Nautilus Environmental, LLC

Whole Effluent Toxicity Test Report: Shell Seattle Terminal; Harbor Island

July 2008

Report date: August 5, 2008

Submitted to:

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1.0 INTRODUCTION

Acute and chronic whole effluent toxicity tests were conducted using effluent samples collected from Shell Harbor Island Oil Refinery in July 2008. Acute bioassays were conducted using the test organisms *Menidia beryllina* (inland silverside) and *Americamysis bahia* (a mysid shrimp and formerly known as *Mysidopsis bahia*). Chronic testing was conducted using *A. bahia* and *Atherinops affinis* (Pacific topsmelt). Testing was performed at Nautilus Environmental's Washington Laboratory located in Tacoma, Washington.

2.0 METHODS

2.1 Sample Collection and Transport

Effluent samples were collected into LDPE cubitainers by PES Environmental personnel. The samples were packed in coolers containing ice and transported to Nautilus the days of collection. Appropriate chain-of-custody procedures were employed during collection and transport.

2.2 Sample Receipt

Nautilus staff checked the samples immediately after arrival at the laboratory and verified they were in good condition and matched information provided on the chain-of-custody forms. Receipt temperatures were measured and recorded on the chain-of-custody form for each sample. Standard water quality parameters consisting of dissolved oxygen (DO), pH, conductivity, alkalinity, hardness, total chlorine, and total ammonia were measured and recorded on a sample check-in sheet provided in Appendix F. Samples were stored at 4°C in the dark until used for testing.

2.3 Test Methods

Acute toxicity tests were conducted using *M. beryllina* and *A. bahia* according to procedures presented by USEPA (2002a). Chronic toxicity tests were conducted according to USEPA (2002b) procedures for *A. bahia* and USEPA (1995) procedures for *A. affinis*. Test methods are summarized in Tables 1 through 4. The methods are the most recently published EPA methods.

Table 1. Summary of methods for the 48h *Americamysis bahia* acute survival test.

Test initiation date and time	7/15/2008; 1500h
Test termination date and time	7/17/2008; 1400h
Test organism	*Americamysis Bahia*
Test organism source	Aquatic BioSystems; Fort Collins, CO
Test organism age	3 days post hatch
Test duration	48 hours with solution renewal at 24 hours
Feeding	*Artemia* nauplii during holding time and 2 hours prior to solution renewal
Test chamber	250 mL plastic cup
Test solution volume	200 mL
Test temperature	25 ± 1°C
Dilution water	Crystal Sea Marine Mix artificial seawater
Salinity	30 ± 2 ppt
Test concentrations (% sample)	100, 50, 25, 12.5, 6.25, laboratory control
Number of organisms/chamber	10
Number of replicates	4
Photoperiod	16 hours light/8 hours dark
Aeration	Sample aerated prior to dilution preparation
Test protocol	EPA-821-R-02-012
Test acceptability criterion for controls	≥ 90% survival
Reference toxicant	Copper chloride

Table 2. Summary of methods for the 96h *Menidia beryllina* acute survival test.

Test initiation date and time	7/15/2008; 1615h
Test termination date and time	7/19/2008; 1630h
Test organism	*Menidia beryllina*
Test organism source	Aquatic BioSystems; Fort Collins, CO
Test organism age	10 days post hatch
Test duration	96 hours with solution renewal at 48 hours
Feeding	*Artemia* nauplii during holding time and 2 hours prior to solution renewal
Test chamber	1 L glass jar
Test solution volume	250 mL
Test temperature	20 ± 1°C
Dilution water	Crystal Sea Marine Mix artificial seawater
Salinity	30 ± 2 ppt
Test concentrations (% sample)	100, 50, 25, 12.5, 6.25, laboratory control
Number of organisms/chamber	10
Number of replicates	4
Photoperiod	16 hours light/8 hours dark
Aeration	Sample aerated prior to dilution preparation
Test protocol	EPA-821-R-02-012
Test acceptability criterion for controls	≥ 90% survival
Reference toxicant	Copper chloride

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Table 3. Summary of methods for the *Americamysis bahia* 7-day survival and growth test.

Test initiation date and time	7/15/2008; 1430h
Test termination date and time	7/22/2008; 1430h
Test Type	Static renewal
Endpoint	Survival and growth at 7 days
Test organism	*Americamysis bahia*
Test organism source	Aquatic BioSystems; Fort Collins, CO
Test organism age	7 days post-hatch
Feeding	*Artemia* nauplii, twice daily
Test chamber and solution volume	250 mL plastic cup
Test solution volume	200 mL
Test temperature	26 ± 1°C
Dilution water	Crystal Sea Marine Mix artificial seawater
Salinity	30 ± 2 ppt
Test concentrations (% sample)	100, 50, 25, 12.5, 6.25, laboratory control
Number of organisms/chamber	5
Number of replicates	8
Photoperiod	16 hours light/8 hours dark
Aeration	Sample aerated prior to dilution preparation
Test protocol	EPA-821-R-02-014
Test acceptability criteria for controls	≥ 80% survival; average dry weight ≥ 0.20 mg
Reference toxicant	Copper chloride

Table 4. Summary of methods for the *Atherinops affinis* 7-day survival and growth test.

Test initiation date and time	7/15/2008; 1445h
Test termination date and time	7/22/2008; 1330h
Test Type	Static renewal
Endpoint	Survival and growth at 7 days
Test organism	*Atherinops affinis*
Test organism source	Aquatic BioSystems; Fort Collins, CO
Test organism age	10 days post-hatch
Feeding	*Artemia* nauplii, twice daily
Test chamber	1 L plastic cups
Test solution volume	500 mL
Test temperature	20 ± 1°C
Dilution water	Crystal Sea Marine Mix artificial seawater
Salinity	30 ± 2 ppt
Test concentrations (% sample)	100, 50, 25, 12.5, 6.25, laboratory control
Number of organisms/chamber	5
Number of replicates	5
Photoperiod	16 hours light/8 hours dark
Aeration	Sample aerated prior to dilution preparation
Test protocol	EPA-600-R-95-136
Test acceptability criteria for controls	≥ 80% survival; average dry weight ≥ 0.85 mg
Reference toxicant	Copper chloride

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3.0 RESULTS

Details of standard water quality measurements conducted upon receipt of samples are provided in Table 5.

Table 5. Sample information.

Sample ID	Wet Test-1-071408	Wet Test-2-071608	Wet Test-3-071808
Nautilus Log-In Number	08-194	08-195	08-203
Collection date and time	7/14/2008; 1000h	7/16/2008; 0900h	7/18/2008; 0915h
Receipt date and time	7/14/2008; 1040h	7/16/2008; 1005h	7/18/2008; 1055h
Receipt temperature (°C)	19.8	9.1	10.0
Dissolved oxygen (mg/L)	4.8	4.5	4.4
pH	6.94	6.76	6.93
Conductivity (µS/cm)	90	86	85
Hardness (mg/L $CaCO_3$)	44	56	56
Alkalinity (mg/L $CaCO_3$)	56	48	52
Total Chlorine (mg/L)	<0.03	<0.03	<0.03
Total Ammonia (mg/L)	<1.0	<1.0	<1.0

Survival was evaluated in the acute toxicity tests after 48 and 96 hours of exposure for *A. bahia* and *M. beryllina,* respectively. Results are summarized in Table 6.

The mysid shrimp test involved a 48-hour static-renewal exposure to the effluent. The endpoint for this test was survival at the end of the 48-hour exposure. Mysid survival was significantly reduced in the 100 percent sample concentration as compared to the laboratory control. However, the difference was less than 10 percent, and adjusting the level of significance to 0.01 for the small difference in response indicates the difference was not statistically significant. There was no toxicity at any concentration in the *Menidia* acute test.

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Table 6. Summary of results for the acute toxicity tests.

Species	Concentration (%)	Percent Survival	Significance Level	NOEC[a] (% effluent)	LOEC[b] (% effluent)	LC_{50} (% effluent)
A. bahia (mysid shrimp)	0.0	100	α=0.05	50	100	>100
	6.25	100	α=0.01	100	>100	>100
	12.5	97.5				
	25	95.0				
	50	97.5				
	100	92.5				
M. beryllina (silverside minnows)	0.0	97.5	α=0.05	100	>100	>100
	6.25	92.5				
	12.5	95.0				
	25	97.5				
	50	97.5				
	100	90.0				

[a]No Observed Effect Concentration, [b]Lowest Observed Effect Concentration

Results for the chronic toxicity tests are summarized in Table 7. The *A.bahia* and *A. affinis* tests involved a 7-day static-renewal exposure to the effluent. The endpoints for these tests were survival and growth (evaluated on the basis of dry weight divided by initial count) at the end of the 7-day exposure.

For the mysid shrimp test, the highest concentration with no observed effect (NOEC) was 100 percent sample for survival. Mysid growth was significantly reduced in the 50 percent test concentration compared to the laboratory control. The difference was less than 20 percent, however, and adjusting the level of significance to 0.01 for the small difference in response indicates the difference was not statistically significant.

For the Pacific topsmelt test, no statistically significant difference in response occurred in any concentration relative to control data for either survival or growth, including the acute and chronic critical effluent concentrations (ACEC and CCEC) of 100 percent sample.

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Table 7. Summary of results for the chronic toxicity tests.

Test Species	Endpoint	NOEC[a] (% effluent)	LOEC[b] (% effluent)
A. bahia (mysid shrimp)	Survival	100	>100
	Growth (α=0.05)	25	50
	Growth (α=0.01)	100	>100
A. affinis (topsmelt)	Survival	100	>100
	Growth	100	>100

[a]No Observed Effect Concentration, [b] Lowest Observed Effect Concentration

4.0 QA/QC

The samples were received in good condition and within the temperature range specified by WDOE (2005). The toxicity tests met all acceptability criteria for performance of control organisms. The sample was aerated prior to test initiation and renewals, in order to avert low dissolved oxygen concentration in the tests due to low dissolved oxygen levels in the client sample. For the mysid chronic test, replicate 6 in the 6.25 percent sample concentration was analyzed with a starting number of 4 instead of 5 animals, due to one fatality not related to sample toxicity. There were no other deviations from the protocols and all water quality parameters remained within the ranges specified in the corresponding test methods throughout the tests.

Results for the reference toxicant tests used to monitor laboratory performance and test organism sensitivity are summarized in Table 8. The results for the reference toxicant tests fell within the acceptable range of mean ± two standard deviations of historical test results, indicating that the tests organisms were of an appropriate degree of sensitivity. The coefficients of variation (CV) for the tests are also shown in the table.

Table 8. Reference toxicant test results.

Species	Date initiated	Endpoint	EC_{50} (µg/L copper)	Acceptable Range (µg/L copper)	CV (%)
A. bahia	7/16/2008	48h Survival	269	33.2-825	46.1
M. beryllina	7/16/2008	96h Survival	283	44.2-413	40.3
A. bahia	7/23/2008	7d Survival	238	131-475	28.4
A. bahia	7/23/2008	Growth	221	144-372	22.1
A. affinis	7/23/2008	7d Survival	48.7	31.7-143	31.9
A. affinis	7/23/2008	Growth	53.6	26.9-153	35.0

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REFERENCES

Tidepool Scientific Software. 2000-2007. CETIS Comprehensive Environmental Toxicity Information System Software, Version 1.6.3revG.

USEPA. 2002a. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition. EPA-821-R-02-012.

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

USEPA. 1995. Short-Term Method for Estimating the Chronic Toxicity of Effluents and Receiving Waters to the West Coast Marine and Estuarine Organisms. EPA-600-R-95-136.

WDOE. 2005. Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria. Washington State Department of Ecology. Water Quality Program. Publication number: WQ-R-95-80, Revised June 2005.

