Toxicity testing on NWTPH-Dx

Environmental Assessment Program
(EAP)

Final Report

January 23, 2018

Submitted to: **Washington Department of Ecology**
PO BOX 47600
Olympia, WA

8664 Commerce Court, Burnaby, BC V5A 4N7

TABLE OF CONTENTS

List of Tables

TABLE OF CONTENTS

List of Figures

List of Appendices

SIGNATURE PAGE

Report By:
Emma Marus, B.Sc.
Laboratory Biologist

Reviewed By:
Curtis Eickhoff, Ph.D.
Senior Reviewer

This report has been prepared by Nautilus Environmental Company Inc. based on data and/or samples provided by our client and the results of this study are for their sole benefit. Any reliance on the data by a third party is at the sole and exclusive risk of that party. The results presented here relate only to the samples tested.

SUMMARY

Sample Information and Test Type

Sample ID	NWTPH-Dx
Sample information	Custom Diesel Fuel #2 Composite Standard No. 572563, Lot# A0127308, 50,000 µg/mL each in acetone, 5 mL ampoules
Sample manufacturer	Restek Corporation 110 Benner Circle • Bellefonte, PA 16823
Sample supplier	Chromatographic Specialties Inc. 300 Laurier Blvd. Brockville, ON. K6V 5W1
Sample receipt date	May 19, 2017
Sample receipt temperature	N/A
Sample storage temperature	Store at 25 °C nominal
Test types	Fathead minnow *(Pimephales promelas*) survival and growth Topsmelt *(Atherinops affinis*) survival and growth *Ceriodaphnia dubia* survival and reproduction Echinoderm *(Strongylocentrotus purpuratus)* fertilization

Summary of Results

Endpoint *	mg/L diesel (95% CL)	NOEC	LOEC
Fathead minnow			
survival LC50	1.87 (1.43 – 2.45)	1.30	2.70
biomass IC25	0.87 (0.70 – 1.23)	0.65	1.30
biomass IC50	1.39 (1.03 – 1.84)		
Topsmelt			
survival LC50	0.68 (0.55 – 0.83)	0.26	0.57
biomass IC25	0.74 (0.37 – 0.80)	0.57	1.60
biomass IC50	0.99 (0.76 – 1.04)		
Ceriodaphnia dubia			
survival LC50	0.23 (0.20 – 0.26)	0.22	0.34
reproduction IC25	0.17 (0.16 – 0.19)	0.15	0.22
reproduction IC50	0.20 (0.19 – 0.24)		
Echinoderm			
fertilization IC25	0.19 (0.12 – 0.22)	<0.05	0.05
fertilization IC50	0.34 (0.29 – 0.38)		

LC = Lethal Concentration, IC = Inhibition Concentration, NOEC = No Observed Effect Concentration, LOEC = Lowest Observed Effect Concentration, * = result was calculated using the solvent control as the negative control

1.0 INTRODUCTION

Nautilus Environmental Company Inc. conducted toxicity tests for the Washington Department of Ecology's (WDOE) Environmental Assessment Program (EAP) as part of a study to determine environmental effects-based concentrations of total petroleum hydrocarbons (TPHs) associated with diesel fuel #2 for aquatic organisms. Customized Diesel samples were ordered by the WDOE and provided by Chromatographic Specialties Inc. These diesel fuel #2 samples were specially prepared in acetone rather than dichloromethane to provide maximum solubility in water for the toxicity tests. The diesel fuel standards were received at the Nautilus laboratory in Burnaby, BC on May 19, 2017. The following toxicity tests were performed on the Custom Diesel Fuel #2 Composite Standards:

- Fathead minnow survival and growth (EPA-821-R-02_013)
- Topsmelt survival and growth (EPA/600/R-95/136)
- *Ceriodaphnia dubia* survival and reproduction (EPA-821-R-02-013)
- Echinoderm fertilization (EPA/600/R-95/136)

This report describes the results of these toxicity tests conducted on the diesel fuel #2 composite standard sample. Copies of laboratory data sheets and printouts of statistical analyses are provided in Appendices A to E. The chain-of-custody form is provided in Appendix F.

2.0 METHODS

Methods for the toxicity tests are summarized in Tables 1 to 4. Testing was conducted according to procedures described by the US EPA (1995, 2002) and the Washington Department of Ecology (WDOE, 2016). To determine an appropriate concentration series that would capture NOEC and LOEC values, range-finding tests were performed for each test species prior to initiation of the definitive tests. Definitive tests using Fathead minnow, Topsmelt and *C. dubia* were initiated on July 6, 2017. Tests using Echinoderms were initiated on July 7, 2017.

Table 1. Summary of test conditions: 7-d fathead minnow (*Pimephales promelas*) survival and growth test.

Test species	*Pimephales promelas*
Organism source	Commercial supplier
Organism age	<24 hours post-hatch
Test type	Static-renewal
Test duration	7 days
Test vessel	375-mL glass container with lid
Test volume	375 mL
Test concentrations	Six concentrations, plus laboratory and methanol control
Test replicates	4 per treatment
Number of organisms	10 per replicate
Control/dilution water	Moderately-hard reconstituted water
Test solution renewal	Daily (80% renewal)
Test temperature	25 ± 1°C
Feeding	Twice a day with approximately 1500-2250 newly hatched brine shrimp nauplii (*Artemia sp.*) in each test container
Light intensity	100 to 500 lux
Photoperiod	16 hours light / 8 hours dark
Aeration	None, unless dissolved oxygen falls to <40% saturation
Test measurements	Temperature, dissolved oxygen, pH and conductivity measured daily; hardness and alkalinity of undiluted sample measured at test initiation; survival checked daily
Test protocol	EPA-821-R-02_013
Statistical software	CETIS Version 1.8.7
Test endpoints	Survival and biomass
Test acceptability criteria for controls	≥80% survival; ≥250 µg mean dry weight
Reference toxicant	Sodium chloride (NaCl)

Table 2. Summary of test conditions: 7-d topsmelt (*Atherinops affinis*) survival and growth test.

Test species	*Atherinops affinis*
Organism source	Commercial supplier
Organism age	9-to 15-days post-hatch
Test type	Static-renewal
Test duration	7 days
Test vessel	500-mL glass container with lid
Test volume	500 mL
Test concentrations	Six concentrations, plus laboratory and methanol control
Test replicates	5 per treatment
Number of organisms	5 per replicate
Control/dilution water	Natural seawater
Test solution renewal	Daily (80% renewal)
Test temperature	20 ± 1°C
Test salinity	30 ± 2 ppt; sample salinity adjusted by addition of H_2Ocean Pro+ marine salts
Feeding	Twice a day with newly hatched brine shrimp nauplii (*Artemia sp.*)
Light intensity	Ambient laboratory lighting
Photoperiod	16 hours light / 8 hours dark
Aeration	None, unless dissolved oxygen falls below 4.0 mg/L
Test measurements	Temperature, dissolved oxygen, pH and salinity measured daily; survival checked daily
Test protocol	EPA/600/R-95/136
Statistical software	CETIS Version 1.8.7
Test endpoints	Survival and biomass
Test acceptability criteria for controls	≥80% survival; ≥0.85 mg mean dry weight
Reference toxicant	Copper (added as $CuCl_2$)

Table 3. Summary of test conditions: *Ceriodaphnia dubia* survival and reproduction test.

Test species	*Ceriodaphnia dubia*
Organism source	In-house culture
Organism age	<24 hour old neonates, produced within a 8 hour window
Test type	Static-renewal
Test duration	7 ± 1 day
Test vessel	20-mL glass test tube with snap cap
Test volume	20 mL
Test concentrations	Six concentrations, plus laboratory and methanol control
Test replicates	10 per treatment
Number of organisms	1 per replicate
Control/dilution water	20% Perrier water and 80% deionized water + 5 µg/L Se and 2 µg/L vitamin B12
Test solution renewal	Daily (100% renewal)
Test temperature	25 ± 1°C
Feeding	Daily with *Pseudokirchneriella subcapitata* and YCT (3:1 ratio)
Light intensity	100 to 600 lux at water surface
Photoperiod	16 hours light / 8 hours dark
Aeration	None
Test measurements	Temperature, dissolved oxygen, pH and conductivity measured daily; hardness and alkalinity of undiluted sample measured at test initiation; survival and reproduction checked daily
Test protocol	EPA-821-R-02-013
Statistical software	CETIS Version 1.8.7
Test endpoints	Survival and reproduction
Test acceptability criteria for controls	≥80% survival; ≥15 young per surviving control producing three broods; ≥60% of controls producing three or more broods; no ephippia present
Reference toxicant	Sodium chloride (NaCl)

Table 4. Summary of test conditions: 40-min echinoderm fertilization test.

Test species	*Strongylocentrotus purpuratus*
Organism source	Commercial supplier
Organism age	< 3 hours post gamete collection
Test type	Static
Test duration	10 minutes sperm exposure; 10 minutes egg fertilization
Test vessel	30-mL glass vials with snap cap
Test volume	30 mL
Test concentrations	Six concentrations, plus laboratory and methanol control
Test replicates	4 per treatment
Number of organisms	2000 eggs per replicate
Control/dilution water	Natural seawater
Test solution renewal	None
Test temperature	12 ± 1°C
Test salinity	30 ± 2 ppt; sample salinity adjusted by addition of H_2Ocean Pro+ marine salts
Feeding	None
Light intensity	Ambient laboratory lighting
Photoperiod	None
Aeration	None
Test measurements	Temperature, dissolved oxygen, pH and salinity measured at test initiation
Test protocol	EPA/600/R-95/136
Statistical software	CETIS Version 1.8.7
Test endpoint	Fertilization
Test acceptability criterion for controls	≥60% and <98% mean fertilization
Reference toxicant	Copper (added as $CuCl_2$)

2.1 Preparation of Stock Dilution Series

The nominal concentration series that was used for the definitive tests with Topsmelt and Echinoderms was 16, 8.0, 4.0, 2.0, 1.0, 0.5 mg diesel/L. The nominal concentration series for fathead minnows was 30, 15, 7.5, 3.8, 1.9, 0.9 mg diesel/L and for *C. dubia* was 6, 3, 1.5, 0.75, 0.38, 0.19, 0.09, 0.045 mg diesel/L. A stock solution of 50 mg diesel/mL was prepared first and used to prepare the dilutions. The stock was prepared by using approximately 1.77 to 1.79 mL of the diesel standard in approximately 5.53 to 5.58 L of dilution water. Stock solutions were made separately for each test species with respective control water. The 50 mg diesel/mL stock solutions in the

aspirator bottles were stirred overnight and allowed to settle for 1 hour prior to use in preparing dilutions. Aspirator bottles were capped with rubber stoppers covered with aluminum foil to prevent evaporation. 5-L bottles were filled close to the top to avoid loss of the diesel from volatilization. Some space was left to avoid adsorption of the diesel to the foil covered stopper. Aspirator bottles were drained using the port at the bottom of the bottle to avoid using the undissolved fraction at the top of the vessels. Stock solutions for the *C. dubia* tests were prepared in 2L Erlenmeyer flasks and were siphoned using hydrocarbon resistant tubing.

Samples of test solutions were taken immediately after dilutions were prepared and were collected in 40-mL sample vials provided by the Manchester Environmental Laboratory, MEL, with no head space.

2.2 Subsampling Test Solutions

Subsamples of the fresh stock water were taken during the filling of the test chambers. Subsamples of the stale test chamber solutions were collected as a composite of the chamber replicates prior to renewal or at the end of the tests.

Test solutions were prepared by the addition of diesel to achieve a range of test concentrations in laboratory control water specific to each test species. Test solutions were prepared and renewed daily for tests with Fathead minnow, Topsmelt and *C. dubia*; the 40 minute echinoderm test did not require renewal of test solution. In addition, an acetone control was tested concurrently with all four tests. The acetone control was used to calculate all test endpoints.

2.3 Acetone Control

An acetone control was prepared as a solution of acetone in dilution water. Each set of tests had a separate acetone control based on the amount of acetone used in the top concentration of the test. For Topsmelt and Echinoderms tests, a 0.036% acetone solution was used. For fathead minnow and *C. dubia*, the percentage of acetone used in the solvent control solution was 0.067 and 0.012% acetone, respectively. The acetone control was prepared in separate containers on the day that the tests were set up and for all test solution renewals.

2.4 Statistical Analysis

Statistical analyses were performed using the acetone control as the negative control with CETIS (Tidepool Scientific Software, 2013).

2.5 Test Lighting

Natural lighting was provided to the toxicity tests using full spectrum UV fluorescent light tubes, Ushio 3000417, F32T8/960 - 32W T8 Fluorescent 6000K 95CRI. Full spectrum fluorescent tubes were purchased from Top Bulb 5204 Indianapolis Blvd. East Chicago, IN 46312.

3.0 RESULTS

3.1 Analytical Results

Samples of the fresh and "stale" test solutions were analyzed by the Manchester Environmental Laboratory. The nominal and measured concentrations are presented in Tables 5 to 8.

Table 5. Results: Fathead minnow nominal and measured test concentrations (mg diesel/L).

Nominal (mg/L)	Measured (mg/L)									
	t=0	t=24		t=48	t=72	t=96		t=120	t=144	t=168
	fresh	stale	fresh	fresh	fresh	stale	fresh	fresh	fresh	stale
30	3.03	0.74	3.26	1.75	1.79	0.59	2.86	2.72	3.68	1.37
15	-	-	-	-	-	-	-	-	-	-
7.5	0.29	0.18	0.48	0.37	0.42	0.19	0.57	0.62	1.05	0.42
7.5 dupl.	0.49	-	-	-	0.43	-	0.54	-	1.05	-
3.8	-	-	-	-	-	-	-	-	-	-
1.9	0.13	0.06	0.12	0.13	0.18	0.18	0.17	0.14	0.21	0.28
0.9	0.08	0.06	0.11	0.14	0.10	0.15	0.10	0.10	0.12	0.29
0	0.05	-	-	-	-	-	-	-	-	-
0	0.05	-	-	-	-	-	-	-	-	-
0	0.05	-	-	-	-	-	-	-	-	-

t = time, Detection Limit = 0.05

Table 6. Results: Topsmelt nominal and measured test concentrations (mg diesel/L).

Nominal (mg/L)	Measured (mg/L)									
	t=0	t=24		t=48	t=72	t=96		t=120	t=144	t=168
	fresh	stale	fresh	fresh	fresh	stale	fresh	fresh	fresh	stale
16	0.62	0.58	2.02	3.31	0.96	x	x	x	x	x
8	-	-	-	-	-	-	-	-	-	-
4	0.07	0.14	0.38	0.31	0.32	0.15	0.17	0.20	0.29	0.20
4 dupl.	0.29	-	0.40	-	0.31	-	0.14	-	0.32	-
2	-	-	-	-	-	-	-	-	-	-
1	0.07	0.05	0.13	0.10	0.11	0.16	0.11	0.18	0.18	0.16
0.5	0.05	0.05	0.07	0.06	0.06	0.12	0.13	0.14	0.13	0.17
0	0.05	-	-	-	-	-	-	-	-	-
0	0.05	-	-	-	-	-	-	-	-	-
0	0.06	-	-	-	-	-	-	-	-	-

* Result of reanalysed sample, t = time, Detection Limit = 0.05, x = sample not taken due to 100% mortality in test concentration.

Table 7. Results: *Ceriodaphnia dubia* nominal and measured test concentrations (mg diesel/L).

Nominal (mg/L)	Measured (mg/L)						
	t = 0	t=24	t=48	t=72	t = 96	t = 120	t=144
	fresh	fresh	fresh	fresh	fresh	fresh	fresh
6	1.19	x	x	x	x	x	x
3	0.55	x	x	x	x	x	x
3 dupl.	0.24	x	x	x	x	x	x
1.5	0.29	1.22	0.36	0.53	x	x	x
0.75	0.11	0.52	0.23	0.15	0.40	0.19	0.42
0.38	0.08	0.24	0.14	0.14	0.21	0.10	0.21
0.19	0.05	-	0.08	-	0.12	-	0.14
0.09	-	-	-	-	-	-	-
0.045	-	-	-	-	-	-	-
0	0.05	-	-	-	-	-	-
0	0.05	-	-	-	-	-	-
0	0.06	-	-	-	-	-	-

*Result of reanalysed sample, t = time, Detection Limit = 0.05, x = sample not taken due to 100% mortality in test concentration.

Table 8. Results: Echinoderm nominal and measured test concentrations (mg diesel/L).

Nominal (mg/L)	Measured (mg/L)
	t=0
	fresh
16	0.62
8	-
4	0.07
4 dupl.	0.29
2	-
1	0.07
0.5	0.05
0	0.05
0	0.05
0	0.06

t = time

3.2 Calculation of Test Solution Concentrations

Due to the poor solubility of diesel in water, the majority of the measured concentrations were >20% lower than nominal concentration. The nominal and measured test concentrations are presented in Tables E-1 - E-3 in Appendix E. The nominal concentrations therefore, could not be used to calculate test endpoints. In order to calculate the average measured test concentrations over the duration of the test and to estimate non-measured test concentrations, nominal and measured concentrations were plotted and linear regression lines were fitted for each test. Test concentrations were calculated from the nominal concentrations using the equation of the regression lines plotted for each definitive test.

Figures 1 to 4 show the regression lines and equations used to calculate the test concentrations, which were used to calculate the test endpoints for the Fathead minnow, Topsmelt, *C. dubia*, and echinoderm tests. For the fathead minnow and Topsmelt test concentrations, two regression lines were fit to provide the best estimate of the average measured concentrations. Concentrations that

were not measured were calculated from the regression equations. Table 9 provides a summary of the calculated diesel test concentrations from the regression equations.

Table 9. Results: Summary of nominal and calculated diesel test concentrations (mg diesel/L).

Fathead minnow		**Topsmelt**		**Echinoderm**		***C. dubia***	
Nominal	Calculated	Nominal	Calculated	Nominal	Calculated	Nominal	Calculated
30	2.7	16	1.6	16	0.62	6	0.75
15	1.3	8	0.57	8	0.33	3	0.50
7.5	0.65	4	0.26	4	0.18	1.5	0.34
3.8	0.31	2	0.16	2	0.11	0.75	0.22
1.9	0.13	1	0.12	1	0.07	0.38	0.15
0.9	0.04	0.5	0.10	0.5	0.05	0.19	0.10
						0.09	0.07
						0.045	0.04

Figure 1. Linear Regression of Nominal vs. Measured Concentrations – Fathead Minnow

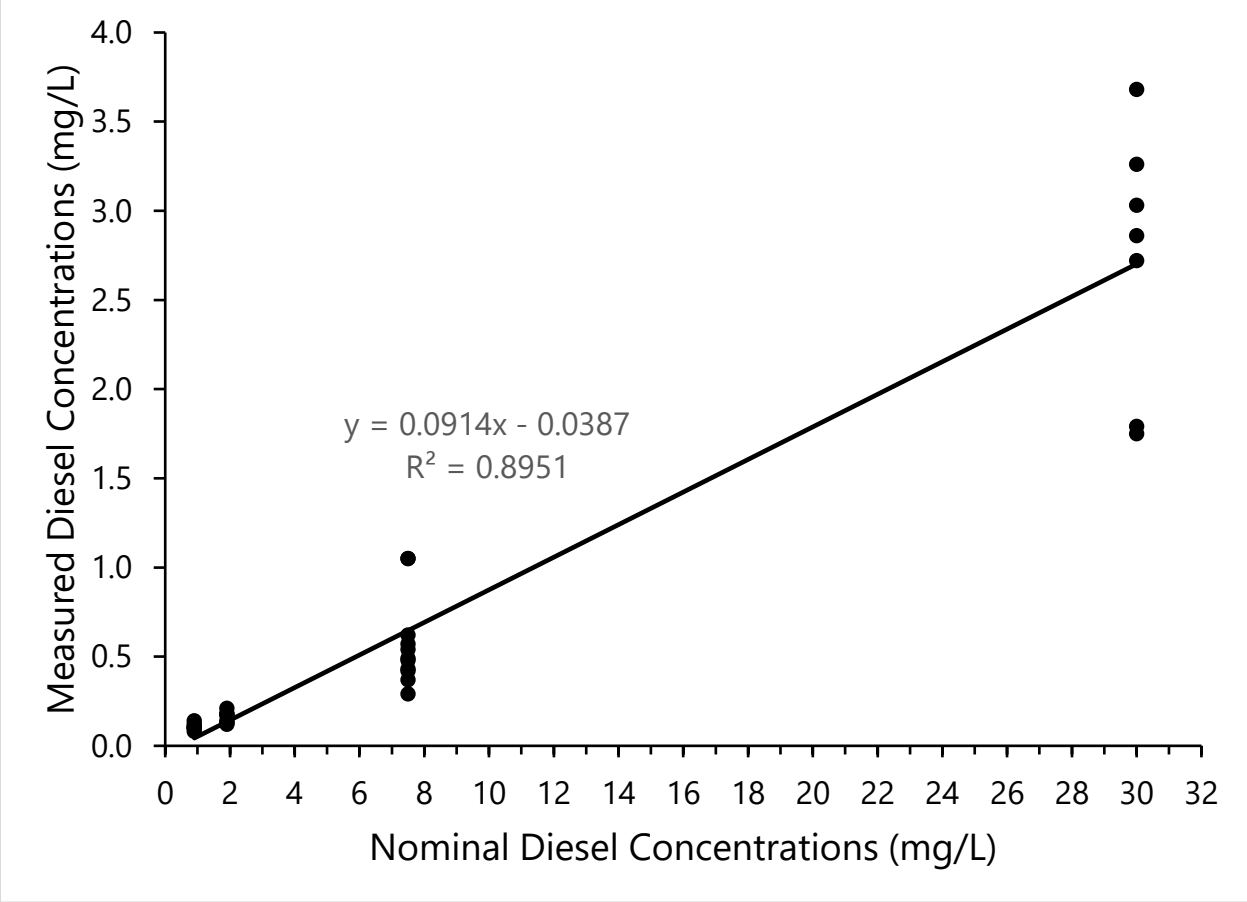


Figure 2. Polynomial Regression of Nominal vs. Measured Concentrations – Topsmelt

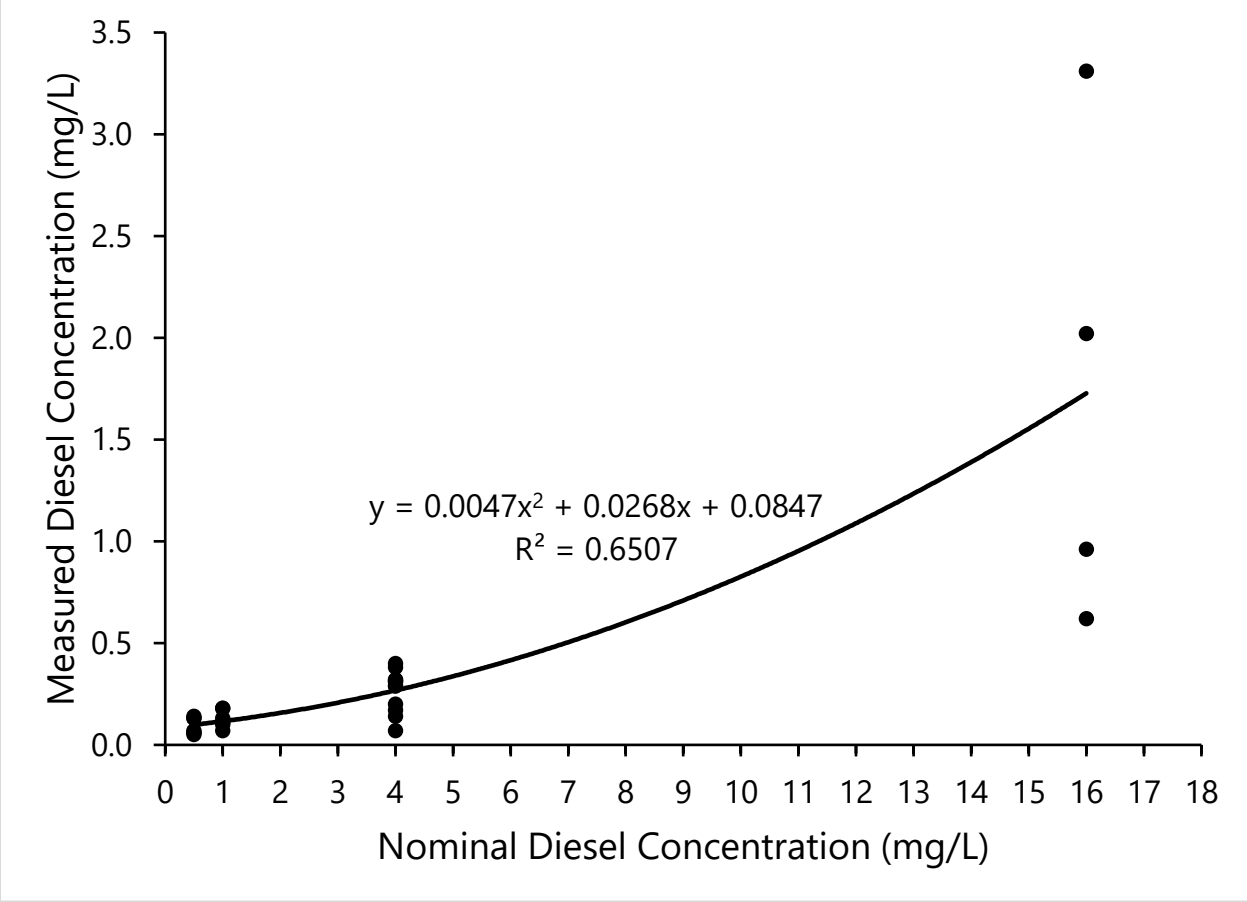


Figure 3. Power Regression of Nominal vs. Measured Concentrations – *C. dubia*

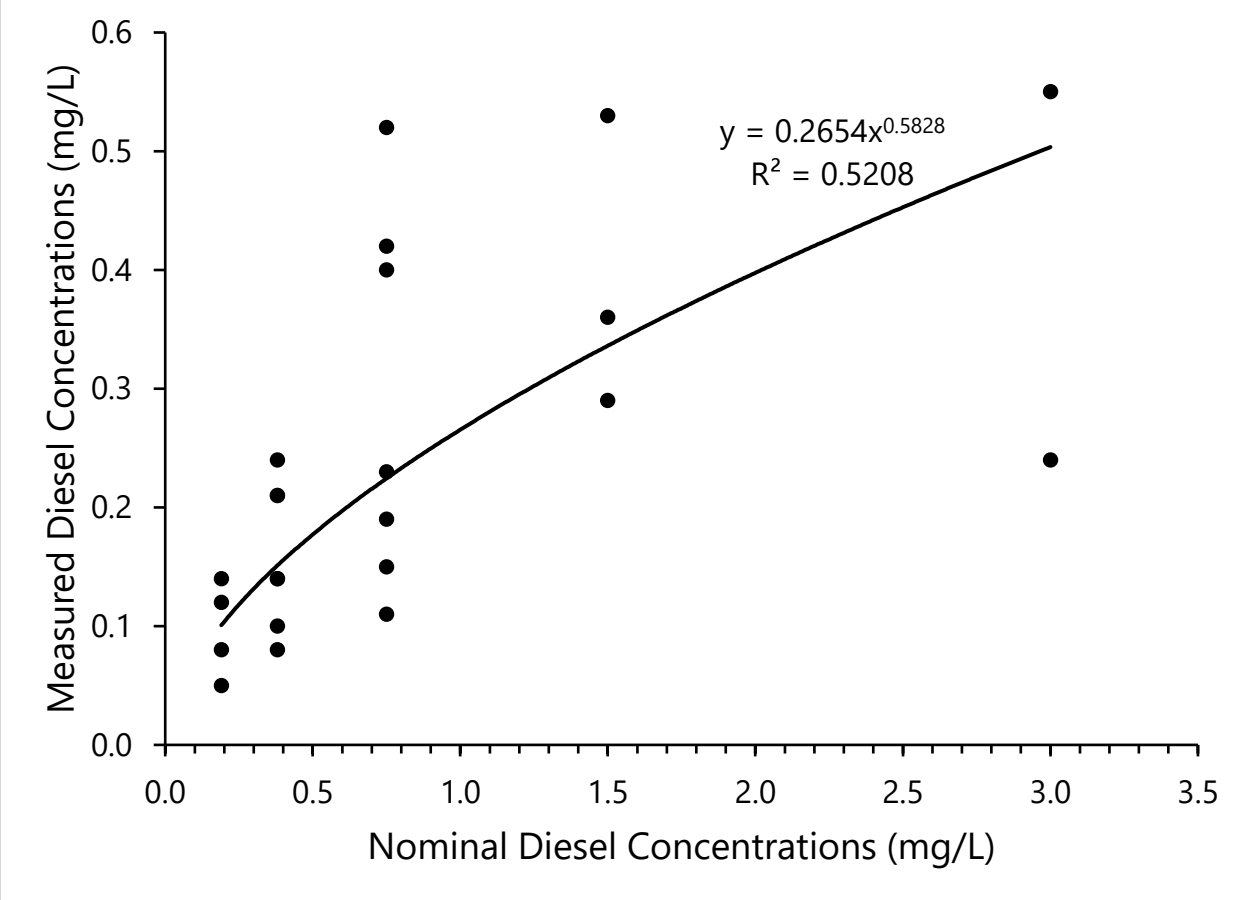
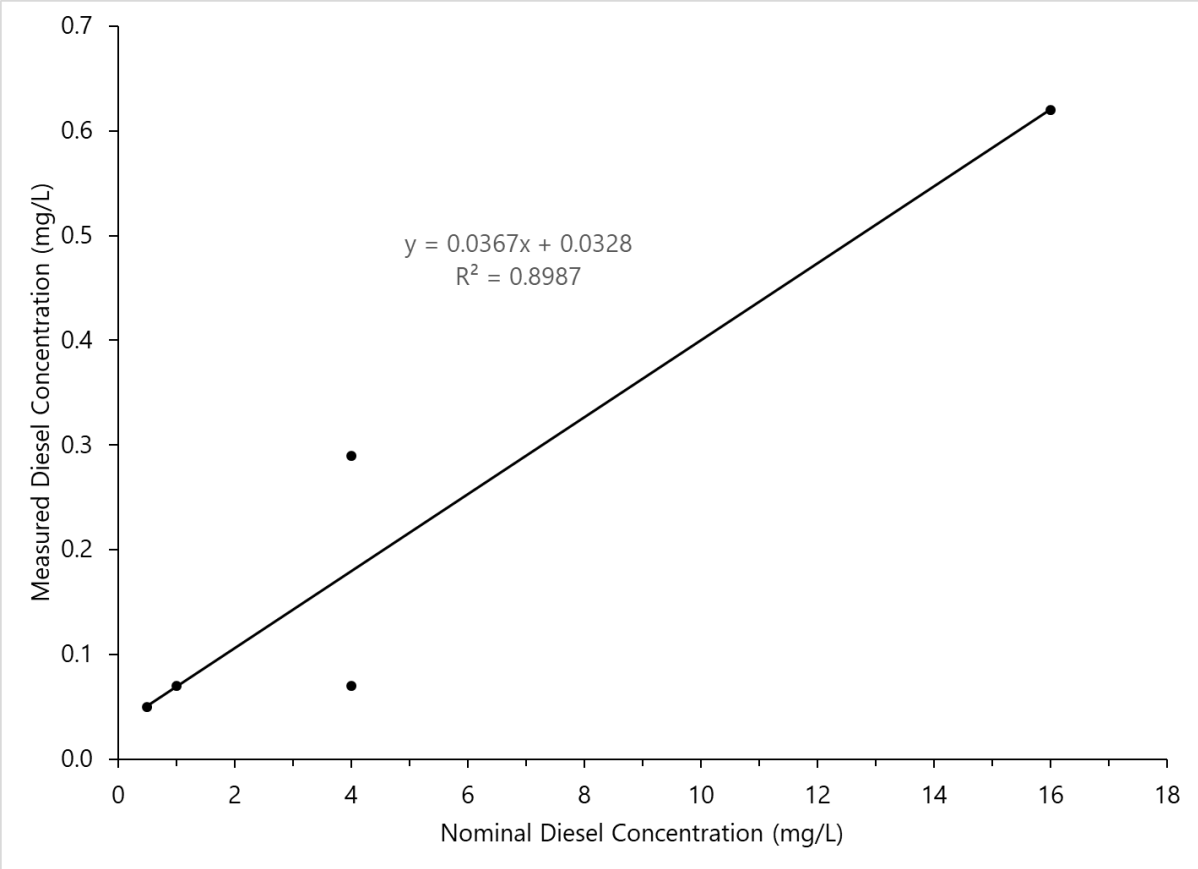


Figure 4. Linear Regression of Nominal vs. Measured Concentrations – Echinoderm



3.3 Toxicity Test Results

The results of the toxicity tests are summarized in Tables 10 to 13. All endpoints were calculated in CETIS using the solvent control as the negative control.

