

## **APPENDIX H**

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# Bioassay Laboratory Report



**Sample SH-05-SS-00 was misidentified as  
sample SH-06-SS-00 in the following  
documents.**



***BIOLOGICAL TESTING RESULTS FOR  
OAKLAND BAY SEDIMENT CHARACTERIZATION,  
OAKLAND BAY, WASHINGTON***

REVISED APRIL 2010

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## 1.0 INTRODUCTION

NewFields conducted toxicity tests with sediment samples collected in Oakland Bay as part of a sediment characterization study. This study was performed under the Washington State Department of Ecology's Toxics Cleanup Program as part of the Puget Sound Initiative. Sediment samples were collected by Herrera Environmental Consultants, Inc. Biological effects were evaluated relative to the biological criteria defined in the Sediment Management Standards (SMS). This report presents the results of the toxicity testing portion of the Oakland Bay sediment characterization.

## 2.0 METHODS

Test methods followed guidance provided by the Puget Sound Estuary Program (PSEP 1995), the WDOE Sampling and Analysis Plan Appendix (SAPA; Ecology 2008), and the various updates presented during the Annual Sediment Management Review meetings (SMARM). Sediment toxicity was evaluated using three standard PSEP bioassays, the 10-day amphipod test, the 20-day juvenile polychaete test, the 48 to 96-hour larval development test, and the Microtox® porewater test. NewFields performed the amphipod, juvenile polychaete and benthic larval tests. The Microtox® test was performed by Nautilus Environmental LLC.

### 2.1 SAMPLE COLLECTION

Test sediment was collected from Oakland Bay by Herrera personnel between September 29 and October 5, 2008. Reference sediment was collected from three sites on October 9, 2008.

### 2.2 SAMPLE AND ANIMAL RECEIPT

Fifty test sediment samples and three reference sediment samples were received by NewFields and stored in a walk-in cold room at  $4 \pm 2^{\circ}\text{C}$  in the dark until testing. Test sediment was not sieved prior to testing. All tests were conducted within the 8-week holding time.

The amphipods *Ampelisca abdita* were supplied by Brezina and Associates in Dillon Beach, California and held in native sediment at  $20^{\circ}\text{C}$  prior to test initiation. The amphipods *Eohaustorius estuarius* were supplied by Northwest Aquatic Sciences in Newport, Oregon and held in native sediment at  $15^{\circ}\text{C}$  prior to test initiation. Juvenile polychaete worms (*Neanthes arenaceodentata*) were supplied by Donald Reish, Ph.D., Long Beach, California. Juvenile polychaetes were held in seawater at  $20^{\circ}\text{C}$  (*Neanthes* are cultured in water-only and are not held in sediment prior to testing). *Mytilus* sp. (mussel) broodstock were supplied by Carlsbad Aquafarms, Carlsbad, California. Broodstock were held in unfiltered seawater from Hood Canal prior to spawning.

Native *E. estuarius* sediment from Yaquina Bay, Oregon was also provided by Northwest Aquatic Sciences for use as control sediment treatments for the amphipod test with *E. estuarius* and the juvenile polychaete test. Native *A. abdita* sediment from Tomales Bay, California was also provided by Brezina and Associates for use as a control sediment treatment for the amphipod test with *A. abdita*.

### 2.3 ULTRA-VIOLET LIGHT EXPOSURE

Test sediment samples from locations with water depths of less than 12 ft were exposed to ultra-violet (UV) light during the entire test exposure. All sediments evaluated in this study were tested under these conditions for all species (except Microtox). The UV light regime followed

guidance provided by Sub-Appendix D and in consultation with Ecology. UV light was provided by a fluorescent light ballast containing one Duro-Test Vita-Lite® (40W, 5500°K, 91 CRI) fluorescent bulb and one standard fluorescent bulb (Phillips F40CW). The UV bulbs were placed within 12” above the sediment surface. All test chambers in the UV exposures were left uncovered to prevent any UV loss. Tests were conducted on water-tables to ensure that the additional lighting did not alter water temperatures in the test chambers. In all other respects, the methods followed the standard testing protocols are summarized below.

2.4 SAMPLE BATCHING

The fifty sediment samples and the three reference samples were run in batches as shown in Table 1. Two batches were run for all tests except for Microtox®, which was conducted in 14 batches due to limitations in the testing apparatus. Each batch included the appropriate reference samples for the respective sediment treatments tested.

The two amphipod test batches utilized different species that were selected based on the grain size of the respective samples. Amphipod test Batch 1 included 20 samples having ≥ 60% fines as determined by the wet sieve grain size method (PSEP 1997) and utilized the species *A. abdita*. Reference sediments RF-01-SS-00 and RF-02-SS-00 were run concurrently with this batch. Amphipod test Batch 2 included 30 samples having < 60% fines as determined by the wet sieve grain size method and utilized the species *E. estuarius*. Reference sediments RF-02-SS-00 and RF-03-SS-00 were run concurrently with this batch.

**Table 1. List of Samples with Collection Date, Receipt Date, and Test Initiation Date.**

Sample	Date Collected	Date Received	Test Batch Number (Initiation Dates Shown Below)			
			Amphipod <sup>1,2</sup>	Polychaete	Larval	Microtox®
				<i>N. arenaceodentata</i>	<i>Mytilus sp.</i>	<i>V. fisheri</i>
RF-01-SS-00	10/9/2008	10/14/2008	1	1,2	1,2	6, 7, 8
RF-02-SS-00	10/9/2008	10/14/2008	1,2	1,2	1,2	9, 10, 11, 12, 13, 14
RF-03-SS-00	10/9/2008	10/14/2008	2	1,2	1,2	1, 2, 3, 4, 5
HI-02-SS-00	10/5/2008	10/6/2008	2	1	2	2
HI-03-SS-00	10/5/2008	10/6/2008	2	2	2	2
HI-04-SS-00	10/5/2008	10/6/2008	2	1	2	2
HI-05-SS-00	10/2/2008	10/3/2008	2	2	1	3
HI-06-SS-00	10/3/2008	10/6/2008	2	1	2	2
HI-07-SS-00	10/2/2008	10/3/2008	2	1	1	3
OB-01-SS-00	10/2/2008	10/3/2008	2	1	1	4
OB-02-SS-00	10/2/2008	10/3/2008	2	1	1	10
OB-03-SS-00	10/2/2008	10/3/2008	2	2	1	10
OB-04-SS-00	10/3/2008	10/6/2008	2	2	2	13
OB-05-SS-00	10/3/2008	10/6/2008	1	1	2	6
OB-06-SS-00	10/3/2008	10/6/2008	1	1	2	7
OB-07-SS-00	10/5/2008	10/6/2008	2	2	2	1
OB-08-SS-00	10/5/2008	10/6/2008	1	2	2	6
OB-09-SS-00	10/3/2008	10/6/2008	1	2	2	8
OB-10-SS-00	10/4/2008	10/6/2008	1	2	2	7
OB-11-SS-00	10/3/2008	10/6/2008	1	1	2	8
OB-12-SS-00	10/4/2008	10/6/2008	1	1	2	6
OB-13-SS-00	10/4/2008	10/6/2008	1	1	2	12

Sample	Date Collected	Date Received	Test Batch Number (Initiation Dates Shown Below)			
			Amphipod <sup>1, 2</sup>	Polychaete	Larval	Microtox®
				<i>N. arenaceodentata</i>	<i>Mytilus sp.</i>	<i>V. fisheri</i>
OB-14-SS-00	10/4/2008	10/6/2008	2	1	2	12
OB-17-WS-00	10/3/2008	10/6/2008	2	1	2	12
OB-18-WS-00	10/3/2008	10/6/2008	1	2	2	7
OB-19-WS-00	10/4/2008	10/6/2008	1	1	2	8
SH-01-SS-00	9/29/2008	10/3/2008	2	1	1	3
SH-02-SS-00	9/30/2008	10/3/2008	2	2	1	11
SH-03-SS-00	10/1/2008	10/3/2008	1	1	1	12
SH-04-SS-00	10/1/2008	10/3/2008	1	1	1	9
SH-06-SS-00	10/1/2008	10/3/2008	2	2	1	14
SH-07-SS-00	10/5/2008	10/6/2008	2	2	2	13
SH-09-SS-00	10/1/2008	10/3/2008	2	1	1	9
SH-10-SS-00	9/30/2008	10/3/2008	2	1	1	4
SH-11-SS-00	10/2/2008	10/3/2008	1	1	2	10
SH-12-SS-00	10/2/2008	10/3/2008	1	2	2	13
SH-13-SS-00	10/5/2008	10/6/2008	1	1	2	13
SH-14-SS-00	9/30/2008	10/3/2008	2	1	1	9
SH-15-SS-00	10/1/2008	10/3/2008	2	2	1	4
SH-16-SS-00	10/1/2008	10/3/2008	2	2	1	4
SH-18-WS-00	10/2/2008	10/3/2008	1	2	2	8
SH-19-WS-00	10/1/2008	10/3/2008	1	2	1	10
SH-20-WS-00	10/1/2008	10/3/2008	1	2	1	6
SH-21-WS-00	10/1/2008	10/3/2008	1	1	1	7
SH-22-WS-00	9/30/2008	10/3/2008	2	1	1	5
SH-23-WS-00	9/30/2008	10/3/2008	1	2	1	11
SH-24-WS-00	10/1/2008	10/3/2008	2	2	1	11
SH-25-WS-00	10/2/2008	10/3/2008	2	2	2	11
SH-26-WS-00	9/30/2008	10/3/2008	2	2	1	3
SH-27-WS-00	9/30/2008	10/3/2008	2	2	1	1
SH-28-WS-00	9/30/2008	10/3/2008	2	1	1	9
SH-29-WS-00	10/1/2008	10/3/2008	2	2	1	1
SH-30-WS-00	10/1/2008	10/3/2008	2	2	1	1
Batch Initiation Date	1	11/11/08	10/30/08	11/20/28	11/6/08 –	
	2	11/14/08	11/5/08	11/25/08	11/26/08	

<sup>1</sup> Amphipod test Batch 1 utilized the species *A. abdita*

<sup>2</sup> Amphipod test Batch 2 utilized the species *E. estuarius*

## 2.5 10-DAY AMPHIPOD BIOASSAY (*A. abdita*)

The 10-day acute toxicity test with *A. abdita* was initiated on November 11, 2008. Test exposures were prepared with approximately 175 mL of sediment placed in clean, acid and solvent-rinsed 1-L glass jars, which were then filled with 775 mL of 0.45-µm filtered seawater at 28 ppt. Seven replicate chambers were prepared for each test treatment, the two reference sediments, and the native control sediment. Five replicates were used to evaluate sediment toxicity while the remaining two replicates were designated as sacrificial surrogate chambers. One surrogate chamber was sacrificed at test initiation to measure porewater and overlying ammonia and sulfides. The remaining surrogate chamber was used for measuring daily water quality throughout the test, as well as porewater and overlying ammonia and sulfides at test

termination. Total ammonia as nitrogen was monitored using an Orion meter fitted with an ammonia ion-specific probe. Total sulfides as  $S^{2-}$  were monitored using a HACH DR/4000V Spectrophotometer.

