

August 4, 2020

Mr. Christopher Bittner  
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**Subject: Results of Analytical Data for Experiment #24**

Mr. Bittner/ Dr. Belovsky:

Below is a summary of the copper analytical data for the acute brine shrimp experiments initiated on June 24, 2020. Total copper samples were collected in old solutions.

**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#13962	8.0	NM	NM	133,200	NM	NM	114

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

<sup>b</sup>Total residual chlorine

**Results of Copper Analysis**

Nominal Value	Total Copper (µg/L)		Percent of Nominal
	Nominal Value	Measured Value	
0 (rGSL)		U	---
312		297	95
625		628	100
1,250		1,386	111
2,500		2,586	103
5,000		4,554	91

U= below method detection limit (100 µg/L)

Measured copper values were similar to nominal values (~100%). Average measured copper concentrations were then used to recalculate the test endpoint on a measured basis. Both

nominal and measured median lethal concentrations are presented in the following table for comparison.

**Test Endpoints**

<b>Test</b>	<b>Survival 96-hour LC<sub>50</sub></b>	<b>Value (µg/L Copper)</b>
<b><i>D. viridis</i> / YTC Mix</b>	Nominal	997.9 (C.L. 908.2 - 1,096)
	Measured	1,092 (C.L. 961.2 - 1,218)
<b><i>D. viridis</i> Only</b>	Nominal	1,956 (C.L. 1,773 - 2,157)
	Measured	2,066 (C.L. 1,898 - 2,248)

We greatly appreciate the opportunity to complete these studies 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.  
Manager / Environmental Toxicologist  
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17001-474-082,083

Attachment

cc: David Pillard, TRE

**CETIS Analytical Report**

Brine shrimp ①

Report Date: 31 Jul-20 14:25 (p 1 of 3)

Test Code: 474-082 | 04-4377-1973

**Fathead Minnow 96-h Acute Survival Test**

TRE Environmental Strategies

<b>Analysis ID:</b> 04-3453-2377	<b>Endpoint:</b> 96h Survival Rate	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 31 Jul-20 14:25	<b>Analysis:</b> Linear Regression (MLE)	<b>Official Results:</b> Yes
<b>Batch ID:</b> 16-6256-6365	<b>Test Type:</b> Survival (96h)	<b>Analyst:</b> Lab Tech
<b>Start Date:</b> 24 Jun-20 14:40	<b>Protocol:</b> EPA/821/R-02-012 (2002)	<b>Diluent:</b> rGSL
<b>Ending Date:</b> 28 Jun-20 14:35	<b>Species:</b> <del>Pimephales promelas</del> <i>Artemia franciscana</i>	<b>Brine:</b> Crystal Sea
<b>Duration:</b> 96h	<b>Source:</b> In-House Culture ①	<b>Age:</b> 48h
<b>Sample ID:</b> 20-5517-8434	<b>Code:</b> 7A7F88C2	<b>Client:</b> Notre Dame
<b>Sample Date:</b> 24 Jun-20 11:20	<b>Material:</b> Copper chloride	<b>Project:</b> Special Studies
<b>Receive Date:</b> 24 Jun-20 14:40	<b>Source:</b> research	
<b>Sample Age:</b> 3h	<b>Station:</b>	

**Linear Regression Options**

Model Function	Threshold Option	Threshold	Optimized	Pooled	Het Corr	Weighted
Log-Normal [NED=A+B*log(X)]	Control Threshold	1E-07	Yes	No	No	Yes

**Regression Summary**

Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	F Stat	Critical	P-Value	Decision(α:5%)
86	-24.74	56.67	59.01	3.038	0.1213	0.9637	0.2077	3.16	0.8897	Non-Significant Lack of Fit

**Point Estimates**

Level	μg/L	95% LCL	95% UCL
LC5	689.5	518.8	811
LC10	763.2	598.6	881.2
LC15	817.3	658.4	933.1
LC20	863	709.4	977.6
LC25	904.3	755.6	1018
LC40	1017	880.9	1135
LC50	1092	961.2	1218

**Regression Parameters**

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
Threshold	5.11E-08	3.52E-05	-6.9E-05	6.91E-05	0.001452	0.9989	Non-Significant Parameter
Slope	8.242	1.341	5.613	10.87	6.146	<0.0001	Significant Parameter
Intercept	-25.04	4.126	-33.13	-16.95	-6.069	<0.0001	Significant Parameter

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	206.0453	206.0453	1	612.6	<0.0001	Significant
Lack of Fit	0.236310	0.078770	3	0.2077	0.8897	Non-Significant
Pure Error	6.826923	0.379274	18			
Residual	7.063233	0.336345	21			

**Residual Analysis**

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Goodness-of-Fit	Pearson Chi-Sq GOF	7.063	32.67	0.9980	Non-Significant Heterogeneity
	Likelihood Ratio GOF	8.527	32.67	0.9925	Non-Significant Heterogeneity
Variances	Mod Levene Equality of Variance	1.727	2.773	0.1794	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.7044	0.9169	<0.0001	Non-normal Distribution
	Anderson-Darling A2 Normality	3.514	2.492	<0.0001	Non-normal Distribution

① DAP 8/1/20 E

**CETIS Analytical Report**

*Brine shrimp*

Report Date: 31 Jul-20 14:25 (p 2 of 3)

Test Code: 474-082 | 04-4377-1973

① **Fathead Minnow 96-h Acute Survival Test**

TRE Environmental Strategies

Analysis ID: 04-3453-2377      Endpoint: 96h Survival Rate      CETIS Version: CETISv1.8.7  
 Analyzed: 31 Jul-20 14:25      Analysis: Linear Regression (MLE)      Official Results: Yes

96h Survival Rate Summary			Calculated Variate(A/B)									
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B	
100	Dilution Water	4	1	1	1	0	0	0.0%	0.0%	40	40	
297		4	1	1	1	0	0	0.0%	0.0%	40	40	
628		4	0.975	0.9	1	0.025	0.05	5.13%	2.5%	39	40	
1386		4	0.2	0	0.3	0.07071	0.1414	70.7%	80.0%	8	40	
2586		4	0	0	0	0	0		100.0%	0	40	
4554		4	0	0	0	0	0		100.0%	0	40	

