

June 12, 2020

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**Subject: Results of Analytical Data for Experiment #14**

Mr. Bittner/ Dr. Belovsky:

Below is a summary of the analytical data for the short-term chronic brine shrimp experiment initiated on May 14, 2020. Total lead samples were collected in new solutions at test initiation and on day 6. Total lead 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#13930	7.9	NM	NM	138,500	NM	NM	120

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

<sup>b</sup>Total residual chlorine

**Results of Lead Analysis**

Nominal Value (mg/L)	Total Lead (mg/L)			Mean Value	Percent of Nominal
	Day 0 New Solution	Day 6 New Solution	Day 1 Old Solution		
0	0.05 U	0.174	0.05 U	0.09	---
29	9.72	10.3	6.93	8.98	31
48	15.3	12.2	13.7	13.7	29
80	24.7	17.7	14.8	19.1	24
134	36.8	31.4	32.8	33.7	25
222	73.0	71.8	54.3	66.4	30

U= below method detection limit (0.05 mg/L)

Average measured lead concentrations were then used to recalculate test endpoints on a measured basis (table below)

**Test Endpoints (mg/L Lead)**

<b>Basis</b>	<b>Survival NOEC</b>	<b>Survival IC20</b>	<b>Growth NOEC</b>	<b>Growth IC20</b>
Nominal	80	87.71 (51.48-161.8)	80	97.84 (48.51-159.4)
Measured	19.07	21.16 (14.16-46.99)	19.07	23.89 (12.66-40.69)

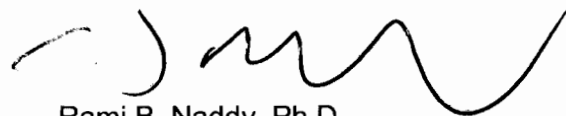
Measured values were significantly reduced compared to nominal values (~28% of nominal). The endpoints reached using the measured values are more in line with the previous studies conducted with lead.

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,



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17001-474-068

Attachment

cc: David Pillard, TRE

**CETIS Analytical Report**

Report Date: 10 Jun-20 09:46 (p 1 of 2)  
 Test Code: 474-068 | 19-2324-3261

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

**TRE Environmental Strategies**

<b>Analysis ID:</b> 17-1291-4378	<b>Endpoint:</b> 7d Survival Rate	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 10 Jun-20 9:46	<b>Analysis:</b> Nonparametric-Control vs Treatments	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-1642-5731	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b> Lab Tech
<b>Start Date:</b> 14 May-20 13:50	<b>Protocol:</b> EPA/821/R-02-013 (2002)	<b>Diluent:</b> rGSL
<b>Ending Date:</b> 21 May-20 14:05	<b>Species:</b> Artemia franciscana	<b>Brine:</b> Crystal Sea
<b>Duration:</b> 7d 0h	<b>Source:</b> In-House Culture	<b>Age:</b> 48h
<b>Sample ID:</b> 09-1873-9497	<b>Code:</b> 36C2DA29	<b>Client:</b> Internal Lab
<b>Sample Date:</b> 14 May-20 10:30	<b>Material:</b> Lead Nitrate	<b>Project:</b> Special Studies
<b>Receive Date:</b> 14 May-20 10:30	<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	18.9%	19.07	33.67	25.34	

**Steel Many-One Rank Sum Test**

Control	vs C-mg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
0.09	8.983	18	10	1	6	0.8333	Asymp	Non-Significant Effect
0.09	13.73	18	10	1	6	0.8333	Asymp	Non-Significant Effect
0.09	19.07	12	10	1	6	0.1424	Asymp	Non-Significant Effect
0.09	33.67*	10	10	0	6	0.0417	Asymp	Significant Effect
0.09	66.37*	10	10	0	6	0.0417	Asymp	Significant Effect

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.094211	0.2188421	5	7.476	0.0006	Significant Effect
Error	0.5269114	0.02927286	18			
Total	1.621122		23			

**Distributional Tests**

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	3.654	4.25	0.0186	Equal Variances
Variances	Levene Equality of Variance	7.087	4.25	0.0008	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8797	0.884	0.0082	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
0.09	Dilution Water	4	1	1	1	1	1	1	0	0.0%	0.0%
8.983		4	1	1	1	1	1	1	0	0.0%	0.0%
13.73		4	1	1	1	1	1	1	0	0.0%	0.0%
19.07		4	0.825	0.4722	1	0.9	0.5	1	0.1109	26.9%	17.5%
33.67		4	0.65	0.2712	1	0.65	0.4	0.9	0.119	36.6%	35.0%
66.37		4	0.625	0.3532	0.8968	0.65	0.4	0.8	0.08539	27.3%	37.5%

**Angular (Corrected) Transformed Summary**

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.09	Dilution Water	4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
8.983		4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
13.73		4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
19.07		4	1.174	0.744	1.604	1.249	0.7854	1.412	0.1351	23.0%	16.9%
33.67		4	0.9566	0.5342	1.379	0.9463	0.6847	1.249	0.1327	27.8%	32.3%
66.37		4	0.9173	0.6318	1.203	0.9386	0.6847	1.107	0.08971	19.6%	35.0%

*6/11/20*

# CETIS Analytical Report

Report Date: 10 Jun-20 09:46 (p 2 of 2)  
 Test Code: 474-068 | 19-2324-3261

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

TRE Environmental Strategies

Analysis ID: 17-1291-4378      Endpoint: 7d Survival Rate      CETIS Version: CETISv1.8.7  
 Analyzed: 10 Jun-20 9:46      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
0.09	Dilution Water	1	1	1	1
8.983		1	1	1	1
13.73		1	1	1	1
19.07		0.9	1	0.5	0.9
33.67		0.9	0.4	0.5	0.8
66.37		0.8	0.6	0.4	0.7

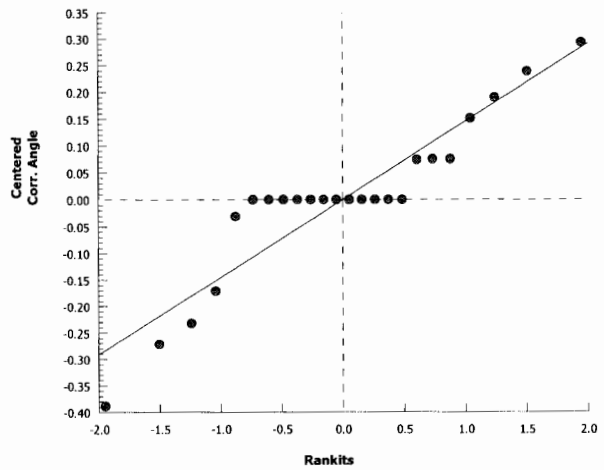
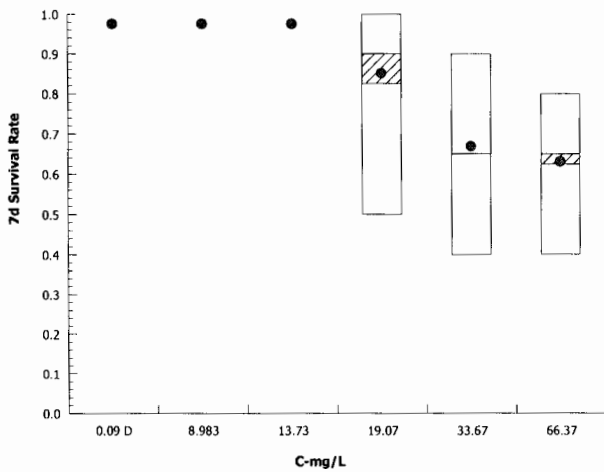
### Angular (Corrected) Transformed Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.09	Dilution Water	1.412	1.412	1.412	1.412
8.983		1.412	1.412	1.412	1.412
13.73		1.412	1.412	1.412	1.412
19.07		1.249	1.412	0.7854	1.249
33.67		1.249	0.6847	0.7854	1.107
66.37		1.107	0.8861	0.6847	0.9912

### 7d Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.09	Dilution Water	10/10	10/10	10/10	10/10
8.983		10/10	10/10	10/10	10/10
13.73		10/10	10/10	10/10	10/10
19.07		9/10	10/10	5/10	9/10
33.67		9/10	4/10	5/10	8/10
66.37		8/10	6/10	4/10	7/10

### Graphics



6/11/20

**CETIS Analytical Report**

*D. B. B. B. B.*

Report Date: 10 Jun-20 09:46 (p 1 of 2)  
 Test Code: 474-068 | 19-2324-3261

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

TRE Environmental Strategies

Analysis ID: 04-3019-9400	Endpoint: <u>7d Survival Rate</u>	CETIS Version: CETISv1.8.7
Analyzed: 10 Jun-20 9:46	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 08-1642-5731	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 14 May-20 13:50	Protocol: EPA/821/R-02-013 (2002)	Diluent: rGSL
Ending Date: 21 May-20 14:05	Species: Artemia franciscana	Brine: Crystal Sea
Duration: 7d 0h	Source: In-House Culture	Age: 48h
Sample ID: 09-1873-9497	Code: 36C2DA29	Client: Internal Lab
Sample Date: 14 May-20 10:30	Material: Lead Nitrate	Project: Special Studies
Receive Date: 14 May-20 10:30	Source: research	
Sample Age: 3h	Station:	

