APPENDIX C. TOXICITY TESTING REPORT FROM NAS

LAMPREY (*LAMPETRA* SP.) DEFINITIVE 96-HR FLOW-THROUGH ACUTE TOXICITY TEST OF COPPER

NAS Test No. 686-38

Submitted to

Windward Environmental LLC
200 West Mercer Street, Suite 401
Seattle, WA 98119

Submitted by

Northwestern Aquatic Sciences
3814 Yaquina Bay Road
P.O. Box 1437
Newport, OR 97365

April 3, 2008

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 686-38
Title: Lamprey (*Lampetra* sp.) definitive 96-hr flow-through acute toxicity test of copper.
Protocol No.: NAS-686-Lamprey FT acute, September 12, 2007

STUDY MANAGEMENT

Study Sponsor: Windward Environmental, 200 West Mercer Street, Suite 401, Seattle, WA 98119.
Sponsor's Study Monitor: Ms. Helle Andersen.
Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365.
Test Location: Newport Laboratory.
Laboratory's Study Personnel: G. A. Buhler, B.S., Proj. Manager; R. S. Caldwell, Ph.D., Study Director; L.K. Nemeth, B.A., M.B.A., QA Officer; G.J. Irissarri, B.S., Aq. Toxicol.; L.P. Sandoval, B.S., Tech.
Study Schedule:
Test Beginning: 9-14-07, 1320 hrs.
Test Ending: 9-18-07, 1330 hrs.
Disposition of Study Records: All specimens, raw data, reports and other study records are stored according to Good Laboratory Practice regulations at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.
Good Laboratory Practices: The test was conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).
Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Description: Copper prepared from $CuSO_4 \cdot 5H_2O$ (Argent Chemical Laboratories, Inc., Redmond, WA, Lot No. 0195).
Preparation of Working Stock Solution and Test Concentrations: Test solutions were prepared for continuous supply to the test aquaria using a Benoit constant flow diluter (Benoit *et al.*, 1982). The diluter was set up to deliver a 0.5 dilution series with a flow rate of approximately 80 ml/min per toxicant concentration. The diluter design was such that 160 ml/min of the toxicant solution at the highest test concentration (nominal 0.2 mg/L as copper) was required to be continuously delivered to the toxicant supply chamber of the diluter. To accomplish this, a large mixing reservoir, approximately 54L capacity, of nominal 0.2 mg/L copper solution in dilution water was maintained at test temperature. This was done by metering, at a rate of 1.0 ml/min, a constant supply of a 32 mg/L working copper stock solution into the large toxicant reservoir with vigorous mixing, while also replacing, under float valve control, dilution water to replace delivery of the nominal 0.2 mg/L copper solution to the diluter. The 32 mg/L working copper stock solution was prepared daily by dilution of a 1,000 mg/L concentrated copper stock solution with dilution water.

DILUTION WATER

Source: City of Newport municipal tap water, treated as below, was supplied continuously to the toxicant diluter throughout the study.
Pretreatment: The water was dechlorinated by carbon filtration, particle filtered to 1 µm, and adjusted to a target water hardness of 25-30 mg/L as $CaCO_3$. Hardness adjustment was accomplished by passage of a portion of the dilution water through deionization cartridges.

TEST ORGANISMS

Species: Lamprey (*Lampetra* sp.)
Age/Size: Ammocoetes; target size range, 4-6 cm; actual size of 10 ammocoete subsample, 4.9 ± 0.7 cm.
Source: Ammocoetes were field collected in approximately equal numbers from the Trask, Nestucca, Siletz, and Alsea Rivers, Oregon. Field collection was by electoshocking and only 4-6 cm size ammocoetes were retained. Personnel of Windward Environmental performed the ammocoete collections.

Acclimation: Upon receipt, animals were removed from the collection site sediment and placed in 10-gallon aquaria supplied under flow-through conditions with dilution water. Water supply was sufficient (30-50 ml/min) to accomplish two volume changes of water per day. Approximately 50 lamprey were placed in each tank. Tanks contained an approximate 8 cm deep layer of sediment (Sandtastic Play Sand, Waupaca Materials) covered with approximately 26L of test water and were supplied with aeration. Normal behavior for the lamprey ammocoetes was to immediately bury in sediment and remain there. Any that did not immediately bury were discarded as potentially damaged. Ammocoetes were not fed during acclimation. Water quality conditions during the two weeks prior to testing (8-31-07 to 9-14-07) were: temperature, 16.8 ± 0.3°C; dissolved oxygen, 8.8 ± 0.5 mg/L; pH, 7.5 ± 0.4; specific conductance, 169 ± 24 µmhos/cm; hardness, 43 ± 13 mg/L as $CaCO_3$; and alkalinity, 31 ± 3 mg/L as $CaCO_3$.

TEST PROCEDURES AND CONDITIONS

Test Chambers: The test chambers were two-gallon glass aquaria capable of holding 5.8 L of test solution. Test chamber placement in the test area was random.

Test Concentrations: The nominal test concentrations were 0.20, 0.10, 0.05, 0.025, 0.0125, and 0 mg/L (control) copper.

Replicates/Treatment: 4

Organisms/Treatment: 40

Loading: 0.38 g/L

Aeration: None.

Feeding: None.

Water Volume Changes: The diluter supplied approximately 80 ml/min of test solution, or 115 L/day, at each test concentration. At this rate of flow, test solution supply to each replicate aquarium was 5.0 volumes per day.

Effects Criteria: The effect criterion used in the lamprey ammocoete acute toxicity test was mortality, defined as a lack of visible respiratory movement and absence of response to tactile stimulation. In addition to mortality, qualitative observations of sublethal responses were recorded when observed.

Target Water Quality Conditions: Water quality measurements were taken daily as specified in the protocol (Physical and Chemical Data, Items 1 and 2). The target water quality conditions were as follows: Temperature, 17 ± 1°C; dissolved oxygen, ≥4.0 mg/L; hardness, 25-30 mg/L as $CaCO_3$. Actual test conditions are reported in the Test Results section.

Photoperiod: The photoperiod was total darkness except for approximately 2-3 hours per day for observations and test exposure system maintenance. Light intensity at the aquarium level was 15.1 foot-candles.

DATA ANALYSIS METHODS

The 96-hr proportion survival was calculated for each treatment replicate from the raw survival data and the measured copper concentrations for each treatment level. The LC50 (survival) was calculated by the Trimmed Spearman-Karber method. An arcsine square root (angular) transformation was performed on the data prior to statistical analysis. The NOEC and LOEC were calculated according to U.S. Environmental Protection Agency (2002) methods. In this procedure, the NOEC and LOEC values were computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test was selected after evaluating the data for normality and homogeneity of variance. The statistical software employed for these calculations was ToxCalc, v.5.0.23N, Tidepool Scientific Software.

TOXICANT AND OTHER CHEMISTRY ANALYSES

Test solutions were sampled once daily for copper from each test concentration by pooling samples from each of the four test replicate aquaria. Subsamples were pooled into new disposable plastic beakers from which the pooled sample was transferred to the analytical laboratory supplied sample bottles containing acid preservative and shipped under chain-of-custody procedures to the analytical chemistry laboratory (Columbia Analytical Services) at Kelso, WA. Both filtered and unfiltered samples were prepared for shipping. Samples were kept refrigerated at ≤4°C. Sample volumes were 100 ml. Filtration consisted of collecting two 50 ml aliquots of pooled sample in a B-D Lot No. 7179829 disposable 60-ml plastic Luer-Loc syringe. The sample was then expelled through a PALL 0.45µm ion chromatography Acrodisc filter directly into the sample bottle.

Additional samples were taken of the dilution water on day 0 of the test for analysis of particulate matter, TOC, and ammonia-N, and from the control and highest test concentration treatments after 96-hr for measurement of ammonia-N. These samples also were shipped to Columbia Analytical Services for analysis.

PROTOCOL DEVIATIONS

1. The protocol specified "constant dark, except during monitoring activities", and also that light intensity should be 50-75 ft-c. when light was on. These specifications were judged to be contradictory and led to the following action. Based on best professional judgment of the investigators, we reduced the light intensity from that given in the protocol in order to minimize fright behavior responses of the ammocoetes during the short period of lighting associated with daily observations.
2. The protocol states: "At the end of the test, all surviving control ammocoetes are weighed and measured for length by replicate." Through a laboratory error, a subsample of 10 ammocoetes were weighed and measured at the beginning of the test instead. This is a minor deviation and is without consequence for interpretation of the study results.

TEST RESULTS

Water quality conditions measured in test aquaria during the 96-hr toxicity test are summarized in Table 1. The temperature specification was met during the study. Dissolved oxygen remained near saturation throughout the test. The pH ranged from 7.0 - 8.5. Hardness also was in the specified range of 25-30 mg/L as $CaCO_3$.

The concentrations of dissolved copper measured during the toxicity test are summarized in Table 2. Mean measured concentrations in the test ranged from 0.013 ± 0.001 mg/L at the lowest level to 0.164 ± 0.036 mg/L at the highest exposure level. Generally, the dilution series was in good agreement with nominal concentrations.

A summary of the 96-hr ammocoetes survival results is shown in Table 3. In this study, the 96-hr control survival (100%) met the test acceptability criteria of ≥90%. Therefore, the test meets the protocol criterion of a valid test. Additionally, 100% mortality occurred in the two highest, and 0% mortality was observed in the two lowest, test concentrations. Partial mortality was observed in the middle (0.043 mg/L) test concentration.

The toxicity of dissolved copper to lamprey (*Lampetra* sp.) ammocoetes as the 96-hr LC50 was 0.046 mg/L (95% confidence limits, 0.042 - 0.051 mg/L). The NOEC and LOEC concentrations were 0.023 and 0.043 mg/L copper, respectively.

LITERATURE CITED

Benoit, D., V. Mattson, and D. Olson. 1982. A continuous-flow mini-diluter system for toxicity testing. Water Res. 16:457-464.

EPA. 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. Environmental Protection Agency, Washington, DC.

STUDY APPROVAL

Project Manager	Date	Study Director	Date
Laboratory Director	Date	Quality Assurance Unit	Date

Table 1. Measured water quality conditions during the 96-hr acute toxicity test of copper to lamprey ammocoetes.

Parameter	Mean ± S.D.	N	Range
Temperature (°C)	16.9 ± 0.2	30	16.6 – 17.4
Dissolved oxygen (mg/L)	8.6 ± 0.5	30	7.6 – 9.6
pH	7.8 ± 0.4	30	7.0 – 8.5
Specific conductance (µmhos/cm)	102 ± 7	10	90 – 110
Hardness (mg/L as $CaCO_3$)	27 ± 1	10	25 – 28
Alkalinity (mg/L as $CaCO_3$)	18 ± 4	10	12 – 24

Table 2. Measured concentrations of dissolved copper during the 96-hr acute toxicity test of copper to lamprey ammocoetes. Measurements performed by Columbia Analytical Services.

Nominal (mg/L)	Measured (mg/L)		
	Mean ± S.D.	N	Range
0.2	0.164 ± 0.036	5	0.120 – 0.201
0.1	0.088 ± 0.009	5	0.077 – 0.099
0.05	0.043 ± 0.003	5	0.038 – 0.046
0.025	0.023 ± 0.001	5	0.022 – 0.024
0.0125	0.013 ± 0.001	5	0.012 – 0.014
0	0.001 ± 0.001	5	0.000 – 0.002

Table 3. Survival of lamprey ammocoetes, *Lampetra* sp. exposed for 96 hours to dissolved copper.

Dissolved Copper (mg/L)	Replicate	Ammocoetes exposed	Ammocoetes Surviving	Percent Survival	Mean % Survival	
0.164	A	10	0	0.0		
	B	10	0	0.0		
	C	10	0	0.0		
	D	10	0	0.0	0.0	*
0.088	A	10	0	0.0		
	B	10	0	0.0		
	C	10	0	0.0		
	D	10	0	0.0	0.0	*
0.043	A	10	4	40.0		
	B	10	8	80.0		
	C	10	7	70.0		
	D	10	4	40.0	57.5	*
0.023	A	10	10	100.0		
	B	10	10	100.0		
	C	10	10	100.0		
	D	10	10	100.0	100.0	
0.013	A	10	10	100.0		
	B	10	10	100.0		
	C	10	10	100.0		
	D	10	10	100.0	100.0	
0.001 (Control)	A	10	10	100.0		
	B	10	10	100.0		
	C	10	10	100.0		
	D	10	10	100.0	100.0	

* Mean significantly ($p=0.05$) different from the control.

APPENDIX I

PROTOCOL

STUDY PROTOCOL

LAMPREY (*LAMPETRA* sp.)
FLOW THROUGH ACUTE TOXICITY TEST

INTRODUCTION

Purpose of Study: The purpose of this study is to determine the acute toxicity of six chemicals, representing a range of toxic modes of action, to lamprey (*Lampetra* sp.) ammocoetes. The study will be performed using methods based on the methods for measuring acute toxicity with other fish species as described in U.S. Environmental Protection Agency (2002) and American Society for Testing and Materials (ASTM) (1996) guidance.

Summary of Method: In definitive tests, lamprey ammocoetes are exposed for 96 hours to five concentrations of test material and a dilution water control. The test concentrations are arranged in a logarithmic series based on the results of range finding tests. Four replicates, each with 10 organisms, are employed at each test concentration, if there are sufficient numbers of ammocoetes. Flow-through exposures are employed. Test chambers are glass aquaria less than or equal to two gallons in volume. Mortality is the effect criterion. The data analysis consists of calculating means and standard deviations for survival. An LC50 with 95% confidence intervals may be calculated along with the NOEC and LOEC concentrations.

STUDY MANAGEMENT

Sponsor's Name and Address:
Windward Environmental LLC
200 West Mercer Street, Suite 401
Seattle, WA 98119

Sponsor's Study Monitor:
Ms. Helle Andersen

Name of Testing Laboratory:
Northwestern Aquatic Sciences
3814 Yaquina Bay Road
P.O. Box 1437
Newport, OR 97365

Test Location: Newport, OR.

Laboratory's Key Personnel to be Assigned to the Study:
Laboratory Director: Richard S. Caldwell, Ph.D.
Project Manager/Study Director: Gary A. Buhler, B.S.
QA Officer/Toxicology Program Mgr: Linda K. Nemeth, B.A., M.B.A.
Aquatic Toxicologist: Gerald J. Irissarri, B.S.

Proposed Study Schedule: July 2007 through September 2007.

Good Laboratory Practices: The test is conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

TEST MATERIAL

Description: Chemicals to be tested include aniline, copper, pentachlorophenol, lindane, diazinon and naphthalene.

Preparation of Working Stock Solution and Test Concentrations: Test materials will be administered using a constant-flow diluter based on the design of Benoit *et al.* (1982). Stock solutions of the sparingly soluble organic compounds, e.g. naphthalene, pentachlorophenol, lindane, diazinon, will be prepared continuously by recirculation of dilution water/toxicant stock solution through a column of toxicant coated glass beads (Veith and Comstock, 1975). This is a conceptual approach in which the specific column, flow rates, glass bead sizes, etc. need to be developed empirically, with analytical feedback, for each study considering such variables as chemical solubility, test temperature, desired highest test concentration, stock toxicant consumption rate, etc. Given the above, some modifications are anticipated as the studies progress. Initial system characteristics are as follows: 1) the toxicant mixing tank will have a 60-liter capacity; 2) a 20 x 280-cm glass column will be employed (10 x 200-cm and 40 x 560-cm columns are also available); 3) the column packing will employ 5-mm glass beads coated with the test compound; 4) particulates will be filtered out through glass wool; 5) flow through column will be controlled by a variable-speed peristaltic pump or by intermittent pumping; and 6) tubing will be glass and silicone rubber. GC/MS analysis of toxicant in the toxicant mixing tank will be used to optimize the system for each chemical noted above prior to test initiation.The contents of the stock toxicant reservoir will be constantly replenished with fresh dilution water as toxicant solution is supplied to the diluter. The aniline stock solution will be prepared similarly, but by volumetric addition of aniline, using a metering pump, to the stock toxicant reservoir *in lieu* of the Veith column. The copper working stock solution will be prepared in the same apparatus by metering pump addition of a concentrated copper stock solution to the stock toxicant reservoir. All toxicant working stock solutions will be prepared and maintained under the same conditions as in the test exposures and delivered continuously to the toxicant chamber of the diluter at an appropriate rate.

DILUTION WATER

Dilution water will be dechlorinated City of Newport municipal tap water adjusted to a water hardness of 25-30 mg/L as $CaCO_3$.

TEST ORGANISMS

Species: Lamprey, *Lampetra* sp.

Source: Ammocoetes will be collected by Windward staff and delivered to the laboratory each day of collection. The ammocoetes will be collected from several rivers including the Siletz River, which was the site for Phase I ammocoetes.

Age: ammocoetes (juvenile prior to metamorphosis)

Acclimation and Pretest Observation: Test organisms will be held in the laboratory in order to acclimate to test temperature. Upon receipt, animals are removed from the sediment they are received in and placed in 10-gallon tanks under flow-through conditions (~35 mL/minute, or ~ 2 volume changes per day, with one additional daily siphon and replacement of 2/3 volume of each tank). Approximately 50 lamprey are placed in each tank. Tanks will contain at least 3 inches of sediment (Sandtastic Play Sand, Waupaca Materials, or similar material) covered with approximately 26L of test water and supplied with aeration. The maximum temperature variation during holding will be ± 1° C. The ammocoetes will be acclimated to the laboratory test conditions for a minimum of 5 to 7 days prior to test initiation. This minimum number of days applies only to the first toxicity test conducted on each batch of field-collected organisms. For all other tests, the ammocoetes will be held for at least 13 days prior to test initiation. When a temperature adjustment of more than 3 to 5°C is required, the transfer from field collection water to laboratory dilution water will be gradual. Normal behavior for lamprey ammocoetes is to immediately bury in sediment and remain there. Any dead animals observed on the sediment surface are removed daily. Past experience suggests that mortality is minimal during short-term holding and any mortality is likely due to damage from field collection. If ammocoetes do not bury or there is excessive

mortality in any tank, animals from that tank will not be used for testing. Ammocoetes will not be fed since they are being held short-term.

DESCRIPTION OF TEST SYSTEM

Preparation of Test Concentrations: Test materials will be administered using a constant-flow diluter based on the design of Benoit *et al.* (1982). The diluter will be calibrated to deliver a 0.5 dilution series. Flow rates will be adjusted to deliver at least five test chamber volumes per concentration per day. Diluter flow rates will be measured and documented at the beginning and conclusion of each 96-hour test. This will be done by measuring individual diluter capillary tube flows. When the flow rates are entered into an Excel® spreadsheet, the actual dilutions of the toxicant stock can be computed. Actual test chemical concentrations (organic chemicals) in the toxicant mixing chamber (toxicant stock solution) and in exposure aquaria will periodically be measured on a real-time basis at NAS to verify that nominal concentrations are being routinely achieved. Definitive concentration documentation in test aquaria will result from daily samples shipped for analysis to CAS.

Test Chambers and Environmental Control: Test chambers used in the toxicity study are glass aquaria less than or equal to two gallons in volume. The test system is housed in a constant-temperature, photoperiod-controlled room. Aeration is not employed. The photoperiod is constant dark except during monitoring activities when ambient (50-75 ft-c) lighting is used.

Cleaning: All laboratory glassware, including test chambers and toxicant dilution system, will be cleaned based on the methods described in U.S. Environmental Protection Agency (2002). Initial cleaning incorporates the use of a laboratory detergent followed by multiple tap water rinses. Subsequently, either dilute (10%, V:V) acid (hydrochloric or nitric) or acetone is used to remove metals or organic contaminants, respectively. Further multiple tap water rinses are employed after each acid or acetone treatment. Sodium hypochlorite bleach may be employed on a limited basis to ensure that microorganisms that could metabolize organic toxicants, e.g. naphthalene, are not present. Exhaustive tap water rinsing is always employed following any chemical cleaning treatment. A final dilution water rinse of the exposure apparatus will precede initiation of each study.

EXPERIMENTAL DESIGN AND TEST PROCEDURES

Experimental Design: The definitive test involves exposure of lamprey ammocoetes to five test concentrations, using a 0.5 dilution series, and a dilution water control. Exposures are for 96 hours. Each treatment consists of four replicates, each containing 10 ammocoetes, if there are sufficient numbers of ammocoetes. If there are not enough ammocoetes for 10 per replicate, then at least 20 per treatment (5 per replicate) is the minimum number preferred. Test chambers are randomly distributed in the test area. Ammocoetes are impartially distributed to each aquarium by adding one or two animals to each test replicate and repeating the process until each contains 10 organisms.

Effect Criterion: The effect criterion used in the lamprey ammocoete acute toxicity test is mortality, defined as a lack of visible respiratory movement and absence of response to tactile stimulation. The mortality data will be used to compute LC50s for each exposure period. In addition to mortality, qualitative observations of sublethal responses consisting of erratic swimming, hyperventilation, hemorrhaging, and hyper/hypoactivity (compared to the activity of the organisms in the control group) will be recorded if observed.

Test Conditions: The dissolved oxygen concentration in each test container must be > 4.0 mg/L throughout the test. The test temperature employed is 17 ± 1°C. A photoperiod of constant darkness is used, except during monitoring activities when illumination is supplied by daylight fluorescent lamps at an intensity of 50-75 ft candles. Loading should not exceed 6.1g/L at 17°C according to EPA. ASTM recommends loading not exceed 1g/L of solution passing through the chamber in 24 hours or 10g/L of solution in the chamber at any time.

Beginning the Test: Adding the organisms to the equilibrated test containers as previously described begins the test.

Feeding: None.

Analysis of Test Concentrations: During each test, samples will be collected for chemical analysis, held at 4°C and shipped by overnight delivery to Columbia Analytical Services in Kelso, WA. The sampling schedule and procedure will follow the project QAPP.

Test Duration, Type and Frequency of Observations, and Methods: The test duration of the acute toxicity test is 96 hours. The type and frequency of observations to be made during the test are summarized as follows:

Type of Observation	Times of observation
Biological Data	
1. mortality (each test container)	0, 24, 48, 72, and 96 hr
2. sublethal observations (qualitative)	0, 24, 48, 72, and 96 hr
Physical and Chemical Data	
1. Dissolved oxygen, pH, & temperature, (in one replicate of each test level and the control)	0, 24, 48, 72, and 96 hr
2. Conductivity, hardness & alkalinity (in one replicate of highest concentration and control)	0, 24, 48, 72, and 96 hr
3. Toxicant concentration sampling (in one replicate of each test concentration and the control)	0, 24, 48, 72, and 96 hr
4. Dilution water sample for particulate matter, TOC and ammonia	0 hr
5. Sample for ammonia taken from one replicate of control and highest test concentration	96 hr

During the test, dead organisms are removed every 24 hours and frozen by concentration for possible tissue analysis. Dissolved oxygen is measured using a polarographic oxygen probe calibrated according to the manufacturer's recommendations. The pH and temperature are measured using a pH probe and a properly calibrated meter with scale divisions of 0.1 pH units. Conductivity is measured using a calibrated conductivity meter. Samples for chemical analysis will be collected, preserved, and sent to Columbia Analytical Services following the recommended procedures of the analytical laboratory.

Test Termination: At the end of the test, all surviving control ammocoetes are weighed and measured for length by replicate. All surviving or dead test organisms will be frozen by concentration for possible later tissue analysis by Columbia Analytical Services.

Criteria of Test Acceptance: The test results are acceptable if survival in the controls is at least 90%. The toxicity tests will be performed as a 50% dilution series, it is anticipated that each test will have at least one or possibly two partial mortality concentrations. Nominal concentrations of each toxicity test have be reviewed and approved by EPA (EPA 2007). However, because of the experimental nature of the study and the limited supply of ammocoetes, the acceptability of the toxicity tests will be based only on the ≥ 90% survival in control group.

DATA ANALYSIS

The percent of live ammocoetes is calculated for each treatment replicate from the raw data and the means are obtained for each treatment level. An LC50 (mortality) is calculated using Probit or Trimmed Spearman-Karber methods. The NOEC and LOEC are calculated for each endpoint according to U.S. Environmental Protection Agency (2002) methods. In this procedure, the NOEC and LOEC values are computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test is selected after evaluating the data for normality and homogeneity of variance. An arcsine-square root transformation is performed on the percentage effect data prior to statistical analysis. The statistical software employed for these calculations is Toxcalc, v.5.0.23N, Tidepool Scientific Software.

REPORTING

The final report of the study results must include all of the following standard information at a minimum:

1. Name and identification of the test;
2. The investigator and laboratory;
3. Information on the toxicants;
4. Information on the dilution water;
5. Detailed information about the test organisms;
6. Description of the test system and its calibration, including test chambers and dilution system;
7. A description of the experimental design and other test conditions including water quality;
8. Definition of the effect criteria and any other observations;
9. Responses, if any, in the control treatment;
10. Tabulation and statistical analysis of measured responses;
11. A description of the statistical methods used;
12. Any unusual information about the test or deviations from procedures;

The final study report will include the study protocol and all study raw data as appendices.

STUDY DESIGN ALTERATION

Amendments made to the protocol must be approved by the sponsor and study director and should include a description of the change; the reason for the change; the date the change took effect; and the dated signatures of the study director and sponsor. Any deviations in the protocol must be described and recorded in the study raw data.

REFERENCED GUIDELINES

ASTM. 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.

Benoit, D., V. Mattson, and D. Olson. 1982. A continuous-flow mini-diluter system for toxicity testing. Water Res. 16:457-464.

EPA. 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. Environmental Protection Agency, Washington, DC.

EPA. 2007. Letter to Lower Willamette Group from C. Humphrey and E. Blischke dated August 17, 2007 regarding Portland Harbor RI/FS: Lamprey toxicity test field sampling plan and QAPP Phase 2 addenda (approval letter). US Environmental Protection Agency Region 10, Oregon Operations Office, Portland, OR.

Veith G.D. and V.M. Comstock. 1975. Apparatus for continuously saturating water with hydrophobic organic chemicals. J Fish Res Board Can 32:1849-1851.

13. APPROVALS

_____ for **Windward Environmental**

Name Date

_____ for **Northwestern Aquatic Sciences**

Name Date

Appendix A
Test Conditions Summary

1. Test species:	*Lampetra* sp.
2. Test type:	Flow-through
3. Test duration:	96 hr
4. Temperature:	17 ± 1°C
5. Dissolved oxygen:	≥ 4.0 mg/L
6. Light quality & intensity:	Ambient laboratory light (50-75 ft-c)
7. Photoperiod:	Constant dark, except during monitoring activities
8. Test chambers:	glass aquaria less than or equal to two gallons in volume.
9. Test chamber volumes per day:	At least 5
10. Replicates per treatment:	4
11. Organisms per replicate:	10
12. Age of test organisms:	ammocoete
13. Feeding regime:	None
14. Aeration:	None
15. Dilution water:	Dechlorinated City of Newport tap water: hardness adjusted to 25-30 mg/L as $CaCO_3$
16. Test concentrations:	5 and a dilution water control
17. Water quality:	Temperature, pH, & dissolved oxygen in one replicate of each test concentration daily. Conductivity, hardness & alkalinity in one replicate of highest concentration and control daily
18. Special controls:	None
19. Endpoint:	Mortality
20. Test acceptability criteria:	≥90% control survival

APPENDIX II

RAW DATA

RSC 2/13/08

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-38 Client Windward Investigator

STUDY MANAGEMENT:

Client: Windward Environmental, 200 West Mercer Street, Suite 401, Seattle, WA 98119
Client's Study Monitor: Ms. Helle Andersen
Testing Laboratory: Northwestern Aquatic Sciences
Test Location: 3814 Yaquina Bay Road, Newport, OR 97365
Laboratory's Study Personnel:
Project Manager: Gary A. Buhler GAB Study Director: Richard S.Caldwell RSC
QA Officer: L.K. Nemeth 1. E.J. TRISSARELLI EJT
2. L. Sudeen LRS 3.
4. 5.
Study Schedule:
Test Beginning: 9-14-07 1320 Test Ending: 9-18-07 1330

TEST MATERIAL:

Description: Copper as $CuSO_4 \bullet 5H_2O$, Argent Lot No. 0195.

DILUTION WATER:

Description: Dechlorined City of Newport tap water
Date of Preparation/Collection: Continuous flow
Water Quality: Cond. (µmhos/cm) 102 ± 8 pH 7.9 ± 0.4
Hardness (mg/L as $CaCO_3$) 27.0 ± 1.4 Alkalinity (mg/L as $CaCO_3$) 15.5 ± 3.5
Treatments: Hardness adjusted to 25-30 mg/L with a Culligan® water treatment system.

TEST ORGANISMS:

Species: Lamprey ammocoetes (*Lampetra* sp.) Age: Size: 4-6 cm
Source: Trask, Nestucca, Siletz and Alsea rivers
Acclimation Data: See pages: 6, 7, + 11

TEST PROCEDURES AND CONDITIONS:

Test Concentrations (nominal): 0.2, 0.1, 00.05, 0.025, 0.0125 & 0 mg/L RSC 2/14/08
Test Chambers (description): 2 gallon glass aquaria Test Volume (ml): 5.80 L
Replicates/treatment: 4 Organisms/treatment: 40 (10/replicate)
Test water changes: flow through
Aeration during test: none unless DO drops below 60% saturation DO ≥ 4.0 mg/L GB 9/5/08
Temperature (°C): 17 ± 1°C
Feeding: None Photoperiod: Darkness except for WQ & chemistry measurements
Test Duration (hr) 96 hours Tank Placement: Complete randomization

Randomization Chart:

Diluter

0.025	0.20	0.025	0.10	0	0.20	0	0.025	0.0125	0.05	0	0.20	0.10	0.0125	0.0125	0.10	0.025	0.05	0.05	0.20	0	[illegible]	0.10	0.0125

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-38 Client Windward Investigator

TEST SOLUTION PREPARATION:

See page 5 for details

Weight of 10 animals at test initiation 9-14-07 CM

	weight (g)	Length (cm)	Comments
1	0.24	5-1	
2	0.14	4-4	
3	0.33	5-7	
4	0.29	5-5	
5	0.10	4-1	
6	0.15	4.5	
7	0.23	5-1	
8	0.32	5-8	
9	0.14	4-3	
10	0.30	4.1	
Mean	0.22	4.9	
S.D.	0.09	0.7	
(N)	10	10	

$$\text{loading} = \frac{0.22\ g \times 10}{5.8\ L} = 0.38\ g/L$$

MISCELLANEOUS NOTES:

Room light intensity (fc): 15-1 9-14-07 CM

Chemistry samples for copper both filtered and unfiltered taken daily. 100 ml each

Chemistry samples for particulate matter, TOC & ammonia in dilution water taken on test day 0.

Chemistry samples for ammonia taken in control & 0.20 A-replicate on test day 4.

Copper [illegible] and sediment [illegible] and replicate aqueous

Samples for filtration drawn with [illegible] and [illegible] dispensed into syringe and expelled into [illegible] through a PALL [illegible] Acrodisc (0.45 µm).

Samples (aliquots) shipped under chain of custody procedures to Columbia Analytical Services, Kelso, WA

Error codes:

1) Correction of handwriting error
2) Written in wrong location; entry deleted
3) Wrong date deleted; replaced with correct date
4) Error found in measurement; measurement repeated

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-38 Client Windward Investigator

WATER QUALITY DATA SHEET

DAY 0 (9/14/07) WD

* Conc. (mg/L)	Temp. (°C)	pH	Cond. µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
0.20	17.0	7.8	110	8.7	28	24	10	10	10	10	
0.10	17.1	7.9		8.8			10	10	10	10	
0.05	17.2	8.2		8.5			10	10	10	10	
0.025	16.9	8.2		8.6			10	10	10	10	
0.0125	17.1	8.2		8.5			10	10	10	10	
0	17.4	8.2	110	8.4	28	23	10	10	10	10	

Filtered/unfiltered chemistry samples taken (9/14/07) WD

Particulate matter, TOC, and ammonia samples taken in dilution water (9/14/07) WD

Hours of light: 4.0

DAY 1 (9/15/07) WD

Conc. (mg/L)	Temp. (°C)	pH	Cond. µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
0.20	16.9	7.8	95	8.1	26	17	10	10	10	10	
0.10	16.8	8.0		8.3			10	10	10	10	
0.05	16.7	8.3		8.1			10	10	10	10	
0.025	16.6	8.3		8.2			10	10	10	10	
0.0125	16.7	8.5		8.0			10	10	10	10	
0	17.0	8.4	100	7.6	26	19	10	10	10	10	

Filtered/unfiltered chemistry samples taken (9/15/07) WD

Hours of light: 2.0

DAY 2 (9/16/07) WD

Conc. (mg/L)	Temp. (°C)	pH	Cond. µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
0.20	17.6	7.2	105	9.4	28	16	10	10	10	10	ANIMALS APPEARED LETHARGIC
0.10	16.9	7.7		8.8			10	10	10	10	
0.05	16.9	7.9		8.6			10	10	10	10	
0.025	16.6	7.9		8.4			10	10	10	10	
0.0125	16.9	8.0		8.2			10	10	10	10	
0	17.2	7.9	105	8.0	28	18	10	10	10	10	

Filtered/unfiltered chemistry samples taken (9/16/07) WD

Hours of light: 10

* Concentrations are nominal and are mg/L as Cu. See 9/20/07

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-38 Client Windward Investigator

WATER QUALITY DATA SHEET

DAY 3 (9/17/07 [illegible]

Conc. (mg/L)	Temp. (°C)	pH	Cond. µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
0.20	17.0	7.2	105	9.6	28	18	0(100)	2(80)	1(90)	1(90)	All survivors lethargic
0.10	17.0	7.0		9.1			6(40)	9(10)	5(50)	7(30)	All survivors lethargic
0.05	17.0	7.4		9.1			10	10	10	10	
0.025	17.1	7.4		8.8			10	10	10	10	
0.0125	17.1	7.6		8.6			10	10	10	10	
0	17.4	7.6	105	8.3	28	21	10	10	10	10	

Filtered/unfiltered chemistry samples taken (9/17/07 [illegible]
Hours of light: 12

DAY 4 (9/18/07) [illegible]

Conc. (mg/L)	Temp. (°C)	pH	Cond. µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
0.20	17.0	7.1	95	9.5	26	12	0	0(20)	0(10)	0(10)	
0.10	16.8	7.2		9.3			0(40)	0(40)	0(50)	0(70)	[illegible]
0.05	16.6	7.4		9.2			4(60)	8(20)	7(30)	[illegible] 4(60)	All survivors lethargic, pale color
0.025	16.6	7.4		9.1			10	10	10	10	lethargic pale color
0.0125	16.8	7.3		8.8			10	10	10	10	
0	17.1	7.5	90	8.4	25	13	10	10	10	10	

Filtered/unfiltered chemistry samples taken (9/18/07) [illegible]
Ammonia samples taken in control and 0.2 mg/L (9/18/07) [illegible]
Hours of light: 12

Dilution-water quality (from control ~~tank~~ [illegible])

Cond.	102 ± 8 (5)	[illegible]
pH	7.9 ± 0.4 (5)	7/[illegible]/08
hardness	27.0 ± 1.4 (5)	
alkalinity	18.8 ± 3.8 (5)	

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-38 Client Windward Investigator

NOTES ON PREPARATION OF 0.2 MG/L COPPER STOCK

SYSTEM DESIGN

The objective is to deliver 0.2 mg/L of copper, prepared from reagent grade $CuSO_4 \cdot 5H_2O$, to the diluter toxicant chamber at a nominal constant rate of 160 ml/min. To accomplish this, a large mixing reservoir, approximately 54L capacity, of 0.2 mg/L Copper solution in dilution water is maintained at test temperature. This is done by metering a constant supply of concentrated copper stock solution into the large toxicant reservoir with vigorous mixing, while also replacing, under float valve control, dilution water to replace delivery of the 0.2 mg/L copper solution to the diluter. Concentrated copper stock solutions will be maintained by daily preparation of 1-2L volumes of the stock solution.

To replace 160 ml/min of 0.2 mg/L Cu withdrawn from the toxicant mixing reservoir, the concentrated stock solution will be prepared at a concentration of 32 mg/L and metered into the mixing reservoir at a rate of 1.0 ml/min. The concentrated stock copper solution will be prepared as needed by dilution of 32 ml/L of 1.00 mg/ml copper solution.

RECORD OF SOLUTION PREPARATIONS

1.0 mg/ml Cu prep'd 9/13/07 RSE - 1 liter

32 mg/L Cu Conc. Stock

RSE 9/13/07 17:30 hr - 3L
RSE 9/14/07 16:50 hr - 2L
RSE 9/15/07 10:55 hr - 1L
RSE 9/16/07 10:55 hr - 1L
RSE 9/17/07 14:30 hr - 2L

DILUTER CALIBRATIONS RECORD

DILUTER CAPILLARY FLOWS

Date 9/13/07	Time 14:00 hr	Init. RSE	Date 9/18/07	Time 1455	Init. RSE
Drain #	Supply Tank	Diluter Tank	Drain #	Supply Tank	Diluter Tank
1	160	80	1	160	77
2	80	81	2	81	80
3	84	76	3	86	76
4	72	78	4	82	79
5	79	80	5	78	79
6	110	78	6	110	79

Flow rates in ml/min

FLOWS TO REPLICATE AQUARIA

Date 9/13/07 Time 16:00 hr Init. RSE

Replicate	Relative Concentration (%) 100	50	25	12.5	6.25	0
A	240	290	310	275	430	210
B	260	280	370	410	280	300
C	320	320	250	265	335	260
D	300	270	220	200	275	350

Date 9/18/07 Time 1600 Init. RSE

Replicate	Relative Concentration (%) 100	50	25	12.5	6.25	0
A	345	240	280	160 ~~80~~	385	245
B	200	270	330	~~285~~ 420	340	285
C	255	320	240	320	320	230
D	310	280	305	285	220	335

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
8-3-07	18.1	6.7	8.6	82	40	35	[illegible]	[illegible]	constant flow		[illegible]	[illegible]	[illegible]
8-4-07	16.8	7.7	9.6	85	26	30		"	"			[illegible]	[illegible]
8-6-07	17.1	6.6	9.8	68	28	10	[illegible]	[illegible]	"			[illegible]	[illegible]
8-7-07	16.4	7.6	9.7	162	34	30	[illegible]	[illegible]	"			[illegible]	[illegible]
8-8-07	18.1	6.1	7.2	60	22	16	[illegible]	[illegible]	"			[illegible]	[illegible]
8-9-07	17.0	7.0	7.4	160	34	30						Holding	[illegible]
8-10-07	16.9	6.6	9.0	60	31	25	Alsea River		"			[illegible]	[illegible]
8-11-07	16.8	7.2	7.5	135	34	30			"			[illegible]	[illegible]
9-1-07	16.9	7.1	7.4	140	—	—			"				
9-2-07	16.7	7.6	9.1	160	—	—			"			Holding	[illegible]
9-3-07	16.7	7.7	9.2	160	—	—			"			Holding	[illegible]
9-4-07	16.2	7.5	8.8	185	34	30			"			"	[illegible]
9-5-07	16.3	7.2	9.1	180	34	30			"			"	[illegible]
9-6-07	16.8	7.3	9.0	185	34	30			"			"	
9-7-07	16.9	7.0	9.1	155	26	30			"			"	
9-8-07	17.0	7.5	9.3	160	—	—			"			"	
9-9-07	16.7	7.5	8.8	170	-	-			"			Holding	[illegible]
9-10-07	16.7	7.2	8.9	175	—	—			"			"	[illegible]
9-11-07	17.4	7.6	9.0	235	60	35			"			"	[illegible]
9-12-07	17.0	8.0	9.4	185	60	38			"			"	[illegible]

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
9-13-07	17.1	8.0	9.0	170	55	30			constant flow			holding	[illegible]
9-14-07	17.1	8.2	9.1	155	48	29			"		[illegible]	"	[illegible]
9-15-07	17.1	8.3	9.2	170	—				"			"	
9-16-07	17.0	8.1	9.2	115	41	30			"			"	[illegible]
9-17-07	17.1	7.7	8.8	120	41	30			"			"	[illegible]
9-18-07	17.0	7.8	9.1	130	39	21			"			"	[illegible]
9-19-07	17.7	7.6	9.1	125	39	34			"			"	[illegible]
9-20-07	17.1	7.9	9.0	125	36	34	Taken for [illegible]		"			"	[illegible]
9-21-07	17.0	8.2	9.5	140	44	31			"			"	[illegible]
9-22-07	17.1	7.9	9.4	130	42	29			"			"	
9-23-07	17.1	7.7	9.3	140	46	28			"			"	[illegible]
9-24-07	16.8	7.9	9.3	140	44	29			"			"	
9-25-07	17.1	7.7	8.8	145	44	30			"			"	[illegible]
9-26-07	17.2	7.8	9.3	140	48	36			"			"	[illegible]
9-27-07	17.4	7.7	9.3	145	48	37			"			"	[illegible]
9-28-07	17.2	7.8	9.3	150	48	36			"			"	[illegible]
9-29-07	16.9	7.5	8.8	150	48	36			"			"	[illegible]
9-30-07	17.0	7.7	8.8	140	48	37			"			"	[illegible]
10-1-07	17.0	7.8	9.4	145	48	36			"		[illegible]	"	[illegible]
10-2-07	17.3	8.1	9.2	135	—	—			"			"	[illegible]

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
10-3-07	17.0	7.8	9.1	130	46	55			constant flow			Holding	GH
10-4-07	16.9	7.9	9.2	120	42	38						"	[illegible]
10-5-07	16.9	7.4	9.0	130	not Taken LPS							"	LPS
10-6-07	16.6	7.2	9.4	130	44	41						"	GH
10-7-07	17.0	7.4	9.5	135	60	50						"	GH
10-8-07	17.0	7.9	9.2	145	50	48						"	GH
10-9-07	17.0	7.6	9.5	150	54	52						"	GH
10-10-07	16.9	7.4	9.2	155	54	49						"	GH
10-11-07	17.0	7.1	9.5	150	54	40						"	[illegible]
10-12-07	17.1	8.0	9.4	155	50	39						"	[illegible]
10-13-07	17.1	7.1	9.1	150	52	40						"	GH
10-14-07	16.9	7.5	9.6	150	52	38						"	[illegible]
10-15-07	17.0	7.6	9.5	155	50	38						"	[illegible]
10-5-9	13.0	6.7	10.6	97	30	30						Precision 4.0	[illegible]
10-16-07	13.2	7.9	10.1	145	60	57						Holding Rm #4	[illegible]
10-16-07	17.1	6.9	9.4	65	20	12						Test animals Rm #1	[illegible]
10-16-07	12.4	6.2	9.9	65	18	14						NEWTON CREEK BEGINNING	GH
10-17-07	13.3	8.3	10.1	135	60	55					[illegible]	Holding Rm #1	GH
10-17-07	13.3	6.7	8.5	75	22	14						Holding Rm #1	GH
10-18-07	13.1	6.8	10.2	57	20	14						NEWTON CREEK RECEIVED	

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
10-18-07	13.2	8.3	9.9	130	48	36			constant flow			Holding	[illegible]
10-19-07	13.3	7.5	9.5	120	48	36						"	LPS
10-20-07	13.2	7.7	10.0	125	46	38							
10-21-07	14.2	7.6	9.9	130	48	37							
10-22-07	14.2	7.7	9.4	135	50	40						"	[illegible]
10-23-07	15.1	7.9	9.7	145	54	44						"	[illegible]
10-24-07	15.6	8.3	9.7	155	58	47						"	[illegible]
10-25-07	16.1	8.5	9.5	160	66	53						"	[illegible]
10-26-07	16.8	8.1	9.6	165	60	47						"	[illegible]
10-27-07	16.8	8.3	9.9	155	62	49						"	[illegible]
10-28-07	16.6	8.3	9.9	165	62	47						"	[illegible]
10-29-07	17.0	8.1	9.5	165	62	48						"	[illegible]
10-30-07	16.9	8.2	9.6	170	64	46						"	[illegible]
10-31-07	16.8	8.2	9.9	175	70	54						"	[illegible]
11-1-07	16.8	8.2	9.8	165	62	45						"	[illegible]
11-2-07	16.8	8.2	9.5	165	62	47						"	[illegible]
11-3-07	16.6	8.1	9.2	160	56	38						"	GH
11-4-07	16.8	8.3	9.6	180	64	48						"	LPS
11-5-07	16.9	8.1	9.9	180	66	49						"	[illegible]
11-6-07	16.9	8.2	9.8	155	52	35						"	[illegible]

Holding Records

G. of 14

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
11-7-07	17.0	8.1	9.4	155	54	37			constant flow			Holding	
11-8-07	16.9	8.1	9.3	155	56	39						"	
11-9-07	16.7	8.6	9.9	160	62	46						"	
11-10-07	16.7	8.1	9.6	155	56	43						"	
11-11-07	16.7	8.1	9.9	145	52	39						"	
11-12-07	16.8	8.0	9.7	155	56	38						"	
11-13-07	16.8	7.6	9.7	155	54	38						"	
11-14-07	16.8	7.9	9.7	150	52	36						"	
11-15-07	16.8	8.0	9.7	160	60	43						"	
11-16-07	16.9	8.2	9.9	160	58	39						"	
11-17-07	16.7	8.3	10.1	165	62	43						"	
11-18-07	16.9	8.1	9.8	150	54	36						"	
11-19-07	16.8	7.9	9.7	175	64	48						"	
11-20-07	16.7	7.9	9.9	170	64	46						"	
11-21-07	16.6	8.0	9.9	175	64	48						"	
11-22-07	16.5	7.9	10.0	165	62	39						"	
11-23-07	16.5	7.8	9.5	170	62	44						"	
11-24-07	16.5	8.0	9.7	190	70	48						"	
11-25-07	16.5	7.9	9.6	175	64	45						"	
11-26-07	16.6	7.5	9.6	175	64	46						"	

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
11-29-07	16.1	7.9	9.6	180	62	44			constant flow			Holding	
11-30-07	16.0	7.7	10.0	175	56	44						"	
12-1-07	16.0	7.8	9.7	170	60	43						"	
12-2-07	16.0	8.0	9.7	165	62	46						"	
12-3-07	16.2	7.9	9.7	185	70	47						"	
12-4-07	15.8	7.5	9.4	165	60	43						"	
12-5-07	15.7	8.2	9.6	155	60	39						"	
12-6-07	15.8	7.7	9.8	170	60	42						"	
12-7-07	16.2	7.6	9.9	165	60	42						"	
12-8-07	15.7	7.6	9.9	160	58	40						"	
12-9-07	17.8	7.4	9.0	175	64	43						"	
12-10-07	17.1	7.3	8.8	165	62	42						"	
12-11-07	17.1	7.2	9.4	170	56	41						"	
12-12-07	17.3	7.4	9.5	160	58	40						"	
12-13-07	16.7	7.2	9.4	170	64	43						"	
12-14-07	16.8	7.3	9.1	170	64	44						"	
12-15-07	17.0	7.2	9.0	165	56	44						"	
12-16-07	16.5	7.2	9.5	170	70	45						"	
12-17-07	16.7	7.2	9.3	170	66	44						"	
12-18-07	16.5	7.4	9.6	195	74	53						"	

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
12-19-07	15.6	7.3	9.9	190	76	51			constant flow			HOLDING	
12-20-07	16.2	7.2	10.3	175	56	46						"	
12-21-07	15.4	8.2	10.0	190	72	48						"	
12-22-07	16.0	7.8	9.8	145	70	46							
12-23-07	17.1	8.0	9.5	195	68	54						"	
12-24-07	17.1	8.0	9.4	190	72	48						"	
12-25-07	16.8	8.1	9.6	190	72	51						"	
12-26-07	16.8	8.3	10.1	200	74	50						"	
12-27-07	17.1	8.2	9.7	195	68	49						"	
12-28-07	17.0	8.0	9.8	170	56	32						"	
12-29-07	17.1	8.1	9.9	175	52	29						"	
12-30-07	16.9	8.0	9.2	195	68	50						"	
12-31-07	16.8	8.3	9.8	205	72	50						"	
1-1-08	17.0	9.0	9.6	180	64	43						"	
1-2-08	16.8	8.2	9.4	185	66	41						"	
1-3-08	16.8	8.3	9.7	180	60	39						"	
1-4-08	16.9	8.2	9.8	175	62	36						"	
1-5-08	16.7	8.4	9.7	155	48	30						"	
1-7-08	16.7	8.4	9.9	160	54	32						"	
1-8-08	16.9	8.1	9.5	165	50	33						"	

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
1-9-08	15.6	8.4	10.6	165	56	30			constant flow			HOLDING	
1-10-08	15.9	8.0	10.2	145	44	24						"	
1-11-08	16.3	8.1	10.3	145	42	24							
1-12-08	16.0	8.4	10.2	165	56	31						"	
1-13-08	16.2	8.1	9.6	170	54	32						"	
1-14-08	16.5	8.2	9.9	160	52	32						"	
1-15-08	16.2	8.2	9.7	160	50	29						"	
1-16-08	16.4	8.2	9.9	125	40	15						"	
1-17-08	15.6	8.0	9.9	165	86	63						"	

Lamprey Holding Records

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd., P.O. Box 1437, Newport, OR 97365
Tel. 541-265-7225, Fax 541-265-2799, www.nwaquatic.com

Client Name: Windward Environmental
UPS#Y3489E
Project No: 07-28-04-53
Address: 200 West Mercer St., Suite 401
Phone No: 206-577-1287
City, State, Zip code: Seattle, WA 98119
Report Attention: Helle Anderson
Sampled by: G.J. Irissarri
Shipping Information: Carrier: UPS
Airbill No:

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	9-14-07	1205	NAS# 1205G Day-0 - 0.005 mg/L Cu	1	X						
	9-14-07		NAS# 1206G Day-0 - 0.005 mg/L Cu Filtered	1	X						
	9-14-07		NAS# 1208G Day-0 - 100	1							
	9-14-07		NAS# 1209G Day-0 - 250	1							
	9-14-07		NAS# 1221G Day-0 - Ammonia	1							

Signature	Print Name	Company	Date	Time	Cooler Custody Seal
Relinquished by: [signature]	Gerald Irissarri	Northwestern Aquatic Sciences	9-14-07	1015	Present / Not Present
Received by:					Intact / Not Intact
Relinquished by:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received by:					
Relinquished by:					
Received by laboratory:					

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd., P.O. Box 1437, Newport, OR 97365
Tel. 541-265-7225, Fax 541-265-2799, www.nwaquatic.com

Client Name: Windward Environmental
UPS#Y3489E
Project No: 07-28-04-53
Address: 200 West Mercer St., Suite 401
Phone No: 206-577-1287
City, State, Zip code: Seattle, WA 98119
Report Attention: Helle Anderson
Sampled by: G.J. Irissarri
Shipping Information: Carrier: UPS
Airbill No:

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	9-16-07	0945	NAS# 1234G Day-2 - 0.0 mg/L Cu	1	X						
	9-16-07		NAS# 1235G Day-2 - 0.0125 mg/L Cu	1	X						
	9-16-07		NAS# 1236G Day-2 - 0.025 mg/L Cu	1	X						
	9-16-07		NAS# 1237G Day-2 - 0.005 mg/L Cu	1	X						
	9-16-07		NAS# 1238G Day-2 - 0.0 mg/L Cu	1	X						
	9-16-07		NAS# 1239G Day-2 - 0.2 mg/L Cu	1	X						
	9-16-07		NAS# 1240G Day-2 - 0 mg/L Cu Filtered	1	X						
	9-16-07		NAS# 1241G Day-2 - 0.0125 mg/L Cu Filtered	1	X						
	9-16-07		NAS# 1242G Day-2 - 0.025 mg/L Cu Filtered	1	X						
	9-16-07		NAS# 1243G Day-2 - 0.05 mg/L Cu Filtered	1	X						
	9-16-07		NAS# 1244G Day-2 - 0.1 mg/L Cu Filtered	1	X						
	9-16-07		NAS# 1245G Day-2 - 0.2 mg/L Cu Filtered	1	X						

Signature	Print Name	Company	Date	Time	Cooler Custody Seal
Relinquished by: [signature]	GERALD IRISSARRI	Northwestern Aquatic Sciences	9-17-07	0940	Present / Not Present
Received by:					Intact / Not Intact
Relinquished by:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received by:					
Relinquished by:					
Received by laboratory:					

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd., P.O. Box 1437, Newport, OR 97365
Tel. 541-265-7225, Fax 541-265-2799, www.nwaquatic.com

Client Name: Windward Environmental
UPS#Y3489E
Project No: 07-28-04-53
Address: 200 West Mercer St., Suite 401
Phone No: 206-577-1287
City, State, Zip code: Seattle, WA 98119
Report Attention: Helle Anderson
Sampled by: G.J. Irissarri
Shipping Information: Carrier: UPS
Airbill No:

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	9-14-07	1205	NAS# 1203G Day-0 - 0.0 mg/L Cu	1	X						
	9-14-07		NAS# 1206G Day-0 - 0.0125 mg/L Cu	1	X						
	9-14-07		NAS# 1207G Day-0 - 0.025 mg/L Cu	1	X						
	9-14-07		NAS# 1208G Day-0 - 0.05 mg/L Cu	1	X						
	9-14-07		NAS# 1209G Day-0 - 0.1 mg/L Cu	1	X						
	9-14-07		NAS# 1210G Day-0 - 0.2 mg/L Cu	1	X						
	9-14-07		NAS# 1211G Day-0 - 0 mg/L Cu Filtered	1	X						
	9-14-07		NAS# 1212G Day-0 - 0.0125 mg/L Cu Filtered	1	X						
	9-14-07		NAS# 1213G Day-0 - 0.025 mg/L Cu Filtered	1	X						
	9-14-07		NAS# 1214G Day-0 - 0.05 mg/L Cu Filtered	1	X						
	9-14-07		NAS# 1215G Day-0 - 0.1 mg/L Cu Filtered	1	X						
	9-14-07		NAS# 1216G Day-0 - 0.2 mg/L Cu Filtered	1	X						

Signature	Print Name	Company	Date	Time	Cooler Custody Seal
Relinquished by: [signature]	Gerald Irissarri	Northwestern Aquatic Sciences	9-14-07	1015	Present / Not Present
Received by:					Intact / Not Intact
Relinquished by:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received by:					
Relinquished by:					
Received by laboratory:					

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd., P.O. Box 1437, Newport, OR 97365
Tel. 541-265-7225, Fax 541-265-2799, www.nwaquatic.com

Client Name: Windward Environmental
UPS#Y3489E
Project No: 07-28-04-53
Address: 200 West Mercer St., Suite 401
Phone No: 206-577-1287
City, State, Zip code: Seattle, WA 98119
Report Attention: Helle Anderson
Sampled by: G.A. Buhler
Shipping Information: Carrier: UPS
Airbill No:

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	9-15-07	1015	NAS# 1222G Day-1 - 0.0 mg/L Cu	1	X						
	9-15-07		NAS# 1223G Day-1 - 0.0125 mg/L Cu	1	X						
	9-15-07		NAS# 1224G Day-1 - 0.025 mg/L Cu	1	X						
	9-15-07		NAS# 1225G Day-1 - 0.05 mg/L Cu	1	X						
	9-15-07		NAS# 1226G Day-1 - 0.1 mg/L Cu	1	X						
	9-15-07		NAS# 1227G Day-1 - 0.2 mg/L Cu	1	X						
	9-15-07		NAS# 1228G Day-1 - 0 mg/L Cu Filtered	1	X						
	9-15-07		NAS# 1229G Day-1 - 0.0125 mg/L Cu Filtered	1	X						
	9-15-07		NAS# 1230G Day-1 - 0.025 mg/L Cu Filtered	1	X						
	9-15-07		NAS# 1231G Day-1 - 0.05 mg/L Cu Filtered	1	X						
	9-15-07		NAS# 1232G Day-1 - 0.1 mg/L Cu Filtered	1	X						
	9-15-07		NAS# 1233G Day-1 - 0.2 mg/L Cu Filtered	1	X						

Signature	Print Name	Company	Date	Time	Cooler Custody Seal
Relinquished by: [signature]	Gerald Irissarri	Northwestern Aquatic Sciences	9-17-07	0940	Present / Not Present
Received by:					Intact / Not Intact
Relinquished by:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received by:					
Relinquished by:					
Received by laboratory:					

Samples shipped to CAS

Test No. 656-38

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd., P.O. Box 1437, Newport, OR 97365
Tel. 541-265-7225, Fax 541-265-2799, www.nwaquatic.com

Client Name: Windward Environmental
UPS#Y3489E
Address: 200 West Mercer St., Suite 401
City, State, Zip code: Seattle, WA 98119
Project No: 07-28-04-53
Phone No: 206-577-1287
Report Attention: Helle Anderson
Shipping Information — Carrier: UPS; Airbill No: 1Z 849 V6 01 0053 2994
Sampled by: G. Irissarri

Testing Required: Copper, Lindane, Pentachlorophenol, Aniline, Diazinon, Naphthalene

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	9-18-07	1010	NAS# 1260G Day-4 - 0.0 mg/L Cu	1	X						
	9-18-07		NAS# 1261G Day-4 - 0.0125 mg/L Cu	1	X						
	9-18-07		NAS# 1262G Day-4 - 0.025 mg/L Cu	1	X						
	9-18-07		NAS# 1263G Day-4 - 0.05 mg/L Cu	1	X						
	9-18-07		NAS# 1264G Day-4 - 0.1 mg/L Cu	1	X						
	9-18-07		NAS# 1265G Day-4 - 0.2 mg/L Cu	1	X						
	9-18-07		NAS# 1266G Day-4 - 0.0 mg/L Cu Filtered	1	X						
	9-18-07		NAS# 1267G Day-4 - 0.0125 mg/L Cu Filtered	1	X						
	9-18-07		NAS# 1268G Day-4 - 0.025 mg/L Cu Filtered	1	X						
	9-18-07		NAS# 1269G Day-4 - 0.05 mg/L Cu Filtered	1	X						
	9-18-07		NAS# 1270G Day-4 - 0.1 mg/L Cu Filtered	1	X						
	9-18-07		NAS# 1271G Day-4 - 0.2 mg/L Cu Filtered	1	X						
	9-18-07	1035	NAS# 1272G Day-4 - Ammonia (Control)	1							
	9-18-07	1035	NAS# 1273G Day-4 Ammonia (0.2 mg/L Cu)	1							

Signature	Print Name	Company	Date	Time	Cooler Custody Seal
Relinquished by: [signature]	GERALD IRISSARRI	Northwestern Aquatic Sciences	9-18-07	1400	Present / Not Present
Received by:					Intact / Not Intact
Relinquished by:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received by Laboratory:					

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd., P.O. Box 1437, Newport, OR 97365
Tel. 541-265-7225, Fax 541-265-2799, www.nwaquatic.com

