High-Salinity Sensitivity Study

Short-and Long-Term Exposure Assessments

Prepared For:

West Basin Municipal District
17140 South Avalon Blvd, Ste. 210
Carson, CA 90746-1296

Prepared By:

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Oakland, CA 94612

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EXECUTIVE SUMMARY

Overview

The West Basin Municipal Water District (WBMWD) High Salinity Sensitivity Study (HSS Study) comprehensively evaluated the potential short-term and long-term exposure effects of high salinity discharges from the WBMWD ocean water desalination demonstration facility (OWDDF) on aquatic organisms representative of communities indigenous to various near shore environments in Southern California. Study parameters are summarized in Table ES-1. Short-term effects were evaluated using Whole Effluent Toxicity (WET) bioassays developed by the U.S. Environmental Protection Agency (USEPA) to quantify the magnitude and threshold of potential biological effects of discharges (e.g. treated wastewater). Both acute toxicity (mortality effects) and chronic toxicity (mortality + sublethal effects) bioassays were performed by a state accredited bioassay laboratory. Long-term effects were evaluated using mesocosm procedures performed at the OWDDF by exposing multiple organisms for eight weeks to ambient seawater and diluted brine flows from the OWDDF in large aquaria constructed to simulate the OWDDF discharge environment.

Short-Term WET Testing

The short-term WET component of the HSS study (Volume I) consisted of a two-phase screening process involving initial acute and chronic toxicity range-finding bioassays followed by definitive bioassays (i.e. a narrower salinity dilution series). The objective of the WET component of the study was to determine which species and early life-stages among those available under USEPA guidelines were the most sensitive to hypersaline conditions, and what salinity levels will elicit adverse effects to those organisms. One objective of the WET testing is to provide toxicity data for the most sensitive species expected to reside in the OWDDF discharge environment. Modeling this data in conjunction with other factors such as water depth and ocean mixing conditions will determine the appropriate salinity thresholds for acute and chronic exposures to OWDDF brine discharges.

WET Phase I (i.e. range-finding) chronic toxicity testing using OWDDF brine discharge samples involved a single test episode and was conducted with the following USEPA authorized test organisms: giant kelp spores (*Macrocystis pyrifera*), purple sea urchin embryos (*Strongylocentrotus purpuratus*), red abalone embryos (*Haliotis rufescens*), larval mysid shrimp (*Americamysis bahia*), and larval topsmelt (*Atherinops affinis*). Each bioassay method evaluated sensitive life stages (e.g. growth or embryo development) over a period of four to seven days. Since the suite of available USEPA *acute* toxicity test methods (i.e. methods that measure mortality only) is significantly more limited (i.e. only available for fish and shrimp species), Phase I acute toxicity testing was conducted with just three species: larval mysid shrimp, larval topsmelt and juvenile sand dabs (*Citharichthys stigmaeus*). The objective of Phase I testing was to determine relative species sensitivities in order to identify a narrower brine dilution range to be used in the Phase II definitive bioassays.

WET Phase II chronic toxicity testing involved two consecutive test episodes using the three species from each trophic level (plant, invertebrate, and vertebrate) most likely to reside within the soft bottom OWDDF discharge environment: giant kelp (kelp spores), mysid shrimp (larvae) and topsmelt (larvae). A purple urchin bioassay was included in Phase II as urchins were more sensitive in Phase I than abalone, the other hard-bottom habitat species, and because a substantial amount of urchin data has been generated with other high salinity studies. The Phase II acute toxicity was performed with the two most sensitive species from Phase I: larval mysid shrimp and larval topsmelt.

Table ES-1. Study Summary

<table>
<tr><th colspan="4">Short-Term WET Testing</th></tr>
<tr><th>Overview</th><th colspan="2">Chronic Toxicity
Effects on mortality and sub-lethal metrics (e.g. embryo development, growth)</th><th>Acute Toxicity
Evaluation of mortality after acute exposure (typically 96-hours)</th></tr>
<tr><td rowspan="3">• Lab-based biological effects testing (bioassays)
• EPA approved species for monitoring effluents
• Focus on early life-stages (i.e. embryo-larval)
• Mix of species native to both soft-bottom and hard-bottom habitats
• Organisms exposed to multiple brine dilutions
• One range-finder testing episode and two definitive episodes
• Most sensitive species used for definitive episodes
• Statistical analyses performed to identify ‘no observed effect levels’</td><td colspan="3">Range-Finder</td></tr>
<tr><td colspan="2">• Bioassays
o 7-day mysid shrimp survival & growth
o 96-hr kelp germination & germ-tube growth
o 72-hr purple urchin embryo development
o 48-hr red abalone embryo development
o 7-day topsmelt larval survival & growth
• Dilutions: 33, 42, 51, 60 and 70 ppt</td><td>• Bioassays
o 96-hour mysid shrimp survival
o 96-hour sand dab survival
o 96-hour topsmelt survival
• Dilutions: 33, 42, 51, 60 and 70 ppt</td></tr>
<tr><td colspan="3">Definitive Testing</td></tr>
<tr><td colspan="2">• Bioassays
o 7-day mysid shrimp survival & growth
o 72-hr purple urchin embryo development
o 7-day topsmelt larval survival & growth
• Dilutions
o Purple urchin: 35, 37, 39, 41, 43 ppt
o Episode 1 Fish: 36.5, 39, 41, 45, 50 ppt
o Episode 1 Fish: 36.5, 39, 41, 45, 60 ppt</td><td>• Bioassays
o 96-hour mysid shrimp survival
o 96-hour topsmelt survival
• Dilutions
o Episode 1: 36.5, 39, 41, 45 & 50 ppt
o Episode 2: 36.5, 39, 41, 45 & 60 ppt</td></tr>
<tr><th colspan="4">Long-Term Mesocosm Testing</th></tr>
<tr><th>Overview</th><th>Species</th><th>Exposure Levels</th><th>Parameters Evaluated</th></tr>
<tr><td>• Testing performed on-site
• Expanded variety of Southern California species
• Juvenile & adult life stages
• 1 ambient and 1 elevated salinity test chambers
• Organisms exposed to both salinities under flow through conditions
• Three 8-week trials
• Each trial comprised of 3 successively higher salinity exposure periods in ‘elevated salinity’ chamber
• Mortality, behavior and post-exposure, sub-lethal parameters evaluated</td><td>• Sand dabs
• White sea bass
• Rockfish (multiple species)
• Shiner perch
• 3-spined sticklebacks
• Tube snouts
• Olive snails
• Purple urchins
• Red abalone
• Blue mussels
• Bat stars
• Sand crabs
• Slender crabs
• Kelp crabs</td><td>• Trial 1
o Ambient
o Low salinity: 37 ppt
o Mid salinity: 42.5 ppt
o High salinity: 47 ppt
• Trial 2
o Ambient
o Low salinity: 37 ppt
o Mid salinity: 42.5 ppt
o High salinity: 44.5 ppt
• Trial 3
o Ambient
o Low salinity: 37 ppt
o Mid salinity: 41 ppt
o High salinity: 44.5 ppt</td><td>• Mortality
• Behavior
• Juvenile fish growth after high salinity exposures
o Sand dabs and/or
o White sea bass
• Shellfish embryo development after mid and high salinity exposures
o Blue mussels or
o Purple urchins
• Purple urchin fertilization after mid salinity exposure (Trial 3 only)</td></tr>
</table>

ppt: Parts salt per thousand parts water

Results of Phase II chronic toxicity testing performed under the short-term WET component of the HSS Study showed that the most sensitive organism among the three test species most representative of the organisms indigenous to the OWDDF discharge environment was the mysid shrimp. The highest salinity level that resulted in no statistically significant effects to this species was 41 parts salt per thousand parts water (ppt). As expected, purple urchins, the more sensitive hard-bottom habitat species, were somewhat more susceptible to the chronic toxicity effects of high salinity than the mysid shrimp. The average Phase II no effects concentration calculated for the purple urchin was 36 ppt. The chronic toxicity results for the most sensitive hard-bottom and soft-bottom species are presented in Figure ES-1.

Results of the Phase II *acute* toxicity testing showed that the most sensitive organism tested was also the mysid shrimp. The highest salinity level that resulted in no statistically significant *acute* toxicity (i.e. mortality after acute exposure) to this species was 45 ppt.

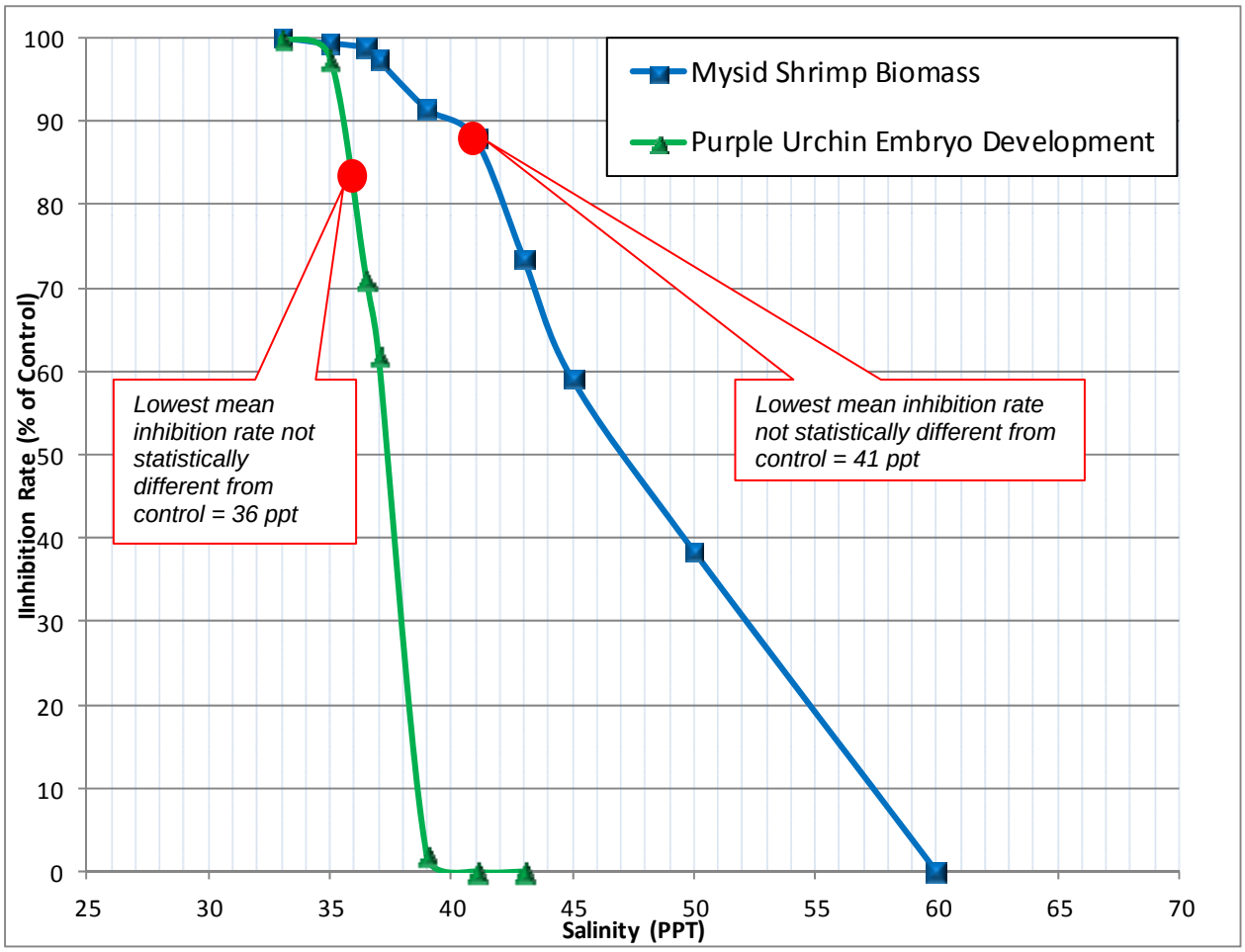

Figure ES-1. Chronic Toxicity Effects of High Salinity on Mysids and Urchins (Phase II Averages)

Long-Term Mesocosm Testing

Objectives for the long-term mesocosm component of the HSS Study are to corroborate the results of the WET component of the study and further inform the overall assessment of potential impacts of the OWDDF brine discharge. The mesocosm component of the study was performed at the OWDDF in Redondo Beach, CA. A mesocosm (i.e. mid-scale habitat simulation) was created with a split-chamber flow-through aquarium of sufficient capacity (300 gallons) to house an assembly of juvenile and adult aquatic organisms representative of the biological community of southern California. Several invertebrate and vertebrate species were acquired from organism providers permitted by the California Department of Fish and Game. Equal numbers of each organism were placed on both sides of the aquarium, and exposed to filtered ambient seawater on one side and high salinity flows on the other. Three 8-week exposure trials were performed. After every two weeks, the salinity level in the high-salinity chamber was reduced to ambient for up to one week and then raised to a higher salinity level. This approach resulted in three different salinity level exposure periods within each trial: low salinity (37 ppt), mid-level salinity (41 or 42.5 ppt), and high salinity (44.5 or 47.5 ppt).

Organisms used in the mesocosm component of the HSS Study in both the ambient and high salinity test chambers were monitored daily throughout each exposure trial for mortalities and variations in behavior. In addition, sub-lethal impacts to select biological endpoints were also assessed at the end of the mid and high salinity exposure periods for each trial. The first post-exposure endpoint measured was mussel (Trial 1) or urchin (Trials 2 and 3) embryo development. Half of the adult invertebrate mussels or urchins were removed after the mid salinity exposure period and used to perform the U.S. EPA chronic toxicity bioassay that assesses embryo fertilization and/or development (fertilization was only measured after the mid-salinity exposure of trial 3). Gametes and/or embryos harvested from adults exposed to both ambient and elevated salinities were placed in test vials containing either ambient seawater or water with an elevated salinity matching that in which the adult organisms had been exposed, and then assessed for fertilization rate and/or embryo development success. This procedure was then repeated after the high salinity exposure period for all three trials. The other sublethal endpoints assessed were weight and length achieved by one or two species of juvenile fish (white sea bass and speckled sanddabs).

Results of the long-term exposure trials (Table ES-2) show that *all* vertebrate and invertebrate organisms exposed to the low and mid salinity levels in the elevated salinity chamber did not exhibit behavior patterns or mortality rates any different from the ambient seawater organisms. The urchins and abalone began showing signs of stress when exposed to the trial 1 high salinity level of 47.5. Most of these urchins and abalone ultimately perished. However, no other species showed any sign of stress throughout the entire 2-week high-salinity exposure period. With the high-salinity level lowered by three ppt in trials 2 and 3, the abalone were not visibly affected, and only three of the 15 urchins suffered mortality. None of the urchins were affected in trial 3.

Results of the mesocosm sub-lethal endpoint evaluations show that there were no significant differences in weight gain or length between fish exposed to high salinity flows and those exposed to ambient seawater throughout all three exposure periods for all three trials. The post-exposure invertebrate bioassays showed that exposure of adult shellfish to mid or high salinity levels did not result in an increased tolerance of their embryos in

elevated salinities. However, embryos from adult urchins exposed at 41 ppt did develop normally in ambient seawater. Additionally, the urchin fertilization bioassay performed after the trial 3 mid-salinity exposure period showed that adults first exposed to 41 ppt resulted in normal fertilization rates for urchin gametes exposed to both ambient *and* 41 ppt salinities.

Table ES-2. Long-Term Mesocosm Results Summary

Salinity Exposure Level	Trial (Salinity)	Post-Exposure Parameters			Mortality
		Urchin Fertilization	Urchin/Mussel Embryo Development	Fish Growth	
Low	Trial 3 (37 ppt)	Not Measured	Not Measured	Not Measured	No significant mortality among 10 different species
	Trial 2 (37 ppt)	Not Measured	Not Measured	Not Measured	No significant mortality among 12 different species
	Trial 1 (37 ppt)	Not Measured	Not Measured	Not Measured	No significant mortality among 9 different species
Mid	Trial 3 (41 ppt)	No significant inhibition for urchin gametes exposed to 41 ppt solution or ambient solutions	Significant inhibition in **urchin** embryos exposed to 41 ppt solution ***but not*** ambient solution	Not Measured	No significant mortality among 10 different species
	Trial 2 (42.5 ppt)	Not Measured	Significant inhibition in **urchin** embryos exposed to ambient and 42.5 ppt solutions	Not Measured	No significant mortality among 12 different species
	Trial 1 (42.5 ppt)	Not Measured	Not Measured	Not Measured	No significant mortality among 9 different species
High	Trial 3 (44.5 ppt)	Not Measured	Significant inhibition in **urchin** embryos exposed to ambient and 44.5 ppt solutions	No significant effect on length or weight measured for 1 fish species: white sea bass	No significant mortality among 10 different species
	Trial 2 (44.5 ppt)	Not Measured	Significant inhibition in **urchin** embryos exposed to ambient and 44.5 ppt solutions	No significant effect on length or weight measured for 2 fish species: sand dabs & white sea bass	Slightly significant mortality among 1 out of 12 different species: 88.5% urchin survival
	Trial 1 (47.5 ppt)	Not Measured	Significant inhibition in **mussel** embryos exposed to ambient and 47.5 ppt solutions	No significant effect on length or weight measured for 1 fish species: sand dabs	Significant mortality among 2 out of 9 different species: 16.7% urchin survival 57.2% abalone survival

No significant effects
Slightly significant effects
Significant effects

The results of the HSS Study are summarized in the Table below. In general, the mesocosm component of the study demonstrated that most organisms are tolerant of long-term exposure to salinities at least as high as 47.5 ppt. The only exceptions were purple urchins and red abalone. that showed tolerance of long-term exposures as high as 42.5 ppt. The long-term mesocosm tolerances were higher than those observed with the short-term WET component of the study. The 'no effects levels' established by the WET bioassays were 41 ppt for the most sensitive soft-bottom organism (mysid shrimp), and 36 ppt for the most sensitive hard-bottom organism (purple urchin). Long-term exposure of adult purple urchins and mussels above 41 ppt did not result in improved embryo-development sensitivity in elevated or ambient salinities. However, embryos developed normally in ambient seawater after the adults were exposed at the 41 ppt salinity level. Additionally, purple urchin fertilization rates were not affected for purple urchin gametes (i.e. pre-embryo eggs and sperm) from adults exposed to ambient or mid-salinity (41 ppt) waters when the gametes were subsequently exposed to ambient *and* 41 ppt salinities.

High Salinity Sensitivity Study Summary

Study Component	Observed Salinity Thresholds		Other Considerations
	Soft-Bottom Organisms	Hard-Bottom Organisms	
Short-Term WET (chronic toxicity)	41 ppt	36 ppt	• No significant effect on urchin embryo development rates in ambient seawater when adults are first exposed to 41 ppt salinities. • Fertilization rate not effected at 41 ppt whether or not adults are previously exposed to this salinity. • Acute toxicity threshold observed with the WET study component = 45 ppt
Long-Term Mesocosm	47.5 ppt	42.5 ppt*	

* Mortality effect

1.0 SHORT-TERM EXPOSURE ASSESSMENT

1.1 INTRODUCTION

In order to characterize the impacts of high-salinity discharges to marine life residing in the discharge environment of its desalination facility, the West Basin Municipal Water District (WBMWD) initiated a two-phase Whole Effluent Toxicity (WET) screening study. Results of the toxicity screening study are evaluated to determine which U.S. Environmental Protection Agency (USEPA) authorized test organisms are most sensitive to hypersaline conditions under acute and chronic exposure conditions, and what salinity levels are expected to elicit adverse effects to these organisms. The toxicity data from the most sensitive species expected to reside in the discharge environment will then be factored with naturally occurring factors such as water depth and ocean mixing conditions, and used to determine an appropriate salinity threshold for the desalination facility brine discharge.

In April 2011, Weston Solutions, Inc. (WESTON) performed Phase I chronic and acute toxicity testing with a WBMWD brine discharge sample using a select suite of USEPA approved test species. All toxicity testing followed USEPA approved test methods. Phase I chronic toxicity tests included the 48-hour giant kelp, *Macrocystis pyrifera,* germination and germ-tube length test (USEPA, 1995); 72-hour purple sea urchin, *Strongylocentrotus purpuratus,* larval development test (USEPA, 1995); 48-hour red abalone, *Haliotis rufescens,* larval development test (USEPA, 1995); 7-day opossum shrimp, *Americamysis bahia*, survival, growth and fecundity test (USEPA, 2002a); and the 7-day topsmelt, *Atherinops affinis,* larval survival and growth test (USEPA, 1995). Phase I acute toxicity tests included the 96-hr speckled sand dab, *Citharichthys stigmaeus*, acute survival test (USEPA, 2002); and the 96-hr topsmelt, *Atherinops affinis,* acute survival test (USEPA, 2002). The results of Phase I bioassay testing were evaluated to select species for Phase II confirmation testing.

Beginning in August 2011, WESTON performed Phase II testing with WBMWD effluent using the three Phase I species representative of the three different trophic levels (plant, invertebrate, and vertebrate) most likely to reside in the WBMWD discharge environment. These bioassays included the 48-hour *Macrocystis pyrifera* test, the 7-day *Americamysis bahia* test, and the 7-day *Atherinops affinis* test. Due to the significant amount of testing performed by other agencies and researchers using the purple urchin, WBMWD decided to also include the 72-hour *Strongylocentrotus purpuratus* test during Phase II testing. Again, all toxicity testing followed USEPA approved test methods. Phase II acute toxicity testing included bioassays performed with the two most sensitive Phase I species: the 96-hr speckled sand dab, *Citharichthys stigmaeus* test; and the 96-hr topsmelt, *Atherinops affinis* test. The results of all Phase I and Phase II testing are presented and discussed herein.

To determine whether consistent patterns of chronic or acute toxicity exists among the species selected from Phase I, Phase II included two consecutive test episodes using the test methods described above. If evaluation of the data generated from both phases indicates a consistent sensitivity hierarchy among the four methods, the most sensitive will be selected as the WBMWD chronic toxicity endpoint to be used with hydrographic modeling measures to determine the potential for adverse impacts within the brine discharge environment.