The results of the 7-day Fathead minnow test are shown in Table 10. The median lethal concentration (LC50) estimate for survival was 1.87 mg diesel/L, and the 25% inhibitive concentration (IC25) estimate for biomass was 0.87 mg diesel/L. The resulting LOEC for survival and biomass was 2.70 and 1.30 mg diesel/L, respectively.

The results of the 7-day Topsmelt test are shown in Table 11. The LC50 estimate for survival was 0.68 mg/L diesel and the IC25 estimate for biomass was 0.74 mg diesel/L. The resulting LOEC estimate for survival and biomass was 0.57 and 1.60 mg diesel/L, respectively.

The results of the *C. dubia* survival and reproduction test are shown in Table 12. Adverse effects were observed on survival, resulting in an LC50 estimate of 0.23 mg diesel/L and an LOEC of

0.34 mg diesel/L. An inhibitory effect was observed on reproduction, resulting in an IC25 estimate of 0.17 mg diesel/L, and an LOEC of 0.22 mg/L diesel.

The results of the Echinoderm fertilization test are shown in Table 13. The IC25 estimate was 0.34 mg diesel/L, and a resulting LOEC of 0.05 mg diesel/L.

Table 10. Results: Fathead minnow 7-day survival and growth test.

Diesel Concentrations		Survival (%) (Mean ± SD)	Biomass (mg) (Mean ± SD)	Dry Weight (mg) (Mean ± SD)
Nominal (mg/L)	Test solutions (mg/L)			
Laboratory control	0	100 ± 0.0	0.7 ± 0.1	0.7 ± 0.1
Acetone control	0	100 ± 0.0	0.6 ± 0.0 [1]	0.6 ± 0.0 [1]
0.9	0.04	97.5 ± 5.0	0.6 ± 0.1	0.6 ± 0.1
1.9	0.13	100 ± 0.0	0.6 ± 0.1	0.6 ± 0.1
3.8	0.31	97.5 ± 5.0	0.6 ± 0.1	0.6 ± 0.0
7.5	0.65	97.5 ± 5.0	0.6 ± 0.0	0.6 ± 0.0
15	1.3	77.5 ± 17.1	0.3 ± 0.1	0.4 ± 0.1
30	2.7	22.5 ± 5.0	0.0 ± 0.0	0.2 ± 0.1
Test endpoint (mg/L) *				
LC50 (95% CL)		1.87 (1.43 – 2.45)	--	--
IC25 (95% CL)		--	0.87 (0.70 – 1.23)	1.19 (0.99 – 1.39)
IC50 (95% CL)		--	1.39 (1.03 – 1.84)	2.02 (1.79 – 2. 29)
NOEC		1.30	0.65	0.65
LOEC		2.70	1.30	1.30

SD = Standard Deviation, LC = Lethal Concentration, IC = Inhibition Concentration, CL = Confidence Limits, N/A = Not Available, NOEC = No Observed Effect Concentration, LOEC = Lowest Observed Effect Concentration
[1] = result was significantly different than the laboratory control, * = result was calculated using the solvent control as the negative control

Table 11. Results: Topsmelt 7-day survival and growth test.

Diesel Concentrations		Survival (%) (Mean ± SD)	Biomass (mg) (Mean ± SD)	Dry Weight (mg) (Mean ± SD)
Nominal (mg/L)	Test solutions (mg/L)			
Laboratory control	0	100 ± 0.0	1.3 ± 0.1	1.3 ± 0.1
Acetone control	0	100 ± 0.0	1.1 ± 0.1 [1]	1.1 ± 0.1 [1]
0.5	0.1	96.0 ± 8.9	1.0 ± 0.1	1.1 ± 0.1
1.0	0.12	92.0 ± 11.0	1.2 ± 0.2	1.3 ± 0.2
2.0	0.16	96.0 ± 8.9	1.2 ± 0.2	1.3 ± 0.1
4.0	0.26	80.0 ± 28.3	1.1 ± 0.3	1.3 ± 0.2
8.0	0.57	76.0 ± 16.7	1.1 ± 0.3	1.4 ± 0.2
16.0	1.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Test endpoint (mg/L) *				
LC50 (95% CL)		0.68 (0.55 – 0.83)	--	--
IC25 (95% CL)		--	0.74 (0.37 – 0.80)	>0.57
IC50 (95% CL)		--	0.99 (0.76 – 1.04)	>0.57
NOEC		0.26	0.57	0.57
LOEC		0.57	1.60	>0.57

SD = Standard Deviation, LC = Lethal Concentration, IC = Inhibition Concentration, CL = Confidence Limits, NOEC = No Observed Effect Concentration, LOEC = Lowest Observed Effect Concentration

[1] = result was significantly different than the laboratory control, * = result was calculated using the solvent control as the negative control

Table 12. Results: *C. dubia* survival and reproduction test.

Diesel Concentrations		Survival (%)	Reproduction (Mean ± SD)
Nominal (mg/L)	**Test solutions (mg/L)**		
Laboratory control	0	100	20.2 ± 2.1
Acetone control	0	100	16.8 ± 4.4 [1]
0.045	0.04	100	17.9 ± 2.5
0.09	0.07	100	18.6 ± 2.4
0.19	0.10	100	18.2 ± 1.5
0.38	0.15	100	17.1 ± 2.8
0.75	0.22	60	6.4 ± 6.4
1.5	0.34	0	0.0 ± 0.0
3.0	0.50	0	0.0 ± 0.0
6.0	0.75	0	0.0 ± 0.0
Test endpoint (mg/L) *			
LC50 (95% CL)		0.23 (0.20 – 0.26)	--
IC25 (95% CL)		--	0.17 (0.16 – 0.19)
IC50 (95% CL)		--	0.20 (0.19 – 0.24)
NOEC		0.22	0.15
LOEC		0.34	0.22

SD = Standard Deviation, LC = Lethal Concentration, IC = Inhibition Concentration, CL = Confidence Limits, NOEC = No Observed Effect Concentration, LOEC = Lowest Observed Effect Concentration

[1] = result was significantly different than the laboratory control, * = result was calculated using the solvent control as the negative control

Table 13. Results: Echinoderm (*Strongylocentrotus purpuratus)* fertilization test.

Diesel Concentrations		Fertilized eggs (%) (Mean ± SD)
Nominal (mg/L)	**Test solutions (mg/L)**	
Laboratory control	0	78.0 ± 2.6
Acetone control	0	72.5 ± 1.0
0.5	0.05	64.8 ± 5.1
1.0	0.07	58.0 ± 2.7
2.0	0.11	58.8 ± 4.1
4.0	0.18	56.0 ± 4.3
8.0	0.33	37.2 ± 4.3
16.0	0.62	12.2 ± 3.3
Test endpoint (mg/L) *		
IC25 (95% CL)		0.19 (0.12 – 0.22)
IC50 (95% CL)		0.34 (0.29 – 0.38)
NOEC		<0.05
LOEC		0.05

SD = Standard Deviation, IC = Inhibition Concentration, CL = Confidence Limits, NOEC = No Observed Effect Concentration, LOEC = Lowest Observed Effect Concentration, * = result was calculated using the solvent control as the negative control

4.0 QA/QC

The health history of the test organisms used in the exposures was acceptable and met the requirements of the US EPA protocols. There was a statistically significant difference between the laboratory control and the solvent control for most of the growth endpoints in the tests. Therefore, the solvent control was used for statistical analyses to calculate endpoints in all tests.

Water quality parameters remained within ranges specified in the protocol throughout the tests and there were no deviations from the test methodologies. Uncertainty associated with these tests is best described by the standard deviation around the mean and/or the confidence intervals around the point estimates.

Results of the reference toxicant tests conducted during the testing program are summarized in Table 13. Results for these tests fell within the range for organism performance of the mean and two standard deviations, based on historical results obtained by the laboratory with these tests. Thus, the sensitivity of the organisms used in these tests was appropriate. The reference toxicant tests were performed under the same conditions as those used for the tests.

Table 14. Reference toxicant test results.

Test Species	Endpoint	Historical Mean (2 SD Range)	CV (%)	Test Number[1]	Test Date
P. promelas	Survival (EC50): 4.6 g/L NaCl	5.0 (3.7 – 6.8)	16	124	July 6, 2017
	Growth (IC50): 4.7 g/L NaCl	4.7 (2.9 – 7.5)	27		
A. affinis	Survival (EC50): 91.2 µg/L Cu	99.9 (64.1 – 155.6)	25	30	July 6, 2017
	Growth (IC50): 87.7 µg/L Cu	93.5 (58.6 – 149.2)	26		
C. dubia	Survival (LC50): 2.1 g/L NaCl	2.0 (1.8 – 2.2)	5	161	July 21, 2017
	Reproduction (IC50): 1.8 g/L NaCl	1.5 (1.1 – 2.1)	18		
S. purpuratus	Fertilization (IC50): 16.4 µg/L Cu	18.9 (7.7 – 46.0)	56	5	July 7, 2017

SD = Standard Deviation, CV = Coefficient of Variation, LC = Lethal Concentration, IC = Inhibition Concentration, EC = Effect Concentration, 1= number of tests used to calculate historical mean and standard deviation

5.0 REFERENCES

USEPA. 2002. Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. 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.

Tidepool Scientific Software. 2013. CETIS comprehensive environmental toxicity information system, version 1.8.7.16 Tidepool Scientific Software, McKinleyville, CA. 275 pp.

WDOE. 2016. 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 2016.

APPENDIX A – Fathead minnow Toxicity Test Data

Fathead Minnow Test Summary Sheet

Client: WDCE

Start Date & Time: July 6/17 @ 1200

Work Order No.: 1705138

Test Species: *Pimephales promelas*

Sample Information:

Sample ID: NWTPH-DX
Sample Date: May 19/17
Date Received: May 19/17
Sample Volume: 50 x 5mL

Dilution Water:

Type: Moderately - hard water
Hardness (mg/L $CaCO_3$): 98
Alkalinity (mg/L $CaCO_3$): 88

Test Organism Information:

Batch No.: 070617
Source: Aquatic BioSystems, CO
Loading Density: 10 fish / 250mL

NaCl Reference Toxicant Results:

Reference Toxicant ID: PP124
Stock Solution ID: NaCl
Date Initiated: July 6/17
7-d EC50 (95% CL): 4.6 (3.9 - 5.4) g/L NaCl
7-d IC50 (95% CL): 4.7 (4.1 - 5.5) g/L NaCl

Survival:

Reference Toxicant Mean ± 2 SD: 5.0 (3.7 - 6.8) g/L NaCl CV (%): 16

Biomass

Reference Toxicant Mean ± 2 SD: 4.7 (2.9 - 7.5) g/L NaCl CV (%): 27

Test Results:

	Survival	Biomass	Dry Weight
NOEC % (v/v)	1.30	0.65	0.65
LOEC % (v/v)	2.70	1.30	1.30
EC25 % (v/v) (95% CL)	1.87 (1.43 - 2.45) ①		
EC50 % (v/v) (95% CL)	>max		
IC25 % (v/v) (95% CL)		0.87 (0.70 - 1.23)	1.19 (0.99 - 1.39)
IC50 % (v/v) (95% CL)		1.39 (1.03 - 1.84)	2.02 (1.79 - 2.29)

① EC50 estimate.

Reviewed by: JCh

Date reviewed: Jan-11/18

7-d Chronic Freshwater Toxicity Test
Initial and Final Water Quality Measurements

Client: WDOE
Sample ID: NWTPH-Dx
Work Order #: 1705136

Start Date & Time: July 6/17 1200h
Stop Date & Time: July 13/17 1315h
Test Species: *Pimephales promelas*

mg/L Diesel Concentration control	Days													
	0	1		2		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	24.0	24.0	25.0	25.0	25.0	24.0	24.5	24.0	24.5	24.0	24.5	24.5	24.5	24.0
DO (mg/L)	8.3	7.2	8.2	5.3	8.2	5.6	8.1	5.2	8.0	5.7	8.1	6.0	8.0	6.2
pH	7.7	7.4	7.5	7.2	7.0	7.4	7.6	7.3	7.8	7.2	7.6	7.0	7.8	6.9
Cond. (µS/cm)	333	335		331		332		331		332		338		339
Initials	KJL	KJL		A		A		YML		ICH		KJL		KJL

Concentration acetone control	Days													
	0	1		2		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	24.0	24.0	24.0	25.0	24.0	24.0	24.5	24.0	24.5	24.0	24.0	24.5	24.0	24.0
DO (mg/L)	7.7	6.8	7.9	5.3	8.1	5.2	8.1	5.5	7.9	5.4	7.9	4.7	7.8	5.7
pH	7.9	7.4	7.4	7.2	7.4	7.4	7.6	7.3	7.8	7.1	7.6	6.9	7.6	6.8
Cond. (µS/cm)	331	331		331		332		332		336		336		332
Initials	KJL	KJL		A		A		YML		ICH		KJL		KJL

Concentration 0.9	Days													
	0	1		2		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	24.0	24.0	24.0	25.0	24.0	24.0	24.0	24.0	24.5	24.0	24.0	24.5	24.0	24.0
DO (mg/L)	7.9	7.0	8.0	5.7	8.1	5.4	8.2	5.4	7.9	4.6	7.9	4.5	7.8	4.5
pH	7.9	7.4	7.5	7.2	7.0	7.4	7.5	7.2	7.8	7.0	7.6	6.9	7.6	6.8
Cond. (µS/cm)	338	331		332		333		337		336		342		347
Initials	KJL	KJL		A		A		YML		KJL		KJL		KJL

Concentration 1.9	Days													
	0	1		2		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	24.0	24.0	24.0	25.0	24.0	24.0	24.0	24.0	24.5	24.0	24.0	24.5	24.0	24.0
DO (mg/L)	7.8	6.8	7.9	5.8	8.1	5.3	8.1	5.1	7.9	4.5	7.9	4.4	7.8	4.6
pH	7.9	7.5	7.6	7.2	7.5	7.4	7.5	7.3	7.8	7.0	7.6	6.9	7.6	6.7
Cond. (µS/cm)	335	338		333		331		334		336		337		338
Initials	KJL	KJL		A		A		YML		KJL		KJL		KJL

WQ Ranges: T (°C) = 25 ± 1; DO (mg/L) = 3.3 to 8.4 (mg/L); pH = 6.5 to 8.5

DO meter: 4 **pH meter:** 4 **Conductivity meter:** 4

	Control			
Hardness*	98			
Alkalinity*	82			

* mg/L as CaCO3

Analysts: KJL, AWD, YML

Reviewed by: JCL

Date reviewed: Aug. 1/17

Sample Description: Diesel standard in moderately hard water

Comments:

7-d Chronic Freshwater Toxicity Test
Initial and Final Water Quality Measurements

Client: WDOE
Sample ID: NWTPH-Dx
Work Order #: 1705136

Start Date & Time: July 6/17 1200h
Stop Date & Time: July 13/17 1315h
Test Species: *Pimephales promelas*

mg/L Diesel Concentration 3.8	Days													
	0	1		2		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	24.0	24.0	24.0	25.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.5	24.0	24.0
DO (mg/L)	7.8	6.9	8.0	6.0	8.1	5.1	8.0	4.9	7.9	4.4	7.9	4.3	7.8	4.1
pH	7.9	7.5	7.6	7.3	7.5	7.5	7.5	7.3	7.9	7.0	7.6	6.9	7.6	6.8
Cond. (µS/cm)	327	331		335		334		333		335		335		336
Initials	KJL	KJL		A		A		YYL		KJL		KJL		KJL

Concentration 7.5	Days													
	0	1		2		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	24.0	24.0	24.0	25.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.5	24.0	24.0
DO (mg/L)	7.8	6.9	8.1	6.0	8.1	5.2	8.1	4.9	7.9	4.5	7.8	4.3	7.9	4.2
pH	7.9	7.5	7.6	7.2	7.5	7.5	7.5	7.3	7.9	7.0	7.6	6.9	7.6	6.8
Cond. (µS/cm)	330	331		335		331		333		333		336		343
Initials	KJL	KJL		A		A		YYL		KJL		KJL		KJL

Concentration 15	Days													
	0	1		2		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	24.0	24.0	24.0	25.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.5	24.0	24.0
DO (mg/L)	7.9	6.9	8.1	6.3	8.1	5.3	8.1	5.0	7.8	4.3	7.8	4.2	7.8	4.1
pH	7.9	7.5	7.6	7.2	7.5	7.5	7.6	7.3	7.9	7.0	7.6	6.9	7.6	6.8
Cond. (µS/cm)	330	337		333		332		333		334		335		338
Initials	KJL	KJL		A		A		YYL		KJL		KJL		KJL

Concentration 30	Days													
	0	1		2		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	24.0	24.0	24.0	25.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.5	24.0	24.0
DO (mg/L)	7.9	6.9	8.1	6.8	8.1	5.0	8.1	4.9	7.8	4.4	7.9	4.2	7.9	4.0
pH	7.9	7.5	7.6	7.4	7.5	7.5	7.5	7.3	7.8	7.0	7.6	7.0	7.6	6.8
Cond. (µS/cm)	329	329		332		332		332		332		334		337
Initials	KJL	KJL		A		A		YYL		KJL		KJL		KJL

WQ Ranges: T (°C) = 25 ± 1; DO (mg/L) = 3.3 to 8.4 (mg/L); pH = 6.5 to 8.5

DO meter: 4 **pH meter:** 4 **Conductivity meter:** 4

	Control			
Hardness*				
Alkalinity*				

* mg/L as CaCO3

Analysts: KJL, AWD, YYL

Reviewed by: JGH

Date reviewed: Aug-1/17

Sample Description:

Comments:

7-d Fathead Minnow Toxicity Test
Daily Survival

Client: WDOE
Sample ID: NWTPH - Dx
Work Order #: 17051366

Start Date & Time: July 6/17 1200h
Stop Date & Time: July 13/17 1515h
Test Species: *Pimephales promelas*

Concentration mg/L Diesel	Rep	Day of Test - No. of Survivors							Comments
		1	2	3	4	5	6	7	
Control	A	10	10	10	10	10	10	10	
	B	10			10	10		10	
	C	10			10	10		10	
	D	10			10	10	↓	10	
acetone control	A	10			10	10	10	10	
	B	10			10	10		10	
	C	10			10	10		10	
	D	10			10	10	↓	10	
0.9	A	10			10	10	10	10	
	B	10			10	10		10	
	C	10			10	10	↓	10	
	D	9	9	9	9	9	9	9	
1.9	A	10	10	10	10	10	10	10	
	B	10			10	10		10	
	C	10			10	10		10	
	D	10			10	10		10	
3.8	A	10			10	10		10	
	B	10			10	10		10	
	C	10			10	10	↓	10	
	D	10			9	9	9	9	
7.5	A	10			10	10	10	10	
	B	10			10	10	10	10	
	C	9	9	9	9	9	9	9	
	D	10	10	10	10	10	10	10	
15	A	10			10	10	10	10	
	B	10			10	10	9	8	
	C	10			10	10	7	7	
	D	10			9	8	8	6	
30	A	10		9	5	3	2	2	
	B	9	9	9	8	6	4	3	
	C	8	8	7	3	2	2	2	
	D	10	10	7	6	3	3	2	
Tech Initials		KJL	A	A	MHL	CSL	KJL	KJL	

Comments:

Reviewed by: JBL

Date reviewed: Aug. 1/17

Fathead Minnow Toxicity Test Data Sheet

Dry Weight Data

Client: WDOE

Sample ID: ~~T705~~ NWTPH-Dx

Work Order No.: 1705136

Start Date & Time: July 6/17 1200h

Termination Date & Time: July 13/17 1315h

Sample ID	Rep	Pan No. Black D	No. alive	Initials	Pan weight (mg)	Pan + organism (mg)	No. weighed	Initials
Control	A	1	10	JCL	1016.44	1023.98	10	KL/NY
	B	2	10		1021.32	1028.99	10	
	C	3	10		~~1016.~~ 1017.18	1024.19	10	
	D	4	10		1023.15	1029.51	10	
Acetone Control	A	5	10		994.35	1001.20	10	
	B	6	10		1000.04	1006.52	10	
	C	7	10		983.34	989.78	10	
	D	8	10		1017.00	1022.96	10	
0.9	A	9	10		1014.671	1021.84	10	
	B	10	10		1023.35	1030.20	10	
	C	11	10		1012.13	1017.55	10	
	D	12	9		999.71	1004.93	9	
1.9	A	13	10		986.30	992.24	10	
	B	14	10		990.57 61	996.03	10	
	C	15	10		1023.69	1030.34	10	
	D	16	10		1029.63	1035.41	10	
3.8	A	17	10		1023.23	1029.26	10	
	B	18	10		1001.83	1008.48	10	
	C	19	10		1030.25	1036.31	10	
	D	20	9		1031.46	1036.76	9	
7.5	A	21	10		1026.84	1032.59	10	
	B	22	10		1024.01	1029.41	10	
	C	23	9		991.72	997.15	9	
	D	24	10		990.13	996.14	10	

Comments: 10% reweigh pans (mg):
pan # 2 - 1028.99 19 - 1036.23
11 - 1017.52 26 - 995.77

Reviewed by: JCL

Date Reviewed: Aug. 1/17

Generated: April 9, 2008

Client: WDOE

W.O.#: 1705136

Hardness and Alkalinity Datasheet

	Alkalinity							Hardness			
Sample ID	Subsample Date	Date Measured	Sample Volume (mL)	(mL) 0.02N HCL/H_2SO_4 used to pH 4.5	(mL) of 0.02N HCL/H_2SO_4 used to pH 4.2	Total Alkalinity ($mg/LCaCO_3$)		Sample Volume (mL)	Volume of 0.01M EDTA Used (mL)	Total Hardness ($mg/L\ CaCO_3$)	Technician
070417	Jul6/17	Jul6/17	50	4.2	4.3	82		50	4.9	98	MC
MHW MHW											

Notes:

Reviewed by: Jon Date Reviewed: Aug. 1/17

CETIS Summary Report

Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental**

Batch ID:	19-7015-5488	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Karen Lee
Start Date:	06 Jul-17 12:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	13 Jul-17 13:15	**Species:**	Pimephales promelas	**Brine:**	
Duration:	7d 1h	**Source:**	Aquatic Biosystems, CO	**Age:**	<24h
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 1h	**Station:**	NWTPH-Dx		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
20-5662-2302	7d Survival Rate	1.3	2.7	1.873	11.0%		Steel Many-One Rank Sum Test
15-9677-8465	7d Survival Rate	0	>0		NA		Wilcoxon Rank Sum Two-Sample Test
09-2509-3875	Mean Dry Biomass-mg	0	>0		10.5%		Equal Variance t Two-Sample Test
14-4325-9303	Mean Dry Biomass-mg	0.65	1.3	0.9192	17.3%		Dunnett Multiple Comparison Test
20-7342-9890	Mean Dry Weight-mg	0	>0		10.5%		Equal Variance t Two-Sample Test
17-4909-5625	Mean Dry Weight-mg	0.65	1.3	0.9192	14.1%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	mg/L	95% LCL	95% UCL	TU	Method
15-5811-1680	7d Survival Rate	EC50	1.873	1.432	2.451		Trimmed Spearman-Kärber
17-7609-5240	Mean Dry Biomass-mg	IC5	0.06182	N/A	0.7039		Linear Interpolation (ICPIN)
		IC10	0.4949	N/A	0.846		
		IC15	0.6956	0.02384	0.8734		
		IC20	0.7802	0.6203	1.022		
		IC25	0.8691	0.6962	1.234		
		IC40	1.163	0.8999	1.666		
		IC50	1.39	1.032	1.844		
10-2749-7402	Mean Dry Weight-mg	IC5	0.487	0.2248	0.6587		Nonlinear Regression
		IC10	0.699	0.4793	0.8795		
		IC15	0.8743	0.662	1.065		
		IC20	1.035	0.8283	1.231		
		IC25	1.189	0.9885	1.389		
		IC40	1.663	1.463	1.878		
		IC50	2.023	1.787	2.29		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
15-5811-1680	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
15-9677-8465	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
20-5662-2302	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
09-2509-3875	Mean Dry Biomass-mg	Control Resp	0.6433	0.25 - NL	Yes	Passes Acceptability Criteria
	Mean Dry Biomass-mg	Control Resp	0.7162	0.25 - NL	Yes	Passes Acceptability Criteria
14-4325-9303	Mean Dry Biomass-mg	Control Resp	0.6433	0.25 - NL	Yes	Passes Acceptability Criteria
17-7609-5240	Mean Dry Biomass-mg	Control Resp	0.6433	0.25 - NL	Yes	Passes Acceptability Criteria
09-2509-3875	Mean Dry Biomass-mg	PMSD	0.105	0.12 - 0.3	Yes	Below Acceptability Criteria
14-4325-9303	Mean Dry Biomass-mg	PMSD	0.1729	0.12 - 0.3	Yes	Passes Acceptability Criteria