Client Name: Windward Environmental
UPS#Y3489E
Address: 200 West Mercer St., Suite 401
City, State, Zip code: Seattle, WA 98119
Project No: 07-28-04-53
Phone No: 206-577-1287
Report Attention: Helle Anderson
Shipping Information — Carrier: UPS; Airbill No: 1Z 849 V6 01 0053 2994
Sampled by: G. Irissarri

Testing Required: Copper, Lindane, Pentachlorophenol, Aniline, Diazinon, Naphthalene

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	9-17-07	1100	NAS# 1246G Day-3 - 0.0 mg/L Cu	1	X						
	9-17-07		NAS# 1247G Day-3 - 0.0125 mg/L Cu	1	X						
	9-17-07		NAS# 1248G Day-3 - 0.025 mg/L Cu	1	X						
	9-17-07		NAS# 1249G Day-3 - 0.05 mg/L Cu	1	X						
	9-17-07		NAS# 1250G Day-3 - 0.1 mg/L Cu	1	X						
	9-17-07		NAS# 1251G Day-3 - 0.2 mg/L Cu	1	X						
	9-17-07		NAS# 1252G Day-3 - 0.0 mg/L Cu Filtered	1	X						
	9-17-07		NAS# 1253G Day-3 - 0.0125 mg/L Cu Filtered	1	X						
	9-17-07		NAS# 1254G Day-3 - 0.025 mg/L Cu Filtered	1	X						
	9-17-07		NAS# 1255G Day-3 - 0.05 mg/L Cu Filtered	1	X						
	9-17-07		NAS# 1256G Day-3 - 0.1 mg/L Cu Filtered	1	X						
	9-17-07		NAS# 1257G Day-3 - 0.2 mg/L Cu Filtered	1	X						
	9-17-07		NAS# 1258G Day-3 - 0.1 mg/L Cu	1	X						
	9-17-07		NAS# 1259G Day-3 - 0.1 mg/L Cu Filtered	1	X						

Signature	Print Name	Company	Date	Time	Cooler Custody Seal
Relinquished by: [signature]	GERALD IRISSARRI	Northwestern Aquatic Sciences	9-17-07	1400	Present / Not Present
Received by:					Intact / Not Intact
Relinquished by:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received by Laboratory:					

Samples: 5 of 7 pages

Test No. 0827-38

9 of 14

Water Quality Computation - Test No. 686-38

	Temp	DO	pH	Cond	Hard	Alk
1	17.0	8.7	7.8	110	28	24
2	17.1	8.8	7.9	110	28	23
3	17.2	8.5	8.2	95	26	17
4	16.9	8.6	8.2	100	26	19
5	17.1	8.5	8.2	105	28	16
6	17.4	8.4	8.2	105	28	18
7	16.9	8.1	7.8	105	28	18
8	16.8	8.3	8.0	105	28	21
9	16.7	8.1	8.3	95	26	12
10	16.6	8.2	8.3	90	25	13
11	16.7	8.0	8.5			
12	17.0	7.6	8.4			
13	17.0	9.4	7.2			
14	16.9	8.8	7.7			
15	16.9	8.6	7.9			
16	16.6	8.4	7.9			
17	16.9	8.2	8.0			
18	17.2	8.0	7.9			
19	17.0	9.6	7.2			
20	17.0	9.1	7.0			
21	17.0	9.1	7.4			
22	17.0	8.8	7.4			
23	17.1	8.6	7.6			
24	17.4	8.3	7.6			
25	17.0	9.5	7.1			
26	16.8	9.3	7.2			
27	16.6	9.2	7.4			
28	16.6	9.1	7.4			
29	16.8	8.8	7.3			
30	17.1	8.4	7.5			
31						
32						
mean	16.9	8.6	7.8	102	27	18
s.d.	0.2	0.5	0.4	7	1	4
N	30	30	30	10	10	10
min	16.6	7.6	7.0	90	25	12
max	17.4	9.6	8.5	110	28	24

2/21/08

10 of 14

WQ Computation - acclimation (8-31-07 to 9-14-07) - Test No. 686-38

	Temp	DO	pH	Cond	Hard	Alk
1	16.8	7.5	7.2	135	34	30
2	16.9	7.4	7.1	140	34	30
3	16.7	9.1	7.6	160	34	30
4	16.7	9.2	7.7	160	34	30
5	16.2	8.8	7.5	185	26	30
6	16.3	9.1	7.2	170	60	35
7	16.8	9.0	7.3	185	60	38
8	16.9	9.1	7.0	155	55	30
9	17.0	9.3	7.5	160	48	29
10	16.7	8.8	7.5	170		
11	16.7	8.9	7.2	175		
12	17.4	9.0	7.6	235		
13	17.0	9.4	8.0	185		
14	17.1	9.0	8.0	170		
15	17.1	9.1	8.2	155		
16						
17						
mean	16.8	8.8	7.5	169	43	31
s.d.	0.3	0.6	0.4	24	13	3
N	15	15	15	15	9	9
min	16.2	7.4	7.0	135	26	29
max	17.4	9.4	8.2	235	60	38

CISE
2/21/08

11 of 14

SampleDate	SampleTim	Matrix	LabMatrix	SampleID	arentSamp	SampleTyp	Method	AnalysisDate	nalysis Tim	nalyteGrou	cas_rn	Analyte	l_or_disso	detect_flag	VALUE	Qualifiers	Units
9/14/2007	12 05	WQ	WQ	NAS# 1211G Day 0-0 0 mg/L Cu Filtered		N	E200 8	10/7/2007	19 17	Metals	7440-50-8	Copper	D	Y	1 8		ug/l
9/14/2007	12 05	WQ	WQ	NAS# 1212G Day 0-0 0125 mg/L Cu Filtered		N	E200 8	10/7/2007	19 19	Metals	7440-50-8	Copper	D	Y	12 9		ug/l
9/14/2007	12 05	WQ	WQ	NAS# 1213G Day 0-0 025 mg/L Cu Filtered		N	E200 8	10/7/2007	19 22	Metals	7440-50-8	Copper	D	Y	21 6		ug/l
9/14/2007	12 05	WQ	WQ	NAS# 1214G Day 0-0 05 mg/L Cu Filtered		N	E200 8	10/7/2007	19 24	Metals	7440-50-8	Copper	D	Y	39		ug/l
9/14/2007	12 05	WQ	WQ	NAS# 1218G Day 0-0 05 mg/L Cu Filtered		N	E200 8	10/7/2007	19 40	Metals	7440-50-8	Copper	D	Y	38 1		ug/l
9/14/2007	12 05	WQ	WQ	NAS# 1218G Day 0-0 05 mg/L Cu FilteredD	NAS# 1218	LR	E200 8	10/7/2007	19 42	Metals	7440-50-8	Copper	D	Y	38 1		ug/l
9/14/2007	12 05	WQ	WQ	NAS# 1215G Day 0-0 1 mg/L Cu Filtered		N	E200 8	10/7/2007	19 26	Metals	7440-50-8	Copper	D	Y	82 2		ug/l
9/14/2007	12 05	WQ	WQ	NAS# 1216G Day 0-0 2 mg/L Cu Filtered		N	E200 8	10/7/2007	19 28	Metals	7440-50-8	Copper	D	Y	120		ug/l
9/15/2007	10 15	WQ	WQ	NAS# 1228G Day 1-0 0 mg/L Cu Filtered		N	E200 8	10/7/2007	16 49	Metals	7440-50-8	Copper	D	Y	0 54		ug/l
9/15/2007	10 15	WQ	WQ	NAS# 1228G Day 1-0 0 mg/L Cu FilteredD	NAS# 1228	LR	E200 8	10/7/2007	16 51	Metals	7440-50-8	Copper	D	Y	0 51		ug/l
9/15/2007	10 15	WQ	WQ	NAS# 1229G Day 1-0 0125 mg/L Cu Filtered		N	E200 8	10/7/2007	16 55	Metals	7440-50-8	Copper	D	Y	12 3		ug/l
9/15/2007	10 15	WQ	WQ	NAS# 1230G Day 1-0 025 mg/L Cu Filtered		N	E200 8	10/7/2007	16 57	Metals	7440-50-8	Copper	D	Y	21 9		ug/l
9/15/2007	10 15	WQ	WQ	NAS# 1231G Day 1-0 05 mg/L Cu Filtered		N	E200 8	10/7/2007	16 59	Metals	7440-50-8	Copper	D	Y	41 6		ug/l
9/15/2007	10 15	WQ	WQ	NAS# 1232G Day 1-0 1 mg/L Cu Filtered		N	E200 8	10/7/2007	17 01	Metals	7440-50-8	Copper	D	Y	77 4		ug/l
9/15/2007	10 15	WQ	WQ	NAS# 1233G Day 1-0 2 mg/L Cu Filtered		N	E200 8	10/7/2007	17 03	Metals	7440-50-8	Copper	D	Y	133		ug/l
9/16/2007	09 45	WQ	WQ	NAS# 1240G Day 2-0 0 mg/L Cu Filtered		N	E200 8	10/7/2007	17 22	Metals	7440-50-8	Copper	D	Y	0.3		ug/l
9/16/2007	09 45	WQ	WQ	NAS# 1241G Day 2-0 0125 mg/L Cu Filtered		N	E200 8	10/7/2007	17 24	Metals	7440-50-8	Copper	D	Y	12.5		ug/l
9/16/2007	09 45	WQ	WQ	NAS# 1242G Day 2-0 025 mg/L Cu Filtered		N	E200 8	10/7/2007	19 47	Metals	7440-50-8	Copper	D	Y	22 8		ug/l
9/16/2007	09 45	WQ	WQ	NAS# 1243G Day 2-0 05 mg/L Cu Filtered		N	E200 8	10/7/2007	19 49	Metals	7440-50-8	Copper	D	Y	41 7		ug/l
9/16/2007	09 45	WQ	WQ	NAS# 1244G Day 2-0 1 mg/L Cu Filtered		N	E200 8	10/7/2007	19 51	Metals	7440-50-8	Copper	D	Y	87 1		ug/l
9/16/2007	09 45	WQ	WQ	NAS# 1245G Day 2-0 2 mg/L Cu Filtered		N	E200 8	10/7/2007	19 53	Metals	7440-50-8	Copper	D	Y	175		ug/l
9/17/2007	11 00	WQ	WQ	NAS #1252G Day 3-0 0 mg/L Cu Filtered		N	E200 8	10/25/2007	18 22	Metals	7440-50-8	Copper	D	N	1 08	U	ug/l
9/17/2007	11 00	WQ	WQ	NAS #1253G Day 3-0 0125 mg/L Cu Filtered		N	E200 8	10/7/2007	17 48	Metals	7440-50-8	Copper	D	Y	13 9		ug/l
9/17/2007	11 00	WQ	WQ	NAS #1254G Day 3-0 025 mg/L Cu Filtered		N	E200 8	10/7/2007	17 51	Metals	7440-50-8	Copper	D	Y	24 3		ug/l
9/17/2007	11 00	WQ	WQ	NAS #1255G Day 3-0 05 mg/L Cu Filtered		N	E200 8	10/7/2007	17 53	Metals	7440-50-8	Copper	D	Y	45 6		ug/l
9/17/2007	11 00	WQ	WQ	NAS #1256G Day 3-0 1 mg/L Cu Filtered		N	E200 8	10/7/2007	17 55	Metals	7440-50-8	Copper	D	Y	96 2		ug/l
9/17/2007	11 00	WQ	WQ	NAS #1259G Day 3-0 1 mg/L Cu Filtered		N	E200 8	10/7/2007	18 09	Metals	7440-50-8	Copper	D	Y	96 2		ug/l
9/17/2007	11 00	WQ	WQ	NAS #1259G Day 3-0 1 mg/L Cu FilteredD	NAS #1259	LR	E200 8	10/7/2007	18 11	Metals	7440-50-8	Copper	D	Y	95 6		ug/l
9/17/2007	11 00	WQ	WQ	NAS #1257G Day 3-0 2 mg/L Cu Filtered		N	E200 8	10/7/2007	18 00	Metals	7440-50-8	Copper	D	Y	190		ug/l
9/18/2007	10 10	WQ	WQ	NAS #1266G Day 4-0 0 mg/L Cu Filtered		N	E200 8	10/7/2007	18 36	Metals	7440-50-8	Copper	D	Y	0 39		ug/l
9/18/2007	10 10	WQ	WQ	NAS #1266G Day 4-0 0 mg/L Cu FilteredD	NAS #1266	LR	E200 8	10/7/2007	18 38	Metals	7440-50-8	Copper	D	Y	0 4		ug/l
9/18/2007	10 10	WQ	WQ	NAS #1267G Day 4-0 0125 mg/L Cu Filtered		N	E200 8	10/7/2007	18 42	Metals	7440-50-8	Copper	D	Y	12.9		ug/l
9/18/2007	10 10	WQ	WQ	NAS #1268G Day 4-0 025 mg/L Cu Filtered		N	E200 8	10/7/2007	18 44	Metals	7440-50-8	Copper	D	Y	23.5		ug/l
9/18/2007	10 10	WQ	WQ	NAS #1269G Day 4-0 05 mg/L Cu Filtered		N	E200 8	10/7/2007	18.50	Metals	7440-50-8	Copper	D	Y	46		ug/l
9/18/2007	10 10	WQ	WQ	NAS #1270G Day 4-0 1 mg/L Cu Filtered		N	E200 8	10/7/2007	18.53	Metals	7440-50-8	Copper	D	Y	98 6		ug/l
9/18/2007	10 10	WQ	WQ	NAS #1271G Day 4-02 mg/L Cu Filtered		N	E200 8	10/7/2007	18 55	Metals	7440-50-8	Copper	D	Y	201		ug/l

Dissolved Copper (mg/L)	Day 0	Day 1	Day 2	Day 3	Day 4	Average	Standard Deviation
0 0	0 002	0 001	0 000	0 001	0.000	0 001	0 001
0.0125	0 013	0 012	0 013	0.014	0 013	0 013	0 001
0.025	0 022	0.022	0 023	0 024	0.024	0 023	0.001
0.05	0 038	0.042	0 042	0.046	0.046	0.043	0.003
0 1	0 082	0 077	0.087	0 096	0 099	0.088	0 009
0.2	0 120	0 133	0 175	0 190	0 201	0 164	0 036

Validated Copper Analyses

Reported by Windward
RSB 2/18/08

686-38

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Acute 96-hr Toxicity Test-96 Hr Survival

Start Date:	9/14/07 13:20	Test ID:	686-38	Sample ID:	
End Date:	9/18/07 13:30	Lab ID:	ORNAS-Northwestern Aquati	Sample Type:	CUSO-Copper sulfate
Sample Date:		Protocol:	NAS-686-Lamprey Ft acute	Test Species:	LY-Lampetra sp.
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
0.013	1.0000	1.0000	1.0000	1.0000
0.023	1.0000	1.0000	1.0000	1.0000
0.043	0.4000	0.8000	0.7000	0.4000
0.088	0.0000	0.0000	0.0000	0.0000
0.164	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
0.013	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
0.023	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*0.043	0.5750	0.5750	0.8669	0.6847	1.1071	24.877	4	10.00	10.00	17	40
0.088	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4			40	40
0.164	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4			40	40

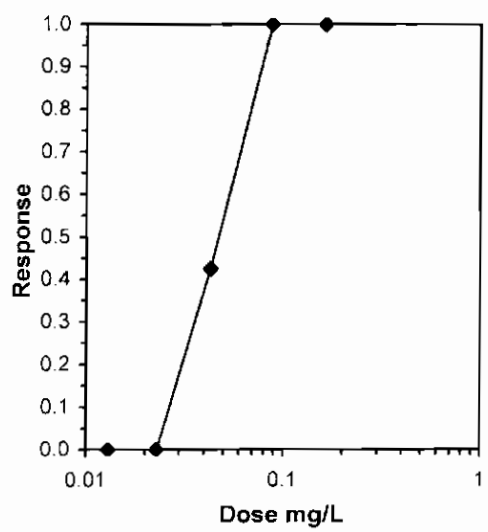
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.70838	0.844	0.31227	2.93727
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	0.023	0.043	0.03145	

LC50 Pbe

Trimmed Spearman-Karber

Trim Level	~~EC50~~	95% CL	
0.0%	0.0463	0.0416	0.0514
5.0%	0.0464	0.0413	0.0521
10.0%	0.0465	0.0407	0.0531
20.0%	0.0468	0.0390	0.0561
Auto-0.0%	0.0463	0.0416	0.0514



ToxCalc v5.0.23N

Test: AT-Acute 96-hr Toxicity Test | Test ID: 686-38
Species: LY-Lampetra sp. | Protocol: NAS-686-Lamprey Ft acute
Sample ID: | Sample Type: CUSO-Copper sulfate
Start Date: 9/14/07 13:20 End Date: 9/18/07 13:30 | Lab ID: ORNAS-Northwestern Aquatic Sciences

Pos	ID	Rep	Group	Start	24 Hr	48 Hr	72 Hr	96 Hr	Notes
	1	1	D-Control	10				10	
	2	2	D-Control	10				10	
	3	3	D-Control	10				10	
	4	4	D-Control	10				10	
	5	1	0.013	10				10	
	6	2	0.013	10				10	
	7	3	0.013	10				10	
	8	4	0.013	10				10	
	9	1	0.023	10				10	
	10	2	0.023	10				10	
	11	3	0.023	10				10	
	12	4	0.023	10				10	
	13	1	0.043	10				4	
	14	2	0.043	10				8	
	15	3	0.043	10				7	
	16	4	0.043	10				4	
	17	1	0.088	10				0	
	18	2	0.088	10				0	
	19	3	0.088	10				0	
	20	4	0.088	10				0	
	21	1	0.164	10				0	
	22	2	0.164	10				0	
	23	3	0.164	10				0	
	24	4	0.164	10				0	

Comments:

LAMPREY (*LAMPETRA* SP.) DEFINITIVE 96-HR FLOW-THROUGH ACUTE TOXICITY TEST OF ANILINE

NAS Test No. 686-39

Submitted to

Windward Environmental LLC
200 West Mercer Street, Suite 401
Seattle, WA 98119

Submitted by

Northwestern Aquatic Sciences
3814 Yaquina Bay Road
P.O. Box 1437
Newport, OR 97365

April 3, 2008

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 686-39
Title: Lamprey (*Lampetra* sp.) definitive 96-hr flow-through acute toxicity test of aniline.
Protocol No.: NAS-686-Lamprey FT acute, September 12, 2007

STUDY MANAGEMENT

Study Sponsor: Windward Environmental, 200 West Mercer Street, Suite 401, Seattle, WA 98119.
Sponsor's Study Monitor: Ms. Helle Andersen.
Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365.
Test Location: Newport Laboratory.
Laboratory's Study Personnel: G. A. Buhler, B.S., Proj. Manager; R. S. Caldwell, Ph.D., Study Director; L.K. Nemeth, B.A., M.B.A., QA Officer; G.J. Irissarri, B.S., Aq. Toxicol.
Study Schedule:
Test Beginning: 10-1-07, 1615 hrs.
Test Ending: 10-5-07, 1600 hrs.
Disposition of Study Records: All specimens, raw data, reports and other study records are stored according to Good Laboratory Practice regulations at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.
Good Laboratory Practices: The test was conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).
Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Description: Aniline (Fisher Scientific, Lot No. 072020).
Preparation of Working Stock Solution and Test Concentrations: Test solutions were prepared for continuous supply to the test aquaria using a Benoit constant flow diluter (Benoit *et al.*, 1982). The diluter was set up to deliver a 0.5 dilution series with a flow rate of approximately 80 ml/min per toxicant concentration. The diluter design was such that 160 ml/min of the toxicant solution at the highest test concentration (nominal 1200 mg/L aniline) was required to be continuously delivered to the toxicant supply chamber of the diluter. To accomplish this, a large mixing reservoir, approximately 54L capacity, of nominal 1200 mg/L aniline solution in dilution water, was maintained at test temperature. This was done by metering, at a rate of 5.5 ml/min, a constant supply of a saturated solution (ca. 35 g/L) of aniline in deionized water into the large toxicant reservoir with vigorous mixing, while also replacing, under float valve control, dilution water to replace delivery of the nominal 1200 mg/L aniline solution to the diluter. The saturated aniline solution was prepared daily.

DILUTION WATER

Source: City of Newport municipal tap water, treated as below, was supplied continuously to the toxicant diluter throughout the study.
Pretreatment: The water was dechlorinated by carbon filtration, particle filtered to 1 µm, and adjusted to a target water hardness of 25-30 mg/L as $CaCO_3$. Hardness adjustment was accomplished by passage of a portion of the dilution water through deionization cartridges.

TEST ORGANISMS

Species: Lamprey (*Lampetra* sp.)
Age/Size: Ammocoetes; target size range, 4-6 cm; actual size of 10 ammocoete subsample, 5.0 ± 0.5 cm.
Source: Ammocoetes were field collected in approximately equal numbers from the Trask, Nestucca, Siletz, and Alsea Rivers, Oregon. Field collection was by electoshocking and only 4-6 cm size ammocoetes were retained. Personnel of Windward Environmental performed the ammocoete collections.
Acclimation: Upon receipt, animals were removed from the collection site sediment and placed in 10-gallon aquaria supplied under flow-through conditions with dilution water. Water supply was sufficient (30-50

ml/min) to accomplish two volume changes of water per day. Approximately 50 lamprey were placed in each tank. Tanks contained an approximate 8 cm deep layer of sediment (Sandtastic Play Sand, Waupaca Materials) covered with approximately 26L of test water and were supplied with aeration. Normal behavior for the lamprey ammocoetes was to immediately bury in sediment and remain there. Any that did not immediately bury were discarded as potentially damaged. Ammocoetes were not fed during acclimation. Water quality conditions during the two weeks prior to testing (9-15-07 to 9-30-07) were: temperature, 17.1 ± 0.2°C; dissolved oxygen, 9.1 ± 0.2 mg/L; pH, 7.8 ± 0.3; specific conductance, 134 ± 12 µmhos/cm; hardness, 44 ± 4 mg/L as $CaCO_3$; and alkalinity, 32 ± 4 mg/L as $CaCO_3$.

TEST PROCEDURES AND CONDITIONS

Test Chambers: The test chambers were two-gallon glass aquaria capable of holding 5.8 L of test solution. Test chamber placement in the test area was random.

Test Concentrations: The nominal test concentrations were 1200, 600, 300, 150, 75, and 0 mg/L (control) aniline.

Replicates/Treatment: 4

Organisms/Treatment: 40

Loading: 0.40 g/L

Aeration: None.

Feeding: None.

Water Volume Changes: The diluter supplied approximately 80 ml/min of test solution, or 115 L/day, at each test concentration. At this rate of flow, test solution supply to each replicate aquarium was 5.0 volumes per day.

Effects Criteria: The effect criterion used in the lamprey ammocoete acute toxicity test was mortality, defined as a lack of visible respiratory movement and absence of response to tactile stimulation. In addition to mortality, qualitative observations of sublethal responses were recorded when observed.

Target Water Quality Conditions: Water quality measurements were taken daily as specified in the protocol (Physical and Chemical Data, Items 1 and 2). The target water quality conditions were as follows: Temperature, 17 ± 1°C; dissolved oxygen, ≥4.0 mg/L; hardness, 25-30 mg/L as $CaCO_3$. Actual test conditions are reported in the Test Results section.

Photoperiod: The photoperiod was total darkness except for approximately 2-3 hours per day for observations and test exposure system maintenance. Light intensity at the aquarium level was 15.7 foot-candles.

DATA ANALYSIS METHODS

The 96-hr proportion survival was calculated for each treatment replicate from the raw survival data and the measured aniline concentrations for each treatment level. The LC50 (survival) was calculated by the probit method. An arcsine square root (angular) transformation was performed on the data prior to statistical analysis. The NOEC and LOEC were calculated according to U.S. Environmental Protection Agency (2002) methods. In this procedure, the NOEC and LOEC values were computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test was selected after evaluating the data for normality and homogeneity of variance. The statistical software employed for these calculations was ToxCalc, v.5.0.23N, Tidepool Scientific Software.

TOXICANT AND OTHER CHEMISTRY ANALYSES

Test solutions were sampled once daily for aniline from each test concentration by pooling samples from each of the four test replicate aquaria. Subsamples were pooled into new disposable plastic beakers from which the pooled sample was transferred to the analytical laboratory supplied sample bottles and shipped under chain-of-custody procedures to the analytical chemistry laboratory (Columbia Analytical Services) at Kelso, WA. No preservative was employed. Samples were kept refrigerated at ≤4°C. Sample volumes were 1 L. Additional samples were taken of the dilution water on day 0 of the test for analysis of particulate matter, TOC, and ammonia-N, and from the control and highest test concentration treatments after 96-hr for measurement of ammonia-N. These samples also were shipped to Columbia Analytical Services for analysis.

PROTOCOL DEVIATIONS

1. The protocol specified "constant dark, except during monitoring activities", and also that light intensity should be 50-75 ft-c. when light was on. These specifications were judged to be contradictory and led to the following action. Based on best professional judgment of the investigators, we reduced the light intensity from that given in the protocol in order to minimize fright behavior responses of the ammocoetes during the short period of lighting associated with daily observations.
2. The protocol states: "At the end of the test, all surviving control ammocoetes are weighed and measured for length by replicate." Through a laboratory error, a subsample of 10 ammocoetes were weighed and measured at the beginning of the test instead. This is a minor deviation and is without consequence for interpretation of the study results.

TEST RESULTS

Water quality conditions measured in test aquaria during the 96-hr toxicity test are summarized in Table 1. The temperature specification was met during the study. Dissolved oxygen remained near saturation throughout the test. The pH ranged from 6.2 – 6.7. Mean hardness also was in the specified range of 25-30 mg/L as $CaCO_3$.

The concentrations of aniline measured during the toxicity test are summarized in Table 2. Mean measured concentrations in the test ranged from 113 ± 16 mg/L at the lowest level to 1300 ± 187 mg/L at the highest exposure level. Generally, the dilution series was in good agreement with nominal concentrations.

A summary of the 96-hr ammocoetes survival results is shown in Table 3. In this study, the 96-hr control survival (100%) met the test acceptability criteria of ≥90%. Therefore, the test meets the protocol criterion of a valid test. Additionally, 100% mortality occurred in the highest, and 0% mortality was observed in the two lowest, test concentrations. Partial mortality was observed in the middle (318 mg/L) and second highest (634 mg/L) test concentrations.

The toxicity of aniline to lamprey (*Lampetra* sp.) ammocoetes as the 96-hr LC50 was 430 mg/L (95% confidence limits, 381 – 486 mg/L). The NOEC and LOEC concentrations were 318 and 634 mg/L aniline, respectively.

LITERATURE CITED

Benoit, D., V. Mattson, and D. Olson. 1982. A continuous-flow mini-diluter system for toxicity testing. Water Res. 16:457-464.

EPA. 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. Environmental Protection Agency, Washington, DC.

STUDY APPROVAL

Project Manager	Date	Study Director	Date
Laboratory Director	Date	Quality Assurance Unit	Date

Table 1. Measured water quality conditions during the 96-hr acute toxicity test of aniline to lamprey ammocoetes.

Parameter	Mean ± S.D.	N	Range
Temperature (°C)	16.9 ± 0.4	30	16.2 – 17.6
Dissolved oxygen (mg/L)	9.2 ± 0.2	30	8.8 – 9.6
pH	6.4 ± 0.2	30	6.2 – 6.7
Specific conductance (μmhos/cm)	98 ± 2	10	97 – 100
Hardness (mg/L as $CaCO_3$)	26 ± 1	10	24 – 28
Alkalinity (mg/L as $CaCO_3$)	21 ± 1	5	20 – 23

Table 2. Measured concentrations of aniline during the 96-hr acute toxicity test of aniline to lamprey ammocoetes. Measurements performed by Columbia Analytical Services.

Nominal (mg/L)	Measured (mg/L)		
	Mean ± S.D.	N	Range
1200	1300 ± 187	5	1100 – 1500
600	634 ± 85	5	530 – 730
300	318 ± 25	5	300 – 360
150	176 ± 23	5	150 – 200
75	113 ± 16	5	95 – 130
0	0.5 ± 0.7	5	0.1 – 1.8

Table 3. Survival of lamprey ammocoetes, *Lampetra* sp. exposed for 96 hours to aniline.

Aniline (mg/L)	Replicate	Ammocoetes exposed	Ammocoetes Surviving	Percent Survival	Mean % Survival	
1300	A	10	0	0.0		
	B	10	0	0.0		
	C	10	0	0.0		
	D	10	0	0.0	0.0	*
634	A	10	2	20.0		
	B	10	2	20.0		
	C	10	0	0.0		
	D	10	3	30.0	17.5	*
318	A	10	7	70.0		
	B	10	7	70.0		
	C	10	6	60.0		
	D	10	10	100.0	75.0	
176	A	10	10	100.0		
	B	10	10	100.0		
	C	10	10	100.0		
	D	10	10	100.0	100.0	
113	A	10	10	100.0		
	B	10	10	100.0		
	C	10	10	100.0		
	D	10	10	100.0	100.0	
0.5 (Control)	A	10	10	100.0		
	B	10	10	100.0		
	C	10	10	100.0		
	D	10	10	100.0	100.0	

* Mean significantly (p=0.05) different from the control.

APPENDIX I

PROTOCOL

STUDY PROTOCOL

LAMPREY (*LAMPETRA* sp.)
FLOW THROUGH ACUTE TOXICITY TEST

INTRODUCTION

Purpose of Study: The purpose of this study is to determine the acute toxicity of six chemicals, representing a range of toxic modes of action, to lamprey (*Lampetra* sp.) ammocoetes. The study will be performed using methods based on the methods for measuring acute toxicity with other fish species as described in U.S. Environmental Protection Agency (2002) and American Society for Testing and Materials (ASTM) (1996) guidance.

Summary of Method: In definitive tests, lamprey ammocoetes are exposed for 96 hours to five concentrations of test material and a dilution water control. The test concentrations are arranged in a logarithmic series based on the results of range finding tests. Four replicates, each with 10 organisms, are employed at each test concentration, if there are sufficient numbers of ammocoetes. Flow-through exposures are employed. Test chambers are glass aquaria less than or equal to two gallons in volume. Mortality is the effect criterion. The data analysis consists of calculating means and standard deviations for survival. An LC50 with 95% confidence intervals may be calculated along with the NOEC and LOEC concentrations.

STUDY MANAGEMENT

Sponsor's Name and Address:
Windward Environmental LLC
200 West Mercer Street, Suite 401
Seattle, WA 98119

Sponsor's Study Monitor:
Ms. Helle Andersen

Name of Testing Laboratory:
Northwestern Aquatic Sciences
3814 Yaquina Bay Road
P.O. Box 1437
Newport, OR 97365

Test Location: Newport, OR.

Laboratory's Key Personnel to be Assigned to the Study:
Laboratory Director: Richard S. Caldwell, Ph.D.
Project Manager/Study Director: Gary A. Buhler, B.S.
QA Officer/Toxicology Program Mgr: Linda K. Nemeth, B.A., M.B.A.
Aquatic Toxicologist: Gerald J. Irissarri, B.S.

Proposed Study Schedule: July 2007 through September 2007.

Good Laboratory Practices: The test is conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

TEST MATERIAL

Description: Chemicals to be tested include aniline, copper, pentachlorophenol, lindane, diazinon and naphthalene.

Preparation of Working Stock Solution and Test Concentrations: Test materials will be administered using a constant-flow diluter based on the design of Benoit *et al.* (1982). Stock solutions of the sparingly soluble organic compounds, e.g. naphthalene, pentachlorophenol, lindane, diazinon, will be prepared continuously by recirculation of dilution water/toxicant stock solution through a column of toxicant coated glass beads (Veith and Comstock, 1975). This is a conceptual approach in which the specific column, flow rates, glass bead sizes, etc. need to be developed empirically, with analytical feedback, for each study considering such variables as chemical solubility, test temperature, desired highest test concentration, stock toxicant consumption rate, etc. Given the above, some modifications are anticipated as the studies progress. Initial system characteristics are as follows: 1) the toxicant mixing tank will have a 60-liter capacity; 2) a 20 x 280-cm glass column will be employed (10 x 200-cm and 40 x 560-cm columns are also available); 3) the column packing will employ 5-mm glass beads coated with the test compound; 4) particulates will be filtered out through glass wool; 5) flow through column will be controlled by a variable-speed peristaltic pump or by intermittent pumping; and 6) tubing will be glass and silicone rubber. GC/MS analysis of toxicant in the toxicant mixing tank will be used to optimize the system for each chemical noted above prior to test initiation.The contents of the stock toxicant reservoir will be constantly replenished with fresh dilution water as toxicant solution is supplied to the diluter. The aniline stock solution will be prepared similarly, but by volumetric addition of aniline, using a metering pump, to the stock toxicant reservoir *in lieu* of the Veith column. The copper working stock solution will be prepared in the same apparatus by metering pump addition of a concentrated copper stock solution to the stock toxicant reservoir. All toxicant working stock solutions will be prepared and maintained under the same conditions as in the test exposures and delivered continuously to the toxicant chamber of the diluter at an appropriate rate.

DILUTION WATER

Dilution water will be dechlorinated City of Newport municipal tap water adjusted to a water hardness of 25-30 mg/L as $CaCO_3$.

TEST ORGANISMS

Species: Lamprey, *Lampetra* sp.

Source: Ammocoetes will be collected by Windward staff and delivered to the laboratory each day of collection. The ammocoetes will be collected from several rivers including the Siletz River, which was the site for Phase 1 ammocoetes.

Age: ammocoetes (juvenile prior to metamorphosis)

Acclimation and Pretest Observation: Test organisms will be held in the laboratory in order to acclimate to test temperature. Upon receipt, animals are removed from the sediment they are received in and placed in 10-gallon tanks under flow-through conditions (~35 mL/minute, or ~ 2 volume changes per day, with one additional daily siphon and replacement of 2/3 volume of each tank). Approximately 50 lamprey are placed in each tank. Tanks will contain at least 3 inches of sediment (Sandtastic Play Sand, Waupaca Materials, or similar material) covered with approximately 26L of test water and supplied with aeration. The maximum temperature variation during holding will be ± 1° C. The ammocoetes will be acclimated to the laboratory test conditions for a minimum of 5 to 7 days prior to test initiation. This minimum number of days applies only to the first toxicity test conducted on each batch of field-collected organisms. For all other tests, the ammocoetes will be held for at least 13 days prior to test initiation. When a temperature adjustment of more than 3 to 5°C is required, the transfer from field collection water to laboratory dilution water will be gradual. Normal behavior for lamprey ammocoetes is to immediately bury in sediment and remain there. Any dead animals observed on the sediment surface are removed daily. Past experience suggests that mortality is minimal during short-term holding and any mortality is likely due to damage from field collection. If ammocoetes do not bury or there is excessive

mortality in any tank, animals from that tank will not be used for testing. Ammocoetes will not be fed since they are being held short-term.

DESCRIPTION OF TEST SYSTEM

Preparation of Test Concentrations: Test materials will be administered using a constant-flow diluter based on the design of Benoit *et al.* (1982). The diluter will be calibrated to deliver a 0.5 dilution series. Flow rates will be adjusted to deliver at least five test chamber volumes per concentration per day. Diluter flow rates will be measured and documented at the beginning and conclusion of each 96-hour test. This will be done by measuring individual diluter capillary tube flows. When the flow rates are entered into an Excel® spreadsheet, the actual dilutions of the toxicant stock can be computed. Actual test chemical concentrations (organic chemicals) in the toxicant mixing chamber (toxicant stock solution) and in exposure aquaria will periodically be measured on a real-time basis at NAS to verify that nominal concentrations are being routinely achieved. Definitive concentration documentation in test aquaria will result from daily samples shipped for analysis to CAS.

Test Chambers and Environmental Control: Test chambers used in the toxicity study are glass aquaria less than or equal to two gallons in volume. The test system is housed in a constant-temperature, photoperiod-controlled room. Aeration is not employed. The photoperiod is constant dark except during monitoring activities when ambient (50-75 ft-c) lighting is used.

Cleaning: All laboratory glassware, including test chambers and toxicant dilution system, will be cleaned based on the methods described in U.S. Environmental Protection Agency (2002). Initial cleaning incorporates the use of a laboratory detergent followed by multiple tap water rinses. Subsequently, either dilute (10%, V:V) acid (hydrochloric or nitric) or acetone is used to remove metals or organic contaminants, respectively. Further multiple tap water rinses are employed after each acid or acetone treatment. Sodium hypochlorite bleach may be employed on a limited basis to ensure that microorganisms that could metabolize organic toxicants, e.g. naphthalene, are not present. Exhaustive tap water rinsing is always employed following any chemical cleaning treatment. A final dilution water rinse of the exposure apparatus will precede initiation of each study.

EXPERIMENTAL DESIGN AND TEST PROCEDURES

Experimental Design: The definitive test involves exposure of lamprey ammocoetes to five test concentrations, using a 0.5 dilution series, and a dilution water control. Exposures are for 96 hours. Each treatment consists of four replicates, each containing 10 ammocoetes, if there are sufficient numbers of ammocoetes. If there are not enough ammocoetes for 10 per replicate, then at least 20 per treatment (5 per replicate) is the minimum number preferred. Test chambers are randomly distributed in the test area. Ammocoetes are impartially distributed to each aquarium by adding one or two animals to each test replicate and repeating the process until each contains 10 organisms.

Effect Criterion: The effect criterion used in the lamprey ammocoete acute toxicity test is mortality, defined as a lack of visible respiratory movement and absence of response to tactile stimulation. The mortality data will be used to compute LC50s for each exposure period. In addition to mortality, qualitative observations of sublethal responses consisting of erratic swimming, hyperventilation, hemorrhaging, and hyper/hypoactivity (compared to the activity of the organisms in the control group) will be recorded if observed.

Test Conditions: The dissolved oxygen concentration in each test container must be > 4.0 mg/L throughout the test. The test temperature employed is 17 ± 1°C. A photoperiod of constant darkness is used, except during monitoring activities when illumination is supplied by daylight fluorescent lamps at an intensity of 50-75 ft candles. Loading should not exceed 6.1g/L at 17°C according.to EPA. ASTM recommends loading not exceed 1g/L of solution passing through the chamber in 24 hours or 10g/L of solution in the chamber at any time.

Beginning the Test: Adding the organisms to the equilibrated test containers as previously described begins the test.

Feeding: None.

Analysis of Test Concentrations: During each test, samples will be collected for chemical analysis, held at 4°C and shipped by overnight delivery to Columbia Analytical Services in Kelso, WA. The sampling schedule and procedure will follow the project QAPP.

Test Duration, Type and Frequency of Observations, and Methods: The test duration of the acute toxicity test is 96 hours. The type and frequency of observations to be made during the test are summarized as follows:

Type of Observation	Times of observation
Biological Data	
1. mortality (each test container)	0, 24, 48, 72, and 96 hr
2. sublethal observations (qualitative)	0, 24, 48, 72, and 96 hr
Physical and Chemical Data	
1. Dissolved oxygen, pH, & temperature, (in one replicate of each test level and the control)	0, 24, 48, 72, and 96 hr
2. Conductivity, hardness & alkalinity (in one replicate of highest concentration and control)	0, 24, 48, 72, and 96 hr
3. Toxicant concentration sampling (in one replicate of each test concentration and the control)	0, 24, 48, 72, and 96 hr
4. Dilution water sample for particulate matter, TOC and ammonia	0 hr
5. Sample for ammonia taken from one replicate of control and highest test concentration	96 hr

During the test, dead organisms are removed every 24 hours and frozen by concentration for possible tissue analysis. Dissolved oxygen is measured using a polarographic oxygen probe calibrated according to the manufacturer's recommendations. The pH and temperature are measured using a pH probe and a properly calibrated meter with scale divisions of 0.1 pH units. Conductivity is measured using a calibrated conductivity meter. Samples for chemical analysis will be collected, preserved, and sent to Columbia Analytical Services following the recommended procedures of the analytical laboratory.

Test Termination: At the end of the test, all surviving control ammocoetes are weighed and measured for length by replicate. All surviving or dead test organisms will be frozen by concentration for possible later tissue analysis by Columbia Analytical Services.

Criteria of Test Acceptance: The test results are acceptable if survival in the controls is at least 90%. The toxicity tests will be performed as a 50% dilution series, it is anticipated that each test will have at least one or possibly two partial mortality concentrations. Nominal concentrations of each toxicity test have be reviewed and approved by EPA (EPA 2007). However, because of the experimental nature of the study and the limited supply of ammocoetes, the acceptability of the toxicity tests will be based only on the ≥ 90% survival in control group.

DATA ANALYSIS

The percent of live ammocoetes is calculated for each treatment replicate from the raw data and the means are obtained for each treatment level. An LC50 (mortality) is calculated using Probit or Trimmed Spearman-Karber methods. The NOEC and LOEC are calculated for each endpoint according to U.S. Environmental Protection Agency (2002) methods. In this procedure, the NOEC and LOEC values are computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test is selected after evaluating the data for normality and homogeneity of variance. An arcsine-square root transformation is performed on the percentage effect data prior to statistical analysis. The statistical software employed for these calculations is Toxcalc, v.5.0.23N, Tidepool Scientific Software.

REPORTING

The final report of the study results must include all of the following standard information at a minimum:

1. Name and identification of the test;
2. The investigator and laboratory;
3. Information on the toxicants;
4. Information on the dilution water;
5. Detailed information about the test organisms;
6. Description of the test system and its calibration, including test chambers and dilution system;
7. A description of the experimental design and other test conditions including water quality;
8. Definition of the effect criteria and any other observations;
9. Responses, if any, in the control treatment;
10. Tabulation and statistical analysis of measured responses;
11. A description of the statistical methods used;
12. Any unusual information about the test or deviations from procedures;

The final study report will include the study protocol and all study raw data as appendices.

STUDY DESIGN ALTERATION

Amendments made to the protocol must be approved by the sponsor and study director and should include a description of the change; the reason for the change; the date the change took effect; and the dated signatures of the study director and sponsor. Any deviations in the protocol must be described and recorded in the study raw data.

REFERENCED GUIDELINES

ASTM. 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.

Benoit, D., V. Mattson, and D. Olson. 1982. A continuous-flow mini-diluter system for toxicity testing. Water Res. 16:457-464.

EPA. 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. Environmental Protection Agency, Washington, DC.

EPA. 2007. Letter to Lower Willamette Group from C. Humphrey and E. Blischke dated August 17, 2007 regarding Portland Harbor RI/FS: Lamprey toxicity test field sampling plan and QAPP Phase 2 addenda (approval letter). US Environmental Protection Agency Region 10, Oregon Operations Office, Portland, OR.

Veith G.D. and V.M. Comstock. 1975. Apparatus for continuously saturating water with hydrophobic organic chemicals. J Fish Res Board Can 32:1849-1851.

13. APPROVALS

_____ for **Windward Environmental**
Name Date

_____ for **Northwestern Aquatic Sciences**
Name Date

Appendix A
Test Conditions Summary

1. Test species:	*Lampetra* sp.
2. Test type:	Flow-through
3. Test duration:	96 hr
4. Temperature:	17 ± 1°C
5. Dissolved oxygen:	≥ 4.0 mg/L
6. Light quality & intensity:	Ambient laboratory light (50-75 ft-c)
7. Photoperiod:	Constant dark, except during monitoring activities
8. Test chambers:	glass aquaria less than or equal to two gallons in volume.
9. Test chamber volumes per day:	At least 5
10. Replicates per treatment:	4
11. Organisms per replicate:	10
12. Age of test organisms:	ammocoete
13. Feeding regime:	None
14. Aeration:	None
15. Dilution water:	Dechlorinated City of Newport tap water; hardness adjusted to 25-30 mg/L as $CaCO_3$
16. Test concentrations:	5 and a dilution water control
17. Water quality:	Temperature, pH, & dissolved oxygen in one replicate of each test concentration daily. Conductivity, hardness & alkalinity in one replicate of highest concentration and control daily
18. Special controls:	None
19. Endpoint:	Mortality
20. Test acceptability criteria:	≥90% control survival

APPENDIX II

RAW DATA

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-39 Client Windward Investigator 2/23/08

STUDY MANAGEMENT:

Client: Windward Environmental, 200 West Mercer Street, Suite 401, Seattle, WA 98119
Client's Study Monitor: Ms. Helle Andersen
Testing Laboratory: Northwestern Aquatic Sciences
Test Location: 3814 Yaquina Bay Road, Newport, OR 97365
Laboratory's Study Personnel:
Project Manager: Gary A. Buhler Study Director: Richard S.Caldwell
QA Officer: L.K. Nemeth 1. G.J. Irissarri
2. 3.
4. 5.
Study Schedule:
Test Beginning: 10-4-07 1615 Test Ending: 10-5-07 1600

TEST MATERIAL:

Description: Aniline Fisher Scientific Lot # 072020

DILUTION WATER:

Description: Dechlorined City of Newport tap water
Date of Preparation/Collection: Continuous flow
Water Quality: Cond. (µmhos/cm) 98.2 ± 1.6 pH 6.5 ± 0.1
Hardness (mg/L as $CaCO_3$) 30.2 ± 1.8 Alkalinity (mg/L as $CaCO_3$) 21.2 ± 1.1
Treatments: Hardness adjusted to 25-30 mg/L with a Culligan® water treatment system.

TEST ORGANISMS:

Species: Lamprey ammocoetes (*Lampetra* sp.) Age: Size: 4-6 cm
Sources: Trask, Nestucca, Siletz and Alsea rivers
Acclimation Data: See pages: 6, 7, 5-11

TEST PROCEDURES AND CONDITIONS:

Test Concentrations (nominal): 1200, 600, 300, 150, 75 & 0 mg/L
Test Chambers (description): 2.8 gallon glass aquaria Test Volume (ml): 5.80 L 2/21/08
Replicates/treatment: 4 Organisms/treatment: 40 (10/replicate)
Test water changes: flow through
Aeration during test: none unless DO drops below 60% saturation DO [illegible] ≥ 4.0 mg/L 4/3/08
Temperature (°C): 17 ± 1°C
Feeding: None Photoperiod: Darkness except for WQ & chemistry measurements
Test Duration (hr) 96 hours Tank Placement: Complete randomization

Randomization Chart:

Diluter

150	1200	150	600	0	1200	0	150	75	300	0	1200	600	75	75	600	150	300	300	1200	0	300	600	75

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-39 Client Windward Investigator

TEST SOLUTION PREPARATION:
See page 5 for details

Weight of 10 animals at test initiation
10-1-07 GAB

	weight (g)	Length (cm)	Comments
1	0.21	5.0	
2	0.24	5.5	
3	0.31	5.3	
4	0.19	5.1	
5	0.20	4.5	
6	0.24	4.8	
7	0.14	4.1	
8	0.33	5.6	
9	0.19	4.8	
10	0.24	5.0	
Mean	0.23	5.0	
S.D.	0.06	0.5	
(N)	(10)	(10)	

MISCELLANEOUS NOTES:

Room light intensity (fc): 15.7 10-4-07 GAB

Chemistry samples for aniline taken daily.

Chemistry samples for particulate matter, TOC & ammonia in dilution water taken on test day 0.

Chemistry samples for ammonia taken in control & 1200 A-replicate on test day 4.

$$\text{loading} = \frac{0.23\,g \times 10}{5.8\,L} = 0.40\,g/L$$

Error codes:
1) Correction of handwriting error
2) Written in wrong location; entry deleted
3) Wrong date deleted; replaced with correct date
4) Error found in measurement; measurement repeated

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-39 Client Windward Investigator

WATER QUALITY DATA SHEET

DAY 0 (10/1/07) GAB

Conc. (mg/L)	Temp. (°C)	pH	Cond. µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
1200	16.8	6.7	100	9.1	26	>500	10	10	10	10	All animals convulsing
600	16.4	6.6		8.9			10	10	10	10	All animals convulsing
300	16.5	6.6		8.8			10	10	10	10	
150	16.2	6.6		8.9			10	10	10	10	
75	16.4	6.7		9.0			10	10	10	10	
0	16.5	6.7	100	8.8	24	20	10	10	10	10	

Chemistry samples taken (10/1/07) GAB
Particulate matter, TOC, and ammonia samples taken in dilution water (10/1/07) GAB
Hours of light: 1-9

DAY 1 (10/2/07) GAB

Conc. (mg/L)	Temp. (°C)	pH	Cond. µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
1200	17.5	6.6	100	9.0	26	>500	0(10)	0(10)	0(10)	0(10)	
600	17.4	6.4		9.2			10	10	10	10	Twitching, pale color, swimming [illegible]
300	17.4	6.4		9.0			10	10	10	10	twitching, swimming erratically
150	17.2	6.3		9.3			10	10	10	10	
75	17.5	6.3		9.1			10	10	10	10	
0	17.6	6.6	100	9.1	26	21	10	10	10	10	

Chemistry samples taken (10/2/07) GAB
Hours of light: 1-0

DAY 2 (10/3/07) GAB

Conc. (mg/L)	Temp. (°C)	pH	Cond. µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
1200	17.1	6.4	97	9.1	26	>500	0	0	0	0	
600	16.9	6.4		9.0			9(10)	10	8(20)	9(10)	light colored, tetanus, mucus shedding *
300	17.0	6.3		8.9			10	10	10	10	light colored, twitching, mucus shedding *
150	16.9	6.3		9.2			10	10	10	10	lethargic or tetanus, some kinked
75	17.0	6.3		9.0			10	10	10	10	most kinked or bent
0	17.3	6.4	97	8.8	25	21	10	10	10	10	

Chemistry samples taken (10/3/07) GAB
Hours of light: 1-4

* Fish "kinked" or bent

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-39 Client Windward Investigator

WATER QUALITY DATA SHEET

DAY 3 (10/4/07) WM

Conc. (mg/L)	Temp. (°C)	pH	Cond. µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
1200	16.7	6.4	97	9.6	25	>500	0	0	0	0	
600	16.6	6.4		9.6			2(70)	5(50)	2(60)	4(50)	light color, mucous sloughing, hemorrhage, tetanus
300	16.6	6.3		9.5			10	9(10)	8(20)	10	light color, mucous sloughing, tetanus, hemorrhage
150	16.6	6.2		9.3			10	10	10	10	twitching of tetanus, kinked
75	16.7	6.2		9.4			10	10	10	10	some kinked
0	17.2	6.4	97	9.2	25	21	10	10	10	10	

Chemistry samples taken (10/4/07) WM
Hours of light: 1.0

DAY 4 (10/5/07) WM

Conc. (mg/L)	Temp. (°C)	pH	Cond. µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
1200	16.9	6.4	97	9.5	26	>500	0	0	0	0	
600	16.9	6.3		9.3			2(30)	2(30)	0(20)	3(10)	light color, mucous sloughing, tetanus, hemorrhage
300	16.9	6.2		9.4			7(30)	7(20)	6(20)	10	light color, tetanus, mucous sloughing, hemorrhage
150	16.8	6.3		9.2			10	10	10	10	kinked, hemorrhage, light color
75	17.0	6.4		9.4			10	10	10	10	kinked
0	17.2	6.5	97	9.1	25	23	10	10	10	10	

Chemistry samples taken (10/5/07) WM
Ammonia samples taken in control and 1200 mg/L (10/5/07) WM
Hours of light: 1.0

Dilution water HQ (Fish control water)

Cond	98.2 ± 1.6 (5)	BSN
pH	6.5 ± 0.1 (5)	2/2/07
~~Sp. Cond~~		
Hardness	26.2 ± 1.8 (5)	
Alkalinity	21.2 ± 1.1 (5)	

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-39 Client Windward Investigator

NOTES ON PREPARATION OF 1200 MG/L ANILINE STOCK

(Ca. 35 g/L) 2/21/08

SYSTEM DESIGN

The objective is to deliver 1200 mg/L of aniline (the highest test concentration) to the diluter toxicant chamber at a nominal constant rate of 160 ml/min. To accomplish this, a large mixing reservoir, approximately 54L capacity, of 1200 mg/L aniline solution in dilution water is maintained at test temperature. This is done by metering a constant supply of a saturated solution ~~(35 g/L (Merck))~~ of aniline in water into the large toxicant reservoir with vigorous mixing, at a rate of 192 mg/min (or 5.5 ml/min) as aniline. Makeup dilution water is also added as needed under float valve control. Approximately 9.5 L of aniline saturated stock solution is prepared daily.

