

July 2, 2020

Mr. Christopher Bittner
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 Utah Dept. of Environmental Quality
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Dr. Gary Belovsky
 Environ. Res. Center & Dept. Biol Sci.
 University of Notre Dame
 Notre Dame, IN 46556

Subject: Results of Analytical Data for Experiment #20

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) ^a	Alk. (mg/L) ^a	Spec. Cond. (µS/cm)	TRC (mg/L) ^b	NH ₃ -N (mg/L)	Salinity (ppt)
RW#13948	7.9	NM	NM	129,700	NM	NM	120

^aAs CaCO₃

^bTotal residual chlorine

Results of Zinc Analysis

Nominal Value (mg/L)	Total Zinc (mg/L)			Mean Value	Percent of Nominal
	Day 0 New Solution	Day 1 Old Solution	Day 6 New Solution		
0	0.021 U	0.021 U	0.021 U	0.021	---
0.5	0.386	0.401	0.398	0.395	79%
1	0.819	0.920	0.692	0.810	81%
2	1.62	1.81	1.43	1.62	81%
4	3.15	3.66	2.80	3.20	80%
8	6.80	7.76	5.34	6.63	83%
16	13.6	15.6	11.0	13.40	84%

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

Average measured zinc concentrations were then used to recalculate test survival and growth endpoints on a measured basis.

Test Endpoints

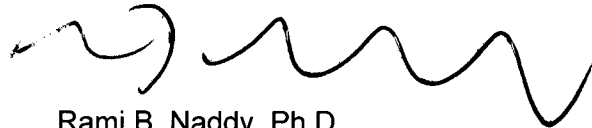
Basis	Survival NOEC	Survival IC20	Growth NOEC	Growth IC20
Nominal	>16	>16	1	1.31 (0.518-1.78)
Measured	>13.4	>13.4	0.81	1.061 (0.442-1.403)

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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Manager / Environmental Toxicologist
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17001-474-076

Attachment

cc: David Pillard, TRE

Zinc Chronic (mg/L)						
Nominal Value (mg)	Day 0 New (mg)	Day 1 Old (mg)	Day 6 New (mg)	Mean (mg)	Percent of Nominal	
0	<0.21 (ND)	<0.21 (ND)	<0.21 (ND)	0.21	100%	
0.5	0.386	0.401	0.398	0.395	79.00%	
1	0.819	0.92	0.692	0.81	81.03%	
2	1.62	1.81	1.43	1.62	81.00%	
4	3.15	3.66	2.8	3.20	80.08%	
8	6.8	7.76	5.34	6.63	82.83%	
16	13.6	15.6	11	13.40	83.75%	

QA: PAP 7/1/20

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Species: Artemia franciscana

Project Number: 14001-474

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		1.12211	1.12335	0.00124	0.00124	10	0.124	0.1065	10	0.124	0.1092
	B		1.13459	1.13558	0.00099	0.00099	10	0.099		9	0.110	
	C		1.13562	1.13669	0.00107	0.00107	10	0.107		10	0.107	
	D		1.12979	1.13075	0.00096	0.00096	10	0.096		10	0.096	
0.5 mg/L	A		1.13844	1.13936	0.00092	0.00092	10	0.092	0.1015	10	0.092	0.1069
	B		1.12469	1.12569	0.00100	0.00100	10	0.100		9	0.111	
	C		1.12873	1.12966	0.00093	0.00093	10	0.093		9	0.103	
	D		1.12899	1.13020	0.00121	0.00121	10	0.121		10	0.121	
1 mg/L	A		1.13584	1.13671	0.00087	0.00087	10	0.087	0.0933	10	0.087	0.0933
	B		1.14575	1.14679	0.00104	0.00104	10	0.104		10	0.104	
	C		1.13565	1.13659	0.00094	0.00094	10	0.094		10	0.094	
	D		1.13027	1.13115	0.00088	0.00088	10	0.088		10	0.088	
2 mg/L	A		1.13153	1.13229	0.00076	0.00076	10	0.076	0.0673	10	0.076	0.0673
	B		1.13680	1.13742	0.00062	0.00062	10	0.062		10	0.062	
	C		1.14278	1.14340	0.00062	0.00062	10	0.062		10	0.062	
	D		1.13326	1.13395	0.00069	0.00069	10	0.069		10	0.069	
4 mg/L	A		1.11502	1.11539	0.00037	0.00037	10	0.037	0.0378	10	0.037	0.0389
	B		1.13572	1.13612	0.00040	0.00040	10	0.040		10	0.040	
	C		1.15180	1.15220	0.00040	0.00040	10	0.040		9	0.044	
	D		1.13434	1.13468	0.00034	0.00034	10	0.034		10	0.034	
8 mg/L	A		1.14068	1.14087	0.00019	0.00019	10	0.019	0.0215	10	0.019	0.0215
	B		1.12880	1.12908	0.00028	0.00028	10	0.028		10	0.028	
	C		1.13670	1.13688	0.00018	0.00018	10	0.018		10	0.018	
	D		1.13615	1.13636	0.00021	0.00021	10	0.021		10	0.021	

A	1.13263	1.13288	0.00025	0.00025	10	0.025	0.0215	10	0.025	0.0229
B	1.14322	1.14341	0.00019	0.00019	10	0.019		8	0.024	
C	1.13565	1.13598	0.00033	0.00033	10	0.033		10	0.033	
D	1.14116	1.14125	0.00009	0.00009	10	0.009		9	0.010	
Blank	1.13452	1.13454	0.00002							

Project Number: 14001-474

Species: Artemia franciscana

QA: DRP 7/1/20

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%
0.5 mg/L	4	0.9	1.0	0.9500	0.0577	6.077%
1 mg/L	4	1.0	1.0	1.0000	0.0000	0.000%
2 mg/L	4	1.0	1.0	1.0000	0.0000	0.000%
4 mg/L	4	0.9	1.0	0.9750	0.0500	5.128%
8 mg/L	4	1.0	1.0	1.0000	0.0000	0.000%
16 mg/L	4	0.8	1.0	0.9250	0.0957	10.351%

Summary Statistics for Growth Data (dry wt per original)

Treatment	N	Min	Max	Mean	SD	C.V.
rGSL	4	0.096	0.124	0.1065	0.0126	11.790%
0.5 mg/L	4	0.092	0.121	0.1015	0.0135	13.279%
1 mg/L	4	0.087	0.104	0.0933	0.0078	8.370%
2 mg/L	4	0.062	0.076	0.0673	0.0067	9.966%
4 mg/L	4	0.034	0.040	0.0378	0.0029	7.609%
8 mg/L	4	0.018	0.028	0.0215	0.0045	20.973%
16 mg/L	4	0.009	0.033	0.0215	0.0101	47.051%

