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# Calleguas Creek Watershed Copper Water-Effects Ratio (WER) Study

Prepared by:  
Larry Walker Associates

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## GLOSSARY OF ACRONYMS

<b>AMEL</b>	Average Monthly Effluent Limit
<b>BLM</b>	Biotic Ligand Model
<b>CCC</b>	Criterion Continuous Concentration
<b>CDA</b>	Copper Development Association
<b>CEQA</b>	California Environmental Quality Act
<b>cfs</b>	cubic feet per second (measure of flow)
<b>CMC</b>	Criterion Maximum Concentration
<b>CSJ</b>	City of San Jose
<b>Cu</b>	Copper
<b>Cu'</b>	Complexed Copper
<b>Cu<sup>2+</sup></b>	Free Copper Ion
<b>CV</b>	Coefficient of Variance
<b>CWA</b>	Clean Water Act
<b>DFG</b>	Department of Fish and Game
<b>DIC</b>	Dissolved Inorganic Carbon
<b>DO</b>	Dissolved Oxygen
<b>DOC</b>	Dissolved Organic Carbon
<b>EC50</b>	50% Effect Concentration
<b>EO</b>	Executive Officer
<b>FACR</b>	Final Acute-Chronic Ratio
<b>FB</b>	Field Blank
<b>FDPE</b>	Fluorocarbon-lined High-Density Polyethylene
<b>GPS</b>	Global Positioning System
<b>HDPE</b>	High Density Polyethylene
<b>ICP-MS</b>	Inductively Coupled Plasma – Mass Spectrometer
<b>LB</b>	Laboratory Blank
<b>LC50</b>	50% Lethal Concentration
<b>LOEC</b>	Lowest Observable Effect Concentration
<b>LWA</b>	Larry Walker Associates
<b>MDEL</b>	Maximum Daily Effluent Limit
<b>mg/L</b>	milligrams per liter (aka: ppm)
<b>Mn</b>	Manganese
<b>MSD</b>	Minimum Significant Difference
<b>neat water</b>	Site or Lab water without salinity adjustment
<b>ng/L</b>	nanograms per liter (aka: ppt)
<b>Ni</b>	Nickel
<b>NOEC</b>	No Observable Effect Concentration
<b>NPDES</b>	National Pollutant Discharge Elimination System
<b>OBS</b>	Optical Backscatterance
<b>PB</b>	Procedure Blank
<b>PER</b>	Pacific EcoRisk Environmental Consulting and Testing
<b>POTW</b>	Publicly Owned Treatment Works

<b>ppb</b>	parts per billion
<b>ppm</b>	parts per million
<b>ppt</b>	parts per thousand (salinity)
<b>QA/QC</b>	Quality Assurance/Quality Control
<b>RPD</b>	Relative Percent Difference
<b>RWQCB</b>	Regional Water Quality Control Board (Los Angeles Region)
<b>SOP</b>	Standard Operating Procedures
<b>SSO</b>	Site-Specific Objective
<b>SWRCB</b>	State Water Resource Control Board
<b>TAC</b>	Technical Advisory Committee
<b>TMDL</b>	Total Maximum Daily Load
<b>TOC</b>	Total Organic Carbon
<b>TSS</b>	Total Suspended Solids
<b>ug/L</b>	micrograms per liter (aka: ppb, parts per billion)
<b>USEPA</b>	United States Environmental Protection Agency
<b>USGS</b>	United States Geological Survey
<b>WER</b>	Water-Effect Ratio
<b>WQO</b>	Water Quality Objective
<b>WWTP</b>	Wastewater Treatment Plant

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Appendix 4: Initial Water Quality Characteristics

Appendix 5: Initial versus Final Analysis of Copper Concentrations

Appendix 6: Toxicity Laboratory Acceptance Criteria

Appendix 7: Total Copper Data

# INTRODUCTION

## Background

In accordance with Section 303(d) of the Clean Water Act (CWA), States are required to list waters that will not comply with adopted water quality objectives after imposition of technology-based controls on point source discharges. Mugu Lagoon (Lagoon) and Lower Calleguas Creek (Creek) were listed on the 1998 303(d) list for California due to levels of copper which exceeded 1986 Basin Plan total recoverable metals objectives and/or United States Environmental Protection Agency (USEPA) national criteria. These exceedances were the basis for a concern that copper was impairing aquatic uses in the Lagoon and Creek by producing either acute or chronic toxicity in sensitive aquatic organisms.

Bioavailability and toxicity of copper are dependent on site-specific factors such as pH, hardness, suspended solids, dissolved oxygen (i.e., Redox state), dissolved carbon compounds, salinity, and other constituents. Because of the potential for site-specific conditions to vary from the conditions used to derive the national aquatic-life criterion, USEPA has provided guidance concerning three procedures that may be used to convert a national criterion into a site-specific criterion (USEPA, 1994). One of these, the Indicator Species procedure, is based on the assumption that characteristics of ambient water may influence the bioavailability and toxicity of a pollutant. Under this procedure, acute toxicity in site water and laboratory water is determined in concurrent toxicity tests using either resident species or acceptable sensitive non-resident species, which can be used as surrogates for the resident species. The ratio of the ambient to the laboratory water toxicity values, deemed a water-effect ratio (WER), can be used to convert a national concentration criterion for a pollutant to a site-specific concentration criterion (or site-specific objective (SSO) in California terminology).

The California Toxics Rule (CTR) defines the chronic criterion for dissolved copper as 3.1 ug/L for marine water and 9.0 ug/L (at hardness of 100 mg/L) for freshwater, *multiplied by a Water-Effect Ratio or WER* (40 CFR 131.38 (b) and (c)(4)(i) and (iii)). The default value for the WER is 1.0 unless a WER has been developed using methods as set forth in US EPA's WER guidance (US EPA, 1994<sup>1</sup>). EPA has, in effect, streamlined SSOs for trace metals given this CTR adopted wording.

## Study Purpose and Approach

The purpose of this study is to develop a WER for copper using methods set forth in the US EPA's guidance. The WER is being developed as part of a comprehensive approach to addressing metals impairments in the Calleguas Creek watershed. The WER study was designed to work in conjunction with the metals TMDL for the watershed to develop an effective implementation strategy for copper. The Work Plan and Sampling & Analysis Plan were developed during 2003 through a stakeholder process that included regulators, dischargers, researchers, and environmental advocates. In particular, the Work Plan was reviewed by Technical Advisory Committee member Russ Flegal of the University of California Santa Cruz, Technical Working Group member Sam Unger of the Los Angeles Regional Water Quality Control Board, and Lucie McGovern of the City of Camarillo. This approach is consistent with the WER guidance manual (USEPA, 1994) that recommends that a multi-disciplinary "design team" with site-specific knowledge be used. The guidance also recommends including the regulatory authority on the team from

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<sup>1</sup>US EPA, 1994. Interim Guidance on Determination and Use of Water-Effect Ratios, USEPA Office of Water, EPA-823-B-94-001, February 1994.

the beginning. Local RWQCB and EPA staff with knowledge of the Calleguas Creek Watershed have been active participants since the beginning.

The final Work Plan (“Calleguas Creek Watershed Metals TMDL Work Plan [2003]”) included in Appendix 1 summarizes the rationale for selecting the sampling sites, monitoring and analytical procedures, and QA/QC protocols.

The primary purpose of the study outlined in the Work Plan was to collect data to improve understanding of the aquatic toxicity of copper in the Lagoon and Creek. The study included (a) the collection of water column data to broaden the knowledge regarding spatial and temporal variability of ambient concentrations of copper and associated chemical parameters and (b) the collection of copper toxicity data for a sensitive saltwater species (*Mytilus edulis*) in the Lagoon and Creek as well as for a sensitive freshwater species (*Ceriodaphnia dubia*) in the Creek to allow calculation of WERs for these reaches. Both saltwater and freshwater species were studied in Lower Calleguas Creek water due to the tidal influence in this zone. Performing toxicity tests on both species allowed the most sensitive and conservative WERs to be developed. The study was designed to help provide a scientific basis for site-specific objectives, the copper TMDL, and future 303(d) lists.

This study was intended to:

- (1) provide technically sound analytical data (i.e., accurate, reproducible, etc.),
- (2) provide data which impartially characterizes chemical and toxicological conditions at various locations in the Lagoon,
- (3) provide data that will be useful in the evaluation of possible copper impairment in the water column of Mugu Lagoon and Lower Calleguas Creek, and
- (4) provide data that will be useful in the development of site-specific water quality objectives (WQO) for copper in the Lagoon and Creek, through the use of water-effect ratios.

Sampling sites were selected to provide representative spatial coverage of the Lagoon and Reach 2 of Calleguas Creek (Figure 1). The sampling schedule captured both wet and dry season conditions, with two sampling events conducted for dry weather, one event under wet conditions in the Lagoon, and two events under wet conditions in Calleguas Creek. Sample runs included four Lagoon sample sites and two Creek sample sites sampled each event, during outgoing tidal conditions.

The WER guidance recommends that data from one sampling event be analyzed prior to the next sampling event, with the goal of improving the sampling design as the study progresses. Following the first sampling event, the data was evaluated to help determine any change in direction. No changes were made in study design, as the original sites appeared to capture any variability in the Lagoon and Creek.

## **Related Analyses**

The primary emphasis of this study was on the development of WERs for copper and on characterizing ambient total and dissolved copper. Additional analyses for various conventional water quality parameters (total suspended solids (TSS), total organic carbon (TOC), dissolved organic carbon (DOC), salinity) were also conducted for each site during each of the events in the study. This information will be used to augment existing data, and to aid in the interpretation of toxicity test results.

## **Biotic Ligand Model**

Some constituents not included in previous monitoring efforts in the Watershed were added to this study to provide information useful to the national effort to develop a Biotic Ligand Model (BLM). The BLM was created to evaluate bioavailability and toxicity of metals that have been discharged into surface water. The model takes into consideration several water quality parameters, including hardness, DOC, chloride, pH, and alkalinity. The USEPA is currently reviewing the BLM as a potentially less resource intensive option to WER studies for the development of site-specific criteria. The Water Environment Research Foundation (WERF) is working closely with the USEPA in the development of this model. At this stage, the model has been developed and is being calibrated and beta-tested for copper and silver. Water quality constituents required as inputs into the model were collected as part of this study in the hopes of providing useful data to BLM researchers and to ensure the data set collected could be used in the BLM at a later date. This BLM work was funded and coordinated by the Copper Development Association (CDA) and results will be reported independently.

## **Technical Working Group & Technical Review Committee**

A Technical Working Group (TWG) was established to review documents and provide input on decisions pertaining to the metals TMDL work. The TWG members are listed below:

- Carolyn Greene - City of Thousand Oaks
- Damon Wing - Ventura Coastkeeper
- John Bejhan - City of Simi Valley
- Morgan Wehtje - Department of Fish and Game
- Rick Farris - US Fish & Wildlife Service
- Sally Coleman - Ventura County Watershed Protection District
- Sam Unger - Los Angeles Regional Water Quality Control Board
- Steve Granade - US Navy

As part of this project, a Technical Advisory Committee (TAC) was convened to provide an independent outside critique of the project design and results. A list of TAC members proposed for review of the technical documents is provided in Table 1.

**Table 1. Technical Advisory Committee Members**

<b>Area of Expertise</b>	<b>TAC Member</b>
Modeling	
Regulatory/TMDL Process/Standards	William Walker
Toxicity - Metals - Pesticides	Russ Flegel, UC Santa Cruz Ronald Tjeerderma, UC Davis
Habitat - Wetlands - Riparian	Eric Stein, SCCWRP Michael Josselyn, WRA
Bioaccumulation/Risk Assessment	David Sedlak, UC Berkeley
Agriculture - Standards - BMP implementation	Donald Suarez, USDA-ARS George E Brown Jr. Salinity Laboratory Stephen Grattan, UC Davis
Bacteria	Stanley Grant, UC Irvine
Treatment Technology Expertise	Michael Stenstrom, UCLA

## Acknowledgements

This project has been a broad, stakeholder based effort from its beginnings. The project was developed as part of the Calleguas Creek Watershed Management Plan that includes the following groups.