Fathead Minnow 7-d Larval Survival and Growth Test **Nautilus Environmental**

7d Survival Rate Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	4	1	1	1	1	1	0	0	0.0%	0.0%
0	Negative Control	4	1	1	1	1	1	0	0	0.0%	0.0%
0.04		4	0.975	0.8954	1	0.9	1	0.025	0.05	5.13%	2.5%
0.13		4	1	1	1	1	1	0	0	0.0%	0.0%
0.31		4	0.975	0.8954	1	0.9	1	0.025	0.05	5.13%	2.5%
0.65		4	0.975	0.8954	1	0.9	1	0.025	0.05	5.13%	2.5%
1.3		4	0.775	0.5032	1	0.6	1	0.08539	0.1708	22.04%	22.5%
2.7		4	0.225	0.1454	0.3046	0.2	0.3	0.025	0.05	22.22%	77.5%

Mean Dry Biomass-mg Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	4	0.6433	0.5852	0.7013	0.596	0.685	0.01825	0.03651	5.68%	0.0%
0	Negative Control	4	0.7162	0.6221	0.8104	0.636	0.767	0.02959	0.05919	8.26%	-11.35%
0.04		4	0.6155	0.4605	0.7705	0.522	0.713	0.04872	0.09744	15.83%	4.32%
0.13		4	0.5948	0.5126	0.6769	0.542	0.665	0.02582	0.05163	8.68%	7.54%
0.31		4	0.601	0.5131	0.689	0.53	0.665	0.02764	0.05527	9.2%	6.57%
0.65		4	0.5648	0.5188	0.6107	0.54	0.601	0.01445	0.02889	5.12%	12.2%
1.3		4	0.3455	0.1755	0.5155	0.255	0.5	0.05343	0.1069	30.93%	46.29%
2.7		4	0.049	0.03309	0.06491	0.038	0.062	0.004999	0.009999	20.4%	92.38%

Mean Dry Weight-mg Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	4	0.6433	0.5852	0.7013	0.596	0.685	0.01825	0.03651	5.68%	0.0%
0	Negative Control	4	0.7162	0.6221	0.8104	0.636	0.767	0.02959	0.05919	8.26%	-11.35%
0.04		4	0.63	0.4996	0.7604	0.542	0.713	0.04099	0.08197	13.01%	2.06%
0.13		4	0.5948	0.5126	0.6769	0.542	0.665	0.02582	0.05163	8.68%	7.54%
0.31		4	0.6157	0.5621	0.6693	0.5889	0.665	0.01684	0.03368	5.47%	4.28%
0.65		4	0.5798	0.5329	0.6268	0.54	0.6033	0.01475	0.0295	5.09%	9.86%
1.3		4	0.4416	0.3611	0.522	0.3812	0.5	0.02528	0.05055	11.45%	31.35%
2.7		4	0.2242	0.1241	0.3243	0.1667	0.31	0.03146	0.06292	28.06%	65.15%

Solvent blank = acetone control

7d Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	1	1	1	1
0	Negative Control	1	1	1	1
0.04		1	1	1	0.9
0.13		1	1	1	1
0.31		1	1	1	0.9
0.65		1	1	0.9	1
1.3		1	0.8	0.7	0.6
2.7		0.2	0.3	0.2	0.2

Mean Dry Biomass-mg Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	0.685	0.648	0.644	0.596
0	Negative Control	0.754	0.767	0.708	0.636
0.04		0.713	0.685	0.542	0.522
0.13		0.594	0.542	0.665	0.578
0.31		0.603	0.665	0.606	0.53
0.65		0.575	0.54	0.543	0.601
1.3		0.5	0.305	0.322	0.255
2.7		0.04601	0.05	0.038	0.062

Mean Dry Weight-mg Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	0.685	0.648	0.644	0.596
0	Negative Control	0.754	0.767	0.708	0.636
0.04		0.713	0.685	0.542	0.58
0.13		0.594	0.542	0.665	0.578
0.31		0.603	0.665	0.606	0.5889
0.65		0.575	0.54	0.6033	0.601
1.3		0.5	0.3812	0.46	0.425
2.7		0.23	0.1667	0.19	0.31

7d Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	10/10	10/10	10/10	10/10
0	Negative Control	10/10	10/10	10/10	10/10
0.04		10/10	10/10	10/10	9/10
0.13		10/10	10/10	10/10	10/10
0.31		10/10	10/10	10/10	9/10
0.65		10/10	10/10	9/10	10/10
1.3		10/10	8/10	7/10	6/10
2.7		2/10	3/10	2/10	2/10

solvent blank = acetone control

CETIS Analytical Report

Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental**

Analysis ID:	15-5811-1680	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:08	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes
Batch ID:	19-7015-5488	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Karen Lee
Start Date:	06 Jul-17 12:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	13 Jul-17 13:15	**Species:**	Pimephales promelas	**Brine:**	
Duration:	7d 1h	**Source:**	Aquatic Biosystems, CO	**Age:**	<24h
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 1h	**Station:**	NWTPH-Dx		

Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	22.50%	0.2727	0.05839	1.873	1.432	2.451

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria

7d Survival Rate Summary

			Calculated Variate(A/B)								
C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Solvent Blank	4	1	1	1	0	0	0.0%	0.0%	40	40
0.04		4	0.975	0.9	1	0.025	0.05	5.13%	2.5%	39	40
0.13		4	1	1	1	0	0	0.0%	0.0%	40	40
0.31		4	0.975	0.9	1	0.025	0.05	5.13%	2.5%	39	40
0.65		4	0.975	0.9	1	0.025	0.05	5.13%	2.5%	39	40
1.3		4	0.775	0.6	1	0.08539	0.1708	22.04%	22.5%	31	40
2.7		4	0.225	0.2	0.3	0.025	0.05	22.22%	77.5%	9	40

7d Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	1	1	1	1
0.04		1	1	1	0.9
0.13		1	1	1	1
0.31		1	1	1	0.9
0.65		1	1	0.9	1
1.3		1	0.8	0.7	0.6
2.7		0.2	0.3	0.2	0.2

7d Survival Rate Binomials

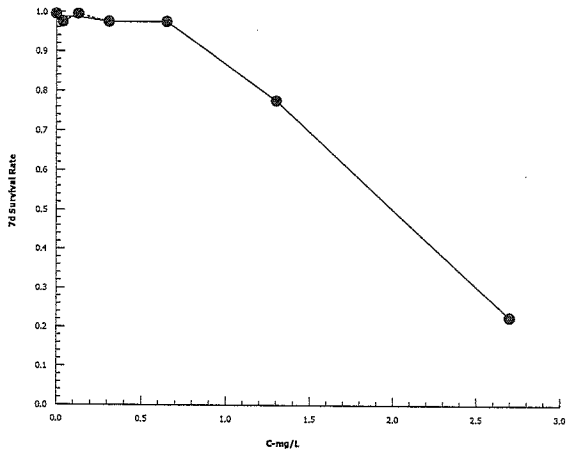
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	10/10	10/10	10/10	10/10
0	Negative Control	10/10	10/10	10/10	10/10
0.04		10/10	10/10	10/10	9/10
0.13		10/10	10/10	10/10	10/10
0.31		10/10	10/10	10/10	9/10
0.65		10/10	10/10	9/10	10/10
1.3		10/10	8/10	7/10	6/10
2.7		2/10	3/10	2/10	2/10

solvent blank = acetone control

Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental**

Analysis ID:	15-5811-1680	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:08	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes

Graphics



Analyst: KSL QA: JGL 12/19/17

CETIS Analytical Report

Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental**

Analysis ID:	20-5662-2302	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:12	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	19-7015-5488	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Karen Lee
Start Date:	06 Jul-17 12:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	13 Jul-17 13:15	**Species:**	Pimephales promelas	**Brine:**	
Duration:	7d 1h	**Source:**	Aquatic Biosystems, CO	**Age:**	<24h
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 1h	**Station:**	NWTPH-Dx		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	11.0%	1.3	2.7	1.873	

Steel Many-One Rank Sum Test

Control	vs	C-mg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Solvent Blank		0.04	16	10	1	6	0.6451	Asymp	Non-Significant Effect
		0.13	18	10	1	6	0.8571	Asymp	Non-Significant Effect
		0.31	16	10	1	6	0.6451	Asymp	Non-Significant Effect
		0.65	16	10	1	6	0.6451	Asymp	Non-Significant Effect
		1.3	12	10	1	6	0.1598	Asymp	Non-Significant Effect
		2.7*	10	10	0	6	0.0480	Asymp	Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.744149	0.4573582	6	42.71	<0.0001	Significant Effect
Error	0.2248609	0.01070766	21			
Total	2.96901		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1.879	3.812	0.1320	Equal Variances
Variances	Levene Equality of Variance	3.511	3.812	0.0145	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8258	0.8975	0.0003	Non-normal Distribution

7d Survival Rate Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	4	1	1	1	1	1	1	0	0.0%	0.0%
0.04		4	0.975	0.8954	1	1	0.9	1	0.025	5.13%	2.5%
0.13		4	1	1	1	1	1	1	0	0.0%	0.0%
0.31		4	0.975	0.8954	1	1	0.9	1	0.025	5.13%	2.5%
0.65		4	0.975	0.8954	1	1	0.9	1	0.025	5.13%	2.5%
1.3		4	0.775	0.5032	1	0.75	0.6	1	0.08539	22.04%	22.5%
2.7		4	0.225	0.1454	0.3046	0.2	0.2	0.3	0.025	22.22%	77.5%

Angular (Corrected) Transformed Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
0.04		4	1.371	1.242	1.501	1.412	1.249	1.412	0.04074	5.94%	2.89%
0.13		4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
0.31		4	1.371	1.242	1.501	1.412	1.249	1.412	0.04074	5.94%	2.89%
0.65		4	1.371	1.242	1.501	1.412	1.249	1.412	0.04074	5.94%	2.89%
1.3		4	1.099	0.7374	1.461	1.049	0.8861	1.412	0.1137	20.68%	22.16%
2.7		4	0.4926	0.4004	0.5849	0.4636	0.4636	0.5796	0.029	11.77%	65.11%

Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental**

Analysis ID:	20-5662-2302	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:12	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

7d Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	1	1	1	1
0.04		1	1	1	0.9
0.13		1	1	1	1
0.31		1	1	1	0.9
0.65		1	1	0.9	1
1.3		1	0.8	0.7	0.6
2.7		0.2	0.3	0.2	0.2

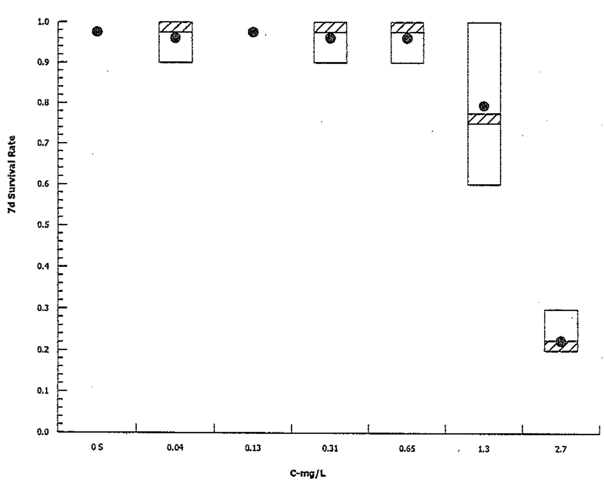
Angular (Corrected) Transformed Detail

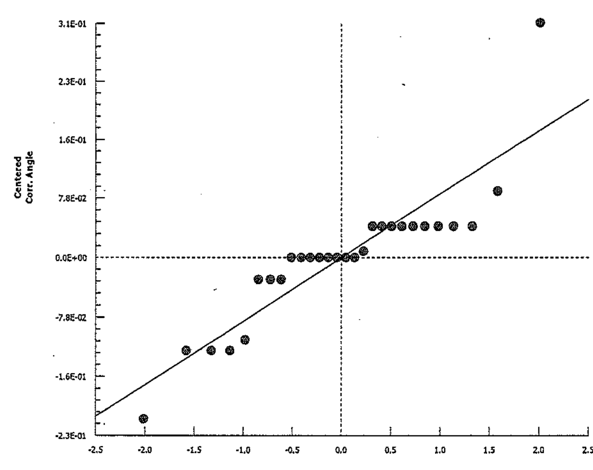
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	1.412	1.412	1.412	1.412
0.04		1.412	1.412	1.412	1.249
0.13		1.412	1.412	1.412	1.412
0.31		1.412	1.412	1.412	1.249
0.65		1.412	1.412	1.249	1.412
1.3		1.412	1.107	0.9912	0.8861
2.7		0.4636	0.5796	0.4636	0.4636

7d Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	10/10	10/10	10/10	10/10
0	Negative Control	10/10	10/10	10/10	10/10
0.04		10/10	10/10	10/10	9/10
0.13		10/10	10/10	10/10	10/10
0.31		10/10	10/10	10/10	9/10
0.65		10/10	10/10	9/10	10/10
1.3		10/10	8/10	7/10	6/10
2.7		2/10	3/10	2/10	2/10

Graphics





CETIS Analytical Report

Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental**

Analysis ID:	15-9677-8465	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:10	**Analysis:**	Nonparametric-Two Sample	**Official Results:**	Yes
Batch ID:	19-7015-5488	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Karen Lee
Start Date:	06 Jul-17 12:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	13 Jul-17 13:15	**Species:**	Pimephales promelas	**Brine:**	
Duration:	7d 1h	**Source:**	Aquatic Biosystems, CO	**Age:**	<24h
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 1h	**Station:**	NWTPH-Dx		

Data Transform	Zeta	Alt Hyp	Trials	Seed	Test Result
Angular (Corrected)	NA	C > T	NA	NA	Passes 7d survival rate

Wilcoxon Rank Sum Two-Sample Test

Control	vs	Control	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Solvent Blank		Negative Control	18	NA	1	6	1.0000	Exact	Non-Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0	0	1	65540	<0.0001	Significant Effect
Error	0	0	6			
Total	0		7			

7d Survival Rate Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	4	1	1	1	1	1	1	0	0.0%	0.0%
0	Negative Control	4	1	1	1	1	1	1	0	0.0%	0.0%

Angular (Corrected) Transformed Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
0	Negative Contr	4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%

7d Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	1	1	1	1
0	Negative Control	1	1	1	1

Angular (Corrected) Transformed Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	1.412	1.412	1.412	1.412
0	Negative Control	1.412	1.412	1.412	1.412

7d Survival Rate Binomials

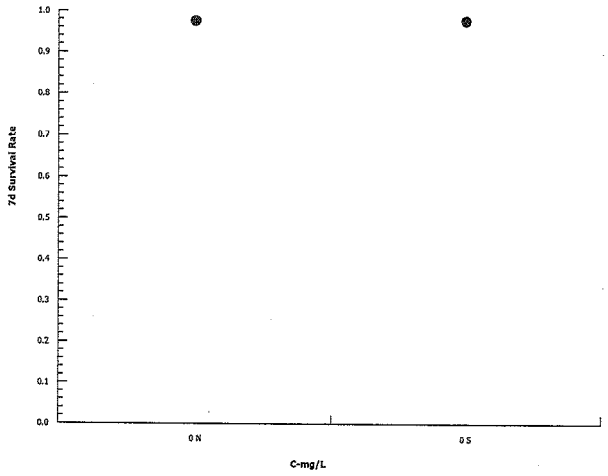
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	10/10	10/10	10/10	10/10
0	Negative Control	10/10	10/10	10/10	10/10

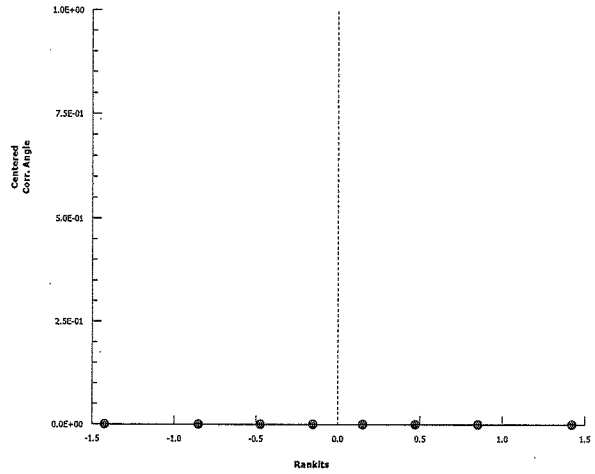
Solvent blank = acetone control

Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental**

Analysis ID:	15-9677-8465	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:10	**Analysis:**	Nonparametric-Two Sample	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental**

Analysis ID:	17-7609-5240	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:09	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	19-7015-5488	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Karen Lee
Start Date:	06 Jul-17 12:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	13 Jul-17 13:15	**Species:**	Pimephales promelas	**Brine:**	
Duration:	7d 1h	**Source:**	Aquatic Biosystems, CO	**Age:**	<24h
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 1h	**Station:**	NWTPH-Dx		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1765519	200	Yes	Two-Point Interpolation

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	0.6433	0.25 - NL	Yes	Passes Acceptability Criteria

Point Estimates

Level	mg/L	95% LCL	95% UCL
IC5	0.06182	N/A	0.7039
IC10	0.4949	N/A	0.846
IC15	0.6956	0.02384	0.8734
IC20	0.7802	0.6203	1.022
IC25	0.8691	0.6962	1.234
IC40	1.163	0.8999	1.666
IC50	1.39	1.032	1.844

Mean Dry Biomass-mg Summary

			Calculated Variate						
C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	4	0.6433	0.596	0.685	0.01825	0.03651	5.68%	0.0%
0.04		4	0.6155	0.522	0.713	0.04872	0.09744	15.83%	4.32%
0.13		4	0.5948	0.542	0.665	0.02582	0.05163	8.68%	7.54%
0.31		4	0.601	0.53	0.665	0.02764	0.05527	9.2%	6.57%
0.65		4	0.5648	0.54	0.601	0.01445	0.02889	5.12%	12.2%
1.3		4	0.3455	0.255	0.5	0.05343	0.1069	30.93%	46.29%
2.7		4	0.049	0.038	0.062	0.004999	0.009999	20.4%	92.38%

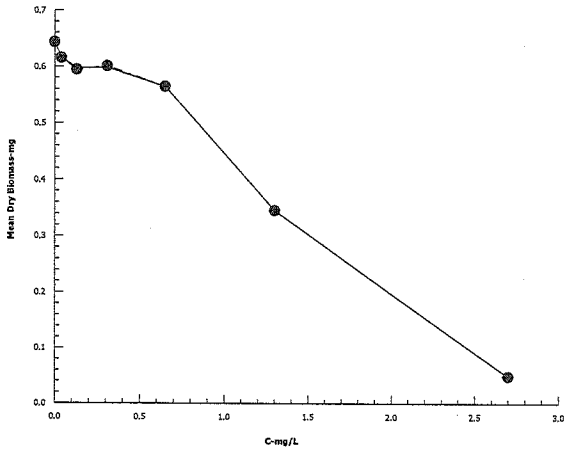
Mean Dry Biomass-mg Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	0.685	0.648	0.644	0.596
0.04		0.713	0.685	0.542	0.522
0.13		0.594	0.542	0.665	0.578
0.31		0.603	0.665	0.606	0.53
0.65		0.575	0.54	0.543	0.601
1.3		0.5	0.305	0.322	0.255
2.7		0.04601	0.05	0.038	0.062

Solvent blank = acetone control

Analysis ID:	17-7609-5240	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:09	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



CETIS Analytical Report

Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental**

Analysis ID:	14-4325-9303	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:12	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	19-7015-5488	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Karen Lee
Start Date:	06 Jul-17 12:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	13 Jul-17 13:15	**Species:**	Pimephales promelas	**Brine:**	
Duration:	7d 1h	**Source:**	Aquatic Biosystems, CO	**Age:**	<24h
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 1h	**Station:**	NWTPH-Dx		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	17.3%	0.65	1.3	0.9192	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Solvent Blank		0.04	0.6109	2.448	0.111	6	0.6325	CDF	Non-Significant Effect
		0.13	1.068	2.448	0.111	6	0.4233	CDF	Non-Significant Effect
		0.31	0.9299	2.448	0.111	6	0.4860	CDF	Non-Significant Effect
		0.65	1.728	2.448	0.111	6	0.1783	CDF	Non-Significant Effect
		1.3*	6.553	2.448	0.111	6	<0.0001	CDF	Significant Effect
		2.7*	13.08	2.448	0.111	6	<0.0001	CDF	Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	0.6433	0.25 - NL	Yes	Passes Acceptability Criteria
PMSD	0.1729	0.12 - 0.3	Yes	Passes Acceptability Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.13376	0.18896	6	45.77	<0.0001	Significant Effect
Error	0.08669976	0.00412856	21			
Total	1.22046		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	14.21	16.81	0.0274	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9571	0.8975	0.2963	Normal Distribution

Mean Dry Biomass-mg Summary

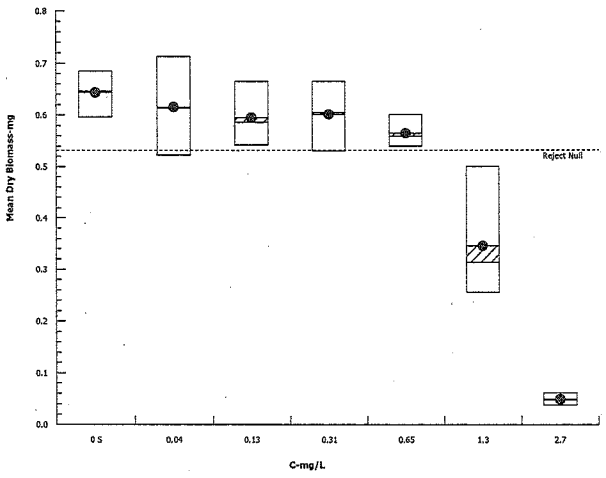
C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	4	0.6433	0.5852	0.7013	0.646	0.596	0.685	0.01825	5.68%	0.0%
0.04		4	0.6155	0.4605	0.7705	0.6135	0.522	0.713	0.04872	15.83%	4.32%
0.13		4	0.5948	0.5126	0.6769	0.586	0.542	0.665	0.02582	8.68%	7.54%
0.31		4	0.601	0.5131	0.689	0.6045	0.53	0.665	0.02764	9.2%	6.57%
0.65		4	0.5648	0.5188	0.6107	0.559	0.54	0.601	0.01445	5.12%	12.2%
1.3		4	0.3455	0.1755	0.5155	0.3135	0.255	0.5	0.05343	30.93%	46.29%
2.7		4	0.049	0.03309	0.06491	0.048	0.038	0.062	0.004999	20.4%	92.38%

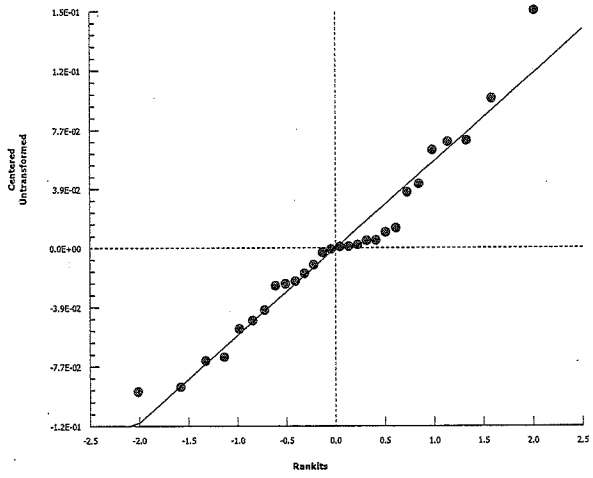
Mean Dry Biomass-mg Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	0.685	0.648	0.644	0.596
0.04		0.713	0.685	0.542	0.522
0.13		0.594	0.542	0.665	0.578
0.31		0.603	0.665	0.606	0.53
0.65		0.575	0.54	0.543	0.601
1.3		0.5	0.305	0.322	0.255
2.7		0.04601	0.05	0.038	0.062

Analysis ID:	14-4325-9303	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:12	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental**

Analysis ID:	09-2509-3875	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:11	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes
Batch ID:	19-7015-5488	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Karen Lee
Start Date:	06 Jul-17 12:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	13 Jul-17 13:15	**Species:**	Pimephales promelas	**Brine:**	
Duration:	7d 1h	**Source:**	Aquatic Biosystems, CO	**Age:**	<24h
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 1h	**Station:**	NWTPH-Dx		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C > T	NA	NA	10.5%	Passes mean dry biomass-mg

Equal Variance t Two-Sample Test

Control	vs	Control	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Solvent Blank		Negative Control	-2.099	1.943	0.068	6	0.9597	CDF	Non-Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	0.6433	0.25 - NL	Yes	Passes Acceptability Criteria
Control Resp	0.7162	0.25 - NL	Yes	Passes Acceptability Criteria
PMSD	0.105	0.12 - 0.3	Yes	Below Acceptability Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01065654	0.01065654	1	4.407	0.0805	Non-Significant Effect
Error	0.0145076	0.002417933	6			
Total	0.02516414		7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	2.628	47.47	0.4485	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9227	0.6451	0.4518	Normal Distribution

Mean Dry Biomass-mg Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	4	0.6433	0.5852	0.7013	0.646	0.596	0.685	0.01825	5.68%	0.0%
0	Negative Control	4	0.7162	0.6221	0.8104	0.731	0.636	0.767	0.02959	8.26%	-11.35%

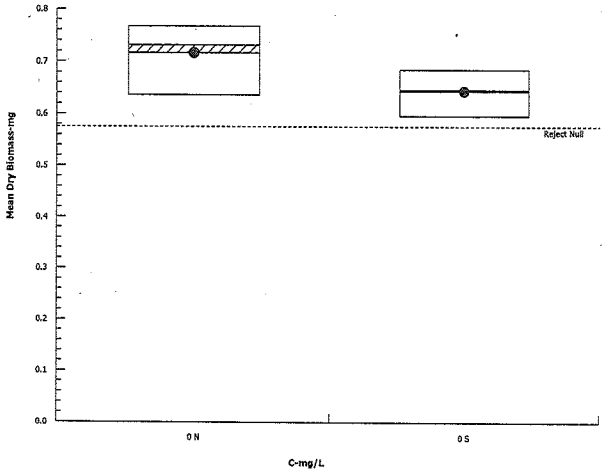
Mean Dry Biomass-mg Detail

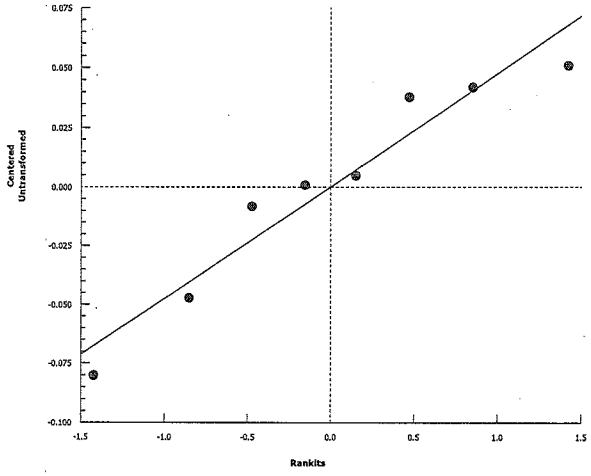
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	0.685	0.648	0.644	0.596
0	Negative Control	0.754	0.767	0.708	0.636

Solvent blank = acetone control

Analysis ID:	09-2509-3875	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:11	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental**

Analysis ID:	10-2749-7402	**Endpoint:**	Mean Dry Weight-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:09	**Analysis:**	Nonlinear Regression	**Official Results:**	Yes
Batch ID:	19-7015-5488	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Karen Lee
Start Date:	06 Jul-17 12:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	13 Jul-17 13:15	**Species:**	Pimephales promelas	**Brine:**	
Duration:	7d 1h	**Source:**	Aquatic Biosystems, CO	**Age:**	<24h
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 1h	**Station:**	NWTPH-Dx		

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Log-Logistic EV [Y=A/(1+(X/D)^C)]	None	None	Normal [W=1]	Off [Y*=Y]

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
5	71.4	-135.8	-132.8	0.8889	Yes	0.4628	2.84	0.7622	Non-Significant Lack of Fit

Point Estimates

Level	mg/L	95% LCL	95% UCL
IC5	0.487	0.2248	0.6587
IC10	0.699	0.4793	0.8795
IC15	0.8743	0.662	1.065
IC20	1.035	0.8283	1.231
IC25	1.189	0.9885	1.389
IC40	1.663	1.463	1.878
IC50	2.023	1.787	2.29