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

Treatment	N	Min	Max	Mean	SD	C.V.
rGSL	4	0.096	0.124	0.1092	0.0115	10.553%
0.5 mg/L	4	0.092	0.121	0.1069	0.0123	11.477%
1 mg/L	4	0.087	0.104	0.0933	0.0078	8.370%
2 mg/L	4	0.062	0.076	0.0673	0.0067	9.966%
4 mg/L	4	0.034	0.044	0.0389	0.0045	11.466%
8 mg/L	4	0.018	0.028	0.0215	0.0045	20.973%
16 mg/L	4	0.010	0.033	0.0229	0.0095	41.630%

CETIS Analytical Report

Report Date: 30 Jun-20 10:35 (p 1 of 2)

Test Code: 474-076 | 07-1158-8845

Brine shrimp

Fathead Minnow 7-d Larval Survival and Growth Test

TRE Environmental Strategies

Analysis ID: 19-6092-5070	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv1.8.7
Analyzed: 30 Jun-20 10:34	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 04-2472-1259	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 04 Jun-20 13:45	Protocol: EPA/821/R-02-013 (2002)	Diluent: rGSL
Ending Date: 11 Jun-20 13:20	Species: Artemia franciscana <i>(i)</i>	Brine: Crystal Sea
Duration: 7d	Source: Hog Island Oyster Co. <i>TRE</i>	Age: 48h
Sample ID: 00-8320-5339	Code: 4F59CDB	Client: Notre Dame
Sample Date: 04 Jun-20 10:35	Material: Zinc sulfate	Project: Special Studies
Receive Date: 13 Jun-20 13:45	Source: Discharge Monitoring Report	
Sample Age: 3h	Station:	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	586445	200	Yes	Two-Point Interpolation

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC5	0.4113	0.1772	1.133
IC10	0.6792	0.1045	1.168
IC15	0.8949	0.0631	1.256
IC20	1.061	0.4422	1.403
IC25	1.227	0.7176	1.563
IC40	1.799	1.271	2.292
IC50	2.37	1.723	2.721

Mean Dry Biomass-mg Summary

Calculated Variate

C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0.21	Dilution Water	4	0.1065	0.096	0.124	0.006278	0.01256	11.8%	0.0%
0.395		4	0.1015	0.092	0.121	0.006739	0.01348	13.3%	4.69%
0.81		4	0.09325	0.087	0.104	0.003902	0.007805	8.37%	12.4%
1.62		4	0.06725	0.062	0.076	0.003351	0.006702	9.97%	36.9%
3.2		4	0.03775	0.034	0.04	0.001436	0.002872	7.61%	64.6%
6.63		4	0.0215	0.018	0.028	0.002255	0.004509	21.0%	79.8%
13.4		4	0.0215	0.009	0.033	0.005058	0.01012	47.1%	79.8%

Mean Dry Biomass-mg Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.21	Dilution Water	0.124	0.099	0.107	0.096
0.395		0.092	0.1	0.093	0.121
0.81		0.087	0.104	0.094	0.088
1.62		0.076	0.062	0.062	0.069
3.2		0.037	0.04	0.04	0.034
6.63		0.019	0.028	0.018	0.021
13.4		0.025	0.019	0.033	0.009

DAP 7/1/20 E

CETIS Analytical Report

Report Date: 30 Jun-20 10:35 (p 2 of 2)

Test Code: 474-076 | 07-1158-8845

① *Brine shrimp*

Fathead Minnow 7-d Larval Survival and Growth Test

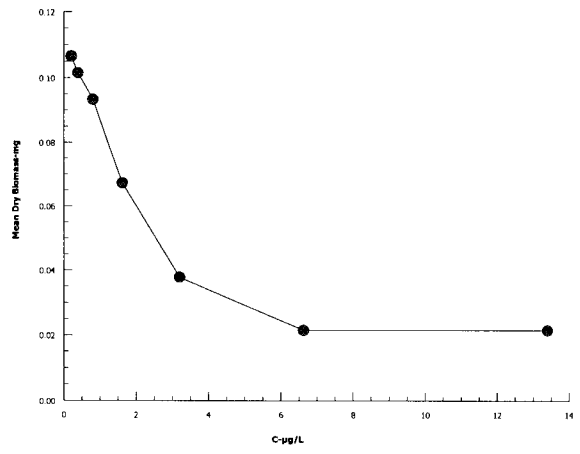
TRE Environmental Strategies

Analysis ID: 19-6092-5070
Analyzed: 30 Jun-20 10:34

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

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



① *DAP 7/1/20 E*

June 17, 2020

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Dr. Gary Belovsky
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Notre Dame, IN 46556

Subject: Results of Short-term Chronic Brine Shrimp Experiment #20

Mr. Bittner/ Dr. Belovsky:

Below is a summary of the short-term chronic brine shrimp experiment initiated on June 4, 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 chronic test as sublethal endpoints were significantly lower than survival endpoints:

- 0.5, 1, 2, 4, 8, and 16 mg/L

The results of these studies will help understand the sub lethal toxicity of zinc to brine shrimp. 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 YTC¹
- 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.

¹ 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) ^a	Alk. (mg/L) ^a	Spec. Cond. (μ S/cm)	TRC (mg/L) ^b	NH ₃ -N (mg/L)	Salinity (ppt)
RW#13948	7.9	NM	NM	129,700	NM	NM	120