Phase I toxicity testing was performed using samples collected on February 7 and February 11, 2011. Phase II, Episode 1 was performed using samples collected on August 3 and August 5, 2011. Phase II, Episode 2 was performed using a sample collected on September 22, 2011. Laboratory benchsheets from Phase I toxicity testing are provided in Appendix A. Laboratory benchsheets from Phase II, Episodes 1 and 2, are provided in Appendix B.

This study was conducted by WESTON at the Bioassay Laboratory in Carlsbad, California under the management of Dr. David Moore, Ph.D.

1.2 METHODS

1.2.1 TEST PROTOCOLS

The five chronic toxicity test methods are listed below:

- The 7-day *A. bahia* survival and growth test was performed in accordance with *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Marine and Estuarine Organisms, Third Edition* (USEPA, 2002a);
- The 48-hour *M. pyrifera* germination and germ-tube length test was performed in accordance with *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms, First Edition* (USEPA, 1995);
- The 72-hour *S. purpuratus* larval development test was performed in accordance with *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms, First Edition* (USEPA, 1995);
- The 48-hour *H. rufescens* larval development test was performed in accordance with *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms, First Edition* (USEPA, 1995);
- The 7-day *A. affinis* larval survival and growth test was performed in accordance with *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms, First Edition* (USEPA, 1995).

The three 96-hour acute toxicity test methods using *C. stigmaeus*, *A. affinis* and *A. bahia* were all performed in accordance with *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition* (USEPA, 2002).

1.2.2 TEST SOLUTION PREPARATION

1.2.2.1 *Sample Receipt*

Brine discharge samples were collected by WBMWD personnel and delivered on ice (0 – 6°C) under chain-of-custody to the WESTON bioassay laboratory. Standard water quality measurements (i.e. dissolved oxygen [DO], temperature, pH, salinity, total ammonia, and total chlorine) were taken upon sample arrival. Phase I bioassay testing was staggered with testing initiated on February 8, February 11, March 2, and April 1, 2011. Upon receipt, all samples used for Phase I testing met recommended initial water quality objectives for DO and pH. Temperatures exceeded the recommended values (0 – 6°C) by 5.9 – 6.4°C. Phase II, Episode 1 bioassays were also staggered; testing was initiated on August 4, August 10, August 15, and August 23, 2011. Upon receipt, all samples required for Episode 1 of Phase II testing met recommended initial water quality objectives for DO and pH. Temperatures slightly exceeded recommended values (0 – 6°C) by 0.1 – 7.6°C. Phase II, Episode 2 bioassays were staggered as well; testing was initiated on September 27, October 4-5, and October 19, 2011. Upon receipt, all samples required for Episode 2 of Phase II testing met recommended initial water quality objectives for DO and pH. Temperatures exceeded the recommended values by 5.1 – 8.8°C. Copies of chain-of-custody forms, organism receipt forms, and sample receipt forms summarizing initial water quality measurements are provided in Appendix C.

1.2.2.2 *Test Dilutions*

For all toxicity testing, organisms were exposed to a series of dilutions created by blending the stock WBMWD brine discharge solution with dilution water. The dilution water was filtered, UV-treated seawater collected from the Scripps Institute of Oceanography. The dilution series selected for Phase I testing was chosen to determine the range of biological effects and, therefore, included ambient seawater and straight 70 parts per thousand (ppt) brine discharge. The three dilutions in between were evenly spaced so that organisms were exposed to a laboratory control treatment (Scripps seawater) and five brine effluent dilutions: 33, 42, 51, 60, 70 ppt. During Phase II Episode 1 testing, exposures were modified to attain greater statistical certainty around the toxic thresholds. The more sensitive purple urchin was exposed to lower salinity dilutions (35, 37, 39, 41 and 43 ppt) while the other three Phase II chronic toxicity and the two acute toxicity test organisms were exposed to dilutions ranging somewhat higher (36.5, 39, 41, 45, 50 ppt). The test organisms exhibited higher tolerance in Phase II, Episode I. Thus during Phase II, Episode 2 testing, the highest dilution was increased to 60 ppt. All other dilutions, including all purple urchin dilutions, remained the same.

1.2.3 Summary of Chronic Bioassay Test Procedures

1.2.3.1 *Americamysis bahia Test*

Seven-day old mysid shrimp were obtained from Aquatic BioSystems, Fort Collins, CO. Prior to test initiation, mysids were received and acclimated to test temperature of (26 ± 1°C) and dilution water salinity. The test was run with eight replicates of 400 milliliter (mL) plastic containers containing 150 mL of test solution and five mysids per container under a 16-hour light: 8-hour dark photoperiod. Eighty percent of the test solution was replaced each day of the test. The mysids were fed a concentrated *Artemia* suspension (~750 nauplii) two times daily. Daily temperature, DO, pH, and salinity were measured for final (previous day) and initial (renewal) solutions. Mortality and behavior were recorded daily. Upon test termination, surviving mysids in the control treatment were evaluated for fecundity. If ≥50% of surviving control female mysids were found to be fecund, all replicates were evaluated for this endpoint. Mysids were then dried at 100°C in an oven for at least 6 hours and weighed to determine average weight. Test conditions for the *A. bahia* survival and growth test are summarized in Table 1-1.

Test Acceptability Criteria

The criteria used to determine test acceptability were the following:

1. Control survival to equal or exceed 80%,
2. Average control dry weight of at least 0.20 mg per mysid.

1.2.3.2 *Macrocystis pyrifera Test*

Kelp was obtained from Dave Gutoff of San Diego, CA. Approximately 30 blades were rinsed in seawater and placed in 1 L of 0.2 µm filtered Scripps seawater until the water turned slightly cloudy, indicating the presence of zoospores. Spores were viewed under a microscope to verify motility and to determine zoospore density. Plastic petri dishes (60 mL) containing a one glass microscope slide and 40 mL of test solution were randomly placed in a temperature controlled room at 15 ± 1°C. A quantity of 7,500 spores per mL was added to each test container. After 48 hours, the slides were removed and examined

by a light microscope. At the end of the test, a minimum of 100 spores per replicate were counted and scored as germinated or not germinated to determine the percent germination. Ten of the germinated spores were used to determine growth by measuring germination-tube lengths. Test conditions for the *M. pyrifera* proportion germinated and germination tube growth-length test are summarized in Table 1-1.

Test Acceptability Criteria

The criteria used to determine test acceptability were the following:

1. Mean control germination of at least 70%,
2. Mean germination-tube length in control of at least 10 µm,
3. Reference toxicant no-observed-effect concentration (NOEC) must be less than 110 µg/L for germination and less than 35 µg/L for growth,
4. The analysis of variance (ANOVA) Mean Square Error in the reference toxicant test must not exceed 70% for the germination endpoint and 12 um for the growth endpoint.

1.2.3.3 *Atherinops affinis Test*

Test animals were supplied by Aquatic BioSystems, Fort Collins, CO. Prior to test initiation, animals were received and acclimated to test temperature and dilution water salinity. The test was run at 20 ± 1°C with five replicates of 600 mL plastic containers containing 200 mL of test solution. Five larvae were added to each test chamber under a 16-hour light: 8-hour dark photoperiod. Renewals were performed daily and test organisms were fed a concentrated *Artemia* suspension (~200 nauplii) twice daily. DO, temperature, salinity, and pH were measured daily. All instruments used by WESTON were calibrated daily and calibration curves were documented in equipment calibration logs. Upon test termination, survival counts were performed and the EC_{50} for survival was calculated. Topsmelt larvae were then dried at 100°C in an oven for a minimum of 6 hours and weighed to determine the mean weight. Test conditions for the *A. affinis* survival and growth test are summarized in Table 1-1.

Test Acceptability Criteria

The criteria used to determine test acceptability were the following:

1. Control survival to equal or exceed 80%,
2. Mean weight per larva must exceed 0.85 mg in the reference and brine controls,
3. The LC_{50} must be within two standard deviations of the laboratory control chart mean,
4. Minimum significant difference (%MSD) of less than 25% relative to the control for survival for the reference toxicant test, and less than 50% relative to the control for growth for the reference toxicant test.

1.2.3.4 *Strongylocentrotus purpuratus Test*

Adult test animals were supplied by Dave Gutoff of San Diego, CA. Sea urchins were acclimated to dilution water salinity at a rate not exceeding 1 ppt per hour. Spawning was attempted using urchins from salinities containing surviving adults, and echinoderm development tests were conducted using animals from salinities with successful spawns. Observations during acclimation and spawning were recorded. To initiate the echinoderm development test, fertilized sea urchin eggs from salinities with successful spawns were allowed to begin dividing. Approximately 300 larvae were targeted for addition to each test chamber. The test was run for 96 hours in pre-cleaned 20 mL glass scintillation vials under

ambient light with a 16-hour light: 8-hour dark photoperiod. DO, salinity, and hydrogen ion concentration (pH) were measured at test initiation and termination; temperature was monitored daily. All instruments used by WESTON were calibrated daily and calibration curves were documented in equipment calibration logs. At 48 hours, half the replicates from each salinity were preserved for counts. At 96 hours, larvae in the remaining half of the replicates from each salinity were preserved. After counts were performed, statistics were run and the median effects concentration (EC_{50}) for survival and normality were calculated. Test conditions for the *S. purpuratus* proportion alive and proportion normal test are summarized in Table 1-1.

Test Acceptability Criteria

The criteria used to determine test acceptability were the following:

1. Larval normality to equal or exceed 80% in the controls,
2. Minimum significant difference (%MSD) is less than or equal to 20% relative to the controls.

1.2.3.5 *Haliotis rufescens Test*

Test animals were supplied by The Cultured Abalone, Goleta, CA. Adult animals were allowed to acclimate to test conditions for approximately 24 to 48 hours prior to test initiation to minimize stress-related spawning difficulties. Abalone eggs were fertilized and allowed to begin dividing, and the test was run at 15 ± 1°C for 48 hours in 600 mL containers containing 200 mL test solution in five separate replicates. Approximately 5 to 10 larvae per mL test solution were targeted for addition to each test chamber. Testing occurred under ambient laboratory light with a 16-hour light: 8-hour dark photoperiod. DO, salinity, and pH were measured at test initiation and termination, and temperature was monitored constantly. At 48 hours, larvae were preserved to be counted at a later date. After counts were performed, statistics were run and the median effective concentration (EC_{50}) for normality was calculated. Test conditions for the *H. rufescens* development test are summarized in Table 1-1.

Test Acceptability Criteria

The criteria used to determine test acceptability were the following:

1. Mean larval normality to equal or exceed 80% in the controls,
2. Response from 56 µg/L zinc treatment must be significantly different from the control response,
3. Minimum significant difference (%MSD) is less than or equal to 20% relative to the control for the reference toxicant.

1.2.4 SUMMARY OF ACUTE BIOASSAY TEST PROCEDURES

1.2.4.1 *A. affinis Test*

Test animals were supplied by Aquatic BioSystems, Fort Collins, CO. Prior to test initiation, animals were received and acclimated to test temperature and dilution water salinity. Five organisms were added to each test chamber and exposed to 200 mL of test solution in five replicates. The test was run for 96 hours at 20 ± 1°C in 250 mL plastic containers under ambient light with a 16-hour light: 8-hour dark photoperiod. Renewals were performed daily and test organisms were fed newly hatched *Artemia* (~100 nauplii) once daily prior to renewal. DO, temperature, salinity, and pH were measured daily. All instruments used by

WESTON were calibrated daily and calibration curves were documented in equipment calibration logs. At 96 hours, mortality counts were performed and statistics were run in which the EC_{50} for survival was calculated. Test conditions for the *A. affinis* acute survival test are summarized in Table 1-2.

Test Acceptability Criteria

The criteria used to determine test acceptability were the following:

1. Control survival to equal or exceed 90%.

1.2.4.2 *Citharichthys stigmaeus Test*

Test animals were supplied by John Brezina, Dillon Beach, CA. Prior to test initiation, mysids were received and acclimated to test temperature and dilution water salinity. Ten organisms were added to each test chamber in four replicates. The test was run for 96 hours in 250 mL plastic containers containing 200 mL test solution under ambient light with a 16-hour light: 8-hour dark photoperiod. Test temperature was maintained at 12 ± 1°C throughout testing. Renewals were performed daily and test organisms were fed newly hatched *Artemia* (~100 nauplii) once daily prior to renewal. DO, temperature, salinity, and pH were measured daily. All instruments used by WESTON were calibrated daily and calibration curves were documented in equipment calibration logs. At 96 hours, mortality counts were performed and statistics were run in which the EC_{50} for survival was calculated. Test conditions for the *C. stigmaeus* acute survival test are summarized in Table 1-2.

Test Acceptability Criteria

The criteria used to determine test acceptability were the following:

1. Control survival to equal or exceed 90%.

1.2.4.3 *A. bahia Test*

Mysid shrimp were obtained from Aquatic BioSystems, Fort Collins, CO. Prior to test initiation, mysids were received and acclimated to test temperature of (25 ± 1°C) and dilution water salinity. The test was run with eight replicates of 250 mL plastic containers containing 200 mL of test solution and five mysids per container under a 16-hour light: 8-hour dark photoperiod. Eighty percent of the test solution was replaced each day of the test. The mysids were fed 0.2 mL of a concentrated newly-hatched *Artemia* suspension (~500 nauplii) once daily. Daily temperature, DO, pH, and salinity were measured for final (previous day) and initial (renewal) solutions. Mortality and behavior were recorded daily. Test conditions for the *A. bahia* acute survival test are summarized in Table 1-17.

Test Acceptability Criteria

The criteria used to determine test acceptability were the following:

1. Control survival to equal or exceed 90%.

1.2.5 STATISTICAL ANALYSIS

At the conclusion of all tests, test species data were evaluated statistically using ToxCalc™ to determine EC_p, NOEC, and Chronic Toxicity Unit (TU_C) values where appropriate. ToxCalc™ is a comprehensive statistical application that follows standard guidelines for acute and chronic toxicity data analysis.

Statistical effects can be measured by the EC_p, the estimated concentration that causes any effect, either lethal (LC) or sublethal (IC), on p% of the test population. The LC_{50} or LC_{25} is the point estimate of the concentration at which a lethal effect is observed in 50 or 25% of the test organisms. The IC_{50} or IC_{25} values are calculated with chronic toxicity bioassays and are point estimates of the concentration at which an inhibitory effect in a sublethal parameter (i.e. growth, reproduction) is observed in 50 or 25% of the organisms. In the case of high salinity testing, the concentration refers to salinity and not percent dilution. EC_p values include 95% confidence limits where available.

The NOEC is the highest tested concentration at which mortality and other sublethal measured effects are not significantly different from the same parameters in the control. TU_C values are defined as $100\%/EC_{25}$ or 100%/NOEC, when an EC_{25} is not calculable.

1.3 RESULTS

1.3.1 PHASE I: SAMPLES COLLECTED FEBRUARY 7 & FEBRUARY 11, 2011

Procedure and organism data for *A. bahia*, *M. pyrifera, S. purpuratus, H. rufescens, A. affinis* and *C. stigmaeus* tests performed during Episode 1 are summarized in Table 1-1 and Table 1-2. Copies of Phase I laboratory benchsheets are provided in Appendix A.

1.3.1.1 *A. bahia Chronic and Acute Bioassays*

Water Quality and Test Acceptability Criteria

For the duration of testing, all water quality parameters were within acceptable limits. All test acceptability criteria were met. Salinity study toxicity test results are presented in Table 1-3 and reference toxicant test results are presented in Table 1-4. Acute toxicity test results are presented in Table 1-5.

Survival

The average 7-day survival rates of *A. bahia* in the laboratory control treatments was 97.5%. Mean survival rates in the 33, 42, 51, 60, and 70 ppt concentrations were 97.5, 87.5, 0.0, 0.0, and 0.0%, respectively. Statistically significant effects on *A. bahia* survival were observed in the 51, 60, and 70 ppt dilutions. Consequently, the NOEC for survival was 42 ppt. The LC_{50} value was 45.3 ppt, and the survival TU_C (100% / NOEC) was 2.38.

Biomass

The mean dry weight of the laboratory control mysids was 0.35 mg. Mean biomass values in the 33, 42, 51, 60, and 70 ppt concentrations were 0.38, 0.34, 0.0, 0.0, and 0.0 mg, respectively. Statistically significant effects on *A. bahia* growth were observed in the 51, 60, and 70 ppt dilutions. Consequently, the biomass NOEC was 42 ppt. The IC_{25} and IC_{50} values were 43.8 ppt and 46.2 ppt, respectively. The TU_C (100% / IC_{25}) for the growth endpoint was 2.29.

Acute Survival

The average 96-hour survival rate for the laboratory control treatment was 100%. Mean survival rates in the 33, 42, 51, 60, 70 ppt concentrations were 100, 92.5, 0.0, 0.0, and 0.0%, respectively. A statistically significant effect on survival was seen in the 51 ppt treatment relative to the laboratory control. Consequently, the NOEC for survival was 42 ppt. The EC_{50} value was 45.5 ppt, and the TU_A (100% / NOEC) for the acute survival endpoint was 2.38.

Reference Toxicant Test (Copper Sulfate)

The reference toxicant test produced a LC_{50} value of 147.1 ppb $CuSO_4$. This value is within two standard deviations of the laboratory mean (251.1 ± 117.8 ppb $CuSO_4$), indicating normal sensitivity of the test organisms for survival. The IC_{50} for biomass was 156.4 ppb $CuSO_4$. This value is within two standard deviations of the laboratory mean (229.7 ± 130.6 ppb $CuSO_4$), indicating normal sensitivity for growth.

Table 1-1. Phase I Chronic Bioassays: Procedure and Organism Data

Parameter	Test Species		
	Americamysis bahia	***Macrocystis pyrifera***	***Strongylocentrotus purpuratus***
Sample Properties			
Dates Sampled	February 7 & February 11, 2011	February 7 & February 11, 2011	February 7 & February 11, 2011
Dates Received	February 9 & February 11, 2011	February 9 & February 11, 2011	February 9 & February 11, 2011
Volume Received	~ 20 L per sample (80 L total)	~ 20 L per sample (80 L total)	~ 20 L per sample (80 L total)
Storage Conditions	4°C, dark	4°C, dark	4°C, dark
Test Species Information			
Supplier	Aquatic BioSystems, Fort Collins, CO	Dave Gutoff, San Diego, CA	Dave Gutoff, San Diego, CA
Date Acquired	February 8, 2011	February 8, 2011	February 16, 2011
Acquired Temperature	18.6°C	N/A	N/A
Age Group	7 days old	Mature	Embryos
Test Procedures			
Type/Duration	Chronic/Renewal; 7 days	Chronic/Static; 48 hours	Chronic/Static; 96 hours
Test Dates	February 8 - 15, 2011	February 8 - 10, 2011	February 16 - 20, 2011
Control/Dilution H_2O	Filtered, UV-treated seawater from Scripps Institute of Oceanography, La Jolla, CA	Filtered, UV-treated seawater from Scripps Institute of Oceanography, La Jolla, CA	Filtered, UV-treated seawater from Scripps Institute of Oceanography, La Jolla, CA
Temperature (recommended/actual)	26 ± 1°C / 24.5 – 27.2°C	15 ± 1°C / 14.5 – 16.0°C	15 ± 1°C / 15.4 - 16.1°C
Photoperiod	16-hour light: 8-hour dark	16-hour light: 8-hour dark	Ambient laboratory
Salinity (recommended/actual)	20 – 30 ± 2 ppt / NA	34 ± 2 ppt / NA	30 ± 2 ppt / NA
Test Chamber	400 mL containers	60 mL petri dish	20 mL scintillation vial
Exposure Volume	150 mL	40 mL	5 mL
Animals/Replicate	5	7500 spores / mL	2000 eggs, 5 x 10^6 sperm
Replicates/Treatment	8	5	4
Feeding	Newly hatched *Artemia* nauplii (~750 nauplii, twice daily)	None	None
Deviations From Protocol	None	None	None

Table 1-1. Phase I Chronic Bioassays: Procedure and Organism Data (Continued)

Parameter	Test Species	
	Haliotis rufescens	***Atherinops affinis***
Sample Properties		
Dates Sampled	February 7 & February 11, 2011	February 7 & February 11, 2011
Dates Received	February 9 & February 11, 2011	February 9 & February 11, 2011
Volume Received	~ 20 L per sample (80 L total)	~ 20 L per sample (80 L total)
Storage Conditions	4°C, dark	4°C, dark
Test Species Information		
Supplier	The Cultured Abalone, Goleta, CA	Aquatic BioSystems, Fort Collins, CO
Date Acquired	March 1, 2011	March 30, 2011
Acquired Temperature	N/A	18.4 – 18.7°C
Age Group	Embryos	13 days old
Test Procedures		
Type/Duration	Chronic/Static; 48 hours	Chronic/Renewal; 7 days
Test Dates	March 2 - 4, 2011	April 1 - 8, 2011
Control/Dilution H_2O	Filtered, UV-treated seawater from Scripps Institute of Oceanography, La Jolla, CA	Filtered, UV-treated seawater from Scripps Institute of Oceanography, La Jolla, CA
Temperature (recommended/actual)	15 ± 1°C / 14.5 – 16.0°C	20 ± 1°C / 18.7 – 20.6°C
Photoperiod	16-hour light: 8-hour dark	16-hour light: 8-hour dark
Salinity (recommended/actual)	34 ± 2 ppt / NA	32 ± 2 ppt / NA
Test Chamber	600 mL containers	600 mL containers
Exposure Volume	200 mL	200 mL
Animals/Replicate	5 – 10 larvae / mL	5
Replicates/Treatment	5	5
Feeding	None	Newly hatched *Artemia* nauplii (~200 nauplii, twice daily)
Deviations From Protocol	None	None

