Monitoring Antifouling Paint

Active Ingredients in California Marinas

State Water Resources Control Board Agreement No. 05-218-250-0

Quality Assurance Project Plan

California Department of Pesticide Regulation
Environmental Monitoring Branch
1001 "I" Street, 3rd Floor
Sacramento, CA 95812

July 2006 (version 1.4)

Funding for this project has been provided in part by the U.S. Environmental Protection Agency (U.S. EPA) pursuant to Assistance Agreement No. C9-96906801 and any amendments thereto which have been awarded to the State Water Board for the implementation of California's Nonpoint Source (NPS) Pollution Control Program. The contents of this document do not necessarily reflect the views and policies of the U.S. EPA or the State Water Board, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Quality Assurance Project Plan

California Department of Pesticide Regulation

PROJECT: Monitoring Antifouling Paint Active Ingredients in California Marinas
State Water Resources Control Board Agreement No. 05-218-250-0

PREPARED BY: California Department of Pesticide Regulation

1. APPROVED BY:

Department of Pesticide Regulation (DPR)

_____ _____
Nan Singhasemanon, Project Manager Date
Department of Pesticide Regulation

_____ _____
Carissa Ganapathy, QA Officer Date
Department of Pesticide Regulation

Analytical Laboratories

_____ _____
Dirk Holstege, Lab Manager Date
UC Davis ANR Analytical Laboratory

_____ _____
Ken Schiff, Lab Manager Date
Southern California Coastal Water Research Project

_____ _____
Ed Wirth, Lab Manager Date
NOAA - Hollings Marine Laboratory

State Water Resources Control Board (SWRCB)

_____ _____
Melenee Emanuel, Contract Manager Date
State Water Resources Control Board

_____ _____
William Ray, QA Officer Date
State Water Resources Control Board

2. TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

3. DISTRIBUTION LIST

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

Nan Singhasemanon
Department of Pesticide Regulation
1001 I Street
Sacramento, CA 95812
nsinghasemanon@cdpr.ca.gov
Phone: (916) 324-4122

Juanita Bacey
Department of Pesticide Regulation
1001 I Street
Sacramento, CA 95812
nbacey@cdpr.ca.gov
Phone: (916) 445-3759

Carissa Ganapathy
Department of Pesticide Regulation
1001 I Street
Sacramento, CA 95812
cgana@cdpr.ca.gov
Phone: (916) 322-3082

Aijun Wang
Department of Pesticide Regulation
1001 I Street
Sacramento, CA 95812
awang@cdpr.ca.gov
Phone: (916) 324-4087

Dirk Holstege
ANR Analytical Laboratory
University of California
207 Hoagland Hall
One Shield Ave
Davis, CA 95616
dmholstege@ucdavis.edu
Phone: (530) 752-0148

Ken Schiff
Southern California Coastal Water Research Project
7171 Fenwick Lane
Westminster, CA 92683
kens@sccwrp.org
Phone: (714) 372-9202

Ed Wirth
Marine Ecotoxicology Branch
NOAA - Hollings Marine Laboratory
331 Fort Johnson Road
Charleston, SC 29412
ed.wirth@noaa.gov
Phone: (843) 762-8903

Melenee Emanuel
State Water Resources Control Board
1001 I Street
Sacramento, CA 95812
memanuel@waterboards.ca.gov
Phone: (916) 341-5271

William Ray
State Water Resources Control Board
1001 I Street
Sacramento, CA 95812
BRay@waterboards.ca.gov
Phone: (916) 341-5583

4. PROJECT/TASK ORGANIZATION

4.1 Involved parties and roles.

Department of Pesticide Regulation (DPR) is a State Government agency interested in the assessment of California Marina Antifouling Paints Pollution. As the lead agency, DPR will organize the sample collection, field and in-house analysis of samples, and the initiation and maintenance of a contract with the analytical laboratories.

Agriculture and Natural Resources Analytical Laboratory, University of California, Davis (UCD-ANRAL) will perform the majority of the chemical analysis.

Southern California Coastal Water Research Project (SCCWRP)'s laboratory in Westminster, California will analyze the development toxicity of water samples and perform Toxicant Identification Evaluation on a subset of study samples.

National Oceanic and Atmospheric Administration (NOAA)'s Hollings Marine Laboratory (HML) in Charleston, South Carolina will analyze water and sediment samples for Irgarol 1051.

The three analytical laboratories, UCD-ANRAL, SCCWRP laboratory and NOAA-HML, hereafter will be collectively referred to as "the Laboratories" in this QAPP. They will analyze submitted samples in accordance with all method and quality assurance requirements found in this QAPP.

4.2 Personnel Responsibilities

Project Manager role:
Nan Singhasemanon is DPR's Project Manager. Mr. Singhasemanon will be responsible for all aspects of the project including planning, coordination, and implementation. Nan will also organize and direct field staff and interact with all contract laboratories involved in this project. Moreover, he is responsible for the completion and submittal of deliverables to the SWRCB per agreement # 05-218-250-0.

Juanita Bacey is DPR's Co-Project Manager. Ms. Bacey will be primarily responsible for the field coordination aspects of the project, which involves activities such as scheduling, field sampling, and field measurements.

Contract Manager role:
Melenee Emanuel (SWRCB) is the Contract Manager. Ms. Emanuel is responsible for obtaining all services and deliverables for the study and for overseeing budgetary expenses.

DPR QA Officer role:
Carissa Ganapathy is DPR's Quality Assurance Officer. Carissa'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. Carissa will also work with the Quality Assurance Officers for the Laboratories by communicating all quality assurance and quality control issues contained in this QAPP to the Laboratories.

Carissa Ganapathy will also review and assess all procedures during the life of the contract against QAPP requirements. Carissa Ganapathy will report all findings to Nan Singhasemanon,

including all requests for corrective action. Carissa Ganapathy may stop all actions, including those conducted by the Laboratories if there are significant deviations from required practices or if there is evidence of a systematic failure.

UCD-ANRAL Lab Manager role:
Dirk Holstege is the UCD-ANRAL Lab Manager. Mr. Holstege will maintain all records associated with the receipt and analysis of samples and will verify that the measurement process meet the data quality objectives specified in this QAPP or acceptable deviations explained for each batch of samples before proceeding with analysis of a subsequent batch.

SCCWRP Lab Manager role:
Ken Schiff is the SCCWRP Lab Manager. Mr. Schiff will maintain all records associated with the receipt and analysis of samples and will verify that the measurement process meet the data quality objectives specified in this QAPP or acceptable deviations explained for each batch of samples before proceeding with analysis of a subsequent batch.

NOAA-HML Lab Manager role:
Ed Wirth is the NOAA-HML Lab Manager. Mr. Wirth will maintain all records associated with the receipt and analysis of samples and will verify that the measurement process meet the data quality objectives specified in this QAPP or acceptable deviations explained for each batch of samples before proceeding with analysis of a subsequent batch.

SWRCB QA Officer role:
Bill Ray is the SWRCB Project Quality Assurance Officer. Bill Ray will be responsible for verifying that the quality assurance and quality control procedures found in this QAPP meet the standards developed for Surface Water Ambient Monitoring Program (SWAMP) QAPPs as set forth in the Electronic Template for SWAMP-Compatible Quality Assurance Project Plans (Nichol and Reyes, 2004).

In addition, the following personnel will act as technical advisors to DPR staff but are not responsible for delivery of any product:

Frank Spurlock, Senior Environmental Research Scientist, Dept. of Pesticide Regulation, Environmental Monitoring Branch, Sacramento, CA

Ray Arnold, Copper Development Association Inc., New York, NY

Paul Salop, Marine Ecologist, Applied Marine Sciences, Inc., Livermore, CA

Ken Schiff, Deputy Director, Southern California Coastal Water Research Project, Westminster, CA

Members of the Non-Point Source Interagency Coordinating Committee's Marina and Recreational Boating Workgroup - Copper Antifouling Paint Sub-Workgroup.

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 DPR's Project Manager and Quality Assurance Officer, and with the concurrence of both the

