TCEQ NELAP Accredited

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Bio-Aquatic Testing
2501 Mayes Rd
Suite 100
Carrollton, TX 75006
(972) 242-7750

Aqueous Plant Extract Ref: 16765 Product Test

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

Using:
Aqueous Plant Extract Ref: 16765
#2 Fuel Oil
Aqueous Plant Extract Ref: 16765 /#2 Fuel Oil Mixture

Prepared by: _____ 6/9/2010
Vice President **Date**

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BIO-AQUATIC TESTING, INC.

2501 Mayes Road, Suite 100
Carrollton, Texas 75006
Tel: (972) 242-7750
Fax: (972) 242-7749

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, Aqueous Plant Extract Ref: 16765. 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 Aqueous Plant Extract Ref: 16765 product, #2 Fuel Oil, and Aqueous Plant Extract Ref: 16765 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
Aqueous Plant Extract Ref: 16765	Menidia beryllina	548.66	2
	Mysidopsis bahia	703.43	1
No. 2 Fuel Oil	Menidia beryllina	2.51	4
	Mysidopsis bahia	2.24	5.5
Aqueous Plant Extract Ref: 16765 & No. 2 FO	Menidia beryllina	2.54	3
	Mysidopsis bahia	2.24	5.5
Reference Toxicant: (Sodium Laurel Sulfate)	Menidia beryllina	12.25	
	Mysidopsis bahia	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: Aqueous Plant Extract Ref: 16765, #2 Fuel Oil, and Aqueous Plant Extract Ref: 16765 /#2 Fuel Oil

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SAMPLE PREPARATION:

Exploratory tests were conducted on the Aqueous Plant Extract Ref: 16765 product to determine a final definitive test dilution series. The Aqueous Plant Extract Ref: 16765 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/ Aqueous Plant Extract Ref: 16765 mix was made in the same manner as the #2 fuel oil stock solution with the ratio of Aqueous Plant Extract Ref: 16765 to oil being 1:10 (.5 mLs #2 fuel oil to 0.05 mLs Aqueous Plant Extract Ref: 16765 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 Aqueous Plant Extract Ref: 16765 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 Aqueous Plant Extract Ref: 16765 / #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 Aqueous Plant Extract Ref: 16765 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/ Aqueous Plant Extract Ref: 16765 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 Aqueous Plant Extract Ref: 16765 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

Aqueous Plant Extract Ref: 16765 /#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 Aqueous Plant Extract Ref: 16765 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
LC_{50}: 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
LC_{50}: 2.24 ppm

The *Mysidopsis bahia* survival data for the #2 Fuel Oil/ Aqueous Plant Extract Ref: 16765 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
LC_{50}: 2.24 ppm

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2501 Mayes Road, Suite 100
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TOXICITY TEST REPORT - 96 Hour Acute – *Menidia beryllina*

Client: Em's Ecological Products, LLC Blending and Packaging
Sample: Aqueous Plant Extract Ref: 16765, #2 Fuel Oil, and Aqueous Plant Extract Ref: 16765 /#2 Fuel Oil

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SAMPLE PREPARATION:

Exploratory tests were conducted on the Aqueous Plant Extract Ref: 16765 product to determine a final definitive test dilution series. The Aqueous Plant Extract Ref: 16765 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/ Aqueous Plant Extract Ref: 16765 mix was made in the same manner as the #2 fuel oil stock solution with the ratio of Aqueous Plant Extract Ref: 16765 to oil being 1:10 (.5 mLs #2 fuel oil to 0.05 mLs Aqueous Plant Extract Ref: 16765 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 Aqueous Plant Extract Ref: 16765 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 Aqueous Plant Extract Ref: 16765 / #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 Aqueous Plant Extract Ref: 16765 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/ Aqueous Plant Extract Ref: 16765 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 nine to eleven 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 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 Aqueous Plant Extract Ref: 16765 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 Aqueous Plant Extract Ref: 16765 /#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 Aqueous Plant Extract Ref: 16765 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
LC_{50}: 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
LC_{50}: 2.51 ppm

The *Menidia beryllina* survival data for the #2 Fuel Oil/ Aqueous Plant Extract Ref: 16765 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
LC_{50}: 2.54 ppm

APPENDIX A

BIO-AQUATIC TESTING, INC.

TOXICITY TEST

48 Hr Acute *Mysidopsis bahia*

Lab ID: 45132

Client: Em's Ecological Products, Aqueous Plant Extract Ref: 16765

Test Temperature (oC): 25 ± 1

Permit Number: N/A N/A

Photo Period: 16 hours light 8 hours dark

Sample Type: Product **Outfall Name:** Product Only

Begin Date: 6/3/2010

Receiving Water Name: N/A

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	
Synthetic Control	10	10				10	10				10	10				100.0%
100	10	10				10	10				10	10				100.0%
250	10	10				10	10				10	10				100.0%
500	10	10				10	10				10	10				100.0%
750	10	10				8	6				4	4				40.0%
1000	10	10				6	7				0	0				0.0%

BIO-AQUATIC TESTING, INC.

