

Bio-Aquatic Testing

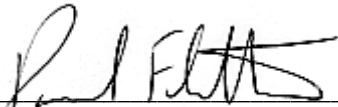
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BioGrass Extra Product Test

48 Hour Acute *Mysidopsis bahia* Toxicity Test
and
96-Hour Acute *Menidia beryllina* Toxicity Test

Using:
BioGrass Extra
#2 Fuel Oil
BioGrass Extra /#2 Fuel Oil Mixture

Prepared by:


Vice President

6/9/2010

Date

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BIO-AQUATIC TESTING, INC.

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EXECUTIVE SUMMARY

Bio-Aquatic Testing Inc. located at 2501 Mayes Rd. Suite 100, Carrollton, Texas 75006 was contracted by Em's Ecological Products, LLC to test the toxicity of their surface-washing product, BioGrass Extra. Bio-Aquatic Testing used the Environmental Protection Agency (EPA) protocol listed in 40 CFR Chapter 1 (7-1-99) Pt. 300 Appendix C, Item 3.0. Revised Standard Dispersant Toxicity Test.

Test protocols call for testing the toxicity of the BioGrass Extra product, #2 Fuel Oil, and BioGrass Extra product / fuel oil mix. The marine invertebrate species, *Mysidopsis bahia* (*Americamysis bahia*) and the marine vertebrate species, *Menidia beryllina* were used in the tests. The test duration using *M. bahia* and *M. beryllina* was 48 hours and 96 hours, respectively.

A summary of all the LC-50 values is given below:

MATERIAL TESTED	SPECIES	LC50 (PPM)	Least to Most Toxic
BioGrass Extra	<i>Menidia beryllina</i>	548.66	2
	<i>Mysidopsis bahia</i>	703.43	1
No. 2 Fuel Oil	<i>Menidia beryllina</i>	2.51	4
	<i>Mysidopsis bahia</i>	2.24	5.5
BioGrass Extra & No. 2 FO	<i>Menidia beryllina</i>	2.54	3
	<i>Mysidopsis bahia</i>	2.24	5.5
Reference Toxicant: (Sodium Laurel Sulfate)	<i>Menidia beryllina</i>	12.25	
	<i>Mysidopsis bahia</i>	11.71	

BIO-AQUATIC TESTING, INC.

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TOXICITY TEST REPORT - 48 Hour Acute – *Mysidopsis bahia*

Client: Em's Ecological Products, LLC Blending and Packaging

Sample: BioGrass Extra, #2 Fuel Oil, and BioGrass Extra /#2 Fuel Oil

SAMPLE PREPARATION:

Exploratory tests were conducted on the BioGrass Extra product to determine a final definitive test dilution series. The BioGrass Extra product stock solutions were prepared by diluting 0.55 mLs of concentrated product into a 550 mLs of synthetic laboratory saltwater. This ratio produces a 1000 ppm stock solution. For the #2 fuel oil stock solution, 0.55 ml of #2 fuel oil was diluted into 550 mls of synthetic saltwater. The 1000 ppm #2 fuel oil/ BioGrass Extra mix was made in the same manner as the #2 fuel oil stock solution with the ratio of BioGrass Extra to oil being 1:10 (.5 mLs #2 fuel oil to 0.05 mLs BioGrass Extra into 550 mLs). A Gastight™ syringe was used to introduce the #2 fuel oil into stock solution flasks. Stock solutions were blended for 5 seconds between serial dilutions. Serial dilutions were made from the stock BioGrass Extra only at concentrations of 100 ppm, 250 ppm, 500 ppm, 750 ppm and 1000 ppm. The #2 fuel oil only stock solution had serial dilutions of 0.1 ppm, 1 ppm, 5 ppm, 10 ppm, and 20 ppm. Serial dilutions were made from the BioGrass Extra / #2 fuel oil stock solution at concentrations of 0.1 ppm, 1 ppm, 5 ppm, 10 ppm and 20 ppm. Total volume of each dilution made was 2000 mls.

