

## Attachment C-2: Sediment Toxicity Testing

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*Dinnel Marine Resources*

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**QUALITY ASSURANCE EVALUATIONS OF  
LARVAL MUSSEL BIOASSAYS OF LOWER  
DUWAMISH RIVER SEDIMENTS**

**Round 1**

**Final Report**

**13 June 2005**

**For**

**Windward Environmental LLC  
Seattle, Washington**

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## 1.0 INTRODUCTION

Northwestern Aquatic Sciences (NAS), Newport, Oregon was contracted by Windward Environmental LLC to conduct amphipod and polychaete bioassays of sediments collected from the Lower Duwamish Waterway in January 2005. NAS is a State of Washington accredited laboratory (Lab accreditation number C042, expiration: 30 September 2005) and is certified to perform amphipod and polychaete tests using the Puget Sound Estuary Program (PSEP 1995) protocols. A copy of NAS' accreditation certificate and Scope of Accreditation appears in Appendix 1.

This report summarizes the Quality Assurance/Quality Control (QA/QC) evaluations of the first of two rounds of amphipod (*Eohaustorius estuarius*) and polychaete (*Neanthes arenaceodentata*) bioassays of sediments collected from the Lower Duwamish Waterway in January 2005. Testing during Round 1 was completed in three test batches; these were initiated on 3, 8 and 11 March 2005, respectively.

The QA steps taken to ensure high quality data and maximum data completeness before, during and after this round of testing are described in this report. Major QA tasks by Dinnel Marine Resources (DMR) included the following:

- A pre-test review of NAS' Standard Operating Procedures (SOPs)
- Pre-test coordination with NAS and Windward Environmental
- An initial evaluation of all data for completeness, correct data entries, and accurate transcription to electronic formats
- A final QA evaluation of overall data quality and usability (this report)

## 2.0 QUALITY ASSURANCE AUDIT RESULTS

### 2.1 LABORATORY PROTOCOLS AND SOPs

The PSEP protocols (PSEP 1995) for conducting amphipod and polychaete bioassays and NAS' laboratory SOPs (NAS-XXX-EE4, Rev. 3 and NAS-XXX-NA4, Rev. 4) for the *Eohaustorius* and *Neanthes* tests were reviewed in detail prior to the initiation of testing. NAS' SOPs were in excellent condition, and no changes were needed other than the addition of some project-specific provisions requested by Windward Environmental. A letter detailing the project-specific test provisions was sent to NAS on 23 February 2005 (Appendix 2).

### 2.2 TEST-IN-PROGRESS AUDITS

An unannounced test-in-progress audit of all three batches of tests was conducted by Dr. Paul Dinnel on 11 March 2005 (two of each test type in progress, two others being set up). All PSEP and project-specific protocol provisions were being followed without any apparent deviations. Completed test-in-progress audit checklists for the amphipod and polychaete bioassays appear in Appendix 3.

## **2.3 INITIAL DATA EVALUATIONS**

All raw data forms and electronic database files were reviewed for completeness and fidelity of transcription to electronic formats. A 100% check was made of all data entered into NAS' internal electronic database. All errors, omissions, clarifications, or changes needed to NAS' draft report were documented and communicated to NAS. A copy of the initial data evaluation report to NAS appears in Appendix 4. All needed corrections to the data report were made by NAS and subsequently verified by DMR (see NAS response letter in Appendix 4).

PSEP (1995) water quality assurance parameters were primarily used for QA assessments of the sediment tests. Where specific values were lacking in PSEP, PSDDA (1994) values were used. These values are summarized in Table 1 for the *Eohaustorius* and *Neanthes* tests. Note that these protocol provisions have occasionally been modified via clarification and issue papers prepared by the U.S. Army Corps of Engineers (USACOE) and the other relevant sediment management agencies (USACOE 1991-2005).

## **2.4 FINAL QA EVALUATION OF OVERALL DATA QUALITY AND USABILITY**

Following corrections to the data reports by NAS personnel, a 100% check was made to verify each correction. Following this, an overall evaluation of data completeness and quality was accomplished (this report). Conclusions regarding data completeness and quality follow below.

### **2.4.1 Chain of Custody and Sample Holding**

All chain of custody protocols were properly observed in transfers of sediment samples from Windward Environmental to NAS. The test and reference sediments were stored at 4° C in a locked cold room until testing was initiated. If samples contained significant headspaces, these head spaces were purged of air with nitrogen gas prior to storage.

Table 1. PSEP (1995) and PSDDA (1994) water quality assurance parameters for the *Eohaustorius* and *Neanthes* tests, as occasionally modified by USACOE clarification and issue papers.

Parameter	<i>Eohaustorius</i>	<i>Neanthes</i>
Temperature, °C	14 – 16	19 - 21
Salinity, ppt	Ambient Interstitial*	28 – 35 (PSDDA) 26 – 30 (PSEP)
Dissolved oxygen, mg/liter	>4.0	>4.5
pH	7 – 9	7 - 9
Total Ammonia, mg/liter	60**	≤10*** 10-20****
Total Sulfide, mg/liter	0.5 <sup>#</sup>	3.4***

\* A project-specific salinity of  $28 \pm 1$  ppt was chosen for the *Eohaustorius* tests

\*\* U.S. EPA No Effect Concentration (EPA 1994) (Discussed in Barton 2002)

\*\*\* DMMP No effects ( $\leq 10$ ) and possible minor effects (10-20) thresholds (Kendall and Barton 2004)

<sup>#</sup> Based on values for *Rhepoxynius* and *Ampelisca*

#### 2.4.2 *Eohaustorius* Test 725-1

1. A *Eohaustorius estuarius* bioassay was conducted on 13 Lower Duwamish River sediment samples, plus 3 reference sediments. A negative control, and two positive (toxic) controls with cadmium and ammonia were run concurrently with the Lower Duwamish test sediments.
2. Testing was initiated within 46 days following sediment collection, which was within the 8 week limit specified in Windward Environmental's Quality Assurance Project Plan (QAPP) (Windward 2005).
3. This test was completed with no protocol deviations and one water quality deviation. Beginning on Day 5, several overlying water salinity measurements exceeded the project-specific guideline of  $28 \pm 1$  ppt, with the maximum measured value being 30.5 ppt. These minor salinity deviations are probably of no concern for two reasons: 1) the PSEP/PSDDA protocol guidelines for salinity are vague for *Eohaustorius* (use interstitial salinity of the test animal collection site) and 2) percent effective mortality in the control and reference sediments were all  $< 10\%$ . Note: the test animals were gradually acclimated to 28 ppt salinity following their collection from Yaquina Bay, where the interstitial salinity at the time of collection was 29 ppt.

4. The reference toxicant 50% Lethal Concentration (LC50) for the cadmium test was 2.04 mg/liter. This result was within NAS' control chart warning limits of 0.56 – 5.89 mg Cd/liter. The ammonia EC50 was 101 mg/liter total ammonia. This result was within NAS' control chart warning limits of 82.7 – 191 mg/liter total ammonia.
5. Negative control mean mortality (2.0 %) was <10% for this test and thus acceptable by present PSEP and PSDDA criteria. The mean mortality responses for the reference sediments ranged from 1.0 to 7.0 %, which were within the PSDDA limit of  $\leq 25$  %.
6. The maximum ammonia concentration measured in the overlying water during this test in any one sample was 13.4 mg/liter total ammonia. This is well below the EPA NOEC threshold concentration of 60 mg/liter and well below NAS' reference toxicant test LC50 of 101 mg/liter. Thus, ammonia concentrations were not high enough to cause significant stresses in this test.
7. No sulfides were detected in any of the test sample overlying waters (detection limit 0.02 mg/liter). Thus, sulfide levels in this test were not high enough to interfere.
8. Replication was five-fold for all samples as specified by PSDDA.
9. Data completeness for the 16 (plus control) samples tested with *Eohaustorius* was >99 %.
10. **Final QA determination:** All data are of excellent quality and fully usable for any purpose.

**Table 2. Summary of *Eohaustorius* Test 725-1.**

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*Eohaustorius estuarius*, 4 -14 March 2005:

Number of test samples, including reference sediments: 16

Sediment holding time <8 weeks?: Yes

Protocol deviations?: No

Average negative control mortality: 2.0 %

Average reference sediment mortality  $\leq 25$  %?: Yes

Reference toxicant LC50: 2.04 mg/liter cadmium and 101 mg/liter total ammonia. These values are within NAS' control chart warning limits.

Water quality parameter deviations: Salinity in some beakers was as high as 30.5 ppt from Day 5 to Day 10. These minor salinity deviations did not appear to affect the results of this test.

Ammonia and sulfide concentrations < critical limits?: Yes, for both ammonia and sulfide.

**QA reviewer conclusion:** All data are of excellent quality and fully usable for any purpose.

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**2.4.3 *Eohaustorius* Test 725-3**

1. A *Eohaustorius estuarius* bioassay was conducted on 13 Lower Duwamish River sediment samples, plus 3 reference sediments. A negative control, and two positive (toxic) controls with cadmium and ammonia were run concurrently with the Lower Duwamish test sediments.
2. Testing was initiated within 48 days following sediment collection, which was within the 8 week limit specified in Windward Environmental's Quality Assurance Project Plan (QAPP) (Windward 2005).
3. This test was completed with one protocol deviation and two water quality deviations. The protocol deviation was that the test water was three days old, slightly exceeding the  $\leq 2$  days specified in the protocol. The water quality deviations were:
  - Day zero overlying water salinity was slightly below the project-specified  $28.0 \pm 1.0$  ppt by up to 1 ppt for samples 9781F, 9822F, and 9827F. All three of these sediments had low interstitial salinity (5-9 ppt), and the slightly low overlying water salinity was attributed to the salinity adjustment procedure used for these sediments.
  - Beginning on Day 3, several overlying water salinity measurements exceeded the project-specified salinity range by up to 2.5 ppt.

None of these protocol or water quality deviations are deemed to be significant. *Eohaustorius* is tolerant of a wide range of salinities (it is the recommended amphipod species for tests where salinities are  $<25$  ppt), the control/reference sediment mortalities were very low ( $< 10$  %) and mean daily emergence values were also very low ( $< 2$ ).

4. The reference toxicant 50% Lethal Concentration (LC50) for the cadmium test was 3.47 mg/liter. This result was within NAS' control chart warning limits of 0.43 – 5.84 mg Cd/liter. The ammonia LC50 was 151 mg/liter total ammonia. This result was within NAS' control chart warning limits of 75.4 – 188 mg/liter total ammonia.
5. Negative control mean mortality (2.0 %) was  $<10$  % for this test and thus acceptable by present PSEP and PSDDA criteria. The mean mortality responses for the reference sediments ranged from 0 to 7.0 %, which were within the PSDDA limit of  $\leq 25$  %.
6. The maximum ammonia concentration measured in the overlying water during this test in any one sample was 4.2 mg/liter total ammonia. This is well below the EPA NOEC threshold concentration of 60 mg/liter and well below NAS' reference toxicant test LC50 of 151 mg/liter. Thus, ammonia concentrations were not high enough to cause significant stresses in this test.
7. The maximum sulfide concentration measured in overlying water during the test in any one sample was 0.18 mg/liter. This is well below the limit of 0.5 mg/liter set by PSDDA/PSEP (based on two other amphipod species). Thus, sulfide levels in this test were probably not high enough to interfere.



