45 µm filtered)
Water quality: DO, pH and salinity on highest sample concentration of each treatment
Reference toxicant: Optional (usually not performed)

Exposures are conducted in the usual 5 dram glass shell vials with 10 ml volumes per replicate. Normally each treatment is tested at the highest concentration possible after salinity adjustment and at half of that concentration. Other concentrations or the addition of more concentrations may be appropriate depending on the goals of the experiment.

TIE MANIPULATIONS

EDTA

The concentration of EDTA within the exposures is 60 mg/l. If multiple concentrations of a sample are to be tested, each sample concentration will have 60 mg/L of EDTA. Therefore, all dilutions should be made before addition of the EDTA stock. The EDTA treatment is performed on samples that have been

salinity adjusted, but not manipulated in any other manner. The stock solution is added to the sample at a rate of 24 µl of stock for every 10 ml of sample. After the addition of EDTA, the pH of the samples should be checked. If the pH is below 7.5, use NaOH (0.1 N or less) to adjust the pH to between 7.8 and 8.3. After addition of the EDTA the samples should be given at least 3 hr for interactions to occur before addition of the gametes begins. A sample of laboratory seawater must be treated with 60 mg/l EDTA and tested as a blank to verify that the treatment is not causing toxicity.

STS

The concentration of STS within the exposures is 50 mg/l. If multiple concentrations of a sample are to be tested, each sample concentration will have 50 mg/L of STS. Therefore, all dilutions should be made before addition of the STS stock. The STS treatment is performed on samples that have been salinity adjusted, but not manipulated in any other manner. The stock solution is added to the sample at a rate of 34 µl of stock for every 10 ml of sample. After addition of the STS the samples should be given at least 1 hr for interactions to occur before addition of the gametes begins. A sample of laboratory seawater must be treated with 50 mg/l STS and tested as a blank to verify that the treatment is not causing toxicity.

Particle Removal (Centrifugation)

Samples for centrifugation should be placed in 500 ml polycarbonate centrifuge tubes. Pairs of tubes should be balanced within 1 gram for placement on opposite sides of the rotor. The samples should be spun at 3000 X G for 30 minutes. The temperature in the centrifuge should be set at about 10 °C. A sample of laboratory seawater must also be centrifuged and tested as a blank to verify that the treatment is not causing toxicity.

The volume of sample to be centrifuged is dependent on the goals of the experiment. For testing of particle removal only, with 3 replicates and 2 concentrations, only 100 ml of sample are needed. However, sample that has gone through the centrifugation process is also used for application to C-18 and cation exchange columns. Samples for chemical analysis may also be centrifuged.

After the centrifuge stops, carefully remove the tubes from the rotor avoiding disruption of the pellet. Using Teflon coated tubing, siphon the supernatant into an appropriate container, again avoiding disruption of the pellet. The type of container will depend on what the sample will be used for (i.e. plastic for a metals sample or an Erlenmeyer flask for toxicity testing).

C-18 Column Extraction

Samples must go through the particle removal process before being applied to the column. The volume of sample to be passed through the column is dependent on the goals of the experiment. For merely testing what passes through the column only about 150 ml of sample needs to be applied. However,

if further study will involve elution of the column, more sample should be applied to increase the amount of the substances adhering to the column. We have 3 sizes of C-18 column available. The volume of sample that can be passed through before exceeding the capacity of the column is based on the concentration of extractable materials, which is an unknown. Therefore, to minimize the chances of exceeding column capacity we will use the 1 gram columns for samples less than 500 ml; the 2 gram for samples between 500 ml and 1 L and the 10 gram column for samples greater than 1 L. Each column size will have a different procedure for preparation and use. Before passing the sample through the column, a sample of laboratory seawater must me passed through and tested as a blank to verify that the treatment is not causing toxicity.

Before using the C-18 columns, verify that all tubing in the Masterflex system is in good working order. Tubing in the pump head should be replaced after a couple of months, whether it has been used or not. Run at least 500 ml of DIW through the tubing, using the pump, before attaching to any columns. Set the flow to the desired rate.

For 1 g columns: Prepare the column by passing 10 ml of isopropanol through at 5 ml/min. Before the sorbant dries, pass 10 ml of DIW. As the last of the DIW passes through, add 100 ml of seawater, discarding the first 25 ml and collecting the remainder for the C-18 blank. The column must then be reconditioned by adding 10 ml of isopropanol. Before sorbant dries, pass 10 ml of DIW, then 20 ml of seawater. Then pass up to 500 ml of sample, discarding the first 25 ml. If the timing of the start of the fertilization test necessitates, a 100 ml subsample of what has passed through the column can be taken as soon as it is available. After all the sample has passed through the column, let the pump run dry for at least 2 minutes to get as much liquid as possible out of the sorbant. The column should then be labeled appropriately, have both ends covered with aluminum foil and stored in the refrigerator for potential elution at a later time.

For 2 g columns: Prepare the column by passing 20 ml of isopropanol through at 7 ml/min. Before the sorbant dries, pass 20 ml of DIW. As the last of the DIW passes through, add 125 ml of seawater, discarding the first 50 ml and collecting the remainder for the C-18 blank. The column must then be reconditioned by adding 20 ml of isopropanol. Before sorbant dries, pass 20 ml of DIW, then 40 ml of seawater. Then pass up to 1000 ml of sample, discarding the first 50 ml. . If the timing of the start of the fertilization test necessitates, a 100 ml subsample of what has passed through the column can be taken as soon as it is available. After all the sample has passed through the column, let the pump run dry for at least 2 minutes to get as much liquid as possible out of the sorbant. The column should then be labeled appropriately, have both ends covered with aluminum foil and stored in the refrigerator for potential elution at a later time.