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Appendix A

***Americamysis bahia* (Mysid Shrimp) Acute Toxicity Test**

Statistical Summaries and Raw Bench Sheets

CETIS Summary Report

Report Date: 31 Jul-08 16:07 (p 1 of 1)
Link/Link Code: 18-2956-1945/0807-T024

Mysid Acute — **Nautilus Environmental WA**

Test Run No:	14-2399-4093	**Test Type:**	Survival (48h)	**Analyst:**	Indira Santiago
Start Date:	15 Jul-08 15:00	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Artificial Saltwater
Ending Date:	17 Jul-08 14:00	**Species:**	Americamysis bahia	**Brine:**	Crystal Sea Marine Mix
Duration:	47h	**Source:**	Aquatic Biosystems, CO	**Age:**	3d

Sample No:	21-2313-1954	**Code:**	08-194	**Client:**	
Sample Date:	14 Jul-08 10:00	**Material:**	Oil Refinery Effluent	**Project:**	
Receive Date:	14 Jul-08 10:40	**Source:**	Shell Seattle Terminal (WA0001791)		
Sample Age:	29h (19.8 °C)	**Station:**			

Comparison Summary

Analysis No	Endpoint	NOEL	LOEL	TOEL	PMSD	Method
02-8730-8475	48h Survival Rate	α 0.01 100	> 100	N/A	9.64%	Dunnett's Multiple Comparison Test
05-6868-5388		α 0.05 50	100	70.7	7.46%	Dunnett's Multiple Comparison Test

Point Estimate Summary

Analysis No	Endpoint	Effect-%	Conc-%	95% LCL	95% UCL	Method
02-3772-4859	48h Survival Rate	25	> 100	N/A	N/A	Linear Interpolation (ICPIN)
		50	> 100	N/A	N/A	

48h Survival Rate Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	4	1	1	1	1	1	0	0	0.0%	0.0%
6.25		4	1	1	1	1	1	0	0	0.0%	0.0%
12.5		4	0.975	0.956	0.994	0.9	1	0.00913	0.05	5.13%	2.5%
25		4	0.95	0.928	0.972	0.9	1	0.0105	0.0577	6.08%	5.0%
50		4	0.975	0.956	0.994	0.9	1	0.00913	0.05	5.13%	2.5%
100		4	0.925	0.906	0.944	0.9	1	0.00913	0.05	5.41%	7.5%

48h Survival Rate Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
6.25		1	1	1	1
12.5		1	0.9	1	1
25		1	0.9	1	0.9
50		1	1	1	0.9
100		0.9	0.9	1	0.9

CETIS Analytical Report

Report Date: 31 Jul-08 16:07 (p 1 of 4)
Link/Link Code: 18-2956-1945/0807-T024

Mysid Acute — **Nautilus Environmental WA**

Analysis No:	02-8730-8475	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv1.6.3
Analyzed:	31 Jul-08 16:06	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Monte Carlo	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)		C > T	Not Run	100	>100	N/A	1	9.64%

Dunnett's Multiple Comparison Test

Control	vs	Conc-%	Test Stat	Critical	MSD	P-Value	Decision(1%)
Dilution Water		6.25	0	3.21	0.157	0.8330	Non-Significant Effect
		12.5	0.832	3.21	0.157	0.4980	Non-Significant Effect
		25	1.66	3.21	0.157	0.1790	Non-Significant Effect
		50	0.832	3.21	0.157	0.4980	Non-Significant Effect
		100	2.5	3.21	0.157	0.0422	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(5%)
Between	0.0453722	0.0090744	5	1.89	0.1460	Non-Significant Effect
Error	0.0863178	0.0047954	18			
Total	0.13169	0.0138699	23			

ANOVA Assumptions

Attribute	Test	Test Stat	Critical	P-Value	Decision(1%)
Variances	Mod Levene Equality of Varianc	1.13	4.25	0.3790	Equal Variances
Distribution	Shapiro-Wilk Normality	0.943		0.1880	Normal Distribution

48h Survival Rate Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	4	1	1	1	1	1	0	0	0.0%	0.0%
6.25		4	1	1	1	1	1	0	0	0.0%	0.0%
12.5		4	0.975	0.956	0.994	0.9	1	0.00928	0.05	5.13%	2.5%
25		4	0.95	0.928	0.972	0.9	1	0.0107	0.0577	6.08%	5.0%
50		4	0.975	0.956	0.994	0.9	1	0.00928	0.05	5.13%	2.5%
100		4	0.925	0.906	0.944	0.9	1	0.00928	0.05	5.41%	7.5%

Angular (Corrected) Transformed Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	4	1.41	1.41	1.41	1.41	1.41	0	0	0.0%	0.0%
6.25		4	1.41	1.41	1.41	1.41	1.41	0	0	0.0%	0.0%
12.5		4	1.37	1.34	1.4	1.25	1.41	0.0151	0.0815	5.94%	2.89%
25		4	1.33	1.29	1.37	1.25	1.41	0.0175	0.0941	7.07%	5.77%
50		4	1.37	1.34	1.4	1.25	1.41	0.0151	0.0815	5.94%	2.89%
100		4	1.29	1.26	1.32	1.25	1.41	0.0151	0.0815	6.32%	8.66%

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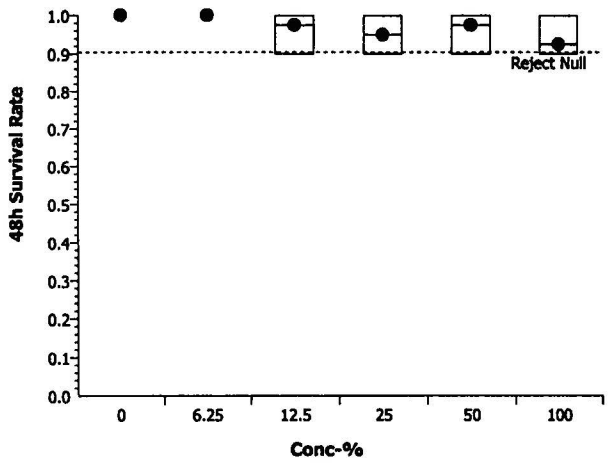
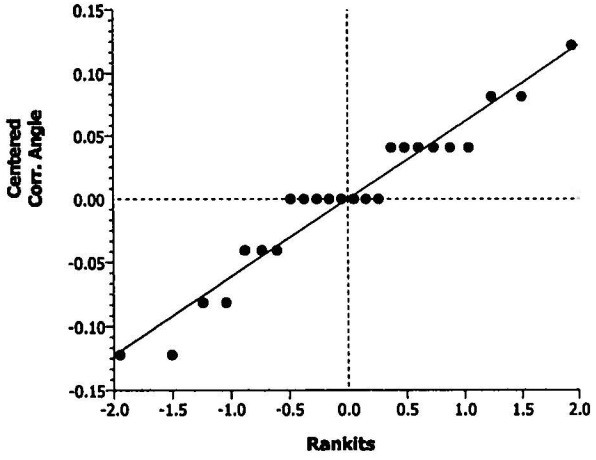
Mysid Acute — **Nautilus Environmental WA**

Analysis No:	02-8730-8475	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv1.6.3
Analyzed:	31 Jul-08 16:06	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





Mysid Acute — **Nautilus Environmental WA**

Analysis No:	05-6868-5388	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv1.6.3
Analyzed:	31 Jul-08 13:52	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Monte Carlo	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)		C > T	Not Run	50	100	70.7	2	7.46%

Dunnett's Multiple Comparison Test

Control	vs	Conc-%	Test Stat	Critical	MSD	P-Value	Decision(5%)
Dilution Water		6.25	0	2.41	0.118	0.8330	Non-Significant Effect
		12.5	0.832	2.41	0.118	0.4980	Non-Significant Effect
		25	1.66	2.41	0.118	0.1790	Non-Significant Effect
		50	0.832	2.41	0.118	0.4980	Non-Significant Effect
		100*	2.5	2.41	0.118	0.0422	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(5%)
Between	0.0453722	0.0090744	5	1.89	0.1460	Non-Significant Effect
Error	0.0863178	0.0047954	18			
Total	0.13169	0.0138699	23			

ANOVA Assumptions

Attribute	Test	Test Stat	Critical	P-Value	Decision(1%)
Variances	Mod Levene Equality of Varianc	1.13	4.25	0.3790	Equal Variances
Distribution	Shapiro-Wilk Normality	0.943		0.1880	Normal Distribution

48h Survival Rate Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	4	1	1	1	1	1	0	0	0.0%	0.0%
6.25		4	1	1	1	1	1	0	0	0.0%	0.0%
12.5		4	0.975	0.956	0.994	0.9	1	0.00928	0.05	5.13%	2.5%
25		4	0.95	0.928	0.972	0.9	1	0.0107	0.0577	6.08%	5.0%
50		4	0.975	0.956	0.994	0.9	1	0.00928	0.05	5.13%	2.5%
100		4	0.925	0.906	0.944	0.9	1	0.00928	0.05	5.41%	7.5%

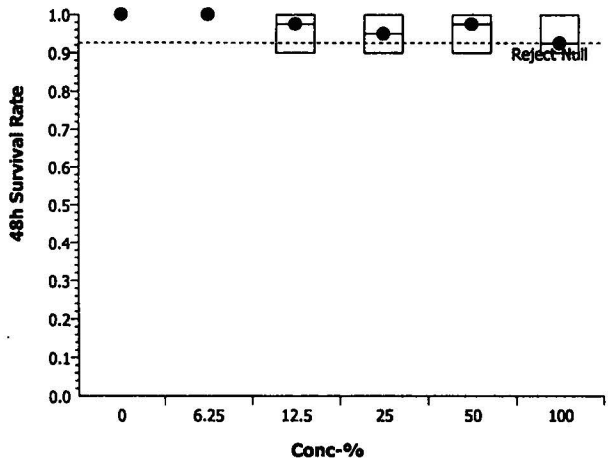
Angular (Corrected) Transformed Summary

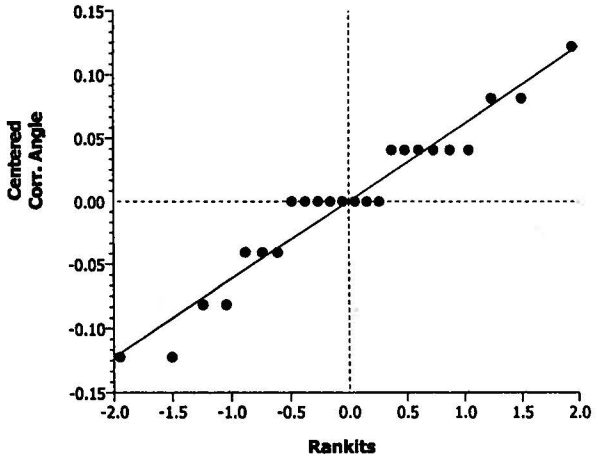
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	4	1.41	1.41	1.41	1.41	1.41	0	0	0.0%	0.0%
6.25		4	1.41	1.41	1.41	1.41	1.41	0	0	0.0%	0.0%
12.5		4	1.37	1.34	1.4	1.25	1.41	0.0151	0.0815	5.94%	2.89%
25		4	1.33	1.29	1.37	1.25	1.41	0.0175	0.0941	7.07%	5.77%
50		4	1.37	1.34	1.4	1.25	1.41	0.0151	0.0815	5.94%	2.89%
100		4	1.29	1.26	1.32	1.25	1.41	0.0151	0.0815	6.32%	8.66%

Mysid Acute — **Nautilus Environmental WA**

Analysis No:	05-6868-5388	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv1.6.3
Analyzed:	31 Jul-08 13:52	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Report Date: 31 Jul-08 16:07 (p 1 of 1)
Link/Link Code: 18-2956-1945/0807-T024

Mysid Acute — **Nautilus Environmental WA**

Analysis No:	02-3772-4859	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv1.6.3
Analyzed:	31 Jul-08 13:53	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	7055475	280	Yes	Two-Point Interpolation

Point Estimates

Effect-%	Conc-%	95% LCL	95% UCL
25	> 100	N/A	N/A
50	> 100	N/A	N/A

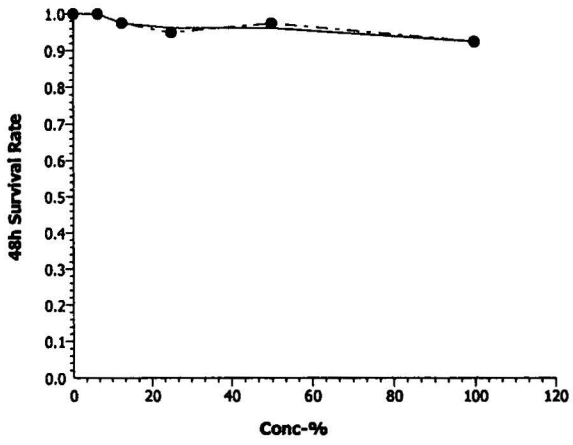
48h Survival Rate Summary

			Calculated Variate(A/B)								
Conc-%	**Control Type**	**Count**	**Mean**	**Min**	**Max**	**Std Err**	**Std Dev**	**CV%**	**Diff%**	**A**	**B**
0	Dilution Water	4	1	1	1	0	0	0.0%	0.0%	40	40
6.25		4	1	1	1	0	0	0.0%	0.0%	40	40
12.5		4	0.975	0.9	1	0.00913	0.05	5.13%	2.5%	39	40
25		4	0.95	0.9	1	0.0105	0.0577	6.08%	5.0%	38	40
50		4	0.975	0.9	1	0.00913	0.05	5.13%	2.5%	39	40
100		4	0.925	0.9	1	0.00913	0.05	5.41%	7.5%	37	40