Test chambers were placed in randomly assigned positions in a 20°C water bath and allowed to equilibrate overnight. Trickle-flow aeration was provided to prevent dissolved oxygen concentrations from dropping below acceptable levels.

Immediately prior to test initiation, water quality parameters were measured in the surrogate chamber for each treatment. Dissolved oxygen (DO), temperature, pH, and salinity were then monitored in the surrogate chambers daily until test termination. Target test parameters were:

Dissolved Oxygen:	≥5.0 mg/L
pH:	7.8 ± 0.5 units
Temperature:	20 ± 1°C
Salinity:	28 ± 2‰

The tests were initiated by randomly allocating 20 *A. abdita* into each test chamber, ensuring that each of the amphipods successfully buried into the sediment. Amphipods that did not bury within approximately one hour were replaced with healthy amphipods. The 10-day amphipod bioassay was conducted as a static test with no feeding during the exposure period. At test termination, sediment from each test chamber was sieved through a 0.5-mm screen and all recovered amphipods transferred into a Petri dish. The number of surviving and dead amphipods was then determined under a dissecting microscope. A water-only, 4-day reference-toxicant test was conducted concurrently with the sediment tests, using cadmium chloride. The cadmium reference-toxicant test was used to ensure animals used in the test were healthy and of similar sensitivity to prior tests.

## 2.6 10-DAY AMPHIPOD BIOASSAY (*E. estuarius*)

The 10-day acute toxicity test with *E. estuarius* was initiated on November 14, 2008. Test exposures were prepared with approximately 175 mL of sediment placed in clean, acid and solvent-rinsed 1-L glass jars, which were then filled with 775 mL of 0.45-µm filtered seawater at 28 ppt. Seven replicate chambers were prepared for each test treatment, the two reference sediments, and the native control sediment. Five replicates were used to evaluate sediment toxicity while the remaining two replicates were designated as sacrificial surrogate chambers. One surrogate chamber was sacrificed at test initiation to measure porewater and overlying ammonia and sulfides. The remaining surrogate chamber was used for measuring daily water quality throughout the test, as well as porewater and overlying ammonia and sulfides at test termination. Total ammonia as nitrogen was monitored using an Orion meter fitted with an ammonia ion-specific probe. Total sulfides as  $S^{2-}$  were monitored using a HACH DR/4000V Spectrophotometer.

Test chambers were placed in randomly assigned positions in a 15°C water bath and allowed to equilibrate overnight. Trickle-flow aeration was provided to prevent dissolved oxygen concentrations from dropping below acceptable levels.

Immediately prior to test initiation, water quality parameters were measured in the surrogate chamber for each treatment. Dissolved oxygen (DO), temperature, pH, and salinity were then monitored in the surrogate chambers daily until test termination. Target test parameters were:

Dissolved Oxygen:	≥5.0 mg/L
pH:	7.8 ± 0.5 units
Temperature:	15 ± 1°C
Salinity:	28 ± 2‰

The tests were initiated by randomly allocating 20 *E. estuarius* into each test chamber, ensuring that each of the amphipods successfully buried into the sediment. Amphipods that did not bury within approximately one hour were replaced with healthy amphipods. The 10-day amphipod bioassay was conducted as a static test with no feeding during the exposure period. At test termination, sediment from each test chamber was sieved through a 0.5-mm screen and all recovered amphipods transferred into a Petri dish. The number of surviving and dead amphipods was then determined under a dissecting microscope. A water-only, 4-day reference-toxicant test was conducted concurrently with the sediment tests, using cadmium chloride. The cadmium reference-toxicant test was used to ensure animals used in the test were healthy and of similar sensitivity to prior tests.

## 2.7 20-DAY JUVENILE POLYCHAETE BIOASSAY

The 20-day chronic toxicity tests with *N. arenaceodentata* were initiated in two batches on October 30 and November 5, 2008. Test exposures were prepared with approximately 175 mL of sediment placed in clean, acid and solvent-rinsed 1-L glass jars, which were then filled with 775 mL of 0.45- $\mu$ m filtered seawater at 28 ppt. Seven replicate chambers were prepared for each test treatment, the three reference sediments, and control sediment. The control sediment and reference treatments were tested with each batch of test treatments. Five replicates were used to evaluate sediment toxicity while the remaining two replicates were designated as sacrificial surrogate chambers. One surrogate chamber was sacrificed at test initiation to measure porewater and overlying ammonia and sulfides. The remaining surrogate chamber was used for measuring daily water quality throughout the test, as well as porewater and overlying ammonia and sulfides at test termination. Total ammonia as nitrogen was monitored using an Orion meter fitted with an ammonia ion-specific probe. Total sulfides as S<sup>2-</sup> were monitored using a HACH DR/4000V Spectrophotometer.

Test chambers were placed in randomly assigned positions in a water bath at 20°C and allowed to equilibrate overnight. Trickle-flow aeration was provided to prevent dissolved oxygen concentrations from dropping below acceptable levels.

Immediately prior to test initiation, water quality parameters were measured. Dissolved oxygen, temperature, pH, and salinity were then monitored in the surrogates daily until test termination. Target test parameters were:

Dissolved Oxygen:	≥5.5 mg/L
pH:	7.8 ± 0.5 units
Temperature:	20 ± 1°C
Salinity:	28 ± 2‰

The juvenile polychaete test was initiated by randomly allocating five *N. arenaceodentata* into each test chamber, and observing whether each of the worms successfully buried into the sediment. Worms that did not bury within approximately one hour were replaced with healthy worms. The 20-day test was conducted as a static-renewal test, with exchanges of 300 mL of water occurring every third day. *N. arenaceodentata* were fed every other day with 40 mg of TetraMarin® (approximately 8 mg dry weight per worm). At test termination, sediment from each test chamber was sieved through a 0.5-mm screen and all recovered worms transferred

into a Petri dish. The number of surviving and dead worms was determined. All surviving worms were then transferred to pre-weighed, aluminum foil weigh-boats, and then dried in a drying oven at 60°C for approximately 24 hours. Each weigh-boat was removed, cooled in a dessicator, and then weighed on a microbalance to 0.001 mg. A water-only, 4-day reference-toxicant test was conducted concurrently with the sediment tests, using cadmium chloride. The cadmium reference-toxicant test was used to ensure animals used in the test were healthy and of similar sensitivity to prior tests.

## 2.8 LARVAL DEVELOPMENTAL BIOASSAY

Test sediment was evaluated using the benthic larval development test with the mussel, *Mytilus* sp. The larval tests were initiated in two batches on November 20 and 25, 2008. To prepare the test exposures, 18 g ( $\pm$  0.5 g) of test sediment were placed in clean, acid and solvent-rinsed 1-L glass jars, which were then filled with 900 mL of 0.45- $\mu$ m of filtered seawater. Six replicate chambers were prepared for each test treatment, the three reference sediments, and a seawater control. The six control chambers contained filtered seawater without sediment. Five of the replicates were used to evaluate the test; the sixth replicate was used as a water quality surrogate. Each chamber was shaken for 10 seconds and then placed in predetermined randomly-assigned positions in a water bath at 16°C.

To collect gametes for each test, mussels were placed in clean seawater and acclimated at 12°C for approximately 20 minutes. The water bath temperature was then increased over a period of 15 minutes to 20°C. Mussels were held at 20°C and monitored for spawning individuals. Spawning females and males were removed from the water bath and placed in individual containers with seawater. These individuals were allowed to spawn until sufficient gametes were available to initiate the test. After the spawning period, eggs are transferred to fresh seawater and filtered through a .5 mm Nitex® mesh screen to remove large debris, feces, and excess gonadal matter. A composite is made of the sperm and diluted with fresh seawater. The fertilization process was initiated by adding sperm to the isolated egg containers. Egg-sperm solutions were periodically homogenized with a perforated plunger during the fertilization process and sub-samples observed under the microscope for egg and sperm viability. Approximately one to one and a half hours after fertilization, embryo solutions were checked for fertilization rate. Only those embryo stocks with >90% fertilization were used to initiate the tests. Embryo solutions were rinsed free of excess sperm and then combined to create one embryo stock solution. Density of the embryo stock solution was determined by counting the number of embryos in a subsample of homogenized stock solution. This was used to determine the volume of embryo stock solution to deliver approximately 27,000 embryos to each test chamber.

The test was initiated by randomly allocating an aliquot of the embryo stock solution into each test chamber four hours after sediments were shaken and within two hours of egg fertilization. Embryos were held in suspension during initiation using a perforated plunger. The actual stocking densities for the two batches were 36.6 and 28.9 embryos/mL respectively, within the target stocking density of 20 - 40 embryos/mL.

Dissolved oxygen, temperature, pH, and salinity were monitored in water quality surrogates to prevent loss or transfer of larvae by adhesion to water-quality probes. Overlying water ammonia and sulfides were measured on Day 0 and Day 2. Total ammonia as nitrogen was monitored using an Orion meter fitted with an ammonia ion-specific probe. Total sulfides as S<sup>2-</sup> were monitored using a HACH DR/4000V Spectrophotometer. Target test parameters were as follows:

Dissolved Oxygen:	≥4.0 mg/L
pH:	7.8 ± 0.5 units
Temperature:	16 ± 1°C
Salinity:	28 ± 1‰

The 48-96 hour test was conducted as a static test without aeration. The test was terminated approximately 48 hours after initiation, when 90% of the control larvae had achieved the prodissoconch I stage. At termination, the overlying seawater was decanted into a clean 1-L jar and mixed with a perforated plunger. From this container, a 10 mL subsample was transferred to a scintillation vial and preserved in 5% buffered formalin. Larvae were subsequently stained with a dilute solution of Rose Bengal in 70% alcohol to help visualization of larvae. The number of normal and abnormal larvae was enumerated on an inverted microscope. Normal larvae included all D-shaped prodissoconch I stage larvae. Abnormal larvae included abnormally shaped prodissoconch I larvae and all early stage larvae. A 48-hour water-only reference-toxicant test with copper sulfate was conducted concurrently with the sediment test.