TEST PROCEDURES:

Mysidopsis bahia

A test control, using untreated synthetic seawater ran concurrently with the test. The volume used for each of the concentration replicates was 1000 mls. The 48-Hour Acute *Mysidopsis bahia* survival test using the BioGrass Extra only was initiated at 1643 hours on June 3, 2010. The 48-Hour Acute *Mysidopsis bahia* survival test using #2 fuel oil was initiated at 1558 hours on June 3, 2010. The 48-Hour Acute *Mysidopsis bahia* survival test using #2 fuel oil/ BioGrass Extra mix was initiated at 1625 hours on June 3, 2010. Tests were set up with 1000 ml Pyrex™ beakers containing 1000 ml of test solution. Each concentration included two replicate beakers. Ten organisms were placed in each replicate according to test protocol. Test organisms were five to seven day old, laboratory-cultured juveniles. Surviving larvae in each test chamber were fed freshly hatched brine shrimp after dead organisms and debris were removed from each test container. Daily chemical parameters were analyzed from one replicate in each dilution. Test solutions were kept at 25° +/- 1° C, with no unacceptable deviations. The test proceeded for 48 hours after which final survival data were collected. The test using the BioGrass Extra only ended at 1639 hours on June 5, 2010. The test using #2 fuel oil was ended at 1530 hours on June 5, 2010. The test using the BioGrass Extra /#2 fuel oil mix was ended at 1550 hours on June 5, 2010.

TEST RESULTS: Toxstat Version 3.4 (University of Wyoming) and the Environmental Protection Agency's Trimmed Spearman-Karber statistical programs were used to analyze all data.

The *Mysidopsis bahia* survival data for the BioGrass Extra product test data were not normally distributed at the alpha level of 0.01 (13.277) using the Shapiro-Wilk's test for normality. Bartlett's test for homogeneity is not run on non-normal data. ANOVA-Dunnett's test on *Mysidopsis bahia* survival data demonstrated statistically significant differences between the control and the 750 ppm and 1000 ppm concentrations. The no observed effect concentration (NOEC) was 500 ppm. The 48-Hour LC-50 (concentration at which 50% mortality is expected to occur) calculated by the Spearman-Karber program, was 703.43 ppm.

LOEC: 750 ppm
NOEC: 500 ppm
LC₅₀: 703.43 ppm

The *Mysidopsis bahia* survival data for #2 Fuel Oil test data were not normally distributed at the alpha level of 0.01 (13.277) using the Shapiro-Wilk's test for normality. Bartlett's test for homogeneity is not run on non-normal data. ANOVA-Dunnett's test on *Mysidopsis bahia* survival data demonstrated statistically significant differences between the control and the 5 ppm, 10 ppm, and 20 ppm concentrations. The no observed effect concentration (NOEC) was 1 ppm. The 48-Hour LC-50 (concentration at which 50% mortality is expected to occur) calculated by the Spearman-Karber program, was 2.24 ppm.

LOEC: 5 ppm
NOEC: 1 ppm
LC₅₀: 2.24 ppm

The *Mysidopsis bahia* survival data for the #2 Fuel Oil/ BioGrass Extra test data were not normally distributed at the alpha level of 0.01 (13.277) using the Shapiro-Wilk's test for normality. Bartlett's test for homogeneity is not run on non-normal data. ANOVA-Dunnett's test on *Mysidopsis bahia* survival data demonstrated statistically significant differences between the control and the 5 ppm, 10 ppm, and 20 ppm concentrations. The no observed effect concentration (NOEC) was 1 ppm. The 48-Hour LC-50 (concentration at which 50% mortality is expected to occur) calculated by the Spearman-Karber program, was 2.24 ppm.

LOEC: 5 ppm
NOEC: 1 ppm
LC₅₀: 2.24 ppm

BIO-AQUATIC TESTING, INC.