^aAs CaCO₃

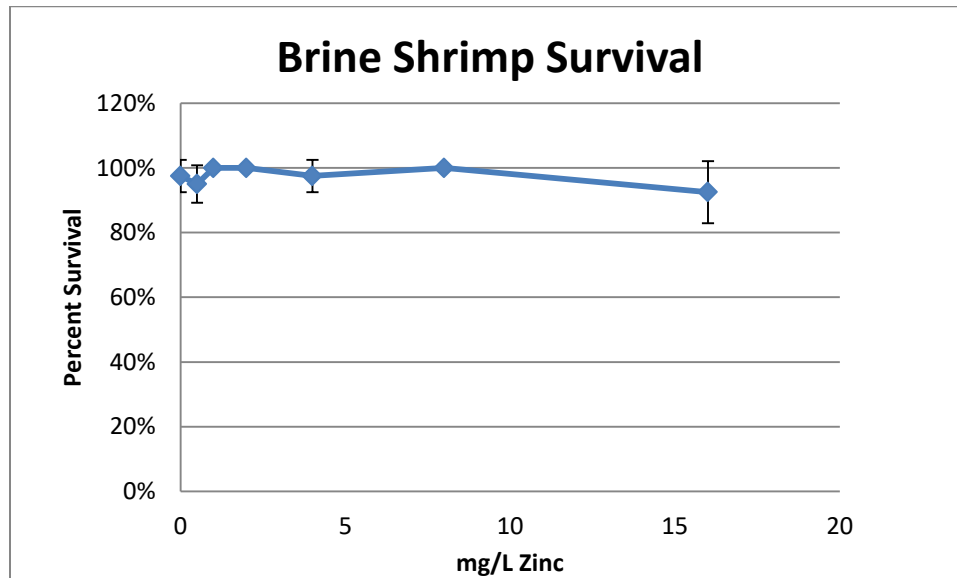
^bTotal residual chlorine

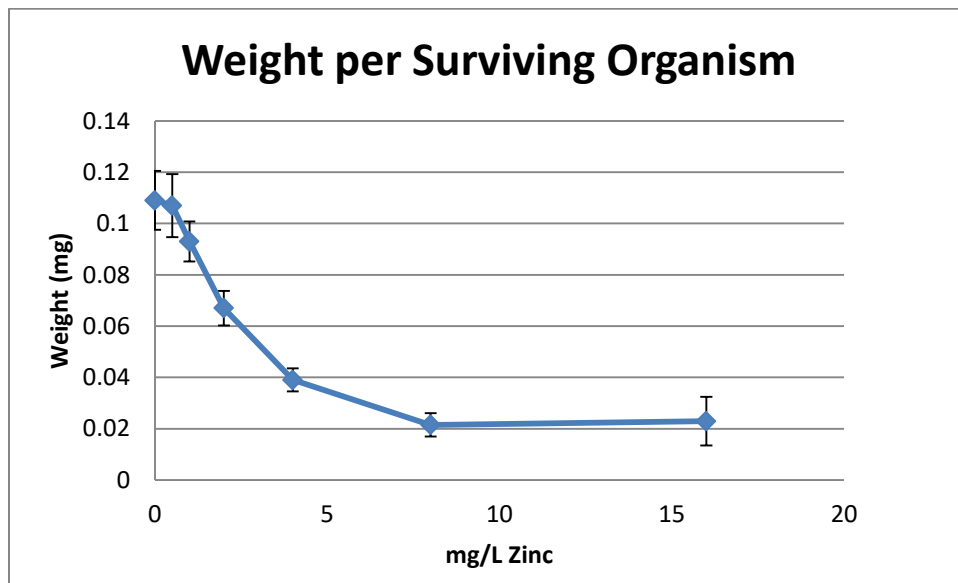
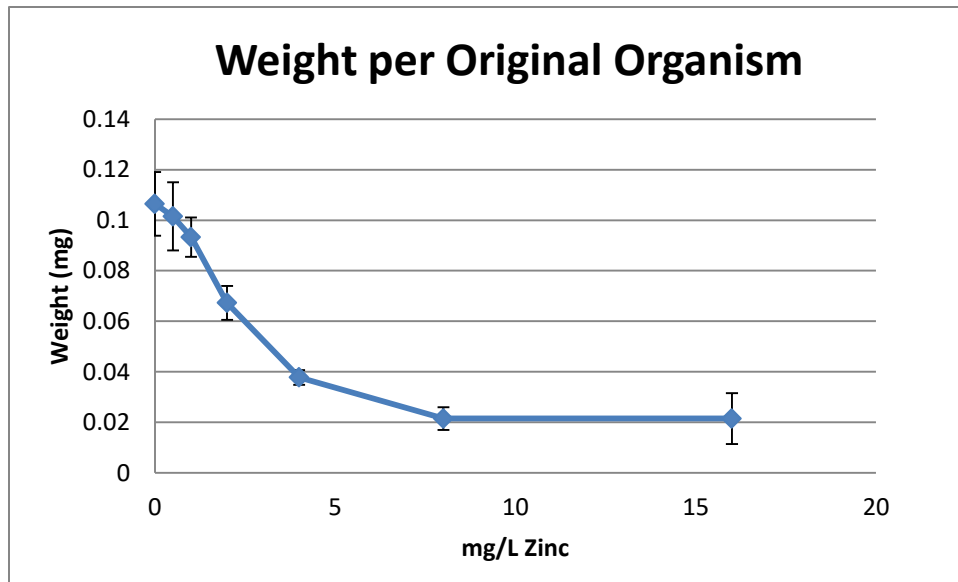
Test activities:

- 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.





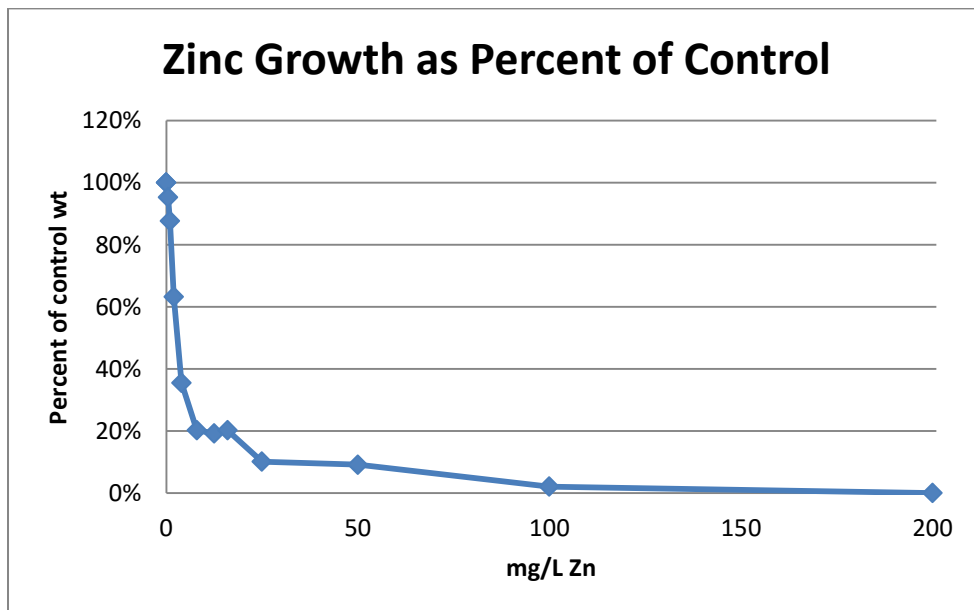
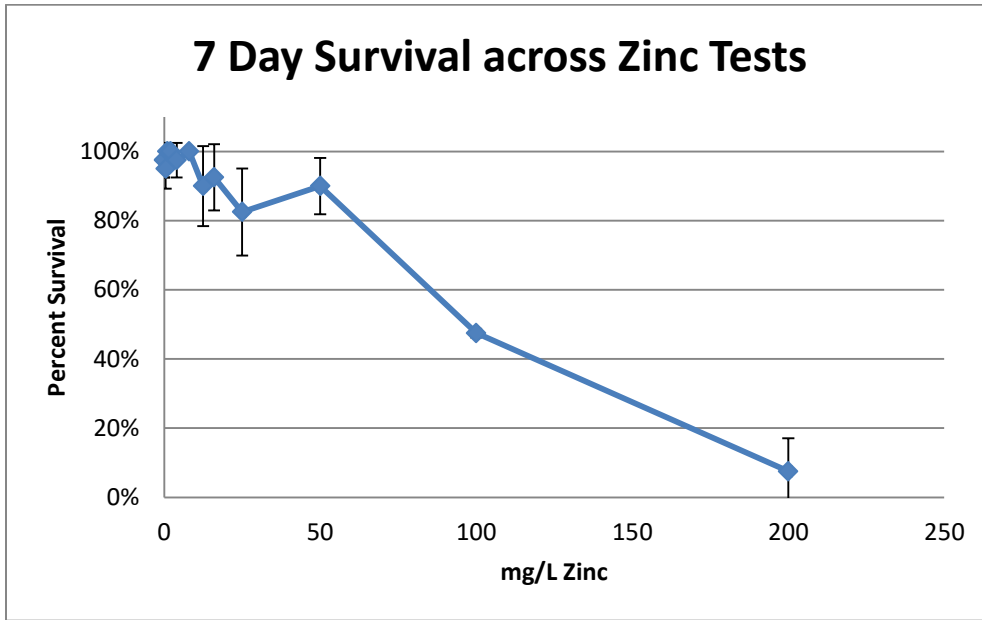
Test Endpoints

Study	Test Endpoints (mg Zn/L, nominal)					
	Survival NOEC	Survival LOEC	Survival IC20	Growth NOEC	Growth LOEC	Growth IC20
7-Day	16	>16	>16	1	2	1.31 (0.518-1.78)

Survival endpoints were determined in the previous zinc test (17001-474-071) and are summarized in the table below:

Study	Test Endpoints (mg Zn/L, nominal)		
	Survival NOEC	Survival LOEC	Survival IC20
7-Day	50	100	60.65 (45.53-71.92)

Survival and growth data from both tests are presented in the graphs below. Growth has been normalized as percentage of control organism weight:



Summary and findings:

- Organism survival was $\geq 90\%$ for the control.
- No survival effect was apparent in the zinc treatments used in this test, which is consistent with the previous zinc studies.
- Growth was significantly more sensitive than survival for zinc.

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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Rami B. Naddy, Ph.D.
Manager / Environmental Toxicologist
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17001-474-076
Attachment

cc: David Pillard, TRE

TOXICITY DATA PACKAGE COVER SHEET

QA: DAP 6/16/20

Test Type: Chronic Project Number: 17001-474-076
Test Substance: Zinc (ZnSO4) Species: Artemia franciscana
Dilution Water: rGSL Organism Lot or Batch Number: 060220
Concurrent Control Water: NA Age: 48HR (48 hr) Supplier: TRE
Date and Time Test Began: 6/4/20 @ 1345 Date and Time Test Ended: 6/11/20 @ 1320
Protocol Number: _____ Investigator(s): EG/CPM/HR/JAF/ES/EN

Background Information

Type of Test: Static-Renewal (Daily) pH control?: Yes No
If yes, give % CO₂: 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 YTC Feeding Frequency: Initiation and Renewals

Test Substance Characterization Parameters and Frequency:

Hardness: Test Initiation Alkalinity: Test Initiation NH₃: Test Initiation TRC: Test Initiation
pH: Daily Conductivity: Daily
Test Concentrations (Volume:Volume): rGSL, 0.5, 1, 2, 4, 8, and 16 mg/L as Zn
Agency Summary Sheet(s)?: None

Reference Toxicant Data: Test Dates: _____ to _____ IC₂₅: _____
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
New Analytical on Days 0 and 6, old analytical on Day 1

Appropriate correction factors have been applied to all temperatures recorded in this data package
Study Director Initials: AB Date: 6/3/20

TEST SUBSTANCE USAGE LOG

Project Number: 17001-474-076

QA: ~~HR~~ 6/16/20

	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	13948			
Concurrent Control Water RW#	NA			
Date(s) Used	6/4/20	6/8/20		
	6/5/20	6/9/20		
	6/6/20	6/10/20		
	6/7/20			

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						
0.5	11	339	350						
1	22	328	350						
2	44	306	350						
4	88	263	350						
8	175	175	350						
16	350	0	350						
	689	1761	2450						
Initials / Date	By 6/4/20 Mixed BS								
Initials / Date	HR 6/5/20 " "								
Initials / Date	AF 6/6/20 " "								
Initials / Date	HR 6/7/20 " "								
Initials / Date	By 6/8/20 " "								
Initials / Date	HR 6/9/20 " "								
Initials / Date	By 6/10/20 " "								
Initials / Date									

Artemia franciscana
CHRONIC BIOLOGICAL DATA

QA: Ver 6/16/20

Project Number: 17001-474-076

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	10	10	10	10	10	97.5%	
	B	10	10	9	9	9	9	9	9		
	C	10	10	10	10	10	10	10	10		
	D	10	10	10	10	10	10	10	10		
0.5	A	10	10	10	10	10	10	10	10	95%	
	B	10	10	10	9	9	9	9	9		
	C	10	9	9	9	9	9	9	9		
	D	10	10	10	10	10	10	10	10		
1	A	10	10	10	10	10	10	10	10*	*1 weak org 100%	
	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		
2	A	10	10	10	10	10	10	10	10	100%	
	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		
4	A	10	10	10	10	10	10	10	10	97.5%	
	B	10	10	10	10	10	10	10	10		
	C	10	10	10	9	10-	10-	10-	9		-1 weak org
	D	10	10	10	10	10	10	10	10		
8	A	10	10	10	10	10	10	10	10	100%	
	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		
16	A	10	10	10	10	10	10	10	10	92.5%	
	B	10	9	9	9	9	9	9	8		
	C	10	10	10	10	10	10	10	10		
	D	10	10	10	10	10	10	10	9		
Date:	6/4/20	6/5/20	6/6/20	6/7/20	6/8/20	6/9/20	6/10/20	6/11/20			
Time:	1345	1450	1440	1145	1430	1305	1340	1320			
Initials:	CP/AS	AF	CP	ES	EN	RF	EN	EN			

CHRONIC CHEMICAL DATA (INITIAL)

QA: DAF 6/16/20

Project Number:	17001-474-076
Test Species:	<i>Artemia franciscana</i>

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	7.8	7.9	8.1	7.9	8.0	7.8		FM27	
D.O. (mg/L)	5.0	5.0	5.0	5.1	4.8	4.9	5.0		17	
Temp. (°C)	20	20	20	20	20	20	20		IR1	
Cond. (µS/cm)	129,700	132,300	128,100	129,700	133,100	140,500	138,100		15	
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH ₃ (mg/L)										
Conc.: 0.5										
pH	7.9	7.8	7.9	8.0	7.9	8.0	7.8			
D.O. (mg/L)	4.9	4.9	4.9	5.0	4.8	4.9	5.0			
Temp. (°C)	20	20	20	20	20	20	20			
Cond. (µS/cm)	130,800	133,500	128,000	130,300	133,400	135,900	138,900			
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH ₃ (mg/L)										
Conc.: 1										
pH	7.9	7.8	7.8	7.9	7.9	7.9	7.8			
D.O. (mg/L)	4.9	4.9	4.9	5.0	4.8	4.9	5.0			
Temp. (°C)	20	20	20	20	20	20	20			
Cond. (µS/cm)	130,700	133,700	127,700	131,100	133,400	134,400	138,900			
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH ₃ (mg/L)										
Conc.: 2										
pH	7.8	7.8	7.8	7.9	7.9	7.9	7.8			
D.O. (mg/L)	4.9	4.9	4.9	5.0	4.8	4.9	5.0			
Temp. (°C)	20	20	20	20	20	20	20			
Cond. (µS/cm)	130,600	133,700	127,700	130,700	133,600	133,800	138,800			
Date:	6/4/20	6/5/20	6/6/20	6/7/20	6/8/20	6/9/20	6/10/20			
Time:	1335	1425	1425	1125	1420	1155	1325			
Initials:	CP	AF	CP	ES	EN	AF	EN			

Note: Hardness, alkalinity, TRC, and NH₃ 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. DAF 6/8/20 E @ EN 6/10/20 E

CHRONIC CHEMICAL DATA (INITIAL)

QA: DAP 6/16/20

Project Number:	17001-474-076
Test Species:	<i>Artemia franciscana</i>

mg/L	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter #	Remarks
Conc.: 4									All Conc.	
pH	7.8	7.8	7.8	7.9	7.8	7.9	7.8			
D.O. (mg/L)	4.9	4.9	4.9	5.0	4.8	4.9	5.0			
Temp. (°C)	20	20	20	20	20	20	20			
Cond. (µS/cm)	130,700	133,400	129,400	132,300	133,200	134,100	139,100			
Conc.: 8										
pH	7.8	7.8	7.8	7.9	7.8	7.8	7.8			
D.O. (mg/L)	4.9	4.9	4.9	5.0	4.8	5.0	5.0			
Temp. (°C)	20	20	20	20	20	20	20			
Cond. (µS/cm)	130,500	133,700	127,600	130,200	133,500	134,000	139,000			
Conc.: 16										
pH	7.8	7.8	7.7	7.8	7.8	7.8	7.7			
D.O. (mg/L)	4.9	4.9	4.9	5.0	4.8	5.0	5.0			
Temp. (°C)	20	20	20	20	20	20	20			
Cond. (µS/cm)	130,000	133,200	127,700	131,000	13300	135,000	138,800			
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH ₃ (mg/L)										
Date:	6/4/20	6/5/20	6/6/20	6/7/20	6/8/20	6/9/20	6/10/20			
Time:	1335	1425	1425	1125	1420	1155	1325			
Initials:	CP	AF	CP	ES	EN	PS	EN			

Note: Hardness, alkalinity, TRC, and NH₃ 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.

CAF 6/10/20E

CHRONIC CHEMICAL DATA (FINAL)

QA: WP 6/16/20

Project Number:	17001-474-076
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								120700		* conductivity 15
pH	7.8	7.9	7.6	7.7	7.8	7.7	7.7		FM27	
D.O. (mg/L)	4.7	4.8	4.8	4.8	4.6	5.1	5.0		17	
Temp (°C)	20	20	21	20	21	19	20		413	
Conc.: 0.5								120900		* conductivity
pH	7.8	7.8	7.6	7.6	7.8	7.8	7.7			
D.O. (mg/L)	4.7	4.7	4.8	4.7	4.5	5.1	4.6			
Temp (°C)	20	21	21	21	21	19	20			
Conc.: 1								121100		* conductivity
pH	7.8	7.8	7.6	7.6	7.8	7.8	7.7			
D.O. (mg/L)	4.6	4.6	4.6	4.6	4.5	5.0	4.6			
Temp (°C)	20	21	21	21	21	19	20			
Conc.: 2								121700		* conductivity
pH	7.8	7.8	7.6	7.6	7.8	7.8	7.7			
D.O. (mg/L)	4.7	4.6	4.6	4.5	4.5	5.0	4.5			
Temp (°C)	20	21	21	21	21	19	20			
Conc.: 4								121500		* conductivity
pH	7.8	7.8	7.7	7.7	7.8	7.8	7.7			
D.O. (mg/L)	4.7	4.7	4.6	4.6	4.6	5.0	4.5			
Temp (°C)	20	21	21	21	21	19	20			
Conc.: 8								120800		* conductivity
pH	7.8	7.8	7.7	7.6	7.8	7.9	7.8			
D.O. (mg/L)	4.8	4.7	4.7	4.6	4.7	5.0	4.7			
Temp (°C)	20	21	21	21	21	19	20			
Conc.: 16								119000		* conductivity
pH	7.8	7.8	7.6	7.6	7.7	7.9	7.7			
D.O. (mg/L)	4.8	4.7	4.8	4.6	4.8	5.0	4.8			
Temp (°C)	20	21	21	21	21	19	20			
Date:	6/5/20	6/6/20	6/7/20	6/8/20	6/9/20	6/10/20	6/11/20			
Time:	1455	1515	1210	1415	1205	1335	1310			
Initials:	AF	CP	ES	EN	PE	EN	EN			

① PE 6/9/20
 ② EN 6/11/20 WP

DAILY TOXICITY TEST LOG

QA: *ASP* 6/10/20

Project Number:	17001-474-076
Test Species:	<i>Artemia franciscana</i>

General Comments		Feeding 72.5 ug/l Chla 0.3 ml YTC	Initials/Date
	Random Chart: "D" Min/Max Thermometer # M-15		
Test Day 0	Test Solution Mixed at: 1320 Test Organisms Added at: 1345 Spiked @ 1635 ✓	Fed @ 1635 ✓	CP 6/4/20
Test Day 1	Real Time: 22 °C Min-Max Range: 21-22 °C Spiked @ 1145	Fed @ 1145 HR	AF 6/5/20
Test Day 2	Real Time: 22 °C Min-Max Range: 21-22 °C Spiked @ 1115	Fed @ 1115 AF	CP 6/6/20
Test Day 3	Real Time: 22 °C Min-Max Range: 21-22 °C Spiked @ 0845	Fed @ 0845 HR	ES 6/7/20
Test Day 4	Real Time: 22 °C Min-Max Range: 21-22 °C Spiked @ 0750 BS transitioned in all concentrations b/n day 3&day 4	Fed @ 0750 R✓	EN 6/8/20
Test Day 5	Real Time: 21 °C Min-Max Range: 21-22 °C Spiked @ 0750	Fed @ 0750 HR	Rg 6/9/20
Test Day 6	Real Time: 20 °C Min-Max Range: 20-21 °C Spiked @ 0730	Fed @ 0730 ✓	EN 6/10/20
Test Day 7	Real Time: 21 °C Min-Max Range: 20-21 °C	Fed @ None	EN 6/11/20

QA: 644 6/16/20

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: <u>17001-474-076</u>		Test Substance: <u>ZINC (ZnSO4)</u>		Comments:								
Species: <u>Artemia franciscana</u>		Analyst Tare: <u>Slk</u>		Analytical Balance ID: <u>SU17#1</u>								
Date/Time of Tare Wt.: <u>6/11/20 @ 0940</u>		Date/Time of Gross Wt.: <u>6/13/20 @ 1000</u>		Dried in Oven # <u>3</u> from Date: <u>6/11/20</u> Time: <u>1505</u> to Date: <u>6/13/20</u> Time: <u>0810</u>								
Boat No.	Treatment	Rep.	Length Units:	Weight Type (Circle):	Wet	Blot Dry	Dry (60-90°C)	Dry (>100°C)	AFDW (>500°C)	Mean Wt. per Treatment (mg) (Original)	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)
				Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g) ¹	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	No. of Surv. Organisms	Mean Wt. per Treatment (mg) (Surviving)
	0	A		1.12211	1.12335	0.00124					10	
		B		1.13459	1.13558	0.00099					9	
		C		1.13562	1.13669	0.00107					10	
		D		1.12979	1.13075	0.00096					10	
	0.5	A		1.13844	1.13936	0.00092					10	
		B		1.12469	1.12569	0.00100					9	
		C		1.12873	1.12966	0.00093					9	
		D		1.12899	1.13020	0.00121					10	
	1	A		1.13584	1.13671	0.00087					10	
		B		1.14575	1.14679	0.00104					10	
		C		1.13565	1.13659	0.00094					10	
		D		1.13027	1.13115	0.00088					10	
				1.13452	1.13454	0.00002						
Blank												
Range												
Mean												
Test Solution Volume:										Loading Rate:		

Add in weight loss of blank boat, if appropriate.

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 17001-474-076		Test Substance: Zinc (ZnSO4)		Comments:					
Species: <i>Artemia franciscana</i>		Analyst Tare: Sk		Analytical Balance ID: Surf #1					
Date/Time of Tare Wt.: 6/11/20 @ 0940		Date/Time of Gross Wt.: 6/13/20 @ 1000		Dried in Oven # 3 from Date: 6/11/20 Time: 1505 to Date: 6/13/20 Time: 0800					
Boat No.	Treatment	Rep.	Length Units:	Weight Type (Circle):			Lot or Batch Number:	Mean Wt. per Treatment (mg) (Surviving)	
				Tare Weight (g)	Wet	Blot Dry Dry (60-90°C) Dry (>100°C) AFDW (>500°C)			No. of Surv. Organisms
				Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g) ¹	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)
	2	A		1.13153	1.13229	0.00070			
		B		1.13680	1.13742	0.00062			
		C		1.14278	1.14340	0.00062			
		D		1.13326	1.13395	0.00069			
	4	A		1.11502	1.11539	0.00037			
		B		1.13572	1.13612	0.00040			
		C		1.15180	1.15220	0.00040			
	8	D		1.13434	1.13468	0.00034			
		A		1.14068	1.14087	0.00019			
		B		1.12880	1.12908	0.00028			
		C		1.13670	1.13688	0.00018			
	D		1.13615	1.13634	0.00021				
	Blank								
	Range								
	Mean								

Test Solution Volume: _____ Loading Rate: _____

¹ Add in weight loss of blank boat, if appropriate.

CAF 6/13/20

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 14001-474 Species: Artemia franciscana

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		1.12211	1.12335	0.00124	0.00124	10	0.124	0.1065	10	0.124	0.1092
	B		1.13459	1.13558	0.00099	0.00099	10	0.099		9	0.110	
	C		1.13562	1.13669	0.00107	0.00107	10	0.107		10	0.107	
	D		1.12979	1.13075	0.00096	0.00096	10	0.096		10	0.096	
0.5 mg/L	A		1.13844	1.13936	0.00092	0.00092	10	0.092	0.1015	10	0.092	0.1069
	B		1.12469	1.12569	0.00100	0.00100	10	0.100		9	0.111	
	C		1.12873	1.12966	0.00093	0.00093	10	0.093		9	0.103	
	D		1.12899	1.13020	0.00121	0.00121	10	0.121		10	0.121	
1 mg/L	A		1.13584	1.13671	0.00087	0.00087	10	0.087	0.0933	10	0.087	0.0933
	B		1.14575	1.14679	0.00104	0.00104	10	0.104		10	0.104	
	C		1.13565	1.13659	0.00094	0.00094	10	0.094		10	0.094	
	D		1.13027	1.13115	0.00088	0.00088	10	0.088		10	0.088	
2 mg/L	A		1.13153	1.13229	0.00076	0.00076	10	0.076	0.0673	10	0.076	0.0673
	B		1.13680	1.13742	0.00062	0.00062	10	0.062		10	0.062	
	C		1.14278	1.14340	0.00062	0.00062	10	0.062		10	0.062	
	D		1.13326	1.13395	0.00069	0.00069	10	0.069		10	0.069	
4 mg/L	A		1.11502	1.11539	0.00037	0.00037	10	0.037	0.0378	10	0.037	0.0389
	B		1.13572	1.13612	0.00040	0.00040	10	0.040		10	0.040	
	C		1.15180	1.15220	0.00040	0.00040	10	0.040		9	0.044	
	D		1.13434	1.13468	0.00034	0.00034	10	0.034		10	0.034	
8 mg/L	A		1.14068	1.14087	0.00019	0.00019	10	0.019	0.0215	10	0.019	0.0215
	B		1.12880	1.12908	0.00028	0.00028	10	0.028		10	0.028	
	C		1.13670	1.13688	0.00018	0.00018	10	0.018		10	0.018	
	D		1.13615	1.13636	0.00021	0.00021	10	0.021		10	0.021	

A	1.13263	1.13288	0.00025	0.00025	10	0.025	0.0215	10	0.025	0.0229
B	1.14322	1.14341	0.00019	0.00019	10	0.019		8	0.024	
C	1.13565	1.13598	0.00033	0.00033	10	0.033		10	0.033	
D	1.14116	1.14125	0.00009	0.00009	10	0.009		9	0.010	
Blank	1.13452	1.13454	0.00002							

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%
0.5 mg/L	4	0.9	1.0	0.9500	0.0577	6.077%
1 mg/L	4	1.0	1.0	1.0000	0.0000	0.000%
2 mg/L	4	1.0	1.0	1.0000	0.0000	0.000%
4 mg/L	4	0.9	1.0	0.9750	0.0500	5.128%
8 mg/L	4	1.0	1.0	1.0000	0.0000	0.000%
16 mg/L	4	0.8	1.0	0.9250	0.0957	10.351%

Summary Statistics for Growth Data (dry wt per original)

Treatment	N	Min	Max	Mean	SD	C.V.
rGSL	4	0.096	0.124	0.1065	0.0126	11.790%
0.5 mg/L	4	0.092	0.121	0.1015	0.0135	13.279%
1 mg/L	4	0.087	0.104	0.0933	0.0078	8.370%
2 mg/L	4	0.062	0.076	0.0673	0.0067	9.966%
4 mg/L	4	0.034	0.040	0.0378	0.0029	7.609%
8 mg/L	4	0.018	0.028	0.0215	0.0045	20.973%
16 mg/L	4	0.009	0.033	0.0215	0.0101	47.051%

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

Treatment	N	Min	Max	Mean	SD	C.V.
rGSL	4	0.096	0.124	0.1092	0.0115	10.553%
0.5 mg/L	4	0.092	0.121	0.1069	0.0123	11.477%
1 mg/L	4	0.087	0.104	0.0933	0.0078	8.370%
2 mg/L	4	0.062	0.076	0.0673	0.0067	9.966%
4 mg/L	4	0.034	0.044	0.0389	0.0045	11.466%
8 mg/L	4	0.018	0.028	0.0215	0.0045	20.973%
16 mg/L	4	0.010	0.033	0.0229	0.0095	41.630%

CETIS Analytical Report

Report Date: 15 Jun-20 15:23 (p 1 of 2)
 Test Code: 474-076 | 07-1158-8845

① Brine shrimp

Fathead Minnow 7-d Larval Survival and Growth Test

TRE Environmental Strategies

Analysis ID: 05-2669-5393	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv1.8.7
Analyzed: 15 Jun-20 15:23	Analysis: Parametric-Control vs Treatments	Official Results: Yes
Batch ID: 04-2472-1259	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 04 Jun-20 13:45	Protocol: EPA/821/R-02-013 (2002)	Diluent: rGSL
Ending Date: 11 Jun-20 13:20	Species: Artemia franciscana ①	Brine: Crystal Sea
Duration: 7d	Source: Hog Island Oyster Co. TRE	Age: 48h
Sample ID: 00-8320-5339	Code: 4F59CDB	Client: Notre Dame
Sample Date: 04 Jun-20 10:35	Material: Zinc sulfate	Project: Special Studies
Receive Date: 13 Jun-20 13:45	Source: Discharge Monitoring Report	
Sample Age: 3h	Station:	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	14.7%	1	2	1.414	

Dunnett Multiple Comparison Test

Control	vs C- ^{mg} / _{μg} L ①	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water	0.5	0.7795	2.45	0.016	6	0.5557	CDF	Non-Significant Effect
	1	2.066	2.45	0.016	6	0.1019	CDF	Non-Significant Effect
	2*	6.119	2.45	0.016	6	<0.0001	CDF	Significant Effect
	4*	10.72	2.45	0.016	6	<0.0001	CDF	Significant Effect
	8*	13.25	2.45	0.016	6	<0.0001	CDF	Significant Effect
	16*	13.25	2.45	0.016	6	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.03351986	0.005586643	6	67.88	<0.0001	Significant Effect
Error	0.00172825	8.229762E-05	21			
Total	0.03524811		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	7.839	16.8	0.2502	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9442	0.897	0.1416	Normal Distribution

Mean Dry Biomass-mg Summary

C- ^{mg} / _{μg} L ①	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	0.1065	0.08652	0.1265	0.103	0.096	0.124	0.006278	11.8%	0.0%
0.5		4	0.1015	0.08005	0.1229	0.0965	0.092	0.121	0.006739	13.3%	4.69%
1		4	0.09325	0.08083	0.1057	0.091	0.087	0.104	0.003902	8.37%	12.4%
2		4	0.06725	0.05659	0.07791	0.0655	0.062	0.076	0.003351	9.97%	36.9%
4		4	0.03775	0.03318	0.04232	0.0385	0.034	0.04	0.001436	7.61%	64.6%
8		4	0.0215	0.01432	0.02868	0.02	0.018	0.028	0.002255	21.0%	79.8%
16		4	0.0215	0.005403	0.0376	0.022	0.009	0.033	0.005058	47.1%	79.8%