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	0.6259	0.01335	0.5997	0.652	46.88	<0.0001	Significant Parameter
C	2.068	0.3089	1.462	2.673	6.693	<0.0001	Significant Parameter
D	2.023	0.1372	1.754	2.292	14.75	<0.0001	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	0.5474977	0.5474977	1	218	<0.0001	Significant
Lack of Fit	0.0050878	0.0012719	4	0.4628	0.7622	Non-Significant
Pure Error	0.0577108	0.0027481	21			
Residual	0.0627985	0.0025119	25			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Bartlett Equality of Variance	4.265	12.59	0.6409	Equal Variances
	Mod Levene Equality of Variance	1.606	2.573	0.1947	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9717	0.9264	0.6256	Normal Distribution
	Anderson-Darling A2 Normality	0.2537	2.492	0.7589	Normal Distribution

Mean Dry Weight-mg Summary

			Calculated Variate						
C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	4	0.6433	0.596	0.685	0.01825	0.03651	5.68%	0.0%
0.04		4	0.63	0.542	0.713	0.04099	0.08197	13.01%	2.06%
0.13		4	0.5948	0.542	0.665	0.02582	0.05163	8.68%	7.54%
0.31		4	0.6157	0.5889	0.665	0.01684	0.03368	5.47%	4.28%
0.65		4	0.5798	0.54	0.6033	0.01475	0.0295	5.09%	9.86%
1.3		4	0.4416	0.3812	0.5	0.02528	0.05055	11.45%	31.35%
2.7		4	0.2242	0.1667	0.31	0.03146	0.06292	28.06%	65.15%

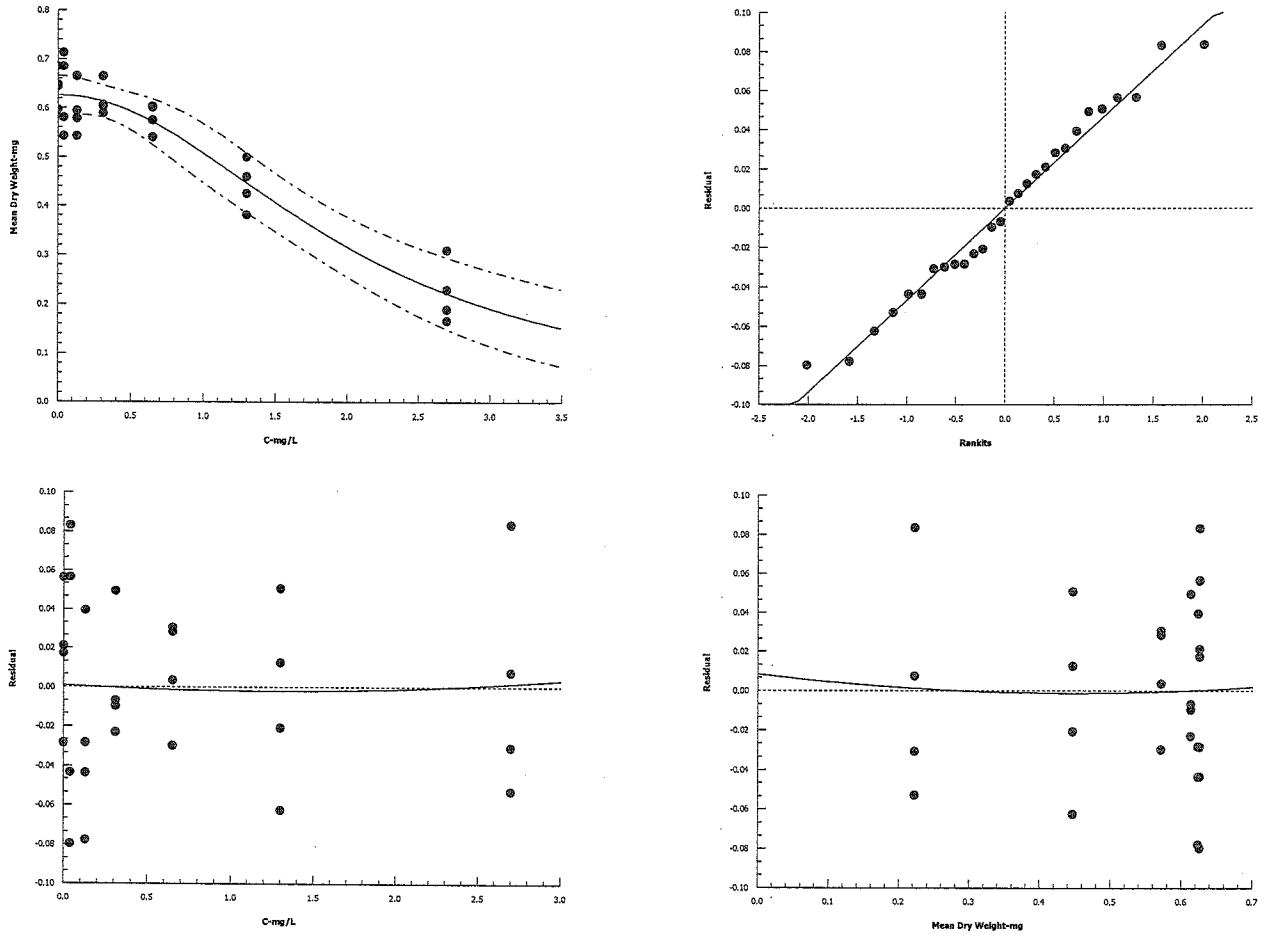
Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental**

Analysis ID:	10-2749-7402	**Endpoint:**	Mean Dry Weight-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:09	**Analysis:**	Nonlinear Regression	**Official Results:**	Yes

Mean Dry Weight-mg Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	0.685	0.648	0.644	0.596
0.04		0.713	0.685	0.542	0.58
0.13		0.594	0.542	0.665	0.578
0.31		0.603	0.665	0.606	0.5889
0.65		0.575	0.54	0.6033	0.601
1.3		0.5	0.3812	0.46	0.425
2.7		0.23	0.1667	0.19	0.31

Graphics — 3P Log-Logistic EV [Y=A/(1+(X/D)^C)]



Solvent blank = acetone control

CETIS Analytical Report

Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental**

Analysis ID:	17-4909-5625	**Endpoint:**	Mean Dry Weight-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:12	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	19-7015-5488	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Karen Lee
Start Date:	06 Jul-17 12:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	13 Jul-17 13:15	**Species:**	Pimephales promelas	**Brine:**	
Duration:	7d 1h	**Source:**	Aquatic Biosystems, CO	**Age:**	<24h
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 1h	**Station:**	NWTPH-Dx		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	14.1%	0.65	1.3	0.9192	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Solvent Blank		0.04	0.3576	2.448	0.091	6	0.7388	CDF	Non-Significant Effect
		0.13	1.308	2.448	0.091	6	0.3207	CDF	Non-Significant Effect
		0.31	0.7426	2.448	0.091	6	0.5727	CDF	Non-Significant Effect
		0.65	1.711	2.448	0.091	6	0.1830	CDF	Non-Significant Effect
		1.3*	5.441	2.448	0.091	6	<0.0001	CDF	Significant Effect
		2.7*	11.31	2.448	0.091	6	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.5525855	0.09209758	6	33.51	<0.0001	Significant Effect
Error	0.05771077	0.002748132	21			
Total	0.6102962		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.265	16.81	0.6409	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.973	0.8975	0.6625	Normal Distribution

Mean Dry Weight-mg Summary

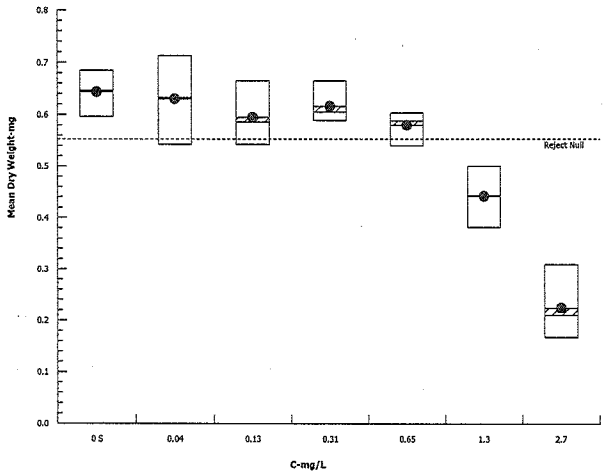
C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	4	0.6433	0.5852	0.7013	0.646	0.596	0.685	0.01825	5.68%	0.0%
0.04		4	0.63	0.4996	0.7604	0.6325	0.542	0.713	0.04099	13.01%	2.06%
0.13		4	0.5948	0.5126	0.6769	0.586	0.542	0.665	0.02582	8.68%	7.54%
0.31		4	0.6157	0.5621	0.6693	0.6045	0.5889	0.665	0.01684	5.47%	4.28%
0.65		4	0.5798	0.5329	0.6268	0.588	0.54	0.6033	0.01475	5.09%	9.86%
1.3		4	0.4416	0.3611	0.522	0.4425	0.3812	0.5	0.02528	11.45%	31.35%
2.7		4	0.2242	0.1241	0.3243	0.21	0.1667	0.31	0.03146	28.06%	65.15%

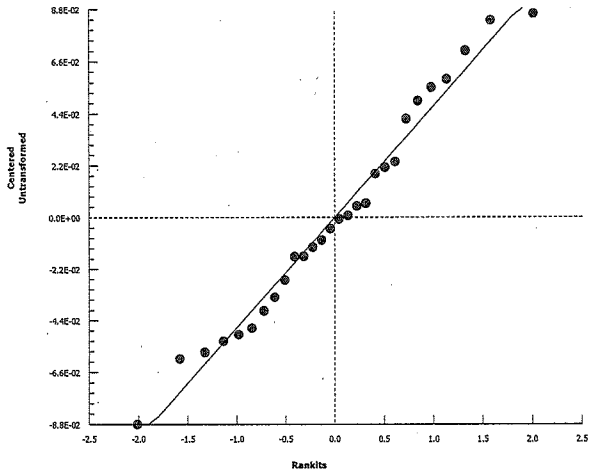
Mean Dry Weight-mg Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	0.685	0.648	0.644	0.596
0.04		0.713	0.685	0.542	0.58
0.13		0.594	0.542	0.665	0.578
0.31		0.603	0.665	0.606	0.5889
0.65		0.575	0.54	0.6033	0.601
1.3		0.5	0.3812	0.46	0.425
2.7		0.23	0.1667	0.19	0.31

Analysis ID:	17-4909-5625	**Endpoint:**	Mean Dry Weight-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:12	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental**

Analysis ID:	20-7342-9890	**Endpoint:**	Mean Dry Weight-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:11	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes
Batch ID:	19-7015-5488	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Karen Lee
Start Date:	06 Jul-17 12:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	13 Jul-17 13:15	**Species:**	Pimephales promelas	**Brine:**	
Duration:	7d 1h	**Source:**	Aquatic Biosystems, CO	**Age:**	<24h
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 1h	**Station:**	NWTPH-Dx		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C > T	NA	NA	10.5%	Passes mean dry weight-mg

Equal Variance t Two-Sample Test

Control	vs	Control	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Solvent Blank		Negative Control	-2.099	1.943	0.068	6	0.9597	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01065654	0.01065654	1	4.407	0.0805	Non-Significant Effect
Error	0.0145076	0.002417933	6			
Total	0.02516414		7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	2.628	47.47	0.4485	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9227	0.6451	0.4518	Normal Distribution

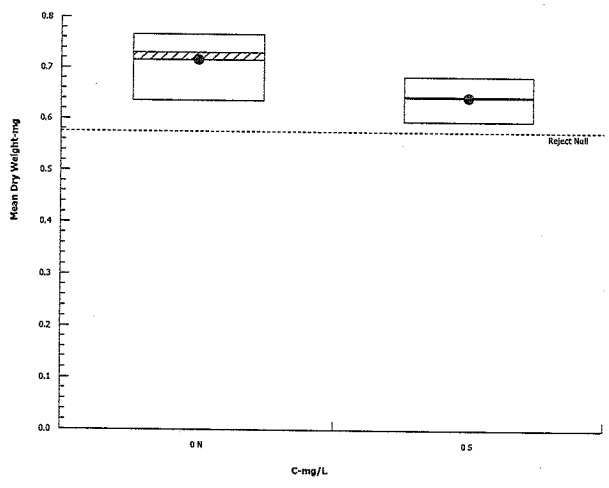
Mean Dry Weight-mg Summary

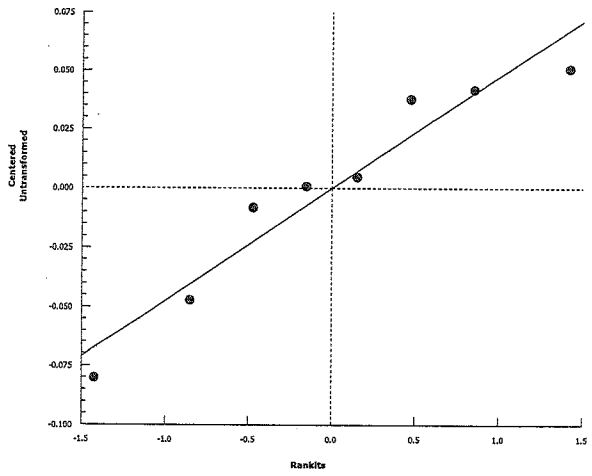
C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	4	0.6433	0.5852	0.7013	0.646	0.596	0.685	0.01825	5.68%	0.0%
0	Negative Control	4	0.7162	0.6221	0.8104	0.731	0.636	0.767	0.02959	8.26%	-11.35%

Mean Dry Weight-mg Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	0.685	0.648	0.644	0.596
0	Negative Control	0.754	0.767	0.708	0.636

Graphics





Solvent blank = acetone control

APPENDIX B - Topsmelt Toxicity Test Data

Topsmelt Test Summary Sheet

Client: WDOE

Start Date & Time: July 6, 2017 @ 1500h

Work Order No.: 170515b

Test Species: *Atherinops affinis*

Sample Information:

Sample ID: NWTPH-Dx
Sample Date: 19-May-17
Date Received: 19-May-17
Sample Volume: 50x 5mL

Dilution Water:

Type: Natural Seawater
Source: Vancouver Aquarium, Vancouver, BC

Test Organism Information:

Batch No.: 070617
Source: Aquatic Biosystems, CO
Age: 10-days

Copper Reference Toxicant Results:

Reference Toxicant ID: AAC30
Stock Solution ID: 17Cu02
Date Initiated: 6-Jul-17
7-d EC50 (95% CL): 91.2 (79.7 - 104.4)
7-d IC50 (95% CL): 87.7 (79.0 - 97.1)

EC50 Reference Toxicant Mean (Acceptable Range): 99.9 (64.1 - 155.6) CV (%): 25
IC50 Reference Toxicant Mean (Acceptable Range): 93.5 (58.6 - 149.2) CV (%): 26

Test Results:

	Survival	Dry Biomass	Dry Weight
NOEC	~~0.27~~ 0.26	~~0.80~~ 0.57	~~0.80~~ 0.57
LOEC	~~0.80~~ 0.57	~~1.70~~ 1.60	~~>0.80~~ >0.57
EC25 % (v/v) (95% CL)	n/a		
EC50 % (v/v) (95% CL)	~~0.68 (0.55 - 0.83)~~ ~~0.81 (0.65 - 1.01)~~	0.74 (0.37 - 0.80)	
IC25 % (v/v) (95% CL)		~~0.95 (0.55 - 1.01)~~	~~>0.8~~ >0.57
IC50 % (v/v) (95% CL)		~~1.18 (0.97 - 1.22)~~ 0.99 (0.76 - 1.04)	~~>0.8~~ >0.57

Reviewed by: JBh

Date reviewed: Aug. 28/17

Chronic Marine Toxicity Test
Initial and Final Water Quality Measurements

Client: ~~In-House~~ (For) WDOE
Sample ID: ~~Cu Reftox~~ NWTPH-Dx
Work Order #: ~~n/a~~ 1705156

Start Date & Time: July 6/17 1500h
Stop Date & Time: July 13/17 1600h
Test Species: *Atherinops affinis*

mg/L Diesel ~~mg/L Cu~~

Concentration Control	Day 0 init.	Day 1 old	Day 1 new	Day 2 old	Day 2 new	Day 3 old	Day 3 new	Day 4 old	Day 4 new	Day 5 old	Day 5 new	Day 6 old	Day 6 new	Day 7 final
Temperature (°C)	19.0	19.5	20.0	20.0	20.0	20.0	19.5	19.5	19.5	20.0	20.0	19.5	20.0	19.5
DO (mg/L)	7.7	6.5	6.75	6.1	7.4	5.4	7.5	5.0	7.6	5.0	7.4	6.5	7.4	5.6
pH	7.6	7.7	7.8	7.5	7.8	7.6	7.8	7.4	7.8	7.5	7.8	7.4	7.8	7.3
Salinity (ppt)	28	28		28		28		28		28		28		28
Initials	KJL	KJL		A		A		YYL		MLT		KJL		KJL

Concentration acetone control	Day 0 init.	Day 1 old	Day 1 new	Day 2 old	Day 2 new	Day 3 old	Day 3 new	Day 4 old	Day 4 new	Day 5 old	Day 5 new	Day 6 old	Day 6 new	Day 7 final
Temperature (°C)	19.0	19.5	20.0	20.0	20.0	20.0	19.5	19.5	19.5	20.0	20.0	19.5	20.0	19.5
DO (mg/L)	7.5	~~6.6~~ 5.7	7.5	5.9	7.4	5.5	7.5	4.5	7.5	4.6	7.4	5.3	7.5	5.3
pH	7.6	7.7	7.8	7.6	7.8	7.6	7.8	7.5	7.8	7.5	7.8	7.4	7.8	7.3
Salinity (ppt)	28	28		28		28		28		28		28		28
Initials	KJL	KJL		A		A		YYL		MLT		KJL		KJL

Concentration 0.5	Day 0 init.	Day 1 old	Day 1 new	Day 2 old	Day 2 new	Day 3 old	Day 3 new	Day 4 old	Day 4 new	Day 5 old	Day 5 new	Day 6 old	Day 6 new	Day 7 final
Temperature (°C)	19.0	19.5	20.0	20.0	20.0	20.0	19.5	19.5	19.5	20.0	20.0	19.5	20.0	19.5
DO (mg/L)	7.4	5.6	7.3	5.8	7.4	5.7	7.6	4.9	7.5	4.3	7.4	4.6	7.5	~~5.2~~ 4.7
pH	7.6	7.7	7.8	7.6	7.8	7.6	7.8	7.6	7.8	7.5	7.8	7.4	7.8	7.2
Salinity (ppt)	28	28		28		28		28		28		28		28
Initials	KJL	KJL		A		A		YYL		MLT		KJL		KJL

Concentration 1	Day 0 init.	Day 1 old	Day 1 new	Day 2 old	Day 2 new	Day 3 old	Day 3 new	Day 4 old	Day 4 new	Day 5 old	Day 5 new	Day 6 old	Day 6 new	Day 7 final
Temperature (°C)	19.0	19.5	20.0	20.0	20.0	20.0	19.5	19.5	19.5	20.0	20.0	19.5	20.0	19.5
DO (mg/L)	7.4	5.6	7.2	5.8	7.4	5.7	7.6	4.9	7.4	4.2	7.4	4.3	7.5	~~5.4~~ 4.5
pH	7.6	7.7	7.8	7.6	7.8	7.6	7.8	7.6	7.8	7.5	7.8	7.3	7.8	7.2
Salinity (ppt)	28	28		28		28		28		28		28		28
Initials	KJL	KJL		A		A		YYL		MLT		KJL		KJL

Analysts: KJL, AWD, YYL, MLT

Reviewed by: JGh
Date reviewed: Aug 2/17

Sample Description: Diesel standard into seawater

Comments:

Chronic Marine Toxicity Test
Initial and Final Water Quality Measurements

Client: WDOE
Sample ID: NWTPH-Dx
Work Order #: 1705154

Start Date & Time: July 6/17 1500h
Stop Date & Time: July 13/17 1600h
Test Species: *Atherinops affinis*

mg/L Diesel Concentration 2	Days													
	0	1		2		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	19.0	19.5	20.0	20.0	20.0	20.0	19.5	19.5	19.5	20.0	20.0	19.5	20.0	19.5
DO (mg/L)	7.4	5.3	7.2	5.9	7.3	5.3	7.5	5.0	7.4	4.5	7.4	4.2	7.5	4.6
pH	7.7	7.2	7.8	7.6	7.8	7.6	7.8	7.6	7.8	7.5	7.8	7.7	7.8	7.2
Salinity (ppt)	28	28		28		28		28		28		28		28
Initials	KJL	KJL		A		A		YYL		MLT		KJL		KJL

Concentration 4	Days													
	0	1		2		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	19.0	19.5	20.0	20.0	20.0	20.0	19.5	19.5	19.5	20.0	20.0	19.5	20.0	19.5
DO (mg/L)	7.3	5.4	7.2	5.9	7.3	5.5	7.5	5.2	7.4	4.6	7.3	4.2	7.5	4.3
pH	7.6	7.7	7.8	7.6	7.8	7.7	7.8	7.5	7.8	7.5	7.8	7.7	7.8	7.2
Salinity (ppt)	28	28		28		28		28		28		28		28
Initials	KJL	KJL		A		A		YYL		MLT		KJL		KJL

Concentration 8	Days													
	0	1		2		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	19.0	19.5	20.0	20.0	20.0	20.0	19.5	19.5	19.5	20.0	20.0	19.5	20.0	19.5
DO (mg/L)	7.3	5.6	7.2	5.9	7.3	5.9	7.5	5.4	7.2	4.7	7.3	4.2	7.5	4.3
pH	7.6	7.7	7.8	7.6	7.8	7.6	7.8	7.6	7.8	7.5	7.8	7.4	7.8	7.2
Salinity (ppt)	28	28		28		28		28		28		28		28
Initials	KJL	KJL		A		A		YYL		MLT		KJL		KJL

Concentration 16	Days													
	0	1		2		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	19.0	19.5	20.0	20.0	20.0	20.0	19.5	19.5						
DO (mg/L)	7.2	5.6	7.2	6.1	7.3	5.4	7.4	5.3						
pH	7.6	7.7	7.8	7.6	7.8	7.6	7.8	7.7						
Salinity (ppt)	28	28		28		28								
Initials	KJL	KJL		A		A		YYL						

Analysts: KJL, AW, YYL, MLT

Reviewed by: JTH

Date reviewed: Aug. 2/17

Sample Description:

Comments:

7-d Topsmelt Toxicity Test
Daily Survival

Client: WDOE
Sample ID: NWTPH-Dx
Work Order #: 1707154

Start Date & Time: July 6/17 ~~1530h~~ 1500h
Stop Date & Time: July 13/17 1600h 1600h
Test Species: *Atherinops affinis*

Concentration mg/L Diesel	Rep	Day of Test - No. of Survivors 1	2	3	4	5	6	7	Comments
Control	A	5	5	5	5	5	5	5	
	B								
	C								
	D								
	E								
Acetone Control	A								
	B								
	C								
	D								
	E							↓	
0.5	A							5	
	B								
	C				↓	↓	↓	↓	
	D		4	4	4	4	4	4	
	E	↓	5	5	5	5	5	5	
1	A	4	4	4	4	4	4	4	
	B	5	5	5	5	5	5	5	
	C		5	5	5	5	5	5	
	D		4	4	4	4	4	4	
	E	↓	5	5	5	5	5	5	
2	A	4	4	4	4	4	4	4	
	B	5	5	5	5	5	5	5	
	C				5	5		5	
	D				5	5		5	
	E	↓	↓		5	5		5	
4	A	5	5	5	5	5		5	
	B	5	5	5	5	5	↓	5	
	C	4	3	3	3	3	3	3	
	D	5	5	5	5	5	5	5	
	E	3	2	2	2	2	2	2	
8	A	5	~~[illegible]~~ 5	4	4	3	3	3	
	B		4	4	4	4	4	4	
	C	↓	5	5	5	5	5	5	
	D	4	3	3	3	3	3	3	
	E	4	4	4	4	4	4	4	
16	A	0	—	—	—	—	—		
	B	3	0	—	—	—			
	C	2	0	—	—	—			
	D	3	0	—	—				
	E	3	2	1	0	—	—		
Tech Initials		KJL	[illegible]	A	[illegible]	MLT	KJL	KJL	

Comments:

Reviewed by: JGb

Date reviewed: Aug. 2/17

Topsmelt Toxicity Test Data Sheet

Dry Weight Data

Client: WDOE

Work Order # : 1705156

Sample ID: NWTPH-DX

Start Date & Time: July 6/17 1500h

Termination Date & Time: July 13/17 1600h

mg/L Diesel	Rep	TS Pan No. purple	No. alive	Initials	Pan weight (mg)	Pan + organism (mg)	No. weighed	Initials
control	A	1	5	KJ-	1022.77	1029.76	5	KJ/NY
	B	2	5		1041.96	1048.18	5	
	C	3	5		1025.15	1031.04	5	
	D	4	5		1030.48	1036.79	5	
	E	5	5		1007.52	1014.45	5	
acetone control	A	6	5		1026.50	1032.77	5	
	B	7	5		1001.90	1007.06	5	
	C	8	5		1000.70	1006.29	5	
	D	9	5		1003.06	1008.08	5	
	E	10	5		1011.60	1017.84	5	
~~16~~ 19 0.5	A	11	5		1006.36	1011.41	5	
	B	12	5		1014.35	1019.32	5	
	C	13	5		1020.01 th	1026.61	5	
	D	14	4		1019.56	1024.16	4	
	E	15	5		1018.54	1024.11	5	
1	A	16	4		1021.59	1027.29	4	
	B	17	5		~~1004~~ 1010.03	1015.32	5	
	C	18	5		1014.91	1021.96	5	
	D	19	4		1016.25	1020.92	4	
	E	20	5		~~1022~~ 1022.06	1028.15	5	
2	A	21	4		1032.78	1037.19	4	
	B	22	5		1037.50	1043.79	5	
	C	23	5		1040.94	1047.05	5	
	D	24	5		1027.90	1034.60	5	
	E	25	5		1031.04	1038.27	5	
4	A	26	5		1034.73	1040.17	5	
	B	27	5		1016.37	1023.31	5	
	C	28	3		1034.72	1039.61	3	
	D	29	5		1019.~~55~~ th	1026.08	5	
	E	30	2		1026.57	1029.11	2	

Comments: 10% reweigh pans (mg):
pan # 5 - 1014.53 29 - 1026.06
17 - 1015.36 32 - 1028.52

Reviewed by: JGR

Date Reviewed: Aug. 2/17

CETIS Summary Report

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

Batch ID:	16-6555-7986	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Karen Lee
Start Date:	06 Jul-17 15:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural seawater
Ending Date:	13 Jul-17 16:00	**Species:**	Atherinops affinis	**Brine:**	
Duration:	7d 1h	**Source:**	Aquatic Biosystems, CO	**Age:**	10-d
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 4h	**Station:**	NWTPH-Dx		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
07-5014-2290	7d Survival Rate	0	>0		NA		Wilcoxon Rank Sum Two-Sample Test
06-0433-8171	7d Survival Rate	0.26	0.57	0.385	21.4%		Dunnett Multiple Comparison Test
18-8308-1078	Mean Dry Biomass-mg	0	>0		11.1%		Equal Variance t Two-Sample Test
05-9686-4041	Mean Dry Biomass-mg	0.57	1.6	0.955	31.2%		Dunnett Multiple Comparison Test
18-1870-0532	Mean Dry Weight-mg	0	>0		11.1%		Equal Variance t Two-Sample Test
19-9222-9790	Mean Dry Weight-mg	0.57	>0.57	NA	19.9%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	mg/L	95% LCL	95% UCL	TU	Method
07-6289-7116	7d Survival Rate	EC50	0.6779	0.5512	0.8337		Trimmed Spearman-Kärber
07-9187-0629	Mean Dry Biomass-mg	IC5	0.2395	N/A	0.7954		Linear Interpolation (ICPIN)
		IC10	0.6021	N/A	0.6758		
		IC15	0.6458	0.005465	0.7172		
		IC20	0.6906	0.02129	0.7597		
		IC25	0.7367	0.3741	0.8032		
		IC40	0.8827	0.6325	0.9402		
		IC50	0.9868	0.765	1.037		
06-8394-4426	Mean Dry Weight-mg	IC5	>0.57	N/A	N/A		Linear Interpolation (ICPIN)
		IC10	>0.57	N/A	N/A		
		IC15	>0.57	N/A	N/A		
		IC20	>0.57	N/A	N/A		
		IC25	>0.57	N/A	N/A		
		IC40	>0.57	N/A	N/A		
		IC50	>0.57	N/A	N/A		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
06-0433-8171	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
07-5014-2290	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
07-6289-7116	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
05-9686-4041	Mean Dry Biomass-mg	Control Resp	1.131	0.85 - NL	Yes	Passes Acceptability Criteria
07-9187-0629	Mean Dry Biomass-mg	Control Resp	1.131	0.85 - NL	Yes	Passes Acceptability Criteria
18-8308-1078	Mean Dry Biomass-mg	Control Resp	1.131	0.85 - NL	Yes	Passes Acceptability Criteria
	Mean Dry Biomass-mg	Control Resp	1.294	0.85 - NL	Yes	Passes Acceptability Criteria
06-0433-8171	7d Survival Rate	PMSD	0.2139	NL - 0.25	No	Passes Acceptability Criteria
05-9686-4041	Mean Dry Biomass-mg	PMSD	0.3119	NL - 0.5	No	Passes Acceptability Criteria
18-8308-1078	Mean Dry Biomass-mg	PMSD	0.111	NL - 0.5	No	Passes Acceptability Criteria