TOXICITY TEST

96 Hr Acute *Menidia beryllina*

Client: Em's Ecological Products, LLC Aqueous Plant Extract Ref: 16765 **Lab ID:** 45132

Permit Number: N/A N/A

Outfall Name: Product Only **Sample Type:** Product

Receiving Water Name: N/A

Test Temperature (oC): 25 ± 1

Photo Period: 16 Hours Light 8 Hours Dark

Test Start Time: 16:38 Test End Time: 16:09

Begin Date: 6/3/2010

End Date: 6/7/2010

SURVIVAL

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

* = Spilled cup

BIO-AQUATIC TESTING, INC.

Effluent Concentration		Number Of Alive 6/3	6/4	6/5	6/6	6/7	Avg% Surv.
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*

Lab ID: 45126

Client: #2 Fuel oil

Test Temperature (oC): 25 ± 1

Permit Number: N/A N/A

Photo Period: 16 hours light 8 hours dark

Sample Type: **Outfall Name:** #2 fuel oil

Begin Date: 6/3/2010

Receiving Water Name: N/A

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

Outfall Name: #2 fuel oil

Sample Type:

Receiving Water Name: N/A

Test Temperature (oC): 25 ± 1

Photo Period: 16 Hours Light
8 Hours Dark

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

* = Spilled cup

BIO-AQUATIC TESTING, INC.

Effluent Concentration		Number Of Alive 6/3	6/4	6/5	6/6	6/7	Avg% Surv.
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*

Lab ID: 45130

Client: Em's Ecological Products, Aqueous Plant Extract Ref: 16765

Test Temperature (oC): 25 ± 1

Permit Number: N/A N/A

Photo Period: 16 hours light 8 hours dark

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

Begin Date: 6/3/2010

Receiving Water Name: N/A

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	
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: Em's Ecological Products, LLC Aqueous Plant Extract Ref: 16765 **Lab ID:** **45130**

Permit Number: N/A N/A

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

Receiving Water Name: N/A

Test Temperature (oC): 25 ± 1

Photo Period: 16 Hours Light 8 Hours Dark

Test Start Time: 16:20 Test End Time: 16:10

Begin Date: 6/3/2010

End Date: 6/7/2010

SURVIVAL

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

* = Spilled cup

BIO-AQUATIC TESTING, INC.

Effluent Concentration		Number Of Alive 6/3	6/4	6/5	6/6	6/7	Avg% Surv.
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

48 Hr Acute **Mysidopsis bahia**

Client: Em's Ecological Products, LLC **Lab ID: 45132**

Facility: Aqueous Plant Extract Ref:

Outfall: Product Only **Dilution Water(s):** Synthetic Lab

Test Begin Date: June 3, 2010

NR indicates that the test is non-renewal.

					Concentration							
ANALYST	DATE	TIME	SX#	UNIT	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	8.0	8.0	8.0	8.0	8.0	8.0		
		25 ± 1		DO (mg/L)	7.5	7.4	7.4	7.3.	7.3	7.3		
		Renew		pH								
				DO (mg/L)								
ME	6/5	48 Hr		pH	7.6	7.8	7.8	7.8	7.8	7.8		
		25 ± 1		DO (mg/L)	4.9	4.1	3.9	3.9	3.8	3.8		
		Renew		pH								
				DO (mg/L)								
	6/6	72 Hr		pH								
		25 ± 1		DO (mg/L)								
		Renew		pH								
				DO (mg/L)								
	6/7	96 Hr		pH								
		25 ± 1		DO (mg/L)								
		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)								

BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen

96 Hr Acute **Menidia beryllina**

Client: Em's Ecological Products, LLC **Lab Number:** 45132

Facility: Aqueous Plant Extract Ref: **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)								

BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen

48 Hr Acute **Mysidopsis bahia**

Client: **#2 Fuel oil** **Lab ID: 45126**

Facility:

Outfall: **#2 fuel oil** **Dilution Water(s): Synthetic Lab**

Test Begin Date: June 3, 2010

NR indicates that the test is non-renewal.

					Concentration							
ANALYST	DATE	TIME	SX#	UNIT	Synthetic	.1	1	5	10	20		
TS	6/3	Start		pH	8.2	8.2	8.2	8.2	8.1	8.1		
		25 ± 1		DO (mg/L)	7.6	7.6	7.5	7.5	7.5	7.5		
ME	6/4	24 Hr		pH	8.0	8.0	7.9	7.9	7.9	7.9		
		25 ± 1		DO (mg/L)	7.8	7.8	7.8	7.8	7.6	7.6		
		Renew		pH								
				DO (mg/L)								
ME	6/5	48 Hr		pH	7.6	7.8	7.8	7.8	7.8	7.8		
		25 ± 1		DO (mg/L)	4.9	4.6	5.2	5.2	5.3	5.3		
		Renew		pH								
				DO (mg/L)								
	6/6	72 Hr		pH								
		25 ± 1		DO (mg/L)								
		Renew		pH								
				DO (mg/L)								
	6/7	96 Hr		pH								
		25 ± 1		DO (mg/L)								
		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)								

BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen

96 Hr Acute **Menidia beryllina**

Client: #2 Fuel oil **Lab Number:** 45126

Facility: **Dilution Water(s):** Synthetic Lab

Outfall: #2 fuel oil **Test Begin Date:** June 3, 2010

NR indicates that the test is non-renewal.