Table 1-2. Phase I Acute Bioassays: Procedure and Organism Data

Parameter	Test Species		
	Atherinops affinis	***Citharichthys stigmaeus***	***Americamysis bahia***
Sample Properties			
Dates Sampled	February 7 & February 11, 2011	February 7 & February 11, 2011	February 7 & February 11, 2011
Dates Received	February 9 & February 11, 2011	February 9 & February 11, 2011	February 9 & February 11, 2011
Volume Received	~ 20 L per sample (80 L total)	~ 20 L per sample (80 L total)	~ 20 L per sample (80 L total)
Storage Conditions	4°C, dark	4°C, dark	4°C, dark
Test Species Information			
Supplier	Aquatic BioSystems, Fort Collins, CO	John Brezina, Dillon Beach, CA	Aquatic BioSystems, Fort Collins, CO
Date Acquired	March 30, 2011	February 9, 2011	February 8, 2011
Acquired Temperature	18.4 – 18.7°C	12.3°C	18.6°C
Age Group	13 days old	Juvenile	7 days old
Test Procedures			
Type/Duration	Acute/Renewal; 96 hours	Acute/Renewal; 96 hours	Acute/Renewal; 96 hours
Test Dates	April 1 - 5, 2011	February10 -14, 2011	February 8 -12, 2011
Control/Dilution H_2O	Filtered, UV-treated seawater from Scripps Institute of Oceanography, La Jolla, CA	Filtered, UV-treated seawater from Scripps Institute of Oceanography, La Jolla, CA	Filtered, UV-treated seawater from Scripps Institute of Oceanography, La Jolla, CA
Temperature (recommended/actual)	20 ± 1°C / 18.7 – 20.6°C	12 ± 1°C / 10.6 – 13.3°C	26 ± 1°C / 24.5 – 27.2°C
Photoperiod	16-hour light: 8-hour dark	16-hour light: 8-hour dark	16-hour light: 8-hour dark
Salinity (recommended/actual)	10 – 30 ppt / NA	32 – 34 ppt / NA	5 – 30 ± 1 ppt / NA
Test Chamber	250 mL containers	250 mL containers	250 mL containers
Exposure Volume	200 mL	200 mL	200 mL
Animals/Replicate	5	10	5
Replicates/Treatment	5	4	8
Feeding	Newly hatched *Artemia* nauplii (~100 nauplii, once prior to renewal)	Newly hatched *Artemia* nauplii (~100 nauplii, once prior to renewal)	Newly hatched *Artemia* nauplii (~100 nauplii, daily)
Deviations From Protocol	None	None	None

Table 1-3. Phase I Test Results: 7-day Chronic Toxicity Bioassay using *Americamysis bahia*

Sample Information
Sample Collected: February 7 & February 11, 2011
Sample Received: February 9 & February 11, 2011
Test Dates: February 8 - 15, 2011

Test Parameters
Concentrations (ppt): 33, 42, 51, 60, 70
Species Common Name: Mysid shrimp
Test Endpoints: Survival, Growth
Test Protocol: EPA/821/R-02/014

SURVIVAL ENDPOINT

Concentration (ppt)	(Mean ± SD)	% Survival in Replicates							
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
70	0.0 ± 0.0	0	0	0	0	0	0	0	0
60	0.0 ± 0.0	0	0	0	0	0	0	0	0
51	0.0 ± 0.0	0	0	0	0	0	0	0	0
42	87.5 ± 14.9	60	100	100	80	100	100	80	80
33	97.5 ± 7.1	100	100	100	100	100	80	100	100
Lab Control	97.5 ± 7.1	100	100	100	100	100	100	80	100

Statistical Summary	
Parameter	Saline Concentration (ppt)
LC_{25}	43.5
LC_{50}	45.3
NOEC	42
LOEC	51
TU_C ($100/LC_{25}$)	2.30

BIOMASS ENDPOINT

Concentration (ppt)	Mean Weight (mg)
70	0.00
60	0.00
51	0.00
42	0.34
33	0.38
Laboratory Control	0.35
Statistical Summary	
Parameter	Salinity (ppt)
IC_{15}	42.8
IC_{25}	43.8
IC_{40}	45.2
IC_{50}	46.2
NOEC	42
LOEC	51
TU_C ($100/IC_{25}$)	2.29

Table 1-4. Phase I Reference Toxicant Test Results for the 7-day Chronic Toxicity Bioassay using *A. bahia*

Sample Information
Sample Collected: February 7 & February 11, 2011
Sample Received: February 9 & February 11, 2011
Test Dates: February 8 - 15, 2011

Test Parameters
Concentrations (ppb): 62.5, 125, 250, 500, 1000
Species Common Name: Mysid shrimp
Test Protocol: EPA/821/R-02/014

SURVIVAL ENDPOINT

Concentration (ppb $CuSO_4$)	Percent Survival (Mean ± SD)	% Survival in Replicates							
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
1000	2.5 ± 7.1	0	0	20	0	0	0	0	0
500	0.0 ± 0.0	0	0	0	0	0	0	0	0
250	2.5 ± 7.1	0	0	0	20	0	0	0	0
125	72.5 ± 26.0	20	60	80	80	100	80	60	100
62.5	100.0 ± 0.0	100	100	100	100	100	100	100	100
Control	100.0 ± 0.0	100	100	100	100	100	100	100	100

Statistical Summary	
Parameter	Concentration (ppb $CuSO_4$)
LC_{25}	122.7
LC_{50}	147.1
NOEC	62.5
LOEC	125

BIOMASS ENDPOINT

Concentration (ppb $CuSO_4$)	Mean Weight (mg)
1000	0.00
500	0.00
250	0.03
125	0.26
62.5	0.43
Control	0.38
Statistical Summary	
Parameter	Concentration (ppb $CuSO_4$)
IC_{15}	88.9
IC_{25}	106.4
IC_{40}	134.6
IC_{50}	156.4
NOEC	62.5
LOEC	125

Table 1-5. Phase I Test Results: 96-hr Acute Toxicity Bioassay using *A. bahia*

Sample Information

Sample Collected: February 7 & February 11, 2011
Sample Received: February 9 & February 11, 2011
Test Dates: February 8 - 12, 2011

Test Parameters:

Concentrations (ppt): 33, 42, 51, 60, 70
Common Name: Mysid shrimp
Test Endpoints: Acute survival
Test Protocol: EPA/600/R-95/136

SURVIVAL ENDPOINT

Saline Concentration (ppt)	Proportion Alive (Mean ± SD)	Proportion Alive in Replicates							
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
70	0.0 ± 0.0	0	0	0	0	0	0	0	0
60	0.0 ± 0.0	0	0	0	0	0	0	0	0
51	0.0 ± 0.0	0	0	0	0	0	0	0	0
42	92.5 ± 10.4	80	100	100	80	100	100	80	100
33	100.0 ± 0.0	100	100	100	100	100	100	100	100
Laboratory Control	100.0 ± 0.0	100	100	100	100	100	100	100	100

Statistical Summary

Parameter	Saline Concentration (ppt)
EC_{25}	–
EC_{50}	45.5
NOEC	42
LOEC	51
TU_C (100/NOEC)	2.38

1.3.1.2 *Macrocystis pyrifera Chronic Bioassay*

Water Quality and Test Acceptability Criteria

All water quality parameters were within test acceptability limits throughout the test, and all test acceptability criteria were met. Salinity toxicity test results are presented in Table 1-5 and reference toxicant test results are presented in Table 1-6.

Proportion Germinated

The mean proportion germinated in the laboratory control treatment was 89.6%. The mean proportions that germinated in the 33, 42, 51, 60 and 70 ppt concentrations were 90.8, 83.8, 73.0, 21.6, and 0.0%, respectively. Statistically significant effects on *M. pyrifera* germination were observed in the 51, 60, and 70 ppt concentrations relative to the laboratory control. Consequently, the NOEC for the proportion germinated endpoint was 42 ppt. The LC_{25} and LC_{50} values were 51.9 ppt and 54.7 ppt, respectively. The TU_C (100% / EC_{25}) for the proportion germinated endpoint was 1.93.

Growth-Length

The mean germination tube length for the laboratory control was 13.0 µm. The mean germination tube length values in the 33, 42, 51, 60, and 70 ppt concentrations were 13.1, 12.7, 9.0, 5.6 and 0.0 µm, respectively. Statistically significant effects were observed in the 51, 60, and 70 ppt concentrations when compared to the laboratory control. Consequently, the growth NOEC was 42 ppt. The IC_{25} and IC_{50} values were 49.1 ppt and 57.5 ppt, respectively. The TU_C (100% / IC_{25}) for the growth endpoint was 2.04.

Reference Toxicant Test (Copper Chloride)

The *M. pyrifera* reference toxicant test produced an EC_{50} value of 102.1 ppb $CuCl_2$ for the proportion germinated endpoint. This value is within two standard deviations of the laboratory mean (96.7 ± 78.6 ppb $CuCl_2$), indicating normal sensitivity of the test organisms based on germination. The EC_{50} for the germination tube length endpoint was 166.8 ppb $CuCl_2$. This value is also within two standard deviations of the laboratory mean (217.0 ± 349.9 ppb $CuCl_2$), indicating normal sensitivity for growth.

Table 1-6. Phase I High Salinity Test Results for the 48-hr Chronic Toxicity Bioassay using *Macrocystis pyrifera*

Sample Information:
Sample Collected: February 7 & February 11, 2011
Sample Received: February 9 & February 11, 2011
Test Dates: February 8 -10, 2011

Test Parameters:
Concentrations (ppt): 33, 42, 51, 60, 70
Common Name: Giant kelp
Test Endpoints: Germination, Growth
Test Protocol: EPA/600/R-95/136

PROPORTION GERMINATED

Concentration (ppt)	Prop. Germinated (Mean ± SD)	Proportion Germinated in Replicates				
		1	*2*	*3*	*4*	*5*
70	0.0 ± 0.0	0	0	0	0	0
60	21.6 ± 5.3	27	25	17	24	15
51	73.0 ± 3.2	75	77	71	69	73
42	83.8 ± 6.1	85	88	87	86	73
33	90.8 ± 2.9	86	92	93	93	90
Control	89.6 ± 2.9	89	88	92	93	86

Statistical Summary

Parameter	Saline Concentration (ppt)
EC_{25}	51.9
EC_{50}	54.7
NOEC	42
LOEC	51
TU_C ($100/EC_{25}$)	1.93

GERM TUBE GROWTH-LENGTH

Concentration (ppt)	Mean Length (µg)
70	0.00
60	5.55
51	9.00
42	12.70
33	13.05
Control	13.00

Statistical Summary

Parameter	Saline Concentration (ppt)
IC_{15}	46.0
IC_{25}	49.1
IC_{40}	54.1
IC_{50}	57.5
NOEC	42
LOEC	51
TU_C ($100/IC_{25}$)	2.04

Table 1-7. Phase I Reference Toxicant Test Results 48-hr Chronic Toxicity Bioassay using *M. pyrifera*

Sample Information
Sample Collected: February 7 & February 11, 2011
Sample Received: February 9 & February 11, 2011
Test Dates: February 8 -10, 2011

Test Parameters
Concentration (ppb): 5.6, 10, 18, 32, 100, 180
Common Name: Giant kelp
Test Endpoints: Germination, Growth
Test Protocol: EPA/600/R-95/136

PROPORTION GERMINATED

Concentration (ppb $CuCl_2$)	(Mean ± SD)	Proportion Germinated in Replicates				
		1	*2*	*3*	*4*	*5*
180	22.4 ± 9.5	19	20	15	19	39
100	44.0 ± 4.3	44	38	49	47	42
32	79.0 ± 3.1	79	84	77	79	76
18	86.4 ± 2.2	87	86	83	89	87
10	84.8 ± 2.7	83	87	86	81	87
6	85.2 ± 3.3	84	82	83	90	87
Control	89.6 ± 2.9	89	88	92	93	86

Statistical Summary

Parameter	Concentration (ppb $CuCl_2$)
EC_{25}	57.1
EC_{50}	102.1
NOEC	18
LOEC	32
TU_C ($100/EC_{25}$)	1.75

GERM TUBE GROWTH-LENGTH

Concentration (ppb)	Mean Length (µg)
180	6.55
100	7.80
32	11.50
18	12.50
10	12.30
6	13.20
Control	13.00

Statistical Summary

Parameter	Concentration (ppb $CuCl_2$)
EC_{15}	37.1
EC_{25}	62.8
EC_{40}	115.5
EC_{50}	166.8
NOEC	18
LOEC	32
TU_C ($100/EC_{25}$)	1.59

1.3.1.3 *S. purpuratus Chronic Bioassay*

Water Quality and Test Acceptability Criteria

All water quality parameters were within acceptable limits throughout the 96-hour test duration. Upon test termination, the laboratory control met the criteria for test acceptability. Salinity toxicity test results are presented in Table 1-7 and reference toxicant test results are presented in Table 1-8.

Proportion Alive

The average proportion alive for *S. purpuratus* was 62.2% for the laboratory control treatment. Mean survival rates in the 33, 42, 51, 60, and 70 ppt concentrations were 62.0, 76.8, 88.4, 89.8, and 76.2%, respectively. No statistically significant effects on survival were observed relative to the laboratory control treatment. Consequently, the NOEC for survival was 69.1 ppt. The IC_{25} and IC_{50} values were both greater than 69.1 ppt. The TU_C (100% / NOEC) for the proportion alive endpoint was 1.45.

Proportion Normal

The mean proportion normal observed in the laboratory control treatment for the *S. purpuratus* test was 94.8 percent. Mean proportion normal in the 33, 42, 51, 60, and 70 ppt concentrations were 89.2, 0.0, 0.0, 0.0, and 0.0%, respectively. There was not a statistically significant effect observed in the 33 ppt salinity treatment when compared to the laboratory control. Consequently, the NOEC for the proportion normal endpoint was 33 ppt. The IC_{25} value was 34.8 ppt and the IC_{50} value was 36.9 ppt, and the TU_C (100% / IC_{25}) for the proportion normal endpoint was 2.87.

Reference Toxicant Test (Copper Sulfate)

The reference toxicant test produced an EC_{50} value of 7.99 ppb $CuSO_4$ for the proportion normal endpoint. This value is outside two standard deviations from the laboratory mean (16.3 ± 8.04 ppb $CuSO_4$), indicating higher than normal sensitivity of the test organisms.

Table 1-8. Phase I Test Results: 96-hr Chronic Toxicity Bioassay using *Strongylocentrotus. purpuratus*

Sample Information
Sample Collected: February 7 & February 11, 2011
Sample Received: February 9 & February 11, 2011
Test Dates: February 16 - 20, 2011

Test Parameters
Concentrations (ppt): 33, 42, 51, 60, 70
Common Name: Purple urchin
Test Endpoints: Survival, Development
Test Protocol: EPA/600/R-95/136

SURVIVAL ENDPOINT

Saline Concentration (ppt)	Proportion Alive (Mean ± SD)	Proportion Alive in Replicates			
		1	*2*	*3*	*4*
70	76.2 ± 7.7	76	80	83	66
60	89.8 ± 10.3	89	76	100	94
51	88.4 ± 9.8	100	82	93	78
42	76.8 ± 8.3	80	67	74	86
33	62.0 ± 3.8	67	58	60	62
Laboratory Control	62.2 ± 10.4	77	55	54	62

Statistical Summary	
Parameter	Saline Concentration (ppt)
IC_{25}	> 70
IC_{50}	> 70
NOEC	70
LOEC	> 70
TU_c (100/NOEC)	1.45

DEVELOPMENTAL ENDPOINT

Saline Concentration (ppt)	Proportion Normal (Mean ± SD)	Proportion Normal in Replicates			
		1	*2*	*3*	*4*
70	0.0 ± 0.0	0	0	0	0
60	0.0 ± 0.0	0	0	0	0
51	0.0 ± 0.0	0	0	0	0
42	0.0 ± 0.0	0	0	0	0
33	89.2 ± 5.0	92	86	84	95
Laboratory Control	94.8 ± 2.6	95	97	91	96

Statistical Summary	
Parameter	Saline Concentration (ppt)
IC_{25}	34.8
IC_{50}	36.9
NOEC	32.4
LOEC	33
TU_c (100/IC_{25})	2.87

Table 1-9. Phase I Reference Toxicant Test Results for the 96-hr Chronic Toxicity Bioassay using *S. purpuratus*

Sample Information
Sample Collected: February 7 & February 11, 2011
Sample Received: February 9 & February 11, 2011
Test Dates: February 16 - 20, 2011

Test Parameters:
Concentrations (ppb): 3.75, 7.5, 15, 30
Common Name: Purple urchin
Test Endpoints: Developmental
Test Protocol: EPA/600/R-95/136

DEVELOPMENTAL ENDPOINT

Concentration (ppb $CuSO_4$)	Proportion Normal (Mean ± SD)	Proportion Normal in Replicates			
		1	*2*	*3*	*4*
30	0.0 ± 0.0	0	0	0	0
15	4.9 ± 1.5	4	3	7	6
7.5	57.5 ± 5.5	55	65	53	57
3.75	77.3 ± 1.6	79	77	78	75
Laboratory Control	94.4 ± 1.4	96	95	94	93

Statistical Summary	
Parameter	Concentration (ppb $CuSO_4$)
EC_{25}	–
EC_{50}	8.0
NOEC	< 3.75
LOEC	3.75
TU_c (100/NOEC)	26.67

1.3.1.4 *H. rufescens Chronic Bioassay*

Water Quality and Test Acceptability Criteria

All water quality parameters were within acceptable limits throughout the 48-hour test duration. Upon test termination, the laboratory control met the criteria for test acceptability. Salinity toxicity test results are presented in Table 1-9 and reference toxicant test results are presented in Table 1-10.

Proportion Normal

The mean proportion normal observed during the *H. rufescens* test was 90.0% for the laboratory control treatment. Mean proportion normal in the 33, 42, 51, 60, and 70 ppt concentrations were 91.0, 0.0, 0.0, 0.0, and 0.0%, respectively. A statistically significant effect on survival was seen in the 42 ppt salinity treatment relative to the laboratory control. Consequently, the NOEC for survival was 33 ppt. The IC_{25} and IC_{50} values were 35.3 ppt and 37.2 ppt, respectively. The TU_C (100% / IC_{25}) for the proportion normal endpoint was 2.84.

Reference Toxicant Test (Zinc Sulfate)

The reference toxicant test produced an IC_{50} value of 60.6 ppb $ZnSO_4$ for the proportion normal endpoint. This value is within two standard deviations from the laboratory mean (32.2 ± 32.0 ppb $ZnSO_4$), indicating normal sensitivity of the test organisms.

Table 1-10. Phase I Test Results: 48-hr Chronic Toxicity Bioassay using *Haliotis rufescens*

Sample Information
Sample Collected: February 7 & February 11, 2011
Sample Received: February 9 & February 11, 2011
Test Dates: March 2 - 4, 2011

Test Paramenters
Concentrations (ppt): 33, 42, 51, 60, 70
Common Name: Red abalone
Test Endpoints: Developmental
Test Protocol: EPA/600/R-95/136

DEVELOPMENTAL ENDPOINT

Saline Concentration (ppt)	Proportion Normal (Mean ± S.D.)	Proportion Normal in Replicates			
		1	*2*	*3*	*4*
70	90.0 ± 4.5	90	89	85	96
60	91.0 ± 2.8	93	87	91	93
51	0.0 ± 0.0	0	0	0	0
42	0.0 ± 0.0	0	0	0	0
33	0.0 ± 0.0	0	0	0	0
Laboratory Control	0.0 ± 0.0	0	0	0	0

Statistical Summary	
Parameter	Saline Concentration (ppt)
IC_{25}	35.3
IC_{50}	37.5
NOEC	33
LOEC	–
TU_c ($100/IC_{25}$)	2.84

Table 1-11. Phase I Reference Toxicant Test Results for the 48-hr Chronic Toxicity Bioassay using *H. rufescens*

Sample Information
Sample Collected: February 7 & February 11, 2011
Sample Received: February 9 & February 11, 2011
Test Dates: March 2 - 4, 2011

Test Parameters:
Concentrations (ppb): 10, 18, 32, 56, 100
Common Name: Red abalone
Test Endpoints: Developmental
Test Protocol: EPA/600/R-95/136

DEVELOPMENTAL ENDPOINT

Concentration (ppb $ZnSO_4$)	Proportion Normal (Mean ± SD)	Proportion Normal in Replicates			
		1	*2*	*3*	*4*
100	0.0 ± 0.0	0	0	0	0
56	57.3 ± 17.0	57	34	74	64
32	92.0 ± 2.4	90	93	95	90
18	92.3 ± 1.7	90	93	92	94
10	89.8 ± 2.2	91	92	89	87
Laboratory Control	90.3 ± 1.0	89	91	91	90

Statistical Summary	
Parameter	Concentration (ppb $ZnSO_4$)
IC_{15}	41.7
IC_{25}	48.2
IC_{40}	58.0
IC_{50}	65.0
NOEC	32
LOEC	56
TU_c ($100/IC_{25}$)	2.08

1.3.1.5 *A. affinis Chronic and Acute Bioassays*

Water Quality and Test Acceptability Criteria

All water quality parameters were within acceptable limits throughout the 7-day test duration. Upon test termination, the laboratory control failed to meet the survival criteria for test acceptability, while meeting the growth criteria for test acceptability. Salinity toxicity test results are presented in Table 1-11 and reference toxicant test results are presented in Table 1-12. Acute toxicity test results are presented in Table 1-13.

Survival

The average survival rate observed during testing was 68% for the laboratory control treatment, which falls below the protocol test acceptability criterion of 80%. Mean survival rates in the 33, 42, 51, 60, and 70 ppt concentrations were 76.0, 56.0, 28.0, 0.0, and 0.0%, respectively. A statistically significant effect on survival was seen in the 51 ppt treatment relative to the laboratory control. Consequently, the NOEC for survival was 42 ppt. The LC_{25} and LC_{50} values were 45.1 and 48.5 ppt, respectively. The TU_C (100% / LC_{25}) for the survival endpoint was 2.22.

Biomass

The mean biomass measured for *A. affinis* was 0.833 mg for the laboratory control treatment. Mean biomass in the 33, 42, 51, 60, and 70 ppt concentrations were 0.842, 0.735, 0.453, 0.0, and 0.0 mg, respectively. A statistically significant effect on survival was seen in the 51 ppt treatment relative to the laboratory control. Consequently, the NOEC for biomass was 42 ppt. The IC_{25} and IC_{50} values were 45.4 ppt and 51.7 ppt, respectively. The TU_C (100% / IC_{25}) for the biomass endpoint was 2.20.