<b>General Purpose Agencies</b>	<b>Water/Wastewater Management Agencies</b>
City of Camarillo City of Moorpark City of Simi Valley City of Thousand Oaks County of Ventura Ventura County Flood Control District	Berylwood Mutual Water Company Calleguas Municipal Water District Camarillo Sanitary District Camrosa Water District Fox Canyon Groundwater Management Agency Pleasant Valley County Water District United Water Conservation District Ventura County Waterworks Districts: 1, 8, 19 Zone Mutual Water Company Ventura County Association of Water Agencies
<b>Other Property Owners/Business Organizations</b>	<b>Recreational and Open Space Entities</b>
Business Industry Association Naval Base Ventura County Ventura County Economic Development Association Ventura County Farm Bureau	California Department of Parks & Recreation Conejo Valley Park & Recreation District Pleasant Valley Park & Recreation District Rancho Simi Valley Recreation & Park District
<b>Agencies/Organizations</b>	<b>Federal and State Agencies</b>
California Coastal Conservancy California Department of Water Resources California Native Plant Society California Wildlife Conservation Board Caltrans Environmental Defense Center Natural Resources Conservation Service Santa Monica Mountains Conservancy Surfrider Foundation Ventura County Resource Conservation District	California Coastal Conservancy CA Department of Fish and Game Regional Water Quality Control Board- Los Angeles US Army Corps of Engineers US Environmental Protection Agency US Fish and Wildlife Service

RWQCB staff approved the Metals TMDL Work Plan and associated WER Sampling & Analysis Plan and are actively participating in work being conducted under the Work Plan.

# **SAMPLING PROCEDURES**

## **Environmental Setting**

Calleguas Creek and its tributaries are located in southeast Ventura County and a small portion of western Los Angeles County. Calleguas Creek drains an area of approximately 343 square miles from the Santa Susana Pass in the east to Mugu Lagoon in the southwest. The main surface water system drains from the mountains in the northeast part of the watershed toward the southwest where it flows through the Oxnard Plain before emptying into the Pacific Ocean through Mugu Lagoon. The watershed, which is elongated along an east-west axis, is about thirty miles long and fourteen miles wide. The Santa Susana Mountains, South Mountain, and Oak Ridge form the northern boundary of the watershed; the southern boundary is formed by the Simi Hills and Santa Monica Mountains.

Land uses in the Calleguas Creek watershed include agriculture, high and low density residential, commercial, industrial, open space, and a Naval Air Base located around Mugu Lagoon. The watershed includes the cities of Simi Valley, Moorpark, Thousand Oaks, and Camarillo. Most of the agriculture is located in the middle and lower watershed with the major urban areas (Thousand Oaks and Simi Valley) located in the upper watershed. The current land use in the watershed is approximately 26% agriculture, 24% urban, and 50% open space. Patches of high quality riparian habitat are present along the length of Calleguas Creek and its tributaries.

The Calleguas Creek Watershed is generally characterized by three major subwatersheds: Arroyo Simi/Las Posas in the northeast, Conejo Creek in the south, and Revolon Slough in the west. Additionally, the lower watershed including Mugu Lagoon is also drained by several minor agricultural drains in the Oxnard plain. The three major subwatersheds are described below in more detail.

### **Conejo Creek Subwatershed**

Conejo Creek and its tributaries (Arroyo Conejo and Arroyo Santa Rosa) drain the southern portion of the watershed. Flow in the southern portion of the watershed originates in the City of Thousand Oaks and flows through the east side of the City of Camarillo before joining Calleguas Creek upstream of the California State University Channel Islands. The subwatershed supports significant residential and agricultural land uses. The streams and channels of the Conejo Creek subwatershed are described below, in order from uppermost to lower.

#### *Calleguas Creek*

Calleguas Creek runs along the eastern side of Oxnard Plain to Mugu Lagoon. From the headwaters in the hills north of Camarillo to the confluence with the Arroyo Las Posas through to the confluence with Conejo Creek, Calleguas Creek is typically dry due to rapid infiltration and evaporation. During wet weather storm events, the stretch of Calleguas Creek provides a conduit for transporting storm flows from the upper CCW to the Pacific Ocean. The Camrosa WRP is located near California State University, Channel Islands. The Camrosa WRP only discharges to the creek during extreme storm events. Calleguas Creek is tidally influenced from Mugu Lagoon to approximately Potrero Road.

## **Revolon Slough Subwatershed**

Revolon Slough drains the agricultural land in the western portion of the watershed (Oxnard Plain). The slough does not pass through any urban areas, but does receive drainage from tributaries which drain urban areas. Revolon Slough starts as Beardsley Wash in the hills north of Camarillo. The wash is a rip-rapped channel for most of its length and combines with Revolon Slough at Central Avenue in Camarillo. The slough is concrete lined just upstream of Central Avenue and remains lined for approximately 4 miles to Wood Road. From there, the slough is soft bottomed with rip-rapped sides. The lower mile to mile and a half of the slough to above Las Posas Road appears to be tidally influenced by inflows from Mugu Lagoon. Revolon Slough flows into Mugu Lagoon in a channel that runs parallel to Calleguas Creek. The flows from Revolon Slough and Calleguas Creek only converge in the Lagoon. In addition to Revolon Slough, a number of agricultural drains (Oxnard Drain, Mugu Drain, and Duck Pond Drain) serve as conveyances for agricultural and industrial drainage water to the Calleguas Creek estuary and Mugu Lagoon.

## **Mugu Lagoon**

Mugu Lagoon, an estuary at the mouth of Calleguas Creek, supports a diverse wildlife population including migratory birds and endangered species. The Point Mugu Naval Air Weapons Station directly impacts Mugu Lagoon as do the substantial agricultural activities in the Oxnard Plain. The Lagoon consists of approximately 287 acres of open water, 128 acres of tidal flats, 40 acres of tidal creeks, 944 acres of tidal marsh and 77 acres of salt pan (California Resources Agency, 1997). The Lagoon is comprised of a central basin which receives the flow from Revolon Slough and Calleguas Creek, and two arms (eastern and western) that receive some drainage from agricultural and industrial drains. In addition, multiple drainage ditches drain into the Lagoon. Two of these ditches, Oxnard drainage ditches 2 and 3, discharge urban and agricultural runoff originating beyond the Naval Station's boundaries into the central and western portion of the Lagoon. The remaining ditches discharge urban and industrial runoff originating on the Station.

The salinity in the Lagoon is generally between 31 and 33 parts per thousand (ppt) (Granade, 2001). The central basin of the Lagoon has a maximum tidal range of approximately -1.1 to 7 feet (as compared to mean sea level) with smaller ranges in the eastern and western arms of the Lagoon. The western arm of the Lagoon receives less tidal volume because of a bridge culvert that restricts the flows in that area. The velocity of water traveling through the narrow mouth of the Lagoon is approximately 5-6 knots, which is a high velocity for a Lagoon (Grigorian, 2001). The mouth of the Lagoon never closes, apparently as a result of a large canyon present at the mouth of Calleguas Creek. The canyon prevents ocean sand from building up to a high enough level to close the mouth and likely accounts for the high velocities in the Lagoon (Grigorian, 2001).

## **Sampling Locations**

Sampling was conducted at four Mugu Lagoon (Reach 1) stations and two Lower Calleguas Creek (Reach 2) stations (Figure 1). Sites were selected with the intent of providing spatial coverage and representing different hydrodynamic segments of Mugu Lagoon and Lower Calleguas Creek. Mugu Lagoon is located within the Naval Air Weapons Station at Point Mugu, making access to some areas of the Lagoon for sample collection difficult and/or impossible. In addition, the Lagoon serves as the pupping and nesting grounds for harbor seals, clapper rails, snowy plovers and least terns. Access to areas of the Lagoon where pupping and nesting is occurring is limited from February to July, and in some areas this extends

into September. High flows in the Lagoon immediately following a storm event made sampling via boat unsafe and inaccessible during these times.

Site identification nomenclature utilized the following information:

Reach – Study – Site in that Reach

For example, for the first site sampled in Reach 1 (Mugu Lagoon) during the WER study, the name “1-WER-A” was used, with additional sites being “-B,” “-C,” and “-D.” In Lower Calleguas Creek (Reach 2) where two different species were tested, the following notations were added to distinguish between species:

M.e. = *Mytilus edulis*

C.d. = *Ceriodaphnia dubia*

Throughout the remainder of this report, where it is necessary to distinguish between species tested, the notations identified above will be added after the “Site in that Reach” letter. For instance, “2-WER-A-M.e.” identifies samples collected at Site A in Lower Calleguas Creek for *Mytilus edulis* toxicity testing during the WER study.



Figure 1. Map of Monitoring and Wastewater Treatment Plant Discharge Locations

## Sampling Period and Site Water Collection

### Sampling Period

USEPA guidance states that the selection of the number and timing of sampling events should take into account seasonal considerations and should result in at least three WERs determined with the primary test species (in this case, *Mytilus edulis* and *Ceriodaphnia dubia*) (USEPA 1994). In accordance with this guidance, four separate sets of surface-water measurements were included to assess ambient conditions and to calculate saltwater and freshwater copper WERs. The selected frequency also represented a balancing of temporal coverage with the need for extensive spatial coverage to address representative areas of the Lagoon and Creek.

Sampling events were conducted from August 2003 to March 2004, with an additional wet weather event in April 2006 (Table 2). The goal of the sampling and toxicity testing was to produce three successful<sup>2</sup> WER events (two from the dry season and one from the wet season). Based on the results of the wet season event in Lower Calleguas Creek, another wet event was added in April 2006 to further characterize copper toxicity during the wet season. The rationale behind the sampling period was to capture the dominant hydrological conditions observed during the year. The actual selection of sample dates was determined by a balancing of multiple criteria including favorable tidal conditions, coordination with analytical labs, availability of test organisms, and sampling boat and crew availability. Sampling conditions for each of the events included the following:

- Dry weather during late summer (August), low flows and calm conditions.
- Dry weather during winter (January), medium flows and somewhat calm conditions.
- Wet weather during winter (March), increased flows and turbid conditions following a storm event.
- Wet weather during winter (April), increased flows and turbid conditions during a storm event, Lower Calleguas Creek sites only.

**Table 2. Sampling Locations and Dates**

Station Code	Site Location	Event 1	Event 2	Event 3	Event 4*
1-WER-A	Mugu Lagoon at 11 <sup>th</sup> Street Bridge				
1-WER-B	Central Mugu Lagoon	8/26/03			---
1-WER-C	Mugu Lagoon at Mouth		1/27/04	3/1/04	
1-WER-D	Mugu Lagoon at Laguna Road Bridge				
2-WER-A	Calleguas Creek at Potrero Road	8/27/03			4/15/06
2-WER-B	Calleguas Creek above Mugu Lagoon				

\*A fourth event was sampled for only Lower Calleguas Creek to further characterize copper toxicity in the Creek during wet weather.

<sup>2</sup> Samples were obtained and preponderance of test results were acceptable per QA/QC measures.

## Site Water Collection

All samples were collected as grab samples from bridges, a boat or by wading into the sampling stream. In general, samples were taken at approximately mid-stream, mid-depth at the location of greatest flow (where feasible). Clean, powder-free nitrile gloves were worn for collection of all samples.

Upon arrival at the sampling stations, weather conditions, time, and station depth were recorded onto field logs. Using 'clean hands' techniques, samples were collected by direct submersion or using a peristaltic pump with appropriately cleaned tubing. Approximately 500 mL were collected into the cubitainer, the cubitainer was then capped and shaken to pre-rinse (repeated 3 times). The cubitainer was then filled with site water, sealed, and placed on ice.

Clean techniques (EPA Method 1669<sup>3</sup>) were used throughout all phases of the sampling and laboratory analytical work, including equipment preparation, water collection, sample handling and storage, and testing. Site water was collected in 5-gallon containers. All containers were acid-rinsed, with the exception of the scintillation vials used for the WER testing. The scintillation vials were rinsed with ultra pure water rather than acid due to associated toxicity of acid residue. Mugu Lagoon site water was collected at slack high tide to minimize TSS and DOC. In Lower Calleguas Creek, samples were collected to minimize tidal influences. After sampling, site water was placed in ice chests, on wet ice, until reaching the appropriate laboratories.

Upon arrival at the laboratory, water quality of the raw water was measured. Measurements included temperature, pH, total organic carbon (TOC), dissolved organic carbon (DOC), total suspended solids (TSS), total and dissolved copper, alkalinity, hardness, and salinity {see Appendix 4}. Samples were stored at  $4 \pm 2^\circ\text{C}$ . Site water samples were used in the toxicity tests within 24-36 hours of collection.