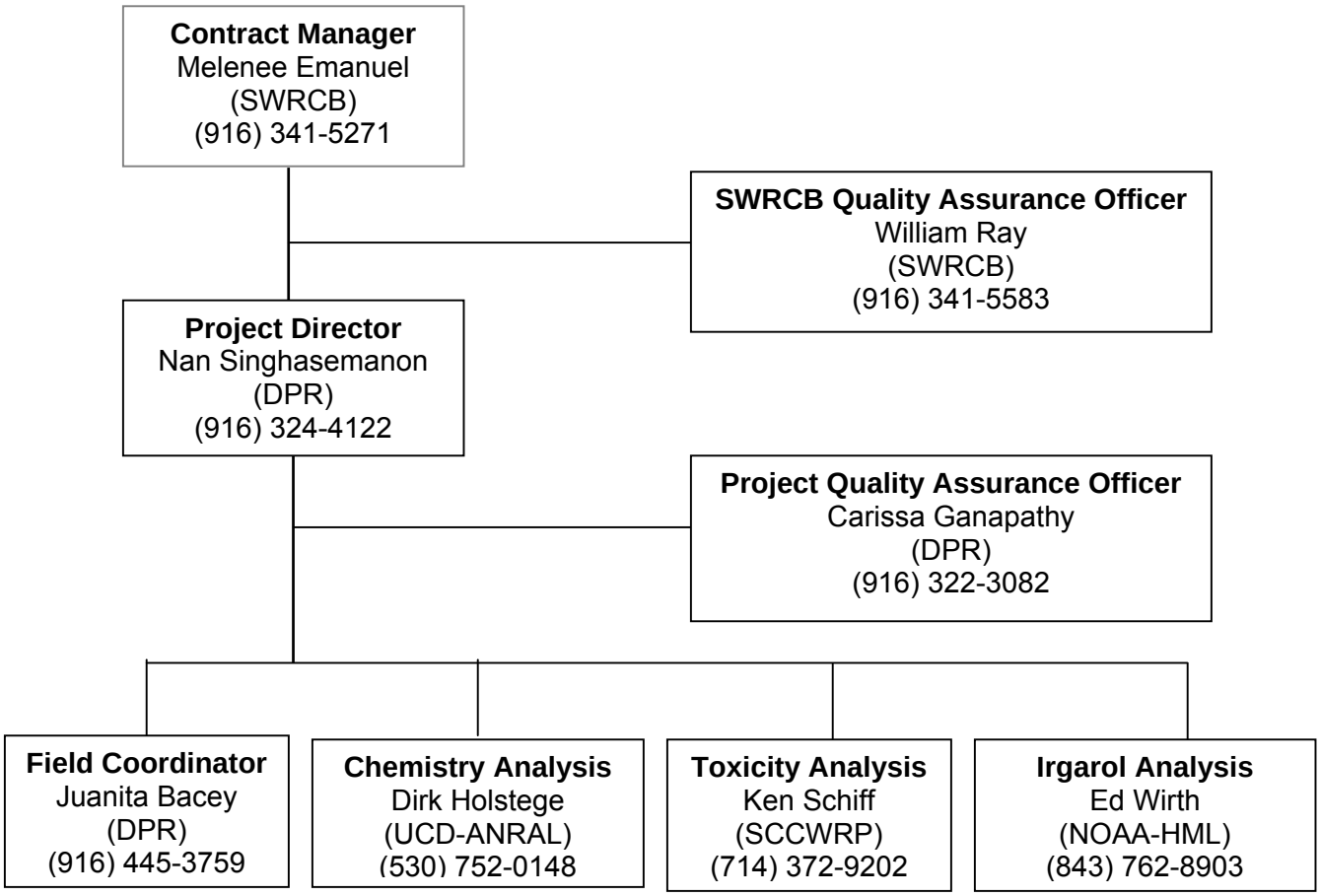
State Board's Contract Manager and Quality Assurance Officer. DPR's Aijun Wang will be responsible for making the changes, submitting drafts for review, preparing a final copy, and submitting the final for signature.

Table 1. (Element 4) Personnel responsibilities.

Name	Organizational Affiliation	Title	Contact Information (Telephone number, fax number, email address.)
Nan Singhasemanon	CDPR	Project Manager	Tel: 916-324-4122 Fax: 916-324-4088 nsinghasemanon@cdpr.ca.gov
Carissa Ganapathy	CDPR	Contractor QA Officer	Tel: 916-322-3082 Fax: 916-324-4088 cgana@cdpr.ca.gov
Juanita Bacey	CDPR	Field Coordinator	Tel: 916-445-3759 Fax: 916-324-4088 nbacey@cdpr.ca.gov
Dirk Holstege	UCD-ANRAL	Lab Manager	Tel: 530-752-0148 Fax: 530-752-9892 dmholstege@ucdavis.edu
Ken Schiff	SCCWRP	Lab Manager	Tel: 714-372-9202 Fax: 714-894-9699 kens@sccwrp.org
Ed Wirth	NOAA-HML	Lab Manager	Tel: 843-762-8903 Fax: 843-762-8737 ed.wirth@noaa.gov
Melenee Emanuel	SWRCB	Contract Manager	Tel: 916-341-5271 Fax: 916-341-5463 memanuel@waterboards.ca.gov
William Ray	SWRCB	QA Officer	Tel: 916-341-5583 Fax: 916-341-5584 BRay@waterboards.ca.gov

4.4 Organizational chart and responsibilities

Figure 1. Organizational chart.



5. PROBLEM DEFINITION/BACKGROUND

5.1 Problem statement.

In 1988, the California Department of Pesticide Regulation (DPR) and the U.S. Environmental Protection Agency (U.S. EPA) established regulations to limit the use of tributyltin antifouling paints (AFPs) because of documented adverse affects to aquatic organisms. Copper-based AFPs, which were also popular at the time, became the dominant class of AFPs in California. Today, cuprous oxide is the most popular of these copper AFPs. It is the primary active ingredient in over 160 AFP products registered in the State. Other active ingredients, such as zinc and Irgarol, are also being used in current AFP products.

In the Shelter Island Yacht Basin (SIYB) Copper Total Maximum Daily Load (TMDL), the San Diego Regional Water Quality Control Board (SD Regional Water Board) concluded that the use of copper AFP pesticides on recreational boats moored at SIYB led to the exceedances of the California Toxics Rule (CTR) standards for copper. High dissolved copper concentrations at SIYB also violated the narrative Water Quality Objectives (WQOs) for toxicity and pesticides as defined in the SD Regional Water Board's Basin Plan.

Additional water column surveys by the SD Regional Water Board of seven other San Diego Bay marinas in 2004 also revealed elevated levels of copper that were above CTR values. These results suggest that 1) elevated dissolved copper levels are not unique to SIYB in San Diego Bay and 2) copper sources are likely to be from within marinas.

Outside of San Diego Bay, the availability of copper data relevant to the evaluation of copper AFP pollution is much more limited. Although a number of aquatic copper studies have been conducted in California, they were not specifically designed to provide an accurate assessment of copper pollution associated with AFP use. Most of these studies did not focus on marinas, or other areas with high boat density and AFP use. Thus, data from more focused studies in water bodies across the State are needed.

The linkage of copper pollution to AFP use at SIYB also raises questions on the pollution potential of other AFPs currently used in California. Zinc pyrithione and Irgarol 1051 are also commonly formulated into AFPs (often as co-biocides to copper). The toxicity of zinc and Irgarol in aquatic systems has been well documented. However, it is not known whether environmental levels of these compounds have reached biologically sensitive levels in California waters. Environmental concentration data of these constituents in areas of high AFP use (or any other areas) are lacking. These unknowns suggest that monitoring studies that will better define the degree and geographical distribution of AFP pollution in areas of high AFP use in California are needed.

5.2 Decisions or outcomes.

This project will: 1) Determine the occurrences and concentrations of selected indicators of AFP pollution (i.e., copper, zinc, and Irgarol) in the water and sediment of selected California marinas and establish whether these levels exceed appropriate water quality standards, guidelines, and other ecologically-relevant values; 2) Quantify copper and zinc in the water and sediment of water areas that are adjacent to each marina to determine if marina pollutant levels are significantly higher than local reference levels; 3) Determine whether AFP indicator levels differ

significantly among salt water, brackish water, and fresh water marinas; 4) Determine the bioavailability and toxicity of copper using EPA's Biotic Ligand Model (BLM); and 5) Measure the toxicity of marina waters on mussel embryo development, compare measured toxicity with copper concentrations and BLM-predicted toxicity, and identify the likely cause of observed toxicity using Toxicity Identification Evaluation (TIE) methods.

The assessments will help DPR determine what types of mitigation and regulatory actions need to be implemented to control antifouling paint pollution, if any is needed.

5.3 Water quality or regulatory criteria

This project uses the following criteria:

- Water quality standards in California Toxics Rule (CTR)
- Sediment quality guidelines (SWRCB Sediment Quality Objectives may be available for data in 2007)
- U.S. EPA's Biotic Ligand Model (BLM) to the Evaluation of Water Quality Criteria for Copper

6. PROJECT/TASK DESCRIPTION

6.1 Work statement and produced products.

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

Sampling will be focused on the water column for copper and zinc concentration. In total, there will be 96 sites in 24 marinas throughout California north of San Diego region. A maximum of 800 water column samples and 250 sediment samples will be collected from the identified marinas and other areas of high boating activities in a number of water body types including coastal bays/harbors, estuaries, and freshwater rivers and lakes.

The second task will involve laboratory analysis. Laboratory analysis includes BLM-associated parameters, chemical measurements of copper, zinc and Irgarol in water and sediment samples. Laboratory analysis also includes toxicity testing using larvae of the bivalve *Mytilus galloprovincialis*. The product for this task will be laboratory analysis reports indicating analytical success for all samples delivered to laboratories.

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 quarterly progress reports describing activities undertaken and accomplishments of each task during the quarter, milestones achieved, and any problems encountered in the performance of the work. The product will also include a final written report at the end of the project, providing a full listing and summary of the data collected including an assessment of AFP pollution in marinas, site-specific estimates of bioavailability, and toxicity to aquatic organisms.

6.2 Constituents to be monitored and measurement techniques.

Monitoring will consist of field measurements for water temperature, pH, Specific conductance (EC), depth and turbidity. Water samples will be collected for analysis of dissolved copper, dissolved zinc, Total Suspended Solids (TSS), Irgarol 1051, dissolved organic carbon (DOC), Salinity (Cl), sulfate, magnesium, calcium, sodium, potassium, alkalinity, developmental toxicity (*M. galloproviancialis*), Toxicity identification evaluation (TIE); Sediment samples will be collected for analysis of total copper, total zinc, grain size, and total organic carbon(TOC).

