

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 #21**

Mr. Bittner/ Dr. Belovsky:

Below is a summary of the analytical data for the acute brine shrimp experiments initiated on June 17, 2020. Total arsenic samples were collected in old solutions on day 2.

**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#13953	7.8	NM	NM	134,200	NM	NM	121

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

<sup>b</sup>Total residual chlorine

**Results of Arsenic Analysis**

<i>D. viridis</i> / YTC Mix			<i>D. viridis</i> Only		
Total Arsenic (mg/L)		Percent of Nominal	Total Arsenic (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)	U	---
5	4.05	81	5	4.29	86
20	16.4	82	20	19.4	97
50	44.4	89	50	43.0	86
100	94.4	94	100	86.4	86
200	181	90	200	177	89

U= below method detection limit (0.11 mg/L)

Measured arsenic values were similar to nominal values (~88%). Average measured arsenic 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 <sub>50</sub>	Value (mg/L Arsenic)
<i>D. viridis</i> / YTC Mix	Nominal	62.71 (C.L. 54.73-71.85)
	Measured	56.52 (C.L. 48.88-65.34)
<i>D. viridis</i> Only	Nominal	66.80 (C.L. 58.33-75.59)
	Measured	57.45 (C.L. 50.03-65.12)

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  
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Rami B. Naddy, Ph.D.  
Manager / Environmental Toxicologist  
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17001-474-088,089

Attachment

cc: David Pillard, TRE

June 24, 2020

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

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

**Subject: Results of Acute Brine Shrimp Bioavailability Experiment #21**

Mr. Bittner/ Dr. Belovsky:

Below is a summary of the acute brine shrimp experiments initiated on June 17, 2020. The purpose of this experiment was to investigate the difference in the bioavailability of arsenic to brine shrimp when fed *D. viridis*/YTC<sup>1</sup> mixture or solely *D. viridis*.

Along with a control, five different nominal arsenic concentrations (prepared with Na<sub>2</sub>HAsO<sub>4</sub>) were tested:

- 5, 20, 50, 100, and 200 mg/L

The results of these studies will help determine the observed toxicity of arsenic 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#13953	7.8	NM	NM	134,200	NM	NM	121

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

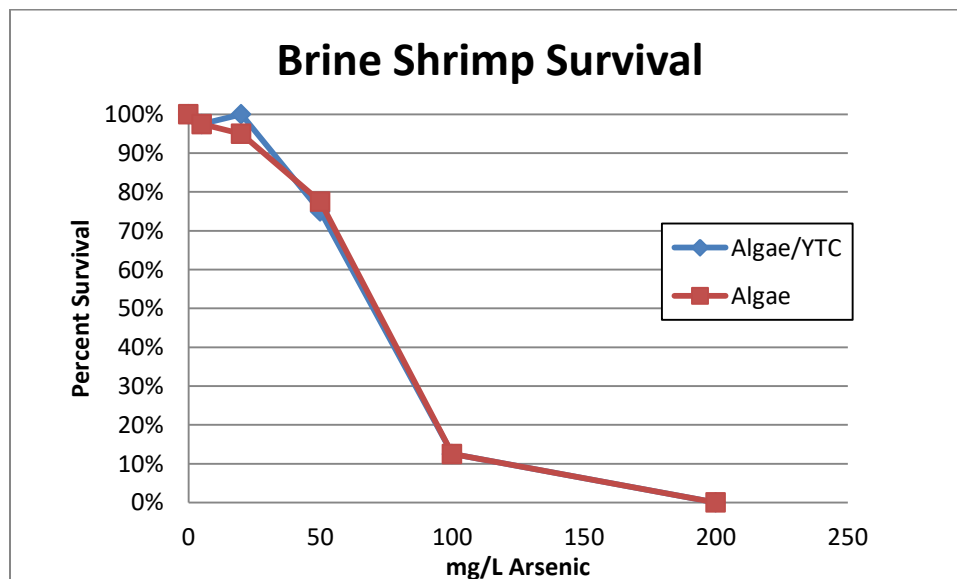
<sup>b</sup>Total residual chlorine

#### 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.
- Arsenic 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 arsenic treatments is illustrated in the following figure:



### Test Endpoints

Food: <i>D. viridis</i> /YTC mix				
Test Concentration (mg/L Arsenic) (nominal)	Percent Survival of <i>Artemia franciscana</i>			
	24 hours	48 hours	72 hours	96 hours
0 (rGSL)	100	100	100	100
5	100	100	100	97.5
20	100	100	100	100
50	97.5	82.5	82.5	75
100	60	22.5	15	12.5
200	10	2.5	0	0
Control Performance		Acceptable		

Food: <i>D. viridis</i> alone				
Test Concentration (mg/L Arsenic) (nominal)	Percent Survival of <i>Artemia franciscana</i>			
	24 hours	48 hours	72 hours	96 hours
0 (rGSL)	100	100	100	100
5	100	97.5	97.5	97.5
20	97.5	97.5	97.5	95
50	90	82.5	82.5	77.5
100	70	30	25	12.5
200	10	2.5	2.5	0
Control Performance		Acceptable		

### Data Analysis and Test Endpoints

Test	Biological Endpoint	Statistical Endpoint	Value (mg/L Arsenic) (nominal)
<i>D. viridis</i> /YTC mix	Survival	96-hour LC <sub>50</sub>	62.71 (C.L. 54.73 -71.85)
<i>D. viridis</i> only	Survival	96-hour LC <sub>50</sub>	66.8 (C.L. 58.33 -75.59)

**Summary and findings:**

- Organism survival was  $\geq 90\%$  for the controls.
- Arsenic toxicity was clearly demonstrated at these testing concentrations.
- Samples were collected for arsenic analysis and measured endpoints will be forthcoming.
- Test end points were similar for both food types, indicating that for arsenic, there is no difference in bioavailability between the two types of food.