## 2.9 MICROTOX® BIOASSAY

The Microtox® test was performed by Nautilus Environmental LLC. The Microtox test exposed the luminescent marine bacterium *Vibrio fischeri* to porewater extracted from test sediments. Bacterial light output was measured using the Microtox Model 500 Analyzer at 5 and 15 minutes of exposure. Light output from the test porewater was compared to that of the reference treatments at both time intervals. A complete description of the Microtox test methods is presented in Appendix A.

## 2.10 DATA ANALYSIS AND QA/QC

All water quality and endpoint data were entered into Excel spreadsheets. Water quality parameters were summarized by calculating the mean, minimum, and maximum values for each test treatment. Endpoint data were calculated for each replicate and the mean and standard deviation were determined for each test treatment.

All hand-entered data were reviewed for data entry errors, any found were corrected prior to summary calculations. A minimum of 10% of all calculations and data sorting were reviewed for errors. Review counts were conducted on any apparent outliers.

The control normalized normal survival endpoint in the larval test was used to evaluate the test sediment. This was based on the number of normal larvae in the treatment and reference divided by the number of normal larvae in the control, as defined in Ecology (2005).

For SMS suitability determinations, comparisons were made according to SAPA (2008) and Fox et al. (1998). All data were tested for normality using the Wilk-Shapiro test and equality of variance using Levene's test. Determinations of statistical significance were based on one-tailed Student's t-tests with an alpha level of 0.05 for the amphipod and polychaete endpoints. A comparison of the larval endpoint, relative to the reference was made using an alpha level of 0.10. For samples failing to meet assumptions of normality, a Mann-Whitney test was conducted to determine significance. For those samples failing to meet the assumptions of normality and equality of variance, a t-test on rankits was used.

### 3.0 RESULTS

The results of the sediment testing, including a summary of test results and water quality observations are presented in this section. Summaries of water quality observations are presented in Appendix B, laboratory data sheets are included as Appendix C and statistical results are provided in Appendix D.

#### 3.1 10-DAY AMPHIPOD BIOASSAY (*A. abdita*)

A summary of *A. abdita* test conditions is presented in Table 2. Mean percent mortality in the control sample was 9%, within the  $\leq 10\%$  mortality acceptance criterion. This indicates that the test conditions were suitable for adequate amphipod survival. The  $LC_{50}$  value for the cadmium reference-toxicant test performed on the organisms was 0.32 mg Cd/L, within the control chart limits (0.13-1.14 mg Cd/L), indicating that the test organisms used in this study were of similar sensitivity of those previously tested at NewFields.

Minor deviations in temperature, salinity, and pH were observed during the test. Temperatures were slightly above limits on day 6 (max 23.8°C). Temperature control system was adjusted and succeeded in maintaining test temperatures within limits for the duration of the test. Salinities ranged between 27 and 35 ppt throughout both tests, rising slightly above the recommended limits of  $28 \pm 2$  ppt. The UV light exposure method required the test jars to be uncovered during the exposure time to not impede light exposure. Some evaporation does occur over the course of the test which may explain the slight increase in salinity. These salinities are still within the tolerance range of the test organisms. The pH values were also above recommended limits, however remained within the tolerance range for these organisms. These deviations should not impact the significance of the test results.

Initial and final interstitial ammonia concentrations were all below the threshold concentration of 30 mg/L total ammonia (Barton 2002) that trigger ammonia reference toxicant testing. Initial and final sulfide concentrations were below 5 mg/L in both overlying and interstitial waters.

Mean mortality in all reference treatments met the SMS performance criteria ( $< 25\%$  mortality) and indicated that the reference sediment was acceptable for comparison. Mean mortality for all samples is shown in Table 3. Test sample SH-21-WS-00 had one replicate in which mortality was 100% whereas all other replicates had 5-25% mortality. It is possible that this replicate was not stocked with organisms at the beginning of the test. This replicate was shown to be a statistical outlier compared to the other replicates using Grubbs test (Grubbs 1969). Due to this anomaly, the mortality results from the water quality surrogate chamber were substituted for the outlier.

**Table 2. Test Condition Summary for *A. abdita*.**

Test Conditions: PSEP <i>A. abdita</i> (SMS)		
Sample Identification	See Table 1	
Date sampled	See Table 1	
Date received	See Table 1	
Sample storage conditions	4°C, dark	
Weeks of holding Recommended: ≤8 weeks (56 days)	Test 37-42 days; Ref 33 days	
Source of control sediment	Brezina and Associates (Tomales Bay, CA)	
<b>Test Species</b>	<b><i>A. abdita</i></b>	
Supplier	Brezina and Associates	
Date acquired	11/7/2008	
Acclimation/holding time	4 days	
Age class	3-5 mm	
<b>Test Procedures</b>	PSEP 1995 with SMARM revisions	
Regulatory Program	SMS	
Test location	NewFields Northwest Laboratory	
Test type/duration	10-Day static	
Test dates	11/11/08 – 11/21/08	
Control water	0.45 µm-filtered, North Hood Canal sweater, adjusted with DI water	
Test temperature	Recommended: 20 ± 1 °C	19.4 – 23.8 °C
Test Salinity	Recommended: 28 ± 2 ppt	27 – 35 ppt
Test dissolved oxygen	Recommended: > 5.0 mg/L	6.6 – 8.7 mg/L
Test pH	Recommended: 7.8 ± 0.5	7.6 – 8.8
SMS control performance standard	Recommended: Control ≤ 10% mortality	9% Pass
SMS reference performance standard	Recommended: Reference mortality < 25%	RF-02-SS-00 15% Pass, RF-03-SS-00 13% Pass
SMS pass/fail SQS	Significant Difference and Treatment – Reference > 25% mortality and statistically significant = <b>FAIL</b>	All Pass
SMS pass/fail CSL	Significant Difference and Treatment – Reference > 30% mortality and statistically significant = <b>FAIL</b>	All Pass
Reference Toxicant LC50	0.32 mg/L	
Acceptable Range	0.135-1.14 mg/L	
Test Lighting	Continuous	
Test chamber	1-Liter Glass Chamber	
Replicates/treatment	5 + 2 surrogates	
Organisms/replicate	20	
Exposure volume	175 mL sediment/ 775 mL water	
Feeding	None	
Water renewal	None	
Deviations from Test Protocol	Minor deviations in temperature, salinity, and pH	

**Table 3. Summary of Test Results for *A. abdita*.**

Sample	<i>A. Abdita</i>	
	Mean Mortality (%)	Standard Deviation
Control	9	5
RF-01-SS-00	15	5
RF-02-SS-00	13	7
OB-05-SS-00	9	7
OB-06-SS-00	14	4
OB-08-SS-00	8	8
OB-09-SS-00	10	11
OB-10-SS-00	6	7
OB-11-SS-00	11	10
OB-12-SS-00	21	19
OB-13-SS-00	10	7
OB-18-WS-00	12	8
OB-19-WS-00	8	8
SH-03-SS-00	14	7
SH-04-SS-00	9	10
SH-11-SS-00	7	6
SH-12-SS-00	11	7
SH-13-SS-00	8	7
SH-18-WS-00	10	9
SH-19-WS-00	15	9
SH-20-WS-00	10	4
SH-21-WS-00 <sup>1</sup>	14	8
SH-23-WS-00	18	8

<sup>1</sup> Surrogate results used for outlier replicate.

### 3.2 10-DAY AMPHIPOD BIOASSAY (*E. estuarius*)

A summary of *E. estuarius* test conditions is presented in Table 4. Mean percent mortality in the control sample was 1%, within the ≤10% mortality acceptance criterion. This indicates that the test conditions were suitable for adequate amphipod survival. The LC<sub>50</sub> values for the cadmium reference-toxicant tests performed on the organisms was 13.4 mg Cd/L, slightly above the control chart limits (4.0-12.2 mg Cd/L), indicating that the test organisms may be less sensitive than those previously tested at NewFields. Reference-toxicant tests performed on the same field population of organisms before and after this event Oct 28 and Dec 23 expressed LC<sub>50</sub> values within limits (6.9 and 8.0 mg Cd/L). Given the historical response of organisms collected from the same field site, and the ability of this able to detect a decrease in survival in one of the treatments (OB-03-SS-00), this deviation should not impact the significance of the test results.

All water quality parameters were within acceptable limits throughout all of the tests except for minor deviations in temperature and pH. The deviations were within the tolerance range for this species and would not be expected to affect the test results. Initial and final interstitial ammonia

concentrations were all below the threshold concentration of 30 mg/L total ammonia (Barton 2002). Sulfide concentrations were below 5 mg/L in both overlying and interstitial waters.

Mean mortality in all reference treatments met the SMS performance criteria (<25% mortality) and indicated that the reference sediment was acceptable for comparison. Mean mortality for all samples is shown in Table 5.

Replicate four of test sample OB-01-SS-00 resulted in 32 animals being recovered at the end of the test, indicating that this chamber may have been double-added (40 animals instead of 20). The water quality surrogate for this sample was sieved and provided 18 organisms (90% survival), similar to the other four replicates, all with 20 animals surviving (100% survival). The survival of the water quality surrogate was used in place of replicate four data for reporting purposes.