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TOXICITY TEST REPORT - 96 Hour Acute – *Menidia beryllina*

Client: Em's Ecological Products, LLC Blending and Packaging

Sample: BioGrass Extra, #2 Fuel Oil, and BioGrass Extra /#2 Fuel Oil

SAMPLE PREPARATION:

Exploratory tests were conducted on the BioGrass Extra product to determine a final definitive test dilution series. The BioGrass Extra product stock solutions were prepared by diluting 0.55 mLs of concentrated product into a 550 mLs of synthetic laboratory saltwater. This ratio produces a 1000 ppm stock solution. For the #2 fuel oil stock solution, 0.55 ml of #2 fuel oil was diluted into 550 mls of synthetic saltwater. The 1000 ppm #2 fuel oil/ BioGrass Extra mix was made in the same manner as the #2 fuel oil stock solution with the ratio of BioGrass Extra to oil being 1:10 (.5 mLs #2 fuel oil to 0.05 mLs BioGrass Extra into 550 mLs). A Gastight™ syringe was used to introduce the #2 fuel oil into stock solution flasks. Stock solutions were capped and vigorously shaken on an orbital shaker at 150 rpm for 5 minutes. Serial dilutions were made from the stock BioGrass Extra only at concentrations of 100 ppm, 250 ppm, 500 ppm, 750 ppm and 1000 ppm. Serial dilutions were made from the #2 fuel oil only and BioGrass Extra / #2 fuel oil stock solutions at concentrations of 1 ppm, 5 ppm, 10 ppm, 20 ppm and 50 ppm. Total volume of each dilution made was 2000 mls.

TEST PROCEDURES:

Menidia beryllina

A test control, using untreated synthetic seawater ran concurrently with the test. The volume for each of the replicates was 1000 mls. The 96-Hour Acute *Menidia beryllina* survival test, using the BioGrass Extra product only was initiated at 1638 hours on June 3, 2010. The 96-Hour Acute *Menidia beryllina* survival test using #2 fuel oil was initiated at 1553 hours on June 3, 2010. The 96-Hour Acute *Menidia beryllina* survival test using #2 fuel oil/ BioGrass Extra mix was initiated at 1620 hours on June 3, 2010. Tests were set up with 1-liter Pyrex™ beakers containing 1000 mls of test solution. Each concentration included two replicates. Ten organisms were placed in each replicate according to protocol, 24 hours after they were initiated. Test organisms were seven day old, laboratory-cultured juveniles. Surviving juveniles in each test chamber were fed freshly hatched brine shrimp after dead organisms and debris were removed from each test container. Daily chemical parameters were analyzed from a replicate of each dilution. Test solutions were kept at 25° +/- 1° C, with no unacceptable deviations. Tests ran for 96 hours after which final survival data were collected. The test using the BioGrass Extra only ended at 1609 hours on June 7, 2010. The test using #2 fuel oil was ended at 1539 hours on June 7, 2010. The test using the BioGrass Extra /#2 Fuel oil mix was ended at 1610 hours on June 7, 2010.

TEST RESULTS: Toxstat Version 3.4 (University of Wyoming) and the Environmental Protection Agency's Trimmed Spearman-Karber statistical programs were used to analyze all data.

The *Menidia beryllina* survival data for the BioGrass Extra test data were not normally distributed at the alpha level of 0.01 (13.277) using the Shapiro-Wilk's test for normality. Bartlett's test for homogeneity is not run on non-normal data. ANOVA-Dunnett's test on *Menidia beryllina* survival data demonstrated a statistically significant difference between the control and the 500 ppm, 750 ppm, and 1000 ppm concentrations. The no observed effect concentration (NOEC) was 250 ppm. The 48-Hour LC-50 (concentration at which 50% mortality is expected to occur) calculated by the Spearman-Karber program, was 548.66 ppm.

LOEC: 500 ppm
NOEC: 250 ppm
LC₅₀: 548.66 ppm

The *Menidia beryllina* survival data for #2 Fuel Oil test data were not normally distributed at the alpha level of 0.01 (13.277) using the Shapiro-Wilk's test for normality. Bartlett's test for homogeneity is not run on non-normal data. ANOVA-Dunnett's test on *Menidia beryllina* survival data demonstrated statistically significant differences between the control and the 5 ppm, 10 ppm, and 20 ppm concentrations. The no observed effect concentration (NOEC) was 1 ppm. The 48-Hour LC-50 (concentration at which 50% mortality is expected to occur) calculated by the Spearman-Karber program, was 2.51 ppm.

