APPENDIX E. TOXICITY TESTING VALIDATION REPORT

DMR-0802

DMR

Dinnel Marine Resources

QUALITY ASSURANCE EVALUATIONS OF LAMPREY AMMOCOETE TOXICITY TESTING FOR PORTLAND HARBOR RI/FS: PHASE 2

Final Report
20 April 2008

For

Windward Environmental LLC
Seattle, Washington

Prepared By

Dinnel Marine Resources
Anacortes, WA

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1.0 INTRODUCTION

Northwestern Aquatic Sciences (NAS) was retained by Windward Environmental LLC to determine the sensitivity of lamprey (*Lampetra* sp.) larvae (ammocoetes) to various chemicals as part of the Portland Harbor RI/FS Round 3 Project. Lamprey tests were conducted in two phases: Phase 1 explored methods for successful holding in the laboratory, generated range-finding data for five chemicals (aniline, lindane, pentachlorophenol, copper and diazinon), and evaluated ammocoete sensitivity to elevated temperatures (17 and 22°C). Phase 2, conducted during the end of 2007 and early 2008, determined the toxicity of the same five chemicals, plus naphthalene, using definitive flow-through tests. NAS is a State of Washington accredited laboratory (Lab ID number C1238, expiration: 30 September 2008) and is certified to perform a wide range of bioassay testing of water, effluents, and sediments. A copy of NAS' accreditation certificate and Scope of Accreditation appears in Appendix 1. There is no similar certification program in the State of Oregon.

This report summarizes the Quality Assurance/Quality Control (QA/QC) evaluations of the Phase 2 testing conducted by NAS. A previous QA/QC report was submitted for the Phase 1 testing (Dinnel Marine Resources 2007) The QA steps taken to ensure high quality data and maximum data completeness before, during and after Phase 2 testing are described in this report. Major QA tasks included the following:

- One audit of a test in progress,
- An initial evaluation of all data for completeness, correct data entries, and accurate transcription to an electronic database,
- A check of all corrections to NAS' data reports,
- A final QA evaluation of overall data quality and usability (this report).

2.0 QUALITY ASSURANCE AUDIT RESULTS

2.1 REVIEW OF THE TEST PROTOCOL AND SOPs

There are no published protocols for conducting toxicity tests with lampreys, other than the ASTM and EPA generic protocols for conducting tests with fish and other aquatic life (ASTM 1996; EPA 2002). For this testing program, general guidance was provided by Windward Environmental (2006) and a draft protocol was written by NAS (Protocol No. NAS-686-Lamprey-rf) and reviewed by Dinnel Marine Resources prior to beginning the Phase 1 chemical range finding and elevated temperature testing. DMR found NAS' draft protocol to be well written and reasonably complete given the lack of previous test experiences with this species.

2.2 TEST-IN-PROGRESS AUDIT

A test-in-progress audit was conducted by Dr. Paul Dinnel on 2 October 2007 during which time an aniline flow-through toxicity test was being run. The findings of this audit appear in Appendix 2. All testing procedures appeared to conform to NAS' lamprey protocol with only one exception: the light intensity in the constant temperature room was less than 50 foot candles during the test monitoring period. The protocol calls for constant dark conditions during testing except that during monitoring the light level should be between 50 and 75 foot candles. This protocol deviation was considered to be of no consequence. Indeed, the lower light levels during monitoring likely served to minimize any stresses that may have been caused by turning on the lights during monitoring.

During the test audit, one additional observation was made: The inflows of toxicant and dilution water from the mixing tanks were situated close to the outflows of the exposure tanks. It was suggested that the toxicant inflows be moved to another area of the exposure tanks to avoid the possibility that some of the new toxicant solutions might make an early exit from the exposure tank. The suggested modification to the plumbing design would help to maintain the toxicant concentrations as close to the nominal concentrations as possible. However, a plumbing change would not affect the calculation of the LC50s since the effect levels were based on the actual measured toxicant concentrations in the exposure tanks.

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 and checks were made of all Excel spreadsheet calculations and formulae. All errors, omissions, clarifications, or changes needed were documented and communicated to NAS. Only a few corrections to the draft data reports were needed. A copy of DMR's two comment letters to NAS appear in Appendix 3.

2.4 FINAL QA EVALUATION OF OVERALL DATA QUALITY AND USABILITY

Following corrections to the data report by NAS personnel, a 100% check of the corrections was made on 4 April 2008 to verify each correction. All corrections made by NAS were deemed satisfactory. Following this, an overall evaluation of data completeness and quality was accomplished. DMR's 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 test animals (from Windward Environmental) and toxicant samples for chemical analyses (to Columbia Analytical Services).

2.4.2 Ammocoete Holding and Feeding

Lamprey ammocoetes were successfully held in a sand substrate in laboratory tanks supplied with flowing seawater. NAS followed the methods outlined in their draft protocol for holding and acclimation of the ammocoetes except that the feeding regime was modified based on information supplied by Mr. William Swink, a lamprey expert with the USGS.

2.4.3 Flow-Through Toxicity Tests

2.4.3.1 Protocol Deviations

Two protocol deviations were apparent for all six of the toxicity tests. First, the light intensity in the constant temperature room was less than 50 foot candles during all test monitoring periods. The protocol calls for constant dark conditions during testing except that during monitoring the light level should be between 50 and 75 foot candles. This protocol deviation was considered to be of no consequence. Indeed, the lower light levels during monitoring likely served to minimize stresses that may have been caused by turning on the lights during monitoring.

The second protocol deviation was that instead of measuring and weighing all control ammocoetes at the end of the test, subsamples of 10 larvae were measured at the beginning of the test. This deviation should have no adverse consequences for the validity of the test data for several reasons: 1) this was an acute test and not a survival and growth test that would have required the final length and weight data, 2) all 60 control larvae (10 each test) measured for the six tests were in the protocol-specified range of 4 to 6 cm length, 3) there would be little, if any, change in larval lengths and weights during the 4-day test periods, and 4) it probably makes more sense to measure the larvae at test initiation so as to ensure that they are in the proper size range.

A third “protocol deviation” occurred for the lindane test (Test No. 686-40) when staff failed to measure the diluter flow rates at the end of the test. This error by staff appears to have no negative consequences for the test results for the following reasons: 1) Staff found that “there was no evidence that flows were impaired and the water quality and lindane concentration data all confirm satisfactory performance of the diluter”, 2) the lindane concentration data used for the final data analyses came from actual measured toxicant concentrations and not from calculated nominal concentrations, and 3) the only mortality observed in the test was an average of 12.5% in the highest test concentration; thus, an LC50 could not be calculated for this test.

2.4.3.2 Water Quality

All water quality measurements for temperature and dissolved oxygen in all six toxicity tests were maintained within the protocol-specified limits of 17 ± 1°C for temperature and >4.0 mg/liter for dissolved oxygen.

2.4.3.3 Loading Densities

The test animal loading densities for all six tests ranged from 0.33 to 0.40 g/liter of tank capacity and approximately 0.07 g/29 liters of solution passing through each exposure tank/day. These loading rates are well below the EPA (2002) loading rate limit of 6.1 g/liter/tank capacity and the ASTM (1996) recommendation of <1 g/liter of solution passing through the exposure tanks in 24 hours.

2.4.3.4 Control Survival

Control survival in all six tests was 100%, which meets the protocol requirement that control survival be ≥90%.

2.4.3.5 Outlier Toxicant Concentration Value for the Diazinon Test

One measured concentration of diazinon from the highest concentration (40 mg/liter nominal) measured 0.04 mg/liter, approximately 1000 times less than what it should have been. NAS staff labeled this an outlier value and deleted it from the daily values that were used to determine an average daily diazinon concentration. This is of little concern in calculation of the test LC50 since there were four other values to average and there was 100% mortality in the highest concentration. The probit methodology for calculating LC50s primarily uses concentrations with partial responses and gives little or no weight to 0 or 100% responses.

2.4.3.6 Data Completeness

Data completeness for all six flow through toxicity tests was >99%. The only measurements missing were a few hardness and alkalinity measurements during the ammocoetes holding and acclimation period and the deleted outlier value for diazinon.

2.5 CONCLUSIONS

NAS successfully completed flow-through toxicity tests of the six toxicants and all test data have been reviewed and validated. However, the test with lindane failed to produce an LC50 because the highest toxicant concentration caused only 12.5% mortality. This test will have to be repeated to determine an LC50, although any future tests may also fail if higher dissolved lindane concentrations cannot be achieved. The test with naphthalene produced a LC50 based on the observation that 50% of the larvae died in the highest concentration and not based on probit (or other) analysis. It is likely that this is a reasonably good estimate of the toxicity of naphthalene, although this LC50 should be used with some caution.

3.0 REFERENCES

ASTM (American Society for Testing and Materials). 1996. Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. E729-96. American Society for Testing and Materials, Philadelphia, PA.

Dinnel Marine Resources. 2007. Quality assurance evaluations of lamprey ammocoete toxicity testing for Portland Harbor RI/FS: Phase 1. Final Report for Windward Environmental LLC, Seattle, WA. DMR-0801. 4 pp. + appendices.

EPA (U.S. Environmental Protection Agency). 2002. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms (Fifth Edition). EPA-821-R-02-012. Office of Water, U.S. EPA, Washington, D.C.

Windward Environmental. 2006. Portland Harbor RI/FS, Round 3: Lamprey ammocoete toxicity testing field sampling plan (draft). Prepared for the Lower Willamette Group by Windward Environmental LLC, Seattle, WA. 20 pp.

Appendix 1

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

The State of Washington
Department 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, 2007, and shall expire September 30, 2008.

Witnessed under my hand on September 17, 2007.

Stewart M. Lombard
Lab Accreditation Unit Supervisor

Laboratory ID
C1238

Matrix Type/Parameter Name	Reference	Method Number	Notes
Daphnia pulex	EPA	2021.0	1,8
Dendraster excentricus	ASTM	E 1563	
Dendraster excentricus	PSEP	1995	
Dendraster excentricus (West Coast)	EPA	1008.0	6,8
Eohaustorius estuarius	ASTM	E 1367	
Eohaustorius estuarius	PSEP	1995	
Eohaustorius estuarius	EPA	100.4	4
Holmesimysis costata	EPA	821-R-02-012	1,8
Holmesimysis costata (West Coast)	EPA	1007.0	6,8
Hyalella azteca	EPA	100.4	5
Hyalella azteca	EPA	100.1	5
Hyalella azteca	ASTM	E 1706	
Leptocheirus plumulosus	ASTM	E 1367	
Leptocheirus plumulosus	EPA	100.4	4
Lumbriculus variegatus	EPA	100.3	5
Lumbriculus variegatus	ASTM	E 1688	
Macoma spp.	ASTM	E 1688	
Menidia beryllina	EPA	1006.0	3,8
Menidia spp.	EPA	2006.0	1,8
Mysidopsis bahia	EPA	1007.0	3,8
Mysidopsis bahia	EPA	2007.0	1,8
Mytilus spp.	PSEP	1995	
Mytilus spp. (West Coast)	EPA	1005.0	6,8
Neanthes arenaceodentata	PSEP	1995	
Nereis/Neanthes spp.	ASTM	E 1688	
Oncorhynchus mykiss	EPA	2019.0	1,8
Pimephales promelas	EPA	2000.0	1,8
Pimephales promelas, Chronic	EPA	1000.0	2,8
Rhepoxynius abronius	EPA	100.4	4
Rhepoxynius abronius	PSEP	1995	

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
Non-potable Water			
Ampelisca abdita	EPA	100.4	4
Ampelisca abdita	PSEP	1995	
Ampelisca abdita	ASTM	E 1367	
Atherinops affinis (West Coast)	EPA	1006.0	6,8
Bioaccumulation, Bedded Sediments	EPA	600/R-93/183	7
Bioaccumulation, Benthic Invert	ASTM	E 1688	
Bioconcentration, Fish, Mollusks	ASTM	E 1022	
Ceriodaphnia dubia	EPA	1002.0	2,8
Ceriodaphnia dubia	EPA	2002.0	1,8
Chironomus tentans	EPA	100.5	5
Chironomus tentans	ASTM	E 1706	
Chironomus tentans	EPA	100.2	5
Corbicula fluminea	ASTM	E 1688	
Crassostrea gigas	PSEP	1995	
Crassostrea gigas (West Coast)	EPA	1005.0	6,8
Cyprinodon variegatus	EPA	1004.0	3,8
Cyprinodon variegatus	EPA	2004.0	1,8
Dangerous Waste Static Salmonid	WDOE	80-12 Part A	
Daphnia magna	EPA	2021.0	1,8

Matrix Type/Parameter Name	Reference	Method Number	Notes
Rhepoxynius abronius	ASTM	E 1367	
Salvelinus fontinalis	EPA	2019.0	1,8
Strongylocentrotus purpuratus	ASTM	E 1563	
Strongylocentrotus purpuratus (WC)	EPA	1008.0	6,8
Strongylocentrotus purpuratus (WC)	EPA	600/R-95/136	6,8
Strongylocentrotus spp.	PSEP	1995	

Accredited Parameter Note Detail

(1) USEPA. "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms." EPA-821-R-02-012. Fifth Edition. Oct 2002. (2) USEPA. "Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms." EPA-821-R-02-013. Fourth Edition. Oct 2002. (3) 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. Oct 2002. (4) USEPA. "Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods." EPA 600/R/R-94/025. June 1994. (5) USEPA. "Methods for Measuriing the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates." EPA 600/R-99/064. Second Edition. March 2000. (6) 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. Aug 1995. (7) USEPA. "Bedded Sediment Bioaccumulation Tests." EPA/600/R-93/183. Sept 1993. (8) Meets requirements of "Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria," Washington Dept. of Ecology, Pub. No. WQ-R-80, Rev. June 2005.

SM Lombard

Authentication Signature

September 17, 2007

Date

Stewart M. Lombard, Lab Accreditation Unit Supervisor

Appendix 2

Results of the Test-in-Progress Audit By Dinnel Marine Resources

CHECKLIST FOR THE 96-hour Lamprey Ammocoetes Flow-Through Bioassay

Project name: PORTLAND HARBOR RI/FS ROUND 3, PHASE 2

Auditor: PAUL DINNEL

Laboratory: NORTHWESTERN AQUATIC SCIENCES NEWPORT, OR

Test type: ANILINE FLOW-THRU

Test personnel: RICHARD CALDWELL, GARY BUHLER, GERALD IRISSARRI

Test Protocol: NAS-686-LAMPREY FT ACUTE

Test initiation Date: 1 OCTOBER 2007

Toxicant being tested ANILINE

Audit date: 2 OCTOBER 2007

Protocol deviations:

Other notes:

Shipping and Holding Conditions

Ammocoetes source: TRASK, NESTUCCA, SILETZ AND ALSEA RIVERS

Date received: 8/27 - 8/30 2007

Holding tanks: 10 GAL. AQUARIA W/ 2-3 INCHES SAND (~8 GAL H_2O)

Feeding: NONE

Loading density ~50/TANK (~10 gm/8 GAL)

Holding temperature: 17°C (RANGE 16.4-17.7)

Water flow rate: 2 1/2 CHANGES/DAY

Minimum holding time:
1ST TEST (COPPER) = 15 DAYS
2ND TEST (ANILINE) = ~30 DAYS

Additional water change? NO

Other notes: NO 2/3 SIPHON REPLACEMENT DUE TO EXCESSIVE STIRRING UP OF SILT IN SEDIMENT

Testing Conditions

Protocol available? YES

Test species: LAMPETRA SP. (SPP?)

Test chamber size: 2 GAL AQUARIA W/ 5.80 LITERS H_2O

Concentrations tested: 5 + CONTROL

Test animal holding time: ~30 DAYS

Dilution factor: 0.5X

Test animal selection criteria: HEALTHY, 4-6 CM ANIMALS FROM MIXED SOURCES

of *ammocoetes*/chamber: 10

Days/hours after test initiation: (FOR AUDIT) 23 HOURS

How toxicant added? AQ. STOCK SOLUTION OF ANILINE ADDED TO MIXING TANK USING A CERAMPUMP METERING PUMP, THEN MIXED SOLUTION ADDED TO TOXICANT HEAD TANK USING A PERISTALTIC PUMP.

Privileged and confidential: Work product prepared in anticipation of possible litigation

CHECKLIST FOR THE 96-hour Lamprey Ammocoetes Flow-Through Bioassay

Freshwater source: NEWPORT CITY WATER

Freshwater treatment: DECHLORINATED W/ CARBON & TREATED W/ A CULLIGAN TREATMENT SYSTEM TO REDUCE HARDNESS

Freshwater holding time: ~ 24 HR.

Number of replicates: 4

Test chamber size: 2.5 GALLON AQUARIA W/ 5.8 LITER H_2O

Flow rate: ~ 5 CHANGES / DAY

Feeding during test? NO

Test animals randomized? YES

Animal loading density: 0.23 g / AQUARIA BASED ON RANDOM SELECTION OF 10 ANIMALS

Test chambers aerated? NO

Photoperiod: 24 HR DARK EXCEPT NORMAL LIGHTING DURING MONITORING

Light intensity during monitoring: 15.1 ft-c.

FROM LAB BENCH SHEETS

Water temperature: 16.2 - 17.6 °C

Water pH: 6.3 - 6.7

Water hardness: NO VALUES AVAILABLE YET

Water alkalinity: NO VALUES AVAILABLE YET

Water DO: 8.8 - 9.3

Toxicant concentrations monitored? YES, DAILY

Toxicant samples collected? YES

Monitoring for conductivity, particulate matter, TOC and ammonia? YES

Daily test records maintained? YES

Test validation criteria: ≥ 90% CONTROL SURVIVAL

Lab QA officer: JULIE FIORE

~~Lab QA Officer~~:

Internal QA checks? YES

Protocol or Water Quality Deviations or Problems Noted:

OUTFLOWS FROM DILUTER BEING ADDED TO TEST CHAMBER NEXT THE OUTFLOWS, SUGGESTED THAT THIS MAY REDUCE THE EFFECTIVE ANILINE CONCENTRATION IN TEST CHAMBERS.

LIGHT INTENSITY DURING MONITORING < 50-75 ft-c IN PROTOCOL (NO PROBLEM)

Other Notes: CHECKED PREVIOUS TEST NOTES FOR COPPER TEST. GOOD PARTIAL KILLS, TOTAL MORTALITY AT HIGH CONC. AND NO KILL AT TWO LOWEST CONCENTRATIONS. PHYSICAL & CHEMICAL DATA LOOK GOOD

QA Auditor: PAUL A. DINNEL **Date:** 2 OCT 2007

Privileged and confidential: Work product prepared in anticipation of possible litigation

Appendix 3

Comments by Dinnel Marine Resources to Northwestern Aquatic Sciences Following DMR's QA Review of the Six Draft Toxicity Test Reports (Two Letters)

DMR

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

26 March 2008

Mr. Gary Buhler
Northwestern Aquatic Sciences
PO Box 1437
Newport, OR 97365

Dear Gary:

I have completed my audit of the first five of your Portland Harbor Lamprey Ammocoete flow-through toxicity test draft reports. As usual, your draft data reports are in excellent shape, with just a few minor items needing further attention.

The following two comments apply to all five of the test reports (copper, aniline, lindane, naphthalene, and pentachlorophenol):

1. NAS' lamprey test protocol (NAS-686-Lamprey FT acute) lists the lower limit for dissolved oxygen as 4.0 mg/liter. However, in each of your draft reports under "Test Procedures and Conditions" you list the lower limit as 6.0 mg/liter. And in another place in several of the reports (Bench Sheet: Lamprey Survival Flow Through Test) you mention the limit as being 60% saturation. I suggest that you correct the draft reports to read ">4.0 mg/liter" unless you have discovered that >6.0 mg/liter is more appropriate.

2. Under "Data Analysis Methods", I suggest that, for the sake of clarification, you change the following wording (end of line 1) "….replicate from the raw data and means…." to "replicate from the raw survival data and the measured [specific toxicant tested] concentrations…. This will clarify that you used the measured toxicant concentration for data analyses instead of the nominal concentrations.

Additional comments for two of the data reports:

Test 686-39 (aniline):

Water Quality Computation table, page 10 of 14: Temperature observation #24 is listed as 16.2 but should be 17.2. The mean and s.d. for temperature are, however, okay as shown.

Test 686-40 (lindane):

Really nit picking here – you might want to add a "c" to "hemicals" in the Veith and Comstock citation on page 4 of 7.

The other three data reports did not appear to need any further corrections. Please forward a record to me of the changes that you make to these five data reports.

Should you have any questions, please call me at 360-299-8468 or contact me via e-mail at padinnel@aol.com. Thank you and your staff for your excellent work.

Sincerely,

Paul Dinnel, Project QA Monitor

CC: Helle Andersen, Windward Environmental

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

30 March 2008

Mr. Gary Buhler
Northwestern Aquatic Sciences
PO Box 1437
Newport, OR 97365

Dear Gary:

I have completed my audit of the sixth and last of your Portland Harbor Lamprey Ammocoete flow-through toxicity test draft reports. As was the case with the first five draft reports, the following two comments apply to the diazinon draft report as well.

3. NAS' lamprey test protocol (NAS-686-Lamprey FT acute) lists the lower limit for dissolved oxygen as 4.0 mg/liter. However, in each of your draft reports under "Test Procedures and Conditions" you list the lower limit as 6.0 mg/liter. I suggest that you correct the draft reports to read ">4.0 mg/liter" unless you have discovered that >6.0 mg/liter is more appropriate.

4. Under "Data Analysis Methods", I suggest that, for the sake of clarification, you change the following wording (end of line 1) "….replicate from the raw data and means…." to "replicate from the raw survival data and the measured diazinon concentrations…. This will clarify that you used the measured toxicant concentration for data analyses instead of the nominal concentrations.

Other than these two comments, I did not detect any need for further changes to this draft report. Should you have any questions, please call me at 360-299-8468 or contact me via e-mail at padinnel@aol.com. Thank you and your staff for your continuing excellent work.

Sincerely,

Paul Dinnel, Project QA Monitor

CC: Helle Andersen, Windward Environmental