96h Survival Rate Detail					
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
100	Dilution Water	1	1	1	1
297		1	1	1	1
628		1	1	0.9	1
1386		0.3	0.3	0	0.2
2586		0	0	0	0
4554		0	0	0	0

96h Survival Rate Binomials					
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
100	Dilution Water	10/10	10/10	10/10	10/10
297		10/10	10/10	10/10	10/10
628		10/10	10/10	9/10	10/10
1386		3/10	3/10	0/10	2/10
2586		0/10	0/10	0/10	0/10
4554		0/10	0/10	0/10	0/10

① DAP 8/1/20 E

① Fathead Minnow 96-h Acute Survival Test

TRE Environmental Strategies

Analysis ID: 04-3453-2377

Endpoint: 96h Survival Rate

CETIS Version: CETISv1.8.7

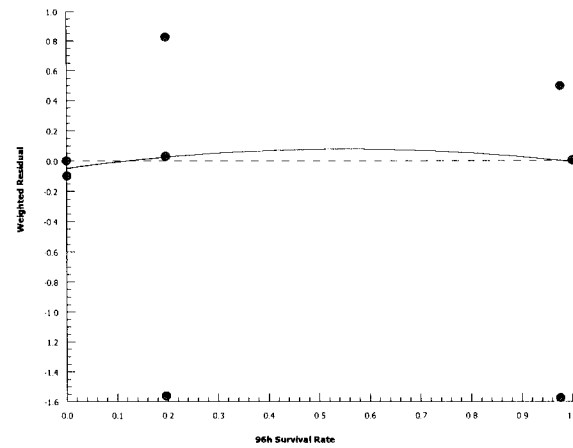
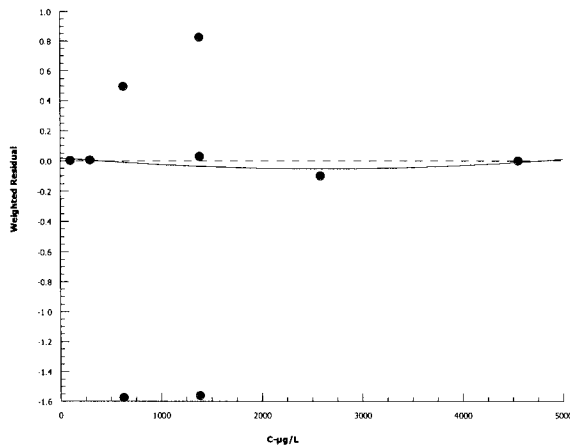
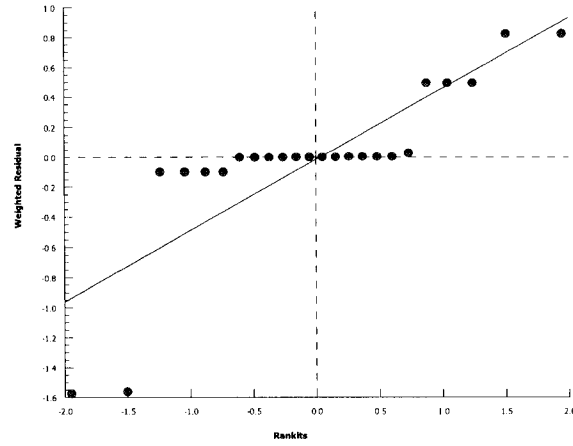
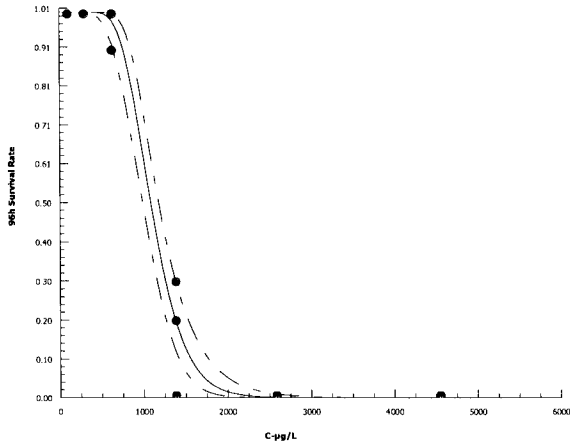
Analyzed: 31 Jul-20 14:25

Analysis: Linear Regression (MLE)

Official Results: Yes

Graphics

Log-Normal [NED=A+B\*log(X)]



① JAP 8/1/20 E

**CETIS Analytical Report**

Report Date: 31 Jul-20 14:27 (p 1 of 2)

Test Code: 474-083 | 11-2056-2513

*Brine shrimp*

**Fathead Minnow 96-h Acute Survival Test**

TRE Environmental Strategies

<b>Analysis ID:</b> 21-2905-7173	<b>Endpoint:</b> 96h Survival Rate	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 31 Jul-20 14:27	<b>Analysis:</b> Trimmed Spearman-Kärber	<b>Official Results:</b> Yes
<b>Batch ID:</b> 04-4329-6666	<b>Test Type:</b> Survival (96h)	<b>Analyst:</b> Lab Tech
<b>Start Date:</b> 24 Jun-20 15:50	<b>Protocol:</b> EPA/821/R-02-012 (2002)	<b>Diluent:</b> rGSL
<b>Ending Date:</b> 28 Jun-20 15:00	<b>Species:</b> Artemia franciscana	<b>Brine:</b> Crystal Sea
<b>Duration:</b> 95h	<b>Source:</b> In-House Culture	<b>Age:</b> 48h
<b>Sample ID:</b> 16-2709-1750	<b>Code:</b> 60FB7326	<b>Client:</b> Notre Dame
<b>Sample Date:</b> 24 Jun-20 12:40	<b>Material:</b> Copper chloride	<b>Project:</b> Special Studies
<b>Receive Date:</b> 28 Jun-20 15:50	<b>Source:</b> research	
<b>Sample Age:</b> 3h	<b>Station:</b>	

**Trimmed Spearman-Kärber Estimates**

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	5.00%	3.315	0.01836	2066	1898	2248

