

June 12, 2020

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**Subject: Results of Analytical Data for Experiment #16**

Mr. Bittner/ Dr. Belovsky:

Below is a summary of the analytical data for the short-term chronic brine shrimp experiment initiated on May 21, 2020. Total zinc samples were collected in new solutions at test initiation and on day 6. Total zinc samples were also collected in old solutions on day 1.

**Characterization of Recon Water**

Sample No.	pH	Hard. (mg/L) <sup>a</sup>	Alk. (mg/L) <sup>a</sup>	Spec. Cond. (µS/cm)	TRC (mg/L) <sup>b</sup>	NH <sub>3</sub> -N (mg/L)	Salinity (ppt)
RW#13937	7.9	NM	NM	136,100	NM	NM	120

<sup>a</sup>As CaCO<sub>3</sub>

<sup>b</sup>Total residual chlorine

**Results of Zinc Analysis**

Nominal Value (mg/L)	Total Zinc (mg/L)			Mean Value	Percent of Nominal
	Day 0 New Solution	Day 6 New Solution	Day 1 Old Solution		
0	0.021 U	--	--	0.02	---
12.5	--	--	--	--	--
25	--	--	--	--	--
50	52.2	56.2	54.6	54.3	109
100	106	110	118	111.3	111
200	214	230	198	214	107

U= below method detection limit (0.021 mg/L)

Average measured zinc concentrations were then used to recalculate test survival endpoints on a measured basis. Due to the large reduction in growth seen in all treatments, zinc analysis was not run for treatments that were not needed for calculating survival end points. Therefore, nominal values were used for the lower treatments since they did not impact the test endpoints.

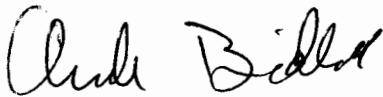
**Test Endpoints**

<b>Basis</b>	<b>Survival NOEC</b>	<b>Survival IC20</b>	<b>Growth NOEC</b>	<b>Growth IC20</b>
Nominal	50	60.65 (45.53-71.92)	<12.5	<12.5 (3.082 (2.8-3.515))
Measured	54.3	66.44 (50.98-78.63)	--	--

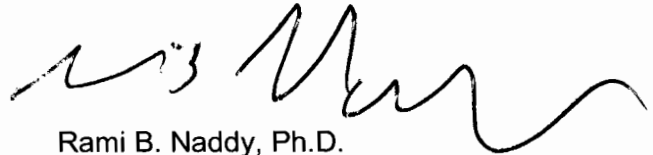
Because the growth endpoints were lower than the lowest zinc concentration tested, a second short-term chronic study will be conducted at lower concentrations to try and obtain more precise growth endpoints.

We greatly appreciate the opportunity to complete this study for you. Please do not hesitate to call if you have any questions or concerns.

Sincerely,



Amanda Bidlack  
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Rami B. Naddy, Ph.D.  
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17001-474-071

Attachment

cc: David Pillard, TRE

474-071

CA rev 6/12/10

Zinc Chronic Original						
Nominal Value (mg)	Day 0 New (mg)	Day 1 Old (mg)	Day 6 New (mg)	Mean (mg)	Percent of Nominal	
0	.021 U			0.02	100%	
12.5				#DIV/0!	#DIV/0!	
25				#DIV/0!	#DIV/0!	
50	52.2	54.6	56.2	54.33	108.67%	
100	106	118	110	111.33	111.33%	
200	214.0	198	230	214.00	107.00%	

**CETIS Analytical Report**

Report Date: 10 Jun-20 13:34 (p 1 of 2)  
 Test Code: 474-071 | 01-6129-1574

*Brine shrimp*

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

TRE Environmental Strategies

<b>Analysis ID:</b> 00-4684-6428	<b>Endpoint:</b> 7d Survival Rate	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 10 Jun-20 13:34	<b>Analysis:</b> Parametric-Control vs Treatments	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-1256-8166	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b> Lab Tech
<b>Start Date:</b> 21 May-20 13:30	<b>Protocol:</b> EPA/821/R-02-013 (2002)	<b>Diluent:</b> rGSL
<b>Ending Date:</b> 28 May-20 13:20	<b>Species:</b> Artemia franciscana	<b>Brine:</b> Crystal Sea
<b>Duration:</b> 7d	<b>Source:</b> In-House Culture	<b>Age:</b> 48h
<b>Sample ID:</b> 17-3344-4662	<b>Code:</b> 67524436	<b>Client:</b> Notre Dame
<b>Sample Date:</b> 21 May-20 10:30	<b>Material:</b> Zinc sulfate	<b>Project:</b> Special Studies
<b>Receive Date:</b> 21 May-20 13:30	<b>Source:</b> research	
<b>Sample Age:</b> 3h	<b>Station:</b>	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	16.4%	54.3	111.3	77.74	

**Dunnett Multiple Comparison Test**

Control	vs C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
0.021	12.5	1.1	2.41	0.245	6	0.3787	CDF	Non-Significant Effect
0.021	25	2.135	2.41	0.245	6	0.0823	CDF	Non-Significant Effect
0.021	54.3	1.151	2.41	0.245	6	0.3569	CDF	Non-Significant Effect
0.021	111.3*	6.027	2.41	0.245	6	<0.0001	CDF	Significant Effect
0.021	214*	10.79	2.41	0.245	6	<0.0001	CDF	Significant Effect

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	3.501526	0.7003053	5	33.94	<0.0001	Significant Effect
Error	0.3714243	0.02063468	18			
Total	3.872951		23			

**Distributional Tests**

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.943	15.1	0.8570	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.939	0.884	0.1546	Normal Distribution

**7d Survival Rate Summary**

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.021	Dilution Water	4	0.975	0.8954	1	1	0.9	1	0.025	5.13%	0.0%
12.5		4	0.9	0.7163	1	0.9	0.8	1	0.05774	12.8%	7.69%
25		4	0.825	0.6248	1	0.8	0.7	1	0.06292	15.3%	15.4%
54.3		4	0.9	0.7701	1	0.9	0.8	1	0.04082	9.07%	7.69%
111.3		4	0.475	0.2748	0.6752	0.5	0.3	0.6	0.06292	26.5%	51.3%
214		4	0.075	0	0.2273	0.05	0	0.2	0.04787	128.0%	92.3%

**Angular (Corrected) Transformed Summary**

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.021	Dilution Water	4	1.371	1.242	1.501	1.412	1.249	1.412	0.04074	5.94%	0.0%
12.5		4	1.26	0.9795	1.54	1.26	1.107	1.412	0.08801	14.0%	8.15%
25		4	1.154	0.8675	1.441	1.107	0.9912	1.412	0.09013	15.6%	15.8%
54.3		4	1.254	1.056	1.453	1.249	1.107	1.412	0.06231	9.93%	8.53%
111.3		4	0.7591	0.5543	0.964	0.7854	0.5796	0.8861	0.06436	17.0%	44.6%
214		4	0.2757	0.0419	0.5096	0.2403	0.1588	0.4636	0.07348	53.3%	79.9%

*Done 6/12/20 ef*

*6/12/20*

**CETIS Analytical Report**

Report Date: 10 Jun-20 13:34 (p 2 of 2)

Test Code: 474-071 | 01-6129-1574

*Done*  
① Fathead Minnow

**7-d Larval Survival and Growth Test**

TRE Environmental Strategies

Analysis ID: 00-4684-6428  
Analyzed: 10 Jun-20 13:34

Endpoint: 7d Survival Rate  
Analysis: Parametric-Control vs Treatments

CETIS Version: CETISv1.8.7  
Official Results: Yes

**7d Survival Rate Detail**

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.021	Dilution Water	0.9	1	1	1
12.5		1	0.8	1	0.8
25		1	0.7	0.8	0.8
54.3		0.9	1	0.9	0.8
111.3		0.5	0.5	0.3	0.6
214		0.1	0	0	0.2