**Table 4. Test Condition Summary for *E. estuarius*.**

Test Conditions: PSEP <i>E. estuarius</i> (SMS)		
Sample Identification	See Table 1	
Date sampled	See Table 1	
Date received	See Table 1	
Sample storage conditions	4°C, dark	
Weeks of holding Recommended: ≤8 weeks (56 days)	Test 40-46 days; Ref 36 days	
Source of control sediment	Northwest Aquatic Sciences (Yaquina Bay, OR)	
<b>Test Species</b>	<b><i>E. estuarius</i></b>	
Supplier	Northwest Aquatic Sciences	
Date acquired	11/12/08	
Acclimation/holding time	2 days	
Age class	3-5 mm	
<b>Test Procedures</b>	PSEP 1995 with SMARM revisions	
Regulatory Program	SMS	
Test location	NewFields Northwest Laboratory	
Test type/duration	10-Day static	
Test dates	11/14/08 – 11/24/08	
Control water	0.45 µm-filtered, North Hood Canal sweater, adjusted with DI water	
Test temperature	Recommended: 15 ± 1 °C	15.0 – 16.9 °C
Test Salinity	Recommended: 28 ± 2 ppt	28 – 30 ppt
Test dissolved oxygen	Recommended: > 5 mg/L	6.4 – 9.7 mg/L
Test pH	Recommended: 7.8 ± 0.5	7.4 – 8.5
SMS control performance standard	Recommended: Control ≤ 10% mortality	1%, Pass
SMS reference performance standard	Recommended: Reference mortality < 25%	RF-02-SS-00 3% Pass, RF-03-SS-00 2% Pass
SMS pass/fail SQS	Significant Difference and Treatment – > 25% mortality and statistically significant = <b>FAIL</b>	OB-03-SS-00
SMS pass/fail CSL	Significant Difference and Treatment – Reference > 30% mortality and statistically significant = <b>FAIL</b>	All Pass
Reference Toxicant LC50	13.4 mg/L	
Acceptable Range	4.0-12.2 mg/L	
Test Lighting	Continuous	
Test chamber	1-Liter Glass Chamber	
Replicates/treatment	5 + 2 surrogates	
Organisms/replicate	20	
Exposure volume	175 mL sediment/ 775 mL water	
Feeding	None	
Water renewal	None	
Deviations from Test Protocol	Minor deviations in temperature and pH	

**Table 5. Summary of Test Results for *E. estuarius*.**

Sample	<i>E. estuarius</i>	
	Mean Mortality (%)	Standard Deviation
Control	1	2
RF-02-SS-00	3	4
RF-03-SS-00	2	4
HI-02-SS-00	4	4
HI-03-SS-00	17	17
HI-04-SS-00	2	4
HI-05-SS-00	1	2
HI-06-SS-00	7	6
HI-07-SS-00	2	3
OB-01-SS-00	11 <sup>1</sup>	25
OB-02-SS-00	21	13
OB-03-SS-00	29	12
OB-04-SS-00	5	6
OB-07-SS-00	14	16
OB-14-SS-00	10	8
OB-17-WS-00	10	5
SH-01-SS-00	10	4
SH-02-SS-00	6	4
SH-06-SS-00	15	12
SH-07-SS-00	5	5
SH-09-SS-00	12	8
SH-10-SS-00	7	3
SH-14-SS-00	18	11
SH-15-SS-00	3	4
SH-16-SS-00	2	3
SH-22-WS-00	10	9
SH-24-WS-00	18	12
SH-25-WS-00	21	8
SH-26-WS-00	13	10
SH-27-WS-00	8	10
SH-28-WS-00	6	4
SH-29-WS-00	4	4
SH-30-WS-00	9	10

<sup>1</sup> Surrogate results used for outlier replicate.

### 3.3 20-DAY JUVENILE POLYCHAETE BIOASSAY

A summary of *N. arenaceodentata* test conditions is shown in Table 6. No mortality was observed in the *N. arenaceodentata* control sediment and mean individual growth (MIG) in the

control sediment was 0.414 and 0.563 mg/ind/day, in the two batches. These values fall within the test acceptability criteria of <10% mean mortality and  $\geq 0.38$  mg/ind/day mean individual growth (Kendall 1996), indicating that the test conditions were suitable for adequate polychaete survival and growth.

Cadmium chloride reference-toxicant tests were performed on each batch of test organisms. LC<sub>50</sub> values for Batches 1 and 2 were 6.8 and 8.0 mg Cd/L, well within control chart limits at the time of testing. This indicates that the test organisms used in this study were of similar sensitivity to those previously tested at NewFields.

Minor deviations in water quality parameters were observed in both batches. The dissolved oxygen concentration in the surrogate chamber of sample OB-02-SS-00 (Batch 1) was 2.9 and 3.3 mg/L on days 19 and 20, respectively. Reference sample RF-02-SS-00 (Batch 2) also had a reduced dissolved oxygen concentration of 3.6 mg/L on day 3. These reduced concentrations were associated with constant airflow interruption to those chambers and was corrected upon discovery. Oxygen levels returned to the appropriate range for the remainder of the test (RF-02-SS-00) or the test reached termination (OB-02-SS-00). Temperatures in Batch 1 fell to approximately 12 degrees Celsius on day 4 of testing due to a malfunction on the temperature control system. Temperature control was restored upon discovery and temperatures were brought back within the recommended range over the course of 2 days to avoid shock. Temperatures remained within range for the remainder of the Batch 1 test. Temperatures were slightly below limits on day 15 of Batch 2 (18.4°C). Temperature control system was increased 0.5 °C and succeeded in maintaining test temperatures for the duration of Batch 2. Salinities ranged between 27 and 32 ppt throughout both tests, rising slightly above the recommended limits of  $28 \pm 2$  ppt. The UV light exposure method required the test jars to be uncovered during the exposure time to not impede light exposure. Some evaporation does occur over the course of the test that may explain the slight increase in salinity. These salinities are still within the tolerance range of the test organisms. Water quality parameters were inadvertently not recorded on day 18 of the Batch 2 test. These deviations should not impact the significance of the test results.

All of the test treatments had ammonia levels below the NOEC (10 mg/L total ammonia) in the initial and final interstitial water. The highest ammonia value observed was 6.75 mg/L in the initial interstitial measurement for sample OB-07-SS-00. Initial sulfide concentrations in interstitial water were below the NOEC (3.47 mg/L; Kendall and Barton 2004) for all samples.

Mean individual growth for the reference treatments compared to the Controls were greater than 80% of the Control, meeting the recommended SMS performance standards (Ecology 2008), except in one instance. Reference RF-03-SS-00 in Batch 1 showed only 65.9% of Control growth. These results indicate that these reference sediments were acceptable for suitability determination with one exception. Mean individual growth for all control, reference, and test sediments are shown in Table 7. Mortality in the test treatments ranged from 0 to 44%; MIG in the test treatments ranged from 0.248 to 0.737 mg/ind/day.

During the course of organism addition at test initiation, some test chambers were incidentally stocked with a differing number of worms than the target of five animals per chamber. Treatments where the initial number of organisms differed from five were: RF-02-SS rep 4 (Batch 1, 6 worms), RF-01-SS rep 4 (Batch 2, 6 worms), OB-06-SS-00 rep 5 (6 worms), OB-11-SS-00 rep 5 (6 worms), and SH-01-SS-00 rep 5 (10 worms). In treatments where the initial number of stocked animals was known to be different, the number of initial animals stocked was adjusted when performing the endpoint calculations. Treatment SH-15-SS-00 rep 1 was not stocked with organisms at test initiation as was confirmed by the lack of technician mark on the test chamber and the absence of worms recovered at test termination. This replicate was

removed from the endpoint calculations. In two instances the number of worms used for determining dry weight differs from the survival count. These two samples, OB-01-SS-00 rep 2 and SH-06-SS-00 rep 5 both had five animals recovered at test termination, but one animal was lost during the transfer to the weight boat for subsequent drying. In these cases five animals were used for calculating the survival endpoint, while the replicate dry weight was divided by four to calculate the individual dry weight.

Indigenous polychaete worms differing from *N. arenaceodentata* were found in the Oakland Bay sediments during test termination. Often the presence of these worms was associated with reduced survival of the *N. arenaceodentata* stocked into the test chambers. The reduction in survival in these chambers was possibly due to predation by the indigenous worms or through competition for food resources. Indigenous worms were not included in the final number alive or in weight determinations. Treatments where indigenous polychaete were found associated with decreased survivors of *N. arenaceodentata* include: SH-03-SS-00 (rep 3), OB-12-SS-00 (rep 4), OB-13-SS-00 (rep 4), SH-25-WS-00 (rep 4), and SH-12-SS-00 (rep 1 and 3)

With the exception of SH-15-SS-00 (rep 1) mentioned above, Instances where zero animals were recovered at the test termination were considered to have 100% mortality. The addition of animals to these test chambers at test initiation was confirmed by the presence of a technician mark on the test chamber. The treatments where zero animals were recovered at test termination include SH-24-WS-00 (rep 1 and 3), OB-08-SS-00 (rep 2), SH-30-WS-00 (rep 4), SH-19-WS-00 (rep 3 and 5), and OB-03-SS-00 (rep 3). In all cases except SH-24-WS-00 replicate 1, indigenous polychaete worms of different species than *N. arenaceodentata* were also recovered from the chambers.

**Table 6. Test Condition Summary for *N. arenaceodentata*.**

Test Conditions: PSEP <i>N. arenaceodentata</i> (SMS)		Batch 1	Batch 2
Sample Identification		See Table 1	
Date sampled		See Table 1	
Date received at NewFields Northwest		See Table 1	
Sample storage conditions		4°C, dark	
Weeks of holding	Recommended: ≤8 weeks (56 days)	Test 25-31 days; Ref 21 days	Test 31-36 days; Ref 27 days
Source of control sediment		Yaquina Bay, OR	
Test Species		<i>N. arenaceodentata</i>	
Supplier		Don Reish/ CalState Long Beach	
Date acquired		10/25/08	11/3-4/08
Acclimation/holding time		5 days	1-2 days
Age class		Juvenile	
Test Procedures		PSEP 1995 with SMARM revisions	
Regulatory Program		SMS	
Test location		NewFields Northwest Laboratory	NewFields Northwest Laboratory
Test type/duration		20-Day static renewal	
Test dates		10/30/08 – 11/19/08	11/5/08 – 11/25/08
Control water		0.45 µm-filtered, North Hood Canal sweater, adjusted with DI water	
Test temperature	Recommended: 20 ± 1 °C	16.9 – 20.7	18.4-23.0
Test Salinity	Recommended: 28 ± 2 ppt	27-32	28-32
Test dissolved oxygen	Recommended: > 6.0 mg/L	2.9 – 10.7	3.6 – 14.2
Test pH	Recommended: 8.0 ± 1.0	7.1-8.8	7.6-8.9
Initial biomass	Recommended: 0.5 - 1.0 mg Minimum: 0.25 mg	0.672 mg	0.478 mg
SMS control performance standard: Mortality	Recommended: Control ≤ 10% mortality	0%	0%
SMS control performance standard: Mean Individual Growth - MIG	Recommended: > 0.72 mg/ind/day Minimum: > 0.38 mg/ind/day	0.563 mg/ind/day	0.414 mg/ind/day
SMS reference performance standard	Recommended: MIG <sub>Reference</sub> /MIG <sub>Control</sub> > 80%	RF-01-SS-00 91.7% Pass, RF-02-SS-00 96.3% Pass, RF-03-SS-00 65.9% Fail	RF-01-SS-00 135.5% Pass, RF-02-SS-00 106.8% Pass, RF-03-SS-00 95.2% Pass
SMS pass/fail SQS	SQS Acceptability: Statistical difference and MIG <sub>Treatment</sub> /MIG <sub>Reference</sub> ≥ 70%	19 Pass, 6 Fail SQS; see Table 11	15 Pass, 10 Fail SQS; see Table 11
SMS pass/fail CSL	CSL Acceptability: Statistical difference and MIG <sub>Treatment</sub> /MIG <sub>Reference</sub> ≥ 50%	25 Pass; see Table 11	22 Pass, 3 Fail CSL; see Table 11
Reference Toxicant LC50		6.8 mg/L	8.0 mg/L
Acceptable Range		2.4-16.9 mg/L	2.4-16.9 mg/L
Test Lighting		Continuous	
Test chamber		1-Liter Glass Chamber	
Replicates/treatment		5 + 2 surrogates for WQ	
Organisms/replicate		5	

Test Conditions: PSEP <i>N. arenaceodentata</i> (SMS)	Batch 1	Batch 2
Exposure volume	175 mL sed/ 775 mL water	
Feeding	40 mg/jar every other day (8mg/ind every other day)	
Water renewal	Water is renewed every third day (1/3 volume of exposure chamber)	
Deviations from Test Protocol	Minor deviations in temperature, salinity, DO	Minor deviations in temperature, salinity, DO

Table 7. Summary of Test Results for *N. arenaceodentata*.