For 10 g columns: (***This method is based on several assumptions regarding volumes for column preparation. As yet we have not used these large cartridges***). Prepare the column by passing 50 ml of isopropanol through at 10 ml/min. Before the sorbant dries, pass 50 ml of DIW. As the last of the DIW passes through, add 150 ml of seawater, discarding the first 75 ml and collecting the remainder for the C-18 blank. The column must then be reconditioned by adding 50 ml of isopropanol. Before sorbant dries, pass 50 ml of DIW, then 50

ml of seawater. Then pass up to 3000 ml of sample, discarding the first 75 ml. . If the timing of the start of the fertilization test necessitates, a 100 ml subsample of what has passed through the column can be taken as soon as it is available. After all the sample has passed through the column, let the pump run dry for at least 2 minutes to get as much liquid as possible out of the sorbant. The column should then be labeled appropriately, have both ends covered with aluminum foil and stored in the refrigerator for potential elution at a later time.

Cation Exchange Column Extraction (optional)

The cation exchange column removes cationic metals and usually provides phase I results similar to the EDTA treatment. The advantage of the cation exchange column is metals removed by the column can be eluted and verification of toxicity and chemical analysis in phase II can be performed.

Samples must go through the particle removal process before being applied to the column. The volume of sample applied to the column is dependant on the goals of the experiment. To merely test the toxicity of the sample after it has passed through the column, only about 150 ml needs to be applied. More sample can be applied to provide a greater amount retained by the column for later elution in phase II testing. Presently, we have only 0.5 g cation exchange columns. To avoid overloading these columns, it would be best to apply no more than 500 ml of sample.

Before using the cation exchange columns verify that all tubing in the Masterflex system is in good working order. Tubing in the pump head should be replaced after a couple of months, whether it has been used or not. Run at least 500 ml of DIW through the tubing, at 7-10 ml/min before attaching to the columns. Then pass 2 ml of 10% HCl through the tubing to remove any metals. Pass at least another 25 ml of DIW through the tubing while setting the flow rate to 2.5 ml/min.

Pass 2 ml of optima grade methanol through the column at 2.5 ml/min. Before sorbant dries, pass 6 ml of DIW. Before the sorbant dries, pass another 70 ml of DIW discarding the first 15 ml and collecting the remainder as a blank. This sample will need to be brined and tested with the fertilization test to verify that the treatment is not causing toxicity. As the last of the DIW passes through the column, between 150 and 500 ml of sample can be applied, discarding the first 25 ml that passes. . If the timing of the start of the fertilization test necessitates, a 100 ml subsample of what has passed through the column can be taken as soon as it is available. After all the sample has passed through the column, let the pump run dry for at least 2 minutes to get as much liquid as possible out of the sorbant. The column should then be labeled appropriately, have both ends covered with parafilm and stored in the refrigerator for potential elution at a later time.

REFERENCE TOXICANT

It is usually not necessary to perform a reference toxicant test concurrently with TIE testing. However, if initial testing and TIE testing are combined, the standard

copper reference toxicant concentration series should be tested (See mussel development test SOP).

DATA ANALYSIS

Enter the percent fertilized data in the Excel spreadsheet by container number. The means and standard deviations of each treatment are calculated automatically. An ANOVA and multiple range test can be run either using the macro built into the spreadsheet or by using Toxstat. If sufficient number of concentrations of each treatment and/or the baseline sample are tested, then EC50 calculations using the probit method can be made using Toxstat.

QUALITY ASSURANCE

Test Acceptability Criteria

The acceptability criteria for TIE testing is much less stringent than for regular testing. Between replicate variability must be low enough that differences between the original sample and the treated sample are discernible. Mean fertilization in the controls should be at least 70% and sperm to egg ratio should not exceed 3000:1.

Deviations from test conditions

While great latitude is allowed in conducting TIE testing, it is very important to record any modifications that are made from the standard operating procedure. Modifications to either TIE manipulations or the fertilization testing methods should be noted.

Use of treatment blanks

Since some of the treatments used in the TIE process can be toxic themselves, it is extremely important that a blank is tested for each treatment. Lack of blanks may render a TIE uninterpretable.

CLEANING PROCEDURES

All glassware used in the TIE process should go through the normal toxicology glassware washing procedure.

The polycarbonate centrifuge tubes should be detergent scrubbed then detergent soaked for 24 hr, followed by 3X tap water rinses, 1X 10% nitric acid rinse, 3X DIW rinse, 2X methanol rinse, 1X hexane rinse. After the hexane rinse the bottles should air dry under a fume hood until all the hexane has evaporated. Then a 24 hr 10% nitric acid soak and finally a 3X Type I water rinse.

REFERENCES

USEPA. 1995. “Short-term methods of estimating the chronic toxicity of effluents and receiving water to west coast marine and estuarine organisms. National Exposure Research Laboratory, Office of Research and Development. Cincinnati, Ohio.

USEPA. 1996. “Marine Toxicity Identification Evaluation (TIE): Phase I Guidance Document”. National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division. Narragansett, Rhode Island.

APPENDIX E
EXAMPLE CHAIN OF CUSTODY FORM

Example Chain-of-Custody Form

Project: ____

Sample ID	Date	Time	# of bottles	Matrix	Container	Analysis

Relinquished		Received
Agency: ______________________________ Name: ______________________________ SIGNATURE:	Date / Time: ____________	Agency: ______________________________ Name: ______________________________ Signature: ____________________________
Agency: ______________________________ Name: ______________________________ Signature: ____________________________	Date / Time: ____________	Agency: ______________________________ Name: ______________________________ Signature: ____________________________
Agency: ______________________________ Name: ______________________________ Signature: ____________________________	Date / Time: ____________	Agency: ______________________________ Name: ______________________________ Signature: ____________________________

Comments
____
____
____
____