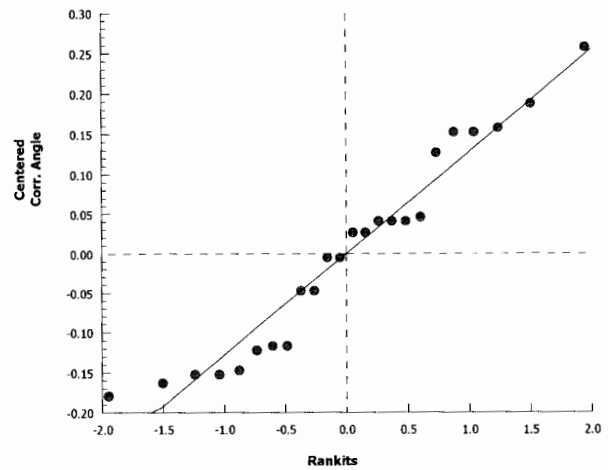
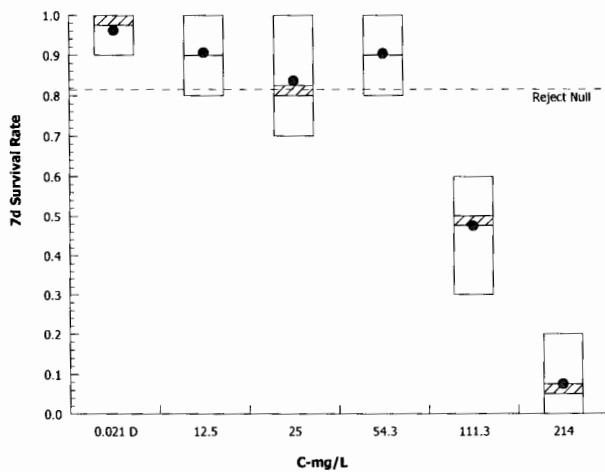
**Angular (Corrected) Transformed Detail**

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.021	Dilution Water	1.249	1.412	1.412	1.412
12.5		1.412	1.107	1.412	1.107
25		1.412	0.9912	1.107	1.107
54.3		1.249	1.412	1.249	1.107
111.3		0.7854	0.7854	0.5796	0.8861
214		0.3218	0.1588	0.1588	0.4636

**7d Survival Rate Binomials**

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.021	Dilution Water	9/10	10/10	10/10	10/10
12.5		10/10	8/10	10/10	8/10
25		10/10	7/10	8/10	8/10
54.3		9/10	10/10	9/10	8/10
111.3		5/10	5/10	3/10	6/10
214		1/10	0/10	0/10	2/10

**Graphics**



*Done 6/12/20 CF*

*6/12/20*

**CETIS Analytical Report**  
*① B.N. SLIMP*

Report Date: 10 Jun-20 16:43 (p 1 of 2)  
 Test Code: 474-071 | 01-6129-1574

Fathead Minnow 7-d Larval Survival and Growth Test TRE Environmental Strategies

Analysis ID: 08-0802-4389	Endpoint: <u>7d Survival Rate</u>	CETIS Version: CETISv1.8.7
Analyzed: 10 Jun-20 16:42	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 08-1256-8166	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 21 May-20 13:30	Protocol: EPA/821/R-02-013 (2002)	Diluent: rGSL
Ending Date: 28 May-20 13:20	Species: Artemia franciscana	Brine: Crystal Sea
Duration: 7d	Source: In-House Culture	Age: 48h
Sample ID: 17-3344-4662	Code: 67524436	Client: Notre Dame
Sample Date: 21 May-20 10:30	Material: Zinc sulfate	Project: Special Studies
Receive Date: 21 May-20 13:30	Source: research	
Sample Age: 3h	Station:	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
<u>Linear</u>	Linear	1454137	200	Yes	Two-Point Interpolation

Point Estimates

Level	mg/L	95% LCL	95% UCL
LC5	8.132	1.81	27.79
LC10	20	1.345	84.61
LC15	59.26	N/A	71.93
LC20	66.44	50.98	78.63
LC25	73.61	58.81	86.04
LC40	95.12	78.88	117.2
LC50	109.5	87.93	139.8

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
0.021	Dilution Water	4	0.975	0.9	1	0.025	0.05	5.13%	0.0%	39	40
12.5		4	0.9	0.8	1	0.05774	0.1155	12.8%	7.69%	36	40
25		4	0.825	0.7	1	0.06292	0.1258	15.3%	15.4%	33	40
54.3		4	0.9	0.8	1	0.04082	0.08165	9.07%	7.69%	36	40
111.3		4	0.475	0.3	0.6	0.06292	0.1258	26.5%	51.3%	19	40
214		4	0.075	0	0.2	0.04787	0.09574	128.0%	92.3%	3	40

7d Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.021	Dilution Water	0.9	1	1	1
12.5		1	0.8	1	0.8
25		1	0.7	0.8	0.8
54.3		0.9	1	0.9	0.8
111.3		0.5	0.5	0.3	0.6
214		0.1	0	0	0.2

7d Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.021	Dilution Water	9/10	10/10	10/10	10/10
12.5		10/10	8/10	10/10	8/10
25		10/10	7/10	8/10	8/10
54.3		9/10	10/10	9/10	8/10
111.3		5/10	5/10	3/10	6/10
214		1/10	0/10	0/10	2/10

*① NW 6/12/20 CF*

**CETIS Analytical Report**

*DBMre Sluimp*

Report Date: 10 Jun-20 16:43 (p 2 of 2)

Test Code: 474-071 | 01-6129-1574

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

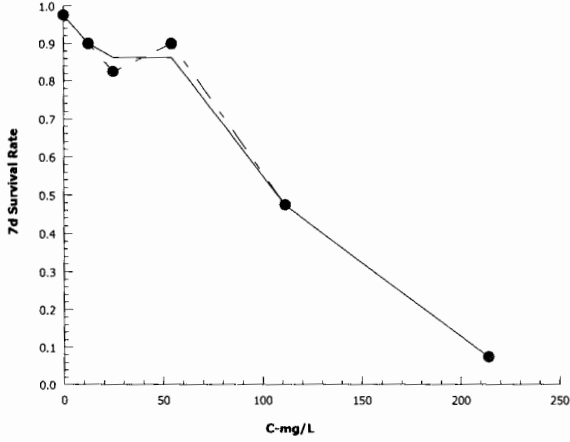
TRE Environmental Strategies

Analysis ID: 08-0802-4389  
Analyzed: 10 Jun-20 16:42

Endpoint: 7d Survival Rate  
Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7  
Official Results: Yes

**Graphics**



*Done 6/12/20 ef*

*6/12/20*

**CETIS Analytical Report**

*D. B. Schump*

Report Date: 10 Jun-20 16:43 (p 1 of 2)  
 Test Code: 474-071 | 01-6129-1574

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

TRE Environmental Strategies

<b>Analysis ID:</b> 09-4990-2357	<b>Endpoint:</b> Mean Dry Biomass-mg	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 10 Jun-20 16:43	<b>Analysis:</b> Linear Interpolation (ICPIN)	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-1256-8166	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b> Lab Tech
<b>Start Date:</b> 21 May-20 13:30	<b>Protocol:</b> EPA/821/R-02-013 (2002)	<b>Diluent:</b> rGSL
<b>Ending Date:</b> 28 May-20 13:20	<b>Species:</b> Artemia franciscana	<b>Brine:</b> Crystal Sea
<b>Duration:</b> 7d	<b>Source:</b> In-House Culture	<b>Age:</b> 48h
<b>Sample ID:</b> 17-3344-4662	<b>Code:</b> 67524436	<b>Client:</b> Notre Dame
<b>Sample Date:</b> 21 May-20 10:30	<b>Material:</b> Zinc sulfate	<b>Project:</b> Special Studies
<b>Receive Date:</b> 21 May-20 13:30	<b>Source:</b> research	
<b>Sample Age:</b> 3h	<b>Station:</b>	

**Linear Interpolation Options**

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1285547	200	Yes	Two-Point Interpolation

**Point Estimates**

Level	mg/L	95% LCL	95% UCL
IC5	0.7903	0.7166	0.9048
IC10	1.56	1.412	1.789
IC15	2.329	2.108	2.673
IC20	3.098	2.804	3.556
IC25	3.867	3.499	4.44
IC40	6.175	5.586	7.092
IC50	7.714	6.977	8.859

*2/2.5*

**Mean Dry Biomass-mg Summary**

**Calculated Variate**

C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0.021	Dilution Water	4	0.09925	0.079	0.119	0.008189	0.01638	16.5%	0.0%
12.5		4	0.01875	0.013	0.028	0.003224	0.006449	34.4%	81.1%
25		4	0.0105	0.007	0.014	0.001756	0.003512	33.4%	89.4%
54.3		4	0.00925	0.005	0.015	0.002175	0.004349	47.0%	90.7%
111.3		4	0.00175	0	0.004	0.000854	0.001708	97.6%	98.2%
214		4	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
0.021	Dilution Water	0.079	0.101	0.098	0.119
12.5		0.028	0.017	0.017	0.013
25		0.014	0.007	0.013	0.008
54.3		0.01	0.015	0.007	0.005
111.3		0.004	0.001	0.002	0
214		0	0	0	0