Acute Survival

The average 96-hour survival rate for the laboratory control treatment was 68%, which is below the protocol acceptability criterion of 90%. The mean survival rates in the 33, 42, 51, 60, and 70 ppt concentrations were 76.0, 56.0, 28.0, 0.0, and 0.0%, respectively. A statistically significant effect on survival was seen in the 51 ppt treatment relative to the laboratory control. Consequently, the NOEC for survival was 42 ppt. The IC_{25} and IC_{50} values were 45.1 and 48.5 ppt, respectively. The TU_A (100% / IC_{25}) for the survival endpoint was 2.22.

Reference Toxicant Test (Copper Sulfate)

The reference toxicant test produced an IC_{50} value of 93.3 ppb $CuSO_4$ for the survival endpoint. This value is within two standard deviations from the laboratory mean (121.0 ± 50.8 ppb $CuSO_4$), indicating normal sensitivity of the test organisms for the survival endpoint. The IC_{50} for biomass was 97.7 ppb $CuSO_4$. This value is within two standard deviations of the laboratory mean (132.6 ± 52.7 ppb $CuSO_4$), indicating normal sensitivity for the biomass endpoint.

Table 1-12. Phase I Test Results: 7-day Chronic Toxicity Bioassay using *Atherinops affinis*

Sample Information
Sample Collected: February 7 & February 11, 2011
Sample Received: February 9 & February 11, 2011
Test Dates: April 1 - 8, 2011

Test Parameters
Concentrations (ppt): 33, 42, 51, 60, 70
Common Name: Topsmelt
Test Endpoints: Survival and Growth
Test Protocol: EPA/600/R-95/136

SURVIVAL ENDPOINT

Concentration (ppt)	Percent Survival (Mean ± SD)	% Survival in Replicates				
		1	*2*	*3*	*4*	*5*
70	0.0 ± 0.0	0	0	0	0	0
60	0.0 ± 0.0	0	0	0	0	0
51	28.0 ± 17.9	40	40	0	20	40
42	56.0 ± 21.9	80	80	40	40	40
33	76.0 ± 21.9	80	40	80	100	80
Control	68.0 ± 22.8	60	60	40	100	80

Statistical Summary

Parameter	Saline Concentration (ppt)
LC_{25}	45.1
LC_{50}	48.5
NOEC	42
LOEC	51
TU_C ($100/LC_{25}$)	2.22

BIOMASS ENDPOINT

Concentration (ppt)	Mean Weight/Individual (mg)
70	0.00
60	0.00
51	0.45
42	0.74
33	0.84
Control	0.83

Statistical Summary

Parameter	Saline Concentration (ppt)
IC_{15}	42.7
IC_{25}	45.4
IC_{40}	49.4
IC_{50}	51.7
NOEC	42
LOEC	51
TU_C ($100/IC_{25}$)	2.20

Table 1-13. Phase I Reference Toxicant Test Results for the 7-day Chronic Toxicity Bioassay using *A. affinis*

Sample Information
Sample Collected: February 7 & February 11, 2011
Sample Received: February 9 & February 11, 2011
Test Dates: April 1 - 8, 2011

Test Paramenters
Concentration (ppb): 25, 50, 100, 200, 400
Common Name: Topsmelt
Test Endpoints: Survival and Growth
Test Protocol: EPA/600/R-95/136

SURVIVAL ENDPOINT

Concentration (ppb $CuSO_4$)	(Mean ± SD)	% Survival in Replicates				
		1	*2*	*3*	*4*	*5*
400	0.0 ± 0.0	0	0	0	0	0
200	4.0 ± 8.9	20	0	0	0	0
100	32.0 ± 17.9	20	20	60	40	20
50	72.0 ± 17.9	80	60	60	100	60
25	76.0 ± 21.9	80	100	40	80	80
Control	76.0 ± 8.9	80	80	80	60	80
Statistical Summary						
Parameter	Concentration (ppb $CuSO_4$)					
LC_{25}	68.9					
LC_{50}	93.3					
NOEC	50					
LOEC	100					
TU_C ($100/LC_{25}$)	1.45					

BIOMASS ENDPOINT

Concentration (ppb)	Mean Weight/Individual (mg)
400	0.00
200	0.00
100	0.45
50	0.74
25	0.84
Control	0.83
Statistical Summary	
Parameter	Concentration (ppb $CuSO_4$)
IC_{15}	42.7
IC_{25}	45.4
IC_{40}	49.4
IC_{50}	51.7
NOEC	42
LOEC	51
TU_C ($100/IC_{25}$)	2.20

Table 1-14. Phase I Test Results: 96-hr Acute Toxicity Bioassay using *A. affinis*

Sample Information
Sample Collected: February 7 & February 11, 2011
Sample Received: February 9 & February 11, 2011
Test Dates: April 1 - 4, 2011

Test Parameters
Concentrations (ppt): 33, 42, 51, 60, 70
Test Endpoints: Acute survival
Test Protocol: EPA/600/R-95/136

SURVIVAL ENDPOINT

Saline Concentration (ppt)	Percent Survival (Mean ± SD)	% Survival in Replicates				
		1	*2*	*3*	*4*	*5*
70	0.0 ± 0.0	0	0	0	0	0
60	0.0 ± 0.0	0	0	0	0	0
51	28.0 ± 17.9	40	40	0	20	40
42	56.0 ± 21.9	80	80	40	40	40
33	76.0 ± 21.9	80	40	80	100	80
Laboratory Control	68.0 ± 22.8	60	60	40	100	80

Statistical Summary

Parameter	Saline Concentration (ppt)
IC_{25}	45.1
IC_{50}	48.5
NOEC	42
LOEC	51
TU_A ($100/IC_{25}$)	2.22

1.3.1.6 *Citharichthys stigmaeus Acute Bioassay*

Water Quality and Test Acceptability Criteria

All water quality parameters were within acceptable limits throughout the 96-hour test duration. Upon test termination, the laboratory control met the criteria for test acceptability. Salinity acute toxicity test results are presented in Table 1-14 and reference toxicant test results are presented in Table 1-15.

Acute Survival

The average 96-hour survival rate for the laboratory control treatment was 100%, and the mean survival rates in the 33, 42, 51, 60, and 70 ppt concentrations were 100, 100, 100, 0.0, and 0.0%, respectively. A statistically significant effect on survival was seen in the 60 ppt treatment relative to the laboratory control. Consequently, the NOEC for survival was 51 ppt. The IC_{25} and IC_{50} values were 53.3 ppt and 55.5 ppt, respectively. The TU_A (100% / IC_{25}) for the acute survival endpoint was 1.88.

Reference Toxicant Test (Sodium Dodecyl Sulfate)

The reference toxicant test produced an IC_{25} and IC_{50} value of 2.98 and 2.98 mg SDS/L, respectively. Due to a lack of data points, a control chart could not be generated by statistical software package.

Table 1-15. Phase I Test Results: 96-hr Acute Toxicity Bioassay using *Citharichthys stigmaeus*

Sample Information

Sample Collected: February 7 & February 11, 2011
Sample Received: February 9 & February 11, 2011
Test Dates: February 11 - 14, 2011

Test Parameters

Concentrations (ppt): 33, 42, 51, 60, 70
Common Name: Sanddab
Test Endpoints: Acute survival
Test Protocol: EPA/821/R-02/014

SURVIVAL ENDPOINT

Saline Concentration (ppt)	Percent Survival (Mean ± SD)	% Survival in Replicates		
		1	*2*	*3*
70	0.0 ± 0.0	0	0	0
60	0.0 ± 0.0	0	0	0
51	100.0 ± 0.0	100	100	100
42	100.0 ± 0.0	100	100	100
33	100.0 ± 0.0	100	100	100
Laboratory Control	100.0 ± 0.0	100	100	100

Statistical Summary	
Parameter	Saline Concentration (ppt)
IC_{25}	53.3
IC_{50}	55.5
NOEC	51
LOEC	60
TU_A ($100/IC_{25}$)	1.88

Table 1-16. Phase I Reference Toxicant Test Results for the 96-hr Acute Toxicity Bioassay using *C. stigmaeus*

Sample Information
Sample Collected: February 7 & February 11, 2011
Sample Received: February 9 & February 11, 2011
Test Dates: February 10 - 14, 2011

Test Parameters
Concentrations (ppb): 0.5, 1, 2, 4, 8
Common Name: Sanddab
Test Endpoints: Acute survival
Test Protocol: EPA/821/R-02/012

SURVIVAL ENDPOINT

Concentration (ppb SDS)	Percent Survival (Mean ± SD)	% Survival in Replicates			
		1	*2*		
8	78.0 ± 2.8	76	80		
4	82.4 ± 9.1	89	76		
2	91.2 ± 12.4	100	82		
1	73.6 ± 9.1	80	67		
0.5	62.8 ± 6.2	67	58		
Laboratory Control	66.0 ± 15.3	77	55		

Statistical Summary

Parameter	Concentration (ppb Sodium Dodecyl Sulfate)
IC_{25}	2.5
IC_{50}	3.0
NOEC	2
LOEC	4

1.3.2 PHASE II, EPISODE 1: SAMPLES COLLECTED AUGUST 3 & AUGUST 5, 2011

As discussed in Section 1.1, Phase II chronic toxicity testing was performed with one Phase I species from each trophic level (i.e. plant, invertebrate and vertebrate) most likely to reside in the WBMWD discharge environment. This suite included the 7-day *A. bahia* (mysid shrimp) and *A. affinis* (topsmelt) bioassays, and the 48-hour *M. pyrifera* (giant kelp) bioassay. The 96-hour *S. purpuratus* (purple urchin) test was also included based on the significant volume of high salinity data generated with this species by previous studies.

Phase II acute toxicity testing was performed with the two most sensitive Phase I species: *A. bahia* and *A. affinis*. Phase II procedure and organism data for all tests performed during Phase II, Episodes 1 is summarized in Table 1-16 and Table 1-17. Copies of laboratory benchsheets for all tests are provided in Appendix B.

1.3.2.1 *A. bahia Chronic and Acute Bioassays*

Water Quality and Test Acceptability Criteria

All water quality parameters were consistently within acceptable limits, and all test acceptability criteria were met. Test results are provided in Table 1-18 and reference toxicant test results are presented in Table 1-19. Acute toxicity test results are presented in Table 1-20.

Survival

The mean 7-day survival rate observed for *A. bahia* in the lab control was 97.5%; mean survival rates in the 37, 39, 41, 45, 50 ppt concentrations were 92.5, 92.5, 95.0, 90.0, and 47.5%, respectively. Significant effects on *A. bahia* survival were seen in the 50 ppt treatment compared to the control. Consequently, the NOEC for survival was 45 ppt. The LC_{25} and LC_{50} values were 48 ppt and 50 ppt, and the survival TU_C (100%/EC_{25}) was 2.09.

Biomass

The mean dry weight measured for the control mysids was 0.39 mg. Mean biomass values in the 37, 39, 41, 45, 50 ppt concentrations were 0.39, 0.36, 0.35, 0.27, and 0.17 mg, respectively. Statistically significant effects were observed in the 45 and 50 ppt treatments relative to the lab control. Consequently, the growth NOEC was 41 ppt. The IC_{25} and IC_{50} values were 43.6 ppt and 48.1 ppt, respectively. The growth TU_C (100% / IC_{25}) was 2.29.

Acute Survival

The average 96-hour survival rate for the laboratory control treatment was 97.5%. Mean survival rates in the 37, 39, 41, 45, 50 ppt concentrations were 95.0, 95.0, 97.5, 90.0, and 55.0%, respectively. A statistically significant effect on survival was seen in the 50 ppt treatment relative to the laboratory control. Consequently, the NOEC for survival was 45 ppt. The EC_{25} and EC_{50} values were 48.1 ppt and 50.7 ppt, respectively. The TU_A (100% / EC_{25}) for the survival endpoint was 2.08.

Reference Toxicant Test (Copper Sulfate)

The reference toxicant test produced an LC_{50} value of 209.6 ppb $CuSO_4$. This value is within two standard deviations of the laboratory mean (255.6 ± 117.7 ppb $CuSO_4$) indicating normal sensitivity of the test organisms based on survival. The IC_{50} for biomass was 209.6 ppb $CuSO_4$. This value is within two standard deviations of the laboratory mean (235.5 ± 133.1 ppb $CuSO_4$), indicating normal sensitivity for the growth endpoint.

Table 1-17. Phase II, Episode 1 Chronic Bioassays: Procedure and Organism Data

Parameter	Test Species	
	Americamysis bahia	***Macrocystis pyrifera***
Sample Properties		
Dates Sampled	August 3 & August 5, 2011	August 3 & August 5, 2011
Dates Received	August 4 & August 5, 2011	August 4 & August 5, 2011
Volume Received	~ 20 L per sample (160 L total)	~ 20 L per sample (160 L total)
Storage Conditions	4°C, dark	4°C, dark
Test Species Information		
Supplier	Aquatic BioSystems, Fort Collins, CO	Dave Gutoff, San Diego, CA
Date Acquired	August 4, 2011	August 15, 2011
Acquired Temperature	19.2°C	N/A
Age Group	7 days old	Mature
Test Procedures		
Type/Duration	Chronic/Renewal; 7 days	Chronic/Static; 48 hours
Test Dates	August 4 - 11, 2011	August 15 - 17, 2011
Control/Dilution H_2O	Filtered, UV-treated seawater from Scripps Institute of Oceanography, La Jolla, CA	Filtered, UV-treated seawater from Scripps Institute of Oceanography, La Jolla, CA
Temperature (recommended/actual)	26 ± 1°C / 24.2 – 26.2°C	15 ± 1°C / 15.3 – 16.0°C
Photoperiod	16-hour light: 8-hour dark	16-hour light: 8-hour dark
Salinity (recommended/actual)	20 – 30 ± 2 ppt / NA	34 ± 2 ppt / NA
Test Chamber	400 mL containers	60 mL petri dish
Exposure Volume	150 mL	40 mL
Animals/Replicate	5	7500 spores / mL
Replicates/Treatment	8	5
Feeding	Newly hatched *Artemia* nauplii (~750 nauplii, twice daily)	None
Deviations From Protocol	None	None

Table 1-17. Phase II, Episode 1 Chronic Bioassays: Procedure and Organism Data (Continued)

Parameter	Test Species	
	Strongylocentrotus purpuratus	***Atherinops affinis***
Sample Properties		
Dates Sampled	August 3 & August 5, 2011	August 3 & August 5, 2011
Dates Received	August 4 & August 5, 2011	August 4 & August 5, 2011
Volume Received	~ 20 L per sample (160 L total)	~ 20 L per sample (160 L total)
Storage Conditions	4°C, dark	4°C, dark
Test Species Information		
Supplier	Dave Gutoff, San Diego, CA	Aquatic BioSystems, Fort Collins, CO
Date Acquired	August 23, 2011	August 9, 2011
Acquired Temperature	N/A	19.8°C
Age Group	Adult	12 days old
Test Procedures		
Type/Duration	Chronic/Static; 96 hours	Chronic/Renewal; 7 days
Test Dates	August 23 - 27, 2011	August 10 - 17, 2011
Control/Dilution H_2O	Filtered, UV-treated seawater from Scripps Institute of Oceanography, La Jolla, CA	Filtered, UV-treated seawater from Scripps Institute of Oceanography, La Jolla, CA
Temperature (recommended/actual)	15 ± 1°C / 14.5 - 16.2°C	20 ± 1°C / 19.4 – 21.4°C
Photoperiod	Ambient laboratory	16-hours light: 8-hours dark
Salinity (recommended/actual)	30 ± 2 ppt / NA	32 ± 2 ppt / NA
Test Chamber	20 mL scintillation vial	600 mL containers
Exposure Volume	5 mL	200 mL
Animals/Replicate	2000 eggs, 5 x 10^6 sperm	5
Replicates/Treatment	4	5
Feeding	None	Newly hatched *Artemia* nauplii (~200 nauplii, twice daily)
Deviations From Protocol	None	None

Table 1-18. Phase II, Episode 1 Acute Bioassays: Procedure and Organism Data

Parameter	Test Species	
	Americamysis bahia	***Atherinops affinis***
Sample Properties		
Dates Sampled	August 3 & August 5, 2011	August 3 & August 5, 2011
Dates Received	August 4 & August 5, 2011	August 4 & August 5, 2011
Volume Received	~ 20 L per sample (160 L total)	~ 20 L per sample (160 L total)
Storage Conditions	4°C, dark	4°C, dark
Test Species Information		
Supplier	Aquatic BioSystems, Fort Collins, CO	Aquatic BioSystems, Fort Collins, CO
Date Acquired	August 4, 2011	August 9, 2011
Acquired Temperature	19.2°C	19.8°C
Age Group	7 days old	12 days old
Test Procedures		
Type/Duration	Acute/Renewal; 96 hours	Acute/Renewal; 96 hours
Test Dates	August 4 - 8, 2011	August 10 - 14, 2011
Control/Dilution H_2O	Filtered, UV-treated seawater from Scripps Institute of Oceanography, La Jolla, CA	Filtered, UV-treated seawater from Scripps Institute of Oceanography, La Jolla, CA
Temperature (recommended/actual)	26 ± 1°C / 24.2 – 26.2°C	20 ± 1°C / 19.4 – 21.4°C
Photoperiod	16-hour light: 8-hour dark	16-hour light: 8-hour dark
Salinity (recommended/actual)	5 – 30 ± 1 ppt / NA	10 – 30 ppt / NA
Test Chamber	250 mL containers	250 mL containers
Exposure Volume	200 mL	200 mL
Animals/Replicate	5	5
Replicates/Treatment	8	5
Feeding	Newly hatched *Artemia* nauplii (~100 nauplii, daily)	Newly hatched *Artemia* nauplii (~100 nauplii, once prior to renewal)
Deviations From Protocol	None	None

Table 1-19. Phase II, Episode 1 Results: 7-day Chronic Toxicity Bioassay using *A. bahia*

Sample Information
Sample Collected: August 3 & August 5, 2011
Sample Received: August 4 & August 5, 2011
Test Dates: August 4 - 11, 2011

Test Parameters
Concentrations (ppt): 36.5, 39, 41, 45, 50
Species Common Name: Mysid shrimp
Test Endpoints: Survival, Growth
Test Protocol: EPA/821/R-02/014

SURVIVAL ENDPOINT

Concentration (ppt)	(Mean ± SD)	% Survival in Replicates							
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
50	47.5 ± 26.0	20	40	60	80	80	60	20	20
45	90.0 ± 15.1	100	100	100	100	80	80	60	100
41	95.0 ± 9.3	100	100	80	100	100	100	80	100
39	92.5 ± 14.9	100	80	100	100	60	100	100	100
36.5	92.5 ± 10.4	100	100	80	100	80	100	80	100
Lab Control	97.5 ± 7.1	100	100	100	100	100	100	80	100

Statistical Summary	
Parameter	Saline Concentration (ppt)
LC_{25}	47.9
LC_{50}	50.0
NOEC	45
LOEC	50
TU_C (100/LC_{25})	2.09

BIOMASS ENDPOINT

Saline Concentration (ppt)	Mean Weight (mg)
50	0.15
45	0.27
41	0.35
39	0.36
36.5	0.39
Laboratory Control	0.39
Statistical Summary	
Parameter	Saline Concentration (ppt)
IC_{15}	41.7
IC_{25}	43.7
IC_{40}	46.4
IC_{50}	48.1
NOEC	41
LOEC	45
TU_C (100/IC_{25})	2.29

Table 1-20. Phase II, Episode 1 Reference Toxicant Test Results for the 7-day Chronic Toxicity Bioassay using *A. bahia*

Sample Information
Sample Collected: August 3 & August 5, 2011
Sample Received: August 4 & August 5, 2011
Test Dates: August 4 - 11, 2011

Test Parameters
Concentrations (ppb): 62.5, 125, 250, 500, 1000
Species Common Name: Mysid shrimp
Test Endpoints: Survival and Growth
Test Protocol: EPA/821/R-02/014

SURVIVAL ENDPOINT

Concentration (ppb $CuSO_4$)	Percent Survival (Mean ± SD)	% Survival in Replicates							
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
1000	0.0 ± 0.0	0	0	0	0	0	0	0	0
500	2.5 ± 7.1	20	0	0	0	0	0	0	0
250	20.0 ± 28.3	0	80	20	20	0	40	0	0
125	85.0 ± 20.7	100	80	80	100	40	100	80	100
62.5	85.0 ± 17.7	100	100	80	60	100	60	80	100
Control	80.0 ± 15.1	80	60	80	80	60	80	100	100

Statistical Summary	
Parameter	Concentration (ppb $CuSO_4$)
LC_{25}	164.2
LC_{50}	209.6
NOEC	125
LOEC	250

BIOMASS ENDPOINT

Concentration (ppb $CuSO_4$)	Mean Weight (mg)
1000	0.00
500	0.01
250	0.07
125	0.30
62.5	0.31
Control	0.30
Statistical Summary	
Parameter	Concentration (ppb $CuSO_4$)
IC_{15}	147.7
IC_{25}	163.7
IC_{40}	187.8
IC_{50}	203.9
NOEC	125
LOEC	250

Table 1-21. Phase II, Episode 1 Results: 96-hr Acute Toxicity Bioassay using *A. bahia*

Sample Information

Sample Collected: August 3 & August 5, 2011

Sample Received: August 4 & August 5, 2011

Test Dates: August 4 - 8, 2011

Test Parameters

Concentrations (ppt): 36.5, 39, 41, 45, 50

Common Name: Mysid shrimp

Test Endpoints: Acute survival

Test Protocol: EPA/821/R-02/014

SURVIVAL ENDPOINT

Saline Concentration (ppt)	Percent Survival (Mean ± SD)	% Survival in Replicates							
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
50	55.0 ± 27.8	40	60	60	100	80	60	20	20
45	90.0 ± 15.1	100	100	100	100	80	80	60	100
41	97.5 ± 7.1	100	100	80	100	100	100	100	100
39	95.0 ± 14.1	100	100	100	100	60	100	100	100
36.5	95.0 ± 9.3	100	100	80	100	80	100	100	100
Laboratory Control	97.5 ± 7.1	100	100	100	100	100	100	80	100

Statistical Summary

Parameter	Saline Concentration (ppt)
EC_{25}	48.1
EC_{50}	50.7
NOEC	45
LOEC	50
TU_A ($100/EC_{25}$)	2.08

1.3.2.2 *Macrocystis pyrifera Chronic Test*

Water Quality and Test Acceptability Criteria

All water quality parameters were consistently within acceptable limits, and all test acceptability criteria were met. Salinity toxicity test results are presented in Table 1-21 and reference toxicant test results are presented in Table 1-22.