Aniline metering pump flows (ml/min)

10/1/07	5.6
10/2/07	5.5
10/3/07	5.5
10/4/07	5.5

DILUTER CALIBRATIONS RECORD

DILUTER CAPILLARY FLOWS (ml/min)

Date 9/29/07	Time 1620	Init. BSL	Date 10/5/07	Time 1700	Init. BSL
Drain #	Supply Tank	Diluter Tank	Drain #	Supply Tank	Diluter Tank
1	165	80	1	160	78
2	82	81	2	80	78
3	85	82	3	84	78
4	82	78	4	82	78
5	78	81	5	86	79
6	105	78	6	100	76

Flow rates in ml/min

FLOWS TO REPLICATE AQUARIA (ml/cycle)

Date 9/29/07 Time 1640 Init. BSL

Replicate	Relative Concentration (%) 100	50	25	12.5	6.25	0
A	300	285	285	335	300	275
B	290	360	330	265	370	205
C	238	200	255	325	365	345
D	325	300	290	260	265	320

Date 10/5/07 Time 1730 Init. BSL

Replicate	Relative Concentration (%) 100	50	25	12.5	6.25	0
A	210	305	245	215	320	250
B	255	290	350	400	320	325
C	275	285	275	290	345	170
D	375	285	320	225	255	340

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (Lampetra sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
8-2-07	16.1	6.7	8.6	82	20	25	[illegible]	[illegible]	constant flow		[illegible]	[illegible]	[illegible]
8-2-07	16.8	7.7	9.6	83	26	30		"	"			[illegible]	GB
8-2-07	17.1	6.6	9.3	68	28	10	[illegible]	[illegible]	"			[illegible]	[illegible]
8-3-07	16.4	7.6	9.7	162	34	30	[illegible]	[illegible]	"			[illegible]	GB
8-3-07	18.1	6.1	7.2	60	22	16	[illegible]	[illegible]	"			[illegible]	GB
8-3-07	17.0	7.0	7.4	160	34	30						Holding	GB
8-3-07	16.9	6.6	9.0	60	31	25	Alder Creek		"			[illegible]	GB
8-3-07	16.8	7.2	7.5	135	34	30			"			[illegible]	[illegible]
9-1-07	16.9	7.1	7.4	140	—	—			"				
9-2-07	16.7	7.6	9.1	160	—	—			"			Holding	GJ
9-3-07	16.7	7.7	7.2	160	—	—			"			Holding	GJ
9-4-07	16.2	7.5	8.8	185	34	30			"			"	GB
9-5-07	16.3	7.2	9.1	170	34	30			"			"	GB
9-6-07	16.8	7.3	9.0	185	34	30			"			"	
9-7-07	16.8	7.0	9.1	155	26	30			"			"	
9-7-07	17.0	7.5	9.3	160	—	—			"			"	
9-9-07	16.7	7.5	8.8	170	—	—			"			Holding	LBS
9-10-07	16.7	7.2	8.9	175	—	—			"			"	AJ
9-11-07	17.4	7.6	9.0	235	60	35			"			"	LBS
9-12-07	17.0	8.0	9.4	185	60	38			"			"	AJ

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (Lampetra sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
9-13-07	17.1	8.0	9.0	170	55	30			constant flow			Holding	AJ
9-14-07	17.1	8.2	9.1	155	48	29			"		[illegible]	"	AJ
9-17-07	17.1	8.3	9.2	170	—	—			"			"	
9-16-07	17.0	8.1	9.2	115	41	30			"			"	GJ
9-17-07	17.1	7.7	8.8	120	41	30			"			"	LBS
9-18-07	17.0	7.8	9.1	120	39	21			"			"	GH
9-19-07	17.7	7.6	9.1	125	39	34			"			"	AJ
9-20-07	17.1	7.9	9.0	125	36	34	Taken for [illegible]		"			"	GH
9-21-07	17.0	8.2	9.5	140	44	31			"			"	AJ
9-22-07	17.1	7.7	9.4	130	42	29			"			"	
9-23-07	17.1	7.7	9.3	140	46	28			"			"	AJ
9-24-07	16.8	7.9	9.3	140	44	29			"			"	
9-25-07	17.1	7.7	9.8	145	44	30			"			"	AJ
9-26-07	17.2	7.8	9.3	140	48	36			"			"	AJ
9-27-07	17.4	7.7	9.3	145	48	37			"			"	AJ
9-28-07	17.2	7.8	9.3	150	48	36			"			"	AJ
9-29-07	16.9	7.5	8.8	150	48	30			"			"	MLR
9-30-07	17.0	7.2	8.8	140	48	37			"			"	MLR
10-1-07	17.0	7.8	9.4	145	48	36			"		[illegible]	"	GB
10-2-07	17.3	8.1	9.2	135	—	—			"			"	GH

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (Lampetra sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
10-3-07	17.0	7.8	9.1	130	46	55			constant flow			Holding	GH
10-4-07	16.9	7.9	9.2	120	42	38						"	AJ
10-5-07	16.9	7.4	9.0	130	not Taken LBS							"	LBS
10-6-07	16.6	7.2	9.4	130	44	41						"	GH
10-7-07	17.0	7.4	9.5	135	60	50						"	GH
10-8-07	17.0	7.9	9.2	145	50	48						"	GH
10-9-07	17.0	7.6	9.5	150	54	52						"	GH
10-10-07	16.9	7.4	9.2	155	54	49						"	GH
10-11-07	17.0	7.1	9.5	150	54	40						"	AJ
10-12-07	17.1	8.0	9.4	155	50	39						"	AJ
10-13-07	17.1	7.1	9.1	150	52	40						"	GH
10-14-07	16.9	7.5	9.6	150	52	38						"	AJ
10-15-07	17.0	7.6	9.5	155	50	38						"	AJ
10-5-07	13.0	6.7	10.6	97	30	30						Receiving Tank	[illegible]
10-16-07	13.2	7.9	10.1	145	60	57						Holding Room #4	AJ
10-16-07	17.1	6.9	9.4	65	20	12						Test animals from Room #1	AJ
10-16-07	12.4	6.2	8.7	65	18	14						NEWTON CREEK DECEMBER	GJ
10-17-07	13.5	8.3	10.1	135	60	55						Holding [illegible]	GH
10-17-07	13.3	6.7	8.5	75	22	14						Holding from #1 Siletz River [illegible]	GH
10-18-07	13.1	6.8	10.2	57	20	14							

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (Lampetra sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
10-18-07	13.2	8.3	9.9	130	48	36			constant flow			Holding	AJ
10-19-07	13.3	7.5	9.5	120	48	36						"	LBS
10-20-07	13.2	7.7	10.0	125	46	38							
10-21-07	14.2	7.6	9.9	130	48	37						"	AJ
10-22-07	14.2	7.7	9.4	135	50	40						"	AJ
10-23-07	15.1	7.9	9.7	145	54	44						"	AJ
10-24-07	15.6	8.3	9.7	155	58	47						"	AJ
10-25-07	16.1	8.5	9.5	160	66	53						"	AJ
10-26-07	16.8	8.1	9.6	165	60	47						"	AJ
10-27-07	16.8	8.3	9.9	155	62	49						"	AJ
10-28-07	16.6	8.3	9.9	165	62	47						"	AJ
10-29-07	17.0	8.1	9.5	165	62	48						"	AJ
10-30-07	16.9	8.2	9.6	170	64	46						"	AJ
10-31-07	16.8	8.2	9.9	175	70	54						"	AJ
11-1-07	16.8	8.2	9.8	165	62	45						"	AJ
11-2-07	16.8	8.2	9.5	165	62	47						"	AJ
11-3-07	16.6	8.1	9.2	160	56	38						"	GJ
11-4-07	16.8	8.3	9.6	180	64	48						"	LBS
11-5-07	16.9	8.1	9.9	180	66	49						"	AJ
11-6-07	16.9	8.2	9.8	155	52	35						"	AJ

Acclimation Records

Cont

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
11-7-07	17.0	8.1	9.4	155	54	37			constant flow			Holding	AP
11-8-07	16.9	8.1	9.3	155	56	39						"	CB
11-9-07	16.7	8.6	9.9	160	62	46						"	CB
11-10-07	16.7	8.1	9.6	155	56	43						"	CB
11-11-07	16.7	8.1	9.9	145	52	39						"	GJ
11-12-07	16.8	8.0	9.7	155	56	38						"	CB
11-13-07	16.8	7.6	9.7	155	54	38						"	AP
11-14-07	16.8	7.9	9.7	150	52	36						"	AP
11-15-07	16.8	8.0	9.7	160	60	43						"	AP
11-16-07	16.9	8.2	9.9	160	58	39						"	GJ
11-17-07	16.7	8.3	10.1	165	62	43						"	AP
11-18-07	16.9	8.1	9.8	150	54	36						"	CB
11-19-07	16.8	7.9	9.7	175	64	48						"	AP
11-20-07	16.7	7.9	9.9	170	64	46						"	AP
11-21-07	16.6	8.0	9.9	175	64	48						"	AP
11-22-07	16.5	7.9	10.0	165	62	39						"	AP
11-23-07	16.5	7.8	9.5	170	62	44						"	CB
11-24-07	16.5	8.0	9.7	190	70	48						"	AP
11-25-07	16.5	7.9	9.6	175	64	45						"	CB
11-26-07	16.6	7.5	9.6	175	64	46						"	AP

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
11-29-07	16.1	7.9	9.6	180	62	44			constant flow			Holding	AP
11-30-07	16.0	7.7	10.0	175	56	44						"	AP
12-1-07	16.0	7.8	9.7	170	60	43						"	CB
12-2-07	16.0	8.0	9.7	175	62	46						"	GJ
12-3-07	16.2	7.9	9.7	185	70	47						"	AP
12-4-07	15.8	7.5	9.4	165	60	43						"	AP
12-5-07	15.7	8.2	9.6	155	60	39						"	AP
12-6-07	15.8	7.7	9.8	170	60	42						"	GJ
12-7-07	16.2	7.6	9.9	165	60	42						"	AP
12-8-07	15.7	7.6	9.9	160	58	40						"	CB
12-9-07	17.8	7.4	9.0	175	64	43						"	GJ
12-10-07	17.1	7.3	8.8	165	62	42						"	CB
12-11-07	17.1	7.2	9.4	170	56	41						"	GJ
12-12-07	17.3	7.4	9.5	160	58	40						"	CB
12-13-07	16.7	7.2	9.4	170	64	43						"	GJ
12-14-07	16.8	7.3	9.1	170	64	44						"	GJ
12-15-07	17.0	7.2	9.0	165	56	44						"	CB
12-16-07	16.5	7.2	9.5	190	70	45						"	GJ
12-17-07	16.7	7.2	9.3	170	66	44						"	CB
12-18-07	16.5	7.4	9.6	195	74	53						"	AP

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
12-19-07	15.6	7.3	9.9	190	76	51			constant flow			HOLDING	GJ
12-20-07	16.2	7.2	10.3	175	56	46						"	CB
12-21-07	15.4	8.2	10.0	190	72	48						"	GJ
12-22-07	16.0	7.8	9.8	145	70	46							
12-23-07	17.1	8.0	9.5	195	68	54						"	GJ
12-24-07	17.1	8.0	9.4	190	72	48						"	GJ
12-25-07	16.8	8.1	9.6	190	72	51						"	GJ
12-26-07	16.8	8.3	10.1	200	74	50						"	AP
12-27-07	17.1	8.2	9.7	195	68	49						"	AP
12-28-07	17.0	8.0	9.8	170	56	32						"	CB
12-29-07	17.1	8.1	9.9	175	52	29						"	CB
12-30-07	16.9	8.0	9.2	195	68	50						"	GJ
12-31-07	16.8	8.3	9.8	205	72	50						"	CB
1-1-08	17.0	9.0	9.6	[illegible]	64	43						"	GJ
1-2-08	16.8	8.2	9.4	185	66	41						"	AP
1-3-08	16.8	8.3	9.7	180	60	39						"	CB
1-4-08	16.9	8.2	9.8	175	62	36						"	CB
1-5-08	16.7	8.4	9.7	155	48	30						"	AP
1-6-08	16.7	8.4	9.9	160	54	32						"	AP
1-7-08	16.9	8.1	9.5	165	50	33						"	AP

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
1-9-08	15.6	8.4	10.6	165	56	30			constant flow			HOLDING	GJ
1-10-08	15.9	8.0	10.2	145	44	24						"	CB
1-11-08	16.3	8.1	10.3	145	42	24							GJ
1-12-08	16.0	8.4	10.2	165	56	31						"	GJ
1-13-08	16.2	8.1	9.6	170	54	32						"	AP
1-14-08	16.5	8.2	9.9	160	52	32						"	CB
1-15-08	16.2	8.2	9.7	160	50	29						"	CB
1-16-08	16.4	8.2	9.9	175	70	15						"	CB
1-17-08	15.6	8.0	9.9	165	86	63						"	CB

Acclimation Records

Fig 9.1

Samples shipped to CAS

Test in 656-39

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd., P.O. Box 1437, Newport, OR 97365
Tel. 541-265-7225, Fax. 541-265-2799, www.nwaquatic.com

Client Name: Integral Consulting, Inc
Address: 319 SW Washington St., Suite 1150
City, State, Zip code: Portland, OR 97204
Project No: Integral B01-01-58C; Windward 07-28-04-53
Phone No: 503-284-5545
Report Attention: Abbie Spielman
Sampled by: D. S. Caldwell
Shipping Information: Carrier: UPS; UPS#Y3489E; Airbill No: 1Z8F9167010353258
Testing Required: Copper, Lindane, Pentachlorophenol, Aniline, Diazinon, Naphthalene

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	10-2-07	1030	NAS# 1310G Day 1 - 0 mg/L Aniline	1				X			
	10-2-07		NAS# 1311G Day 1 - 75 mg/L Aniline	1				X			
	10-2-07		NAS# 1312G Day 1 - 150 mg/L Aniline	1				X			
	10-2-07		NAS# 1313G Day 1 - 300 mg/L Aniline	1				X			
	10-2-07		NAS# 1314G Day 1 - 600 mg/L Aniline	1				X			
	10-2-07		NAS# 1315G Day 1 - 1200 mg/L Aniline	1				X			

Relinquished by (Signature)	Print Name	Company	Date	Time	Cooler Custody Seal
Gary Bible	Gary Bible	Northwestern Aquatic Sciences	10-2-07	1110	Present / Not Present; Intact / Not Intact
Received by					Internal Cooler Temperature Upon Lab Receipt (°C)
Relinquished by					
Received by					
Relinquished by					
Received by laboratory					

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd., P.O. Box 1437, Newport, OR 97365
Tel. 541-265-7225, Fax. 541-265-2799, www.nwaquatic.com

Client Name: Integral Consulting, Inc
Address: 319 SW Washington St., Suite 1150
City, State, Zip code: Portland, OR 97204
Project No: Integral B01-01-58C; Windward 07-28-04-53
Phone No: 503-284-5545
Report Attention: Abbie Spielman
Sampled by: GAO, WJ
Shipping Information: Carrier: UPS; UPS#Y3489E; Airbill No: 1Z8F9167010353258
Testing Required: Copper, Lindane, Pentachlorophenol, Aniline, Diazinon, Naphthalene

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	10-4-07	0930	NAS# 1324G Day 3 - 0 mg/L Aniline	1				X			
	10-4-07		NAS# 1325G Day 3 - 75 mg/L Aniline	1				X			
	10-4-07		NAS# 1326G Day 3 - 150 mg/L Aniline	1				X			
	10-4-07		NAS# 1327G Day 3 - 300 mg/L Aniline	1				X			
	10-4-07		NAS# 1328G Day 3 - 600 mg/L Aniline	1				X			
	10-4-07		NAS# 1329G Day 3 - 1200 mg/L Aniline	1				X			

Relinquished by (Signature)	Print Name	Company	Date	Time	Cooler Custody Seal
Gerald Irisarri	GERALD IRISARRI	Northwestern Aquatic Sciences	10-4-07	1500	Present / Not Present; Intact / Not Intact
Received by					Internal Cooler Temperature Upon Lab Receipt (°C)
Relinquished by					
Received by					
Relinquished by					
Received by laboratory					

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd., P.O. Box 1437, Newport, OR 97365
Tel. 541-265-7225, Fax. 541-265-2799, www.nwaquatic.com

Client Name: Integral Consulting, Inc
Address: 319 SW Washington St., Suite 1150
City, State, Zip code: Portland, OR 97204
Project No: Integral B01-01-58C; Windward 07-28-04-53
Phone No: 503-284-5545
Report Attention: Abbie Spielman
Sampled by: D. S. Caldwell
Shipping Information: Carrier: UPS; UPS#Y3489E; Airbill No: 1Z8F9167010353258
Testing Required: Copper, Lindane, Pentachlorophenol, Aniline, Diazinon, Naphthalene

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	10-1-07	1600	NAS# 1301G Day 0 - 0 mg/L Aniline	1				X			
	10-1-07		NAS# 1302G Day 0 - 75 mg/L Aniline	1				X			
	10-1-07		NAS# 1303G Day 0 - 150 mg/L Aniline	1				X			
	10-1-07		NAS# 1304G Day 0 - 300 mg/L Aniline	1				X			
	10-1-07		NAS# 1305G Day 0 - 600 mg/L Aniline	1				X			
	10-1-07		NAS# 1306G Day 0 - 1200 mg/L Aniline	1				X			
	10-1-07		NAS# 1307G Day 0 - TSS	1							
	10-1-07		NAS# 1308G Day 0 - TSS	1							
	10-1-07		NAS# 1309G Day 0 - Ammonia	1							

Relinquished by (Signature)	Print Name	Company	Date	Time	Cooler Custody Seal
Gary Bible	Gary Bible	Northwestern Aquatic Sciences	10-2-07	1110	Present / Not Present; Intact / Not Intact
Received by					Internal Cooler Temperature Upon Lab Receipt (°C)
Relinquished by					
Received by					
Relinquished by					
Received by laboratory					

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd., P.O. Box 1437, Newport, OR 97365
Tel. 541-265-7225, Fax. 541-265-2799, www.nwaquatic.com

Client Name: Integral Consulting, Inc
Address: 319 SW Washington St., Suite 1150
City, State, Zip code: Portland, OR 97204
Project No: Integral B01-01-58C; Windward 07-28-04-53
Phone No: 503-284-5545
Report Attention: Abbie Spielman
Sampled by: GAO, WJ
Shipping Information: Carrier: UPS; UPS#Y3489E; Airbill No: 1Z8F9167010353258
Testing Required: Copper, Lindane, Pentachlorophenol, Aniline, Diazinon, Naphthalene

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	10-3-07	1510	NAS# 1316G Day 2 - 0 mg/L Aniline	1				X			
	10-3-07		NAS# 1317G Day 2 - 75 mg/L Aniline	1				X			
	10-3-07		NAS# 1318G Day 2 - 150 mg/L Aniline	1				X			
	10-3-07		NAS# 1319G Day 2 - 300 mg/L Aniline	1				X			
	10-3-07		NAS# 1320G Day 2 - 600 mg/L Aniline	1				X			
	10-3-07		NAS# 1321G Day 2 - 1200 mg/L Aniline	1				X			

Relinquished by (Signature)	Print Name	Company	Date	Time	Cooler Custody Seal
Gerald Irisarri	GERALD IRISARRI	Northwestern Aquatic Sciences	10-4-07	1500	Present / Not Present; Intact / Not Intact
Received by					Internal Cooler Temperature Upon Lab Receipt (°C)
Relinquished by					
Received by					
Relinquished by					
Received by laboratory					

Samples Shipped to CAS

Test 7/6 656-39

9 of 19

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
1814 Yaquina Bay Rd., P.O. Box 1437, Newport, OR 97365
Tel 541-265-7225, Fax 541-265-2709, www.nwaquatic.com

Client Name: Integral Consulting, Inc
Address: 319 SW Washington St., Suite 1150
City, State, Zip code: Portland, OR 97204
Project No: Integral B01-0-58C Windward 07-28-04-53
Phone No: 503-284-5545
Report Attention: Abbie Spielman
Shipping Information: Carrier: UPS UPS#V3489E
Airbill No: 1Z999E9 01203 3276
Testing Required

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Cyanide	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	10-5-07	1450	NASS 1330G Day 4 - 0 mg/L Aniline	1				X			
	10-5-07		NASS 1331G Day 4 - 75 mg/L Aniline	1				X			
	10-5-07		NASS 1332G Day 4 - 150 mg/L Aniline	1				X			
	10-5-07		NASS 1333G Day 4 - 300 mg/L Aniline	1				X			
	10-5-07		NASS 1334G Day 4 - 600 mg/L Aniline	1				X			
	10-5-07		NASS 1335G Day 4 - 1200 mg/L Aniline	1				X			
	10-5-07		NASS 1336G Day 4 Antimony	1							
	10-5-07		NASS 1337G Day 4 300 mg/L Aniline, Diesel	1				X			

Signature	Print Name	Company	Date	Time	Cooler Custody Seal
Relinquished by: [signature]	GERALD EISGACER	Northwestern Aquatic Sciences	10-7-07	1500	Present / Not Present
Received by:					Intact / Not Intact
Relinquished by:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received by:					
Relinquished by:					
Received by laboratory:					

Water Quality Computation - Test No. 686-39

	Temp	DO	pH	Cond	Hard	Alk
1	16.8	9.1	6.7	100	26	20
2	16.4	8.9	6.6	100	24	21
3	16.5	8.8	6.6	100	26	21
4	16.2	8.9	6.6	100	26	21
5	16.4	9.0	6.7	97	26	23
6	16.5	8.8	6.7	97	25	
7	17.5	9.0	6.6	97	25	
8	17.4	9.2	6.4	97	28	
9	17.4	9.0	6.4	97	26	
10	17.2	9.3	6.3	97	28	
11	17.5	9.1	6.3			
12	17.6	9.1	6.6			
13	17.1	9.1	6.4			
14	16.9	9.0	6.4			
15	17.0	8.9	6.3			
16	16.9	9.2	6.3			
17	17.0	9.0	6.3			
18	17.3	8.8	6.4			
19	16.7	9.6	6.4			
20	16.6	9.6	6.4			
21	16.6	9.5	6.3			
22	16.6	9.3	6.2			
23	16.7	9.4	6.2			
24	17.2	9.2	6.4			
25	16.9	9.5	6.4			
26	16.9	9.3	6.3			
27	16.9	9.4	6.2			
28	16.8	9.2	6.3			
29	17.0	9.4	6.4			
30	17.2	9.1	6.5			
31						
32						
mean	16.9	9.2	6.4	98	26	21
s.d.	0.4	0.2	0.2	2	1	1
N	30	30	30	10	10	5
min	16.2	8.8	6.2	97	24	20
max	17.6	9.6	6.7	100	28	23

4/3/08

WQ Computation - acclimation (9-15-07 to 9-30-07) - Test No. 686-39

	Temp	DO	pH	Cond	Hard	Alk
1	17 1	9.2	8.3	120		
2	17.0	9.2	8.1	115	41	30
3	17 1	8.8	7 7	120	41	30
4	17.0	9.1	7.8	120	39	21
5	17 7	9.1	7.6	125	39	34
6	17 1	9.0	7 9	125	36	34
7	17.0	9.5	8.2	140	44	31
8	17 1	9.4	7.9	130	42	29
9	17 1	9.3	7 7	140	46	28
10	16.8	9.3	7.9	140	44	29
11	17 1	8.8	7 7	145	44	30
12	17.2	9.3	7.8	140	48	36
13	17.4	9.3	7.7	145	48	37
14	17.2	9.3	7.8	150	48	36
15	16.9	8.8	7.5	150	48	36
16	17.0	8 8	7.2	140	48	37
17						
mean	17.1	9.1	7.8	134	44	32
s.d.	0.2	0.2	0.3	12	4	4
N	16	16	16	16	15	15
min	16.8	8.8	7.2	115	36	21
max	17 7	9.5	8.3	150	48	37

RSW
2/21/08

SampleDate	SampleTime	Matrix	LabMatrix	SampleID	arentSamp	SampleType	Method	AnalysisDate	nalysisTim	nalyteGrou	cas_rn	Analyte	l_or_dissol	detect_flag	VALUE	Qualifiers	Units
10/1/2007	16:00	WQ	WQ	NAS#1301G Day 0 - 0mg/L Aniline		N	SW8270C	10/12/2007	13:15	SVOCs	62-53-3	Aniline	N	Y	1800		ug/l
10/1/2007	16:00	WQ	WQ	NAS#1302G Day 0 - 75mg/L Aniline		N	SW8270C	10/15/2007	13:21	SVOCs	62-53-3	Aniline	N	Y	100000		ug/l
10/1/2007	16:00	WQ	WQ	NAS#1303G Day 0 - 150mg/L Aniline		N	SW8270C	10/15/2007	14:01	SVOCs	62-53-3	Aniline	N	Y	200000		ug/l
10/1/2007	16:00	WQ	WQ	NAS#1304G Day 0 - 300mg/L Aniline		N	SW8270C	10/15/2007	14:41	SVOCs	62-53-3	Aniline	N	Y	320000		ug/l
10/1/2007	16:00	WQ	WQ	NAS#1305G Day 0 - 600mg/L Aniline		N	SW8270C	10/15/2007	15:20	SVOCs	62-53-3	Aniline	N	Y	730000		ug/l
10/1/2007	16:00	WQ	WQ	NAS#1306G Day 0 - 1200mg/L Aniline		N	SW8270C	10/15/2007	18:40	SVOCs	62-53-3	Aniline	N	Y	1500000		ug/l
10/2/2007	10:30	WQ	WQ	NAS#1310G Day 1 - 0mg/L Aniline		N	SW8270C	10/12/2007	12:35	SVOCs	62-53-3	Aniline	N	Y	400		ug/l
10/2/2007	10:30	WQ	WQ	NAS#1311G Day 1 - 75mg/L Aniline		N	SW8270C	10/15/2007	10:41	SVOCs	62-53-3	Aniline	N	Y	95000		ug/l
10/2/2007	10:30	WQ	WQ	NAS#1312G Day 1 - 150mg/L Aniline		N	SW8270C	10/15/2007	11:21	SVOCs	62-53-3	Aniline	N	Y	200000		ug/l
10/2/2007	10:30	WQ	WQ	NAS#1313G Day 1 - 300mg/L Aniline		N	SW8270C	10/15/2007	16:00	SVOCs	62-53-3	Aniline	N	Y	360000		ug/l
10/2/2007	10:30	WQ	WQ	NAS#1314G Day 1 - 600mg/L Aniline		N	SW8270C	10/15/2007	12:41	SVOCs	62-53-3	Aniline	N	Y	710000		ug/l
10/2/2007	10:30	WQ	WQ	NAS#1315G Day 1 - 1200mg/l Aniline		N	SW8270C	10/15/2007	18:00	SVOCs	62-53-3	Aniline	N	Y	1500000		ug/l
10/3/2007	15:10	WQ	WQ	NAS#1316G Day 2 - 0 mg/L Aniline		N	SW8270C	10/30/2007	12:22	SVOCs	62-53-3	Aniline	N	Y	170		ug/l
10/3/2007	15:10	WQ	WQ	NAS#1317G Day 2 - 75 mg/L Aniline		N	SW8270C	10/31/2007	17:01	SVOCs	62-53-3	Aniline	N	Y	130000		ug/l
10/3/2007	15:10	WQ	WQ	NAS#1318G Day 2 - 150 mg/L Aniline		N	SW8270C	10/31/2007	17:39	SVOCs	62-53-3	Aniline	N	Y	160000		ug/l
10/3/2007	15:10	WQ	WQ	NAS#1319G Day 2 - 300 mg/L Aniline		N	SW8270C	10/31/2007	18:17	SVOCs	62-53-3	Aniline	N	Y	310000		ug/l
10/3/2007	15:10	WQ	WQ	NAS#1320G Day 2 - 600 mg/L Aniline		N	SW8270C	10/31/2007	18:55	SVOCs	62-53-3	Aniline	N	Y	580000		ug/l
10/3/2007	15:10	WQ	WQ	NAS#1321G Day 2 - 1200 mg/L Aniline		N	SW8270C	11/7/2007	17:00	SVOCs	62-53-3	Aniline	N	Y	1200000		ug/l
10/4/2007	09:30	WQ	WQ	NAS#1324G Day 3 - 0 mg/L Aniline		N	SW8270C	10/31/2007	15:26	SVOCs	62-53-3	Aniline	N	Y	280		ug/l
10/4/2007	09:30	WQ	WQ	NAS#1325G Day 3 - 75 mg/L Aniline		N	SW8270C	11/7/2007	17:40	SVOCs	62-53-3	Aniline	N	Y	110000		ug/l
10/4/2007	09:30	WQ	WQ	NAS#1326G Day 3 - 150 mg/L Aniline		N	SW8270C	11/1/2007	18:07	SVOCs	62-53-3	Aniline	N	Y	170000		ug/l
10/4/2007	09:30	WQ	WQ	NAS#1327G Day 3 - 300 mg/L Aniline		N	SW8270C	11/7/2007	18:19	SVOCs	62-53-3	Aniline	N	Y	300000		ug/l
10/4/2007	09:30	WQ	WQ	NAS#1328G Day 3 - 600 mg/L Aniline		N	SW8270C	11/7/2007	18:58	SVOCs	62-53-3	Aniline	N	Y	620000		ug/l
10/4/2007	09:30	WQ	WQ	NAS#1329G Day 3 - 1200 mg/L Aniline		N	SW8270C	11/1/2007	17:29	SVOCs	62-53-3	Aniline	N	Y	1200000		ug/l
10/5/2007	14:20	WQ	WQ	NAS# 1330G Day 4--0 mg/L Aniline		N	SW8270C	10/30/2007	13:38	SVOCs	62-53-3	Aniline	N	Y	65		ug/l
10/5/2007	14:20	WQ	WQ	NAS# 1331G Day 4--75 mg/L Aniline		N	SW8270C	11/8/2007	12:34	SVOCs	62-53-3	Aniline	N	Y	130000		ug/l
10/5/2007	14:20	WQ	WQ	NAS# 1332G Day 4--150 mg/L Aniline		N	SW8270C	11/1/2007	18:45	SVOCs	62-53-3	Aniline	N	Y	150000		ug/l
10/5/2007	14:20	WQ	WQ	NAS# 1333G Day 4- 300 mg/L Aniline		N	SW8270C	11/3/2007	21:05	SVOCs	62-53-3	Aniline	N	Y	280000		ug/l
10/5/2007	14:20	WQ	WQ	NAS# 1337G Day 4--300 mg/L Aniline Dupl.		N	SW8270C	11/8/2007	13:53	SVOCs	62-53-3	Aniline	N	Y	320000		ug/l
10/5/2007	14:20	WQ	WQ	NAS# 1334G Day 4--600 mg/L Aniline		N	SW8270C	11/3/2007	21:43	SVOCs	62-53-3	Aniline	N	Y	530000		ug/l
10/5/2007	14:20	WQ	WQ	NAS# 1335G Day 4--1200 mg/L Aniline		N	SW8270C	11/8/2007	13:14	SVOCs	62-53-3	Aniline	N	Y	1100000		ug/l

Aniline (mg/L)	Day 0	Day 1	Day 2	Day 3	Day 4	Average	Standard Deviation
0	1.8	0.4	0.2	0.3	0.1	0.5	0.7
75	100	95	130	110	130	113	16
150	200	200	160	170	150	176	23
300	320	360	310	300	300	318	25
600	730	710	580	620	530	634	85
1200	1500	1500	1200	1200	1100	1300	187

Validated Aniline Analyses
Reported by Windward
PSM 2/18/08

686-39

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Acute 96-hr Toxicity Test-96 Hr Survival

Start Date:	10/1/07 16:15	Test ID:	686-39	Sample ID:	
End Date:	10/5/07 16:00	Lab ID:	ORNAS-Northwestern Aquati	Sample Type:	Aniline
Sample Date:		Protocol:	NAS-686-Lamprey Ft acute	Test Species:	LY-Lampetra sp.
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
113	1.0000	1.0000	1.0000	1.0000
176	1.0000	1.0000	1.0000	1.0000
318	0.7000	0.7000	0.6000	1.0000
634	0.2000	0.2000	0.0000	0.3000
1300	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical		Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4				0	40
113	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00		0	40
176	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00		0	40
318	0.7500	0.7500	1.0701	0.8861	1.4120	21.798	4	12.00	10.00		10	40
*634	0.1750	0.1750	0.4164	0.1588	0.5796	43.287	4	10.00	10.00		33	40
1300	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4				40	40

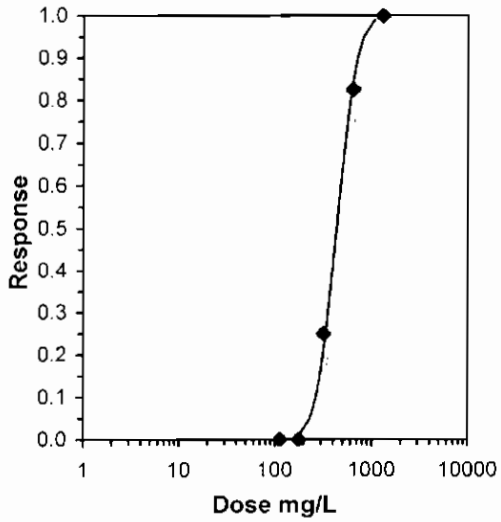
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.79057	0.868	0.73529	4.14079
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	318	634	449.012	

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	6.01036	0.81925	4.40464	7.61608	0	0.88158	7.81472	0.83	2.63301	0.16638	4
Intercept	-10.825	2.15221	-15.044	-6.607							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	176.181	125.131	217.282
EC05	3.355	228.742	176.868	269.738
EC10	3.718	262.9	212.002	303.692
EC15	3.964	288.784	239.043	329.71
EC20	4.158	311.162	262.499	352.606
EC25	4.326	331.737	283.974	374.133
EC40	4.747	389.819	343.031	438.412
EC50	5.000	429.551	381.302	486.105
EC60	5.253	473.333	421.177	542.397
EC75	5.674	556.206	491.045	658.522
EC80	5.842	592.984	520.273	713.426
EC85	6.036	638.934	555.664	784.47
EC90	6.282	701.84	602.52	885.627
EC95	6.645	806.649	677.532	1062.86
EC99	7.326	1047.3	840.06	1504.18



Test: AT-Acute 96-hr Toxicity Test
Test ID: 686-39
Species: LY-Lampetra sp.
Protocol: NAS-686-Lamprey Ft acute
Sample ID:
Sample Type: Aniline
Start Date: 10/1/07 16:15
End Date: 10/5/07 16:00
Lab ID: ORNAS-Northwestern Aquatic Sciences

Pos	ID	Rep	Group	Start	24 Hr	48 Hr	72 Hr	96 Hr	Notes
	1	1	D-Control	10				10	
	2	2	D-Control	10				10	
	3	3	D-Control	10				10	
	4	4	D-Control	10				10	
	5	1	113.000	10				10	
	6	2	113.000	10				10	
	7	3	113.000	10				10	
	8	4	113.000	10				10	
	9	1	176.000	10				10	
	10	2	176.000	10				10	
	11	3	176.000	10				10	
	12	4	176.000	10				10	
	13	1	318.000	10				7	
	14	2	318.000	10				7	
	15	3	318.000	10				6	
	16	4	318.000	10				10	
	17	1	634.000	10				2	
	18	2	634.000	10				2	
	19	3	634.000	10				0	
	20	4	634.000	10				3	
	21	1	1300.000	10				0	
	22	2	1300.000	10				0	
	23	3	1300.000	10				0	
	24	4	1300.000	10				0	

Comments:

LAMPREY (*LAMPETRA* SP.) DEFINITIVE 96-HR FLOW-THROUGH ACUTE TOXICITY TEST OF LINDANE

NAS Test No. 686-40

Submitted to

Windward Environmental LLC
200 West Mercer Street, Suite 401
Seattle, WA 98119

Submitted by

Northwestern Aquatic Sciences
3814 Yaquina Bay Road
P.O. Box 1437
Newport, OR 97365

April 3, 2008

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 686-40
Title: Lamprey (*Lampetra* sp.) definitive 96-hr flow-through acute toxicity test of lindane.
Protocol No.: NAS-686-Lamprey FT acute, September 12, 2007

STUDY MANAGEMENT

Study Sponsor: Windward Environmental, 200 West Mercer Street, Suite 401, Seattle, WA 98119.
Sponsor's Study Monitor: Ms. Helle Andersen.
Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365.
Test Location: Newport Laboratory.
Laboratory's Study Personnel: G. A. Buhler, B.S., Proj. Manager; R. S. Caldwell, Ph.D., Study Director; L.K. Nemeth, B.A., M.B.A., QA Officer; G.J. Irissarri, B.S., Aq. Toxicol.
Study Schedule:
Test Beginning: 10-18-07, 1100 hrs.
Test Ending: 10-22-07, 1215 hrs.
Disposition of Study Records: All specimens, raw data, reports and other study records are stored according to Good Laboratory Practice regulations at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.
Good Laboratory Practices: The test was conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).
Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Description: Lindane (1,2,3,4,5,6-Hexachlorocyclohexane, γ-isomer, 97%) Aldrich Lot No. 07325DD.
Preparation of Working Stock Solution and Test Concentrations: Test solutions were prepared for continuous supply to the test aquaria using a Benoit constant flow diluter (Benoit *et al.*, 1982). The diluter was set up to deliver a 0.5 dilution series with a flow rate of approximately 80 ml/min per toxicant concentration. The diluter design was such that 160 ml/min of the toxicant solution at the highest test concentration (i.e. the water saturation level of lindane initially estimated to be approximately 8 mg/L at the test temperature) was required to be continuously delivered to the toxicant supply chamber of the diluter. To accomplish this, a large mixing reservoir, approximately 54L capacity, to contain the saturated solution of lindane in dilution water, was maintained at test temperature. The approach to preparing the saturated lindane solution in the mixing tank involved continuous recirculation of the tank contents through one or more columns of lindane coated glass beads (Veith and Comstock, 1975). The tank contents were continually mixed using a stainless steel stirring paddle, while also replacing, under float valve control, dilution water to replace delivery of the nominal 8 mg/L lindane solution to the diluter. Actual saturation of the tank contents with lindane could probably not be fully achieved due a limited rate of recirculation through the Veith columns, and to the constant addition of make-up water to replace solution delivered to the diluter. Note: From numerous unvalidated direct measurements by NAS of lindane concentration in the Veith column eluates during progress of the study, and in the mixing tank following a full 24 hour cessation of solution delivery to the diluter at the end of the study, we have evidence that the lindane water saturation level under test conditions may be approximately 5 mg/L.

DILUTION WATER

Source: City of Newport municipal tap water, treated as below, was supplied continuously to the toxicant diluter throughout the study.
Pretreatment: The water was dechlorinated by carbon filtration, particle filtered to 1 μm, and adjusted to a target water hardness of 25-30 mg/L as $CaCO_3$. Hardness adjustment was accomplished by passage of a portion of the dilution water through deionization cartridges.

TEST ORGANISMS

Species: Lamprey (*Lampetra* sp.)

Age/Size: Ammocoetes; target size range, 4-6 cm; actual size of 10 ammocoete subsample, 4.8 ± 0.6 cm.

Source: Ammocoetes were field collected in approximately equal numbers from the Trask, Nestucca, Siletz, and Alsea Rivers, Oregon. Field collection was by electoshocking and only 4-6 cm size ammocoetes were retained. Personnel of Windward Environmental performed the ammocoete collections.

Acclimation: Upon receipt, animals were removed from the collection site sediment and placed in 10-gallon aquaria supplied under flow-through conditions with dilution water. Water supply was sufficient (30-50 ml/min) to accomplish two volume changes of water per day. Approximately 50 lamprey were placed in each tank. Tanks contained an approximate 8 cm deep layer of sediment (Sandtastic Play Sand, Waupaca Materials) covered with approximately 26L of test water and were supplied with aeration. Normal behavior for the lamprey ammocoetes was to immediately bury in sediment and remain there. Any that did not immediately bury were discarded as potentially damaged. Ammocoetes were not fed during acclimation. Water quality conditions during the two weeks prior to testing (10-3-07 to 10-17-07) were: temperature, 17.0 ± 0.2°C; dissolved oxygen, 9.3 ± 0.3 mg/L; pH, 7.4 ± 0.4; specific conductance, 133 ± 28 µmhos/cm; hardness, 46 ± 12 mg/L as $CaCO_3$; and alkalinity, 40 ± 13 mg/L as $CaCO_3$.

TEST PROCEDURES AND CONDITIONS

Test Chambers: The test chambers were two-gallon glass aquaria capable of holding 5.8 L of test solution. Test chamber placement in the test area was random.

Test Concentrations: The nominal test concentrations were 8, 4, 2, 1, 0.5, and 0 mg/L (control) lindane.

Replicates/Treatment: 4

Organisms/Treatment: 40

Loading: 0.34 g/L

Aeration: None.

Feeding: None.

Water Volume Changes: The diluter supplied approximately 80 ml/min of test solution, or 115 L/day, at each test concentration. At this rate of flow, test solution supply to each replicate aquarium was 5.0 volumes per day.

Effects Criteria: The effect criterion used in the lamprey ammocoete acute toxicity test was mortality, defined as a lack of visible respiratory movement and absence of response to tactile stimulation. In addition to mortality, qualitative observations of sublethal responses were recorded when observed.

Target Water Quality Conditions: Water quality measurements were taken daily as specified in the protocol (Physical and Chemical Data, Items 1 and 2). The target water quality conditions were as follows: Temperature, 17 ± 1°C; dissolved oxygen, ≥4.0 mg/L; hardness, 25-30 mg/L as $CaCO_3$. Actual test conditions are reported in the Test Results section.

Photoperiod: The photoperiod was total darkness except for approximately 2-3 hours per day for observations and test exposure system maintenance. Light intensity at the aquarium level was 14.1 foot-candles.

DATA ANALYSIS METHODS

The 96-hr proportion survival was calculated for each treatment replicate from the raw survival data and the measured lindane concentrations for each treatment level. No attempt was made to compute an LC50 because less than 50% mortality occurred at the highest test concentration employed. The NOEC and LOEC were calculated according to U.S. Environmental Protection Agency (2002) methods. In this procedure, the NOEC and LOEC values were computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test was selected after evaluating the data for normality and homogeneity of variance. The statistical software employed for these calculations was ToxCalc, v.5.0.23N, Tidepool Scientific Software.

TOXICANT AND OTHER CHEMISTRY ANALYSES

Test solutions were sampled once daily for lindane from each test concentration by pooling samples from each of the four test replicate aquaria. Subsamples were pooled into new disposable plastic beakers from which the pooled sample was transferred to the analytical laboratory supplied sample bottles and shipped under chain-of-custody procedures to the analytical chemistry laboratory (Columbia Analytical Services) at Kelso, WA. No

preservative was employed. Samples were kept refrigerated at ≤4°C. Sample volumes were 1 L. Additional samples were taken of the dilution water on day 0 of the test for analysis of particulate matter, TOC, and ammonia-N, and from the control and highest test concentration treatments after 96-hr for measurement of ammonia-N. These samples also were shipped to Columbia Analytical Services for analysis.

PROTOCOL DEVIATIONS

1. The protocol specified "constant dark, except during monitoring activities", and also that light intensity should be 50-75 ft-c. when light was on. These specifications were judged to be contradictory and led to the following action. Based on best professional judgment of the investigators, we reduced the light intensity from that given in the protocol in order to minimize fright behavior responses of the ammocoetes during the short period of lighting associated with daily observations.
2. The protocol states: "At the end of the test, all surviving control ammocoetes are weighed and measured for length by replicate." Through a laboratory error, a subsample of 10 ammocoetes were weighed and measured at the beginning of the test instead. This is a minor deviation and is without consequence for interpretation of the study results.
3. Diluter flows were not performed at the end of the test through a lab oversight. There was no evidence that flows were impaired and water quality and lindane concentration data all confirm satisfactory performance of the diluter.

TEST RESULTS

Water quality conditions measured in test aquaria during the 96-hr toxicity test are summarized in Table 1. The temperature specification was met during the study. Dissolved oxygen remained near saturation throughout the test. The pH ranged from 6.4 – 7.2. Mean hardness also was in the specified range of 25-30 mg/L as $CaCO_3$.

The concentrations of lindane measured during the toxicity test are summarized in Table 2. Mean measured concentrations in the test ranged from 0.21 ± 0.05 mg/L at the lowest level to 2.68 ± 0.73 mg/L at the highest exposure level. Measured dilutions were roughly 25-35% of nominal test concentrations. Higher test concentrations could not be achieved because of solubility limits of lindane along with limitations on the rate of operation of the Veith columns, and the high rate of utilization of toxicant stock by the diluter.

A summary of the 96-hr ammocoetes survival results is shown in Table 3. In this study, the 96-hr control survival (100%) met the test acceptability criteria of ≥90%. Therefore, the test meets the protocol criterion of a valid test. Partial mortality was observed in the highest (2.68 mg/L) test concentration. No mortality occurred in any other test concentrations.

The toxicity of lindane to lamprey (*Lampetra* sp.) ammocoetes as the 96-hr LC50 was >2.68 mg/L. The NOEC and LOEC concentrations were 2.68 and >2.68 mg/L lindane, respectively.

LITERATURE CITED

Benoit, D., V. Mattson, and D. Olson. 1982. A continuous-flow mini-diluter system for toxicity testing. Water Res. 16:457-464.

EPA. 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. Environmental Protection Agency, Washington, DC.

Veith G.D. and V.M. Comstock. 1975. Apparatus for continuously saturating water with hydrophobic organic chemicals. J Fish Res Board Can 32:1849-1851.

STUDY APPROVAL

Gary Buhler	4/11/08	Richard S. Caldwell	4/11/08
Project Manager	Date	Study Director	Date
Richard S. Caldwell	4/11/08	Linda K. Jenseth	4/7/08
Laboratory Director	Date	Quality Assurance Unit	Date

Table 1. Measured water quality conditions during the 96-hr acute toxicity test of lindane to lamprey ammocoetes.

Parameter	Mean ± S.D.	N	Range
Temperature (°C)	17.3 ± 0.2	30	16.8 – 17.6
Dissolved oxygen (mg/L)	8.9 ± 0.4	30	7.9 – 9.7
pH	6.9 ± 0.2	30	6.4 – 7.2
Specific conductance (µmhos/cm)	96 ± 4	10	89 – 102
Hardness (mg/L as $CaCO_3$)	26 ± 1	10	26 – 28
Alkalinity (mg/L as $CaCO_3$)	15 ± 1	10	13 – 17

Table 2. Measured concentrations of lindane during the 96-hr acute toxicity test of lindane to lamprey ammocoetes. Measurements performed by Columbia Analytical Services.

Nominal (mg/L)	Measured (mg/L)		
	Mean ± S.D.	N	Range
8	2.68 ± 0.73	5	1.60 – 3.60
4	1.15 ± 0.34	5	0.76 – 1.60
2	0.56 ± 0.08	5	0.44 – 0.67
1	0.28 ± 0.12	5	0.07 – 0.36
0.5	0.21 ± 0.05	5	0.15 – 0.26
0	0.0 ± 0.0	5	0.0 – 0.0

Table 3. Survival of lamprey ammocoetes, *Lampetra* sp. exposed for 96 hours to lindane.

Lindane (mg/L)	Replicate	Ammocoetes exposed	Ammocoetes Surviving	Percent Survival	Mean % Survival
2.68	A	10	6	60.0	
	B	10	9	90.0	
	C	10	10	100.0	
	D	10	10	100.0	87.5
1.15	A	10	10	100.0	
	B	10	10	100.0	
	C	10	10	100.0	
	D	10	10	100.0	100.0
0.56	A	10	10	100.0	
	B	10	10	100.0	
	C	10	10	100.0	
	D	10	10	100.0	100.0
0.28	A	10	10	100.0	
	B	10	10	100.0	
	C	10	10	100.0	
	D	10	10	100.0	100.0
0.21	A	10	10	100.0	
	B	10	10	100.0	
	C	10	10	100.0	
	D	10	10	100.0	100.0
0.0 (Control)	A	10	10	100.0	
	B	10	10	100.0	
	C	10	10	100.0	
	D	10	10	100.0	100.0

* Mean significantly (p=0.05) different from the control.

APPENDIX I

PROTOCOL

STUDY PROTOCOL

LAMPREY (*LAMPETRA* sp.)
FLOW THROUGH ACUTE TOXICITY TEST

INTRODUCTION

Purpose of Study: The purpose of this study is to determine the acute toxicity of six chemicals, representing a range of toxic modes of action, to lamprey (*Lampetra* sp.) ammocoetes. The study will be performed using methods based on the methods for measuring acute toxicity with other fish species as described in U.S. Environmental Protection Agency (2002) and American Society for Testing and Materials (ASTM) (1996) guidance.

Summary of Method: In definitive tests, lamprey ammocoetes are exposed for 96 hours to five concentrations of test material and a dilution water control. The test concentrations are arranged in a logarithmic series based on the results of range finding tests. Four replicates, each with 10 organisms, are employed at each test concentration, if there are sufficient numbers of ammocoetes. Flow-through exposures are employed. Test chambers are glass aquaria less than or equal to two gallons in volume. Mortality is the effect criterion. The data analysis consists of calculating means and standard deviations for survival. An LC50 with 95% confidence intervals may be calculated along with the NOEC and LOEC concentrations.

STUDY MANAGEMENT

Sponsor's Name and Address:
Windward Environmental LLC
200 West Mercer Street, Suite 401
Seattle, WA 98119

Sponsor's Study Monitor:
Ms. Helle Andersen

Name of Testing Laboratory:
Northwestern Aquatic Sciences
3814 Yaquina Bay Road
P.O. Box 1437
Newport, OR 97365

Test Location: Newport, OR.

Laboratory's Key Personnel to be Assigned to the Study:
Laboratory Director: Richard S. Caldwell, Ph.D.
Project Manager/Study Director: Gary A. Buhler, B.S.
QA Officer/Toxicology Program Mgr: Linda K. Nemeth, B.A., M.B.A.
Aquatic Toxicologist: Gerald J. Irissarri, B.S.

Proposed Study Schedule: July 2007 through September 2007.

Good Laboratory Practices: The test is conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

TEST MATERIAL

Description: Chemicals to be tested include aniline, copper, pentachlorophenol, lindane, diazinon and naphthalene.

Preparation of Working Stock Solution and Test Concentrations: Test materials will be administered using a constant-flow diluter based on the design of Benoit *et al* (1982). Stock solutions of the sparingly soluble organic compounds, e.g. naphthalene, pentachlorophenol, lindane, diazinon, will be prepared continuously by recirculation of dilution water/toxicant stock solution through a column of toxicant coated glass beads (Veith and Comstock, 1975). This is a conceptual approach in which the specific column, flow rates, glass bead sizes, etc. need to be developed empirically, with analytical feedback, for each study considering such variables as chemical solubility, test temperature, desired highest test concentration, stock toxicant consumption rate, etc. Given the above, some modifications are anticipated as the studies progress. Initial system characteristics are as follows: 1) the toxicant mixing tank will have a 60-liter capacity; 2) a 20 x 280-cm glass column will be employed (10 x 200-cm and 40 x 560-cm columns are also available); 3) the column packing will employ 5-mm glass beads coated with the test compound; 4) particulates will be filtered out through glass wool; 5) flow through column will be controlled by a variable-speed peristaltic pump or by intermittent pumping; and 6) tubing will be glass and silicone rubber. GC/MS analysis of toxicant in the toxicant mixing tank will be used to optimize the system for each chemical noted above prior to test initiation.The contents of the stock toxicant reservoir will be constantly replenished with fresh dilution water as toxicant solution is supplied to the diluter. The aniline stock solution will be prepared similarly, but by volumetric addition of aniline, using a metering pump, to the stock toxicant reservoir *in lieu* of the Veith column. The copper working stock solution will be prepared in the same apparatus by metering pump addition of a concentrated copper stock solution to the stock toxicant reservoir. All toxicant working stock solutions will be prepared and maintained under the same conditions as in the test exposures and delivered continuously to the toxicant chamber of the diluter at an appropriate rate.

DILUTION WATER

Dilution water will be dechlorinated City of Newport municipal tap water adjusted to a water hardness of 25-30 mg/L as $CaCO_3$.

TEST ORGANISMS

Species: Lamprey, *Lampetra* sp.

Source: Ammocoetes will be collected by Windward staff and delivered to the laboratory each day of collection. The ammocoetes will be collected from several rivers including the Siletz River, which was the site for Phase I ammocoetes.

Age: ammocoetes (juvenile prior to metamorphosis)

Acclimation and Pretest Observation: Test organisms will be held in the laboratory in order to acclimate to test temperature. Upon receipt, animals are removed from the sediment they are received in and placed in 10-gallon tanks under flow-through conditions (~35 mL/minute, or ~ 2 volume changes per day, with one additional daily siphon and replacement of 2/3 volume of each tank). Approximately 50 lamprey are placed in each tank. Tanks will contain at least 3 inches of sediment (Sandtastic Play Sand, Waupaca Materials, or similar material) covered with approximately 26L of test water and supplied with aeration. The maximum temperature variation during holding will be ± 1° C. The ammocoetes will be acclimated to the laboratory test conditions for a minimum of 5 to 7 days prior to test initiation. This minimum number of days applies only to the first toxicity test conducted on each batch of field-collected organisms. For all other tests, the ammocoetes will be held for at least 13 days prior to test initiation. When a temperature adjustment of more than 3 to 5°C is required, the transfer from field collection water to laboratory dilution water will be gradual. Normal behavior for lamprey ammocoetes is to immediately bury in sediment and remain there. Any dead animals observed on the sediment surface are removed daily. Past experience suggests that mortality is minimal during short-term holding and any mortality is likely due to damage from field collection. If ammocoetes do not bury or there is excessive

mortality in any tank, animals from that tank will not be used for testing. Ammocoetes will not be fed since they are being held short-term.

DESCRIPTION OF TEST SYSTEM

Preparation of Test Concentrations: Test materials will be administered using a constant-flow diluter based on the design of Benoit *et al.* (1982). The diluter will be calibrated to deliver a 0.5 dilution series. Flow rates will be adjusted to deliver at least five test chamber volumes per concentration per day. Diluter flow rates will be measured and documented at the beginning and conclusion of each 96-hour test. This will be done by measuring individual diluter capillary tube flows. When the flow rates are entered into an Excel® spreadsheet, the actual dilutions of the toxicant stock can be computed. Actual test chemical concentrations (organic chemicals) in the toxicant mixing chamber (toxicant stock solution) and in exposure aquaria will periodically be measured on a real-time basis at NAS to verify that nominal concentrations are being routinely achieved. Definitive concentration documentation in test aquaria will result from daily samples shipped for analysis to CAS.

Test Chambers and Environmental Control: Test chambers used in the toxicity study are glass aquaria less than or equal to two gallons in volume. The test system is housed in a constant-temperature, photoperiod-controlled room. Aeration is not employed. The photoperiod is constant dark except during monitoring activities when ambient (50-75 ft-c) lighting is used.

Cleaning: All laboratory glassware, including test chambers and toxicant dilution system, will be cleaned based on the methods described in U.S. Environmental Protection Agency (2002). Initial cleaning incorporates the use of a laboratory detergent followed by multiple tap water rinses. Subsequently, either dilute (10%, V:V) acid (hydrochloric or nitric) or acetone is used to remove metals or organic contaminants, respectively. Further multiple tap water rinses are employed after each acid or acetone treatment. Sodium hypochlorite bleach may be employed on a limited basis to ensure that microorganisms that could metabolize organic toxicants, e.g. naphthalene, are not present. Exhaustive tap water rinsing is always employed following any chemical cleaning treatment. A final dilution water rinse of the exposure apparatus will precede initiation of each study.

EXPERIMENTAL DESIGN AND TEST PROCEDURES

Experimental Design: The definitive test involves exposure of lamprey ammocoetes to five test concentrations, using a 0.5 dilution series, and a dilution water control. Exposures are for 96 hours. Each treatment consists of four replicates, each containing 10 ammocoetes, if there are sufficient numbers of ammocoetes. If there are not enough ammocoetes for 10 per replicate, then at least 20 per treatment (5 per replicate) is the minimum number preferred. Test chambers are randomly distributed in the test area. Ammocoetes are impartially distributed to each aquarium by adding one or two animals to each test replicate and repeating the process until each contains 10 organisms.

Effect Criterion: The effect criterion used in the lamprey ammocoete acute toxicity test is mortality, defined as a lack of visible respiratory movement and absence of response to tactile stimulation. The mortality data will be used to compute LC50s for each exposure period. In addition to mortality, qualitative observations of sublethal responses consisting of erratic swimming, hyperventilation, hemorrhaging, and hyper/hypoactivity (compared to the activity of the organisms in the control group) will be recorded if observed.

Test Conditions: The dissolved oxygen concentration in each test container must be > 4.0 mg/L throughout the test. The test temperature employed is 17 ± 1°C. A photoperiod of constant darkness is used, except during monitoring activities when illumination is supplied by daylight fluorescent lamps at an intensity of 50-75 ft candles. Loading should not exceed 6.1g/L at 17°C according to EPA. ASTM recommends loading not exceed 1g/L of solution passing through the chamber in 24 hours or 10g/L of solution in the chamber at any time.

Beginning the Test: Adding the organisms to the equilibrated test containers as previously described begins the test.

Feeding: None.

Analysis of Test Concentrations: During each test, samples will be collected for chemical analysis, held at 4°C and shipped by overnight delivery to Columbia Analytical Services in Kelso, WA. The sampling schedule and procedure will follow the project QAPP.

Test Duration, Type and Frequency of Observations, and Methods: The test duration of the acute toxicity test is 96 hours. The type and frequency of observations to be made during the test are summarized as follows:

Type of Observation	Times of observation
Biological Data	
1. mortality (each test container)	0, 24, 48, 72, and 96 hr
2. sublethal observations (qualitative)	0, 24, 48, 72, and 96 hr
Physical and Chemical Data	
1. Dissolved oxygen, pH, & temperature, (in one replicate of each test level and the control)	0, 24, 48, 72, and 96 hr
2. Conductivity, hardness & alkalinity (in one replicate of highest concentration and control)	0, 24, 48, 72, and 96 hr
3. Toxicant concentration sampling (in one replicate of each test concentration and the control)	0, 24, 48, 72, and 96 hr
4. Dilution water sample for particulate matter, TOC and ammonia	0 hr
5. Sample for ammonia taken from one replicate of control and highest test concentration	96 hr

During the test, dead organisms are removed every 24 hours and frozen by concentration for possible tissue analysis. Dissolved oxygen is measured using a polarographic oxygen probe calibrated according to the manufacturer's recommendations. The pH and temperature are measured using a pH probe and a properly calibrated meter with scale divisions of 0.1 pH units. Conductivity is measured using a calibrated conductivity meter. Samples for chemical analysis will be collected, preserved, and sent to Columbia Analytical Services following the recommended procedures of the analytical laboratory.

Test Termination: At the end of the test, all surviving control ammocoetes are weighed and measured for length by replicate. All surviving or dead test organisms will be frozen by concentration for possible later tissue analysis by Columbia Analytical Services.

Criteria of Test Acceptance: The test results are acceptable if survival in the controls is at least 90%. The toxicity tests will be performed as a 50% dilution series, it is anticipated that each test will have at least one or possibly two partial mortality concentrations. Nominal concentrations of each toxicity test have be reviewed and approved by EPA (EPA 2007). However, because of the experimental nature of the study and the limited supply of ammocoetes, the acceptability of the toxicity tests will be based only on the ≥ 90% survival in control group.

DATA ANALYSIS

The percent of live ammocoetes is calculated for each treatment replicate from the raw data and the means are obtained for each treatment level. An LC50 (mortality) is calculated using Probit or Trimmed Spearman-Karber methods. The NOEC and LOEC are calculated for each endpoint according to U.S. Environmental Protection Agency (2002) methods. In this procedure, the NOEC and LOEC values are computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test is selected after evaluating the data for normality and homogeneity of variance. An arcsine-square root transformation is performed on the percentage effect data prior to statistical analysis. The statistical software employed for these calculations is Toxcalc, v.5.0.23N, Tidepool Scientific Software.

REPORTING

The final report of the study results must include all of the following standard information at a minimum:

1. Name and identification of the test;
2. The investigator and laboratory;
3. Information on the toxicants;
4. Information on the dilution water;
5. Detailed information about the test organisms;
6. Description of the test system and its calibration, including test chambers and dilution system;
7. A description of the experimental design and other test conditions including water quality;
8. Definition of the effect criteria and any other observations;
9. Responses, if any, in the control treatment;
10. Tabulation and statistical analysis of measured responses;
11. A description of the statistical methods used;
12. Any unusual information about the test or deviations from procedures;

The final study report will include the study protocol and all study raw data as appendices.

STUDY DESIGN ALTERATION

Amendments made to the protocol must be approved by the sponsor and study director and should include a description of the change; the reason for the change; the date the change took effect; and the dated signatures of the study director and sponsor. Any deviations in the protocol must be described and recorded in the study raw data.

REFERENCED GUIDELINES

ASTM. 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.

Benoit, D., V. Mattson, and D. Olson. 1982. A continuous-flow mini-diluter system for toxicity testing. Water Res. 16:457-464.

EPA. 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. Environmental Protection Agency, Washington, DC.

EPA. 2007. Letter to Lower Willamette Group from C. Humphrey and E. Blischke dated August 17, 2007 regarding Portland Harbor RI/FS: Lamprey toxicity test field sampling plan and QAPP Phase 2 addenda (approval letter). US Environmental Protection Agency Region 10, Oregon Operations Office, Portland, OR.

Veith G.D. and V.M. Comstock. 1975. Apparatus for continuously saturating water with hydrophobic organic chemicals. J Fish Res Board Can 32:1849-1851.

13. APPROVALS

_____ for **Windward Environmental**

Name Date

_____ for **Northwestern Aquatic Sciences**

Name Date

Appendix A
Test Conditions Summary

1. Test species:	*Lampetra* sp.
2. Test type:	Flow-through
3. Test duration:	96 hr
4. Temperature:	17 ± 1°C
5. Dissolved oxygen:	≥ 4.0 mg/L
6. Light quality & intensity:	Ambient laboratory light (50-75 ft-c)
7. Photoperiod:	Constant dark, except during monitoring activities
8. Test chambers:	glass aquaria less than or equal to two gallons in volume.
9. Test chamber volumes per day:	At least 5
10. Replicates per treatment:	4
11. Organisms per replicate:	10
12. Age of test organisms:	ammocoete
13. Feeding regime:	None
14. Aeration:	None
15. Dilution water:	Dechlorinated City of Newport tap water; hardness adjusted to 25-30 mg/L as $CaCO_3$
16. Test concentrations:	5 and a dilution water control
17. Water quality:	Temperature, pH, & dissolved oxygen in one replicate of each test concentration daily. Conductivity, hardness & alkalinity in one replicate of highest concentration and control daily
18. Special controls:	None
19. Endpoint:	Mortality
20. Test acceptability criteria:	≥90% control survival

APPENDIX II

RAW DATA

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-40 Client Windward Investigator RSC 4/23/08

STUDY MANAGEMENT:

Client: Windward Environmental, 200 West Mercer Street, Suite 401, Seattle, WA 98119
Client's Study Monitor: Ms. Helle Andersen
Testing Laboratory: Northwestern Aquatic Sciences
Test Location: 3814 Yaquina Bay Road, Newport, OR 97365
Laboratory's Study Personnel:
Project Manager: Gary A. Buhler GAB Study Director: Richard S.Caldwell RSC
QA Officer: L.K. Nemeth 1. G.J. Trissarri
2. 3.
4. 5.
Study Schedule:
Test Beginning: 10/18/07 1100 Test Ending: 10/22/07 1215

TEST MATERIAL:

Description: Lindane (1,2,3,4,5,6-Hexachlorocyclohexane, γ-isomer,97%), Aldrich Lot No. 07325DD.

DILUTION WATER:

Description: Dechlorined City of Newport tap water
Date of Preparation/Collection: Continuous flow
Water Quality: Cond. (µmhos/cm) 96.2 ± 4.9 pH 6.8 ± 0.2
Hardness (mg/L as $CaCO_3$) 26.4 ± 0.9 Alkalinity (mg/L as $CaCO_3$) 19.4 ± 0.9
Treatments: Hardness adjusted to 25-30 mg/L with a Culligan® water treatment system.

TEST ORGANISMS:

Species: Lamprey ammocoetes (*Lampetra* sp.) Age: Size: 4-6 cm
Source: Trask, Nestucca, and Siletz rivers and Alsea
Acclimation Data: See pages: 6, 7, & 11

TEST PROCEDURES AND CONDITIONS:

Test Concentrations (nominal): 8, 4, 2, 1, 0.5, & 0 mg/L
Test Chambers (description): 2½ gallon glass aquaria Test Volume (ml): 5.80 L RSC 2/21/08
Replicates/treatment: 4 Organisms/treatment: 40 (10/replicate)
Test water changes: flow through
Aeration during test: none DO limit ≥ 4.0 mg/L GAB 4/21/08
Temperature (°C): 17 ± 1°C
Feeding: None Photoperiod: Darkness except for WQ & chemistry measurements
Test Duration (hr) 96 hours Tank Placement: Complete randomization
Randomization Chart.