Batch 1				Batch 2			
Sample	Mean Mortality (%)	MIG (mg/ind/day)	MIG Std Dev	Sample	Mean Mortality (%)	MIG (mg/ind/day)	MIG Std Dev
Control	0	0.563	0.089	Control	0	0.414	0.105
RF-01-SS-00	0	0.516	0.087	RF-01-SS-00	8	0.561	0.127
RF-02-SS-00	0	0.542	0.127	RF-02-SS-00	0	0.442	0.242
RF-03-SS-00	4	0.371	0.169	RF-03-SS-00	20	0.394	0.198
HI-02-SS-00	8	0.338	0.192	SH-06-SS-00	0	0.277	0.114
HI-04-SS-00	0	0.575	0.286	SH-25-WS-00	12	0.405	0.277
HI-06-SS-00	16	0.445	0.142	SH-12-SS-00	16	0.449	0.251
HI-07-SS-00	0	0.510	0.158	OB-10-SS-00	0	0.516	0.104
OB-01-SS-00	8	0.443	0.095	SH-20-WS-00	0	0.517	0.252
OB-02-SS-00	4	0.418	0.135	SH-26-WS-00	0	0.248	0.089
OB-05-SS-00	4	0.307	0.109	SH-15-SS-00	5	0.365	0.203
OB-06-SS-00	0	0.373	0.149	OB-04-SS-00	0	0.516	0.125
OB-11-SS-00	4	0.425	0.099	SH-24-WS-00	44	0.347	0.211
OB-12-SS-00	12	0.398	0.097	HI-05-SS-00	4	0.329	0.109
OB-13-SS-00	16	0.369	0.101	SH-23-WS-00	16	0.322	0.167
OB-14-SS-00	12	0.423	0.152	SH-27-WS-00	24	0.307	0.188
OB-17-WS-00	16	0.546	0.162	OB-07-SS-00	8	0.289	0.102
OB-19-WS-00	8	0.473	0.103	OB-09-SS-00	0	0.460	0.067
SH-01-SS-00	4	0.400	0.125	SH-18-WS-00	4	0.424	0.078
SH-03-SS-00	16	0.450	0.242	OB-08-SS-00	20	0.420	0.207
SH-04-SS-00	8	0.479	0.180	SH-30-WS-00	20	0.394	0.104
SH-09-SS-00	0	0.488	0.134	SH-19-WS-00	28	0.367	0.157
SH-10-SS-00	0	0.403	0.109	OB-18-WS-00	4	0.432	0.101
SH-11-SS-00	12	0.552	0.064	SH-16-SS-00	8	0.271	0.110
SH-13-SS-00	4	0.346	0.066	SH-02-SS-00	8	0.410	0.137
SH-14-SS-00	4	0.451	0.160	HI-03-SS-00	0	0.404	0.127
SH-21-WS-00	4	0.368	0.066	OB-03-SS-00	20	0.330	0.154
SH-22-WS-00	12	0.348	0.115	SH-29-WS-00	8	0.320	0.166
SH-28-WS-00	0	0.374	0.078	SH-07-SS-00	4	0.389	0.099

### 3.4 LARVAL DEVELOPMENT BIOASSAY

A summary of the test condition results from the *Mytilus* sp. test is presented in Table 8. Stocking densities were 36.6 and 28.9 embryos/ml for Batches 1 and 2, respectively. The larval

test was validated by mean normal survival in the control treatments of 84 and 100% (102.9%), within the acceptability criterion of  $\geq 70\%$ . Water quality parameters remained within the recommended limits throughout the ~48-hour test. Ammonia and sulfide values detected in the test chambers were below the NOEC values for *Mytilus* sp.

The EC<sub>50</sub> values for the copper reference-toxicant tests for proportion normal were 7.4 and 8.3  $\mu\text{g Cu/L}$  for the two batches of test organisms, within the control chart limits at the time of testing. The results of the reference-toxicant test indicate that the test organisms used in this study were similar in sensitivity to those previously tested at NewFields. Mean control normalized normal survival in the reference sediments ranged from 40.3% to 100%. Test sample normal survival was variable and ranged from 21% to 100% as shown in Table 9.

**Table 8. Test Condition Summary for *Mytilus* sp.**

Test Conditions: PSEP <i>Mytilus</i> sp.		Batch 1	Batch 2
Sample Identification		See Table 1	
Date sampled		See Table 1	
Date received at NewFields Northwest		See Table 1	
Sample storage conditions		4°C, dark	
Weeks of holding	Recommended: $\leq 8$ weeks (56 days)	Test 49-52 days; Ref 42 days	Test 51-53 days; Ref 47 days
Test Species		<i>Mytilus</i> sp.	
Supplier		Carlsbad Aquafarms	
Date acquired		11/18/08	11/22/08
Acclimation/holding time		2 day	4 day
Age class		<2-h old embryos	<2-h old embryos
Test Procedures		PSEP 1995 with SMARM revisions	
Regulatory Program		SMS	
Test location		NewFields Northwest Laboratory	
Test type/duration	Recommended: 48-96 Hour static test	48 hr	47 hr
Test dates		11/20/2008-11/22/2008	11/26/2008-11/27/2008
Control water		0.45 $\mu\text{m}$ -filtered, North Hood Canal sweater, adjusted with DI water	
Test temperature	Recommended: $16 \pm 1$ °C	15.1-16.5	15.2-16.6
Test Salinity	Recommended: $28 \pm 1$ ppt	28	27-28
Test dissolved oxygen	Recommended: > 4.8 mg/L	5.2 – 9.7	6.0 – 8.8
Test pH	Recommended: $7.8 \pm 0.5$	7.3-8.0	7.5-8.2
Stocking Density (embryos/mL)	Recommended: 20 – 40	36.6	28.9
SMS control performance standard	Recommended: Normal survival $\geq 70\%$	84.0, Pass	102.9, Pass
SMS reference performance standard	Recommended: Reference/Control $\geq 65\%$	RF-01-SS-00 51.2% Fail, RF-02-SS-00 45.0% Fail, RF-03-SS-00 70.2% Pass	RF-01-SS-00 85.6% Pass, RF-02-SS-00 65.7% Pass, RF-03-SS-00 78.6% Pass
SMS pass/fail SQS (NSCA=Normal Survival Control Adjusted)	Statistical difference and $\text{NSCA}_{\text{Treatment}} / \text{NSCA}_{\text{Reference}} > 0.85$	17 Pass, 8 Fail SQS; see Table 12	13 Pass, 11 Fail SQS; see Table 12
SMS pass/fail CSL	Statistical difference and $\text{NSCA}_{\text{Treatment}} / \text{NSCA}_{\text{Reference}} > 0.70$	24 Pass, 2 Fail CSL; see Table 12	18 Pass, 6 Fail CSL; see Table 12
Reference Toxicant EC <sub>50</sub>		7.4 $\mu\text{g Cu/L}$	8.3 $\mu\text{g Cu/L}$
Acceptable Range		3.4 – 18.7 $\mu\text{g Cu/L}$	3.5 – 18.0 $\mu\text{g Cu/L}$
Test Lighting		14 light:10 Dark	
Test chamber		1-Liter Glass Chamber	

Test Conditions: PSEP <i>Mytilus</i> sp.	Batch 1	Batch 2
Replicates/treatment	5 + 1 WQ surrogate	
Exposure volume	18 g sed/900 mL water	
Feeding	None	
Water renewal	None	
Deviations from Test Protocol	None	None

**Table 9. Summary of Test Results for *Mytilus* sp.**

Batch 1				Batch 2			
Sample	Normalized Percent Normal	NCMA	Std Dev	Sample	Normalized Percent Normal	NCMA	Std Dev
Control	84.0	16.0	3.6	Control	102.9	0.7	0.2
RF-01-SS-00	51.2	48.8	4.1	RF-01-SS-00	85.6	14.4	0.8
RF-02-SS-00	45.0	55.0	6.3	RF-02-SS-00	65.7	34.3	1.2
RF-03-SS-00	70.2	29.8	12.1	RF-03-SS-00	78.6	21.4	2.0
HI-05-SS-00	92.4	7.6	9.1	HI-02-SS-00	87.4	12.6	0.5
HI-07-SS-00	88.2	11.8	9.6	HI-03-SS-00	81.6	18.4	0.2
OB-01-SS-00	78.2	21.8	2.8	HI-04-SS-00	83.0	17.0	0.4
OB-02-SS-00	73.8	26.2	6.9	HI-06-SS-00	76.1	23.9	0.7
OB-03-SS-00	69.6	30.4	7.9	OB-04-SS-00	49.9	50.1	0.9
SH-01-SS-00	65.7	34.3	7.7	OB-05-SS-00	63.0	37.0	0.8
SH-02-SS-00	34.4	65.6	15.7	OB-06-SS-00	58.1	41.9	1.4
SH-03-SS-00	34.4	65.6	13.6	OB-07-SS-00	81.9	18.1	0.6
SH-04-SS-00	44.1	55.9	5.3	OB-08-SS-00	79.8	20.2	2.7
SH-06-SS-00	18.1	81.9	4.3	OB-09-SS-00	67.7	32.3	0.8
SH-09-SS-00	71.2	28.8	5.6	OB-10-SS-00	46.4	53.6	4.7
SH-10-SS-00	75.0	25.0	8.9	OB-11-SS-00	76.7	23.3	0.5
SH-14-SS-00	56.0	44.0	11.5	OB-12-SS-00	71.8	28.2	0.7
SH-15-SS-00	85.4	14.6	14.3	OB-13-SS-00	45.1	54.9	0.8
SH-16-SS-00	78.9	21.1	6.5	OB-14-SS-00	51.3	48.7	2.4
SH-19-WS-00	40.9	59.1	6.8	OB-17-WS-00	75.8	24.2	0.2
SH-20-WS-00	73.0	27.0	6.0	OB-18-WS-00	47.8	52.2	5.3
SH-21-WS-00	37.5	62.5	4.5	OB-19-WS-00	47.5	52.5	6.8
SH-22-WS-00	44.2	55.8	4.7	SH-07-SS-00	43.8	56.2	4.5
SH-23-WS-00	60.7	39.3	12.9	SH-11-SS-00	75.9	24.1	1.3
SH-24-WS-00	32.8	67.2	5.9	SH-12-SS-00	66.1	33.9	2.1
SH-26-WS-00	58.1	41.9	7.1	SH-13-SS-00	73.8	26.2	2.3
SH-27-WS-00	87.5	12.5	9.0	SH-18-WS-00	72.6	27.4	2.0
SH-28-WS-00	69.3	30.7	9.0	SH-25-WS-00	71.0	29.0	1.6
SH-29-WS-00	90.2	9.8	7.2				
SH-30-WS-00	63.9	36.1	5.3				
Stocking Density	366.0			Stocking Density	289.0		