8. Replication was five-fold for all samples as specified by PSDDA.
9. Data completeness for the 16 (plus control) samples tested with *Eohaustorius* was >99 %.
10. **Final QA determination:** All data are of excellent quality and fully usable for any purpose.

**Table 3. Summary of *Eohaustorius* Test 725-3.**

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*Eohaustorius estuarius*, 8 -18 March 2005:

Number of test samples, including reference sediments: 16

Sediment holding time <8 weeks?: Yes

Protocol deviations?: Yes. Age of test water was 3 days instead of  $\leq 2$  days

Average negative control mortality: 2.0 %

Average reference sediment mortality  $\leq 25$  %?: Yes

Reference toxicant LC50: 3.47 mg/liter cadmium and 151 mg/liter total ammonia. These values are within NAS' control chart warning limits.

Water quality parameter deviations: Salinity in some beakers was up to 2.5 ppt high or low during the test and the test water was 3 days old instead of  $\leq 2$  days. These minor salinity and water age deviations did not appear to affect the results of this test.

Ammonia and sulfide concentrations < critical limits?: Yes, for both ammonia and sulfide.

**QA reviewer conclusion:** All data are of excellent quality and fully usable for any purpose. The slight salinity and water age deviations are deemed insignificant.

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#### **2.4.4 *Eohaustorius* Test 725-5**

1. A *Eohaustorius estuarius* bioassay was conducted on 1 Lower Duwamish River sediment sample, plus 1 reference sediment. A negative control, and two positive (toxic) controls with cadmium and ammonia were run concurrently with the Lower Duwamish test sediment.
2. Testing was initiated 49 days following sediment collection, which was within the 8 week limit specified in Windward Environmental's Quality Assurance Project Plan (QAPP) (Windward 2005).
3. This test was completed with no protocol deviations and one water quality deviation. The water quality deviation was that on Days 7-10, several overlying salinity measurements exceeded the project-specified  $28.0 \pm 1.0$  ppt by up to 1.5 ppt.
4. The reference toxicant 50% Lethal Concentration (LC50) for the cadmium test was 2.27 mg/liter. This result was within NAS' control chart warning limits of 0.51 – 5.87 mg Cd/liter. The ammonia LC50 was 226 mg/liter total ammonia. This result exceeded NAS' control

chart warning limits of 80.3 – 188 mg/liter total ammonia. However, NAS' ammonia control chart for *Eohaustorius* only had 8 data points; thus, variability may be somewhat greater than presently indicated.

5. Negative control mean mortality (1.0 %) was <10% for this test and thus acceptable by present PSEP and PSDDA criteria. The mean mortality response for the reference sediment was 5.0 %, which was within the PSDDA limit of  $\leq 25$  %.
6. The maximum ammonia concentration measured in the overlying water during this test in any one sample was 22.5 mg/liter total ammonia. This is well below the EPA NOEC threshold concentration of 60 mg/liter and well below NAS' reference toxicant test LC50 of 226 mg/liter. Thus, ammonia concentrations were not high enough to cause significant stresses in this test.
7. No sulfides were detected in any of the test sample overlying waters (detection limit 0.02 mg/liter). Thus, sulfide levels in this test were not high enough to interfere.
8. Replication was five-fold for all samples as specified by PSDDA.
9. Data completeness for the 2 (plus control) samples tested with *Eohaustorius* was 100 %.
10. **Final QA determination:** All data are of excellent quality and fully usable for any purpose.

**Table 4. Summary of *Eohaustorius* Test 725-5.**

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*Eohaustorius estuarius*, 11 - 21 March 2005:

Number of test samples, including reference sediments: 2

Sediment holding time <8 weeks?: Yes

Protocol deviations?: No

Average negative control mortality: 1.0 %

Average reference sediment mortality  $\leq 25$  %?: Yes

Reference toxicant LC50: 2.27 mg/liter cadmium and 226 mg/liter total ammonia. The cadmium LC50 is within NAS' control chart warning limits but the ammonia response is higher than the control chart upper limit; however, the control chart only had eight data points at the time.

Water quality parameter deviations: Salinity in some beakers was as high as 1.5 ppt above the project-specified range. These minor salinity deviations probably did not significantly affect the results of the test.

Ammonia and sulfide concentrations < critical limits?: Yes, for both ammonia and sulfide.

**QA reviewer conclusion:** All data are of excellent quality and fully usable for any purpose.

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#### 2.4.5 *Neanthes* Test 725-2

1. A *Neanthes arenaceodentata* bioassay was conducted on 13 Lower Duwamish River sediment samples, plus 3 reference sediments. A negative control, and two positive (toxic) controls with cadmium and ammonia were run concurrently with the Lower Duwamish test sediments.
2. Testing was initiated within 46 days following sediment collection, which was within the 8 week limit specified in Windward Environmental's Quality Assurance Project Plan (QAPP) (Windward 2005).
3. This test was completed with no protocol deviations and one water quality deviation. NAS' report mentions a protocol deviation about not holding test animals under constant light. To this reviewer's knowledge, neither PSEP nor PSDDA has any requirement for holding *Neanthes* under constant light during acclimation, nor is it a provision in NAS' SOP for the *Neanthes* test. The one water quality deviation was that one salinity measurement on Day 3 exceeded the protocol-specified  $28.0 \pm 2.0$  ppt by 0.5 ppt. This salinity deviation is deemed insignificant.
4. The reference toxicant 50% Lethal Concentration (LC50) for the cadmium test was 8.63 mg/liter. This result was within NAS' control chart warning limits of 4.4 – 11.1 mg Cd/liter. The ammonia LC50 was 183 mg/liter total ammonia. This result was below NAS' control chart warning limits of 286 - 477 mg/liter total ammonia. However, NAS' ammonia control chart for *Neanthes* only had 5 data points; thus, variability may be somewhat greater than presently indicated. In addition, the mean control survival in the ammonia reference toxicant test was only 85 % (100 % and 70 % for the two replicates), which was less than the protocol-specified 90%. Neither the low ammonia test LC50 or the slightly low survival in the ammonia test controls are deemed significant since the test negative control performance was excellent (100 % survival and an individual growth rate of 1.12 mg/day/worm).
5. Negative control mean mortality (0 %) was <10% for this test and thus acceptable by present PSEP and PSDDA criteria. The control mean individual growth rate was 1.12 mg/day/worm, which is substantially greater than the 0.72 mg/day/worm recommended by PSDDA/PSEP. The mean mortality responses for the 3 reference sediments were also 0 %, which was within the PSDDA limit of  $\leq 25$  %. The mean individual growth rates for the 3 reference sediments were 87.5, 100 and 76.8 % of the negative control growth rate. The growth rate in the third reference sediment (LDW-SSMSMP43-010) did not quite meet the PSDDA requirement that mean growth should be  $\geq 80$  % of the negative control growth. However, it should be noted that the control growth rate was substantially higher than the recommended rate of 0.72 mg/day/worm and that the reference sediment in question also had a growth rate (0.86 mg/day/worm) higher than that recommended for the control. Thus, the slightly low growth rate in the one reference sediment should pose no problems for data interpretation.

6. The maximum ammonia concentration measured in the overlying water during the test in any one sample was 9.9 mg/liter total ammonia. This is below the DMMP no effects threshold concentration of 10 mg/liter and well below NAS' reference toxicant test LC50 of 183 mg/liter. Thus, ammonia concentrations were not high enough to cause significant stresses in this test.
7. No sulfides were detected in any of the test sample overlying waters (detection limit 0.02 mg/liter). Thus, sulfide levels in this test were not high enough to interfere.
8. Replication was five-fold for all samples as specified by PSDDA.
9. Data completeness for the 16 (plus control) samples tested with *Neanthes* was 100 %.
10. **Final QA determination:** All data are of good quality and fully usable for any purpose. Note should be taken of the slightly low mean individual growth rate for reference sediment LDW-SSMSMP43-010, although this slightly low growth rate should be of little consequence for data interpretation.

**Table 5. Summary of *Neanthes* Test 725-2.**

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*Neanthes arenaceodentata*, 4 -24 March 2005:

Number of test samples, including reference sediments: 16

Sediment holding time <8 weeks?: Yes

Protocol deviations?: No

Average negative control mortality: 0 %

Average control individual growth rate  $\geq 0.72$  mg/day/worm?: Yes

Average reference sediment mortality  $\leq 10$  %?: Yes

Average reference sediment individual growth rates  $\geq 80$  % compared to the negative control?: Yes, for two of the three samples. The third sample (LDW-SSMSMP43-010) was slightly less than 80 % (76.8 %). Although the growth rate in this reference sample is probably satisfactory, some care should be used in the data interpretation.

Reference toxicant LC50: 8.63 mg/liter cadmium and 183 mg/liter total ammonia. The cadmium LC50 is within control chart warning limits but the ammonia response is lower than the control chart lower limit; however, the ammonia control chart only had five data points at the time.

Water quality parameter deviations: Salinity in one beaker was 0.5 ppt above the protocol-specified range. This minor salinity deviation should not have significantly affected the results of the test.

Ammonia and sulfide concentrations < critical limits?: Yes, for both ammonia and sulfide.

**QA reviewer conclusion:** All data are of good quality and fully usable for any purpose. Note should be taken of the slightly low mean individual growth rate for reference sediment LDW-

SSMSMP43-010, although this slightly low growth rate should be of little consequence to data interpretation.

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#### 2.4.6 *Neanthes* Test 725-4

1. A *Neanthes arenaceodentata* bioassay was conducted on 13 Lower Duwamish River sediment samples, plus 3 reference sediments. A negative control, and two positive (toxic) controls with cadmium and ammonia were run concurrently with the Lower Duwamish test sediments.
2. Testing was initiated within 48 days following sediment collection, which was within the 8 week limit specified in Windward Environmental's Quality Assurance Project Plan (QAPP) (Windward 2005).
3. This test was completed with minor protocol deviations and two water quality deviation. The minor protocol deviations were that some water quality measurements were missed or mistakenly taken following water renewal changes instead of before. In one case, interstitial salinity and pH could not be measured due to insufficient amount of sample. These deviations resulted in a few missing water quality data. NAS' report mentions a protocol deviation about not holding test animals under constant light. To this reviewer's knowledge, neither PSEP nor PSDDA has any requirement for holding *Neanthes* under constant light during acclimation, nor is it a provision in NAS' SOP for the *Neanthes* test.

The two water quality deviations were: 1) two overlying water salinity measurements on Day 12 exceeded the protocol-specified  $28.0 \pm 2.0$  ppt by 0.5 ppt and 2) Day 0 overlying salinity was up to 1.0 ppt low in 5 of the samples. These samples all underwent salinity adjustment due to low interstitial salinities and the slightly low overlying water salinities are likely the result of the initial low interstitial salinities. It is likely that these slightly low salinities did not affect the test results significantly. In one case NAS points out an apparent aberrant salinity measurement (Day 6, sample 9842F = 25.0 ppt). The salinity in this beaker was 29.0 and 30.0 on Days 3 and 9 respectively; thus, the likely salinity in this beaker was 29-30 ppt on Day 6.