Diluter

1	8	1	4	0	8	0	1	0.5	2	0	8	4	0.5	0.5	4	1	2	2	8	0	2	4	0.5

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-40 Client Windward Investigator

WEIGHT OF 10 ANIMALS AT TEST INITIATION

10-18-07 651

	weight (mg)	Length (cm)	Comments
1	0.26	5.4	
2	0.12	4.0	
3	0.21	5.6	
4	0.21	5.1	
5	0.17	4.3	
6	0.16	4.4	
7	0.12	4.1	
8	0.20	4.8	
9	0.25	5.1	
10	0.26	5.6	
~~Total~~ Mean	1.20	4.84	
~~Average animal~~ 5.2	0.06	0.61	
N	10	10	

MISCELLANEOUS NOTES:

Room light intensity (fc): 14.1 10-18-07 651

Chemistry samples taken daily.

Chemistry samples for particulate matter, TOC & ammonia in dilution water taken on test day 0.

Chemistry samples for ammonia taken in control & 8 mg/L A replicate on test day 4.

$$\text{Loadi}_I = \frac{0.20\,g \times 10}{5.86\,L} = 0.34\,g/L$$

Error codes

1) Correction of handwriting error
2) Written in wrong location, entry deleted
3) Wrong date deleted; replaced with correct data
4) Error found in measurement; measurement repeated

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-40 Client ____ Windward ____ Investigator ____

WATER QUALITY DATA SHEET

DAY 0 (10/18/07) RSC/GJI

Conc (mg/L)	Temp (°C)	pH	Cond µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
8	17.3	7.2	93	9.7	26	16	10	10	10	10	
4	17.1	7.2		9.6			10	10	10	10	
2	17.1	7.2		9.5			10	10	10	10	
1	16.8	7.2		9.4			10	10	10	10	
0.5	16.9	7.1		9.3			10	10	10	10	
0	17.0	6.8	89	9.3 ~~89~~	26	13	10	10	10	10	

Chemistry samples taken (10/18/07) GJI ← ② RSC
Particulate matter, TOC, and ammonia samples taken in dilution water (10/18/07) GJI
Hours of light: 1.0

DAY 1 (10/19/07) GJI/RSC

Conc (mg/L)	Temp (°C)	pH	Cond µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
8	17.5	7.0	97	9.2	26	17	10	10	10	10	red color, extremely lethargic
4	17.4	6.9		9.1			10	10	10	10	mostly lethargic
2	17.4	6.9		9.9			10	10	10	10	mild, lethargic, faster
1	17.4	6.8		9.7			10	10	10	10	
0.5	17.4	6.8		9.7			10	10	10	10	
0	17.6	6.7	99	9.8	26	15	10	10	10	10	

Chemistry samples taken (10/19/07) GJI
Hours of light: 1.0 HR 46 MIN GJI

DAY 2 (10/20/07) GJI/RSC

Conc (mg/L)	Temp (°C)	pH	Cond µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
8	17.6	7.0	94	9.5	26	15	10	10	10	10	red color, extremely lethargic
4	17.4	6.9		9.3			10	10	10	10	lethargic, mild fin fanning
2	17.3	6.9		9.2			10	10	10	10	mild, lethargic, faster
1	17.4	6.8		9.0			10	10	10	10	mild, lethargic, faster
0.5	17.3	6.8		8.8			10	10	10	10	
0	17.4	6.7	96	8.9	26	15	10	10	10	10	

Chemistry samples taken (10/20/07) GJI
Hours of light: 2.0 HRS 10 MIN GJI

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-40 Client Windward Investigator

WATER QUALITY DATA SHEET

DAY 3 (10 21 07) [illegible]

Conc (mg/L)	Temp (°C)	pH	Cond µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
8	17.4	6.8	100	8.8	26	14	6 (4D)	9 (1D)	10	10	[illegible] DEAD ANIMALS EXHIBIT A DIFFERENT RED PIGMENTATION AT MOUTH
4	17.3	6.8		8.6			10	10	10	10	[illegible] mild tw tox
2	17.2	6.8		8.4			10	10	10	10	[illegible] mild tw tox
1	17.2	6.8		8.2			10	10	10	10	[illegible] tw tox
0.5	17.2	6.9		8.2			10	10	10	10	mild tw tox
0	17.0	7.0	95	8.3	28	14	10	10	10	10	

Chemistry samples taken (10 21 07) [illegible]
Hours of light: 2 0 HRS 15 MIN [illegible]

DAY 4 (10 22 07) [illegible]

Conc (mg/L)	Temp (°C)	pH	Cond µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
8	17.4	6.8	91	8.9	26	15	6	9	10	10	[illegible]
4	17.2	6.8		8.8			10	10	10	10	[illegible] mild tw tox
2	17.2	6.8		8.8			10	10	10	10	[illegible] mild tw tox
1	17.0	6.8		8.6			10	10	10	10	[illegible] mild tw tox
0.5	17.2	6.8		8.7			10	10	10	10	[illegible] mild tw tox
0	17.5	6.9	102	8.6	26	15	10	10	10	10	

Chemistry samples taken (10 22 07) [illegible]
Ammonia samples taken in control and 8 mg/L (10 22 07) [illegible]
Hours of light: 1.0

Dilution water WQ (for control [illegible])

Cond.	96.2 ± 4.9 (5)	0286
pH	6.8 ± 0.2 (5)	2/8/08
Hardness	26.4 ± 0.9 (5)	
Alkalinity	14.4 ± 0.9 (5)	

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-40 Client ___ Windward Investigator ___

NOTES ON PREPARATION OF 8 mg/L LINDANE STOCK

System Design

The objective is to deliver 8 mg/L of lindane to the diluter toxicant chamber at a nominal constant rate of 160 ml/min. To accomplish this, a large reservoir, approximately 54L capacity, of lindane solution in dilution water is maintained at test temperature. As solution is removed to the diluter, makeup water is introduced under float valve control. The concentration of lindane in the reservoir is maintained by circulation of the contents through a column of glass beads coated with lindane (Veith column).

NOTES ON MAINTENANCE OF THE LINDANE DELIVERY SYSTEM

Added a second Veith column in parallel to increase flow rate from ≈ 160 ml/min to ≈ 300 ml/min. GSC 10/18/07

DILUTER CALIBRATIONS RECORD

DILUTER CAPILLARY FLOWS

Date 10-18-07	Time 1150	Init. GSC		Date	Time	Init.
Drain #	Supply Tank	Diluter Tank		Drain #	Supply Tank	Diluter Tank
1	158	78		1		
2	78	81		2		
3	80	74		3		
4	80	78		4		
5	78	74		5		
6	165	75		6		

Flow rates in ml/min

FLOWS TO REPLICATE AQUARIA

Date 10/18/07 Time 1200 Init. GSC

Replicate	Relative Concentration (%) 100	50	25	12.5	6.25	0
A	310	210	260	280	310	260
B	290	300	270	460	300	300
C	350	350	210	200	310	220
D	220	310	320	260	200	350

Date ___ Time ___ Init. ___

Replicate	Relative Concentration (%) 100	50	25	12.5	6.25	0
A						
B						
C						
D						

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (Lampetra sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
8-2-07	18.1	6.7	8.0	82	20	35	[illegible]	[illegible]	constant flow		[illegible]	[illegible]	[illegible]
8-2-07	16.8	7.7	9.6	85	26	30		"	"			[illegible]	[illegible]
8-2-07	17.1	6.6	9.8	65	28	10	[illegible]	[illegible]	"			[illegible]	[illegible]
8-2-07	16.4	7.6	9.7	162	34	30	[illegible]	[illegible]	"			[illegible]	[illegible]
8-2-07	18.1	6.1	7.2	60	22	16	Stillwater	[illegible]	"			[illegible]	[illegible]
8-3-07	17.0	7.0	7.4	160	34	30						Holding	[illegible]
8-3-07	16.9	6.6	9.0	60	31	25	Alder Creek		"			[illegible]	[illegible]
8-3-07	16.8	7.2	7.5	135	34	30			"				
9-1-07	16.9	7.1	7.4	140	—	—			"			Holding	
9-2-07	16.7	7.6	9.1	160	—	—			"			Holding	GH
9-3-07	16.7	7.7	7.2	160	—	—			"				
9-4-07	16.2	7.5	8.8	185	34	30			"			"	[illegible]
9-5-07	16.3	7.2	9.1	170	34	30			"			"	[illegible]
9-6-07	16.8	7.3	9.0	185	34	30			"			"	
9-7-07	16.9	7.0	9.1	155	26	30			"			"	
9-7-07	17.0	7.5	9.3	160	—	—			"				
9-9-07	16.7	7.5	8.8	170	-	-			"			holding	LPS
9-10-07	16.7	7.2	8.9	175	—	—			"			"	[illegible]
9-11-07	17.4	7.6	9.0	235	60	35			"			"	LPS
9-12-07	17.0	8.0	9.4	185	60	38			"			"	[illegible]

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (Lampetra sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
9-13-07	17.1	8.0	9.0	170	55	30			constant flow			Holding	[illegible]
9-14-07	17.1	8.2	9.1	155	48	29			"		[illegible]	"	[illegible]
9-15-07	17.1	8.3	9.2	170	—	—			"			"	
9-16-07	17.0	8.1	9.2	115	41	30			"			"	GH
9-17-07	17.1	7.7	8.8	120	41	30			"			"	LPS
9-18-07	17.0	7.8	9.1	120	39	21			"			"	GH
9-19-07	17.7	7.6	9.1	125	39	34			"			"	[illegible]
9-20-07	17.1	7.9	9.0	125	36	34	Taken for [illegible]		"			"	GH
9-21-07	17.0	8.2	9.5	140	44	31			"			"	[illegible]
9-22-07	17.1	7.7	9.4	130	42	29			"			"	
9-23-07	17.1	7.7	9.3	140	46	28			"			"	[illegible]
9-24-07	16.8	7.9	9.3	140	44	29			"			"	
9-25-07	17.1	7.7	8.8	145	44	30			"			"	[illegible]
9-26-07	17.2	7.8	9.3	140	48	36			"			"	[illegible]
9-27-07	17.4	7.7	9.3	145	48	37			"			"	[illegible]
9-28-07	17.2	7.8	9.3	150	48	36			"			"	[illegible]
9-29-07	16.9	7.5	8.8	150	48	36			"			"	[illegible]
9-30-07	17.0	7.7	8.8	140	48	37			"		[illegible]	"	[illegible]
10-1-07	17.0	7.8	9.4	145	48	36			"			"	GH
10-2-07	17.3	8.1	9.2	135	—	—			"			"	GH

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (Lampetra sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
10-3-07	17.0	7.8	9.1	130	46	55			constant flow			Holding	GH
10-4-07	16.9	7.9	9.2	120	42	38						"	[illegible]
10-5-07	16.9	7.4	9.0	130	not Taken LPS							"	LPS
10-6-07	16.6	7.2	9.4	130	44	41						"	GH
10-7-07	17.0	7.4	9.5	135	60	50						"	GH
10-8-07	17.0	7.9	9.2	145	50	48						"	GH
10-9-07	17.0	7.6	9.5	150	54	52						"	GH
10-10-07	16.9	7.4	9.2	155	54	49						"	GH
10-11-07	17.0	7.1	9.5	150	54	40						"	[illegible]
10-12-07	17.1	8.0	9.4	155	50	39						"	[illegible]
10-13-07	17.1	7.1	9.1	150	52	40						"	GH
10-14-07	16.9	7.5	9.4	150	52	38						"	[illegible]
10-15-07	17.0	7.6	9.5	155	50	38						"	[illegible]
10-5-07	13.0	6.7	10.6	97	30	30						Receive Hw	[illegible]
10-16-07	13.2	7.9	10.1	145	60	57						Holding R#4	[illegible]
10-16-07	17.1	6.9	9.4	65	20	12						Test aquarelle	[illegible]
10-16-07	12.4	6.2	9.7	65	18	14						Northern River Receiving	GH
10-17-07	13.3	8.3	10.1	135	60	55					[illegible]	Holding [illegible]	GH
10-17-07	17.3	6.7	9.5	75	22	14						Hold in Room #1	GH
10-17-07	13.1	6.8	10.2	57	20	14						Sheep River Receiving	

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (Lampetra sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
10-18-07	13.2	8.3	9.9	130	48	36			constant flow			Holding	[illegible]
10-19-07	13.3	7.5	9.5	120	48	36						"	LPS
10-20-07	13.2	7.7	10.0	125	46	38							
10-21-07	14.2	7.6	9.9	130	48	37						"	[illegible]
10-22-07	14.2	7.7	9.4	135	50	40						"	[illegible]
10-23-07	15.1	7.9	9.7	145	54	44						"	[illegible]
10-24-07	15.6	8.3	9.7	155	58	47						"	[illegible]
10-25-07	16.1	8.5	9.5	160	66	53						"	[illegible]
10-26-07	16.8	8.1	9.6	165	60	47						"	[illegible]
10-27-07	16.8	8.3	9.9	155	62	49						"	[illegible]
10-28-07	16.6	8.3	9.9	165	62	47						"	[illegible]
10-29-07	17.0	8.1	9.5	165	62	48	[illegible]					"	[illegible]
10-30-07	16.9	8.2	9.6	170	64	46						"	[illegible]
10-31-07	16.8	8.2	9.9	175	70	54						"	[illegible]
11-1-07	16.8	8.2	9.8	165	62	45						"	[illegible]
11-2-07	16.8	8.2	9.5	165	62	47						"	[illegible]
11-3-07	16.6	8.1	9.2	160	56	38						"	[illegible]
11-4-07	16.8	8.3	9.6	180	64	48						"	[illegible]
11-5-07	16.9	8.1	9.9	180	66	49						"	[illegible]
11-6-07	16.9	8.2	9.8	155	52	35							

ANIMAL RECEIPT/HOLDING, FRESHWATER
SPECIES Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init
11-7-07	17.0	8.1	9.4	155	54	37			constant flow			holding	
11-8-07	16.9	8.1	9.3	155	56	39						"	
11-9-07	16.7	8.6	9.7	160	62	46						"	
11-10-07	16.7	8.1	9.6	155	56	43						"	
11-11-07	16.7	8.1	9.9	145	52	39						"	
11-12-07	16.8	8.0	9.7	155	56	38						"	
11-13-07	16.8	7.6	9.7	155	54	38						"	
11-14-07	16.8	7.9	9.7	150	52	36						"	
11-15-07	16.8	8.0	9.7	160	60	43						"	
11-16-07	16.9	8.2	9.9	160	58	39						"	
11-17-07	16.7	8.3	10.1	165	62	43						"	
11-18-07	16.9	8.1	9.8	150	54	36						"	
11-19-07	16.8	7.9	9.7	175	64	48						"	
11-20-07	16.7	7.9	9.9	170	64	46						"	
11-21-07	16.6	8.0	9.9	175	64	48						"	
11-22-07	16.5	7.9	10.0	165	62	39						"	
11-23-07	16.5	7.8	9.5	170	62	44						"	
11-24-07	16.5	8.0	9.7	190	70	48						"	
11-25-07	16.5	7.9	9.6	175	64	45						"	
11-26-07	16.6	7.5	9.6	175	64	46						"	

ANIMAL RECEIPT/HOLDING, FRESHWATER
SPECIES Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init
11-29-07	16.1	7.9	9.6	180	62	44			constant flow			holding	
11-30-07	16.0	7.7	10.0	175	56	44						"	
12-1-07	16.0	7.8	9.7	170	60	43						"	
12-2-07	16.0	8.0	9.7	175	62	46						"	
12-3-07	16.2	7.9	9.7	185	70	47						"	
12-4-07	15.8	7.5	9.4	165	60	43						"	
12-5-07	15.7	8.2	9.6	155	60	39						"	
12-6-07	15.8	7.7	9.8	170	60	42						"	
12-7-07	16.2	7.6	9.9	165	60	42						"	
12-8-07	15.7	7.6	9.9	160	58	40							
12-9-07	17.8	7.4	9.0	175	64	43						"	
12-10-07	17.1	7.3	8.8	165	62	42						"	
12-11-07	17.1	7.2	9.4	170	56	41						"	
12-12-07	17.3	7.4	9.5	160	58	40						"	
12-13-07	16.7	7.2	9.1	170	64	43						"	
12-14-07	16.8	7.3	9.1	170	64	44						"	
12-15-07	17.0	7.2	9.0	165	56	44						"	
12-16-07	16.5	7.2	9.5	180	70	45						"	
12-17-07	16.7	7.2	9.3	170	66	44						"	
12-18-07	16.5	7.4	9.6	195	74	53						"	

ANIMAL RECEIPT/HOLDING, FRESHWATER
SPECIES Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init
12-19-07	15.6	7.3	9.9	170	76	51			constant flow			HOLDING	
12-20-07	16.2	7.2	10.3	175	56	46						"	
12-21-07	15.4	7.2	10.0	190	72	48						"	
12-22-07	16.0	7.8	9.8	145	70	46							
12-23-07	17.1	7.0	9.5	195	68	54						"	
12-24-07	17.1	7.0	9.4	190	72	48						"	
12-25-07	16.9	7.1	9.6	190	72	51						"	
12-26-07	16.8	7.3	10.1	200	74	50						"	
12-27-07	17.1	7.2	9.7	195	68	49						"	
12-28-07	17.0	8.0	9.8	170	56	32						"	
12-29-07	17.1	8.1	9.9	175	52	39						"	
12-30-07	16.9	8.0	9.2	195	68	50						"	
12-31-07	16.8	8.3	9.8	205	72	50						"	
1-1-08	17.0	8.0	9.6	[illegible]	64	43						"	
1-2-08	16.8	8.2	9.6	185	66	41						"	
1-3-08	16.8	8.3	9.7	180	60	39						"	
1-4-08	16.9	8.2	9.8	175	62	36						"	
1-5-08	16.7	8.4	9.7	155	48	30						"	
1-7-08	16.7	8.4	9.9	160	54	32						"	
1-8-08	16.9	8.1	9.5	145	50	33						"	

ANIMAL RECEIPT/HOLDING, FRESHWATER
SPECIES Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init
1-9-08	15.6	8.4	10.6	165	56	30			constant flow			HOLDING	
1-10-08	15.9	8.0	10.2	145	44	24						"	
1-11-08	16.3	8.1	10.3	145	42	24							
1-12-08	16.0	8.4	10.2	165	56	31						"	
1-13-08	16.2	8.1	9.6	170	54	32						"	
1-14-08	16.5	8.2	9.9	160	52	32						"	
1-15-08	16.2	8.2	9.7	160	50	29						"	
1-16-08	16.4	8.2	9.9	175	40	15						"	
1-20-08	15.6	8.0	9.9	165	86	63						"	

Samples shipped to CAS

Test No 656-40

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd., P.O. Box 1437, Newport, OR 97365
Tel 541-265-7225, Fax 541-265-2799, www.nwaquatic.com

Client Name: Integral Consulting, Inc
Address: 319 SW Washington St. Suite 1150
City, State, Zip code: Portland, OR 97204
Project No: Integral B01-01-58C Windward 07-28-04-53
Phone No: 503-284-5545
Report Attention: Abbie Spielman
Sampled by: GS
Shipping Information: Carrier UPS; UPS#Y3489E; Airbill No
Testing Required: Copper; Lindane; Pentachlorophenol; Aniline; Diazinon; Naphthalene; Comments

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	10-19-07	1120	NAS# 1355G Day 1 - 0 mg/L Lindane			X					
	10-19-07		NAS# 1356G Day 1 - 0.5 mg/L Lindane			X					
	10-19-07		NAS# 1357G Day 1 - 1.0 mg/L Lindane			X					
	10-19-07		NAS# 1358G Day 1 - 2.0 mg/L Lindane	1		X					
	10-19-07		NAS# 1359G Day 1 - 4.0 mg/L Lindane	1		X					
	10-19-07		NAS# 1360G Day 1 - 8.0 mg/L Lindane	1		X					
	10-19-07		NAS# 1361G Day 1 - 1.0 mg/L Lindane Dupl	1		X					

Signature	Print Name	Company	Date	Time	Cooler Custody Seal
Relinquished by: [signature]	GERALD IRISSARRI	Northwestern Aquatic Sciences	10-22-07	1330	Present / Not Present
Received by:					Intact / Not Intact
Relinquished by:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received by:					
Relinquished by:					
Received by laboratory:					

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd., P.O. Box 1437, Newport, OR 97365
Tel 541-265-7225, Fax 541-265-2799, www.nwaquatic.com

Client Name: Integral Consulting, Inc
Address: 319 SW Washington St., Suite 1150
City, State, Zip code: Portland, OR 97204
Project No: Integral B01-01-58C Windward 07-28-04-53
Phone No: 503-284-5545
Report Attention: Abbie Spielman
Sampled by:
Shipping Information: Carrier UPS; UPS#Y3489E; Airbill No
Testing Required: Copper; Lindane; Pentachlorophenol; Aniline; Diazinon; Naphthalene; Comments

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	10-21-07	0950	NAS# 1365G Day 3 - 0 mg/L Lindane	1		X					
	10-21-07		NAS# 1366G Day 3 - 0.5 mg/L Lindane	1		X					
	10-21-07		NAS# 1367G Day 3 - 1.0 mg/L Lindane	1		X					
	10-21-07		NAS# 1368G Day 3 - 2.0 mg/L Lindane	1		X					
	10-21-07		NAS# 1369G Day 3 - 4.0 mg/L Lindane	1		X					
	10-21-07		NAS# 1370G Day 3 - 8.0 mg/L Lindane	1		X					
	10-21-07		NAS# 1371G Day 3 - 4.0 mg/L Lindane Dupl	1		X					

Signature	Print Name	Company	Date	Time	Cooler Custody Seal
Relinquished by: [signature]	GERALD IRISSARRI	Northwestern Aquatic Sciences	10-22-07	1330	Present / Not Present
Received by:					Intact / Not Intact
Relinquished by:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received by:					
Relinquished by:					
Received by laboratory:					

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd., P.O. Box 1437, Newport, OR 97365
Tel 541-265-7225, Fax 541-265-2799, www.nwaquatic.com

Client Name: Integral Consulting, Inc
Address: 319 SW Washington St., Suite 1150
City, State, Zip code: Portland, OR 97204
Project No: Integral B01-01-58C Windward 07-28-04-53
Phone No: 503-284-5545
Report Attention: Abbie Spielman
Sampled by: GS
Shipping Information: Carrier UPS; UPS#Y3489E; Airbill No
Testing Required: Copper; Lindane; Pentachlorophenol; Aniline; Diazinon; Naphthalene; Comments

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	10-18-07	0930	NAS# 1342G Day 0 - 0 mg/L Lindane	1		X					
	10-18-07		NAS# 1343G Day 0 - 0.5 mg/L Lindane	1		X					
	10-18-07		NAS# 1344G Day 0 - 1.0 mg/L Lindane	1		X					
	10-18-07		NAS# 1345G Day 0 - 2.0 mg/L Lindane	1		X					
	10-18-07		NAS# 1346G Day 0 - 4.0 mg/L Lindane	1		X					
	10-18-07		NAS# 1347G Day 0 - 8.0 mg/L Lindane	1		X					
	10-18-07		NAS# 1348G Day 0 - [illegible]	1							
	10-18-07		NAS# 1349G Day 0 - [illegible]	1							
	10-18-07		NAS# 1350G Day 0 - Ammonia	1							

Signature	Print Name	Company	Date	Time	Cooler Custody Seal
Relinquished by: [signature]	GERALD IRISSARRI	Northwestern Aquatic Sciences	10-22-07	1330	Present / Not Present
Received by:					Intact / Not Intact
Relinquished by:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received by:					
Relinquished by:					
Received by laboratory:					

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd., P.O. Box 1437, Newport, OR 97365
Tel 541-265-7225, Fax 541-265-2799, www.nwaquatic.com

Client Name: Integral Consulting, Inc
Address: 319 SW Washington St., Suite 1150
City, State, Zip code: Portland, OR 97204
Project No: Integral B01-01-58C Windward 07-28-04-53
Phone No: 503-284-5545
Report Attention: Abbie Spielman
Sampled by:
Shipping Information: Carrier UPS; UPS#Y3489E; Airbill No
Testing Required: Copper; Lindane; Pentachlorophenol; Aniline; Diazinon; Naphthalene; Comments

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	10-20-07	1010	NAS# 1359G Day 2 - 0 mg/L Lindane	1		X					
	10-20-07		NAS# 1360G Day 2 - 0.5 mg/L Lindane	1		X					
	10-20-07		NAS# 1361G Day 2 - 1.0 mg/L Lindane	1		X					
	10-20-07		NAS# 1362G Day 2 - 2.0 mg/L Lindane	1		X					
	10-20-07		NAS# 1363G Day 2 - 1.0 mg/L Lindane	1		X					
	10-20-07		NAS# 1364G Day 2 - 8.0 mg/L Lindane	1		X					

Signature	Print Name	Company	Date	Time	Cooler Custody Seal
Relinquished by: [signature]	GERALD IRISSARRI	Northwestern Aquatic Sciences	10-22-07	1330	Present / Not Present
Received by:					Intact / Not Intact
Relinquished by:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received by:					
Relinquished by:					
Received by laboratory:					

Sample shipped to CAS

Test No. C86-40

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd., P.O. Box 1407 Newport, OR 97365
Tel: 541-265-7225 Fax: 541-265-2709, www.nwaquatic.com

Client Name: Integral Consulting, Inc
Address: 319 SW Washington St., Suite 1150
City, State, Zip Code: Portland, OR 97204
Project No: Integral B01-01-SBC; Windward 07-28-04.53
Phone No: 503-284-5545
Report Attention: Abbie Spielman
Shipping Information: Carrier: UPS; UPS#Y3489F
Testing Required: Copper, Lindane, Pentachlorophenol, Aniline, Diazinon, Naphthalene
Comments

Lab Sample No.	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene
	10-22-07	0840	NAS# 1372G Day 4 - 0 mg/L Lindane	1		X				
	10-22-07		NAS# 1373G Day 4 - 0.5 mg/L Lindane	1		X				
	10-22-07		NAS# 1374G Day 4 - 1.0 mg/L Lindane	1		X				
	10-22-07		NAS# 1375G Day 4 - 2.0 mg/L Lindane			X				
	10-22-07		NAS# 1376G Day 4 - 4.0 mg/L Lindane			X				
	10-22-07		NAS# 1377G Day 4 - 8.0 mg/L Lindane	1		X				
	10-22-07		NAS# 1378G Day 4 - Ammonia from 0 mg/L Lindane concentration	1						
	10-22-07		NAS# 1379G Day 4 - Ammonia from 8 mg/L Lindane concentration	1						

	Signature	Print Name	Company	Date	Time	Cooler Custody Seal
Relinquished by	[signature]	GERALD IRISSARI	Northwestern Aquatic Sciences	10-22-07	1330	Present / Not Present
Received by						Intact / Not Intact
Relinquished by						Internal Cooler Temperature Upon Lab Receipt (°C)
Received by						
Relinquished by						
Received by laboratory						

Water Quality Computation - Test No. 686-40

	Temp	DO	pH	Cond	Hard	Alk
1	17.3	9.7	7.2	93	26	16
2	17.1	9.6	7.2	89	26	13
3	17.1	9.5	7.2	97	26	17
4	16.8	9.4	7.2	99	26	15
5	16.9	9.3	7.1	94	26	15
6	17.0	9.3	6.8	96	26	15
7	17.5	9.2	7.0	100	26	14
8	17.4	9.1	6.9	95	28	14
9	17.4	7.9	6.9	99	26	15
10	17.4	8.7	6.8	102	26	15
11	17.4	8.7	6.8			
12	17.6	8.8	6.4			
13	17.6	9.5	7.0			
14	17.4	9.3	6.9			
15	17.3	9.2	6.9			
16	17.4	9.0	6.8			
17	17.3	8.8	6.8			
18	17.4	8.9	6.7			
19	17.4	8.8	6.8			
20	17.3	8.6	6.8			
21	17.2	8.6	6.8			
22	17.2	8.2	6.8			
23	17.2	8.2	6.9			
24	17.0	8.3	7.0			
25	17.4	8.9	6.8			
26	17.2	8.8	6.8			
27	17.2	8.8	6.8			
28	17.0	8.6	6.8			
29	17.2	8.7	6.8			
30	17.5	8.6	6.9			
31						
32						
mean	17.3	8.9	6.9	96	26	15
s.d.	0.2	0.4	0.2	4	1	1
N	30	30	30	10	10	10
min	16.8	7.9	6.4	89	26	13
max	17.6	9.7	7.2	102	28	17

RSL
2/21/08

WQ Computation - acclimation (10-3-07 to 10-17-07) - Test No. 686-40

	Temp	DO	pH	Cond	Hard	Alk
1	17.0	9.1	7.8	130	46	55
2	16.9	9.2	7.9	120	42	38
3	16.9	9.0	7.4	130		
4	16.6	9.4	7.2	130	44	41
5	17.0	9.5	7.4	135	60	50
6	17.0	9.2	7.9	145	50	48
7	17.0	9.5	7.6	150	54	52
8	16.9	9.2	7.4	155	54	49
9	17.0	9.5	7.1	150	54	40
10	17.1	9.4	8.0	155	50	39
11	17.1	9.1	7.1	150	52	40
12	16.9	9.6	7.5	150	52	38
13	17.0	9.5	7.6	155	50	38
14	17.1	9.4	6.9	65	20	12
15	17.3	8.5	6.7	75	22	14
16						
17						
mean	17.0	9.3	7.4	133	46	40
s.d.	0.2	0.3	0.4	28	12	13
N	15	15	15	15	14	14
min	16.6	8.5	6.7	65	20	12
max	17.3	9.6	8.0	155	60	55

RSL
2/21/08

SampleDate	SampleTim	Matrix	LabMatrix	SampleID	arentSamp	SampleTyp	Method	nalysisDat	nalysisTim	nalyteGrou	cas_rn	Analyte	l_or_dissol	detect_flag	VALUE	Qualifiers	Units
10/18/2007	09:30	WQ	WQ	NAS#1342G Day 0-0 mg/L Lindane		N	SW8081A	########	00.43	Pesticides	58-89-9	gamma-He	N	Y	8 8		ug/l
10/18/2007	09:30	WQ	WQ	NAS#1343G Day 0-0.5 mg/L Lindane		N	SW8081A	########	01 09	Pesticides	58-89-9	gamma-He	N	Y	150		ug/l
10/18/2007	09:30	WQ	WQ	NAS#1344G Day 0-1 0 mg/L Lindane		N	SW8081A	########	01 35	Pesticides	58-89-9	gamma-He	N	Y	330		ug/l
10/18/2007	09:30	WQ	WQ	NAS#1345G Day 0-2.0 mg/L Lindane		N	SW8081A	########	02 01	Pesticides	58-89-9	gamma-He	N	Y	670		ug/l
10/18/2007	09:30	WQ	WQ	NAS#1346G Day 0-4 0 mg/L Lindane		N	SW8081A	########	02.27	Pesticides	58-89-9	gamma-He	N	Y	760		ug/l
10/18/2007	09.30	WQ	WQ	NAS#1347G Day 0-8 0 mg/L Lindane		N	SW8081A	########	02 53	Pesticides	58-89-9	gamma-He	N	Y	2500		ug/l
10/19/2007	11:20	WQ	WQ	NAS#1352G Day 1-0 mg/L Lindane		N	SW8081A	########	07 40	Pesticides	58-89-9	gamma-He	N	Y	1 1		ug/l
10/19/2007	11:20	WQ	WQ	NAS#1353G Day 1 0 5 mg/L Lindane		N	SW8081A	########	08 06	Pesticides	58-89-9	gamma-He	N	Y	170		ug/l
10/19/2007	11:20	WQ	WQ	NAS#1354G Day 1-1 0 mg/L Lindane		N	SW8081A	########	08:32	Pesticides	58-89-9	gamma-He	N	Y	340		ug/l
10/19/2007	11.20	WQ	WQ	NAS#1358G Day 1-1 0 mg/L Lindane Dupl		N	SW8081A	########	10 17	Pesticides	58-89-9	gamma-He	N	Y	330		ug/l
10/19/2007	11:20	WQ	WQ	NAS#1355G Day 1-2 0 mg/L Lindane		N	SW8081A	########	08 58	Pesticides	58-89-9	gamma-He	N	Y	440		ug/l
10/19/2007	11 20	WQ	WQ	NAS#1356G Day 1-4 0 mg/L Lindane		N	SW8081A	########	09 25	Pesticides	58-89-9	gamma-He	N	Y	1000		ug/l
10/19/2007	11:20	WQ	WQ	NAS#1357G Day 1-8 0 mg/L Lindane		N	SW8081A	########	09 51	Pesticides	58-89-9	gamma-He	N	Y	2700		ug/l
10/20/2007	10:10	WQ	WQ	NAS#1359G Day 2-0 mg/L Lindane		N	SW8081A	########	10 43	Pesticides	58-89-9	gamma-He	N	Y	8 7		ug/l
10/20/2007	10:10	WQ	WQ	NAS#1360G Day 2-0.5 mg/L Lindane		N	SW8081A	########	11:09	Pesticides	58-89-9	gamma-He	N	Y	260		ug/l
10/20/2007	10:10	WQ	WQ	NAS#1361G Day 2-1.0 mg/L Lindane		N	SW8081A	########	11 35	Pesticides	58-89-9	gamma-He	N	Y	360		ug/l
10/20/2007	10:10	WQ	WQ	NAS#1362G Day 2-2.0 mg/L Lindane		N	SW8081A	########	14 12	Pesticides	58-89-9	gamma-He	N	Y	560		ug/l
10/20/2007	10:10	WQ	WQ	NAS#1363G Day 2-4 0 mg/L Lindane		N	SW8081A	########	14 38	Pesticides	58-89-9	gamma-He	N	Y	1600		ug/l
10/20/2007	10:10	WQ	WQ	NAS#1364G Day 2-8 0 mg/L Lindane		N	SW8081A	########	15 05	Pesticides	58-89-9	gamma-He	N	Y	3600		ug/l
10/21/2007	09:50	WQ	WQ	NAS#1365G Day 3-0 mg/L Lindane		N	SW8081A	########	15 31	Pesticides	58-89-9	gamma-He	N	Y	0 47	J	ug/l
10/21/2007	09 50	WQ	WQ	NAS#1366G Day 3-0 5 mg/L Lindane		N	SW8081A	########	15 57	Pesticides	58-89-9	gamma-He	N	Y	250		ug/l
10/21/2007	09:50	WQ	WQ	NAS#1367G Day 3-1 0 mg/L Lindane		N	SW8081A	########	16 23	Pesticides	58-89-9	gamma-He	N	Y	71		ug/l
10/21/2007	09:50	WQ	WQ	NAS#1368G Day 3-2 0 mg/L Lindane		N	SW8081A	########	16 49	Pesticides	58-89-9	gamma-He	N	Y	570		ug/l
10/21/2007	09 50	WQ	WQ	NAS#1369G Day 3-4 0 mg/L Lindane		N	SW8081A	########	17 15	Pesticides	58-89-9	gamma-He	N	Y	1400		ug/l
10/21/2007	09:50	WQ	WQ	NAS#1371G Day 3 4.0 mg/L Lindane Dupl		N	SW8081A	########	18.08	Pesticides	58-89-9	gamma-He	N	Y	590		ug/l
10/21/2007	09:50	WQ	WQ	NAS#1370G Day 3-8.0 mg/L Lindane		N	SW8081A	########	17:41	Pesticides	58-89-9	gamma-He	N	Y	3000		ug/l
10/22/2007	08:40	WQ	WQ	NAS#1372G Day 4-0 mg/L Lindane		N	SW8081A	########	01 35	Pesticides	58-89-9	gamma-He	N	Y	1 1		ug/l
10/22/2007	08:40	WQ	WQ	NAS#1373G Day 4 0.5 mg/L Lindane		N	SW8081A	########	02.01	Pesticides	58-89-9	gamma-He	N	Y	220		ug/l
10/22/2007	08:40	WQ	WQ	NAS#1374G Day 4 1 0 mg/L Lindane		N	SW8081A	########	02 27	Pesticides	58-89-9	gamma-He	N	Y	310		ug/l
10/22/2007	08:40	WQ	WQ	NAS#1375G Day 4-2.0 mg/L Lindane		N	SW8081A	########	02:53	Pesticides	58-89-9	gamma-He	N	Y	580		ug/l
10/22/2007	08:40	WQ	WQ	NAS#1376G Day 4-4 0 mg/L Lindane		N	SW8081A	########	03.19	Pesticides	58 89 9	gamma-He	N	Y	1400		ug/l
10/22/2007	08:40	WQ	WQ	NAS#1377G Day 4-8.0 mg/L Lindane		N	SW8081A	########	03 45	Pesticides	58-89-9	gamma-He	N	Y	1600		ug/l

Lindane (mg/L)	Day 0	Day 1	Day 2	Day 3	Day 4	Average	Standard Deviation
0	0.0	0.0	0.0	0.0	0 0	0.0	0.0
0.5	0.15	0 17	0 26	0.25	0.22	0 21	0.05
1 0	0.33	0 34	0.36	0.07	0.31	0.28	0.12
2.0	0.67	0.44	0 56	0.57	0 58	0.56	0.08
4.0	0.76	1 00	1 60	1.00	1.40	1 15	0.34
8.0	2.50	2 70	3.60	3.00	1 60	2.68	0.73

Validated Lindane Analyses
Reported by Windward
Rec 2/14/08

686-40

12 of 14

Acute 96-hr Toxicity Test-96 Hr Survival

Start Date:	10/18/07 11:00	Test ID:	686-40	Sample ID:	
End Date:	10/22/07 12:15	Lab ID:	ORNAS-Northwestern Aquati	Sample Type:	LINDANE
Sample Date:		Protocol:	NAS-686-Lamprey Ft acute	Test Species:	LY-Lampetra sp.
Comments:					

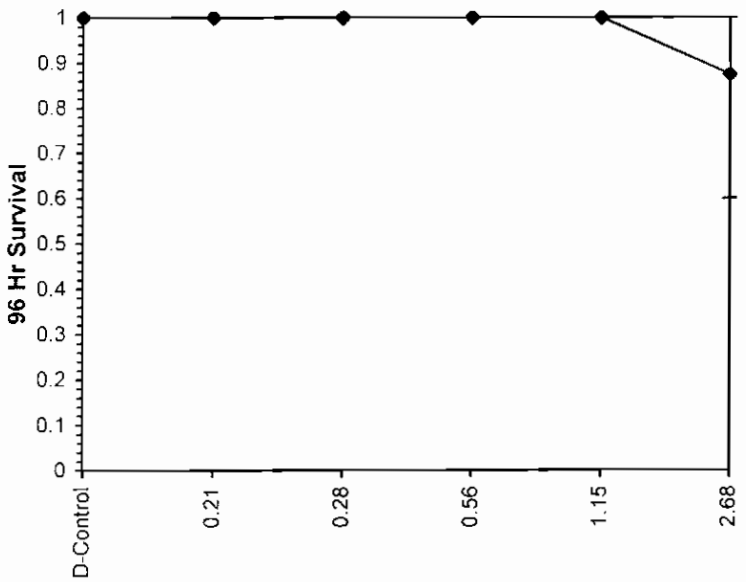
Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
0.21	1.0000	1.0000	1.0000	1.0000
0.28	1.0000	1.0000	1.0000	1.0000
0.56	1.0000	1.0000	1.0000	1.0000
1.15	1.0000	1.0000	1.0000	1.0000
2.68	0.6000	0.9000	1.0000	1.0000

			Transform: Arcsin Square Root					Rank	1-Tailed		
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical		
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
0.21	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
0.28	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
0.56	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
1.15	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
2.68	0.8750	0.8750	1.2398	0.8861	1.4120	20.004	4	14.00	10.00	5	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.46216	0.884	-2.2465	11.8408
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2.68	>2.68		

Dose-Response Plot



LC50 > 2.68 mg/L
RKL 2/18/08

ToxCalc v5.0.23N

Test: AT-Acute 96-hr Toxicity Test
Test ID: 686-40
Species: LY-Lampetra sp.
Protocol: NAS-686-Lamprey Ft acute
Sample ID:
Sample Type: Lindane
Start Date: 10/18/07 11:00
End Date: 10/22/07 12:15
Lab ID: ORNAS-Northwestern Aquatic Sciences

Pos	ID	Rep	Group	Start	24 Hr	48 Hr	72 Hr	96 Hr	Notes
	1	1	D-Control	10				10	
	2	2	D-Control	10				10	
	3	3	D-Control	10				10	
	4	4	D-Control	10				10	
	5	1	0.210	10				10	
	6	2	0.210	10				10	
	7	3	0.210	10				10	
	8	4	0.210	10				10	
	9	1	0.280	10				10	
	10	2	0.280	10				10	
	11	3	0.280	10				10	
	12	4	0.280	10				10	
	13	1	0.560	10				10	
	14	2	0.560	10				10	
	15	3	0.560	10				10	
	16	4	0.560	10				10	
	17	1	1.150	10				10	
	18	2	1.150	10				10	
	19	3	1.150	10				10	
	20	4	1.150	10				10	
	21	1	2.680	10				6	
	22	2	2.680	10				9	
	23	3	2.680	10				10	
	24	4	2.680	10				10	

Comments:

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-686-Lamprey-rf

3/4/08

LAMPREY SURVIVAL RANGE-FINDING TEST

Test No. 686-41 Client Windward Investigator

STUDY MANAGEMENT:

Client: Windward Environmental, 200 West Mercer Street, Suite 401, Seattle, WA 98119
Client's Study Monitor: Ms. Helle Andersen
Testing Laboratory: Northwestern Aquatic Sciences
Test Location: Newport Laboratory
Laboratory's Study Personnel:
Project Manager/Study Director: G.A. Buhler/R.S. Caldwell
QA Officer: L.K. Nemeth 1. G.J. TRISSARRI
2. 3.
4. 5.
Study Schedule:
Test Beginning: 10-30-07 1520 Test Ending: 11/3/07 1500

TEST MATERIAL:

Description: Naphthalene, Fisher Scientific, Certified, Lot No. 056112
NAS Sample No.:
Date of Preparation: See pg 2

DILUTION WATER:

Description: City of Newport tap water
Date of Preparation/Collection: 10-30-07
Water Quality: Cond. (µmhos/cm) 90 pH 7.2
Hardness (mg/L as $CaCO_3$) 22 Alkalinity (mg/L as $CaCO_3$) 8
Total Chlorine (mg/L, DL 0.02 mg/L) <0.02
Treatments: Dechlorinated, aerated ≥ 24 hours

TEST ORGANISMS:

Species: *Lampetra* sp. Age/Size: 4-6 cm
Source: Trask, Nestucca, Siletz & Alsea rivers
Acclimation Data:

Date	Temp. (°C)	pH	DO (mg/L)	Conductivity (µmhos/cm)	Hardness (mg/L)	Alkalinity (mg/L)
10-16-07	12.4 13.3	6.9	9.4	145	60	57
10-17-07	[illegible]	6.7	8.5	135	60	55
10-18-07	13.2	8.3	9.9	130	48	36
10-19-07	13.3	7.5	9.5	120	48	36
10-20-07	13.2	7.7	10.0	125	46	38
10-21-07	14.2	7.6	9.9	130	48	37
10-22-07	14.2	7.7	9.4	135	50	40
10-23-07	15.1	7.9	9.7	145	54	44
10-24-07	15.6	8.3	9.7	155	58	47
10-25-07	16.1	8.5	9.5	160	66	53
10-26-07	16.8	8.1	9.6	165	60	47
10-27-07	16.8	8.3	9.9	155	62	49
10-28-07	16.6	8.3	9.9	165	62	47
10-29-07	17.0	8.1	9.5	165	62	48
10-30-07	16.9	8.2	9.6	170	64	49
Mean	15.0	7.9	9.6	147	56	46
S.D.	1.7	0.5	0.4	17	7	7
(N)	15	15	15	15	15	15

← Nestucca R. receiving N_2O temperature

LAMPREY SURVIVAL RANGE-FINDING TEST

Test No. 686-41 Client Windward Investigator

TEST PROCEDURES AND CONDITIONS:

Test Concentrations (10% series recommended): 30, 3.0, 0.3, 0.03 and 0 mg/L (control) All concentrations are nominal.
Test Chambers (description): 2.5 gallon glass aquaria covered with plastic wrap and plastic lids
Test Volume (mL): 2.8 L Replicates/treatment: 1
Organisms/treatment: 5 (5/repl) Temperature (°C) 17 ± 1
Test water changes: daily Aeration during test: None
Feeding: None. Test Duration: 96 hr
Photoperiod (L/D): Constant darkness (except for daily observations ≈ 1 hr)
Light intensity (ft.c.): 15.4
Beaker Placement: Stratified randomization

Randomization chart: Location: Room 1

Ø	0.3	30	0.03	3.0	–

STOCK SOLUTION PREPARATION:

- Day 0: Prep'd 10/29/07 1720 116.4 mg/4.0 L RSC
- Day 1 " 10/30 1400 124 mg/4.0 L RSC
- Day 2 " 10/31 1430 120.8 mg/4.0 L RSC
- Day 3 " 11/1 1530 119.3 " RSC

TEST SOLUTION PREPARATION:

- Day 0: 10/29/07 1400 RSC
- Day 1 10/31/07 1410 RSC
- Day 2 11/1/07 1530 RSC
- Day 3 11/2/07 1530 RSC

LOADING (g/L):

(weigh and measure length ~~of controls~~ at end of test) RSC 3/4/08

Length (~~mm~~ cm)	Weight (g)
5.0	0.23
5.6	0.32
5.5	0.33
4.5	0.23
4.5	0.16

	Length (~~mm~~ cm)	Weight (g)
mean	5.0	0.25
S.D.	0.5	0.07
N	5	5

Error codes:
1) Correction of handwriting error
2) Written in wrong location; entry deleted
3) Wrong date deleted; replaced with correct date
4) Error found in measurement; measurement repeated

$$\text{Fish loading} = \frac{0.25\ g/fish \times 5\ fish}{2.8\ L} = 0.45\ g/L$$

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-686-Lamprey-rf

LAMPREY SURVIVAL RANGE-FINDING TEST

Test No. 686-41 Client Windward Investigator

WATER QUALITY DATA SHEET

DAY 0 (10/30/07) GAB

Conc. (mg/L)	Temp (°C)	pH	DO (mg/L)	Conductivity (µmhos/cm)	Hardness (mg/L)	Alkalinity (mg/L)	# Survivors	Comments
30	16.4	7.3	8.0	105	22	14	5	
3.0	16.3	7.2	8.2				5	
0.3	16.4	7.2	8.4				5	
0.03	16.3	7.2	8.4				5	
Control	16.5	7.1	8.4	90	22	8	5	

NOTES: Sample new solutions at each concentration and ship to CAS.

DAY 1 (10/31/07) GAB

Conc. (mg/L)	Temp (°C)	pH	DO (mg/L)	Conductivity (µmhos/cm)	Hardness (mg/L)	Alkalinity (mg/L)	# Survivors	Comments
30	16.1	7.2	7.7				5	all animals lethargic
3.0	16.2	7.2	7.6				5	
0.3	16.2	7.1	7.8				5	
0.03	16.2	7.1	7.9				5	
Control	16.2	7.2	7.9				5	

NOTES: Sample new solutions at each concentration and ship to CAS.

DAY 2 (11/1/07) GAB

Conc. (mg/L)	Temp (°C)	pH	DO (mg/L)	Conductivity (µmhos/cm)	Hardness (mg/L)	Alkalinity (mg/L)	# Survivors	Comments
30	16.3	7.3	8.1	98	22	15	5	All animals inactive, moves, lethargic, pale color, spasm twitch
3.0	16.4	7.3	8.2				5	
0.3	16.4	7.2	8.3				5	
0.03	16.4	7.2	8.2				5	
Control	16.4	7.2	8.4	82	22	10	5	

NOTES: Sample new and old solutions at each concentration and ship to CAS.

LAMPREY SURVIVAL RANGE-FINDING TEST

Test No. 686-41 Client Windward Investigator

WATER QUALITY DATA SHEET

DAY 3 (11 / 2 / 07) WB/GSI

Conc. (mg/L)	Temp (°C)	pH	DO (mg/L)	Conductivity (µmhos/cm)	Hardness (mg/L)	Alkalinity (mg/L)	# Survivors	Comments
30	16.8	7.2	7.8				4 (1D)	inactive, moved slightly when gently prodded, pale color, rusty hemorrhaged spots on body
3.0	16.7	7.2	7.9				5	
0.3	16.6	7.2	8.3				5	
0.03	16.9	7.2	8.6				5	
Control	17.2	7.2	9.4				5	

NOTES: Sample new solutions at each concentration and ship to CAS.

DAY 4 (11 / 3 / 07) WB/GSI

Conc. (mg/L)	Temp (°C)	pH	DO (mg/L)	Conductivity (µmhos/cm)	Hardness (mg/L)	Alkalinity (mg/L)	# Survivors	Comments
30	16.9	7.3	7.9	92	23	13	2 (2D)	survivors inactive, little movement when gently prodded
3.0	16.8	7.2	8.1				5	
0.3	16.9	7.2	8.1				5	
0.03	16.9	7.3	8.0				5	
Control	16.6	7.3	8.2	90	23	11	5	

NOTES: Sample old solutions at each concentration and ship to CAS. Weigh and measure lengths on control animals.

Water Quality Summary							
				93	—	—	
Mean	16.5	7.2	8.1	8	22	12	
S.D	0.3	0.1	0.3		1	3	
N	25	25	25	6	6	6	
low	16.1	7.1	7.6	82	22	8	
high	17.2	7.3	8.6	105	23	15	

LAMPREY SURVIVAL RANGE-FINDING TEST

Test No. 686-41 Client Windward Investigator

WATER SAMPLING RECORD

NAS Sample #	Description Day	Conc. (mg/L)	Date	Time	Initials	Comments
1390G	Day 0-new	30	10-30-07	1600	GSI	
1389G	Day 0-new	3.0				
1388G	Day 0-new	0.3				
1387G	Day 0-new	0.03				
1386G	Day 0-new	control				
1395G	Day 1-new	30	10-31-07	1500	GSI	
1394G	Day 1-new	3.0				
1393G	Day 1-new	0.3				
1392G	Day 1-new	0.03				
1391G	Day 1-new	control				
1405G	Day 2-new	30	GSI ~~10~~ 11-1-07	1540	GSI	
1404G	Day 2-new	3.0				
1403G	Day 2-new	0.3				
1402G	Day 2-new	0.03				
1401G	Day 2-new	control				
1400G	Day 2-old	30		1430		
1399G	Day 2-old	3.0				
1398G	Day 2-old	0.3				
1397G	Day 2-old	0.03				
1396G	Day 2-old	control				
1412G	Day 3-new	30	11-2-07	1530	GSI	1413G SAMPLE
1411G	Day 3-new	3.0				COLLECTED
1410G	Day 3-new	0.3				AS DAY3-NEW
1409G	Day 3-new	0.03				30 mg/L DUPLICATE
1408G	Day 3-new	control				
1418G	Day 4-old	30	11-3-07	1200	GSI	
1417G	Day 4-old	3.0				
1416G	Day 4-old	0.3				
1415G	Day 4-old	0.03				
1414G	Day 4-old	control				

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (Lampetra sp.)

DATES	TEMP (°C)	pH	DO (mg/L)	COND (umhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Ini.
9-2-2007	18.1	6.7	8.6	82	10	25	[illegible]	[illegible]	[illegible]		[illegible]	Rec'd [illegible]	[illegible]
9-3-07	16.8	7.7	9.6	85	26	30		"	"			[illegible]	[illegible]
9-5-07	17.1	6.6	9.8	68	28	10	[illegible]	[illegible]	"			Rec'd [illegible]	[illegible]
9-7-07	16.4	7.6	9.7	162	34	30	[illegible]	[illegible]	"			[illegible]	[illegible]
9-9-07	18.2	6.1	7.2	60	22	16	[illegible]	[illegible]	"			[illegible]	[illegible]
9-10-07	17.0	7.0	7.4	160	34	30						[illegible]	[illegible]
9-10-07	16.9	6.6	9.0	60	31	25	[illegible]		"			[illegible]	[illegible]
9-11-07	16.8	7.2	7.5	135	34	30			"			[illegible]	[illegible]
9-12-07	16.9	7.1	7.4	140	—	—			"				
9-12-07	16.7	7.6	9.1	160	-	—			"			[illegible]	[illegible]
9-13-07	16.7	7.7	7.6	160	-	—			"			[illegible]	[illegible]
9-14-07	16.2	7.5	8.8	185	34	30			"			"	[illegible]
9-15-07	16.3	7.2	9.1	190	34	30			"			"	[illegible]
9-16-07	16.8	7.3	9.0	185	34	30			"			"	
9-17-07	16.8	7.0	9.1	155	26	30			"			"	
9-18-07	17.0	7.5	9.3	160	—	—			"			"	
9-19-07	16.7	7.5	8.8	170	-	—			"			Holding	[illegible]
9-20-07	16.7	7.2	8.9	175	—	—			"			"	[illegible]
9-21-07	17.4	7.6	9.0	235	60	35			"			"	[illegible]
9-22-07	17.0	8.0	9.4	185	60	38			"			"	[illegible]

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (Lampetra sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (umhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Ini.
9-13-07	17.1	8.0	9.0	170	55	30			[illegible]			Holding	[illegible]
9-14-07	17.1	8.2	9.1	155	48	29			"		[illegible]	"	[illegible]
9-17-07	17.1	8.3	9.2	120	—	—			"			"	
9-18-07	17.0	8.1	9.2	115	41	30			"			"	[illegible]
9-19-07	17.1	7.7	8.8	120	41	30			"			"	[illegible]
9-20-07	17.0	7.9	9.1	120	39	21			"			"	[illegible]
9-21-07	17.7	7.6	9.1	125	39	34			"			"	[illegible]
9-22-07	17.1	7.9	9.0	125	36	34	[illegible]		"			"	[illegible]
9-23-07	17.0	8.2	9.5	140	44	31	"		"			"	[illegible]
9-24-07	17.1	7.9	9.4	130	42	29			"			"	[illegible]
9-25-07	17.1	7.7	9.3	140	46	28			"			"	[illegible]
9-26-07	16.8	7.9	9.3	140	44	29			"			"	
9-27-07	17.1	7.7	9.8	145	44	30			"			"	[illegible]
9-28-07	17.2	7.8	9.3	140	48	36			"			"	[illegible]
9-29-07	17.4	7.7	9.3	145	48	37			"			"	[illegible]
9-30-07	17.2	7.8	9.3	150	48	36			"			"	[illegible]
9-31-07	16.9	7.5	8.8	150	48	30			"			"	[illegible]
9-10-07	17.0	7.7	8.8	140	48	37			"			"	[illegible]
10-1-07	17.0	7.8	9.4	145	48	36			"		[illegible]	"	[illegible]
10-2-07	17.3	8.1	9.2	135	—	—			"			"	[illegible]

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (Lampetra sp.)