48h Survival Rate Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
6.25		1	1	1	1
12.5		1	0.9	1	1
25		1	0.9	1	0.9
50		1	1	1	0.9
100		0.9	0.9	1	0.9

Graphics



Saltwater Acute
48 Hour Toxicity Test Data Sheet
Nautilus Environmental

Client: Shell- Harbor Island
Sample ID: Wet Test 1-071408
Test # 0807- T024

Start Date & Time: 7/15/08 1500
End Date & Time: 7/17/08 1400
Test Organisms: Americamysis bahia

Conc. or %	Rep #	Cont. #	Number of Live Organisms 0	24	48	Dissolved Oxygen (mg/L) 0	Fin. 24	Init. 24	48	pH (units) 0	Fin. 24	Init. 24	48	Salinity (ppt) 0	Fin. 24	Init. 24	48	Temperature (°C) 0	Fin. 24	Init. 24	48	Mean Percent Survival
CON	1	22	10	10	10	6.0	5.7	6.6	6.0	8.57	8.14	8.50	8.24	28.3	29.0	29.2	28.7	24.3	24.8	25.2	25.0	
	2	11	10	10	10																	
	3	18	10	10	10																	
	4	23	10	10	10																	
6.25	1	21	10	10	10	6.3	5.7	6.7	5.9	8.55	8.13	8.52	8.20	28.6	29.2	28.8	29.4	24.5	24.3	25.1	25.0	
	2	15	10	10	10																	
	3	7	10	10	10																	
	4	16	10	10	10																	
12.5	1	13	10	10	10	6.4	5.8	6.7	6.0	8.52	8.11	8.55	8.24	28.8	29.2	28.8	29.8	24.3	24.5	25.3	24.9	
	2	20	10	9	9																	
	3	24	10	10	10																	
	4	6	10	10	10																	
25	1	14	10	10	10	6.2	5.8	6.7	5.9	8.49	8.12	8.55	8.26	28.9	29.0	28.3	29.3	24.2	24.5	25.3	25.0	
	2	1	10	9	9																	
	3	9	10	10	10																	
	4	3	10	10	9																	
50	1	5	10	10	10	6.3	5.7	6.7	5.9	8.37	8.12	8.55	8.27	29.2	29.5	28.6	29.4	24.3	24.4	25.2	25.1	
	2	4	10	10	10																	
	3	2	10	10	10																	
	4	8	10	9	9																	
100	1	17	10	9	9	6.2	5.6	6.8	5.7	8.19	8.10	8.57	8.35	29.9	29.9	30.8	30.9	24.0	24.6	25.2	25.1	
	2	10	10	9	9																	
	3	19	10	10	10																	
	4	12	10	9	9																	
Technician Initials			BP	MM	ST	CC	MM	ST	ST													

Dilution Water Batch #: 039 ASW

Sample Description:

Comments: 0 hrs: Sample aerated prior to dilutions
24 hrs.
48 hrs.

Organism source: ABS
Date Received: 7/15/08
Date of Hatch: 7/12/08

QA check: LS

Nautilus Environmental
5009 Pacific Hwy. E. Suite 2
Tacoma, WA 98424
(253) 922-4296

Appendix B

***Menidia beryllina* (Silverside Minnows) Acute Toxicity Test**

Statistical Summaries and Raw Bench Sheets

CETIS Summary Report

Report Date: 31 Jul-08 14:06 (p 1 of 1)
Link/Link Code: 07-9006-9783/0807-T025

Inland Silverside 96-h Acute Survival Test — **Nautilus Environmental WA**

Test Run No:	19-4002-8835	**Test Type:**	Survival (96h)	**Analyst:**	Indira Santiago
Start Date:	15 Jul-08 16:15	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Artificial Saltwater
Ending Date:	19 Jul-08 16:30	**Species:**	Menidia beryllina	**Brine:**	Crystal Sea Marine Mix
Duration:	4d 0h	**Source:**	Aquatic Biosystems, CO	**Age:**	10d

Sample No:	21-2313-1954	**Code:**	08-194	**Client:**	
Sample Date:	14 Jul-08 10:00	**Material:**	Oil Refinery Effluent	**Project:**	
Receive Date:	14 Jul-08 10:40	**Source:**	Shell Seattle Terminal (WA0001791)		
Sample Age:	30h (19.8 °C)	**Station:**			

Comparison Summary

Analysis No	Endpoint	NOEL	LOEL	TOEL	PMSD	Method
18-3539-2395	96h Survival Rate	100	> 100	N/A	12.8%	Dunnett's Multiple Comparison Test

Test Acceptability

Analysis No	Endpoint	Attribute	Test Stat	Acceptabilty Limits	Overlap	Decision
18-3539-2395	96h Survival Rate	Control Resp	0.975	0.9 - NL	Yes	Passes acceptability criteria

96h Survival Rate Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	4	0.975	0.956	0.994	0.9	1	0.00913	0.05	5.13%	0.0%
6.25		4	0.925	0.889	0.961	0.8	1	0.0175	0.0957	10.4%	5.13%
12.5		4	0.95	0.928	0.972	0.9	1	0.0105	0.0577	6.08%	2.56%
25		4	0.975	0.956	0.994	0.9	1	0.00913	0.05	5.13%	0.0%
50		4	0.975	0.956	0.994	0.9	1	0.00913	0.05	5.13%	0.0%
100		4	0.9	0.857	0.943	0.8	1	0.0211	0.115	12.8%	7.69%

96h Survival Rate Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	0.9
6.25		1	0.8	0.9	1
12.5		0.9	1	1	0.9
25		1	0.9	1	1
50		0.9	1	1	1
100		0.8	1	0.8	1

Nautilus Environmental
Washington Laboratory
5009 Pacific Hwy. E., Suite 2
Tacoma, WA 98424

96 Hour Toxicity Test Data Sheet
Saltwater 96-hr Acute with Renewal

Client: Shell-Harbor Island
Sample ID: WET Test 1 - 071408
Test # ~~0706~~ 0807-T025
Nautilus Check-In #: 08-194

Start Date & Time: 7/15/08 1615
End Date & Time: 7/19/08 1630
Test Organism: Menidia beryllina

Sample Conc. or %	D.O. (mg/L) Init. 0	24	Fin. 48	Init. 48	72	Fin. 96	pH (mg/L) Init. 0	24	Fin. 48	Init. 48	72	Fin. 96
CON	6.4	6.2	6.5	6.8	6.8	6.9	8.54	8.26	8.09	8.73	8.41	8.04
6.25	6.1	6.0	6.7	6.7	6.8	6.8	8.54	8.27	8.10	8.74	8.45	8.11
12.5	6.4	6.2	6.2	6.7*	~~7.0~~	6.8	8.53	8.31	8.11	8.71	8.43	8.12
25	6.4	6.1	6.1	6.8	6.8	6.9	8.50	8.28	8.14	8.66	8.36	8.12
50	6.5	6.2	6.0	6.8*	~~6.8~~	6.8	8.42	8.22	8.15	8.55	8.33	8.14
100	6.1	6.2	6.3	6.8	6.8	6.8	8.23	8.15	8.17	8.22	8.16	8.15

*6.8

Sample Conc. or %	Salinity ppt Init. 0	24	Fin. 48	Init. 48	~~Fin.~~ 72	Fin. 96	Test Temperature (°C) Init. 0	24	Fin. 48	Init. 48	72	Fin. 96
CON	28.3	28.4	28.3	28.4	29.0	28.5	20.4	19.9	20.3	20.5	20.1	20.0
6.25	28.5	28.4	28.3	28.6	29.1	28.8	20.0	20.3	20.3	20.4	20.0	20.0
12.5	28.6	28.7	28.4	28.6	29.1	28.9	20.2	20.2	20.0	20.4	20.1	19.9
25	28.7	28.9	29.0	28.6	29.0	29.9	19.9	19.8	19.8	20.4	20.1	19.9
50	28.9	28.8	29.7	28.6	29.0	29.5	19.5	20.2	19.8	20.3	20.1	19.7
100	29.9	29.9	29.8	28.5	28.8	29.5	19.4	20.2	19.7	20.4	20.1	19.7
Tech. Initials	BP	[illegible]	MM	MM	[illegible]	[illegible]						
Sample Used:	#1 08-194			08-195								

Sample Conc. or %	Rep #	Cont #	Number of Live Organisms 0	24	48	72	96
CON	1	1	10	10	10	10	~~9~~ 10
	2	23	10	10	10	10	~~9~~ 10
	3	15	10	10	10	10	10
	4	7	10	10	9	9	9
6.25	1	9	10	10	10	10	10
	2	13	10	10	9	9	8
	3	14	10	9	9	9	9
	4	19	10	10	10	10	10
12.5	1	3	10	10	9	9	9
	2	20	10	10	10	10	10
	3	17	10	10	10	10	10
	4	22	10	9	9	9	9
25	1	11	10	10	10	10	10
	2	16	10	10	9	9	9
	3	4	10	10	10	10	10
	4	5	10	10	10	10	10
50	1	18	10	10	10	10	9
	2	12	10	10	10	10	10
	3	21	10	10	10	10	10
	4	24	10	10	10	10	10
100	1	2	10	10	10	10	8
	2	16	10	10	10	10	10
	3	6	10	10	8	8	8
	4	8	10	10	10	10	10
Tech. Initials			[illegible]	[illegible]	MM	[illegible]	[illegible]

Dilution Water Batch #: ASW 039

Sample Description: Sample aerated prior to dilutions
Comments:
QA Check: 105

Organism Source: ABS
Date Received: 7/15/08
Date of Hatch: 7/15/08

Appendix C

***Americamysis bahia* (Mysid Shrimp) Chronic Test**

Statistical Summaries and Raw Bench Sheets

CETIS Summary Report

Report Date: 05 Aug-08 07:37 (p 1 of 2)
Link/Link Code: 04-0973-9523/0807-T026

Mysidopsis 7-d Survival, Growth and Fecundity Test — **Nautilus Environmental WA**

Test Run No:	04-0345-8018	**Test Type:**	Growth-Survival-Fec (7d)	**Analyst:**	Indira Santiago
Start Date:	15 Jul-08 14:30	**Protocol:**	EPA/821/R-02-014 (2002)	**Diluent:**	Artificial Saltwater
Ending Date:	22 Jul-08 14:30	**Species:**	Americamysis bahia	**Brine:**	Crystal Sea Marine Mix
Duration:	7d 0h	**Source:**	Aquatic Biosystems, CO	**Age:**	7d

Sample No:	21-2313-1954	**Code:**	08-194	**Client:**	
Sample Date:	14 Jul-08 10:00	**Material:**	Oil Refinery Effluent	**Project:**	
Receive Date:	14 Jul-08 10:40	**Source:**	Shell Seattle Terminal (WA0001791)		
Sample Age:	28h (19.8 °C)	**Station:**			

Sample Renewals

Renewal	Sample Code	Sample Date	Receive Date	Renewal Date	Temp °C
1	08-195	16 Jul-08 09:00	16 Jul-08 10:05	17 Jul-08 13:00	9.1
2	08-203	18 Jul-08 09:15	18 Jul-08 10:55	19 Jul-08 13:00	10

Comments: Replicate 6 in the 6.25 percent sample concentration analyzed with starting count of 4 due to one fatality not related to sample toxicity.