4. The reference toxicant 50% Lethal Concentration (LC50) for the cadmium test was 8.06 mg/liter. This result was within NAS' control chart warning limits of 4.4 – 11.1 mg Cd/liter. The ammonia LC50 was 344 mg/liter total ammonia. This result was within NAS' control chart warning limits of 286 - 477 mg/liter total ammonia.
5. Negative control mean mortality (4 %) was <10% for this test and thus acceptable by present PSEP and PSDDA criteria. The control mean individual growth rate was 0.95 mg/day/worm, which is greater than the 0.72 mg/day/worm recommended by PSDDA/PSEP. The mean mortality responses for the 3 reference sediments were all 0 %, which was within the PSDDA limit of  $\leq 25$  %. The mean individual growth rates for the 3 reference sediments ranged from

- 96.8 to 102 % of the negative control growth rate, which meets the PSDDA requirement that mean reference sample growth should be  $\geq 80$  % of the negative control growth.
6. The maximum ammonia concentration measured in the overlying water during this test in any one sample was 10.3 mg/liter total ammonia. This is very slightly above the DMMP no effects threshold concentration of 10 mg/liter but at the bottom end of the possible DMMP minor effects threshold of 10-20 mg/liter total ammonia. It is also well below NAS' reference toxicant test LC50 of 344 mg/liter. Thus, ammonia concentrations were likely not high enough to cause significant stresses in this test.
  7. The maximum overlying water sulfide concentration measured in this test was 0.02 mg/liter, which is substantially below the DMMP limit of 3.4 mg/liter. Thus, sulfide levels in this test were not high enough to interfere.
  8. Replication was five-fold for all samples as specified by PSDDA.
  9. Data completeness for the 16 (plus control) samples tested with *Neanthes* was about 99 %.
  10. **Final QA determination:** All data are of good quality and fully usable for any purpose. The slight salinity deviations (0.5 to 1.0 ppt) in a few of the samples on some days and a few cases of missing water quality data should pose no significant problems for data interpretations.

**Table 6. Summary of *Neanthes* Test 725-4.**

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*Neanthes arenaceodentata*, 8 -28 March 2005:

- Number of test samples, including reference sediments: 16  
Sediment holding time <8 weeks?: Yes  
Protocol deviations?: Yes. A few water quality measurements were missed or collected following water renewal (affected measurements on Day 3 only). These deviations are very minor.  
Average negative control mortality: 4 %  
Average control individual growth rate  $\geq 0.72$  mg/day/worm?: Yes  
Average reference sediment mortality  $\leq 10$  %?: Yes  
Average reference sediment individual growth rates  $\geq 80$  % compared to the negative control?: Yes  
Reference toxicant LC50: 8.06 mg/liter cadmium and 344 mg/liter total ammonia. Both values are within NAS' control chart warning limits.  
Water quality parameter deviations: A few overlying salinity values were up to 1.0 ppt high or low on a few days during the test.  
Ammonia and sulfide concentrations < critical limits?: Yes for sulfide and probably for ammonia.

**QA reviewer conclusion:** All data are of good quality and fully usable for any purpose. The minor salinity deviations should not have affected the quality of the results.

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#### 2.4.7 *Neanthes* Test 725-6

1. A *Neanthes arenaceodentata* bioassay was conducted on 1 Lower Duwamish River sediment sample, plus 1 reference sediment. A negative control, and two positive (toxic) controls with cadmium and ammonia were run concurrently with the Lower Duwamish test sediment.
2. Testing was initiated within 49 days following sediment collection, which was within the 8 week limit specified in Windward Environmental's Quality Assurance Project Plan (QAPP) (Windward 2005).
3. This test was completed with one protocol deviation and one water quality deviation. The protocol deviation was that water quality measurements on Day 15 were taken after water renewal instead of before. This resulted in a few missing data. The one water quality deviation was that two overlying salinity measurements on Day 18 exceeded the protocol-specified  $28.0 \pm 2.0$  ppt by 1.0 ppt. These salinity deviations are deemed insignificant.
4. The reference toxicant 50% Lethal Concentration (LC50) for the cadmium test was 7.70 mg/liter. This result was within NAS' control chart warning limits of 4.43 – 11.2 mg Cd/liter. The ammonia LC50 was 350 mg/liter total ammonia. This result was within NAS' control chart warning limits of 284 - 466 mg/liter total ammonia.
5. Negative control mean mortality (0 %) was <10% for this test and thus acceptable by present PSEP and PSDDA criteria. The control mean individual growth rate was 0.87 mg/day/worm, which is greater than the 0.72 mg/day/worm recommended by PSDDA/PSEP. The mean mortality response for the reference sediment was also 0 %, which was within the PSDDA limit of  $\leq 25$  %. The mean individual growth rate for the reference sediment was 93.1 % of the negative control growth rate, which meets the PSDDA requirement that mean growth should be  $\geq 80$  % of the negative control growth.
6. The maximum ammonia concentration measured in the overlying water during this test in any one sample was 5.0 mg/liter total ammonia. This is below the DMMP no effects threshold concentration of 10 mg/liter and well below NAS' reference toxicant test LC50 of 350 mg/liter. Thus, ammonia concentrations were not high enough to cause significant stresses in this test.
7. No sulfides were detected in any of the test sample overlying waters (detection limit 0.02 mg/liter). Thus, sulfide levels in this test were not high enough to interfere.
8. Replication was five-fold for all samples as specified by PSDDA.
9. Data completeness for the 2 (plus control) samples tested with *Neanthes* was about 99 %.

10. **Final QA determination:** All data are of excellent quality and fully usable for any purpose. The minor salinity deviations (up to 1.0 ppt) in two samples are of no consequence.

**Table 7. Summary of *Neanthes* Test 725-6.**

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*Neanthes arenaceodentata*, 11 - 31 March 2005:

Number of test samples, including reference sediments: 2

Sediment holding time <8 weeks?: Yes

Protocol deviations?: Yes. On Day 15, water quality measurements were collected after water renewals instead of before, resulting in missing data on that day.

Average negative control mortality: 0 %

Average control individual growth rate  $\geq 0.72$  mg/day/worm?: Yes

Average reference sediment mortality  $\leq 10$  %?: Yes

Average reference sediment individual growth rate  $\geq 80$  % compared to the negative control?: Yes

Reference toxicant LC50: 7.70 mg/liter cadmium and 350 mg/liter total ammonia. Both values are within NAS' control chart warning limits.

Water quality parameter deviations: Two overlying salinity measurements on Day 18 exceeded the protocol-specified  $28.0 \pm 2.0$  ppt by 1.0 ppt. These salinity deviations are deemed insignificant.

Ammonia and sulfide concentrations < critical limits?: Yes, for both ammonia and sulfide.

**QA reviewer conclusion:** All data are of excellent quality and fully usable for any purpose.

---

### 3.0 REFERENCES

- Barton, J. 2002. Ammonia and amphipod toxicity testing. Dredged Material Management Program (DMMP) clarification Paper dated 6/15/02. U.S. Army Corps of Engineers, Seattle District, Seattle, WA. 6 pp.
- EPA (U.S. Environmental Protection Agency). 1994. Methods for assessing the toxicity of sediment-associated contaminants with estuarine amphipods. EPA/600/R-94/025. Pp 80-82.



Kendall, D. and J. Barton. 2004. Ammonia and sulfide guidance relative to *Neanthes* growth bioassay. Dredged Material Management Program (DMMP) clarification paper dated 6/15/04. U.S. Army Corps of Engineers, Seattle District, Seattle, WA. 9 pp.

PSDDA (Puget Sound Dredged Disposal Analysis). 1994. Dredged Analysis Information System (DAIS), Version 4.4. Electronic database from Seattle District, U. S. Army Corps of Engineers.

PSEP (Puget Sound Estuary Program). 1995. Recommended guidelines for conducting laboratory bioassays on Puget Sound Sediments. Final Report by PTI Environmental Services for U. S. Environmental Protection Agency, Region 10, Office of Puget Sound, Seattle, WA.

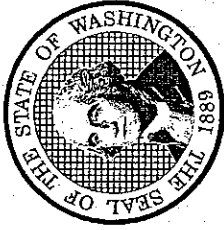
USACOE (U.S. Army Corps of Engineers). 1991-2005. Periodic Clarification and Issue papers issued by the U.S. Army Corps of Engineers, Seattle District, Dredged Materials Management Office (DMMO) from 1991 through 2005 (See DMMO website).

Windward (Windward Environmental LLC). 2005. Quality assurance project plan: Surface sediment sampling for chemical analyses and toxicity testing of the Lower Duwamish Waterway. Final Report for the U.S. Environmental Protection Agency, Region 10, Seattle, WA and the Washington Department of Ecology, Northwest Office, Bellevue, WA. 89 pp. + maps.

## **Appendix 1**

### **Northwestern Aquatic Sciences' State of Washington Accreditation Certificate and Scope of Accreditation**

The State of  
Department



Washington  
of Ecology

This is to certify that

Northwestern Aquatic Sciences  
Newport, OR

has complied with provisions set forth in Chapter 173-50 WAC and is hereby recognized by the Department of Ecology as an ACCREDITED LABORATORY for the analytical parameters listed on the accompanying Scope of Accreditation. This certificate is effective October 1, 2004, and shall expire September 30, 2005.

Witnessed under my hand on October 7, 2004.

Perry F. Brake, Chemist  
Lab Accreditation Section Manager

Lab Accreditation Number

**C042**

# Scope of Accreditation

## Northwestern Aquatic Sciences

### Newport, OR

is accredited by the State of Washington Department of Ecology to perform analyses for the parameters listed below using the analytical methods indicated. This Scope of Accreditation may apply to any of the following matrix types: non-potable water, drinking water, solid and chemical materials, and air and emissions. Accreditation for all parameters is final unless indicated otherwise in a note. Accreditation is for the latest version of a method unless otherwise specified in a note. EPA refers to the U.S. Environmental Protection Agency. SM refers to American Public Health Association's publication, Standard Methods for the Examination of Water and Wastewater, 18th, 19th or 20th Edition, unless otherwise noted. ASTM stands for the American Society for Testing and Materials. PSEP stands for Puget Sound Estuary Program. Other references are detailed in the notes section.