LOEC: 5 ppm
NOEC: 1 ppm
LC₅₀: 2.51 ppm

The *Menidia beryllina* survival data for the #2 Fuel Oil/ BioGrass Extra test data were normally distributed at the alpha level of 0.01 (13.277) using the Shapiro-Wilk's test for normality. Bartlett's test for homogeneity could not be run because at least on concentration had zero variance. ANOVA-Dunnett's test on *Menidia beryllina* survival data demonstrated statistically significant differences between the control and the 5 ppm, 10 ppm and 20 ppm concentrations. The no observed effect concentration (NOEC) was 1 ppm. The 48-Hour LC-50 (concentration at which 50% mortality is expected to occur) calculated by the Spearman-Karber program, was 2.54 ppm.

LOEC: 5 ppm
NOEC: 1 ppm
LC₅₀: 2.54 ppm

APPENDIX A

BIO-AQUATIC TESTING, INC.

TOXICITY TEST

48 Hr Acute *Mysidopsis bahia*

Client: Em's Ecological Products, BioGrass Extra

Permit Number: N/A

Sample Type: Product

Receiving Water Name: N/A

N/A

Outfall Name: Product Only

Lab ID: 45132

Test Temperature (oC): 25 ± 1

Photo Period: 16 hours light
8 hours dark

Begin Date: 6/3/2010

End Date: 6/5/2010

Test Start Time: 16:43

Test End Time: 16:39

SURVIVAL

Effluent
Con.
ppm

Number Of Alive Per Replicate

Avg%
Surv.

	6/3															6/4															6/5															
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E																										
Synthetic Control	10	10				10	10				10	10				10	10				100.0%																									
100	10	10				10	10				10	10				10	10				100.0%																									
250	10	10				10	10				10	10				10	10				100.0%																									
500	10	10				10	10				10	10				10	10				100.0%																									
750	10	10				8	6				4	4				4	4				40.0%																									
1000	10	10				6	7				0	0				0	0				0.0%																									

BIO-AQUATIC TESTING, INC.

TOXICITY TEST

96 Hr Acute *Menidia beryllina*

Client: Em's Ecological Products, LLC BioGrass Extra

Lab ID: 45132

Permit Number: N/A N/A

Test Temperature (oC): 25 ± 1

Outfall Name: Product Only

Sample Type: Product

Photo Period: 16 Hours Light
8 Hours Dark

Receiving Water Name: N/A

Test Start Time:

Test End Time:

Begin Date: 6/3/2010

End Date: 6/7/2010

SURVIVAL

Effluent Concentration	Number Of Alive					Avg% Surv.
	6/3	6/4	6/5	6/6	6/7	
Synthetic Control	A	10	10	10	10	100.0%
	B	10	10	10	10	
	C					
	D					
	E					
100	A	10	10	10	10	100.0%
	B	10	10	10	10	
	C					
	D					
	E					
250	A	10	10	10	10	100.0%
	B	10	10	10	10	
	C					
	D					
	E					

BIO-AQUATIC TESTING, INC.

Effluent Concentration	Number Of Alive					Avg% Surv.	
	6/3	6/4	6/5	6/6	6/7		
500	A	10	7	7	7	7	80.0%
	B	10	9	9	9	9	
	C						
	D						
	E						
750	A	10	0	0	0	0	0.0%
	B	10	0	0	0	0	
	C						
	D						
	E						
1000	A	10	0	0	0	0	0.0%
	B	10	0	0	0	0	
	C						
	D						
	E						
	A						
	B						
	C						
	D						
	E						

BIO-AQUATIC TESTING, INC.