Comparison Summary

Analysis No	Endpoint		NOEL	LOEL	TOEL	PMSD	Method
13-5330-3560	7d Survival Rate		100	> 100	N/A	13.1%	Steel Many-One Rank Test
12-0613-3514	Mean Dry Biomass-mg	α 0.05	25	50	35.4	16.7%	Dunnett's Multiple Comparison Test
12-1064-7806		α 0.01	100	> 100	N/A	21.6%	Dunnett's Multiple Comparison Test

Point Estimate Summary

Analysis No	Endpoint	Effect-%	Conc-%	95% LCL	95% UCL	Method
08-0617-7939	Mean Dry Biomass-mg	25	> 100	N/A	N/A	Linear Interpolation (ICPIN)
		50	> 100	N/A	N/A	

Test Acceptability

Analysis No	Endpoint	Attribute	Test Stat	Acceptability Limits	Overlap	Decision
13-5330-3560	7d Survival Rate	Control Resp	0.975	0.8 - NL	Yes	Passes acceptability criteria
08-0617-7939	Mean Dry Biomass-mg	Control Resp	0.41	0.2 - NL	Yes	Passes acceptability criteria
12-0613-3514	Mean Dry Biomass-mg	Control Resp	0.41	0.2 - NL	Yes	Passes acceptability criteria
12-1064-7806	Mean Dry Biomass-mg	Control Resp	0.41	0.2 - NL	Yes	Passes acceptability criteria
12-0613-3514	Mean Dry Biomass-mg	PMSD	0.167	0.11 - 0.37	Yes	Passes acceptability criteria
12-1064-7806	Mean Dry Biomass-mg	PMSD	0.216	0.11 - 0.37	Yes	Passes acceptability criteria

7d Survival Rate Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	8	0.975	0.949	1	0.8	1	0.0129	0.0707	7.25%	0.0%
6.25		8	0.975	0.949	1	0.8	1	0.0129	0.0707	7.25%	0.0%
12.5		8	0.95	0.897	1	0.6	1	0.0258	0.141	14.9%	2.56%
25		8	0.925	0.869	0.981	0.6	1	0.0272	0.149	16.1%	5.13%
50		8	0.925	0.886	0.964	0.8	1	0.0189	0.104	11.2%	5.13%
100		8	0.95	0.915	0.985	0.8	1	0.0169	0.0926	9.75%	2.56%

Mean Dry Biomass-mg Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	8	0.41	0.396	0.423	0.352	0.448	0.00668	0.0366	8.93%	0.0%
6.25		8	0.396	0.383	0.409	0.34	0.436	0.00627	0.0343	8.67%	3.42%
12.5		8	0.381	0.35	0.411	0.192	0.472	0.0149	0.0816	21.5%	7.14%
25		8	0.406	0.376	0.436	0.268	0.556	0.0148	0.0811	20.0%	0.92%
50		8	0.33	0.31	0.351	0.264	0.42	0.01	0.0548	16.6%	19.4%
100		8	0.349	0.33	0.367	0.26	0.426	0.00888	0.0486	13.9%	14.9%

Mysidopsis 7-d Survival, Growth and Fecundity Test — **Nautilus Environmental WA**

7d Survival Rate Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Dilution Water	1	1	1	1	1	1	0.8	1
6.25		1	1	1	0.8	1	1	1	1
12.5		1	1	1	1	1	0.6	1	1
25		1	0.6	1	1	0.8	1	1	1
50		1	1	1	0.8	0.8	1	1	0.8
100		1	1	0.8	1	1	1	1	0.8

Mean Dry Biomass-mg Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Dilution Water	0.448	0.382	0.378	0.4	0.444	0.432	0.352	0.442
6.25		0.376	0.392	0.366	0.436	0.34	0.42	0.4	0.436
12.5		0.394	0.394	0.38	0.472	0.394	0.192	0.426	0.392
25		0.556	0.268	0.412	0.43	0.362	0.384	0.394	0.442
50		0.346	0.288	0.286	0.39	0.264	0.42	0.346	0.302
100		0.38	0.342	0.26	0.368	0.342	0.356	0.426	0.314

CETIS Analytical Report

Report Date: 31 Jul-08 16:00 (p 1 of 1)
Link/Link Code: 04-0973-9523/0807-T026

Mysidopsis 7-d Survival, Growth and Fecundity Test — **Nautilus Environmental WA**

Analysis No:	08-0617-7939	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.6.3
Analyzed:	31 Jul-08 14:39	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	5334240	280	Yes	Two-Point Interpolation

Point Estimates

Effect-%	Conc-%	95% LCL	95% UCL
25	> 100	N/A	N/A
50	> 100	N/A	N/A

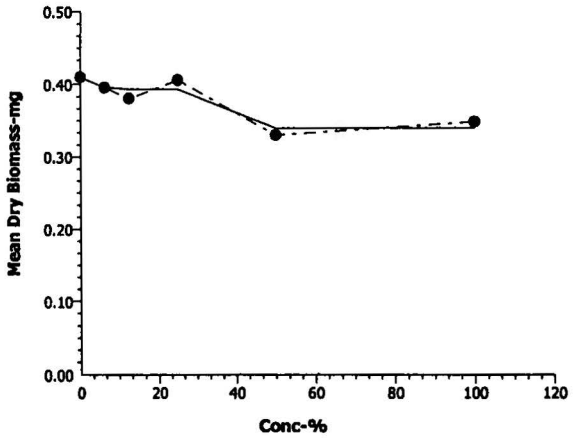
Mean Dry Biomass-mg Summary

			Calculated Variate						
Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	8	0.41	0.352	0.448	0.00668	0.0366	8.93%	0.0%
6.25		8	0.396	0.34	0.436	0.00627	0.0343	8.67%	3.42%
12.5		8	0.381	0.192	0.472	0.0149	0.0816	21.5%	7.14%
25		8	0.406	0.268	0.556	0.0148	0.0811	20.0%	0.92%
50		8	0.33	0.264	0.42	0.01	0.0548	16.6%	19.4%
100		8	0.349	0.26	0.426	0.00888	0.0486	13.9%	14.9%

Mean Dry Biomass-mg Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Dilution Water	0.448	0.382	0.378	0.4	0.444	0.432	0.352	0.442
6.25		0.376	0.392	0.366	0.436	0.34	0.42	0.4	0.436
12.5		0.394	0.394	0.38	0.472	0.394	0.192	0.426	0.392
25		0.556	0.268	0.412	0.43	0.362	0.384	0.394	0.442
50		0.346	0.288	0.286	0.39	0.264	0.42	0.346	0.302
100		0.38	0.342	0.26	0.368	0.342	0.356	0.426	0.314

Graphics



CETIS Analytical Report

Report Date: 31 Jul-08 16:00 (p 1 of 3)
Link/Link Code: 04-0973-9523/0807-T026

Mysidopsis 7-d Survival, Growth and Fecundity Test — **Nautilus Environmental WA**

Analysis No:	12-1064-7806	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.6.3
Analyzed:	31 Jul-08 15:58	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Monte Carlo	NOEL	LOEL	TOEL	TU	PMSD
Untransformed		C > T	Not Run	100	>100	N/A	1	21.6%

Dunnett's Multiple Comparison Test

Control	vs	Conc-%	Test Stat	Critical	MSD	P-Value	Decision(1%)
Dilution Water		6.25	0.472	2.99	0.0886	0.6580	Non-Significant Effect
		12.5	0.986	2.99	0.0886	0.4240	Non-Significant Effect
		25	0.126	2.99	0.0886	0.7930	Non-Significant Effect
		50	2.68	2.99	0.0886	0.0214	Non-Significant Effect
		100	2.06	2.99	0.0886	0.0820	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(5%)
Between	0.0420994	0.0084199	5	2.39	0.0537	Non-Significant Effect
Error	0.147887	0.0035211	42			
Total	0.1899864	0.011941	47			

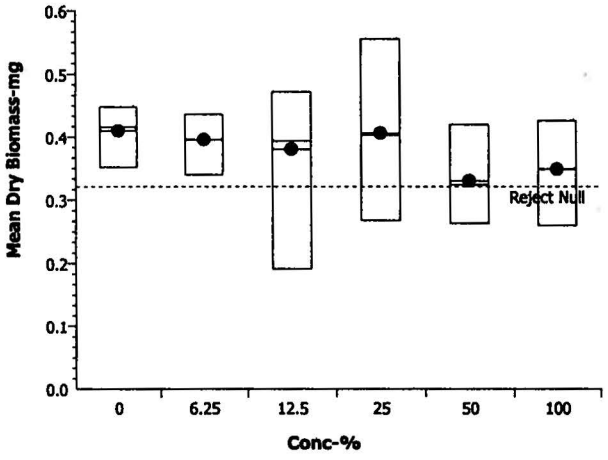
ANOVA Assumptions

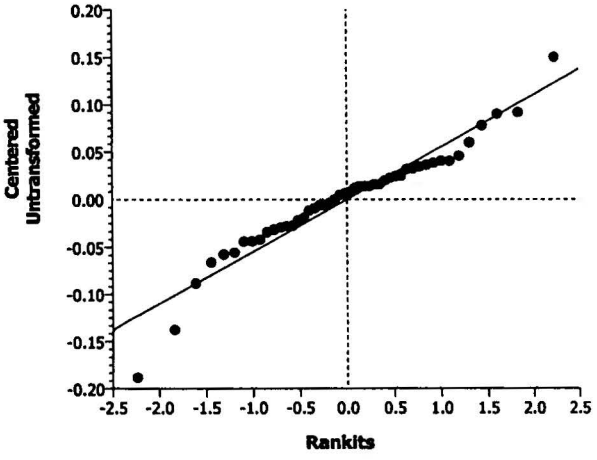
Attribute	Test	Test Stat	Critical	P-Value	Decision(1%)
Variances	Bartlett Equality of Variance	8.99	15.1	0.1090	Equal Variances
Distribution	Shapiro-Wilk Normality	0.945		0.0246	Normal Distribution

Mean Dry Biomass-mg Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	8	0.41	0.396	0.424	0.352	0.448	0.0068	0.0366	8.93%	0.0%
6.25		8	0.396	0.383	0.409	0.34	0.436	0.00637	0.0343	8.67%	3.42%
12.5		8	0.381	0.349	0.412	0.192	0.472	0.0152	0.0816	21.5%	7.14%
25		8	0.406	0.375	0.437	0.268	0.556	0.0151	0.0811	20.0%	0.92%
50		8	0.33	0.309	0.351	0.264	0.42	0.0102	0.0548	16.6%	19.4%
100		8	0.349	0.33	0.367	0.26	0.426	0.00903	0.0486	13.9%	14.9%

Graphics





CETIS Analytical Report

Report Date: 01 Aug-08 09:58 (p 1 of 1)
Link/Link Code: 04-0973-9523/0807-T026

Mysidopsis 7-d Survival, Growth and Fecundity Test — **Nautilus Environmental WA**

Analysis No: 12-0613-3514 **Endpoint:** Mean Dry Biomass-mg **CETIS Version:** CETISv1.6.3
Analyzed: 31 Jul-08 16:00 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Monte Carlo	NOEL	LOEL	TOEL	TU	PMSD
Untransformed		C > T	Not Run	25	50	35.4	4	16.7%

Dunnett's Multiple Comparison Test

Control	vs	Conc-%	Test Stat	Critical	MSD	P-Value	Decision(5%)
Dilution Water		6.25	0.472	2.31	0.0684	0.6580	Non-Significant Effect
		12.5	0.986	2.31	0.0684	0.4240	Non-Significant Effect
		25	0.126	2.31	0.0684	0.7930	Non-Significant Effect
		50*	2.68	2.31	0.0684	0.0214	Significant Effect
		100	2.06	2.31	0.0684	0.0820	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(5%)
Between	0.0420994	0.0084199	5	2.39	0.0537	Non-Significant Effect
Error	0.147887	0.0035211	42			
Total	0.1899864	0.011941	47			

ANOVA Assumptions

Attribute	Test	Test Stat	Critical	P-Value	Decision(1%)
Variances	Bartlett Equality of Variance	8.99	15.1	0.1090	Equal Variances
Distribution	Shapiro-Wilk Normality	0.945		0.0246	Normal Distribution

Mean Dry Biomass-mg Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	8	0.41	0.396	0.424	0.352	0.448	0.0068	0.0366	8.93%	0.0%
6.25		8	0.396	0.383	0.409	0.34	0.436	0.00637	0.0343	8.67%	3.42%
12.5		8	0.381	0.349	0.412	0.192	0.472	0.0152	0.0816	21.5%	7.14%
25		8	0.406	0.375	0.437	0.268	0.556	0.0151	0.0811	20.0%	0.92%
50		8	0.33	0.309	0.351	0.264	0.42	0.0102	0.0548	16.6%	19.4%
100		8	0.349	0.33	0.367	0.26	0.426	0.00903	0.0486	13.9%	14.9%