---

Matrix Type/Parameter Name	Reference	Method Number	Notes
<b>Non-potable Water</b>			
Ampelisca abdita	ASTM	E 1367	1
Ampelisca abdita	EPA	100.4	13
Ampelisca abdita	PSEP	1995	2
Atherinops affinis (West Coast)	EPA	1006.0	3,4
Bioaccumulation, Benthic Invert	ASTM	E 1688	5
Bioconcentration, Fish, Mollusks	ASTM	E 1022	6
Ceriodaphnia dubia	EPA	1002.0	4,7
Ceriodaphnia dubia	EPA	2002.0	4,8
Chironomus tentans	EPA	100.5	14
Chironomus tentans	ASTM	E 1706	9
Chironomus tentans	EPA	100.2	14
Crassostrea gigas	PSEP	1995	2
Crassostrea gigas (West Coast)	EPA	1005.0	3,4
Cyprinodon variegatus	EPA	1004.0	4,10
Cyprinodon variegatus	EPA	2004.0	4,8
Dangerous Waste Static Salmonid	WDOE	80-12 Part A	11
Daphnia magna	EPA	2021.0	4,8
Daphnia pulex	EPA	2021.0	4,8

Matrix Type/Parameter Name	Reference	Method Number	Notes
Dendraster excentricus	ASTM	E 1563	12
Dendraster excentricus	PSEP	1995	2
Dendraster excentricus (West Coast)	EPA	1008.0	3,4
Eohaustorius estuarius	ASTM	E 1367	1
Eohaustorius estuarius	PSEP	1995	2
Eohaustorius estuarius	EPA	100.4	13
Holmesimysis costata	EPA	821-R-02-012	4,8
Holmesimysis costata (West Coast)	EPA	1007.0	3,4
Hyalella azteca	EPA	100.1	14
Hyalella azteca	ASTM	E 1706	9
Hyalella azteca	EPA	100.4	14
Leptocheirus plumulosus	ASTM	E 1367	1
Leptocheirus plumulosus	EPA	100.4	13
Menidia beryllina	EPA	1006.0	4,10
Menidia spp.	EPA	2006.0	4,8
Mysidopsis bahia	EPA	1007.0	4,10
Mysidopsis bahia	EPA	2007.0	8
Mytilus spp.	PSEP	1995	2
Mytilus spp. (West Coast)	EPA	1005.0	3,4
Neanthes arenaceodentata	PSEP	1995	2
Salvelinus fontinalis	EPA	2019.0	4,8
Oncorhynchus mykiss	EPA	2019.0	4,8
Pimephales promelas, Chronic	EPA	1000.0	4,7
Pimephales promelas	EPA	2000.0	4,8
Rhepoxynius abronius	ASTM	E 1367	1
Rhepoxynius abronius	PSEP	1995	2
Rhepoxynius abronius	EPA	100.4	13
Strongylocentrotus purpuratus	ASTM	E 1563	12
Strongylocentrotus purpuratus (WC)	EPA	1008.0	3,4
Strongylocentrotus purpuratus (WC)	EPA	600/R-95/136	3,4

Matrix Type/Parameter Name	Reference	Method Number	Notes
Strongylocentrotus spp.	PSEP	1995	2

#### Accredited Parameter Note Detail

(1) ASTM. "Standard Guide for Conducting 10-day Static Sediment Toxicity Tests with Marine and Estuarine Amphipods," E 1367-99. (2) Puget Sound Estuary Program, "Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments," July 1995. (3) USEPA. "Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms," EPA 600/R-95/136 (Third edition) August 1995. (4) Meets requirements of "Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria," Washington Department of Ecology, Publication Number WQ-R-80, Revised December 2001. (5) ASTM. "Standard Guide for Determination of the Bioaccumulation of Sediment Associated Contaminants by Benthic Invertebrates," E 1688-00a. (6) ASTM. "Practice for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Mollusks," E 1022-94. (7) USEPA. "Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms," EPA-821-R-02-013 (Fourth Edition) October 2002. (8) USEPA. "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms," EPA-821-R-02-012 (Fifth Edition) October 2002. (9) ASTM. "Test Method for Measuring the Toxicity of Sediment-associated Contaminants with Freshwater Invertebrates," E 1706-00. (10) USEPA. "Short-term Methods for Measuring the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms," EPA-821-R-02-014 (Fourth Edition) October 2002. (11) Washington Department of Ecology. "Biological Testing Methods," WDOE 80-12 Revised April 1997. (12) ASTM. "Guide for Conducting Static Acute Toxicity Tests with Echinoid Embryos," E 1563-98. (13) USEPA. "Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods," EPA 600/R-94/025 June 1994. (14) USEPA. "Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates." EPA 600/R-99/064 (Second Edition) March 2000.



10/7/04

Authentication Signature

Perry Brake -- Section Manager, Washington State Department of Ecology -- Lab Accreditation Section

## **Appendix 2**

### **Dinnel Marine Resources' Letter to Northwestern Aquatic Sciences Detailing Project-Specific Testing Requirements**

# DMR

---

Dinnel Marine Resources  
1519 13<sup>th</sup> St.  
Anacortes, WA 98221  
360-299-8468

23 February 2005

Ms. Michelle Redmond  
Northwestern Aquatic Sciences  
PO Box 1437  
Newport, OR 97365

Dear Michelle:

Thank you for providing copies of NAS' protocols for the *Eohaustorius estuarius* and *Neanthes arenaceodentata* bioassay tests to be used to assay Lower Duwamish Waterway Group sediment samples. I found the protocols to be very well written and in conformance with PSEP/SMS guidelines developed for these tests (PSEP 1995, with periodic modifications by the Dredged Material Management Program). I see no need to modify anything in your current protocols for these two test species.

As noted in both of your protocols, there are a number of variables that can change from client to client depending on their testing needs. I take this opportunity to highlight these project-specific items and encourage you to append this letter to your working protocols being used for the Duwamish Waterway testing. Project-specific testing requirements noted here are specified by the client, Windward Environmental LLC (Windward 2005).

**For the *Eohaustorius estuarius* testing:**

1. The salinity of the dilution water is specified as "...at ambient interstitial salinity for the sediment collection site for the *E. estuarius* test." Windward (2005) does not specify a salinity for this test. Thus, the question is still open as to what salinity will be used for testing. Since most amphipod bioassays use 28 ppt, I suggest that the dilution salinity for the *E. estuarius* be 28 ppt, unless the ambient salinity at the amphipod collection site is markedly different from this (see NAS protocol sections 4 and 7.5).
2. For the Duwamish testing, please note that interstitial sediment sulfide will be measured in all sediment samples at test initiation (see table in protocol section 7.7).
3. Windward has requested that the reference toxicant be cadmium chloride (see protocol section 7.10).



4. The reference sediment performance criterion for this project is "mean percent mortality in the reference sediment must be  $\leq 25$  % over the negative control (see protocol section 7.9).
5. The 8-week sediment holding time is in effect for this project. Sediment samples must be stored in the dark at  $4 \pm 2$  °C with no headspace or headspace filled with nitrogen gas (see protocol section 2.6).
6. Any ammonia purging must be authorized by Windward (see protocol section 7.2).

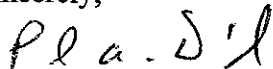
**For the *Neanthes arenaceodentata* testing:**

1. The 8-week sediment holding time is in effect for this project. Sediment samples must be stored in the dark at  $4 \pm 2$  °C with no headspace or headspace filled with nitrogen gas (see protocol section 2.6 and 3).
2. Any ammonia purging must be authorized by Windward (see section 7.2).
3. For the Duwamish testing, please note that interstitial sediment sulfides will be measured at test initiation (see protocol table in section 7.7).
4. Windward has requested that the reference toxicant be cadmium chloride (see section 7.10).

Please advise me if you have any concerns about these testing provisions.

I look forward to working with you and NAS on this project. As usual, I will conduct several unannounced test-in-progress audits during this project.

Sincerely,



Paul Dinnel, Project QA Monitor

CC: Helle Andersen, Windward Environmental LLC

## References

PSEP (Puget Sound Estuary Program). 1995. Recommended guidelines for conducting laboratory bioassays on Puget Sound sediments. Final Report. Prepared for the Puget Sound Estuary Program, U.S. Environmental Protection Agency, Region 10, Office of Puget Sound, and U.S. Army Corps of Engineers, Seattle District, Seattle, WA. Prepared by PTI Environmental Services, Inc., Bellevue, WA.

Windward (Windward Environmental LLC). 2005. Quality assurance project plan: surface sediment sampling for chemical analyses and toxicity testing of the Lower Duwamish Waterway. Final Report submitted to the U.S. Environmental Protection Agency, Region 10, Seattle, WA and the Washington State Department of Ecology, Northwest Regional Office, Bellevue, WA. 89 pp + maps.

## **Appendix 3**

### **Results of the Test-in-Progress Audits of Northwestern Aquatic Sciences' Round 1 Tests**

**CHECKLIST FOR 10-DAY AMPHIPOD BIOASSAY - ROUND 1**

Project Name: LOWER DUWAMISH RIVER Auditor: PAUL DINNELL  
 Laboratory: NORTHWESTERN AQUATIC SCIENCES, NEWPORT, OR Test Type: AMPHIPOD SURVIVAL & REPRODUCTION  
 Test Personnel: MICHELLE REDMOND, GERALD FRISSARI, GARY BUNLER Test SOP: NAS-XXX-EE4, REV. 3  
 Test Date: 3/11/05 Number of Samples: 27  
 SOP Deviations: NONE

Other Notes: \_\_\_\_\_

**Shipping and Holding Conditions**

# Samples Received: 27 # Samples Tested: 27, IN 3 BATCHES  
 Holding Time at Test Initiation: ≤ 47 Days  
 Holding Conditions: 4°C, Dark, NO HEAD SPACE, OR PURGED w/ N<sub>2</sub>  
 Problems Noted in Shipping and Holding: NONE

**Testing Conditions**

Protocol Used: NAS-XXX-EE4, REV. 3 Protocol Available?: YES  
 Deviations?: NO Test Initiation Date: 1 = 3/4  
 Number of Samples: 27 Multiple Batches?: 2 = 3/8  
 Test Species: ECHAUSTORIUS ESTUARINUS Animal Source: YARQUINA BAY  
 Holding Conditions: 15°C, CONSTANT LIGHT, SALINITY ADJUSTED TO 28‰ Holding Time: 2-6 DAYS  
 Feeding During Holding?: NO Size Selection Criteria: ADULTS

Other Notes: \_\_\_\_\_

**CHECKLIST FOR 10-DAY AMPHIPOD BIOASSAY****Quality Assurance Audit**

Audit date: 3/11/05 Days/Hours After Initiation: VARIOUS

Source of Neg. Control Sediment: YAQUINA Bay # of Reference Sediments: 3

Amount of Sediment Used: 175 ml Final Water Volume: 950 ml

Seawater Source YAQUINA Bay Seawater Treatment: 0.4  $\mu$ m FILTER, SALINITY ADJUSTED w/ MILLI-Q, AERATION

Seawater Holding Time: 2-3 DAYS Number of Replicates: 5 + 1 WATER QUALITY

Sediment Equilibration Period?: OVERNIGHT Beakers/Amphipods Randomized?: YES

No. Amphipods/Beaker: 20 Feeding During Test?: NO

Interstitial Salinities Checked?: YES Interstitial Salinities Adjusted?: YES

All Beakers Aerated?: YES Water Temperature: 14.9 - 15.9 °C

Water Salinity: 26.0 - 30.0 ‰ Water DO: 7.2 - 8.2 mg/l

Water pH: 7.2 - 8.3 Photoperiod: CONSTANT LIGHT

Positive Controls Used?: YES Positive Control Toxicant: Cd & AMMONIA

Daily Test Records Maintained?: YES Emergence Data Collected?: YES

QA Officer: LINDA NEMETH Internal QA Checks?: YES

Will Reburial Test Be Conducted at End of Test?: YES

Sulfides and Ammonia Measured at Initiation and End?: YES

**SOP Deviations or Problems Noted:** NONE

my SPOT CHECK: TEMP. = 15.5 °C  
 SALINITY = 28 ‰  
 ALL AERATION GOOD  
 VOLUMES GOOD

QA Officer: PAUL A. DINNELL

Audit Date: 3/11/05

**CHECKLIST FOR 20-DAY JUVENILE POLYCHAETE BIOASSAY - Round 1**

Project Name: LOWER DUWAMISH RIVER Auditor: PAUL DINNELL  
Laboratory: NORTHWESTERN AQUATIC SCIENCES, NEWPORT, OR Test Type: NEANTHES SURVIVAL & GROWTH  
Test Personnel: MICHELE REDMOND, GERARD IRISSARRI, GARY BULLER Test SOP: NAS-XXX-NA4, REV. 4  
Test Date: 3/11/05 Number of Samples: 27 in 3 BATCHES  
SOP Deviations: No