*6/12/20*



# CETIS Analytical Report

① Braushorn

Report Date: 10 Jun-20 16:43 (p 2 of 2)  
Test Code: 474-071 | 01-6129-1574

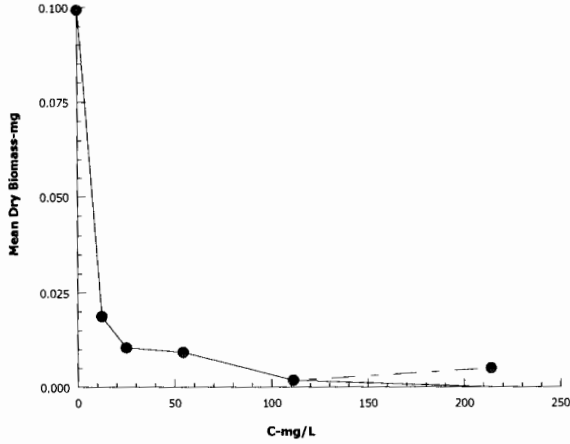
## Fathead Minnow 7-d Larval Survival and Growth Test

TRE Environmental Strategies

Analysis ID: 09-4990-2357      Endpoint: Mean Dry Biomass-mg  
Analyzed: 10 Jun-20 16:43      Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7  
Official Results: Yes

### Graphics



① new 6/12/20 c

6/12/20

June 5, 2020

Mr. Christopher Bittner  
Standards Coordinator  
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Salt Lake City, UT 84116

Dr. Gary Belovsky  
Environ. Res. Center & Dept. Biol Sci.  
University of Notre Dame  
Notre Dame, IN 46556

**Subject: Results of Short-term Chronic Brine Shrimp Experiment #16**

Mr. Bittner/ Dr. Belovsky:

Below is a summary of the short-term chronic brine shrimp experiment initiated on May 21, 2020. The purpose of this experiment was to investigate the effect of zinc on *Artemia franciscana* toxicity in a short-term chronic test.

Along with a control, five different zinc concentrations were tested, based off of the previously conducted acute test:

- 12.5, 25, 50, 100, and 200 mg/L

The results of these studies will help determine the experimental design of the definitive short-term chronic toxicity tests. The test volume was consistent at 50 ml.

**Species:** *Artemia franciscana*

**Test type:**

- Test duration: 7 days
- Test type: static-renewal (solutions and food renewed daily)
- Algae: *Dunaliella viridis*
- Food concentration: 72.5 µg/L Ch1a and 0.3 ml YTC<sup>1</sup>
- Temperature: 20°C
- Test volume(s): 50 ml
- Replicates: 4
- Organisms/Rep: 10
- Test media: 120 ppt rGSL media (per Notre Dame recipe)

**Pretest conditions:** <24-h old *A. franciscana* were hatched out in ~29 ppt artificial seawater (Crystal Sea Marine Mix) and ~200 organisms were placed in 120 ppt rGSL water and fed *Dunaliella viridis* at a density of 72.5 µg/L Ch1a and 0.3 ml YTC. Solutions were gently aerated.

<sup>1</sup> yeast-trout chow-cerophyl mixture used as a typical food for water fleas in whole effluent toxicity testing (USEPA 2002)

### Characterization of Recon Water

Sample No.	pH	Hard. (mg/L) <sup>a</sup>	Alk. (mg/L) <sup>a</sup>	Spec. Cond. ( $\mu$ S/cm)	TRC (mg/L) <sup>b</sup>	NH <sub>3</sub> -N (mg/L)	Salinity (ppt)
RW#13937	7.9	NM	NM	136,100	NM	NM	120

<sup>a</sup>As CaCO<sub>3</sub>

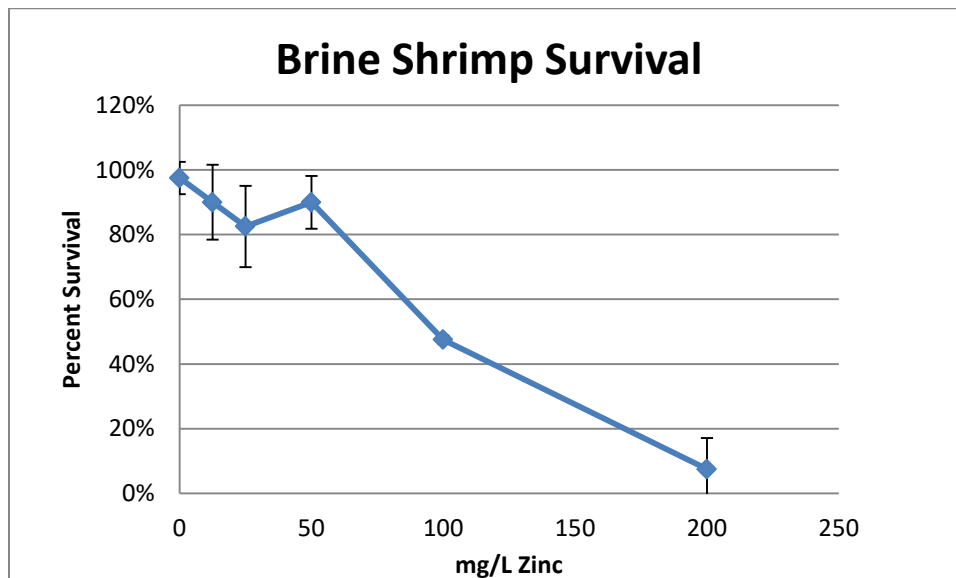
<sup>b</sup>Total residual chlorine

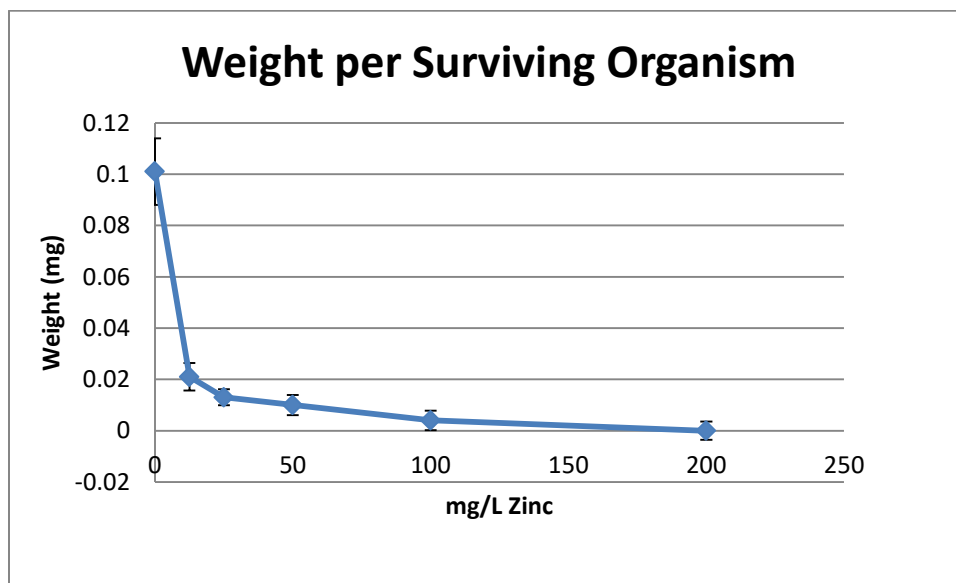
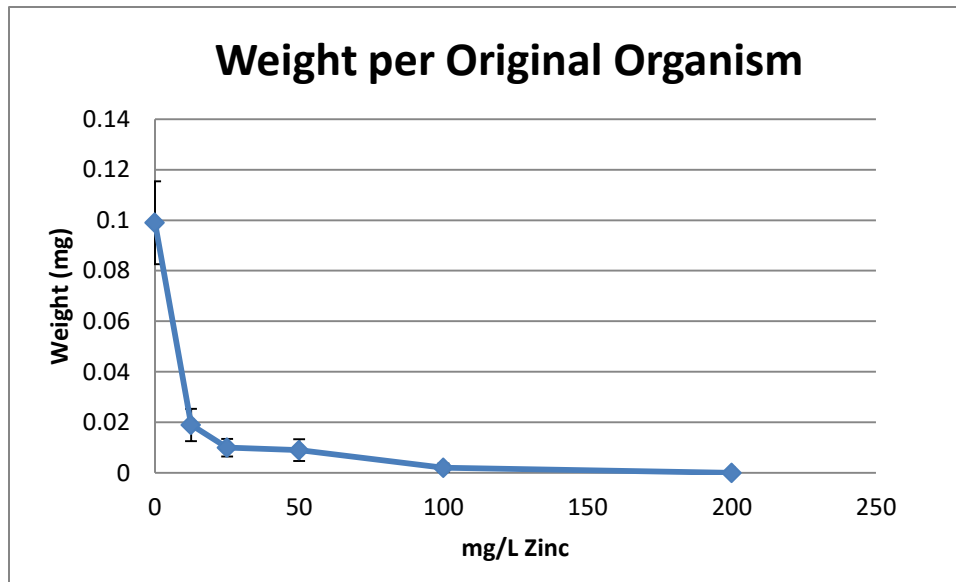
#### Test activities:

- Biological observations (primarily survival) taken daily.
- Chemistries taken on renewal days (i.e., pH, dissolved oxygen, and temperature).
- Conductivity was measured at test termination or when there was 0% survival in that treatment.
- Dry weights were determined at test termination.
- Zinc was added to 120 rGSL media containing food and allowed to equilibrate for 3 hours prior to use in the toxicity tests.