DATES	TEMP (°C)	pH	DO (mg/L)	COND (umhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Ini.
10-3-07	17.0	7.8	9.1	130	46	55			[illegible]			Holding	[illegible]
10-4-07	16.9	7.9	9.2	120	42	38						"	[illegible]
10-5-07	16.9	7.4	9.0	130	hoT Tokens							"	[illegible]
10-6-07	16.6	7.2	9.4	130	44	41						"	[illegible]
10-7-07	17.0	7.4	9.5	135	60	50						"	[illegible]
10-8-07	17.0	7.9	9.2	145	50	48						"	[illegible]
10-9-07	17.0	7.6	9.5	150	54	52						"	[illegible]
10-10-07	16.9	7.4	9.2	155	54	49						"	[illegible]
10-11-07	17.0	7.1	9.5	150	54	40						"	[illegible]
10-12-07	17.1	8.0	9.4	155	50	39						"	[illegible]
10-13-07	17.1	7.1	9.1	150	52	40						"	[illegible]
10-14-07	16.9	7.5	9.4	150	52	38						"	[illegible]
10-15-07	17.0	7.6	9.5	155	50	38						"	[illegible]
10-15-07	13.0	6.7	10.6	97	30	30						[illegible]	[illegible]
10-16-07	13.2	7.9	10.1	145	60	57						Holding [illegible]	[illegible]
10-16-07	17.1	6.9	9.4	165	20	12						Test [illegible]	[illegible]
10-16-07	12.4	6.2	8.9	65	18	14						[illegible]	[illegible]
10-17-07	13.5	8.3	10.1	135	60	55					[illegible]	[illegible]	[illegible]
10-17-07	13.3	6.7	8.5	75	22	14						Holding [illegible]	[illegible]
10-19-07	18.1	6.9	10.2	57	20	14						[illegible]	

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (Lampetra sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (umhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Ini.
10-18-07	13.2	8.3	9.9	130	48	36			[illegible]			Holding	[illegible]
10-19-07	13.3	7.5	9.5	120	48	36						"	[illegible]
10-20-07	13.2	7.9	10.0	125	46	38							
10-21-07	14.2	7.6	9.9	130	49	37							
10-22-07	14.2	7.7	9.4	135	50	40						"	[illegible]
10-23-07	15.1	7.9	9.7	145	54	44						"	[illegible]
10-24-07	15.6	8.3	9.7	155	58	47						"	[illegible]
10-25-07	16.1	8.5	9.5	160	66	53						"	[illegible]
10-26-07	16.8	8.1	9.6	165	60	47						"	[illegible]
10-27-07	16.8	8.3	9.9	155	62	49						"	[illegible]
10-28-07	16.6	8.3	9.9	165	62	47						"	[illegible]
10-29-07	17.0	8.1	9.5	165	62	48						"	[illegible]
10-30-07	16.9	8.2	9.6	170	64	46						"	[illegible]
10-31-07	16.8	8.2	9.9	175	70	54						"	[illegible]
11-1-07	16.8	8.2	9.8	165	62	45						"	[illegible]
11-2-07	16.8	8.2	9.5	165	62	47						"	[illegible]
11-3-07	16.6	8.1	9.2	160	56	38						"	[illegible]
11-4-07	16.8	8.3	9.6	180	64	48						"	[illegible]
11-5-07	16.9	8.1	9.9	180	66	49						"	[illegible]
11-6-07	16.9	8.2	9.8	155	52	35						"	[illegible]

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (Lampetra sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (umhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
1-17-07	17.0	8.1	9.4	155	54	37						Holding	
1-18-07	16.9	8.1	9.3	155	56	39						"	
1-19-07	16.7	8.6	9.7	160	62	46						"	
1-20-07	16.7	8.1	9.6	155	56	43						"	
1-21-07	16.7	8.1	9.9	145	52	39						"	
1-22-07	16.8	8.0	9.7	155	56	38						"	
1-23-07	16.8	7.6	9.7	155	54	38						"	
1-24-07	16.8	7.9	9.7	150	52	36						"	
1-25-07	16.8	8.0	9.7	160	60	43						"	
1-26-07	16.9	8.2	9.9	160	58	39						"	
1-27-07	16.7	8.3	10.1	165	62	43						"	
1-28-07	16.9	8.1	9.8	150	54	36						"	
1-29-07	16.8	7.9	9.7	175	64	48						"	
1-30-07	16.7	7.9	9.9	170	64	46						"	
1-31-07	16.6	8.0	9.9	175	64	48						"	
2-01-07	16.5	7.9	10.0	165	62	39						"	
2-02-07	16.5	7.8	9.5	170	62	44						"	
2-03-07	16.5	8.0	9.7	190	70	48						"	
2-04-07	16.5	7.9	9.6	175	64	45						"	
2-05-07	16.6	7.5	9.4	175	64	46						"	

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (Lampetra sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (umhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
1-29-07	16.1	7.9	9.6	180	62	44						Holding	
1-30-07	16.0	7.7	10.0	175	56	44						"	
1-31-07	16.0	7.8	9.7	170	60	43						"	
2-1-07	16.0	8.0	9.8	175	62	46						"	
2-2-07	16.2	7.9	9.7	185	70	47						"	
2-3-07	15.8	7.5	9.4	165	60	45						"	
2-5-07	15.7	8.2	9.6	155	60	39						"	
2-6-07	15.8	7.8	9.8	170	60	42						"	
2-7-07	16.3	7.6	9.9	165	60	42						"	
2-8-07	15.7	7.6	9.9	160	58	40						"	
2-9-07	17.7	7.9	9.0	175	64	43						"	
2-10-07	17.1	7.9	8.8	165	62	42						"	
2-11-07	17.1	7.6	9.4	170	56	41						"	
2-12-07	17.3	7.4	9.5	160	58	40						"	
2-13-07	16.7	7.6	9.4	170	64	43						"	
2-14-07	16.8	7.3	9.1	170	64	44						"	
2-15-07	17.0	7.2	9.0	165	56	44						"	
2-16-07	16.5	7.2	9.5	180	70	45						"	
2-17-07	16.7	7.2	9.3	170	66	44						"	
2-18-07	16.5	7.4	9.6	175	74	53						"	

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (Lampetra sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (umhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
12-31-07	15.6	7.3	9.9	190	76	51						Holding	
1-2-08	16.2	7.2	10.3	175	56	46						"	
1-3-08	15.4	7.2	10.0	190	72	48						"	
1-4-08	16.0	7.8	9.8	175	70	46							
1-5-08	17.1	7.0	9.5	185	68	54						"	
1-6-08	17.1	7.0	9.4	190	72	48						"	
1-7-08	16.8	7.1	9.0	190	72	51						"	
1-8-08	16.8	7.3	10.1	200	74	50						"	
1-9-08	17.1	7.2	9.7	195	68	49						"	
1-10-08	17.0	7.0	9.8	170	56	32						"	
1-11-08	17.1	9.1	9.9	175	52	29						"	
1-12-08	16.9	8.0	9.2	195	68	50						"	
1-13-08	16.8	7.3	9.5	205	72	50						"	
1-14-08	17.0	7.0	9.0	[illegible]	64	43						"	
1-15-08	16.8	8.4	9.6	185	56	41						"	
1-16-08	16.8	7.3	9.7	180	60	39						"	
1-17-08	16.9	8.2	9.8	175	62	36						"	
1-18-08	16.7	8.4	9.7	155	48	30						"	
1-19-08	16.7	8.4	9.9	160	54	32						"	
1-20-08	16.9	8.1	9.5	165	50	33						"	

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (Lampetra sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (umhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
1-7-08	15.6	8.1	10.4	165	56	30						Holding	
1-10-08	15.4	7.0	10.2	145	44	24						"	
1-11-08	16.3	8.1	10.3	145	42	24							
1-12-08	16.0	7.4	10.2	145	56	31						"	
1-13-08	16.2	8.1	9.6	170	54	32						"	
1-14-08	16.5	8.2	9.9	160	52	32						"	
1-15-08	16.2	8.2	9.7	160	50	29						"	
1-16-08	16.8	8.2	9.9	175	40	15						"	
1-17-08	15.6	8.0	9.9	165	56	63						"	

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences

Client Name: Integral Consulting Inc.
Project: Integral DEI 01-501
Address: 2135 SW Abingdon Dr., Suite 110
Portland, OR 97201
Phone: 503-284-5545
Project Manager: Abbie Spielman
Sampler: G.J. IRISSARRI

Lab Sample No.	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Fluoranthene	Phenanthrene/phenol	Aniline	Dibenzofuran	Naphthalene	Comments
	10-31-07	1600	NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	
	10-31-07		NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	
	10-31-07		NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	
	10-31-07		NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	
	10-31-07		NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	

Relinquished by (Signature)	Print Name	Company	Date	Time	Cooler Custody Seal
	GERALD IRISSARRI	Northwestern Aquatic Sciences	11-5-07	1400	Present / Not Present
Received by:					Intact / Not Intact
Relinquished by:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received by:					
Relinquished by:					
Received for laboratory by:					

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences

Client Name: Integral Consulting, Inc.
Project: Integral DEI 01-501
Address: 2135 SW Abingdon Dr., Suite 110
Portland, OR 97201
Phone: 503-284-5545
Project Manager: Abbie Spielman
Sampler: G.J. IRISSARRI

Lab Sample No.	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Fluoranthene	Phenanthrene/phenol	Aniline	Dibenzofuran	Naphthalene	Comments
	11-1-07	1650	NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	
	11-1-07		NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	
	11-1-07		NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	
	11-1-07		NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	
	11-1-07		NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	
	11-1-07	1510	NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	
	11-1-07		NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	
	11-1-07		NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	
	11-1-07		NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	

Relinquished by (Signature)	Print Name	Company	Date	Time	Cooler Custody Seal
	GERALD IRISSARRI	Northwestern Aquatic Sciences	11-5-07	1400	Present / Not Present
Received by:					Intact / Not Intact
Relinquished by:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received by:					
Relinquished by:					
Received for laboratory by:					

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences

Client Name: Integral Consulting, Inc.
Project: Integral DEI 01-501
Address: 2135 SW Abingdon Dr., Suite 110
Portland, OR 97201
Phone: 503-284-5545
Project Manager: Abbie Spielman
Sampler: G.J. IRISSARRI

Lab Sample No.	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Fluoranthene	Phenanthrene/phenol	Aniline	Dibenzofuran	Naphthalene	Comments
	11-2-07	1550	NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	
	11-2-07		NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	
	11-2-07		NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	
	11-2-07		NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	
	11-2-07		NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	
	11-2-07		NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	

Relinquished by (Signature)	Print Name	Company	Date	Time	Cooler Custody Seal
	GERALD IRISSARRI	Northwestern Aquatic Sciences	11-5-07	1400	Present / Not Present
Received by:					Intact / Not Intact
Relinquished by:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received by:					
Relinquished by:					
Received for laboratory by:					

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences

Client Name: Integral Consulting, Inc.
Project: Integral DEI 01-501
Address: 2135 SW Abingdon Dr., Suite 110
Portland, OR 97201
Phone: 503-284-5545
Project Manager: Abbie Spielman
Sampler: G.J. IRISSARRI

Lab Sample No.	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Fluoranthene	Phenanthrene/phenol	Aniline	Dibenzofuran	Naphthalene	Comments
	11-2-07	1550	NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	
	11-2-07		NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	
	11-2-07		NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	
	11-2-07		NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	
	11-2-07		NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	
	11-2-07		NASF 13106 Dry 2 - 0 mg/L Naphthalene - 0	3						X	

Relinquished by (Signature)	Print Name	Company	Date	Time	Cooler Custody Seal
	GERALD IRISSARRI	Northwestern Aquatic Sciences	11-5-07	1400	Present / Not Present
Received by:					Intact / Not Intact
Relinquished by:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received by:					
Relinquished by:					
Received for laboratory by:					

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences

Sample Date	Time Sampled	Time Received	Sample Description	Number of Containers	Copper	Lead	Pentachlorophenol	Arsenic	Dieldrin	Naphthalene	Comments
11-5-07	13:00		NAS-14[illegible] Bay 4 - [illegible]	3						X	
11-5-07			NAS-14[illegible] Bay 4 - [illegible]	3						X	
11-5-07			NAS-14[illegible] Bay 4 - [illegible]	3						X	
11-5-07			NAS-14[illegible] Bay 4 - [illegible]	3						X	
11-5-07			NAS-14[illegible] Bay 4 - [illegible]	3						X	

Relinquished by:	Signature	Print Name	Company	Date	Time
		GERALD IRISCHRAI	Northwestern Aquatic Sciences	11-5-07	1400
Received by:					
Relinquished by:					
Received by:					
Relinquished by:					
Received by laboratory:					

Date Results Sent: Phone / Fax / Email

Method / Recipient

Internal Chain Documentation: Hyna Lab Receipt [illegible]

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SampleDate	SampleTime	CMatrix	CQMatrix	SampleID	PermitSampleTypeID	MethodID	AnalysisDate	AnalysisTypeID	CategoryID	CAS-RN	AnalyteID	CQ-Code	DetectionFlag	VALUE	QualifierID	Units
10/30/2007	16:00	WQ	WQ	NAS#1386G Day0-0mg/L Naphthalene-NEW	N	SW8260B	11/12/2007	13:19	PAHs	91-20-3	Naphthalen	N	Y	0.72	J	ug/l
10/30/2007	16:00	WQ	WQ	NAS#1387G Day0-0.03mg/L Naphthalene-NEW	N	SW8260B	11/13/2007	20:31	PAHs	91-20-3	Naphthalen	N	Y	100		ug/l
10/30/2007	16:00	WQ	WQ	NAS#1388G Day0-0.3mg/L Naphthalene-NEW	N	SW8260B	11/13/2007	20:51	PAHs	91-20-3	Naphthalen	N	Y	93		ug/l
10/30/2007	16:00	WQ	WQ	NAS#1389G Day0-3.0mg/L Naphthalene-NEW	N	SW8260B	11/12/2007	17:05	PAHs	91-20-3	Naphthalen	N	Y	1100		ug/l
10/30/2007	16:00	WQ	WQ	NAS#1390G Day0-30.0mg/L Naphthalene-NEW	N	SW8260B	11/12/2007	17:25	PAHs	91-20-3	Naphthalen	N	Y	13000		ug/l
10/31/2007	15:00	WQ	WQ	NAS#1391G Day1-0mg/L Naphthalene-NEW	N	SW8260B	11/13/2007	02:18	PAHs	91-20-3	Naphthalen	N	N	0.29	U	ug/l
10/31/2007	15:00	WQ	WQ	NAS#1392G Day1-0.03mg/L Naphthalene-NEW	N	SW8260B	11/13/2007	02:37	PAHs	91-20-3	Naphthalen	N	Y	19		ug/l
10/31/2007	15:00	WQ	WQ	NAS#1393G Day1-0.3mg/L Naphthalene-NEW	N	SW8260B	11/14/2007	19:56	PAHs	91-20-3	Naphthalen	N	Y	170		ug/l
10/31/2007	15:00	WQ	WQ	NAS#1394G Day1-3.0mg/L Naphthalene-NEW	N	SW8260B	11/13/2007	06:32	PAHs	91-20-3	Naphthalen	N	Y	2100		ug/l
10/31/2007	15:00	WQ	WQ	NAS#1395G Day1-30.0mg/L Naphthalene-NEW	N	SW8260B	11/13/2007	06:52	PAHs	91-20-3	Naphthalen	N	Y	17000		ug/l
11/1/2007	14:30	WQ	WQ	NAS#1396G Day2-0mg/L Naphthalene-OLD	N	SW8260B	11/14/2007	17:57	PAHs	91-20-3	Naphthalen	N	Y	0.3	J	ug/l
11/1/2007	14:30	WQ	WQ	NAS#1397G Day2-0.03mg/L Naphthalene-OLD	N	SW8260B	11/14/2007	18:37	PAHs	91-20-3	Naphthalen	N	Y	15		ug/l
11/1/2007	14:30	WQ	WQ	NAS#1398G Day2-0.3mg/L Naphthalene-OLD	N	SW8260B	11/14/2007	19:36	PAHs	91-20-3	Naphthalen	N	Y	66		ug/l
11/1/2007	14:30	WQ	WQ	NAS#1399G Day2-3.0mg/L Naphthalene-OLD	N	SW8260B	11/14/2007	03:44	PAHs	91-20-3	Naphthalen	N	Y	630		ug/l
11/1/2007	14:30	WQ	WQ	NAS#1400G Day2-30.0mg/L Naphthalene-OLD	N	SW8260B	11/14/2007	04:03	PAHs	91-20-3	Naphthalen	N	Y	8900		ug/l
11/1/2007	15:40	WQ	WQ	NAS#1401G Day2-0mg/L Naphthalene-NEW	N	SW8260B	11/14/2007	01:46	PAHs	91-20-3	Naphthalen	N	N	0.23	U	ug/l
11/1/2007	15:40	WQ	WQ	NAS#1402G Day2-0.03mg/L Naphthalene-NEW	N	SW8260B	11/14/2007	02:06	PAHs	91-20-3	Naphthalen	N	Y	23		ug/l
11/1/2007	15:40	WQ	WQ	NAS#1403G Day2-0.3mg/L Naphthalene-NEW	N	SW8260B	11/14/2007	20:15	PAHs	91-20-3	Naphthalen	N	Y	200		ug/l
11/1/2007	15:40	WQ	WQ	NAS#1404G Day2-3.0mg/L Naphthalene-NEW	N	SW8260B	11/14/2007	04:23	PAHs	91-20-3	Naphthalen	N	Y	2000		ug/l
11/1/2007	15:40	WQ	WQ	NAS#1405G Day2-30.0mg/L Naphthalene-NEW	N	SW8260B	11/14/2007	04:42	PAHs	91-20-3	Naphthalen	N	Y	22000		ug/l
11/2/2007	15:30	WQ	WQ	NAS#1408G Day2-0mg/L Naphthalene	N	SW8260B	11/14/2007	19:17	PAHs	91-20-3	Naphthalen	N	Y	0.37	J	ug/l
11/2/2007	15:30	WQ	WQ	NAS#1409G Day2-0.03mg/L Naphthalene	N	SW8260B	11/14/2007	00:04	PAHs	91-20-3	Naphthalen	N	Y	23		ug/l
11/2/2007	15:30	WQ	WQ	NAS#1410G Day2-0.3mg/L Naphthalene	N	SW8260B	11/14/2007	05:02	PAHs	91-20-3	Naphthalen	N	Y	200		ug/l
11/2/2007	15:30	WQ	WQ	NAS#1411G Day2-3.0mg/L Naphthalene	N	SW8260B	11/14/2007	05:22	PAHs	91-20-3	Naphthalen	N	Y	1900		ug/l
11/2/2007	15:30	WQ	WQ	NAS#1412G Day2-30.0mg/L Naphthalene	N	SW8260B	11/14/2007	05:41	PAHs	91-20-3	Naphthalen	N	Y	16000		ug/l
11/2/2007	15:30	WQ	WQ	NAS#1413G Day2-30.0mg/L Naphthalene-DUPL	N	SW8260B	11/14/2007	06:01	PAHs	91-20-3	Naphthalen	N	Y	19000		ug/l
11/3/2007	12:00	WQ	WQ	NAS#1414G Day4-0mg/L Naphthalene-OLD	N	SW8260B	11/14/2007	20:35	PAHs	91-20-3	Naphthalen	N	Y	1.5	J	ug/l
11/3/2007	12:00	WQ	WQ	NAS#1415G Day4-0.03mg/L Naphthalene-OLD	N	SW8260B	11/14/2007	03:24	PAHs	91-20-3	Naphthalen	N	Y	15		ug/l
11/3/2007	12:00	WQ	WQ	NAS#1416G Day4-0.3mg/L Naphthalene	N	SW8260B	11/14/2007	06:40	PAHs	91-20-3	Naphthalen	N	Y	86		ug/l
11/3/2007	12:00	WQ	WQ	NAS#1417G Day4-3.0mg/L Naphthalene-OLD	N	SW8260B	11/14/2007	07:00	PAHs	91-20-3	Naphthalen	N	Y	890		ug/l
11/3/2007	12:00	WQ	WQ	NAS#1418G Day4-30.0mg/L Naphthalene-OLD	N	SW8260B	11/14/2007	07:19	PAHs	91-20-3	Naphthalen	N	Y	11000		ug/l

Day 3 not day 2?

Naphthalene (mg/L)	Day 0	Day 1	Day 2 Old	Day 2 New	Day 3	Day 4	Average	Standard Deviation
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.03	0.10	0.02	0.02	0.02	0.02	0.02	0.03	0.03
0.3	0.03	0.17	0.07	0.20	0.20	0.09	0.14	0.06
3.0	1.10	2.10	0.63	2.00	1.90	0.89	1.44	0.64
30.0	13.00	17.00	8.90	22.00	17.50	11.00	14.90	4.62

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Reviewed by Whitford 2/18/08

Acute 96-hr Toxicity Test-96 Hr Survival

Start Date:	10/30/07 15:20	Test ID:	686-41	Sample ID:	
End Date:	11/3/07 15:00	Lab ID:	ORNAS-Northwestern Aquati	Sample Type:	Naphthalene
Sample Date:		Protocol:	NAS-686-Lamprey-rf	Test Species:	LY-Lampetra sp.
Comments:					

Conc-mg/L	1
D-Control	1.0000
0.03	1.0000
0.14	1.0000
1.44	1.0000
14.9	0.4000

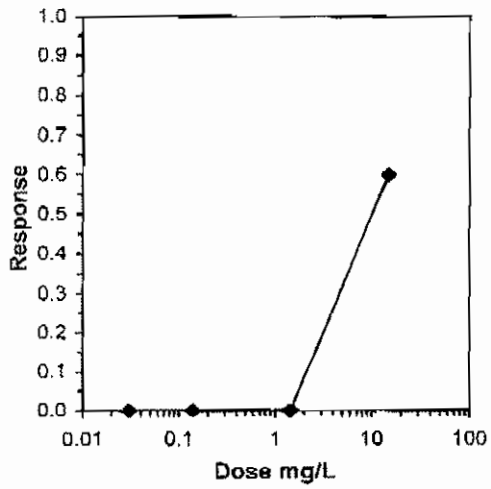
Conc-mg/L	Mean	N-Mean	Resp	Not Resp	Total	N	Fisher's Exact P	1-Tailed Critical	Number Resp	Total Number
D-Control	1.0000	1.0000	0	5	5	1			0	5
0.03	1.0000	1.0000	0	5	5	1	1.0000	0.0500	0	5
0.14	1.0000	1.0000	0	5	5	1	1.0000	0.0500	0	5
1.44	1.0000	1.0000	0	5	5	1	1.0000	0.0500	0	5
14.9	0.4000	0.4000	3	2	5	1	0.0833	0.0500	3	5

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Fisher's Exact Test	14.9	>14.9		

RS 3/4/08

Trim Level	~~EC50~~ LC50	95% CL	
0.0%			
5.0%			
10.0%			
20.0%			
Auto-40.0%	10.094	2.435	41.846

Trimmed Spearman-Karber



ToxCalc v5.0.23N

Test: AT-Acute 96-hr Toxicity Test
Test ID: 686-41
Species: LY-Lampetra sp.
Protocol: NAS-686-Lamprey-rf
Sample ID:
Sample Type: Naphthalene
Start Date: 10/30/07 15:20 End Date: 11/3/07 15:00
Lab ID: ORNAS-Northwestern Aquatic Sciences

Pos	ID	Rep	Group	Start	24 Hr	48 Hr	72 Hr	96 Hr	Notes
	1	1	D-Control	5				5	
	2	1	0.030	5				5	
	3	1	0.140	5				5	
	4	1	1.440	5				5	
	5	1	14.900	5				2	

Comments:

LAMPREY (*LAMPETRA* SP.) DEFINITIVE 96-HR FLOW-THROUGH ACUTE TOXICITY TEST OF NAPHTHALENE

NAS Test No. 686-42

Submitted to

Windward Environmental LLC
200 West Mercer Street, Suite 401
Seattle, WA 98119

Submitted by

Northwestern Aquatic Sciences
3814 Yaquina Bay Road
P.O. Box 1437
Newport, OR 97365

April 3, 2008

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 686-42
Title: Lamprey (*Lampetra* sp.) definitive 96-hr flow-through acute toxicity test of naphthalene.
Protocol No.: NAS-686-Lamprey FT acute, September 12, 2007

STUDY MANAGEMENT

Study Sponsor: Windward Environmental, 200 West Mercer Street, Suite 401, Seattle, WA 98119.
Sponsor's Study Monitor: Ms. Helle Andersen.
Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365.
Test Location: Newport Laboratory.
Laboratory's Study Personnel: G. A. Buhler, B.S., Proj. Manager; R. S. Caldwell, Ph.D., Study Director; L.K. Nemeth, B.A., M.B.A., QA Officer; G.J. Irissarri, B.S., Aq. Toxicol.
Study Schedule:
Test Beginning: 11-14-07, 1330 hrs.
Test Ending: 11-18-07, 1430 hrs.
Disposition of Study Records: All specimens, raw data, reports and other study records are stored according to Good Laboratory Practice regulations at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.
Good Laboratory Practices: The test was conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).
Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Description: Naphthalene (Fisher Scientific, Lot No. 056112).
Preparation of Working Stock Solution and Test Concentrations: Test solutions were prepared for continuous supply to the test aquaria using a Benoit constant flow diluter (Benoit *et al.*, 1982). The diluter was set up to deliver a 0.5 dilution series with a flow rate of approximately 80 ml/min per toxicant concentration. The diluter design was such that 160 ml/min of the toxicant solution at the highest test concentration (i.e. the water saturation level of naphthalene estimated to be approximately 20 mg/L at the test temperature) was required to be continuously delivered to the toxicant supply chamber of the diluter. To accomplish this, a large mixing reservoir, approximately 54L capacity, to contain the saturated solution of naphthalene in dilution water, was maintained at test temperature. The approach to preparing the saturated naphthalene solution in the mixing tank involved continuous recirculation of the tank contents, at an estimated rate of 1000 ml/min, through a commercial carbon water filter in which the activated carbon contents were replaced with a large quantity (>100 g) of crystalline naphthalene. The tank contents were continually mixed using a stainless steel stirring paddle, while also replacing, under float valve control, dilution water to replace delivery of the nominal 20 mg/L naphthalene solution to the diluter. Unvalidated GCMS analysis of the mixing tank contents by the testing laboratory showed that concentrations ranging from 14.1 to 17.5 mg/L were achieved during the study, but that highest test concentrations in the test aquaria were 35.9 ± 3.7% less than measured in the mixing tank. It is presumed that the loss was due to volatilization of naphthalene during passage through the diluter and distribution system.

DILUTION WATER

Source: City of Newport municipal tap water, treated as below, was supplied continuously to the toxicant diluter throughout the study.
Pretreatment: The water was dechlorinated by carbon filtration, particle filtered to 1 μm, and adjusted to a target water hardness of 25-30 mg/L as $CaCO_3$. Hardness adjustment was accomplished by passage of a portion of the dilution water through deionization cartridges.

TEST ORGANISMS

Species: Lamprey (*Lampetra* sp.)
Age/Size: Ammocoetes; target size range, 4-6 cm; actual size of 10 ammocoete subsample, 4.8 ± 0.6 cm.
Source: Ammocoetes were field collected in approximately equal numbers from the Trask, Nestucca, Siletz, and Alsea Rivers, Oregon. Field collection was by electoshocking and only 4-6 cm size ammocoetes were retained. Personnel of Windward Environmental performed the ammocoete collections.
Acclimation: Upon receipt, animals were removed from the collection site sediment and placed in 10-gallon aquaria supplied under flow-through conditions with dilution water. Water supply was sufficient (30-50 ml/min) to accomplish two volume changes of water per day. Approximately 50 lamprey were placed in each tank. Tanks contained an approximate 8 cm deep layer of sediment (Sandtastic Play Sand, Waupaca Materials) covered with approximately 26L of test water and were supplied with aeration. Normal behavior for the lamprey ammocoetes was to immediately bury in sediment and remain there. Any that did not immediately bury were discarded as potentially damaged. Ammocoetes were not fed during acclimation. Water quality conditions during the two weeks prior to testing (11-1-07 to 11-14-07) were: temperature, 16.8 ± 0.1°C; dissolved oxygen, 9.6 ± 0.2 mg/L; pH, 8.1 ± 0.2; specific conductance, 160 ± 10 µmhos/cm; hardness, 57 ± 5 mg/L as $CaCO_3$; and alkalinity, 41 ± 5 mg/L as $CaCO_3$.

TEST PROCEDURES AND CONDITIONS

Test Chambers: The test chambers were two-gallon glass aquaria capable of holding 5.8 L of test solution. Test chamber placement in the test area was random.
Test Concentrations: The nominal test concentrations were 20, 10, 5, 2.5, 1.25, and 0 mg/L (control) naphthalene.
Replicates/Treatment: 4
Organisms/Treatment: 40
Loading: 0.38 g/L
Aeration: None.
Feeding: None.
Water Volume Changes: The diluter supplied approximately 80 ml/min of test solution, or 115 L/day, at each test concentration. At this rate of flow, test solution supply to each replicate aquarium was 5.0 volumes per day.
Effects Criteria: The effect criterion used in the lamprey ammocoete acute toxicity test was mortality, defined as a lack of visible respiratory movement and absence of response to tactile stimulation. In addition to mortality, qualitative observations of sublethal responses were recorded when observed.
Target Water Quality Conditions: Water quality measurements were taken daily as specified in the protocol (Physical and Chemical Data, Items 1 and 2). The target water quality conditions were as follows: Temperature, 17 ± 1°C; dissolved oxygen, ≥4.0 mg/L; hardness, 25-30 mg/L as $CaCO_3$. Actual test conditions are reported in the Test Results section.
Photoperiod: The photoperiod was total darkness except for approximately 2-3 hours per day for observations and test exposure system maintenance. Light intensity at the aquarium level was 13.2 foot-candles.

DATA ANALYSIS METHODS

The 96-hr proportion survival was calculated for each treatment replicate from the raw survival data and the measured naphthalene concentrations for each treatment level. The statistical software could not compute an LC50 because only 50% mortality occurred at the highest test concentration employed. The NOEC and LOEC were calculated according to U.S. Environmental Protection Agency (2002) methods. In this procedure, the NOEC and LOEC values were computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test was selected after evaluating the data for normality and homogeneity of variance. The statistical software employed for these calculations was ToxCalc, v.5.0.23N, Tidepool Scientific Software.

TOXICANT AND OTHER CHEMISTRY ANALYSES

Test solutions were sampled once daily for naphthalene from each test concentration by pooling samples from each of the four test replicate aquaria. Subsamples were pooled into new disposable plastic beakers from which the pooled sample was transferred to the analytical laboratory supplied sample bottles and shipped under chain-

of-custody procedures to the analytical chemistry laboratory (Columbia Analytical Services) at Kelso, WA. No preservative was employed. Samples were kept refrigerated at ≤4°C. Sample volumes were 1 L. Additional samples were taken of the dilution water on day 0 of the test for analysis of particulate matter, TOC, and ammonia-N, and from the control and highest test concentration treatments after 96-hr for measurement of ammonia-N. These samples also were shipped to Columbia Analytical Services for analysis.

PROTOCOL DEVIATIONS

1. The protocol specified "constant dark, except during monitoring activities", and also that light intensity should be 50-75 ft-c. when light was on. These specifications were judged to be contradictory and led to the following action. Based on best professional judgment of the investigators, we reduced the light intensity from that given in the protocol in order to minimize fright behavior responses of the ammocoetes during the short period of lighting associated with daily observations.
2. The protocol states: "At the end of the test, all surviving control ammocoetes are weighed and measured for length by replicate." Through a laboratory error, a subsample of 10 ammocoetes were weighed and measured at the beginning of the test instead. This is a minor deviation and is without consequence for interpretation of the study results.

TEST RESULTS

Water quality conditions measured in test aquaria during the 96-hr toxicity test are summarized in Table 1. The temperature specification was met during the study. Dissolved oxygen remained near saturation throughout the test. The pH ranged from 7.0 to 7.6. Mean hardness also was in the specified range of 25-30 mg/L as $CaCO_3$.

The concentrations of naphthalene measured during the toxicity test are summarized in Table 2. Mean measured concentrations in the test ranged from 0.70 ± 0.10 mg/L at the lowest level to 10.18 ± 1.93 mg/L at the highest exposure level. Measured dilutions were roughly 50% of nominal test concentrations. Higher test concentrations could not be achieved because of solubility limits and volatility of naphthalene and the high rate of utilization of toxicant stock by the diluter.

A summary of the 96-hr ammocoetes survival results is shown in Table 3. In this study, the 96-hr control survival (100%) met the test acceptability criteria of ≥90%. Therefore, the test meets the protocol criterion of a valid test. A 50% mortality was observed in the highest (10.18 mg/L) test concentration. No mortality occurred in any other test concentrations.

The toxicity of naphthalene to lamprey (*Lampetra* sp.) ammocoetes as the 96-hr LC50 was estimated by data inspection to be 10.18 mg/L. The NOEC and LOEC concentrations were 5.32 and 10.18 mg/L naphthalene, respectively.

LITERATURE CITED

Benoit, D., V. Mattson, and D. Olson. 1982. A continuous-flow mini-diluter system for toxicity testing. Water Res. 16:457-464.

EPA. 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. Environmental Protection Agency, Washington, DC.

STUDY APPROVAL

Project Manager	4/11/08 Date	Study Director	4/11/08 Date
Laboratory Director	4/11/08 Date	Quality Assurance Unit	4/7/08 Date

Table 1. Measured water quality conditions during the 96-hr acute toxicity test of naphthalene to lamprey ammocoetes.

Parameter	Mean ± S.D.	N	Range
Temperature (°C)	17.2 ± 0.4	30	16.8 – 17.8
Dissolved oxygen (mg/L)	9.4 ± 0.2	30	9.0 – 9.8
pH	7.3 ± 0.2	30	7.0 – 7.6
Specific conductance (μmhos/cm)	109 ± 7	10	101 – 120
Hardness (mg/L as $CaCO_3$)	28 ± 2	10	24 – 32
Alkalinity (mg/L as $CaCO_3$)	16 ± 1	10	13 – 18

Table 2. Measured concentrations of naphthalene during the 96-hr acute toxicity test of naphthalene to lamprey ammocoetes. Measurements performed by Columbia Analytical Services.

Nominal (mg/L)	Measured (mg/L)		
	Mean ± S.D.	N	Range
20	10.18 ± 1.93	5	7.40 – 12.00
10	5.32 ± 0.63	5	4.50 – 6.20
5	2.64 ± 0.28	5	2.30 – 2.90
2.5	1.24 ± 0.18	5	1.00 – 1.40
1.25	0.70 ± 0.10	5	0.58 – 0.81
0	0.0 ± 0.0	5	0.0 – 0.0

Table 3. Survival of lamprey ammocoetes, *Lampetra* sp. exposed for 96 hours to naphthalene.

Naphthalene (mg/L)	Replicate	Ammocoetes exposed	Ammocoetes Surviving	Percent Survival	Mean % Survival	
10.18	A	10	5	50.0		
	B	10	4	40.0		
	C	10	5	50.0		
	D	10	6	60.0	50.0	*
5.32	A	10	10	100.0		
	B	10	10	100.0		
	C	10	10	100.0		
	D	10	10	100.0	100.0	
2.64	A	10	10	100.0		
	B	10	10	100.0		
	C	10	10	100.0		
	D	10	10	100.0	100.0	
1.24	A	10	10	100.0		
	B	10	10	100.0		
	C	10	10	100.0		
	D	10	10	100.0	100.0	
0.70	A	10	10	100.0		
	B	10	10	100.0		
	C	10	10	100.0		
	D	10	10	100.0	100.0	
0.0 (Control)	A	10	10	100.0		
	B	10	10	100.0		
	C	10	10	100.0		
	D	10	10	100.0	100.0	

* Mean significantly (p=0.05) different from the control.

APPENDIX I

PROTOCOL

STUDY PROTOCOL

LAMPREY (*LAMPETRA* sp.)
FLOW THROUGH ACUTE TOXICITY TEST

INTRODUCTION

Purpose of Study: The purpose of this study is to determine the acute toxicity of six chemicals, representing a range of toxic modes of action, to lamprey (*Lampetra* sp.) ammocoetes. The study will be performed using methods based on the methods for measuring acute toxicity with other fish species as described in U.S. Environmental Protection Agency (2002) and American Society for Testing and Materials (ASTM) (1996) guidance.

Summary of Method: In definitive tests, lamprey ammocoetes are exposed for 96 hours to five concentrations of test material and a dilution water control. The test concentrations are arranged in a logarithmic series based on the results of range finding tests. Four replicates, each with 10 organisms, are employed at each test concentration, if there are sufficient numbers of ammocoetes. Flow-through exposures are employed. Test chambers are glass aquaria less than or equal to two gallons in volume. Mortality is the effect criterion. The data analysis consists of calculating means and standard deviations for survival. An LC50 with 95% confidence intervals may be calculated along with the NOEC and LOEC concentrations.

STUDY MANAGEMENT

Sponsor's Name and Address:
Windward Environmental LLC
200 West Mercer Street, Suite 401
Seattle, WA 98119

Sponsor's Study Monitor:
Ms. Helle Andersen

Name of Testing Laboratory:
Northwestern Aquatic Sciences
3814 Yaquina Bay Road
P.O. Box 1437
Newport, OR 97365

Test Location: Newport, OR.

Laboratory's Key Personnel to be Assigned to the Study:
Laboratory Director: Richard S. Caldwell, Ph.D.
Project Manager/Study Director: Gary A. Buhler, B.S.
QA Officer/Toxicology Program Mgr: Linda K. Nemeth, B.A., M.B.A.
Aquatic Toxicologist: Gerald J. Irissarri, B.S.

Proposed Study Schedule: July 2007 through September 2007.

Good Laboratory Practices: The test is conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

TEST MATERIAL

Description: Chemicals to be tested include aniline, copper, pentachlorophenol, lindane, diazinon and naphthalene.

Preparation of Working Stock Solution and Test Concentrations: Test materials will be administered using a constant-flow diluter based on the design of Benoit *et al.* (1982). Stock solutions of the sparingly soluble organic compounds, e.g. naphthalene, pentachlorophenol, lindane, diazinon, will be prepared continuously by recirculation of dilution water/toxicant stock solution through a column of toxicant coated glass beads (Veith and Comstock, 1975). This is a conceptual approach in which the specific column, flow rates, glass bead sizes, etc. need to be developed empirically, with analytical feedback, for each study considering such variables as chemical solubility, test temperature, desired highest test concentration, stock toxicant consumption rate, etc. Given the above, some modifications are anticipated as the studies progress. Initial system characteristics are as follows: 1) the toxicant mixing tank will have a 60-liter capacity; 2) a 20 x 280-cm glass column will be employed (10 x 200-cm and 40 x 560-cm columns are also available); 3) the column packing will employ 5-mm glass beads coated with the test compound; 4) particulates will be filtered out through glass wool; 5) flow through column will be controlled by a variable-speed peristaltic pump or by intermittent pumping; and 6) tubing will be glass and silicone rubber. GC/MS analysis of toxicant in the toxicant mixing tank will be used to optimize the system for each chemical noted above prior to test initiation.The contents of the stock toxicant reservoir will be constantly replenished with fresh dilution water as toxicant solution is supplied to the diluter. The aniline stock solution will be prepared similarly, but by volumetric addition of aniline, using a metering pump, to the stock toxicant reservoir *in lieu* of the Veith column. The copper working stock solution will be prepared in the same apparatus by metering pump addition of a concentrated copper stock solution to the stock toxicant reservoir. All toxicant working stock solutions will be prepared and maintained under the same conditions as in the test exposures and delivered continuously to the toxicant chamber of the diluter at an appropriate rate.

DILUTION WATER

Dilution water will be dechlorinated City of Newport municipal tap water adjusted to a water hardness of 25-30 mg/L as $CaCO_3$.

TEST ORGANISMS

Species: Lamprey, *Lampetra* sp.

Source: Ammocoetes will be collected by Windward staff and delivered to the laboratory each day of collection. The ammocoetes will be collected from several rivers including the Siletz River, which was the site for Phase 1 ammocoetes.

Age: ammocoetes (juvenile prior to metamorphosis)

Acclimation and Pretest Observation: Test organisms will be held in the laboratory in order to acclimate to test temperature. Upon receipt, animals are removed from the sediment they are received in and placed in 10-gallon tanks under flow-through conditions (~35 mL/minute, or ~ 2 volume changes per day, with one additional daily siphon and replacement of 2/3 volume of each tank). Approximately 50 lamprey are placed in each tank. Tanks will contain at least 3 inches of sediment (Sandtastic Play Sand, Waupaca Materials, or similar material) covered with approximately 26L of test water and supplied with aeration. The maximum temperature variation during holding will be ± 1° C. The ammocoetes will be acclimated to the laboratory test conditions for a minimum of 5 to 7 days prior to test initiation. This minimum number of days applies only to the first toxicity test conducted on each batch of field-collected organisms. For all other tests, the ammocoetes will be held for at least 13 days prior to test initiation. When a temperature adjustment of more than 3 to 5°C is required, the transfer from field collection water to laboratory dilution water will be gradual. Normal behavior for lamprey ammocoetes is to immediately bury in sediment and remain there. Any dead animals observed on the sediment surface are removed daily. Past experience suggests that mortality is minimal during short-term holding and any mortality is likely due to damage from field collection. If ammocoetes do not bury or there is excessive

mortality in any tank, animals from that tank will not be used for testing. Ammocoetes will not be fed since they are being held short-term.

DESCRIPTION OF TEST SYSTEM

Preparation of Test Concentrations: Test materials will be administered using a constant-flow diluter based on the design of Benoit *et al* (1982). The diluter will be calibrated to deliver a 0.5 dilution series. Flow rates will be adjusted to deliver at least five test chamber volumes per concentration per day. Diluter flow rates will be measured and documented at the beginning and conclusion of each 96-hour test. This will be done by measuring individual diluter capillary tube flows. When the flow rates are entered into an Excel® spreadsheet, the actual dilutions of the toxicant stock can be computed. Actual test chemical concentrations (organic chemicals) in the toxicant mixing chamber (toxicant stock solution) and in exposure aquaria will periodically be measured on a real-time basis at NAS to verify that nominal concentrations are being routinely achieved. Definitive concentration documentation in test aquaria will result from daily samples shipped for analysis to CAS.

Test Chambers and Environmental Control: Test chambers used in the toxicity study are glass aquaria less than or equal to two gallons in volume. The test system is housed in a constant-temperature, photoperiod-controlled room. Aeration is not employed. The photoperiod is constant dark except during monitoring activities when ambient (50-75 ft-c) lighting is used.

Cleaning: All laboratory glassware, including test chambers and toxicant dilution system, will be cleaned based on the methods described in U.S. Environmental Protection Agency (2002). Initial cleaning incorporates the use of a laboratory detergent followed by multiple tap water rinses. Subsequently, either dilute (10%, V:V) acid (hydrochloric or nitric) or acetone is used to remove metals or organic contaminants, respectively. Further multiple tap water rinses are employed after each acid or acetone treatment. Sodium hypochlorite bleach may be employed on a limited basis to ensure that microorganisms that could metabolize organic toxicants, e.g. naphthalene, are not present. Exhaustive tap water rinsing is always employed following any chemical cleaning treatment. A final dilution water rinse of the exposure apparatus will precede initiation of each study.

EXPERIMENTAL DESIGN AND TEST PROCEDURES

Experimental Design: The definitive test involves exposure of lamprey ammocoetes to five test concentrations, using a 0.5 dilution series, and a dilution water control. Exposures are for 96 hours. Each treatment consists of four replicates, each containing 10 ammocoetes, if there are sufficient numbers of ammocoetes. If there are not enough ammocoetes for 10 per replicate, then at least 20 per treatment (5 per replicate) is the minimum number preferred. Test chambers are randomly distributed in the test area. Ammocoetes are impartially distributed to each aquarium by adding one or two animals to each test replicate and repeating the process until each contains 10 organisms.

Effect Criterion: The effect criterion used in the lamprey ammocoete acute toxicity test is mortality, defined as a lack of visible respiratory movement and absence of response to tactile stimulation. The mortality data will be used to compute LC50s for each exposure period. In addition to mortality, qualitative observations of sublethal responses consisting of erratic swimming, hyperventilation, hemorrhaging, and hyper/hypoactivity (compared to the activity of the organisms in the control group) will be recorded if observed.

Test Conditions: The dissolved oxygen concentration in each test container must be > 4.0 mg/L throughout the test. The test temperature employed is 17 + 1°C. A photoperiod of constant darkness is used, except during monitoring activities when illumination is supplied by daylight fluorescent lamps at an intensity of 50-75 ft candles. Loading should not exceed 6.1g/L at 17°C according to EPA. ASTM recommends loading not exceed 1g/L of solution passing through the chamber in 24 hours or 10g/L of solution in the chamber at any time.

Beginning the Test: Adding the organisms to the equilibrated test containers as previously described begins the test.

Feeding: None.

Analysis of Test Concentrations: During each test, samples will be collected for chemical analysis, held at 4°C and shipped by overnight delivery to Columbia Analytical Services in Kelso, WA. The sampling schedule and procedure will follow the project QAPP.

Test Duration, Type and Frequency of Observations, and Methods: The test duration of the acute toxicity test is 96 hours. The type and frequency of observations to be made during the test are summarized as follows:

Type of Observation	Times of observation
Biological Data	
1. mortality (each test container)	0, 24, 48, 72, and 96 hr
2. sublethal observations (qualitative)	0, 24, 48, 72, and 96 hr
Physical and Chemical Data	
1. Dissolved oxygen, pH, & temperature, (in one replicate of each test level and the control)	0, 24, 48, 72, and 96 hr
2. Conductivity, hardness & alkalinity (in one replicate of highest concentration and control)	0, 24, 48, 72, and 96 hr
3. Toxicant concentration sampling (in one replicate of each test concentration and the control)	0, 24, 48, 72, and 96 hr
4. Dilution water sample for particulate matter, TOC and ammonia	0 hr
5. Sample for ammonia taken from one replicate of control and highest test concentration	96 hr

During the test, dead organisms are removed every 24 hours and frozen by concentration for possible tissue analysis. Dissolved oxygen is measured using a polarographic oxygen probe calibrated according to the manufacturer's recommendations. The pH and temperature are measured using a pH probe and a properly calibrated meter with scale divisions of 0.1 pH units. Conductivity is measured using a calibrated conductivity meter. Samples for chemical analysis will be collected, preserved, and sent to Columbia Analytical Services following the recommended procedures of the analytical laboratory.

Test Termination: At the end of the test, all surviving control ammocoetes are weighed and measured for length by replicate. All surviving or dead test organisms will be frozen by concentration for possible later tissue analysis by Columbia Analytical Services.

Criteria of Test Acceptance: The test results are acceptable if survival in the controls is at least 90%. The toxicity tests will be performed as a 50% dilution series, it is anticipated that each test will have at least one or possibly two partial mortality concentrations. Nominal concentrations of each toxicity test have be reviewed and approved by EPA (EPA 2007). However, because of the experimental nature of the study and the limited supply of ammocoetes, the acceptability of the toxicity tests will be based only on the ≥ 90% survival in control group.

DATA ANALYSIS

The percent of live ammocoetes is calculated for each treatment replicate from the raw data and the means are obtained for each treatment level. An LC50 (mortality) is calculated using Probit or Trimmed Spearman-Karber methods. The NOEC and LOEC are calculated for each endpoint according to U.S. Environmental Protection Agency (2002) methods. In this procedure, the NOEC and LOEC values are computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test is selected after evaluating the data for normality and homogeneity of variance. An arcsine-square root transformation is performed on the percentage effect data prior to statistical analysis. The statistical software employed for these calculations is Toxcalc, v.5.0.23N, Tidepool Scientific Software.

REPORTING

The final report of the study results must include all of the following standard information at a minimum:

1. Name and identification of the test;
2. The investigator and laboratory;
3. Information on the toxicants;
4. Information on the dilution water;
5. Detailed information about the test organisms;
6. Description of the test system and its calibration, including test chambers and dilution system;
7. A description of the experimental design and other test conditions including water quality;
8. Definition of the effect criteria and any other observations;
9. Responses, if any, in the control treatment;
10. Tabulation and statistical analysis of measured responses;
11. A description of the statistical methods used;
12. Any unusual information about the test or deviations from procedures;

The final study report will include the study protocol and all study raw data as appendices.

STUDY DESIGN ALTERATION

Amendments made to the protocol must be approved by the sponsor and study director and should include a description of the change; the reason for the change; the date the change took effect; and the dated signatures of the study director and sponsor. Any deviations in the protocol must be described and recorded in the study raw data.

REFERENCED GUIDELINES

ASTM. 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.

Benoit, D., V. Mattson, and D. Olson. 1982. A continuous-flow mini-diluter system for toxicity testing. Water Res. 16:457-464.

EPA. 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. Environmental Protection Agency, Washington, DC.

EPA. 2007. Letter to Lower Willamette Group from C. Humphrey and E. Blischke dated August 17, 2007 regarding Portland Harbor RI/FS: Lamprey toxicity test field sampling plan and QAPP Phase 2 addenda (approval letter). US Environmental Protection Agency Region 10, Oregon Operations Office, Portland, OR.

Veith G.D. and V.M. Comstock. 1975. Apparatus for continuously saturating water with hydrophobic organic chemicals. J Fish Res Board Can 32:1849-1851.

13. APPROVALS

_____ _____ for **Windward Environmental**
Name Date

_____ _____ _____ for **Northwestern Aquatic Sciences**
Name Date

Appendix A
Test Conditions Summary

1. Test species:	*Lampetra* sp.
2. Test type:	Flow-through
3. Test duration:	96 hr
4. Temperature:	17 ± 1°C
5. Dissolved oxygen:	≥ 4.0 mg/L
6. Light quality & intensity:	Ambient laboratory light (50-75 ft-c)
7. Photoperiod:	Constant dark, except during monitoring activities
8. Test chambers:	glass aquaria less than or equal to two gallons in volume.
9. Test chamber volumes per day:	At least 5
10. Replicates per treatment:	4
11. Organisms per replicate:	10
12. Age of test organisms:	ammocoete
13. Feeding regime:	None
14. Aeration:	None
15. Dilution water:	Dechlorinated City of Newport tap water: hardness adjusted to 25-30 mg/L as $CaCO_3$
16. Test concentrations:	5 and a dilution water control
17. Water quality:	Temperature, pH, & dissolved oxygen in one replicate of each test concentration daily. Conductivity, hardness & alkalinity in one replicate of highest concentration and control daily
18. Special controls:	None
19. Endpoint:	Mortality
20. Test acceptability criteria:	≥90% control survival

APPENDIX II

RAW DATA

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-42 Client Windward Investigator 2/23/08

STUDY MANAGEMENT:

Client: Windward Environmental, 200 West Mercer Street, Suite 401, Seattle, WA 98119
Client's Study Monitor: Ms. Helle Andersen
Testing Laboratory: Northwestern Aquatic Sciences
Test Location: 3814 Yaquina Bay Road, Newport, OR 97365
Laboratory's Study Personnel:
Project Manager: Gary A. Buhler Study Director: Richard S.Caldwell
QA Officer: L.K. Nemeth 1. G.J. IRISSARRI GJI
2. 3.
4. 5.
Study Schedule:
Test Beginning: 11-14-07 1330 Test Ending: 11-18-07 1430

TEST MATERIAL:

Description: Naphthalene ($C_{10}H_8$) Fisher Scientific lot # 056112.

DILUTION WATER:

Description: Dechlorined City of Newport tap water
Date of Preparation/Collection: Continuous Flow
Water Quality: Cond. (µmhos/cm) 109 ± 8 pH 7.2 ± 0.2
Hardness (mg/L as $CaCO_3$) 28.5 ± 3.0 Alkalinity (mg/L as $CaCO_3$) 16.4 ± 1.1
Treatments: Hardness adjusted to 25-30 mg/L with a Culligan® water treatment system.

TEST ORGANISMS:

Species: Lamprey ammocoetes (*Lampetra* sp.) Age: Size: 4-6 cm
Source: Trask, Nestucca, Siletz & Alsea rivers
Acclimation Data: See pages: 6, 7, + 11

TEST PROCEDURES AND CONDITIONS:

Test Concentrations (nominal): 20, 10, 5.0, 2.5, 1.25, & 0 mg/L
Test Chambers (description): 2.5 gallon glass aquaria Test Volume (ml): 5.80 L
Replicates/treatment: 4 Organisms/treatment: 40 (10/replicate)
Test water changes: flow through
Aeration during test: none DO limit ≥ 40 mg/L 4/3/08
Temperature (°C): 17 ± 1°C
Feeding: None Photoperiod: Darkness except for WQ & chemistry measurements
Test Duration (hr) 96 hours Tank Placement: Complete randomization
Randomization Chart:

Diluter

2.5	20	2.5	10	0	20	0	2.5	1.25	5	0	20	10	1.25	1.25	10	2.5	5	5	20	0	5	10	1.25

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-42 Client Windward Investigator

WEIGHT OF 10 ANIMALS AT TEST INITIATION 11-14-07 CB

	weight (g)	Length (cm)	Comments
1	0.14	4.1	
2	0.23	5.2	
3	0.30	5.4	
4	0.15	4.2	
5	0.23	5.0	
6	0.31	5.8	
7	0.17	4.3	
8	0.21	4.8	
9	0.19	4.6	
10	0.23	4.4	
~~Total~~ Mean	0.22	4.8	
~~Average animal~~ S.D	0.06	0.6	
N	10	10	

MISCELLANEOUS NOTES:

Room light intensity (fc): 132 11-16-07 CB

Chemistry samples taken daily.

Chemistry samples for particulate matter, TOC & ammonia in dilution water taken on test day 0.

Chemistry samples for ammonia taken in control & 8 mg/L A replicate on test day 4.

$$\text{Loading} = \frac{0.22\ g \times 10}{5.8\ L} = 0.38\ g/L$$

Error codes:

1) Correction of handwriting error
2) Written in wrong location; entry deleted
3) Wrong date deleted; replaced with correct data
4) Error found in measurement; measurement repeated

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-42 Client Windward Investigator

WATER QUALITY DATA SHEET

DAY 0 (11/14/07) GAS

Conc. (%)	Temp. (°C)	pH	Cond. µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
20	17.3	7.6	110	9.5	28	16	10	10	10	10	twitching in all animals
10	17.0	7.5		9.6			10	10	10	10	
5.0	17.1	7.5		9.7			10	10	10	10	
2.5	17.1	7.5		9.5			10	10	10	10	
1.25	17.1	7.5		9.6			10	10	10	10	
0	16.8	7.5	110	9.8	30	16	10	10	10	10	

Chemistry samples taken (11/14/07) GAS/RSC
Particulate matter, TOC, and ammonia samples taken in dilution water (11/14/07) GAS/RSC
Hours of light: 1.8

DAY 1 (11/15/07) GAS

Conc. (mg/L)	Temp. (°C)	pH	Cond. µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
20	17.8	7.4	120	9.2	30	15	10	10	10	10	motionless unless gently probed then a slight twitch
10	17.4	7.4		9.5			10	10	10	10	not active, all twitching
5.0	17.7	7.5		9.3			10	10	10	10	≈50% twitching
2.5	17.8	7.4		9.6			10	10	10	10	
1.25	17.7	7.5		9.5			10	10	10	10	
0	17.3	7.5	120	9.4	32	16	10	10	10	10	

Chemistry samples taken (11/15/07) GAS/
Hours of light: 1.0

DAY 2 (11/16/07) GAS

Conc. (mg/L)	Temp. (°C)	pH	Cond. µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
20	17.8	7.2	115	9.6	30	17	10	9(20)	9(10)	10	motionless, little movement when gently probed
10	17.7	7.2		9.4			10	10	10	10	motionless unless gently probed then a slight twitch
5.0	17.5	7.2		9.5			10	10	10	10	
2.5	17.6	7.2		9.4			10	10	10	10	
1.25	17.6	7.2		9.4			10	10	10	10	
0	17.4	7.2	110	9.4	30	18	10	10	10	10	

Chemistry samples taken (11/16/07) GAS/
Hours of light: 1.0

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-42 Client Windward Investigator

WATER QUALITY DATA SHEET

DAY 3 (11/17/07) RSC

Conc. (mg/L)	Temp. (°C)	pH	Cond. µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	Survivors B	Survivors C	Survivors D	Comments
20	17.2	7.4	104	9.6	26	16	9(10)	8	8(10)	9(10)	MOTIONLESS, SOME TWITCHING WHEN PROBED
10	17.0	7.4		9.6			10	10	10	10	MOTIONLESS, SLIGHTLY MORE TWITCHING WHEN PROBED THAN ANIMALS IN 20 mg/L CONCENTRATION
5.0	16.9	7.3		9.4			10	10	10	10	
2.5	16.7	7.2		9.4			10	10	10	10	
1.25	16.9	7.2		9.5			10	10	10	10	
0	17.0	7.0	101	9.4	28	17	10	10	10	10	

Chemistry samples taken (11/17/07) 657

Hours of light: 1.0

DAY 4 (11/18/07) 657

Conc. (mg/L)	Temp. (°C)	pH	Cond. µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	Survivors B	Survivors C	Survivors D	Comments
20	17.1	7.1	101	9.4	26	13	5(40)	4(40)	5(30)	6(30)	MOTIONLESS, SOME VERY SLIGHT TWITCHING WHEN PROBED, DEAD WHITE, HEMORRHAGING
10	16.9	7.2		9.2			10	10	10	10	MOTIONLESS, SLIGHTLY MORE TWITCHING WHEN PROBED THAN 20 mg/L GROUP
5.0	16.8	7.2		9.3			10	10	10	10	SLIGHTLY LESS ACTIVE
2.5	16.8	7.0		9.1			10	10	10	10	
1.25	16.9	7.1		9.0			10	10	10	10	
0	16.8	7.0	102	9.2	24	15	10	10	10	10	

Chemistry samples taken (11/18/07) 657

Ammonia samples taken in control and 20 mg/L (11/18/07) 657

Hours of light: 2.0

Dilution water WQ (from control aquaria)

Cond.	109	± 8	(5)
pH	7.2	± 0.2	(5)
Hardness	28.8	± 3.0	(5)
Alkalinity	16.4	± 1.1	(5)

RSC 2/21/08

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-42 Client Windward Investigator

2 RSC 2/10/08

NOTES ON PREPARATION OF ~~3~~0 mg/L NAPHTHALENE STOCK

System Design

The objective is to deliver 20 mg/L of naphthalene to the diluter toxicant chamber at a nominal constant rate of 160 ml/min. To accomplish this, a large reservoir, approximately 54L capacity, of naphthalene solution in dilution water is maintained at test temperature. As solution is removed to the diluter, makeup water is introduced under float valve control. The concentration of naphthalene in the reservoir is maintained by high volume circulation of the contents through a cartridge containing crystalline naphthalene.

Naphthalene RSC 2/10/08

NOTES ON MAINTENANCE OF THE ~~LINDANE~~ DELIVERY SYSTEM

Cartridge filled with > 100 g naphthalene crystals after removal of previous filter material & reflushing with tap water.

Cartridge flow rate adjusted to ~~approx~~ an estimated rate of ≈ 1000 ml/min

Measurement (GC/MS) of mixing tank naphthalene concentration confirmed that level of ≈ 16 mg/L was being delivered.