Mean Dry Biomass-mg Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Dilution Water	0.448	0.444	0.442	0.432	0.4	0.382	0.378	0.352
6.25		0.436	0.436	0.42	0.4	0.392	0.376	0.366	0.34
12.5		0.472	0.426	0.394	0.394	0.394	0.392	0.38	0.192
25		0.556	0.442	0.43	0.412	0.394	0.384	0.362	0.268
50		0.42	0.39	0.346	0.346	0.302	0.288	0.286	0.264
100		0.426	0.38	0.368	0.356	0.342	0.342	0.314	0.26

HI-SHELL001201

Mysidopsis 7-d Survival, Growth and Fecundity Test — **Nautilus Environmental WA**

Analysis No:	13-5330-3560	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.6.3
Analyzed:	31 Jul-08 14:38	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Monte Carlo	NOEL	LOEL	TOEL	TU	PMSD
Rank		C > T	Not Run	100	>100	N/A	1	13.1%

Steel Many-One Rank Test

Control	vs	Conc-%	Test Stat	Critical	Ties	P-Value	Decision(5%)
Dilution Water		6.25	68	46	2	0.8330	Non-Significant Effect
		12.5	67.5	46	1	0.8170	Non-Significant Effect
		25	63.5	46	2	0.6570	Non-Significant Effect
		50	60	46	2	0.4880	Non-Significant Effect
		100	64	46	2	0.6800	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(5%)
Between	0.0258848	0.005177	5	0.319	0.8990	Non-Significant Effect
Error	0.6809601	0.0162133	42			
Total	0.706845	0.0213903	47			

ANOVA Assumptions

Attribute	Test	Test Stat	Critical	P-Value	Decision(1%)
Variances	Bartlett Equality of Variance	6.25	15.1	0.2830	Equal Variances
Distribution	Shapiro-Wilk Normality	0.692		0.0000	Non-normal Distribution

7d Survival Rate Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	8	0.975	0.948	1	0.8	1	0.0131	0.0707	7.25%	0.0%
6.25		8	0.975	0.948	1	0.8	1	0.0131	0.0707	7.25%	0.0%
12.5		8	0.95	0.896	1	0.6	1	0.0263	0.141	14.9%	2.56%
25		8	0.925	0.868	0.982	0.6	1	0.0276	0.149	16.1%	5.13%
50		8	0.925	0.886	0.964	0.8	1	0.0192	0.104	11.2%	5.13%
100		8	0.95	0.915	0.985	0.8	1	0.0172	0.0926	9.75%	2.56%

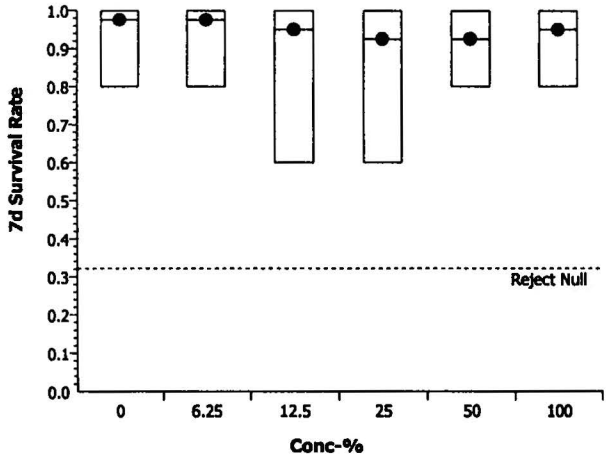
Rank Transformed Summary

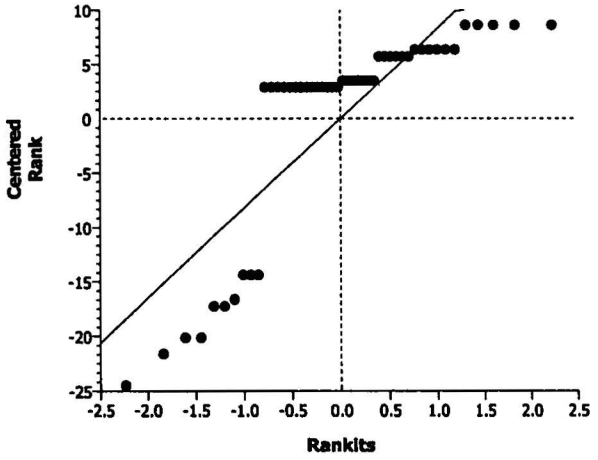
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	8	26.6	23.5	29.7	6.5	29.5	1.51	8.13	30.5%	0.0%
6.25		8	26.6	23.5	29.7	6.5	29.5	1.51	8.13	30.5%	0.0%
12.5		8	26	22.2	29.8	1.5	29.5	1.84	9.9	38.1%	2.35%
25		8	23.1	18.6	27.6	1.5	29.5	2.21	11.9	51.4%	13.1%
50		8	20.9	16.3	25.4	6.5	29.5	2.21	11.9	57.0%	21.6%
100		8	23.8	19.7	27.8	6.5	29.5	1.98	10.6	44.8%	10.8%

Mysidopsis 7-d Survival, Growth and Fecundity Test — **Nautilus Environmental WA**

Analysis No:	13-5330-3560	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.6.3
Analyzed:	31 Jul-08 14:38	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





Client: Shell Harbor Island
Sample ID: Wet Test-1-071408
Test No: 0807-T026
Nautilus Check-In #: 08-194 08-195 08-203

Start Date & Time: 7/15/08 1430
Stop Date & Time: 7/22/08 1430
Test species: Americamysis bahia

Conc. or % CON	Days 0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	8.57	8.09	8.48	8.02	8.70	8.00	8.48	7.87	8.43	7.81	8.29	7.59	8.65	7.85
DO (mg/l)	6.3	5.4	6.4	5.8	6.7	6.6	6.9	6.7	7.0	5.2	6.3	4.3	6.0	5.6
Salinity (ppt)	28.4	28.4	30.7	31.1	29.1	29.5	29.0	29.1	29.4	29.4	28.9	29.3	29.7	29.7
Temperature (°C)	25.1	25.3	25.3	25.3	25.0	25.2	25.1	25.0	25.6	25.3	25.5	25.6	25.3	25.3

6.25	Days 0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	8.57	8.11	8.49	8.03	8.69	8.05	8.47	7.96	8.44	7.88	8.33	7.82	8.53	7.99
DO (mg/l)	6.3	5.5	6.7	5.7	6.6	6.5	6.9	6.9	7.2	5.2	6.4	4.9	5.9	5.5
Salinity (ppt)	28.4	28.7	25.7	30.0	29.0	29.2	28.8	29.5	29.3	29.6	29.2	29.5	29.7	29.7
Temperature (°C)	25.6	25.7	25.9	25.6	26.1	25.1	25.2	25.1	25.4	25.3	26.4	25.8	25.4	25.4

12.5	Days 0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	8.55	8.10	8.51	8.03	8.67	8.05	8.46	7.99	8.44	7.91	8.37	7.85	8.52	8.00
DO (mg/l)	6.0	5.3	6.5	5.8	6.6	6.5	6.8	6.9	7.2	5.1	6.4	4.8	6.2	5.3
Salinity (ppt)	28.6	28.6	28.3	28.6	28.9	29.1	28.7	29.5	29.3	29.6	29.2	29.4	29.9	29.8
Temperature (°C)	25.5	25.4	25.6	25.5	25.2	25.2	25.3	25.2	25.5	25.4	25.3	25.10	25.3	25.5

25	Days 0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	8.52	8.12	8.51	8.06	8.63	8.04	8.43	7.96	8.44	7.96	8.41	7.96	8.47	8.05
DO (mg/l)	5.8	5.4	6.4	5.6	6.4	6.4	6.8	6.0	7.3	5.1	6.3	4.6	6.0	5.4
Salinity (ppt)	28.6	28.8	28.9	29.3	28.7	29.1	28.9	29.7	29.3	29.6	29.0	29.3	29.9	30.2
Temperature (°C)	25.10	25.3	25.6	25.5	25.2	25.2	25.0	25.1	25.4	25.5	26.5	25.6	25.5	25.4

50	Days 0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	8.47	8.14	8.51	8.08	8.55	8.03	8.37	7.99	8.41	8.01	8.47	8.07	8.39	8.06
DO (mg/l)	6.1	5.5	6.2	6.0	6.6	6.4	6.8	6.1	7.2	5.0	6.4	5.0	6.0	5.4
Salinity (ppt)	28.9	29.2	28.6	29.2	28.6	29.4	29.1	29.9	29.3	30.2	29.0	29.9	30.1	30.2
Temperature (°C)	25.6	25.4	25.6	25.3	25.1	25.2	25.0	25.1	25.5	25.4	25.4	25.3	25.2	25.4

100	Days 0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	8.37	8.16	8.53	8.15	8.38	8.00	8.25	8.00	8.37	8.09	8.56	8.18	8.25	8.08
DO (mg/l)	6.0	5.4	6.5	5.1	6.5	6.4	6.8	6.1	7.1	4.9	6.4	4.1	5.9	5.4
Salinity (ppt)	29.6	29.7	29.9	30.2	28.4	29.1	29.4	29.9	30.4	30.5	28.8	29.3	30.6	30.4
Temperature (°C)	25.7	25.3	29.9	25.5	25.1	25.2	25.2	25.1	25.4	25.5	25.4	25.6	25.3	25.4
Tech Initials:	CC	LES	LES			BP	BP							BP

Nautilus Environmental
Washington Laboratory
5009 Pacific Hwy. E., Suite 2
Tacoma, WA 98424

Dilution Water Batch #: 039 ASW

QA Check: LES

Sample Description: Sample aerated prior to dilutions
Organism Source: ABS
Date Received: 7/15/08
Date of Hatch: 7/8/08

Comments:

Nautilus Environmental
Washington Laboratory
5009 Pacific Hwy. E., Suite 2
Tacoma, WA 98424

Raw Data Sheet
Mysid Shrimp
(Americamysis bahia)
Mysid Survival

Client: Shell- Harbor Island **Test Number:** 0807 - T026

Sample ID: Wet Test 1 -07~~1~~08

Conc. or %	Cont.	Rep.	Days 0	1	2	3	4	5	6	7	Mean % Survival
CON	19	1	5	5	5	5	5	5	5	5	
	3	2	5	5	5	5	5	5	5	5	
	5	3	5	5	5	5	5	5	5	5	
	25	4	5	5	5	5	5	5	5	5	
	17	5	5	5	5	5	5	5	5	5	
	21	6	5	5	5	5	5	5	5	5	
	45	7	5	5	5	5	5	5	4	4	
	36	8	5	5	5	5	5	5	5	5	
6.25	9	1	5	5	5	5	5	5	5	5	
	48	2	5	5	5	5	5	5	5	5	
	41	3	5	5	5	5	5	5	5	5	
	43	4	5	4	4	4	4	4	4	4	
	23	5	5	5	5	5	5	5	5	5	
	12	6	5	5	5	5	5	5	5	4*	
	15	7	5	5	5	5	5	5	5	5	
	37	8	5	5	5	5	5	5	5	5	
12.5	24	1	5	5	5	5	5	5	5	5	
	39	2	5	5	5	5	5	5	5	5	
	14	3	5	5	5	5	5	5	5	5	
	47	4	5	5	5	5	5	5	5	5	
	11	5	5	5	5	5	5	5	5	5	
	29	6	5	5	5	5	5	4	3	3	
	6	7	5	5	5	5	5	5	5	5	
	31	8	5	5	5	5	5	5	5	5	
Technician Initials			RS	RS	[illegible]	BP	[illegible]	[illegible]	[illegible]	BP	

Feeding Times: 0 1700 1 815 1645 2 815 1600 3 900 1645 4 830 1715 5 845 1530 6 815

QA check RS

Comments: * one Dead above water line

Nautilus Environmental
Washington Laboratory
5009 Pacific Hwy. E., Suite 2
Tacoma, WA 98424