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

7d Survival Rate Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	5	1	1	1	1	1	0	0	0.0%	0.0%
0	Negative Control	5	1	1	1	1	1	0	0	0.0%	0.0%
0.1		5	0.96	0.8489	1	0.8	1	0.04	0.08944	9.32%	4.0%
0.12		5	0.92	0.784	1	0.8	1	0.04899	0.1095	11.91%	8.0%
0.16		5	0.96	0.8489	1	0.8	1	0.04	0.08944	9.32%	4.0%
0.26		5	0.8	0.4488	1	0.4	1	0.1265	0.2828	35.36%	20.0%
0.57		5	0.76	0.5522	0.9678	0.6	1	0.07483	0.1673	22.02%	24.0%
1.6		5	0	0	0	0	0	0	0		100.0%

Mean Dry Biomass-mg Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	5	1.131	0.9857	1.277	1.004	1.254	0.0524	0.1172	10.36%	0.0%
0	Negative Control	5	1.294	1.175	1.412	1.178	1.398	0.04258	0.0952	7.36%	-14.36%
0.1		5	1.032	0.926	1.137	0.92	1.12	0.03804	0.08505	8.25%	8.81%
0.12		5	1.152	0.9304	1.374	0.934	1.41	0.07982	0.1785	15.49%	-1.84%
0.16		5	1.23	0.9657	1.494	0.882	1.446	0.09506	0.2126	17.29%	-8.7%
0.26		5	1.054	0.6234	1.484	0.508	1.388	0.1549	0.3464	32.88%	6.86%
0.57		5	1.077	0.6527	1.501	0.648	1.556	0.1528	0.3416	31.72%	4.81%
1.6		5	0	0	0	0	0	0	0		100.0%

Mean Dry Weight-mg Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	5	1.131	0.9857	1.277	1.004	1.254	0.0524	0.1172	10.36%	0.0%
0	Negative Control	5	1.294	1.175	1.412	1.178	1.398	0.04258	0.0952	7.36%	-14.36%
0.1		5	1.078	0.99	1.165	0.994	1.15	0.03156	0.07058	6.55%	4.74%
0.12		5	1.256	1.059	1.453	1.058	1.425	0.07098	0.1587	12.64%	-11.01%
0.16		5	1.274	1.114	1.434	1.102	1.446	0.05758	0.1288	10.11%	-12.6%
0.26		5	1.336	1.091	1.582	1.088	1.63	0.08829	0.1974	14.77%	-18.14%
0.57		5	1.396	1.156	1.636	1.08	1.556	0.08648	0.1934	13.85%	-23.44%

Solvent blank = acetone control

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

7d Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Solvent Blank	1	1	1	1	1
0	Negative Control	1	1	1	1	1
0.1		1	1	1	0.8	1
0.12		0.8	1	1	0.8	1
0.16		0.8	1	1	1	1
0.26		1	1	0.6	1	0.4
0.57		0.6	0.8	1	0.6	0.8
1.6		0	0	0	0	0

Mean Dry Biomass-mg Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Solvent Blank	1.254	1.032	1.118	1.004	1.248
0	Negative Control	1.398	1.244	1.178	1.262	1.386
0.1		1.01	0.994	1.12	0.92	1.114
0.12		1.14	1.058	1.41	0.934	1.218
0.16		0.882	1.258	1.222	1.34	1.446
0.26		1.088	1.388	0.978	1.306	0.508
0.57		0.648	1.212	1.556	0.89	1.078
1.6		0	0	0	0	0

Mean Dry Weight-mg Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Solvent Blank	1.254	1.032	1.118	1.004	1.248
0	Negative Control	1.398	1.244	1.178	1.262	1.386
0.1		1.01	0.994	1.12	1.15	1.114
0.12		1.425	1.058	1.41	1.167	1.218
0.16		1.102	1.258	1.222	1.34	1.446
0.26		1.088	1.388	1.63	1.306	1.27
0.57		1.08	1.515	1.556	1.483	1.348
1.6						

7d Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Solvent Blank	5/5	5/5	5/5	5/5	5/5
0	Negative Control	5/5	5/5	5/5	5/5	5/5
0.1		5/5	5/5	5/5	4/5	5/5
0.12		4/5	5/5	5/5	4/5	5/5
0.16		4/5	5/5	5/5	5/5	5/5
0.26		5/5	5/5	3/5	5/5	2/5
0.57		3/5	4/5	5/5	3/5	4/5
1.6		0/5	0/5	0/5	0/5	0/5

Solvent blank= acetone control

CETIS Analytical Report

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

Analysis ID:	07-6289-7116	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:28	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes
Batch ID:	16-6555-7986	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Karen Lee
Start Date:	06 Jul-17 15:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural seawater
Ending Date:	13 Jul-17 16:00	**Species:**	Atherinops affinis	**Brine:**	
Duration:	7d 1h	**Source:**	Aquatic Biosystems, CO	**Age:**	10-d
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 4h	**Station:**	NWTPH-Dx		

Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	4.00%	-0.1689	0.04493	0.6779	0.5512	0.8337

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria

7d Survival Rate Summary

			Calculated Variate(A/B)								
C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Solvent Blank	5	1	1	1	0	0	0.0%	0.0%	25	25
0.1		5	0.96	0.8	1	0.04	0.08944	9.32%	4.0%	24	25
0.12		5	0.92	0.8	1	0.04899	0.1095	11.91%	8.0%	23	25
0.16		5	0.96	0.8	1	0.04	0.08944	9.32%	4.0%	24	25
0.26		5	0.8	0.4	1	0.1265	0.2828	35.36%	20.0%	20	25
0.57		5	0.76	0.6	1	0.07483	0.1673	22.02%	24.0%	19	25
1.6		5	0	0	0	0	0		100.0%	0	25

7d Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Solvent Blank	1	1	1	1	1
0.1		1	1	1	0.8	1
0.12		0.8	1	1	0.8	1
0.16		0.8	1	1	1	1
0.26		1	1	0.6	1	0.4
0.57		0.6	0.8	1	0.6	0.8
1.6		0	0	0	0	0

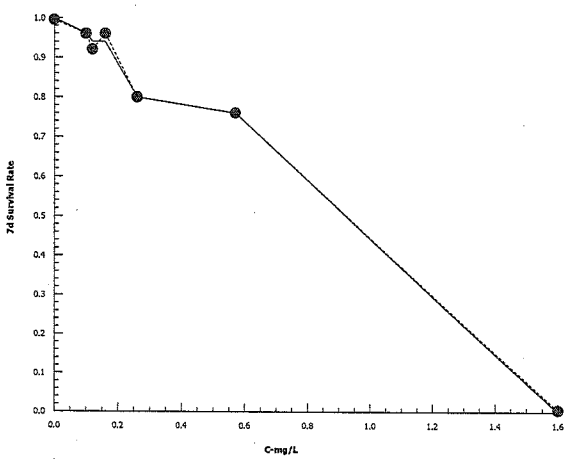
7d Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Solvent Blank	5/5	5/5	5/5	5/5	5/5
0	Negative Control	5/5	5/5	5/5	5/5	5/5
0.1		5/5	5/5	5/5	4/5	5/5
0.12		4/5	5/5	5/5	4/5	5/5
0.16		4/5	5/5	5/5	5/5	5/5
0.26		5/5	5/5	3/5	5/5	2/5
0.57		3/5	4/5	5/5	3/5	4/5
1.6		0/5	0/5	0/5	0/5	0/5

Solvent blank = acetone control

Analysis ID:	07-6289-7116	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:28	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes

Graphics



Analyst: KJL QA: Jan 9/18

CETIS Analytical Report

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

Analysis ID:	06-0433-8171	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:38	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	16-6555-7986	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Karen Lee
Start Date:	06 Jul-17 15:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural seawater
Ending Date:	13 Jul-17 16:00	**Species:**	Atherinops affinis	**Brine:**	
Duration:	7d 1h	**Source:**	Aquatic Biosystems, CO	**Age:**	10-d
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 4h	**Station:**	NWTPH-Dx		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	21.4%	0.26	0.57	0.385	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Solvent Blank		0.1	0.4405	2.362	0.255	8	0.6726	CDF	Non-Significant Effect
		0.12	0.881	2.362	0.255	8	0.4737	CDF	Non-Significant Effect
		0.16	0.4405	2.362	0.255	8	0.6726	CDF	Non-Significant Effect
		0.26	2.071	2.362	0.255	8	0.0873	CDF	Non-Significant Effect
		0.57*	2.58	2.362	0.255	8	0.0320	CDF	Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
PMSD	0.2139	NL - 0.25	No	Passes Acceptability Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.3071862	0.06143725	5	2.102	0.1001	Non-Significant Effect
Error	0.701349	0.02922288	24			
Total	1.008535		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1.39	4.248	0.2747	Equal Variances
Variances	Levene Equality of Variance	8.658	3.895	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.9157	0.9031	0.0208	Normal Distribution

7d Survival Rate Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	5	1	1	1	1	1	1	0	0.0%	0.0%
0.1		5	0.96	0.8489	1	1	0.8	1	0.04	9.32%	4.0%
0.12		5	0.92	0.784	1	1	0.8	1	0.04899	11.91%	8.0%
0.16		5	0.96	0.8489	1	1	0.8	1	0.04	9.32%	4.0%
0.26		5	0.8	0.4488	1	1	0.4	1	0.1265	35.36%	20.0%
0.57		5	0.76	0.5522	0.9678	0.8	0.6	1	0.07483	22.02%	24.0%
1.6		5	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	5	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
0.1		5	1.298	1.165	1.43	1.345	1.107	1.345	0.04763	8.21%	3.54%
0.12		5	1.25	1.088	1.412	1.345	1.107	1.345	0.05833	10.43%	7.08%
0.16		5	1.298	1.165	1.43	1.345	1.107	1.345	0.04763	8.21%	3.54%
0.26		5	1.121	0.7304	1.512	1.345	0.6847	1.345	0.1408	28.08%	16.65%
0.57		5	1.066	0.829	1.304	1.107	0.8861	1.345	0.08548	17.92%	20.73%
1.6		5	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	83.24%

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

Analysis ID:	06-0433-8171	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:38	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

7d Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Solvent Blank	1	1	1	1	1
0.1		1	1	1	0.8	1
0.12		0.8	1	1	0.8	1
0.16		0.8	1	1	1	1
0.26		1	1	0.6	1	0.4
0.57		0.6	0.8	1	0.6	0.8
1.6		0	0	0	0	0

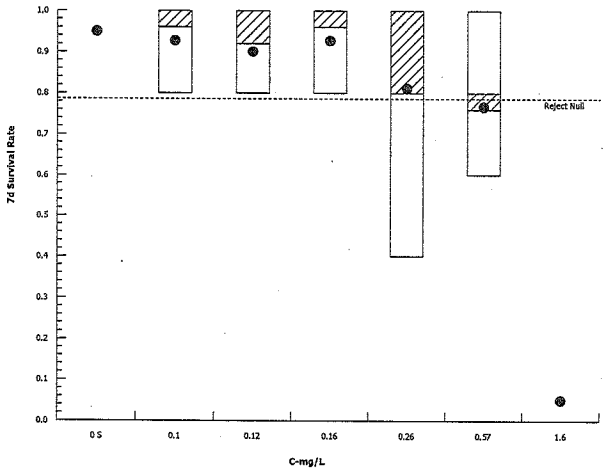
Angular (Corrected) Transformed Detail

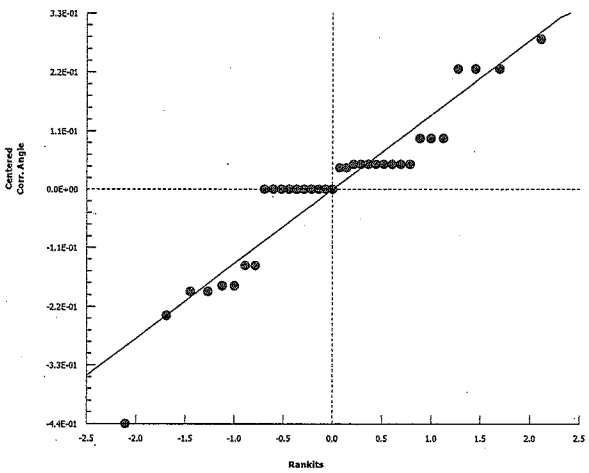
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Solvent Blank	1.345	1.345	1.345	1.345	1.345
0.1		1.345	1.345	1.345	1.107	1.345
0.12		1.107	1.345	1.345	1.107	1.345
0.16		1.107	1.345	1.345	1.345	1.345
0.26		1.345	1.345	0.8861	1.345	0.6847
0.57		0.8861	1.107	1.345	0.8861	1.107
1.6		0.2255	0.2255	0.2255	0.2255	0.2255

7d Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Solvent Blank	5/5	5/5	5/5	5/5	5/5
0	Negative Control	5/5	5/5	5/5	5/5	5/5
0.1		5/5	5/5	5/5	4/5	5/5
0.12		4/5	5/5	5/5	4/5	5/5
0.16		4/5	5/5	5/5	5/5	5/5
0.26		5/5	5/5	3/5	5/5	2/5
0.57		3/5	4/5	5/5	3/5	4/5
1.6		0/5	0/5	0/5	0/5	0/5

Graphics





CETIS Analytical Report

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

Analysis ID:	07-5014-2290	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:35	**Analysis:**	Nonparametric-Two Sample	**Official Results:**	Yes
Batch ID:	16-6555-7986	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Karen Lee
Start Date:	06 Jul-17 15:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural seawater
Ending Date:	13 Jul-17 16:00	**Species:**	Atherinops affinis	**Brine:**	
Duration:	7d 1h	**Source:**	Aquatic Biosystems, CO	**Age:**	10-d
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 4h	**Station:**	NWTPH-Dx		

Data Transform	Zeta	Alt Hyp	Trials	Seed	Test Result
Angular (Corrected)	NA	C > T	NA	NA	Passes 7d survival rate

Wilcoxon Rank Sum Two-Sample Test

Control	vs	Control	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Solvent Blank		Negative Control	27.5	NA	1	8	1.0000	Exact	Non-Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0	0	1	65540	<0.0001	Significant Effect
Error	0	0	8			
Total	0		9			

7d Survival Rate Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	5	1	1	1	1	1	1	0	0.0%	0.0%
0	Negative Control	5	1	1	1	1	1	1	0	0.0%	0.0%

Angular (Corrected) Transformed Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	5	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
0	Negative Contr	5	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%

7d Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Solvent Blank	1	1	1	1	1
0	Negative Control	1	1	1	1	1

Angular (Corrected) Transformed Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Solvent Blank	1.345	1.345	1.345	1.345	1.345
0	Negative Control	1.345	1.345	1.345	1.345	1.345

7d Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Solvent Blank	5/5	5/5	5/5	5/5	5/5
0	Negative Control	5/5	5/5	5/5	5/5	5/5

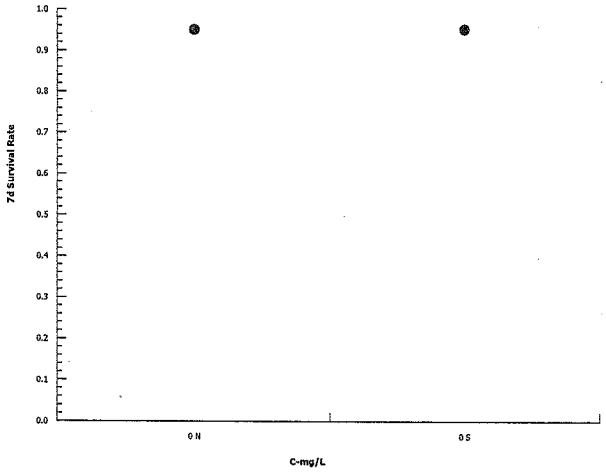
Solvent blank = Acetone control

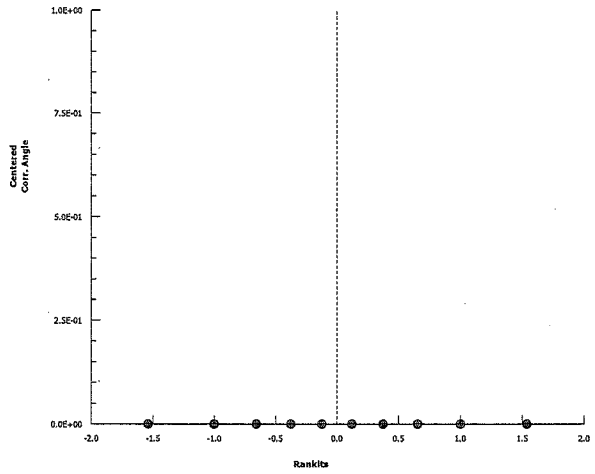
Analyst: KJL QA: JGL Jan. 9/18

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

Analysis ID:	07-5014-2290	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:35	**Analysis:**	Nonparametric-Two Sample	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	07-9187-0629	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:28	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	16-6555-7986	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Karen Lee
Start Date:	06 Jul-17 15:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural seawater
Ending Date:	13 Jul-17 16:00	**Species:**	Atherinops affinis	**Brine:**	
Duration:	7d 1h	**Source:**	Aquatic Biosystems, CO	**Age:**	10-d
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 4h	**Station:**	NWTPH-Dx		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1494352	200	Yes	Two-Point Interpolation

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1.131	0.85 - NL	Yes	Passes Acceptability Criteria

Point Estimates

Level	mg/L	95% LCL	95% UCL
IC5	0.2395	N/A	0.7954
IC10	0.6021	N/A	0.6758
IC15	0.6458	0.005465	0.7172
IC20	0.6906	0.02129	0.7597
IC25	0.7367	0.3741	0.8032
IC40	0.8827	0.6325	0.9402
IC50	0.9868	0.765	1.037

Mean Dry Biomass-mg Summary

			Calculated Variate						
C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	5	1.131	1.004	1.254	0.0524	0.1172	10.36%	0.0%
0.1		5	1.032	0.92	1.12	0.03804	0.08505	8.25%	8.81%
0.12		5	1.152	0.934	1.41	0.07982	0.1785	15.49%	-1.84%
0.16		5	1.23	0.882	1.446	0.09506	0.2126	17.29%	-8.7%
0.26		5	1.054	0.508	1.388	0.1549	0.3464	32.88%	6.86%
0.57		5	1.077	0.648	1.556	0.1528	0.3416	31.72%	4.81%
1.6		5	0	0	0	0	0		100.0%

Mean Dry Biomass-mg Detail

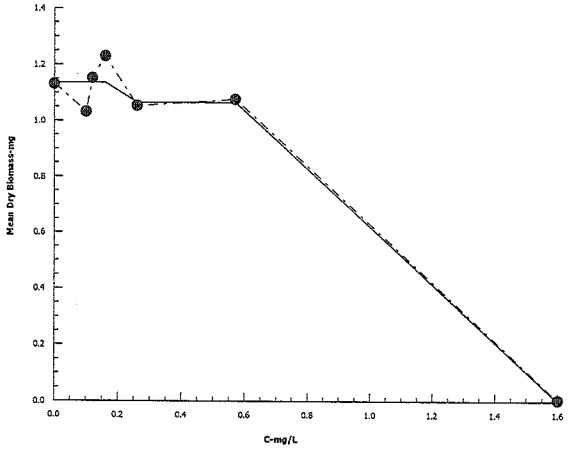
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Solvent Blank	1.254	1.032	1.118	1.004	1.248
0.1		1.01	0.994	1.12	0.92	1.114
0.12		1.14	1.058	1.41	0.934	1.218
0.16		0.882	1.258	1.222	1.34	1.446
0.26		1.088	1.388	0.978	1.306	0.508
0.57		0.648	1.212	1.556	0.89	1.078
1.6		0	0	0	0	0

Solvent blank= acetone control

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

Analysis ID:	07-9187-0629	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:28	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



Analyst: KJL QA: Jan 9/18

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

Analysis ID:	05-9686-4041	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:38	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	16-6555-7986	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Karen Lee
Start Date:	06 Jul-17 15:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural seawater
Ending Date:	13 Jul-17 16:00	**Species:**	Atherinops affinis	**Brine:**	
Duration:	7d 1h	**Source:**	Aquatic Biosystems, CO	**Age:**	10-d
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 4h	**Station:**	NWTPH-Dx		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	31.2%	0.57	1.6	0.955	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Solvent Blank		0.1	0.6668	2.362	0.353	8	0.5722	CDF	Non-Significant Effect
		0.12	-0.1393	2.362	0.353	8	0.8712	CDF	Non-Significant Effect
		0.16	-0.6587	2.362	0.353	8	0.9590	CDF	Non-Significant Effect
		0.26	0.5195	2.362	0.353	8	0.6384	CDF	Non-Significant Effect
		0.57	0.3642	2.362	0.353	8	0.7043	CDF	Non-Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1.131	0.85 - NL	Yes	Passes Acceptability Criteria
PMSD	0.3119	NL - 0.5	No	Passes Acceptability Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.1345526	0.02691051	5	0.4824	0.7859	Non-Significant Effect
Error	1.338785	0.05578272	24			
Total	1.473338		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	9.885	15.09	0.0786	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9718	0.9031	0.5901	Normal Distribution

Mean Dry Biomass-mg Summary

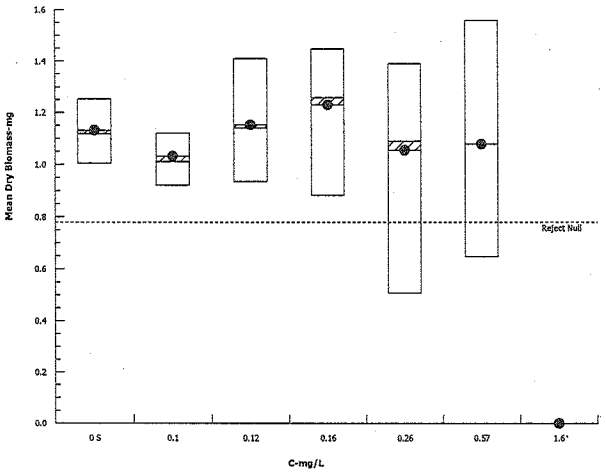
C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	5	1.131	0.9857	1.277	1.118	1.004	1.254	0.0524	10.36%	0.0%
0.1		5	1.032	0.926	1.137	1.01	0.92	1.12	0.03804	8.25%	8.81%
0.12		5	1.152	0.9304	1.374	1.14	0.934	1.41	0.07982	15.49%	-1.84%
0.16		5	1.23	0.9657	1.494	1.258	0.882	1.446	0.09506	17.29%	-8.7%
0.26		5	1.054	0.6234	1.484	1.088	0.508	1.388	0.1549	32.88%	6.86%
0.57		5	1.077	0.6527	1.501	1.078	0.648	1.556	0.1528	31.72%	4.81%
1.6		5	0	0	0	0	0	0	0		100.0%

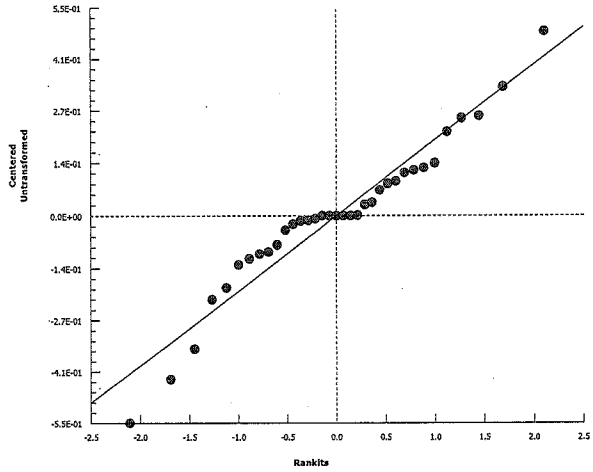
Mean Dry Biomass-mg Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Solvent Blank	1.254	1.032	1.118	1.004	1.248
0.1		1.01	0.994	1.12	0.92	1.114
0.12		1.14	1.058	1.41	0.934	1.218
0.16		0.882	1.258	1.222	1.34	1.446
0.26		1.088	1.388	0.978	1.306	0.508
0.57		0.648	1.212	1.556	0.89	1.078
1.6		0	0	0	0	0

Analysis ID:	05-9686-4041	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:38	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	18-8308-1078	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:35	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes
Batch ID:	16-6555-7986	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Karen Lee
Start Date:	06 Jul-17 15:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural seawater
Ending Date:	13 Jul-17 16:00	**Species:**	Atherinops affinis	**Brine:**	
Duration:	7d 1h	**Source:**	Aquatic Biosystems, CO	**Age:**	10-d
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 4h	**Station:**	NWTPH-Dx		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C > T	NA	NA	11.1%	Passes mean dry biomass-mg

Equal Variance t Two-Sample Test

Control	vs	Control	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Solvent Blank		Negative Control	-2.405	1.86	0.126	8	0.9786	CDF	Non-Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1.131	0.85 - NL	Yes	Passes Acceptability Criteria
Control Resp	1.294	0.85 - NL	Yes	Passes Acceptability Criteria
PMSD	0.111	NL - 0.5	No	Passes Acceptability Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.06593832	0.06593832	1	5.786	0.0428	Significant Effect
Error	0.09117568	0.01139696	8			
Total	0.157114		9			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	1.515	23.15	0.6972	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8703	0.7411	0.1009	Normal Distribution

Mean Dry Biomass-mg Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	5	1.131	0.9857	1.277	1.118	1.004	1.254	0.0524	10.36%	0.0%
0	Negative Control	5	1.294	1.175	1.412	1.262	1.178	1.398	0.04258	7.36%	-14.36%

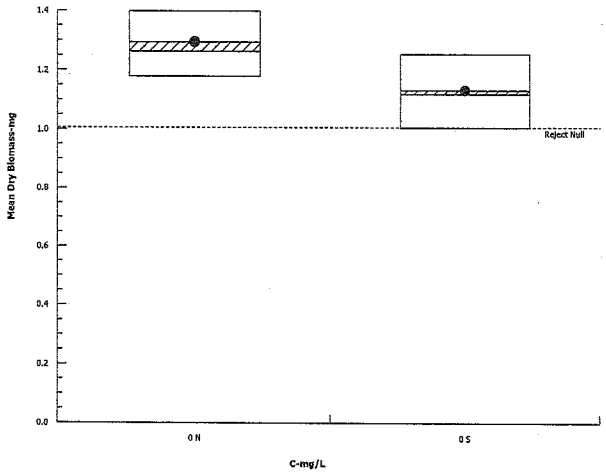
Mean Dry Biomass-mg Detail

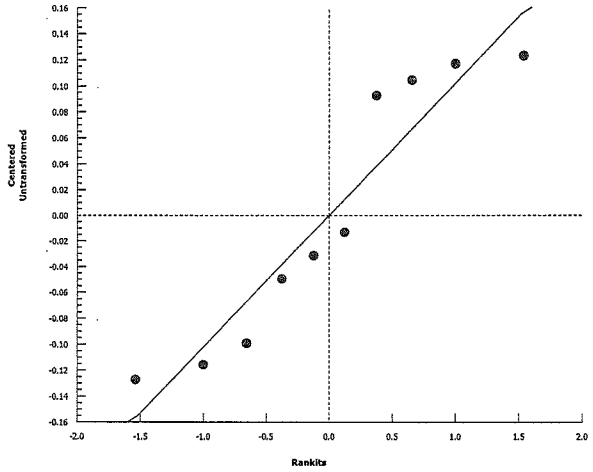
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Solvent Blank	1.254	1.032	1.118	1.004	1.248
0	Negative Control	1.398	1.244	1.178	1.262	1.386

Solvent blank = acetone control

Analysis ID:	18-8308-1078	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:35	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	06-8394-4426	**Endpoint:**	Mean Dry Weight-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:34	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	16-6555-7986	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Karen Lee
Start Date:	06 Jul-17 15:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural seawater
Ending Date:	13 Jul-17 16:00	**Species:**	Atherinops affinis	**Brine:**	
Duration:	7d 1h	**Source:**	Aquatic Biosystems, CO	**Age:**	10-d
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 4h	**Station:**	NWTPH-Dx		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1980132	200	Yes	Two-Point Interpolation