Other Notes: \_\_\_\_\_

**Shipping and Holding Conditions**

# Samples Received: 27 # Samples Tested: 27, in 3 BATCHES  
Holding Time at Test Initiation: ≤ 47 DAYS  
Holding Conditions: 4°C, DARK, NO HEAD SPACE, OR PURGED w/ N<sub>2</sub>  
Problems Noted in Shipping and Holding: NONE

**Testing Conditions**

Protocol Used: NAS-XXX-NA4, REV. 4 Protocol Available?: YES  
Deviations?: No Test Initiation Date: 1 = 3/4  
2 = 3/8  
3 = 3/11  
Number of Samples: 27 Multiple Batches?: YES  
Test Species: NEANTHES AENACEODENTATA Animal Source: DON REISH, LONG BEACH STATE UNIV.  
Holding Conditions: 20°C, CONSTANT LIGHT, 34 → 28‰ SALINITY Holding Time: 3-7 DAYS  
Feeding During Holding?: YES - TERTAMIN Average Weight at T<sub>0</sub>: IN PROCESS

Other Notes:

**CHECKLIST FOR 20-DAY JUVENILE POLYCHAETE BIOASSAY****Quality Assurance Audit**

Audit date: 3/11/05	Days/Hours After Initiation: VARIOUS
Source of Neg. Control Sediment: YAQUINA BAY	# of Reference Sediments: 3
Amount of Sediment Used: 175 ml	Final Water Volume: 950 ml
Seawater Source: YAQUINA BAY	Seawater Treatment: 0.4 $\mu$ m FILTER, SALINITY ADJUSTED w/ MILLI Q + AERATION
Seawater Holding Time: 2-3 Days	Number of Replicates: 5 + 1 WATER QUALITY
Sediment Equilibration Period?: OVERNIGHT	Beakers Randomized?: YES
No. Worms/Beaker: 5	Feeding During Test: 40 mg / 2 DAYS
Interstitial Salinities Checked?: YES	Interstitial Salinities Adjusted?: YES
All Beakers Aerated?: YES	Water Temperature: 19.3 - 20.9 °C
Water Salinity: 25.0 - 30.5 ‰	Water DO: 4.7 - 6.9 mg/l
Water pH: 7.6 - 8.4	Seawater Renewal Schedule: 1/3 EVERY 3 DAYS
Positive Controls Used?: YES	Photoperiod: CONSTANT LIGHT
Positive Control Toxicant: Cd & AMMONIA	Daily Test Records Maintained?: YES
Internal QA Checks?: YES	QA Officer: LINDA NEMETH
Sulfides and Ammonia Measured at Initiation and End?: YES	

**SOP Deviations or Problems Noted:** NONE

my SPOT CHECK:

TEMPERATURE = 20 °C  
 SALINITY = 28 ‰  
 AERATION GOOD  
 VOLUMES GOOD

QA Officer: PAUL A. DINNELL

Audit Date: 3/11/05

## **Appendix 4**

### **Comments to Northwestern Aquatic Sciences by Dinnel Marine Resources following DMR's QA Review of NAS' Six Round 1 Data Reports and NAS' Response**



# DMR

---

Dinnel Marine Resources  
1519 13<sup>th</sup> St.  
Anacortes, WA 98221  
360-299-8468

19 April 2005

Ms. Michele Redmond  
Lower Duwamish River Bioassay Project Manager  
Northwestern Aquatic Sciences  
PO Box 1437  
Newport, OR 97365

Dear Michele:

I have finished my audits of your six draft data reports for the Batch 1 testing of Lower Duwamish River test sediments. As usual, your data reports were in excellent condition and reflect your usual high degree of attention to detail. There are only two minor corrections needed to the reports. My audit findings are noted below. Please provide me with copies of any corrections made to your draft data reports.

### ***Eohaustorius estuarius* Reports**

**Test No. 725-1:** Table 2, Summary of interstitial water quality.... The maximum value for Dissolved Sulfide should apparently be 7.7 mg/l instead of 15.4 mg/l. 7.7 mg/l is the value listed on your Excel spreadsheet summary. Note that this sample apparently had a dilution factor of 10 instead of the usual 5. Please also verify that the final value is 7.7 (which is half of the measured value) instead of 30.8, which would be twice the measured value.

**Test No. 725-3:** No corrections needed.

**Test No. 725-5:** No corrections needed.

### ***Neanthes arenaceodentata* Reports**

**Test No. 725-2:** No corrections needed.

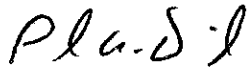
**Test No. 725-4:** No corrections needed.

Test No. 725-6: Endpoints Data Entry and Calculations File – Please delete the extra “5” in the “MORT” column for beaker # 13 (index # 6), which is the water quality replicate (unless you actually did assess mortality in this beaker for some unstated reason).

Also, you might want to make a note in your records that my business name changed several years ago from Dinnel Marine Research to Dinnel Marine Resources to reflect the fact that not all of my consulting work is “research.”

Once again, thank you and your staff for all of your excellent work on this and other bioassay projects.

Sincerely,



Paul A. Dinnel

Quality Assurance Manager

CC: Helle Andersen, Windward Environmental

# NORTHWESTERN AQUATIC SCIENCES

A Division of NAS Associates, Inc.

P.O. Box 1437, Newport, Oregon 97365 • (541) 265-7225 • Fax: (541) 265-2799 • [contact@nwaquatic.com](mailto:contact@nwaquatic.com)



April 22, 2005

Dr. Paul Dinnel  
Dinnel Marine Resources  
1519 13<sup>th</sup> St.  
Anacortes, WA 98221

Dear Paul:

Enclosed are copies of all six final reports for the first round of the Lower Duwamish testing, along with those pages of the raw data that were changed following your quality assurance review. Changes are as follows:

1. In all reports and page 1 of the raw data, your company name was corrected to Dinnel Marine Resources.
2. For report 725-1, with *Eohaustorius*, your comment was:

Table 2, Summary of interstitial water quality.... The maximum value for Dissolved Sulfide should apparently be 7.7 mg/l instead of 15.4 mg/l. 7.7 mg/l is the value listed on your Excel spreadsheet summary. Note that this sample apparently had a dilution factor of 10 instead of the usual 5. Please also verify that the final value is 7.7 (which is half of the measured value) instead of 30.8, which would be twice the measured value.

The error was in the spreadsheet. Raw data (pages 42-43) indicate that the true value is 15.4 mg/L. The spreadsheet was corrected, both where the value was entered on day zero and in the maximum for the summary statistics at the bottom. The report did not require correction.

3. For report 725-6, with *Neanthes*, your comment was:

Endpoints Data Entry and Calculations File – Please delete the extra “5” in the “MORT” column for beaker # 13 (index # 6), which is the water quality replicate (unless you actually did assess mortality in this beaker for some unstated reason).

The “5” was removed.

Sincerely,

A handwritten signature in black ink that reads "Michele S. Redmond". The signature is written in a cursive, flowing style.

Michele S. Redmond  
Cc: Helle Andersen, Windward Environmental



*Dinnel Marine Resources*

---

**QUALITY ASSURANCE EVALUATIONS OF  
LARVAL MUSSEL BIOASSAYS OF LOWER  
DUWAMISH RIVER SEDIMENTS**

**Round 1**

**Final Report**

**13 June 2005**

**For**

**Windward Environmental LLC  
Seattle, Washington**

**Prepared By**

**Dinnel Marine Resources  
Anacortes, WA**

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## Appendices:

1. MEC-Weston's State of Washington Accreditation Certificate and Scope of Accreditation
2. Pre-test Laboratory Inspection Checklist and Communications Related to Pre-Test Refinements of MEC-Weston's Standard Operating Procedures
3. Comments by Dinnel Marine Resources to MEC-Weston Following DMR's QA Review of the Final Data (Batch 1 and Batch 2)

## **1.0 INTRODUCTION**

MEC Analytical Systems-Weston Solutions (MEC-Weston), Tiburon, California was contracted by Windward Environmental LLC to conduct larval mussel bioassays of sediments collected from the Lower Duwamish Waterway in January 2005. MEC-Weston is a State of Washington accredited laboratory (Lab accreditation number C284, expiration: 3 September 2005) and is certified to perform larval tests using the Puget Sound Estuary Program (PSEP 1995) protocols. A copy of MEC-Weston's accreditation certificate and Scope of Accreditation appears in Appendix 1.

This report summarizes the Quality Assurance/Quality Control (QA/QC) evaluations of the first of two rounds of mussel (*Mytilus galloprovincialis*) larval development bioassays of sediments collected from the Lower Duwamish Waterway in January 2005. Testing during Round 1 was completed in two test batches, the first initiated on 9 March and the second initiated on 15 March 2005.

The QA steps taken to ensure high quality data and maximum data completeness before, during and after this round of testing are described in this report. Major QA tasks by Dinnel Marine Resources (DMR) included the following:

- A pre-test inspection of MEC-Weston's laboratory capabilities, equipment and personnel
- A pre-test review of MEC-Weston's Standard Operating Procedures (SOPs)
- Pre-test coordination with the MEC-Weston and Windward Environmental
- An initial evaluation of all data for completeness, correct data entries, and accurate transcription to electronic formats
- A final QA evaluation of overall data quality and usability (this report).

## **2.0 QUALITY ASSURANCE AUDIT RESULTS**

### **2.1 PRE-TEST LABORATORY INSPECTION**

A pre-test inspection of MEC-Weston's Tiburon bioassay laboratory, equipment, and personnel qualifications was conducted by Dr. Paul Dinnel on 28 February 2005. A summary of the inspection was recorded in checklist form (Appendix 2). MEC-Weston's bioassay laboratory, equipment, and credentials of the testing personnel all appeared to be in excellent order. No modifications to the laboratory or equipment were required.

### **2.2 LABORATORY PROTOCOLS AND SOPs**

The PSEP protocol (PSEP 1995) for conducting larval mussel bioassays and MEC-Weston's laboratory SOP (MEC-Weston SOP BIO073.00) for this test were reviewed in detail prior to the initiation of testing. MEC-Weston's SOP was in excellent condition, and my suggestions for revisions/clarifications were minor. My comments to MEC-Weston were contained in a letter to Mr. Bill Gardiner dated 26 February 2005 (Appendix 2). Comments received from Bill Gardiner in an e-mail message dated 27 February 2005 (Appendix 2) clarified several questions about their SOP regarding randomization and use of a perforated plunger for sampling larvae. Subsequent discussion with Windward Environmental staff clarified the issue related to aeration during testing.