Proportion Germinated

The mean proportion germinated in the laboratory control treatment was 84.8%. The mean proportion germinated in the 37, 39, 41, 45, 50 ppt concentrations was 85.4, 84.2, 80.0, 72.0 and 70.4%, respectively. Statistically significant effects on *M. pyrifera* germination were observed in the 45 ppt and 50 ppt concentrations relative to the laboratory control. Consequently, the NOEC for the proportion germinated endpoint was 41 ppt. The EC_{25} and EC_{50} values were both greater than 50 ppt, and the TU_C (100% / NOEC) for the proportion germinated endpoint was 2.44.

Growth-Length

The mean germination tube length for the laboratory control was 14.1 µm. Mean germination tube length values in the 37, 39, 41, 45, 50 ppt concentrations were 13.3, 13.1, 9.3, 10.7 and 9.3 µm, respectively. Statistically significant effects were observed in the 41, 45, and 50 ppt concentrations when compared to the laboratory control. Consequently, the growth NOEC was 39 ppt. The IC_{25} and IC_{50} values were 40.6 ppt and greater than 50 ppt, respectively. The TU_C (100% / IC_{25}) for the growth endpoint was 2.46.

Reference Toxicant Test (Copper Chloride)

The *M. pyrifera* reference toxicant test produced an EC_{50} value of 92.8 ppb $CuCl_2$ for the proportion germinated endpoint. This value is within two standard deviations of the laboratory mean (92.7 ± 77.2 ppb $CuCl_2$), indicating normal sensitivity of the test organisms based on germination. The IC_{50} for the germination tube length endpoint was 272.5 ppb $CuCl_2$. This value is also within two standard deviations of the laboratory mean (193.4 ± 181.9 ppb $CuCl_2$), indicating normal sensitivity for growth.

Table 1-22. Phase II, Episode 1 Results: 48-hr Chronic Toxicity Bioassay using *Macrocystis pyrifera*

Sample Information
Sample Collected: August 3 & August 5, 2011
Sample Received: August 4 & August 5, 2011
Test Dates: August 15 - 17, 2011

Test Parameters
Concentrations (ppt): 36.5, 39, 41, 45, 50
Common Name: Giant kelp
Test Endpoints: Germination, Growth
Test Protocol: EPA/600/R-95/136

PROPORTION GERMINATED

Concentration (ppt)	(Mean ± SD)	Proportion Germinated in Replicates				
		1	*2*	*3*	*4*	*5*
50	70.4 ± 4.6	66	72	74	65	75
45	72.0 ± 6.0	63	69	76	78	74
41	80.0 ± 4.8	77	75	83	85	
39	84.2 ± 1.3	84	83	83	85	86
36.5	85.4 ± 4.2	82	85	91	88	81
Control	84.8 ± 4.7	88	83	78	90	85

Statistical Summary

Parameter	Saline Concentration (ppt)
EC_{25}	> 50
EC_{50}	> 50
NOEC	41
LOEC	45
TU_C (100/NOEC)	2.44

GERMTUBE GROWTH-LENGTH

Concentration (ppt)	Mean Length (µg)
50	9.30
45	10.65
41	9.30
39	13.10
36.5	13.25
Control	14.05

Statistical Summary

Parameter	Saline Concentration (ppt)
IC_{15}	39.7
IC_{25}	40.6
IC_{40}	> 50
IC_{50}	> 50
NOEC	39
LOEC	41
TU_C (100/IC_{25})	2.46

Table 1-23. Phase II, Episode 1 Reference Toxicant Test Results for the 48-hr Chronic Toxicity Bioassay using *M. pyrifera*

Sample Information
Sample Collected: August 3 & August 5, 2011
Sample Received: August 4 & August 5, 2011
Test Dates: August 15 - 17, 2011

Test Parameters
Concentration (ppb): 5.6, 10, 18, 32, 100, 180, 300
Common Name: Giant kelp
Test Endpoints: Germination, Growth
Test Protocol: EPA/600/R-95/136

PROPORTION GERMINATED

Concentration (ppb $CuCl_2$)	(Mean ± SD)	Proportion Germinated in Replicates				
		1	*2*	*3*	*4*	*5*
300	8.2 ± 2.8	7	4	10	11	9
180	29.0 ± 4.1	28	25	28	36	28
100	49.8 ± 2.6	53	51	49	46	50
32	68.0 ± 2.2	68	69	65	70	--
18	73.3 ± 4.1	73	69	71	73	80
10	76.4 ± 3.9	77	76	82	71	76
5.6	88.4 ± 1.9	89	86	91	89	87
Control	84.8 ± 4.7	88	83	78	90	85

Statistical Summary

Parameter	Concentration (ppb $CuCl_2$)
EC_{25}	--
EC_{50}	92.8
NOEC	5.6
LOEC	10
TU_C (100/NOEC)	17.9

GERM TUBE GROWTH-LENGTH

Concentration (ppb)	Mean Length (µg)
300	6.75
180	7.95
100	8.95
32	10.13
18	11.10
10	12.15
6	12.80
Control	14.05

Statistical Summary

Parameter	Concentration (ppb $CuCl_2$)
IC_{15}	11.6
IC_{25}	26.1
IC_{40}	141.6
IC_{50}	272.5
NOEC	5.6
LOEC	10
TU_C (100/IC_{25})	3.83

1.3.2.3 *S. purpuratus Test*

Water Quality and Test Acceptability Criteria

All water quality parameters were within acceptable limits throughout the 96-hour test duration. Upon test termination, the laboratory control met the criteria for test acceptability. Salinity toxicity test results are presented in Table 1-23 and reference toxicant test results are presented in Table 1-24.

Proportion Alive

The average proportion alive for *S. purpuratus* was 90.2% for the laboratory control treatment. The mean proportion alive in the 35, 37, 39, 41, and 43 ppt concentrations were 89.8, 89.0, 91.4, 87.7, and 93.5%, respectively. No statistically significant effects on survival were observed relative to the laboratory control treatment. Consequently, the NOEC for the proportion alive endpoint was 43 ppt. The IC_{25} and IC_{50} values were both greater than 43 ppt. The TU_C (100% / NOEC) for the proportion alive endpoint was 2.33.

Proportion Normal

The mean proportion normal observed in the laboratory control treatment for the *S. purpuratus* test was 94.4%. The mean proportion normal in the 35, 37, 39, 41, and 43 ppt concentrations were 93.3, 27.8, 2.4, 0.0, and 0.0%, respectively. A statistically significant effect was observed in the 37 ppt salinity treatment when compared to the laboratory control. Consequently, the NOEC for the proportion normal endpoint was 35 ppt. The IC_{25} and IC_{50} values were 35.7 ppt and 36.4 ppt, respectively. The TU_C (100% / IC_{25}) for the proportion normal endpoint was 2.80.

Reference Toxicant Test (Copper Sulfate)

The reference toxicant test produced an EC_{50} value of 12.9 ppb $CuSO_4$ for the proportion normal endpoint. This value is within two standard deviations of the laboratory mean (16.0 ± 7.76 ppb $CuSO_4$), indicating normal sensitivity of the test organisms.

Table 1-24. Phase II, Episode 1 Results: 96-hr Chronic Toxicity Bioassay using *S. purpuratus*

Sample Information
Sample Collected: August 3 & August 5, 2011
Sample Received: August 4 & August 5, 2011
Test Dates: August 23 - 27, 2011

Test Parameters
Concentrations (ppt): 35, 37, 39, 41, 43
Common Name: Purple urchin
Test Endpoints: Survival, Development
Test Protocol: EPA/600/R-95/136

SURVIVAL ENDPOINT

Saline Concentration (ppt)	Proportion Alive (Mean ± SD)	Proportion Alive in Replicates			
		1	*2*	*3*	*4*
43	93.5 ± 5.7	97	100	89	88
41	87.7 ± 5.6	94	86	81	90
39	91.4 ± 3.2	90	88	93	95
37	88.9 ± 6.8	95	82	84	95
35	89.8 ± 6.8	87	100	86	87
Laboratory Control	90.2 ± 6.7	92	87	83	99

Statistical Summary	
Parameter	Saline Concentration (ppt)
IC_{25}	> 43
IC_{50}	> 43
NOEC	43
LOEC	> 43
TU_c (100/IC_{25})	2.33

DEVELOPMENTAL ENDPOINT

Saline Concentration (ppt)	Proportion Normal (Mean ± SD)	Proportion Normal in Replicates			
		1	*2*	*3*	*4*
43	0.0 ± 0.0	0	0	0	0
41	0.0 ± 0.0	0	0	0	0
39	2.4 ± 0.8	3	2	3	2
37	27.8 ± 22.8	28	59	5	19
35	93.3 ± 2.3	91	95	91	95
Laboratory Control	94.4 ± 0.8	95	94	94	94

Statistical Summary	
Parameter	Saline Concentration (ppt)
IC_{25}	35.7
IC_{50}	36.4
NOEC	35
LOEC	37
TU_c (100/NOEC)	2.80

Table 1-25. Phase II, Episode 1 Reference Toxicant Test Results for the 96-hr Chronic Toxicity Bioassay using *S. purpuratus*

Sample Information
Sample Collected: August 3 & August 5, 2011
Sample Received: August 4 & August 5, 2011
Test Dates: August 23 - 27, 2011

Test Parameters
Concentrations (ppb): 3.75, 7.5, 15, 30, 60
Common Name: Purple urchin
Test Endpoints: Developmental
Test Protocol: EPA/600/R-95/136

DEVELOPMENTAL ENDPOINT

Concentration (ppb $CuSO_4$)	Proportion Normal (Mean ± SD)	Proportion Normal in Replicates			
		1	*2*	*3*	*4*
60	0.0 ± 0.0	0	0	0	0
30	0.0 ± 0.0	0	0	0	0
15	26.8 ± 35.0	9	78	19	1
7.5	94.3 ± 2.5	95	91	97	94
3.75	95.3 ± 3.3	96	91	95	99
Laboratory Control	97.0 ± 1.8	98	96	99	95

Statistical Summary	
Parameter	Concentration (ppb $CuSO_4$)
EC_{25}	10.9
EC_{50}	12.9
NOEC	7.5
LOEC	15
TU_c ($100/EC_{25}$)	9.21

1.3.2.4 *A. affinis Chronic and Acute Tests*

Water Quality and Test Acceptability Criteria

All water quality parameters were within acceptable limits throughout the 7-day test duration. Salinity toxicity test results are presented in Table 1-25 and reference toxicant test results are presented in Table 1-26. Acute toxicity test results are presented in Table 1-27.

Survival

The average survival rate observed during testing was 96% for the laboratory control treatment. Mean survival rates in the 37, 39, 41, 45, 50 ppt concentrations were 92.0, 88.0, 88.0, 100.0, and 72.0%, respectively. No statistically significant effects on survival were observed when compared to the laboratory control. Consequently, the NOEC for survival was 50 ppt. The LC_{25} and LC_{50} values were both greater than 50 ppt, and the TU_C (100% / NOEC) for the survival endpoint was 2.0.

Biomass

The mean biomass measured for *A. affinis* was 1.02 mg for the laboratory control treatment. Mean biomass in the 37, 39, 41, 45, 50 ppt concentrations were 1.03, 1.03, 1.03, 1.04, and 0.86 mg, respectively. No statistically significant effects on biomass were observed relative to the laboratory control. Consequently, the NOEC for biomass was 50 ppt. The IC_{25} and IC_{50} values were both greater than 50 ppt, and the TU_C (100% / NOEC) for the biomass endpoint was 2.0.

Acute Survival

The average 96-hour survival rate for the laboratory control treatment was 96.0%. The mean survival rates in the 37, 39, 41, 45, 50 ppt concentrations were 92.0, 92.0, 88.0, 100, and 72.0%, respectively. No statistically significant effects on acute survival were observed relative to the laboratory control. Consequently, the NOEC for survival was 50 ppt. The IC_{25} and IC_{50} values were both greater than 50 ppt, and the TU_A (100% / NOEC) for the survival endpoint was 2.0.

Reference Toxicant Test (Copper Sulfate)

The reference toxicant test produced an LC_{50} value of 69.5 ppb $CuSO_4$ for the survival endpoint. This value is within two standard deviations from the laboratory mean (117.9 ± 55.4 ppb $CuSO_4$), indicating normal sensitivity of the test organisms for the survival endpoint. The IC_{50} for biomass was 80.6 ppb $CuSO_4$. This value is within two standard deviations of the laboratory mean (130.4 ± 57.5 ppb $CuSO_4$), indicating normal sensitivity for the biomass endpoint.

Table 1-26. Phase II, Episode 1 Results: 7-day Chronic Toxicity Bioassay using *A. affinis*

Sample Information
Sample Collected: August 3 & August 5, 2011
Sample Received: August 4 & August 5, 2011
Test Dates: August 10 - 17, 2011

Test Parameters
Concentrations (ppt): 36.5, 39, 41, 45, 50
Common Name: Topsmelt
Test Endpoints: Survival and Growth
Test Protocol: EPA/600/R-95/136

SURVIVAL ENDPOINT

Concentration (ppt)	(Mean ± SD)	% Survival in Replicates				
		1	*2*	*3*	*4*	*5*
50	72.0 ± 22.8	60	80	100	80	40
45	100.0 ± 0.0	100	100	100	100	100
41	88.0 ± 11.0	80	100	80	80	100
39	88.0 ± 11.0	80	100	80	100	80
36.5	92.0 ± 11.0	100	100	80	100	80
Control	96.0 ± 8.9	80	100	100	100	100

Statistical Summary

Parameter	Saline Concentration (ppt)
LC_{25}	> 50
LC_{50}	> 50
NOEC	50
LOEC	> 50
TU_C (100/NOEC)	2.00

BIOMASS ENDPOINT

Concentration (ppt)	Mean Weight/Individual (mg)
50	0.86
45	1.04
41	1.03
39	1.03
37	1.03
Control	1.02

Statistical Summary

Parameter	Saline Concentration (ppt)
IC_{15}	49.5
IC_{25}	> 50
IC_{40}	> 50
IC_{50}	> 50
NOEC	50
LOEC	> 50
TU_C (100/NOEC)	2.00

Table 1-27. Phase II, Episode 1 Reference Toxicant Test Results for the 7-day Chronic Toxicity Bioassay using *A. affinis*

Sample Information
Sample Collected: August 3 & August 5, 2011
Sample Received: August 4 & August 5, 2011
Test Dates: August 10 - 17, 2011

Test Parameters
Concentration (ppb): 25, 50, 100, 200, 400
Common Name: Topsmelt
Test Endpoints: Survival and Growth
Test Protocol: EPA/600/R-95/136

SURVIVAL ENDPOINT

Concentration (ppb $CuSO_4$)	(Mean ± SD)	% Survival in Replicates				
		1	*2*	*3*	*4*	*5*
400	0.0 ± 0.0	0	0	0	0	0
200	0.0 ± 0.0	0	0	0	0	0
100	16.0 ± 26.1	20	60	0	0	0
50	88.0 ± 11.0	80	100	80	80	100
25	96.0 ± 8.9	100	100	100	100	80
Control	100.0 ± 0.0	100	100	100	100	100
Statistical Summary						
Parameter	Concentration (ppb $CuSO_4$)					
LC_{25}	52.4					
LC_{50}	69.5					
NOEC	50					
LOEC	100					
TU_C ($100/LC_{25}$)	1.91					

BIOMASS ENDPOINT

Concentration (ppb)	Mean Weight/Individual (mg)
400	0.00
200	0.00
100	1.74
50	1.21
25	1.17
Control	1.12
Statistical Summary	
Parameter	Concentration (ppb $CuSO_4$)
IC_{15}	> 100
IC_{25}	> 100
IC_{40}	> 100
IC_{50}	> 100
NOEC	100
LOEC	> 100
TU_C (100/NOEC)	1.00

Table 1-28. Phase II, Episode 1 Results: 96-hr Acute Toxicity Bioassay using *A. affinis*

Sample Information
Sample Collected: August 3 & August 5, 2011
Sample Received: August 4 & August 5, 2011
Test Dates: August 10 - 14, 2011

Test Parameters
Concentrations (ppt): 36.5, 39, 41, 45, 50
Common Name: Topsmelt
Test Endpoints: Acute survival
Test Protocol: EPA/600/R-95/136

SURVIVAL ENDPOINT

Saline Concentration (ppt)	Proportion Alive (Mean ± SD)	Proportion Alive in Replicates				
		1	*2*	*3*	*4*	*5*
50	72.0 ± 22.8	60	80	100	80	40
45	100.0 ± 0.0	100	100	100	100	100
41	88.0 ± 11.0	80	100	80	80	100
39	92.0 ± 11.0	80	100	80	100	100
36.5	92.0 ± 11.0	100	100	80	100	80
Laboratory Control	96.0 ± 8.9	80	100	100	100	100

Statistical Summary

Parameter	Saline Concentration (ppt)
IC_{25}	> 50
IC_{50}	> 50
NOEC	50
LOEC	> 50
TU_c (100/NOEC)	2.00

1.3.3 PHASE II, EPISODE 2: SAMPLES COLLECTED SEPTEMBER 22, 2011

Procedure and organism data for *A. bahia*, *M. pyrifera*, *S. purpuratus*, and *A. affinis* tests performed during Phase II, Episode 2 are summarized in Table 1-28 and Table 1-29. Copies of laboratory benchsheets for all tests are provided in Appendix B.

1.3.3.1 *A. bahia Chronic and Acute Tests*

Water Quality and Test Acceptability Criteria

All water quality parameters were consistently within acceptable limits, and all test acceptability criteria were met. Salinity toxicity test results are presented in Table 1-30 and reference toxicant test results are presented in Table 1-31. Acute toxicity test results are presented in Table 1-32.

Survival

The mean 7-day survival rate observed for *A. bahia* in the laboratory control was 97.5%. The mean survival rates in the 37, 39, 41, 45, 60 ppt concentrations were 95.0, 97.5, 97.5, 87.5, and 0.0%, respectively. Statistically significant effects on *A. bahia* survival were seen in the 60 ppt treatment compared to the laboratory control. Consequently, the NOEC for survival was 45 ppt. The LC_{50} value was 51.6 ppt, and the survival TU_C (100% / LC_{25}) was 2.11.

Biomass

The mean dry weight measured for the laboratory control mysids was 0.43 mg. The mean biomass values in the 37, 39, 41, 45, 60 ppt concentrations were 0.42, 0.39, 0.37, 0.21, and 0.0 mg, respectively. Statistically significant effects were observed in the 45 and 60 ppt treatments relative to the laboratory control. Consequently, the biomass NOEC was 41 ppt. The IC_{25} and IC_{50} values were 42.3 ppt and 44.8 ppt, respectively, and the biomass TU_C (100% / IC_{25}) was 2.37.

Acute Survival

The average 96-hour survival rate for the laboratory control treatment was 100%. The mean survival rates in the 37, 39, 41, 45, 50 ppt concentrations were 100, 100, 100, 92.5, and 0.0%, respectively. A statistically significant effect on survival was seen in the 60 ppt treatment relative to the laboratory control. Consequently, the NOEC for survival was 45 ppt. The EC_{50} value was 51.2 ppt, and the TU_A (100% / NOEC) for the acute survival endpoint was 2.22.

Reference Toxicant Test (Copper Sulfate)

The reference toxicant test produced an LC_{50} value of 163.7 ppb $CuSO_4$. This value is within two standard deviations of the laboratory mean (247.6 ± 119.9 ppb $CuSO_4$) indicating normal sensitivity of the test organisms based on survival. The IC_{50} for biomass was 173.7 ppb $CuSO_4$. This value is within two standard deviations of the laboratory mean (230.5 ± 134.6 ppb $CuSO_4$), indicating normal sensitivity for the growth endpoint.