**Linear Interpolation Options**

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1521383	200	Yes	Two-Point Interpolation

**Point Estimates**

Level	mg/L	95% LCL	95% UCL
LC5	15.26	14.04	22.66
LC10	16.78	14.34	27.45
LC15	18.31	14.65	32.88
LC20	21.16	14.16	46.99
LC25	25.33	12.87	57.95
LC40	>66.37	N/A	N/A
LC50	>66.37	N/A	N/A

**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
0.09	Dilution Water	4	1	1	1	0	0	0.0%	0.0%	40	40
8.983		4	1	1	1	0	0	0.0%	0.0%	40	40
13.73		4	1	1	1	0	0	0.0%	0.0%	40	40
19.07		4	0.825	0.5	1	0.1109	0.2217	26.9%	17.5%	33	40
33.67		4	0.65	0.4	0.9	0.119	0.238	36.6%	35.0%	26	40
66.37		4	0.625	0.4	0.8	0.08539	0.1708	27.3%	37.5%	25	40

**7d Survival Rate Detail**

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.09	Dilution Water	1	1	1	1
8.983		1	1	1	1
13.73		1	1	1	1
19.07		0.9	1	0.5	0.9
33.67		0.9	0.4	0.5	0.8
66.37		0.8	0.6	0.4	0.7

**7d Survival Rate Binomials**

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.09	Dilution Water	10/10	10/10	10/10	10/10
8.983		10/10	10/10	10/10	10/10
13.73		10/10	10/10	10/10	10/10
19.07		9/10	10/10	5/10	9/10
33.67		9/10	4/10	5/10	8/10
66.37		8/10	6/10	4/10	7/10

*Over 6/10/20 CP*

*6/10/20*

# CETIS Analytical Report

*DBase stamp*

Report Date: 10 Jun-20 09:46 (p 2 of 2)

Test Code: 474-068 | 19-2324-3261

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

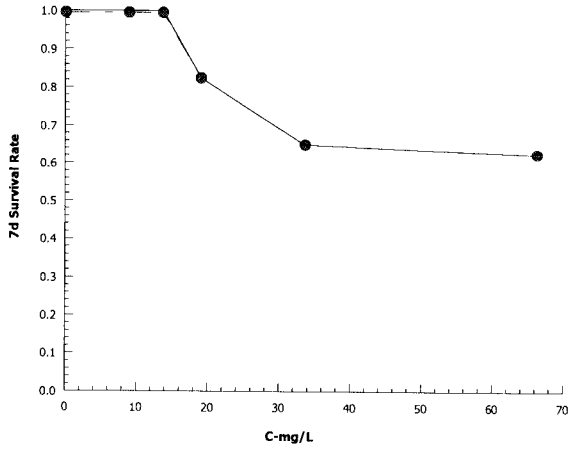
TRE Environmental Strategies

Analysis ID: 04-3019-9400  
Analyzed: 10 Jun-20 9:46

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

CETIS Version: CETISv1.8.7  
Official Results: Yes

### Graphics



*Done 6/11/20 CF*

**CETIS Analytical Report**

Report Date: 10 Jun-20 09:47 (p 1 of 2)  
 Test Code: 474-068 | 19-2324-3261

*Brushup*

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

TRE Environmental Strategies

<b>Analysis ID:</b> 04-1775-2505	<b>Endpoint:</b> Mean Dry Biomass-mg	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 10 Jun-20 9:47	<b>Analysis:</b> Nonparametric-Control vs Treatments	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-1642-5731	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b> Lab Tech
<b>Start Date:</b> 14 May-20 13:50	<b>Protocol:</b> EPA/821/R-02-013 (2002)	<b>Diluent:</b> rGSL
<b>Ending Date:</b> 21 May-20 14:05	<b>Species:</b> Artemia franciscana	<b>Brine:</b> Crystal Sea
<b>Duration:</b> 7d 0h	<b>Source:</b> In-House Culture	<b>Age:</b> 48h
<b>Sample ID:</b> 09-1873-9497	<b>Code:</b> 36C2DA29	<b>Client:</b> Internal Lab
<b>Sample Date:</b> 14 May-20 10:30	<b>Material:</b> Lead Nitrate	<b>Project:</b> Special Studies
<b>Receive Date:</b> 14 May-20 10:30	<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	27.3%	19.07	33.67	25.34	

**Steel Many-One Rank Sum Test**

Control	vs C-mg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision( $\alpha:5\%$ )
0.09	8.983	12	10	0	6	0.1424	Asymp	Non-Significant Effect
0.09	13.73	12.5	10	1	6	0.1834	Asymp	Non-Significant Effect
0.09	19.07	16.5	10	1	6	0.6742	Asymp	Non-Significant Effect
0.09	33.67*	10	10	0	6	0.0417	Asymp	Significant Effect
0.09	66.37*	10	10	0	6	0.0417	Asymp	Significant Effect

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision( $\alpha:5\%$ )
Between	0.01278871	0.002557742	5	7.033	0.0008	Significant Effect
Error	0.006546251	0.0003636806	18			
Total	0.01933496		23			

**Distributional Tests**

Attribute	Test	Test Stat	Critical	P-Value	Decision( $\alpha:1\%$ )
Variances	Bartlett Equality of Variance	15.24	15.1	0.0094	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.9317	0.884	0.1064	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
0.09	Dilution Water	4	0.119	0.112	0.126	0.119	0.114	0.124	0.002198	3.69%	0.0%
8.983		4	0.109	0.09654	0.1215	0.108	0.101	0.119	0.003916	7.18%	8.4%
13.73		4	0.112	0.1041	0.1199	0.1115	0.107	0.118	0.002483	4.43%	5.88%
19.07		4	0.1083	0.07083	0.1457	0.1195	0.073	0.121	0.01176	21.7%	9.03%
33.67		4	0.06875	0.01576	0.1217	0.0735	0.03	0.098	0.01665	48.4%	42.2%
66.37		4	0.05925	0.02684	0.09166	0.065	0.03	0.077	0.01018	34.4%	50.2%

**Mean Dry Biomass-mg Detail**

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.09	Dilution Water	0.114	0.124	0.121	0.117
8.983		0.101	0.119	0.111	0.105
13.73		0.118	0.107	0.109	0.114
19.07		0.119	0.12	0.073	0.121
33.67		0.095	0.03	0.052	0.098
66.37		0.077	0.063	0.03	0.067

*Done 6/11/20 CF*

*6/11/20*

① *Base shrimp*

~~Flathead Minnow~~ 7-d Larval Survival and Growth Test

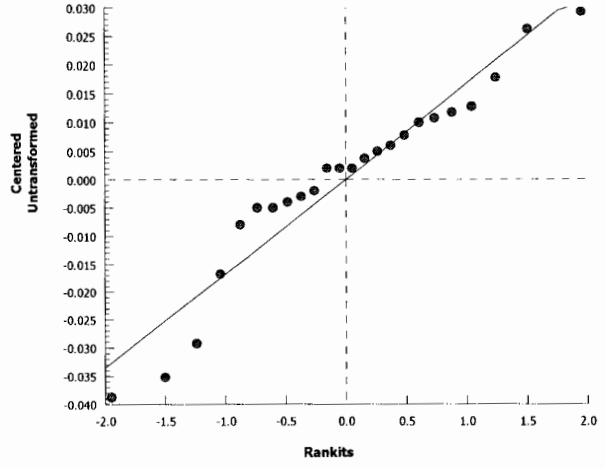
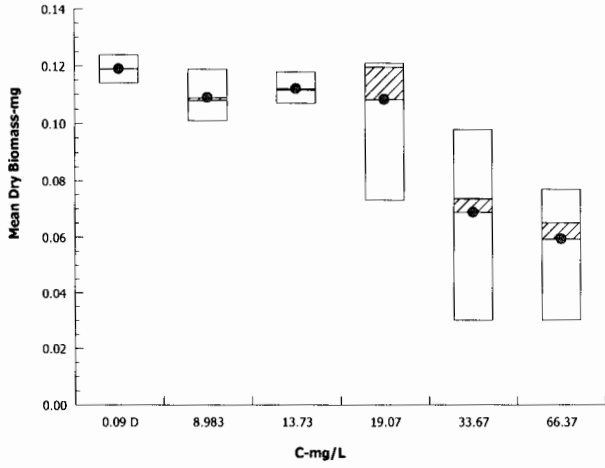
TRE Environmental Strategies

Analysis ID: 04-1775-2505  
Analyzed: 10 Jun-20 9:47

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

CETIS Version: CETISv1.8.7  
Official Results: Yes

Graphics



*Onw 6/11/20 CF*



**CETIS Analytical Report**

*① Brine shrimp*

Report Date: 10 Jun-20 09:47 (p 1 of 2)  
 Test Code: 474-068 | 19-2324-3261

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

TRE Environmental Strategies

<b>Analysis ID:</b> 06-3320-2147	<b>Endpoint:</b> Mean Dry Biomass-mg	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 10 Jun-20 9:47	<b>Analysis:</b> Linear Interpolation (ICPIN)	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-1642-5731	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b> Lab Tech
<b>Start Date:</b> 14 May-20 13:50	<b>Protocol:</b> EPA/821/R-02-013 (2002)	<b>Diluent:</b> rGSL
<b>Ending Date:</b> 21 May-20 14:05	<b>Species:</b> Artemia franciscana	<b>Brine:</b> Crystal Sea
<b>Duration:</b> 7d 0h	<b>Source:</b> In-House Culture	<b>Age:</b> 48h
<b>Sample ID:</b> 09-1873-9497	<b>Code:</b> 36C2DA29	<b>Client:</b> Internal Lab
<b>Sample Date:</b> 14 May-20 10:30	<b>Material:</b> Lead Nitrate	<b>Project:</b> Special Studies
<b>Receive Date:</b> 14 May-20 10:30	<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	413993	200	Yes	Two-Point Interpolation

**Point Estimates**

Level	mg/L	95% LCL	95% UCL
IC5	6.315	3.137	28.63
IC10	19.5	2.011	24.4
IC15	21.69	11.91	30.41
IC20	23.89	12.66	40.69
IC25	26.09	13.25	48.07
IC40	32.69	24.12	N/A
IC50	65.51	9.761	N/A

**Mean Dry Biomass-mg Summary**

**Calculated Variate**

C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0.09	Dilution Water	4	0.119	0.114	0.124	0.002198	0.004397	3.69%	0.0%
8.983		4	0.109	0.101	0.119	0.003916	0.007832	7.18%	8.4%
13.73		4	0.112	0.107	0.118	0.002483	0.004967	4.43%	5.88%
19.07		4	0.1083	0.073	0.121	0.01176	0.02351	21.7%	9.03%
33.67		4	0.06875	0.03	0.098	0.01665	0.0333	48.4%	42.2%
66.37		4	0.05925	0.03	0.077	0.01018	0.02037	34.4%	50.2%

**Mean Dry Biomass-mg Detail**

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.09	Dilution Water	0.114	0.124	0.121	0.117
8.983		0.101	0.119	0.111	0.105
13.73		0.118	0.107	0.109	0.114
19.07		0.119	0.12	0.073	0.121
33.67		0.095	0.03	0.052	0.098
66.37		0.077	0.063	0.03	0.067

*Over 6/11/20 cf*

CETIS Analytical Report

*Bracon*

Report Date: 10 Jun-20 09:47 (p 2 of 2)

Test Code: 474-068 | 19-2324-3261

Pathead Mimosa 7-d Larval Survival and Growth Test

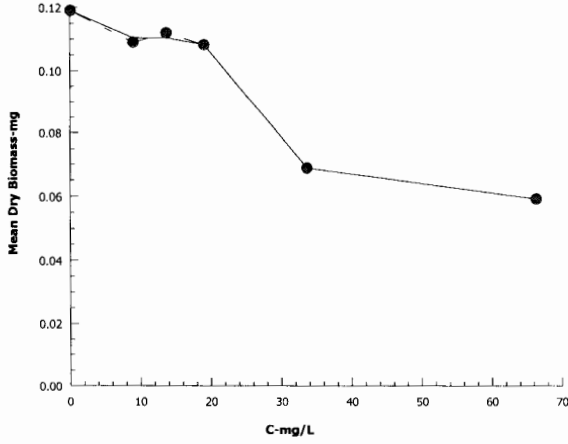
TRE Environmental Strategies

Analysis ID: 06-3320-2147  
Analyzed: 10 Jun-20 9:47

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

CETIS Version: CETISv1.8.7  
Official Results: Yes

Graphics



May 29, 2020

Mr. Christopher Bittner  
Standards Coordinator  
Utah Dept. of Environmental Quality  
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University of Notre Dame  
Notre Dame, IN 46556

**Subject: Results of Short-term Chronic Brine Shrimp Experiment #14**

Mr. Bittner/ Dr. Belovsky:

Below is a summary of the short-term chronic brine shrimp experiment initiated on May 14, 2020. The purpose of this experiment was to investigate the effect of lead on *Artemia franciscana* toxicity in a short-term chronic test.

Along with a control, five different lead concentrations were tested:

- 29, 48, 80, 134, and 222 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 Chla 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 Chla 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. (μS/cm)	TRC (mg/L) <sup>b</sup>	NH <sub>3</sub> -N (mg/L)	Salinity (ppt)
RW#13930	7.9	NM	NM	138,500	NM	NM	120

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

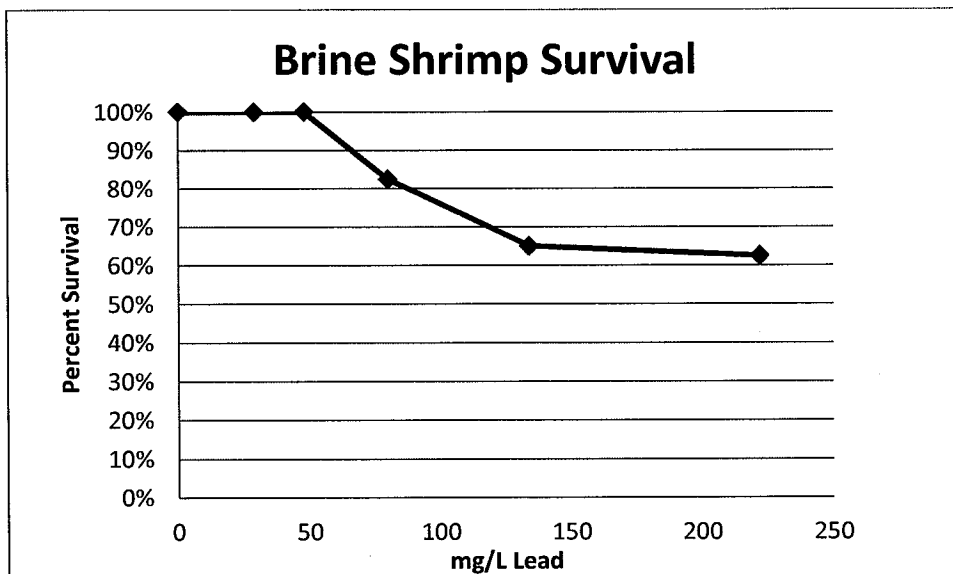
<sup>b</sup>Total residual chlorine

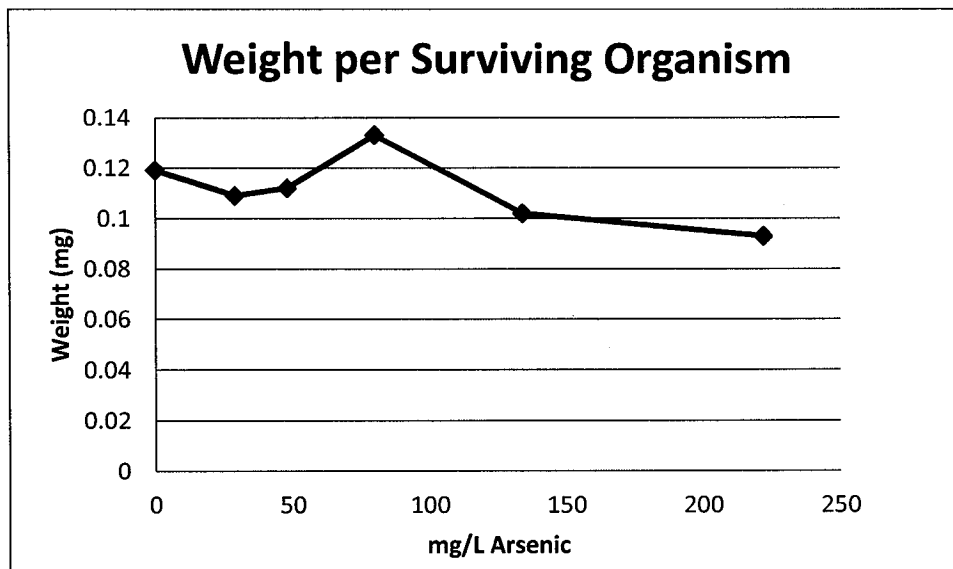
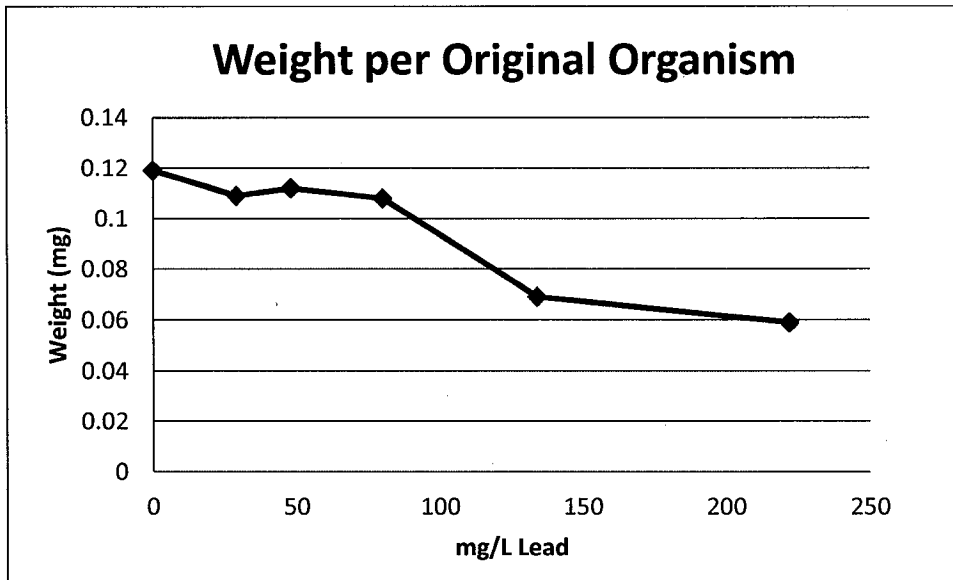
#### Test activities:

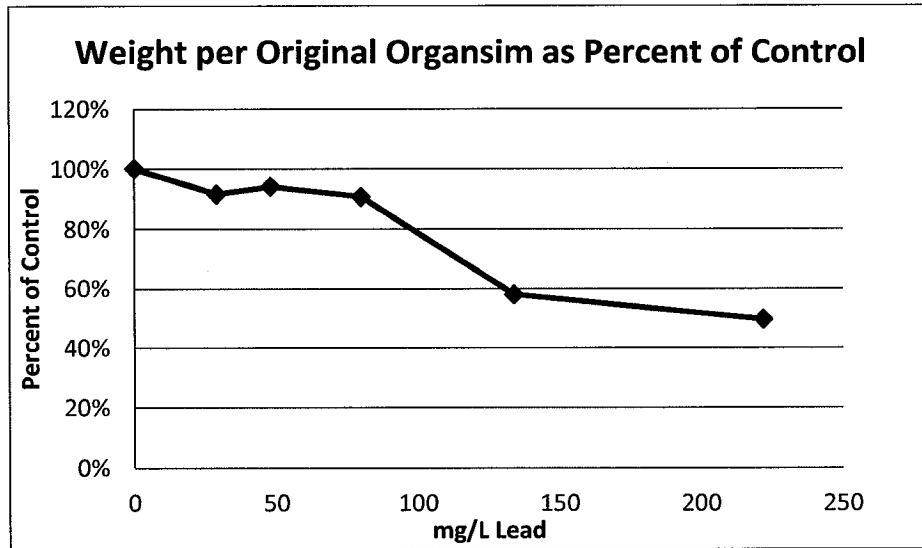
- Biological observations (primarily survival) taken daily.
- Chemistries taken on renewal days (i.e., pH, dissolved oxygen, and temperature).
- Conductivity was measured at test termination or when there was 0% survival in that treatment.
- Dry weights were determined at test termination.
- Lead was 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 lead treatments are illustrated in the following figures.







**Test Endpoints**

Study	Test Endpoints (mg Pb/L, nominal)					
	Survival NOEC	Survival LOEC	Survival IC20	Growth NOEC	Growth LOEC	Growth IC20
7-Day	80	134	87.74 (51.48-161.8)	80	134	97.84 (48.51-159.4)

**Summary and findings:**

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

Analytical samples from each treatment have been collected and sent in for lead 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  
[bidlackac.tre@gmail.com](mailto:bidlackac.tre@gmail.com)

17001-474-068

Attachment

cc: David Pillard, TRE

Rami B. Naddy, Ph.D.  
 Manager / Environmental Toxicologist  
[naddyrb.tre@gmail.com](mailto:naddyrb.tre@gmail.com)

**TOXICITY DATA PACKAGE COVER SHEET**

QA: RDW 5/28/20

Test Type: Chronic Project Number: 17001-474-068  
Test Substance: Lead (Pb(NO3)2) Species: Artemia franciscana  
Dilution Water: rGSL Organism Lot or Batch Number: 051220  
Concurrent Control Water: NA Age: 48 hr (48 hr) Supplier: TRE  
Date and Time Test Began: 5/14/20 @ 1350 Date and Time Test Ended: 5/21/20 @ 1405  
Protocol Number: \_\_\_\_\_ Investigator(s): CP/NJ/HR/EN

**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 YT1 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, 29, 48, 80, 134, and 222 mg/L as Pb

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: 5/21/20

**TEST SUBSTANCE USAGE LOG**

QA: ~~AS~~ 5/28/20

Project Number: 17001-474-068

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number	CV7-026			
Test Substance Collection Date and Time	From:	From:	From:	From:
	@	@	@	@
Sample Type (Grab or Comp)	To:	To:	To:	To:
	@	@	@	@
Date Test Substance Received				
Dilution Water Number RW# or TRE#, circle one	RW# 13930			
Concurrent Control Water RW#				
Date(s) Used	5/14/20	5/18/20		
	5/15/20	5/19/20		
	5/16/20	5/20/20		
	5/17/20			

**Preparation of Test Solutions**

Test Substance Conc. (% Effluent)	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						
29	46	304	350						
48	76	274	350						
80	126	224	350						
134	211	139	350						
222	350	0	350						
	809	1291	2100						
Initials / Date	AL 5/14/20 Mixed BS								
Initials / Date	AB 5/15/20 " "								
Initials / Date	CP 5/16/20 " "								
Initials / Date	CP 5/17/20 " "								
Initials / Date	EN 5/18/20 " "								
Initials / Date	R 5/19/20 " "								
Initials / Date	HR 5/20/20 " "								
Initials / Date									



**Artemia franciscana**  
**CHRONIC BIOLOGICAL DATA**

QA: MP 5/28/20

Project Number: 17001-474-068

mg/L	Test Replicate	Number of Surviving Organisms								% Survival 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	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	
29	A	10	10	10*	10*	10	10	10	10	* 1 weak org
	B	10	10	10	10	10	10	10	10	100
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10*	10*	* 1 weak org
48	A	10	10	10	10	10	10	10	10	
	B	10	10	10	10	10	10	10	10	100
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
80	A	10	10	10	10	10	10*	10*	9	* 1 weak org
	B	10	10	10	10	10	10	10	10	82.5
	C	10	10	10	10	10	10	5	5	
	D	10	10	10	10	10	10	10	9	
134	A	10	10	10	10	10*	9	9	9	* 1 weak org 65
	B	10	10	10	10	10	10*	6	4	* 1 weak org
	C	10	10	10	10	10	10	7*	5	* 3 weak orgs
	D	10	10	10	10	10	10*	9	8	* 2 weak orgs
222	A	10	10	10	10	9	9	8	8	62.5
	B	10	10	10	10	10	9	7	6	
	C	10	10	10	10	10	10*	7	4	* 2 weak orgs
	D	10	10	10	10	10	10	8*	7	* 1 weak org
	A									
	B									
	C									
	D									
Date:	5/14/20	5/15/20	5/16/20	5/17/20	5/18/20	5/19/20	5/20/20	5/21/20		
Time:	1350	1425	1450	1435	1415	1415	1345	1405		
Initials:	CP/MS	AB	CP	HR	EN	CP	CP	AB		

CHRONIC CHEMICAL DATA (INITIAL)

QA: ~~DA~~ 5/28/20

Project Number:	17001-474-068
Test Species:	<i>Artemia franciscana</i>

%	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	8.0	8.0	8.0	8.0	7.8	7.9		FM3627	
D.O. (mg/L)	5.2	5.8	5.5	5.8	5.2	5.1	5.2		17	
Temp. (°C)	20	20	20	20	20	20	20		IR1	
Cond. (µS/cm)	138,500	147,400	138,200	135,000	137,900	131,700	130,900		15	
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH <sub>3</sub> (mg/L)										
Conc.:	29									
pH	7.8	8.0	8.0	7.9	7.9	7.8	7.9			
D.O. (mg/L)	5.2	5.3	5.5	5.2	5.1	5.1	5.2			
Temp. (°C)	20	20	20	20	20	20	20			
Cond. (µS/cm)	137,900	143,900	138,100	134,700	139,100	132,800	131,600			
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH <sub>3</sub> (mg/L)										
Conc.:	48									
pH	7.7	7.9	7.9	7.8	7.9	7.7	7.8			
D.O. (mg/L)	5.2	5.3	5.5	5.8	5.1	5.1	5.1			
Temp. (°C)	20	20	20	20	20	20	20			
Cond. (µS/cm)	138,100	141,500	138,300	135,000	138,800	133,500	131,000			
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH <sub>3</sub> (mg/L)										
Conc.:	80									
pH	7.7	7.9	7.8	7.7	7.8	7.7	7.8			
D.O. (mg/L)	5.2	5.6	5.6	5.4	5.1	5.2	5.3			
Temp. (°C)	20	20	20	20	20	20	20			
Cond. (µS/cm)	138,300	140,900	138,600	135,000	138,900	133,400	130,700			
Date:	5/14/20	5/15/20	5/16/20	5/17/20	5/18/20	5/19/20	5/20/20			
Time:	1335	1400	1445	1420	1405	1400	1340			
Initials:	CP	AB	CP	HR	EN	CP	CP			

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 CP 5/14/20 E

② HR 5/17/20 E

CHRONIC CHEMICAL DATA (INITIAL)

QA: DAP 4/28/20

Project Number:	17001-474-068
Test Species:	<i>Artemia franciscana</i>

%	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter #	Remarks
Conc.:	134								All Conc.	
pH	7.4	7.2	7.6	7.0	7.7	7.5	7.7			
D.O. (mg/L)	5.2	5.4	5.5	5.4	5.1	5.1	5.1			
Temp. (°C)	20	20	20	20	20	20	20			
Cond. (µS/cm)	138,100	136,500	138,200	135,200	138,800	133,300	130,700			
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.:	222									
pH	7.1	7.4	7.3	7.3	7.4	7.3	7.5			
D.O. (mg/L)	5.1	5.5	5.4	5.4	5.0	5.1	5.2			
Temp. (°C)	20	20	20	20	20	20	20			
Cond. (µS/cm)	138,000	141,900	138,200	135,400	138,100	133,200	130,600			
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH <sub>3</sub> (mg/L)										
Date:	5/14/20	5/15/20	5/16/20	5/17/20	5/18/20	5/19/20	5/20/20			
Time:	1335	1400	1445	1420	1405	1400	1340			
Initials:	CP	AB	CP	HR	EN	CP	CP			

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: DAP 5/28/20

Project Number:	17001-474-068
Test Species:	<i>Artemia franciscana</i>

%	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter #	Remarks
Conc.:	0									
								133700		* conductivity
pH	7.9	8.0	7.9	8.0	7.8	7.8	7.7		FM27	
D.O. (mg/L)	5.8	5.1	5.9	5.0	4.9	5.4.8	5.4		17	
Temp (°C)	20	22 <sup>Δ</sup>	20	23 <sup>Δ</sup>	22 <sup>Δ</sup>	21	20		413	
Conc.:	29									
								135200		* conductivity
pH	7.9	8.0	7.9	7.9	7.7	7.8	7.7			
D.O. (mg/L)	5.5	5.6	5.0	5.2	4.7	4.9	5.6			
Temp (°C)	20	22 <sup>Δ</sup>	20	23 <sup>Δ</sup>	22 <sup>Δ</sup>	21	20			
Conc.:	48									
								135400		* conductivity
pH	7.9	7.9	7.9	7.9	7.7	7.8	7.7			
D.O. (mg/L)	5.9	5.3	5.2	4.8	4.7	4.7	5.1			
Temp (°C)	20	22 <sup>Δ</sup>	20	23 <sup>Δ</sup>	22 <sup>Δ</sup>	21	20			
Conc.:	80									
								135200		* conductivity
pH	7.8	7.9	7.8	7.8	7.6	7.7	7.7			
D.O. (mg/L)	5.7	5.6	5.3	4.7	4.6	4.7	5.4			
Temp (°C)	20	22 <sup>Δ</sup>	20	23 <sup>Δ</sup>	22 <sup>Δ</sup>	21	20			
Conc.:	134									
								135400		* conductivity
pH	7.8	7.8	7.7	7.8	7.6	7.7	7.7			
D.O. (mg/L)	5.4	5.6	5.4	5.0	4.7	4.8	5.2			
Temp (°C)	20	22 <sup>Δ</sup>	20	23 <sup>Δ</sup>	22 <sup>Δ</sup>	21	20			
Conc.:	222									
								134800		* conductivity
pH	7.6	7.6	7.0	7.7	7.5	7.6	7.7			
D.O. (mg/L)	5.5	5.1	5.2	4.8	4.8	4.8	5.1			
Temp (°C)	20	22 <sup>Δ</sup>	20	23 <sup>Δ</sup>	22 <sup>Δ</sup>	21	20			
Conc.:										
pH										
D.O. (mg/L)										
Temp (°C)										
Date:	5/15/20	5/16/20	5/17/20	5/18/20	5/19/20	5/20/20	5/21/20			
Time:	1435	1520	1430	1410	1435	1420	1500			
Initials:	AB	CP	HR	EN	CP	CP	AM			

Δ checked all reps  
 ① CP 5/20/20 E

DAILY TOXICITY TEST LOG

QA: DLR 5/28/20

Project Number:	17001-474-068
Test Species:	<i>Artemia franciscana</i>

General Comments		Feeding	Initials/Date
	Random Chart: "D" Min/Max Thermometer # <sup>L-29</sup> <del>M-15</del> ①	72.5 ug/l Chla 0.3 ml YTC	
Test Day 0	Test Solution Mixed at: 1320 Test Organisms Added at: 1350 Spiked @ 1030	Fed @ 1050 <sup>cp</sup>	cp 5/14/20
Test Day 1	Real Time: 21 °C Min-Max Range: 21-21 °C Spiked @ 1100	Fed @ 1100 <sup>As</sup>	As 5/15/20
Test Day 2	Real Time: 21 °C Min-Max Range: 21-21 °C Spiked @ 1130 * Moved test from Bath 2 to Bath 1 due to high temps	Fed @ 1130 <sup>cp</sup>	cp 5/16/20
Test Day 3	Real Time: 20 °C Min-Max Range: 20-22 °C Spiked @ 1040	Fed @ 1040 <sup>cp</sup>	HR 5/17/20
Test Day 4	Real Time: 20 °C Min-Max Range: 20-22 °C Spiked @ 1050	Fed @ 1050	EN 5/18/20
Test Day 5	Real Time: <del>20</del> ② 21 °C Min-Max Range: <del>20-22</del> ② 20-22 °C Spiked @ 1040	Fed @ 1040	cp 5/19/20
Test Day 6	Real Time: 22 °C Min-Max Range: 21-22 °C Spiked @ 1050	Fed @ 1050	cp 5/20/20
Test Day 7	Real Time: 22 °C Min-Max Range: 21-22 °C	Fed @ None	As 5/21/20

① cp 5/14/20 E  
 ② EN 5/18/20 w/p

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

Project Number: 17001-474-068		Test Substance: Lead (Pb (No.03)2)		Comments:								
Species: <i>Artemia franciscana</i>		Analyst Tare: <i>JK</i>		Analytical Balance ID: Sart # 1								
Date/Time of Tare Wt.: 5/21/20 @ 1210		Date/Time of Gross Wt.: 5/23/20 @ 1000		Dried in Oven # 3 from Date: 5/21/20 Time: 1515 to Date: 5/23/20 Time: 0910								
Boat No.	Treatment	Rep.	Length Units:	Weight Type (Circle):			Lot or Batch Number: 051220					
				Wet	Blot Dry	Dry (60-90°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) <sup>1</sup>	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	No. of Surv. Organisms	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)	
	0	A	1.13801	1.13915	0.00114				10			
		B	1.13613	1.13737	0.00124				10			
		C	1.13921	1.14042	0.00121				10			
		D	1.13225	1.13442	0.00117				10			
	29	A	1.11468	1.11569	0.00101				10			
		B	1.12399	1.12518	0.00119				10			
		C	1.12797	1.12908	0.00111				10			
		D	1.12525	1.12630	0.00105				10			
	48	A	1.12954	1.13072	0.00118				10			
		B	1.14947	1.15054	0.00107				10			
		C	1.15019	1.15128	0.00109				10			
		D	1.14278	1.14392	0.00114				10			
			1.14234	1.14236	±0.00002							
	Blank											
	Range											
	Mean											
Test Solution Volume:				Loading Rate:								

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

QA: DAF 5/28/20

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

Project Number: 17001-474-068		Test Substance: Lead (Pb(N <sub>2</sub> O <sub>3</sub> ) <sub>2</sub> )		Comments: Analytical Balance ID: <u>Sart #1</u> Dried in Oven # <u>3</u> from Date: <u>5/21/20</u> Time: <u>1515</u> to Date: <u>5/23/20</u> Time: <u>0905</u>										
Species: <u>Artemia franciscana</u>		Analyst Tare: <u>SK</u>		Analyst Gross: <u>AF</u>										
Date/Time of Tare Wt.: <u>5/21/20 @ 1210</u>		Date/Time of Gross Wt.: <u>5/23/20 @ 1000</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)	No. of Surv. Organisms	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)
				Tare Weight (g)	Gross Weight (g)									
	80	A		1.13935	1.14054	0.00119						9		
		B		1.13190	1.13310	0.00120						10		
		C		1.11215	1.11288	0.00073						5		
		D		1.14890	1.15011	0.00121						9		
	134	A		1.12289	1.12384	0.00095						<del>9</del>		
		B		1.14711	1.14741	0.00030						4		
		C		1.14813	1.14865	0.00052						5		
		D		1.15119	1.15217	0.00098						8		
	222	A		1.12338	1.12415	0.00077						8		
		B		1.14456	1.14519	0.00063						10		
		C		1.13522	1.13552	0.00030						4		
		D		1.14792	1.14859	0.00067						7		
	Blank													
	Range													
	Mean													
Test Solution Volume:												Loading Rate:		

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

DAF 5/28/20

QA: *ddp 5/29/20*

**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		1.13801	1.13915	0.00114	0.00114	10	0.114	0.1190	10	0.114	0.1190
	B		1.13613	1.13737	0.00124	0.00124	10	0.124		10	0.124	
	C		1.13921	1.14042	0.00121	0.00121	10	0.121		10	0.121	
	D		1.13325	1.13442	0.00117	0.00117	10	0.117		10	0.117	
29 mg/L	A		1.11468	1.11569	0.00101	0.00101	10	0.101	0.1090	10	0.101	0.1090
	B		1.12399	1.12518	0.00119	0.00119	10	0.119		10	0.119	
	C		1.12797	1.12908	0.00111	0.00111	10	0.111		10	0.111	
	D		1.12525	1.12630	0.00105	0.00105	10	0.105		10	0.105	
48 mg/L	A		1.12954	1.13072	0.00118	0.00118	10	0.118	0.1120	10	0.118	0.1120
	B		1.14947	1.15054	0.00107	0.00107	10	0.107		10	0.107	
	C		1.15019	1.15128	0.00109	0.00109	10	0.109		10	0.109	
	D		1.14278	1.14392	0.00114	0.00114	10	0.114		10	0.114	
80 mg/L	A		1.13935	1.14054	0.00119	0.00119	10	0.119	0.1083	9	0.132	0.1332
	B		1.13190	1.13310	0.00120	0.00120	10	0.120		10	0.120	
	C		1.11215	1.11288	0.00073	0.00073	10	0.073		5	0.146	
	D		1.14890	1.15011	0.00121	0.00121	10	0.121		9	0.134	
134 mg/L	A		1.12289	1.12384	0.00095	0.00095	10	0.095	0.0687	9	0.106	0.1018
	B		1.14711	1.14741	0.00030	0.00030	10	0.030		4	0.075	
	C		1.14813	1.14865	0.00052	0.00052	10	0.052		5	0.104	
	D		1.15119	1.15217	0.00098	0.00098	10	0.098		8	0.122	
222 mg/L	A		1.12338	1.12415	0.00077	0.00077	10	0.077	0.0592	8	0.096	0.0930
	B		1.14456	1.14519	0.00063	0.00063	10	0.063		6	0.105	
	C		1.13522	1.13552	0.00030	0.00030	10	0.030		4	0.075	
	D		1.14792	1.14859	0.00067	0.00067	10	0.067		7	0.096	
Blank			1.14234	1.14236	0.00002							



QA: Dep 5/29/20

Project Number: 14001-474 Species: Artemia franciscana

**Summary Statistics for Survival Data**

Treatment	N	Min	Max	Mean	SD	C.V.
rGSL	4	1.0	1.0	1.0000	0.0000	0.0000%
29 mg/L	4	1.0	1.0	1.0000	0.0000	0.0000%
48 mg/L	4	1.0	1.0	1.0000	0.0000	0.0000%
80 mg/L	4	0.5	1.0	0.8250	0.2217	26.877%
134 mg/L	4	0.4	0.9	0.6500	0.2380	36.623%
222 mg/L	4	0.4	0.8	0.6250	0.1708	27.325%

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

Treatment	N	Min	Max	Mean	SD	C.V.
rGSL	4	0.114	0.124	0.1190	0.0044	3.695%
29 mg/L	4	0.101	0.119	0.1090	0.0078	7.185%
48 mg/L	4	0.107	0.118	0.1120	0.0050	4.434%
80 mg/L	4	0.073	0.121	0.1083	0.0235	21.722%
134 mg/L	4	0.030	0.098	0.0687	0.0333	48.437%
222 mg/L	4	0.030	0.077	0.0592	0.0204	34.379%

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

Treatment	N	Min	Max	Mean	SD	C.V.
rGSL	4	0.114	0.124	0.1190	0.0044	3.695%
29 mg/L	4	0.101	0.119	0.1090	0.0078	7.185%
48 mg/L	4	0.107	0.118	0.1120	0.0050	4.434%
80 mg/L	4	0.120	0.146	0.1332	0.0107	8.001%
134 mg/L	4	0.075	0.122	0.1018	0.0197	19.370%
222 mg/L	4	0.075	0.105	0.0930	0.0127	13.686%

**CETIS Analytical Report**

*Brine Shrimp*

Report Date: 26 May-20 14:14 (p 1 of 2)

Test Code: 474-068 | 19-2324-3261

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

TRE Environmental Strategies

<b>Analysis ID:</b> 19-4492-9365	<b>Endpoint:</b> 7d Survival Rate	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 26 May-20 14:13	<b>Analysis:</b> Nonparametric-Control vs Treatments	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-1642-5731	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b> Lab Tech
<b>Start Date:</b> 14 May-20 13:50	<b>Protocol:</b> EPA/821/R-02-013 (2002)	<b>Diluent:</b> rGSL
<b>Ending Date:</b> 21 May-20 14:05	<b>Species:</b> Artemia franciscana	<b>Brine:</b> Crystal Sea
<b>Duration:</b> 7d 0h	<b>Source:</b> In-House Culture	<b>Age:</b> 48h
<b>Sample ID:</b> 09-1873-9497	<b>Code:</b> 36C2DA29	<b>Client:</b> Internal Lab
<b>Sample Date:</b> 14 May-20 10:30	<b>Material:</b> Lead Nitrate	<b>Project:</b> Special Studies
<b>Receive Date:</b> 14 May-20 10:30	<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	18.9%	80	134	103.5	

**Steel Many-One Rank Sum Test**

Control	vs C-mg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water	29	18	10	1	6	0.8333	Asymp	Non-Significant Effect
	48	18	10	1	6	0.8333	Asymp	Non-Significant Effect
	80	12	10	1	6	0.1424	Asymp	Non-Significant Effect
	134*	10	10	0	6	0.0417	Asymp	Significant Effect
	222*	10	10	0	6	0.0417	Asymp	Significant Effect

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.094211	0.2188421	5	7.476	0.0006	Significant Effect
Error	0.5269114	0.02927286	18			
Total	1.621122		23			

**Distributional Tests**

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	3.654	4.25	0.0186	Equal Variances
Variances	Levene Equality of Variance	7.087	4.25	0.0008	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8797	0.884	0.0082	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	Dilution Water	4	1	1	1	1	1	1	0	0.0%	0.0%
29		4	1	1	1	1	1	1	0	0.0%	0.0%
48		4	1	1	1	1	1	1	0	0.0%	0.0%
80		4	0.825	0.4722	1	0.9	0.5	1	0.1109	26.9%	17.5%
134		4	0.65	0.2712	1	0.65	0.4	0.9	0.119	36.6%	35.0%
222		4	0.625	0.3532	0.8968	0.65	0.4	0.8	0.08539	27.3%	37.5%

**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	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
29		4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
48		4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
80		4	1.174	0.744	1.604	1.249	0.7854	1.412	0.1351	23.0%	16.9%
134		4	0.9566	0.5342	1.379	0.9463	0.6847	1.249	0.1327	27.8%	32.3%
222		4	0.9173	0.6318	1.203	0.9386	0.6847	1.107	0.08971	19.6%	35.0%

① *DATA 5/28/20 E*

**CETIS Analytical Report**

**Brine Shrimp**

Report Date: 26 May-20 14:14 (p 2 of 2)

Test Code: 474-068 | 19-2324-3261

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

TRE Environmental Strategies

Analysis ID: 19-4492-9365      Endpoint: 7d Survival Rate  
 Analyzed: 26 May-20 14:13      Analysis: Nonparametric-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	Dilution Water	1	1	1	1
29		1	1	1	1
48		1	1	1	1
80		0.9	1	0.5	0.9
134		0.9	0.4	0.5	0.8
222		0.8	0.6	0.4	0.7

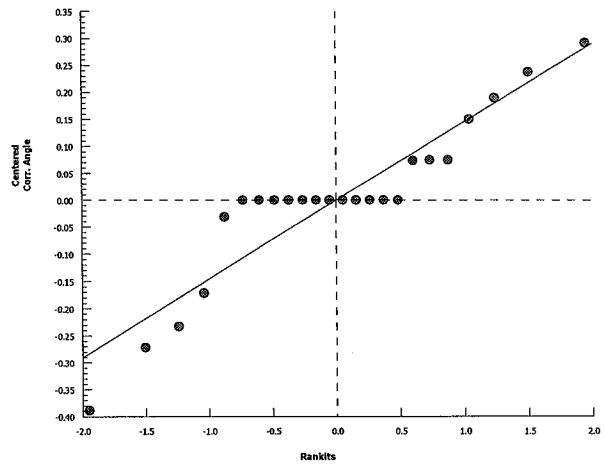
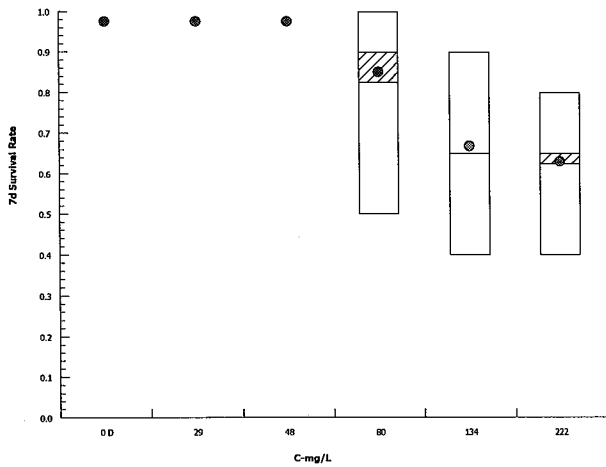
**Angular (Corrected) Transformed Detail**

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.412	1.412	1.412	1.412
29		1.412	1.412	1.412	1.412
48		1.412	1.412	1.412	1.412
80		1.249	1.412	0.7854	1.249
134		1.249	0.6847	0.7854	1.107
222		1.107	0.8861	0.6847	0.9912

**7d Survival Rate Binomials**

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	10/10	10/10	10/10	10/10
29		10/10	10/10	10/10	10/10
48		10/10	10/10	10/10	10/10
80		9/10	10/10	5/10	9/10
134		9/10	4/10	5/10	8/10
222		8/10	6/10	4/10	7/10

**Graphics**



① DAP 5/28/20

**CETIS Analytical Report**

Report Date: 26 May-20 14:15 (p 1 of 2)

Test Code: 474-068 | 19-2324-3261

*Brine shrimp*

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

TRE Environmental Strategies

<b>Analysis ID:</b> 03-5205-4252	<b>Endpoint:</b> 7d Survival Rate	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 26 May-20 14:14	<b>Analysis:</b> Linear Interpolation (ICPIN)	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-1642-5731	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b> Lab Tech
<b>Start Date:</b> 14 May-20 13:50	<b>Protocol:</b> EPA/821/R-02-013 (2002)	<b>Diluent:</b> rGSL
<b>Ending Date:</b> 21 May-20 14:05	<b>Species:</b> Artemia franciscana	<b>Brine:</b> Crystal Sea
<b>Duration:</b> 7d 0h	<b>Source:</b> In-House Culture	<b>Age:</b> 48h
<b>Sample ID:</b> 09-1873-9497	<b>Code:</b> 36C2DA29	<b>Client:</b> Internal Lab
<b>Sample Date:</b> 14 May-20 10:30	<b>Material:</b> Lead Nitrate	<b>Project:</b> Special Studies
<b>Receive Date:</b> 14 May-20 10:30	<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	978205	200	Yes	Two-Point Interpolation

**Point Estimates**

Level	mg/L	95% LCL	95% UCL
LC5	57.14	49.34	99.29
LC10	66.29	50.68	112.9
LC15	75.43	52.02	132.1
LC20	87.71	51.48	161.8
LC25	103.1	49.05	212.9
LC40	>222	N/A	N/A
LC50	>222	N/A	N/A

**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	1	1	1	0	0	0.0%	0.0%	40	40
29		4	1	1	1	0	0	0.0%	0.0%	40	40
48		4	1	1	1	0	0	0.0%	0.0%	40	40
80		4	0.825	0.5	1	0.1109	0.2217	26.9%	17.5%	33	40
134		4	0.65	0.4	0.9	0.119	0.238	36.6%	35.0%	26	40
222		4	0.625	0.4	0.8	0.08539	0.1708	27.3%	37.5%	25	40

**7d Survival Rate Detail**

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
29		1	1	1	1
48		1	1	1	1
80		0.9	1	0.5	0.9
134		0.9	0.4	0.5	0.8
222		0.8	0.6	0.4	0.7

**7d Survival Rate Binomials**

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	10/10	10/10	10/10	10/10
29		10/10	10/10	10/10	10/10
48		10/10	10/10	10/10	10/10
80		9/10	10/10	5/10	9/10
134		9/10	4/10	5/10	8/10
222		8/10	6/10	4/10	7/10

*QAP 5/28/20 E*

CETIS Analytical Report

Brine shrimp

Report Date: 26 May-20 14:15 (p 2 of 2)

Test Code: 474-068 | 19-2324-3261

① Fathead Minnow 7-d Larval Survival and Growth Test

TRE Environmental Strategies

Analysis ID: 03-5205-4252

Endpoint: 7d Survival Rate

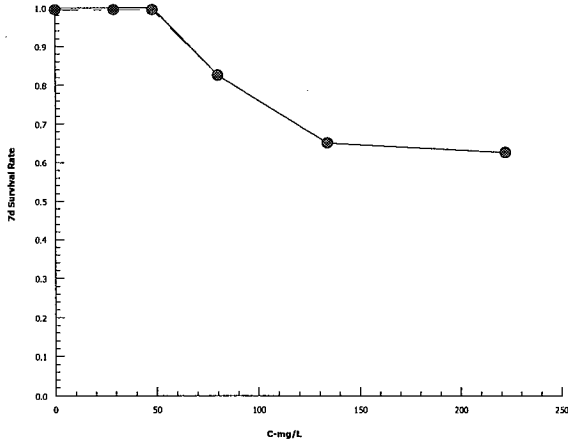
CETIS Version: CETISv1.8.7

Analyzed: 26 May-20 14:14

Analysis: Linear Interpolation (ICPIN)

Official Results: Yes

Graphics



① DAA 5/28/20 E

**CETIS Analytical Report**

*Brine Shrimp*

Report Date: 26 May-20 14:15 (p 1 of 2)

Test Code: 474-068 | 19-2324-3261

① **Eathead Minnow 7-d Larval Survival and Growth Test**

TRE Environmental Strategies

<b>Analysis ID:</b> 13-5366-9313	<b>Endpoint:</b> Mean Dry Biomass-mg	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 26 May-20 14:15	<b>Analysis:</b> Nonparametric-Control vs Treatments	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-1642-5731	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b> Lab Tech
<b>Start Date:</b> 14 May-20 13:50	<b>Protocol:</b> EPA/821/R-02-013 (2002)	<b>Diluent:</b> rGSL
<b>Ending Date:</b> 21 May-20 14:05	<b>Species:</b> Artemia franciscana	<b>Brine:</b> Crystal Sea
<b>Duration:</b> 7d 0h	<b>Source:</b> In-House Culture	<b>Age:</b> 48h
<b>Sample ID:</b> 09-1873-9497	<b>Code:</b> 36C2DA29	<b>Client:</b> Internal Lab
<b>Sample Date:</b> 14 May-20 10:30	<b>Material:</b> Lead Nitrate	<b>Project:</b> Special Studies
<b>Receive Date:</b> 14 May-20 10:30	<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	17.4%	80	>80	NA	

**Steel Many-One Rank Sum Test**

Control	vs	C-mg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		29	12	10	0	6	0.1003	Asymp	Non-Significant Effect
		48	12.5	10	1	6	0.1315	Asymp	Non-Significant Effect
		80	16.5	10	1	6	0.5715	Asymp	Non-Significant Effect

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0002881875	9.606249E-05	3	0.5837	0.6370	Non-Significant Effect
Error	0.00197475	0.0001645625	12			
Total	0.002262938		15			

**Distributional Tests**

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	10.05	11.3	0.0182	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8157	0.841	0.0044	Non-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	0.119	0.112	0.126	0.119	0.114	0.124	0.002198	3.69%	0.0%
29		4	0.109	0.09654	0.1215	0.108	0.101	0.119	0.003916	7.18%	8.4%
48		4	0.112	0.1041	0.1199	0.1115	0.107	0.118	0.002483	4.43%	5.88%
80		4	0.1083	0.07083	0.1457	0.1195	0.073	0.121	0.01176	21.7%	9.03%

**Mean Dry Biomass-mg Detail**

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.114	0.124	0.121	0.117
29		0.101	0.119	0.111	0.105
48		0.118	0.107	0.109	0.114
80		0.119	0.12	0.073	0.121

① *DAF 5/28/20 E*

CETIS Analytical Report

Brine shrimp

Report Date: 26 May-20 14:15 (p 2 of 2)

Test Code: 474-068 | 19-2324-3261

① Fathead Minnow 7-d Larval Survival and Growth Test

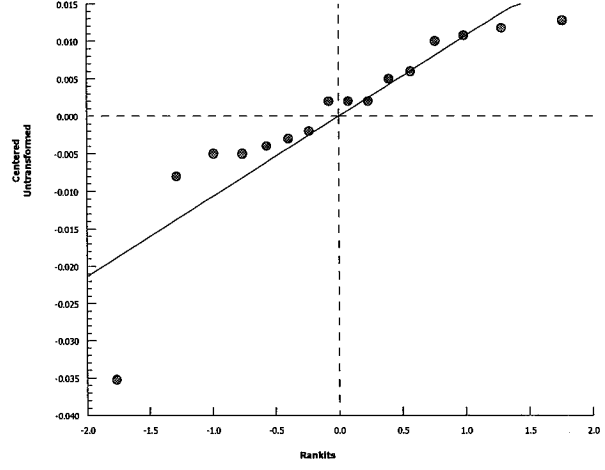
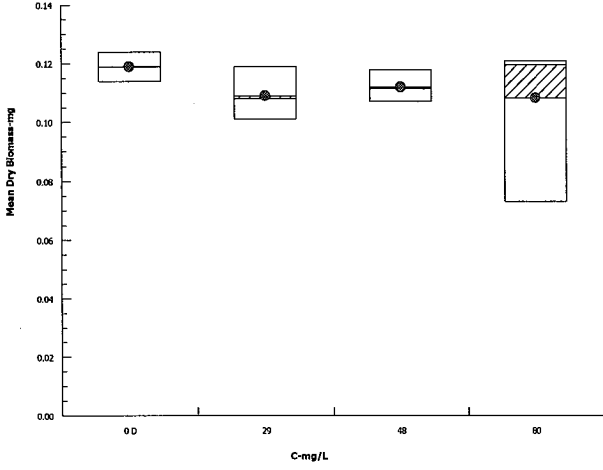
TRE Environmental Strategies

Analysis ID: 13-5366-9313  
Analyzed: 26 May-20 14:15

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

CETIS Version: CETISv1.8.7  
Official Results: Yes

Graphics



① DAP 5/28/20 E

**CETIS Analytical Report**

*Brine Shrimp*

Report Date: 26 May-20 14:16 (p 1 of 2)