DILUTER CALIBRATIONS RECORD

DILUTER CAPILLARY FLOWS

Date 11/13/07	Time	Init. RSC		Date 11/19/07	Time	Init. RSC
Drain #	Supply Tank	Diluter Tank		Drain #	Supply Tank	Diluter Tank
1	160	78		1	158	80
2	78	70		2	77	73
3	80	78		3	76	76
4	82	79		4	80	79
5	79	76		5	73	70
6	108	82		6	100	79
Flow rates in ml/min						

FLOWS TO REPLICATE AQUARIA

Date 11/13/07 Time Init. RSC

Replicate	Relative Concentration (%)					
	100	50	25	12.5	6.25	0
A	240	315	270	345	255	270
B	285	240	275	290	255	250
C	275	265	255	315	270	235
D	300	275	275	320	345	260

Date 11/19/07 Time Init. RSC

Replicate	Relative Concentration (%)					
	100	50	25	12.5	6.25	0
A	240	355	285	355	235	330
B	175	240	280	330	250	250
C	325	275	230	275	270	245
D	385	285	275	330	250	210

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
8-2-07	16.1	6.7	8.6	82	20	25	[illegible]	[illegible]	constant flow		[illegible]	[illegible]	[illegible]
8-3-07	16.8	7.7	9.6	85	26	30		"	"			[illegible]	[illegible]
8-6-07	17.1	6.6	9.3	65	28	10	[illegible]	[illegible]	"			[illegible]	[illegible]
8-7-07	16.4	7.6	9.7	162	34	30	[illegible]	[illegible]	"			[illegible]	[illegible]
8-8-07	18.1	6.1	7.2	60	22	16	[illegible]	[illegible]	"			[illegible]	[illegible]
8-9-07	17.0	7.0	7.4	160	34	30						Holding	[illegible]
8-10-07	16.9	6.6	9.0	60	31	25	Alder Creek		"			[illegible]	[illegible]
8-13-07	16.8	7.2	7.5	135	34	30			"			[illegible]	[illegible]
9-1-07	16.9	7.1	7.4	140	—	—			"				
9-2-07	16.7	7.6	9.1	160	—	—			"			Holding	GH
9-3-07	16.7	7.7	9.2	160	—	—			"			Holding	GH
9-4-07	16.2	7.5	8.8	185	34	30			"			"	[illegible]
9-5-07	16.3	7.2	9.1	190	34	30			"			"	[illegible]
9-6-07	16.8	7.3	9.0	185	34	30			"			"	
9-7-07	16.9	7.0	9.1	155	26	30			"			"	
9-8-07	17.0	7.5	9.3	160	—	—			"			"	
9-9-07	16.7	7.5	8.8	170	—	—			"			holding	LPS
9-10-07	16.7	7.2	8.9	175	—	—			"			"	[illegible]
9-11-07	17.4	7.6	9.0	235	60	35			"			"	LPS
9-12-07	17.0	8.0	9.4	185	60	38			"			"	[illegible]

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
9-13-07	17.1	8.0	9.0	170	55	30			constant flow			holding	[illegible]
9-14-07	17.1	8.2	9.1	155	48	29			"		[illegible]	"	[illegible]
9-15-07	17.1	8.3	9.2	120	—	—			"			"	
9-16-07	17.0	8.1	9.2	115	41	30			"			"	GH
9-17-07	17.1	7.7	8.8	120	41	30			"			"	LPS
9-18-07	17.0	7.8	9.1	120	39	21			"			"	GH
9-19-07	17.7	7.6	9.1	125	39	34			"			"	[illegible]
9-20-07	17.1	7.9	9.0	125	36	34	Taken [illegible]		"			"	GH
9-21-07	17.0	8.2	9.5	140	44	31	"		"			"	[illegible]
9-22-07	17.1	7.7	9.1	130	42	29			"			"	
9-23-07	17.1	7.7	9.3	140	46	28			"			"	[illegible]
9-24-07	16.8	7.9	9.3	140	44	29			"			"	
9-25-07	17.1	7.7	8.8	145	44	30			"			"	[illegible]
9-26-07	17.2	7.8	9.3	140	48	36			"			"	[illegible]
9-27-07	17.4	7.7	9.3	145	48	37			"			"	[illegible]
9-28-07	17.2	7.8	9.3	150	48	36			"			"	[illegible]
9-29-07	16.9	7.5	8.8	150	48	36			"			"	[illegible]
9-30-07	17.0	7.2	8.8	140	48	37			"			"	[illegible]
10-1-07	17.0	7.8	9.4	145	48	36			"		[illegible]	"	GH
10-2-07	17.3	8.1	9.2	135	—	—			"			"	GH

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
10-3-07	17.0	7.8	9.1	130	46	55			constant flow			Holding	GH
10-4-07	16.9	7.9	9.2	120	42	38						"	[illegible]
10-5-07	16.9	7.4	9.0	130	not Taken LPS							"	LPS
10-6-07	16.6	7.2	9.4	130	44	41						"	GH
10-7-07	17.0	7.4	9.5	135	60	50						"	GH
10-8-07	17.0	7.9	9.2	145	50	48						"	GH
10-9-07	17.0	7.6	9.5	150	54	52						"	GH
10-10-07	16.9	7.4	9.2	155	54	49						"	GH
10-11-07	17.0	7.1	9.5	150	54	40						"	[illegible]
10-12-07	17.1	8.0	9.4	155	50	39						"	[illegible]
10-13-07	17.1	7.1	9.1	150	52	40						"	GH
10-14-07	16.9	7.5	9.6	150	52	38						"	[illegible]
10-15-07	17.0	7.6	9.5	155	50	38						"	[illegible]
10-5-9	13.0	6.7	10.6	97	30	30						Receiving Lab	[illegible]
10-16-07	13.2	7.9	10.1	145	60	57						Holding Rm #4	[illegible]
10-16-07	17.1	6.9	9.4	165	20	12						Test aquarium Rm #1	[illegible]
10-16-07	12.4	6.2	8.9	65	18	14						Northern Rock Darter	GH
10-17-7	13.3	8.3	10.1	135	60	55					[illegible]	Hold in [illegible] Rm #1	GH
10-17-7	17.3	6.7	8.5	75	22	14						Hold in Rm #1	GH
10-18-07	13.1	6.5	10.2	57	20	14						Sweetwater Darter	

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
10-18-07	13.2	8.3	9.9	130	48	36			constant flow			Holding	[illegible]
10-19-07	13.3	7.5	9.5	120	48	36						"	LPS
10-20-07	13.2	7.7	10.0	125	46	38							
10-21-07	14.2	7.6	9.9	130	48	37							
10-22-07	14.2	7.7	9.4	135	50	40						"	[illegible]
10-23-07	15.1	7.9	9.7	145	54	44						"	[illegible]
10-24-07	15.6	8.3	9.7	155	58	47						"	[illegible]
10-25-07	16.1	8.5	9.5	160	66	53						"	[illegible]
10-26-07	16.8	8.1	9.6	165	60	47						"	[illegible]
10-27-07	16.8	8.3	9.9	155	62	49						"	[illegible]
10-28-07	16.6	8.3	9.9	165	62	47						"	[illegible]
10-29-07	17.0	8.1	9.5	165	62	48						"	[illegible]
10-30-07	16.9	8.2	9.6	170	64	46						"	LPS
10-31-07	16.8	8.2	9.9	175	70	54						"	[illegible]
11-1-07	16.8	8.2	9.8	165	62	45						"	[illegible]
11-2-07	16.8	8.2	9.5	165	62	47						"	[illegible]
11-3-07	16.6	8.1	9.2	160	56	38						"	GH
11-4-07	16.8	8.3	9.6	180	64	48						"	LPS
11-5-07	16.9	8.1	9.9	180	66	49						"	[illegible]
11-6-07	16.9	8.7	9.8	155	52	35						"	[illegible]

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
11-7-07	17.0	8.1	9.4	155	54	37			constant flow			Holding	
11-8-07	16.9	8.1	9.3	155	56	39						"	
11-9-07	16.7	8.6	9.9	160	62	46						"	
11-10-07	16.7	8.1	9.6	155	56	43						"	
11-11-07	16.7	8.1	9.9	145	52	39						"	
11-12-07	16.8	8.0	9.7	155	56	38						"	
11-13-07	16.8	7.6	9.7	155	54	38						"	
11-14-07	16.8	7.9	9.7	150	52	36						"	
11-15-07	16.8	8.0	9.7	160	60	43						"	
11-16-07	16.9	8.2	9.9	160	58	39						"	
11-17-07	16.7	8.3	10.1	165	62	43						"	
11-18-07	16.9	8.1	9.8	150	54	36						"	
11-19-07	16.8	7.9	9.7	175	64	48						"	
11-20-07	16.7	7.9	9.9	170	64	46						"	
11-21-07	16.6	8.0	9.9	175	64	48						"	
11-22-07	16.5	7.9	10.0	165	62	39						"	
11-23-07	16.5	7.8	9.5	170	62	44						"	
11-24-07	16.5	8.0	9.7	190	70	48						"	
11-25-07	16.5	7.9	9.6	175	64	45						"	
11-26-07	16.6	7.5	9.6	175	64	46						"	

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
11-29-07	16.1	7.9	9.6	180	62	44			constant flow			Holding	
11-30-07	16.0	7.7	10.0	175	56	44						"	
12-1-07	16.0	7.8	9.7	170	60	43						"	
12-2-07	16.0	8.0	9.7	185	62	46						"	
12-3-07	16.2	7.9	9.7	185	70	47						"	
12-4-07	15.8	7.5	9.4	165	60	43						"	
12-5-07	15.7	8.2	9.6	155	60	39						"	
12-6-07	15.8	7.7	9.8	170	60	42						"	
12-7-07	16.3	7.6	9.9	165	60	42						"	
12-8-07	15.7	7.6	9.9	160	58	40						"	
12-9-07	17.8	7.4	9.0	175	64	43						"	
12-10-07	17.1	7.3	8.8	165	62	42						"	
12-11-07	17.1	7.2	9.4	170	56	41						"	
12-12-07	17.3	7.4	9.5	160	58	40						"	
12-13-07	16.7	7.2	9.1	170	64	43						"	
12-14-07	16.8	7.3	9.1	170	64	44						"	
12-15-07	17.0	7.2	9.0	165	56	44						"	
12-16-07	16.5	7.2	9.5	170	70	45						"	
12-17-07	16.7	7.2	9.3	170	66	44						"	
12-18-07	16.5	7.4	9.6	195	74	53						"	

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
12-19-07	15.6	7.3	9.9	190	76	51			constant flow			HOLDING	
12-20-07	16.2	7.2	10.3	175	56	46						"	
12-21-07	15.4	8.2	10.0	190	72	48						"	
12-22-07	16.0	7.8	9.8	145	70	46							
12-23-07	17.1	8.0	9.5	195	68	54						"	
12-24-07	17.1	8.0	9.4	190	72	48						"	
12-25-07	16.8	8.1	9.6	190	72	51						"	
12-26-07	16.8	8.3	10.1	200	74	50						"	
12-27-07	17.1	8.2	9.7	195	68	49						"	
12-28-07	17.0	8.0	9.8	170	56	32						"	
12-29-07	17.1	8.1	9.9	175	52	29						"	
12-30-07	16.9	8.0	9.6	195	68	50						"	
12-31-07	16.8	8.3	9.8	205	72	50						"	
1-1-08	17.0	8.0	9.6	[illegible]	64	43						"	
1-2-08	16.8	8.2	9.6	185	66	41						"	
1-3-08	16.8	8.3	9.7	180	60	39						"	
1-4-08	16.9	8.2	9.8	175	62	36						"	
1-5-08	16.7	8.4	9.7	155	48	30						"	
1-7-08	16.7	8.4	9.9	160	54	32						"	
1-8-08	16.9	8.1	9.5	165	50	33						"	

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
1-9-08	15.6	8.4	10.6	165	56	30			constant flow			HOLDING	
1-10-08	15.9	8.0	10.2	145	44	24						"	
1-11-08	16.3	8.1	10.3	145	42	24							
1-12-08	16.0	8.4	10.2	165	56	31						"	
1-13-08	16.2	8.1	9.6	170	54	32						"	
1-14-08	16.5	8.2	9.9	160	52	32						"	
1-15-08	16.2	8.2	9.7	160	50	29						"	
1-16-08	16.4	8.2	9.9	125	40	15						"	
1-17-08	15.6	8.0	9.9	165	86	63						"	

Sample stopped to GAS 2/18/08 (10:00 AM)

Test No. 656-42

Samples shipped to CAS

Test 7/2 CEC. 97

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd., P.O. Box 1437, Newport, OR 97365
Tel: 541-265-7225, Fax: 541-265-2799, www.nwaquatic.com

Client Name: Integral Consulting, Inc
Address: 319 SW Washington St., Suite 1150
City, State, Zip code: Portland, OR 97204
Project No: Integral B01-01-58C Windward 07-28-04-33
Phone No: 503-284-5545
Report Attention: Abbie Spielman
Shipping Information: Carrier: UPS UPS#Y3489E
Airbill No: 1Z999 16601685 3427
Sampled by: G. J. IRISSARRI

Testing Required: Copper, Lindane, Pentachlorophenol, Aniline, Diazinon, Naphthalene

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	11-15-07	1430	NAS# 1428G Day 0 - 0 mg/L Naphthalene	3						X	
	11-15-07		NAS# 1429G Day 0 - 1.25 mg/L Naphthalene	3						X	
	11-15-07		NAS# 1430G Day 0 - 2.5 mg/L Naphthalene	3						X	
	11-15-07		NAS# 1431G Day 0 - 5.0 mg/L Naphthalene	3						X	
	11-15-07		NAS# 1432G Day 0 - 10.0 mg/L Naphthalene	3						X	
	11-15-07		NAS# 1433G Day 0 - 20.0 mg/L Naphthalene	3						X	

Signature	Print Name	Company	Date	Time
Relinquished by: [signature]	GERALD IRISSARRI	Northwestern Aquatic Sciences	11-19-07	1430
Received by:				
Relinquished by:				
Received by:				
Relinquished by:				
Received by laboratory:				

Cooler Custody Seal: Present / Not Present; Intact / Not Intact
Internal Cooler Temperature Upon Lab Receipt (°C):

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd., P.O. Box 1437, Newport, OR 97365
Tel: 541-265-7225, Fax: 541-265-2799, www.nwaquatic.com

Client Name: Integral Consulting, Inc
Address: 319 SW Washington St., Suite 1150
City, State, Zip code: Portland, OR 97204
Project No: Integral B01-01-58C Windward 07-28-04-33
Phone No: 503-284-5545
Report Attention: Abbie Spielman
Shipping Information: Carrier: UPS UPS#Y3489E
Airbill No: 1Z999 16601685 3427
Sampled by: G. J. IRISSARRI

Testing Required: Copper, Lindane, Pentachlorophenol, Aniline, Diazinon, Naphthalene

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	11-17-07	1400	NAS# 1428G Day 2 - 0 mg/L Naphthalene	3						X	
	11-17-07		NAS# 1429G Day 2 - 1.25 mg/L Naphthalene	3						X	
	11-17-07		NAS# 1430G Day 2 - 2.5 mg/L Naphthalene	3						X	
	11-17-07		NAS# 1431G Day 2 - 5.0 mg/L Naphthalene	3						X	
	11-17-07		NAS# 1432G Day 2 - 10.0 mg/L Naphthalene	3						X	
	11-17-07		NAS# 1433G Day 2 - 20.0 mg/L Naphthalene	3						X	

Signature	Print Name	Company	Date	Time
Relinquished by: [signature]	GERALD IRISSARRI	Northwestern Aquatic Sciences	11-19-07	1430
Received by:				
Relinquished by:				
Received by:				
Relinquished by:				
Received by laboratory:				

Cooler Custody Seal: Present / Not Present; Intact / Not Intact
Internal Cooler Temperature Upon Lab Receipt (°C):

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd., P.O. Box 1437, Newport, OR 97365
Tel: 541-265-7225, Fax: 541-265-2799, www.nwaquatic.com

Client Name: Integral Consulting, Inc
Address: 319 SW Washington St., Suite 1150
City, State, Zip code: Portland, OR 97204
Project No: Integral B01-01-58C Windward 07-28-04-33
Phone No: 503-284-5545
Report Attention: Abbie Spielman
Shipping Information: Carrier: UPS UPS#Y3489E
Airbill No: 1Z999 16601685 3427
Sampled by: RSC/GJI

Testing Required: Copper, Lindane, Pentachlorophenol, Aniline, Diazinon, Naphthalene

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	11-14-07	1600	NAS# 1428G Day 0 - 0 mg/L Naphthalene	3						X	
	11-14-07	1600	NAS# 1429G Day 0 - 1.25 mg/L Naphthalene	3						X	
	11-14-07	1600	NAS# 1430G Day 0 - 2.5 mg/L Naphthalene	3						X	
	11-14-07	1600	NAS# 1431G Day 0 - 5.0 mg/L Naphthalene	3						X	
	11-14-07	1600	NAS# 1432G Day 0 - 10.0 mg/L Naphthalene	3						X	
	11-14-07	1600	NAS# 1433G Day 0 - 20.0 mg/L Naphthalene	3						X	
	11-14-07	1600	NAS# 1434G Day-0 - TOC	1							
	11-14-07	1600	NAS# 1435G Day-0 - TSS	1							
	11-14-07	1600	NAS# 1436G Day-0 - Ammonia	1							

Signature	Print Name	Company	Date	Time
Relinquished by: [signature]	GERALD IRISSARRI	Northwestern Aquatic Sciences	11-19-07	1430
Received by:				
Relinquished by:				
Received by:				
Relinquished by:				
Received by laboratory:				

Cooler Custody Seal: Present / Not Present; Intact / Not Intact
Internal Cooler Temperature Upon Lab Receipt (°C):

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd., P.O. Box 1437, Newport, OR 97365
Tel: 541-265-7225, Fax: 541-265-2799, www.nwaquatic.com

Client Name: Integral Consulting, Inc
Address: 319 SW Washington St., Suite 1150
City, State, Zip code: Portland, OR 97204
Project No: Integral B01-01-58C Windward 07-28-04-33
Phone No: 503-284-5545
Report Attention: Abbie Spielman
Shipping Information: Carrier: UPS UPS#Y3489E
Airbill No: 1Z999 16601685 3427
Sampled by: G. J. IRISSARRI

Testing Required: Copper, Lindane, Pentachlorophenol, Aniline, Diazinon, Naphthalene

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	11-16-07	1400	NAS# 1428G Day 2 - 0 mg/L Naphthalene	3						X	
	11-16-07		NAS# 1429G Day 2 - 1.25 mg/L Naphthalene	3						X	
	11-16-07		NAS# 1430G Day 2 - 2.5 mg/L Naphthalene	3						X	
	11-16-07		NAS# 1431G Day 2 - 5.0 mg/L Naphthalene	3						X	
	11-16-07		NAS# 1432G Day 2 - 10.0 mg/L Naphthalene	3						X	
	11-16-07		NAS# 1433G Day 2 - 20.0 mg/L Naphthalene	3						X	
	11-16-07		NAS# 1434G Day 2 - 1.25 mg/L Naphthalene Duplicate	3						X	

Signature	Print Name	Company	Date	Time
Relinquished by: [signature]	GERALD IRISSARRI	Northwestern Aquatic Sciences	11-19-07	1430
Received by:				
Relinquished by:				
Received by:				
Relinquished by:				
Received by laboratory:				

Cooler Custody Seal: Present / Not Present; Intact / Not Intact
Internal Cooler Temperature Upon Lab Receipt (°C):

Samples shipped to CAS

Test No. 656-42

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
5814 Yaquina Bay Rd., P.O. Box 1437, Newport, OR 97365
Tel: 541-265-7225, Fax: 541-265-2799, www.nwaquatic.com

Client Name: Integral Consulting, Inc.
Address: 319 SW Washington St., Suite 1450
City, State, Zip Code: Portland, OR 97204

Project No: Integral R01.01 SBC Windward 07-28-04-53
Phone No: 503-284-5545
Report Attention: Abbie Spielmann

Shipping Information: Carrier: UPS UPS459348011
Airbill No: 1Z8449470190180332

Sampled by: GG

Testing Required: Copper, Lead, Polycyclic Aromatic Hydrocarbons, Ammonia, Dioxinon, Naphthalene

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lead	Polycyclic Aromatic Hydrocarbons	Ammonia	Dioxinon	Naphthalene	Comments
	11-18-07	1400	NASS-2900 Day 4 - 0 mg/L Naphthalene	3						X	
	11-18-07	1400	NASS-2900 Day 4 - 1.25 mg/L Naphthalene	3						X	
	11-18-07	1400	NASS-2900 Day 4 - 2.5 mg/L Naphthalene	3						X	
	11-18-07	1400	NASS-2900 Day 4 - 5.0 mg/L Naphthalene	3						X	
	11-18-07	1400	NASS-2900 Day 4 - 10.0 mg/L Naphthalene	3						X	
	11-18-07	1400	NASS-2900 Day 4 - 20.0 mg/L Naphthalene	3						X	
	11-18-07	1400	NASS-2900 Day 4 - Ammonia 0 mg/L Naphthalene concentration	1							
	11-18-07	1400	NASS-2900 Day 4 - Ammonia 20.0 mg/L Naphthalene concentration	1							
	11-18-07	1000	Synthetic playground leachate standards	1							

Relinquished by: Signature	Print Name	Company	Date	Time	Cooler Custody Seal
Shall Alvarez	GERALD ALVAREZ	Northwestern Aquatic Sciences	11-19-07	1450	Present / Not Present
Received by:					Intact / Not Intact
Relinquished by:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received by:					
Relinquished by:					
Received by laboratory:					

Water Quality Computation - Test No. 686-42

	Temp	DO	pH	Cond	Hard	Alk
1	17.3	9.5	7.6	110	28	16
2	17.0	9.6	7.5	110	30	16
3	17 1	9.7	7 5	120	30	15
4	17 1	9.5	7.5	120	32	16
5	17 1	9.6	7 5	115	30	17
6	16.8	9.8	7 5	110	30	18
7	17.8	9.2	7.4	104	26	16
8	17.4	9.5	7.4	101	28	17
9	17.7	9.3	7.5	101	26	13
10	17.8	9.6	7.4	102	24	15
11	17.7	9.5	7.5			
12	17.3	9.4	7.5			
13	17.8	9.6	7.2			
14	17.7	9.4	7.2			
15	17.5	9 5	7.2			
16	17.6	9.4	7.2			
17	17.6	9.4	7.2			
18	17.4	9.4	7.2			
19	17.2	9.6	7.4			
20	17.0	9.6	7.4			
21	16.9	9.4	7.3			
22	16.8	9 4	7.2			
23	16.9	9.5	7.2			
24	17.0	9.4	7.0			
25	17.1	9.4	7 1			
26	16.9	9.2	7.2			
27	16.8	9.3	7.2			
28	16.8	9.1	7.0			
29	16.9	9.0	7.1			
30	16 8	9.2	7.0			
31						
32						
mean	17.2	9.4	7.3	109	28	16
s.d.	0.4	0.2	0.2	7	2	1
N	30	30	30	10	10	10
min	16.8	9.0	7.0	101	24	13
max	17.8	9.8	7.6	120	32	18

2/21/08

WQ Computation - acclimation (11-1-07 to 11-14-07) - Test No. 686-42

	Temp	DO	pH	Cond	Hard	Alk
1	16.8	9.8	8.2	165	62	45
2	16.8	9.5	8.2	165	62	47
3	16.6	9.2	8.1	160	56	38
4	16.8	9.6	8.3	180	64	48
5	16.9	9.9	8.1	180	66	49
6	16.9	9.8	8.2	155	52	35
7	17.0	9.4	8.1	155	54	37
8	16.9	9.3	8.1	155	56	39
9	16.7	9.9	8.6	160	62	46
10	16.7	9.6	8.1	155	56	43
11	16.7	9.9	8.1	145	52	39
12	16.8	9.7	8.0	155	56	38
13	16.8	9.7	7.6	155	54	38
14	16.8	9.7	7.9	150	52	36
15						
16						
17						
mean	16.8	9.6	8.1	160	57	41
s.d.	0.1	0.2	0.2	10	5	5
N	14	14	14	14	14	14
min	16.6	9.2	7.6	145	52	35
max	17.0	9.9	8.6	180	66	49

RSL
2/21/08

SampleDate	SampleTim	Matrix	LabMatrix	SampleID	arentSamp	SampleTyp	Method	AnalysisDatc	nalysisTim	nalyteGrou	cas_m	Analyte	l_or_disso	detect_flag	VALUE	Qualifiers	Units
11/14/2007	16:00	WQ	WQ	NAS# 1428G Day 0-0 mg/L Napnthalene		N	SW8260B	11/28/2007	15:04	PAHs	91-20-3	Naphthaler	N	Y	41		ug/l
11/14/2007	16:00	WQ	WQ	NAS# 1429G Day 0-1 25 mg/L Naphthalene		N	SW8260B	11/23/2007	21 36	PAHs	91-20-3	Naphthaler	N	Y	630		ug/l
11/14/2007	16:00	WQ	WQ	NAS# 1430G Day 0-2 5 mg/L Naphthalene		N	SW8260B	11/23/2007	21 56	PAHs	91-20-3	Naphthaler	N	Y	1100		ug/l
11/14/2007	16:00	WQ	WQ	NAS# 1431G Day 0-5.0 mg/L Naphthalene		N	SW8260B	11/23/2007	22 35	PAHs	91-20-3	Naphthaler	N	Y	2400		ug/l
11/14/2007	16:00	WQ	WQ	NAS# 1432G Day 0-10.0 mg/L Naphthalene		N	SW8260B	11/23/2007	22 55	PAHs	91-20-3	Naphthaler	N	Y	5000		ug/l
11/14/2007	16:00	WQ	WQ	NAS# 1433G Day 0-20 0 mg/L Naphthalene		N	SW8260B	11/23/2007	22 16	PAHs	91-20-3	Naphthaler	N	Y	7400		ug/l
11/15/2007	14 30	WQ	WQ	NAS# 1438G Day 1-0 mg/L Naphthalene		N	SW8260B	11/29/2007	01 25	PAHs	91-20-3	Naphthaler	N	Y	41		ug/l
11/15/2007	14 30	WQ	WQ	NAS# 1439G Day 1-1 25 mg/L Naphthalene		N	SW8260B	11/27/2007	23:57	PAHs	91-20-3	Naphthaler	N	Y	810		ug/l
11/15/2007	14 30	WQ	WQ	NAS# 1440G Day 1-2.5 mg/L Naphthalene		N	SW8260B	11/28/2007	00 24	PAHs	91-20-3	Naphthaler	N	Y	1400		ug/l
11/15/2007	14 30	WQ	WQ	NAS# 1441G Day 1-5 0 mg/L Naphthalene		N	SW8260B	11/28/2007	00 52	PAHs	91-20-3	Naphthaler	N	Y	2900		ug/l
11/15/2007	14 30	WQ	WQ	NAS# 1442G Day 1-10 0 mg/L Naphthalene		N	SW8260B	11/28/2007	01 19	PAHs	91-20-3	Naphthaler	N	Y	6200		ug/l
11/15/2007	14 30	WQ	WQ	NAS# 1443G Day 1-20.0 mg/L Naphthalene		N	SW8260B	11/28/2007	01 46	PAHs	91-20-3	Naphthaler	N	Y	12000		ug/l
11/15/2007	14 30	WQ	WQ	NAS# 1444G Day 1-20.0 mg/L Naphthalene-D		N	SW8260B	11/28/2007	02 14	PAHs	91-20-3	Naphthaler	N	Y	12000		ug/l
11/16/2007	14 00	WQ	WQ	NAS# 1445G Day 2-0 mg/L Naphthalene		N	SW8260B	11/29/2007	00 30	PAHs	91-20-3	Naphthaler	N	Y	37		ug/l
11/16/2007	14 00	WQ	WQ	NAS# 1446G Day 2-1 25 mg/L Naphthalene		N	SW8260B	11/28/2007	03 09	PAHs	91-20-3	Naphthaler	N	Y	780		ug/l
11/16/2007	14 00	WQ	WQ	NAS# 1451G Day 2-1 25 mg/L Naphthalene-D		N	SW8260B	11/28/2007	05.26	PAHs	91-20-3	Naphthaler	N	Y	830		ug/l
11/16/2007	14 00	WQ	WQ	NAS# 1447G Day 2-2.5 mg/L Naphthalene		N	SW8260B	11/28/2007	03 36	PAHs	91-20-3	Naphthaler	N	Y	1400		ug/l
11/16/2007	14 00	WQ	WQ	NAS# 1448G Day 2-5 0 mg/L Naphthalene		N	SW8260B	11/28/2007	04 04	PAHs	91-20-3	Naphthaler	N	Y	2900		ug/l
11/16/2007	14 00	WQ	WQ	NAS# 1449G Day 2-10 0 mg/L Naphthalene		N	SW8260B	11/28/2007	04 31	PAHs	91-20-3	Naphthaler	N	Y	5500		ug/l
11/16/2007	14 00	WQ	WQ	NAS# 1450G Day 2-20 0 mg/L Napnthalene		N	SW8260B	11/28/2007	04 59	PAHs	91-20-3	Naphthaler	N	Y	12000		ug/l
11/17/2007	14 00	WQ	WQ	NAS# 1453G Day 3-0 mg/L Nachtha ene		N	SW8260B	11/29/2007	04 37	PAHs	91-20 3	Naphthaler	N	Y	38		ug/l
11/17/2007	14 00	WQ	WQ	NAS# 1454G Day 3-1 25 mg/L Naphthalene		N	SW8260B	11/29/2007	05 04	PAHs	91-20-3	Naphthaler	N	Y	690		ug/l
11/17/2007	14 00	WQ	WQ	NAS# 1455G Day 3-2 5 mg/L Naphthalene		N	SW8260B	11/29/2007	05 31	PAHs	91-20-3	Naphthaler	N	Y	1300		ug/l
11/17/2007	14 00	WQ	WQ	NAS# 1456G Day 3-5 0 mg/L Naphthalene		N	SW8260B	11/29/2007	05 59	PAHs	91-20-3	Naphthaler	N	Y	2700		ug/l
11/17/2007	14 00	WQ	WQ	NAS# 1457G Day 3-10 0 mg/L Naphthalene		N	SW8260B	11/29/2007	06 26	PAHs	91-20-3	Naphthaler	N	Y	5400		ug/l
11/17/2007	14 00	WQ	WQ	NAS# 1458G Day 3-20 0 mg/L Naphthalene		N	SW8260B	11/29/2007	06 53	PAHs	91-20-3	Naphthaler	N	Y	10000		ug/l
11/18/2007	14 00	WQ	WQ	NAS# 1498G Day4-0 mg/L Napnthalene		N	SW8260B	11/29/2007	01 52	PAHs	91-20-3	Naphthaler	N	Y	37		ug/l
11/18/2007	14 00	WQ	WQ	NAS# 1499G Day4-1 25 mg/L Naphthalene		N	SW8260B	11/29/2007	02 20	PAHs	91-20-3	Naphthaler	N	Y	580		ug/l
11/18/2007	14 00	WQ	WQ	NAS# 1500G Day 4-2 5 mg/L Naphthalene		N	SW8260B	11/29/2007	02 47	PAHs	91-20-3	Naphthaler	N	Y	1000		ug/l
11/18/2007	14 00	WQ	WQ	NAS# 1501G Day 4-5 0 mg/L Naphthalene		N	SW8260B	11/29/2007	03 14	PAHs	91-20-3	Naphthaler	N	Y	2300		ug/l
11/18/2007	14 00	WQ	WQ	NAS# 1502G Day 4-10 0 mg/L Naphthalene		N	SW8260B	11/29/2007	03 42	PAHs	91-20-3	Naphthaler	N	Y	4500		ug/l
11/18/2007	14 00	WQ	WQ	NAS# 1503G Day 4-20 0 mg/L Naphthalene		N	SW8260B	11/29/2007	04 09	PAHs	91-20-3	Naphthaler	N	Y	9500		ug/l

Naphthalene (mg/L)	Day 0	Day 1	Day 2	Day 3	Day 4	Average	Standard Deviation
0	0 0	0 0	0 0	0.0	0 0	0 0	0 0
1 25	0.63	0 81	0 81	0 69	0 58	0 70	0 10
2 5	1 10	1 40	1 40	1 30	1.00	1 24	0 18
5 0	2 40	2 90	2 90	2 70	2 30	2 64	0 28
10 0	5 00	6.20	5 50	5 40	4 50	5 32	0 63
20 0	7 40	12 00	12 00	10 00	9 50	10 18	1 93

Validated Naphthalene Analyses Reported by Windward 2/18/08

686-42

12 of 14

Acute 96-hr Toxicity Test-96 Hr Survival

Start Date:	11/14/07 13:30	Test ID:	686-42	Sample ID:	
End Date:	11/18/07 14:30	Lab ID:	ORNAS-Northwestern Aquati	Sample Type:	Naphthalene
Sample Date:		Protocol:	NAS-686-Lamprey Ft acute	Test Species:	LY-Lampetra sp.
Comments:					

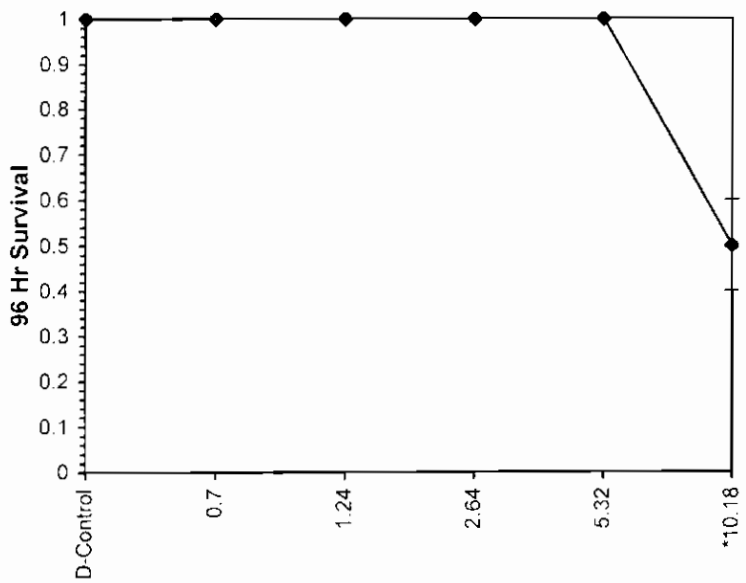
Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
0.7	1.0000	1.0000	1.0000	1.0000
1.24	1.0000	1.0000	1.0000	1.0000
2.64	1.0000	1.0000	1.0000	1.0000
5.32	1.0000	1.0000	1.0000	1.0000
10.18	0.5000	0.4000	0.5000	0.6000

			Transform: Arcsin Square Root					Rank	1-Tailed
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4		
0.7	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00
1.24	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00
2.64	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00
5.32	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00
*10.18	0.5000	0.5000	0.7854	0.6847	0.8861	10.467	4	10.00	10.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.40374	0.884	-6E-15	11.5
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	5.32	10.18	7.35918	

Dose-Response Plot



LC50 = 10.18
by data inspection
RSe 2/15/08

No 95% confidence limits can be estimated.

ToxCalc v5.0.23N

Test: AT-Acute 96-hr Toxicity Test
Test ID: 686-42
Species: LY-Lampetra sp.
Protocol: NAS-686-Lamprey Ft acute
Sample ID:
Sample Type: Naphthalene
Start Date: 11/14/07 13:30
End Date: 11/18/07 14:30
Lab ID: ORNAS-Northwestern Aquatic Sciences

Pos	ID	Rep	Group	Start	24 Hr	48 Hr	72 Hr	96 Hr	Notes
	1	1	D-Control	10				10	
	2	2	D-Control	10				10	
	3	3	D-Control	10				10	
	4	4	D-Control	10				10	
	5	1	0.700	10				10	
	6	2	0.700	10				10	
	7	3	0.700	10				10	
	8	4	0.700	10				10	
	9	1	1.240	10				10	
	10	2	1.240	10				10	
	11	3	1.240	10				10	
	12	4	1.240	10				10	
	13	1	2.640	10				10	
	14	2	2.640	10				10	
	15	3	2.640	10				10	
	16	4	2.640	10				10	
	17	1	5.320	10				10	
	18	2	5.320	10				10	
	19	3	5.320	10				10	
	20	4	5.320	10				10	
	21	1	10.180	10				5	
	22	2	10.180	10				4	
	23	3	10.180	10				5	
	24	4	10.180	10				6	

Comments:

LAMPREY (*LAMPETRA* SP.) DEFINITIVE 96-HR FLOW-THROUGH ACUTE TOXICITY TEST OF PENTACHLOROPHENOL

NAS Test No. 686-43

Submitted to

Windward Environmental LLC
200 West Mercer Street, Suite 401
Seattle, WA 98119

Submitted by

Northwestern Aquatic Sciences
3814 Yaquina Bay Road
P.O. Box 1437
Newport, OR 97365

April 3, 2008

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 686-43
Title: Lamprey (*Lampetra* sp.) definitive 96-hr flow-through acute toxicity test of pentachlorophenol.
Protocol No.: NAS-686-Lamprey FT acute, September 12, 2007

STUDY MANAGEMENT

Study Sponsor: Windward Environmental, 200 West Mercer Street, Suite 401, Seattle, WA 98119.
Sponsor's Study Monitor: Ms. Helle Andersen.
Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365.
Test Location: Newport Laboratory.
Laboratory's Study Personnel: G. A. Buhler, B.S., Proj. Manager; R. S. Caldwell, Ph.D., Study Director; L.K. Nemeth, B.A., M.B.A., QA Officer; G.J. Irissarri, B.S., Aq. Toxicol.
Study Schedule:
Test Beginning: 12-16-07, 1130 hrs.
Test Ending: 12-20-07, 1300 hrs.
Disposition of Study Records: All specimens, raw data, reports and other study records are stored according to Good Laboratory Practice regulations at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.
Good Laboratory Practices: The test was conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).
Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Description: Pentachlorophenol, 99.2% (Chem Service, WestChester, PA, Lot No. 360-83B).
Preparation of Working Stock Solution and Test Concentrations: Test solutions were prepared for continuous supply to the test aquaria using a Benoit constant flow diluter (Benoit *et al.*, 1982). The diluter was set up to deliver a 0.5 dilution series with a flow rate of approximately 80 ml/min per toxicant concentration. The diluter design was such that 160 ml/min of the toxicant solution at the highest test concentration (nominal 0.2 mg/L pentachlorophenol) was required to be continuously delivered to the toxicant supply chamber of the diluter. To accomplish this, a large mixing reservoir, approximately 54L capacity, of nominal 0.2 mg/L pentachlorophenol solution in dilution water, was maintained at test temperature. This was done by metering, at a rate of 1.6 ml/min, a constant supply of 20 mg/L pentachlorophenol concentrated stock solution in dilution water into the large toxicant reservoir with vigorous mixing, while also replacing, under float valve control, dilution water to replace delivery of the nominal 0.2 mg/L pentachlorophenol solution to the diluter. The pentachlorophenol concentrated stock solution was prepared daily.

DILUTION WATER

Source: City of Newport municipal tap water, treated as below, was supplied continuously to the toxicant diluter throughout the study.
Pretreatment: The water was dechlorinated by carbon filtration, particle filtered to 1 µm, and adjusted to a target water hardness of 25-30 mg/L as $CaCO_3$. Hardness adjustment was accomplished by passage of a portion of the dilution water through deionization cartridges.

TEST ORGANISMS

Species: Lamprey (*Lampetra* sp.)
Age/Size: Ammocoetes; target size range, 4-6 cm; actual size of 10 ammocoete subsample, 5.0 ± 0.7 cm.
Source: Ammocoetes were field collected in approximately equal numbers from the Trask, Nestucca, Siletz, and Alsea Rivers, Oregon. Field collection was by electoshocking and only 4-6 cm size ammocoetes were retained. Personnel of Windward Environmental performed the ammocoete collections.

Acclimation: Upon receipt, animals were removed from the collection site sediment and placed in 10-gallon aquaria supplied under flow-through conditions with dilution water. Water supply was sufficient (30-50 ml/min) to accomplish two volume changes of water per day. Approximately 50 lamprey were placed in each tank. Tanks contained an approximate 8 cm deep layer of sediment (Sandtastic Play Sand, Waupaca Materials) covered with approximately 26L of test water and were supplied with aeration. Normal behavior for the lamprey ammocoetes was to immediately bury in sediment and remain there. Any that did not immediately bury were discarded as potentially damaged. Ammocoetes were not fed during acclimation. Water quality conditions during the two weeks prior to testing (12-1-07 to 12-16-07) were: temperature, 16.5 ± 0.7°C; dissolved oxygen, 9.5 ± 0.3 mg/L; pH, 7.5 ± 0.3; specific conductance, 169 ± 9 µmhos/cm; hardness, 62 ± 4 mg/L as $CaCO_3$; and alkalinity, 43 ± 2 mg/L as $CaCO_3$.

TEST PROCEDURES AND CONDITIONS

Test Chambers: The test chambers were two-gallon glass aquaria capable of holding 5.8 L of test solution. Test chamber placement in the test area was random.

Test Concentrations: The nominal test concentrations were 0.2, 0.1, 0.05, 0.025, 0.0125, and 0 mg/L (control) pentachlorophenol.

Replicates/Treatment: 4

Organisms/Treatment: 40

Loading: 0.33 g/L

Aeration: None.

Feeding: None.

Water Volume Changes: The diluter supplied approximately 80 ml/min of test solution, or 115 L/day, at each test concentration. At this rate of flow, test solution supply to each replicate aquarium was 5.0 volumes per day.

Effects Criteria: The effect criterion used in the lamprey ammocoete acute toxicity test was mortality, defined as a lack of visible respiratory movement and absence of response to tactile stimulation. In addition to mortality, qualitative observations of sublethal responses were recorded when observed.

Target Water Quality Conditions: Water quality measurements were taken daily as specified in the protocol (Physical and Chemical Data, Items 1 and 2). The target water quality conditions were as follows: Temperature, 17 ± 1°C; dissolved oxygen, ≥4.0 mg/L; hardness, 25-30 mg/L as $CaCO_3$. Actual test conditions are reported in the Test Results section.

Photoperiod: The photoperiod was total darkness except for approximately 2-3 hours per day for observations and test exposure system maintenance. Light intensity at the aquarium level was 14.9 foot-candles.

DATA ANALYSIS METHODS

The 96-hr proportion survival was calculated for each treatment replicate from the raw survival data and the measured pentachlorophenol concentrations for each treatment level. The LC50 (survival) was calculated by the probit method. An arcsine square root (angular) transformation was performed on the data prior to statistical analysis. The NOEC and LOEC were calculated according to U.S. Environmental Protection Agency (2002) methods. In this procedure, the NOEC and LOEC values were computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test was selected after evaluating the data for normality and homogeneity of variance. The statistical software employed for these calculations was ToxCalc, v.5.0.23N, Tidepool Scientific Software.

TOXICANT AND OTHER CHEMISTRY ANALYSES

Test solutions were sampled once daily for pentachlorophenol from each test concentration by pooling samples from each of the four test replicate aquaria. Subsamples were pooled into new disposable plastic beakers from which the pooled sample was transferred to the analytical laboratory supplied sample bottles and shipped under chain-of-custody procedures to the analytical chemistry laboratory (Columbia Analytical Services) at Kelso, WA. No preservative was employed. Samples were kept refrigerated at ≤4°C. Sample volumes were 1 L. Additional samples were taken of the dilution water on day 0 of the test for analysis of particulate matter, TOC, and ammonia-N, and from the control and highest test concentration treatments after 96-hr for measurement of ammonia-N. These samples also were shipped to Columbia Analytical Services for analysis.

PROTOCOL DEVIATIONS

1. The protocol specified "constant dark, except during monitoring activities", and also that light intensity should be 50-75 ft-c. when light was on. These specifications were judged to be contradictory and led to the following action. Based on best professional judgment of the investigators, we reduced the light intensity from that given in the protocol in order to minimize fright behavior responses of the ammocoetes during the short period of lighting associated with daily observations.
2. The protocol states: "At the end of the test, all surviving control ammocoetes are weighed and measured for length by replicate." Through a laboratory error, a subsample of 10 ammocoetes were weighed and measured at the beginning of the test instead. This is a minor deviation and is without consequence for interpretation of the study results.

TEST RESULTS

Water quality conditions measured in test aquaria during the 96-hr toxicity test are summarized in Table 1. The temperature specification was met during the study. Dissolved oxygen remained near saturation throughout the test. The pH ranged from 6.3 – 6.9. Mean hardness also was in the specified range of 25-30 mg/L as $CaCO_3$.

The concentrations of pentachlorophenol measured during the toxicity test are summarized in Table 2. Mean measured concentrations in the test ranged from 0.017 ± 0.004 mg/L at the lowest level to 0.230 ± 0.025 mg/L at the highest exposure level. Generally, the dilution series was in good agreement with nominal concentrations.

A summary of the 96-hr ammocoetes survival results is shown in Table 3. In this study, the 96-hr control survival (100%) met the test acceptability criteria of ≥90%. Therefore, the test meets the protocol criterion of a valid test. Additionally, 100% mortality occurred in the two highest test concentrations, and 0% mortality was observed in the lowest. test concentration. Partial mortality was observed in the third (0.056 mg/L) and fourth highest (0.030 mg/L) test concentrations.

The toxicity of pentachlorophenol to lamprey (*Lampetra* sp.) ammocoetes as the 96-hr LC50 was 0.031 mg/L (95% confidence limits, 0.028 0.034 mg/L). The NOEC and LOEC concentrations were 0.017 and 0.030 mg/L pentachlorophenol, respectively.

LITERATURE CITED

Benoit, D., V. Mattson, and D. Olson. 1982. A continuous-flow mini-diluter system for toxicity testing. Water Res. 16:457-464.

EPA. 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. Environmental Protection Agency, Washington, DC.

STUDY APPROVAL

Project Manager	4/11/08	Study Director	4/11/08
Laboratory Director	4/11/08	Quality Assurance Unit	4/7/08

Table 1. Measured water quality conditions during the 96-hr acute toxicity test of pentachlorophenol to lamprey ammocoetes.

Parameter	Mean ± S.D.	N	Range
Temperature (°C)	17.0 ± 0.3	30	16.3 – 17.6
Dissolved oxygen (mg/L)	9.9 ± 0.1	30	9.7 – 10.1
pH	6.6 ± 0.2	30	6.3 – 6.9
Specific conductance (µmhos/cm)	125 ± 2	10	120 – 128
Hardness (mg/L as $CaCO_3$)	30 ± 2	10	26 – 32
Alkalinity (mg/L as $CaCO_3$)	15 ± 1	10	13 – 15

Table 2. Measured concentrations of pentachlorophenol during the 96-hr acute toxicity test of pentachlorophenol to lamprey ammocoetes. Measurements performed by Columbia Analytical Services.

Nominal (mg/L)	Measured (mg/L)		
	Mean ± S.D.	N	Range
0.2	0.230 ± 0.025	5	0.200 – 0.260
0.1	0.110 ± 0.014	5	0.095 – 0.130
0.05	0.056 ± 0.012	5	0.045 – 0.073
0.025	0.030 ± 0.006	5	0.023 – 0.038
0.0125	0.017 ± 0.004	5	0.014 – 0.022
0	0.000 ± 0.000	5	0.000 – 0.000

Table 3. Survival of lamprey ammocoetes, *Lampetra* sp. exposed for 96 hours to pentachlorophenol.

Pentachlorophenol (mg/L)	Replicate	Ammocoetes exposed	Ammocoetes Surviving	Percent Survival	Mean % Survival	
0.230	A	10	0	0.0		
	B	10	0	0.0		
	C	10	0	0.0		
	D	10	0	0.0	0.0	*
0.110	A	10	0	0.0		
	B	10	0	0.0		
	C	10	0	0.0		
	D	10	0	0.0	0.0	*
0.056	A	10	0	0.0		
	B	10	0	0.0		
	C	10	0	0.0		
	D	10	1	10.0	2.5	*
0.030	A	10	3	30.0		
	B	10	8	80.0		
	C	10	4	40.0		
	D	10	5	50.0	50.0	*
0.017	A	10	10	100.0		
	B	10	10	100.0		
	C	10	10	100.0		
	D	10	10	100.0	100.0	
0.000 (Control)	A	10	10	100.0		
	B	10	10	100.0		
	C	10	10	100.0		
	D	10	10	100.0	100.0	

* Mean significantly (p=0.05) different from the control.

APPENDIX I

PROTOCOL

STUDY PROTOCOL

LAMPREY (*LAMPETRA* sp.)
FLOW THROUGH ACUTE TOXICITY TEST

INTRODUCTION

Purpose of Study: The purpose of this study is to determine the acute toxicity of six chemicals, representing a range of toxic modes of action, to lamprey (*Lampetra* sp.) ammocoetes. The study will be performed using methods based on the methods for measuring acute toxicity with other fish species as described in U.S. Environmental Protection Agency (2002) and American Society for Testing and Materials (ASTM) (1996) guidance.

Summary of Method: In definitive tests, lamprey ammocoetes are exposed for 96 hours to five concentrations of test material and a dilution water control. The test concentrations are arranged in a logarithmic series based on the results of range finding tests. Four replicates, each with 10 organisms, are employed at each test concentration, if there are sufficient numbers of ammocoetes. Flow-through exposures are employed. Test chambers are glass aquaria less than or equal to two gallons in volume. Mortality is the effect criterion. The data analysis consists of calculating means and standard deviations for survival. An LC50 with 95% confidence intervals may be calculated along with the NOEC and LOEC concentrations.

STUDY MANAGEMENT

Sponsor's Name and Address:
Windward Environmental LLC
200 West Mercer Street, Suite 401
Seattle, WA 98119

Sponsor's Study Monitor:
Ms. Helle Andersen

Name of Testing Laboratory:
Northwestern Aquatic Sciences
3814 Yaquina Bay Road
P.O. Box 1437
Newport, OR 97365

Test Location: Newport, OR.

Laboratory's Key Personnel to be Assigned to the Study:
Laboratory Director: Richard S. Caldwell, Ph.D.
Project Manager/Study Director: Gary A. Buhler, B.S.
QA Officer/Toxicology Program Mgr: Linda K. Nemeth, B.A., M.B.A.
Aquatic Toxicologist: Gerald J. Irissarri, B.S.

Proposed Study Schedule: July 2007 through September 2007.

Good Laboratory Practices: The test is conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

TEST MATERIAL

Description: Chemicals to be tested include aniline, copper, pentachlorophenol, lindane, diazinon and naphthalene.

Preparation of Working Stock Solution and Test Concentrations: Test materials will be administered using a constant-flow diluter based on the design of Benoit *et al.* (1982). Stock solutions of the sparingly soluble organic compounds, e.g. naphthalene, pentachlorophenol, lindane, diazinon, will be prepared continuously by recirculation of dilution water/toxicant stock solution through a column of toxicant coated glass beads (Veith and Comstock, 1975). This is a conceptual approach in which the specific column, flow rates, glass bead sizes, etc. need to be developed empirically, with analytical feedback, for each study considering such variables as chemical solubility, test temperature, desired highest test concentration, stock toxicant consumption rate, etc. Given the above, some modifications are anticipated as the studies progress. Initial system characteristics are as follows: 1) the toxicant mixing tank will have a 60-liter capacity; 2) a 20 x 280-cm glass column will be employed (10 x 200-cm and 40 x 560-cm columns are also available); 3) the column packing will employ 5-mm glass beads coated with the test compound; 4) particulates will be filtered out through glass wool; 5) flow through column will be controlled by a variable-speed peristaltic pump or by intermittent pumping: and 6) tubing will be glass and silicone rubber. GC/MS analysis of toxicant in the toxicant mixing tank will be used to optimize the system for each chemical noted above prior to test initiation.The contents of the stock toxicant reservoir will be constantly replenished with fresh dilution water as toxicant solution is supplied to the diluter. The aniline stock solution will be prepared similarly, but by volumetric addition of aniline, using a metering pump, to the stock toxicant reservoir *in lieu* of the Veith column. The copper working stock solution will be prepared in the same apparatus by metering pump addition of a concentrated copper stock solution to the stock toxicant reservoir. All toxicant working stock solutions will be prepared and maintained under the same conditions as in the test exposures and delivered continuously to the toxicant chamber of the diluter at an appropriate rate.

DILUTION WATER

Dilution water will be dechlorinated City of Newport municipal tap water adjusted to a water hardness of 25-30 mg/L as $CaCO_3$.

TEST ORGANISMS

Species: Lamprey, *Lampetra* sp.

Source: Ammocoetes will be collected by Windward staff and delivered to the laboratory each day of collection. The ammocoetes will be collected from several rivers including the Siletz River, which was the site for Phase 1 ammocoetes.

Age: ammocoetes (juvenile prior to metamorphosis)

Acclimation and Pretest Observation: Test organisms will be held in the laboratory in order to acclimate to test temperature. Upon receipt, animals are removed from the sediment they are received in and placed in 10-gallon tanks under flow-through conditions (~35 mL/minute, or ~ 2 volume changes per day, with one additional daily siphon and replacement of 2/3 volume of each tank). Approximately 50 lamprey are placed in each tank. Tanks will contain at least 3 inches of sediment (Sandtastic Play Sand, Waupaca Materials, or similar material) covered with approximately 26L of test water and supplied with aeration. The maximum temperature variation during holding will be ± 1° C. The ammocoetes will be acclimated to the laboratory test conditions for a minimum of 5 to 7 days prior to test initiation. This minimum number of days applies only to the first toxicity test conducted on each batch of field-collected organisms. For all other tests, the ammocoetes will be held for at least 13 days prior to test initiation. When a temperature adjustment of more than 3 to 5°C is required, the transfer from field collection water to laboratory dilution water will be gradual. Normal behavior for lamprey ammocoetes is to immediately bury in sediment and remain there. Any dead animals observed on the sediment surface are removed daily. Past experience suggests that mortality is minimal during short-term holding and any mortality is likely due to damage from field collection. If ammocoetes do not bury or there is excessive

mortality in any tank, animals from that tank will not be used for testing. Ammocoetes will not be fed since they are being held short-term.

DESCRIPTION OF TEST SYSTEM

Preparation of Test Concentrations: Test materials will be administered using a constant-flow diluter based on the design of Benoit *et al.* (1982). The diluter will be calibrated to deliver a 0.5 dilution series. Flow rates will be adjusted to deliver at least five test chamber volumes per concentration per day. Diluter flow rates will be measured and documented at the beginning and conclusion of each 96-hour test. This will be done by measuring individual diluter capillary tube flows. When the flow rates are entered into an Excel® spreadsheet, the actual dilutions of the toxicant stock can be computed. Actual test chemical concentrations (organic chemicals) in the toxicant mixing chamber (toxicant stock solution) and in exposure aquaria will periodically be measured on a real-time basis at NAS to verify that nominal concentrations are being routinely achieved. Definitive concentration documentation in test aquaria will result from daily samples shipped for analysis to CAS.

Test Chambers and Environmental Control: Test chambers used in the toxicity study are glass aquaria less than or equal to two gallons in volume. The test system is housed in a constant-temperature, photoperiod-controlled room. Aeration is not employed. The photoperiod is constant dark except during monitoring activities when ambient (50-75 ft-c) lighting is used.

Cleaning: All laboratory glassware, including test chambers and toxicant dilution system, will be cleaned based on the methods described in U.S. Environmental Protection Agency (2002). Initial cleaning incorporates the use of a laboratory detergent followed by multiple tap water rinses. Subsequently, either dilute (10%, V:V) acid (hydrochloric or nitric) or acetone is used to remove metals or organic contaminants, respectively. Further multiple tap water rinses are employed after each acid or acetone treatment. Sodium hypochlorite bleach may be employed on a limited basis to ensure that microorganisms that could metabolize organic toxicants, e.g. naphthalene, are not present. Exhaustive tap water rinsing is always employed following any chemical cleaning treatment. A final dilution water rinse of the exposure apparatus will precede initiation of each study.

EXPERIMENTAL DESIGN AND TEST PROCEDURES

Experimental Design: The definitive test involves exposure of lamprey ammocoetes to five test concentrations, using a 0.5 dilution series, and a dilution water control. Exposures are for 96 hours. Each treatment consists of four replicates, each containing 10 ammocoetes, if there are sufficient numbers of ammocoetes. If there are not enough ammocoetes for 10 per replicate, then at least 20 per treatment (5 per replicate) is the minimum number preferred. Test chambers are randomly distributed in the test area. Ammocoetes are impartially distributed to each aquarium by adding one or two animals to each test replicate and repeating the process until each contains 10 organisms.

Effect Criterion: The effect criterion used in the lamprey ammocoete acute toxicity test is mortality, defined as a lack of visible respiratory movement and absence of response to tactile stimulation. The mortality data will be used to compute LC50s for each exposure period. In addition to mortality, qualitative observations of sublethal responses consisting of erratic swimming, hyperventilation, hemorrhaging, and hyper/hypoactivity (compared to the activity of the organisms in the control group) will be recorded if observed.

Test Conditions: The dissolved oxygen concentration in each test container must be > 4.0 mg/L throughout the test. The test temperature employed is 17 ± 1°C. A photoperiod of constant darkness is used, except during monitoring activities when illumination is supplied by daylight fluorescent lamps at an intensity of 50-75 ft candles. Loading should not exceed 6.1g/L at 17°C according to EPA. ASTM recommends loading not exceed 1g/L of solution passing through the chamber in 24 hours or 10g/L of solution in the chamber at any time.

Beginning the Test: Adding the organisms to the equilibrated test containers as previously described begins the test.

Feeding: None.

Analysis of Test Concentrations: During each test, samples will be collected for chemical analysis, held at 4°C and shipped by overnight delivery to Columbia Analytical Services in Kelso, WA. The sampling schedule and procedure will follow the project QAPP.

Test Duration, Type and Frequency of Observations, and Methods: The test duration of the acute toxicity test is 96 hours. The type and frequency of observations to be made during the test are summarized as follows:

Type of Observation	Times of observation
Biological Data	
1. mortality (each test container)	0, 24, 48, 72, and 96 hr
2. sublethal observations (qualitative)	0, 24, 48, 72, and 96 hr
Physical and Chemical Data	
1. Dissolved oxygen, pH, & temperature, (in one replicate of each test level and the control)	0, 24, 48, 72, and 96 hr
2. Conductivity, hardness & alkalinity (in one replicate of highest concentration and control)	0, 24, 48, 72, and 96 hr
3. Toxicant concentration sampling (in one replicate of each test concentration and the control)	0, 24, 48, 72, and 96 hr
4. Dilution water sample for particulate matter, TOC and ammonia	0 hr
5. Sample for ammonia taken from one replicate of control and highest test concentration	96 hr

During the test, dead organisms are removed every 24 hours and frozen by concentration for possible tissue analysis. Dissolved oxygen is measured using a polarographic oxygen probe calibrated according to the manufacturer's recommendations. The pH and temperature are measured using a pH probe and a properly calibrated meter with scale divisions of 0.1 pH units. Conductivity is measured using a calibrated conductivity meter. Samples for chemical analysis will be collected, preserved, and sent to Columbia Analytical Services following the recommended procedures of the analytical laboratory.

Test Termination: At the end of the test, all surviving control ammocoetes are weighed and measured for length by replicate. All surviving or dead test organisms will be frozen by concentration for possible later tissue analysis by Columbia Analytical Services.

Criteria of Test Acceptance: The test results are acceptable if survival in the controls is at least 90%. The toxicity tests will be performed as a 50% dilution series, it is anticipated that each test will have at least one or possibly two partial mortality concentrations. Nominal concentrations of each toxicity test have be reviewed and approved by EPA (EPA 2007). However, because of the experimental nature of the study and the limited supply of ammocoetes, the acceptability of the toxicity tests will be based only on the ≥ 90% survival in control group.

DATA ANALYSIS

The percent of live ammocoetes is calculated for each treatment replicate from the raw data and the means are obtained for each treatment level. An LC50 (mortality) is calculated using Probit or Trimmed Spearman-Karber methods. The NOEC and LOEC are calculated for each endpoint according to U.S. Environmental Protection Agency (2002) methods. In this procedure, the NOEC and LOEC values are computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test is selected after evaluating the data for normality and homogeneity of variance. An arcsine-square root transformation is performed on the percentage effect data prior to statistical analysis. The statistical software employed for these calculations is Toxcalc, v.5.0.23N, Tidepool Scientific Software.

REPORTING

The final report of the study results must include all of the following standard information at a minimum:

1. Name and identification of the test;
2. The investigator and laboratory;
3. Information on the toxicants;
4. Information on the dilution water;
5. Detailed information about the test organisms;
6. Description of the test system and its calibration, including test chambers and dilution system;
7. A description of the experimental design and other test conditions including water quality;
8. Definition of the effect criteria and any other observations;
9. Responses, if any, in the control treatment;
10. Tabulation and statistical analysis of measured responses;
11. A description of the statistical methods used;
12. Any unusual information about the test or deviations from procedures;

The final study report will include the study protocol and all study raw data as appendices.

STUDY DESIGN ALTERATION

Amendments made to the protocol must be approved by the sponsor and study director and should include a description of the change; the reason for the change; the date the change took effect; and the dated signatures of the study director and sponsor. Any deviations in the protocol must be described and recorded in the study raw data.

REFERENCED GUIDELINES

ASTM. 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.

Benoit, D., V. Mattson, and D. Olson. 1982. A continuous-flow mini-diluter system for toxicity testing. Water Res. 16:457-464.

EPA. 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. Environmental Protection Agency, Washington, DC.

EPA. 2007. Letter to Lower Willamette Group from C. Humphrey and E. Blischke dated August 17, 2007 regarding Portland Harbor RI/FS: Lamprey toxicity test field sampling plan and QAPP Phase 2 addenda (approval letter). US Environmental Protection Agency Region 10, Oregon Operations Office, Portland, OR.