Point Estimates

Level	mg/L	95% LCL	95% UCL
IC5	>0.57	N/A	N/A
IC10	>0.57	N/A	N/A
IC15	>0.57	N/A	N/A
IC20	>0.57	N/A	N/A
IC25	>0.57	N/A	N/A
IC40	>0.57	N/A	N/A
IC50	>0.57	N/A	N/A

Mean Dry Weight-mg Summary

			Calculated Variate						
C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	5	1.131	1.004	1.254	0.0524	0.1172	10.36%	0.0%
0.1		5	1.078	0.994	1.15	0.03156	0.07058	6.55%	4.74%
0.12		5	1.256	1.058	1.425	0.07098	0.1587	12.64%	-11.01%
0.16		5	1.274	1.102	1.446	0.05758	0.1288	10.11%	-12.6%
0.26		5	1.336	1.088	1.63	0.08829	0.1974	14.77%	-18.14%
0.57		5	1.396	1.08	1.556	0.08648	0.1934	13.85%	-23.44%

Mean Dry Weight-mg Detail

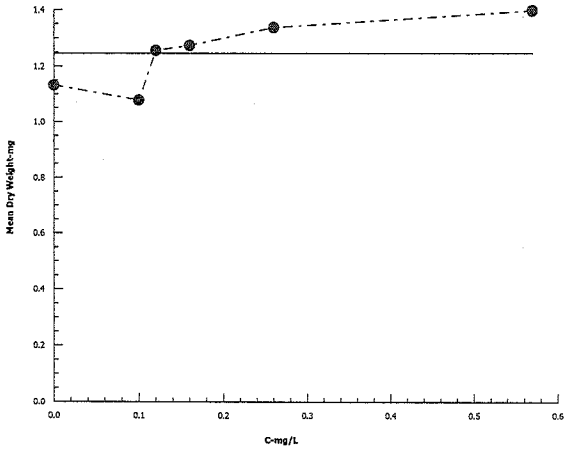
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Solvent Blank	1.254	1.032	1.118	1.004	1.248
0.1		1.01	0.994	1.12	1.15	1.114
0.12		1.425	1.058	1.41	1.167	1.218
0.16		1.102	1.258	1.222	1.34	1.446
0.26		1.088	1.388	1.63	1.306	1.27
0.57		1.08	1.515	1.556	1.483	1.348

Solvent blank = acetone control

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

Analysis ID:	06-8394-4426	**Endpoint:**	Mean Dry Weight-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:34	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



Analyst: KR QA: Jan 9/18

CETIS Analytical Report

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

Analysis ID:	19-9222-9790	**Endpoint:**	Mean Dry Weight-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:38	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	16-6555-7986	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Karen Lee
Start Date:	06 Jul-17 15:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural seawater
Ending Date:	13 Jul-17 16:00	**Species:**	Atherinops affinis	**Brine:**	
Duration:	7d 1h	**Source:**	Aquatic Biosystems, CO	**Age:**	10-d
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 4h	**Station:**	NWTPH-Dx		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	19.9%	0.57	>0.57	NA	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Solvent Blank		0.1	0.5611	2.362	0.226	8	0.6200	CDF	Non-Significant Effect
		0.12	-1.303	2.362	0.226	8	0.9931	CDF	Non-Significant Effect
		0.16	-1.492	2.362	0.226	8	0.9961	CDF	Non-Significant Effect
		0.26	-2.148	2.362	0.226	8	0.9996	CDF	Non-Significant Effect
		0.57	-2.776	2.362	0.226	8	0.9999	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.3658784	0.07317568	5	3.208	0.0234	Significant Effect
Error	0.5474073	0.02280864	24			
Total	0.9132857		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.442	15.09	0.4876	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9854	0.9031	0.9438	Normal Distribution

Mean Dry Weight-mg Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	5	1.131	0.9857	1.277	1.118	1.004	1.254	0.0524	10.36%	0.0%
0.1		5	1.078	0.99	1.165	1.114	0.994	1.15	0.03156	6.55%	4.74%
0.12		5	1.256	1.059	1.453	1.218	1.058	1.425	0.07098	12.64%	-11.01%
0.16		5	1.274	1.114	1.434	1.258	1.102	1.446	0.05758	10.11%	-12.6%
0.26		5	1.336	1.091	1.582	1.306	1.088	1.63	0.08829	14.77%	-18.14%
0.57		5	1.396	1.156	1.636	1.483	1.08	1.556	0.08648	13.85%	-23.44%

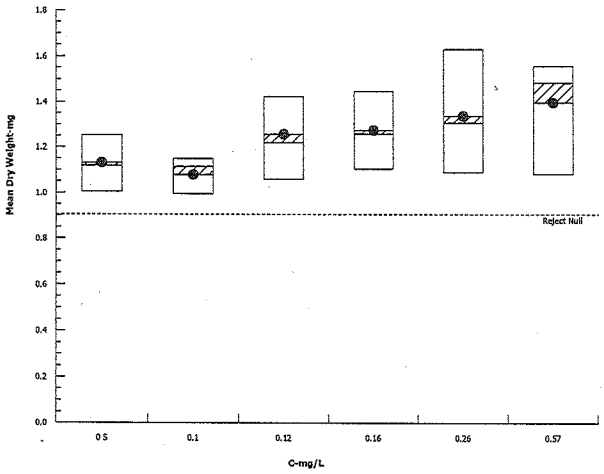
Mean Dry Weight-mg Detail

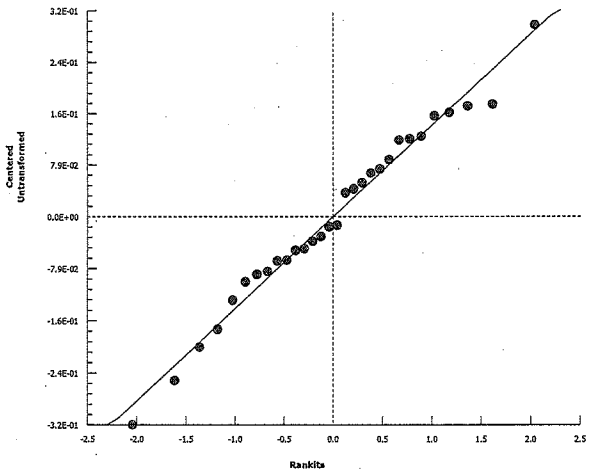
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Solvent Blank	1.254	1.032	1.118	1.004	1.248
0.1		1.01	0.994	1.12	1.15	1.114
0.12		1.425	1.058	1.41	1.167	1.218
0.16		1.102	1.258	1.222	1.34	1.446
0.26		1.088	1.388	1.63	1.306	1.27
0.57		1.08	1.515	1.556	1.483	1.348

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

Analysis ID:	19-9222-9790	**Endpoint:**	Mean Dry Weight-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:38	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	18-1870-0532	**Endpoint:**	Mean Dry Weight-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:35	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes
Batch ID:	16-6555-7986	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Karen Lee
Start Date:	06 Jul-17 15:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural seawater
Ending Date:	13 Jul-17 16:00	**Species:**	Atherinops affinis	**Brine:**	
Duration:	7d 1h	**Source:**	Aquatic Biosystems, CO	**Age:**	10-d
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 4h	**Station:**	NWTPH-Dx		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C > T	NA	NA	11.1%	Passes mean dry weight-mg

Equal Variance t Two-Sample Test

Control	vs	Control	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Solvent Blank		Negative Control	-2.405	1.86	0.126	8	0.9786	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.06593832	0.06593832	1	5.786	0.0428	Significant Effect
Error	0.09117568	0.01139696	8			
Total	0.157114		9			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	1.515	23.15	0.6972	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8703	0.7411	0.1009	Normal Distribution

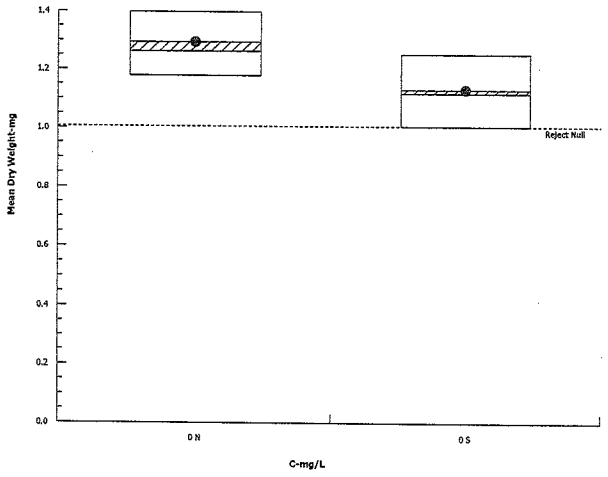
Mean Dry Weight-mg Summary

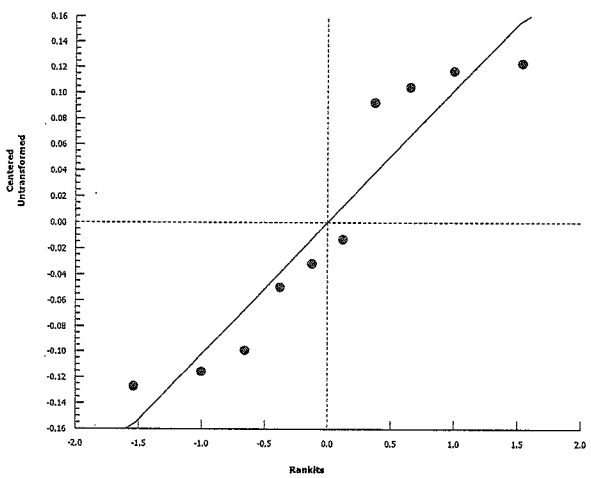
C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	5	1.131	0.9857	1.277	1.118	1.004	1.254	0.0524	10.36%	0.0%
0	Negative Control	5	1.294	1.175	1.412	1.262	1.178	1.398	0.04258	7.36%	-14.36%

Mean Dry Weight-mg Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Solvent Blank	1.254	1.032	1.118	1.004	1.248
0	Negative Control	1.398	1.244	1.178	1.262	1.386

Graphics





Solvent blank = acetone control

CETIS Analytical Report

Nautilus Environmental

Pacific Topsmelt 7-d Survival and Growth Test

Analysis ID:	18-1870-0532	**Endpoint:**	Mean Dry Weight-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:35	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes
Batch ID:	16-6555-7986	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Karen Lee
Start Date:	06 Jul-17 15:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural seawater
Ending Date:	13 Jul-17 16:00	**Species:**	Atherinops affinis	**Brine:**	
Duration:	7d 1h	**Source:**	Aquatic Biosystems, CO	**Age:**	10-d
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 4h	**Station:**	NWTPH-Dx		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C > T	NA	NA	11.1%	Passes mean dry weight-mg

Equal Variance t Two-Sample Test

Control	vs	Control	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Solvent Blank		Negative Control	-2.405	1.86	0.126	8	0.9786	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.06593832	0.06593832	1	5.786	0.0428	Significant Effect
Error	0.09117568	0.01139696	8			
Total	0.157114		9			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	1.515	23.15	0.6972	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8703	0.7411	0.1009	Normal Distribution

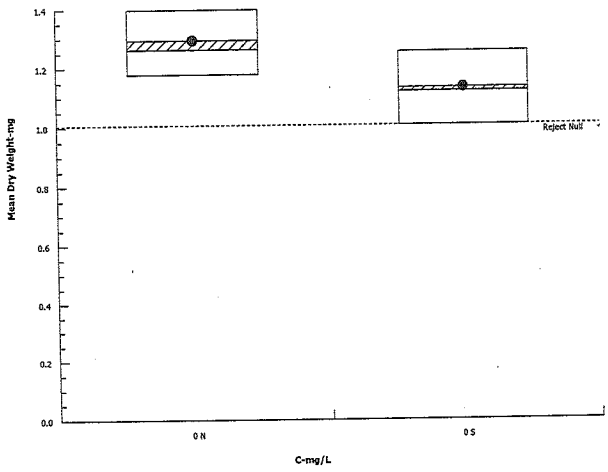
Mean Dry Weight-mg Summary

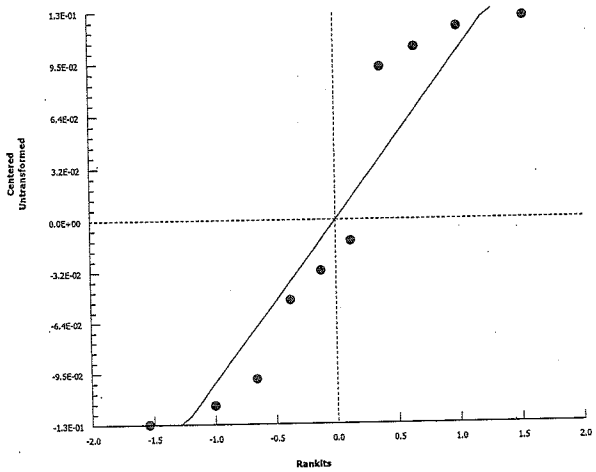
C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	5	1.131	0.9857	1.277	1.118	1.004	1.254	0.0524	10.36%	0.0%
0	Negative Control	5	1.294	1.175	1.412	1.262	1.178	1.398	0.04258	7.36%	-14.36%

Mean Dry Weight-mg Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Solvent Blank	1.254	1.032	1.118	1.004	1.248
0	Negative Control	1.398	1.244	1.178	1.262	1.386

Graphics





Analyst: EMM QA: JGL Jan-11/18

APPENDIX C – *Ceriodaphnia dubia* Toxicity Test Data

Ceriodaphnia dubia Summary Sheet

Client: WDOE
Work Order No.: 170516

Start Date/Time: July 6/17 @ 1200
Set up by: YYL (AMM)

Sample Information:

Sample ID: NWTPH-DX
Sample Date: May 9/17
Date Received: May 9/17
Sample Volume: USEPA Standards 50 x 5mL

Test Validity Criteria:

1) Mean survival of first generation controls is ≥80 %
2) At least 60% of controls have produced three broods within 8 days
3) An average of ≥15 live young produced per surviving female in the control solutions during the first three broods.
4) Invalid if ephippia observed in any control solution at any time.

WQ Ranges:

T (°C) = 25 ± 1; DO (mg/L) = 4.0 to 8.4 ; pH = 6.0 to 9.0

Test Organism Information:

Broodstock No.: 063017B
Age of young (Day 0): <24-h (within 8-h)
Avg No. young in first 3 broods of previous 7 d: 20
Mortality (%) in previous 7 d: 9
Individual female # used ≥8 young on test day: 21 → 30

NaCl Reference Toxicant Results:

Reference Toxicant ID: Ca(6)
Stock Solution ID: June 21/17
Date Initiated: 17Nov02

7-d LC50 (95% CL): 2.1 (1.5-3.0) g/L NaCL
7-d IC50 (95% CL): 1.8 (1.7-1.9) g/L NaCL

7-d LC50 Reference Toxicant Mean and Historical Range: 2.0 (1.8-2.2) g/L NaCL CV (%): 5
7-d IC50 Reference Toxicant Mean and Historical Range: 1.5 (1.1-2.1) g/L NaCL CV (%): 18

Test Results: (1) (mg/L DieCl)

	Survival	Reproduction
LC50 %~~v/v (95% CL)~~	(1) ~~0.20 (0.17-0.23)~~ 0.23 (0.20-0.26)	
IC25 %~~v/v (95% CL)~~		~~0.14 (0.13-0.15)~~ 0.17 (0.16-0.19)
IC50 %~~v/v (95% CL)~~		~~0.17 (0.15-0.20)~~ 0.20 (0.19-0.24)
NOEC %~~v/v~~	~~0.18~~ 0.22	~~0.12~~ 0.15
LOEC %~~v/v~~	~~0.31~~ 0.34	~~0.18~~ 0.22

(1) endpoint calculated using acetone control (solvent) as negative control

Reviewed by: JGM
Date reviewed: Aug. 25/17

Chronic Freshwater Toxicity Test
Initial and Final Water Quality Measurements

Client: WDOE
Sample ID: NWTPH-Dx (Diesel)
Work Order #: 17C516

Start Date & Time: July 6/17 @ 1200h
Stop Date & Time: July 13/17 @ 0745h
Test Species: *Ceriodaphnia dubia*

(mg/L Diesel)

Concentration: control	Days													
	0	1		2		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0
DO (mg/L)	8.1	7.6	8.0	7.8	8.0	7.3	8.0	7.7	8.0	7.7	8.0	7.3	8.0	7.7
pH	8.1	7.7	8.0	7.7	7.8	7.6	7.7	7.6	8.0	7.5	8.0	7.6	8.0	7.6
Cond. (µS/cm)	206	210		205		204		204		208		208		211
Initials	EMM	EMM		JW		A		EMM		EMM		EMM		AM

Concentration: acetone control	Days													
	0	1		2		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0
DO (mg/L)	8.1	7.6	8.2	7.9	8.1	7.2	7.9	7.3	7.9	7.3	8.0	7.3	8.2	7.3
pH	8.1	7.8	8.1	7.87	8.0	7.5	7.8	7.6	8.0	7.5	7.9	7.6	7.9	7.6
Cond. (µS/cm)	206	209		JW 205		204		205		209		208		211
Initials	EMM	EMM		A		A		EMM		EMM		EMM		AM

Concentration: 0.045	Days													
	0	1		2		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0
DO (mg/L)	8.1	7.6	8.2	7.9	8.1	7.1	7.8	7.3	8.1	7.3	8.1	7.4	8.2	7.3
pH	8.1	7.8	8.2	7.75	8.0	7.3	7.8	7.6	8.0	7.5	7.9	7.6	7.9	7.6
Cond. (µS/cm)	206	209		JW 217		214		209		210		209		206
Initials	EMM	EMM		A		A		EMM		EMM		EMM		AM

Concentration: 0.09	Days													
	0	1		2		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0
DO (mg/L)	8.1	7.3	8.2	7.9	8.2	7.3	7.9	7.2	8.1	7.3	8.1	7.4	8.2	7.2
pH	8.1	7.8	8.1	7.6	8.0	7.6	7.8	7.6	8.0	7.5	8.0	7.6	7.9	7.6
Cond. (µS/cm)	208	209		217		216		209		216		209		208
Initials	EMM	EMM		A		A		EMM		EMM		EMM		EMM

Thermometer: 4 DO meter: 2 pH meter: 4 Conductivity meter: 2

	Control			
Hardness*	100			
Alkalinity*	98			

* mg/L as CaCO3

Analysts: EMM, YYL, AWD
Reviewed by: JW JGL
Date reviewed: Aug. 2/17

Sample Description: Diesel Standard

Comments: Broodboard Used: 063017B (#21 → 30)

Chronic Freshwater Toxicity Test
Initial and Final Water Quality Measurements

Client: WDOE
Sample ID: NWTPH-Dx (Diesel)
Work Order #: 170516
(mg/L Diesel)

Start Date & Time: July 6/17 @ 1200h
Stop Date & Time: July 13/17 @ 1300h 0745h
Test Species: *Ceriodaphnia dubia*

Concentration 0.19	Days 0	1		2		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0
DO (mg/L)	8.1	7.3	7.7	7.8	8.3	7.2	8.0	7.2	8.1	7.1	8.1	7.2	8.1	7.1
pH	8.1	7.8	8.1	7.6	8.1	7.5	7.7	7.6	7.7	7.7	7.8	7.7	7.9	7.6
Cond. (µS/cm)	208	208		217		215		208		209		208		211
Initials	EMM	EMM		JW		A		EMM		EMM		EMM		EMM

Concentration 0.38	Days 0	1		2		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0	24.0	25.0
DO (mg/L)	8.1	7.3	7.7	7.8	8.3	7.1	7.6	7.1	8.0	7.1	8.1	7.2	8.1	7.1
pH	8.1	7.8	8.1	7.6	8.0	7.5	7.7	7.6	7.7	7.7	7.8	7.7	7.9	7.6
Cond. (µS/cm)	208	208		216		214		209		210		209		211
Initials	EMM	EMM		JW		A		EMM		EMM		EMM		EMM

Concentration 0.75	Days 0	1		25.0 2 24.0		25.0 3 24.0		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	24.0	25.0	24.0	~~25.0~~	~~24.0~~	~~25.0~~	~~24.0~~	25.0	24.0	25.0	24.0	25.0	24.0	25.0
DO (mg/L)	8.1	7.3	7.7	7.8	8.3	7.2	7.6	7.3	7.8	7.2	8.0	7.3	8.1	7.2
pH	8.1	7.5	8.1	7.8	8.1	7.5	7.7	7.6	7.7	7.6	7.8	7.6	7.9	7.6
Cond. (µS/cm)	209	209		216		215		210		211		209		210
Initials	EMM	EMM		JW		A		EMM		EMM		EMM		EMM

Concentration 1.5	Days 0	1		25.0 2 24.0		3		4		5		6		7
	init.	old	new	old	new	old	new	old	new	old	new	old	new	final
Temperature (°C)	24.0	25.0	24.0	~~25.0~~	~~24.0~~	~~25.0~~	~~24.0~~	25.0	24.0					
DO (mg/L)	8.1	7.4	7.6	7.7	8.3	7.3	7.5	7.1	7.9					
pH	8.1	7.9	8.1	7.7	8.0	7.5	7.7	7.5	7.7					
Cond. (µS/cm)	209	208		215		213		210 219						
Initials	EMM	EMM		JW		A		EMM						

Thermometer: 4 DO meter: 2 pH meter: 4 Conductivity meter: 2

	Control			
Hardness*	100			
Alkalinity*	98			

* mg/L as CaCO3

Analysts: EMM, YYL, AWD JW
Reviewed by: JGA
Date reviewed: Aug. 2/17

Sample Description: Diesel standard

Comments: Broodboard Used: 060178 (#21 → 30)

Chronic Freshwater Toxicity Test
Initial and Final Water Quality Measurements

Client: WDOE
Sample ID: NWTPH – Dx (Diesel)
Work Order #: 170516

Start Date & Time: July 6/17 @ 1200h
Stop Date & Time: July 13/17 @ 0745h
Test Species: *Ceriodaphnia dubia*

(mg/L diesel)

Concentration 3	Day 0 init.	Day 1 old	Day 1 new	Day 2 old	Day 2 new	Day 3 old	Day 3 new	Day 4 old	Day 4 new	Day 5 old	Day 5 new	Day 6 old	Day 6 new	Day 7 final
Temperature (°C)	24.0	25.0												
DO (mg/L)	8.1	7.8												
pH	8.1	8.2												
Cond. (µS/cm)	210	210												
Initials	EMM	EMM												

Concentration 6	Day 0 init.	Day 1 old	Day 1 new	Day 2 old	Day 2 new	Day 3 old	Day 3 new	Day 4 old	Day 4 new	Day 5 old	Day 5 new	Day 6 old	Day 6 new	Day 7 final
Temperature (°C)	24.0	25.0												
DO (mg/L)	8.0	7.8												
pH	8.2	8.3												
Cond. (µS/cm)	212	213												
Initials	EMM	EMM												

Concentration	Day 0 init.	Day 1 old	Day 1 new	Day 2 old	Day 2 new	Day 3 old	Day 3 new	Day 4 old	Day 4 new	Day 5 old	Day 5 new	Day 6 old	Day 6 new	Day 7 final
Temperature (°C)														
DO (mg/L)														
pH														
Cond. (µS/cm)														
Initials														

Concentration	Day 0 init.	Day 1 old	Day 1 new	Day 2 old	Day 2 new	Day 3 old	Day 3 new	Day 4 old	Day 4 new	Day 5 old	Day 5 new	Day 6 old	Day 6 new	Day 7 final
Temperature (°C)														
DO (mg/L)														
pH														
Cond. (µS/cm)														
Initials														

Thermometer: 4 DO meter: 2 pH meter: 4 Conductivity meter: 2

	Control			
Hardness*	100			
Alkalinity*	98			

* mg/L as CaCO3

Analysts: EMM, YML, AWD, TW
Reviewed by: JW
Date reviewed: Aug. 2/17

Sample Description: Diesel standard

Comments: Broodboard Used: 063017B (# 21730)

Chronic Freshwater Toxicity Test
C. dubia Reproduction Data

Client: WDOE
Sample ID: NWTPH-DX (Diesel)
Work Order: 17C516

nominal (mg/L Diesel)

Start Date & Time: July 6/17 @ 1200h
Stop Date & Time: July 13/17 @ 0745h
Set up by: EMM/YYL

Concentration: control lab

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	EMM
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	JW
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	A
4	3	3	4	4	3	3	2	3	3	3	EMM
5	7	7	8	8	6	6	5	✓	6	8	EMM
6	✓	✓	✓	✓	9	✓	✓	8	✓	10	EMM
7	9	10	11	10	✓	9	10	11	13	✓	EMM
8											
Total	19	20	23	22	18	18	17	22	22	21	EMM

Concentration: control acetone

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	EMM
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	JW
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	A
4	3	3	3	4	4	3	2	2	3	3	EMM
5	6	5	✓	8	7	7	6	✓	6	5	EMM
6	8	✓	6	✓	✓	9	✓	3	9	✓	EMM
7	✓	8	9	9	8	✓	9	✓	✓	10	EMM
8											
Total	17	16	18	21	19	19	17	5	18	18	EMM

Concentration: 0.045

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	EMM
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	JW
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	A
4	3	3	2	3	3	3	3	2	3	3	EMM
5	6	6	7	6	7	8	8	7	6	6	EMM
6	✓	✓	✓	✓	✓	10	✓	✓	✓	✓	EMM
7	10	11	9	9	10	✓	9	6	8	8	EMM
8											
Total	19	20	18	18	17	21	20	12	17	17	EMM

Concentration: 0.09

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	EMM
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	JW
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	A
4	3	2	3	4	4	3	3	2	2	2	EMM
5	7	6	7	7	8	7	6	6	7	✓	EMM
6	✓	8	✓	✓	11	10	✓	✓	✓	4	EMM
7	10	✓	8	8	✓	✓	9	11	10	8	EMM
8											
Total	20	16	18	19	23	20	18	19	19	14	EMM

Concentration: 0.19

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	EMM
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	JW
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	A
4	3	3	3	2	2	3	3	2	3	3	EMM
5	6	7	7	8	6	✓	✓	6	7	7	EMM
6	✓	✓	✓	6	✓	6	7	✓	✓	✓	EMM
7	8	9	9	✓	8	9	10	10	9	10	EMM
8											
Total	17	19	19	16	16	18	20	18	19	20	EMM

Concentration: 0.38

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	EMM
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	JW
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	A
4	2	3	3	3	3	4	3	2	2	2	EMM
5	7	6	6	✓	7	3	8	6	6	✓	EMM
6	✓	✓	✓	9	✓	✓	✓	✓	✓	4	EMM
7	✓	✓	7	8	9	10	10	8	7	5	EMM
8											
Total	18	18	16	20	19	17	21	16	15	11	EMM

Concentration: 0.75

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	EMM
2	✓	✓	✓	✓	X	✓	✓	✓	✓	X	JW
3	✓	✓	✓	X	|	✓	X	✓	✓	|	A
4	2	✓	✓	|	|	2	|	3	✓	|	EMM
5	4	6	2	|	|	6	|	4	2	|	EMM
6	✓	3	✓	|	|	✓	|	✓	2	|	EMM
7	8	✓	6	|	|	7	|	7	✓	|	EMM
8											
Total	14	9	8	CX	0X	15	CX	14	4	0X	EMM

Concentration: 1.5

Days	A	B	C	D	E	F	G	H	I	J	Init
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	EMM
2	✓	✓	✓	X	✓	✓	✓	X	X	X	JW
3	X	X	X	|	X	X	✓	|	|	|	A
4	|	|	|	|	|	|	X	|	|	|	EMM
5	|	|	|	|	|	|	|	|	|	|	
6	|	|	|	|	|	|	|	|	|	|	
7	|	|	|	|	|	|	|	|	|	|	
8											
Total	CX	CX	CX	0X	CX	CX	0X	0X	0X	0X	JW

Concentration: 3.0

Days	A	B	C	D	E	F	G	H	I	J	Init
1	X	X	X	X	X	X	X	X	X	X	EMM
2	|	|	|	|	|	|	|	|	|	|	
3	|	|	|	|	|	|	|	|	|	|	
4	|	|	|	|	|	|	|	|	|	|	
5	|	|	|	|	|	|	|	|	|	|	
6	|	|	|	|	|	|	|	|	|	|	
7	|	|	|	|	|	|	|	|	|	|	
8											
Total	CX	CX	CX	CX	CX	CX	CX	CX	CX	CX	EMM

Notes: X = mortality.

Sample Description: See water quality sheet

Comments: Total # Young only based on the first 3 Broods. Fourth and subsequent broods not included in total count.