Raw Data Sheet
Mysid Shrimp
(Americamysis bahia)
Mysid Survival

Client: Shell Harbor Island

Test Number: 0807-T026

Sample ID: WET Test-1-071408

Conc. or %	Cont.	Rep.	Days 0	1	2	3	4	5	6	7	Mean % Survival
25	33	1	5	5	5	5	5	5	5	5	
	40	2	5	5	5	5	5	5	3	3	
	13	3	5	5	5	5	5	5	5	5	
	16	4	5	5	5	5	5	5	5	5	
	8	5	5	5	4	4	4	4	4	4	
	30	6	5	5	5	5	5	5	5	5	
	7	7	5	5	5	5	5	5	5	5	
	27	8	5	5	5	5	5	5	5	5	
50	42	1	5	5	5	5	5	5	5	5	
	10	2	5	5	5	5	5	5	5	5	
	32	3	5	5	5	5	5	5	4	4	
	34	4	5	5	4	4	4	4	4	4	
	18	5	5	5 4 LS	4	4	4	4	4	4	
	46	6	5	5	5	5	5	5	5	5	
	26	7	5	5	5	5	5	5	5	5	
	2	8	5	4	4	4	4	4	4	4	
100	1	1	5	5	5	5	5	5	5	5	
	38	2	5	5	5	5	5	5	5	5	
	20	3	5	4	4	4	4	4	4	4	
	22	4	5	5	5	5	5	5	5	5	
	44	5	5	5	5	5	5	5	5	5	
	4	6	5	5	5	5	5	5	5	5	
	35	7	5	5	5	5	5	5	5	5	
	28	8	5	5	5	5	5	5	4	4	
Technician Initials			LS	LS	[illegible]	BP	[illegible]	[illegible]	[illegible]	BP	

Feeding Times: 0 1700 1 815 1645 2 815 1600 3 900 1645 4 830 1715 5 845 1530 6 815

QA check LS

Comments:

Nautilus Environmental
Washington Laboratory
5009 Pacific Hwy. E., Suite 2
Tacoma, WA 98424

Raw Data Sheet
Mysid Weights
Seven Day Chronic Bioassay

Client: Shell Harbor Island

Species: *Americamysis bahia*

Sample ID: Wet Test - 1 - 071408

Test Number: 0807 - T026

Conc. or (%)	Cont.	Rep.	pan wt. (gm)	pan + mysid (gm)	mysid wt. (mg)	# mysids	avg. per mysid (mg)	avg. per conc.
CON	19	1	0.04130	0.04354		5		
	3	2	0.04126	0.04317		5		
	5	3	0.04419	0.04608		5		
	25	4	0.04066	0.04266		5		
	17	5	0.04262	0.04484		5		
	21	6	0.04236	0.04452		5		
	45	7	0.04009	0.04185		4		
	36	8	0.04266	0.04487		5		
6.25	9	1	0.04286	0.04474		5		
	48	2	0.04061	0.04257		5		
	41	3	0.04067	0.04250		5		
	43	4	0.03725	0.03943		4		
	23	5	0.04001	0.04171		5		
	12	6	0.04105	0.04273		4		
	15	7	0.04131	0.04331		5		
	37	8	0.04006	0.04224		5		
12.5	24	1	0.03~~8~~4141	0.04338		5		
	39	2	0.04253	0.04450		5		
	14	3	0.04404	0.04594		5		
	47	4	0.03991	0.04227		5		
	11	5	0.04236	0.04433		5		
	29	6	0.04124	0.04220		3		
	6	7	0.04228	0.04441		5		
	31	8	0.03409	0.03605		5		
	Tech Initials:		JS	JCS				

Date/Time in: 7/22/08 1430 **Oven temp. (°C):** 65.0 **QA Check:** JCS

Date/Time out: 7/23/08 1500 **Oven temp. (°C):** 65.7

Nautilus Environmental
Washington Laboratory
5009 Pacific Hwy. E., Suite 2
Tacoma, WA 98424

Raw Data Sheet
Mysid Weights
Seven Day Chronic Bioassay

Client: Shell Harbor Island

Species: *Americamysis bahia*

Sample ID: WET Test -1-071408

Test Number: 0807-T026

Conc. or %	Cont.	Rep.	pan wt. (gm)	pan + mysid (gm)	mysid wt. (mg)	# mysids	avg. per mysid (mg)	avg. per conc.
25	33	1	0.04069	0.04347		5		
	40	2	0.04188	0.04322		3		
	13	3	0.04123	0.04329		5		
	16	4	0.04259	0.04474		5		
	8	5	0.03858	0.04039		4		
	30	6	0.04225	0.04417		5		
	7	7	0.03986	0.04183		5		
	27	8	0.04114	0.04335		5		
50	42	1	0.03939	0.04112		5		
	10	2	0.04169	0.04313		5		
	32	3	0.03995	0.04138		4		
	34	4	0.04094	0.04289		4		
	18	5	0.04230	0.04362		4		
	46	6	0.03985	0.04195		5		
	26	7	0.04225	0.04398		5		
	2	8	0.04465	0.04616		4		
100	1	1	0.04178	0.04368		5		
	38	2	0.04074	0.04245		5		
	20	3	0.04125	0.04255		4		
	22	4	0.04162	0.04346		5		
	44	5	0.04251	0.04422		5		
	4	6	0.04365	0.04543		5		
	35	7	0.04046	0.04259		5		
	28	8	0.04071	0.04228		4		
	Tech Initials:		[illegible]	RS				

Date/Time in: 7/22/08 1430 **Oven temp. (°C):** 65.0 **QA Check:** RS

Date/Time out: 7/23/08 1500 **Oven temp. (°C):** 65.7

Appendix D

Atherinops affinis **(Pacific Topsmelt) Chronic Test**

Statistical Summaries and Raw Bench Sheets

CETIS Summary Report

Report Date: 31 Jul-08 14:58 (p 1 of 1)
Link/Link Code: 12-2691-4583/0807-T027

Pacific Topsmelt 7-d Survival and Growth Test — **Nautilus Environmental WA**

Test Run No:	17-1737-0808	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Indira Santiago
Start Date:	15 Jul-08 14:45	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Artificial Saltwater
Ending Date:	22 Jul-08 13:30	**Species:**	Atherinops affinis	**Brine:**	Crystal Sea Marine Mix
Duration:	6d 23h	**Source:**	Aquatic Biosystems, CO	**Age:**	10d

Sample No:	21-2313-1954	**Code:**	08-194	**Client:**	
Sample Date:	14 Jul-08 10:00	**Material:**	Oil Refinery Effluent	**Project:**	
Receive Date:	14 Jul-08 10:40	**Source:**	Shell Seattle Terminal (WA0001791)		
Sample Age:	29h (19.8 °C)	**Station:**			

Comparison Summary

Analysis No	Endpoint	NOEL	LOEL	TOEL	PMSD	Method
17-1439-0657	7d Survival Rate	100	> 100	N/A	9.1%	Steel Many-One Rank Test
04-2277-9743	Mean Dry Biomass-mg	100	> 100	N/A	14.8%	Dunnett's Multiple Comparison Test

Test Acceptability

Analysis No	Endpoint	Attribute	Test Stat	Acceptability Limits	Overlap	Decision
17-1439-0657	7d Survival Rate	Control Resp	0.96	0.8 - NL	Yes	Passes acceptability criteria
04-2277-9743	Mean Dry Biomass-mg	Control Resp	1.65	0.85 - NL	Yes	Passes acceptability criteria
17-1439-0657	7d Survival Rate	PMSD	0.091	NL - 0.25	No	Passes acceptability criteria
04-2277-9743	Mean Dry Biomass-mg	PMSD	0.148	NL - 0.5	No	Passes acceptability criteria

7d Survival Rate Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	5	0.96	0.927	0.993	0.8	1	0.0163	0.0894	9.32%	0.0%
6.25		5	1	1	1	1	1	0	0	0.0%	-4.17%
12.5		5	1	1	1	1	1	0	0	0.0%	-4.17%
25		5	1	1	1	1	1	0	0	0.0%	-4.17%
50		5	0.96	0.927	0.993	0.8	1	0.0163	0.0894	9.32%	0.0%
100		5	1	1	1	1	1	0	0	0.0%	-4.17%

Mean Dry Biomass-mg Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Dilution Water	5	1.65	1.59	1.71	1.5	1.9	0.0289	0.158	9.63%	0.0%
6.25		5	1.71	1.64	1.77	1.47	1.9	0.0321	0.176	10.3%	-3.72%
12.5		5	1.67	1.64	1.7	1.58	1.78	0.0143	0.0783	4.69%	-1.43%
25		5	1.75	1.68	1.82	1.47	1.98	0.0336	0.184	10.5%	-6.39%
50		5	1.56	1.48	1.64	1.23	1.79	0.0391	0.214	13.7%	4.98%
100		5	1.57	1.52	1.62	1.45	1.73	0.0252	0.138	8.81%	4.76%

7d Survival Rate Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Dilution Water	1	0.8	1	1	1
6.25		1	1	1	1	1
12.5		1	1	1	1	1
25		1	1	1	1	1
50		1	1	0.8	1	1
100		1	1	1	1	1

Mean Dry Biomass-mg Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Dilution Water	1.58	1.5	1.56	1.7	1.9
6.25		1.73	1.6	1.9	1.47	1.84
12.5		1.69	1.61	1.58	1.78	1.69
25		1.75	1.98	1.47	1.79	1.76
50		1.56	1.71	1.23	1.79	1.54
100		1.47	1.73	1.45	1.48	1.7

HI-SHELL001211

Initial and Final Chemistries | **Seven Day Chronic Saltwater Bioassay**

Client: Shell Harbor Island
Sample ID: Wet-Test-1-071408
Test No: 0807-T027
Nautilus Check-In #: 08-194 08-195

Start Date & Time: 7/15/08 1445
Stop Date & Time: 7/22/08 1330
Test species: Atherinops affinis
08-203

Conc. or % CON	Days 0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	8.59	8.21	8.54	8.18	8.69	8.17	8.51	8.15	8.38	7.96	8.31	7.90	8.57	7.99
DO (mg/l)	6.6	6.0	6.9	6.8	7.1	7.0	7.1	7.0	7.1	5.6	6.7	5.4	6.7	5.9
Salinity (ppt)	28.0	28.5	29.0	29.0	28.5	28.7	28.5	28.6	28.9	28.9	29.0	29.3	29.1	29.3
Temperature (°C)	20.1	20.3	19.9	20.7	20.1	20.6	19.7	20.4	20.7	20.3	20.8	21.0	21.0	21.0

6.25	Days 0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	8.59	8.23	8.54	8.18	8.68	8.17	8.47	7.92	8.48	7.97	8.35	7.86	8.56	7.99
DO (mg/l)	6.7	6.2	7.0	6.7	7.0	7.0	7.1	7.0	7.2	5.6	7.0	5.4	6.8	6.0
Salinity (ppt)	28.4	28.5	28.7	28.9	28.7	28.8	29.0	28.9	28.8	29.0	29.1	29.2	29.5	29.1
Temperature (°C)	20.1	20.3	20.0	20.7	20.1	20.5	19.9	20.4	20.5	20.5	20.9	21.0	21.0	21.0

12.5	Days 0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	8.57	8.24	8.57	8.19	8.67	8.15	8.47	8.01	8.48	8.00	8.38	7.90	8.54	8.06
DO (mg/l)	6.6	6.1	6.8	6.7	7.0	7.1	7.2	7.1	7.2	5.7	7.0	5.7	6.8	5.8
Salinity (ppt)	28.3	28.5	29.5	28.7	28.7	28.8	28.7	28.6	29.0	29.0	29.1	29.2	29.5	29.3
Temperature (°C)	20.1	20.2	19.9	20.6	19.9	20.4	19.6	20.4	20.5	20.5	20.8	21.0	20.9	20.9

25	Days 0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	8.55	8.25	8.56	8.20	8.62	8.12	8.44	8.02	8.47	8.07	8.42	7.96	8.50	8.04
DO (mg/l)	6.9	6.2	6.9	6.7	7.0	7.0	7.2	7.1	7.1	5.9	6.9	5.4	6.9	6.1
Salinity (ppt)	28.3	28.6	28.6	29.3	28.7	28.9	29.1	29.1	28.9	29.2	28.9	29.1	29.5	29.1
Temperature (°C)	19.8	20.3	20.0	20.5	19.6	20.4	19.6	20.2	20.1	20.6	20.7	21.0	20.8	21.0

50	Days 0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	8.49	8.21	8.56	8.21	8.48	8.11	8.38	8.00	8.43	8.07	8.47	8.00	8.40	8.05
DO (mg/l)	6.9	6.2	7.1	6.5	6.8	6.7	7.2	7.0	7.1	5.8	6.9	5.4	6.8	6.1
Salinity (ppt)	28.3	28.4	28.3	28.6	28.9	29.1	29.5	29.3	29.0	29.2	28.8	29.1	29.3	29.1
Temperature (°C)	19.8	20.2	20.0	20.5	19.6	20.3	19.6	20.0	20.1	20.7	20.5	20.9	21.0	21.0