Veith G.D. and V.M. Comstock. 1975. Apparatus for continuously saturating water with hydrophobic organic chemicals. J Fish Res Board Can 32:1849-1851.

13. APPROVALS

_____ _____ for **Windward Environmental**
Name Date

_____ _____ ___ for **Northwestern Aquatic Sciences**
Name Date

Appendix A
Test Conditions Summary

1. Test species:	*Lampetra* sp.
2. Test type:	Flow-through
3. Test duration:	96 hr
4. Temperature:	17 ± 1°C
5. Dissolved oxygen:	≥ 4.0 mg/L
6. Light quality & intensity:	Ambient laboratory light (50-75 ft-c)
7. Photoperiod:	Constant dark, except during monitoring activities
8. Test chambers:	glass aquaria less than or equal to two gallons in volume.
9. Test chamber volumes per day:	At least 5
10. Replicates per treatment:	4
11. Organisms per replicate:	10
12. Age of test organisms:	ammocoete
13. Feeding regime:	None
14. Aeration:	None
15. Dilution water:	Dechlorinated City of Newport tap water; hardness adjusted to 25-30 mg/L as $CaCO_3$
16. Test concentrations:	5 and a dilution water control
17. Water quality:	Temperature, pH, & dissolved oxygen in one replicate of each test concentration daily. Conductivity, hardness & alkalinity in one replicate of highest concentration and control daily
18. Special controls:	None
19. Endpoint:	Mortality
20. Test acceptability criteria:	≥90% control survival

APPENDIX II

RAW DATA

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-43 Client Windward Investigator

STUDY MANAGEMENT:

Client: Windward Environmental, 200 West Mercer Street, Suite 401, Seattle, WA 98119
Client's Study Monitor: Ms. Helle Andersen
Testing Laboratory: Northwestern Aquatic Sciences
Test Location: 3814 Yaquina Bay Road, Newport, OR 97365
Laboratory's Study Personnel:
Project Manager: Gary A. Buhler Study Director: Richard S.Caldwell
QA Officer: L.K. Nemeth 1. G.J. BISSONETTE
2. 3.
4. 5.
Study Schedule:
Test Beginning: 12/16/07 11:30 AM Test Ending: 12/20/07 13:00

TEST MATERIAL:

Description: Pentachlorophenol (PCP) ChemService, West Chester, PA (Lot No. 362-83B)

DILUTION WATER:

Description: Dechlorined City of Newport tap water
Date of Preparation/Collection: Continuous Flow
Water Quality: Cond. (µmhos/cm) 12.4 ± 7 pH 6.5 ± 0.2
Hardness (mg/L as $CaCO_3$) 31.6 ± 0.4 Alkalinity (mg/L as $CaCO_3$) 14.4 ± 0.4
Treatments: Hardness adjusted to 25-30 mg/L with a Culligan® water treatment system.

TEST ORGANISMS:

Species: Lamprey ammocoetes (*Lampetra* sp.) Age: Size: 4-6 cm
Source: Trask, Nestucca, Siletz & Alsea Rivers
Acclimation Data: See pages:

TEST PROCEDURES AND CONDITIONS:

Test Concentrations (nominal): 0.2, 0.1, 0.05, 0.025, 0.0125, & 0 mg/L
Test Chambers (description): 2.5 gallon glass aquaria Test Volume (ml): 5.80 L
Replicates/treatment: 4 Organisms/treatment: 40 (10 replicate)
Test water changes: flow through
Aeration during test: none DO limit ≥ 4.0 mg/L
Temperature (°C): 17 + 1°C
Feeding: None Photoperiod: Darkness except for WQ & chemistry measurements
Test Duration (hr) 96 hours Tank Placement: Complete randomization
Randomization Chart:

Diluter

.025	2	.025	1	0	2	0	.025	.0125	.05	0	2	1	.0125	.0125	1	.025	.05	.05	2	0	.05	1	.0125

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-43 Client Windward Investigator

WEIGHT OF 10 ANIMALS AT TEST INITIATION

	weight (g)	Length (cm)	Comments
1	0.21	5.3	
2	0.07	4.0	
3	0.11	4.1	
4	0.20	5.5	
5	0.26	5.8	
6	0.17	4.4	
7	0.13	4.3	
8	0.21	5.2	
9	0.28	5.6	
10	0.23	5.5	
~~Total~~ Mean	0.19	5.0	
~~Average animal~~ SD	0.06	0.7	
N	10	10	

MISCELLANEOUS NOTES:

Room light intensity (fc): 14.9 [illegible] 12/20/07

Chemistry samples taken daily.

Chemistry samples for particulate matter, TOC & ammonia in dilution water taken on test day 0.

Chemistry samples for ammonia taken in control & 0.4 mg/L A replicate on test day 4.

$$\text{Loading} = \frac{0.19\,g \times 10}{5.8\,L} = 0.33\ g/L$$

Error codes:

1) Correction of handwriting error
2) Written in wrong location; entry deleted
3) Wrong date deleted; replaced with correct data
4) Error found in measurement; measurement repeated

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-43 Client_____ Windward_____ _____ Investigator_____

WATER QUALITY DATA SHEET

DAY 0 (12 16 07) [illegible]

Conc (mg/L)	Temp (°C)	pH	Cond µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
0.2	[illegible]	6.6	125	9.7	30	14	10	10	10	10	
0.1	17.0	6.6		9.8			10	10	10	10	
0.05	16.7	6.5		9.8			10	10	10	10	
0.025	[illegible]	6.4		9.7			10	10	10	10	
0.0125	[illegible]	[illegible]		9.8			10	10	10	10	
0	16.3	6.5	122	9.5	30	13	10	10	10	10	

Chemistry samples taken (12 16 07) [illegible]
Particulate matter, TOC, and ammonia samples taken in dilution water ([illegible]) [illegible]
Hours of light: 2

DAY 1 (12 17 07) [illegible]

Conc (mg/L)	Temp (°C)	pH	Cond µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
0.2	17.0	6.7	128	10.1	32	15	0(10)	0(10)	0(10)	0(10)	
0.1	16.8	6.7		10.1			6(40)	9(40)	10	8(20)	
0.05	16.7	6.7		10.0			10	10	10	10	
0.025	17.0	6.5		10.0			10	10	10	10	
0.0125	16.5	6.5		9.6			10	10	10	10	
0	17.3	6.4	126	10.0	32	15	10	10	10	10	

Chemistry samples taken (12 17 07) 12 [illegible]
Hours of light: 2

DAY 2 (12 18 07) [illegible]

Conc (mg/L)	Temp (°C)	pH	Cond µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
0.2	17.0	6.6	125	9.9	30	15	0	0	0	0	
0.1	16.7	6.6		10.0			0(60)	0(40)	0(100)	0(80)	
0.05	16.8	6.5		10.1			9(10)	8(20)	8(20)	10	
0.025	16.7	6.5		9.9			10	10	10	10	
0.0125	16.6	6.5		10.0			10	10	10	10	
0	16.8	6.5	120	10.0	32	15	10	10	10	10	

Chemistry samples taken (12 18 07) 1145
Hours of light: 2

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-43 Client Windward Investigator

WATER QUALITY DATA SHEET

DAY 3 (12 19 2003)

Conc (mg/L)	Temp (°C)	pH	Cond µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
0.2	17.4	6.6	124	10.1	28	15	0	0	0	0	
0.1	17.2	6.6		9.9			0	0	0	0	
0.05	17.0	6.5		9.7			4(50)	2(60)	2(60)	3(70)	
0.025	17.5	6.5		9.9			10	10	10	10	
0.0125	17.2	6.5		9.8			10	10	10	10	
0	16.8	6.5	125	10.0	32	15	10	10	10	10	

Chemistry samples taken (12 19 03) 1900
Hours of light. 3

DAY 4 (12 20 2003)

Conc (mg/L)	Temp (°C)	pH	Cond µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
0.2	17.6	6.9	125	9.8	26	15	0	0	0	0	
0.1	16.9	6.9		9.7			0	0	0	0	
0.05	16.9	6.9		9.8			0(40)	0(70)	0(20)	1(20)	
0.025	17.5	6.8		9.7			3(70)	8(20)	9(60)	5(50)	
0.0125	17.2	6.8		9.8			10	10	10	10	
0	16.7	6.8	125	9.9	32	14	10	10	10	10	

Chemistry samples taken (12 20 03) 1900
Ammonia samples taken in control and 0.2 mg/L (12 20 03) 1900
Hours of light. 3

Dilution Water WQ (from control aquaria)

Cond	124	± 2.5	(5)	
pH	6.5	± 0.2	(5)	
Hardness	31.6	± 0.9	(5)	
Alkalinity	14.4	± 0.9	(5)	

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-43 Client Windward Investigator_____

NOTES ON PREPARATION OF 0.2 mg/L PENTACHLOROPHENOL STOCK

System Design

The objective is to deliver 0.2 mg/L of pentachlorophenol to the diluter toxicant chamber at a nominal constant rate of 160 ml/min. To accomplish this, a large reservoir, approximately 54L capacity, of pentachlorophenol solution in dilution water is maintained at test temperature. As solution is removed to the diluter, makeup water is introduced under float valve control. The concentration of pentachlorophenol in the reservoir is maintained by metering 1.6 ml/min of a 20 mg/L pentachlorophenol concentrated stock solution into the mixing tank.

NOTES ON MAINTENANCE OF THE PENTACHLOROPHENOL DELIVERY SYSTEM

Concentrated PCP stock prepd daily 20 hr prior to use
Cons combined concentration ≈ 20 mg/L 12/15 – 12/18
Cons combined 0.33 mg/L ~~conc~~ aq/mix ≈ 0.23 mg/L 12/19 – 12/20

DILUTER CALIBRATIONS RECORD

DILUTER CAPILLARY FLOWS

Date 12/15/08	Time 1630	Init. [illegible]	Date 12/2 1/08	Time 1500	Init. [illegible]
Drain #	Supply Tank	Diluter Tank	Drain #	Supply Tank	Diluter Tank
1	160	78	1	161	79
2	78	78	2	79	83
3	79	77	3	80	77
4	80	76	4	83	75
5	79	75	5	78	76
6	81	78	6	81	80

Flow rates in ml/min

FLOWS TO REPLICATE AQUARIA

Date 12/15/08 Time 1630 Init. [illegible]

Replicate	Relative Concentration (%) 100	50	25	12.5	6.25	0
A	315	325	325	290	295	325
B	280	280	315	335	280	270
C	325	295	255	315	310	275
D	300	310	260	260	340	275

Date 12/21/08 Time 1630 Init. [illegible]

Replicate	Relative Concentration (%) 100	50	25	12.5	6.25	0
A	320	340	310	350	260	320
B	275	270	290	315	250	260
C	275	270	270	270	280	250
D	315	285	245	290	330	285

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Int.
8-2-07	18.1	6.7	8.6	82	40	25	[illegible]	[illegible]	constant flow		[illegible]	[illegible]	[illegible]
8-4-07	16.8	7.7	9.6	85	26	30		"	"			[illegible]	[illegible]
8-6-07	17.1	6.6	9.8	68	28	10	[illegible]	[illegible]	"			[illegible]	[illegible]
8-7-07	16.4	7.6	9.7	162	34	30	[illegible]	[illegible]	"			[illegible]	[illegible]
8-14-07	18.1	6.1	7.2	60	22	16	[illegible]	[illegible]	"			[illegible]	[illegible]
8-30-07	17.0	7.0	7.4	160	34	30						Holding	[illegible]
8-30-07	16.9	6.6	9.0	60	31	25	Alder Creek		"			[illegible]	[illegible]
8-31-07	16.8	7.2	7.5	135	34	30			"			[illegible]	[illegible]
9-1-07	16.9	7.1	7.4	140	—	—			"				
9-2-07	16.7	7.6	9.1	160	—	—			"		HOLDING		GH
9-3-07	16.7	7.7	7.2	160	—	—			"		HOLDING		GH
9-4-07	16.2	7.5	8.8	185	34	30			"			"	[illegible]
9-5-07	16.3	7.2	9.1	180	34	30			"			"	[illegible]
9-6-07	16.8	7.3	9.0	185	34	30			"			"	
9-7-07	16.8	7.0	9.1	155	26	30			"			"	
9-7-07	17.0	7.5	9.3	160	—	—			"			"	
9-9-07	16.7	7.5	8.8	170	—	—			"			Holding	LPS
9-10-07	16.7	7.2	8.9	175	—	—			"			"	[illegible]
9-11-07	17.4	7.6	9.0	235	60	35			"			"	LPS
9-12-07	17.0	8.0	9.4	185	60	38			"			"	[illegible]

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Int.
9-13-07	17.1	8.0	9.0	170	55	30			constant flow			Holding	[illegible]
9-14-07	17.1	8.2	9.1	155	48	29			"		[illegible]	"	[illegible]
9-15-07	17.1	8.3	9.2	170	—				"			"	
9-16-07	17.0	8.1	9.2	115	41	30			"			"	GH
9-17-07	17.1	7.7	8.8	120	41	30			"			"	LPS
9-18-07	17.0	7.8	9.1	130	39	21			"			"	GH
9-19-07	17.7	7.6	9.1	125	39	34			"			"	[illegible]
9-20-07	17.1	7.9	9.0	125	36	34	Taken for [illegible]		"			"	GH
9-21-07	17.0	8.2	9.5	140	44	31	"		"			"	[illegible]
9-22-07	17.1	7.7	9.4	130	42	29			"			"	
9-23-07	17.1	7.7	9.3	140	46	28			"			"	[illegible]
9-24-07	16.8	7.9	9.3	140	44	29			"			"	
9-25-07	17.1	7.7	9.8	145	44	30			"			"	[illegible]
9-26-07	17.2	7.8	9.3	140	48	36			"			"	[illegible]
9-27-07	17.4	7.7	9.3	145	48	37			"			"	[illegible]
9-28-07	17.2	7.8	9.3	150	48	36			"			"	[illegible]
9-29-07	16.9	7.5	8.8	150	48	30			"			"	[illegible]
9-30-07	17.0	7.7	8.8	140	48	37			"			"	[illegible]
10-1-07	17.0	7.8	9.4	145	48	36			"		[illegible]	"	OD
10-2-07	17.3	8.1	9.2	135	—	—			"			"	GH

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Int.
10-3-07	17.0	7.8	9.1	130	46	55			constant flow			Holding	GH
10-4-07	16.9	7.9	9.2	120	42	38						"	[illegible]
10-5-07	16.9	7.4	9.0	130	not Taken LPS							"	LPS
10-6-07	16.6	7.2	9.4	130	44	41						"	GH
10-7-07	17.0	7.4	9.5	135	60	50						"	GH
10-8-07	17.0	7.9	9.2	145	50	48						"	GH
10-9-07	17.0	7.6	9.5	150	54	52						"	GH
10-10-07	16.9	7.4	9.2	155	54	49						"	GH
10-11-07	17.0	7.1	9.5	150	54	40						"	[illegible]
10-12-07	17.1	8.0	9.4	155	50	39						"	[illegible]
10-13-07	17.1	7.1	9.1	150	52	40						"	GH
10-14-07	16.9	7.5	9.6	150	52	38						"	[illegible]
10-15-07	17.0	7.6	9.5	155	50	38						"	[illegible]
10-5-9	13.0	6.7	10.6	97	30	30						Receiving Lab	[illegible]
10-16-07	13.2	7.9	10.1	145	60	57						Holding Rm #4	[illegible]
10-16-07	17.1	6.9	9.4	165	20	12						Test animals Rm #1	[illegible]
10-16-07	12.4	6.2	8.9	65	18	14						NEWTUCK RIVER RECEIVING	GH
10-9-7	13.3	8.3	10.1	135	60	55					[illegible]	Holding test animals Room #1	GH
10-17-7	17.3	6.7	8.5	75	22	14						Holding Room #1	GH
10-17-07	13.1	6.8	10.2	57	20	14						SHELTER ISLAND RECEIVING	

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Int.
10-18-07	13.2	8.3	9.9	130	48	36			constant flow			Holding	[illegible]
10-19-07	13.3	7.5	9.5	120	48	36						"	LPS
10-20-07	13.2	7.7	10.0	125	46	38							
10-21-07	14.2	7.6	9.9	130	48	37							
10-22-07	14.2	7.7	9.4	135	50	40						"	[illegible]
10-23-07	15.1	7.9	9.7	145	54	44						"	[illegible]
10-24-07	15.6	8.3	9.7	155	58	47						"	[illegible]
10-25-07	16.1	8.5	9.5	160	66	53						"	[illegible]
10-26-07	16.8	8.1	9.6	165	60	47						"	[illegible]
10-27-07	16.8	8.3	9.9	155	62	49						"	[illegible]
10-28-07	16.6	8.3	9.9	165	62	47						"	[illegible]
10-29-07	17.0	8.1	9.5	165	62	48						"	[illegible]
10-30-07	16.9	8.2	9.6	170	64	49						"	[illegible]
10-31-07	16.8	8.2	9.9	175	70	54						"	[illegible]
11-1-07	16.8	8.2	9.8	165	62	45						"	[illegible]
11-3-07	16.8	8.2	9.5	165	62	47						"	[illegible]
11-3-07	16.6	8.1	9.2	160	56	38						"	OD
11-4-07	16.8	8.3	9.6	180	64	48						"	LPS
11-5-07	16.9	8.1	9.9	180	66	49						"	[illegible]
11-6-07	16.9	8.2	9.8	155	52	35						"	[illegible]

Holding Records

686-43

ANIMAL RECEIPT/HOLDING FRESHWATER
SPECIES Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (umhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init
11-7-07	17.0	8.1	9.4	155	54	37			constant flow			Holding	AF
11-8-07	16.9	8.1	9.3	155	56	39						"	CB
11-9-07	16.7	8.6	9.7	160	62	46						"	CB
11-10-07	16.7	8.1	9.6	155	56	43						"	CB
11-11-07	16.7	8.1	9.9	145	52	39						"	GJZ
11-12-07	16.8	8.0	9.7	155	56	38						"	AF
11-13-07	16.8	7.6	9.7	155	54	38						"	AF
11-14-07	16.8	7.9	9.7	150	52	36						"	AF
11-15-07	16.8	8.0	9.7	160	60	43						"	AF
11-16-07	16.9	8.2	9.9	160	58	39						"	GJZ
11-17-07	16.7	8.3	10.1	165	62	43						"	AF
11-18-07	16.9	8.1	9.8	150	54	36						"	CB
11-19-07	16.8	7.9	9.7	175	64	48						"	AF
11-20-07	16.7	7.9	9.9	170	64	46						"	AF
11-21-07	16.6	8.0	9.9	175	64	48						"	AF
11-22-07	16.5	7.9	10.0	165	62	39						"	AF
11-23-07	16.5	7.8	9.5	170	62	44						"	CB
11-24-07	16.5	8.0	9.7	190	70	48						"	AF
11-25-07	16.5	7.9	9.6	175	64	45						"	CB
11-26-07	16.6	7.5	9.6	175	64	46						"	AF

ANIMAL RECEIPT/HOLDING FRESHWATER
SPECIES Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (umhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init
11-27-07	16.1	7.9	9.6	180	62	44			constant flow			Holding	AF
11-30-07	16.0	7.7	10.0	175	56	44						"	AF
12-1-07	16.0	7.8	9.7	170	60	43						"	CB
12-2-07	16.0	8.0	9.7	175	62	46						"	GJZ
12-3-07	16.2	7.9	9.7	185	70	47						"	AF
12-4-07	15.8	7.5	9.4	165	60	43						"	AF
12-5-07	15.7	8.2	9.6	155	60	39						"	AF
12-6-07	15.8	7.7	9.8	170	60	42						"	GJZ
12-7-07	16.2	7.6	9.9	165	60	42						"	AF
12-8-07	15.7	7.6	9.9	160	58	40							CB
12-9-07	17.8	7.4	9.0	175	64	43						"	GJZ
12-10-07	17.1	7.3	8.8	165	62	42						"	CB
12-11-07	17.1	7.2	9.4	170	56	41						"	GJZ
12-12-07	17.3	7.4	9.5	160	58	40						"	CB
12-13-07	16.7	7.2	9.4	170	64	43						"	GJZ
12-14-07	16.8	7.3	9.1	170	64	44						"	GJZ
12-17-07	17.0	7.2	9.0	165	56	44						"	CB
12-18-07	16.5	7.2	9.5	180	70	45						"	GJZ
12-19-07	16.7	7.2	9.3	170	66	44						"	CB
12-20-07	16.5	7.4	9.6	195	74	53						"	AF

ANIMAL RECEIPT/HOLDING FRESHWATER
SPECIES Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (umhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init
12-19-07	15.6	7.3	9.9	170	76	51			constant flow			HOLDING	GJZ
12-20-07	16.2	7.2	10.3	175	56	46						"	CB
12-21-07	15.4	8.2	10.0	190	72	48						"	GJZ
12-22-07	16.0	7.8	9.8	145	70	46							
12-23-07	17.1	8.0	9.5	195	68	54						"	GJZ
12-24-07	17.1	8.0	9.4	190	72	48						"	GJZ
12-25-07	16.8	8.1	9.6	190	72	51						"	GJZ
12-26-07	16.8	8.3	10.1	200	74	50						"	AF
12-27-07	17.1	8.2	9.7	195	68	49						"	AF
12-28-07	17.0	8.0	9.8	170	56	32						"	CB
12-29-07	17.1	8.1	9.9	175	52	29						"	CB
12-30-07	16.9	8.0	9.2	195	68	50						"	GJZ
12-31-07	16.8	8.3	9.8	205	72	50						"	CB
1-1-08	17.0	8.0	9.6	[illegible]	64	43						"	GJZ
1-2-08	16.8	8.2	9.4	185	66	41						"	AF
1-3-08	16.8	8.3	9.7	180	60	39						"	CB
1-4-08	16.9	8.2	9.8	175	62	36						"	CB
1-5-08	16.7	8.4	9.7	155	48	30						"	AF
1-7-08	16.7	8.4	9.9	160	54	32						"	AF
1-8-08	16.9	8.1	9.5	145	50	33						"	AF

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (umhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init
1-9-08	15.6	8.4	10.6	165	56	30			constant flow			HOLDING	GJZ
1-10-08	15.4	8.0	10.2	145	44	24						"	CB
1-11-08	16.4	8.1	10.3	145	42	24							CB
1-12-08	16.0	8.4	10.2	165	56	31						"	GJZ
1-13-08	16.2	8.1	9.6	170	54	32						"	AF
1-14-08	16.5	8.2-8.9	9.9	160	52	32						"	CB
1-15-08	16.2	8.2-8.7	9.7	160	50	29						"	CB
1-16-08	16.4	8.2	9.9	175	40	15						"	CB
1-17-08	15.6	8.0	9.9	165	86	63						"	CB

Holding Records

GFG-42

Sample shipped to GAS

Test on 12/6-13

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd. P.O. Box 1437 Newport, OR 97365
Tel 541-265-7225 Fax 541-265-2799 www.nwaquatic.com

Client Name: Integral Consulting, Inc
Address: 319 SW Washington St Suite 1150
City, State, Zip code: Portland, OR 97204
Project No: Integral B01-01-58C Windward 07-28-04-53
Phone No: 503-284-5545
Report Attention: Abbie Spielman
Sampled by: RSC
Shipping Information: Carrier UPS; UPS#Y3489E; Airbill No 1Z949R6101033418
Testing Required: Copper, Lindane, Pentachlorophenol, Aniline, Diazinon, Naphthalene

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	12/16/07	1200	NAS# 1536G Day 0 - 0 mg/L Pentachlorophenol	1			X				
			NAS# 1537G Day 0 - 0.0125 mg/L Pentachlorophenol	1			X				
			NAS# 1538G Day 0 - 0.025 mg/L Pentachlorophenol	1			X				
			NAS# 1539G Day 0 - 0.05 mg/L Pentachlorophenol	1			X				
			NAS# 1540G Day 0 - 0.1 mg/L Pentachlorophenol	1			X				
			NAS# 1541G Day 0 - 0.2 mg/L Pentachlorophenol	1			X				
			NAS# 1542G Day 0 - TOC	1							
			NAS# 1543G Day 0 - TSS	1							
			NAS# 1544G Day 0 - Ammonia	1							

Signature	Print Name	Company	Date	Time	Cooler Custody Seal
Relinquished by:	GERALD IRISSARRI	Northwestern Aquatic Sciences	12-21-07	1400	Present / Not Present
Received by:					Intact / Not Intact
Relinquished by:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received by:					
Relinquished by:					
Received by Laboratory:					

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CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd. P.O. Box 1437 Newport, OR 97365
Tel 541-265-7225 Fax 541-265-2799 www.nwaquatic.com

Client Name: Integral Consulting, Inc
Address: 319 SW Washington St Suite 1150
City, State, Zip code: Portland, OR 97204
Project No: Integral B01-01-58C Windward 07-28-04-53
Phone No: 503-284-5545
Report Attention: Abbie Spielman
Sampled by: [illegible]
Shipping Information: Carrier UPS; UPS#Y3489E; Airbill No 1Z949R6101033418
Testing Required: Copper, Lindane, Pentachlorophenol, Aniline, Diazinon, Naphthalene

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	12/19/07	1400	NAS# 1550G Day 1 - 0 mg/L Pentachlorophenol	1			X				
	12/19/07		NAS# 1560G Day 1 - 0.0125 mg/L Pentachlorophenol	1			X				
	12/19/07		NAS# 1561G Day 1 - 0.025 mg/L Pentachlorophenol	1			X				
	12/19/07		NAS# 1562G Day 1 - 0.05 mg/L Pentachlorophenol	1			X				
	12/19/07		NAS# 1563G Day 1 - 0.1 mg/L Pentachlorophenol	1			X				
	12/19/07		NAS# 1564G Day 1 - 0.2 mg/L Pentachlorophenol	1			X				

Signature	Print Name	Company	Date	Time	Cooler Custody Seal
Relinquished by:	GERALD IRISSARRI	Northwestern Aquatic Sciences	12-21-07	1400	Present / Not Present
Received by:					Intact / Not Intact
Relinquished by:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received by:					
Relinquished by:					
Received by Laboratory:					

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd. P.O. Box 1437 Newport, OR 97365
Tel 541-265-7225 Fax 541-265-2799 www.nwaquatic.com

Client Name: Integral Consulting, Inc
Address: 319 SW Washington St Suite 1150
City, State, Zip code: Portland, OR 97204
Project No: Integral B01-01-58C Windward 07-28-04-53
Phone No: 503-284-5545
Report Attention: Abbie Spielman
Sampled by: [illegible]
Shipping Information: Carrier UPS; UPS#Y3489E; Airbill No 1Z949R6101033418
Testing Required: Copper, Lindane, Pentachlorophenol, Aniline, Diazinon, Naphthalene

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	12/18/07	1400	NAS# 1555G Day 2 - 0 mg/L Pentachlorophenol	1			X				
	12/18/07		NAS# 1556G Day 2 - 0.0125 mg/L Pentachlorophenol	1			X				
	12/18/07		NAS# 1557G Day 2 - 0.025 mg/L Pentachlorophenol	1			X				
	12/18/07		NAS# 1558G Day 2 - 0.05 mg/L Pentachlorophenol	1			X				
	12/18/07		NAS# 1559G Day 2 - 0.1 mg/L Pentachlorophenol	1			X				
	12/18/07		NAS# 1560G Day 2 - 0.2 mg/L Pentachlorophenol	1			X				

Signature	Print Name	Company	Date	Time	Cooler Custody Seal
Relinquished by:	GERALD IRISSARRI	Northwestern Aquatic Sciences	12-21-07	1400	Present / Not Present
Received by:					Intact / Not Intact
Relinquished by:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received by:					
Relinquished by:					
Received by Laboratory:					

Samples shipped to CAS

Tot # No. 466 - 43

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
38[illegible] Yaquina Bay Rd. P.O. Box 1437, Newport, OR 97365
Tel 541-265-7225, Fax 541-265-2799 www.nwaquatic.com

Client Name: Integral Consulting Inc.
Address: 319 SW Washington St. Suite 1150
City, State, Zip Code: Portland, OR 97204
Project No.: Integral B01-01-58C; Windward 03-28-04-53
Phone No.: 503-284-5545
Report Attention: Abbie Spielman
Sampled by:
Carrier: UPS
UPS#V54801
Sample Delivery Group: [illegible]
Analysis Requested

Lab Sample No.	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Cadmium	Pentachlorophenol	Acrylamide	Dioxin/Furan	Naphthalene	Comments
	12/20/07	1430	NAS# 1565G Day 4 - 0 mg/l Pentachlorophenol	1			X				
	12/20/07		NAS# 1566G Day 4 - 0.0125 mg/l Pentachlorophenol	1			X				
	12/20/07		NAS# 1567G Day 4 - 0.025 mg/l Pentachlorophenol	1			X				
	12/20/07		NAS# 1568G Day 4 - 0.05 mg/l Pentachlorophenol	1			X				
	12/20/07		NAS# 1569G Day 4 - 0.1 mg/l Pentachlorophenol	1			X				
	12/20/07		NAS# 1570G Day 4 - 0.2 mg/l Pentachlorophenol	1			X				
	12/20/07		NAS# 1571G Day 4 - 0.0125 mg/l Pentachlorophenol DUPLICATE	1			X				
	12/20/07		NAS# 1572G Day 4 - Ammonia From 0 mg/l Pentachlorophenol concentration	1							
	12/20/07		NAS# 1573G Day 4 - Ammonia From 0.2 mg/l Pentachlorophenol concentration	1							

Signature | Print Name | Company | Date | Time | Cooler Custody Seal

Relinquished by: [signature] GERALD REISSNER — Northwestern Aquatic Sciences — 12-21-07 1400 — Present / Not Present
Received by: — Intact / Not Intact
Relinquished by: — Internal Cooler Temperature Upon Receipt (°C)
Received by laboratory:

9 of 14

Water Quality Computation - Test No. 686-43

	Temp	DO	pH	Cond	Hard	Alk
1	17.2	9.7	6.6	125	30	14
2	17.0	9.8	6.6	122	30	13
3	16.7	9.8	6.5	128	32	15
4	17.2	9.8	6.4	126	32	15
5	16.9	9.8	6.5	125	30	15
6	16.3	9.8	6.3	120	32	15
7	17.0	10.1	6.8	124	28	15
8	16.8	10.1	6.7	125	32	15
9	16.7	10.0	6.7	125	26	15
10	17.0	10.0	6.5	125	32	14
11	16.5	9.9	6.5			
12	17.3	10.0	6.4			
13	17.0	9.9	6.6			
14	16.7	10.0	6.6			
15	16.8	10.1	6.5			
16	16.9	9.9	6.5			
17	16.6	10.0	6.5			
18	16.8	10.0	6.5			
19	17.4	10.1	6.6			
20	17.2	9.9	6.6			
21	17.0	9.7	6.5			
22	17.5	9.9	6.5			
23	17.2	9.8	6.5			
24	16.8	10.0	6.5			
25	17.6	9.8	6.9			
26	16.9	9.7	6.9			
27	16.9	9.8	6.9			
28	17.5	9.7	6.8			
29	17.2	9.8	6.8			
30	16.7	9.9	6.8			
31						
32						
mean	17.0	9.9	6.6	125	30	15
s.d.	0.3	0.1	0.2	2	2	1
N	30	30	30	10	10	10
min	16.3	9.7	6.3	120	26	13
max	17.6	10.1	6.9	128	32	15

RSL
2/21/08

WQ Computation - acclimation (12-1-07 to 12-16-07) - Test No. 686-43

	Temp	DO	pH	Cond	Hard	Alk
1	16.0	9.7	7.8	170	60	43
2	16.0	9.7	8.0	185	62	46
3	16.2	9.7	7.9	185	70	47
4	15.8	9.4	7.5	165	60	43
5	15.7	9.6	8.2	155	60	39
6	15.8	9.8	7.7	170	60	42
7	16.2	9.9	7.6	165	60	42
8	15.7	9.9	7.6	160	58	40
9	17.8	9.0	7.4	175	64	43
10	17.1	8.8	7.3	165	62	42
11	17.1	9.4	7.2	170	56	41
12	17.3	9.5	7.4	160	58	40
13	16.7	9.4	7.2	170	64	43
14	16.8	9.1	7.3	170	64	44
15	17.0	9.0	7.2	165	56	44
16	16.5	9.5	7.2	180	70	45
17						
mean	16.5	9.5	7.5	169	62	43
s.d.	0.7	0.3	0.3	9	4	2
N	16	16	16	16	16	16
min	15.7	8.8	7.2	155	56	39
max	17.8	9.9	8.2	185	70	47

RSe
2/21/08

SampleDate	SampleTim	Matrix	LabMatrix	SampleID	arentSamp	SampleType	Method	AnalysisDate	nalysisTim	nalyteGrou	cas_m	Analyte	l_or_dissol	detect_flag	VALUE	Qualifiers	Units
12/16/2007	12:00	WQ	WQ	NAS#1536G Day0-0mg/l Pentachlorophenol		N	SW8151M	1/18/2008	19:23	Phenols	87-86-5	Pentachlor	N	N	0.08	U	ug/l
12/16/2007	12:00	WQ	WQ	NAS#1537G Day0-0.0125mg/L Pentachlorophe		N	SW8151M	1/17/2008	14:28	Phenols	87-86-5	Pentachlor	N	Y	22		ug/l
12/16/2007	12:00	WQ	WQ	NAS#1538G Day0-0.025mg/l Pentachlopher		N	SW8151M	1/17/2008	14:47	Phenols	87-86-5	Pentachlor	N	Y	38		ug/l
12/16/2007	12:00	WQ	WQ	NAS#1539G Day0-0.05mg/L Pentachlopheno		N	SW8151M	1/17/2008	15:06	Phenols	87-86-5	Pentachlor	N	Y	73		ug/l
12/16/2007	12:00	WQ	WQ	NAS#1540G Day0-0.1mg/L Pentachlorophenol		N	SW8151M	1/17/2008	15:25	Phenols	87-86-5	Pentachlor	N	Y	130		ug/l
12/16/2007	12:00	WQ	WQ	NAS#1541G Day0-0.2mg/L Pentachlorophenol		N	SW8151M	1/17/2008	15:45	Phenols	87-86-5	Pentachlor	N	Y	250		ug/l
12/17/2007	12:15	WQ	WQ	NAS#1545G Day1-0mg/l Pentachlorophenol		N	SW8151M	1/23/2008	02:42	Phenols	87-86-5	Pentachlor	N	N	0.08	U	ug/l
12/17/2007	12:15	WQ	WQ	NAS#1546G Day1-0.0125mg/L Pentachlorophe		N	SW8151M	1/23/2008	03:39	Phenols	87-86-5	Pentachlor	N	Y	20		ug/l
12/17/2007	12:15	WQ	WQ	NAS#1547G Day1-0.025mg/l Pentachlopher		N	SW8151M	1/23/2008	03:58	Phenols	87-86-5	Pentachlor	N	Y	29		ug/l
12/17/2007	12:15	WQ	WQ	NAS#1548G Day1-0.05mg/L Pentachlopheno		N	SW8151M	1/23/2008	04:18	Phenols	87-86-5	Pentachlor	N	Y	50		ug/l
12/17/2007	12:15	WQ	WQ	NAS#1549G Day1-0.1mg/L Pentachlorophenol		N	SW8151M	1/23/2008	04:37	Phenols	87-86-5	Pentachlor	N	Y	110		ug/l
12/17/2007	12:15	WQ	WQ	NAS#1551G Day1-0.1mg/L Pentachlopheno		N	SW8151M	1/23/2008	05:15	Phenols	87-86-5	Pentachlor	N	Y	120		ug/l
12/17/2007	12:15	WQ	WQ	NAS#1550G Day1-0.2mg/L Pentachlorophenol		N	SW8151M	1/23/2008	04:56	Phenols	87-86-5	Pentachlor	N	Y	260		ug/l
12/18/2007	11:45	WQ	WQ	NAS#1552G Day2-0mg/l Pentachlorophenol		N	SW8151M	1/23/2008	05:34	Phenols	87-86-5	Pentachlor	N	N	0.08	U	ug/l
12/18/2007	11:45	WQ	WQ	NAS#1553G Day2-0.0125mg/L Pentachlorophe		N	SW8151M	1/23/2008	06:51	Phenols	87-86-5	Pentachlor	N	Y	15		ug/l
12/18/2007	11:45	WQ	WQ	NAS#1554G Day2-0.025mg/l Pentachlopher		N	SW8151M	1/23/2008	07:10	Phenols	87-86-5	Pentachlor	N	Y	28		ug/l
12/18/2007	11:45	WQ	WQ	NAS#1555G Day2-0.05mg/L Pentachlopheno		N	SW8151M	1/23/2008	07:29	Phenols	87-86-5	Pentachlor	N	Y	50		ug/l
12/18/2007	11:45	WQ	WQ	NAS#1556G Day2-0.1mg/L Pentachlorophenol		N	SW8151M	1/23/2008	07:49	Phenols	87-86-5	Pentachlor	N	Y	110		ug/l
12/18/2007	11:45	WQ	WQ	NAS#1557G Day2-0.2mg/L Pentachlorophenol		N	SW8151M	1/23/2008	08:08	Phenols	87-86-5	Pentachlor	N	Y	230		ug/l
12/19/2007	19:00	WQ	WQ	NAS#1559G Day3-0mg/l Pentachlorophenol		N	SW8151M	1/19/2008	07:14	Phenols	87-86-5	Pentachlor	N	N	0.08	U	ug/l
12/19/2007	19:00	WQ	WQ	NAS#1560G Day3-0.0125mg/L Pentachlorophe		N	SW8151M	1/19/2008	07:33	Phenols	87-86-5	Pentachlor	N	Y	14		ug/l
12/19/2007	19:00	WQ	WQ	NAS#1561G Day3-0.025mg/l Pentachlopher		N	SW8151M	1/19/2008	07:52	Phenols	87-86-5	Pentachlor	N	Y	23		ug/l
12/19/2007	19:00	WQ	WQ	NAS#1562G Day3-0.05mg/L Pentachlopheno		N	SW8151M	1/19/2008	09:28	Phenols	87-86-5	Pentachlor	N	Y	45		ug/l
12/19/2007	19:00	WQ	WQ	NAS#1563G Day3-0.1mg/L Pentachlorophenol		N	SW8151M	1/19/2008	09:48	Phenols	87-86-5	Pentachlor	N	Y	95		ug/l
12/19/2007	19:00	WQ	WQ	NAS#1564G Day3-0.2mg/L Pentachlorophenol		N	SW8151M	1/19/2008	10:07	Phenols	87-86-5	Pentachlor	N	Y	210		ug/l
12/20/2007	14:00	WQ	WQ	NAS#1565G Day4-0mg/l Pentachlorophenol		N	SW8151M	1/23/2008	08:27	Phenols	87-86-5	Pentachlor	N	N	0.08	U	ug/l
12/20/2007	14:00	WQ	WQ	NAS#1566G Day4-0.0125mg/L Pentachlorophe		N	SW8151M	1/23/2008	08:46	Phenols	87-86-5	Pentachlor	N	Y	14		ug/l
12/20/2007	14:00	WQ	WQ	NAS#1571G Day4-0.0125mg/l Pentachlorophe		N	SW8151M	1/19/2008	11:24	Phenols	87-86-5	Pentachlor	N	Y	18		ug/l
12/20/2007	14:00	WQ	WQ	NAS#1567G Day4-0.025mg/l Pentachlopher		N	SW8151M	1/23/2008	09:05	Phenols	87-86-5	Pentachlor	N	Y	30		ug/l
12/20/2007	14:00	WQ	WQ	NAS#1568G Day4-0.05mg/L Pentachlopheno		N	SW8151M	1/19/2008	10:26	Phenols	87-86-5	Pentachlor	N	Y	55		ug/l
12/20/2007	14:00	WQ	WQ	NAS#1569G Day4-0.1mg/L Pentachlorophenol		N	SW8151M	1/19/2008	10:45	Phenols	87-86-5	Pentachlor	N	Y	100		ug/l
12/20/2007	14:00	WQ	WQ	NAS#1570G Day4-0.2mg/L Pentachlorophenol		N	SW8151M	1/19/2008	11:04	Phenols	87-86-5	Pentachlor	N	Y	200		ug/l

Pentachlorophenol (ug/L)	Day 0	Day 1	Day 2	Day 3	Day 4	Average	Standard Deviation
0	0.08	0.08	0.08	0.08	0.08	0.08	0.00
12.5	22	20	15	14	16	17	4
25	38	29	28	23	30	30	6
50	73	50	50	45	55	58	12
100	130	115	110	95	100	110	14
200	250	260	230	210	200	230	25

Results converted to mg/L for reporting and tox test data analysis
RSC 2/27/08

Validated Pentachlorophenol Analysis
Reported by Windward
RSC 2/27/08

686-43

12 of 14

686-43

Acute 96-hr Toxicity Test-96 Hr Survival

Start Date:	12/16/07 11:30	Test ID:	686-43	Sample ID:	Pentachlorophenol
End Date:	12/20/07 13:00	Lab ID:	ORNAS-Northwestern Aquati	Sample Type:	
Sample Date:		Protocol:	-NAS-686-LAMPREY FT ACI	Test Species:	LY-Lampetra sp.
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
0.017	1.0000	1.0000	1.0000	1.0000
0.03	0.3000	0.8000	0.4000	0.5000
0.056	0.0000	0.0000	0.0000	0.1000
0.11	0.0000	0.0000	0.0000	0.0000
0.23	0.0000	0.0000	0.0000	0.0000

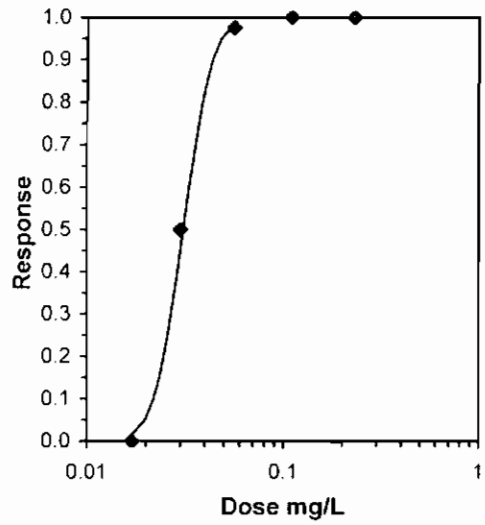
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
0.017	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*0.03	0.5000	0.5000	0.7892	0.5796	1.1071	28.888	4	10.00	10.00	20	40
*0.056	0.0250	0.0250	0.1995	0.1588	0.3218	40.840	4	10.00	10.00	39	40
0.11	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4			40	40
0.23	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4			40	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.76072	0.844	1.40502	5.34963
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	0.017	0.03	0.02258	

					Maximum Likelihood-Probit						
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	8.58843	1.45508	5.73646	11.4404	0	1.24762	7.81472	0.74	-1.5109	0.11644	4
Intercept	17.9763	2.21935	13.6263	22.3262							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	0.01653	0.01201	0.0196
EC05	3.355	0.01984	0.01566	0.02265
EC10	3.718	0.02187	0.018	0.02454
EC15	3.964	0.02336	0.01974	0.02594
EC20	4.158	0.02461	0.0212	0.02716
EC25	4.326	0.02574	0.02251	0.0283
EC40	4.747	0.02881	0.02594	0.03164
~~EC50~~ LC50	5.000	0.03084	0.02803	0.03411
EC60 OB	5.253	0.03301	0.0301	0.03701
EC75 2-27-08	5.674	0.03695	0.03348	0.04288
EC80	5.842	0.03865	0.03483	0.04559
EC85	6.036	0.04072	0.03642	0.04903
EC90	6.282	0.04348	0.03847	0.05382
EC95	6.645	0.04793	0.04162	0.06191
EC99	7.326	0.05754	0.04806	0.08085



6F6-43

Test: AT-Acute 96-hr Toxicity Test						Test ID: 686-43			
Species: LY-Lampetra sp.						Protocol: -NAS-686-LAMPREY FT ACUTE			
Sample ID: Pentachlorophenol						Sample Type:			
Start Date: 12/16/07 11:30				End Date: 12/20/07 13:00		Lab ID: ORNAS-Northwestern Aquatic Sciences			
Pos	ID	Rep	Group	Start	24 Hr	48 Hr	72 Hr	96 Hr	Notes
	1	1	D-Control	10				10	
	2	2	D-Control	10				10	
	3	3	D-Control	10				10	
	4	4	D-Control	10				10	
	5	1	0.017	10				10	
	6	2	0.017	10				10	
	7	3	0.017	10				10	
	8	4	0.017	10				10	
	9	1	0.030	10				3	
	10	2	0.030	10				8	
	11	3	0.030	10				4	
	12	4	0.030	10				5	
	13	1	0.056	10				0	
	14	2	0.056	10				0	
	15	3	0.056	10				0	
	16	4	0.056	10				1	
	17	1	0.110	10				0	
	18	2	0.110	10				0	
	19	3	0.110	10				0	
	20	4	0.110	10				0	
	21	1	0.230	10				0	
	22	2	0.230	10				0	
	23	3	0.230	10				0	
	24	4	0.230	10				0	

Comments:

LAMPREY (*LAMPETRA* SP.) DEFINITIVE 96-HR FLOW-THROUGH ACUTE TOXICITY TEST OF DIAZINON

NAS Test No. 686-44

Submitted to

Windward Environmental LLC
200 West Mercer Street, Suite 401
Seattle, WA 98119

Submitted by

Northwestern Aquatic Sciences
3814 Yaquina Bay Road
P.O. Box 1437
Newport, OR 97365

April 3, 2008

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 686-44
Title: Lamprey (*Lampetra* sp.) definitive 96-hr flow-through acute toxicity test of diazinon.
Protocol No.: NAS-686-Lamprey FT acute, September 12, 2007

STUDY MANAGEMENT

Study Sponsor: Windward Environmental, 200 West Mercer Street, Suite 401, Seattle, WA 98119.
Sponsor's Study Monitor: Ms. Helle Andersen.
Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365.
Test Location: Newport Laboratory.
Laboratory's Study Personnel: G. A. Buhler, B.S., Proj. Manager; R. S. Caldwell, Ph.D., Study Director; L.K. Nemeth, B.A., M.B.A., QA Officer; G.J. Irissarri, B.S., Aq. Toxicol.
Study Schedule:
Test Beginning: 1-16-08, 1600 hrs.
Test Ending: 1-20-08, 1600 hrs.
Disposition of Study Records: All specimens, raw data, reports and other study records are stored according to Good Laboratory Practice regulations at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.
Good Laboratory Practices: The test was conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).
Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Description: Diazinon, 99.5% (Chem Service, WestChester, PA, Lot No. 393-91A).
Preparation of Working Stock Solution and Test Concentrations: Test solutions were prepared for continuous supply to the test aquaria using a Benoit constant flow diluter (Benoit *et al.*, 1982). The diluter was set up to deliver a 0.5 dilution series with a flow rate of approximately 80 ml/min per toxicant concentration. The diluter design was such that 160 ml/min of the toxicant solution at the highest test concentration (nominal 40 mg/L diazinon) was required to be continuously delivered to the toxicant supply chamber of the diluter. To accomplish this, a large mixing reservoir, approximately 54L capacity, of nominal 40 mg/L diazinon solution in dilution water, was maintained at test temperature. This was achieved using a Cole-Parmer single channel syringe pump, fitted with a 10 mL gas-tight Hamilton syringe and refilled daily, to deliver 0.4 ml/hr of pure diazinon to the mixing tank. The pure diazinon was injected through 0.042 in I.D. PTFE tubing into a recirculating stream of water from the mixing reservoir. Make-up dilution water was replaced, under float valve control, to replace delivery of the nominal 40 mg/L diazinon solution to the diluter. The mixing tank contents were vigorously stirred using a stainless steel paddle to facilitate rapid solution of diazinon.

DILUTION WATER

Source: City of Newport municipal tap water, treated as below, was supplied continuously to the toxicant diluter throughout the study.
Pretreatment: The water was dechlorinated by carbon filtration, particle filtered to 1 μm, and adjusted to a target water hardness of 25-30 mg/L as $CaCO_3$. Hardness adjustment was accomplished by passage of a portion of the dilution water through deionization cartridges.

TEST ORGANISMS

Species: Lamprey (*Lampetra* sp.)
Age/Size: Ammocoetes; target size range, 4-6 cm; actual size of 10 ammocoete subsample, 4.9 ± 0.8 cm.
Source: Ammocoetes were field collected in approximately equal numbers from the Trask, Nestucca, Siletz, and Alsea Rivers, Oregon. Field collection was by electoshocking and only 4-6 cm size ammocoetes were retained. Personnel of Windward Environmental performed the ammocoete collections.

Acclimation: Upon receipt, animals were removed from the collection site sediment and placed in 10-gallon aquaria supplied under flow-through conditions with dilution water. Water supply was sufficient (30-50 ml/min) to accomplish two volume changes of water per day. Approximately 50 lamprey were placed in each tank. Tanks contained an approximate 8 cm deep layer of sediment (Sandtastic Play Sand, Waupaca Materials) covered with approximately 26L of test water and were supplied with aeration. Normal behavior for the lamprey ammocoetes was to immediately bury in sediment and remain there. Any that did not immediately bury were discarded as potentially damaged. Ammocoetes were not fed during acclimation. Water quality conditions during the two weeks prior to testing (1-1-08 to 1-16-08) were: temperature, 16.5 ± 0.4°C; dissolved oxygen, 9.9 ± 0.3 mg/L; pH, 8.2 ± 0.1; specific conductance, 163 ± 17 µmhos/cm; hardness, 54 ± 7 mg/L as $CaCO_3$; and alkalinity, 31 ± 7 mg/L as $CaCO_3$.

TEST PROCEDURES AND CONDITIONS

Test Chambers: The test chambers were two-gallon glass aquaria capable of holding 5.8 L of test solution. Test chamber placement in the test area was random.
Test Concentrations: The nominal test concentrations were 40, 20, 10, 5, 2.5, and 0 mg/L (control) diazinon.
Replicates/Treatment: 4
Organisms/Treatment: 40
Loading: 0.36 g/L
Aeration: None.
Feeding: None.
Water Volume Changes: The diluter supplied approximately 80 ml/min of test solution, or 115 L/day, at each test concentration. At this rate of flow, test solution supply to each replicate aquarium was 5.0 volumes per day.
Effects Criteria: The effect criterion used in the lamprey ammocoete acute toxicity test was mortality, defined as a lack of visible respiratory movement and absence of response to tactile stimulation. In addition to mortality, qualitative observations of sublethal responses were recorded when observed.
Target Water Quality Conditions: Water quality measurements were taken daily as specified in the protocol (Physical and Chemical Data, Items 1 and 2). The target water quality conditions were as follows: Temperature, 17 ± 1°C; dissolved oxygen, ≥4.0 mg/L; hardness, 25-30 mg/L as $CaCO_3$. Actual test conditions are reported in the Test Results section.
Photoperiod: The photoperiod was total darkness except for approximately 2-3 hours per day for observations and test exposure system maintenance. Light intensity at the aquarium level was 15.2 foot-candles.

DATA ANALYSIS METHODS

The 96-hr proportion survival was calculated for each treatment replicate from the raw survival data and the measured diazinon concentrations for each treatment level. The LC50 (survival) was calculated by the Trimmed Spearman-Karber method. An arcsine square root (angular) transformation was performed on the data prior to statistical analysis. The NOEC and LOEC were calculated according to U.S. Environmental Protection Agency (2002) methods. In this procedure, the NOEC and LOEC values were computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test was selected after evaluating the data for normality and homogeneity of variance. The statistical software employed for these calculations was ToxCalc, v.5.0.23N, Tidepool Scientific Software.

TOXICANT AND OTHER CHEMISTRY ANALYSES

Test solutions were sampled once daily for diazinon from each test concentration by pooling samples from each of the four test replicate aquaria. Subsamples were pooled into new disposable plastic beakers from which the pooled sample was transferred to the analytical laboratory supplied sample bottles and shipped under chain-of-custody procedures to the analytical chemistry laboratory (Columbia Analytical Services) at Kelso, WA. No preservative was employed. Samples were kept refrigerated at ≤4°C. Sample volumes were 1 L. Additional samples were taken of the dilution water on day 0 of the test for analysis of particulate matter, TOC, and ammonia-N, and from the control and highest test concentration treatments after 96-hr for measurement of ammonia-N. These samples also were shipped to Columbia Analytical Services for analysis.

PROTOCOL DEVIATIONS

1. The protocol specified "constant dark, except during monitoring activities", and also that light intensity should be 50-75 ft-c. when light was on. These specifications were judged to be contradictory and led to the following action. Based on best professional judgment of the investigators, we reduced the light intensity from that given in the protocol in order to minimize fright behavior responses of the ammocoetes during the short period of lighting associated with daily observations.
2. The protocol states: "At the end of the test, all surviving control ammocoetes are weighed and measured for length by replicate." Through a laboratory error, a subsample of 10 ammocoetes were weighed and measured at the beginning of the test instead. This is a minor deviation and is without consequence for interpretation of the study results.

TEST RESULTS

Water quality conditions measured in test aquaria during the 96-hr toxicity test are summarized in Table 1. The temperature specification was met during the study. Dissolved oxygen remained near saturation throughout the test. The pH ranged from 6.9 – 7.5. Mean hardness also was in the specified range of 25-30 mg/L as $CaCO_3$.

The concentrations of diazinon measured during the toxicity test are summarized in Table 2. An outlier measurement in the nominal 40 mg/L concentration on Day 3 was omitted from the computation of the mean and standard deviation. No explanation for the outlier could be found. Mean measured concentrations in the test ranged from 1.7 ± 0.1 mg/L at the lowest level to 28.8 ± 7.7 mg/L at the highest exposure level. Measured test concentrations were from 63% to 72% of nominal.

A summary of the 96-hr ammocoetes survival results is shown in Table 3. In this study, the 96-hr control survival (100%) met the test acceptability criteria of ≥90%. Therefore, the test meets the protocol criterion of a valid test. Additionally, 100% mortality occurred in the highest test concentration, and 0% mortality was observed in the two lowest test concentrations. Partial mortality was observed in the second (12.5 mg/L) and third highest (6.3 mg/L) test concentrations.

The toxicity of diazinon to lamprey (*Lampetra* sp.) ammocoetes as the 96-hr LC50 was 8.90 mg/L (95% confidence limits, 8.46 – 9.36 mg/L). The NOEC and LOEC concentrations were 6.31 and 12.5 mg/L diazinon, respectively.

LITERATURE CITED

Benoit, D., V. Mattson, and D. Olson. 1982. A continuous-flow mini-diluter system for toxicity testing. Water Res. 16:457-464.

EPA. 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. Environmental Protection Agency, Washington, DC.

STUDY APPROVAL

Project Manager	4/11/08	Study Director	4/11/08
Laboratory Director	4/11/08	Quality Assurance Unit	4/7/08

Table 1. Measured water quality conditions during the 96-hr acute toxicity test of diazinon to lamprey ammocoetes.

Parameter	Mean ± S.D.	N	Range
Temperature (°C)	16.9 ± 0.4	30	16.2 – 17.6
Dissolved oxygen (mg/L)	9.8 ± 0.1	30	9.6 – 10.0
pH	7.3 ± 0.1	30	6.9 – 7.5
Specific conductance (µmhos/cm)	114 ± 6	10	100 – 120
Hardness (mg/L as $CaCO_3$)	30 ± 1	10	28 – 32
Alkalinity (mg/L as $CaCO_3$)	10 ± 1	10	9 – 11

Table 2. Measured concentrations of diazinon during the 96-hr acute toxicity test of diazinon to lamprey ammocoetes. Measurements performed by Columbia Analytical Services.

Nominal (mg/L)	Measured (mg/L)		
	Mean ± S.D.	N	Range
40	28.8 ± 7.7	4	23 – 40
20	12.5 ± 2.8	5	8.5 – 16
10	6.3 ± 0.5	5	5.5– 6.7
5	3.2 ± 0.2	5	3.0 – 3.5
2.5	1.7 ± 0.1	5	1.5 – 1.8
0	0.0 ± 0.0	5	0.0 – 0.0

Table 3. Survival of lamprey ammocoetes, *Lampetra* sp. exposed for 96 hours to diazinon.

Diazinon (mg/L)	Replicate	Ammocoetes exposed	Ammocoetes Surviving	Percent Survival	Mean % Survival	
28.8	A	10	0	0.0		
	B	10	0	0.0		
	C	10	0	0.0		
	D	10	0	0.0	0.0	*
12.5	A	10	0	0.0		
	B	10	1	10.0		
	C	10	0	0.0		
	D	10	0	0.0	2.5	*
6.3	A	10	9	90.0		
	B	10	10	100.0		
	C	10	10	100.0		
	D	10	10	100.0	97.5	
3.2	A	10	10	100.0		
	B	10	10	100.0		
	C	10	10	100.0		
	D	10	10	100.0	100.0	
1.7	A	10	10	100.0		
	B	10	10	100.0		
	C	10	10	100.0		
	D	10	10	100.0	100.0	
0.0 (Control)	A	10	10	100.0		
	B	10	10	100.0		
	C	10	10	100.0		
	D	10	10	100.0	100.0	

* Mean significantly (p=0.05) different from the control.

APPENDIX I

PROTOCOL

STUDY PROTOCOL

LAMPREY (*LAMPETRA* sp.)
FLOW THROUGH ACUTE TOXICITY TEST

INTRODUCTION

Purpose of Study: The purpose of this study is to determine the acute toxicity of six chemicals, representing a range of toxic modes of action, to lamprey (*Lampetra* sp.) ammocoetes. The study will be performed using methods based on the methods for measuring acute toxicity with other fish species as described in U.S. Environmental Protection Agency (2002) and American Society for Testing and Materials (ASTM) (1996) guidance.

Summary of Method: In definitive tests, lamprey ammocoetes are exposed for 96 hours to five concentrations of test material and a dilution water control. The test concentrations are arranged in a logarithmic series based on the results of range finding tests. Four replicates, each with 10 organisms, are employed at each test concentration, if there are sufficient numbers of ammocoetes. Flow-through exposures are employed. Test chambers are glass aquaria less than or equal to two gallons in volume. Mortality is the effect criterion. The data analysis consists of calculating means and standard deviations for survival. An LC50 with 95% confidence intervals may be calculated along with the NOEC and LOEC concentrations.

STUDY MANAGEMENT

Sponsor's Name and Address:
Windward Environmental LLC
200 West Mercer Street, Suite 401
Seattle, WA 98119

Sponsor's Study Monitor:
Ms. Helle Andersen

Name of Testing Laboratory:
Northwestern Aquatic Sciences
3814 Yaquina Bay Road
P.O. Box 1437
Newport, OR 97365

Test Location: Newport, OR.