Water temperature will be measured using multicomponent meter;

Water pH will be measured using portable pH meter;

Specific conductance (EC) will be measured using Dissolved Oxygen meter;

Dissolved copper will be analyzed using EPA 220.2 Graphite Furnace Atomic Absorption method;

Dissolved zinc will be analyzed using EPA 200.7 Inductively-Coupled Plasma method;

Total copper and total zinc in sediment will be analyzed using EPA 3051 Microwave assisted acid digestion of sediments, sludges, soild, and oils;

Total Suspended Solids (TSS) will be determined using EPA 160.2 Gravimetric Method.

Irgarol 1051 will be analyzed at NOAA's Hollings Marine Laboratory using an high performance liquid chromatography (HPLC) electro spray ionization tandem mass spectrometry method as published in *Thomas et al, 2002*;

DOC will be determined using EPA 415.1-.2 Total organic carbon in water;

TOC will be determined using DPR SOP METH005.00 Total Organic Carbon (DC-85A) Instrument;

Salinity(Cl) and Sulfate will be determined using EPA 300.0 ion chromatography

SCCWRP's laboratory in Westminster, CA will test the salt and brackish water samples for sub-chronic developmental toxicity on the mussel *Mydulis galloprovincialis* (EPA/600/R-95/136);

SCCWRP will also perform follow up TIEs on a sub-set of toxic samples using methods in EPA/600/R-96/054. The toxicity threshold that will be the trigger for TIE consideration will be 50% abnormal embryo development relative to control. SCCWRP will perform a maximum of 4 TIEs for this study.

Grain size will be determined using DPR method SOPMETH 004.00.

6.3 Project schedule

A project schedule follows.

Table 2. (Element 6) Project schedule timeline.

Activity	Date (MM/DD/YY)		Deliverable	Deliverable Due Date
	Anticipated Date of Initiation	Anticipated Date of Completion		
Start Project	3/3/06	3/3/06	None	
			Quarterly reports	4/20/06
Collect Samples	7/10/06	10/6/06	Quarterly reports	7/20/06
Analyze Samples	7/10/06	12/1/06	Quarterly reports	10/20/06
			Quarterly reports	1/20/07
			Quarterly reports	4/20/07
Draft Final Report	1/20/07	5/15/07	Draft final report	5/15/07
			Project Summary Form	6/15/07
Final Report	5/25/07	6/15/07	Final Project Report	6/15/07

6.4 Geographical setting

The study areas will be at marinas scattered across California in which environmental AFP data have not been well documented. These marinas represent areas of high boating activities in a number of water body types including coastal bays/harbors, estuaries, and freshwater rivers and lakes.

Since AFP levels (particularly copper) in marinas have been documented in San Diego Bay, Mission Bay, Oceanside Harbor, and Dana Point Harbor, these regions will be excluded from the geographic scope of this study. Moreover, the Santa Ana Regional Water Quality Control Board has recently initiated a monitoring study of metals in Newport Bay area marinas. Therefore, this region will also be excluded.

6.5 Constraints

The sampling period will be constrained to California's summer months (July through September) to avoid confounding hydrologic factors that would be introduced by storm events. Processes such as flushing, dilution, mixing and sediment resuspension will certainly affect the water and sediment levels of AFP indicators and other analytes. Moreover, during storm periods, non-marina input of AFP indicators into the marina could be significantly larger than sources from within the marina itself. Since DPR is interested in evaluating AFP pollution (via leaching and hull cleaning) from moored vessels and considering that the highest density of boats in marinas tends to occur during the warmest months, the chosen study period is most appropriate.

There are also some limitations to the chosen study design. In some cases, it will not be possible to relate all in-marina concentrations of AFP pollutants completely to AFP sources. This is potentially an issue with zinc. Zinc is commonly used as the boat sacrificial anode. Moreover, the use of zinc pyrithione AFP products is not as prevalent as the use of copper AFP products. Therefore, the interpretation of in-marina water and sediment levels of zinc will have to account for this limitation. In other words, in-marina data for zinc is likely more representative of the combined effects of zinc used in sacrificial anodes, AFP products, background, and other potential sources. Thus, zinc data collected in this study may be best utilized as reference values for future investigations.

The interpretation of sediment results will also have to be done with caution due to the tendency of sediment to act as a sink for metals. Copper and zinc that are present in the sediment may not solely originate from AFP sources, but may come from other sources inside or outside of the marina. So, although we can make a strong argument that copper in marina waters during dry periods can be primarily attributed to the combination of reference levels of copper and copper stemming from the discharge of AFPs from boats in the marina, it would be more difficult to make this assertion with copper in the marina sediment.

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 Tables 3 and 4, respectively.

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 3, 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 3, 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 3, 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 3, 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 3, 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 disassociate any bias associated with seasonality.

The following table summarizes the Data Quality Objectives.

Measurement or Analyses Type	Applicable Data Quality Objective
Field Measurement	Accuracy, Precision, Completeness
Laboratory Analyses	Accuracy, Precision, Recovery, Completeness

Field and Laboratory Measurements Data Quality Objectives are shown on Tables 3 and 4.

Table 3. (Element 7) Data quality objectives for field measurements.

Group	Parameter	Accuracy	Precision (RPD)	Recovery	Target Reporting Limit	Completeness
Field Measurement	Dissolved Oxygen	± 0.5 mg/L	± 0.5 or 10%	NA	NA	90%
	Temperature	± 0.5 °C	± 0.5 or 5%	NA	NA	90%
	Conductivity	± 5%	± 5%	NA	NA	90%
	pH by meter	± 0.5 units	± 0.5 or 5%	NA	NA	90%
	Depth	± 0.2 meters	NA	NA	NA	NA
	Turbidity	± 10% or 0.1,	± 10% or 0.1	NA	NA	90%

Table 4. (Element 7) Data quality objectives for laboratory measurements.

Group	Parameter	Accuracy	Precision (RPD)	Recovery	Target Reporting Limits	Completeness
Laboratory Analyses	Copper (dis.)	75%-125%	± 25%	MS ± 25%	0.5 – 2.0 µg/L	90%
	Copper (tot.)	75%-125%	± 25%	MS ± 25%	0.5 – 2.0 µg/L	90%
	Zinc (dis.)	75%-125%	± 25%	± 25%	1.0 – 5.0 µg/L	90%
	Irgarol 1051	75%-125%	± 25%	84-112%	0.1 ng/L	90%
	DOC	75%-125%	± 25%	± 25%	0.05 mg/L	90%
	Salinity(Cl)	80%-120%	Lab duplicate, blind field duplicate, MS/MSD 25% RPD	Matrix spike 80% - 120% or control limit at ± 3 Standard Deviation	2.0 mg/L	90%
	Sulfate				2.0 mg/L	
	Magnesium				2.0 mg/L	
	Calcium				2.0 mg/L	
	Sodium				2.0 mg/L	
	Potassium				1.0 mg/L	
	Alkalinity				< 2.0 mg/L	
	Toxicity, TIE	± 2 SD[1]	± 2 SD[1]	N/A	30%[2]	90%
	Total Suspended Solids	75% - 125%	± 25%	± 25%	4.0 mg/L	90%
	Copper (tot.)[3]	75% - 125%	MS/MSD ± 25 RPD	MS 75% - 125%	10 mg/kg	90%
	Zinc (tot.)[3]				10 mg/kg	
	TOC[3]	± 20% to 25%.	Replicates within ± 20%	± 25%	5.0 mg/L	90%
	grain size[3]	NA	± 20%	± 25%	2 µm	90%

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

8. SPECIAL TRAINING NEEDS/CERTIFICATION

8.1 Specialized training or certifications.

No specialized training or certifications is required for this project. DPR will hold in-house training for field staff on project sampling equipment and study orientation.

8.2 Training and certification documentation.

DPR, UCD-ANR, SCCWRP and NOAA-HML maintain records of their training. Those records can be obtained, if needed, through the Laboratory Managers. The Contractor's Quality Assurance Officer is responsible for overseeing training.

8.3 Training personnel.

DPR, UCD-ANR, SCCWRP and NOAA-HML maintain field/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. The QA Officers of DPR, UCD-ANRAL, SCCWRP and NOAA-HML provide training to the Laboratories' personnel, respectively.

Table 5. (Element 8) Specialized personnel training or certification.

Specialized Training Course Title or Description	Training Provider	Personnel Receiving Training/ Organizational Affiliation	Location of Records & Certificates *
N/A	N/A	N/A	N/A

9. DOCUMENTS AND RECORDS

DPR will collect records for sample collection, laboratory analyses and toxicity testing. Samples sent to the Laboratories will include a Chain of Custody form. The Laboratories generate records for sample receipt and storage, analyses, and reporting.

The Project Manager, Nan Singhasemanon, maintains the database of information collected in this project. For any records stored electronically, an extra copy will be produced each month as a backup measure.

All records generated by this project will be stored at DPR's main office. The Laboratories records pertinent to this project will be maintained at their respective main offices. Copies of all records held by the Laboratories will be provided to DPR and stored in the project file.

Copies of this QAPP will be distributed to all parties involved with the project, including field collectors and Managers of the Laboratories. Copies will be sent to the Laboratories' Manager for distribution within the laboratories. Any future amended QAPPs will be held and distributed in the same fashion. All originals of the first and subsequent amended QAPPs will be held at DPR. Copies of versions, other than the most current, will be discarded so as not to create confusion.