NCMA = Normalized Percent Combined Mortality and Abnormality

### 3.5 MICROTOX® BIOASSAY

See Table 3 In Appendix A.

## 4.0 DISCUSSION

Sediments were evaluated based on Sediment Management Standards (SMS) criteria. The biological criteria are based on both statistical significance (a statistical comparison) and the degree of biological response (a numerical comparison). The SMS criteria are stated in the Washington Department of Ecology Sampling and Analysis Plan Appendix (WDOE 2008). Comparisons were made for each treatment against each of the reference samples. Two numerical comparisons were made under SMS, the Sediment Quality Standards (SQS) and the Cleanup Standards Limit (CSL). All treatments were compared to each of the reference sediment treatments.

### 4.1 AMPHIPOD TEST SUITABILITY DETERMINATION

Under the SMS program, a test treatment fails SQS if mean mortality is statistically ( $p \leq 0.05$ ) greater than that of the reference treatment and mean mortality in the test sediment is greater than 25%. Treatments fail the CSL if mean mortality is statistically ( $p \leq 0.05$ ) greater than that of the reference treatment and mean mortality in the test sediment is more than 30% greater than the reference sediment mean mortality.

Sample OB-03-SS-00 failed to meet the SQS criteria when compared to the associated reference samples RF-02-SS-00 and RF-03-SS-00 (Table 10). This sample did not fail the CSL criteria.

**Table 10. Suitability Comparisons for Amphipods**

Test Batch	Sample	Mean Mortality (%)			Mortality Compared to Reference (%)			Statistically Significant ( $p \leq 0.05$ )			
		Sample	RF-01	RF-02	RF-03	RF-01	RF-02	RF-03	RF-01	RF-02	RF-03
2	HI-02-SS-00	4	NA	3	2	NA	1	2			
2	HI-03-SS-00	17	NA	3	2	NA	14	15			Y
2	HI-04-SS-00	2	NA	3	2	NA	-1	0			
2	HI-05-SS-00	1	NA	3	2	NA	-2	-1			
2	HI-06-SS-00	7	NA	3	2	NA	4	5			
2	HI-07-SS-00	2	NA	3	2	NA	-1	0			
2	OB-01-SS-00	11	NA	3	2	NA	8	9			
2	OB-02-SS-00	21	NA	3	2	NA	18	19		Y	Y
2	OB-03-SS-00	<b>29</b>	NA	3	2	NA	<b>26</b>	<b>27</b>		Y	Y
2	OB-04-SS-00	5	NA	3	2	NA	2	3			
1	OB-05-SS-00	9	15	13	NA	-6	-4	NA			
1	OB-06-SS-00	14	15	13	NA	-1	1	NA			
2	OB-07-SS-00	14	NA	3	2	NA	11	12			Y
1	OB-08-SS-00	8	15	13	NA	-7	-5	NA			
1	OB-09-SS-00	10	15	13	NA	-5	-3	NA			
1	OB-10-SS-00	6	15	13	NA	-9	-7	NA			
1	OB-11-SS-00	11	15	13	NA	-4	-2	NA			
1	OB-12-SS-00	21	15	13	NA	6	8	NA			
1	OB-13-SS-00	10	15	13	NA	-5	-3	NA			
2	OB-14-SS-00	10	NA	3	2	NA	7	8			Y
2	OB-17-WS-00	10	NA	3	2	NA	7	8		Y	Y
1	OB-18-WS-00	12	15	13	NA	-3	-1	NA			
1	OB-19-WS-00	8	15	13	NA	-7	-5	NA			
2	SH-01-SS-00	10	NA	3	2	NA	7	8		Y	Y
2	SH-02-SS-00	6	NA	3	2	NA	3	4			
1	SH-03-SS-00	14	15	13	NA	-1	1	NA			
1	SH-04-SS-00	9	15	13	NA	-6	-4	NA			
2	SH-06-SS-00	15	NA	3	2	NA	12	13		Y	Y
2	SH-07-SS-00	5	NA	3	2	NA	2	3			
2	SH-09-SS-00	12	NA	3	2	NA	9	10		Y	Y
2	SH-10-SS-00	7	NA	3	2	NA	4	5		Y	
1	SH-11-SS-00	7	15	13	NA	-8	-6	NA			
1	SH-12-SS-00	11	15	13	NA	-4	-2	NA			
1	SH-13-SS-00	8	15	13	NA	-7	-5	NA			
2	SH-14-SS-00	18	NA	3	2	NA	15	16		Y	Y
2	SH-15-SS-00	3	NA	3	2	NA	0	1			
2	SH-16-SS-00	2	NA	3	2	NA	-1	0			
1	SH-18-WS-00	10	15	13	NA	-5	-3	NA			
1	SH-19-WS-00	15	15	13	NA	0	2	NA			
1	SH-20-WS-00	10	15	13	NA	-5	-3	NA			
1	SH-21-WS-00	14	15	13	NA	-1	1	NA			
2	SH-22-WS-00	10	NA	3	2	NA	7	8			Y
1	SH-23-WS-00	18	15	13	NA	3	5	NA			
2	SH-24-WS-00	18	NA	3	2	NA	15	16		Y	Y
2	SH-25-WS-00	21	NA	3	2	NA	18	19		Y	Y
2	SH-26-WS-00	13	NA	3	2	NA	10	11		Y	Y
2	SH-27-WS-00	8	NA	3	2	NA	5	6			
2	SH-28-WS-00	6	NA	3	2	NA	3	4			
2	SH-29-WS-00	4	NA	3	2	NA	1	2			
2	SH-30-WS-00	9	NA	3	2	NA	6	7			

Shaded cells indicate samples not meeting SMS Criteria compared to at least one reference sample.  
**Bold indicates SQS Failure (>25)**

4.2 JUVENILE POLYCHAETE TEST SUITABILITY DETERMINATION

Suitability determinations for the juvenile polychaete test were based on mean individual growth (MIG). A test treatment fails SQS criteria if MIG is statistically ( $p \leq 0.05$ ) lower in the test treatment, relative to the reference, and MIG in the test treatment is  $< 70\%$  that of the reference. A test treatment fails CSL criteria if MIG is statistically ( $p \leq 0.05$ ) lower in the test treatment, relative to the reference, and MIG in the test treatment is  $< 50\%$  that of the reference.

Eighteen samples failed to meet SQS criteria and three samples failed to meet CSL criteria relative to at least one of the reference samples as shown by the shaded cells in Table 11.

Reference treatment RF-03-SS-00 tested in Batch 1 did not meet the acceptability criterion of  $\geq 80\%$  mean individual growth compared to control.