### **2.3 TEST-IN-PROGRESS AUDITS**

No test-in-progress audits of the larval mussel tests were conducted due to the travel distance involved and the very short duration of the larval tests (48 hours).

### **2.4 INITIAL DATA EVALUATIONS**

All raw data forms and electronic database files were reviewed for completeness and fidelity of transcription to electronic formats. A 100% check was made of all data entered into MEC-Weston's internal electronic database. All errors, omissions, clarifications, or changes needed to MEC-Weston's draft report were documented and communicated to MEC-Weston. A copy of the initial data evaluation report to MEC-Weston appears in Appendix 3. All needed corrections to the data report were made by MEC-Weston and subsequently verified by DMR.

PSEP (1995) water quality assurance parameters were primarily used for QA assessments of the sediment tests. Where specific values were lacking in PSEP, PSDDA (1994) values were used. These values are summarized in Table 1 for the *Mytilus* test.

### **2.5 FINAL QA EVALUATION OF OVERALL DATA QUALITY AND USABILITY**

Following corrections to the data report by MEC-Weston personnel, a 100% check was made to verify each correction. Following this, an overall evaluation of data completeness and quality was accomplished. Conclusions regarding data completeness and quality follow below (summary details for the *Mytilus* larval tests are given in Tables 2 and 3).

#### **2.5.1 Chain of Custody and Sample Holding**

All chain of custody protocols were properly observed in transfers of sediment samples from Windward Environmental to MEC-Weston. The test and reference sediments were stored at 4° C in a locked cold room until testing was initiated. If samples contained significant headspaces, these headspaces were purged of air with nitrogen gas prior to storage.

Table 1. PSEP (1995) and PSDDA (1994) water quality assurance parameters for the *Mytilus* larval bioassay.

<b>Parameter</b>	<b><i>Mytilus</i> Test</b>
Temperature, °C	15-17
Salinity, ppt	27-29
Dissolved oxygen, mg/liter	60% saturation
pH	7-9
Total Ammonia, mg/liter	None
Total Sulfide, mg/liter	None

### 2.5.2 Larval Mussel Bioassay, Batch 1

1. A mussel larval bioassay was conducted on 20 Lower Duwamish River sediment samples, plus 3 reference sediments. A negative (seawater only) control, and two positive (toxic) controls with copper and ammonia were run concurrently with the Lower Duwamish test sediments.
2. Testing was initiated within 51 days following sediment collection, which was within the 8 week limit specified in Windward Environmental's Quality Assurance Project Plan (QAPP) (Windward 2005).
3. This test was completed with no protocol or water quality deviations. However, two test chambers (SS22, replicate 5 and SS37, replicate 5) were double inoculated with eggs at test initiation and two test chambers (SS89, replicate 1 and SS37, replicate 1) were not inoculated at test initiation. Results from these replicates were treated as statistical outliers and were not included in the mean calculations for their respective samples.
4. The reference toxicant 50% Effective Concentration (EC50) for the copper test was 9.6 µg/liter. This result was within MEC-Weston's control chart warning limits of 5.7 to 15.4 µg Cu/liter. The ammonia EC50 for the mussel larval test was 5.7 mg/liter total ammonia.



MEC-Weston does not have enough data points as yet to establish control chart limits for ammonia for the mussel larval test.

5. Negative (seawater only) control combined mortality/abnormality (1.8 %) was <30% for this test and thus acceptable by present PSEP and PSDDA criteria. The mean combined mortality/abnormality responses (normalized to the seawater control) for the reference sediments ranged from 18.5 to 26.4 %, which were within the PSDDA limit of  $\leq 35$  %.
6. The maximum total ammonia concentration measured in the overlying water during the test in any one sample was 1.24 mg/liter total ammonia. This is well below the EC50 of 5.7 mg/liter and below the NOEC of 2.5 mg/liter measured in the parallel ammonia positive control test. Thus, ammonia concentrations were probably not high enough to cause significant stresses in this test.
7. The maximum total sulfides concentration measured in the overlying water during the test in any one sample was 0.145 mg/liter. There currently are no PSEP or PSDDA protocol guidelines for sulfides, but an equivalent guideline for the echinoderm larval bioassay is a maximum of 0.5 mg/liter sulfides. In addition, the sediment test samples with the highest initial overlying water sulfides (1.45 and 1.40 mg/liter) did not have unusually high mortality or abnormality responses. Thus, sulfide levels in this test probably were not high enough to interfere.
8. Replication was five-fold for all samples as specified by PSDDA.
9. Data completeness for the 23 (plus control) samples tested with mussel larvae was >99 %.
10. **Final QA determination:** All data are of excellent quality and fully usable for any purpose.

**Table 2. Summary of the Batch 1 mussel larval bioassay.**

---

*Mytilus galloprovincialis*, 9 – 11 March 2005:

Number of test samples, including reference sediments: 30

Sediment holding time <8 weeks?: Yes

Protocol deviations? No

Average negative control combined mortality/abnormality: 1.8 %

Average reference sediment combined mortality/abnormality  $\leq 35$ % normalized to the seawater negative control?: Yes

Reference toxicant EC50: 9.6  $\mu\text{g/liter}$  copper and 5.7 mg/liter total ammonia. The copper EC50 is within MEC-Weston's control chart warning limits. There are no established control chart warning limits for ammonia yet.

Water quality parameter deviations: None

Ammonia and sulfide concentrations < critical limits?: Yes for ammonia and probably for sulfides.

**QA reviewer conclusion:** All data are of excellent quality and fully usable for any purpose.

---

### 2.5.3 Larval Mussel Bioassay, Batch 2

1. A second mussel larval bioassay was conducted on 7 Lower Duwamish River sediment samples, plus 3 reference sediments. A negative (seawater only) control, and two positive (toxic) controls with copper and ammonia were run concurrently with the Lower Duwamish test sediments.
2. Testing was initiated within 56 days following sediment collection, which was within the 8 week limit specified in Windward Environmental's Quality Assurance Project Plan (QAPP) (Windward 2005).
3. This test was completed with one protocol deviation and one water quality deviation. The protocol deviation was that the stocking density (13.4 eggs/ml) was about 33 % below the recommended stocking density of 20-40 eggs/ml. To the best knowledge of this reviewer, the larval stocking density was arbitrarily set, and a density of 13.4 larvae/ml will still yield 134 larvae to be counted in the controls (using 10 ml subsamples). This number is quite sufficient for assessing any toxicant effects since most bioassays rely on a sample size of only 5-10 animals/chamber.

The water quality deviation was that water temperatures in the test chambers fell up to 0.6 °C below the specified range of 15-17 °C on test days 1 and 2. These slightly low temperatures may have slightly retarded the development rate of the larvae, but should not have had any significant effects on survival or abnormality rates.

4. The reference toxicant 50% Effective Concentration (EC50) for the copper test was 5.9 µg/liter. This result was within MEC-Weston's control chart warning limits of 5.7 to 15.4 µg Cu/liter. The ammonia EC50 for the mussel larval test was 6.7 mg/liter total ammonia. MEC-Weston does not have enough data points as yet to establish control chart limits for ammonia for the mussel larval test.
5. Negative (seawater only) control combined mortality/abnormality (14.7 %) was <30% for this test and thus acceptable by present PSEP and PSDDA criteria. The mean combined mortality/abnormality responses (normalized to the seawater control) for the reference sediments ranged from 18.1 to 27.0 %, which were within the PSDDA limit of ≤35 %.
6. The maximum total ammonia concentration measured in the overlying water during the test in any one sample was 0.54 mg/liter total ammonia. This is well below the EC50 of 6.7 mg/liter and below the NOEC of 2.5 mg/liter measured in the parallel ammonia positive control test.

Thus, ammonia concentrations were probably not high enough to cause significant stresses in this test.

7. The maximum total sulfides concentration measured in the overlying water during the test in any one sample was 0.155 mg/liter. There currently are no PSEP or PSDDA protocol guidelines for sulfides, but an equivalent guideline for the echinoderm larval bioassay is a maximum of 0.5 mg/liter sulfides. In addition, the sediment test samples with the highest initial overlying water sulfides (two of the reference sediments at 1.50 and 1.55 mg/liter) did not have unusually high mortality or abnormality responses. Thus, sulfide levels in this test probably were not high enough to interfere.
8. Replication was five-fold for all samples as specified by PSDDA.
9. Data completeness for the 10 (plus control) samples tested with mussel larvae was 100 %.
10. **Final QA determination:** All data are of excellent quality and fully usable for any purpose.

**Table 3. Summary of the Batch 2 mussel larval bioassay.**

---

*Mytilus galloprovincialis*, 15 – 17 March 2005:

Number of test samples, including reference sediments: 10

Sediment holding time <8 weeks?: Yes

Protocol deviations? Yes. The initial egg stocking density was about 33% below the protocol-recommended density of 20-40 eggs/ml. This deviation is considered minor since plenty of eggs (about 130 per 10 ml subsample) still remain for accurate assessment of any toxicant effects.

Average negative control combined mortality/abnormality: 14.7 %

Average reference sediment combined mortality/abnormality  $\leq$ 35% normalized to the seawater negative control?: Yes

Reference toxicant EC50: 5.9  $\mu$ g/liter copper and 6.7 mg/liter total ammonia. The copper EC50 is within MEC-Weston's control chart warning limits. There are no established control chart warning limits for ammonia yet.

Water quality parameter deviations: Temperatures on days 1 and 2 were up to 0.6 °C below protocol specifications. These slightly lower temperatures should not have significantly affected the results of the test.

Ammonia and sulfide concentrations < critical limits?: Yes for ammonia and probably for sulfides.

**QA reviewer conclusion:** All data are of good quality and fully usable for any purpose.

---

### 3.0 REFERENCES

- Ecology (Washington State Department of Ecology). 1996. Sediment management standards: Marine bioassays. Task II: Recommended quality assurance and quality control deliverables. Ecology publication No. 96-314:18 pp.
- PSDDA (Puget Sound Dredged Disposal Analysis). 1994. Dredged Analysis Information System (DAIS), Version 4.4. Electronic database from Seattle District, U. S. Army Corps of Engineers.
- PSEP (Puget Sound Estuary Program). 1995. Recommended guidelines for conducting laboratory bioassays on Puget Sound Sediments. Final Report by PTI Environmental Services for U. S. Environmental Protection Agency, Region 10, Office of Puget Sound, Seattle, WA.
- Windward (Windward Environmental LLC). 2005. Quality assurance project plan: Surface sediment sampling for chemical analyses and toxicity testing of the Lower Duwamish Waterway. Final Report for the U.S. Environmental Protection Agency, Region 10, Seattle, WA and the Washington Department of Ecology, Northwest Office, Bellevue, WA. 89 pp. + maps.

## **Appendix 1**

### **MEC-Weston's State of Washington Accreditation Certificate and Scope of Accreditation**

The State of  
Department



Washington  
of Ecology

This is to certify that

**MEC - Weston Solutions Inc. N CA Bioassay Lab  
Tiburon, CA**

has complied with provisions set forth in Chapter 173-50 WAC and is hereby recognized by the Department of Ecology as an ACCREDITED LABORATORY for the analytical parameters listed on the accompanying Scope of Accreditation. This certificate is effective September 4, 2004, and shall expire September 3, 2005.

Witnessed under my hand on September 1, 2004.