Mean Dry Biomass-mg Detail

C- ^{mg} / _{μg} L ①	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.124	0.099	0.107	0.096
0.5		0.092	0.1	0.093	0.121
1		0.087	0.104	0.094	0.088
2		0.076	0.062	0.062	0.069
4		0.037	0.04	0.04	0.034
8		0.019	0.028	0.018	0.021
16		0.025	0.019	0.033	0.009

① DAP 6/16/20 E

Brine shrimp

① Fathead Minnow 7-d Larval Survival and Growth Test

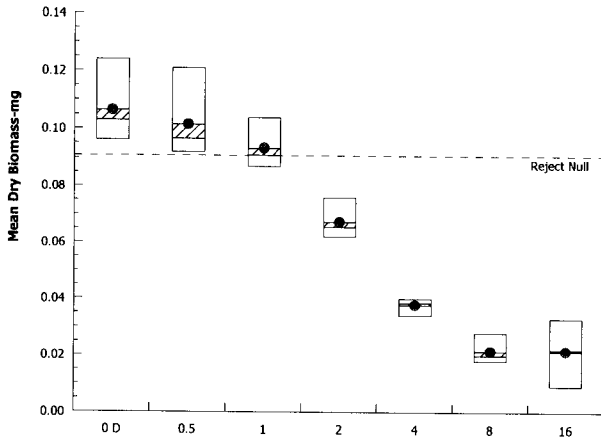
TRE Environmental Strategies

Analysis ID: 05-2669-5393
Analyzed: 15 Jun-20 15:23

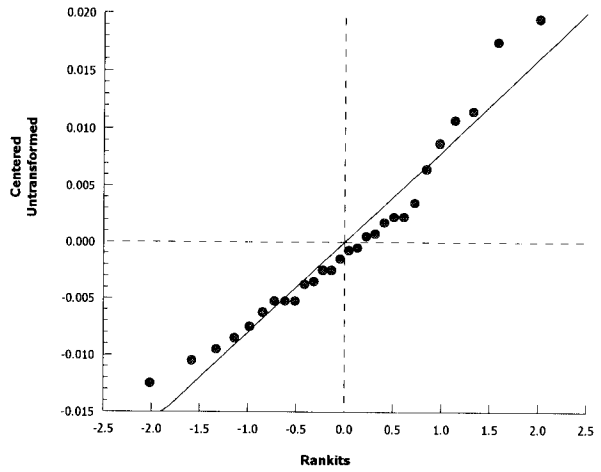
Endpoint: Mean Dry Biomass-mg
Analysis: Parametric-Control vs Treatments

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



① C-10/L
mg



① DAP 6/16/20 E

CETIS Analytical Report

Report Date: 15 Jun-20 15:24 (p 1 of 2)
 Test Code: 474-076 | 07-1158-8845

Brine shrimp

Fathead Minnow 7-d Larval Survival and Growth Test

TRE Environmental Strategies

Analysis ID: 04-2727-0406	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv1.8.7
Analyzed: 15 Jun-20 15:23	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 04-2472-1259	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 04 Jun-20 13:45	Protocol: EPA/821/R-02-013 (2002)	Diluent: rGSL
Ending Date: 11 Jun-20 13:20	Species: Artemia franciscana	Brine: Crystal Sea
Duration: 7d	Source: Hog Island Oyster Co. <i>TRE</i>	Age: 48h
Sample ID: 00-8320-5339	Code: 4F59CDB	Client: Notre Dame
Sample Date: 04 Jun-20 10:35	Material: Zinc sulfate	Project: Special Studies
Receive Date: 13 Jun-20 13:45	Source: Discharge Monitoring Report	
Sample Age: 3h	Station:	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1144646	200	Yes	Two-Point Interpolation

Point Estimates

Level	<i>ms</i> µg/L <i>µg/L</i>	95% LCL	95% UCL
IC5	0.5197	N/A	1.357
IC10	0.8424	N/A	1.448
IC15	1.105	0.001624	1.618
IC20	1.31	0.5185	1.777
IC25	1.514	0.9182	1.969
IC40	2.227	1.574	2.871
IC50	2.949	2.222	3.414

Mean Dry Biomass-mg Summary

Calculated Variate

<i>ms</i> µg/L <i>µg/L</i>	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	0.1065	0.096	0.124	0.006278	0.01256	11.8%	0.0%
0.5		4	0.1015	0.092	0.121	0.006739	0.01348	13.3%	4.69%
1		4	0.09325	0.087	0.104	0.003902	0.007805	8.37%	12.4%
2		4	0.06725	0.062	0.076	0.003351	0.006702	9.97%	36.9%
4		4	0.03775	0.034	0.04	0.001436	0.002872	7.61%	64.6%
8		4	0.0215	0.018	0.028	0.002255	0.004509	21.0%	79.8%
16		4	0.0215	0.009	0.033	0.005058	0.01012	47.1%	79.8%

Mean Dry Biomass-mg Detail

<i>ms</i> µg/L <i>µg/L</i>	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.124	0.099	0.107	0.096
0.5		0.092	0.1	0.093	0.121
1		0.087	0.104	0.094	0.088
2		0.076	0.062	0.062	0.069
4		0.037	0.04	0.04	0.034
8		0.019	0.028	0.018	0.021
16		0.025	0.019	0.033	0.009

① DEP 6/16/20 E

CETIS Analytical Report

Brine shrimp

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① Fathead Minnow 7-d Larval Survival and Growth Test

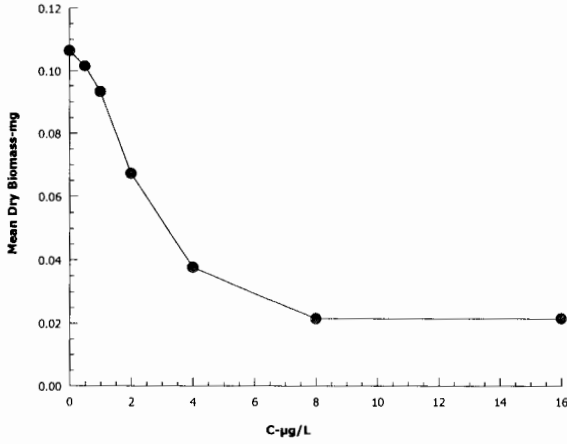
TRE Environmental Strategies

Analysis ID: 04-2727-0406
Analyzed: 15 Jun-20 15:23

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

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



① DSP 6/16/20