**Table 11. Suitability Comparisons for *N. arenaceodentata*.**

Test Batch	Sample	Mean Individual Growth Rate (mg/ind/day)				MIG Relative to Reference			Statistically Significant ( $p \leq 0.05$ )		
		Sample	RF-01	RF-02	RF-03	RF-01	RF-02	RF-03	RF-01	RF-02	RF-03
1	HI-02-SS-00	0.338	0.516	0.542	0.371	<b>0.656</b>	<b>0.625</b>	0.912	Y	Y	
2	HI-03-SS-00	0.404	0.561	0.442	0.394	0.720	0.914	1.028	Y		
1	HI-04-SS-00	0.575	0.516	0.542	0.371	1.115	1.062	1.551			
2	HI-05-SS-00	0.329	0.561	0.442	0.394	<b>0.587</b>	0.745	0.837	Y		
1	HI-06-SS-00	0.445	0.516	0.542	0.371	0.863	0.822	1.200			
1	HI-07-SS-00	0.510	0.516	0.542	0.371	0.988	0.941	1.374			
1	OB-01-SS-00	0.443	0.516	0.542	0.371	0.858	0.817	1.194			
1	OB-02-SS-00	0.418	0.516	0.542	0.371	0.810	0.771	1.127			
2	OB-03-SS-00	0.330	0.561	0.442	0.394	0.588	0.746	0.839			
2	OB-04-SS-00	0.516	0.561	0.442	0.394	0.920	1.167	1.312			
1	OB-05-SS-00	0.307	0.516	0.542	0.371	<b>0.595</b>	<b>0.567</b>	0.828	Y	Y	
1	OB-06-SS-00	0.373	0.516	0.542	0.371	0.724	<b>0.689</b>	1.007		Y	
2	OB-07-SS-00	0.289	0.561	0.442	0.394	<b>0.515</b>	0.653	0.734	Y		
2	OB-08-SS-00	0.420	0.561	0.442	0.394	0.748	0.949	1.067			
2	OB-09-SS-00	0.460	0.561	0.442	0.394	0.820	1.040	1.170			
2	OB-10-SS-00	0.516	0.561	0.442	0.394	0.919	1.166	1.311			
1	OB-11-SS-00	0.425	0.516	0.542	0.371	0.824	0.785	1.147			
1	OB-12-SS-00	0.398	0.516	0.542	0.371	0.771	0.735	1.073	Y	Y	
1	OB-13-SS-00	0.369	0.516	0.542	0.371	0.716	<b>0.681</b>	0.995	Y	Y	
1	OB-14-SS-00	0.423	0.516	0.542	0.371	0.821	0.782	1.142			
1	OB-17-WS-00	0.546	0.516	0.542	0.371	1.059	1.008	1.473			
2	OB-18-WS-00	0.432	0.561	0.442	0.394	0.770	0.977	1.099			
1	OB-19-WS-00	0.473	0.516	0.542	0.371	0.917	0.873	1.275			
1	SH-01-SS-00	0.400	0.516	0.542	0.371	0.776	0.739	1.079			
2	SH-02-SS-00	0.410	0.561	0.442	0.394	0.730	0.926	1.041			
1	SH-03-SS-00	0.450	0.516	0.542	0.371	0.872	0.831	1.213			
1	SH-04-SS-00	0.479	0.516	0.542	0.371	0.928	0.884	1.291			
2	SH-06-SS-00	0.277	0.561	0.442	0.394	<b>0.494*</b>	0.626	0.704	Y		
2	SH-07-SS-00	0.389	0.561	0.442	0.394	<b>0.694</b>	0.880	0.990	Y		
1	SH-09-SS-00	0.488	0.516	0.542	0.371	0.946	0.901	1.317			
1	SH-10-SS-00	0.403	0.516	0.542	0.371	0.781	0.744	1.087			
1	SH-11-SS-00	0.552	0.516	0.542	0.371	1.071	1.020	1.490			
2	SH-12-SS-00	0.449	0.561	0.442	0.394	0.799	1.014	1.140			
1	SH-13-SS-00	0.346	0.516	0.542	0.371	<b>0.671</b>	<b>0.639</b>	0.934	Y	Y	
1	SH-14-SS-00	0.451	0.516	0.542	0.371	0.874	0.833	1.216			
2	SH-15-SS-00	0.365	0.561	0.442	0.394	0.650	0.825	0.928			
2	SH-16-SS-00	0.271	0.561	0.442	0.394	<b>0.483*</b>	0.613	0.690	Y		
2	SH-18-WS-00	0.424	0.561	0.442	0.394	0.755	0.958	1.077	Y		
2	SH-19-WS-00	0.367	0.561	0.442	0.394	<b>0.654</b>	0.830	0.934	Y		
2	SH-20-WS-00	0.517	0.561	0.442	0.394	0.920	1.168	1.313			
1	SH-21-WS-00	0.368	0.516	0.542	0.371	0.714	<b>0.680</b>	0.993	Y	Y	
1	SH-22-WS-00	0.348	0.516	0.542	0.371	<b>0.675</b>	<b>0.643</b>	0.939	Y	Y	
2	SH-23-WS-00	0.322	0.561	0.442	0.394	<b>0.573</b>	0.727	0.817	Y		
2	SH-24-WS-00	0.347	0.561	0.442	0.394	0.618	0.785	0.882			
2	SH-25-WS-00	0.405	0.561	0.442	0.394	0.721	0.914	1.028			
2	SH-26-WS-00	0.248	0.561	0.442	0.394	<b>0.442*</b>	0.561	0.631	Y		

Test Batch	Sample	Mean Individual Growth Rate (mg/ind/day)				MIG Relative to Reference			Statistically Significant (p≤0.05)		
		Sample	RF-01	RF-02	RF-03	RF-01	RF-02	RF-03	RF-01	RF-02	RF-03
2	SH-27-WS-00	0.307	0.561	0.442	0.394	<b>0.547</b>	0.694	0.780	Y		
1	SH-28-WS-00	0.374	0.516	0.542	0.371	0.726	<b>0.691</b>	1.010	Y	Y	
2	SH-29-WS-00	0.320	0.561	0.442	0.394	<b>0.571</b>	0.724	0.814	Y		
2	SH-30-WS-00	0.394	0.561	0.442	0.394	0.702	0.891	1.002	Y		

Shaded cells indicate samples not meeting SMS Criteria compared to at least one reference sample.  
**Bold indicates SQS Failure (Statistical difference and <0.70),**  
**Bold and asterisk (\*) indicates CSL failure (Statistical difference and <0.50)**  
 Note: RF-03 in Batch 1 did not meet Reference Acceptability Criterion

### 4.3 LARVAL TEST SUITABILITY DETERMINATION

Larval test treatments fail SQS criteria if the percentage of normal larvae in the test treatment is significantly ( $p \leq 0.10$ ) lower than that of the reference and if the normal larval development in the test treatment is less than 85% of the normal development in the reference. Treatments fail CSL criteria if the percentage of normal larvae in the test treatment is significantly ( $p \leq 0.10$ ) lower than that of the reference and if the normal larval development in the test treatment is less than 70% of the normal development in the reference. As shown in Table 12, 20 of the project samples failed to meet SQS criteria ( $<0.85$ ) and 15 project samples failed to meet CSL criteria ( $<0.70$ ). Test treatment SH-18-WS-00 had a relative difference of 0.849 when compared to reference RF-01-SS-00, falling just below the 0.85 criterion. This sample is considered as failing to meet the SQS criteria for the purposes of this report.

Reference treatments RF-01-SS-00 and RF-02-SS-00 tested in Batch 1 did not meet the acceptability criterion of  $\geq 65\%$  of control normality.

Note: Samples SH-02-SS-00 and SH-03-SS-00 had identical mean normal survivorship (34.4%); however, the replicate data for these two treatments differed. The similarity between the two samples is coincidental.

Table 12. Suitability Comparisons for *Mytilus* sp.

Test Batch	Sample	Mean Normal Development (%) Control Adjusted				Relative to Reference Development			Statistically Significant (p ≤ 0.1)		
		Sample	RF-01	RF-02	RF-03	RF-01	RF-02	RF-03	RF-01	RF-02	RF-03
2	HI-02-SS-00	87.4	85.6	65.7	78.6	1.02	1.33	1.11			
2	HI-03-SS-00	81.6	85.6	65.7	78.6	0.95	1.24	1.04			
2	HI-04-SS-00	83.0	85.6	65.7	78.6	0.97	1.26	1.06			
1	HI-05-SS-00	92.4	51.2	45.0	70.2	1.81	2.05	1.32			
2	HI-06-SS-00	76.1	85.6	65.7	78.6	0.89	1.16	0.97	Y		
1	HI-07-SS-00	88.2	51.2	45.0	70.2	1.72	1.96	1.26			
1	OB-01-SS-00	78.2	51.2	45.0	70.2	1.53	1.74	1.11			
1	OB-02-SS-00	73.8	51.2	45.0	70.2	1.44	1.64	1.05			
1	OB-03-SS-00	69.6	51.2	45.0	70.2	1.36	1.55	0.99			
2	OB-04-SS-00	49.9	85.6	65.7	78.6	<b>0.58*</b>	<b>0.76</b>	<b>0.64*</b>	Y	Y	Y
2	OB-05-SS-00	63.0	85.6	65.7	78.6	<b>0.74</b>	0.96	<b>0.80</b>	Y		Y
2	OB-06-SS-00	58.1	85.6	65.7	78.6	<b>0.68*</b>	0.88	<b>0.74</b>	Y	Y	Y
2	OB-07-SS-00	81.9	85.6	65.7	78.6	0.96	1.25	1.04	Y		
2	OB-08-SS-00	79.8	85.6	65.7	78.6	0.93	1.21	1.02	Y		
2	OB-09-SS-00	67.7	85.6	65.7	78.6	<b>0.79</b>	1.03	0.86	Y		Y
2	OB-10-SS-00	46.4	85.6	65.7	78.6	<b>0.54*</b>	<b>0.71</b>	<b>0.59*</b>	Y	Y	Y
2	OB-11-SS-00	76.7	85.6	65.7	78.6	0.90	1.17	0.98	Y		
2	OB-12-SS-00	71.8	85.6	65.7	78.6	<b>0.84</b>	1.09	0.91	Y		Y
2	OB-13-SS-00	45.1	85.6	65.7	78.6	<b>0.53*</b>	<b>0.69*</b>	<b>0.57*</b>	Y	Y	Y
2	OB-14-SS-00	51.3	85.6	65.7	78.6	<b>0.60*</b>	<b>0.78</b>	<b>0.65*</b>	Y	Y	Y
2	OB-17-WS-00	75.8	85.6	65.7	78.6	0.89	1.15	0.96	Y		
2	OB-18-WS-00	47.8	85.6	65.7	78.6	<b>0.56*</b>	<b>0.73</b>	<b>0.61*</b>	Y	Y	Y
2	OB-19-WS-00	47.5	85.6	65.7	78.6	<b>0.55*</b>	<b>0.72</b>	<b>0.60*</b>	Y	Y	Y
1	SH-01-SS-00	65.7	51.2	45.0	70.2	1.28	1.46	0.94			
1	SH-02-SS-00	34.4	51.2	45.0	70.2	<b>0.67*</b>	0.76	<b>0.49*</b>	Y		Y
1	SH-03-SS-00	34.4	51.2	45.0	70.2	<b>0.67*</b>	<b>0.76</b>	<b>0.49*</b>	Y	Y	Y
1	SH-04-SS-00	44.1	51.2	45.0	70.2	0.86	0.98	<b>0.63*</b>	Y		Y
1	SH-06-SS-00	18.1	51.2	45.0	70.2	<b>0.35*</b>	<b>0.40*</b>	<b>0.26*</b>	Y	Y	Y
2	SH-07-SS-00	43.8	85.6	65.7	78.6	<b>0.51*</b>	<b>0.67*</b>	<b>0.56*</b>	Y	Y	Y
1	SH-09-SS-00	71.2	51.2	45.0	70.2	1.39	1.58	1.01			
1	SH-10-SS-00	75.0	51.2	45.0	70.2	1.47	1.67	1.07			
2	SH-11-SS-00	75.9	85.6	65.7	78.6	0.89	1.16	0.97	Y		
2	SH-12-SS-00	66.1	85.6	65.7	78.6	<b>0.77</b>	1.01	<b>0.84</b>	Y		Y
2	SH-13-SS-00	73.8	85.6	65.7	78.6	0.86	1.12	0.94	Y		
1	SH-14-SS-00	56.0	51.2	45.0	70.2	1.09	1.24	<b>0.80</b>			Y
1	SH-15-SS-00	85.4	51.2	45.0	70.2	1.67	1.90	1.22			
1	SH-16-SS-00	78.9	51.2	45.0	70.2	1.54	1.75	1.12			
2	SH-18-WS-00	72.6	85.6	65.7	78.6	<b>0.85</b>	1.11	0.92	Y		Y
1	SH-19-WS-00	40.9	51.2	45.0	70.2	<b>0.80</b>	0.91	<b>0.58*</b>	Y		Y
1	SH-20-WS-00	73.0	51.2	45.0	70.2	1.43	1.62	1.04			
1	SH-21-WS-00	37.5	51.2	45.0	70.2	<b>0.73</b>	<b>0.83</b>	<b>0.53*</b>	Y	Y	Y
1	SH-22-WS-00	44.2	51.2	45.0	70.2	0.86	0.98	<b>0.63*</b>	Y		Y
1	SH-23-WS-00	60.7	51.2	45.0	70.2	1.19	1.35	0.86			
1	SH-24-WS-00	32.8	51.2	45.0	70.2	<b>0.64*</b>	<b>0.73</b>	<b>0.47*</b>	Y	Y	Y
2	SH-25-WS-00	71.0	85.6	65.7	78.6	<b>0.83</b>	1.08	0.90	Y		Y
1	SH-26-WS-00	58.1	51.2	45.0	70.2	1.14	1.29	<b>0.83</b>			Y
1	SH-27-WS-00	87.5	51.2	45.0	70.2	1.71	1.94	1.25			
1	SH-28-WS-00	69.3	51.2	45.0	70.2	1.35	1.54	0.99			
1	SH-29-WS-00	90.2	51.2	45.0	70.2	1.76	2.01	1.29			
1	SH-30-WS-00	63.9	51.2	45.0	70.2	1.25	1.42	0.91			