Perry F. Brake, Chemist  
Lab Accreditation Section Manager

Lab Accreditation Number  
**C284**

# Scope of Accreditation

## MEC - Weston Solutions Inc. N CA Bioassay Lab

Tiburon, CA

is accredited by the State of Washington Department of Ecology to perform analyses for the parameters listed below using the analytical methods indicated. This Scope of Accreditation may apply to any of the following matrix types: non-potable water, drinking water, solid and chemical materials, and air and emissions. Accreditation for all parameters is final unless indicated otherwise in a note. Accreditation is for the latest version of a method unless otherwise specified in a note. EPA refers to the U.S. Environmental Protection Agency. SM refers to American Public Health Association's publication, Standard Methods for the Examination of Water and Wastewater, 18th, 19th or 20th Edition, unless otherwise noted. ASTM stands for the American Society for Testing and Materials. PSEP stands for Puget Sound Estuary Program. Other references are detailed in the notes section.

Matrix Type/Parameter Name	Reference	Method Number	Notes
<b>Non-potable Water</b>			
Ampelisca abdita	ASTM	E 1367	2
Ampelisca abdita	EPA	100.4	11
Ampelisca abdita	PSEP	1995	6
Atherinops affinis (West Coast)	EPA	1006.0	7,14
Atherinops affinis	EPA	821-R-02-012	8,14
Bioaccumulation, Benthic Invert	ASTM	E 1688	4
Ceriodaphnia dubia	EPA	1002.0	9,14
Ceriodaphnia dubia	EPA	2002.0	8,14
Crassostrea gigas	PSEP	1995	6
Crassostrea gigas (West Coast)	EPA	1005.0	7,14
Cyprinodon variegatus	EPA	1004.0	10,14
Cyprinodon variegatus	EPA	2004.0	8,14
Dangerous Waste Static Salmonid	WDOE	80-12 Part A	13
Daphnia magna	EPA	2021.0	8,14
Dendraster excentricus	ASTM	E 1563	3
Dendraster excentricus	PSEP	1995	6
Dendraster excentricus (West Coast)	EPA	1008.0	7,14
Eohaustorius estuarius	ASTM	E 1367	2

Matrix Type/Parameter Name	Reference	Method Number	Notes
Eohaustorius estuarius	PSEP	1995	6
Eohaustorius estuarius	EPA	100.4	2
Haliotus rufescens (West Coast)	EPA	600-R-95/136	7,14
Holmesimysis costata	EPA	821-R-02-012	8,14
Holmesimysis costata (West Coast)	EPA	1007.0	7,14
Macrocystis pyrifera (WC)	EPA	1009.0	7,14
Menidia beryllina	EPA	1006.0	10,14
Menidia spp.	EPA	2006.0	8,14
Mysidopsis bahia	EPA	1007.0	10,14
Mysidopsis bahia	EPA	2007.0	8,14
Mytilus spp.	PSEP	1995	6
Mytilus spp. (West Coast)	EPA	1005.0	7,14
Neanthes arenaceodentata	PSEP	1995	6
Oncorhynchus mykiss	EPA	2019.0	8,14
Pimephales promelas, Chronic	EPA	1000.0	9,14
Pimephales promelas	EPA	2000.0	8,14
Rhepoxynius abronius	ASTM	E 1367	2
Rhepoxynius abronius	PSEP	1995	6
Rhepoxynius abronius	EPA	100.4	11
Skeletonema costatum	ASTM	E 1218	1
Selenastrum capricornutum	ASTM	E 1218	1
Selenastrum capricornutum	EPA	1003.0	9,14
Strongylocentrotus purpuratus	ASTM	E 1563	3
Strongylocentrotus purpuratus (WC)	EPA	1008.0	7,14
Strongylocentrotus purpuratus (WC)	EPA	600/R-95/136	7,14
Strongylocentrotus spp.	PSEP	1995	6
Thalassiosira pseudonana	ASTM	E 1218	1



Matrix Type/Parameter Name                      Reference    Method Number    Notes

**Accredited Parameter Note Detail**

(1) ASTM. "Standard Guide for Conducting Static 96-h Toxicity Tests with Microalgae." E 1218-00. (2) ASTM. "Standard Guide for Conducting 10-day Static Sediment Toxicity Tests with Marine and Estuarine Amphipods." E 1367-03. (3) Standard Guide for Conducting Static Acute Toxicity Tests with Echinoid Embryos." E 1563-98(2004). (4) ASTM. "Standard Guide for Determination of the Bioaccumulation of Sediment Associated Contaminants by Benthic Invertebrates." E 1688-00a. (5) ASTM. "Test Method for Measuring the Toxicity of Sediment-associated Contaminants with Freshwater Invertebrates." E 1706-00. (6) PSEP. "Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments." July 1995. (7) USEPA. "Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms." EPA 600/R-95/136. (8) USEPA. "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms." EPA-821-R-02-012. Fifth Edition. October 2002. (9) USEPA. "Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms." EPA-821-R-02-013. Fourth Edition. October 2002. (10) USEPA. "Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms." EPA-821-R-02-014. Third Edition. October 2002. (11) USEPA. "Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods." EPA 600/R-94/025. June 1994. (12) USEPA. "Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates." EPA 600/R-99/064. March 2000. (13) WDOE. "Biological Testing Methods." WDOE 80-12. Revised April 1997. (14) Meets requirements of "Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria." WDOE WQ-R-95-80, Revised December 2001.

 9/1/04

Authentication Signature

Perry Brake – Section Manager, Washington State Department of Ecology – Lab Accreditation Section

## **Appendix 2**

### **Pre-Test Laboratory Inspection Checklist and Communications Related to Pre-Test Refinements of MEC-Weston's Standard Operating Procedures**

## ON-SITE LABORATORY EVALUATION FORMS AND CHECKLISTS

The laboratory must have an informal meeting to introduce the technical staff and other personnel of the toxicity test program to the evaluator. The laboratory must present and discuss aspects of the laboratory QA and toxicity test program. The evaluator must explain the audit or evaluation process if it is not clear to the laboratory.

The completed pre-survey forms (Appendix E) should be used by the evaluator as supplemental on-site checklists with Appendix F.

The following items are covered in this appendix:

- I. On-Site informal Laboratory Presentation
- II. Organization History
- III. Laboratory Staff
- IV. Facilities and General Equipment
- V. Test Equipment, Instruments, and Supplies
- VI. Test Organisms
- VII. Documentation
- VIII. Toxicity Test Methodology
- IX. Quality Assurance and Quality Control
- X. Data Handling
- XI. Summary

APPENDIX F

INFORMAL LABORATORY MEETING AND INTRODUCTION  
OF THE TECHNICAL STAFF

I. Organizational Information

Facility Description: MEL - WESTON SOLUTIONS TIBURON LABORATORY

Name/Affiliation: \_\_\_\_\_

Address: 3150 PARADISE DR., BLDG 36  
TIBURON, CA 94920

Phone Number: 415-435-1847

Laboratory Director/Manager: SCOTT BODENSTEINER

Type of Evaluation: LARVAL BIOMASSAY PRE-TEST AUDIT

Informal meeting and introduction of laboratory staff: Y X N \_\_\_\_\_

Laboratory Personnel Contacted

<u>Name</u>	<u>Title</u>
<u>WILLIAM GARDINER</u>	<u>SENIOR SCIENTIST</u>
<u>MATT ZINKL</u>	<u>LAB MANAGER</u>
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

## ON-SITE LABORATORY FORMS AND CHECKLISTS

Note: The completed pre-survey forms (Appendix E) should be used by the audit or evaluator to supplement the on-site forms and checklists.

### II. Organization History

Item	Yes	No	Comments
Laboratory On-Site Introduction/ Meeting with staff	X		
Laboratory demonstrates active toxicity test program	X		
Performance reference toxicant data available and checked	X		
Organization chart provided	X	<del>X</del>	PaB

### III. Laboratory Staff

Item	Yes	No	Comments
Pre-survey forms verification	X		
Appropriate educational background experience/toxicity testing	X		
Laboratory adequately staffed	X		
All technical staff available during evaluation	X		FOR LABORATORY TEST
QA officer report to senior management	X		LIN CRAFT, CARLSBAD OFFICE
Director/Supervisor/ Manager available during evaluation		X	
QA officer available during evaluation		X	

### IV. Facilities and General Equipment

Item	Yes	No	Comments
Pre-survey forms verification			
Tour lab	X		

IV. Facilities and General Equipment (Continued)

Item	Yes	No	Comments
Tour mobile lab, if available		X	NONE USED
Lab work space adequate	X		
Culture space adequate	X		
Toxicity Test space adequate	X		
Lab has distilled/demineralized water.	X		
Lab has distilled demineralized water checked/recorded.	X		
Analytical balance/calibrated yearly	X		
Balance routinely checked/class S weights/recorded logbook	X		
Exhaust hoods provided	X		
Refrigerator/freezer adequate, etc.	X		
Lab maintained in clean/organized manner	X		
Contamination-free work areas available for handling test materials	X		
Culture and test areas separated	X		Partially
Adequate storage areas available	X		
Temperature of lab adequate	X		
Lighting adequate	X		
Air condition/ventilation adequate	X		
Chemical waste disposal policies/SOPs available	X		
Lab secure	X		

V. Test Equipment, Instruments, and Supplies

Item	Yes	No	Comments
Pre-survey forms verification	X		

V. Test Equipment, Instruments, and Supplies (Continued)

Item	Yes	No	Comments
SOP(s) verification	X		
Calibration checks/log books pre-survey forms	X		
Manual available to operator	X		

VI. Test Organisms

Item	Yes	No	Comments
Pre-survey forms verification	X		
Culture Maintenance SOP(s) available		X	NOT NEEDED, MUSSELS ORDERED AS NEEDED
Disease control/treatment protocols documented		X	NA
Holding/acclimation facilities adequate	X		
Source of test organisms documented	X		
Food and feeding program documented		X	NA
Freshwater supply/source/quantity used/quality documented			NA
Estuarine/marine water supply/ source/quantity used/quality/ documented	X		AVAIL. ON REQUEST

VII. Documentation

Item	Yes	No	Comments
Pre-survey forms verification	X		

VII. Documentation (Continued)

Item	Yes	No	Comments
Sample custodian designated	X		
Sample procedures/ responsibilities documented	X		
Written SOPs available for receipt of samples			
QA procedures documented/ available to staff	X		
Written SOPs developed for compiling/maintaining sample document files	X		
Written SOPs for samples preservation, storage/ are maintained.	X		
Written SOPs for culture/ test methods	X		
Daily activities/toxicity test documented	X		
Bound logbooks available/ general chemistry (pH, DO, etc.)	X		
Bound logbooks used, pages numbered consecutively		X	
Type of work clearly displayed on logbooks	X		
Logbooks maintained in legible manner	X		
Are anomalies recorded routinely			
Are inserts permanently affixed and signed.			
Supervisor inspects notebooks/ for appropriate documentation	X		

VIII. Toxicity Test Methodology (Recommended Toxicity Test Conditions and Test Acceptability Criteria: On-Site Checklists, see Appendix G.)