**96h Survival Rate Summary**

C-µg/L	Control Type	Count	Calculated Variate(A/B)								
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
100	Dilution Water	4	1	1	1	0	0	0.0%	0.0%	40	40
297		4	0.925	0.8	1	0.04787	0.09574	10.4%	7.5%	37	40
628		4	0.95	0.9	1	0.02887	0.05773	6.08%	5.0%	38	40
1386		4	0.975	0.9	1	0.025	0.05	5.13%	2.5%	39	40
2586		4	0.2	0.1	0.3	0.05774	0.1155	57.7%	80.0%	8	40
4554		4	0	0	0	0	0	100.0%	100.0%	0	40

**96h Survival Rate Detail**

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
100	Dilution Water	1	1	1	1
297		1	1	0.8	0.9
628		1	0.9	1	0.9
1386		1	0.9	1	1
2586		0.3	0.1	0.3	0.1
4554		0	0	0	0

**96h Survival Rate Binomials**

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
100	Dilution Water	10/10	10/10	10/10	10/10
297		10/10	10/10	8/10	9/10
628		10/10	9/10	10/10	9/10
1386		10/10	9/10	10/10	10/10
2586		3/10	1/10	3/10	1/10
4554		0/10	0/10	0/10	0/10

*Order 8/1/20 E*

**CETIS Analytical Report**

*Brine shrimp*

Report Date: 31 Jul-20 14:27 (p 2 of 2)

Test Code: 474-083 | 11-2056-2513

① Fathead Minnow 96-h Acute Survival Test

TRE Environmental Strategies

Analysis ID: 21-2905-7173

Endpoint: 96h Survival Rate

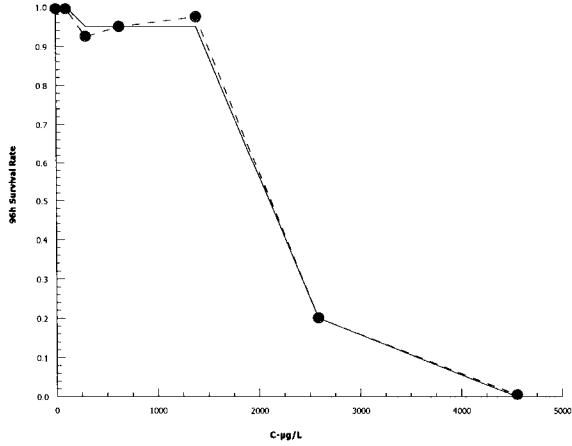
CETIS Version: CETISv1.8.7

Analyzed: 31 Jul-20 14:27

Analysis: Trimmed Spearman-Kärber

Official Results: Yes

**Graphics**



① DAP 8/1/20 E

July 1, 2020

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Standards Coordinator  
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 Acute Brine Shrimp Bioavailability Experiment #24**

Mr. Bittner/ Dr. Belovsky:

Below is a summary of the acute brine shrimp experiments initiated on June 24, 2020. The purpose of these experiments was to investigate the difference in the bioavailability of copper to brine shrimp when fed *D. viridis*/YTC<sup>1</sup> mixture or solely *D. viridis*.

Along with a control, five different nominal copper concentrations (prepared with CuCl<sub>2</sub>) were tested:

- 312.5, 325, 1,250, 3,000, and 5,000 µg/L

The results of these studies will help determine the observed toxicity of copper to brine shrimp fed two different diets. The test volume was consistent at 50 ml.

**Species:** *Artemia franciscana*

**Test type:**

- Test duration: 4 days
- Test type: static-renewal (solutions and food renewed at 48 hours)
- Algae: *Dunaliella viridis*
- Food concentration: 72.5 µg/L Chla and 0.3 ml YTC or 145 µg/L Chla
- Temperature: 20°C
- Test volume(s): 50 ml
- Replicates: 4
- Organisms/Rep: 10
- Test media: 120 ppt rGSL media (per Notre Dame recipe)

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



**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.

### 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#13962	8.0	NM	NM	133,200	NM	NM	114

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

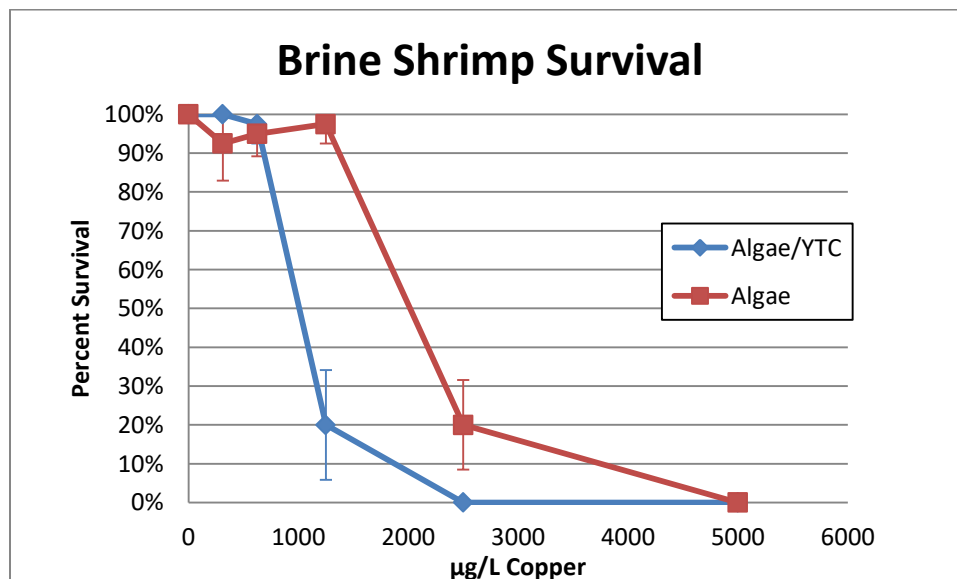
<sup>b</sup>Total residual chlorine

#### Test activities:

- Biological observations (primarily survival) taken daily.
- Chemistries taken daily (i.e., pH, dissolved oxygen, and temperature).
- Conductivity was measured at test initiation, renewal and termination or when there was 0% survival in that treatment.
- Copper 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 (mean ±1 standard deviation) of the brine shrimp in the copper treatments is illustrated in the following figure:



### Test Endpoints

Food: <i>D. viridis</i> /YTC mix				
Test Concentration ( $\mu\text{g/L}$ Copper) (nominal)	Percent Survival of <i>Artemia franciscana</i>			
	24 hours	48 hours	72 hours	96 hours
0 (rGSL)	100	100	100	100
312.5	100	100	100	100
625	100	97.5	97.5	97.5
1,250	27.5	20	20	20
2,500	0	0	0	0
5,000	0	0	0	0
Control Performance		Acceptable		

Food: <i>D. viridis</i> alone				
Test Concentration ( $\mu\text{g/L}$ Copper) (nominal)	Percent Survival of <i>Artemia franciscana</i>			
	24 hours	48 hours	72 hours	96 hours
0 (rGSL)	100	100	100	100
312.5	92.5	92.5	92.5	92.5
625	97.5	95	95	95
1,250	97.5	97.5	97.5	97.5
2,500	65	20	20	20
5,000	0	0	0	0
Control Performance		Acceptable		

### Data Analysis and Test Endpoints

Test	Biological Endpoint	Statistical Endpoint	Value ( $\mu\text{g/L}$ Copper) (nominal)
<i>D. viridis</i> /YTC mix	Survival	96-hour $\text{LC}_{50}$	997.9 (C.L. 908.2 -1,096)
<i>D. viridis</i> only	Survival	96-hour $\text{LC}_{50}$	1,956 (C.L. 1,773 -2,157)

**Summary and findings:**

- Organism survival was  $\geq 90\%$  for the controls.
- Copper toxicity was clearly demonstrated at these testing concentrations.
- Samples were collected for copper analysis and measured endpoints will be forthcoming.
- Test end points were significantly higher (less toxicity) in the *D. viridis* only test. The 96-h  $LC_{50}$  from the *D. viridis*/YTC was 39.2% of the 96-h  $LC_{50}$  from the *D. viridis* only test.

We greatly appreciate the opportunity to complete these studies 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.  
Manager / Environmental Toxicologist  
[naddyrb.tre@gmail.com](mailto:naddyrb.tre@gmail.com)

17001-474-(082,083)

Attachment

cc: David Pillard, TRE

**TRE**

TOXICITY DATA PACKAGE COVER SHEET

QA: DSP 6/29/20

Test Type: Chronic Acute Project Number: 17001-474-082  
Test Substance: Copper (CuCl2) Species: Artemia franciscana  
Dilution Water: rGSL Organism Lot or Batch Number: 062220  
Concurrent Control Water: NA Age: 48 HR (48 hr) Supplier: TRE  
Date and Time Test Began: 6/24/20 @ 1440 Date and Time Test Ended: 6/28/20 @ 1435  
Protocol Number: \_\_\_\_\_ Investigator(s): SC/CP/HB/JS

Background Information

Type of Test: Static-Renewal (48 h) 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: 96 hr 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 NH<sub>3</sub>: Test Initiation TRC: Test Initiation  
pH: Daily Conductivity: Daily

Test Concentrations (Volume:Volume): rGSL, 312.5, 325, 1,250, 2,500, and 5,000 µg/L as Cu  
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: 6/23/20

AS 6/23/20

**TEST SUBSTANCE USAGE LOG**

Project Number: 17001-474-082

QA: NA 6/25/20

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number	ENSR #19122			
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 <u>RW#</u> or TRE#, circle one	13962 / 13970*			
Concurrent Control Water RW#	NA			
Date(s) Used	6/24/20			
	6/26/20*			

**Preparation of Test Solutions**

Test Substance Conc. (µg/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	250	250						
312.5	16	234	250						
625	31	219	250						
1250	63	188	250						
2500	125	125	250						
5000	250	0	250						
	484	1016	1500						
Initials / Date	ge 6/24/20 mixed BS								
Initials / Date	ge 6/26/20 "								
Initials / Date									
Initials / Date									
Initials / Date									
Initials / Date									
Initials / Date									
Initials / Date									

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

QA: RMP 6/30/20

Project Number: 17001-474-082

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	/	/	/	100%
	B	10	10	10	10	10	/	/	/	
	C	10	10	10	10	10	/	/	/	
	D	10	10	10*	10	10	/	/	/	
312.5	A	10	10	10	10	10	/	/	/	100%
	B	10	10	10	10	10	/	/	/	
	C	10	10	10	10	10	/	/	/	
	D	10	10	10	10	10	/	/	/	
625	A	10	10	10	10	10	/	/	/	97.5%
	B	10	10	10	10	10	/	/	/	
	C	10	10	9	9	9	/	/	/	
	D	10	10	10	10	10	/	/	/	
1250	A	10	5	3	3	3	/	/	/	20%
	B	10	4	3	3	3	/	/	/	
	C	10	0	—	—	—	/	/	/	
	D	10	2	2	2	2	/	/	/	
2500	A	10	0	/	/	/	/	/	/	0
	B	10	0	/	/	/	/	/	/	
	C	10	0	/	/	/	/	/	/	
	D	10	0	/	/	/	/	/	/	
5000	A	10	0	/	/	/	/	/	/	0
	B	10	0	/	/	/	/	/	/	
	C	10	0	/	/	/	/	/	/	
	D	10	0	/	/	/	/	/	/	
	A						/	/	/	
	B						/	/	/	
	C						/	/	/	
	D						/	/	/	
Date:		6/24/20	6/25/20	6/26/20	6/27/20	6/28/20				
Time:		1440	1830	1335	1100	1435				
Initials:		CP/MB	CP	CP	CP	ES				

CHRONIC CHEMICAL DATA (INITIAL)

QA: DWP 6/30/20

Project Number:	17001-474-082
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	8.0	/	8.2	/	/	/	/	/	FM27	
D.O. (mg/L)	5.0	/	5.1	/	/	/	/	/	17	
Temp. (°C)	20	/	20	/	/	/	/	/	IR1	
Cond. (µS/cm)	133,200	/	132,900	/	/	/	/	/	15	
Hard. (mg/L)		/		/	/	/	/	/		
Alk. (mg/L)		/		/	/	/	/	/		
TRC (mg/L)		/		/	/	/	/	/		
NH <sub>3</sub> (mg/L)		/		/	/	/	/	/		
Conc.:	312.5									
pH	7.9	/	8.1	/	/	/	/	/		
D.O. (mg/L)	5.0	/	5.1	/	/	/	/	/		
Temp. (°C)	20	/	20	/	/	/	/	/		
Cond. (µS/cm)	134,700	/	133,200	/	/	/	/	/		
Hard. (mg/L)		/		/	/	/	/	/		
Alk. (mg/L)		/		/	/	/	/	/		
TRC (mg/L)		/		/	/	/	/	/		
NH <sub>3</sub> (mg/L)		/		/	/	/	/	/		
Conc.:	625									
pH	8.0	/	8.1	/	/	/	/	/		
D.O. (mg/L)	5.0	/	5.0	/	/	/	/	/		
Temp. (°C)	20	/	20	/	/	/	/	/		
Cond. (µS/cm)	135,100	/	133,000	/	/	/	/	/		
Hard. (mg/L)		/		/	/	/	/	/		
Alk. (mg/L)		/		/	/	/	/	/		
TRC (mg/L)		/		/	/	/	/	/		
NH <sub>3</sub> (mg/L)		/		/	/	/	/	/		
Conc.:	1250									
pH	8.0	/	8.1	/	/	/	/	/		
D.O. (mg/L)	5.0	/	5.0	/	/	/	/	/		
Temp. (°C)	20	/	20	/	/	/	/	/		
Cond. (µS/cm)	135,100	/	133,200	/	/	/	/	/		
Date:	6/24/20		6/26/20							
Time:	1430		1325							
Initials:	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 (INITIAL)

QA: PJP 6/30/20

Project Number:	17001-474-082
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.:	2500								All Conc.	
pH	7.9	/	/	/	/	/	/	/		
D.O. (mg/L)	5.0	/	/	/	/	/	/	/		
Temp. (°C)	20	/	/	/	/	/	/	/		
Cond. (µS/cm)	134,900	/	/	/	/	/	/	/		
Conc.:		/	/	/	/	/	/	/		
pH		/	/	/	/	/	/	/		
D.O. (mg/L)		/	/	/	/	/	/	/		
Temp. (°C)		/	/	/	/	/	/	/		
Cond. (µS/cm)		/	/	/	/	/	/	/		
Conc.:		/	/	/	/	/	/	/		
pH		/	/	/	/	/	/	/		
D.O. (mg/L)		/	/	/	/	/	/	/		
Temp. (°C)		/	/	/	/	/	/	/		
Cond. (µS/cm)		/	/	/	/	/	/	/		
Conc.:	5000									
pH	7.9	/	/	/	/	/	/	/		
D.O. (mg/L)	5.1	/	/	/	/	/	/	/		
Temp. (°C)	20	/	/	/	/	/	/	/		
Cond. (µS/cm)	134,600	/	/	/	/	/	/	/		
Hard. (mg/L)		/	/	/	/	/	/	/		
Alk. (mg/L)		/	/	/	/	/	/	/		
TRC (mg/L)		/	/	/	/	/	/	/		
NH <sub>3</sub> (mg/L)		/	/	/	/	/	/	/		
Date:	6/24/20		6/26/20							
Time:	1430		1325							
Initials:	CP		CP							

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.



CHRONIC CHEMICAL DATA (FINAL)

QA: DP 6/30/20

Project Number:	17001-474-082
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									All Conc.	* conductivity
pH	7.8	8.0	8.0	8.0					FM27	133.9
D.O. (mg/L)	5.0	5.0	4.3	4.6					17	
Temp (°C)	19	19	20	19					L-13	
Conc.: 312.5										* conductivity
pH	7.7	8.0	8.0	8.0						134.9
D.O. (mg/L)	4.9	4.9	4.3	4.3						
Temp (°C)	20	19	20	19						
Conc.: 625										* conductivity
pH	7.7	8.0	8.0	8.0						134.8
D.O. (mg/L)	4.9	4.9	4.4	4.4						
Temp (°C)	20	19	20	20						
Conc.: 1250										* conductivity
pH	7.8	8.0	8.0	8.0						134.1
D.O. (mg/L)	4.9	4.9	4.4	4.6						
Temp (°C)	20	19	20	20						
Conc.: 2500	130,800									* conductivity
pH	7.7									
D.O. (mg/L)	4.9									
Temp (°C)	20									
Conc.: 5000	130,000									* conductivity
pH	7.7									
D.O. (mg/L)	5.0									
Temp (°C)	20									
Conc.:										
pH										
D.O. (mg/L)										
Temp (°C)										
Date:	6/25/20	6/26/20	6/27/20	6/28/20						
Time:	1840	1400	1110	1435						
Initials:	CP	CP	CP	ES						

DAILY TOXICITY TEST LOG

QA: DP 6/30/20

Project Number:	17001-474-082
Test Species:	<i>Artemia franciscana</i>

General Comments		Feeding 72.5 ug/l Chla 0.33ml YTC	Initials/Date
	Random Chart: <u>Pi</u> Min/Max Thermometer # <u>M-15</u>		
Test Day 0	Test Solution Mixed at: <u>1120</u> Test Organisms Added at: <u>1440</u>	Fed @ <u>1120</u>	<u>CP</u> <u>6/24/20</u>
Test Day 1	Real Time: <u>20</u> °C Min-Max Range: <u>20-21</u> °C	<u>NONE</u>	<u>CP</u> <u>6/25/20</u>
Test Day 2	Real Time: <u>20</u> °C Min-Max Range: <u>20-20</u> °C	Fed @ <u>1000</u>	<u>CP</u> <u>6/26/20</u>
Test Day 3	Real Time: <u>21</u> °C Min-Max Range: <u>20-22</u> °C	<u>NONE</u>	<u>CP</u> <u>6/27/20</u>
Test Day 4	Real Time: <u>22</u> °C Min-Max Range: <u>21-24</u> °C	<u>NONE</u>	<u>ES</u> <u>6/28/20</u>

**CETIS Analytical Report**

Report Date: 31 Jul-20 14:39 (p 1 of 2)  
 Test Code: 474-082 | 04-4377-1973

**Fathead Minnow 96-h Acute Survival Test**

**TRE Environmental Strategies**

<b>Analysis ID:</b> 02-5835-2962	<b>Endpoint:</b> 96h Survival Rate	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 31 Jul-20 14:39	<b>Analysis:</b> Untrimmed Spearman-Kärber	<b>Official Results:</b> Yes
<b>Batch ID:</b> 16-6256-6365	<b>Test Type:</b> Survival (96h)	<b>Analyst:</b> Lab Tech
<b>Start Date:</b> 24 Jun-20 14:40	<b>Protocol:</b> EPA/821/R-02-012 (2002)	<b>Diluent:</b> rGSL
<b>Ending Date:</b> 28 Jun-20 14:35	<b>Species:</b> Pimephales promelas	<b>Brine:</b> Crystal Sea
<b>Duration:</b> 96h	<b>Source:</b> In-House Culture	<b>Age:</b> 48h
<b>Sample ID:</b> 20-5517-8434	<b>Code:</b> 7A7F88C2	<b>Client:</b> Notre Dame
<b>Sample Date:</b> 24 Jun-20 11:20	<b>Material:</b> Copper chloride	<b>Project:</b> Special Studies
<b>Receive Date:</b> 24 Jun-20 14:40	<b>Source:</b> research	
<b>Sample Age:</b> 3h	<b>Station:</b>	

**Spearman-Kärber Estimates**

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	0.00%	2.999	0.02044	997.9	908.2	1096

**Test Acceptability Criteria**

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

**96h Survival Rate Summary**

**Calculated Variate(A/B)**

C-µg/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
312.5		4	1	1	1	0	0	0.0%	0.0%	40	40
625		4	0.975	0.9	1	0.025	0.05	5.13%	2.5%	39	40
1250		4	0.2	0	0.3	0.07071	0.1414	70.7%	80.0%	8	40
2500		4	0	0	0	0	0		100.0%	0	40
5000		4	0	0	0	0	0		100.0%	0	40

**96h Survival Rate Detail**

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
312.5		1	1	1	1
625		1	1	0.9	1
1250		0.3	0.3	0	0.2
2500		0	0	0	0
5000		0	0	0	0

**96h Survival Rate Binomials**

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	10/10	10/10	10/10	10/10
312.5		10/10	10/10	10/10	10/10
625		10/10	10/10	9/10	10/10
1250		3/10	3/10	0/10	2/10
2500		0/10	0/10	0/10	0/10
5000		0/10	0/10	0/10	0/10

Fathead Minnow 96-h Acute Survival Test

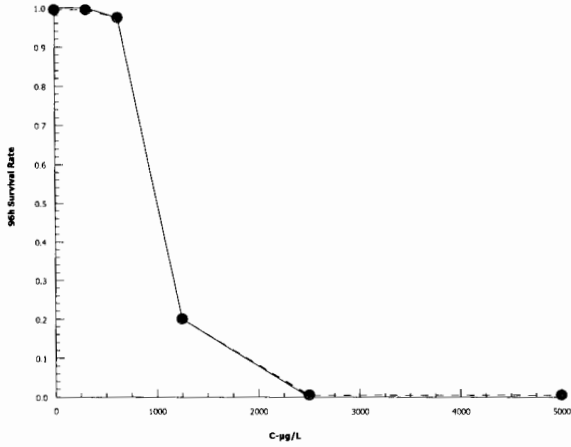
TRE Environmental Strategies

Analysis ID: 02-5835-2962  
Analyzed: 31 Jul-20 14:39

Endpoint: 96h Survival Rate  
Analysis: Untrimmed Spearman-Kärber

CETIS Version: CETISv1.8.7  
Official Results: Yes

Graphics



TOXICITY DATA PACKAGE COVER SHEET

QA: JAP 6/30/20

Test Type: Chronic Acute Project Number: 17001-474-083  
Test Substance: Copper (CuCl<sub>2</sub>) Species: Artemia franciscana  
Dilution Water: rGSL Organism Lot or Batch Number: 062220  
Concurrent Control Water: NA Age: 48 hr (48 hr) Supplier: TRE  
Date and Time Test Began: 6/24/20 @ 1550 Date and Time Test Ended: 6/28/20 @ 1500  
Protocol Number: \_\_\_\_\_ Investigator(s): HR/CP/MS/ES

Background Information

Type of Test: Static-Renewal (48 h) 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: 96 hr Organisms per Replicate: 10  
Type of Food and Quantity per Chamber: 145 ug/L Chla 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, 312.5, 325, 1,250, 2,500, and 5,000 µg/L as Cu

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: 6/23/20

AS 6/23/20

**TEST SUBSTANCE USAGE LOG**

Project Number: 17001-474-083

QA's DAD 6/30/20

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number	ENSR 19122			
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 <u>(RW#)</u> or TRE#, circle one	13962			
Concurrent Control Water RW#	NA			
Date(s) Used	4/24/20			
	4/26/20			

**Preparation of Test Solutions**

Test Substance Conc. (µg/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	250	250						
312.5	16	234	250						
625	31	219	250						
1250	63	188	250						
2500	125	125	250						
5000	250	0	250						
	484	1016	1500						
Initials / Date	HR 4/24/20 Mixed B.S.								
Initials / Date	HR 4/26/20 " "								
Initials / Date									
Initials / Date									
Initials / Date									
Initials / Date									
Initials / Date									
Initials / Date									

Artemia franciscana  
 CHRONIC BIOLOGICAL DATA

QA: DAP 6/30/20

Project Number: 17001-474-083

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	/	/	/	100%	
	B	10	10	10	10	10	/	/	/		
	C	10	10	10	10*	10	/	/	/		* 1 weak org
	D	10	10	10	10	10	/	/	/		
312.5	A	10	10	10*	10	10	/	/	/	* 1 weak org 92.5	
	B	10	10	10	10	10	/	/	/		
	C	10	8	8	8	8	/	/	/		
	D	10	9	9	9	9	/	/	/		
625	A	10	10*	10	10	10	/	/	/	* 1 weak org 95	
	B	10	10	9	9	9	/	/	/		
	C	10	10*	10*	10*	10	/	/	/	* 2 weak orgs	
	D	10	9	9	9	9	/	/	/		
1250	A	10	10	10	10	10	/	/	/	97.5	
	B	10	9	9	9	9	/	/	/		
	C	10	10	10	10	10	/	/	/		
	D	10	10-	10	10	10*	/	/	/		- 1 extra org removed * 1 weak org.
2500	A	10	6*	3	3	3	/	/	/	* 2 weak orgs 20	
	B	10	5*	1	1	1	/	/	/	* 2 weak orgs	
	C	10	6	3	3	3	/	/	/		
	D	10	9	1	1	1	/	/	/		
5000	A	10	0	/	/	/	/	/	/	0	
	B	10	0	/	/	/	/	/	/		
	C	10	0	/	/	/	/	/	/		
	D	10	0	/	/	/	/	/	/		
	A						/	/	/		
	B						/	/	/		
	C						/	/	/		
	D						/	/	/		
Date:		6/24/20	6/25/20	6/26/20	6/27/20	6/28/20					
Time:		1550	1850	1425	1120	1500					
Initials:		CP/AB	CP	CP	CP	ES					

CHRONIC CHEMICAL DATA (INITIAL)

CRA: DMP 6/30/20

Project Number:	17001-474-083
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		8.0	/	8.1	/	/	/	/	/	FM27	
D.O. (mg/L)		5.1	/	5.1	/	/	/	/	/	17	
Temp. (°C)		20	/	20	/	/	/	/	/	IR1	
Cond. (µS/cm)		134,500	/	132,000	/	/	/	/	/	15	
Hard. (mg/L)		/	/	/	/	/	/	/	/		
Alk. (mg/L)		/	/	/	/	/	/	/	/		
TRC (mg/L)		/	/	/	/	/	/	/	/		
NH <sub>3</sub> (mg/L)		/	/	/	/	/	/	/	/		
Conc.:	312.5										
pH		8.0	/	8.1	/	/	/	/	/		
D.O. (mg/L)		5.0	/	5.1	/	/	/	/	/		
Temp. (°C)		20	/	20	/	/	/	/	/		
Cond. (µS/cm)		135,000	/	133,000	/	/	/	/	/		
Hard. (mg/L)		/	/	/	/	/	/	/	/		
Alk. (mg/L)		/	/	/	/	/	/	/	/		
TRC (mg/L)		/	/	/	/	/	/	/	/		
NH <sub>3</sub> (mg/L)		/	/	/	/	/	/	/	/		
Conc.:	625										
pH		8.0	/	8.1	/	/	/	/	/		
D.O. (mg/L)		5.0	/	5.0	/	/	/	/	/		
Temp. (°C)		20	/	20	/	/	/	/	/		
Cond. (µS/cm)		135,000	/	133,000	/	/	/	/	/		
Hard. (mg/L)		/	/	/	/	/	/	/	/		
Alk. (mg/L)		/	/	/	/	/	/	/	/		
TRC (mg/L)		/	/	/	/	/	/	/	/		
NH <sub>3</sub> (mg/L)		/	/	/	/	/	/	/	/		
Conc.:	1250										
pH		8.0	/	8.1	/	/	/	/	/		
D.O. (mg/L)		5.0	/	5.1	/	/	/	/	/		
Temp. (°C)		20	/	20	/	/	/	/	/		
Cond. (µS/cm)		135,200	/	133,200	/	/	/	/	/		
Date:		6/24/20		6/24/20							
Time:		1545		1410							
Initials:		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 (INITIAL)

QA: DDP 6/30/20

Project Number:	17001-474-083
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.:	2500								All Conc.	
pH	8.0	/	8.0	/	/	/	/	/		
D.O. (mg/L)	5.1	/	5.1	/	/	/	/	/		
Temp. (°C)	20	/	20	/	/	/	/	/		
Cond. (µS/cm)	134,700	/	133,300	/	/	/	/	/		
Conc.:		/	/	/	/	/	/	/		
pH	/	/	/	/	/	/	/	/		
D.O. (mg/L)	/	/	/	/	/	/	/	/		
Temp. (°C)	/	/	/	/	/	/	/	/		
Cond. (µS/cm)	/	/	/	/	/	/	/	/		
Conc.:		/	/	/	/	/	/	/		
pH	/	/	/	/	/	/	/	/		
D.O. (mg/L)	/	/	/	/	/	/	/	/		
Temp. (°C)	/	/	/	/	/	/	/	/		
Cond. (µS/cm)	/	/	/	/	/	/	/	/		
Conc.:	5000	/	/	/	/	/	/	/		
pH	7.9	/	/	/	/	/	/	/		
D.O. (mg/L)	5.1	/	/	/	/	/	/	/		
Temp. (°C)	20	/	/	/	/	/	/	/		
Cond. (µS/cm)	134,700	/	/	/	/	/	/	/		
Hard. (mg/L)	/	/	/	/	/	/	/	/		
Alk. (mg/L)	/	/	/	/	/	/	/	/		
TRC (mg/L)	/	/	/	/	/	/	/	/		
NH <sub>3</sub> (mg/L)	/	/	/	/	/	/	/	/		
Date:	6/24/20		6/26/20							
Time:	1545		1410							
Initials:	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 6/30/20

Project Number:	17001-474-083
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					/	/	/	/	All Conc.	* conductivity
pH	7.9	8.1	8.1	8.1	/	/	/	/	FM27	130.6
D.O. (mg/L)	5.4	5.2	4.8	4.5	/	/	/	/	17	
Temp (°C)	20	20	20	20	/	/	/	/	L-13	
Conc.: 312.5					/	/	/	/		* conductivity
pH	7.9	8.1	8.1	8.1	/	/	/	/		133.5
D.O. (mg/L)	5.3	5.2	4.8	4.5	/	/	/	/		
Temp (°C)	20	20	21	20	/	/	/	/		
Conc.: 625					/	/	/	/		* conductivity
pH	7.9	8.0	8.1	8.0	/	/	/	/		133.4
D.O. (mg/L)	5.3	5.2	4.8	4.5	/	/	/	/		
Temp (°C)	20	20	21	20	/	/	/	/		
Conc.: 1250					/	/	/	/		* conductivity
pH	7.8	8.0	8.0	8.0	/	/	/	/		133.3
D.O. (mg/L)	5.3	5.2	4.9	4.5	/	/	/	/		
Temp (°C)	20	20	21	20	/	/	/	/		
Conc.: 2500					/	/	/	/		* conductivity
pH	7.8	8.0	8.0	8.0	/	/	/	/		134.1
D.O. (mg/L)	5.3	5.2	4.5	4.5	/	/	/	/		
Temp (°C)	20	20	21	20	/	/	/	/		
Conc.: 5000	128,100	/	/	/	/	/	/	/		* conductivity
pH	7.9	/	/	/	/	/	/	/		
D.O. (mg/L)	5.4	/	/	/	/	/	/	/		
Temp (°C)	20	/	/	/	/	/	/	/		
Conc.:					/	/	/	/		
pH					/	/	/	/		
D.O. (mg/L)					/	/	/	/		
Temp (°C)					/	/	/	/		
Date:	6/25/20	6/26/20	6/27/20	6/28/20						
Time:	1920	1445	1155	1500						
Initials:	CP	CP	CP	ES						