Table 1-29. Phase II, Episode 2 Chronic Bioassays: Procedure and Organism Data

Parameter	Test Species	
	Americamysis bahia	***Macrocystis pyrifera***
Sample Properties		
Dates Sampled	September 22, 2011	September 22, 2011
Dates Received	September 23, 2011	September 23, 2011
Volume Received	~ 20 L per sample (80 L total)	~ 20 L per sample (80 L total)
Storage Conditions	4°C, dark	4°C, dark
Test Species Information		
Supplier	Aquatic BioSystems, Fort Collins, CO	Dave Gutoff, San Diego, CA
Date Acquired	October 18, 2011	October 4, 2011
Acquired Temperature	15.6°C	N/A
Age Group	7 days old	Mature
Test Procedures		
Type/Duration	Chronic/Renewal; 7 days	Chronic/Static; 48 hours
Test Dates	October 19 - 26, 2011	October 4 -6, 2011
Control/Dilution H_2O	Filtered, UV-treated seawater from Scripps Institute of Oceanography, La Jolla, CA	Filtered, UV-treated seawater from Scripps Institute of Oceanography, La Jolla, CA
Temperature (recommended/actual)	26°C ± 1°C / 24.5 – 26.8°C	15°C ± 1°C / 14.8 – 15.6°C
Photoperiod	16-hour light: 8-hour dark	16-hour light: 8-hour dark
Salinity (recommended/actual)	20 – 30 ± 2 ppt / NA	34 ± 2 ppt / NA
Test Chamber	400 mL containers	60 mL petri dish
Exposure Volume	150 mL	40 mL
Animals/Replicate	5	7500 spores / mL
Replicates/Treatment	8	5
Feeding	Newly hatched *Artemia* nauplii (~750 nauplii, twice daily)	None
Deviations From Protocol	None	None

Table 1-29 Phase II, Episode 2 Chronic Bioassays: Procedure and Organism Data (Continued)

Parameter	Test Species	
	Strongylocentrotus purpuratus	***Atherinops affinis***
Sample Properties		
Dates Sampled	September 22, 2011	September 22, 2011
Dates Received	September 23, 2011	September 23, 2011
Volume Received	~ 20 L per sample (80 L total)	~ 20 L per sample (80 L total)
Storage Conditions	4°C, dark	4°C, dark
Test Species Information		
Supplier	Dave Gutoff, San Diego, CA	Aquatic BioSystems, Fort Collins, CO
Date Acquired	October 5, 2011	September 27, 2011
Acquired Temperature	N/A	19.9 – 20.0°C
Age Group	Adult	12 days old
Test Procedures		
Type/Duration	Chronic/Static; 96 hours	Chronic/Renewal; 7 days
Test Dates	October 5 - 9, 2011	September 27 - October 4, 2011
Control/Dilution H_2O	Filtered, UV-treated seawater from Scripps Institute of Oceanography, La Jolla, CA	Filtered, UV-treated seawater from Scripps Institute of Oceanography, La Jolla, CA
Temperature (recommended/actual)	15 ± 1°C / 14.5 - 16.3°C	20 ± 1°C / 19.2 – 20.6°C
Photoperiod	Ambient laboratory	16-hour light: 8-hour dark
Salinity (recommended/actual)	30 ± 2 ppt / NA	32 ± 2 ppt / NA
Test Chamber	20 mL scintillation vial	600 mL containers
Exposure Volume	5 mL	200 mL
Animals/Replicate	2000 eggs, 5 x 10^6 sperm	5
Replicates/Treatment	4	5
Feeding	None	Newly hatched *Artemia* nauplii (~200 nauplii, twice daily)
Deviations From Protocol	None	None

Table 1-30. Phase II, Episode 2 Acute Bioassays: Procedure and Organism Data

Parameter	Test Species	
	Americamysis bahia	***Atherinops affinis***
Sample Properties		
Dates Sampled	September 22, 2011	September 22, 2011
Dates Received	September 23, 2011	September 23, 2011
Volume Received	~ 20 L per sample (80 L total)	~ 20 L per sample (80 L total)
Storage Conditions	4°C, dark	4°C, dark
Test Species Information		
Supplier	Aquatic BioSystems, Fort Collins, CO	Aquatic BioSystems, Fort Collins, CO
Date Acquired	October 18, 2011	September 27, 2011
Acquired Temperature	15.6°C	19.9 – 20.0°C
Age Group	7 days old	12 days old
Test Procedures		
Type/Duration	Acute/Renewal; 96 hours	Acute/Renewal; 96 hours
Test Dates	October 19 - 23, 2011	September 27 - October 1, 2011
Control/Dilution H_2O	Filtered, UV-treated seawater from Scripps Institute of Oceanography, La Jolla, CA	Filtered, UV-treated seawater from Scripps Institute of Oceanography, La Jolla, CA
Temperature (recommended/actual)	26 ± 1°C / 24.5 – 26.8°C	20 ± 1°C / 19.2 – 20.6°C
Photoperiod	16-hour light: 8-hour dark	16-hour light: 8-hour dark
Salinity (recommended/actual)	5 – 30 ± 1 ppt / NA	10 – 30 ppt / NA
Test Chamber	250 mL containers	250 mL containers
Exposure Volume	200 mL	200 mL
Animals/Replicate	5	5
Replicates/Treatment	8	5
Feeding	Newly hatched *Artemia* nauplii (~100 nauplii, daily)	Newly hatched *Artemia* nauplii (~100 nauplii, once prior to renewal)
Deviations From Protocol	None	None

Table 1-31. Phase II, Episode 2 Results: 7-day Chronic Toxicity Bioassay using *A. bahia*

Sample Information
Sample Collected: September 22, 2011
Sample Received: September 23, 2011
Test Dates: October 19 - 26, 2011

Test Parameters
Concentrations (ppt): 36.5, 39, 41, 45, 60
Species Common Name: Mysid shrimp
Test Endpoints: Survival, Growth
Test Protocol: EPA/821/R-02/014

SURVIVAL ENDPOINT

Concentration (ppt)	(Mean ± SD)	% Survival in Replicates							
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
60	0.0 ± 0.0	0	0	0	0	0	0	0	0
45	87.5 ± 10.4	80	80	100	80	80	80	100	100
41	97.5 ± 7.1	100	100	80	100	100	100	100	100
39	97.5 ± 7.1	100	100	80	100	100	100	100	100
36.5	95.0 ± 9.3	100	80	100	100	80	100	100	100
Lab Control	97.5 ± 7.1	80	100	100	100	100	100	100	100

Statistical Summary	
Parameter	Saline Concentration (ppt)
LC_{25}	47.5
LC_{50}	51.6
NOEC	45
LOEC	60
TU_C ($100/LC_{25}$)	2.11

BIOMASS ENDPOINT

Concentration (ppt)	Mean Weight (mg)
60	0.00
45	0.21
41	0.37
39	0.39
36.5	0.42
Laboratory Control	0.43
Statistical Summary	
Parameter	Saline Concentration (ppt)
IC_{15}	41.3
IC_{25}	42.3
IC_{40}	43.8
IC_{50}	44.8
NOEC	41
LOEC	45
TU_C ($100/IC_{25}$)	2.37

Table 1-32. Phase II, Episode 2 Reference Toxicant Test Results for the 7-day Chronic Toxicity Bioassay using *A. bahia*

Sample Information
Sample Collected: September 22, 2011
Sample Received: September 23, 2011
Test Dates: October 19 - 26, 2011

Test Parameters
Concentrations (ppb): 62.5, 125, 250, 500, 1000
Species Common Name: Mysid shrimp
Test Endpoints: Survival and Growth
Test Protocol: EPA/821/R-02/014

SURVIVAL ENDPOINT

Concentration (ppb $CuSO_4$)	(Mean ± SD)	% Survival in Replicates							
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
1000	0.0 ± 0.0	0	0	0	0	0	0	0	0
500	2.5 ± 7.1	0	0	0	20	0	0	0	0
250	15.0 ± 20.7	20	0	20	0	0	0	60	20
125	72.5 ± 23.8	80	60	100	100	40	80	80	40
62.5	92.5 ± 10.4	80	100	100	100	80	100	80	100
Control	97.5 ± 7.1	100	100	100	100	100	80	100	100

Statistical Summary	
Parameter	Concentration (ppb $CuSO_4$)
LC_{25}	116.4
LC_{50}	163.7
NOEC	62.5
LOEC	125

BIOMASS ENDPOINT

Concentration (ppb $CuSO_4$)	Mean Weight (mg)
1000	0.00
500	0.01
250	0.04
125	0.21
62.5	0.28
Control	0.28
Statistical Summary	
Parameter	Concentration (ppb $CuSO_4$)
IC_{15}	97.1
IC_{25}	120.5
IC_{40}	152.5
IC_{50}	173.7
NOEC	62.5
LOEC	125

Table 1-33. Phase II, Episode 2 Results: 96-hour Acute Toxicity Bioassay using *A. bahia*

Sample Information
Sample Collected: September 22, 2011
Sample Received: September 23, 2011
Test Dates: October 19 - 23, 2011

Test Parameters
Concentrations (ppt): 36.5, 39, 41, 45, 60
Common Name: Mysid shrimp
Test Endpoints: Acute survival
Test Protocol: EPA/821/R-02/014

SURVIVAL ENDPOINT

Saline Concentration (ppt)	Proportion Alive (Mean ± SD)	Proportion Alive in Replicates							
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
60	0.0 ± 0.0	0	0	0	0	0	0	0	0
45	92.5 ± 10.4	100	80	100	100	80	80	100	100
41	100.0 ± 0.0	100	100	100	100	100	100	100	100
39	100.0 ± 0.0	100	100	100	100	100	100	100	100
36.5	100.0 ± 0.0	100	100	100	100	100	100	100	100
Laboratory Control	100.0 ± 0.0	100	100	100	100	100	100	100	100

Statistical Summary

Parameter	Saline Concentration (ppt)
EC_{25}	–
EC_{50}	51.2
NOEC	45
LOEC	60
TU_C (100/NOEC)	2.22

1.3.3.2 *M. pyrifera Chronic Test*

Water Quality and Test Acceptability Criteria

All water quality parameters were consistently within acceptable limits, and all test acceptability criteria were met. Salinity toxicity test results are presented in Table 1-33 and reference toxicant test results are presented in Table 1-34.

Proportion Germinated

The mean proportion germinated in the laboratory control treatment was 83.2%. The mean proportion germinated in the 37, 39, 41, 45, and 60 ppt concentration was 81.8, 77.6, 74.8, 72.4 and 31.6%, respectively. Statistically significant effects on *M. pyrifera* germination were observed in the 41, 45, and 60 ppt concentrations relative to the laboratory control. Consequently, the NOEC for the proportion germinated endpoint was 39 ppt. The EC_{25} and EC_{50} values were 48.6 ppt and 56.3 ppt, respectively. The TU_C (100% / EC_{25}) for the proportion germinated endpoint was 2.06.

Growth-Length

The mean germination tube length for the laboratory control was 14.7 µm. The mean germination tube length values in the 37, 39, 41, 45, 60 ppt concentrations were 13.5, 13.5, 13.7, 11.7 and 6.0 µm, respectively. Statistically significant effects were observed in the 45 ppt and 50 ppt concentrations when compared to the laboratory control. Consequently, the growth NOEC was 41 ppt. The IC_{25} and IC_{50} values were 46.9 ppt and 56.5 ppt, respectively. The TU_C (100% / IC_{25}) for the growth endpoint was 2.13.

Reference Toxicant Test (Copper Chloride)

The *M. pyrifera* reference toxicant test produced an EC_{50} value of 321.74 ppb $CuCl_2$ for the proportion germinated endpoint. This value is within two standard deviations of the laboratory mean (102.0 ± 127.5 ppb $CuCl_2$), indicating less than normal sensitivity of the test organisms based on germination. The EC_{50} for the germination tube length endpoint was 244.1 ppb $CuCl_2$. This value is within two standard deviations of the laboratory mean (202.7 ± 171.8 ppb $CuCl_2$), indicating normal sensitivity for growth.

Table 1-34. Phase II, Episode 2 Results: 48-hr Chronic Toxicity Bioassay using *M. pyrifera*

Sample Information
Sample Collected: September 22, 2011
Sample Received: September 23, 2011
Test Dates: October 4 - 6, 2011

Test Parameters
Concentrations (ppt): 36.5, 39, 41, 45, 60
Common Name: Giant kelp
Test Endpoints: Germination, Growth
Test Protocol: EPA/600/R-95/136

PROPORTION GERMINATED

Concentration (ppt)	(Mean ± SD)	Proportion Germinated in Replicates				
		1	*2*	*3*	*4*	*5*
60	31.6 ± 7.9	39	37	33	30	19
45	72.4 ± 3.5	76	68	71	71	76
41	74.8 ± 4.1	80	73	78	70	73
39	77.6 ± 2.4	75	81	77	79	76
36.5	81.8 ± 2.0	82	85	80	82	80
Control	83.2 ± 2.6	85	86	81	84	80

Statistical Summary	
Parameter	Saline Concentration (ppt)
EC_{25}	48.6
EC_{50}	56.3
NOEC	39
LOEC	41
TU_C (100/EC_{25})	2.06

GERM TUBE GROWTH-LENGTH

Concentration (ppt)	Mean Length (µg)
60	6.00
45	11.70
41	13.65
39	13.50
36.5	13.45
Control	14.65

Statistical Summary	
Parameter	Saline Concentration (ppt)
IC_{15}	43.4
IC_{25}	46.9
IC_{40}	52.7
IC_{50}	56.5
NOEC	41
LOEC	45
TU_C (100/IC_{25})	2.13

Table 1-35. Phase II, Episode 2 Reference Toxicant Test Results for the 48-hr Chronic Toxicity Bioassay using *M. pyrifera*

Sample Information
Sample Collected: September 22, 2011
Sample Received: September 23, 2011
Test Dates: October 4 - 6, 2011

Test Parameters
Concentration (ppb): 5.6, 10, 18, 32, 100, 180, 300
Common Name: Giant kelp
Test Endpoints: Germination, Growth
Test Protocol: EPA/600/R-95/136

PROPORTION GERMINATED

Concentration (ppb $CuCl_2$)	(Mean ± SD)	Proportion Germinated in Replicates				
		1	*2*	*3*	*4*	*5*
300	39.4 ± 10.2	37	48	42	47	23
180	51.8 ± 7.1	59	54	54	52	40
100	61.4 ± 7.1	63	64	64	67	49
32	73.2 ± 3.1	73	76	74	75	68
18	75.4 ± 3.0	79	78	73	72	75
10	77.0 ± 3.3	76	80	72	77	80
5.6	76.6 ± 2.8	74	79	80	74	76
Control	83.2 ± 2.6	85	86	81	84	80

Statistical Summary

Parameter	Concentration (ppb $CuCl_2$)
EC_{25}	100.1
EC_{50}	321.7
NOEC	< 5.6
LOEC	5.6
TU_C (100/NOEC)	17.9

GERM TUBE GROWTH-LENGTH

Concentration (ppb)	Mean Length (µg)
300	6.30
180	8.50
100	9.80
32	12.65
18	13.10
10	12.90
6	13.00
Control	14.65

Statistical Summary

Parameter	Concentration (ppb $CuCl_2$)
EC_{15}	36.7
EC_{25}	71.7
EC_{40}	162.2
EC_{50}	244.1
NOEC	< 5.6
LOEC	5.6
TU_C (100/EC_{25})	17.86

1.3.3.3 *S. purpuratus Test*

Water Quality and Test Acceptability Criteria

All water quality parameters were within acceptable limits throughout the 96-hour test duration. Upon test termination, the laboratory control met the criteria for test acceptability. Salinity toxicity test results are presented in Table 1-35 and reference toxicant test results are presented in Table 1-36.

Proportion Alive

The average proportion alive for *S. purpuratus* was 88.4% for the laboratory control treatment. The mean proportion alive in the 35, 37, 39, 41, and 43 ppt concentrations were 92.3, 74.8, 99.3, 98.8, and 98.3%, respectively. No statistically significant effects on survival were observed relative to the laboratory control treatment. Consequently, the NOEC for the proportion alive endpoint was 43 ppt. The IC_{25} and IC_{50} values were both greater than 43 ppt. The TU_C (100% / NOEC) for the proportion alive endpoint was 2.33.

Proportion Normal

The mean proportion normal observed in the laboratory control treatment for the *S. purpuratus* test was 94.9%. Mean proportion normal in the 35, 37, 39, 41, and 43 ppt concentrations were 91.7, 90.1, 1.3, 0.0, and 0.0%, respectively. A statistically significant effect was observed in the 39 ppt salinity treatment when compared to the laboratory control. Consequently, the NOEC for the proportion normal endpoint was 37 ppt. The IC_{25} and IC_{50} values were 37.4 ppt and 38.0 ppt, respectively. The TU_C (100% / IC_{25}) for the proportion normal endpoint was 2.67.

Reference Toxicant Test (Copper Sulfate)

The reference toxicant test produced an EC_{50} value of 14.2 ppb $CuSO_4$ for the proportion normal endpoint. This value is within two standard deviations of the laboratory mean (15.9 ± 7.60 ppb $CuSO_4$), indicating normal sensitivity of the test organisms.

Table 1-36. Phase II, Episode 2 Results: 96-hr Chronic Toxicity Bioassay using *S. purpuratus*

Sample Information
Sample Collected: September 22, 2011
Sample Received: September 23, 2011
Test Dates: October 5 - 9, 2011

Test Parameters
Concentrations (ppt): 35, 37, 39, 41, 43
Common Name: Purple urchin
Test Endpoints: Survival, Development
Test Protocol: EPA/600/R-95/136

SURVIVAL ENDPOINT

Saline Concentration (ppt)	Proportion Alive (Mean ± SD)	Proportion Alive in Replicates			
		1	*2*	*3*	*4*
43	98.3 ± 2.0	100	97	96	100
41	98.8 ± 2.5	100	95	100	100
39	99.3 ± 0.9	99	98	100	100
37	74.8 ± 9.9	85	67	65	81
35	92.3 ± 10.2	96	100	77	96
Laboratory Control	88.4 ± 8.6	100	79	87	87

Statistical Summary

Parameter	Saline Concentration (ppt)
IC_{25}	> 43
IC_{50}	> 43
NOEC	43
LOEC	> 43
TU_c (100/NOEC)	2.33

DEVELOPMENTAL ENDPOINT

Saline Concentration (ppt)	Proportion Normal (Mean ± SD)	Proportion Normal in Replicates			
		1	*2*	*3*	*4*
43	0.0 ± 0.0	0	0	0	0
41	0.0 ± 0.0	0	0	0	0
39	1.2 ± 1.3	0	3	1	1
37	90.1 ± 7.5	83	96	85	98
35	91.7 ± 4.3	95	89	87	96
Laboratory Control	94.9 ± 6.0	99	86	95	99

Statistical Summary

Parameter	Saline Concentration (ppt)
IC_{25}	37.4
IC_{50}	38.0
NOEC	37
LOEC	39
TU_c (100/IC_{25})	2.67

Table 1-37. Phase II, Episode 2 Reference Toxicant Test Results for the 96-hr Chronic Toxicity Bioassay using *S. purpuratus*

Sample Information
Sample Collected: Septmber 22, 2011
Sample Received: September 23, 2011
Test Dates: October 5 - 9, 2011

Test Parameters
Concentrations (ppb): 3.75, 7.5, 15, 30
Common Name: Purple urchin
Test Endpoints: Developmental
Test Protocol: EPA/600/R-95/136

DEVELOPMENTAL ENDPOINT

Concentration (ppb $CuSO_4$)	Proportion Normal (Mean ± SD)	Proportion Normal in Replicates			
		1	*2*	*3*	*4*
30	0.0 ± 0.0	0	0	0	0
15	37.3 ± 4.9	37	40	31	42
7.5	88.8 ± 6.4	95	91	89	80
3.75	93.0 ± 5.5	97	94	96	85
Laboratory Control	90.7 ± 3.4	86	90	93	94

Statistical Summary

Parameter	Concentration (ppb $CuSO_4$)
EC_{25}	12.2
EC_{50}	14.2
NOEC	7.5
LOEC	15
TU_c ($100/EC_{25}$)	8.22

1.3.3.4 *A. affinis Chronic and Acute Tests*

Water Quality and Test Acceptability Criteria

All water quality parameters were within acceptable limits throughout the 7-day test duration. Salinity toxicity test results are presented in Table 1-37 and reference toxicant test results are presented in Table 1-38. Acute toxicity test results are presented in Table 1-39.

Survival

The average survival rate observed during testing was 88.0% for the laboratory control treatment. The mean survival rates in the 37, 39, 41, 45, 60 ppt concentrations were 92.0, 84.0, 92.0, 80.0, and 12.0%, respectively. Statistically significant effects on survival were observed in the 60 ppt salinity treatment when compared to the laboratory control. Consequently, the NOEC for survival was 45 ppt. The LC_{25} and LC_{50} values were 48.6 ppt and 52.6 ppt, respectively. The TU_C (100% / LC_{25}) for the survival endpoint was 2.06.

Biomass

The mean biomass measured for *A. affinis* was 0.80 mg for the laboratory control treatment. The mean biomass in the 37, 39, 41, 45, 60 ppt concentrations was 0.79, 0.77, 0.87, 0.85, and 0.21 mg, respectively. Statistically significant effects on biomass were observed in the 60 ppt salinity treatment when compared to the laboratory control. Consequently, the NOEC for biomass was 45 ppt. The IC_{25} and IC_{50} values were 50.0 ppt and 55.1 ppt, and the TU_C (100% / IC_{25}) for the biomass endpoint was 2.0.

Acute Survival

The average 96-hour survival rate for the laboratory control treatment was 88.0%. The mean acute survival rates in the 37, 39, 41, 45, 60 ppt concentrations were 92.0, 84.0, 92.0, 80.0, and 12.0%, respectively. Statistically significant effects on acute survival were observed in the 60 ppt salinity treatment relative to the laboratory control. Consequently, the NOEC for acute survival was 45 ppt. The EC_{25} and EC_{50} values were 48.6 ppt and 52.7 ppt, respectively. The TU_A (100% / EC_{25}) for the acute survival endpoint was 2.06.

Reference Toxicant Test (Copper Sulfate)

The reference toxicant test produced an LC_{50} value of 117.1 ppb $CuSO_4$ for the survival endpoint. This value is within two standard deviations from the laboratory mean (115.8 ± 52.0 ppb $CuSO_4$), indicating normal sensitivity of the test organisms for the survival endpoint. The IC_{50} for biomass was 140.2 ppb $CuSO_4$. This value is within two standard deviations of the laboratory mean (129.1 ± 55.2 ppb $CuSO_4$), indicating normal sensitivity for the biomass endpoint.