#### Results:

The survival and average dry weights for the brine shrimp in the zinc (nominal) treatments are illustrated in the following figures.





### Test Endpoints

Study	Test Endpoints (mg Zn/L, nominal)					
	Survival NOEC	Survival LOEC	Survival IC20	Growth NOEC	Growth LOEC	Growth IC20
7-Day	50	100	60.65 (45.53-71.92)	<12.5	12.5	<12.5 (3.082 (2.8–3.515))

### Summary and findings:

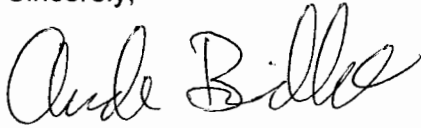
- Organism survival was  $\geq 90\%$  for the control.
- A survival effect was observed in the two highest concentrations.

- There was a large growth effect for all zinc concentrations tested.

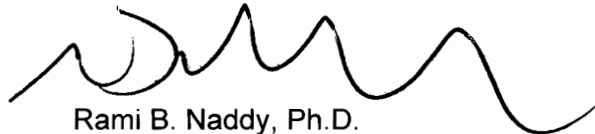
Analytical samples from each treatment have been collected and sent in for zinc measurement. We will provide a summary of those results separately.

We greatly appreciate the opportunity to complete this study for you. Please do not hesitate to call if you have any questions or concerns.

Sincerely,



Amanda Bidlack  
Project Specialist / QA Officer  
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17001-474-071  
Attachment

cc: David Pillard, TRE

TOXICITY DATA PACKAGE COVER SHEET

QA: ASP 6/3/20

Test Type: Chronic Project Number: 17001-474-071  
Test Substance: Zinc (ZnSO4) Species: Artemia franciscana  
Dilution Water: rGSL Organism Lot or Batch Number: 051920  
Concurrent Control Water: NA Age: 48hr (48 hr) Supplier: TRE  
Date and Time Test Began: 5/21/20 @ 1330 Date and Time Test Ended: 5/28/20 @ 1320  
Protocol Number: \_\_\_\_\_ Investigator(s): AS/CP/HP/EN/IG/ES/AF/MB

Background Information

Type of Test: Static-Renewal (Daily) pH control?: Yes No  
If yes, give % CO<sub>2</sub>: NA  
Test Temperature: 20 ± 1 °C Env. Chmbr/Bath #: 25 Test Chmbrs: 147-ml cups  
Photoperiod: 16 h light : 8 h dark Light intensity: 50-100 ft-c.  
Test Solution Vol.: 50 ml Replicates per Treatment: 4  
Length of Test: 7 days Organisms per Replicate: 10  
Type of Food and Quantity per Chamber: 72.5 ug/L Chla/ 0.3 ml YT Feeding Frequency: Initiation and Renewals

Test Substance Characterization Parameters and Frequency:

Hardness: Test Initiation Alkalinity: Test Initiation NH<sub>3</sub>: Test Initiation TRC: Test Initiation

pH: Daily Conductivity: Daily

Test Concentrations (Volume:Volume): rGSL, 12.5, 25, 50, 100, and 200 mg/L as Zn

Agency Summary Sheet(s)?: None

Reference Toxicant Data: Test Dates: \_\_\_\_\_ to \_\_\_\_\_ IC<sub>25</sub>: \_\_\_\_\_  
Hist. 95% Control Limits: \_\_\_\_\_ to \_\_\_\_\_ Method for Determining Ref. Tox. Value: Linear Interpolation

Special Procedures and Considerations:

Organisms hatched 2 days prior to initiation and held in rGSL with 72.5 ug/L Chla/ 0.3 ml YTC

Appropriate correction factors have been applied to all temperatures recorded in this data package

Study Director Initials: AS Date: 5/28/20

**TEST SUBSTANCE USAGE LOG**

Project Number: 17001-474-071

*QA # 1840/3/20*

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number	C99-093			
Test Substance Collection Date and Time	From: @	From: @	From: @	From: @
	To: @	To: @	To: @	To: @
Sample Type (Grab or Comp)				
Date Test Substance Received				
Dilution Water Number RW# or TRE#, circle one	13937			
Concurrent Control Water RW#				
Date(s) Used	5/21/20	5/25/20		
	5/22/20	5/26/20		
	5/23/20	5/27/20		
	5/24/20			

**Preparation of Test Solutions**

Test Substance Conc. (mg/L)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)
0	0	350	350						
12.5	22	328	350						
25	44	306	350						
50	88	263	350						
100	175	175	350						
200	350	0	350						
	678	1422	2100						
Initials / Date	AB 5/21/20 mixed BS								
Initials / Date	HP 5/22/20 " "								
Initials / Date	Ry 5/23/20 " "								
Initials / Date	Ry 5/24/20 " "								
Initials / Date	Ry 5/25/20 " "								
Initials / Date	Ry 5/26/20 " "								
Initials / Date	Ry 5/27/20 " "								
Initials / Date									

Artemia franciscana  
 CHRONIC BIOLOGICAL DATA

QA: WAP 6/3/20

Project Number: 17001-474-071

mg/L	Test Replicate	Number of Surviving Organisms								Remarks
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
0	A	10	10	10	9	9	9	9	9	97.5%
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
12.5	A	10	10	10	10	10	10	10	10	90%
	B	10	10	10	10	10	10	8	8	
	C	10	10	10	10	10	10	10	10	
	D	10	9	9	9	9	9	8	8	
25	A	10	10	10	10	10	10	10	10	82.5%
	B	10	10	10	9	9	9	8	7	
	C	10	9	9	9	9	9	9	8	
	D	10	10	10	10	10	10	9	8	
50	A	10	10	10	10	9	9	9	9	90%
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	9	9	
	D	10	10	10	10	10	10	9	8	
100	A	10	10	10	10	10	9	9	5	① <del>72.5%</del> 47.5%
	B	10	10	10	10	10	8	7	5	
	C	10	10	10	10	9	9	8	3	
	D	10	10	10	10	10	9	8	6	
200	A	10	5	5	4	2	2	1	1	7.5%
	B	10	2	1	1	0	—	—	—	
	C	10	4	4	4	2	2	2	0	
	D	10	5	5	5	4	4	4	2	
	A									
	B									
	C									
	D									
Date:		5/21/20	5/22/20	5/23/20	5/24/20	5/25/20	5/26/20	5/27/20	5/28/20	
Time:		1330	1520	1210	1130	1205	1325	1545	1320	
Initials:		CP/EN	EN	ES	ES	AP	EN	MB	EN	

DATA 6/3/20 B



CHRONIC CHEMICAL DATA (INITIAL)