① CP 6/27/20 E  
 ② ES 6/28/20, E

DAILY TOXICITY TEST LOG

QA: PAA 6/20/20

Project Number:	17001-474-083
Test Species:	<i>Artemia franciscana</i>

General Comments		Feeding 145 ug/l Chla	Initials/Date
	Random Chart: "D" Min/Max Thermometer # M-15		
Test Day 0	Test Solution Mixed at: 1240 Test Organisms Added at: 1550	Fed @ 1240 HR	CP 6/24/20
Test Day 1	Real Time: 20 °C Min-Max Range: 20 - 21 °C	NONE	CP 6/25/20
Test Day 2	Real Time: 20 °C Min-Max Range: 20 - 20 °C	Fed @ 1055 HR	CP 6/26/20
Test Day 3	Real Time: 21 °C Min-Max Range: 20 - 22 °C	NONE	CP 6/27/20
Test Day 4	Real Time: 22 °C Min-Max Range: 21-24 °C	none	6/28/20 ES

**CETIS Analytical Report**

Report Date: 29 Jun-20 10:39 (p 1 of 2)

① Brine shrimp

Test Code: 474-083 | 11-2056-2513

**Fathead Minnow 96-h Acute Survival Test**

TRE Environmental Strategies

<b>Analysis ID:</b> 03-6909-2370	<b>Endpoint:</b> 96h Survival Rate	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 29 Jun-20 10:38	<b>Analysis:</b> Trimmed Spearman-Kärber	<b>Official Results:</b> Yes
<b>Batch ID:</b> 04-4329-6666	<b>Test Type:</b> Survival (96h)	<b>Analyst:</b> Lab Tech
<b>Start Date:</b> 24 Jun-20 15:50	<b>Protocol:</b> EPA/821/R-02-012 (2002)	<b>Diluent:</b> rGSL
<b>Ending Date:</b> 28 Jun-20 15:00	<b>Species:</b> Artemia franciscana	<b>Brine:</b> Crystal Sea
<b>Duration:</b> 95h	<b>Source:</b> In-House Culture	<b>Age:</b> 48h
<b>Sample ID:</b> 16-2709-1750	<b>Code:</b> 60FB7326	<b>Client:</b> Notre Dame
<b>Sample Date:</b> 24 Jun-20 12:40	<b>Material:</b> Copper chloride	<b>Project:</b> Special Studies
<b>Receive Date:</b> 28 Jun-20 15:50	<b>Source:</b> research	
<b>Sample Age:</b> 3h	<b>Station:</b>	

**Trimmed Spearman-Kärber Estimates**

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	5.00%	3.291	0.02129	1956	1773	2157

**96h Survival Rate Summary**

**Calculated Variate(A/B)**

C-µg/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
312.5		4	0.925	0.8	1	0.04787	0.09574	10.4%	7.5%	37	40
625		4	0.95	0.9	1	0.02887	0.05773	6.08%	5.0%	38	40
1250		4	0.975	0.9	1	0.025	0.05	5.13%	2.5%	39	40
2500		4	0.2	0.1	0.3	0.05774	0.1155	57.7%	80.0%	8	40
5000		4	0	0	0	0	0		100.0%	0	40

**96h Survival Rate Detail**

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
312.5		1	1	0.8	0.9
625		1	0.9	1	0.9
1250		1	0.9	1	1
2500		0.3	0.1	0.3	0.1
5000		0	0	0	0

**96h Survival Rate Binomials**

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	10/10	10/10	10/10	10/10
312.5		10/10	10/10	8/10	9/10
625		10/10	9/10	10/10	9/10
1250		10/10	9/10	10/10	10/10
2500		3/10	1/10	3/10	1/10
5000		0/10	0/10	0/10	0/10

① DAP 6/30/20 E

**CETIS Analytical Report**

Report Date: 29 Jun-20 10:39 (p 2 of 2)

Test Code: 474-083 | 11-2056-2513

*Brine shrimp*

① **Fathead Minnow 96-h Acute Survival Test**

TRE Environmental Strategies

Analysis ID: 03-6909-2370

Endpoint: 96h Survival Rate

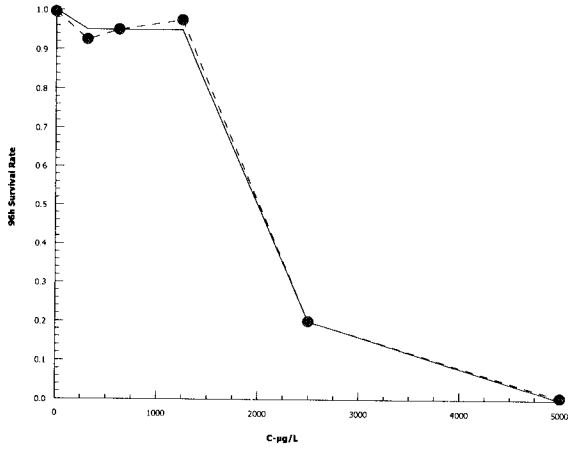
CETIS Version: CETISv1.8.7

Analyzed: 29 Jun-20 10:38

Analysis: Trimmed Spearman-Kärber

Official Results: Yes

**Graphics**



① *DAF 6/30/20 E*