TOXICITY TEST

48 Hr Acute *Mysidopsis bahia*

Client: #2 Fuel oil

Permit Number: N/A

Sample Type:

Receiving Water Name: N/A

N/A

Outfall Name: #2 fuel oil

Lab ID: 45126

Test Temperature (oC): 25 ± 1

Photo Period: 16 hours light
8 hours dark

Begin Date: 6/3/2010

End Date: 6/5/2010

Test Start Time: 15:58

Test End Time: 15:30

SURVIVAL

Effluent Con. ppm	Number Of Alive Per Replicate															Avg% Surv.
	6/3					6/4					6/5					
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	
Synthetic Control	10	10				10	10				10	10				100.0%
.1	10	10				10	10				10	10				100.0%
1	10	10				10	10				10	10				100.0%
5	10	10				0	0				0	0				0.0%
10	10	10				0	0				0	0				0.0%
20	10	10				0	0				0	0				0.0%

BIO-AQUATIC TESTING, INC.

TOXICITY TEST

96 Hr Acute *Menidia beryllina*

Client: #2 Fuel oil

Lab ID: 45126

Permit Number: N/A N/A

Test Temperature (oC): 25 ± 1

Outfall Name: #2 fuel oil

Sample Type:

Photo Period: 16 Hours Light
8 Hours Dark

Receiving Water Name: N/A

Test Start Time: 15:53

Test End Time: 15:39

Begin Date: 6/3/2010

End Date: 6/7/2010

SURVIVAL

Effluent Concentration	Number Of Alive					Avg% Surv.
	6/3	6/4	6/5	6/6	6/7	
Synthetic Control	A	10	10	10	10	100.0%
	B	10	10	10	10	
	C					
	D					
	E					
.1	A	10	10	10	10	100.0%
	B	10	10	10	10	
	C					
	D					
	E					
1	A	10	10	10	10	100.0%
	B	10	10	10	10	
	C					
	D					
	E					

BIO-AQUATIC TESTING, INC.

Effluent Concentration	Number Of Alive					Avg% Surv.	
	6/3	6/4	6/5	6/6	6/7		
5	A	10	8	0	0	0	10.0%
	B	10	7	3	2	2	
	C						
	D						
	E						
10	A	10	1	0	0	0	0.0%
	B	10	2	0	0	0	
	C						
	D						
	E						
20	A	10	1	0	0	0	0.0%
	B	10	1	0	0	0	
	C						
	D						
	E						
	A						
	B						
	C						
	D						
	E						

BIO-AQUATIC TESTING, INC.

TOXICITY TEST

48 Hr Acute *Mysidopsis bahia*

Client: Em's Ecological Products, BioGrass Extra

Permit Number: N/A

Sample Type: Product

Receiving Water Name: N/A

N/A

Outfall Name: Product + #2 Fuel Oil

Lab ID: 45130

Test Temperature (oC): 25 ± 1

Photo Period: 16 hours light
8 hours dark

Begin Date: 6/3/2010

End Date: 6/5/2010

Test Start Time: 16:25

Test End Time: 15:50

SURVIVAL

Effluent
Con.
ppm

Number Of Alive Per Replicate

Avg%
Surv.

	6/3															6/4															6/5															
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E																										
Synthetic Control	10	10				10	10				10	10				10	10				100.0%																									
.1	10	10				10	10				10	10				10	10				100.0%																									
1	10	10				10	10				10	10				10	10				100.0%																									
5	10	10				0	0				0	0				0	0				0.0%																									
10	10	10				0	0				0	0				0	0				0.0%																									
20	10	10				0	0				0	0				0	0				0.0%																									

BIO-AQUATIC TESTING, INC.

TOXICITY TEST

96 Hr Acute *Menidia beryllina*

Client: Em's Ecological Products, LLC BioGrass Extra

Lab ID: 45130

Permit Number: N/A N/A

Test Temperature (oC): 25 ± 1

Outfall Name: Product + #2 Fuel Oil Sample Type: Product

Photo Period: 16 Hours Light
8 Hours Dark

Receiving Water Name: N/A

Test Start Time:

Test End Time:

Begin Date: 6/3/2010

End Date: 6/7/2010

SURVIVAL

Effluent Concentration	Number Of Alive					Avg% Surv.
	6/3	6/4	6/5	6/6	6/7	
Synthetic Control	A	10	10	10	10	100.0%
	B	10	10	10	10	
	C					
	D					
	E					
.1	A	10	10	10	10	95.0%
	B	10	10	9	9	
	C					
	D					
	E					
1	A	10	10	10	10	95.0%
	B	10	10	9	9	
	C					
	D					
	E					

BIO-AQUATIC TESTING, INC.