Item	Yes	No	Comments
Pre-survey forms verification	X		
Required methods used	X		
Any unauthorized deviations	X		
Are written SOPs provided	X		



VIII. Toxicity Test Methodology (Recommended Toxicity Test Conditions and Test Acceptability Criteria: On-Site Checklists, see Appendix G.) (Continued)

Item	Yes	No	Comments
Biologist/technician record bench data in neat accurate manner	X		
Appropriate instrumentation used with each toxicity test	X		

IX. Quality Assurance/Quality Control (QA/QC)

Item	Yes	No	Comments
Pre-survey forms verification	X		
Lab maintains QA/QC manual	X		
Manual addresses elements of QA program, including the following:			
a. Personnel	X		
b. Facilities and equipment	X		
c. Operation of instruments	X		
d. Documentation of SOPs	X		
e. Procurement and inventory practices			NA
f. Project plans/ Data quality objectives	X		PROJECT SPECIFIC PLANS
g. Reliability of data	X		
h. Data validation	X		
i. Feedback and corrective action	X		
j. Instrument calibration	X		

IX. Quality Assurance and Quality Control (Continued)

Item	Yes	No	Comments
k. Recordkeeping	X		
l. Internal QA/QC audits	X		
m. QC responsibilities/ reporting clearly defined	X		
n. QC charts maintained for routine analysis	X		
o. QC records show corrective action to meet QC criteria	X		
p. Supervisory personnel review data and QC results	X		
Chain-of-custody maintained	X		
Record keeping adequate	X		
Instrument Calibration/ logbooks maintained	X		
Reference toxicant evaluations used	X		
Analytical support/ inorganic analyses	X		
Analytical support/ organic analyses	X		

X. Data Handling

Item	Yes	No	Comments
Recommended statistical programs used	X		
Data calculations check/ second person	X		
Data calculations documented	X		
Data analyses capabilities available	X		
Data and records retained	X		
PC computer(s) available	X		

# DMR

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Dinnel Marine Resources  
1519 13<sup>th</sup> St.  
Anacortes, WA 98221  
360-299-8468

26 February 2005

Mr. Bill Gardiner  
MEC-Weston Solutions  
3150 Paradise Drive, Bldg. 36  
Tiburon, CA 94920

Dear Bill:

Thank you for providing copies of MEC-Weston Solutions' protocol for the Mussel embryo bioassay tests to be used to assay Lower Duwamish Waterway Group sediment samples. I found your protocol to be well written and generally in conformance with PSEP/SMS guidelines developed for these tests (PSEP [or PSWQA] 1995, with periodic modifications by the Dredged Material Management Program). There are a few potential minor protocol refinements that I will discuss with you and your staff on my site visit on the morning of 28 February, 2005. Several of these items are noted here:

1. No provision is given in MEC's protocol for randomization. The PSEP (1995) guidelines state that "A random numbering method should be used to distribute the chambers in the water bath (or incubator or cold room)."
2. No mention is made in your protocol about use of a perforated plunger to mix embryos/larvae at test initiation or termination. Use of a perforated plunger is required by the PSEP guidelines to ensure proper mixing during subsampling.

As noted in your protocol, there are a number of variables that can change from client to client depending on their testing needs. I take this opportunity to highlight these project-specific items and encourage you to append this letter to your working mussel embryo protocol being used for the Duwamish Waterway testing. Project-specific testing requirements noted here are specified by the client, Windward Environmental LLC (Windward 2005).

1. "The mean effective mortality (combined mortality and abnormal development) in the negative control should be  $\leq 30\%$  (Windward 2005, pg. 71). In other words, normal development in the negative control must be  $\geq 70\%$  relative to the average embryo density of the Time zero counts.
2. At this time, Windward Environmental requests that "all beakers will be aerated to maintain correct levels of (DO) saturation throughout the tests" (Windward 2005, pg. 69). Normally, the PSEP (1995) mussel embryo development test protocol calls for aeration only if DO levels in one or more test/control beakers falls below 60% saturation. This aeration requirement needs to be verified with Windward prior to test initiation.
3. For the Duwamish testing, please note the following Windward requirements for water quality monitoring: "Temperature, salinity, DO and pH will be measured daily for the amphipod mortality and larval development tests." Additionally, "Ammonia and total sulfides will be measured in overlying water in all three tests at test initiation." (Windward 2005, pg. 73).
4. Windward Environmental (2005, pg. 72) indicates that "For the bivalve test, the negative control seawater will be either ambient seawater from San Francisco Bay flowing into the MEC laboratory or seawater collected from Bodega Bay." At the moment, your protocol specifies collection from Scripps Institution of Oceanography (La Jolla).
5. Windward has requested that the reference toxicant be copper sulfate, which, per your protocol, is your normal reference toxicant for this test.
6. The reference sediment performance criterion for this project is "Mean number of normal developed larvae in the reference sediments divided by the mean number of normal developed larvae in the negative control should be  $\geq 65\%$ ." (Windward 2005, pg. 71).
7. The 8-week sediment holding time is in effect for this project. Sediment samples must be stored in the dark at  $4 \pm 2\text{ }^\circ\text{C}$  with no headspace or headspace filled with nitrogen gas (Windward 2005, pg. 68).
8. "The EC50 for a positive control test (copper sulfate) should be within the mean EC50  $\pm 2$  standard deviations of the control chart" mean for the mussel embryo development test (Windward 2005, pg. 71).
9. Also, please note that Windward requires that "Toxicity test samples will be retested within the (8-week) holding time if the negative control fails to meet the performance criteria." (Windward 2005, pg. 71).

Please advise me if you have any concerns about these testing provisions.

I look forward to working with you and MEC-Weston Solutions on this project. Please be sure to provide me with periodic updates as may be necessary and advise me of any protocol deviations or negative control, positive control or reference sediment QA failures.

Sincerely,



Paul Dinnel, Project QA Monitor

CC: Helle Andersen, Windward Environmental LLC  
Matt Zinkl, MEC-Weston Solutions

## References

PSEP (Puget Sound Estuary Program). 1995. Recommended guidelines for conducting laboratory bioassays on Puget Sound sediments. Final Report. Prepared for the Puget Sound Estuary Program, U.S. Environmental Protection Agency, Region 10, Office of Puget Sound, and U.S. Army Corps of Engineers, Seattle District, Seattle, WA. Prepared by PTI Environmental Services, Inc., Bellevue, WA.

Windward (Windward Environmental LLC). 2005. Quality assurance project plan: surface sediment sampling for chemical analyses and toxicity testing of the Lower Duwamish Waterway. Final Report submitted to the U.S. Environmental Protection Agency, Region 10, Seattle, WA and the Washington State Department of Ecology, Northwest Regional Office, Bellevue, WA. 89 pp + maps.

Subj: **RE: Comments on Mussel Embryo protocol**  
Date: 02/27/2005 1:01:03 PM Pacific Standard Time  
From: [Bill.Gardiner@WestonSolutions.com](mailto:Bill.Gardiner@WestonSolutions.com)  
To: [PADinnel@aol.com](mailto:PADinnel@aol.com)

Paul,

thanks for your comments. Yes we do use a perforated plunger for mixing the larval stock and yes we randomize jar positions. You will be able to see the randomization sheets tomorrow.

I agree we should check with Windward about aeration. We normally do not aerate unless DO drops to unacceptable levels, as PSEP requires. My guess is that Windward does not intend to have this deviation in the protocol.

See you tomorrow - have a safe flight.

Bill

-----Original Message-----

From: [PADinnel@aol.com](mailto:PADinnel@aol.com) [<mailto:PADinnel@aol.com>]  
Sent: Sat 2/26/2005 8:18 PM  
To: Gardiner, William  
Cc: [hellea@windwardenv.com](mailto:hellea@windwardenv.com); Zinkl, Matt  
Subject: Comments on Mussel Embryo protocol  
Dear Bill:

Please see attached.

Paul

## **Appendix 3**

### **Comments by Dinnel Marine Resources to MEC-Weston Following DMR's QA Review of the Final Data (Batch 1 and Batch 2)**

# DMR

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Dinnel Marine Resources  
1519 13<sup>th</sup> St.  
Anacortes, WA 98221  
360-299-8468

20 May 2005

Mr. Bill Gardiner  
MEC-Weston Solutions  
3150 Paradise Drive, Bldg. 36  
Tiburon, CA 94920

Dear Bill:

Thank you and your staff for all of your work on the mussel bioassays of Lower Duwamish sediment samples, for the excellent quality Round 1 data report, and for personally verifying all of the Round 1 larval counts. I have finished my QA audit of your Round 1 data report and found it to be in excellent shape, with the exception of a few minor items that need to be corrected in the final draft. There were a few deviations noted in your report (e.g., Batch 2 stocking density, minor temperature deviations), but none of these deviations should have adversely affected the validity or usability of the test data.

The corrections needed to the final draft report are:

1. Under "Data Analysis and QA/QC" (Page 4): Your formulas should read:

percentage combined mortality:	$1 - (N/I) (100)$ instead of $1 - (N/I)$
percentage mortality:	$1 - (T_i/I) (100)$ instead of $1 - (T/I)$
percentage abnormal:	$1 - (N/T_i) (100)$ instead of $1 - (N/T)$

2. For your Batch 2 summary (Page 5), the temperature deviations were  $\leq 0.6^\circ\text{C}$  instead of  $\leq 0.5^\circ\text{C}$ .
3. Your Batch 2 Ammonia EC50 should be 6.7 to 6.9 (depending on the trim factor used) instead of 9.6 mg/l TAN (Page 5).
4. Table 5: There are transcription errors for three of the temperature values in the table:

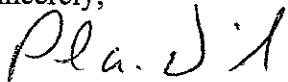
Sample LDW-SS120 Mean temperature should be 14.9 instead of 15.9  
Sample SMP-43 Maximum temperature should be 16.2 instead of 15.2  
Sample LDW-SS63 Maximum temperature should be 15.5 instead of 16.5



5. Table 6: There is one transcription error: The Day 2 sulfide value for sample CR-20 should be 0.020 instead of 0.026.
6. Appendix Table A1: One slight error: For sample CR-20, replicate 2, the number abnormal should be 0 instead of 1. You'll need to see the first count sheet to note that this value was a "0" on that first sheet. The total number of larvae in the sample and the averages will have to be changed as well, although the recalculated average values will be essentially the same.
7. Footnote b to Appendix Table 1: The stocking density in the footnote should read 30.7 embryos/ml instead of 307.2 (which is the average number in a 10 ml subsample).
8. Footnote b (?) to Appendix Table 2: The stocking density in the footnote should read 13.4 embryos/ml instead of 134.0 (which is the average number in a 10 ml subsample).
9. Bench sheet for the Batch 1 termination counts: It would be helpful to add "Batch 1" to these sheets to distinguish from Batch 2 (already labeled as such). Also note that the abnormal count for position 37 should be 0 instead of 1 as per the first count sheet (see comment #6 above)
10. The stocking density raw data counts are missing from the Batch 1 termination count data sheet. They should be appended to the end of this form (as they are for Batch 2)

Thank you for addressing these comments. Please forward any corrections to the Batch 1 data report so that I may verify the changes (or comments as to why they should not be changed).

Sincerely,



Paul Dinnel, Project QA Monitor

CC: Helle Andersen, Windward Environmental LLC  
Matt Zinkl, MEC-Weston Solutions