Reviewed by: JW

Date reviewed: Aug. 2/17

Chronic Freshwater Toxicity Test
C. dubia Reproduction Data

2/2

Client: WSCE
Sample ID: NWTPH-DX (DIESEL)
Work Order: NCS16

nominal (mg/L Diesel)

Start Date & Time: July 6/17 @ 1200 h
Stop Date & Time: July 13/17 @ 0745h
Set up by: tmm/MH

Days	Concentration: 6.0										
	A	B	C	D	E	F	G	H	I	J	Init
1	X	X	X	X	X	X	X	X	X	X	tmm
2	\	\	\	\	\	\	\	\	\	\	
3	\	\	\	\	\	\	\	\	\	\	
4	\	\	\	\	\	\	\	\	\	\	
5	\	\	\	\	\	\	\	\	\	\	
6	\	\	\	\	\	\	\	\	\	\	
7	\	\	\	\	\	\	\	\	\	\	
8	\	\	\	\	\	\	\	\	\	\	
Total	0X	0X	0X	0X	0X	0X	0X	0X	0X	0X	tmm

Days	Concentration:										
	A	B	C	D	E	F	G	H	I	J	Init
1											
2											
3											
4											
5											
6											
7											
8											
Total											

Days	Concentration:										
	A	B	C	D	E	F	G	H	I	J	Init
1											
2											
3											
4											
5											
6											
7											
8											
Total											

Notes: X = mortality.

Sample Description: See water quality sheet
Comments: Total # Young only based on the first 3 Broods. Fourth and subsequent broods not included in total count.

Reviewed by: JOL
Date reviewed: Aug. 2/17

Client: WDOE

W.O.#: 170516

Hardness and Alkalinity Datasheet

	Alkalinity							Hardness			
Sample ID	Subsample Date	Date Measured	Sample Volume (mL)	(mL) 0.02N HCL/H_2SO_4 used to pH 4.5	(mL) of 0.02N HCL/H_2SO_4 used to pH 4.2	Total Alkalinity ($mg/LCaCO_3$)		Sample Volume (mL)	Volume of 0.01M EDTA Used (mL)	Total Hardness ($mg/L\ CaCO_3$)	Technician
(1) NWTPH-DX	may 19/17	may 19/17	—	—	—	—		—	—	—	—
201.1 ferric Ctrl	↓	↓	50	5.0	5.1	98		50	5.0	100	tmm

Notes: (1) Standard, chemical

Reviewed by: JGr Date Reviewed: Aug. 2, 17

CETIS Summary Report

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental**

Batch ID:	17-4821-2249	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Emma Marus
Start Date:	06 Jul-17 12:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	20% Perrier Water
Ending Date:	13 Jul-17 07:45	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	6d 20h	**Source:**	In-House Culture	**Age:**	<8h
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 1h	**Station:**	NWTPH-Dx		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
03-4172-7690	7d Survival Rate	0	>0		NA		Fisher Exact Test
00-4799-0384	7d Survival Rate	0.22	0.34	0.2735	NA		Fisher Exact/Bonferroni-Holm Test
04-4584-0796	Reproduction	0.15	0.22	0.1817	22.6%		Steel Many-One Rank Sum Test
06-8611-0723	Reproduction	0	>0		15.8%		Wilcoxon Rank Sum Two-Sample Test

Point Estimate Summary

Analysis ID	Endpoint	Level	mg/L	95% LCL	95% UCL	TU	Method
05-4281-5112	7d Survival Rate	EC50	0.2322	0.2046	0.2636		Spearman-Kärber
19-2673-4690	Reproduction	IC5	0.1508	0.09271	0.1563		Linear Interpolation (ICPIN)
		IC10	0.1564	0.1291	0.1635		
		IC15	0.1622	0.1478	0.1705		
		IC20	0.1679	0.1565	0.1797		
		IC25	0.1737	0.1632	0.1891		
		IC40	0.1912	0.1794	0.2219		
		IC50	0.203	0.1884	0.2408		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
00-4799-0384	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
03-4172-7690	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
05-4281-5112	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
04-4584-0796	Reproduction	Control Resp	16.8	15 - NL	Yes	Passes Acceptability Criteria
06-8611-0723	Reproduction	Control Resp	16.8	15 - NL	Yes	Passes Acceptability Criteria
	Reproduction	Control Resp	20.2	15 - NL	Yes	Passes Acceptability Criteria
19-2673-4690	Reproduction	Control Resp	16.8	15 - NL	Yes	Passes Acceptability Criteria
04-4584-0796	Reproduction	PMSD	0.2259	0.13 - 0.47	Yes	Passes Acceptability Criteria
06-8611-0723	Reproduction	PMSD	0.1581	0.13 - 0.47	Yes	Passes Acceptability Criteria

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental**

7d Survival Rate Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	10	1	1	1	1	1	0	0	0.0%	0.0%
0	Negative Control	10	1	1	1	1	1	0	0	0.0%	0.0%
0.04		10	1	1	1	1	1	0	0	0.0%	0.0%
0.07		10	1	1	1	1	1	0	0	0.0%	0.0%
0.1		10	1	1	1	1	1	0	0	0.0%	0.0%
0.15		10	1	1	1	1	1	0	0	0.0%	0.0%
0.22		10	0.6	0.2306	0.9694	0	1	0.1633	0.5164	86.07%	40.0%
0.34		10	0	0	0	0	0	0	0		100.0%
0.5		10	0	0	0	0	0	0	0		100.0%
0.75		10	0	0	0	0	0	0	0		100.0%

Reproduction Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	10	16.8	13.68	19.92	5	21	1.381	4.367	25.99%	0.0%
0	Negative Control	10	20.2	18.7	21.7	17	23	0.6633	2.098	10.38%	-20.24%
0.04		10	17.9	16.1	19.7	12	21	0.7951	2.514	14.05%	-6.55%
0.07		10	18.6	16.87	20.33	14	23	0.763	2.413	12.97%	-10.71%
0.1		10	18.2	17.14	19.26	16	20	0.4667	1.476	8.11%	-8.33%
0.15		10	17.1	15.06	19.14	11	21	0.9	2.846	16.64%	-1.79%
0.22		10	6.4	1.823	10.98	0	15	2.023	6.398	99.97%	61.9%
0.34		10	0	0	0	0	0	0	0		100.0%
0.5		10	0	0	0	0	0	0	0		100.0%
0.75		10	0	0	0	0	0	0	0		100.0%

7d Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Solvent Blank	1	1	1	1	1	1	1	1	1	1
0	Negative Control	1	1	1	1	1	1	1	1	1	1
0.04		1	1	1	1	1	1	1	1	1	1
0.07		1	1	1	1	1	1	1	1	1	1
0.1		1	1	1	1	1	1	1	1	1	1
0.15		1	1	1	1	1	1	1	1	1	1
0.22		1	1	1	0	0	1	0	1	1	0
0.34		0	0	0	0	0	0	0	0	0	0
0.5		0	0	0	0	0	0	0	0	0	0
0.75		0	0	0	0	0	0	0	0	0	0

Reproduction Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Solvent Blank	17	16	18	21	19	19	17	5	18	18
0	Negative Control	19	20	23	22	18	18	17	22	22	21
0.04		19	20	18	18	17	21	20	12	17	17
0.07		20	16	18	19	23	20	18	19	19	14
0.1		17	19	19	16	16	18	20	18	19	20
0.15		18	18	16	20	19	17	21	16	15	11
0.22		14	9	8	0	0	15	0	14	4	0
0.34		0	0	0	0	0	0	0	0	0	0
0.5		0	0	0	0	0	0	0	0	0	0
0.75		0	0	0	0	0	0	0	0	0	0

Solvent blank = acetone control

Ceriodaphnia 7-d Survival and Reproduction Test **Nautilus Environmental**

7d Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Solvent Blank	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0	Negative Control	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0.04		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0.07		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0.1		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0.15		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0.22		1/1	1/1	1/1	0/1	0/1	1/1	0/1	1/1	1/1	0/1
0.34		0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
0.5		0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
0.75		0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1

CETIS Analytical Report

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental**

Analysis ID:	05-4281-5112	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:55	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes
Batch ID:	17-4821-2249	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Emma Marus
Start Date:	06 Jul-17 12:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	20% Perrier Water
Ending Date:	13 Jul-17 07:45	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	6d 20h	**Source:**	In-House Culture	**Age:**	<8h
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 1h	**Station:**	NWTPH-Dx		

Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	0.00%	-0.6341	0.02753	0.2322	0.2046	0.2636

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria

7d Survival Rate Summary

			Calculated Variate(A/B)								
C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Solvent Blank	10	1	1	1	0	0	0.0%	0.0%	10	10
0.04		10	1	1	1	0	0	0.0%	0.0%	10	10
0.07		10	1	1	1	0	0	0.0%	0.0%	10	10
0.1		10	1	1	1	0	0	0.0%	0.0%	10	10
0.15		10	1	1	1	0	0	0.0%	0.0%	10	10
0.22		10	0.6	0	1	0.1633	0.5164	86.07%	40.0%	6	10
0.34		10	0	0	0	0	0		100.0%	0	10
0.5		10	0	0	0	0	0		100.0%	0	10
0.75		10	0	0	0	0	0		100.0%	0	10

7d Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Solvent Blank	1	1	1	1	1	1	1	1	1	1
0.04		1	1	1	1	1	1	1	1	1	1
0.07		1	1	1	1	1	1	1	1	1	1
0.1		1	1	1	1	1	1	1	1	1	1
0.15		1	1	1	1	1	1	1	1	1	1
0.22		1	1	1	0	0	1	0	1	1	0
0.34		0	0	0	0	0	0	0	0	0	0
0.5		0	0	0	0	0	0	0	0	0	0
0.75		0	0	0	0	0	0	0	0	0	0

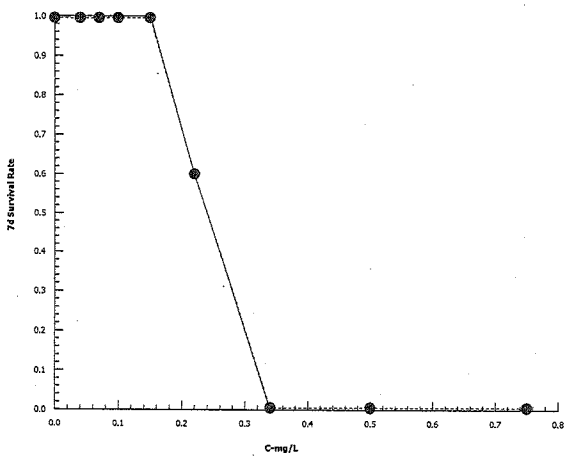
7d Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Solvent Blank	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0	Negative Control	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0.04		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0.07		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0.1		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0.15		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0.22		1/1	1/1	1/1	0/1	0/1	1/1	0/1	1/1	1/1	0/1
0.34		0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
0.5		0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
0.75		0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental**

Analysis ID:	05-4281-5112	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:55	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes

Graphics



Analyst: EMM QA: Jan. 11/18

CETIS Analytical Report

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental**

Analysis ID:	00-4799-0384	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:58	**Analysis:**	STP 2x2 Contingency Tables	**Official Results:**	Yes
Batch ID:	17-4821-2249	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Emma Marus
Start Date:	06 Jul-17 12:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	20% Perrier Water
Ending Date:	13 Jul-17 07:45	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	6d 20h	**Source:**	In-House Culture	**Age:**	<8h
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 1h	**Station:**	NWTPH-Dx		

Data Transform	Zeta	Alt Hyp	Trials	Seed	NOEL	LOEL	TOEL	TU
Untransformed		C > T	NA	NA	0.22	0.34	0.2735	

Fisher Exact/Bonferroni-Holm Test

Control	vs	C-mg/L	Test Stat	P-Value	P-Type	Decision(α:5%)
Solvent Blank		0.04	1	1.0000	Exact	Non-Significant Effect
		0.07	1	1.0000	Exact	Non-Significant Effect
		0.1	1	1.0000	Exact	Non-Significant Effect
		0.15	1	1.0000	Exact	Non-Significant Effect
		0.22	0.04334	0.2167	Exact	Non-Significant Effect
		0.34	5.413E-06	<0.0001	Exact	Significant Effect
		0.5	5.413E-06	<0.0001	Exact	Significant Effect
		0.75	5.413E-06	<0.0001	Exact	Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria

Data Summary

C-mg/L	Control Type	NR	R	NR + R	Prop NR	Prop R	%Effect
0	Solvent Blank	10	0	10	1	0	0.0%
0.04		10	0	10	1	0	0.0%
0.07		10	0	10	1	0	0.0%
0.1		10	0	10	1	0	0.0%
0.15		10	0	10	1	0	0.0%
0.22		6	4	10	0.6	0.4	40.0%
0.34		0	10	10	0	1	100.0%
0.5		0	10	10	0	1	100.0%
0.75		0	10	10	0	1	100.0%

7d Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Solvent Blank	1	1	1	1	1	1	1	1	1	1
0.04		1	1	1	1	1	1	1	1	1	1
0.07		1	1	1	1	1	1	1	1	1	1
0.1		1	1	1	1	1	1	1	1	1	1
0.15		1	1	1	1	1	1	1	1	1	1
0.22		1	1	1	0	0	1	0	1	1	0
0.34		0	0	0	0	0	0	0	0	0	0
0.5		0	0	0	0	0	0	0	0	0	0
0.75		0	0	0	0	0	0	0	0	0	0

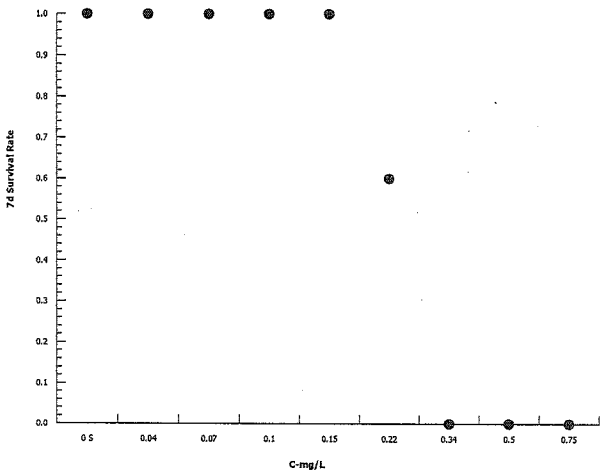
Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental**

Analysis ID:	00-4799-0384	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:58	**Analysis:**	STP 2x2 Contingency Tables	**Official Results:**	Yes

7d Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Solvent Blank	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0	Negative Control	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0.04		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0.07		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0.1		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0.15		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0.22		1/1	1/1	1/1	0/1	0/1	1/1	0/1	1/1	1/1	0/1
0.34		0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
0.5		0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
0.75		0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1

Graphics



Analyst: EWM QA: JGH Jan-4/18

CETIS Analytical Report

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental**

Analysis ID:	03-4172-7690	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:56	**Analysis:**	Single 2x2 Contingency Table	**Official Results:**	Yes
Batch ID:	17-4821-2249	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Emma Marus
Start Date:	06 Jul-17 12:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	20% Perrier Water
Ending Date:	13 Jul-17 07:45	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	6d 20h	**Source:**	In-House Culture	**Age:**	<8h
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 1h	**Station:**	NWTPH-Dx		

Data Transform	Zeta	Alt Hyp	Trials	Seed	Test Result
Untransformed		C > T	NA	NA	Passes 7d survival rate

Fisher Exact Test

Control	vs	Control	Test Stat	P-Value	P-Type	Decision(α:5%)
Solvent Blank		Negative Control	1	1.0000	Exact	Non-Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria

Data Summary

C-mg/L	Control Type	NR	R	NR + R	Prop NR	Prop R	%Effect
0	Solvent Blank	10	0	10	1	0	0.0%
0	Negative Contr	10	0	10	1	0	0.0%

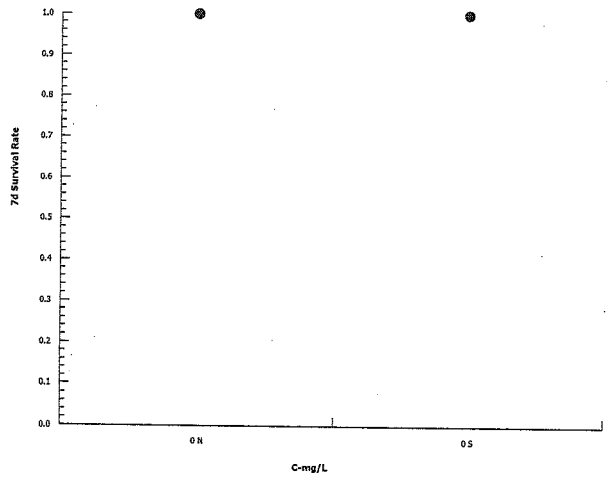
7d Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Solvent Blank	1	1	1	1	1	1	1	1	1	1
0	Negative Control	1	1	1	1	1	1	1	1	1	1

7d Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Solvent Blank	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0	Negative Control	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1

Graphics



Solvent blank = acetone control

Analyst: KJL QA: JGL Jan. 9/18

CETIS Analytical Report

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental**

Analysis ID:	19-2673-4690	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:56	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	17-4821-2249	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Emma Marus
Start Date:	06 Jul-17 12:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	20% Perrier Water
Ending Date:	13 Jul-17 07:45	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	6d 20h	**Source:**	In-House Culture	**Age:**	<8h
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 1h	**Station:**	NWTPH-Dx		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	547535	200	Yes	Two-Point Interpolation

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	16.8	15 - NL	Yes	Passes Acceptability Criteria

Point Estimates

Level	mg/L	95% LCL	95% UCL
IC5	0.1508	0.09271	0.1563
IC10	0.1564	0.1291	0.1635
IC15	0.1622	0.1478	0.1705
IC20	0.1679	0.1565	0.1797
IC25	0.1737	0.1632	0.1891
IC40	0.1912	0.1794	0.2219
IC50	0.203	0.1884	0.2408

Reproduction Summary

			Calculated Variate						
C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	10	16.8	5	21	1.381	4.367	25.99%	0.0%
0.04		10	17.9	12	21	0.7951	2.514	14.05%	-6.55%
0.07		10	18.6	14	23	0.763	2.413	12.97%	-10.71%
0.1		10	18.2	16	20	0.4667	1.476	8.11%	-8.33%
0.15		10	17.1	11	21	0.9	2.846	16.64%	-1.79%
0.22		10	6.4	0	15	2.023	6.398	99.97%	61.9%
0.34		10	0	0	0	0	0		100.0%
0.5		10	0	0	0	0	0		100.0%
0.75		10	0	0	0	0	0		100.0%

Reproduction Detail

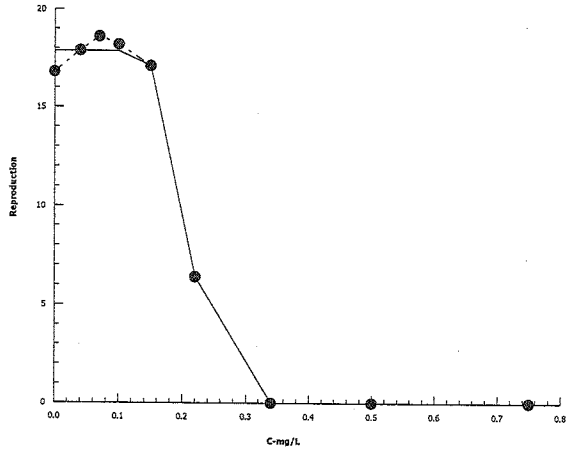
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Solvent Blank	17	16	18	21	19	19	17	5	18	18
0.04		19	20	18	18	17	21	20	12	17	17
0.07		20	16	18	19	23	20	18	19	19	14
0.1		17	19	19	16	16	18	20	18	19	20
0.15		18	18	16	20	19	17	21	16	15	11
0.22		14	9	8	0	0	15	0	14	4	0
0.34		0	0	0	0	0	0	0	0	0	0
0.5		0	0	0	0	0	0	0	0	0	0
0.75		0	0	0	0	0	0	0	0	0	0

Solvent blank = acetone control

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental**

Analysis ID:	19-2673-4690	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:56	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



Analyst: KDL QA: JGL Jan 9/18

CETIS Analytical Report

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental**

Analysis ID:	04-4584-0796	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:58	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	17-4821-2249	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Emma Marus
Start Date:	06 Jul-17 12:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	20% Perrier Water
Ending Date:	13 Jul-17 07:45	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	6d 20h	**Source:**	In-House Culture	**Age:**	<8h
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 1h	**Station:**	NWTPH-Dx		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	22.6%	0.15	0.22	0.1817	

Steel Many-One Rank Sum Test

Control	vs	C-mg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Solvent Blank		0.04	111.5	75	4	18	0.9403	Asymp	Non-Significant Effect
		0.07	120.5	75	3	18	0.9913	Asymp	Non-Significant Effect
		0.1	114	75	4	18	0.9629	Asymp	Non-Significant Effect
		0.15	100.5	75	5	18	0.7129	Asymp	Non-Significant Effect
		0.22*	60	75	0	18	0.0016	Asymp	Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	16.8	15 - NL	Yes	Passes Acceptability Criteria
PMSD	0.2259	0.13 - 0.47	Yes	Passes Acceptability Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1090.533	218.1067	5	15.88	<0.0001	Significant Effect
Error	741.8	13.73704	54			
Total	1832.333		59			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	22.3	15.09	0.0005	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.9315	0.9459	0.0023	Non-normal Distribution

Reproduction Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	10	16.8	13.68	19.92	18	5	21	1.381	25.99%	0.0%
0.04		10	17.9	16.1	19.7	18	12	21	0.7951	14.05%	-6.55%
0.07		10	18.6	16.87	20.33	19	14	23	0.763	12.97%	-10.71%
0.1		10	18.2	17.14	19.26	18.5	16	20	0.4667	8.11%	-8.33%
0.15		10	17.1	15.06	19.14	17.5	11	21	0.9	16.64%	-1.79%
0.22		10	6.4	1.823	10.98	6	0	15	2.023	99.97%	61.9%
0.34		10	0	0	0	0	0	0	0		100.0%
0.5		10	0	0	0	0	0	0	0		100.0%
0.75		10	0	0	0	0	0	0	0		100.0%

Analyst: EMM QA: JGH Jan 11/18

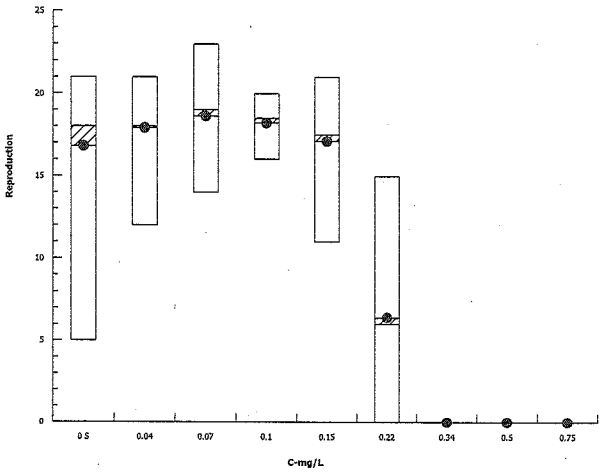
Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental**

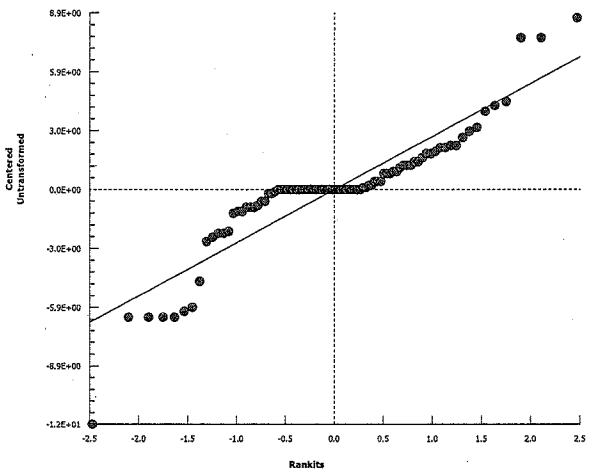
Analysis ID:	04-4584-0796	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:58	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Reproduction Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Solvent Blank	17	16	18	21	19	19	17	5	18	18
0.04		19	20	18	18	17	21	20	12	17	17
0.07		20	16	18	19	23	20	18	19	19	14
0.1		17	19	19	16	16	18	20	18	19	20
0.15		18	18	16	20	19	17	21	16	15	11
0.22		14	9	8	0	0	15	0	14	4	0
0.34		0	0	0	0	0	0	0	0	0	0
0.5		0	0	0	0	0	0	0	0	0	0
0.75		0	0	0	0	0	0	0	0	0	0

Graphics





CETIS Analytical Report

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental**

Analysis ID:	06-8611-0723	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:57	**Analysis:**	Nonparametric-Two Sample	**Official Results:**	Yes

Batch ID:	17-4821-2249	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Emma Marus
Start Date:	06 Jul-17 12:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	20% Perrier Water
Ending Date:	13 Jul-17 07:45	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	6d 20h	**Source:**	In-House Culture	**Age:**	<8h

Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	48d 1h	**Station:**	NWTPH-Dx		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C > T	NA	NA	15.8%	Passes reproduction

Wilcoxon Rank Sum Two-Sample Test

Control	vs	Control	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Solvent Blank		Negative Control	135.5	NA	4	18	0.9924	Exact	Non-Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	16.8	15 - NL	Yes	Passes Acceptability Criteria
Control Resp	20.2	15 - NL	Yes	Passes Acceptability Criteria
PMSD	0.1581	0.13 - 0.47	Yes	Passes Acceptability Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	57.8	57.8	1	4.926	0.0395	Significant Effect
Error	211.2	11.73333	18			
Total	269		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	4.333	6.541	0.0398	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.7662	0.866	0.0003	Non-normal Distribution

Reproduction Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	10	16.8	13.68	19.92	18	5	21	1.381	25.99%	0.0%
0	Negative Control	10	20.2	18.7	21.7	20.5	17	23	0.6633	10.38%	-20.24%

Reproduction Detail

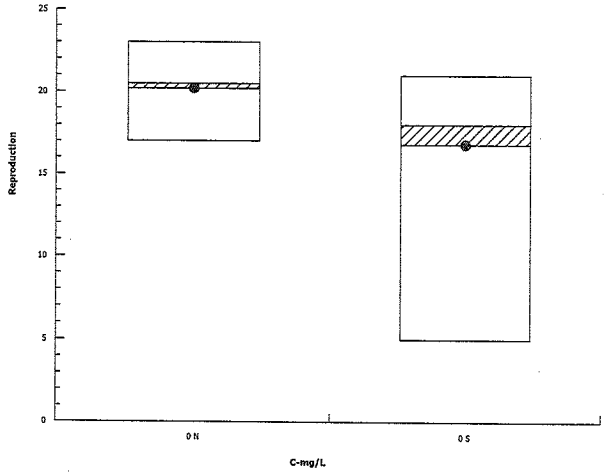
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Solvent Blank	17	16	18	21	19	19	17	5	18	18
0	Negative Control	19	20	23	22	18	18	17	22	22	21

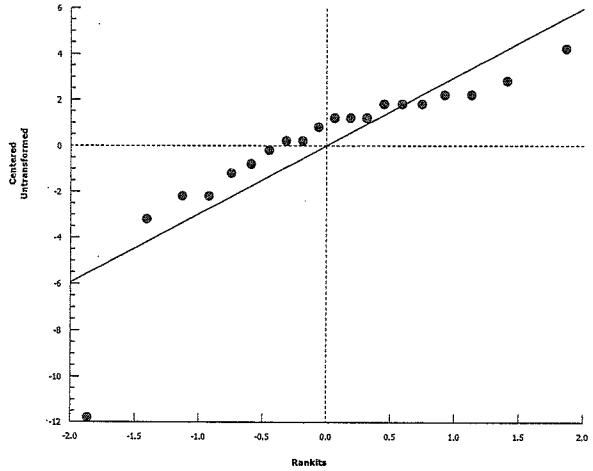
solvent blank = acetone control

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental**

Analysis ID:	06-8611-0723	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 10:57	**Analysis:**	Nonparametric-Two Sample	**Official Results:**	Yes

Graphics





APPENDIX D – Echinoderm Toxicity Test Data

Echinoderm Fertilization Test Summary Sheet

Client: WDOE

Start Date/Time: 7-July-17 @ 1600h

Work Order No.: 170514b

Test Species: *S. purpuratus*

Sample Information:

Sample ID: NWTPH-Dx
Sample Date: 19-May-17
Date Received: 19-May-17
Sample Volume: 50 x 5 mL

Dilution Water:

Type: Natural Seawater
Source: Vancouver Aquarium

Test Organism Information:

Batch No.: 7-Jul-17
Source: Nautilus Environmental San Diego
Sperm:Egg Ratio: 2000 : 1
Exposure Period: 20 min : 20 min

Reference Toxicant Results:

Reference Toxicant ID: SpUS05
Stock Solution ID: 17Cu02
Date Initiated: 7-Jul-17
IC50 (95% CL): 16.4 (15.2 - 17.8) ug/L Cu

Reference Toxicant Mean ± 2 SD: 18.9 (7.7 - 46.0) ug/L Cu
Reference Toxicant CV (%): 56

Test Results:

mg/L	Fertilization Rate
NOEC	<0.05
LOEC	0.05
IC25 (mg/L) (95% CL)	0.2 (0.1 - 0.2)
IC50 (mg/L) (95% CL)	0.3 (0.3 - 0.4)

Reviewed by: JOL

Date reviewed: Sep. 19/17

Echinoderm Fertilization WQ Data Sheet

Client : WDOE

Analysts: JW

Sample ID: NWTPH-Dx

Test Date & Time: July 6/17 @ 1600h (7 JW)

WO No: 170514

Test Species: *S. purpuratus*

Initial Water Quality (JW)

	100% sample
Temp (°C)	
DO (mg/L)	
pH	
Salinity	

Brine/Salt Adjusted Water Quality (JW)

	% sample
Temp (°C)	
DO (mg/L)	
pH	
Salinity	

Concentration ~~% (v/v)~~ mg/L JW	Water Quality			
	Temp. (°C)	DO (mg/L)	pH	Salinity (ppt)
seawater control	13.0	8.2	7.8	28
Acetone control	13.0	8.0	7.0	28
0.5	13.0	8.1	7.8	28
1	13.0	8.0	7.8	28
2	13.0	7.9	7.8	28
4	13.0	7.8	7.8	28
8	13.0	7.8	7.8	28
16	13.0	7.6	7.8	28
Analyst Initials	JW	JW	JW	JW

Thermometer: CER # 2 **DO meter:** 2 **pH meter:** 1 **Salinity:** 2

Sample Description: Diesel Standards.