Table 1-38. Phase II, Episode 2 Results: 7-day Chronic Toxicity Bioassay using *A. affinis*

Sample Information
Sample Collected: September 22, 2011
Sample Received: September 23, 2011
Test Dates: September 27 - October 4, 2011

Test Parameters
Concentrations (ppt): 36.5, 39, 41, 45, 60
Common Name: Topsmelt
Test Endpoints: Survival and Growth
Test Protocol: EPA/600/R-95/136

SURVIVAL ENDPOINT

Concentration (ppt)	(Mean ± SD)	% Survival in Replicates				
		1	*2*	*3*	*4*	*5*
60	12.0 ± 11.0	0	20	0	20	20
45	80.0 ± 14.1	80	80	80	100	60
41	92.0 ± 11.0	100	80	80	100	100
39	84.0 ± 16.7	80	100	100	80	60
36.5	92.0 ± 11.0	80	100	100	100	80
Control	88.0 ± 11.0	80	100	80	80	100

Statistical Summary	
Parameter	Saline Concentration (ppt)
LC_{25}	48.6
LC_{50}	52.6
NOEC	45
LOEC	60
TU_C (100/NOEC)	2.06

BIOMASS ENDPOINT

Concentration (ppt)	Mean Weight/Individual (mg)
60	0.21
45	0.85
41	0.86
39	0.77
37	0.79
Control	0.80

Statistical Summary	
Parameter	Saline Concentration (ppt)
IC_{15}	48.0
IC_{25}	50.0
IC_{40}	53.1
IC_{50}	55.1
NOEC	45
LOEC	60
TU_C (100/IC_{25})	2.00

Table 1-39. Phase II, Episode 2 Reference Toxicant Test Results for the 7-day Chronic Toxicity Bioassay using *A. affinis*

Sample Information
Sample Collected: September 22, 2011
Sample Received: September 23, 2011
Test Dates: September 27 - October 4, 2011

Test Parameters
Concentration (ppb): 25, 50, 100, 200, 400
Common Name: Topsmelt
Test Endpoints: Survival and Growth
Test Protocol: EPA/600/R-95/136

SURVIVAL ENDPOINT

Concentration (ppb $CuSO_4$)	(Mean ± SD)	% Survival in Replicates				
		1	*2*	*3*	*4*	*5*
400	0.0 ± 0.0	0	0	0	0	0
200	4.0 ± 8.9	0	0	0	0	20
100	64.0 ± 26.1	20	80	80	80	60
50	100.0 ± 0.0	100	100	100	100	100
25	88.0 ± 11.0	80	100	80	100	80
Control	92.0 ± 17.9	60	100	100	100	100

Statistical Summary

Parameter	Concentration (ppb $CuSO_4$)
LC_{25}	95.4
LC_{50}	117.1
NOEC	100
LOEC	200

BIOMASS ENDPOINT

Concentration (ppb)	Mean Weight/Individual (mg)
400	0.00
200	0.06
100	0.76
50	1.04
25	0.96
Control	0.86

Statistical Summary

Parameter	Concentration (ppb $CuSO_4$)
IC_{15}	86.6
IC_{25}	106.1
IC_{40}	126.6
IC_{50}	140.2
NOEC	100
LOEC	200

Table 1-40. Phase II, Episode 2 Results: 96-hour Acute Toxicity Bioassay using *A. affinis*

Sample Information
Sample Collected: Septmber 22, 2011
Sample Received: September 23, 2011
Test Dates: September 27 - October 1, 2011

Test Parameters
Concentrations (ppt): 36.5, 39, 41, 45, 60
Common Name: Topsmelt
Test Endpoints: Acute survival
Test Protocol: EPA/600/R-95/136

SURVIVAL ENDPOINT

Saline Concentration (ppt)	Proportion Alive (Mean ± SD)	Proportion Alive in Replicates				
		1	*2*	*3*	*4*	*5*
60	12.0 ± 11.0	0	20	0	20	20
45	80.0 ± 14.1	80	80	80	100	60
41	92.0 ± 11.0	100	80	80	100	100
39	84.0 ± 16.7	80	100	100	80	60
36.5	92.0 ± 11.0	80	100	100	100	80
Laboratory Control	88.0 ± 11.0	80	100	80	80	100

Statistical Summary	
Parameter	Saline Concentration (ppt)
EC_{25}	48.6
EC_{50}	52.6
NOEC	45
LOEC	60
TU_A ($100/EC_{25}$)	2.06

1.4 DISCUSSION

All bioassays conducted adhered to the methods outlined by USEPA protocols. Water quality deviations that occurred during testing were responded to with corrective actions by laboratory technicians, and did not prove to affect test results. Additionally, reference toxicant test results showed that the organisms used for each test were appropriately sensitive. With the exception of the topsmelt tested in Phase I, all bioassays met the protocol acceptance criteria for survival and sublethal response to the laboratory control treatment.

1.4.1 ACUTE TOXICITY RESULTS

Phase I acute toxicity testing was performed to determine what specific range of salinities would elicit both highly significant effects (i.e. 100% mortality) and no significant effects, and to identify the two most sensitive species to expose to salinities within that range during Phase II. As shown in Table 1-40, Phase I acute toxicity testing results indicated that *C. stigmaeus* (sand dab), with an NOEC of 51 ppt, was the least sensitive of the three species tested. The *A. bahia* (mysid shrimp) and *A. affinis* (topsmelt) bioassays resulted in similar statistical endpoint values (e.g. the NOEC for both was 42 ppt) and were, therefore, used for Phase II testing.

As a result of the Phase I testing effects levels, the salinity test dilutions for Phase II were ranged from 36.5 to 50 ppt. The results of Phase II, Episodes 1 and 2, showed that the salinity effects on the two species were consistently similar. The mean LC_{50} values calculated from all three test episodes were 49.2 and 50.7 ppt for *A. bahia* and *A. affinis*, respectively. The *A. affinis* mean LC_{50} however includes a conservative estimate of 51 ppt for the Phase II, Episode 1 bioassay because the LC_{50} was greater than the highest test concentration of 50 ppt, and was therefore not calculable (this why the 50 ppt dilution was substituted with 60 ppt for Episode 2). As a result, the actual difference between the two would have been slightly greater, and since there was a consistent sensitivity hierarchy, *A. bahia* is considered the more sensitive acute toxicity test species. As shown in Figure 1-1, the lowest salinity level to elicit a statistically significant effect on acute survival during the Phase II definitive testing episodes was 50 ppt. The highest salinity level that did not result in toxic effects (NOEC) was 45 ppt. The absence of an effect at this concentration was shown with *A. bahia* in both Phase II episodes and with *A. affinis* in the Episode 2.

1.4.2 CHRONIC TOXICITY RESULTS

Phase I chronic toxicity testing was performed to determine what specific range of salinities would elicit both highly significant effects (i.e. 100% mortality) and no significant effects in order to develop a Phase II dilution range that would provide more definitive results. As a result of the Phase I testing effects levels, the salinity test dilutions for Phase II ranged from 36.5 to 50 ppt for the three species representing trophic levels that would most likely be found in the brine discharge environment: *A. affinis* (topsmelt), *A. bahia* (mysid shrimp), and *M. pyrifera* (giant kelp). The *S. purpuratus* (purple urchin) bioassay, which was the slightly more sensitive of the two other species tested was also promulgated in Phase II in order to compare to the results of several other high salinity studies that have used this bioassay.

Table 1-41. Summary of Acute Toxicity Bioassay Test Results

Test Organism	Endpoint	Phase I				Phase II - Episode 1				Phase II - Episode 2			
		EC_{25}	EC_{50}	NOEC	LOEC	EC_{25}	EC_{50}	NOEC	LOEC	EC_{25}	EC_{50}	NOEC	LOEC
Atherinops affinis	Acute Survival	45.1	48.5	42	51	> 50	> 50	50	>50	48.6	52.6	45	60
Americamysis bahia	Acute Survival	43.8	45.5	42	51	48.1	50.7	45	50	--	51.2	45	60
Citharichthys stigmaeus	Acute Survival	53.3	55.5	51	60								

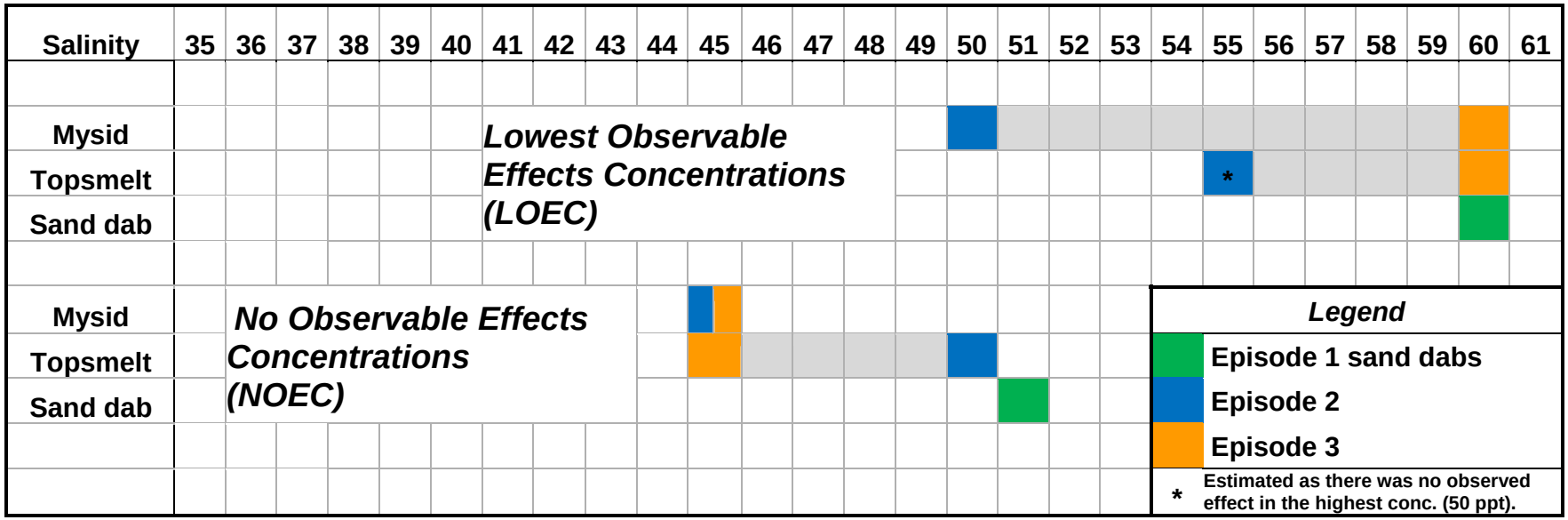

Figure 1-1. Acute Toxicity LOEC and NOEC Species Comparisons

As presented in Table 1-42, the results of Phase II, Episodes 1 and 2, show that the salinity effects were consistently least significant on *A. affinis*. Although the *M. pyrifera* bioassays resulted in NOEC values one dilution lower than the *A. bahia* bioassays, this occurred due to low variations from the mean commonly observed with *M. pyrifera* testing that result in differences from the control group that can be small but still statistically significant. For example, the percent difference in *M. pyrifera* germination rate between the control treatment and the 41 ppt treatment was only 10%, but this was a statistically significant difference (making it the LOEC); whereas the percent difference in *A. bahia* biomass between the control treatment and the 41 ppt treatment was 14%, yet this was *not* a statistically significant difference (making it the NOEC). The more appropriate chronic toxicity endpoint value to use for comparing species sensitivity is the IC_{50}.

The mean IC_{50} values calculated from all three test episodes for *A. bahia* biomass and *M. pyrifera* germ-tube growth (the two more sensitive endpoints for both species) were 46.4 and 55 ppt, respectively. The *A. affinis* mean IC_{50}, however, includes a conservative estimate of 51 ppt for the Phase II, Episode 1 bioassay because the IC_{50} was greater than the highest test concentration of 50 ppt, which was not calculable. As a result, the actual difference between the two would have been even greater. Therefore, *A. bahia* is considered the most sensitive chronic toxicity test organism among the three chosen as representatives of species most likely to reside in the discharge environment. Figure 1-2 shows the results of the most sensitive *A. bahia* test episode (Phase II, Episode 2) and toxicity endpoint (biomass). The lowest salinity level to elicit a significant effect on *A. bahia* biomass was 45 ppt. The highest salinity level that did not result in statistically significant effects (NOEC) was 41 ppt. As expected, the purple urchin was more sensitive to the effects of high salinity than the mysid shrimp. The average Phase II no-effects concentration calculated for the purple urchin was 36 ppt, and the three episode IC_{50} average was 37.1 ppt.

Table 1-42. Summary of Chronic Toxicity Bioassay Test Results

Test Organism	Endpoint	Phase I				Phase II - Episode 1				Phase II - Episode 2			
		EC_{25}	EC_{50}	NOEC	TU_C	EC_{25}	EC_{50}	NOEC	TU_C	EC_{25}	EC_{50}	NOEC	TU_C
Americamysis bahia	Survival	43.5	45.3	42	2.38	47.9	50.0	45	2.09	47.5	51.6	45	2.11
	Biomass	43.8	46.2	42	2.29	43.7	48.1	41	2.29	42.3	44.8	41	2.37
Macrocystis pyrifera	Prop Germ	51.9	54.7	42	1.93	> 50	> 50	41	2.44	48.6	56.3	39	2.06
	Growth	49.1	57.5	42	2.04	40.6	> 50	39	2.46	46.9	56.5	41	2.13
Atherinops affinis	Survival	45.1	48.5	42	2.22	> 50	> 50	50	2.00	48.6	52.6	45	2.06
	Biomass	45.4	51.7	42	2.20	> 50	> 50	50	2.00	50.0	55.1	45	2.00
Strongylocentrotus purpuratus	Prop Alive	> 70	> 70	70	1.45	> 43	> 43	43	2.33	> 43	> 43	43	2.33
	Prop Normal	34.8	36.9	33	2.87	35.7	36.4	35	2.80	37.4	38.0	37	2.67
Haliotis rufescens	Prop Normal	35.3	37.5	33	2.84								

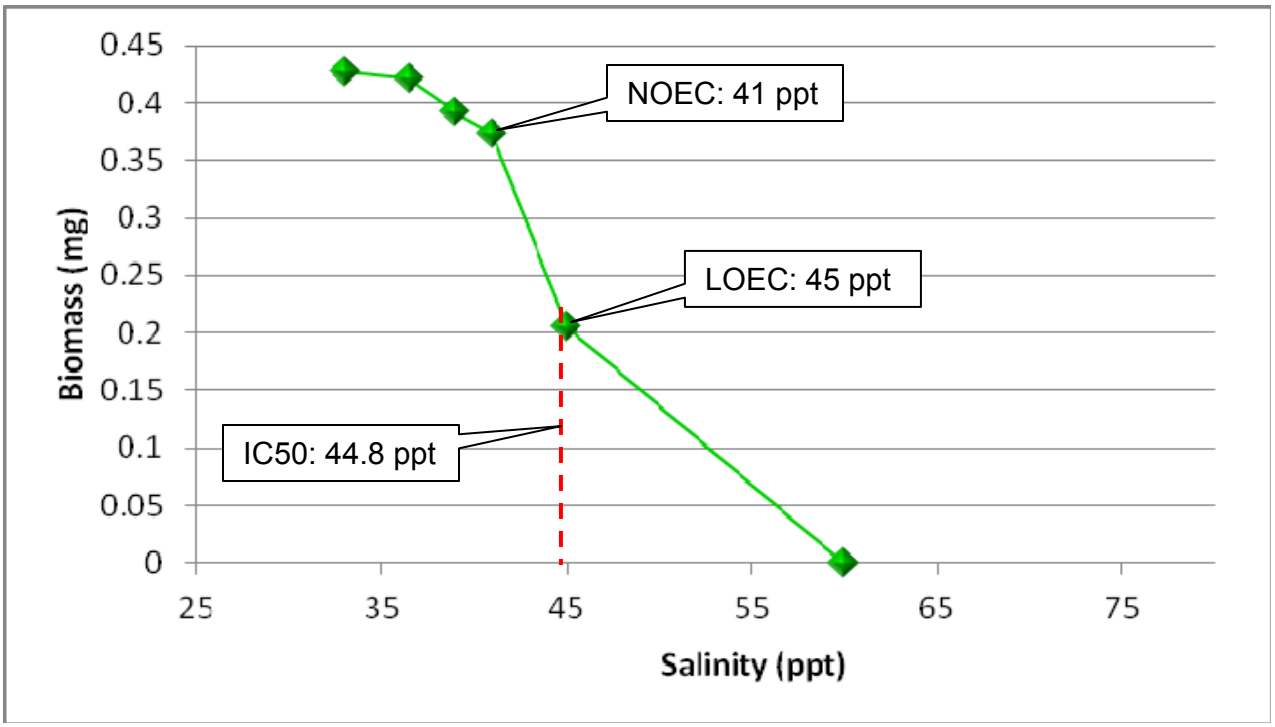

Figure 1-2. Growth Results of Most Sensitive *A. bahia* Bioassay (Phase II, Episode 2)

2.0 LONG-TERM EXPOSURE ASSESSMENT

2.1 INTRODUCTION

To assess the potential long-term effects of elevated salinities exhibited by discharges from the West Basin Municipal Water District (WBMWD) ocean water desalination demonstration facility (OWDDF) on aquatic organisms representative of faunal communities indigenous to various nearshore environments in Southern California, Weston Solutions, Inc. (WESTON) performed a biometric mesocosm study at the OWDDF in Redondo Beach, CA. The mesocosm study was also conducted to corroborate the results of the acute and chronic toxicity testing already completed to assess the *short-term* exposure effects of diluted OWDDF brine samples on various aquatic organisms. A mesocosm (i.e. mid-scale habitat simulation) was created with a split-chamber flow-through aquarium of sufficient capacity (300 gallons) to house an assembly of juvenile and adult aquatic organisms representative of the biological community of southern California.

Several invertebrate and vertebrate species were acquired from organism providers permitted by the California Department of Fish and Game. Equal numbers of each species were placed in the high and ambient salinity sides of the research aquarium, and subsequently monitored for variations in behavior and mortality. The size of the chamber necessary to adequately hold the number and size of organisms acquired prohibited the use of more than one chamber for the ambient or elevated salinity exposures. As such, a multiple replicate testing scenario was not possible. Therefore, a temporal replication approach was taken whereby three eight-week exposure Trials were performed in succession. Each exposure Trial involved successive incremental increases in salinity in the elevated salinity exposure chamber, resulting in three two-week exposures to low (37 ppt), mid (41 or 42.5 ppt) and high (44.5 or 47.5 ppt) salinity levels. In an effort to avoid any confounding influence associated with acclimation in between each of three salinity level exposure periods, Salinities in the brine exposure chamber were slowly reduced back to ambient salinity before being slowly raised to the next exposure level.

The organisms in both the ambient and high salinity test chambers were monitored daily throughout each exposure trial for mortalities and variations in behavior. In addition, sub-lethal impacts to select biological endpoints (i.e. shellfish embryo development, shellfish fertilization, and fish growth) were also assessed at the end of the mid and high salinity exposure periods for each trial. First, half of the adult invertebrate mussels or urchins were removed after the mid salinity exposure period and used to perform the U.S. EPA chronic toxicity bioassay test that assesses embryo fertilization and/or development. Gametes and/or embryos harvested from adults exposed to both ambient and elevated salinities were placed in test vials containing either ambient seawater or water with an elevated salinity matching that in which the adult organisms had been exposed, and then assessed for fertilization rate and/or embryo development success. This procedure was then repeated after the high salinity exposure period for all three Trials. Sub-lethal endpoints were also assessed using two fish species. Juvenile white sea bass and speckled sanddabs big enough not to be preyed on in the exposure chambers, but young enough to exhibit noticeable growth over the eight week exposure Trials were acquired and distributed equally between the two exposure chambers (10 of each on both sides). The fish were removed after completion of the high salinity exposure period and were then measured for weight gain and increases in length relative to the data obtained by measuring a sub-set of the test population prior to study initiation. The mortality, behavior and sub-lethal endpoint data are reported and discussed in the following sections.

2.2 METHODS

2.2.1 TEST EXPOSURE PROCEDURES

All testing was conducted at the OWDDF in a research aquarium partitioned into two 150-gallon test chambers, allowing for housing and monitoring of several test organisms under two simultaneous salinity regimes – ambient salinity and a diluted brine flow (Figure 2-1). The size of the research aquarium, the limited space available at the OWDDF, and the number of organisms desired for testing prohibited the use of multiple, simultaneously performed replicates. Therefore, a temporal replication approach was taken, which involved three eight-week exposure Trials performed in succession. During each exposure Trial, the salinity in the diluted brine flow chamber was increased incrementally twice, resulting in three two-week exposures to low (37 ppt), mid (41 or 42.5 ppt) and high (44.5 or 47.5 ppt) salinity levels. The test dates and three exposure salinities for each Trial are displayed in Table 2-1.

Figure 2-1. High Salinity Study Exposure Aquarium

Table 2-1. High Salinity Test Exposures and Duration

Test Trial	Test Dates	Salinity Regime	Test Salinity (ppt)
Trial 1 October 3, 2011 – November 28, 2011	October 3 – October 17, 2011	Low	37
	October 24 – November 7, 2011	Mid	42.5
	November 14 – November 28, 2011	High	47.5
Trial 2 December 15, 2011 – February 6, 2012	December 15 – December 29, 2011	Low	37
	January 2 – January 16, 2012	Mid	42.5
	January 23 – February 6, 2012	High	44.5
Trial 3 May 18, 2012 – July 9, 2012	May 18 – June 1, 2012	Low	37
	June 5 – June 19, 2012	Mid	41
	June 25 – July 9, 2012	High	44.5

In an effort to avoid any confounding influence associated with acclimation in between each of three salinity level exposure periods, Salinities in the brine exposure chamber were slowly reduced back to ambient salinity before being slowly raised to the next exposure level. The initial transition from ambient to the low end of the three high-salinity levels was done over a 12-hour period. The transition to and from ambient between low and mid salinity was done over 24 hours, and the transition to and from ambient between mid and high salinities was done over 36 hours. The time spent at ambient during the two transition periods was at least three days.

2.2.2 TEST ORGANISMS

The high salinity study long-term exposure test populations were comprised of the following species that were also tested during the short-term exposure testing phase: blue mussels, purple urchins, red abalone, and Pacific sanddabs. The ambient and high-salinity test populations were supplemented with various other selected species expected to occur in both soft and hard-bottom habitats of Southern California. A well-rounded blend of invertebrate and vertebrate species was achieved for each Trial. The number of individuals placed in each test chamber differed among test species; the number of individuals was dependent upon the size and type of species selected. Some species were provided from culturing facilities (i.e. white sea bass form Hubbs Seaworld Research Institute, red abalone from the Monterey Abalone Farm, and blue mussels from the Carlsbad Aquafarm), whereas the rest were wild-caught and provided by suppliers certified by the California Department of Fish and Game. As shown in Table 2-2, the test population make-up varied slightly by testing episode due to seasonality and culture availability.