Laboratory's Key Personnel to be Assigned to the Study:
Laboratory Director: Richard S. Caldwell, Ph.D.
Project Manager/Study Director: Gary A. Buhler, B.S.
QA Officer/Toxicology Program Mgr: Linda K. Nemeth, B.A., M.B.A.
Aquatic Toxicologist: Gerald J. Irissarri, B.S.

Proposed Study Schedule: July 2007 through September 2007.

Good Laboratory Practices: The test is conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

TEST MATERIAL

Description: Chemicals to be tested include aniline, copper, pentachlorophenol, lindane, diazinon and naphthalene.

Preparation of Working Stock Solution and Test Concentrations: Test materials will be administered using a constant-flow diluter based on the design of Benoit *et al.* (1982). Stock solutions of the sparingly soluble organic compounds, e.g. naphthalene, pentachlorophenol, lindane, diazinon, will be prepared continuously by recirculation of dilution water/toxicant stock solution through a column of toxicant coated glass beads (Veith and Comstock, 1975). This is a conceptual approach in which the specific column, flow rates, glass bead sizes, etc. need to be developed empirically, with analytical feedback, for each study considering such variables as chemical solubility, test temperature, desired highest test concentration, stock toxicant consumption rate, etc. Given the above, some modifications are anticipated as the studies progress. Initial system characteristics are as follows: 1) the toxicant mixing tank will have a 60-liter capacity; 2) a 20 x 280-cm glass column will be employed (10 x 200-cm and 40 x 560-cm columns are also available); 3) the column packing will employ 5-mm glass beads coated with the test compound; 4) particulates will be filtered out through glass wool; 5) flow through column will be controlled by a variable-speed peristaltic pump or by intermittent pumping; and 6) tubing will be glass and silicone rubber. GC/MS analysis of toxicant in the toxicant mixing tank will be used to optimize the system for each chemical noted above prior to test initiation.The contents of the stock toxicant reservoir will be constantly replenished with fresh dilution water as toxicant solution is supplied to the diluter. The aniline stock solution will be prepared similarly, but by volumetric addition of aniline, using a metering pump, to the stock toxicant reservoir *in lieu* of the Veith column. The copper working stock solution will be prepared in the same apparatus by metering pump addition of a concentrated copper stock solution to the stock toxicant reservoir. All toxicant working stock solutions will be prepared and maintained under the same conditions as in the test exposures and delivered continuously to the toxicant chamber of the diluter at an appropriate rate.

DILUTION WATER

Dilution water will be dechlorinated City of Newport municipal tap water adjusted to a water hardness of 25-30 mg/L as $CaCO_3$.

TEST ORGANISMS

Species: Lamprey, *Lampetra* sp.

Source: Ammocoetes will be collected by Windward staff and delivered to the laboratory each day of collection. The ammocoetes will be collected from several rivers including the Siletz River, which was the site for Phase 1 ammocoetes.

Age: ammocoetes (juvenile prior to metamorphosis)

Acclimation and Pretest Observation: Test organisms will be held in the laboratory in order to acclimate to test temperature. Upon receipt, animals are removed from the sediment they are received in and placed in 10-gallon tanks under flow-through conditions (~35 mL/minute, or ~ 2 volume changes per day, with one additional daily siphon and replacement of 2/3 volume of each tank). Approximately 50 lamprey are placed in each tank. Tanks will contain at least 3 inches of sediment (Sandtastic Play Sand, Waupaca Materials, or similar material) covered with approximately 26L of test water and supplied with aeration. The maximum temperature variation during holding will be + 1° C. The ammocoetes will be acclimated to the laboratory test conditions for a minimum of 5 to 7 days prior to test initiation. This minimum number of days applies only to the first toxicity test conducted on each batch of field-collected organisms. For all other tests, the ammocoetes will be held for at least 13 days prior to test initiation. When a temperature adjustment of more than 3 to 5°C is required, the transfer from field collection water to laboratory dilution water will be gradual. Normal behavior for lamprey ammocoetes is to immediately bury in sediment and remain there. Any dead animals observed on the sediment surface are removed daily. Past experience suggests that mortality is minimal during short-term holding and any mortality is likely due to damage from field collection. If ammocoetes do not bury or there is excessive

mortality in any tank, animals from that tank will not be used for testing. Ammocoetes will not be fed since they are being held short-term.

DESCRIPTION OF TEST SYSTEM

Preparation of Test Concentrations: Test materials will be administered using a constant-flow diluter based on the design of Benoit *et al* (1982). The diluter will be calibrated to deliver a 0.5 dilution series. Flow rates will be adjusted to deliver at least five test chamber volumes per concentration per day. Diluter flow rates will be measured and documented at the beginning and conclusion of each 96-hour test. This will be done by measuring individual diluter capillary tube flows. When the flow rates are entered into an Excel® spreadsheet, the actual dilutions of the toxicant stock can be computed. Actual test chemical concentrations (organic chemicals) in the toxicant mixing chamber (toxicant stock solution) and in exposure aquaria will periodically be measured on a real-time basis at NAS to verify that nominal concentrations are being routinely achieved. Definitive concentration documentation in test aquaria will result from daily samples shipped for analysis to CAS.

Test Chambers and Environmental Control: Test chambers used in the toxicity study are glass aquaria less than or equal to two gallons in volume. The test system is housed in a constant-temperature, photoperiod-controlled room. Aeration is not employed. The photoperiod is constant dark except during monitoring activities when ambient (50-75 ft-c) lighting is used.

Cleaning: All laboratory glassware, including test chambers and toxicant dilution system, will be cleaned based on the methods described in U.S. Environmental Protection Agency (2002). Initial cleaning incorporates the use of a laboratory detergent followed by multiple tap water rinses. Subsequently, either dilute (10%, V:V) acid (hydrochloric or nitric) or acetone is used to remove metals or organic contaminants, respectively. Further multiple tap water rinses are employed after each acid or acetone treatment. Sodium hypochlorite bleach may be employed on a limited basis to ensure that microorganisms that could metabolize organic toxicants, e.g. naphthalene, are not present. Exhaustive tap water rinsing is always employed following any chemical cleaning treatment. A final dilution water rinse of the exposure apparatus will precede initiation of each study.

EXPERIMENTAL DESIGN AND TEST PROCEDURES

Experimental Design: The definitive test involves exposure of lamprey ammocoetes to five test concentrations, using a 0.5 dilution series, and a dilution water control. Exposures are for 96 hours. Each treatment consists of four replicates, each containing 10 ammocoetes, if there are sufficient numbers of ammocoetes. If there are not enough ammocoetes for 10 per replicate, then at least 20 per treatment (5 per replicate) is the minimum number preferred. Test chambers are randomly distributed in the test area. Ammocoetes are impartially distributed to each aquarium by adding one or two animals to each test replicate and repeating the process until each contains 10 organisms.

Effect Criterion: The effect criterion used in the lamprey ammocoete acute toxicity test is mortality, defined as a lack of visible respiratory movement and absence of response to tactile stimulation. The mortality data will be used to compute LC50s for each exposure period. In addition to mortality, qualitative observations of sublethal responses consisting of erratic swimming, hyperventilation, hemorrhaging, and hyper/hypoactivity (compared to the activity of the organisms in the control group) will be recorded if observed.

Test Conditions: The dissolved oxygen concentration in each test container must be > 4.0 mg/L throughout the test. The test temperature employed is 17 ± 1°C. A photoperiod of constant darkness is used, except during monitoring activities when illumination is supplied by daylight fluorescent lamps at an intensity of 50-75 ft candles. Loading should not exceed 6.1g/L at 17°C according to EPA. ASTM recommends loading not exceed 1g/L of solution passing through the chamber in 24 hours or 10g/L of solution in the chamber at any time.

Beginning the Test: Adding the organisms to the equilibrated test containers as previously described begins the test.

Feeding: None.

Analysis of Test Concentrations: During each test, samples will be collected for chemical analysis, held at 4°C and shipped by overnight delivery to Columbia Analytical Services in Kelso, WA. The sampling schedule and procedure will follow the project QAPP.

Test Duration, Type and Frequency of Observations, and Methods: The test duration of the acute toxicity test is 96 hours. The type and frequency of observations to be made during the test are summarized as follows:

Type of Observation	Times of observation
Biological Data	
1. mortality (each test container)	0, 24, 48, 72, and 96 hr
2. sublethal observations (qualitative)	0, 24, 48, 72, and 96 hr
Physical and Chemical Data	
1. Dissolved oxygen, pH, & temperature, (in one replicate of each test level and the control)	0, 24, 48, 72, and 96 hr
2. Conductivity, hardness & alkalinity (in one replicate of highest concentration and control)	0, 24, 48, 72, and 96 hr
3. Toxicant concentration sampling (in one replicate of each test concentration and the control)	0, 24, 48, 72, and 96 hr
4. Dilution water sample for particulate matter, TOC and ammonia	0 hr
5. Sample for ammonia taken from one replicate of control and highest test concentration	96 hr

During the test, dead organisms are removed every 24 hours and frozen by concentration for possible tissue analysis. Dissolved oxygen is measured using a polarographic oxygen probe calibrated according to the manufacturer's recommendations. The pH and temperature are measured using a pH probe and a properly calibrated meter with scale divisions of 0.1 pH units. Conductivity is measured using a calibrated conductivity meter. Samples for chemical analysis will be collected, preserved, and sent to Columbia Analytical Services following the recommended procedures of the analytical laboratory.

Test Termination: At the end of the test, all surviving control ammocoetes are weighed and measured for length by replicate. All surviving or dead test organisms will be frozen by concentration for possible later tissue analysis by Columbia Analytical Services.

Criteria of Test Acceptance: The test results are acceptable if survival in the controls is at least 90%. The toxicity tests will be performed as a 50% dilution series. it is anticipated that each test will have at least one or possibly two partial mortality concentrations. Nominal concentrations of each toxicity test have be reviewed and approved by EPA (EPA 2007). However, because of the experimental nature of the study and the limited supply of ammocoetes, the acceptability of the toxicity tests will be based only on the ≥ 90% survival in control group.

DATA ANALYSIS

The percent of live ammocoetes is calculated for each treatment replicate from the raw data and the means are obtained for each treatment level. An LC50 (mortality) is calculated using Probit or Trimmed Spearman-Karber methods. The NOEC and LOEC are calculated for each endpoint according to U.S. Environmental Protection Agency (2002) methods. In this procedure, the NOEC and LOEC values are computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test is selected after evaluating the data for normality and homogeneity of variance. An arcsine-square root transformation is performed on the percentage effect data prior to statistical analysis. The statistical software employed for these calculations is Toxcalc, v.5.0.23N, Tidepool Scientific Software.

REPORTING

The final report of the study results must include all of the following standard information at a minimum:

1. Name and identification of the test;
2. The investigator and laboratory;
3. Information on the toxicants;
4. Information on the dilution water;
5. Detailed information about the test organisms;
6. Description of the test system and its calibration, including test chambers and dilution system;
7. A description of the experimental design and other test conditions including water quality;
8. Definition of the effect criteria and any other observations;
9. Responses, if any, in the control treatment;
10. Tabulation and statistical analysis of measured responses;
11. A description of the statistical methods used;
12. Any unusual information about the test or deviations from procedures;

The final study report will include the study protocol and all study raw data as appendices.

STUDY DESIGN ALTERATION

Amendments made to the protocol must be approved by the sponsor and study director and should include a description of the change; the reason for the change; the date the change took effect; and the dated signatures of the study director and sponsor. Any deviations in the protocol must be described and recorded in the study raw data.

REFERENCED GUIDELINES

ASTM. 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.

Benoit, D., V. Mattson, and D. Olson. 1982. A continuous-flow mini-diluter system for toxicity testing. Water Res. 16:457-464.

EPA. 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. Environmental Protection Agency, Washington, DC.

EPA. 2007. Letter to Lower Willamette Group from C. Humphrey and E. Blischke dated August 17, 2007 regarding Portland Harbor RI/FS: Lamprey toxicity test field sampling plan and QAPP Phase 2 addenda (approval letter). US Environmental Protection Agency Region 10, Oregon Operations Office, Portland, OR.

Veith G.D. and V.M. Comstock. 1975. Apparatus for continuously saturating water with hydrophobic organic chemicals. J Fish Res Board Can 32:1849-1851.

13. APPROVALS

_____ _____ for **Windward Environmental**
Name Date

_____ _____ _ for **Northwestern Aquatic Sciences**
Name Date

Appendix A
Test Conditions Summary

1. Test species:	*Lampetra* sp.
2. Test type:	Flow-through
3. Test duration:	96 hr
4. Temperature:	17 ± 1°C
5. Dissolved oxygen:	≥ 4.0 mg/L
6. Light quality & intensity:	Ambient laboratory light (50-75 ft-c)
7. Photoperiod:	Constant dark, except during monitoring activities
8. Test chambers:	glass aquaria less than or equal to two gallons in volume.
9. Test chamber volumes per day:	At least 5
10. Replicates per treatment:	4
11. Organisms per replicate:	10
12. Age of test organisms:	ammocoete
13. Feeding regime:	None
14. Aeration:	None
15. Dilution water:	Dechlorinated City of Newport tap water; hardness adjusted to 25-30 mg/L as $CaCO_3$
16. Test concentrations:	5 and a dilution water control
17. Water quality:	Temperature, pH, & dissolved oxygen in one replicate of each test concentration daily. Conductivity, hardness & alkalinity in one replicate of highest concentration and control daily
18. Special controls:	None
19. Endpoint:	Mortality
20. Test acceptability criteria:	≥90% control survival

APPENDIX II

RAW DATA

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-44 Client Windward Investigator

STUDY MANAGEMENT:

Client: Windward Environmental, 200 West Mercer Street, Suite 401, Seattle, WA 98119
Client's Study Monitor: Ms. Helle Andersen
Testing Laboratory: Northwestern Aquatic Sciences
Test Location: 3814 Yaquina Bay Road, Newport, OR 97365
Laboratory's Study Personnel:
Project Manager: Gary A. Buhler Study Director: Richard S.Caldwell
QA Officer: L.K. Nemeth 1.
2. 3.
4. 5.
Study Schedule:
Test Beginning: Test Ending:

TEST MATERIAL:

Description: Diazinon Chem Service Lot # 393-91A

DILUTION WATER:

Description: Dechlorinated City of Newport tap water
Date of Preparation/Collection:
Water Quality: Cond. (µmhos/cm) pH
Hardness (mg/L as $CaCO_3$) Alkalinity (mg/L as $CaCO_3$)
Treatments: Hardness adjusted to 25-30 mg/L with a Culligan water treatment system.

TEST ORGANISMS:

Species: Lamprey ammocoetes (*Lampetra* sp.) Age: Size: 4-6 cm
Source:
Acclimation Data: See pages:

TEST PROCEDURES AND CONDITIONS:

Test Concentrations (nominal): 40, 20, 10, 5, 2.5 & 0 mg/L
Test Chambers (description): 2 gallon glass aquaria Test Volume (ml): 5.80 L
Replicates/treatment: 4 Organisms/treatment: 40 (10/replicate)
Test water changes: flow through
Aeration during test: none
Temperature (°C): 17 ± 1°C
Feeding: None Photoperiod: Darkness except for WQ & chemistry measurements
Test Duration (hr) 96 hours Tank Placement: Complete randomization
Randomization Chart

Diluter

5	40	5	20	0	40	0	5	2.5	10	0	40	20	2.5	2.5	20	5	10	10	40	0	10	20	2.5

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-44 Client_____ Windward_____ _____ Investigator___ _____

WEIGHT OF 10 ANIMALS AT TEST INITIATION

	weight (g)	Length (cm)	Comments
1	~~4.4~~ 0.12	4.4	
2	0.28	5.5	
3	0.33	5.9	
4	0.31	5.8	
5	0.26	5.5	
6	0.27	5.4	
7	0.03	4.1	
8	0.08	4.0	
9	0.13	4.2	
10	0.21	4.1	
Total Mean	0.21	4.9	
Average animal S.D.	0.10	0.8	
n	10	10	

MISCELLANEOUS NOTES:

Room light intensity (fc): 15.2 [illegible] 1/20/08

Chemistry samples taken daily.

Chemistry samples for particulate matter, TOC & ammonia in dilution water taken on test day 0.

Chemistry samples for ammonia taken in control & 40 mg/L. A replicate on test day 4.

$$\text{Loading} = \frac{0.21\ g \times 10}{5.8\ L} = 0.36\ g/L$$

Error codes:

1) Correction of handwriting error
2) Written in wrong location; entry deleted
3) Wrong date deleted; replaced with correct data
4) Error found in measurement, measurement repeated

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-44 Client Windward Investigator

WATER QUALITY DATA SHEET

DAY 0 (/ 16 / 8) 1622

Conc (mg/L)	Temp (°C)	pH	Cond µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
40	16.5	7.2	113	9.8	32	10	10	10	10	10	
20	16.6	7.2		9.8			10	10	10	10	
10	16.5	7.2		9.8			10	10	10	10	
5	16.6	7.2		9.8			10	10	10	10	
2.5	16.6	7.1		9.8			10	10	10	10	
0	16.5	6.9	113	9.7	30	10	10	10	10	10	

Chemistry samples taken (/ 16 / 8) 16:20
Particulate matter, TOC, and ammonia samples taken in dilution water (/ 16 / 8) 16:20
Hours of light: 9.0

DAY 1 (/ 17 / 8) 1205

Conc (mg/L)	Temp (°C)	pH	Cond µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
40	16.5	7.3	115	9.8	~~12~~ 30	10	10	10	10	10	lethargic
20	16.6	7.2		9.8			10	10	10	10	lethargic
10	16.5	7.2		9.9	0-10		10	10	10	10	lethargic
5	16.2	7.2		9.7	1-18		10	10	10	10	lethargic
2.5	16.4	7.2		9.8			10	10	10	10	slightly lethargic
0	16.6	7.1	110	9.8	12 28	9	10	10	10	10	

Chemistry samples taken (/ 17 / 8) 1230
Hours of light: 1.5

DAY 2 (/ 18 / 8) 1305

Conc (mg/L)	Temp (°C)	pH	Cond µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
40	17.6	7.4	120	9.6	30	10	0(100)	0(100)	0(100)	0(100)	
20	17.2	7.5		9.8			10	10	10	10	lethargic
10	17.4	7.5		9.7			10	10	10	10	lethargic
5	17.5	7.4		9.6			10	10	10	10	lethargic
2.5	17.4	7.4		9.6			10	10	10	10	slightly lethargic
0	17.3	7.2	120	9.8	30	10	10	10	10	10	

Chemistry samples taken (/ 18 / 8) 1300
Hours of light: 0.5

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-44 Client Windward Investigator

WATER QUALITY DATA SHEET

DAY 3 (1/14 [illegible])

Conc (mg/L)	Temp (°C)	pH	Cond µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
40	17.3	7.3	115	9.8	30	9	0	0	0	0	
20	16.9	7.3		9.8			5(50)	3(70)	6(40)	6(40)	lethargic
10	16.8	7.3		9.7			10	10	10	10	lethargic
5	16.9	7.3		9.8			10	10	10	10	lethargic
2.5	16.8	7.3		9.9			10	10	10	10	
0	16.9	7.3	100	9.8	30	11	10	10	10	10	

Chemistry samples taken (1/14 [illegible]) 1230

Hours of light: 15

DAY 4 (1/15/03) 0[illegible]

Conc (mg/L)	Temp (°C)	pH	Cond µmhos/cm	DO (mg/L)	Total Hardness (mg/L)	Total Alkalinity (mg/L)	Survivors A	B	C	D	Comments
40	17.3	7.3	115	9.8	30	10	0	0	0	0	
20	16.8	7.3		10.0			0(50)	1(40)	0(60)	0(60)	
10	16.9	7.3		9.9			9(10)	10	10	10	lethargic, [illegible]
5	16.8	7.2		9.9			10	10	10	10	not active, lethargic
2.5	16.8	7.2		9.9			10	10	10	10	not lethargic
0	17.0	7.2	115	9.8	30	10	10	10	10	10	not lethargic (normal act.)

Chemistry samples taken (1/15 [illegible]) 1330

Ammonia samples taken in control and 40 mg/L (1/15 [illegible]) 1[illegible]

Hours of light: 1

Dilution water WQ (from control aquaria)

Cond 112 ± 1 (5)

pH 7.1 ± 0.2 (5)

Hardness 29.6 ± 0.1 (5)

Alkalinity 10.0 ± 0.7 (5)

2/21/07

LAMPREY SURVIVAL FLOW-THROUGH TEST

Test No. 686-44 Client Windward Investigator

NOTES ON PREPARATION OF 40 mg/L DIAZINON STOCK

System Design

The objective is to deliver 40 mg/L of diazinon to the diluter toxicant chamber at a nominal constant rate of 160 ml/min. To accomplish this, a large reservoir, approximately 54L capacity, of diazinon solution in dilution water is maintained at test temperature. As solution is removed to the diluter, makeup water is introduced under float valve control. The concentration of diazinon in the reservoir is to be maintained by metering 0.344 ml/hr of pure diazinon into the mixing tank. This addition was accomplished using a single channel syringe pump (Cole-Parmer) fitted with a 10 ml gas-tight Hamilton syringe which is refilled daily. The actual delivery rate is set slightly in excess at 0.4 ml/hr since a setting of 0.344 ml/hr is not available. The pure diazinon is injected through 0.042 in I.D. PTFE tubing into a recirculating stream of water from the mixing reservoir. The mixing tank contents are vigorously stirred to facilitate rapid solution of diazinon.

NOTES ON MAINTENANCE OF THE DIAZINON DELIVERY SYSTEM

Morning of 1/20/08, the glass wool filter through which recirculating mixing tank stream passes was found to be clogged & water was overflowing top of tank. Wool was removed & excess undissolved diazinon was at the bottom of mixing tank. Brief analysis performed that concentration was elevated in ~~mixing~~ 40 mg test aquaria

RSC 1/20/08

DILUTER CALIBRATIONS RECORD

DILUTER CAPILLARY FLOWS

Date 1/16/08	Time 1310	Init. RSC	Date 1/20/08	Time 1645	Init. RSC
Drain #	Supply Tank	Diluter Tank	Drain #	Supply Tank	Diluter Tank
1	162	79	1	161	78
2	79	78	2	80	79
3	81	77	3	80	78
4	80	79	4	78	77
5	78	80	5	77	79
6	108	77	6	105	76

Flow rates in ml/min

FLOWS TO REPLICATE AQUARIA

Date 1/16/08 Time 1320 Init. RSC

Replicate	Relative Concentration (%) 100	50	25	12.5	6.25	0
A	310	280	325	290	285	280
B	250	260	290	285	270	325
C	265	305	275	310	315	285
D	325	295	310	260	320	320

Date 1/20/08 Time 1100 Init. RSC

Replicate	Relative Concentration (%) 100	50	25	12.5	6.25	0
A	315	295	315	295	340	270
B	265	255	320	310	290	315
C	270	290	250	320	305	275
D	305	315	290	285	310	305

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
8-2-07	16.1	6.7	8.6	82	40	25	[illegible]	[illegible]	constant flow		[illegible]	[illegible]	[illegible]
8-3-07	16.8	7.7	9.6	83	26	30		"	"			[illegible]	[illegible]
8-6-07	17.1	6.6	9.3	68	28	10	[illegible]	[illegible]	"			[illegible]	[illegible]
8-7-07	16.4	7.6	9.7	162	34	30	[illegible]					[illegible]	[illegible]
8-8-07	18.1	6.1	7.2	60	22	16	[illegible]	[illegible]				[illegible]	[illegible]
8-9-07	17.0	7.0	7.4	160	34	30						Holding	[illegible]
8-10-07	16.9	6.6	9.0	60	31	25	Alsea River		"			[illegible]	[illegible]
8-11-07	16.8	7.2	7.5	135	34	30			"			[illegible]	[illegible]
9-1-07	16.9	7.1	7.4	140	—	—			"				
9-2-07	16.7	7.6	9.1	160	—	—			"			Holding	[illegible]
9-3-07	16.7	7.7	9.2	160	—	—			"			Holding	[illegible]
9-4-07	16.2	7.5	8.8	185	34	30			"			"	[illegible]
9-5-07	16.3	7.2	9.1	170	34	30			"			"	[illegible]
9-6-07	16.8	7.3	9.0	185	34	30			"			"	
9-7-07	16.9	7.0	9.1	155	26	30			"			"	
9-8-07	17.0	7.5	9.3	160	—	—			"			"	
9-9-07	16.7	7.5	8.8	170	—	—			"			Holding	[illegible]
9-10-07	16.7	7.2	8.9	175	—	—			"			"	[illegible]
9-11-07	17.4	7.6	9.0	235	60	35			"			"	[illegible]
9-12-07	17.0	8.0	9.4	185	60	38			"			"	[illegible]

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
9-13-07	17.1	8.0	9.0	170	55	30			constant flow			holding	[illegible]
9-14-07	17.1	8.2	9.1	155	48	29			"		[illegible]	"	[illegible]
9-15-07	17.1	8.3	9.2	120	—	—			"			"	
9-16-07	17.0	8.1	9.2	115	41	30			"			"	[illegible]
9-17-07	17.1	7.7	8.8	120	41	30			"			"	[illegible]
9-18-07	17.0	7.8	9.1	120	39	21			"			"	[illegible]
9-19-07	17.7	7.6	9.1	125	39	34			"			"	[illegible]
9-20-07	17.1	7.9	9.0	125	36	34	taken for [illegible]		"			"	[illegible]
9-21-07	17.0	8.2	9.5	140	44	31			"			"	[illegible]
9-22-07	17.1	7.7	9.4	130	42	29			"			"	
9-23-07	17.1	7.7	9.3	140	46	28			"			"	[illegible]
9-24-07	16.8	7.9	9.3	140	44	29			"			"	
9-25-07	17.1	7.7	8.8	145	44	30			"			"	[illegible]
9-26-07	17.2	7.8	9.3	140	48	36			"			"	[illegible]
9-27-07	17.4	7.7	9.3	145	48	37			"			"	[illegible]
9-28-07	17.2	7.8	9.3	150	48	36			"			"	[illegible]
9-29-07	16.9	7.5	8.8	150	48	36			"			"	[illegible]
9-30-07	17.0	7.2	8.8	140	48	37			"			"	[illegible]
10-1-07	17.0	7.8	9.4	145	48	36			"		[illegible]	"	[illegible]
10-2-07	17.3	8.1	9.2	135	—	—			"			"	[illegible]

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
10-3-07	17.0	7.8	9.1	130	46	55			constant flow			Holding	GH
10-4-07	16.9	7.9	9.2	120	42	38						"	[illegible]
1050	16.9	7.4	9.0	130	not Taken							"	LPS
10-6-07	16.6	7.2	9.4	130	44	41						"	GH
10-7-07	17.0	7.4	9.5	135	60	50						"	GH
10-8-07	17.0	7.9	9.2	145	50	48						"	GH
10-9-07	17.0	7.6	9.5	150	54	52						"	GH
10-10-07	16.9	7.4	9.2	155	54	49						"	GH
10-11-07	17.0	7.1	9.5	150	54	40						"	[illegible]
10-12-07	17.1	8.0	9.4	155	50	39						"	[illegible]
10-13-07	17.1	7.1	9.1	150	52	40						"	GH
10-14-07	16.9	7.5	9.6	150	52	38						"	[illegible]
10-15-07	17.0	7.6	9.5	155	50	38						"	[illegible]
10-5-9	13.0	6.7	10.6	97	30	30						Receiving Tank	[illegible]
10-16-07	13.2	7.9	10.1	145	60	57						Holding Rm #4	[illegible]
10-16-07	17.1	6.9	9.4	165	30	12						Test aquarium Rm #1	[illegible]
10-16-07	12.4	6.2	8.9	65	18	14						NORTH FORK COQUILLE	GH
10-9-7	13.3	8.3	10.1	135	60	55					[illegible]	Holding Rm #1	GH
10-17-7	17.3	6.7	8.5	75	22	14						Holding Rm #1	GH
10-17-07	13.1	6.8	10.2	57	20	14						NORTH FORK COQUILLE	

ANIMAL RECEIPT/HOLDING: FRESHWATER
SPECIES: Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No. of Animals	Water Change	Feeding	Disposition	Comments	Init.
10-18-07	13.2	8.3	9.9	130	48	36			constant flow			Holding	[illegible]
10-19-07	13.3	7.5	9.5	120	48	36						"	LPS
10-20-07	13.2	7.7	10.0	125	46	38							
10-21-07	14.2	7.6	9.9	130	48	37							
10-22-07	14.2	7.7	9.4	135	50	40						"	[illegible]
10-23-07	15.1	7.9	9.7	145	54	44						"	[illegible]
10-24-07	15.6	8.3	9.7	155	58	47						"	[illegible]
10-25-07	16.1	8.5	9.5	160	66	53						"	[illegible]
10-26-07	16.8	8.1	9.6	165	60	47						"	[illegible]
10-27-07	16.8	8.3	9.9	155	62	49						"	[illegible]
10-28-07	16.6	8.3	9.9	165	62	47						"	[illegible]
10-29-07	17.0	8.1	9.5	165	62	48						"	[illegible]
10-30-07	16.9	8.2	9.6	170	64	46						"	[illegible]
10-31-07	16.8	8.2	9.9	175	70	54						"	[illegible]
11-1-07	16.8	8.2	9.8	165	62	45						"	[illegible]
11-2-07	16.8	8.2	9.5	165	62	47						"	[illegible]
11-3-07	16.6	8.1	9.2	160	56	38						"	GH
11-4-07	16.8	8.3	9.6	180	64	48						"	LPS
11-5-07	16.9	8.1	9.9	180	66	49						"	[illegible]
11-6-07	16.9	8.7	9.8	155	52	35						"	[illegible]

Holding Records

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ANIMAL RECEIPT/HOLDING FRESHWATER
SPECIES Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No of Animals	Water Change	Feeding	Disposition	Comments	Init
11-7-07	17.0	8.1	9.4	155	54	37			constant flow			Holding	
11-8-07	16.9	8.1	9.3	155	56	39						"	
11-9-07	16.7	8.6	9.9	160	62	46						"	
11-10-07	16.7	8.1	9.6	155	56	43						"	
11-11-07	16.7	8.1	9.9	145	52	39						"	
11-12-07	16.8	8.0	9.7	155	56	38						"	
11-13-07	16.8	7.6	9.7	155	54	38						"	
11-14-07	16.8	7.9	9.7	150	52	36						"	
11-15-07	16.8	8.0	9.7	160	60	43						"	
11-16-07	16.9	8.2	9.9	160	58	39						"	
11-17-07	16.7	8.3	10.1	165	62	43						"	
11-18-07	16.9	8.1	9.8	150	54	36						"	
11-19-07	16.8	7.9	9.7	175	64	48						"	
11-20-07	16.7	7.9	9.9	170	64	46						"	
11-21-07	16.6	8.0	9.9	175	64	48						"	
11-22-07	16.5	7.9	10.0	165	62	39						"	
11-23-07	16.5	7.8	9.5	170	62	44						"	
11-24-07	16.5	8.0	9.7	190	70	48						"	
11-25-07	16.5	7.9	9.6	175	64	45						"	
11-26-07	16.6	7.5	9.6	175	64	46						"	

ANIMAL RECEIPT/HOLDING FRESHWATER
SPECIES Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No of Animals	Water Change	Feeding	Disposition	Comments	Init
11-29-07	16.1	7.9	9.6	180	62	44			constant flow			Holding	
11-30-07	16.0	7.7	10.0	175	56	44						"	
12-1-07	16.0	7.8	9.7	170	60	43						"	
12-2-07	16.0	8.0	9.7	170	62	46						"	
12-3-07	16.2	7.9	9.7	185	70	47						"	
12-4-07	15.8	7.5	9.4	165	60	43						"	
12-5-07	15.7	8.2	9.6	155	60	39						"	
12-6-07	15.8	7.7	9.8	170	60	42						"	
12-7-07	16.2	7.6	9.9	165	60	42						"	
12-8-07	15.7	7.6	9.9	160	58	40							
12-9-07	17.8	7.4	9.0	175	64	43						"	
12-10-07	17.1	7.3	8.8	165	62	42						"	
12-11-07	17.1	7.2	9.4	170	56	41						"	
12-12-07	17.3	7.4	9.5	160	58	40						"	
12-13-07	16.7	7.2	9.1	170	64	43						"	
12-14-07	16.8	7.3	9.1	170	64	44						"	
12-15-07	17.0	7.2	9.0	165	56	44						"	
12-16-07	16.5	7.2	9.5	190	70	45						"	
12-17-07	16.7	7.2	9.3	170	66	44						"	
12-18-07	16.5	7.4	9.6	195	74	53						"	

Holding Records

ANIMAL RECEIPT/HOLDING FRESHWATER
SPECIES Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No of Animals	Water Change	Feeding	Disposition	Comments	Init
12-19-07	15.6	7.3	9.9	190	76	51			constant flow			HOLDING	
12-20-07	16.2	7.2	10.3	175	56	46						"	
12-21-07	15.4	8.2	10.0	190	72	48						"	
12-22-07	16.0	7.8	9.8	145	70	46							
12-23-07	17.1	8.0	9.5	195	68	54						"	
12-24-07	17.1	8.0	9.4	190	72	48						"	
12-25-07	16.9	8.1	9.6	190	72	51						"	
12-26-07	16.8	8.3	10.1	200	74	50						"	
12-27-07	17.1	8.2	9.7	195	68	49						"	
12-28-07	17.0	8.0	9.8	170	56	32						"	
12-29-07	17.1	8.1	9.9	175	52	29						"	
12-30-07	16.9	8.0	9.6	195	68	50						"	
12-31-07	16.8	8.5	9.8	205	72	50						"	
1-1-08	17.0	9.0	9.6	[illegible]	64	43						"	
1-2-08	16.8	8.2	9.4	185	66	41						"	
1-3-08	16.8	8.3	9.7	180	60	39						"	
1-4-08	16.9	8.2	9.8	175	62	36						"	
1-5-08	16.7	8.4	9.7	155	48	30						"	
1-7-08	16.7	8.4	9.9	160	54	32						"	
1-8-08	16.9	8.1	9.5	165	50	33						"	

ANIMAL RECEIPT/HOLDING FRESHWATER
SPECIES Lamprey (*Lampetra* sp.)

DATE	TEMP (°C)	pH	DO (mg/L)	COND (µmhos/cm)	HARD (mg/L)	ALK (mg/L)	Source	No of Animals	Water Change	Feeding	Disposition	Comments	Init
1-9-08	15.6	8.4	10.6	165	56	30			constant flow			HOLDING	
1-10-08	15.9	8.0	10.2	145	44	24						"	
1-11-08	16.3	8.1	10.3	145	42	24							
1-12-08	16.0	8.4	10.2	165	56	31						"	
1-13-08	16.2	8.1	9.6	170	54	32						"	
1-14-08	16.5	8.2	9.9	160	52	32						"	
1-15-08	16.2	8.2	9.7	160	50	29						"	
1-16-08	16.4	8.2	9.9	175	70	15						"	
1-17-08	15.6	8.0	9.9	165	86	63						"	

Samples shipped to CAS

Test No. 686-44

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CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd. P.O. Box 1437, Newport, OR 97365
Tel 541-265-7225 Fax 541-265-2799 www.nwaquatic.com

Client Name: Integral Consulting, Inc
Address: 319 SW Washington St. Suite 1150
City, State, Zip code: Portland, OR 97204
Project No: Integral B01-01-58C Windward 07-28-04 53
Phone No: 503-284-5545
Report Attention: Abbie Spielman
Sampled by: RSC
Shipping Information: Carrier: UPS UPS#Y2489E
Testing Required: Copper, Lindane, Pentachlorophenol, Aroclors, Diazinon, Naphthalene

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aroclors	Diazinon	Naphthalene	Comments
	1-17-08	1200	NAS# 1593G Day 1 - 0 mg/L Diazinon	1					X		
	1-17-08		NAS# 1594G Day 1 - 2.5 mg/L Diazinon	1					X		
	1-17-08		NAS# 1595G Day 1 - 5.0 mg/L Diazinon	1					X		
	1-17-08		NAS# 1596G Day 1 - 10.0 mg/L Diazinon	1					X		
	1-17-08		NAS# 1597G Day 1 - 20.0 mg/L Diazinon	1					X		
	1-17-08		NAS# 1598G Day 1 - 40.0 mg/L Diazinon	1					X		
	1-17-08		NAS# 1599G Day 1 - 40.0 mg/L Diazinon DUPLICATE	1					X		

Signature	Print Name	Company	Date	Time	Cooler Custody Seal
Relinquished By: Gerald Irissarri	GERALD IRISSARRI	Northwestern Aquatic Sciences	1-21-08	1430	Present / Not Present
Received By:					Intact / Not Intact
Relinquished By:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received By:					
Relinquished By:					
Received By (Laboratory):					

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd. P.O. Box 1437, Newport, OR 97365
Tel 541-265-7225 Fax 541-265-2799 www.nwaquatic.com

Client Name: Integral Consulting, Inc
Address: 319 SW Washington St. Suite 1150
City, State, Zip code: Portland, OR 97204
Project No: Integral B01-01-58C Windward 07-28-04 53
Phone No: 503-284-5545
Report Attention: Abbie Spielman
Sampled by: RSC Caldwell
Shipping Information: Carrier: UPS UPS#Y2489E
Testing Required: Copper, Lindane, Pentachlorophenol, Aroclors, Diazinon, Naphthalene

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aroclors	Diazinon	Naphthalene	Comments
	1-19-08	1230	NAS# 1611G Day 3 - 0 mg/L Diazinon	1					X		
	1-19-08		NAS# 1612G Day 3 - 2.5 mg/L Diazinon	1					X		
	1-19-08		NAS# 1613G Day 3 - 5.0 mg/L Diazinon	1					X		
	1-19-08		NAS# 1614G Day 3 - 10.0 mg/L Diazinon	1					X		
	1-19-08		NAS# 1615G Day 3 - 20.0 mg/L Diazinon	1					X		
	1-19-08		NAS# 1616G Day 3 - 40.0 mg/L Diazinon	1					X		

Signature	Print Name	Company	Date	Time	Cooler Custody Seal
Relinquished By: Gerald Irissarri	GERALD IRISSARRI	Northwestern Aquatic Sciences	1-21-08	1430	Present / Not Present
Received By:					Intact / Not Intact
Relinquished By:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received By:					
Relinquished By:					
Received By (Laboratory):					

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd. P.O. Box 1437, Newport, OR 97365
Tel 541-265-7225 Fax 541-265-2799 www.nwaquatic.com

Client Name: Integral Consulting, Inc
Address: 319 SW Washington St. Suite 1150
City, State, Zip code: Portland, OR 97204
Project No: Integral B01-01-58C Windward 07-28-04 53
Phone No: 503-284-5545
Report Attention: Abbie Spielman
Sampled by: RSC
Shipping Information: Carrier: UPS UPS#Y2489E
Testing Required: Copper, Lindane, Pentachlorophenol, Aroclors, Diazinon, Naphthalene

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aroclors	Diazinon	Naphthalene	Comments
	1-16-08	1630	NAS# 1584G Day 0 - 0 mg/L Diazinon	1					X		
	1-16-08		NAS# 1585G Day 0 - 2.5 mg/L Diazinon	1					X		
	1-16-08		NAS# 1586G Day 0 - 5.0 mg/L Diazinon	1					X		
	1-16-08		NAS# 1587G Day 0 - 10.0 mg/L Diazinon	1					X		
	1-16-08		NAS# 1588G Day 0 - 20.0 mg/L Diazinon	1					X		
	1-16-08		NAS# 1589G Day 0 - 40.0 mg/L Diazinon	1					X		
	1-16-08		NAS# 1590G Day 0 - TOC	1							
	1-16-08		NAS# 1591G Day 0 - TSS	1							
	1-16-08		NAS# 1592G Day 0 - Ammonia	1							

Signature	Print Name	Company	Date	Time	Cooler Custody Seal
Relinquished By: Gerald Irissarri	GERALD IRISSARRI	Northwestern Aquatic Sciences	1-21-08	1430	Present / Not Present
Received By:					Intact / Not Intact
Relinquished By:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received By:					
Relinquished By:					
Received By (Laboratory):					

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
3814 Yaquina Bay Rd. P.O. Box 1437, Newport, OR 97365
Tel 541-265-7225 Fax 541-265-2799 www.nwaquatic.com

Client Name: Integral Consulting, Inc
Address: 319 SW Washington St. Suite 1150
City, State, Zip code: Portland, OR 97204
Project No: Integral B01-01-58C Windward 07-28-04 53
Phone No: 503-284-5545
Report Attention: Abbie Spielman
Sampled by: RSC Caldwell
Shipping Information: Carrier: UPS UPS#Y2489E
Testing Required: Copper, Lindane, Pentachlorophenol, Aroclors, Diazinon, Naphthalene

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aroclors	Diazinon	Naphthalene	Comments
	1-18-08	1730	NAS# 1604G Day 2 - 0 mg/L Diazinon	1					X		
	1-18-08		NAS# 1605G Day 2 - 2.5 mg/L Diazinon	1					X		
	1-18-08		NAS# 1606G Day 2 - 5.0 mg/L Diazinon	1					X		
	1-18-08		NAS# 1607G Day 2 - 10.0 mg/L Diazinon	1					X		
	1-18-08		NAS# 1608G Day 2 - 20.0 mg/L Diazinon	1					X		
	1-18-08		NAS# 1609G Day 2 - 40.0 mg/L Diazinon	1					X		
	1-18-08		NAS# 1610G Day 2 - 2.5 mg/L Diazinon DUPLICATE	1					X		

Signature	Print Name	Company	Date	Time	Cooler Custody Seal
Relinquished By: Gerald Irissarri	GERALD IRISSARRI	Northwestern Aquatic Sciences	1-21-08	0142	Present / Not Present
Received By:					Intact / Not Intact
Relinquished By:					Internal Cooler Temperature Upon Lab Receipt (°C)
Received By:					
Relinquished By:					
Received By (Laboratory):					

Samples shipped to CAS

Test No. 656-44

CHAIN OF CUSTODY RECORD

Northwestern Aquatic Sciences
2814 Yaquina Bay Rd., P.O. Box 1437, Newport, OR 97365
Tel. 541-265-7225 Fax 541-265-2799, www.nwaquatic.com

Client Name: Integral Consulting, Inc
Address: 319 SW Washington St., Suite 1150
City, State, Zip code: Portland, OR 97204
Project No: Integra. B01-01-586 Windward 07-28-04-53
Phone No: 503-284-5545
Report attention: Abbie Spielman
Sampled by: C. S. Cotham
Shipping Information: Carrier UPS, UPS# Y3489F
Testing Required

Lab Sample No	Date Sampled	Time Sampled	Sample Description	Number of Containers	Copper	Lindane	Pentachlorophenol	Aniline	Diazinon	Naphthalene	Comments
	1-20-08	1030	NASS 1625G Day 1 - 0 mg/L Diazinon						X		
	1-20-08		NASS 1627G Day 4 - 2.5 mg/L Diazinon						X		
	1-20-08		NASS 1629G Day 4 - 5.0 mg/L Diazinon						X		
	1-20-08		NASS 1624G Day 4 - 10.0 mg/L Diazinon						X		
	1-20-08		NASS 1630G Day 4 - 20.0 mg/L Diazinon						X		
	1-20-08		NASS 1626G Day 4 - 40.0 mg/L Diazinon						X		
	1-20-08		NASS 1628G Day 4 - Ammonia Control mg/L Diazinon concentration								
	1-20-08		NASS 1628G Day 4 - Ammonia Treat 40.0 mg/L Diazinon concentration								

Signature	Print Name	Company	Date	Time	Cooler Custody Seal
Relinquished by: [signature]	GEORGE IRISSARRI	Northwestern Aquatic Sciences	1-21-08	1430	Present / Not Present
Received by:					Intact / Not Intact
Relinquished by:					
Received by:					Internal Custody Temperature Upon Receipt °C
Relinquished by:					
Received for Laboratory by:					

686-44

Water Quality Computation - Test No. 686-44

	Temp	DO	pH	Cond	Hard	Alk
1	16.5	9.8	7.2	113	32	10
2	16.6	9.8	7.2	113	30	10
3	16.5	9.8	7.2	115	30	10
4	16.6	9.8	7.2	110	28	9
5	16.6	9.8	7.1	120	30	10
6	16.5	9.7	6.9	120	30	10
7	16.5	9.8	7.3	115	30	9
8	16.6	9.8	7.2	100	30	11
9	16.5	9.9	7.2	115	30	10
10	16.2	9.7	7.2	115	30	10
11	16.4	9.8	7.2			
12	16.6	9.8	7.1			
13	17.6	9.6	7.4			
14	17.2	9.8	7.5			
15	17.4	9.7	7.5			
16	17.5	9.6	7.4			
17	17.4	9.6	7.4			
18	17.3	9.8	7.2			
19	17.3	9.8	7.3			
20	16.9	9.8	7.3			
21	16.8	9.7	7.3			
22	16.9	9.8	7.3			
23	16.8	9.9	7.3			
24	16.9	9.8	7.3			
25	17.3	9.8	7.3			
26	16.8	10.0	7.3			
27	16.9	9.9	7.3			
28	16.8	9.9	7.2			
29	16.8	9.9	7.2			
30	17.0	9.8	7.2			
31						
32						
mean	16.9	9.8	7.3	114	30	10
s.d.	0.4	0.1	0.1	6	1	1
N	30	30	30	10	10	10
min	16.2	9.6	6.9	100	28	9
max	17.6	10.0	7.5	120	32	11

2/21/08

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WQ Computation - acclimation (1-1-08 to 1-16-08) - Test No. 686-44

	Temp	DO	pH	Cond	Hard	Alk
1	17.0	9.6	8.0	190	64	43
2	16.8	9.6	8.2	185	66	41
3	16.8	9.7	8.3	180	60	39
4	16.9	9.8	8.2	175	62	36
5	16.7	9.7	8.4	155	48	30
6	16.7	9.9	8.4	160	54	32
7	16.9	9.5	8.1	165	50	33
8	15.6	10.6	8.4	165	56	30
9	15.9	10.2	8.0	145	44	24
10	16.3	10.3	8.1	145	42	24
11	16.0	10.2	8.4	165	56	31
12	16.2	9.6	8.1	170	54	32
13	16.5	9.9	8.2	160	52	32
14	16.2	9.7	8.2	160	50	29
15	16.4	9.9	8.2	125	40	15
16						
17						
mean	16.5	9.9	8.2	163	53	31
s.d	0.4	0.3	0.1	17	8	7
N	15	15	15	15	15	15
min	15.6	9.5	8.0	125	40	15
max	17.0	10.6	8.4	190	66	43

SampleDate	ampleTime	Matrix	LabMatrix	SampleID	arentSamp	ampleTyp	Method	nalysisDate	nalysisTim	nalyteGrou	cas_rn	Analyte	l_or_dissol	detect_flag	VALUE	Qualifiers	Units
1/16/08	16 30	WQ	WQ	NAS# 1584G Day 0-0 mg/L Diazinon		N	SW8141A	2/15/08	01 54	Pesticides	333-41-5	Diazinon	N	Y	6 6		ug/l
1/16/08	16 30	WQ	WQ	NAS# 1585G Day 0-2 5 mg/L Diazinon		N	SW8141A	2/16/08	00 29	Pesticides	333-41-5	Diazinon	N	Y	1500		ug/l
1/16/08	16 30	WQ	WQ	NAS# 1586G Day 0-5 0 mg/L Diazinon		N	SW8141A	2/16/08	01 10	Pesticides	333-41-5	Diazinon	N	Y	3000		ug/l
1/16/08	16 30	WQ	WQ	NAS# 1587G Day 0-10 0 mg/L Diazinon		N	SW8141A	2/16/08	01 51	Pesticides	333-41-5	Diazinon	N	Y	5500		ug/l
1/16/08	16 30	WQ	WQ	NAS# 1588G Day 0-20 0 mg/L Diazinon		N	SW8141A	2/16/08	02 32	Pesticides	333-41-5	Diazinon	N	Y	8500	J	ug/l
1/16/08	16 30	WQ	WQ	NAS# 1589G Day 0-40-0 mg/L Diazinon		N	SW8141A	2/21/08	11 14	Pesticides	333-41 5	Diazinon	N	Y	23000		ug/l
1/17/08	12 00	WQ	WQ	NAS# 1593G Day 1-0 mg/L Diazinon		N	SW8141A	2/16/08	06 38	Pesticides	333-41 5	Diazinon	N	Y	12		ug/l
1/17/08	12 00	WQ	WQ	NAS# 1594G Day 1 2 5 mg/L Diazinon		N	SW8141A	2/16/08	07 19	Pesticides	333-41-5	Diazinon	N	Y	1700		ug/l
1/17/08	12 00	WQ	WQ	NAS# 1595G Day 1-5 0 mg/L Diazinon		N	SW8141A	2/16/08	08 00	Pesticides	333-41-5	Diazinon	N	Y	3500		ug/l
1/17/08	12 00	WQ	WQ	NAS# 1596G Day 1-10 0 mg/L Diazinon		N	SW8141A	2/16/08	08 41	Pesticides	333-41-5	Diazinon	N	Y	6900		ug/l
1/17/08	12 00	WQ	WQ	NAS# 1599G Day 1-10 0 mg/L DiazinonDUPLI		N	SW8141A	2/16/08	10 43	Pesticides	333-41-5	Diazinon	N	Y	5800		ug/l
1/17/08	12 00	WQ	WQ	NAS# 1597G Day 1-20 0 mg/L Diazinon		N	SW8141A	2/21/08	11 55	Pesticides	333-41-5	Diazinon	N	Y	12000		ug/l
1/17/08	12 00	WQ	WQ	NAS# 1598G Day 1-40 0 mg/L Diazinon		N	SW8141A	2/21/08	12 37	Pesticides	333-41-5	Diazinon	N	Y	27000		ug/l
1/18/08	13 00	WQ	WQ	NAS# 1604G Day 2-0 mg/L Diazinon		N	SW8141A	2/15/08	13 31	Pesticides	333-41-5	Diazinon	N	Y	5.2		ug/l
1/18/08	13 00	WQ	WQ	NAS# 1605G Day 2-2 5 mg/L Diazinon		N	SW8141A	2/15/08	11 24	Pesticides	333-41-5	Diazinon	N	Y	1600		ug/l
1/18/08	13:00	WQ	WQ	NAS# 1610G Day 2-2 5 mg/L DiazinonDUPLIC		N	SW8141A	2/16/08	17 32	Pesticides	333-41-5	Diazinon	N	Y	1800		ug/l
1/18/08	13:00	WQ	WQ	NAS# 1606G Day 2-5 0 mg/L Diazinon		N	SW8141A	2/16/08	12 05	Pesticides	333-41-5	Diazinon	N	Y	3000		ug/l
1/18/08	13:00	WQ	WQ	NAS# 1607G Day 2-10 0 mg/L Diazinon		N	SW8141A	2/16/08	12 46	Pesticides	333-41-5	Diazinon	N	Y	6400		ug/l
1/18/08	13 00	WQ	WQ	NAS# 1608G Day 2-20 0 mg/L Diazinon		N	SW8141A	2/21/08	13 18	Pesticides	333-41-5	Diazinon	N	Y	12000		ug/l
1/18/08	13 00	WQ	WQ	NAS# 1609G Day 2-40 0 mg/L Diazinon		N	SW8141A	2/21/08	13 59	Pesticides	333-41-5	Diazinon	N	Y	25000		ug/l
1/19/08	12 30	WQ	WQ	NAS# 1611G Day 3-0 mg/L Diazinon		N	SW8141A	2/17/08	03 49	Pesticides	333-41-5	Diazinon	N	Y	8 7		ug/l
1/19/08	12 30	WQ	WQ	NAS# 1612G Day 3-2 5 mg/L Diazinon		N	SW8141A	2/16/08	18 13	Pesticides	333-41-5	Diazinon	N	Y	1800		ug/l
1/19/08	12 30	WQ	WQ	NAS# 1613G Day 3-5 0 mg/L Diazinon		N	SW8141A	2/16/08	18 54	Pesticides	333 41 5	Diazinon	N	Y	3400		ug/l
1/19/08	12 30	WQ	WQ	NAS# 1614G Day 3-10.0 mg/L Diazinon		N	SW8141A	2/16/08	19 35	Pesticides	333-41-5	Diazinon	N	Y	6600		ug/l
1/19/08	12 30	WQ	WQ	NAS# 1615G Day 3-20.0 mg/L Diazinon		N	SW8141A	2/21/08	14 40	Pesticides	333-41-5	Diazinon	N	Y	14000		ug/l
1/19/08	12 30	WQ	WQ	NAS# 1616G Day 3-40 0 mg/L Diazinon		N	SW8141A	2/21/08	17 24	Pesticides	333-41-5	Diazinon	N	Y	37		ug/l
1/20/08	10 30	WQ	WQ	NAS# 1621G Day 4-0 mg/L Diazinon		N	SW8141A	2/21/08	16 43	Pesticides	333-41-5	Diazinon	N	Y	10		ug/l
1/20/08	10 30	WQ	WQ	NAS# 1622G Day 4-2 5 mg/L Diazinon		N	SW8141A	2/16/08	21 39	Pesticides	333-41-5	Diazinon	N	Y	1700		ug/l
1/20/08	10 30	WQ	WQ	NAS# 1623G Day 4-5 0 mg/L Diazinon		N	SW8141A	2/16/08	22 20	Pesticides	333-41-5	Diazinon	N	Y	3300		ug/l
1/20/08	10 30	WQ	WQ	NAS# 1624G Day 4-10 0 mg/L Diazinon		N	SW8141A	2/17/08	01 45	Pesticides	333-41-5	Diazinon	N	Y	6700		ug/l
1/20/08	10:30	WQ	WQ	NAS# 1625G Day 4-20 0 mg/L Diazinon		N	SW8141A	2/21/08	15 21	Pesticides	333-41-5	Diazinon	N	Y	18000		ug/l
1/20/08	10 30	WQ	WQ	NAS# 1626G Day 4-40 0 mg/L Diazinon		N	SW8141A	2/21/08	16.02	Pesticides	333-41-5	Diazinon	N	Y	40000		ug/l

Diazinon (mg/L)	Day 0	Day 1	Day 2	Day 3	Day 4	Average	Standard Deviation
0	0 01	0 01	0 01	0 01	0 01	0 01	0 00
2 5	1 5	1 7	1 7	1 8	1 7	1 7	0 1
5 0	3 0	3 5	3 0	3 4	3 3	3 2	0 2
10 0	5 5	6 4	6 4	6 6	6 7	6 3	0 5
20 0	8 5	12 0	12 0	14 0	16 0	12 5	2 8
40 0	23 0	27 0	25 0	0 04	40 0	23.0	14 5

Includes day 3 outlier

Diazinon (mg/L)	Day 0	Day 1	Day 2	Day 3	Day 4	Average	Standard Deviation
0 00	0 01	0 01	0 01	0 01	0 01	0 01	0 00
2 5	1 5	1 7	1 7	1 8	1 7	1 7	0 1
5 0	3 0	3 5	3 0	3 4	3 3	3.2	0.2
10 0	5 5	6 4	6 4	6 6	6 7	6 3	0 5
20 0	8 5	12 0	12 0	14 0	16 0	12 5	2 8
40 0	23 0	27 0	25 0		40 0	28 8	7 7

Delete Day 3 outlier
used for LC50 calculation
DSR 3/24/08

Validated Diazinon Analyses
Reviewed by [illegible]
3/24/08

GFE-44

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686-44

Acute 96-hr Toxicity Test-96 Hr Survival

Start Date:	1/16/08 16:00	Test ID:	686-44	Sample ID:	
End Date:	1/20/08 16:00	Lab ID:	ORNAS-Northwestern Aquati	Sample Type:	Diazinon
Sample Date:		Protocol:	-NAS-686-LAMPREY FT ACI	Test Species:	LY-Lampetra sp.
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1.7	1.0000	1.0000	1.0000	1.0000
3.2	1.0000	1.0000	1.0000	1.0000
6.3	0.9000	1.0000	1.0000	1.0000
12.5	0.0000	0.1000	0.0000	0.0000
28.8	0.0000	0.0000	0.0000	0.0000

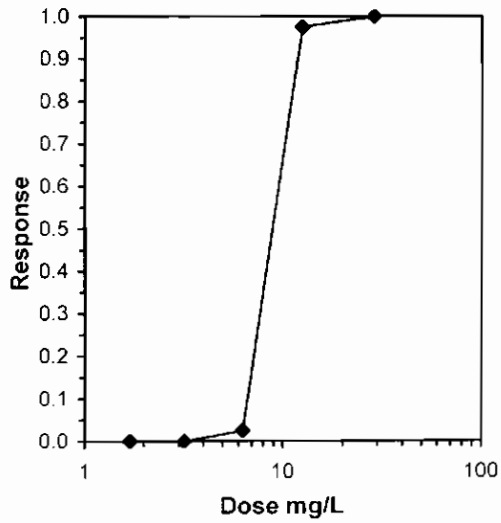
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
1.7	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
3.2	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
6.3	0.9750	0.9750	1.3713	1.2490	1.4120	5.942	4	16.00	10.00	1	40
*12.5	0.0250	0.0250	0.1995	0.1588	0.3218	40.840	4	10.00	10.00	39	40
28.8	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4			40	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.81129	0.868	-6E-14	4.06699
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	6.3	12.5	8.87412	

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	8.8916	8.4547	9.3511
5.0%	8.8741	8.6535	9.1004
10.0%	8.8741	8.6535	9.1004
20.0%	8.8741	8.6535	9.1004
Auto-0.0%	8.8916	8.4547	9.3511



686-44

Test: AT-Acute 96-hr Toxicity Test	Test ID: 686-44
Species: LY-Lampetra sp.	Protocol: -NAS-686-LAMPREY FT ACUTE
Sample ID:	Sample Type: Diazinon
Start Date: 1/16/08 16:00 End Date: 1/20/08 16:00	Lab ID: ORNAS-Northwestern Aquatic Sciences

Pos	ID	Rep	Group	Start	24 Hr	48 Hr	72 Hr	96 Hr	Notes
	1	1	D-Control	10				10	
	2	2	D-Control	10				10	
	3	3	D-Control	10				10	
	4	4	D-Control	10				10	
	5	1	1.700	10				10	
	6	2	1.700	10				10	
	7	3	1.700	10				10	
	8	4	1.700	10				10	
	9	1	3.200	10				10	
	10	2	3.200	10				10	
	11	3	3.200	10				10	
	12	4	3.200	10				10	
	13	1	6.300	10				9	
	14	2	6.300	10				10	
	15	3	6.300	10				10	
	16	4	6.300	10				10	
	17	1	12.500	10				0	
	18	2	12.500	10				1	
	19	3	12.500	10				0	
	20	4	12.500	10				0	
	21	1	28.800	10				0	
	22	2	28.800	10				0	
	23	3	28.800	10				0	
	24	4	28.800	10				0	

Comments: