TRE Environmental Strategies, LLC
100 Racquette Drive, Unit A, Fort Collins, Colorado, 80524
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July 10, 2020

Mr. Christopher Bittner
Standards Coordinator
Utah Dept. of Environmental Quality
195 N 1950 W
Salt Lake City, UT 84116

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

Subject: Results of Analytical Data for Experiment #22

Mr. Bittner/ Dr. Belovsky:

Below is a summary of the analytical data for the acute brine shrimp experiments initiated on June 18, 2020. Total zinc samples were collected in old solutions on day 2.

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#13962	7.7	NM	NM	133,500	NM	NM	114

[a]As CaCO3
[b]Total residual chlorine

Results of Zinc Analysis

D. viridis / YTC Mix			*D. viridis* Only		
Total Zinc (mg/L)		Percent of Nominal	Total Zinc (mg/L)		Percent of Nominal
Nominal Value (mg/L)	Day 2 Old Solution		Nominal Value (mg/L)	Day 2 Old Solution	
0 (rGSL)	U	---	0 (rGSL)	0.026	---
19	13.7	72	19	15.2	80
37.5	27.5	73	37.5	31.5	84
75	57.1	76	75	56.3	75
150	108	72	150	106	71
300	227	76	300	227	76

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

Measured zinc values were slightly reduced from nominal values (~75%). Average measured zinc concentrations were then used to recalculate the test endpoint on a measured basis. Both nominal and measured median lethal concentrations are presented below for comparison.

Test Endpoints

Test	Survival 96-hour LC_{50}	Value (mg/L Zinc)
D. viridis **/ YTC Mix**	Nominal	183.6 (C.L. 128.8-261.6)
	Measured	134.9 (C.L. 94.86-191.7)
D. viridis **Only**	Nominal	157.3 (C.L. 128.4-188.4)
	Measured	115.6 (C.L. 94.82-138.8)

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
Project Specialist / QA Officer
bidlackac.tre@gmail.com

Rami B. Naddy, Ph.D.
Manager / Environmental Toxicologist
naddyrb.tre@gmail.com

17001-474-091,092

Attachment

cc: David Pillard, TRE

TRE Environmental Strategies, LLC
100 Racquette Drive, Unit A, Fort Collins, Colorado, 80524
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June 24, 2020

Mr. Christopher Bittner
Standards Coordinator
Utah Dept. of Environmental Quality
195 N 1950 W
Salt Lake City, UT 84116

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

Subject: Results of Acute Brine Shrimp Bioavailability Experiment #22

Mr. Bittner/ Dr. Belovsky:

Below is a summary of the acute brine shrimp experiments initiated on June 18, 2020. The purpose of these experiments was to investigate the difference in the bioavailability of zinc to brine shrimp when fed *D. viridis*/YTC[1] mixture or solely *D. viridis*.

Along with a control, five different nominal zinc concentrations (prepared with $ZnSO_4$) were tested:

- 19, 37.5, 75, 150, and 300 mg/L

The results of these studies will help determine the observed toxicity of zinc 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 Chl*a* and 0.3 ml YTC or 145 µg/L Chl*a*
- Temperature: 20°C
- Test volume(s): 50 ml
- Replicates: 4
- Organisms/Rep: 10
- Test media: 120 ppt rGSL media (per Notre Dame recipe)

[1] 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 Chl*a* and 0.3 ml YTC. Solutions were gently aerated.

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#13962	7.7	NM	NM	133,500	NM	NM	114

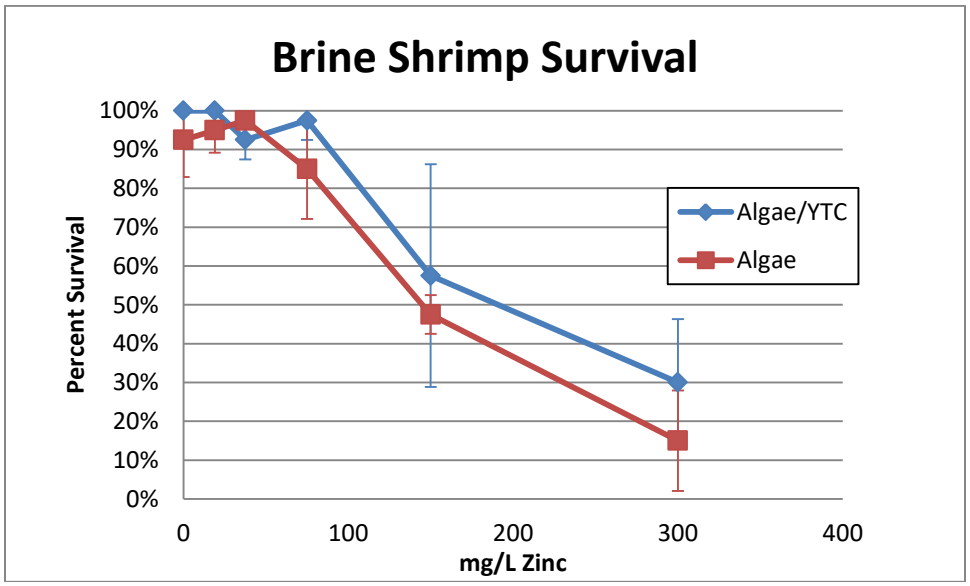
[a]As CaCO3
[b]Total residual chlorine

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.
- 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 of the brine shrimp in the zinc treatments is illustrated in the following figure:



TRE

Test Endpoints

Food: *D. viridis*/YTC mix				
Test Concentration (mg/L Zinc) (nominal)	**Percent Survival of *Artemia franciscana***			
	24 hours	**48 hours**	**72 hours**	**96 hours**
0 (rGSL)	100	100	100	100
19	100	100	100	100
37.5	100	100	97.5	92.5
75	100	97.5	97.5	97.5
150	95	77.5	70	57.5
300	97.5	77.5	50	30
Control Performance	Acceptable			

Food: *D. viridis* alone				
Test Concentration (mg/L Zinc) (nominal)	**Percent Survival of *Artemia franciscana***			
	24 hours	**48 hours**	**72 hours**	**96 hours**
0 (rGSL)	97.5	97.5	92.5	92.5
19	95	95	95	95
37.5	100	100	100	97.5
75	95	92.5	90	85
150	77.5	75	60	47.5
300	40	37.5	35	15
Control Performance	Acceptable			

Data Analysis and Test Endpoints

Test	Biological Endpoint	Statistical Endpoint	Value (mg/L Zinc) (nominal)
D. viridis/YTC mix	Survival	96-hour LC_{50}	183.6 (C.L. 128.8 -261.6)
D. viridis only	Survival	96-hour LC_{50}	157.3 (C.L. 128.4 -188.4)

TRE

Summary and findings:

- Organism survival was ≥ 90% for the controls.
- Zinc toxicity was clearly demonstrated at these testing concentrations.
- Samples were collected for zinc analysis and measured endpoints will be forthcoming.
- Test end points were similar for both food types. Even though the 96-h LC_{50} from the *D. viridis*/YTC mix was higher than the *D. viridis* only test, the substantial overlap of the 95% confidence limits suggests there is no statistical difference in zinc bioavailability between the two types of food.

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

Rami B. Naddy, Ph.D.
Manager / Environmental Toxicologist
naddyrb.tre@gmail.com

17001-474-(091,092)

Attachment

cc: David Pillard, TRE

TOXICITY DATA PACKAGE COVER SHEET

QA: [illegible] 6/23/20

Test Type: ~~Chronic~~ Acute

Project Number: 17001-474-091

Test Substance: Znc (ZnSO4)

Species: *Artemia franciscana*

Dilution Water: rGSL

Organism Lot or Batch Number: 061620

Concurrent Control Water: NA

Age: 48HR (48 hr)

Supplier: TRE

Date and Time Test Began: 6/18/20 @ 1420

Date and Time Test Ended: 6/22/20 @ 1350

Protocol Number:

Investigator(s): HR/CR/EN/AF/ES

Background Information

pH control?: Yes **No**

Type of Test: Static-Renewal (48 h)

If yes, give % CO_2: 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 Renwals

Test Substance Characterization Parameters and Frequency:

Hardness: Test Initiation

Alkalinity: Test Initiation

NH_3: Test Initiation

TRC: Test Initiation

pH: Daily

Conductivity: Daily

Test Concentrations (Volume:Volume): rGSL, 19, 37.5, 75, 150, and 300 mg/L as Zn

Agency Summary Sheet(s)?: None

Reference Toxicant Data:	Test Dates: NA	to NA	IC_{25}: —
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: AB Date: 6/16/20

AB 6/16/20

TEST SUBSTANCE USAGE LOG

QA: DLP 6/23/20

Project Number: 17001-474-091

	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	13962			
Concurrent Control Water RW#	NA			
Date(s) Used	6/18/20			
	6/20/20			

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	250	250						
19	16	234	250						
37.5	31	219	250						
75	63	188	250						
150	125	125	250						
300	250	0	250						
	485	1015	1500						

Initials / Date	HR 6/18/20 Mixed B.S.		
Initials / Date	CP 6/20/20 " "		
Initials / Date			
Initials / Date			
Initials / Date			
Initials / Date			
Initials / Date			
Initials / Date			

QA Form No. 060
Revision 3
Effective 02/14

Artemia franciscana
CHRONIC BIOLOGICAL DATA

QA: DAP 6/23/20

Project Number: 17001-474-091

mg/L	Test Replicate	Number of Surviving Organisms Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Remarks
										% survival
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				
19	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				
37.5	A	10	10	10	10	9				92.5
	B	10	10	10	9	9				
	C	10	10	10	10	9				
	D	10	10	10	10	10				
75	A	10	10	10	10	10				97.5
	B	10	10	10	10	10				
	C	10	10*	9	9	9				*removed 1 extra org
	D	10	10	10	10	10				
150	A	10	9	7	7	5				57.5
	B	10	9	8	7	6				
	C	10	10	9	①67	7				
	D	10	10	7	7	5				
300	A	10	10	9	6	5				30
	B	10	10	7	5	3				
	C	10	10	8	8	3				
	D	10	9	7	1	1				
	A									
	B									
	C									
	D									
Date:		6/18/20	6/19/20	6/20/20	6/21/20	6/22/20				
Time:		1420	1700	1400	0935	1300				
Initials:		CP/EN	AF	CP	ES	EN				

① ES 6/21/20 jf

CHRONIC CHEMICAL DATA (INITIAL)

QA: DAP 6/23/20

Project Number: 17001-474-091

Test Species: ***Artemia franciscana***

%	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter #	Remarks
Conc.: 0									All Conc.	
pH	7.7		7.9						FM27	
D.O. (mg/L)	5.1		5.0						17	
Temp. (°C)	20		20						IR1	
Cond. (µS/cm)	133,500		130,500						15	
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Conc.: 19										
pH	7.6		7.8							
D.O. (mg/L)	5.1		5.0							
Temp. (°C)	20		20							
Cond. (µS/cm)	133,400		131,000							
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Conc.: 37.5										
pH	7.5		7.6							
D.O. (mg/L)	5.1		5.0							
Temp. (°C)	20		20							
Cond. (µS/cm)	132,700		131,000							
Conc.: 75										
pH	7.2		7.3							
D.O. (mg/L)	5.1		5.1							
Temp. (°C)	20		20							
Cond. (µS/cm)	131,900		132,000							
Date:	6/18/20		6/20/20							
Time:	1410		1350							
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.

QA Form No. 058
Revision 4
Effective 02/14

CHRONIC CHEMICAL DATA (INITIAL)

QA: DAP 6/23/20

Project Number:	17001-474-091
Test Species:	***Artemia franciscana***

%	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter #	Remarks
Conc.: 150									All Conc.	
pH	6.9		7.0							
D.O. (mg/L)	5.1		5.1							
Temp. (°C)	20		20							
Cond. (µS/cm)	131,500		130,300							
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.: 300										
pH	6.7		6.7							
D.O. (mg/L)	5.1		5.1							
Temp. (°C)	20		20							
Cond. (µS/cm)	131,700		130,600							
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Date:	6/18/20		6/20/20							
Time:	1410		1350							
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.

QA Form No. 059
Revision 3
Effective 02/14

QA's DAP 6/23/20

CHRONIC CHEMICAL DATA (FINAL)

Project Number:	17001-474-091
Test Species:	***Artemia franciscana***

%		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter #	Remarks
Conc.:	0				182300					All Conc.	* conductivity 15
pH		7.9	7.9	8.0	7.9					FM27	
D.O. (mg/L)		4.9	5.2	5.0	5.0					17	
Temp (°C)		20	20	20	19					29	
Conc.:	19				189900						* conductivity
pH		7.8	7.8	8.0	7.9						
D.O. (mg/L)		5.0	5.3	5.1	5.1						
Temp (°C)		20	20	19	19						
Conc.:	37.5				188600						* conductivity
pH		7.8	7.8	8.0	7.9						
D.O. (mg/L)		15.0	5.3	5.1	5.1						
Temp (°C)		20	20	19	19						
Conc.:	75				186700						* conductivity
pH		7.8	7.6	7.9	7.7						
D.O. (mg/L)		5.0	5.3	5.1	5.0						
Temp (°C)		20	20	19	19						
Conc.:	150				185900						* conductivity
pH		7.5	7.3	7.8	7.4						
D.O. (mg/L)		5.1	5.3	5.1	5.0						
Temp (°C)		20	20	19	19						
Conc.:	300				188000						* conductivity
pH		7.2	7.9	7.4	7.1						
D.O. (mg/L)		5.1	5.3	5.1	5.0						
Temp (°C)		20	20	19	19						
Conc.:											
pH											
D.O. (mg/L)											
Temp (°C)											
Date:		6/19/20	6/20/20	6/21/20	6/22/20						
Time:		1650	1415	0925	1340						
Initials:		AF	CP	ES	EN						

①AF 6/19/20E

QA: DAP 6/23/20

DAILY TOXICITY TEST LOG

Project Number:	17001-474-091
Test Species:	***Artemia franciscana***

General Comments	Random Chart: P Min/Max Thermometer # M-15	Feeding 72.5 ug/l Chla 0.33ml YTC	Initials/Date
Test Day 0	Test Solution Mixed at: 1050 Test Organisms Added at: 1420	Fed @ 1050 HR	CP 6/18/20
Test Day 1	Real Time: 21 °C Min-Max Range: 20-21 °C	NONE	AF 6/19/20
Test Day 2	Real Time: 21 °C Min-Max Range: 20-22 °C	Fed @ 1045 CP	CP 6/20/20
Test Day 3	Real Time: 21 °C Min-Max Range: 20-22 °C	none	ES 6/21/20
Test Day 4	Real Time: 21 °C Min-Max Range: 20-22 °C	None	EN 6/22/20

① ~~Fathead Minnow~~ 96-h Acute Survival Test | **TRE Environmental Strategies**

Analysis ID:	12-3138-9461	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	23 Jun-20 8:32	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes
Batch ID:	13-5627-3287	**Test Type:**	Survival (96h)	**Analyst:**	Lab Tech
Start Date:	18 Jun-20 14:20	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	rGSL
Ending Date:	22 Jun-20 13:50	**Species:**	Artemia franciscana	**Brine:**	Crystal Sea
Duration:	96h	**Source:**	In-House Culture	**Age:**	48h
Sample ID:	06-1181-3621	**Code:**	247788F5	**Client:**	Notre Dame
Sample Date:	18 Jun-20 14:20	**Material:**	Zinc sulfate	**Project:**	Special Studies
Receive Date:	22 Jun-20 13:50	**Source:**	Discharge Monitoring Report		
Sample Age:	NA	**Station:**			

Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	30.00%	2.264	0.0769	183.6	128.8	261.6

96h Survival Rate Summary — Calculated Variate(A/B)

C-mg/L ①	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
19		4	1	1	1	0	0	0.0%	0.0%	40	40
37.5		4	0.925	0.9	1	0.025	0.05	5.41%	7.5%	37	40
75		4	0.975	0.9	1	0.025	0.05	5.13%	2.5%	39	40
150		4	0.575	0.5	0.7	0.04787	0.09574	16.7%	42.5%	23	40
300		4	0.3	0.1	0.5	0.08165	0.1633	54.4%	70.0%	12	40

96h Survival Rate Detail

C-mg/L ①	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
19		1	1	1	1
37.5		0.9	0.9	0.9	1
75		1	1	0.9	1
150		0.5	0.6	0.7	0.5
300		0.5	0.3	0.3	0.1

96h Survival Rate Binomials

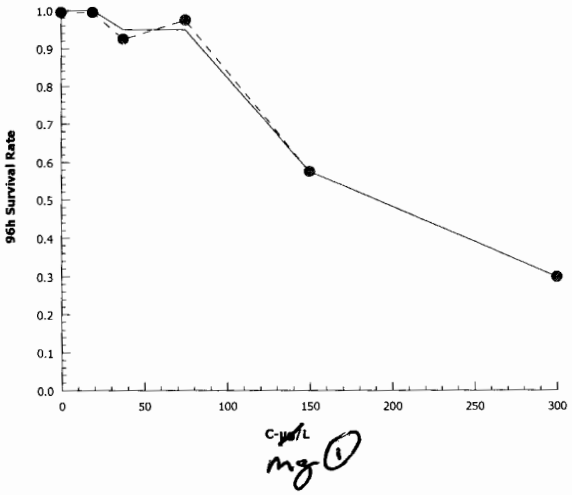
C-mg/L ①	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	10/10	10/10	10/10	10/10
19		10/10	10/10	10/10	10/10
37.5		9/10	9/10	9/10	10/10
75		10/10	10/10	9/10	10/10
150		5/10	6/10	7/10	5/10
300		5/10	3/10	3/10	1/10

① DAP 6/23/20 E

(1) ~~Fathead Minnow~~ **96-h Acute Survival Test** **TRE Environmental Strategies**

Analysis ID:	12-3138-9461	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	23 Jun-20 8:32	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes

Graphics



(1) DAP 6/23/20 E

TOXICITY DATA PACKAGE COVER SHEET

QA: DAP 6/23/20

Test Type: ~~Chronic~~ Acute (1)

Project Number: 17001-474-092

Test Substance: Znc ($ZnSO_4$)

Species: *Artemia franciscana*

Dilution Water: rGSL

Organism Lot or Batch Number: 061620

Concurrent Control Water: NA

Age: 48 HR (48 hr)

Supplier: TRE

Date and Time Test Began: 6/18/20 @ 1535

Date and Time Test Ended: 6/22/20 @ 1500

Protocol Number:

Investigator(s): HR/CP/MB/AF/ES/EN

Background Information

pH control?: Yes **No**

Type of Test: Static-Renewal (48 h)

If yes, give % CO_2: 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 Renwals

Test Substance Characterization Parameters and Frequency:

Hardness: Test Initiation Alkalinity: Test Initiation NH_3: Test Initiation TRC: Test Initiation

pH: Daily Conductivity: Daily

Test Concentrations (Volume:Volume): rGSL, 19, 37.5, 75, 150, and 300 mg/L as Zn

Agency Summary Sheet(s)?: None

Reference Toxicant Data:	Test Dates: NA	to NA	IC_{25}: —
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: AB Date: 6/16/20

AB 6/16/20 E

TEST SUBSTANCE USAGE LOG

QA: DKP 6/23/20

Project Number: 17001-474-092

	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	13962			
Concurrent Control Water RW#	NA			
Date(s) Used	6/18/20			
	6/20/20			

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	250	250						
19	16	234	250						
37.5	31	219	250						
75	63	188	250						
150	125	125	250						
300	250	0	250						
	485	1015	1500						

Initials / Date	HR 6/18/20 Mixed B.S.		
Initials / Date	CP 6/20/20 " "		
Initials / Date			
Initials / Date			
Initials / Date			
Initials / Date			
Initials / Date			
Initials / Date			

Artemia franciscana
CHRONIC BIOLOGICAL DATA

QA: DMA 6/23/20

Project Number: 17001-474-092

mg/L	Test Replicate	Number of Surviving Organisms Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	% Survival Remarks
0	A	10	10	10	10	10				92.5%
	B	10	9	9	9	9				
	C	10	10	10	10	10				
	D	10	10	9	8	8				
19	A	10	10	10	10	10				95%
	B	10	10	10	10	10				
	C	10	9	9	9	9				
	D	10	9	9	9	9				
37.5	A	10	10	10	10	9				87.5%
	B	10	10	10	10	10				
	C	10	10	10	10	10				
	D	10	10	10	10	10				
75	A	10	10	10	10	10				85%
	B	10	9	9	9	9				
	C	10	9	9	8	7				
	D	10	10	9	9	8				
150	A	10	7	7	4	4				47.5%
	B	10	8	7	6	5				
	C	10	7	7	6	5				
	D	10	9	9	8	5				
300	A	10	5	5	4	3				15%
	B	10	2	2	2	1				
	C	10	5	4	4	2				
	D	10	4	4	4	0				
	A									
	B									
	C									
	D									
	Date:	6/18/20	6/19/20	6/20/20	6/21/20	6/22/20				
	Time:	1535	1720	1525	0955	1500				
	Initials:	CP/MB	AF	CP	ES	EN				

CHRONIC CHEMICAL DATA (INITIAL)

QA: DLD 6/23/20

Project Number: 17001-474-092

Test Species: ***Artemia franciscana***

%	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						FM27	
D.O. (mg/L)	4.8		5.0						17	
Temp. (°C)	20		20						IR1	
Cond. (µS/cm)	129,200		132,200						15	
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Conc.: 19										
pH	7.8		7.8							
D.O. (mg/L)	4.8		5.1							
Temp. (°C)	20		20							
Cond. (µS/cm)	130,300		132,800							
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Conc.: 37.5										
pH	7.7		7.6							
D.O. (mg/L)	4.9		5.1							
Temp. (°C)	20		20							
Cond. (µS/cm)	130,400		132,900							
Conc.: 75										
pH	7.3		7.3							
D.O. (mg/L)	5.0		5.0							
Temp. (°C)	20		20							
Cond. (µS/cm)	130,500		133,100							
Date:	6/18/20		6/20/20							
Time:	1525		1500							
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.

QA: DAA 6/23/20

CHRONIC CHEMICAL DATA (INITIAL)

Project Number:	17001-474-092
Test Species:	***Artemia franciscana***

%	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter #	Remarks
Conc.: 150									All Conc.	
pH	7.0		7.0							
D.O. (mg/L)	5.0		5.0							
Temp. (°C)	20		20							
Cond. (µS/cm)	130,800		132,800							
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.: 300										
pH	6.7		6.7							
D.O. (mg/L)	5.1		5.1							
Temp. (°C)	20		20							
Cond. (µS/cm)	131,300		133,600							
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Date:	6/18/20		6/20/20							
Time:	1525		1500							
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.

QA Form No. 059
Revision 3
Effective 02/14

QA: DAP 6/23/20

CHRONIC CHEMICAL DATA (FINAL)

Project Number:	17001-474-092
Test Species:	***Artemia franciscana***

%	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter #	Remarks
Conc.: 0				165000					All Conc.	* conductivity 15
pH	8.0	8.0	8.2	8.1					FM27	
D.O. (mg/L)	4.8	4.9	4.8	4.9					17	
Temp (°C)	20	20	20	19					L9	
Conc.: 19				183600						* conductivity
pH	8.0	7.9	8.1	8.0						
D.O. (mg/L)	5.0	4.9	4.8	5.0						
Temp (°C)	20	20	19	19						
Conc.: 37.5				184700						* conductivity
pH	7.9	7.9	8.1	8.0						
D.O. (mg/L)	5.0	4.9	4.8	5.1						
Temp (°C)	20	20	19	19						
Conc.: 75				185200						* conductivity
pH	7.8	7.7	7.9	7.8						
D.O. (mg/L)	5.1	5.0	4.9	5.0						
Temp (°C)	20	20	19	19						
Conc.: 150				185900						* conductivity
pH	7.6	7.3	7.7	7.6						
D.O. (mg/L)	5.1	5.0	4.9	5.0						
Temp (°C)	20	20	19	19						
Conc.: 300				186400						* conductivity
pH	7.4	6.9	7.3	7.2						
D.O. (mg/L)	5.1	5.0	4.9	5.1						
Temp (°C)	20	20	19	19						
Conc.:										
pH										
D.O. (mg/L)										
Temp (°C)										
Date:	6/19/20	6/20/20	6/21/20	6/22/20						
Time:	1725	1515	0955	1450						
Initials:	AF	CP	ES	EN						

QA Form No. 055
Revision 3
Effective 02/14
QA ; DEP 6/23/20

DAILY TOXICITY TEST LOG

Project Number:	17001-474-092
Test Species:	***Artemia franciscana***

General Comments		Feeding 145 ug/l Chla	Initials/Date
	Random Chart: D Min/Max Thermometer # M15		
Test Day 0	Test Solution Mixed at: 1100 Test Organisms Added at: 1535	Fed @ 1100 HR	CP 6/18/20
Test Day 1	Real Time: 21 °C Min-Max Range: 20-21 °C	NONE	AF 6/19/20
Test Day 2	Real Time: 21 °C Min-Max Range: 20 - 22 °C	Fed @ 1145 CP	CP 6/20/20
Test Day 3	Real Time: 21 °C Min-Max Range: 20-22 °C	none	ES 6/21/20
Test Day 4	Real Time: 21 °C Min-Max Range: 20-22 °C	None	EN 6/22/20

CETIS Analytical Report

Report Date: 23 Jun-20 08:36 (p 1 of 3)
Test Code: 474-092 | 07-2515-4986

(1) Brine Shrimp

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

TRE Environmental Strategies

Analysis ID:	10-3925-7752	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	23 Jun-20 8:35	**Analysis:**	Linear Regression (MLE)	**Official Results:**	Yes
Batch ID:	12-4465-0364	**Test Type:**	Survival (96h)	**Analyst:**	Lab Tech
Start Date:	18 Jun-20 15:35	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	rGSL
Ending Date:	22 Jun-20 15:00	**Species:**	Artemia franciscana	**Brine:**	Crystal Sea
Duration:	95h	**Source:**	In-House Culture	**Age:**	48h
Sample ID:	06-1181-3621	**Code:**	247788F5	**Client:**	Notre Dame
Sample Date:	18 Jun-20 14:20	**Material:**	Zinc sulfate	**Project:**	Special Studies
Receive Date:	22 Jun-20 13:50	**Source:**	research		
Sample Age:	75m	**Station:**			

Linear Regression Options

Model Function	Threshold Option	Threshold	Optimized	Pooled	Het Corr	Weighted
Log-Normal [NED=A+B*log(X)]	Control Threshold	0.075	Yes	No	No	Yes

Regression Summary

Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	F Stat	Critical	P-Value	Decision(α:5%)
13	-85.61	178.4	180.8	2.197	0.2606	0.8213	0.7513	3.16	0.5358	Non-Significant Lack of Fit

Point Estimates

Level	mg/L	95% LCL	95% UCL
LC5	58.62	31.63	80.47
LC10	72.89	43.87	95.42
LC15	84.44	54.56	107.3
LC20	94.91	64.74	118.1
LC25	104.9	74.8	128.5
LC40	135.1	106	161.4
LC50	157.3	128.4	188.4

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
Threshold	0.0492	0.02121	0.007624	0.09078	2.319	0.0305	Significant Parameter
Slope	3.838	0.6879	2.489	5.186	5.579	<0.0001	Significant Parameter
Intercept	-8.43	1.526	-11.42	-5.438	-5.523	<0.0001	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	99.78909	99.78909	1	107.7	<0.0001	Significant
Lack of Fit	2.165107	0.721702	3	0.7513	0.5358	Non-Significant
Pure Error	17.29004	0.960558	18			
Residual	19.45515	0.926436	21			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Goodness-of-Fit	Pearson Chi-Sq GOF	19.46	32.67	0.5560	Non-Significant Heterogenity
	Likelihood Ratio GOF	22.71	32.67	0.3599	Non-Significant Heterogenity
Variances	Bartlett Equality of Variance	5.535	11.07	0.3542	Equal Variances
	Mod Levene Equality of Variance	1.942	2.773	0.1369	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9481	0.9169	0.2458	Normal Distribution
	Anderson-Darling A2 Normality	0.6392	2.492	0.0960	Normal Distribution

(1) DAP 6/23/20 F

① ~~Fathead Minnow~~ 96-h Acute Survival Test — TRE Environmental Strategies

Analysis ID:	10-3925-7752	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	23 Jun-20 8:35	**Analysis:**	Linear Regression (MLE)	**Official Results:**	Yes

96h Survival Rate Summary — Calculated Variate(A/B)

C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Dilution Water	4	0.925	0.8	1	0.04787	0.09574	10.4%	0.0%	37	40
19		4	0.95	0.9	1	0.02887	0.05773	6.08%	-2.7%	38	40
37.5		4	0.975	0.9	1	0.025	0.05	5.13%	-5.41%	39	40
75		4	0.85	0.7	1	0.06455	0.1291	15.2%	8.11%	34	40
150		4	0.475	0.4	0.5	0.025	0.05	10.5%	48.6%	19	40
300		4	0.15	0	0.3	0.06455	0.1291	86.1%	83.8%	6	40