QA: DAP 6/3/10

Project Number: 17001-474-071

Test Species: *Artemia franciscana*

mg/L	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter #	Remarks
Conc.: 0									All Conc.	
pH	7.9	7.7	7.9	8.0	7.9	7.8	7.8		FM27	
D.O. (mg/L)	5.6	5.5	6.0	5.8	6.4	5.1	5.9		17	
Temp. (°C)	20	20	20	20	20	20	20		IR1	
Cond. (µS/cm)	136,100	132300	144900	134400	162,900	138300	131500		15	
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH <sub>3</sub> (mg/L)										
Conc.: 12.5										
pH	7.8	7.6	7.7	7.9	7.7	7.7	7.7			
D.O. (mg/L)	5.5	5.2	5.4	5.5	6.6	5.3	5.4			
Temp. (°C)	20	20	20	20	20	20	20			
Cond. (µS/cm)	136,200	133500	148600	135000	163,600	139900	132200			
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH <sub>3</sub> (mg/L)										
Conc.: 25										
pH	7.7	7.6	7.7	7.8	7.6	7.6	7.6			
D.O. (mg/L)	5.6	5.5	5.7	5.3	6.7	5.4	5.4			
Temp. (°C)	20	20	20	20	20	20	20			
Cond. (µS/cm)	135,900	132000	125400	135000	163,200	140400	133100			
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH <sub>3</sub> (mg/L)										
Conc.: 50										
pH	7.5	7.4	7.6	7.6	7.5	7.4	7.5			
D.O. (mg/L)	5.7	5.5	5.5	5.5	6.6	5.5	5.7			
Temp. (°C)	20	20	20	20	20	20	20			
Cond. (µS/cm)	135,700	132000	137700	134700	163,200	140700	132700			
Date:	5/21/20	5/22/20	5/23/20	5/24/20	5/25/20	5/26/20	5/27/20			
Time:	1325	1510	1120	1200	1140	1315	1525			
Initials:	CP	EN	ES	ES	AF	EN	MB			

Note: Hardness, alkalinity, TRC, and NH<sub>3</sub> data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

\*Dilution/control water and effluent were brought to 25C prior to making the dilution series. The temperature of resulting effluent dilution is assumed to also be 25C.

① EN 5/22/20 E

CHRONIC CHEMICAL DATA (INITIAL)

QA: ~~AW~~ 6/3/20

Project Number: 17001-474-071

Test Species: *Artemia franciscana*

%	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter #	Remarks
Conc.: 100									All Conc.	
pH	7.3	7.1	7.4	7.4	7.2	7.3	7.3			
D.O. (mg/L)	5.9	5.6	6.2	5.1	6.9	5.5	6.0			
Temp. (°C)	20	20	20	20	20	20	20			
Cond. (µS/cm)	135,400	132,000	134,000	135,100	162,800	140,000	132,700			
Conc.: [ ]										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.: [ ]										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.: [ ]										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.: 200										
pH	7.1	6.9	7.1	7.1	7.0	7.0	7.1			
D.O. (mg/L)	5.4	5.4	6.1	5.1	6.7	5.0	5.7			
Temp. (°C)	20	20	20	20	20	20	20			
Cond. (µS/cm)	134,400	131,400	134,500	132,300	160,700	136,000	132,000			
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH <sub>3</sub> (mg/L)										
Date:	5/21/20	5/22/20	5/23/20	5/24/20	5/24/20	5/24/20	5/27/20			
Time:	1325	1510	1130	1205	1140	1315	1525			
Initials:	CP	EW	ES	ES	AF	EW	MB			

Note: Hardness, alkalinity, TRC, and NH<sub>3</sub> data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

\*Dilution/control water and effluent were brought to 25C prior to making the dilution series. The temperature of resulting effluent dilution is assumed to also be 25C.

CHRONIC CHEMICAL DATA (FINAL)

QA: DDA 6/3/20

Project Number:	17001-474-071
Test Species:	<i>Artemia franciscana</i>

mg/L	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter #	Remarks
Conc.: 0								136600	All Conc.	* conductivity 15
pH	7.9	7.9	7.9	7.9	7.7	7.7	7.9		FM27	
D.O. (mg/L)	5.4	5.4	5.1	6.2	5.6	5.0	4.8		17	
Temp (°C)	23 <sup>A</sup>	21	20	20	19	21	20		L-6	
Conc.: 12.5								122000		* conductivity
pH	7.9	7.9	7.9	7.8	7.7	7.8	7.9			
D.O. (mg/L)	5.9	5.3	5.6	6.3	5.8	5.0	5.2			
Temp (°C)	23 <sup>A</sup>	21	20	20	19	21	20			
Conc.: 25								122500		* conductivity
pH	7.8	7.8	7.8	7.8	7.7	7.8	7.9			
D.O. (mg/L)	5.8	5.7	5.2	6.5	5.5	5.1	5.4			
Temp (°C)	23 <sup>A</sup>	21	20	20	19	21	20			
Conc.: 50								123600		* conductivity
pH	7.8	7.8	7.8	7.7	7.4	7.7	7.9			
D.O. (mg/L)	5.4	6.2	5.5	6.7	5.7	5.2	5.5			
Temp (°C)	23 <sup>A</sup>	21	20	20	19	21	20			
Conc.: 100								123100		* conductivity
pH	7.7	7.7	7.7	7.7	7.5	7.7	7.8			
D.O. (mg/L)	5.2	5.2	5.4	6.9	5.7	5.2	5.6			
Temp (°C)	23 <sup>A</sup>	21	20	20	19	21	20			
Conc.: 200								120800		* conductivity
pH	7.2	NM	7.5	7.3	7.2	7.3	7.4			
D.O. (mg/L)	5.6	NM	5.0	6.0	5.7	5.0	5.3			
Temp (°C)	23 <sup>A</sup>	21	20	20	19	21	20			
Conc.:										
pH										
D.O. (mg/L)										
Temp (°C)										
Date:	5/22/20	5/23/20	5/24/20	5/25/20	5/26/20	5/27/20	5/28/20			
Time:	1600	1210	1205	1210	1320	1610	1315			
Initials:	EN	ES	ES	AF	EW	MB	EN			

Checked all reps

DAILY TOXICITY TEST LOG

QA: MAP 6/3/20

Project Number:	17001-474-071
Test Species:	<i>Artemia franciscana</i>

General Comments	Feeding	Initials/Date
Random Chart: "P" Min/Max Thermometer # M-15	72.5 ug/l Chla 0.3 ml YTC	
Test Day 0 Test Solution Mixed at: 1015 Test Organisms Added at: 1330 Spiked @ 1030	Fed @ 1030	CP 5/21/20
Test Day 1 Real Time: 20 °C Min-Max Range: 20-22 °C Spiked @ 1210	Fed @ 1210	EN 5/22/20
Test Day 2 Real Time: <del>25</del> <sup>21</sup> °C Min-Max Range: <del>25-26</del> <sup>21-22</sup> °C Spiked @ 0845	Fed @ 0845	ES 5/23/20
Test Day 3 Real Time: 21 °C Min-Max Range: 21-22 °C Spiked @ 0830	Fed @ 0830	ER 5/24/20
Test Day 4 Real Time: 20 °C Min-Max Range: 20-22 °C Spiked @ 0815	Fed @ 0815	AF 5/25/20
Test Day 5 Real Time: 20 °C Min-Max Range: 19-21 °C Spiked @ 0815	Fed @ 0815	EN 5/26/20
Test Day 6 Real Time: 22 °C Min-Max Range: 19-22 °C Spiked @ 1215	Fed @ 1215	MB 5/27/20
Test Day 7 Real Time: 22 °C Min-Max Range: 21-22 °C	Fed @ None	EN 5/28/20

① ER 5/23/20, E

QA: ~~NA~~ 6/3/20

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 17001-474-071		Test Substance: Zinc (ZnSO <sub>4</sub> )										Comments:			
Species: Artemia franciscana		Analyst Tare: Sk		Analyst Gross: cp								Analytical Balance ID: Sart#1		Dried in Oven # 3	
Date/Time of Tare Wt.: 5/28/20 @ 1230		Date/Time of Gross Wt.: 5/30/20 @ 1440		Wet		Blot Dry		Dry (>100°C)		AFDW (>500°C)		Lot or Batch Number: 051920			
Boat No.	Treatment	Rep.	Length Units:	Weight Type (Circle):		Net Weight (g)	Adjusted Net Weight (g)	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	No. of Surv. Organisms	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)		
				Tare Weight (g)	Gross Weight (g)										
	0	A		1.13286	1.13365	0.00079					9				
		B		1.12104	1.12205	0.00101					10				
		C		1.11673	1.11771	0.00098					10				
		D		1.12536	1.12655	0.00119					10				
	12.5	A		1.14030	1.14058	0.00028					10				
		B		1.12801	1.12818	0.00017					8				
		C		1.14188	1.14205	0.00017					10				
		D		1.12830	1.12843	0.00013					8				
	25	A		1.13821	1.13835	0.00014					10				
		B		1.13875	1.13882	0.00007					7				
		C		1.13980	1.13993	<del>0.00003</del> 0.00013					8				
		D		1.14016	1.14024	0.00008					8				
Blank				1.12820	1.12820										
Range															
Mean															
Test Solution Volume:												Loading Rate:			

Add in weight loss of blank boat, if appropriate.

Sk 5/28/20 WP @ NS 6/1/20 E

QA: ASD 6/3/16

**TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING**

Project Number: <u>17001-474-011</u>		Test Substance: <u>Zinc (ZnSO4)</u>		Comments:													
Species: <u>Artemia franciscana</u>		Analyst Tare: <u>Sk</u>		Analytical Balance ID: <u>Sart#1</u>													
Date/Time of Tare Wt.: <u>5/28/20 @ 1230</u>		Date/Time of Gross Wt.: <u>5/30/20 @ 1640</u>		Dried in Oven # <u>3</u> from Date: <u>5/28/20</u> Time: <u>1250</u>													
Date/Time of Tare Wt.: <u>5/28/20 @ 1230</u>		Analyst Gross: <u>CP</u>		to Date: <u>5/28/20</u> Time: <u>0746</u>													
Boat No.	Treatment	Rep.	Length Units:	Weight Type (Circle):			Net Weight (g)	Blot Dry	Dry (60-90°C)	Dry (>100°C)	AFDW (>500°C)	Mean Wt. per Treatment (mg) (Original)	No. of Surv. Organisms	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)		
				Tare Weight (g)	Gross Weight (g)	Wet										Adjusted Net Weight (g) <sup>1</sup>	No. of Orig. Organisms
	mg/L	A		1.12334	1.12344		0.00010						9				
				1.11704	1.11719		0.00015							10			
				1.14352	1.14359		0.00007								9		
				1.14402	1.14407		0.00005								8		
	100	A		1.13496	1.13500		0.00004						5				
				1.12704	1.12705		0.00001								5		
				1.13968	1.13970		0.00002								3		
				1.13820	1.13820		0								6		
	200	A		1.14870	1.14869		-0.00001						1				
				1.13358										0			
				1.13209	1.13208		-0.00001								2		
	Blank																
	Range																
	Mean																
Test Solution Volume:												Loading Rate:					

<sup>1</sup> Add in weight loss of blank boat, if appropriate.

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 14001-474

Species: Artemia franciscana

QA: D 680 6/3/10

Treatment Rep	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)	No of Orig. Organisms	Mean Wt./ Original Organism (mg)	Mean Wt./ Treatment (mg) (Original)	Number of Surv. Organisms	Mean Wt./ Surviving Organism (mg)	Mean Wt./ Treatment (mg) (Surviving)
rGSL	A	1.13286	1.13355	0.00079	0.00079	10	0.079	0.0993	9	0.088	0.1014
	B	1.12104	1.12205	0.00101	0.00101	10	0.101		10	0.101	
	C	1.11673	1.11771	0.00098	0.00098	10	0.098		10	0.098	
	D	1.12536	1.12655	0.00119	0.00119	10	0.119		10	0.119	
12.5 mg/L	A	1.14030	1.14058	0.00028	0.00028	10	0.028	0.0187	10	0.028	0.0206
	B	1.12801	1.12818	0.00017	0.00017	10	0.017		8	0.021	
	C	1.14188	1.14205	0.00017	0.00017	10	0.017		10	0.017	
	D	1.12830	1.12843	0.00013	0.00013	10	0.013		8	0.016	
25 mg/L	A	1.13821	1.13835	0.00014	0.00014	10	0.014	0.0105	10	0.014	0.0126
	B	1.13875	1.13882	0.00007	0.00007	10	0.007		7	0.010	
	C	1.13980	1.13993	0.00013	0.00013	10	0.013		8	0.016	
	D	1.14016	1.14024	0.00008	0.00008	10	0.008		8	0.010	
50 mg/L	A	1.12334	1.12344	0.00010	0.00010	10	0.010	0.0092	9	0.011	0.0100
	B	1.11704	1.11719	0.00015	0.00015	10	0.015		10	0.015	
	C	1.14352	1.14359	0.00007	0.00007	10	0.007		9	0.008	
	D	1.14402	1.14407	0.00005	0.00005	10	0.005		8	0.006	
100 mg/L	A	1.13496	1.13500	0.00004	0.00004	10	0.004	0.0018	5	0.008	0.0042
	B	1.12704	1.12705	0.00001	0.00001	10	0.001		5	0.002	
	C	1.13968	1.13970	0.00002	0.00002	10	0.002		3	0.007	
	D	1.13820	1.13820	0.00000	0.00000	10	0.000		6	0.000	
200 mg/L	A	1.14870	1.14869	-0.00001	-0.00001	10	-0.001	-0.0005	1	-0.010	-0.0075
	B	0.00000	0.00000	0.00000	0.00000	10	0.000		0	-	
	C	0.00000	0.00000	0.00000	0.00000	10	0.000		0	-	
	D	1.13209	1.13208	-0.00001	-0.00001	10	-0.001		2	-0.005	
Blank		1.12820	1.12820	0.00000							

QA: WSP 6/3/16

Project Number: 14001-474 Species: Artemia franciscana

**Summary Statistics for Survival Data**

Treatment	N	Min	Max	Mean	SD	C.V.
rGSL	4	0.9	1.0	0.9750	0.0500	5.128%
12.5 mg/L	4	0.8	1.0	0.9000	0.1155	12.830%
25 mg/L	4	0.7	1.0	0.8250	0.1258	15.252%
50 mg/L	4	0.8	1.0	0.9000	0.0816	9.072%
100 mg/L	4	0.3	0.6	0.4750	0.1258	26.491%
200 mg/L	4	0.0	0.2	0.0750	0.0957	127.657%

**Summary Statistics for Growth Data (dry wt per original)**

Treatment	N	Min	Max	Mean	SD	C.V.
rGSL	4	0.079	0.119	0.0993	0.0164	16.502%
12.5 mg/L	4	0.013	0.028	0.0187	0.0064	34.392%
25 mg/L	4	0.007	0.014	0.0105	0.0035	33.447%
50 mg/L	4	0.005	0.015	0.0092	0.0043	47.020%
100 mg/L	4	0.000	0.004	0.0018	0.0017	97.590%
200 mg/L	4	-0.001	0.000	-0.0005	0.0006	-115.470%

**Summary Statistics for Growth Data (dry wt per surviving organism)**

Treatment	N	Min	Max	Mean	SD	C.V.
rGSL	4	0.088	0.119	0.1014	0.0130	12.815%
12.5 mg/L	4	0.016	0.028	0.0206	0.0054	26.119%
25 mg/L	4	0.010	0.016	0.0126	0.0031	24.662%
50 mg/L	4	0.006	0.015	0.0100	0.0039	38.695%
100 mg/L	4	0.000	0.008	0.0042	0.0038	90.863%
200 mg/L	2	-0.010	-0.005	-0.0075	0.0035	-47.140%



**CETIS Analytical Report**

Report Date: 02 Jun-20 08:29 (p 1 of 2)  
 Test Code: 474-071 | 01-6129-1574

*Brine shrimp*

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

TRE Environmental Strategies

<b>Analysis ID:</b> 11-8599-4819	<b>Endpoint:</b> 7d Survival Rate	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 02 Jun-20 8:28	<b>Analysis:</b> Parametric-Control vs Treatments	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-1256-8166	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b> Lab Tech
<b>Start Date:</b> 21 May-20 13:30	<b>Protocol:</b> EPA/821/R-02-013 (2002)	<b>Diluent:</b> rGSL
<b>Ending Date:</b> 28 May-20 13:20	<b>Species:</b> Artemia franciscana	<b>Brine:</b> Crystal Sea
<b>Duration:</b> 7d	<b>Source:</b> In-House Culture	<b>Age:</b> 48h
<b>Sample ID:</b> 17-3344-4662	<b>Code:</b> 67524436	<b>Client:</b> Notre Dame
<b>Sample Date:</b> 21 May-20 10:30	<b>Material:</b> Zinc sulfate	<b>Project:</b> Special Studies
<b>Receive Date:</b> 21 May-20 13:30	<b>Source:</b> research	
<b>Sample Age:</b> 3h	<b>Station:</b>	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	16.4%	50	100	70.71	

**Dunnnett Multiple Comparison Test**

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		12.5	1.1	2.41	0.245	6	0.3787	CDF	Non-Significant Effect
		25	2.135	2.41	0.245	6	0.0823	CDF	Non-Significant Effect
		50	1.151	2.41	0.245	6	0.3569	CDF	Non-Significant Effect
		100*	6.027	2.41	0.245	6	<0.0001	CDF	Significant Effect
		200*	10.79	2.41	0.245	6	<0.0001	CDF	Significant Effect

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	3.501526	0.7003053	5	33.94	<0.0001	Significant Effect
Error	0.3714243	0.02063468	18			
Total	3.872951		23			

**Distributional Tests**

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.943	15.1	0.8570	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.939	0.884	0.1546	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	Dilution Water	4	0.975	0.8954	1	1	0.9	1	0.025	5.13%	0.0%
12.5		4	0.9	0.7163	1	0.9	0.8	1	0.05774	12.8%	7.69%
25		4	0.825	0.6248	1	0.8	0.7	1	0.06292	15.3%	15.4%
50		4	0.9	0.7701	1	0.9	0.8	1	0.04082	9.07%	7.69%
100		4	0.475	0.2748	0.6752	0.5	0.3	0.6	0.06292	26.5%	51.3%
200		4	0.075	0	0.2273	0.05	0	0.2	0.04787	128.0%	92.3%

**Angular (Corrected) Transformed Summary**

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.371	1.242	1.501	1.412	1.249	1.412	0.04074	5.94%	0.0%
12.5		4	1.26	0.9795	1.54	1.26	1.107	1.412	0.08801	14.0%	8.15%
25		4	1.154	0.8675	1.441	1.107	0.9912	1.412	0.09013	15.6%	15.8%
50		4	1.254	1.056	1.453	1.249	1.107	1.412	0.06231	9.93%	8.53%
100		4	0.7591	0.5543	0.964	0.7854	0.5796	0.8861	0.06436	17.0%	44.6%
200		4	0.2757	0.0419	0.5096	0.2403	0.1588	0.4636	0.07348	53.3%	79.9%

*ONDA 6/3/20 E*

**CETIS Analytical Report**

Report Date: 02 Jun-20 08:29 (p 2 of 2)  
 Test Code: 474-071 | 01-6129-1574

① *Brine shrimp*  
~~Fathead Minnow 7-d Larval Survival and Growth Test~~

TRE Environmental Strategies

Analysis ID: 11-8599-4819      Endpoint: 7d Survival Rate      CETIS Version: CETISv1.8.7  
 Analyzed: 02 Jun-20 8:28      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
0	Dilution Water	0.9	1	1	1
12.5		1	0.8	1	0.8
25		1	0.7	0.8	0.8
50		0.9	1	0.9	0.8
100		0.5	0.5	0.3	0.6
200		0.1	0	0	0.2

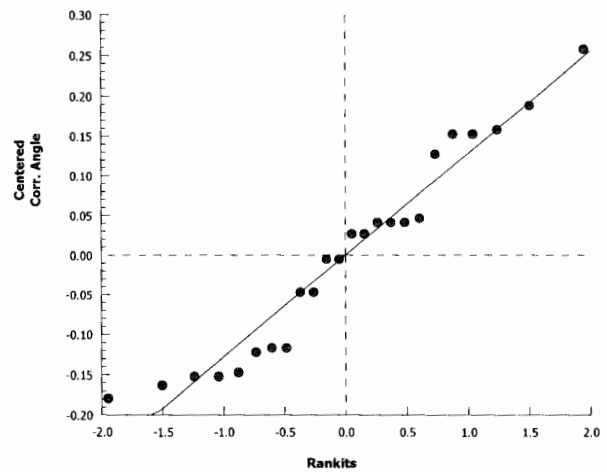
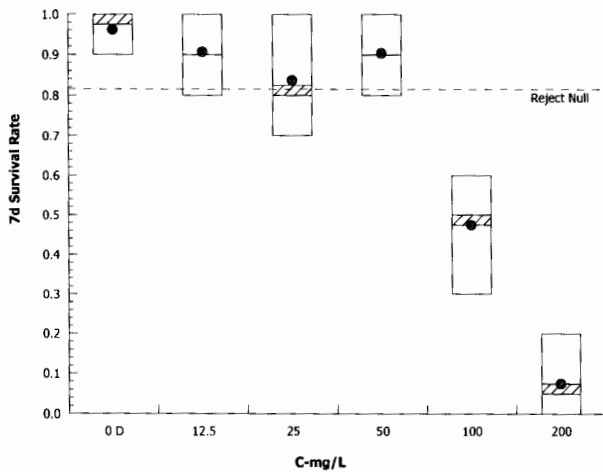
**Angular (Corrected) Transformed Detail**

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.249	1.412	1.412	1.412
12.5		1.412	1.107	1.412	1.107
25		1.412	0.9912	1.107	1.107
50		1.249	1.412	1.249	1.107
100		0.7854	0.7854	0.5796	0.8861
200		0.3218	0.1588	0.1588	0.4636

**7d Survival Rate Binomials**

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	9/10	10/10	10/10	10/10
12.5		10/10	8/10	10/10	8/10
25		10/10	7/10	8/10	8/10
50		9/10	10/10	9/10	8/10
100		5/10	5/10	3/10	6/10
200		1/10	0/10	0/10	2/10

**Graphics**



① *dan 6/3/20E*

**CETIS Analytical Report**

① Brine shrimp

Report Date: 02 Jun-20 08:29 (p 1 of 2)  
 Test Code: 474-071 | 01-6129-1574

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

TRE Environmental Strategies

Analysis ID: 00-5331-5017	Endpoint: 7d Survival Rate	CETIS Version: CETISv1.8.7
Analyzed: 02 Jun-20 8:29	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 08-1256-8166	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 21 May-20 13:30	Protocol: EPA/821/R-02-013 (2002)	Diluent: rGSL
Ending Date: 28 May-20 13:20	Species: Artemia franciscana	Brine: Crystal Sea
Duration: 7d	Source: In-House Culture	Age: 48h
Sample ID: 17-3344-4662	Code: 67524436	Client: Notre Dame
Sample Date: 21 May-20 10:30	Material: Zinc sulfate	Project: Special Studies
Receive Date: 21 May-20 13:30	Source: research	
Sample Age: 3h	Station:	

**Linear Interpolation Options**

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1960014	200	Yes	Two-Point Interpolation

**Point Estimates**

Level	mg/L	95% LCL	95% UCL
LC5	8.125	1.283	33.88
LC10	20	0.3158	76.32
LC15	54.35	N/A	65.39
LC20	60.65	45.53	71.92
LC25	66.94	53.96	79.84
LC40	85.81	70.92	108.5
LC50	98.39	79.33	131

**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	Dilution Water	4	0.975	0.9	1	0.025	0.05	5.13%	0.0%	39	40
12.5		4	0.9	0.8	1	0.05774	0.1155	12.8%	7.69%	36	40
25		4	0.825	0.7	1	0.06292	0.1258	15.3%	15.4%	33	40
50		4	0.9	0.8	1	0.04082	0.08165	9.07%	7.69%	36	40
100		4	0.475	0.3	0.6	0.06292	0.1258	26.5%	51.3%	19	40
200		4	0.075	0	0.2	0.04787	0.09574	128.0%	92.3%	3	40

**7d Survival Rate Detail**

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9	1	1	1
12.5		1	0.8	1	0.8
25		1	0.7	0.8	0.8
50		0.9	1	0.9	0.8
100		0.5	0.5	0.3	0.6
200		0.1	0	0	0.2

**7d Survival Rate Binomials**

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	9/10	10/10	10/10	10/10
12.5		10/10	8/10	10/10	8/10
25		10/10	7/10	8/10	8/10
50		9/10	10/10	9/10	8/10
100		5/10	5/10	3/10	6/10
200		1/10	0/10	0/10	2/10

① N/A 6/3/20 E

① Fathead Minnow 7-d Larval Survival and Growth Test

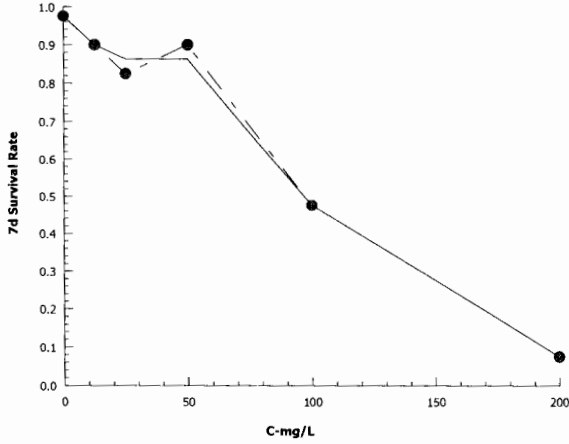
TRE Environmental Strategies

Analysis ID: 00-5331-5017  
Analyzed: 02 Jun-20 8:29

Endpoint: 7d Survival Rate  
Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7  
Official Results: Yes

Graphics



① DAP 6/3/20 E

**CETIS Analytical Report**

*Brine shrimp*

Report Date: 02 Jun-20 08:30 (p 1 of 2)  
 Test Code: 474-071 | 01-6129-1574

① **Fathead Minnow 7-d Larval Survival and Growth Test** TRE Environmental Strategies

Analysis ID: 20-1431-1363	Endpoint: <u>Mean Dry Biomass-mg</u>	CETIS Version: CETISv1.8.7
Analyzed: 02 Jun-20 8:29	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 08-1256-8166	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 21 May-20 13:30	Protocol: EPA/821/R-02-013 (2002)	Diluent: rGSL
Ending Date: 28 May-20 13:20	Species: Artemia franciscana	Brine: Crystal Sea
Duration: 7d	Source: In-House Culture	Age: 48h
Sample ID: 17-3344-4662	Code: 67524436	Client: Notre Dame
Sample Date: 21 May-20 10:30	Material: Zinc sulfate	Project: Special Studies
Receive Date: 21 May-20 13:30	Source: research	
Sample Age: 3h	Station:	

**Linear Interpolation Options**

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
<u>Linear</u>	Linear	1723101	200	Yes	Two-Point Interpolation

**Point Estimates**

Level	mg/L	95% LCL	95% UCL
IC5	0.7706	0.7	0.8789
IC10	1.541	1.4	1.758
IC15	2.312	2.1	2.637
<u>IC20</u>	<u>3.082</u>	<u>2.8</u>	<u>3.515</u>
IC25	3.853	3.5	4.394
IC40	6.165	5.6	7.031
IC50	7.706	7	8.789

*< 1270*

**Mean Dry Biomass-mg Summary**

**Calculated Variate**

C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	0.09925	0.079	0.119	0.008189	0.01638	16.5%	0.0%
12.5		4	0.01875	0.013	0.028	0.003224	0.006449	34.4%	81.1%
25		4	0.0105	0.007	0.014	0.001756	0.003512	33.4%	89.4%
50		4	0.00925	0.005	0.015	0.002175	0.004349	47.0%	90.7%
100		4	0.00175	0	0.004	0.000854	0.001708	97.6%	98.2%
200		4	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
0	Dilution Water	0.079	0.101	0.098	0.119
12.5		0.028	0.017	0.017	0.013
25		0.014	0.007	0.013	0.008
50		0.01	0.015	0.007	0.005
100		0.004	0.001	0.002	0
200		0	0	0	0

*① RRP 6/3/20 E*

*Brine shrimp*

① Fathead Minnow 7-d Larval Survival and Growth Test

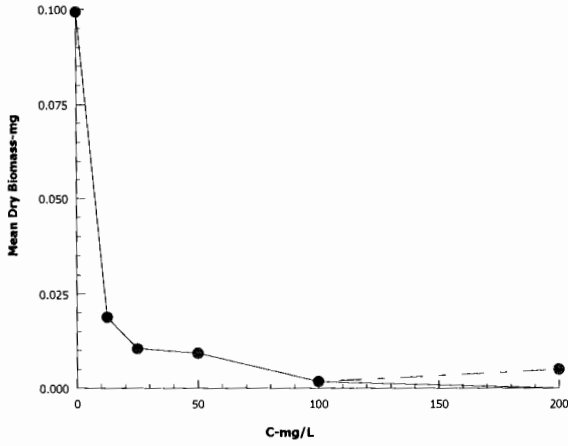
TRE Environmental Strategies

Analysis ID: 20-1431-1363  
Analyzed: 02 Jun-20 8:29

Endpoint: Mean Dry Biomass-mg  
Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7  
Official Results: Yes

Graphics



① *SDP 6/3/20 E*

**CETIS Analytical Report**

Report Date: 02 Jun-20 08:30 (p 1 of 2)  
 Test Code: 474-071 | 01-6129-1574

*Brine Shrimp*

①

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

TRE Environmental Strategies

<b>Analysis ID:</b> 11-2629-5550	<b>Endpoint:</b> Mean Dry Biomass-mg	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 02 Jun-20 8:30	<b>Analysis:</b> Parametric-Control vs Treatments	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-1256-8166	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b> Lab Tech
<b>Start Date:</b> 21 May-20 13:30	<b>Protocol:</b> EPA/821/R-02-013 (2002)	<b>Diluent:</b> rGSL
<b>Ending Date:</b> 28 May-20 13:20	<b>Species:</b> Artemia franciscana	<b>Brine:</b> Crystal Sea
<b>Duration:</b> 7d	<b>Source:</b> In-House Culture	<b>Age:</b> 48h
<b>Sample ID:</b> 17-3344-4662	<b>Code:</b> 67524436	<b>Client:</b> Notre Dame
<b>Sample Date:</b> 21 May-20 10:30	<b>Material:</b> Zinc sulfate	<b>Project:</b> Special Studies
<b>Receive Date:</b> 21 May-20 13:30	<b>Source:</b> research	
<b>Sample Age:</b> 3h	<b>Station:</b>	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	15.0%	<12.5	12.5	NA	

**Dunnett Multiple Comparison Test**

Control	vs C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water	12.5*	12.33	2.29	0.015	6	<0.0001	CDF	Significant Effect
	25*	13.59	2.29	0.015	6	<0.0001	CDF	Significant Effect
	50*	13.78	2.29	0.015	6	<0.0001	CDF	Significant Effect

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.02261669	0.007538896	3	88.41	<0.0001	Significant Effect
Error	0.00102325	8.527083E-05	12			
Total	0.02363994		15			

**Distributional Tests**

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	7.932	11.3	0.0474	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8985	0.841	0.0761	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	Dilution Water	4	0.09925	0.07319	0.1253	0.0995	0.079	0.119	0.008189	16.5%	0.0%
12.5		4	0.01875	0.008489	0.02901	0.017	0.013	0.028	0.003224	34.4%	81.1%
25		4	0.0105	0.004912	0.01609	0.0105	0.007	0.014	0.001756	33.4%	89.4%
50		4	0.00925	0.002329	0.01617	0.0085	0.005	0.015	0.002175	47.0%	90.7%

**Mean Dry Biomass-mg Detail**

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.079	0.101	0.098	0.119
12.5		0.028	0.017	0.017	0.013
25		0.014	0.007	0.013	0.008
50		0.01	0.015	0.007	0.005

① DATA 6/3/20 E

*Brine shrimp*

① Fathead Minnow 7-d Larval Survival and Growth Test

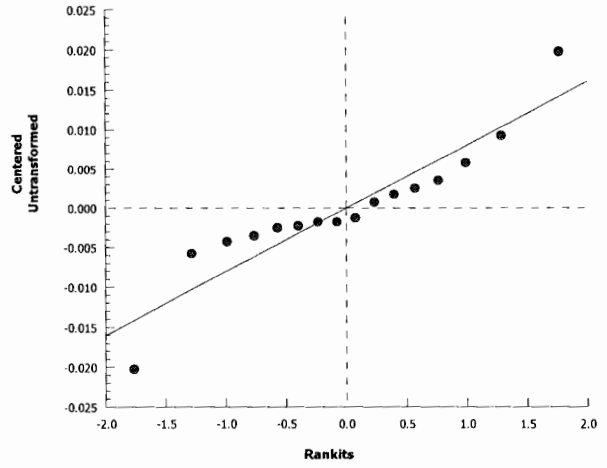
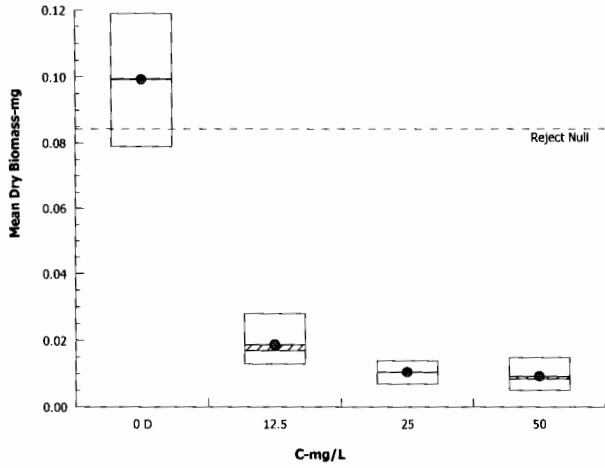
TRE Environmental Strategies

Analysis ID: 11-2629-5550  
Analyzed: 02 Jun-20 8:30

Endpoint: Mean Dry Biomass-mg  
Analysis: Parametric-Control vs Treatments

CETIS Version: CETISv1.8.7  
Official Results: Yes

Graphics



① *DAW* 6/3/20 E