Test Code: 474-068 | 19-2324-3261

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

TRE Environmental Strategies

<b>Analysis ID:</b> 10-5709-1593	<b>Endpoint:</b> Mean Dry Biomass-mg	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 26 May-20 14:15	<b>Analysis:</b> Nonparametric-Control vs Treatments	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-1642-5731	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b> Lab Tech
<b>Start Date:</b> 14 May-20 13:50	<b>Protocol:</b> EPA/821/R-02-013 (2002)	<b>Diluent:</b> rGSL
<b>Ending Date:</b> 21 May-20 14:05	<b>Species:</b> Artemia franciscana	<b>Brine:</b> Crystal Sea
<b>Duration:</b> 7d 0h	<b>Source:</b> In-House Culture	<b>Age:</b> 48h
<b>Sample ID:</b> 09-1873-9497	<b>Code:</b> 36C2DA29	<b>Client:</b> Internal Lab
<b>Sample Date:</b> 14 May-20 10:30	<b>Material:</b> Lead Nitrate	<b>Project:</b> Special Studies
<b>Receive Date:</b> 14 May-20 10:30	<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	27.3%	80	134	103.5	

**Steel Many-One Rank Sum Test**

Control	vs	C-mg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		29	12	10	0	6	0.1424	Asymp	Non-Significant Effect
		48	12.5	10	1	6	0.1834	Asymp	Non-Significant Effect
		80	16.5	10	1	6	0.6742	Asymp	Non-Significant Effect
		134*	10	10	0	6	0.0417	Asymp	Significant Effect
		222*	10	10	0	6	0.0417	Asymp	Significant Effect

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01278871	0.002557742	5	7.033	0.0008	Significant Effect
Error	0.006546251	0.0003636806	18			
Total	0.01933496		23			

**Distributional Tests**

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	15.24	15.1	0.0094	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.9317	0.884	0.1064	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	0.119	0.112	0.126	0.119	0.114	0.124	0.002198	3.69%	0.0%
29		4	0.109	0.09654	0.1215	0.108	0.101	0.119	0.003916	7.18%	8.4%
48		4	0.112	0.1041	0.1199	0.1115	0.107	0.118	0.002483	4.43%	5.88%
80		4	0.1083	0.07083	0.1457	0.1195	0.073	0.121	0.01176	21.7%	9.03%
134		4	0.06875	0.01576	0.1217	0.0735	0.03	0.098	0.01665	48.4%	42.2%
222		4	0.05925	0.02684	0.09166	0.065	0.03	0.077	0.01018	34.4%	50.2%

**Mean Dry Biomass-mg Detail**

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.114	0.124	0.121	0.117
29		0.101	0.119	0.111	0.105
48		0.118	0.107	0.109	0.114
80		0.119	0.12	0.073	0.121
134		0.095	0.03	0.052	0.098
222		0.077	0.063	0.03	0.067

*QDAP 5/28/20 E*



CETIS Analytical Report

Brine shrimp

Report Date: 26 May-20 14:16 (p 2 of 2)

Test Code: 474-068 | 19-2324-3261

① Fathead Minnow 7-d Larval Survival and Growth Test

TRE Environmental Strategies

Analysis ID: 10-5709-1593

Endpoint: Mean Dry Biomass-mg

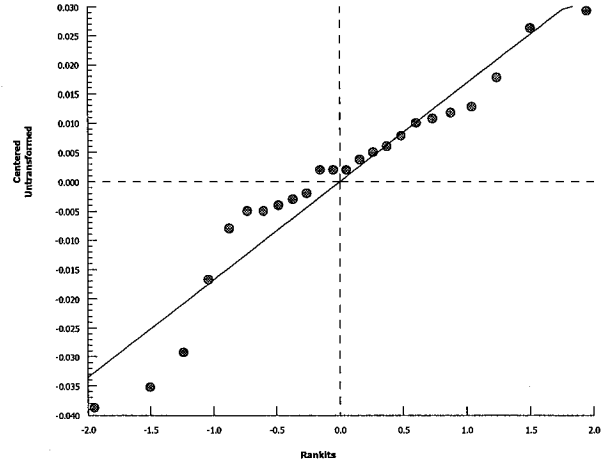
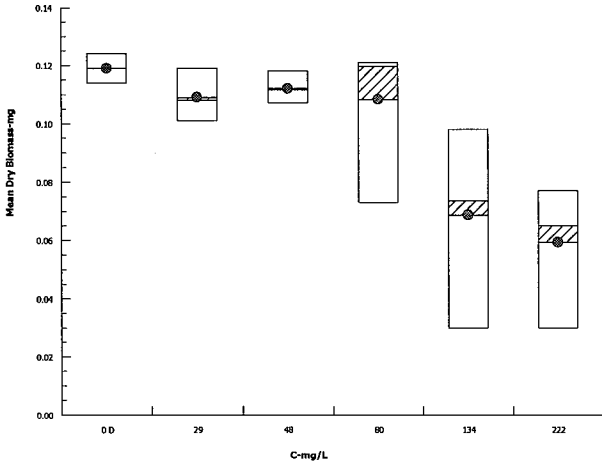
CETIS Version: CETISv1.8.7

Analyzed: 26 May-20 14:15

Analysis: Nonparametric-Control vs Treatments

Official Results: Yes

Graphics



① DAP 5/28/20 E

**CETIS Analytical Report**

Report Date: 26 May-20 14:16 (p 1 of 2)

*Brine shrimp*

Test Code: 474-068 | 19-2324-3261

① ~~Fathad Minnow~~ 7-d Larval Survival and Growth Test

TRE Environmental Strategies

Analysis ID: 00-0714-3453	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv1.8.7
Analyzed: 26 May-20 14:16	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 08-1642-5731	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 14 May-20 13:50	Protocol: EPA/821/R-02-013 (2002)	Diluent: rGSL
Ending Date: 21 May-20 14:05	Species: Artemia franciscana	Brine: Crystal Sea
Duration: 7d 0h	Source: In-House Culture	Age: 48h
Sample ID: 09-1873-9497	Code: 36C2DA29	Client: Internal Lab
Sample Date: 14 May-20 10:30	Material: Lead Nitrate	Project: Special Studies
Receive Date: 14 May-20 10:30	Source: research	
Sample Age: 3h	Station:	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1362251	200	Yes	Two-Point Interpolation

Point Estimates

Level	mg/L	95% LCL	95% UCL
IC5	20.3	10.53	124
IC10	81.57	N/A	105.5
IC15	89.71	41.07	128.3
IC20	97.84	48.51	159.4
IC25	106	55.8	176.5
IC40	130.4	96.27	N/A
IC50	219.7	63.76	N/A

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	0.119	0.114	0.124	0.002198	0.004397	3.69%	0.0%
29		4	0.109	0.101	0.119	0.003916	0.007832	7.18%	8.4%
48		4	0.112	0.107	0.118	0.002483	0.004967	4.43%	5.88%
80		4	0.1083	0.073	0.121	0.01176	0.02351	21.7%	9.03%
134		4	0.06875	0.03	0.098	0.01665	0.0333	48.4%	42.2%
222		4	0.05925	0.03	0.077	0.01018	0.02037	34.4%	50.2%

Mean Dry Biomass-mg Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.114	0.124	0.121	0.117
29		0.101	0.119	0.111	0.105
48		0.118	0.107	0.109	0.114
80		0.119	0.12	0.073	0.121
134		0.095	0.03	0.052	0.098
222		0.077	0.063	0.03	0.067

① DAP 5/28/20 E

# CETIS Analytical Report

*Brine shrimp*

Report Date: 26 May-20 14:16 (p 2 of 2)

Test Code: 474-068 | 19-2324-3261

① Fathead Minnow 7-d Larval Survival and Growth Test

TRE Environmental Strategies

Analysis ID: 00-0714-3453

Endpoint: Mean Dry Biomass-mg

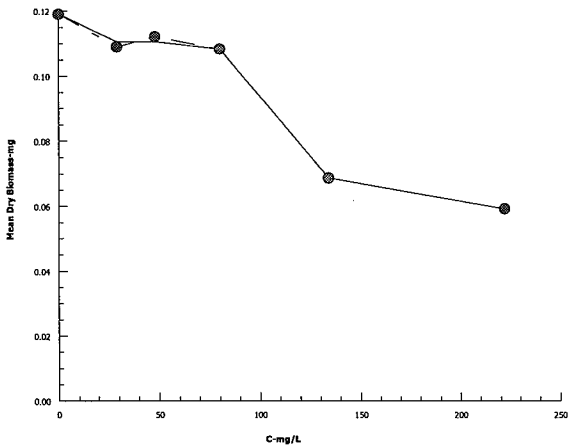
CETIS Version: CETISv1.8.7

Analyzed: 26 May-20 14:16

Analysis: Linear Interpolation (ICPIN)

Official Results: Yes

## Graphics



① DAP 5/28/20 E

**Brine Shrimp Chronic**

**Definitive TEST**

May 2020

Primary stock @ 8000.00 mg/L Pb = 12.78842 g Pb(NO3)2 / L H2O

Volume per treatment (L) 7.0

100 TR  
50 Diss

\_\_\_\_\_ dilution series

Trtmt	Conc. ug/L	Stock (ml)	Total Vol(L)
6	222,000	194.25	7.000
5	-	0.00	7.000
4	-	0.00	7.000
3	-	0.00	7.000
2	-	0.00	7.000
1	0	0.00	7.000
Total		194.25	42.0

2.8% % spike of vol

Take 50 ml for QC dup - D2

126