100	Days 0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	8.36	8.18	8.57	8.27	8.18	8.00	8.25	7.99	8.36	8.09	8.54	8.17	8.24	8.07
DO (mg/l)	6.7	6.1	7.3	6.6	6.9	7.1	7.2	7.0	7.2	5.6	7.2	5.7	6.9	5.9
Salinity (ppt)	28.1	28.4	29.9	29.7	29.4	29.7	30.4	30.1	28.9	29.5	28.4	29.0	29.5	29.1
Temperature (°C)	19.9	20.3	19.6	20.5	19.6	20.1	19.5	20.1	19.9	20.6	20.3	20.9	20.9	21.0
Tech Initials:	MM	BP	MM	BP	BP	MN	MN	[illegible]	[illegible]	MM	MM	[illegible]	[illegible]	[illegible]

Nautilus Environmental
Washington Laboratory
5009 Pacific Hwy. E., Suite 2
Tacoma, WA 98424

Dilution Water Batch #: 039 ASW

QA Check: [illegible]

Sample Description: Sample aerated prior to dilutions
Organism Source: ABS
Date Received: 7/15/08
Date of Hatch: 7/5/08

Comments:

Nautilus Environmental
Washington Laboratory
5009 Pacific Hwy. E., Suite 2
Tacoma, WA 98424

Raw Data Sheet
Pacific Topsmelt
(Atherinops affinis)
Larval Survival

Client Name: Shell Harbor Island **Test No.:** 0807-TD27

Sample ID: WetTest-1-071408

Conc. or %	Cont.	Rep.	Days 0	1	2	3	4	5	6	7	Mean % Survival
CON	15	1	5	5	5	5	5	5	5	5	
	28	2	5	5	5	5	5	5	5	4	
	8	3	5	5	5	5	5	5	5	5	
	13	4	5	5	5	5	5	5	5	5	
	22	5	5	5	5	5	5	5	5	5	
6.25	3	1	5	5	5	5	5	5	5	5	
	18	2	5	5	5	5	5	5	5	5	
	4	3	5	5	5	5	5	5	5	5	
	14	4	5	5	5	5	5	5	5	5	
	23	5	5	5	5	5	5	5	5	5	
12.5	9	1	5	5	5	5	5	5	5	5	
	25	2	5	5	5	5	5	5	5	5	
	16	3	5	5	5	5	5	5	5	5	
	1	4	5	5	5	5	5	5	5	5	
	17	5	5	5	5	5	5	5	5	5	
25	26	1	5	5	5	5	5	5	5	5	
	7	2	5	5	5	5	5	5	5	5	
	24	3	5	5	5	5	5	5	5	5	
	6	4	5	5	5	5	5	5	5	5	
	5	5	5	5	5	5	5	5	5	5	
50	29	1	5	5	5	5	5	5	5	5	
	30	2	5	5	5	5	5	5	5	5	
	20	3	5	5	5	5	4	4	~~5~~ 4	4	
	21	4	5	5	5	5	5	5	5	5	
	11	5	5	5	5	5	5	5	5	5	
100	2	1	5	5	5	5	5	5	5	5	
	27	2	5	5	5	5	5	5	5	5	
	12	3	5	5	5	5	5	5	5	5	
	19	4	5	5	5	5	5	5	5	5	
	10	5	5	5	5	5	5	5	5	5	
Tech Initials			MM	MM	BP	[illegible]	[illegible]	MM	[illegible]	[illegible]	

Feeding Times: 0 ____ / 1700 1 815 / 1645 2 815 / 1600 3 900 / 1645 4 830 / 1715 5 845 / 1530 6 815

Comments: _____ **QA Check** LS

Nautilus Environmental
Washington Laboratory
5009 Pacific Hwy., E. Suite 2
Tacoma, WA 98424

Fish Weights
Seven Day Chronic Bioassay

Client: Shell Harbor Island

Species: *A. affinis*

Sample ID: Wet Test-T-071408

Test No: 0807-T027

Conc. or %	cont. #	rep.	pan wt. (gm)	pan + fish (gm)	fish wt. (mg)	# fish	avg. per fish (mg)	avg. per conc. (mg)
CON	15	1	0.04061	0.04850		5		
	28	2	0.04260	0.05030		4		
	8	3	0.03892	0.04670		5		
	13	4	0.03926	~~0.04776~~ 0.04776		5		
	22	5	0.04302	0.05251		5		
6.25	3	1	0.03815	0.04680		5		
	18	2	0.04301	0.05102		5		
	4	3	0.03746	0.04697		5		
	14	4	0.04150	0.04884		5		
	23	5	0.04476	0.05394		5		
12.5	9	1	0.04121	0.04967		5		
	25	2	0.04184	0.04988		5		
	16	3	0.04150	0.04941		5		
	1	4	0.03733	0.04623		5		
	17	5	0.04145	0.04989		5		
25	26	1	0.04246	0.05121		5		
	7	2	0.03761	0.04753		5		
	24	3	0.04542	0.05277		5		
	6	4	0.03584	0.04481		5		
	5	5	0.03608	0.04488		5		
50	29	1	0.04424	0.05202		5		
	30	2	0.03838	0.04692		5		
	20	3	0.04139	0.04754		4		
	21	4	0.04267	0.05161		5		
	11	5	0.04760	0.04946		5		
100	2	1	0.03978	0.04715		5		
	27	2	0.04264	0.05131		5		
	12	3	0.04071	0.04794		5		
	19	4	0.04342	0.05084		5		
	10	5	0.04168	0.05019		5		
	Tech Initials:		DS	LCS				

Date/Time in: 7/22/08 1330 Oven temp. (°C): 62.3 QA check LCS

Date/Time out: 7/23/08 1500 Oven temp. (°C): 65.7

Appendix E
Bioassay Testing Checklists

WET TESTING BIOASSAY CHECKLIST

MARINE ACUTE TOXICITY - 48-HOUR STATIC-RENEWAL TEST USING *AMERICAMYSIS BAHIA*

Sample ID Number: Wet Test 1-071408
Project Name: Shell Harbor Island NPDES Tests
EPA Test Method: EPA-821-R-02-012
(Circle method to verify)

Date: 7/15/08
Laboratory: Nautilus Environmental, WA
Personnel: Barb Parsons, Megan Murphy, Eric Tollefs, Cat Curran

CHAIN-OF-CUSTODY	Yes	No	Comment
Are all Chain-of-Custody (COC) forms included with the sample(s)?	✓		
Correct number of water samples received?	✓		
Is the COC form completely filled out, signed, and dated?	✓		
Was the sample container temperature recorded and within the control limits (4°C)?	✓		
TEST PROCEDURES			
Equipment was calibrated?	✓		
Test salinity is set to project specific salinity?	✓		
Water samples kept at 4°C?	✓		
Testing initiated within 36 hours of sample collection?	✓		
Mysids are 1-5 days old, within 24 hours of same age?	✓		
Mysids acclimated to 25 ± 1°C?	✓		
Randomization sheets prepared?	✓		
Control prepared?	✓		
Dilution concentrations prepared to correct specifications?	✓		
Test chambers randomized?	✓		
pH, DO, salinity and temperature measured, recorded, and within acceptable parameters?	✓		DO. low-sample aerated prior to dilutions
Ten mysids added to test chamber?	✓		
Environmental chamber at 25 ± 1°C with 16 hours light/8 hours dark photoperiod?	✓		

HI-SHELL001216

	YES	NO	COMMENT
DAILY MONITORING			
Test chambers rearranged according to concentration each day?	✓		
pH, DO, salinity and temperature measured and within acceptable parameters?	✓		
Mysids fed once daily	✓		
Surviving mysids counted?	✓		
Tanks cleaned?	✓		debris pipetted out
Effluent renewal at 24 hours?	✓		
Test ended within 48 ± 2 hours of start time?	✓		
DATA REVIEW			
Test acceptability criteria met? • Mean control survival ≥ 90%	✓		
Records are complete with no missing data?	✓		

Comments:

HI-SHELL001217

WET TESTING BIOASSAY CHECKLIST

MARINE ACUTE TOXICITY - 96-HOUR STATIC-RENEWAL TEST USING *MENIDIA BERYLLINA*

Sample ID Number: Wet Test 1-071408
Project Name: Shell Harbor Island NPDES Tests
EPA Test Method: EPA-821-R-02-012
(Circle method to verify)

Date: 7/15/08
Laboratory: Nautilus Environmental, WA
Personnel: Barbara Parsons, Eric Tollefson, Meghan May, Maria Graffield, Laura Shanks

CHAIN-OF-CUSTODY	Yes	No	Comment
Are all Chain-of-Custody (COC) forms included with the sample(s)?	✓		
Correct number of water samples received?	✓		
Is the COC form completely filled out, signed, and dated?	✓		
Was the sample container temperature recorded and within the control limits (4°C)?	✓		
TEST PROCEDURES			
Equipment was calibrated?	✓		
Test salinity is set to project specific salinity?	✓		
Water samples kept at 4°C?	✓		
Testing initiated within 36 hours of sample collection?	✓		
Fish are 9-14 days old, within 24 hours of same age?	✓		
Fish acclimated to 25 ± 1°C?	•	✓	This test can be run at 20.0°C as per WDOE protocol
Randomization sheets prepared?	✓		
Control prepared?	✓		
Dilution concentrations prepared to correct specifications?	✓		
Test chambers randomized?	✓		
pH, DO, salinity and temperature measured, recorded, and within acceptable parameters?	✓		yes sample ~~water~~ aerated prior to dilutions due to low D.O.
Ten fish added to test chamber?	✓		
Environmental chamber at 25 ± 1°C with 16 hours light/8 hours dark photoperiod?	✓		

	YES	NO	COMMENT
DAILY MONITORING			
Fed animals once prior to 48 hour renewal?	✓		
Test chambers rearranged according to concentration each day?	✓		
pH, DO, salinity and temperature measured and within acceptable parameters?	✓		
80% of test solution renewed at 48 hours?	✓		
Surviving fish counted?	✓		
Tanks cleaned?	✓		Debris pipetted out
Test ended within 96 ± 2 hours of start time?	✓		
DATA REVIEW			
Test acceptability criteria met? • Mean control survival ≥ 90%	✓		
Records are complete with no missing data?	✓		

Comments:

HI-SHELL001219

WET TESTING BIOASSAY CHECKLIST

MARINE CHRONIC TOXICITY - 7 DAY STATIC-RENEWAL TEST WITH *AMERICAMYSIS BAHIA*

Sample ID Number: Wet-Test-1-071408
Project Name: Shell Harbor Island NPDES Tests
EPA Test Method: EPA-821-R-02-014, method 1007.0
(Circle method to verify)

Date: 7/15/08
Laboratory: Nautilus Environmental, WA
Personnel: ~~Medium Murphy~~ Cat Curran, Indira Santiago, Hadra Shanks, Barb. Parsons, Eric Tollerson, Marsha Bray

CHAIN-OF-CUSTODY	Yes	No	Comment
Are all Chain-of-Custody (COC) forms included with the sample(s)?	✓		
Correct number of water samples received?	✓		
Is the COC form completely filled out, signed, and dated?	✓		
Was the sample container temperature recorded and within the control limits (4°C)?	✓		
TEST PROCEDURES			
Equipment was calibrated?	✓		
Test salinity is 30 ± 2ppt?	✓		
Water samples kept at 4°C?	✓		
Testing initiated within 36 hours of sample collection?	✓		
Test animals are 7 days old, within 24 hours of same age?	✓		
Randomization sheets prepared?	✓		
Control prepared?	✓		
Dilution concentrations prepared to correct specifications?	✓		
Test chambers randomized?	✓		
pH, DO, salinity and temperature measured, recorded, and within acceptable parameters?	✓		Sample aerated prior to dilutions due to low D.O.
Mysids added to test chambers?	✓		
Environmental chamber set to 26°C ±1 with 16 hours light/8 hours dark photoperiod?	✓		

	YES	NO	COMMENT
DAILY MONITORING			
Test chambers rearranged according to concentration each day?	✓		
pH, DO, salinity and temperature measured prior to water change and after water change during the 7 day period? Are the measurements within acceptable parameters?	✓		
Tanks cleaned?	✓		Debris pipetted out
90% test solution renewals conducted?	✓		
Surviving mysids counted daily?	✓		
Mysids fed twice a day on Days 1-6?	✓		
Mysids dried on Day 7?	✓		
Mysid weights measured?	✓		
DATA REVIEW			
Test acceptability criteria met? • Mean control survival ≥ 80% • Average dry weight ≥ 0.20 mg per surviving mysid in control	✓		
Records are complete with no missing data?	✓		

Comments:

HI-SHELL001221

WET TESTING BIOASSAY CHECKLIST

MARINE CHRONIC TOXICITY - 7 DAY STATIC-RENEWAL TEST WITH *ATHERINOPS AFFINIS*

Sample ID Number: Wet Test-1-071408
Project Name: Shell Harbor Island NPDES Tests
EPA Test Method: EPA / 600 / R-95 / 136
(Circle method to verify)

Date: 7/15/08
Laboratory: Nautilus Environmental, WA
Personnel: Meghan Murphy, Karl Parsons, Maria Brayfield, Eric Tollefson, Laura Shanks.