Persons responsible for maintaining records for this project are as follows. Juanita Bacey, Field Coordinator will maintain all sample collection, sample transport, chain of custody, and field analyses forms. Carissa Ganapathy, DPR Laboratory Liaison will maintain all records associated with the receipt and analysis of samples analyzed for TOC and sediment grain size, and all records submitted by the Laboratories. Nan Singhasemanon will maintain the database. Dirk Holstege, Laboratory Director for UCD-ANRAL, will maintain UCD-ANRAL's records. Ken Schiff, Director for SCCWRP, will maintain SCCWRP's records. Ed Wirth will maintain NOAA-HML's records. DPR Project Manager Nan Singhasemanon will oversee the actions of these persons and will arbitrate any issues relative to records retention and any decisions to discard records.

All records will be passed to the State Board Contract Manager Melenee Emanuel at project completion. Copies of the records will be maintained at DPR and UCD-ANRAL for at least five years after project completion.

Table 6. (Element 9) Document and record retention, archival, and disposition information.

	Identify Type Needed	Retention	Archival	Disposition
Sample Collection Records	Chains of Custody	Until completion and approval of final reports	5 years	Archivist may continue storage or dispose of at the end of 5 years
Field Records	Field Data Sheets	Same as above	5 years	Same as above
Analytical Records	Sample Reports	Same as above	5 years	Same as above
Data Records	Excel Database	Same as above	Indefinitely	N/A
Assessment Records	Final Data Reports	Same as above	5 years	Archivist may continue storage or dispose of at the end of 5 years

GROUP B DATA GENERATION AND ACQUISITION

10. SAMPLING PROCESS DESIGN

Copper, zinc, and Irgarol will be used as indicators of AFP pollution. Water and sediment samples will be taken from marinas and adjacent areas and analyzed for these indicators as well as a number of other constituents. If marina levels of indicators are consistently higher than ambient reference levels, then it is likely that sources of pollutants from within the marinas (particularly AFPs) are contributing to these locally elevated levels. Since Irgarol is exclusively used in California as an AFP, we will not be looking for this biocide in reference samples.

The sampling period will July through September to avoid confounding hydrologic factors that would be introduced by storm events. Processes such as flushing, dilution, mixing and sediment resuspension will certainly affect the water and sediment levels of AFP indicators and other analytes. Moreover, during storm periods, non-marina input of AFP indicators into the marina could be significantly larger than sources from within the marina itself. Since DPR is interested in evaluating AFP pollution (via leaching and hull cleaning) from moored vessels and considering that the highest density of boats in marinas tends to occur during the warmest months, the chosen study period is most appropriate.

At each marina, concentrations of AFP indicators will be quantified for both marina and reference samples three times during this sampling period. This translates to a sampling frequency of about once every four weeks. Site means for the sampling period can be calculated from these three sampling events. These means can then also be averaged to generate a marina mean for the sampling period. Note that these site means cannot be used for trend analysis although this would be possible if the sampling frequency and duration are longer. However, trends analysis is not an objective of this study.

A number of water quality parameters will also be measured to provide input into EPA's copper BLM. This model determines the bioavailability of copper and predicts its toxicity to aquatic organisms. It is based on the concept that toxicity is determined by the amount of copper that binds onto a biotic ligand site (target organism's biochemical site). The amount of copper that is available to bind to these sites is dependent on the amount of dissolved copper and the presence of various complexing substances in the water. The fresh water BLM has proven to be so reliable that EPA has adopted it to establish future copper water quality criteria for the protection of aquatic organisms in fresh water (EPA, 2003b).

The BLM is currently being evaluated for salt and brackish water applications (Arnold et al., 2005). Thus far, the model appears to require identical input parameters that are needed for the fresh water application. Based on the overall acceptance of the model's scientific basis for fresh water, it will likely be adopted by EPA for use on estuarine and marine waters in the near future. Thus, we will measure for the current BLM water quality input parameters.

Although we anticipate that the BLM will eventually provide an estimate of toxicity for our a large number of our study samples, a subset of salt and brackish marina water samples will still be assessed for actual toxicity using EPA's method for short-term chronic toxicity test on mussel (*Mytilus galloprovincialis*) embryo development. Since this test is fairly sensitive to copper levels, the results will help establish whether elevated levels of dissolved copper correspond to sample toxicity. The determination of actual toxicity will also help DPR evaluate the usability of

the current version of the salt water BLM by comparing actual to estimated toxicity.

The collection of toxicity samples will also allow TIEs to be done to better link chemistry results with biological effects. TIE procedures will be performed on a subset of toxic samples to confirm the cause of the observed toxicity. TIE treatments are designed to selectively remove or neutralize classes of compounds and their associated toxicity to identify the most likely cause of the observed toxicity.

In coming up with the design of this study, it was assumed:

- In dry periods, the level of dissolved copper measured in marina water represents the combined effects of reference levels of copper and copper discharged from AFP-painted boats in the marina.
- All of the Irgarol detected in marina water and sediment comes from AFP-painted boats in the marina.
- In poorly flushed marinas, the dispersal and movement of AFP pollutants tend to be limited and localized.

Site Selection Criteria:

DPR staff used marina-specific information produced by the Marina Mapping Sub-Workgroup of the Non-Point Source Interagency Coordinating Committee's Marina and Recreational Boating Workgroup to generate an initial list of candidate marinas. Aerial photos and maps were also used to evaluate individual marina layouts and nearby anthropogenic, geologic, and hydrologic features.

A more manageable list of candidate marinas was isolated from the larger initial list based on the following considerations (in descending order of importance):

1) Marina contains a relatively high number of slips.
2) Marina contains slip areas that are sufficiently isolated from adjacent or surrounding sources (e.g., boatyards, industrial discharges, mining discharges).
3) Historical and current activities (e.g., dredging, construction) in the marina area will not significantly interfere with the interpretation of results.
4) Marina area experiences relatively poor flushing (the likelihood of finding elevated levels of marina-borne pollutants over an extended period is high.)
5) Marinas are evenly distributed across the study area.
6) Marina is a good candidate from a sampling logistics standpoint.

Since copper levels in marinas have been documented to some extent in San Diego Bay, Mission Bay, Oceanside Harbor, and Dana Point Harbor, these regions will be excluded from the geographic scope of this study (Singhasemanon, 2005) (Schiff, 2006). Moreover, the Santa Ana Regional Water Quality Control Board will be initiating a monitoring study of metals in Newport Bay area marinas in the summer of 2006 (Candelaria, 2006). Therefore, our study will not include sites in the Newport Bay region.

Twenty-four marinas have been chosen as study marinas, including 16 salt water marinas, four brackish water marinas, and four fresh water marinas (listed below as Table 7).

Table 7: (Element 10) Sampling locations

Sampling Location	City	SiteID	Matrix
Folsom Lake Marina	Folsom	FL1-FL8	Fresh Water and Sediment
Tahoe Keys Marina	Lake Tahoe	TK1-TK8	Fresh Water and Sediment
Sacramento City Marina	Sacramento	SA1-SA8	Fresh Water and Sediment
Village West Marina	Stockton	VW1-VW8	Fresh Water and Sediment
Antioch Marina	Antioch	AM1-AM8	Brackish Water and Sediment
Benicia Marina	Benicia	BM1-BM8	Brackish Water and Sediment
Vallejo Municipal Marina	Vallejo	VM1-VM8	Brackish Water and Sediment
Pittsburg Marina	Pittsburg	PM1-PM8	Brackish Water and Sediment
Clipper Yacht Harbor	Sausalito	CY1-CY8	Salt Water and Sediment
San Francisco Marina	San Francisco	SF1-SF8	Salt Water and Sediment
South Beach Harbor	San Francisco	SH1-SH8	Salt Water and Sediment
City of Berkeley Marina	Berkeley	CB1-CB8	Salt Water and Sediment
Marina Bay Yacht Harbor	Richmond	MB1-MB8	Salt Water and Sediment
Loch Lomond Marina	San Rafael	LL1-LL8	Salt Water and Sediment
San Leandro Marina	San Leandro	SL1-SL8	Salt Water and Sediment
Ballena Isle Marina	Alameda	BI1-BI8	Salt Water and Sediment
Coyote Point Marina	San Mateo	CP1-CP8	Salt Water and Sediment
Santa Cruz Harbor	Santa Cruz	SC1-SC8	Salt Water and Sediment
Monterey Harbor	Monterey	MH1-MH8	Salt Water and Sediment
Santa Barbara Waterfront/Harbor	Santa Barbara	SB1-SB8	Salt Water and Sediment
Marina del Rey Back Basins	Marina del Rey	RB1-RB8	Salt Water and Sediment
Marina del Rey Front Basins	Marina del Rey	RF1-RF8	Salt Water and Sediment
Alamitos Bay Marina	Long Beach	AB1-AB8	Salt Water and Sediment
Downtown/ Shoreline Marina	Long Beach	DS1-DS8	Salt Water and Sediment

AFPs are usually not a necessity for boats in fresh water areas because hull fouling is not a major operational concern and because these boats may spend a significant amount of time out of the water when they are not being used. AFP use for boats maintained in brackish water areas tend to be somewhat higher than freshwater areas since these boat do occasionally have to deal with salt water fouling. However, the highest level of fouling by far occurs on boats that regularly operate in salt water regions. In general, these boats also spend more time in the water, therefore experiencing higher fouling pressures. As such, AFPs are widely employed for these vessels.

Since the highest amount of AFP use occurs in salt water areas, there will be more of an emphasis on salt water marinas in this study. The distribution of marina types will be 16 salt water marinas, four brackish water marinas, and four fresh water marinas. This distribution may change once sampling logistics and other factors are considered. If necessary, sites may have to be substituted.

Reference sites will also be determined for each marina to help establish that marinas are likely the source of AFP pollutants. Each marina operator, manager, dock master, or harbor master (these individuals will be collectively referred to, from here fourth, as marina managers) will be consulted to ascertain viable locations near each marina area in which analyzable reference samples can be collected. Reference sites will be identified and selected using the following criteria (in descending order of importance):

1) The site should be located outside the influence of marina activities and potential sources of AFPs, but adjacent to the marina area and within the same body of water.
2) The site should be sufficiently isolated from potentially confounding inputs (e.g., boatyards, industrial discharges, and various historical contamination).
3) Historical and current activities (e.g., dredging, construction) in the immediate area will not significantly interfere with the interpretation of results.
4) The site contains underlying sediment that can be collected and analyzed.
5) There is suitable and safe access to the site.

The exact location of reference sites will have to be determined on the day of sampling when site-specific conditions can be considered.

Sampling Vessels:

DPR staff will work to ensure that a boat and operator will be available for each marina site. DPR aims to initially work with marina managers of the selected marinas to secure an on-site option. Every marina has a unique facility administrator in the marina manager. These individuals almost always operate or have staff that operates at least one vessel on site. Moreover, they are very knowledgeable of each marina's layout, history, hydrology, and site-specific features.

DPR staff will develop a sampling schedule for all of the selected marinas prior to the start of sampling to ensure that necessary sampling vessels and operating staff will be available on specific dates.

Sampling Method and Frequency:

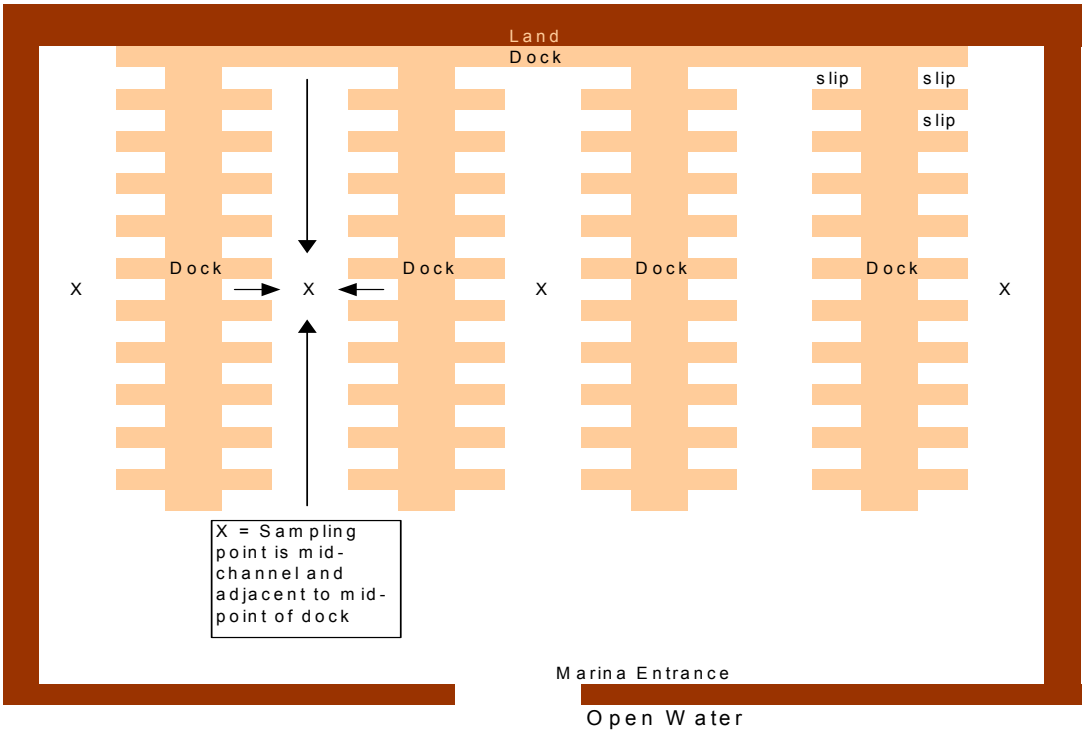
Marina Sites – Copper, Zinc, BLM-Associated Parameters

Water and sediment samples will be taken by boat from four points within each marina for copper, zinc, and BLM-associated parameters. Water samples will be collected once a month at each site over a three-month period for a total of three events. Sediment samples will only be collected in the third sampling event. In this event, water samples will be collected prior to sediment samples at each sampling point; this will minimize contamination of the water samples.

Past copper monitoring suggests that copper levels in both water and sediment tend to be highest near the area of moored vessels and lower toward the entrance of the marina (SDRWQCB, 2005) (Pap, 2004). Marina sampling sites will therefore be located in the vicinity of moored vessels to focus on areas of potentially high AFP indicator levels. To maintain site-to-site consistency, DPR staff will chose sampling sites that are located near the center of the

fairway (common term for the channels between the docks) and adjacent to the midway point of the dock/pier structure (see Figure 2.)

Figure 2. Schematic diagram of sampling points located within the marina structure.



Although each marina will have a unique layout of docks and slips, DPR staff will identify each marina's candidate fairways. Fairways that are adjacent to docks with less than 50% of their slips filled will not be considered viable for the initial sampling event (and therefore excluded from subsequent events). If there are more than four viable fairways, the fairways that will contain the final sampling sites will be randomly chosen.

To accurately revisit sampling sites during subsequent events, each sampling location will be initially identified using a global positioning system (GPS) unit to mark the exact latitudinal and longitudinal coordinates. If a site becomes inaccessible during subsequent events, attempts will be made to collect samples within three days of the visit.

Marina Sites – Irgarol

Both water and sediment samples will be taken for Irgarol. Two water samples will be taken from 12 marinas in the first sampling event and the third sampling event. Holding times constraints and shipping schedules will dictate which marinas the Irgarol samples will come from. Once these marinas are determined, these two sites will be randomly selected from the four sampling sites associated with copper, zinc, and BLM-associated parameters. There will be a maximum of 48 water samples taken for Irgarol. There will be no Irgarol samples taken

during the second sampling event.

One sediment sample will also be taken from each of the same 12 marinas during the third sampling event for a total of 12 Irgarol sediment samples. The sediment sampling sites will be randomly chosen from one of the two Irgarol water sampling sites.

Marina Sites – Toxicity and TIEs

Water samples will be taken for toxicity. Four samples will be taken from 12 marinas in the second sampling event (same 12 marinas as for the Irgarol samples). Toxicity samples will be taken from the four sampling sites associated with copper, zinc, and BLM-associated parameters. There will be a maximum of 48 toxicity samples taken for toxicity. There will be no toxicity samples taken during the first or third sampling event.

A maximum of four TIE samples will be chosen from toxicity samples that exhibit greater than 50% abnormal result. The toxicity testing laboratory will determine which toxicity samples are the best candidates for the TIEs. If possible, the four TIE samples will come from four different marinas.

There will be no sediment samples taken for toxicity or TIE analysis.

Reference Sites - Copper, Zinc, and BLM-Associated Parameters

Water and sediment samples will be taken by boat from four points outside of the marina area. Water samples will be collected once a month at each site over a three-month period for a total of three sampling events. Sediment samples will only be collected in the third sampling event. In this event, water samples will be collected prior to sediment samples at each sampling point; this will minimize contamination of the water samples.

The determination of viable reference sites will have to be done on a site specific basis using the criteria listed under the Site Selection Criteria section.

Reference Sites - Irgarol, Toxicity, and TIEs

There will be no samples taken at reference sites for Irgarol, toxicity, and TIE analysis.

In addition to the previously listed target parameters, the following *in-situ* field measurements will be collected using a variety of water quality meters:

- Specific conductance (EC)
- pH
- Temperature
- Depth
- Turbidity

Among them, specific conductance (EC), depth and turbidity, as well as grain size parameter in the BLM-associated parameters are for informational purpose only; other parameters and analysis for copper, zinc, Irgarol and toxicity/TIEs will be critical.

A summary of sampling locations, number of samples and frequency of sampling is shown below (Table 8).

Table 8. (Element 10) Number and frequency of samples.

	Type of sample	Analyte(s)	Matrix	No. of samples	Frequency of Sampling	Total Number of Samples per marina	Total No. of samples
12 Marinas	In marina	Cu, Zn, BLM	Water	4	1/month X 3	12	144
			Sediment	4	1/month X 1	4	48
	Reference	Cu, Zn, BLM	Water	4	1/month X 3	12	144
			Sediment	4	1/month X 1	4	48
12 Marinas	In marina	Cu, Zn, BLM	Water	4	1/month X 3	12	144
			Sediment	4	1/month X 1	4	48
		Irgarol	Water	2	1/month X 2	4	48
			Sediment	1	1/month X 1	1	12
		Toxicity/TIE*	Water	4	1/month X 1	4	48
	Reference	Cu, Zn, BLM	Water	4	1/month X 3	12	144
			Sediment	4	1/month X 1	4	48

***:** A maximum of four TIE samples will be chosen from 48 toxicity samples**.**

Sample delivery strategy

The samples for chemistry analysis will be transported to DPR warehouse at the end of each sampling trip by field crew for intermediate storage. The samples will then be delivered to UCD-ANRAL Lab in the morning of the next day. Some TSS samples may have to be shipped to UCD-ANRAL lab to stay within holding times.

The samples for Irgarol analysis and toxicity/TIE analysis will be collected and shipped en route through a United Parcel Service office that is close to the sampling locations. Samples will be shipped as soon as possible to stay within holding time constraints. Some toxicity/TIE samples (those taken from Southern California marinas) may be picked up by SCCWRP staff and delivered directly to the SCCWRP lab.

Natural variability

For salt water and brackish water sites, samples will be collected during slack tide or as close to it as possible in order to minimize the variability effect of tidal flows on sample integrity. The inclusion of multiple sites for each marina helps account for spatial variability. The inclusion of multiple sampling events helps account for temporal variability.

11. SAMPLING METHODS

Water Samples - Water samples will be taken from approximately 1 meter below the surface. Schiff et al. (2006) found a depth-related gradient for copper in marinas with the highest concentrations near the surface. Furthermore, to avoid AFP contamination from the sampling vessel itself, samples will be taken approximately 2 meters from the side of the boat.

The water sampling apparatus will consist of plastic tubing attached to a plastic pole. On one end, a peristaltic pump will draw water directly into the sample container. For metals, EPA certified, pre-cleaned 250 ml polyethylene plastic bottles will be used. For Irgarol, EPA-certified, pre-cleaned 1-L amber glass bottles will be used. For BLM-associated parameters 250 ml polyethylene bottles will be used. For toxicity samples, 1-L polyethylene containers will be used.

Samples to be analyzed for metals (dissolved copper and zinc), salts (magnesium, calcium, sodium, and potassium), and dissolved organic carbon will be filtered (in-line 0.45µm filter) and then acidified with Optima®, ultra-pure nitric acid to a pH level of < 2.0.

Samples to be analyzed for sulfate, chloride, and alkalinity will also be filtered but will not be acidified.

Samples to be analyzed for total copper will be unfiltered/acidified.

Samples to be analyzed for TSS, Irgarol, and toxicity/TIE will not be filtered or acidified.

For salt water and brackish water sites, samples will be collected during slack tide or as close to it as possible in order to minimize the possible effect of tidal flows on sample integrity.

Sediment Samples - Sediment will be collected using a Van Veen® grab sampler. The jaws and doors will be coated with Teflon® to achieve metal inertness. Each grab must satisfy the following criteria in order be an acceptable sample:

- Complete closure of the Van Veen sampler
- No evidence of sediment washout through the doors
- Minimum disturbance of the sediment surface

The overlying water in the sampler must first be drained by slightly opening the sampler. Care will be taken to minimize disturbance of the fine-grained top layer of sediment during this process. The top 2 cm of sediment will then be collected with a clean Teflon® coated scoop and placed into a 4 oz., EPA-certified, pre-cleaned polyethylene container. This will be repeated until sufficient sediment has been collected to fill the sample container.

The sample containers will be pre-cleaned using ultra-pure nitric acid. The samplers will be cleansed with DI water on the site before and after each sampling.

When problems occur, the field crew members will take corrective action and the incidence will be recorded on the field data sheet under “comment” column.

12. SAMPLE HANDLING AND CUSTODY

Since low concentrations of metals in the water samples are expected in this study, sample collection and handling will follow EPA Method 1669 *Sampling of Ambient Water for Trace Metals at EPA Water Quality Criteria Levels (EPA, 1996a)*.

After samples are taken, they will be kept in coolers with wet ice and be transferred to the analytical laboratories within the holding times specified in Table 8. Prior to analysis, samples will be kept refrigerated at 4°C until extraction or chemical analysis. Irgarol and toxicity samples will have to be shipped to their respective analytical laboratories due to short holding times requirements. These samples will be carefully packed and shipped via the United Parcel Service in coolers with wet or blue ice to their destinations.

To provide for proper tracking and handling of the samples, 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. The temperature upon receipt should be lower than 4°C.

A customized DPR chain-of-custody form will be completed and will accompany each sample. A example of the Chain-of-Custody form is attached.

Table 9. (Element 12) Sample handling and custody.

Analyte	Container Type/Size	Preservative	Maximum Holding Time
Dissolved Cu and Zn in water	250 ml polyethylene bottle, pre-cleaned	Filter at sample site using 0.45 micron in-line filter. Cool to 4°C, dark. Acidify within 48 hrs., for pH<2.	4 hrs. filtration and acidification, 6 months analysis
Total Cu in water	250 ml polyethylene bottle, pre-cleaned	Unfiltered. Cool to 4°C, dark. Acidify within 48 hrs., for pH<2.	4 hrs. acidification, 6 months analysis
Soluble salts (Mg, Ca, Na, K), dissolved organic carbon	250 ml polyethylene bottle	Filter at sample site using 0.45 micron in-line filter. Cool to 4°C, dark. Acidify within 48 hrs., for pH<2.	4 hrs. filtration and acidification, 6 months analysis
Total Suspended Solids	250 ml polyethylene bottle	Cool to 4°C, dark	7 days from collection; 5 days from receipt at laboratory
Irgarol	1-L amber glass bottle, pre-cleaned	Cool to 4°C, dark	48 hrs.
Alkalinity Salinity (Cl), Sulfate (SO_4)	250 mL polyethylene	Filter at sample site using 0.45 micron in-line filter. Cool to 4°C, dark.	14 days at 4°C, dark
Toxicity in water	1-L polyethylene containers	Cool to 4°C, dark	48 hrs. preferred, 72 hrs. max
Sediment TOC	250 mL polyethylene	Cool to 4°C, dark, up to 28 days	12 months

13. ANALYTICAL METHODS

See Table 9 for analytical methods.

Table 10. (Element 13) Laboratory analytical methods.

Analyte	Laboratory / Organization	Analytical Method/ SOP	Modification to Method	Reporting Limits
Dissolved Copper	UCD-ANRAL	EPA 220.2	None	0.5 – 2.0 µg/L
Dissolved Zinc	UCD-ANRAL	EPA 200.7	None	1.0 – 5.0 µg/L
Total Copper	UCD-ANRAL	220.2	None	0.5 – 2.0 µg/L
Dissolved Organic Carbon	DPR	EPA 415.1-.2	None	50 µg/L
Salinity (Cl)	UCD-ANRAL	EPA 300.0	None	2mg/L
Sulfate	UCD-ANRAL	EPA 300.0	None	2mg/L
Magnesium	UCD-ANRAL	EPA 200.7	None	2mg/L
Calcium	UCD-ANRAL	EPA 200.7	None	2mg/L
Sodium	UCD-ANRAL	EPA 273.1	None	2mg/L
Potassium	UCD-ANRAL	EPA 258.1	None	2mg/L
Alkalinity	UCD-ANRAL	EPA 310.1	None	>2mg/L
Total Copper(sediment)	UCD-ANRAL	EPA 3051 / EPA 200.7	None	10mg/kg (dry weight)
Total Zinc(sediment)	UCD-ANRAL	EPA 3051 / EPA 200.7	None	10mg/kg (dry weight)
Total Suspended Solids	UCD-ANRAL	EPA 160.2	None	4.0mg/L
Irgarol in water	NOAA-HML	Thomas, 2002*	None	0.1 ng/L
Irgarol in sediment	NOAA-HML	Sapozhnikova 2006**		
Toxicity in water	SCCWRP	EPA/600/R-95/136(salt and brackish) EPA 1002.0 (fresh)	None	NA
Toxicity Identification Evaluation	SCCWRP	EPA/600/R-96/054	None	NA
Grain Size	DPR	DPR SOPMETH004.00	None	2 µm smallest particle size
Total Organic Carbon	DPR	DPR SOP METH005.00	None	5 mg/kg (dry weight)

*: Thomas, K.V. *et al*, *The Science of the Total Environment* 293 (2002) 117-127.

**: Sapozhnikova, Y. 2006. *Analytical method for Irgarol in sediment.* Communication to Nan Singhasemanon (DPR).

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 pouring "blank water" (contaminant-free deionized water) into the sample bottle in the field during a sampling event.

Equipment blanks will also be used. 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

For analysis of Irgarol 1051, each batch of 9 samples contained 1 reagent blank, 1 replicate, and 1 matrix spike.

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)

Laboratory Control Sample (LCS) and duplicate will also be used to measure precision and accuracy of the laboratory analytical methods without the inferences from the matrix in each batch.

Standard Reference Materials (SRM) will also be used to measure precision and accuracy of the laboratory analytical method to quantify a known and certified amount of analyte.

For analysis of Irgarol, each batch of 9 samples will contain one reagent blank, one replicate, and one matrix spike.

15. INSTRUMENT/EQUIPMENT TESTING, INSPECTING, AND MAINTENANCE

15.1 Sampling Equipment

Sampling equipment receive regular maintenance based on a combination of manufacturer requirements and the actual amount of equipment use in the field. A second sampler will be taken into the field in case the first sampler fails for any reason.

15.2 Analytical Instruments

The laboratories maintain their equipments in accordance with their SOPs, which include those specified by the manufacturer and those specified by the method.

Problems with the analytical instruments during analysis will require repair, recalibration, and reanalysis of the sample.

16. INSTRUMENT/EQUPMENT CALIBRATION AND FREQUENCY

All laboratory equipments are calibrated based on manufacturer recommendations and accepted laboratory protocol. The laboratories maintain calibration practices as part of the method SOPs. The instruments 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 OF SUPPLIES AND CONSUMABLES

Supplies and consumables that may be used during field investigations include sample bottles, hoses, materials for decontamination activities, deionized water, and potable water. Project team members obtaining supplies and consumables are responsible for assuring that the materials obtained are intact and in good condition, are available in adequate supply, and are stored appropriately until use. Project team members will direct any questions or identification of any problems regarding supplies and consumables to the Project Leaders for resolution.

Gloves, sample containers, and any other consumable equipment used for sampling will be inspected by the sampling crew on receipt and will be rejected/returned if any obvious signs of contamination (torn packages, etc.) are observed.

Laboratory solvents, reagents, and other materials used in sample analysis by the Laboratories are demonstrated to be free from interferences or contamination by running method blanks initially and with each sample lot.

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 (EXISTING DATA)

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 objective of Data Management is to establish procedures to be used during the field investigations for documenting, tracking, and presenting investigative data. Efficient utilization and comprehensive consideration of available data requires that the data be properly organized for review. Organization of the data shall be planned prior to actual collection to assure the generation of identifiable and useable data. This section describes the operating practices to be followed by personnel during the collecting and reporting of data.

Data Recording

Observations made and measurements taken in the field are recorded on appropriate data sheets or in field memoranda. Upon completion of the field investigation, the data will be entered into a spreadsheet and tabulated for evaluation and presentation in the field investigations report. Copies of the selected original data records will be attached to the report as appendixes. Data used for analysis, presentation, and reporting on the project will be stored in an appropriate electronic format.

Each Laboratory Delivery Group will be submitted as a complete and single electronic data deliverable (EDD). It is expected that the laboratory will perform a comparison of electronic data with the hard copy report prior to submittal to ensure that the EDD and hard copy data are identical. The EDD should be submitted on a diskette or via email, with the disk label including the Laboratory Delivery Group, submittal date, laboratory name, and site description. If the EDD is resubmitted to DPR, the EDD will be labeled as "Revised".

Data Verification

Data verification is an integral part of the QA program and consists of reviewing and assessing the quality of data. Data verification provides assurance that the data are of acceptable quality as reported. For validity, the characteristics of importance are precision, accuracy, representativeness, comparability, and completeness. Data usability is the determination of whether or not a data set is sufficiently complete and of sufficient quality to support a decision or action, in terms of the specific data quality objectives (DQOs). Analytical data submitted by the laboratory in EDD form will be verified and, if necessary, exception reports will be produced. The data verification process includes:

- Evaluating against blank criteria—laboratory, field, and trip blanks;
- Evaluating against accuracy criteria—holding times, surrogates, laboratory control samples, and matrix spikes;
- Evaluating against precision criteria—matrix spikes/matrix spike duplicates, and field and laboratory duplicates;
- Confirming that data qualifiers are assigned appropriately; and
- Uploading field sample analytical data only to the central database.

Data Transformation

Transforming data by converting individual data point values into related values or symbols using conversion formulas or a system of replacement is not currently proposed for data evaluation for this project at this time. If data transformation is required at a later date, then conversion procedures will be described in detail in the associated work plan or technical report.

Data Transmittal

The integration of field data is completed by inputting the data from field forms into a spreadsheet format by data entry personnel. The Project Leader reviews the spreadsheet for completeness and accuracy by comparing the electronic spreadsheet to the original field data. Analytical laboratory data are provided in both a hard copy and in EDD format. The electronic data are provided in a specified format that will be uploaded to intermediate files and reviewed for completeness and accuracy by the Project Leader before use.

Data Tracking

The Project Leaders will be responsible for the day-to-day monitoring of data collected in the field. He/she assures that data are collected in the format specified in the task's work plan, assigns sample designations, and routes data to the project files. At least one copy of all project documents will be retained for project use during the investigation. Original documents will be maintained in the project file. The Project Leaders will also be responsible for the day-to-day monitoring of activities related to the generation and reporting of chemical data. He/she ensures that samples are analyzed according to the specified procedures; that data are verified; and that the data are properly coded, checked for accuracy, and entered into the data management system. He/she assures the data are then routed to the project files.

Data Storage and Retrieval

A project file will be established for the storage of original data, historical data, written documents, and data collected or generated during this work. The format for the file may include the following categories:

- Correspondence;
- Budgets;
- Contracts;
- Field Data;
- Figures and Maps;
- Permits;
- Laboratory Data and QA/QC Documents;
- Chains of Custody;
- Photographs;
- Reports; and
- Schedules.

All materials will be dated, carry the initials of the person responsible for the preparation of the document, and bear the project number. All documents relating to the project shall be

controlled to assure proper distribution, filing and retrieval. The document control shall also assure that revisions are properly recorded, distributed, and filed. The Project Leaders maintains overall responsibility for the project files and assures that appropriate documents are filed.

Data will be maintained as established in section 9 above. Copies of field data sheets, copies of chain of custody forms, original preliminary and final lab reports, and electronic media reports will be sent to the Project Manager. The field crew will retain original field logs. The contract laboratory(s) will retain copies of the preliminary and final data reports

Field data sheets are returned to the Project Leader after each sampling event, copied and filed. Sample results from the Laboratories are sent to the Project Leader. 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. After the final QA checks for errors are completed, the data are added to the final database. The production of data tables is generated from this database.

GROUP C: ASSESSMENT AND OVERSIGHT

20. ASSESSMENTS & RESPONSE ACTIONS

Measurement data must be consistently assessed and documented to determine whether project quality assurance objectives (QAOs) have been met, quantitatively assess data quality and identify potential limitations on data use. Assessment and compliance with quality control procedures will be undertaken during the data collection phase of the project:

- Performance assessment of the sampling procedures will be performed by the field sampling crews. Corrective action shall be carried out by the field sampling crew and reported to the quality assurance manager.

- The laboratory is responsible for following the procedures and operating the analytical systems within the statistical control limits. These procedures include proper instrument maintenance, calibration of the instruments, and the laboratory QC sample analyses at the required frequency (i.e., method blanks, laboratory control samples, etc.). Associated QC sample results are reported with all sample results so the project staff can evaluate the analytical process performance.

All project data must be reviewed as part of the data assessment.

Project data review established for this project includes the following steps:

- Initial review of analytical and field data for complete and accurate documentation, chain of custody procedures, analytical holding times compliance, and required frequency of field and laboratory QC samples;
- Evaluation of analytical and field blank results to identify random and systematic contamination;
- Comparison of all spike and duplicate results with project objectives for precision and accuracy;
- Assigning data qualifiers flags to the data as necessary to reflect limitations identified by the process; and
- Calculating completeness by analyte.

Corrective Actions

During the course of sample collection and analysis in this study, the laboratory supervisors and analysts, and laboratory QA officer and team members will make sure that all measurements and procedures are followed as specified in this QAPP, and measurements meet the prescribed and acceptance criteria. If a problem arises, prompt action to correct the immediate problem and identify its root causes is imperative. Any related systematic problems must also be identified.

Problems about analytical data quality that require corrective action are documented in the Laboratories' QA/QC Guidance. Problems about field data quality that may require corrective action are documented in the field data sheets.

Site Management

The project QA officer will observe field activities to ensure tasks are conducted according to the project specifications.

21. REPORTS TO MANAGEMENT

Reports to management may include project status reports, the results of surveillance evaluations, field and/or laboratory audits, and data quality assessments. These reports will be directed to the Project Leader who has ultimate responsibility for assuring that any corrective action response is completed, verified, and documented.

Final reports produced under an approved work plan will include a QA section with the following information:

- Identification of problems that required corrective action and resolution of the problems;
- Data quality assessment in terms of precision and accuracy and how they affect the usability of the analytical results;
- Limitations of any qualified results and a discussion of any rejected results; and
- Discussion of the field and laboratory QA/QC sample results.

Written communications between project team members, including reports to project management, will be maintained in the project files.

Interim and final reports will be issued by DPR according to the following table.

Table 11. (Element 21) QA management reports.

Type of Report	Frequency (daily, weekly, monthly, quarterly, annually, etc.)	Projected Delivery Dates(s)	Person(s) Responsible for Report Preparation	Report Recipients
Quarterly Progress Reports	Quarterly	4/20/06 and quarterly thereafter	Nan Singhasemanon	Melenee Emanuel
Draft Final Report	One time only	5/15/07	Nan Singhasemanon	Melenee Emanuel
Final Report	One time only	6/15/2007	Nan Singhasemanon	Melenee Emanuel

GROUP D: DATA VALIDATION AND USABILITY

22. DATA REVIEW, VERIFICATION, AND VALIDATION REQUIREMENTS

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.

DPR 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 DPR.

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

The usability of the verified data will be assessed by comparing the data to the verification criteria and DQOs. The usability assessment will provide an overall summary of data quality; defining acceptability or problems with accuracy, precision, sensitivity, and representativeness of the results with clear guidance to the data users of the uncertainties in the data that have been qualified as estimated. Because of cumulative effects of QC exceedances, some specific results may be determined to be unusable. Alternatively, based upon the EPA guidelines and best professional judgment, specific results may be determined to be usable for DQOs when they are not significantly outside the QC criteria.

The final activity of the data verification process is to assess whether the data meets the DQOs. The final results, as adjusted for the findings of any data verification/data evaluation, will be checked against the DQOs and an assessment will be made as to whether the data are of sufficient quality to support the DQOs. The decision as to data sufficiency may be affected by the overall precision, accuracy, and completeness of the data as demonstrated by the data validation process. If the data are sufficient to achieve project objectives, the Project Leader will release the data and work can proceed. If the data are insufficient, corrective action will be required.

25. LITERATURE CITED

Arnold, W.R., R.C. Santore, J. S. Cotsifas. 2005. "Predicting Copper Toxicity in Estuarine and Marine Waters Using the Biotic Ligand Model". *Marine Pollution Bulletin*. **50**:1634-1640.

California Department of Pesticide Regulation (DPR). 2005. Product/Label Database. *Summary Report Output: Indexed by Product, Chemical, and Site Data*. December.

Candelaria, L. Santa Ana Regional Water Quality Control Board (SARWQCB) Staff. *Personal Communications with Nan Singhasemanon (DPR)*. April 2006.

Dietrich, H. 2005. "Procedure for Determining Soil Particle Size Using the Hydrometer Method". California Department of Pesticide Regulation. Environmental Monitoring Branch. Standard Operating Procedures. 2005.

Gunasekara, A. 2006. "Instructions for Calibration and Use of Total Organic Carbon (CD-85A) Instrument". California Department of Pesticide Regulation. Environmental Monitoring Branch. Standard Operating Procedures. 2006.

Hall, L. and P. Gardinali, 2004. Ecological Risk Assessment for Irgarol 1051 and Its Major Metabolite in United States Surface Waters. Human and Ecological Risk Assessment. **10**(3): 525-542.

Los Angeles Regional Water Quality Control Board. (LARWQCB). 2005. *Total Maximum Daily Loads for Toxic Pollutants in Marina del Rey Harbor*. Draft Report. August.

Nichol, G. and Reyes, E., 2004. Electronic Template for SWAMP-Compatible Quality Assurance Project Plans. March 2004.

Pap, R. 2004. *San Francisco Bay Marina Water Quality Project.* Prepared for the San Francisco Bay Conservation and Development Commission. August.

San Diego Regional Water Quality Control Board. (SDRWQCB). 2005. *Total Maximum Daily Load for Dissolved Copper in Shelter Island Yacht Basin, San Diego Bay*. Resolution No. R9-2005-0019 Basin Plan Amendment and Technical Report. February.

Schiff, K., J. Brown, and D. Diehl. 2006. *Extent and Magnitude of Copper Contamination in Marinas of the San Diego region, California*. Technical Report. Southern California Coastal Water Research Project, Westminster, CA. March.

Singhasemanon, N. 2005. "California Copper Monitoring Studies with Potential Relevance to the Evaluation of Copper Antifouling Paint Pollution". California Department of Pesticide Regulation. Staff Draft. First Version. January.

Thomas, K.V. et al. 2002. "Antifouling Paint Booster Biocides in UK Coastal Waters; Inputs, Occurrence, and Environmental Fate", *Science of Total Environment.* **293:**117-127.

United States Environmental Protection Agency (EPA). 1983. *Methods for Chemical Analysis of Water and Wastes*. EPA/600/4-79/020. National Exposure Research Laboratory, Cincinnati,

OH.

United States Environmental Protection Agency (EPA). 1993. *Methods for the Determination of Inorganic Substances in Environmental Samples.* EPA/600/R-93/100. Environmental Monitoring Systems Laboratory. Cincinnati, OH..

United States Environmental Protection Agency (EPA). 1994. *Methods for the Determination of Metals in Environmental Samples, Supplement I.* EPA-600/R-94/111. U.S. Environmental Monitoring Systems Laboratory. Cincinnati, OH.

United States Environmental Protection Agency (EPA). 1995. *Short Term Methods for Estimating Chronic Toxicity of Effluents and Receiving Water to West Coast Marine and Estuarine Organisms*. EPA/600/R-96/054. National Exposure Research Laboratory. Cincinnati, OH.

United States Environmental Protection Agency (EPA). 1996a. *Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels*. EPA/821/R-96/011. Office of Water. Engineering and Services Division. Washington, D.C.

United States Environmental Protection Agency (EPA). 1996b. *Toxicity Identification Evaluation Procedures*. EPA/600/R-96/054. National Exposure Research Laboratory, Cincinnati, OH.

United States Environmental Protection Agency (EPA). 2002. *Total Maximum Daily Loads for Toxic Pollutants, San Diego Creek and Newport Bay, California*. EPA Region IX. June.

United States Environmental Protection Agency (EPA). 2003a. “Irgarol: Updated Environmental Risk Assessment for Antifoulant Use”. Memorandum from K. Montague and R. Petrie to D. Edwards through N. Cook. EPA Office of Pesticide Programs, Antimicrobial Division. July 15.

United States Environmental Protection Agency (EPA). 2003b. *Draft Update of Ambient Water Quality Criteria for Copper*. Report No. 822R03026.

United States Environmental Protection Agency (EPA). 2005. *Determination of Total Organic Carbon and Specific UV Absorbance at 254 nm in Source Water and Drinking Water.* EPA-600/R-05/055. National Exposure Research Laboratory. Cincinnati, OH.

Appendix A.
Standard Operation Procedure for Total Organic Carbon (DC-85A) Instrument

Department of Pesticide Regulation

SOP METH005.00

http://www.cdpr.ca.gov/docs/empm/pubs/sops/meth005_00.pdf

Appendix B.

Standard Operation Procedure for Grain Size determination

Department of Pesticide Regulation

SOP METH004.00

http://www.cdpr.ca.gov/docs/empm/pubs/sops/meth004.pdf

Appendix C

Standard Operation Procedure for Mussel Embryo Development Test

Southern California Coastal Water Research Project Toxicology Laboratory

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

Summary of test conditions
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.

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 CuCl2·2·H20 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 subsamples.

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 subsamples 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.

Appendix D.

Standard Operation Procedure for Conducting a Phase I Toxicity Indentification 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 ± 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.

Method for determining Irgarol in water and sediment

Liquid Chromatography

The chromatographic separation of antifouling compounds was achieved using a HPLC (Agilent Technologies, Inc., Palo Alto, CA) and a reverse phase column (Luna C18, 5 µm, 100Å, 50 x 2.0 mm ID Phenomenex) fitted with a guard column (C18 4x2.0 mm).The mobile phase was methanol/ammonium formate/formic acid buffer (pH 3.9) run over a gradient.

Mass spectrometry

Analytical detection and quantitation was performed utilizing ElectroSpray Ionization (ESI) tandem Mass Spectrometry (MS-MS-ESI) with an ABI 4000 triple quadrupole mass spectrometer (Applied Biosystems, MDS Sciex, Framingham, MA) coupled to an Agilent 1100 liquid chromatograph (Agilent Technologies, Inc., Palo Alto, CA). Operating conditions were: positive ionization - capillary voltage 3,000 V, fragmentor 56 V, nebulizer gas pressure 55 psi, source temperature 550°C. Ion transitions were monitored in Multiple Reaction Monitoring Mode (MRM). Two transitions were monitored for each compound: for Irgarol 1051 MRM1: 254→198 and MRM2: 254→83, for M1 MRM1:214→158 and MRM2: 214→85. Chlorotholuron dimethyl d_6 was used as an internal standard. Calibration curves were linear within 5-1,000.0 ng/ml with r^2 being greater than 0.99.

Analytical method for water samples

Upon receiving, the samples were spiked with an internal standard – Chlorotholuron dimethyl d_6, and extracted within 24 hours.

Briefly, 500 ml of sample were extracted using a 1 g C18 Solid Phase Extraction cartridge, conditioned with acetone, methanol, and HPLC water prior to extraction. The compounds of interests (Irgarol 1051, its major metabolite M1 (aka as GS26575), were eluted using methanol, and the resulting extract was evaporated under nitrogen.

Quality assurance/Quality control for water samples

Recovery studies were performed using seawater samples (salinity 30 ppt) spiked with standard solutions at concentrations of 10, 100 and 1,000 ng/L in five replicates. Antifouling compounds recoveries were 86-89%, 89-111% and 86-108% for 10, 100 and 1,000 ng/L spike levels, respectively. For Quality Assurance/Quality Control (QA/QC), each batch of 9 samples contained 1 reagent blank, 1 replicate, and 1 matrix spike. Method detection limits (MDLs) were: 0.1 ng/L for Irgarol and M1.

Analytical method for sediment samples

An analytical method has been developed for analysis of Irgarol 1051, its major metabolite M1, and diuron, and its metabolites: dichlorophenyl urea (DCPU), dichlorophenyl methylurea (DCPMU), chlorophenyl dimethylurea (CPMU). The method utilizes Accelerated Solvent Extraction (ASE; Dionex Inc., Sunnyvale, CA) using an ASE 200 Extraction System. Ten grams of sediment for each sample were mixed and grinded with anhydrous sodium sulfate, and extracted with methylene chloride using the ASE conditions described below:

Extraction Solvent: Methylene Chloride
Temperature: 120°C
Pressure: 2,000 psi
Heat Time: 5min
Static Time: 5min

Flush Volume: 50%
Purge Time: 60s
Static Cycles: 3

The resulting extract was evaporated under nitrogen gas, centrifuged in micro centrifuge filter tubes, and analyzed by LC-MS-MS as described above.

Quality assurance/Quality control for sediment samples

The method was developed using sediments spiked with 1, 10 and 100 ng/g of antifouling standards. The recoveries were: 84-112% with standard deviation no greater than 14%. Each batch of samples contained reagent blank, spike, matrix spike, matrix spike duplicate, and sample replicate.