Effluent Concentration	Number Of Alive					Avg% Surv.	
	6/3	6/4	6/5	6/6	6/7		
5	A	10	7	2	2	2	15.0%
	B	10	9	2	2	1	
	C						
	D						
	E						
10	A	10	2	0	0	0	5.0%
	B	10	3	1	1	1	
	C						
	D						
	E						
20	A	10	0	0	0	0	0.0%
	B	10	0	0	0	0	
	C						
	D						
	E						
	A						
	B						
	C						
	D						
	E						

APPENDIX B

BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen

96 Hr Acute

Menidia beryllina

Client: Em's Ecological Products, LLC

Lab Number: 45132

Facility: BioGrass Extra

Dilution Water(s): Synthetic Lab

Outfall: Product Only

Test Begin Date: June 3, 2010

NR indicates that the test is non-renewal.

ANALYST	DATE	TIME	SX#	UNIT	Concentration								
					Synthetic	100	250	500	750	1000			
TS	6/3	Start		pH	8.2	8.2	8.2	8.2	8.2	8.2			
		25 ± 1		DO (mg/L)	7.6	7.5	7.5	7.5	7.4	7.4			
ME	6/4	24 Hr		pH	8.1	8.1	8.1	8.1	8.1	8.1			
		25 ± 1		DO (mg/L)	7.5	7.5	7.5	7.4	7.3	7.3			
		Renew		pH									
				DO (mg/L)									
ME	6/5	48 Hr		pH	8.0	7.9	7.9	7.9	7.9	7.9			
		25 ± 1		DO (mg/L)	7.2	7.2	7.2	7.2	7.2	6.9			
		Renew		pH									
				DO (mg/L)									
TS	6/6	72 Hr		pH	7.9	7.8	7.8	7.8	7.8	7.8			
		25 ± 1		DO (mg/L)	6.0	6.0	5.9	5.9	5.9	5.9			
		Renew		pH									
				DO (mg/L)									
TS	6/7	96 Hr		pH	7.8	7.8	7.8	7.8	7.7	7.7			
		25 ± 1		DO (mg/L)	5.4	4.7	4.6	4.4	3.9	4.0			
		Renew		pH									
				DO (mg/L)									
	6/8	120 Hr		pH									
		25 ± 1		DO (mg/L)									
		Renew		pH									
				DO (mg/L)									
	6/9	144 Hr		pH									
		25 ± 1		DO (mg/L)									
		Renew		pH									
				DO (mg/L)									
	6/10	168 Hr		pH									
		25 ± 1		DO (mg/L)									

APPENDIX C

STATISTICS SUMMARY

Both the lethal and sub-lethal endpoints were statistically calculated according to their respective EPA guidelines. The Chronic Freshwater organisms were calculated according to EPA/821/R-02/013, October 2002, Fourth Edition. The Chronic Marine and Estuarine organisms were calculated according to EPA/821/R-02/014, October 2002 Third Edition. The Acute Freshwater and Marine organisms were calculated according to EPA/821/R-02/012, October 2002 Fifth Edition. Listed below are the basic principles of these guidelines.

The acute *Menidia beryllina* survival data is analyzed using the Shapiro-Wilk's test for normality and Bartlett's Test for homogeneity. If the data passes both tests (parametric) then the data is run through ANOVA and Dunnett's. If the data fails either test then Steels Many-One Rank test is used, unless the degrees of freedom are not appropriate. The Trimmed Spearman-Karber method is used to calculate the LC50.