CHAIN-OF-CUSTODY	Yes	No	comment
Are all Chain-of-Custody (COC) forms included with the sample(s)?	✓		
Correct number of water samples received?	✓		
Is the COC form completely filled out, signed, and dated?	✓		
Was the sample container temperature recorded and within the control limits (4°C)?	✓		
TEST PROCEDURES			
Equipment was calibrated?	✓		
Test salinity is 30 ± 1ppt or 34 ± 2 ppt?	✓		
Water samples kept at 4°C?	✓		
Testing initiated within 36 hours of sample collection?	✓		
Test animals are 9-15 days old?	✓		
Fish acclimated to 20 ± 1°C?	✓		
Randomization sheets prepared?	✓		
Control prepared?	✓		
Dilution concentrations prepared to correct specifications?	✓		
Test chambers randomized?	✓		
pH, DO, salinity and temperature measured, recorded, and within acceptable parameters?	✓		Sample aerated prior to dilutions due to low DO.
Fish added to test chamber?	✓		
Environmental chamber at 20 ± 1°C with 16 hours light/8 hours dark photoperiod?	✓		

	YES	NO	COMMENT
DAILY MONITORING			
Test chambers rearranged according to concentration each day?	✓		
pH, DO, salinity and temperature measured prior to water change and after water change during the 7 day period? Are the measurements within acceptable parameters?	✓		
Tanks cleaned?	✓		Debris pipetted out
80% test solution renewals conducted?	✓		
Surviving fish counted daily?	✓		
Fish fed once a day on Days 1-6?	✓		
Fish dried on Day 7?	✓		
Fish weights measured?	✓		
DATA REVIEW			
Test acceptability criteria met? • Mean control survival ≥ 80% • Average dry weight ≥0.50 mg for 7-day fish larvae (dried immediately)	✓		
Records are complete with no missing data?	✓		

Comments:

Appendix F
Sample Check-In Sheet

Nautilus Environmental
5009 Pacific Hwy East, Ste. 2
Tacoma, WA 98424

Sample Check-In Information

Client: PES Environmental / Shell Harbor Island
Sample ID: WET TEST 1 - 071408

Tests Performed: My + Mb-a, My-c Aa-c
Test ID No(s).:

Sample Description:

Sample ID:	WET TEST-1 071408	WET Test-2-071408	Wet Test-3-07-18-08	
Log-in No. (07-xxxx):	08-194	08-195	08-203	
Sample Collection Date & Time:	7/14/08 1020	7/16/08 0900	7/18/08 915	
Sample Receipt Date & Time:	7/14/08 1040	7/16/08 1005	7/18/08 1055	
Check-in Temperature (°C)	12.8	9.1	10.0	
Temperature OK?	(Y) N	(Y) N	(Y) N	Y N
DO (mg/L)	4.8	4.5	4.4	
pH (units)	6.94	6.76	6.93	
Conductivity (µS/cm)	90	86	85	
Salinity (ppt)	—	—	—	
Tit. Vol / Sam. Vol. / Alkalinity (mg/L)*	1.4 / 25 / 56	1.2 / 25 / 48	1.3 / 25 / 52	/ /
Tit. Vol. / Sam. Vol. / Hardness (mg/L)* *	1.1 / 25 / 44	1.4 / 25 / 56	1.4 / 25 / 56	/ /
Total Chlorine (mg/L)	<0.03	<0.03	<0.03	
Total Ammonia (mg/L)	<1.0	<1.0	<1.0	
Technician Initials	JS	BP	JS	

* = mg/L as $CaCO_3$, * = Measured for freshwater samples only, NA = Not Applicable, NM = Not Measured

COC Complete? Y or N
1 Y 2 Y 3 Y

Filtration? Y (N)
Pore Size:
Organisms or Debris

Aeration? (Y) N JS
Length of Time: 15 min
Final DO: 6.4
Final pH: 8.23

Hardness Adjustment? Y (N)
If adjusted, please see worksheet for details.

Freshwater Tests:

Control/Dilution Water Source: test type: ____ 8:2 (DMW) MHW Other: ____ Alkalinity: ____ Hardness: ____
Control/Dilution Water Source: test type: ____ 8:2 (DMW) MHW Other: ____ Alkalinity: ____ Hardness: ____
Additional Control? Y N = ____ Alkalinity: ____ Hardness: ____

Marine Tests:

Control/Dilution Water Source: test type: all (ART SW) NAT SW Alkalinity: 124 Salinity: 28.2
Control/Dilution Water Source: test type: ____ ART SW NAT SW Alkalinity: ____ Salinity: ____
Additional Control? Y N = ____ Alkalinity: ____ Salinity: ____
Sample Salted w/ artificial salt? Y (N) If yes, what ppt? 30 test type: all
Sample salted w/brine? Y N If yes, what ppt? ____ test type: ____

Sub-samples for additional chemistry:

Comments: Temperature for grab sample must be 0-20°C if received within 1 hour of collection time, 0-12°C if effluent received within 4 hours of collection time, and 0-6°C for all other samples.

QC Check: LBS

Appendix G
Chain-of-Custody Forms

Nautilus Environmental

☐ **CALIFORNIA**
5550 Morehouse Drive • Suite 150
San Diego, California 92121
Phone 858.587.7333
Fax 858.587.3961

☒ **WASHINGTON**
5009 Pacific Highway East • Suite 2
Tacoma, Washington 98424
Phone 253.922.4296
Fax 253.922.5814

☐ **BRITISH COLUMBIA**
8664 Commerce Court
Burnaby, British Columbia, Canada V5A 4N3
Phone 604.420.8773
Fax 604.357.1361

Chain of Custody

Date 7/14/08 **Page** 1 **of** 1

Sample Collection by: PES Environmental (Russell Stolsen)

Report to:
Company ~~Bill H~~ PES Environmental
Address 1215 Fourth Ave. Ste 1350
City Seattle State WA Zip 98161
Contact Bill Haldeman
Phone/Email (206) 529-3980

Invoice to:
Company PES Environmental
Address Same
City State Zip
Contact
Phone/Email

ANALYSES REQUIRED

SAMPLE ID	DATE	TIME	MATRIX	CONTAINER TYPE	NUMBER OF CONTAINERS	COMMENTS	Wet Test	Americamysis bahia acute + chronic (NS)	Menidia beryllina acute (NS)	Topsmelt chronic (NS)	RECEIPT TEMPERATURE (°C)
~~Shell-wet Test-1~~ 9/8 Wet Test-1-071408	7/14/08	① 0900 1000 MRZ	W	Poly	1		X	X	X	X	19.8

PROJECT INFORMATION	SAMPLE RECEIPT	
CLIENT Shell	TOTAL NO. OF CONTAINERS	1
P.O. NO.	REC'D GOOD CONDITION	✓
SHIPPED VIA: Transported by PES	MATCHES TEST SCHEDULE	✓

SPECIAL INSTRUCTIONS/COMMENTS:
① end of collection time per chain MRZ

RELINQUISHED BY (CLIENT)
(Signature) JR Stolsen (Time) 1045
(Printed Name) J. Russell Stolsen (Date) 7/14/08
(Company) PES Environmental

RECEIVED BY (COURIER)
(Signature) (Time)
(Printed Name) (Date)
(Company)

RELINQUISHED BY (COURIER)
(Signature) (Time)
(Printed Name) (Date)
(Company)

RECEIVED BY (LABORATORY)
(Signature) Laura Shanks (Time) 1040
(Printed Name) Laura Shanks (Date) 7/14/08
Nautilus Environmental Log-in No. 08-194

Additional costs may be required for sample disposal or storage. Net 30 unless otherwise contracted.

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HI-SHELL001227

Nautilus Environmental

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8664 Commerce Court
Burnaby, British Columbia, Canada V5A 4N3
Phone 604.420.8773
Fax 604.357.1361

Chain of Custody

Date 7/16/08 Page 1 of 2

Sample Collection by: Russell Stolsen, PES Environmental

Report to:
Company PES Environmental
Address 1215 4th Ave Ste. 1350
City Seattle State WA Zip 98161
Contact Bill Haldeman
Phone/Email 206.529-3980

Invoice to:
Company PES Environmental
Address Same
City State Zip
Contact
Phone/Email

ANALYSES REQUIRED

SAMPLE ID	DATE	TIME	MATRIX	CONTAINER TYPE	NUMBER OF CONTAINERS	COMMENTS	Wet Test	Mysid & Topsmelt chronic (TBS)	Menidia acute (TBS)									RECEIPT TEMPERATURE (°C)
WetTest-2-071608	7/16/08	0900	W	Poly	1		X	X	X									9.1

PROJECT INFORMATION	
CLIENT	Shell
P.O. NO.	828.001.01
SHIPPED VIA:	PES transport

SAMPLE RECEIPT	
TOTAL NO. OF CONTAINERS	1
REC'D GOOD CONDITION	Y
MATCHES TEST SCHEDULE	Y

SPECIAL INSTRUCTIONS/COMMENTS:

RELINQUISHED BY (CLIENT)
(Signature) J. Russell Stolsen (Time) 1010
(Printed Name) J. Russell Stolsen (Date) 7/16/08
(Company) PES Environmental

RELINQUISHED BY (COURIER)
(Signature) (Time)
(Printed Name) (Date)
(Company)

RECEIVED BY (COURIER)
(Signature) (Time)
(Printed Name) (Date)
(Company)

RECEIVED BY (LABORATORY)
(Signature) Barbara Parsons (Time) 10:05
(Printed Name) Barbara Parsons (Date) 7/16/08
Nautilus Environmental Log-in No. 08-195

Additional costs may be required for sample disposal or storage. Net 30 unless otherwise contracted.

HI-SHELL001228

Nautilus Environmental

Chain of Custody

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8664 Commerce Court
Burnaby, British Columbia, Canada V5A 4N3
Phone 604.420.8773
Fax 604.357.1361

Date 7/18/08 Page 1 of 1

Sample Collection by: Russell Stolsen, PES Environmental

Report to:
Company PES Environmental
Address 1215 4th Ave. Ste. 1350
City Seattle State WA Zip 98161
Contact Bill Haldeman
Phone/Email 206.529.3980

Invoice to:
Company PES Environmental
Address Same
City _____ State _____ Zip _____
Contact _____
Phone/Email _____

ANALYSES REQUIRED

SAMPLE ID	DATE	TIME	MATRIX	CONTAINER TYPE	NUMBER OF CONTAINERS	COMMENTS	Wet Test	Mysid + Topsmelt chronic										RECEIPT TEMPERATURE (°C)
WetTest-3-071808	7/18/08	0915	W	Poly	1		X	X										10.0

PROJECT INFORMATION	SAMPLE RECEIPT	
CLIENT Shell Harbor Island	TOTAL NO. OF CONTAINERS	1
P.O. NO. 828.001.01	REC'D GOOD CONDITION	Y
SHIPPED VIA: Transported by PES	MATCHES TEST SCHEDULE	Y

SPECIAL INSTRUCTIONS/COMMENTS:

RELINQUISHED BY (CLIENT)
(Signature) R Stolsen (Time) 1055
(Printed Name) Russell Stolsen (Date) 7/18/08
(Company) PES Environmental

RECEIVED BY (COURIER)
(Signature) (Time)
(Printed Name) (Date)
(Company)

RELINQUISHED BY (COURIER)
(Signature) (Time)
(Printed Name) (Date)
(Company)

RECEIVED BY (LABORATORY)
(Signature) Indira Santiago (Time) 1055
(Printed Name) Indira Santiago (Date) 7/18/08
Nautilus Environmental Log-in No. 08-203

Additional costs may be required for sample disposal or storage. Net 30 unless otherwise contracted.

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