Routine water quality characteristics (temperature, pH, dissolved oxygen (DO) and salinity) for each event were measured in the field. Clean sampling techniques were used for all fieldwork (USEPA, 1995a). All tubing and sample containers used for the collection of ambient water samples were cleaned following USEPA guidelines (i.e., Alconox<sup>®</sup>, organic solvent, acid and de-ionized water). Methanol was used as the organic solvent, and its use was followed by a minimum of four DI rinses. Methanol was used on field sampling tubing and containers, and, basically, all laboratory glassware and plastic-ware.

## LABORATORY PROCEDURES

### Site Water Preparation and Salinity Adjustment

Previous work has indicated that a salinity of below 25 parts per thousand (ppt) adversely affects the saltwater test species, *Mytilus edulis*. As a result, a toxicity test salinity of  $30 \pm 2$  ppt was chosen. Site waters with a salinity  $<28$  ppt were salinity adjusted to the selected range by adding GP-2 salts (a synthetic sea salt). Test solutions were mixed on a mechanical stir-plate (using a Teflon stir-bar) until the GP-2 salts were dissolved. The target salinity was confirmed by measuring an aliquot of water with a conductivity meter.

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<sup>3</sup> USEPA. April 1995. Method 1669: Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels. EPA 821-R-95-034.

Although the Lower Calleguas Creek has relatively low salinity, the saltwater CTR criteria apply to this reach. As stated in the CTR, the more stringent of the freshwater and saltwater criteria apply if the salinity of the reach is between 1 and 10 ppt more than 5% of the time. Because the Lower Calleguas Creek is tidally influenced and the salinity is between 1 and 10 ppt more than 5% of the time, the more stringent saltwater copper criteria apply. Therefore *Mytilus edulis* testing was conducted on this reach and the samples adjusted to the salinity necessary to test this species.

### Synthetic Sea Salt Preparation

Synthetic sea salts were prepared as described in ASTM E-724-98: *Standard Guide for Conducting Static Acute Toxicity Tests Starting with Embryos of Four Species of Saltwater Bivalve Molluscs*. Reagent grade chemicals were combined in a one-gallon plastic container in the order provided in Table 3. The amount of salt prepared for each event varied by need. After the addition of each chemical, the container was shaken vigorously. Fresh synthetic seawater salts were prepared for each testing event.

**Table 3. Synthetic Seawater Salt Preparation**

Chemical	Amount (mg)	Amount (mg/L)
NaF	3	0.79
SrCl <sub>2</sub> *6H <sub>2</sub> O	20	5.28
NaSiO <sub>3</sub> *9H <sub>2</sub> O <sup>1</sup>	39.4	10.41
H <sub>3</sub> BO <sub>3</sub>	30	7.93
KBr	100	26.42
NaHCO <sub>3</sub>	200	52.84
KCl	700	184.94
CaCl <sub>2</sub> *2H <sub>2</sub> O	1470	388.38
Na <sub>2</sub> SO <sub>4</sub>	4000	1057
NaCl	23500	6209
MgCl <sub>2</sub> *6H <sub>2</sub> O	10780	2848
Total	40842.4	10791

<sup>1</sup>Substitution in place of Na<sub>2</sub>SiO<sub>3</sub>\*H<sub>2</sub>O (20 mg)

### Laboratory Dilution Water Preparation and Salinity Adjustment

Dilution water used in the laboratory water and reference toxicant tests for the saltwater tests was 1 µm sand-filtered natural seawater obtained from the Granite Canyon Marine Laboratory in Carmel, California. Seawater was collected into an appropriately cleaned and labeled 5-gallon FDPE container from a continuously running seawater source. After collection and temporary storage of the samples on wet ice in ice chests, the water was transported overnight to the Pacific EcoRisk (PER) laboratory. Upon receipt at PER, the laboratory water was logged in and placed in cold storage at 4°C ± 2°C until testing was initiated. Prior to the preparation of test solutions, an aliquot of lab water was filtered (0.45 µm) and adjusted (with reverse osmosis, 18.1 MΩ de-ionized water) to the test salinity of 30 ± 2 ppt.

The quality of seawater obtained from Granite Canyon Marine Laboratory met all laboratory standards. Granite Canyon seawater has been used since 1984 by the California Marine Bioassay Project to develop

sensitive methods for testing discharges into California marine waters (USEPA, 1995b). These methods include the development of tests for abalone (*Haliotis rufescens*), topsmelt (*Atherinops affinis*), giant kelp (*Macrocystis pyrifera*) and mysids (*Holmesimysis costata*).

Dilution water used in the laboratory water and reference toxicant tests for the freshwater tests consisted of EPA synthetic freshwater at a hardness of 220 mg/L, prepared just prior to test initiation. This hardness was selected as a conservative estimate of Lower Calleguas Creek ambient hardness, which ranged from 371 – 485 mg/L during the freshwater toxicity testing events.

### Copper Spiking and Test Solution Preparation

To bracket the expected EC50 value and obtain partial effects results for *Mytilus edulis* and *Ceriodaphnia dubia* development, nominal test copper concentrations were selected. Table 4 and Table 5 provide nominal (i.e., calculated) test copper concentrations used in this study. Each toxicity test had between seven and ten concentrations of copper. Test concentrations were prepared by spiking one-liter aliquots of the laboratory and site waters with a certified commercial copper nitrate standard (obtained from Inorganic Ventures of Lakewood, New Jersey). A two-liter volume of test solution was prepared for solutions used as “duplicates”. Prior to analysis, test solutions were allowed to sit for approximately three hours. This allowed copper partitioning to reach equilibrium with site water constituents and is consistent with WER guidance.

**Table 4. Nominal total copper additions to site water and lab water for *Mytilus edulis* tests**

Site	Nominal Test Concentrations (Total Cu ug/L)
Mugu Lagoon Sites	100, 70, 49, 34, 24, 17, 12, 8, 6, 0
Lab Water	34, 24, 17, 12, 8, 6, 4, 0
2-WER-A-M.e.	500, 350, 245, 172, 120, 84, 59, 41, 29 and 0
2-WER-B-M.e.	1000, 700, 490, 343, 240, 168, 118, 82, 58, 40 and 0
Lab Water2	100, 70, 49, 34, 24, 17, 12, 8, 6, 4, and 0

**Table 5. Nominal total copper additions to site waters and lab water for *Ceriodaphnia* tests.**

Site	Nominal Test Concentrations (Total Cu ug/L)
2-WER-A-C.d.	500, 350, 245, 172, 120, 84, 59, 41, 29 and 0
2-WER-B-C.d.	1000, 700, 490, 343, 240, 168, 118, 82, 58, 40 and 0
Lab Water1	100, 50, 35, 24, 17, 12, 8, 6, 4, and 0
Lab Water2	100, 70, 49, 34, 24, 17, 12, 8, 6, 4, and 0

## Toxicity Testing Procedure

### Saltwater

*Mytilus edulis* is the ideal organism for use in saltwater WER studies with copper. When deriving a site-specific criterion, it is critical to use a test species that is sensitive at Criterion Continuous Concentrations (CCC) or Criterion Maximum Concentrations (CMC). The concentrations that affected *Mytilus edulis* approximate the criteria concentrations. *Mytilus edulis* is the most appropriate species to use both as a surrogate for brackish water species and to set a site-specific criterion for copper for a number of important reasons:

- The CTR saltwater criterion for copper is determined exclusively by *Mytilus edulis*. Since it is used exclusively to set the current national criterion, it is appropriate to use it exclusively to set a site-specific criterion for the Lagoon and Creek.
- It is the most sensitive species in the national saltwater database. It therefore is not only a good surrogate for invertebrate species (which tend to be more sensitive to copper than vertebrates) and not only a good surrogate for mollusks (a phylum sensitive to copper – the 3<sup>rd</sup>, 4<sup>th</sup>, and 6<sup>th</sup> most sensitive species in the national copper database are mollusks), but it is a good surrogate for any sensitive saltwater animal (at any salinity above ~ 2 ppt).
- The most sensitive freshwater species to copper are daphnids (water fleas). In soft water, where copper is more bioavailable, they are about as sensitive as *Mytilus edulis* (genus mean acute value (GMAV) of 14.48 ug/L for the genus *Daphnia*, 9.92 ug/L for *Ceriodaphnia* and 9.625 ug/L for *Mytilus edulis*).

The *Mytilus edulis* toxicity test used for this study followed the guidelines established by the USEPA manual (USEPA, 1995b). A summary of test conditions and acceptability criteria used in *Mytilus edulis* toxicity testing is provided in Appendix 6.

The adult, reproductive mussels were obtained from a commercial supplier (Carlsbad Aquafarms, Carlsbad, CA). Upon receipt and prior to spawning, the adult bivalves were stored in filtered seawater at a temperature of 15°C ± 1°C. Bivalve embryos were generated from gravid *Mytilus edulis*. To induce spawning, the gravid adults were placed into clean Bodega Bay seawater (0.45 µm-filtered) at 20°C. This increase in temperature induced the bivalves to release sperm and eggs. When an individual bivalve was observed releasing sperm or eggs, it was transferred to a separate container for isolation and collection of gametes. To evaluate viability and quality, gametes were examined microscopically. The highest quality gametes were then used to prepare freshly-fertilized embryos by mixing a solution of sperm (at the appropriate concentration) to an aliquot of the best quality eggs. The resulting embryos were examined approximately one hour after fertilization to ensure viability.

Toxicity testing required the use of five replicates at each treatment level. Each replicate consisted of a 20-mL glass scintillation vial containing 10 mL of appropriate test solution. To initiate the test, approximately 150 to 300 embryos at or beyond the two-celled stage were inoculated into each test scintillation vial. Initial embryo density numbers were not used to calculate endpoints but to verify that the controls were behaving

normally (i.e., adequate survival). Additional replicates were established to determine initial embryo density, successful embryo development (i.e., to allow monitoring of the test conditions without affecting actual test replicates) and final water quality characteristics. Water quality vials contained 20 mL of test solution at the same embryo density as the test vials. Test and observation/monitoring vials were then placed into a temperature-controlled water bath at  $15^{\circ}\text{C} \pm 1^{\circ}\text{C}$  under a 16L: 8D photoperiod.

After 48 hrs, the "observation" vials were examined to ensure that  $\geq 90\%$  of the surviving embryos achieved normal development to the "D-hinge" stage. If normal embryo development was confirmed, the test was terminated by adding 0.5 mL of 5% glutaraldehyde. At test termination, the water quality vials at each treatment level were composited and analyzed for salinity, D.O., and pH. Each preserved test vial was subsequently examined microscopically to determine the percent of embryos exhibiting normal development.

To determine any developmental impairment or toxicity, the percent normal development results (for each treatment level) were compared to the control treatment results. Determinations of the No Observable Effect Concentration (NOEC), Lowest Observable Effect Concentration (LOEC) and key Effect Concentration (EC) point estimates were made using the CETIS<sup>®</sup> statistical package (Version 1.023, TidePool Scientific, McKinleyville, CA). EC50 values were calculated using either the Maximum Likelihood Probit or Trimmed Spearman-Kärber Method. After an initial statistical evaluation using nominal copper concentrations was conducted, specific copper concentrations test treatments were selected and measured for total and dissolved copper. Test response data were reanalyzed to determine EC50 point estimates based on measured copper concentrations.

## **Freshwater**

The acute survival test with *Ceriodaphnia dubia* was performed only on the two water samples for which the ambient salinity was below a threshold value of  $2,000 + 500 \mu\text{S/cm}$  conductivity (Lower Calleguas Creek stations).

The range-finding tests for *Ceriodaphnia* consisted of acute (48-hr) exposures to test solutions that were prepared by spiking the site waters and "Lab" water with copper from a commercial  $\text{CuNO}_3$  standard at concentrations of 10, 50, 100, 200, 500, and 1000  $\mu\text{g/L}$  Cu. "New" water quality characteristics (pH, D.O., and conductivity) were measured for each test solution prior to use in these tests.

There were 2 replicates for each test treatment, each replicate consisting of 60-mL of test solution in a 100-mL HDPE beaker; a third "water quality" replicate was similarly established for measurement of test solution water quality characteristics. Neonate *Ceriodaphnia* (<24 hrs old), from in-house laboratory cultures, were used to start these acute tests, which were initiated by allocating 10 *Ceriodaphnia* into each of the replicate cups. The cups containing the test treatments were placed in a temperature-controlled water bath so as to maintain the water temperature in each replicate cup at  $20^{\circ}\text{C}$ , under fluorescent lighting on a 16L:8D photoperiod. Routine water quality characteristics (pH and D.O.) of the test waters were measured each day and at the end of the test in the water quality replicate. After 48 hrs, the tests were terminated and the number of live neonates in each replicate cup was determined.

The survival data for the treatments for each site water were analyzed to determine key concentration-response endpoints (e.g., EC50 values); all statistical analyses were performed using the CETIS<sup>®</sup> statistical package. The results of these range-finding tests were then used to determine the nominal definitive test

copper concentrations based upon identification of copper concentrations that would be expected to bracket the potential range of *Ceriodaphnia dubia* acute survival EC50 values.

The control treatment for each of the two site waters consisted of an aliquot of the site water without any added copper. Nominal definitive test copper concentrations (Table 5) were selected based on the results of the copper range-finding tests performed on site waters and Lab waters so as to bracket the expected range of *Ceriodaphnia dubia* acute survival EC50 values. Test solutions at these concentrations were prepared by spiking 1.0-L aliquots of the site waters and Lab water with copper from a commercial CuNO<sub>3</sub> standard. Test solutions were allowed to sit for approximately 3 hours prior to test initiation to allow for copper partitioning to reach equilibrium with the site water constituents. Initial test water quality characteristics (pH, D.O., and salinity) were determined for each treatment test solution prior to use in the tests.

There were 4 replicates for each test treatment, each replicate consisting of 60-mL of test solution in a 100-mL HDPE beaker; an additional “water quality” replicate was similarly established for measurement of test solution water quality characteristics. These acute tests were initiated by allocating 5 neonate *Ceriodaphnia* (< 24 hrs old), from in-house laboratory cultures, into each of the replicate beakers. The test replicates were then placed in a foam board which floated in a temperature-controlled water bath so as to maintain the water temperature in each replicate cup at 20°C, under fluorescent lighting on a 16L:8D photoperiod.

Routine water quality characteristics (pH and DO) of each of the test treatment test solutions were measured in the water quality replicate each day and at the end of the test. After 48 hrs, the tests were terminated and the number of live neonates in each replicate cup was determined. The survival data for each test treatment were analyzed and compared to the appropriate Control treatment to determine key concentration-response endpoints (e.g., EC50 values); all statistical analyses were performed using the CETIS® statistical package.

## **Secondary and Supportive Testing**

In this study, a secondary freshwater and saltwater aquatic test species were not used to verify WER results obtained from *Mytilus edulis* and *Ceriodaphnia dubia*. It was determined to be unnecessary in large part because *Mytilus edulis* is the same (and most sensitive) species used to set the USEPA saltwater quality objective for copper. Likewise, the Streamlined Water-Effect Ratio Procedure for Copper recognizes that daphnids are quite sensitive to copper and have been the most useful organisms for freshwater WER studies (USEPA, 2001). Other species for which approved toxicity tests exist would be less sensitive to copper resulting in less applicable WERs. In addition, Cu WER studies using only one species have been completed and approved in other areas. Additionally, the Streamlined Water-Effect Ratio Procedure for Copper (USEPA, 2001) requires the testing of only one species and states “the 1994 Interim Procedure recommendation for a test with a second species has been dropped, because the additional test has not been found to have value.”

## **Reference Toxicant Testing**

To confirm that the *Mytilus edulis* embryos were responding to toxic stress in a typical fashion, a reference toxicant test was run concurrently with each set of site water (and Lab water) tests. The control water used for reference toxicant testing consisted of 0.45 µm filtered seawater from Bodega Bay at 30 ppt. Test

solutions were prepared by spiking the control water with copper (as  $\text{CuCl}_2$ ) at copper concentrations of 1.25, 2.5, 5, 10, 15 and 20  $\mu\text{g/L}$ .

To confirm that the *Ceriodaphnia dubia* embryos were responding to toxic stress in a typical fashion, a reference toxicant test was run concurrently with each set of site water (and Lab water) tests. The control water used for reference toxicant testing consisted of 80% Arrowhead and 20% Evian commercial spring waters. Test solutions were prepared by spiking the control water with copper (as  $\text{CuCl}_2$ ) at copper concentrations of 4, 8, 16, 32, and 64  $\mu\text{g/L}$ . Test results were used to determine EC50 endpoints to compare to the ongoing laboratory reference toxicant database to ensure that test result responses were consistent with previous test results. Statistical analyses were performed using the CETIS® statistical package.

### **Collection of Site water and Test Solutions**

Prior to analysis, the following samples were collected for chemical analyses: samples of each test solution, “neat” (i.e., without salinity adjustment) ambient site waters and lab water. Samples undergoing copper analyses were collected by directly pouring an aliquot (800 mL to 850 mL) of test solution into a uniquely-labeled and pre-cleaned one-liter HDPE bottle. Collected samples were sealed, placed on ice and shipped to CRG Marine Laboratory in Torrance, California for analysis.

Samples of the “neat” ambient site waters and lab water were similarly collected for analyses of dissolved manganese. Additional samples of salinity-adjusted ambient site and lab waters were collected for analyses of selected major ions and other parameters associated with the bioavailability and/or toxicity of copper. Collected samples were sealed, placed on ice and shipped to CRG Marine Laboratory for ancillary analysis.

### **Collection of Site Waters and Test Solutions for Chemical Analyses**

Immediately prior to test initiation and again at test termination, samples of each test solution were collected for copper analysis. These samples were collected into labeled, pre-cleaned 250-mL HDPE bottles (supplied by the analytical lab), which were sealed and placed within an insulated cooler. At this time, 1-L samples of each of the two site waters and of the “Lab” water were similarly collected for analysis of TSS, TOC, DOC, hardness, alkalinity and ammonia. These samples were immediately shipped via overnight delivery, on ice and under chain of custody, to the analytical laboratory (CRG Laboratories, Inc).

### **Measurement of Toxicity Test Solutions for Total and Dissolved Copper**

Once toxicity testing was completed, guidance found in the USEPA Memorandum *Interim Guidance on the Determination and Use of Water Effect Ratios for Metals* was used to select test solutions for chemical analysis (USEPA, 1994). Rather than measuring all test solutions, this guidance recommends measuring test solutions (for initial and final dissolved copper) that are used in determining the endpoint. This study followed the USEPA recommendation of measuring only values used in determining the endpoint but with one modification. WER calculations were based on EC50s calculated using initial copper concentrations as opposed to a time-weighted average of initial and final values. This is a more conservative approach given that a proportionately greater copper recovery is expected in site water than in lab water when measured at the test conclusion (San Jose, 1998). This is most likely due to the lab water experiencing a greater loss of copper to glassware, as opposed to the site water that has more constituents that can coat the glass and

prevent copper loss. The net effect of using the weighted average instead of the initial concentrations would have a disproportionately lower lab water EC50 that in turn would produce a disproportionately higher WER. Thus it is more conservative to analyze only the initial concentrations. Initial and final results were measured for one station's tests during the first sampling event for comparison.

## **Chemical Analysis of Water Samples and Test Solutions**

Spiked samples were delivered to the analytical laboratory in <24 hours. Samples were handled in this manner so that all of the filtration, preservation, and other sample handling after spiking could be conducted in the analytical laboratory's clean room facilities and using their equipment and distilled acid.

Upon arrival at CRG Marine Laboratory, all samples for copper analyses were split. One of the split aliquots was then filtered (0.45  $\mu$ m) and placed into a separate pre-cleaned HDPE bottle. Both aliquots (filtered and unfiltered) were preserved with ultra-pure HNO<sub>3</sub>. "Neat" (unadjusted salinity) waters, salinity-adjusted ambient site waters, lab water and selected test solutions were analyzed for copper (total and dissolved). Copper analyses were performed using USEPA Method 200.8.

Additional samples of salinity-adjusted ambient site and lab waters were analyzed for selected major ions and other parameters associated with the bioavailability and/or toxicity of copper and nickel. In addition, Pacific EcoRisk performed pH and salinity measurements of the test solutions. Most of these constituents were included to support a parallel study using these data as input into the Biotic Ligand Model (BLM).

**Table 6. Summary of Measured Parameters and Analytical Methods**

Analyte	Laboratory	Method	Holding Time <sup>a</sup>	MDL
Total Suspended Solids (TSS)	CRG	SM 2540-D	7 days	0.1 mg/L
Total Organic Carbon (TOC)	CRG	EPA 415.1	28 days	0.5 mg/L
Dissolved Organic Carbon (DOC)	CRG	EPA 415.1	24 hrs (filter), 28 days	0.5 mg/L
Total Dissolved Solids (TDS)	CRG	SM 2540-C	7 days	0.1 mg/L
Ammonia	CRG	SM 4500-NH3 F	28 days	0.01 mg/L
Chloride	CRG	SM 4500-Cl E	28 days	0.01 mg/L
Total Hardness as CaCO <sub>3</sub>	CRG	SM 2340-B	180 days	1 mg/L
Dissolved Alkalinity	CRG	EPA 310.2	14 days	1 mg/L
Dissolved Calcium (Ca)	CRG	EPA 1640/200.8	24 hrs (filter), 180 days	0.5 mg/L
Dissolved Magnesium (Mg)	CRG	EPA 1640/200.8	24 hrs (filter), 180 days	5 mg/L
Dissolved Sodium (Na)	CRG	EPA 1640/200.8	24 hrs (filter), 180 days	5 mg/L
Dissolved Potassium (K)	CRG	EPA 1640/200.8	24 hrs (filter), 180 days	5 mg/L
Dissolved Sulfate (SO <sub>4</sub> )	CRG	SM 4500-SO <sub>4</sub> F	24 hrs (filter), 28 days	0.01 mg/L
Total Recoverable Copper	CRG	EPA 1640/200.8	180 days	0.005/0.1 ug/L
Dissolved Copper	CRG	EPA 1640/200.8	48 hrs (filter), 180 days	0.005/0.1 ug/L

<sup>a</sup>Holding times are from date/time of sample collection.

## QUALITY ASSURANCE/QUALITY CONTROL

Quality control/quality assurance (QA/QC) practices were maintained during all facets of this study (sampling, testing, chemical analysis). This is evidenced by the high quality, low variability results obtained in compliance with the individual lab's QA/QC criteria. QA/QC data is provided in Appendix 3.

The laboratories used, CRG Marine Laboratory and Pacific Ecorisk are NELAP/NELAC certified, and in addition, they are also certified in California.

### Synthetic Sea Salts

Artificial sea salts were added to site water due to the fact that site waters were either collected from a freshwater environment (Creek) or from areas in the Lagoon that were significantly effected by freshwater inputs into the Lagoon. With respect to *Mytilus* test salinity requirements, this species can not be tested at salinities much lower than 30 ppt, thus requiring the use of artificial sea salts in test experimental design. During non-storm conditions (Events 1 and 2), the presence of freshwater inputs resulted in an increased site water dissolved organic carbon (DOC) concentration relative to areas of the Lagoon which were not significantly impacted by freshwater inputs. This pattern was similarly observed and exacerbated during storm conditions (Events 3 and 4). Studies have demonstrated that copper toxicity to *Mytilus* is inversely proportional to the concentration of DOC in the site water (Arnold et al., 2006). In fact, analytical chemistry data collected as part of this project were used to validate this model. A comparison of model-predicted copper EC50 values to the *Mytilus* copper EC50 values reported in this study were in agreement, indicating that the site water characteristics (i.e. DOC) were driving the decrease in copper toxicity to *Mytilus*. GP-2 sea salt is made from reagent grade salts and as a result does not contribute DOC to the site water matrix. This is substantiated by work performed by Arnold et al. (in press) that evaluated the potential for DOC contribution to test media by artificial sea salts; GP-2 sea salt was evaluated as part of this study. Results of that study indicated that GP-2 salts would not contribute DOC to test media.

Upstream inputs of DOC from Calleguas Creek (freshwater) appears to be driving the bioavailability of copper in the *Mytilus* toxicity tests and thus resulting in higher EC50 concentrations; toxicity testing with *Ceriodaphnia dubia* for this study also support this conclusion.

### Chemistry QA/QC

Extensive QA/QC requirements were designed into this study as part of the agreements with the contract laboratories that performed the physical, chemical, and biological analyses. This QA/QC analysis summarizes the acceptability of data generated during the sampling events. Holding times, analytical accuracy and precision, potential contamination, and conformance to data acceptability criteria were reviewed. Questionable raw data, results or missing data were identified and referred back to the originating lab for further investigation and qualification as appropriate.

Analytical chemistry accuracy and precision were monitored throughout the sampling events of this study using blanks, duplicates and spikes. Accuracy was assessed through percent recovery analysis of external reference standards and matrix-spike experiments. Precision of methods was determined through the calculation of relative percent difference (RPD) between matrix duplicate and field duplicate analyses. Control limits for precision and accuracy for these analyses were 20% maximum RPD, and 75% minimum

to 125% maximum recovery, respectively. The potential for contamination of environmental samples was investigated through the collection and analysis of lab, field, method, filtered, and procedure blanks to determine if contamination arose at the various stages of sampling and analysis.

Analytical results, toxicity test results, and QA/QC results from each sampling event were compared with QA/QC parameters. Limited QA/QC evaluation of hardness, Mg, TOC and TSS values was performed given that precision of these parameters was less critical to the interpretation of results.

## **Chemistry Data Quality**

### **Holding Times**

The USEPA analytical holding time guidelines require metals sample filtration and preservation within 48 hours of sampling and analysis within 6 months. These guidelines were consistently met. A few samples (alkalinity, TDS, TSS) were analyzed outside of the recommended holding times, so these samples were qualified (Appendix 2) as “estimated” values. These qualifications did not affect the WER calculations.

### **Precision**

Laboratory duplicate samples were analyzed and did not require any data qualifications.

### **Accuracy**

Percent recoveries of external reference standard measurements and matrix-spike duplicates were deemed acceptable when measured values were between 75% - 125% of the certified concentration values. One sample (TOC) was qualified as “high bias” because the recovery was greater than 125%. This indicates that the concentration of TOC reported for that sample may be higher than the actual sample concentration. This qualification did not affect the subsequent WER analyses and calculations.

## **Toxicity Test QA/QC**

Test acceptability requirements set forth in the USEPA Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms (USEPA, 1995b) and WER test guidance (USEPA, 1994) were used in the assessment of toxicity data.

### **Standard Test Conditions/Test Acceptability Criteria**

The toxicity testing of the ambient site waters with *Mytilus sp.* and *Ceriodaphnia dubia* incorporated standard QA procedures to ensure that the test results were valid, including the use of negative controls, positive controls, test replicates, and measurement of water quality during testing. These QA procedures are consistent with methods described in the USEPA guidelines. Water samples for the toxicity testing were shipped/stored at  $\leq 4^{\circ}\text{C}$  and were used within the 36 hour holding time period. All measurements of routine water quality characteristics were performed as described in the PER Standard Operating Procedures.

## Lab Water Quality and Holding Times

Table 7 provides sample collection dates and respective test initiations.

**Table 7. Copper WER Study Sample Collection and Test Initiation Dates**

Event	Location	Site Water Collection Date	Lab Water Collection Date	Test Initiation Date <sup>a</sup>
Event 1	Lagoon	8/26/03	8/26/03 <sup>S</sup>	8/27/03
	Creek	8/27/03	8/28/03 <sup>F</sup>	8/28/03
Event 2	Lagoon	1/27/04	1/26/04 <sup>S</sup>	1/28/04
	Creek	1/27/04	1/27/04 <sup>F</sup>	1/28/04
Event 3	Lagoon	3/01/04	2/26/04 <sup>S</sup>	3/02/04 <sup>b</sup>
	Creek	3/01/04	3/02/04 <sup>F</sup>	3/02/04 <sup>b</sup>
Event 4	Creek	4/15/06	4/14/06 <sup>S</sup>	4/15/06

a – Typically, tests were initiated on the day following site water collection.

b – Freshwater toxicity tests were conducted but the analytical laboratory mistakenly did not run the copper analyses.

S – Saltwater

F – Freshwater

## Sea Salt Controls

A “sea salt” control with the maximum salinity addition (salting from zero to  $30 \pm 2$  ppt) was used for each event to evaluate the affects of synthetic sea salts on embryo development. Salt controls were compared to lab control water to test statistical significance. Test results indicated that the addition of sea salts did not effect normal development. A summary of synthetic sea salt control results is provided in Table 8. In addition, initial test water quality characteristics (pH, D.O., and salinity) were determined for each treatment test solution prior to testing.

**Table 8. Summary Results for Synthetic Sea Salt Control**

Treatment	Mean Normal Development (%)
GP2 Control (Event 1)	91.3
GP2 Control (Event 2)	91.6
GP2 Control (Event 3)	98.0
GP2 Control (Event 4)	98.8

## Initial versus Final Copper Concentrations

The CCW Study followed the initial versus final copper test sample analysis protocols established during previous studies (San Francisco Bay, New York Harbor) given the fact that these protocols had been peer reviewed and approved by both the San Francisco Bay Technical Review Committees and EPA specialists.

The 1994 WER guidance conservatively recommends that both initial and final copper measurements be made on all concentrations used in determining the EC50 endpoint. Based on previous results, in this study, only initial total and dissolved copper measurements were made for selected concentrations and the control. Subsequent statistical analyses and EC50 calculations were based on measured copper concentrations at the beginning of the test, rather than on a time-weighted average of initial and final values.

In the San Jose Copper WER study, for example, in which both initial and final copper values were measured for many samples, data showed that laboratory water loses more copper (proportionally) than site water (Appendix 5). This difference in percent lost results in the calculation of a higher WER (i.e., laboratory water, the denominator in the equation, has a smaller value). Therefore, using the final copper concentration, or an average of initial and final, will result in a higher WER value for all samples. Using the initial copper concentration is thus a conservative approach to EC50 and WER calculations. A site-specific copper study conducted in the New York/New Jersey Harbor, analyzed both initial and final copper concentrations and then calculated the mean of the two values. The results of this study found that initial measurements of copper produced more conservative WERs because site water copper concentrations increased from initial to final, while lab water concentrations stayed virtually the same.

Initial and final copper concentrations were measured during one event of the CCW work to verify this conservative assumption. Site data (Table 9) showed a slight average increase in copper from initial to final. Lab water results showed that for spiked samples, there was a decrease in copper concentration in the final samples. Therefore, if there is an average increase in copper concentrations in site water and decrease in lab water concentrations, using the initial copper concentration will be a more conservative option as it will produce lower WER values.

**Table 9. Copper concentrations in site water and lab water (ug/L) before and after toxicity testing.**

Nominal Spike	Dissolved		Total	
	Initial	Final	Initial	Final
Site Water:				
0	2.13	3.54	2.92	3.36
172	125	132	141	143
245	171	180	191	196
350	210	231	263	264
Lab Water:				
0	1.11	1.92	1.34	1.59
17	14.4	14.3	14.8	12.8

Based on the San Francisco Bay and New York/New Jersey data and conclusions, along with initial and final concentrations measured in this study, it was determined that using only initial copper concentrations would be a reasonable and conservative approach for calculating the EC50s used in the WER calculations.

## Comparison to Standard Parameters

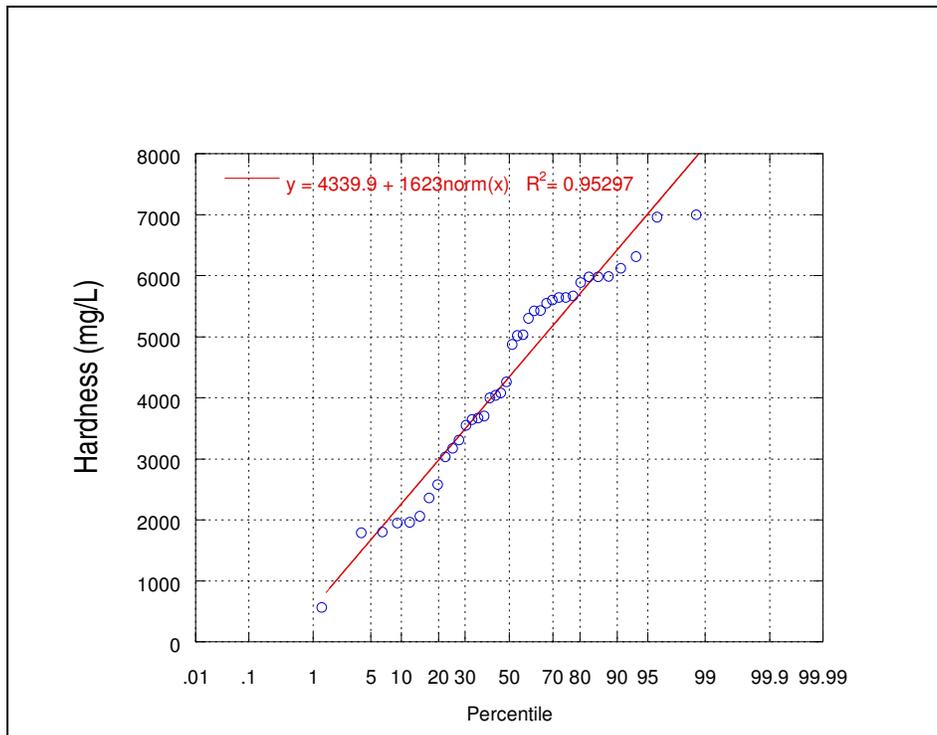
Per the 1994 WER Guidance, standard parameters collected in Mugu Lagoon and Lower Calleguas Creek during the four events were compared to long term average and median concentrations of these same parameters (Table 10, Table 11). These comparisons indicate that conventional parameters were within the expected range for the sites, based on historic data. Additionally, probability plots were created to illustrate the trends of historic hardness and TSS data (Figure 2, Figure 3, Figure 4, and Figure 5).

**Table 10. Comparison of Event Hardness to Average Hardness (mg/L)**

	Event 1	Event 2	Event 3	Event 4
1-WER-A	6120	3550	---	---
1-WER-B	5990	3170	---	---
1-WER-C	6310	5550	1800	---
1-WER-D	5980	5020	3670	---
Reach 1 Average*	3134 mg/L			
Reach 1 Median*	2044 mg/L			
Reach 1 Range*†	1029 – 7650 mg/L			
2-WER-A	264	272	306	156
2-WER-B	451	400	371	157
Reach 2 Average*	534			
Reach 2 Median*	480			
Reach 2 Range*†	146 – 643 mg/L			

\*Reach averages, medians and ranges incorporate data from 1986 – 2004.

†Ranges were calculated using the mean  $\pm$ 2 standard deviations.



**Figure 2. Probability Plot for Reach 1 Hardness**

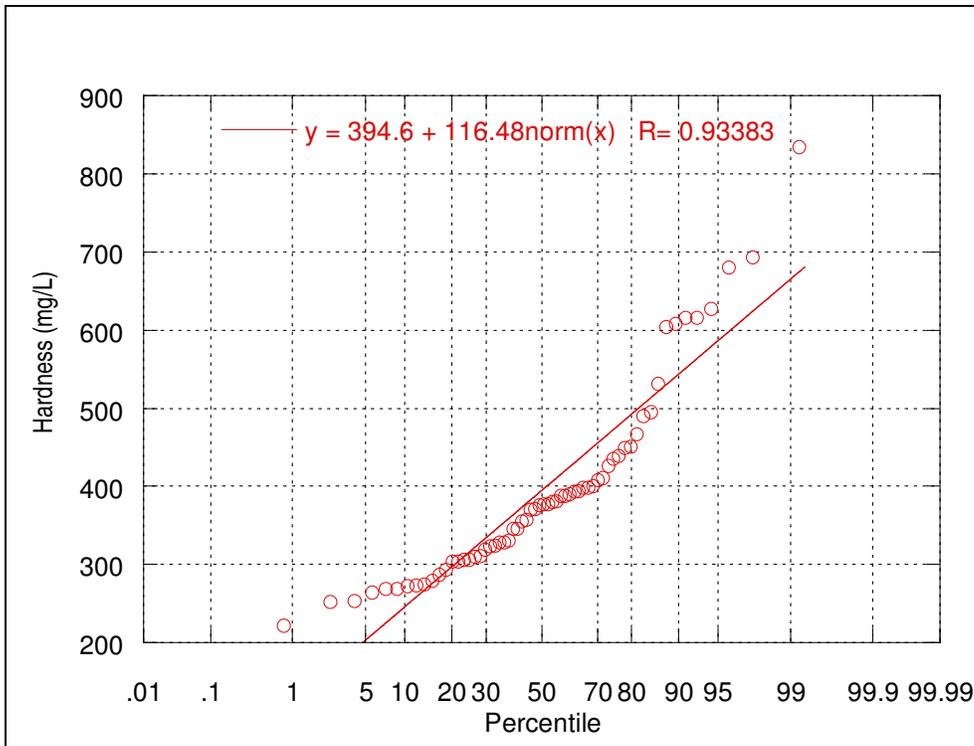


Figure 3. Probability Plot for Reach 2 Hardness

Table 11. Comparison of Event TSS to Average TSS (mg/L)

	Event 1	Event 2	Event 3	Event 4
1-WER-A	6.0	13	78	---
1-WER-B	6.1	9.5	---	---
1-WER-C	8.4	9.8	19	---
1-WER-D	12	6.1	41	---
Reach 1 Average*		77 mg/L		
Reach 1 Median*		12 mg/L		
Reach 1 Range*†		-0 – 629 mg/L		
2-WER-A	5.7	43	222	952
2-WER-B	4.0	14	41	900
Reach 2 Average*		104 mg/L		
Reach 2 Median*		29 mg/L		
Reach 2 Range*†		0 – 574 mg/L		

\*Reach averages, medians and ranges incorporate data from 2003 – 2004.

†Ranges were calculated using the mean  $\pm$  2 standard deviations. A "0" was included where -2SD resulted in a negative number.

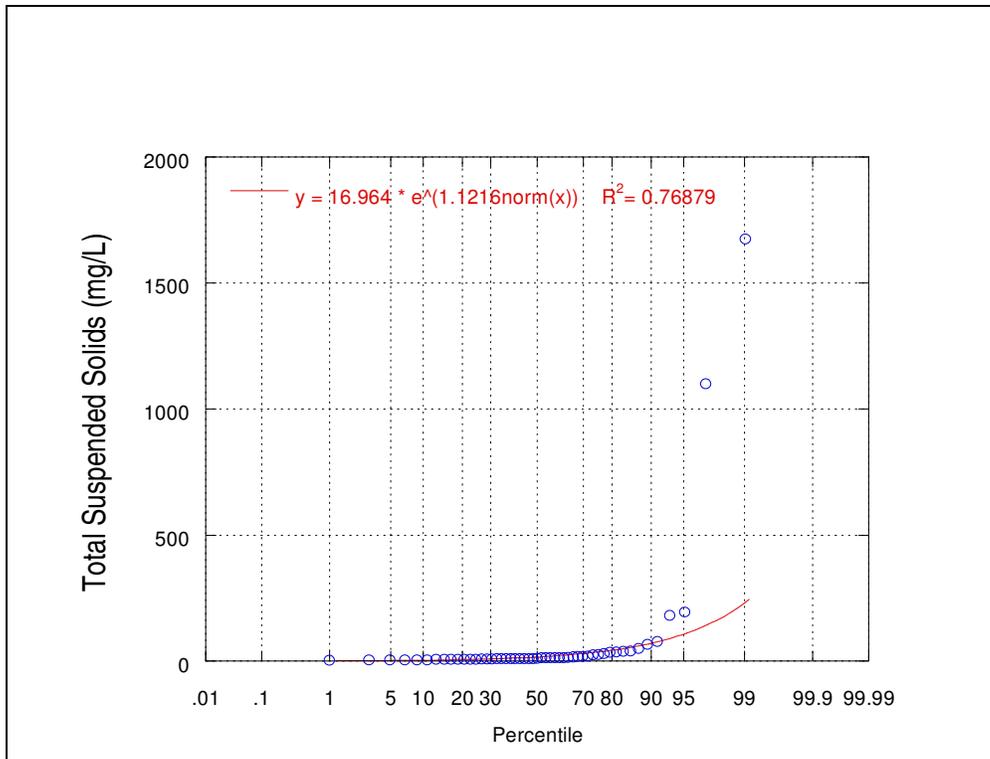


Figure 4. Probability Plot for Reach 1 Total Suspended Solids

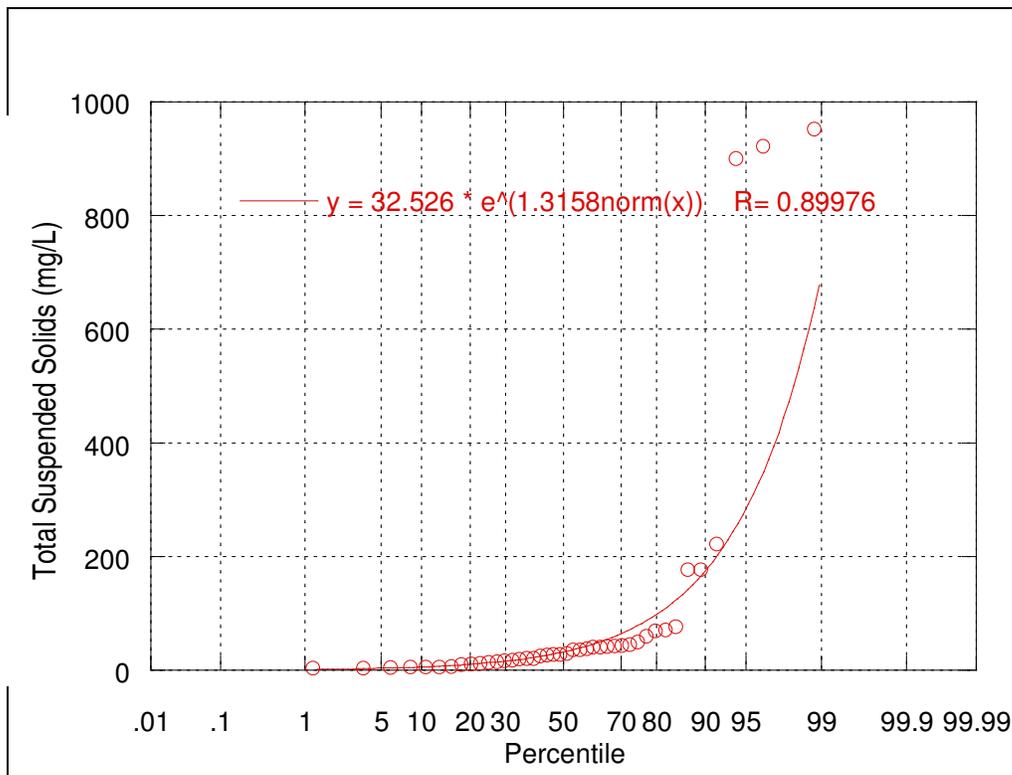


Figure 5. Probability Plot for Reach 2 Total Suspended Solids

## QA/QC Conclusions

The results from all sampling events are complete with sufficient QA data to support the validity of the reported chemical and toxicological data. Only the minor QA issues discussed above were identified. None of these issues impacted the calculation of the WERs.

## RESULTS

Tables of results for all measured parameters are located in Appendix 2. Concentration-response plots for all *Mytilus*, *Ceriodaphnia*, and lab water toxicity tests are presented below. The “% Effect” on the y-axis represents the percentage of test organisms that were not adversely affected. All of the curves show the expected effect that as the organisms are exposed to increasing copper concentrations, adverse effects are observed.

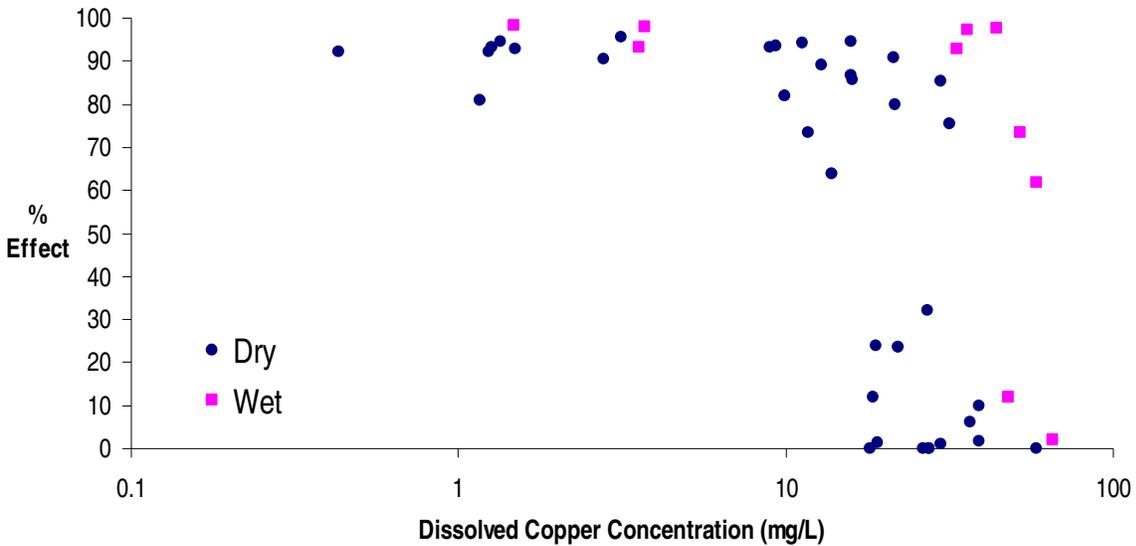


Figure 6. Concentration-response curves for *Mytilus* tests in Mugu Lagoon

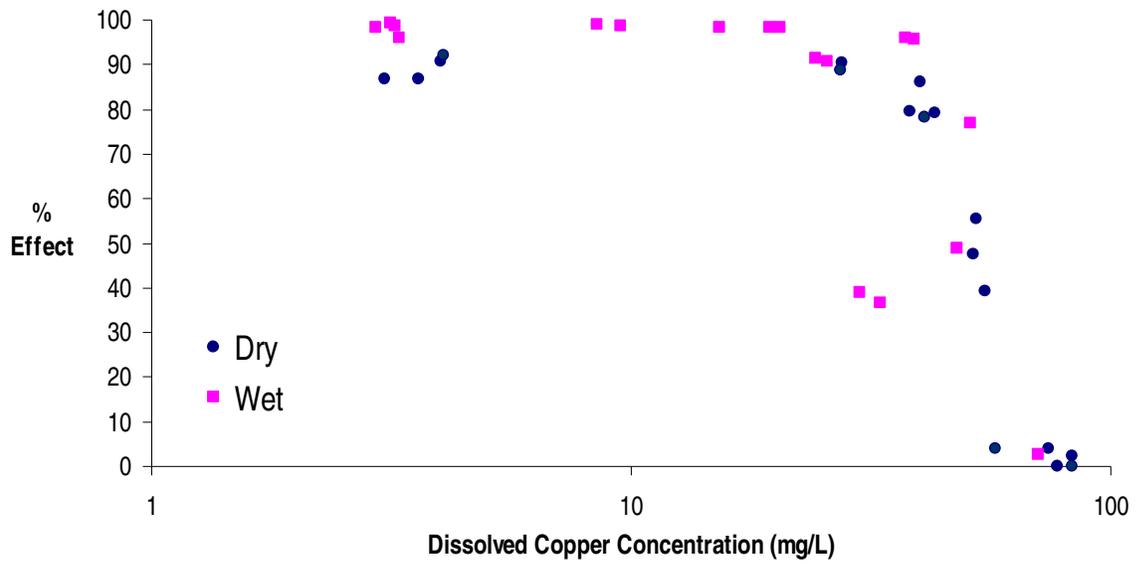


Figure 7. Concentration-response curves for *Mytilus* tests in Lower Calleguas Creek

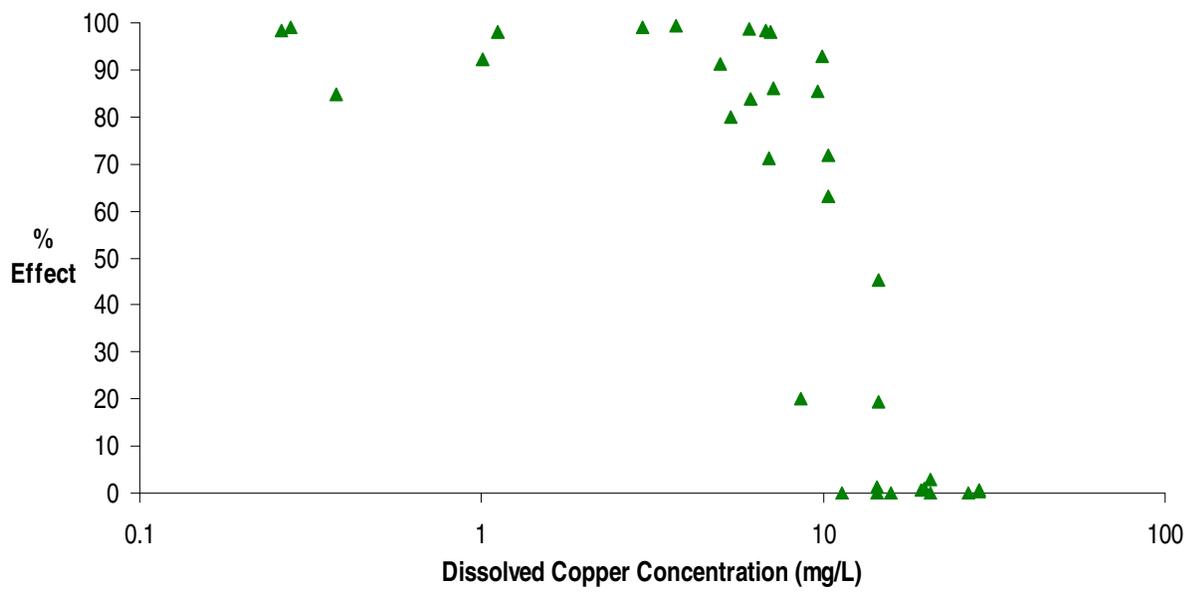


Figure 8. Concentration-response curves for *Mytilus* lab water tests

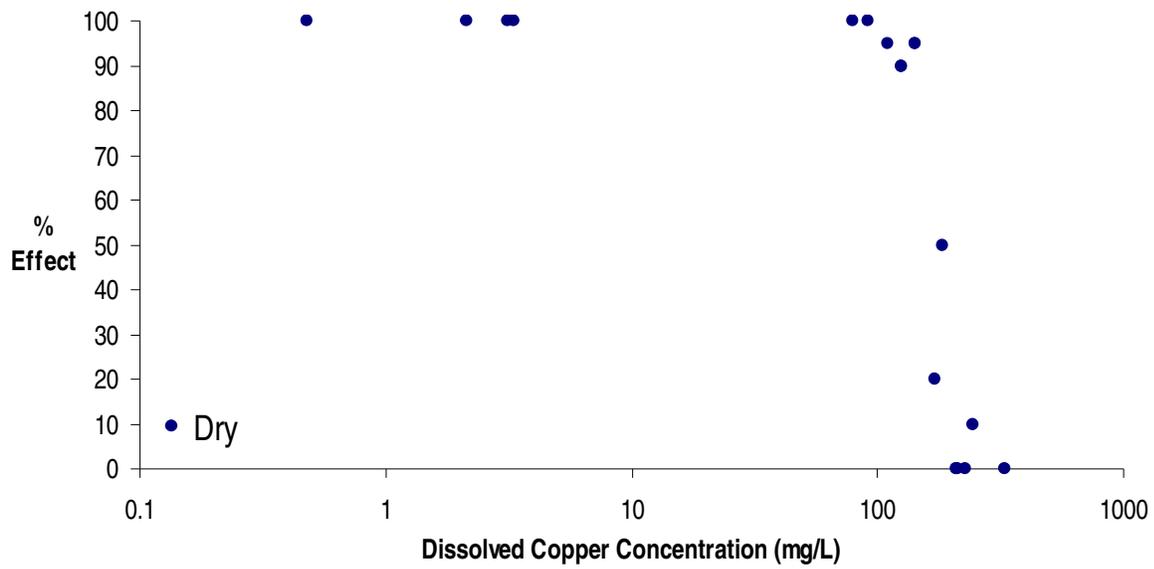


Figure 9. Concentration-response curves for *Ceriodaphnia* tests in Lower Calleguas Creek

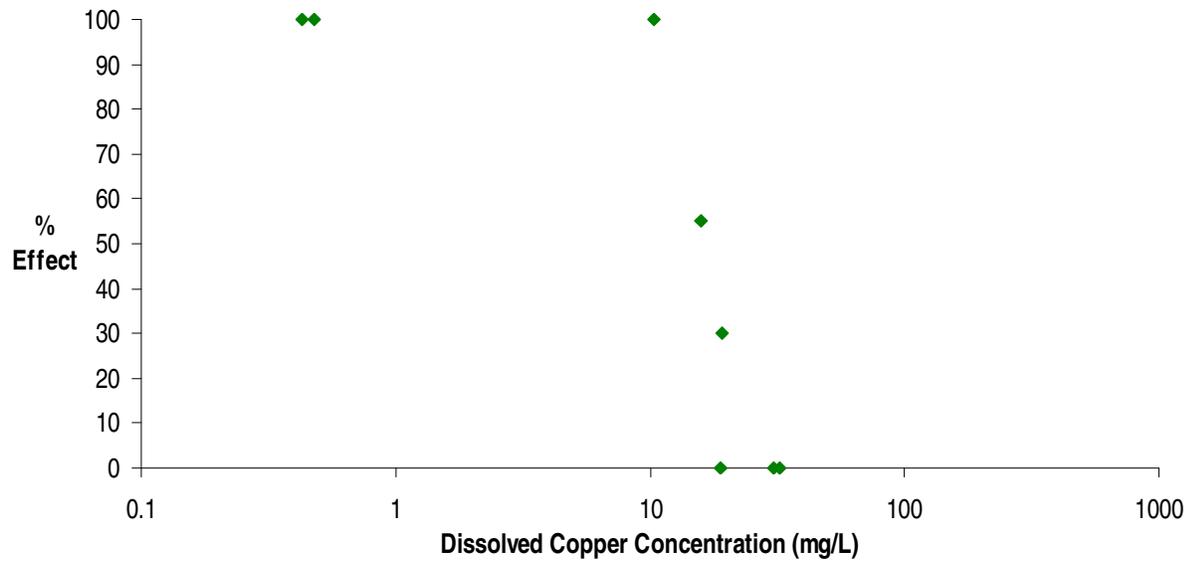


Figure 10. Concentration-response curves for *Ceriodaphnia* lab water tests

**Table 12. Total and dissolved copper EC50 determinations for site water and lab water (*Mytilus* tests).**

Site	Date Initiated*	Dissolved Copper EC50, ug/L (95% confidence limits)	Dissolved Copper WER	Salinity (ppt)
1-WER-A	8/27/03	19.6 (19.3-19.8)	1.68	31.1
1-WER-B	8/27/03	15.9 (15.8-16.0)	1.36	31.0
1-WER-C	8/27/03	14.7 (14.2-15.0)	1.26	30.0
1-WER-D	8/27/03	20.0 (19.5-20.6)	1.71	31.5
"Lab" Water	8/27/03	11.7 (11.5-11.8)	---	---
2-WER-A-M.e.	8/28/03	52.9 (49.5-58.7)	4.49	0.5
2-WER-B-M.e.	8/28/03	48.2 (45.6-49.5)	4.08	1.4
"Lab" Water	8/28/03	11.8 (11.5-12.0)	---	---
1-WER-A	1/28/04	34.4 (34.0-34.8)	2.80	17.5
1-WER-B	1/28/04	33.8 (33.4-34.0)	2.75	15.8
1-WER-C	1/28/04	16.1 (15.3-17.2)	1.31	31.6
1-WER-D	1/28/04	22.5 (16.2-30.6)	1.83	27.5
2-WER-A-M.e.	1/28/04	59.4 (54.3-62.6)	4.83	1.0
2-WER-B-M.e.	1/28/04	54.0 (49.6-57.9)	4.39	1.2
"Lab" Water	1/28/04	12.3 (11.5-13.0)	---	---
1-WER-A	3/2/04	56.8 (55.7-57.8)	4.03	4.7
1-WER-C	3/2/04	41.6 (40.9-42.3)	2.95	7.2
1-WER-D	3/2/04	54.4 (53.8-55.2)	3.86	22.8
2-WER-A-M.e.	3/2/04	47.9 (45.9-50.4)	3.40	0.5
2-WER-B-M.e.	3/2/04	>47.8 (---)	3.39	0.7
"Lab" Water	3/2/04	14.1 (12.5-16.1)	---	---
2-WER-A-M.e.	4/15/06	31.6 (31.2-32.0)	4.20	0.3
2-WER-B-M.e.	4/15/06	28.9 (28.6-29.3)	3.84	0.3
"Lab" Water	4/15/06	7.53 (7.31-7.76)	---	---

\*The dates in the above table represent the day the toxicity tests were initiated. Typically, samples were collected and shipped overnight to Pacific Ecorisk, who initiated the toxicity tests upon arrival.

Note: The species mean acute value (SMAV) for *Mytilus* is 9.625 ug/L for dissolved copper. The SMAV is the geometric mean of the results of all acceptable acute toxicity tests for the most sensitive life stage of the species.

**Table 13. Total and dissolved copper EC50 determinations for site water and lab water (*Ceriodaphnia* tests).**

Site	Date Initiated*	Dissolved Copper EC50, ug/L (95% confidence limits)	Dissolved Copper WER
2-WER-A-C.d.	8/28/03	150 (139-161)	8.93
2-WER-B-C.d.	8/28/03	179 (161-197)	10.6
"Lab" Water	8/28/03	16.8 (15.2-18.4)	---
2-WER-A-C.d.	1/28/04	175 (168-178)	6.81
2-WER-B-C.d.	1/28/04	183 (174-186)	7.12
"Lab" Water	1/28/04	25.7 (25.7-25.7)	---

\*The dates in the above table represent the day the toxicity tests were initiated. Typically, samples were collected and shipped overnight to Pacific Ecorisk, who initiated the toxicity tests upon arrival.

Note: The species mean acute value (SMAV) for *Ceriodaphnia* is 22.1 ug/L for dissolved copper, at a hardness of 100 mg/L. The SMAV is the geometric mean of the results of all acceptable acute toxicity tests for the most sensitive life stage of the species.

## Summary Statistics

Summary statistics for dissolved copper concentrations and dissolved copper EC50s and WERs are presented in this section. Table 14 and Table 15 present the dissolved copper data measured in ambient samples collected in both Mugu Lagoon and Lower Calleguas Creek. Tables 16, 17, 18, and 19 present EC50 values for Mugu Lagoon and Lower Calleguas Creek. Tables 20 and 21 summarize WER results for Mugu Lagoon and Lower Calleguas Creek (*Mytilus* and *Ceriodaphnia*). Summaries are provided for each event, as well as all events combined. Results are not reported for *Ceriodaphnia* for Event 3 because although toxicity samples were collected, the analytical laboratory mistakenly did not run the copper analyses.

**Table 14. Dissolved copper ambient concentrations (ug/L) in Mugu Lagoon.**

Site	Event 1	Event 2	Event 3	All Events
1-WER-A	0.69	4.32	3.74	ave = 2.92
1-WER-B	0.99	3.79	---	ave = 2.39
1-WER-C	0.68	1.85	3.57	ave = 2.03
1-WER-D	0.60	1.90	1.72	ave = 1.41
number	4	4	3	11
minimum	0.60	1.85	1.72	0.60
maximum	0.99	4.32	3.74	4.32
a. mean	0.74	2.97	3.01	2.17
g. mean	0.73	2.75	2.84	1.71
90 <sup>th</sup> Percentile	0.90	4.16	3.71	3.79
5 <sup>th</sup> Percentile	0.61	1.86	1.91	0.64
median	0.69	2.85	3.57	1.85
std. deviation	0.17	1.28	1.12	1.43

**Table 15. Dissolved copper ambient concentrations (ug/L) in Lower Calleguas Creek.**

Site	Event 1	Event 2	Event 3	Event 4	All Events
2-WER-A	6.54	4.04	2.77	2.48	ave = 3.96
2-WER-B	8.67	4.01	2.7	2.4	ave = 4.45
number	2	2	2	2	8
minimum	6.54	4.01	2.70	2.4	2.40
maximum	8.67	4.04	2.77	2.48	8.67
a. mean	7.61	4.03	2.74	2.44	4.20
g. mean	7.53	4.02	2.73	2.44	3.77
90 <sup>th</sup> Percentile	8.46	4.04	2.76	2.47	7.18
5 <sup>th</sup> Percentile	6.65	4.01	2.70	2.40	2.43
median	7.61	4.03	2.74	2.44	3.39
std. deviation	1.51	0.02	0.05	0.06	2.27

Dissolved copper EC50 values and WERs summary statistics are provided below. Dissolved copper EC50 values were used to calculate the WERs for each station and event:

$$\text{WER} = \frac{\text{Site Water EC50}}{\text{Lab Water EC50}}$$

**Table 16. Dissolved copper EC50 values (ug/L) and summary statistics in Mugu Lagoon.**

Site	Event 1	Event 2	Event 3	All Events
Lab Water	11.7	12.3	14.1	ave = 12.7
1-WER-A	19.6	34.4	56.8	ave = 36.9
1-WER-B	15.9	33.8	---	ave = 24.9
1-WER-C	14.7	16.1	41.6	ave = 24.1
1-WER-D	20.0	22.5	54.4	ave = 32.3
number	4	4	3	11
minimum	14.7	16.1	41.6	14.7
maximum	20.0	34.4	56.8	56.8
a. mean	17.5	26.7	50.9	30.0
g. mean	17.4	25.5	50.5	26.7
90 <sup>th</sup> Percentile	19.9	34.2	56.3	54.4
5 <sup>th</sup> Percentile	14.9	17.1	42.9	15.3
median	17.7	28.1	54.4	22.5
std. deviation	2.65	8.94	8.17	15.4