The acute *Mysidopsis bahia* survival data is analyzed using the Shapiro-Wilk's test for normality and Bartlett's Test for homogeneity. If the data passes both tests (parametric) then the data is run through ANOVA and Dunnett's. If the data fails either test then Steels Many-One Rank test is used, unless the degrees of freedom are not appropriate. The Trimmed Spearman-Karber method is used to calculate the LC50.

APPENDIX D

Americamysis bahia

BIO-AQUATIC TESTING, INC.

Carrollton, TX

REFERENCE TOXICANTS

Bio-Aquatic Testing conducts reference toxicant testing monthly for organisms cultured in-house. For studies requiring purchased organisms, reference toxicant testing is performed simultaneously. Reference toxicant testing validates data and measures organism consistency. Only reagent grade chemicals are used of the following choices: sodium laurel sulfate (SLS), copper sulfate, cadmium chloride, and sodium chloride. Organism responses are tracked with control charts for each reference toxicant/organism combination. The data are examined for sensitivity trends and to determine if results are within EPA described limits.

ACUTE REFERENCE TOXICANT TEST RESULTS

DILUTION WATER:	Standard Synthetic Saltwater
CHEMICAL:	Sodium Laurel Sulfate
DURATION:	48 Hour Acute
TEST NUMBER:	194
PROJECT NUMBER:	45082
START DATE:	5/27/2010
START TIME:	1500
TOTAL NUMBER EXPOSED:	40 organisms per concentration
CONCENTRATIONS (mg/L):	CON 2.5 5 10 15 20 40
NUMBER DEAD PER CONCENTRATION:	0 0 1 2 40 40 40
TEST METHODS:	As listed in EPA-821-R-02-012
STATISTICAL METHODS:	SURVIVAL: Trimmed Spearman-Karber
LC50:	11.71 mg/L
95% LOWER CONFIDENCE LIMITS:	11.13 mg/L
95% UPPER CONFIDENCE LIMITS:	12.32 mg/L

Menidia beryllina

BIO-AQUATIC TESTING, INC.

Carrollton, TX

REFERENCE TOXICANTS

Bio-Aquatic Testing conducts reference toxicant testing monthly for organisms cultured in-house. For studies requiring purchased organisms, reference toxicant testing is performed simultaneously. Reference toxicant testing validates data and measures organism consistency. Only reagent grade chemicals are used of the following choices: sodium laurel sulfate (SLS), copper sulfate, cadmium chloride, and sodium chloride. Organism responses are tracked with control charts for each reference toxicant/organism combination. The data are examined for sensitivity trends and to determine if results are within EPA described limits.

ACUTE REFERENCE TOXICANT TEST RESULTS

DILUTION WATER:	Standard Synthetic Saltwater						
CHEMICAL:	Sodium Laurel Sulfate						
DURATION:	96 Hour Acute						
TEST NUMBER:	187						
PROJECT NUMBER:	45080						
START DATE:	5/27/2010						
START TIME:	1330						
TOTAL NUMBER EXPOSED:	40 organisms per concentration						
CONCENTRATIONS (mg/L):	CON	2.5	5.0	10.0	15.0	20.0	40.0
NUMBER DEAD PER CONCENTRATION:	0	0	0	0	40	40	40
STATISTICAL METHODS:	SURVIVAL: Trimmed Spearman-Kärber						
LC50:	12.25	mg/L					
95% LOWER CONFIDENCE LIMITS:	NR	mg/L					
95% UPPER CONFIDENCE LIMITS:	NR	mg/L					

APPENDIX E

LITERATURE REFERENCES

- U.S.E.P.A., 1994. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Marine and Estuarine Organisms (Second Edition) U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio.
- U.S.E.P.A., 1994. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Marine and Estuarine Organisms (Third Edition) U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio.
- U.S.E.P.A., 1991. Technical Support Document for Water Quality-Based Toxic Control, U.S. Environmental Protection Agency, EPA/505/2-90-001.
- Zarr, Jerrold, H., 1984. Biostatistical Analysis, (Second Edition). Prentice-Hall, Inc. Englewood Cliffs, New Jersey.
- Weber, C.I., (ed.) 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (Fourth Edition). U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio. EPA-600/4-90-027