Shaded cells indicate samples not meeting SMS Criteria compared to at least one reference sample.  
**Bold indicates SQS Failure (SD and <0.85), Bold and asterisk (\*) indicates CSL failure (SD and <0.70)**  
 Note: RF-01 and RF-02 in Batch 1 did not meet Reference Acceptability Criterion

#### 4.4 MICROTOX® TEST SUITABILITY DETERMINATION

The SMS program criteria state that a test sediment fails the SQS criteria when the mean light output of the test sediment is less than 80% of the mean light output of the reference sediment and the two means are statistically different ( $p \leq 0.05$ ). No criteria exist for the Microtox® test for CSL.

Test treatments were analyzed in 14 batches run concurrently with the associated reference sample for those respective treatments. Due to this test design, test sediments were compared to one reference only. In instances where the reference sediments did not meet acceptability criteria, the control response was used in its place for the suitability determination.

One sample (OB-05-SS-00) failed to meet SQS criteria and was significantly less than the associated reference RF-01-SS-00.

Table 13. Suitability Comparisons for Microtox® (*V. fischeri*)

Test Batch	Sample	Sample Mean % of Initial Light Output: Minute Reading		Comparison To:	Reference Mean % of Initial Light Output: Minute Reading		% Absolute Difference		Fails SQS: Statistically Different from Reference (or Control) and > 20% Different (Absolute)
		5	15		5	15	5	15	
2	HI-02-SS-00	107	111	RF-03-SS-00	98	98	-9	-13	
2	HI-03-SS-00	108	105	RF-03-SS-00	98	98	-10	-7	
2	HI-04-SS-00	115	121	RF-03-SS-00	98	98	-17	-23	
3	HI-05-SS-00	99	94	Control	91	84	-8	-10	
2	HI-06-SS-00	104	109	RF-03-SS-00	98	98	-6	-11	
3	HI-07-SS-00	97	96	Control	91	84	-6	-12	
4	OB-01-SS-00	98	93	RF-03-SS-00	78	75	-20	-18	
10	OB-02-SS-00	102	98	Control	95	89	-7	-9	
10	OB-03-SS-00	103	101	Control	95	89	-8	-12	
13	OB-04-SS-00	97	90	Control	95	89	-2	-1	
6	OB-05-SS-00	43	35	RF-01-SS-00	101	92	58	57	Y
7	OB-06-SS-00	104	95	RF-01-SS-00	101	94	-3	-1	
1	OB-07-SS-00	104	101	RF-03-SS-00	98	98	-6	-3	
6	OB-08-SS-00	101	92	RF-01-SS-00	101	92	0	0	
8	OB-09-SS-00	99	95	RF-01-SS-00	102	97	3	2	
7	OB-10-SS-00	103	97	RF-01-SS-00	101	94	-2	-3	
8	OB-11-SS-00	94	89	RF-01-SS-00	102	97	8	8	
6	OB-12-SS-00	101	92	RF-01-SS-00	101	92	0	0	
12	OB-13-SS-00	92	86	Control	91	85	-1	-1	
12	OB-14-SS-00	95	90	Control	91	85	-4	-5	
12	OB-17-WS-00	96	91	Control	91	85	-5	-6	
7	OB-18-WS-00	104	96	RF-01-SS-00	101	94	-3	-2	
8	OB-19-WS-00	100	95	RF-01-SS-00	102	97	2	2	
3	SH-01-SS-00	100	96	Control	91	84	-9	-12	
11	SH-02-SS-00	101	98	Control	93	84	-8	-14	
12	SH-03-SS-00	96	92	Control	91	85	-5	-7	
9	SH-04-SS-00	100	97	Control	95	92	-5	-5	
14	SH-06-SS-00	106	100	Control	95	83	-11	-17	
13	SH-07-SS-00	95	86	Control	89	83	-6	-3	
9	SH-09-SS-00	101	98	Control	95	92	-6	-6	
4	SH-10-SS-00	103	98	RF-03-SS-00	78	75	-25	-23	
10	SH-11-SS-00	100	96	Control	95	89	-5	-7	
13	SH-12-SS-00	95	86	Control	89	83	-6	-3	
13	SH-13-SS-00	94	87	Control	89	83	-5	-4	
9	SH-14-SS-00	103	103	Control	95	92	-8	-11	
4	SH-15-SS-00	101	97	RF-03-SS-00	78	75	-23	-22	
4	SH-16-SS-00	101	93	RF-03-SS-00	78	75	-23	-18	
8	SH-18-WS-00	100	95	RF-01-SS-00	102	97	2	2	
10	SH-19-WS-00	102	97	Control	95	89	-7	-8	
6	SH-20-WS-00	102	94	RF-01-SS-00	101	92	-1	-2	
7	SH-21-WS-00	102	94	RF-01-SS-00	101	94	-1	0	
5	SH-22-WS-00	110	104	RF-03-SS-00	100	93	-10	-11	
11	SH-23-WS-00	103	101	Control	93	84	-10	-17	
11	SH-24-WS-00	98	95	Control	93	84	-5	-11	
11	SH-25-WS-00	103	100	Control	93	84	-10	-16	
3	SH-26-WS-00	100	94	Control	91	84	-9	-10	
1	SH-27-WS-00	100	96	RF-03-SS-00	98	98	-2	2	
9	SH-28-WS-00	101	97	Control	95	92	-6	-5	
1	SH-29-WS-00	106	106	RF-03-SS-00	98	98	-8	-8	
1	SH-30-WS-00	106	100	RF-03-SS-00	98	98	-8	-2	

Shaded cells indicate samples not meeting SMS Criteria compare to reference.  
**Bold indicates SQS Failure (Statistical difference and >0.20)**

#### 4.5 SUMMARY

Thirty-four samples failed to meet SQS or CSL performance criteria for one or more of the toxicity tests performed on the Oakland Bay sediments (Table 14). Nine samples expressed failures for more than one species. These samples included OB-06-SS-00, OB-13-SS-00, SH-06-SS-00, SH-07-SS-00, SH-19-WS-00, SH-21-WS-00, SH-22-WS-00, and SH-26-WS-00, (juvenile polychaete and larval development) and OB-05-SS-00 (juvenile polychaete, larval development, and Microtox®).

**Table 14. Summary of Samples Not Meeting SMS Criteria.**

Sample	Sediment Quality Standards										Cleanup Screening Levels								
	Amphipod			Polychaete			Larval			Microtox®	Amphipod			Polychaete			Larval		
	RF-01	RF-02	RF-03	RF-01	RF-02	RF-03	RF-01	RF-02	RF-03		RF-01	RF-02	RF-03	RF-01	RF-02	RF-03	RF-01	RF-02	RF-03
HI-02-SS-00				X	X														
HI-05-SS-00				X															
OB-03-SS-00		X	X																
OB-04-SS-00							X	X	X								X		X
OB-05-SS-00				X	X		X		X	X									
OB-06-SS-00					X		X		X								X		
OB-07-SS-00				X															
OB-09-SS-00							X												
OB-10-SS-00							X	X	X								X		X
OB-12-SS-00							X												
OB-13-SS-00					X		X	X	X								X	X	X
OB-14-SS-00							X	X	X								X		X
OB-18-WS-00							X	X	X								X		X
OB-19-WS-00							X	X	X								X		X
SH-02-SS-00							X <sup>1</sup>		X								X <sup>1</sup>		X
SH-03-SS-00							X <sup>1</sup>	X <sup>1</sup>	X								X <sup>1</sup>		X
SH-04-SS-00									X										X
SH-06-SS-00				X			X <sup>1</sup>	X <sup>1</sup>	X				X				X <sup>1</sup>	X <sup>1</sup>	X
SH-07-SS-00				X			X	X	X								X	X	X
SH-12-SS-00							X		X										
SH-13-SS-00				X	X														
SH-14-SS-00									X										
SH-16-SS-00				X									X						
SH-18-WS-00							X												
SH-19-WS-00				X			X <sup>1</sup>		X										X
SH-21-WS-00					X		X <sup>1</sup>	X <sup>1</sup>	X										X
SH-22-WS-00				X	X				X										X
SH-23-WS-00				X															
SH-24-WS-00							X <sup>1</sup>	X <sup>1</sup>	X								X <sup>1</sup>		X
SH-25-WS-00							X												
SH-26-WS-00				X					X				X						
SH-27-WS-00				X															
SH-28-WS-00					X														
SH-29-WS-00				X															

X = Does not meet criterion

<sup>1</sup> Failure expressed when compared to reference treatment not meeting acceptability criteria.

## 5.0 REFERENCES

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## **Appendix H**

# **BIOLOGICAL TESTING RESULTS FOR OAKLAND BAY SEDIMENT CHARACTERIZATION, OAKLAND BAY, WASHINGTON**

*Newfields, Revised April 2010*

**The appendices to this report are available upon  
request from Department of Ecology, Southwest  
Regional Office, Toxics Cleanup Program**

**Appendix A – Microtox Report**

**Appendix B – Water Quality Summaries**

**Appendix C – Laboratory Data Sheets**

**Appendix D – Statistical Comparisons**