**Table 17. Dissolved copper EC50 values (ug/L) and summary statistics in Lower Calleguas Creek (*Mytilus*).**

Site	Event 1	Event 2	Event 3	Event 4	All Events
Lab Water	11.7	12.3	14.1	7.53	ave = 11.4
2-WER-A-M.e.	53.0	59.4	47.9	31.6	ave = 48.0
2-WER-B-M.e.	48.2	54.0	47.8	28.9	ave = 44.7
number	2	2	2	2	8
minimum	48.2	54.0	47.8	28.9	28.9
maximum	53.0	59.4	47.9	31.6	59.4
a. mean	50.6	56.7	47.8	30.3	46.4
g. mean	50.5	56.6	47.8	30.2	45.1
90 <sup>th</sup> Percentile	52.5	58.9	47.9	31.3	55.6
5 <sup>th</sup> Percentile	48.4	54.2	47.8	29.0	29.8
median	50.6	56.7	47.8	30.3	48.1
std. deviation	3.39	3.82	0.07	1.9	10.7

**Table 18. Dissolved copper EC50 values (ug/L) and summary statistics in Lower Calleguas Creek (*Ceriodaphnia*).**

Site	Event 1	Event 2	All Events
Lab Water	16.8	25.7	ave = 21.3
2-WER-A-C.d.	150	175	ave = 163
2-WER-B-C.d.	179	183	ave = 181
number	2	2	4
minimum	150	175	150
maximum	179	183	183
a. mean	164	179	172
g. mean	163	179	171
90 <sup>th</sup> Percentile	176	182	182
5 <sup>th</sup> Percentile	151	175	154
median	164	179	177
std. deviation	20.5	5.66	14.9

**Table 19. Dissolved copper WER values and summary statistics in Mugu Lagoon.**

Site	Event 1	Event 2	Event 3	All Events
1-WER-A	1.68	2.80	4.03	ave = 2.8
1-WER-B	1.36	2.75	---	ave = 2.1
1-WER-C	1.26	1.31	2.95	ave = 1.8
1-WER-D	1.71	1.83	3.86	ave = 2.5
number	4	4	3	11
minimum	1.26	1.31	2.95	1.26
maximum	1.71	2.80	4.03	4.03
a. mean	1.50	2.17	3.61	2.32
g. mean	1.49	2.07	3.58	2.13
90 <sup>th</sup> Percentile	1.70	2.78	3.99	3.86
5 <sup>th</sup> Percentile	1.27	1.39	3.04	1.28
median	1.52	2.29	3.86	1.83
std. deviation	0.23	0.73	0.58	1.01

**Table 20. Dissolved copper WER values and summary statistics in Lower Calleguas Creek (*Mytilus*).**

Site	Event 1	Event 2	Event 3	Event 4	All Events
2-WER-A-M.e.	4.49	4.83	3.40	4.20	ave = 4.2
2-WER-B-M.e.	4.08	4.39	3.39	3.84	ave = 3.9
number	2	2	2	2	8
minimum	4.08	4.39	3.39	3.84	3.39
maximum	4.49	4.83	3.40	4.20	4.83
a. mean	4.29	4.61	3.39	4.02	4.08
g. mean	4.28	4.60	3.39	4.02	4.05
90 <sup>th</sup> Percentile	4.45	4.79	3.40	4.16	4.59
5 <sup>th</sup> Percentile	4.11	4.41	3.39	3.86	3.39
median	4.29	4.61	3.39	4.02	4.14
std. deviation	0.29	0.31	0.01	0.25	0.51

**Table 21. Dissolved copper WER values and summary statistics in Lower Calleguas Creek (*Ceriodaphnia*).**

Site	Event 1	Event 2	All Events
2-WER-A-C.d.	8.93	6.81	ave = 7.87
2-WER-B-C.d.	10.7	7.12	ave = 8.89
number	2	2	4
minimum	8.93	6.81	6.81
maximum	10.6	7.12	10.6
a. mean	9.79	6.96	8.38
g. mean	9.75	6.96	8.24
90 <sup>th</sup> Percentile	10.5	7.09	10.1
5 <sup>th</sup> Percentile	9.01	6.82	6.86
median	9.79	6.96	8.02
std. deviation	1.22	0.22	1.78

An aspect of spatial variability not directly addressed by WER measurements involves evaluating whether the measured ambient copper concentrations are exceeding toxicity threshold values. However the WER data can be used in an indirect manner to evaluate this issue by conducting what the WER guidance describes a “sample-specific WER approach” (USEPA, 1994).

$$\frac{\text{Measured Copper (ug/L)}}{3.1 \text{ ug/L} * \text{Copper WER}}$$

In this approach, a quotient is calculated by dividing the concentration of dissolved copper (at each station) for each event by the product of the national WQC (3.1 ug/L) times the WER obtained for each station. The WER guidance states that “when the quotient for a sample is less than 1.0, the concentration of the metal in that sample is acceptable, when the quotient for a sample is greater than 1.0, the concentration of metal in that sample is too high (USEPA, 1994).” A table of these values using the data collected during this study shows that all such quotients are less than 1.0 (Table 22), and are therefore acceptable.

**Table 22. Sample Specific WER Approach Results**

Site	Event 1	Event 2	Event 3	Event 4
1-WER-A	0.13	0.50	0.30	---
1-WER-B	0.23	0.44	---	---
1-WER-C	0.17	0.46	0.38	---
1-WER-D	0.11	0.33	0.14	---
2-WER-A-M.e.	0.47	0.27	0.26	0.19
2-WER-B-M.e.	0.69	0.29	0.26	0.20

## CALCULATION OF RECOMMENDED WER AND SSO

The EPA has developed two guidance documents to assist in the development of WERs for copper. The 1994 WER guidance contains two different methods for developing WERs for all metals. The Streamlined Water-Effect Ratio Procedure for Discharges of Copper provides guidance for developing WERs for copper downstream of POTW discharges. Each of the methods is designed to address different waterbody types and discharge conditions. For the purposes of calculating the recommended WERs, the 1994 WER guidance was used as the basis for the analysis because not all of the criteria for using the Streamlined Method were met. For Mugu Lagoon, Method 2 in the 1994 WER guidance is the only applicable method. For Calleguas Creek, either Method 1 or Method 2 could apply for calculating the WER.

The 1994 WER guidance, Method 1, includes a specific calculation method that is basically the calculation of an adjusted geometric mean of the dry weather samples. Additional analysis is included to account for different flow conditions, but the calculations are based on the assumption that samples were collected directly downstream of a POTW discharge. In the case of Calleguas Creek, there are no POTW discharges to the reach for which the WERs were being developed. Therefore, all of the specific calculations outlined in Method 1 could not be directly applied. Additionally, concerns have been raised about potential differences between dry and wet weather samples that are not specifically addressed in the WER guidance.

Method 2 provides less specific guidance about how to calculate the final WERs, but suggests that “a WER is determined for each sample, and the final WER (FWER) is calculated as the geometric mean of some or all of the WERs” (USEPA, 1994). Additionally, the *Streamlined WER Procedure* (though not used as the basis for the study) also specifies that the final WER be calculated as the geometric mean of two (or more) sample WERs.

Because all three WER calculation methods include a discussion of geometric means as possible calculation methods, geometric means were determined to be the most appropriate calculation method for the final WERs. This calculation approach was developed in conjunction with the TAC and Regional Board staff. The geometric mean is a measure of the central tendency of a data set that minimizes the effects of extreme values. The equation for the geometric mean is:

$$\text{Geometric mean} = \sqrt[n]{y_1 * y_2 * y_3 * \dots * y_n}$$

An example of the geometric mean calculation for site 2-WER-A and 2-WER-B for *Mytilus*, using the WERs calculated at the Lower Calleguas Creek sites during the dry weather events is as follows:

$$\text{Geometric mean}_{\text{Mytilus, Lower Calleguas Creek}} = \sqrt[4]{4.49 * 4.08 * 4.83 * 4.39} = 4.44$$

To address concerns that dry and wet weather conditions produce different WERs, the geometric mean of the dry weather WERs and wet weather WERs were calculated separately. To ensure that the selected final WER was protective for all conditions, the lower of the dry and wet weather geometric mean WERs for each reach was selected as the final WER. In Mugu Lagoon, the dry weather results differed based on the degree of freshwater influence on the Lagoon. The sample results indicate that when the freshwater flows were more significant, the WERs in the reaches closest to the freshwater inputs were higher than during other times. Therefore, to provide a conservative estimate of the dry weather WER, the geometric mean of

the dry weather WERs in the Lagoon were calculated without the higher dry weather WERs from periods with more significant freshwater flows.

As can be seen in Table 18 and Table 21, the EC50 and calculated WER values from results of *Ceriodaphnia* tests are much greater than those calculated using *Mytilus* test data. Therefore, to take a conservative approach, only *Mytilus* results are used in subsequent calculations of WERs and site-specific objectives (SSOs).

The wet and dry weather WERs are presented Table 23.

**Table 23. Dissolved copper WER geometric mean values.**

Test	Location	Weather	Geometric Mean
<i>Mytilus edulis</i>	Mugu Lagoon	Dry	1.51*
		Wet	3.58
	Lower Calleguas Creek	Dry	4.44
		Wet	3.69

\* To provide a conservative estimate of the dry weather WER, the geometric mean was calculated using only those samples which did not require the addition of GP-2 salts. The results indicate that when the freshwater flows were more significant, the WERs in the areas of the Lagoon closest to the freshwater inputs were higher than during other times. The samples used for this calculations included Event 1, Sites A, B, C, D and Event 2, Sites C, D.

Based on the results in Table 23, the dry weather WER is the lowest WER for Mugu Lagoon and the wet weather WER is the lowest value for Lower Calleguas Creek. Therefore, the recommended WERs are 1.51 for Mugu Lagoon and 3.69 for Lower Calleguas Creek.

In addition to Mugu Lagoon and Lower Calleguas Creek, the saltwater criteria also applies to Revolon Slough because the salinity of the reach is between 1 and 10 ppt more than 5% of the time. The CTR requires that the lower of the saltwater and freshwater CTR criteria be applied in those situations. The WER for Mugu Lagoon effectively adjusts the saltwater criteria for the most sensitive area of the watershed. Because Revolon Slough flows directly into Mugu Lagoon and the criteria are driven by this connection, the WER developed for the Lagoon will be applied to Revolon Slough as well. As shown by the results of the Lower Calleguas Creek sampling, the WER in waterbodies with lower salinities is higher than in the Lagoon so it is conservative to apply the Lagoon WER to Revolon Slough.

The recommended SSOs are determined by multiplying the CTR saltwater chronic and acute criteria by the final WERs as shown in the equations below:

$$SSO_{\text{general}} = \text{CTR criterion} * \text{Final WER}$$

$$SSO_{\text{chronic}} = 3.1 \text{ ug/L} * \text{Final WER}$$

$$SSO_{\text{acute}} = 4.8 \text{ ug/L} * \text{Final WER}$$

For example:

$$SSO_{\text{chronic Mugu Lagoon}} = 3.1 \text{ ug/L} * 1.51 = 4.68 \text{ ug/L}$$

The final recommended WERs and SSOs are shown in Table 24.

**Table 24. Recommended WERs and SSOs for Mugu Lagoon, Revolon Slough and Lower Calleguas Creek.**

Reach	Final WER	Chronic SSO <sup>1</sup> (ug/L)	Acute SSO <sup>1</sup> (ug/L)
Mugu Lagoon	1.51	4.68	7.25
Lower Calleguas Creek	3.69	11.4	17.7
Revolon Slough	1.51	4.68	7.25

<sup>1</sup> The Saltwater criterion is applied to Mugu Lagoon, Revolon Slough and Lower Calleguas Creek. Mugu Lagoon salinities are >10ppt all of the time, and Lower Calleguas Creek and Revolon Slough salinities are most typically between 1-10ppt, indicating that the more stringent of the saltwater and freshwater criteria should be applied. For copper, the saltwater criterion is more stringent than the freshwater.

## REFERENCES

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