Table 2-2. Test Organism Collection Sources and Use

Test Species	Source	Trial 1	Trial 2	Trial 3
Sanddabs	Brezina and Assoiciates (Dillon Beach, CA)	✓	✓	
White sea bass	Hubbs Seaworld (San Diego, CA)		✓	✓
Rockfish	Marinus Scientific (Newport Beach, CA)	✓	✓	✓
Shiner perch	Marinus Scientific (Newport Beach, CA)			✓
Kelp perch	Brezina and Associates (Dillon Beach, CA)	✓	✓	
Three-spined sticklebacks	Marinus Scientific (Newport Beach, CA)			✓
Tube snouts	Marinus Scientific (Newport Beach, CA)		✓	
Olive snails	Brezina and Associates (Dillon Beach, CA)	✓	✓	✓
Purple urchins	Monterey Abalone Farm (Monterey Bay) or Dave Gutoff (Point Loma, San Diego, CA)	✓	✓	✓
Red abalone	Monterey Abalone Farm (Monterey Bay)	✓	✓	✓
Blue mussels	Carlsbad Aquafarms (Cultured in Carlsbad, CA)	✓	✓	✓
Bat stars	Marinus Scientific (Newport Beach, CA)	✓	✓	✓
Sand crabs	Marinus Scientific (Newport Beach, CA)		✓	
Slender crabs	Sea Lab (Redondo Beach, CA)	✓		
Kelp crabs	Sea Lab (Redondo Beach, CA)		✓	✓

The health of the test organisms included in the long-term high salinity exposure assessment was monitored daily. The aquarium and salinity levels were maintained and controlled by the West Basin's OWDDF operations contractor, Unified Water. Mortality and qualitative assessments of health (i.e., appearance, feeding, and activity) were recorded for the duration of the long-term assessment.

Post-exposure endpoint assessments were also performed to assess potential sub-lethal impacts to certain biological endpoints. Either or both of the two fish test species, the speckled sanddab (*Citharichthys stigmaeus*) and the white seabass (*Atractoscion nobilis*), were exposed as juveniles, so that their growth rates (length and weight) could be measured and compared at the end of each Trial. Additionally, at the completion of the mid and high salinity exposures of each Trial, effects on reproductive health were evaluated with either, the purple urchin (*Strongylocentrotus purpuratus*) or the blue mussel (*Mytilus galloprovincialis*). Embryo development rates were measured for all three Trials. Embryo fertilization rates were also measured after the mid-salinity exposure of Trial 3.

2.2.3 POST-EXPOSURE ENDPOINT ASSESSMENTS

The sub-lethal endpoints assessed with the fish and invertebrate species are outlined in Table 2-3.

Table 2-3. Sub-Lethal Biological Endpoints Measured

Biological Endpoint	Trial 1		Trial 2		Trial 3	
	Mid Salinity	High Salinity	Mid Salinity	High Salinity	Mid Salinity	High Salinity
Sanddab growth		✓		✓		
White sea bass growth				✓		✓
Blue mussel embryo development		✓				
Purple urchin embryo development			✓	✓	✓	✓
Purple urchin fertilization					✓	

2.2.3.1 *Fish Growth*

Sanddabs and white sea bass were used to assess high salinity impacts on fish growth. Weight and standard lengths were measured with ten randomly selected fish of each species batch upon initiation of each Trial. Then another randomly selected ten fish were added to both the ambient and high salinity chambers. Weights and lengths were then measured on all surviving fish at the end of each Trial. White sea bass of the appropriate size-class were not available at the initiation of the first Trial, so only the sanddabs were measured at the end of this Trial. The size difference between the two fish species used in Trial 3 was too great, and the sanddabs fell prey to the sea bass in both exposure treatments, leaving only the sea bass to measure at the end of this Trial.

2.2.3.2 *Invertebrate embryo development*

The mussel and urchin methods used to assess reproductive success are derived from U.S. EPA protocols for assessing chronic toxicity of effluent samples (USEPA 2002). Spawning of the adults harvested after the mid and high-salinity exposure periods were induced by temperature manipulation (bivalves) or by injecting adult organisms with 0.5 mL of 0.5M KCl (urchin). Unfertilized eggs were separated from debris by filtering the suspension through an 80-um Nitex mesh screen. Released gametes were then combined in individual containers of filtered seawater and allowed to fertilize for up to one (urchin) or two (bivalves) hours under gentle aeration. Embryo stock density was estimated by counting an aliquot of dilute stock concentrate. Equal volumes of stock were then added to each test chamber to achieve a density of 150 - 300 embryos/mL. The tests were run using four or five replicates for each of four treatments:

1. Embryos from adults exposed to ambient seawater tested with ambient seawater
2. Embryos from adults exposed to ambient seawater tested with diluted brine
3. Embryos from adults exposed to elevated salinity tested with ambient seawater
4. Embryos from adults exposed to elevated salinity tested with diluted brine

The tests were performed at 16 ± 1^{o}C (bivalves) or 15 ± 1^{o}C (urchin) under a 16-hour light: eight-hour dark photoperiod. Temperature, pH, dissolved oxygen, and salinity were measured at test initiation and termination. After 48 hours (mussel) or 72 (urchin) hours, each treatment replicate was preserved using a 0.5-mL formaldehyde solution. All (mussel) or the first 100 (urchin) larvae in each replicate are counted in a Sedgwick-Rafter cell, and the total number of normally and abnormally developed larvae is determined. The test acceptability criterion for the bivalve tests is >70% lab control survival, whereas the acceptability criterion for the urchin test is >80% lab control survival (only normally developed larvae were enumerated as surviving). A reference toxicant test was also conducted using copper sulfate as a positive control Test acceptability criteria are presented below.

Blue Mussel Test Acceptability Criteria

1. Control survival must meet or exceed 70%,
2. At least 90% normal shell development in surviving controls,
3. Minimum significant difference (MSD) <25%.

Purple Urchin Test Acceptability Criteria

1. Larval normality to equal or exceed 80% in the controls,
2. MSD is less than or equal to 20% relative to the controls.

2.3 Results

2.3.1 MORTALITY AND OBSERVATIONS

2.3.1.1 *Mortality*

Tables 2-4 through 2-6 summarize the effects of high-salinity exposure on survival of all organisms exposed during the three Trials. Significant effects on survival were only observed with the red abalone and the purple urchins during the high-salinity exposure periods. The red abalone survival was affected during the high salinity (47.5 ppt) exposure period of Trial 1, but not the high salinity (44.5 ppt) exposure periods for Trials 2 and 3. Purple urchin survival was affected by the high salinity exposure period of Trial 1, but less affected during the Trial 2 high salinity exposure period, and no significant urchin mortality occurred during the high salinity exposure period of Trial 3. Mortality and other observations were recorded in the benchsheets provided in Appendix B.

Table 2-4. Mortalities Observed During Long-Term Exposure Trial #1

Test Species	Origin al Number of Organisms	Mortalities					
		Low Salinity (37 ppt)		Mid Salinity (42.5 ppt)		High Salinity (47.5 ppt)	
		Ambient	Test	Ambient	Test	Ambient	Test
Sanddabs	10	0	1	0	0	0	0
Rockfish	4	0	0	1	0	0	0
Kelp perch	6	0	0	0	0	1	0
Olive snails	12	0	0	0	0	0	0
Purple urchins	6	1	0	0	0	0	5
Red abalone	7	0	0	0	0	0	3
Blue mussels	30	3	3	1	0	0	0
Bat stars	4	0	0	0	0	0	0
Slender crabs	2	1	0	1	2	0	0

Table 2-5. Mortalities Observed During Long-Term Exposure Trial #2

Test Species	Original Number of Organisms	Mortalities					
		Low Salinity (37 ppt)		Mid Salinity (42.5 ppt)		High Salinity (44.5 ppt)	
		Ambient	Test	Ambient	Test	Ambient	Test
Sanddabs	10	0	0	0	0	0	0
White sea bass	10	0	0	0	0	0	0
Rockfish	4	0	0	0	0	0	0
Kelp perch	9	0	0	0	0	0	0
Olive snails	24	0	0	0	0	0	0
Tube snouts	3	0	1	0	0	1	2
Purple urchins	30	3	3	0	1	0	3
Red abalone	8	0	0	0	0	0	0
Blue mussels	30	12	10	5	5	0	0
Bat stars	4	0	0	0	0	0	0
Sand crabs	10	0	0	0	0	0	0
Slender crabs	1	0	0	0	1	1	-

Table 2-6. Mortalities Observed During Long-Term Exposure Trial #3

Test Species	Original Number of Organisms	Mortalities					
		Low Salinity (37 ppt)		Mid Salinity (41 ppt)		High Salinity (44.5 ppt)	
		Ambient	Test	Ambient	Test	Ambient	Test
Sanddabs	25	0	0	0	0	0	0
White Sea Bass	10	0	1	0	0	0	0
3-Spined Sticklebacks	3	0	0	0	0	0	0
Rockfish	8	0	0	0	0	0	0
Kelp perch	5	0	0	0	0	0	0
Shiner perch	2	0	0	0	0	1	0
Olive snails	15	0	0	0	0	0	0
Purple urchins	34	10	6	1	1	0	0
Red abalone	8	0	0	0	0	0	0
Blue mussels	50	0	0	0	0	0	0
Bat stars	4	0	0	0	0	0	0
Kelp crabs	4	2	0	0	0	0	0

2.3.1.2 *Behavior and Appearance*

With the exception of purple urchins, red abalone and bat stars observed in the elevated salinity chamber during the high salinity exposure periods, the behavior patterns and appearance of all organisms was consistent between the elevated salinity and ambient seawater chambers throughout the low and mid-salinity exposure periods of all three trials. Copies of the observation bench sheets are provided in Appendix B. The fish looked full-bodied and active during all *three* exposure periods for all three trials. All of the invertebrates remained intact throughout the low and mid-level salinity exposure periods. (i.e. no invertebrates were shedding limbs or degrading). During the high salinity exposure periods for all three elevated salinity exposures, some of the invertebrates seemed to be acting lethargic. A few of the purple urchins ($n < 5$) in the elevated salinity chamber during the high salinity exposure periods lost spines and many seemed to rest in place with little spine and/or tube foot movement (see Figure 2-1). The urchins on the ambient seawater chamber consistently showed good tube feet extension and were readily grazing upon supplied kelp. The urchins, abalone and bat stars on the ambient side of the aquarium during the high salinity exposure periods were able to better adhere to the aquarium walls than the same animals exposed to the high salinity organisms. The crabs and other invertebrates appeared and behaved normally in both the ambient and high salinity chambers throughout all three exposure periods of the three Trials.

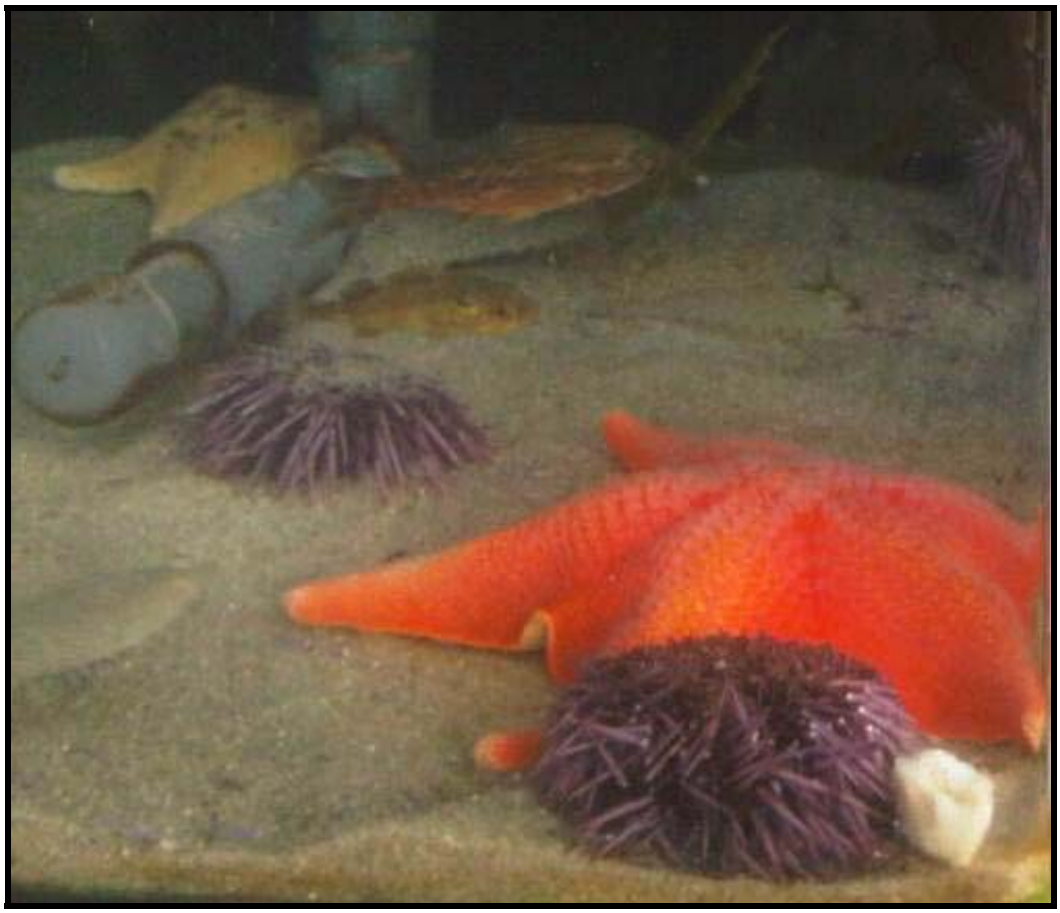

Figure 2-1. Impaired urchins at 47.5 ppt exhibiting down-turned spines

2.3.1.3 *Fish Growth*

The results of the fish growth measurements are presented in Table 2-7. Significant growth was measured among fish exposed to both the ambient and elevated salinity treatments compared to the baseline measurements for all three trials. There were no statistically significant differences in weights or lengths between the fish exposed to the ambient and elevated salinity treatments.

Table 2-7. Long-Term Exposure Fish Growth Results

Sample Identification	Trial 1		Trial 2		Trial 3	
	Mean Length (mm)	Mean Weight (g)	Mean Length (mm)	Mean Weight (g)	Mean Length (mm)	Mean Weight (g)
Pacific Sanddabs						
Baseline	52 +/- 4.7	2.6 +/- 1.2	61 +/- 9.7	2.5 +/- 1.1	NA	NA
Ambient	80 +/- 7.3	5.6 +/- 1.6	56 +/- 10	5.1 +/- 2.3	NA	NA
High Salinity	75 +/- 5.2	4.7 +/- 1.1	55 +/- 8.6	4.5 +/- 1.8	NA	NA
White Sea Bass						
Baseline	NA	NA	56 +/- 10	2.5 +/- 0.8	101 +/- 4.8	10 +/- 1.5
Ambient	NA	NA	61 +/- 7.1	4.9 +/- 1.1	120 +/- 1.6	14 +/- 3.5
High Salinity	NA	NA	62 +/- 3.3	4.5 +/- 1.0	112 +/- 10	12 +/- 3.5

NA: Not Assessed

2.3.1.4 *Invertebrate Embryo Development*

All mussels were collected from both chambers following completion of the Trial 1 high salinity exposure period, and at least 12 purple urchins were collected from both the ambient and elevated salinity chambers after the mid-salinity and high salinity exposure periods of Trials 2 and 3. These organisms were transported to commercial bioassay laboratories, and U.S. EPA approved embryo development bioassays were subsequently performed immediately. In addition, purple urchin *fertilization* bioassays were performed with urchins collected from the ambient and elevated salinity chambers after the mid-salinity exposure period of Trial 3. All water quality parameters measured were within acceptable limits throughout the 96-hour test duration of all post-exposure reproduction assessment bioassays. Upon test termination, the laboratory controls met the criteria for test acceptability for all testing. Laboratory benchsheets and statistics worksheets are provided in Appendix B.

2.3.1.5 *Trial 1 Post-Exposure Embryo Development*

No post-exposure embryo development bioassays were performed after the mid-salinity exposure period of trial 1. Following completion of high salinity (47.5 ppt) exposure period, mussels from the ambient and elevated salinity chambers were used for assessing embryo development under the four scenarios described in Section 2.2.3.2. Results of these bioassays are presented in Table 2-8. Significant effects were observed with embryos from adult mussels collected from the elevated salinity chamber when exposed to either ambient seawater or high salinity (47.5 ppt) solutions. However, the effect was less severe in mussels embryos exposed to ambient seawater, indicating the potential for recovery of mussel embryos discharged in ambient seawater after adults are exposed to salinities above 40 ppt.

Table 2-8. Long-Term High Salinity Toxicity Test Results Summary
Blue Mussel Embryo Developmental Endpoint
First Trial – HIGH Salinity

Adult Treatment	Embryo Exposure Salinity (ppt)	Mean Prop. Normal	Proportion Normal per Replicate			
			1	*2*	*3*	*4*
Ambient Exposure	33	91.5	91.6	91.5	91.5	91.5
	47.5	0.0	0.0	0.0	0.0	0.0
47.5 ppt Exposure	33	59.7	75.0	16.0	68.4	79.2
	47.5	0.21	0.42	0.0	0.42	0.0

2.3.1.6 *Trial 2 Post-Exposure Embryo Development*

Purple urchins from the mid-salinity and high salinity exposure periods were collected and used for assessing embryo development under the four scenarios described in Section 2.2.3.2. Results of these bioassays are presented in Tables 2-9 and 2-10. Significant effects were observed with embryos from adults collected from the elevated salinity chamber after both the mid-salinity (42.5 ppt) and high salinity (44.5 ppt) exposure periods. With normal development rates of 4.4 and 0.8% observed with the ambient salinity bioassays performed with embryos from the adult urchins exposed to the elevated salinities, there may be some potential for recovery of mussel embryos discharged to ambient seawater after adults are exposed to salinities above 40 ppt.

Table 2-9. Long-Term High Salinity Toxicity Test Results Summary
Purple Urchin Embryo Developmental Endpoint
Second Trial – MID Salinity

Adult Treatment	Embryo Exposure Salinity (ppt)	Mean Prop. Normal	Proportion Normal per Replicate			
			1	*2*	*3*	*4*
Ambient Exposure	33	88.7	91.6	91.6	89.9	81.6
	42.5	0.0	0.0	0.0	0.0	0.0
42.5 ppt Exposure	33	4.6	7.5	5.3	2.9	2.9
	42.5	0.0	0.0	0.0	0.0	0.0

Table 2-10. Long-Term High Salinity Toxicity Test Results Summary
Purple Urchin Embryo Developmental Endpoint
Second Trial – HIGH Salinity

Adult Treatment	Embryo Exposure Salinity (ppt)	Mean Prop. Normal	Proportion Normal per Replicate			
			1	*2*	*3*	*4*
Ambient Exposure	33	45.0	45.3	45.0	41.9	47.8
	44.5	0.0	0.0	0.0	0.0	0.0
44.5 ppt Exposure	33	0.8	1.1	1.4	0.9	0.0
	44.5	0.0	0.0	0.0	0.0	0.0

2.3.1.7 *Trial 3 Post-Exposure Embryo Development and Fertilization*

Purple urchins from the mid-salinity and high salinity exposure periods were collected and used for assessing embryo development under the four scenarios described in Section 2.2.3.2. Results of these bioassays are presented in Tables 2-11 and 2-12. Significant effects were observed with embryos from adults collected from the elevated salinity chamber after both the mid-salinity (41 ppt) and high salinity (44.5 ppt) exposure periods. There was *not* a statistically significant reduction in the normal development rate observed with the ambient salinity bioassays performed with embryos from the adult urchins exposed during the mid-salinity (41 ppt) exposure period. Based on the results observed for embryos from adults exposed to the higher mid-salinity level from Trial 2 (42.5 ppt), it appears that the adult exposure threshold for ensuring recovery of urchin embryos discharged to ambient seawater is 41 or 42 ppt.

Fertilization potential for gametes collected from urchins exposed during the mid-salinity exposure period (41 ppt) was also evaluated. The fertilization results are presented in Table 2-13. Fertilization rates were not affected when tested with either ambient or similarly elevated salinity (41 ppt) levels. This single fertilization assessment bioassay seems to indicate that the purple urchin fertilization process is substantially more tolerant to elevated salinity exposures than the post-fertilization embryo development process.

Table 2-11. Long-Term High Salinity Toxicity Test Results Summary
Purple Urchin Developmental Endpoint
Third Trial – MID Salinity

Adult Treatment	Embryo Exposure Salinity (ppt)	Mean Prop. Normal	Proportion Normal per Replicate				
			1	*2*	*3*	*4*	*5*
Ambient Exposure	33	92.8	96.9	92.9	87.7	95.7	90.8
	41	25.6	19.7	15.7	40.0	2.8	49.7
41 ppt Exposure	33	86.9	86.2	91.4	88.4	76.7	91.8
	41	0.0	0.0	0.0	0.0	0.0	0.0

Table 2-12. Long-Term High Salinity Toxicity Test Results Summary
Purple Urchin Embryo Developmental Endpoint
Third Trial – HIGH Salinity

Adult Treatment	Embryo Exposure Salinity (ppt)	Mean Prop. Normal	Proportion Normal per Replicate				
			1	*2*	*3*	*4*	*5*
Ambient Exposure	33	23.2	28.6	18.8	28.1	27.8	12.9
	44.5	0.0	0.0	0.0	0.0	0.0	0.0
44.5 ppt Exposure	33	0.0	0.0	0.0	0.0	0.0	0.0
	44.5	0.0	0.0	0.0	0.0	0.0	0.0

Table 2-13. Long-Term High Salinity Toxicity Test Results Summary
Purple Urchin Fertilization Endpoint
Third Trial – MID Salinity

Adult Treatment	Embryo Exposure Salinity (ppt)	Mean Prop. Normal	Proportion Normal per Replicate				
			1	*2*	*3*	*4*	*5*
Ambient Exposure	33	93.6	97.3	95.1	94.6	84.0	97.0
	41	82.3	81.5	82.9	84.2	76.6	86.4
44.5 ppt Exposure	33	92.0	100	89.6	100	95.7	74.6
	41	88.6	86.5	96.3	90.8	82.6	86.5

3.0 REFERENCES

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USEPA 2002b. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, 3rd Edition. EPA-821/R-02/014.October 2002.