ANALYST	DATE	TIME	SX#	UNIT	Concentration							
					Synthetic	.1	1	5	10	20		
TS	6/3	Start		pH	8.2	8.2	8.2	8.2	8.1	8.1		
		25 ± 1		DO (mg/L)	7.6	7.6	7.5	7.5	7.5	7.5		
ME	6/4	24 Hr		pH	8.1	8.1	8.1	8.1	8.1	8.1		
		25 ± 1		DO (mg/L)	7.6	7.6	7.6	7.6	7.6	7.6		
		Renew		pH								
				DO (mg/L)								
ME	6/5	48 Hr		pH	8.0	8.0	8.0	8.1	8.1	8.1		
		25 ± 1		DO (mg/L)	7.4	7.0	7.0	7.0	7.0	7.0		
		Renew		pH								
				DO (mg/L)								
TS	6/6	72 Hr		pH	8.0	8.0	8.0	8.0	8.0	8.0		
		25 ± 1		DO (mg/L)	6.6	6.5	6.4	6.3	6.3	6.2		
		Renew		pH								
				DO (mg/L)								
TS	6/7	96 Hr		pH	7.8	7.8	7.9	7.8	7.8	7.9		
		25 ± 1		DO (mg/L)	5.4	4.6	4.4	4.3	4.7	5.0		
		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)								

BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen

48 Hr Acute **Mysidopsis bahia**

Client: Em's Ecological Products, LLC **Lab ID: 45130**

Facility: Aqueous Plant Extract Ref: **Dilution Water(s):** Synthetic Lab

Outfall: Product + #2 Fuel Oil **Test Begin Date:** June 3, 2010

NR indicates that the test is non-renewal.

					Concentration							
ANALYST	DATE	TIME	SX#	UNIT	Synthetic	.1	1	5	10	20		
TS	6/3	Start		pH	8.2	8.1	8.1	8.1	8.1	8.1		
		25 ± 1		DO (mg/L)	7.6	7.6	7.6	7.6	7.6	7.6		
ME	6/4	24 Hr		pH	7.8	7.8	7.8	7.8	7.8	7.8		
		25 ± 1		DO (mg/L)	6.5	6.5	6.4	6.3	6.3	6.3		
		Renew		pH								
				DO (mg/L)								
ME	6/5	48 Hr		pH	7.6	7.9	7.9	7.9	7.9	7.9		
		25 ± 1		DO (mg/L)	4.9	3.9	4.0	4.0	4.1	4.1		
		Renew		pH								
				DO (mg/L)								
	6/6	72 Hr		pH								
		25 ± 1		DO (mg/L)								
		Renew		pH								
				DO (mg/L)								
	6/7	96 Hr		pH								
		25 ± 1		DO (mg/L)								
		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)								

BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen

96 Hr Acute **Menidia beryllina**

Client: Em's Ecological Products, LLC **Lab Number:** 45130

Facility: Aqueous Plant Extract Ref: **Dilution Water(s):** Synthetic Lab

Outfall: Product + #2 Fuel Oil **Test Begin Date:** June 3, 2010

NR indicates that the test is non-renewal.

ANALYST	DATE	TIME	SX#	UNIT	Concentration							
					Synthetic	.1	1	5	10	20		
TS	6/3	Start		pH	8.2	8.1	8.1	8.1	8.1	8.1		
		25 ± 1		DO (mg/L)	7.6	7.6	7.6	7.6	7.6	7.6		
ME	6/4	24 Hr		pH	8.1	8.1	8.1	8.1	8.0	8.0		
		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	8.0	8.0	8.0	8.0	8.0		
		25 ± 1		DO (mg/L)	7.2	7.2	7.2	7.1	7.1	7.1		
		Renew		pH								
				DO (mg/L)								
TS	6/6	72 Hr		pH	8.0	8.0	8.0	7.9	7.9	7.9		
		25 ± 1		DO (mg/L)	6.4	6.3	6.3	6.0	5.9	5.8		
		Renew		pH								
				DO (mg/L)								
TS	6/7	96 Hr		pH	7.9	7.9	7.9	7.8	7.8	7.8		
		25 ± 1		DO (mg/L)	5.6	5.5	5.5	5.5	5.5	5.5		
		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-Karber	
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.

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