96h Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	0.9	1	0.8
19		1	1	0.9	0.9
37.5		0.9	1	1	1
75		1	0.9	0.7	0.8
150		0.4	0.5	0.5	0.5
300		0.3	0.1	0.2	0

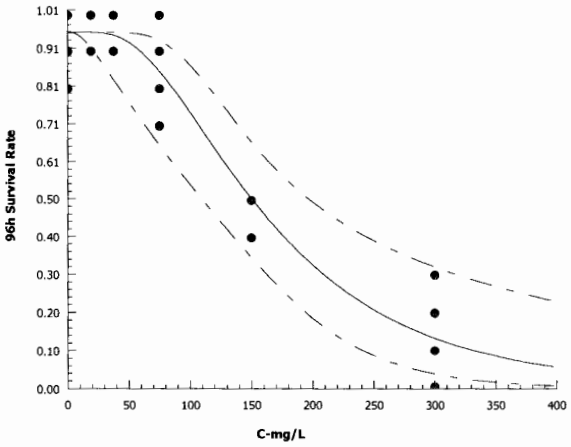
96h Survival Rate Binomials

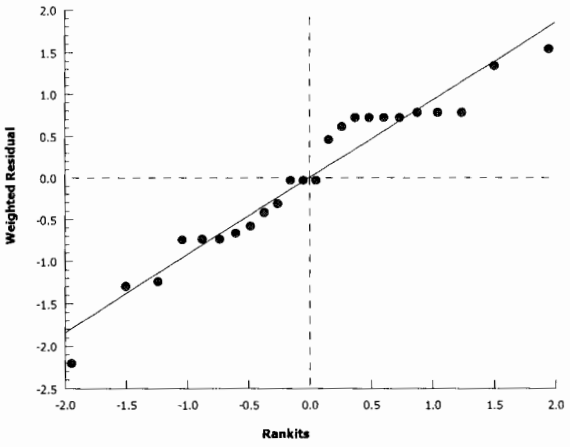
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	10/10	9/10	10/10	8/10
19		10/10	10/10	9/10	9/10
37.5		9/10	10/10	10/10	10/10
75		10/10	9/10	7/10	8/10
150		4/10	5/10	5/10	5/10
300		3/10	1/10	2/10	0/10

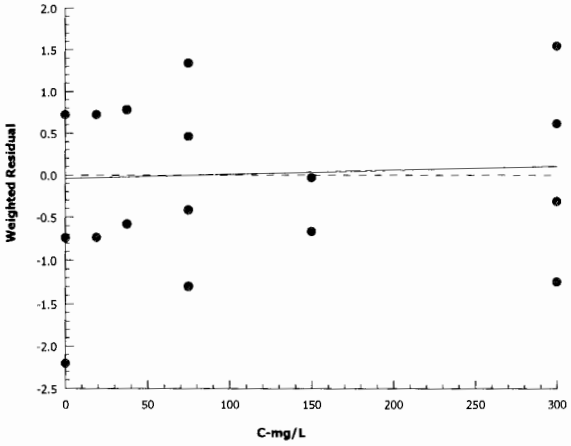
① DAP 6/23/20

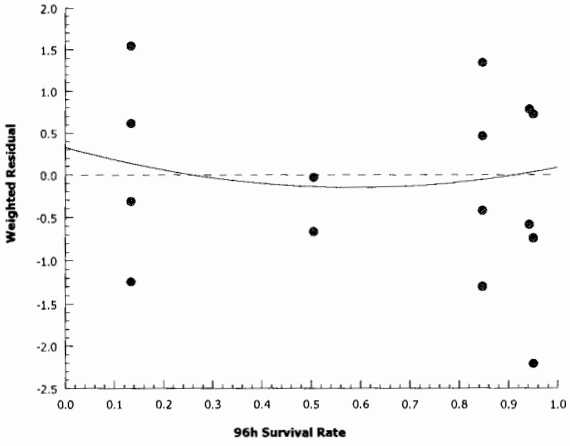
Analysis ID:	10-3925-7752	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	23 Jun-20 8:35	**Analysis:**	Linear Regression (MLE)	**Official Results:**	Yes

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









(1) DSP 6/23/20 E