Echinoderm Source: Nautilus San Diego **Date Received:** July 6/17 (7 JW)

Seawater Source: Vancouver Aquarium **Date Collected:** July 5/17

Comments:

Reviewed: JW **Date Reviewed:** Aug. 2/17

Echinoderm Fertilization Toxicity Test Data Sheet
Fertilized Egg Counts

Client: WDOE

Work Order #: 170514

Sample ID: NWTPH-Dx

Start Date/Time: July 6/17 @ 1600h (7 JW)

Test species: *S. purpuratus*

Test set up by: JW

Test Duration: 20min : 20min

mg/L	Rep	No. Fertilized Eggs	No. Unfertilized Eggs	Comments	Initials
Seawater Control	A	75	25		JW
	B	77	23		↓
	C	81	19		
	D	79	21		
Acetone Control	A	72	28		
	B	72	28		
	C	72	28		
	D	74	26		
0.5	A	64	36		
	B	72	28		
	C	60	40		
	D	63	37		
1	A	59	41		
	B	59	41		
	C	60	40		
	D	54	46		
2	A	56	JW ~~46~~ 44		
	B	55	45		
	C	60	40		
	D	64	36		
4	A	58	42		
	B	50	50		
	C	60	40		
	D	56	44 ~~46~~ JW		
8	A	31	69		
	B	38	62		
	C	40	60		
	D	40	60		
16	A	JW ~~87~~ 13	JW ~~13~~ 87		
	B	↓ ~~84~~ 16	↓ ~~16~~ 84		
	C	↓ ~~8~~ 12	88		
	D	8	92		↓
		A 0	100		
		B 0	100		
		C 0	100		
		D 0	100		

Comments: EGG CONTROL

Reviewed by: JBH

Date Reviewed: Aug. 2/17

CETIS Summary Report

Purple Sea Urchin Sperm Cell Fertilization Test — **Nautilus Environmental**

Batch ID:	18-1547-6078	**Test Type:**	Fertilization	**Analyst:**	Jeslin Wijaya
Start Date:	07 Jul-17 16:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural seawater
Ending Date:	07 Jul-17 16:40	**Species:**	Strongylocentrotus purpuratus	**Brine:**	
Duration:	40m (20:20)	**Source:**	San Diego Lab	**Age:**	
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	49d 5h	**Station:**	NWTPH-Dx		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
08-5894-3439	Fertilization Rate	<0	0		3.84%		Paired Sample t Test
17-9317-6247	Fertilization Rate	<0.05	0.05	NA	9.18%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	mg/L	95% LCL	95% UCL	TU	Method
14-6104-0145	Fertilization Rate	IC5	0.02308	0.01095	0.06835		Linear Interpolation (ICPIN)
		IC10	0.0467	0.02196	0.06441		
		IC15	0.05976	0.0397	0.06918		
		IC20	0.1208	0.03378	0.2265		
		IC25	0.1923	0.124	0.2221		
		IC40	0.278	0.2449	0.3095		
		IC50	0.3405	0.2928	0.3833		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
08-5894-3439	Fertilization Rate	Control Resp	0.725	0.7 - NL	Yes	Passes Acceptability Criteria
	Fertilization Rate	Control Resp	0.78	0.7 - NL	Yes	Passes Acceptability Criteria
14-6104-0145	Fertilization Rate	Control Resp	0.725	0.7 - NL	Yes	Passes Acceptability Criteria
17-9317-6247	Fertilization Rate	Control Resp	0.725	0.7 - NL	Yes	Passes Acceptability Criteria
08-5894-3439	Fertilization Rate	PMSD	0.03838	NL - 0.25	No	Passes Acceptability Criteria
17-9317-6247	Fertilization Rate	PMSD	0.09176	NL - 0.25	No	Passes Acceptability Criteria

Fertilization Rate Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	4	0.725	0.7091	0.7409	0.72	0.74	0.004999	0.009999	1.38%	0.0%
0	Negative Control	4	0.78	0.7389	0.8211	0.75	0.81	0.01291	0.02582	3.31%	-7.59%
0.05 ✓		4	0.6475	0.566	0.729	0.6	0.72	0.02562	0.05123	7.91%	10.69%
0.07 ✓		4	0.58	0.5369	0.6231	0.54	0.6	0.01354	0.02708	4.67%	20.0%
0.11 ✓		4	0.5875	0.5221	0.6529	0.55	0.64	0.02056	0.04113	7.0%	18.97%
0.18 ✓		4	0.56	0.4913	0.6287	0.5	0.6	0.0216	0.0432	7.72%	22.76%
0.33 ✓		4	0.3725	0.3045	0.4405	0.31	0.4	0.02136	0.04272	11.47%	48.62%
0.62 ✓		4	0.1225	0.06993	0.1751	0.08	0.16	0.01652	0.03304	26.97%	83.1%

Fertilization Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	0.72	0.72	0.72	0.74
0	Negative Control	0.75	0.77	0.81	0.79
0.05		0.64	0.72	0.6	0.63
0.07		0.59	0.59	0.6	0.54
0.11		0.56	0.55	0.6	0.64
0.18		0.58	0.5	0.6	0.56
0.33		0.31	0.38	0.4	0.4
0.62		0.13	0.16	0.12	0.08

Purple Sea Urchin Sperm Cell Fertilization Test **Nautilus Environmental**

Fertilization Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	72/100	72/100	72/100	74/100
0	Negative Control	75/100	77/100	81/100	79/100
0.05		64/100	72/100	60/100	63/100
0.07		59/100	59/100	60/100	54/100
0.11		56/100	55/100	60/100	64/100
0.18		58/100	50/100	60/100	56/100
0.33		31/100	38/100	40/100	40/100
0.62		13/100	16/100	12/100	8/100

CETIS Analytical Report

Test Code: 170514b | 03-4856-7405

Purple Sea Urchin Sperm Cell Fertilization Test — **Nautilus Environmental**

Analysis ID:	14-6104-0145	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Sep-17 9:07	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	18-1547-6078	**Test Type:**	Fertilization	**Analyst:**	Jeslin Wijaya
Start Date:	07 Jul-17 16:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural seawater
Ending Date:	07 Jul-17 16:40	**Species:**	Strongylocentrotus purpuratus	**Brine:**	
Duration:	40m (20:20)	**Source:**	San Diego Lab	**Age:**	
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	49d 5h	**Station:**	NWTPH-Dx		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	63407	200	Yes	Two-Point Interpolation

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	0.725	0.7 - NL	Yes	Passes Acceptability Criteria

Point Estimates

Level	mg/L	95% LCL	95% UCL
IC5	0.02308	0.01095	0.06835
IC10	0.0467	0.02196	0.06441
IC15	0.05976	0.0397	0.06918
IC20	0.1208	0.03378	0.2265
IC25	0.1923	0.124	0.2221
IC40	0.278	0.2449	0.3095
IC50	0.3405	0.2928	0.3833

Fertilization Rate Summary

			Calculated Variate(A/B)								
C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Solvent Blank	4	0.725	0.72	0.74	0.004999	0.009999	1.38%	0.0%	290	400
0.05		4	0.6475	0.6	0.72	0.02562	0.05123	7.91%	10.69%	259	400
0.07		4	0.58	0.54	0.6	0.01354	0.02708	4.67%	20.0%	230	400
0.11		4	0.5875	0.55	0.64	0.02056	0.04113	7.0%	18.97%	235	400
0.18		4	0.56	0.5	0.6	0.0216	0.0432	7.72%	22.76%	224	400
0.33		4	0.3725	0.31	0.4	0.02136	0.04272	11.47%	48.62%	149	400
0.62		4	0.1225	0.08	0.16	0.01652	0.03304	26.97%	83.1%	49	400

Fertilization Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	0.72	0.72	0.72	0.74
0.05		0.64	0.72	0.6	0.63
0.07		0.59	0.59	0.6	0.54
0.11		0.56	0.55	0.6	0.64
0.18		0.58	0.5	0.6	0.56
0.33		0.31	0.38	0.4	0.4
0.62		0.13	0.16	0.12	0.08

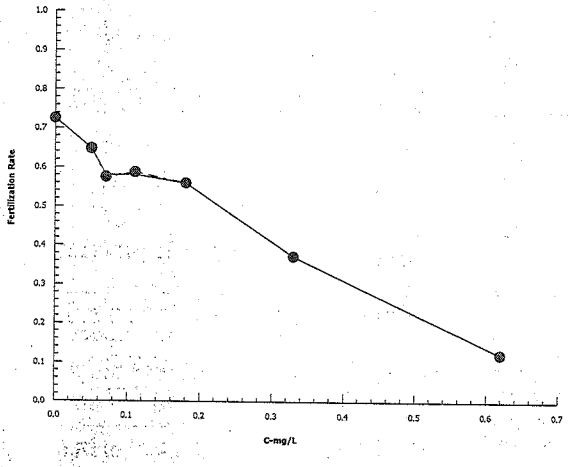
Purple Sea Urchin Sperm Cell Fertilization Test — **Nautilus Environmental**

Analysis ID:	14-6104-0145	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Sep-17 9:07	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Fertilization Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	72/100	72/100	72/100	74/100
0	Negative Control	75/100	77/100	81/100	79/100
0.05		64/100	72/100	60/100	63/100
0.07		59/100	59/100	60/100	54/100
0.11		56/100	55/100	60/100	64/100
0.18		58/100	50/100	60/100	56/100
0.33		31/100	38/100	40/100	40/100
0.62		13/100	16/100	12/100	8/100

Graphics



Analyst: AMM QA: JCL 9/19/17

CETIS Analytical Report

Test Code: 170514b | 03-4856-7405

Purple Sea Urchin Sperm Cell Fertilization Test — **Nautilus Environmental**

Analysis ID:	17-9317-6247	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Sep-17 9:08	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	18-1547-6078	**Test Type:**	Fertilization	**Analyst:**	Jeslin Wijaya
Start Date:	07 Jul-17 16:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural seawater
Ending Date:	07 Jul-17 16:40	**Species:**	Strongylocentrotus purpuratus	**Brine:**	
Duration:	40m (20:20)	**Source:**	San Diego Lab	**Age:**	
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	49d 5h	**Station:**	NWTPH-Dx		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	9.18%	<0.05	0.05	NA	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Solvent Blank		0.05*	2.814	2.448	0.072	6	0.0238	CDF	Significant Effect
		0.07*	5.189	2.448	0.072	6	0.0001	CDF	Significant Effect
		0.11*	4.925	2.448	0.072	6	0.0002	CDF	Significant Effect
		0.18*	5.872	2.448	0.072	6	<0.0001	CDF	Significant Effect
		0.33*	12.3	2.448	0.072	6	<0.0001	CDF	Significant Effect
		0.62*	22.51	2.448	0.072	6	<0.0001	CDF	Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	0.725	0.7 - NL	Yes	Passes Acceptability Criteria
PMSD	0.09176	NL - 0.25	No	Passes Acceptability Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.185588	0.197598	6	113.6	<0.0001	Significant Effect
Error	0.03651587	0.001738851	21			
Total	1.222104		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.013	16.81	0.4217	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9729	0.8975	0.6591	Normal Distribution

Fertilization Rate Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	4	0.725	0.7091	0.7409	0.72	0.72	0.74	0.004999	1.38%	0.0%
0.05		4	0.6475	0.566	0.729	0.635	0.6	0.72	0.02562	7.91%	10.69%
0.07		4	0.58	0.5369	0.6231	0.59	0.54	0.6	0.01354	4.67%	20.0%
0.11		4	0.5875	0.5221	0.6529	0.58	0.55	0.64	0.02056	7.0%	18.97%
0.18		4	0.56	0.4913	0.6287	0.57	0.5	0.6	0.0216	7.72%	22.76%
0.33		4	0.3725	0.3045	0.4405	0.39	0.31	0.4	0.02136	11.47%	48.62%
0.62		4	0.1225	0.06993	0.1751	0.125	0.08	0.16	0.01652	26.97%	83.1%

Angular (Corrected) Transformed Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	4	1.019	1.001	1.037	1.013	1.013	1.036	0.005631	1.11%	0.0%
0.05		4	0.9359	0.8492	1.022	0.9221	0.8861	1.013	0.02722	5.82%	8.14%
0.07		4	0.8658	0.8223	0.9093	0.8759	0.8254	0.8861	0.01367	3.16%	15.02%
0.11		4	0.8736	0.8068	0.9403	0.8658	0.8355	0.9273	0.02097	4.8%	14.25%
0.18		4	0.8457	0.7765	0.9149	0.8556	0.7854	0.8861	0.02173	5.14%	16.99%
0.33		4	0.656	0.5848	0.7272	0.6745	0.5905	0.6847	0.02237	6.82%	35.61%
0.62		4	0.3552	0.2728	0.4376	0.3613	0.2868	0.4115	0.02589	14.58%	65.13%

Analyst: EMM QA: 10/17

Purple Sea Urchin Sperm Cell Fertilization Test — **Nautilus Environmental**

Analysis ID:	17-9317-6247	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Sep-17 9:08	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Fertilization Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	0.72	0.72	0.72	0.74
0.05		0.64	0.72	0.6	0.63
0.07		0.59	0.59	0.6	0.54
0.11		0.56	0.55	0.6	0.64
0.18		0.58	0.5	0.6	0.56
0.33		0.31	0.38	0.4	0.4
0.62		0.13	0.16	0.12	0.08

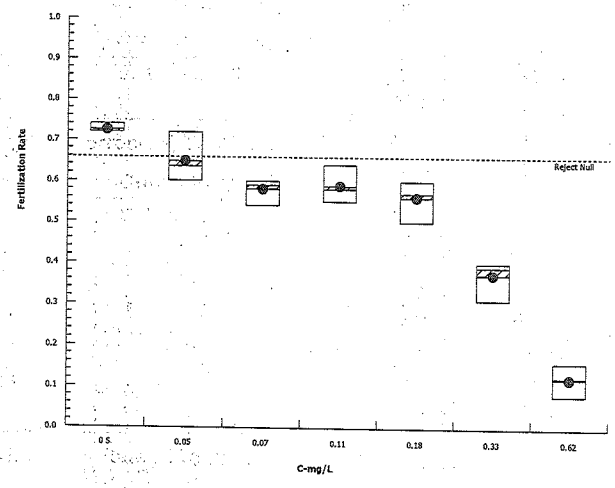
Angular (Corrected) Transformed Detail

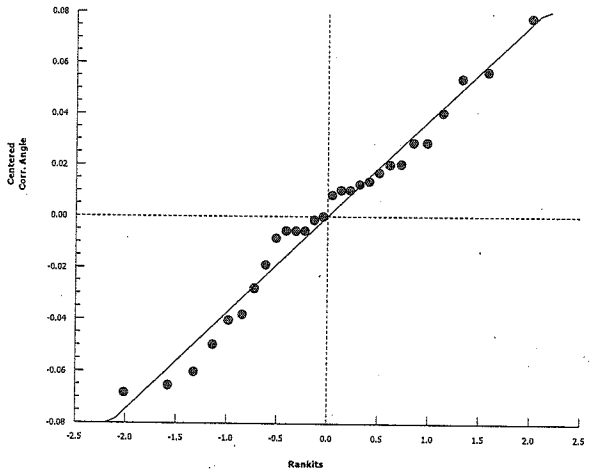
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	1.013	1.013	1.013	1.036
0.05		0.9273	1.013	0.8861	0.9169
0.07		0.8759	0.8759	0.8861	0.8254
0.11		0.8455	0.8355	0.8861	0.9273
0.18		0.8657	0.7854	0.8861	0.8455
0.33		0.5905	0.6642	0.6847	0.6847
0.62		0.3689	0.4115	0.3537	0.2868

Fertilization Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	72/100	72/100	72/100	74/100
0	Negative Control	75/100	77/100	81/100	79/100
0.05		64/100	72/100	60/100	63/100
0.07		59/100	59/100	60/100	54/100
0.11		56/100	55/100	60/100	64/100
0.18		58/100	50/100	60/100	56/100
0.33		31/100	38/100	40/100	40/100
0.62		13/100	16/100	12/100	8/100

Graphics





Purple Sea Urchin Sperm Cell Fertilization Test — **Nautilus Environmental**

Analysis ID:	08-5894-3439	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Sep-17 9:08	**Analysis:**	Parametric-Paired Sample	**Official Results:**	Yes
Batch ID:	18-1547-6078	**Test Type:**	Fertilization	**Analyst:**	Jeslin Wijaya
Start Date:	07 Jul-17 16:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural seawater
Ending Date:	07 Jul-17 16:40	**Species:**	Strongylocentrotus purpuratus	**Brine:**	
Duration:	40m (30:30)	**Source:**	San Diego Lab	**Age:**	
Sample ID:	07-6142-9744	**Code:**	2D627EF0	**Client:**	WDOE
Sample Date:	19 May-17 11:15	**Material:**	Diesel	**Project:**	
Receive Date:	19 May-17 11:15	**Source:**	WDOE		
Sample Age:	49d 5h	**Station:**	NWTPH-Dx		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C > T	NA	NA	3.84%	Fails fertilization rate

Paired Sample t Test

Control	vs	Control	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		Solvent Blank	4.222	2.353	0.036	3	0.0121	CDF	Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	0.725	0.7 - NL	Yes	Passes Acceptability Criteria
Control Resp	0.78	0.7 - NL	Yes	Passes Acceptability Criteria
PMSD	0.03838	NL - 0.25	No	Passes Acceptability Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.008257935	0.008257935	1	14.99	0.0083	Significant Effect
Error	0.003306119	0.0005510199	6			
Total	0.01156405		7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	7.686	47.47	0.1280	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9563	0.6451	0.7746	Normal Distribution

Fertilization Rate Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	4	0.725	0.7091	0.7409	0.72	0.72	0.74	0.004999	1.38%	0.0%
0	Negative Control	4	0.78	0.7389	0.8211	0.78	0.75	0.81	0.01291	3.31%	-7.59%

Angular (Corrected) Transformed Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	4	1.019	1.001	1.037	1.013	1.013	1.036	0.005631	1.11%	0.0%
0	Negative Contr	4	1.083	1.033	1.133	1.083	1.047	1.12	0.01561	2.88%	-6.31%

Fertilization Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	0.72	0.72	0.72	0.74
0	Negative Control	0.75	0.77	0.81	0.79

Angular (Corrected) Transformed Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	1.013	1.013	1.013	1.036
0	Negative Control	1.047	1.071	1.12	1.095

Purple Sea Urchin Sperm Cell Fertilization Test

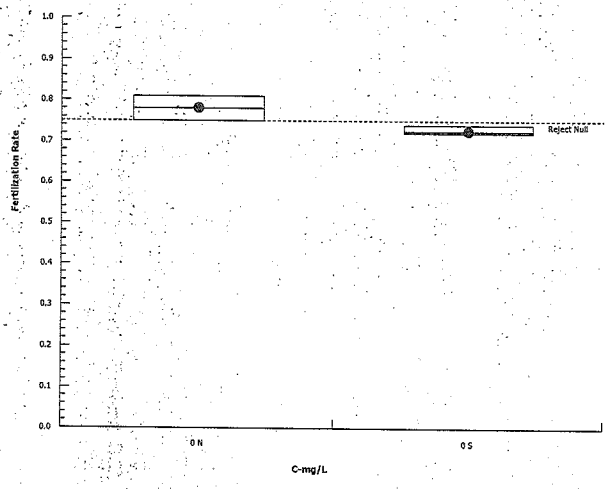
Nautilus Environmental

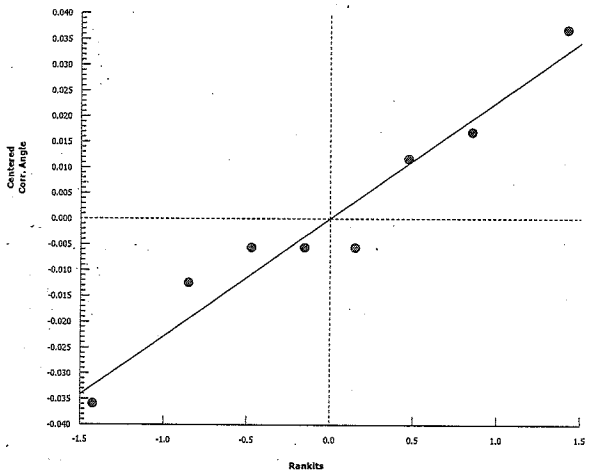
Analysis ID: 08-5894-3439 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 19 Sep-17 9:08 **Analysis:** Parametric-Paired Sample **Official Results:** Yes

Fertilization Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Solvent Blank	72/100	72/100	72/100	74/100
0	Negative Control	75/100	77/100	81/100	79/100

Graphics





APPENDIX E – Nominal and Measured Concentrations, Percent Difference

Table 1 (E-1). Fathead Minnow Nominal and Measured Concentrations with Percent Difference (mg diesel/L).

Nominal (mg/L)	Measured (mg/L)																			
	t=0		t=24				t=48		t=72		t=96				t=120		t=144		t=168	
	F	%D	S	%D	F	%D	F	%D	F	%D	S	%D	F	%D	F	%D	F	%D	S	%D
30	3.03	90%	0.74	98%	3.26	89%	1.75	94%	1.79	94%	0.59	98%	2.86	90%	2.72	91%	3.68	88%	1.37	95%
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7.5	0.29	96%	0.18	98%	0.48	94%	0.37	95%	0.42	94%	0.19	97%	0.57	92%	0.62	92%	1.05	86%	0.42	94%
7.5 dupl.	0.49	93%	-	-	x	-	-	-	0.43	94%	-	-	0.54	93%	-	-	1.05	86%	-	-
3.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1.9	0.13	93%	0.06	97%	0.12	94%	0.13	93%	0.18	91%	0.18	91%	0.17	91%	0.14	93%	0.21	89%	0.28	85%
0.9	0.08	91%	0.06	93%	0.11	88%	0.14	84%	0.10	89%	0.15	83%	0.10	89%	0.10	89%	0.12	87%	0.29	68%

F = fresh, S= Stale, t = time, %D = percent difference

Table 2 (E-2). Topsmelt and Echinoderm Nominal and Measured Concentrations with Percent Difference (mg diesel/L).

Nominal (mg/L)	Measured (mg/L)																			
	t=0		t=24				t=48		t=72		t=96				t=120		t=144		t=168	
	F	%D	S	%D	F	%D	F	%D	F	%D	S	%D	F	%D	F	%D	F	%D	S	%D
16	0.62	96%	0.58	96%	2.02	87%	3.31	79%	0.96	94%	x	-	x	-	x	-	x	-	x	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	0.07	98%	0.14	97%	0.38	91%	0.31	92%	0.32	92%	0.15	96%	0.17	96%	0.20	95%	0.29	93%	0.20	95%
4 dupl.	0.29	93%	-	-	0.40	90%	-	-	0.31	92%	-	-	0.14	97%	-	-	0.32	92%	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	0.07	93%	0.05	95%	0.13	87%	0.10	90%	0.11	89%	0.16	84%	0.11	89%	0.18	82%	0.18	82%	0.16	84%
0.5	0.05	90%	0.05	90%	0.07	86%	0.06	88%	0.06	88%	0.12	76%	0.13	74%	0.14	72%	0.13	74%	0.17	66%

F = fresh, S= Stale, t = time, %D = percent difference

Table 3 (E-3). *Ceriodaphnia dubia* Nominal and Measured Concentrations with Percent Difference (mg diesel/L).

Nominal (mg/L)	Measured (mg/L)													
	t=0		t=24		t=48		t=72		t=96		t=120		t=144	
	F	%D	F	%D	F	%D	F	%D	F	%D	F	%D	F	%D
6	1.19	80%	x	-	x	-	x	-	x	-	x	-	x	-
3	0.55	82%	x	-	x	-	x	-	x	-	x	-	x	-
3 dupl.	0.24	92%	x	-	x	-	x	-	x	-	x	-	x	-
1.5	0.29	81%	1.22	19%	0.36	76%	0.53	65%	x	-	x	-	x	-
0.75	0.11	85%	0.52	31%	0.23	69%	0.15	80%	0.40	47%	0.19	75%	0.42	44%
0.38	0.08	79%	0.24	37%	0.14	63%	0.14	63%	0.21	45%	0.10	74%	0.21	45%
0.19	0.05	74%	-	-	0.08	58%	-	-	0.12	37%	-	-	0.14	26%
0.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.045	-	-	-	-	-	-	-	-	-	-	-	-	-	-

F = fresh, t = time, %D = percent difference

APPENDIX F Chain-of-Custody Form

NAUTILUS ENVIRONMENTAL

TESTING LOCATION (Please Circle)

Burnaby
8664 Commerce Court
Burnaby, British Columbia, Canada
V5A 4N7
Phone 604.420.8773

Calgary
#4, 6125 12 Street SE
Calgary, Alberta, Canada
T2H 2K1
Phone 403.253.7121

Chain of Custody

Date May 8 Page 1 of 1

Report to:		**Invoice To:**	
Company	Washington Department of Ecology	Company	Department of Ecology
Address	P.O. Box 47600	Address	Accounts Payable
City/Prov/PC	Olympia, WA	City/Prov/PC	P.O. Box 47612
Contact	William Hobbs	Contact	Olympia WA 98504-7612
Phone	360-407-7512	Phone	JW Dianne Schieck
Email	whob461@ecy.wa.gov	Email	360 306-407-7020/disc461@
		PO No.	#17-31239 ecy.wa.gov

Sample Collection By: J. John Weakland ordered Sample Type: Grab ○ OR Composite ○

	SAMPLE ID	DATE (DD/MM/YY)	TIME	MATRIX	# OF CONTAINERS AND VOLUME (e.g. 1 x 20 L)	COMMENTS	ANALYSES REQUIRED: Fathead minnow 7d	Echinoderm fertilization	Fathead	Topsmelt 7 day	Ceriodaphnia repro.							Receipt Temperature (°C)
1	NWTPH-Gx	05/04/17	11:26	methanol	gasoline standards (50 x 5 mL)	- use USEPA protocols	X	X		X	X							
2	NWTPH-Dx	19/05/17	11:15	acetone	diesel standards (50 x 5 mL)	- use USEPA protocols	X	X		X	X							
3																		
4																		
5																		
6																		
7																		
8																		
9																		
10																		

WO 170513 170514 170515 170516

Transferred to 2 different separate COCs

SPECIAL INSTRUCTIONS/COMMENTS (CLIENT)	SAMPLE RECEIPT DETAILS (LABORATORY)			
	1. Total No. of Containers		4. Ice Present in Cooler?	Y / N
	2. Courier		5. Seal Present?	Y / N
	3. Good Condition?	Y / N	6. Initials Present on Seal?	Y / N

RELINQUISHED BY (CLIENT)	RECEIVED BY (LABORATORY)
(Printed Name) (Signature)	(Printed Name) (Signature)
(Company) (Date DD/MM/YY and Time)	(Company) (Date DD/MM/YY and Time)

SAMPLE DESCRIPTION AND COMMENTS (LABORATORY)

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

END OF REPORT