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

17001-474-(088,089)

Attachment

cc: David Pillard, TRE

TOXICITY DATA PACKAGE COVER SHEET

QA: ASR 6/23/20

Test Type: Chronic Acute Project Number: 17001-474-088  
Test Substance: Arsenic (Na2HAsO4) Species: Artemia franciscana  
Dilution Water: rGSL Organism Lot or Batch Number: 061520  
Concurrent Control Water: NA Age: 48 hr (48 hr) Supplier: TRE  
Date and Time Test Began: 6/17/20 @ 1500 Date and Time Test Ended: 6/21/20 @ 1445  
Protocol Number: \_\_\_\_\_ Investigator(s): ASR/CP/AE/L/HR

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, 5, 20, 50, 100, and 200 mg/L as As  
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: ASR Date: 6/16/20

ASR 6/16/20

**TEST SUBSTANCE USAGE LOG**

QA : DSP 6/23/20

Project Number: 17001-474-088

	Sample 1	Sample 2	Sample 3	Sample 4
<b>Test Substance Number</b>	C17-021			
<b>Test Substance Collection Date and Time</b>	From: @	From: @	From: @	From: @
	To: @	To: @	To: @	To: @
<b>Sample Type (Grab or Comp)</b>				
<b>Date Test Substance Received</b>				
<b>Dilution Water Number RW# or TRE#, circle one</b>	13953			
<b>Concurrent Control Water RW#</b>				
<b>Date(s) Used</b>	6/17/20			
	6/19/20			

**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						
5	6	244	250						
20	25	225	250						
50	63	188	250						
100	125	125	250						
200	250	0	250						
	469	1031	1500						
Initials / Date	By 6/17/20 M-ved BS								
Initials / Date	HR 6/19/20 " "								
Initials / Date									
Initials / Date									
Initials / Date									
Initials / Date									
Initials / Date									
Initials / Date									



Artemia franciscana  
 CHRONIC BIOLOGICAL DATA

QA: NHP 6/23/20

Project Number: 17001-474-088

mg/L	Test Replicate	Number of Surviving Organisms								% surviving 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	/	/	/	
5	A	10	10	10	10	10	/	/	/	97.5
	B	10	10	10	10	10	/	/	/	
	C	10	10	10	10	9	/	/	/	• dead org stuck to side of cup
	D	10	10	10	10	10	/	/	/	
20	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	/	/	/	
50	A	10	10	10	10	9	/	/	/	• dead org stuck to side of cup
	B	10	10	10	10*	8	/	/	/	* 1 weak org 75
	C	10	10	7	7	7	/	/	/	
	D	10	9	6	6	6	/	/	/	
100	A	10	7	1	1*	1	/	/	/	* weak org 12.1
	B	10	6	3	2	2*	/	/	/	* 2 weak orgs
	C	10	6	2	2	1	/	/	/	
	D	10	5	3	1	1	/	/	/	
200	A	10	1	0	—	—	/	/	/	0
	B	10	1	0	—	—	/	/	/	
	C	10	0	—	—	—	/	/	/	
	D	10	2 <sup>A</sup>	1	0	—	/	/	/	2.1%
	A						/	/	/	
	B						/	/	/	
	C						/	/	/	
	D						/	/	/	
Date:		6/17/20	6/18/20	6/19/20	6/20/20	6/21/20				
Time:		1500	0930	1345	1020	1445				
Initials:		CP/AF	M	CP	CP	HR				

CHRONIC CHEMICAL DATA (INITIAL)

QA: DAP 6/23/20

Project Number:	17001-474-088
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	7.8	/	8.0	/	/	/	/	/	FM27	
D.O. (mg/L)	5.0	/	5.2	/	/	/	/	/	17	
Temp. (°C)	20	/	20	/	/	/	/	/	IR1	
Cond. (µS/cm)	146,700	/	130,500	/	/	/	/	/	15	
Hard. (mg/L)		/		/	/	/	/	/		
Alk. (mg/L)		/		/	/	/	/	/		
TRC (mg/L)		/		/	/	/	/	/		
NH <sub>3</sub> (mg/L)		/		/	/	/	/	/		
Conc.:	5									
pH	7.7	/	8.0	/	/	/	/	/		
D.O. (mg/L)	4.9	/	5.1	/	/	/	/	/		
Temp. (°C)	20	/	20	/	/	/	/	/		
Cond. (µS/cm)	135,300	/	130,100	/	/	/	/	/		
Hard. (mg/L)		/		/	/	/	/	/		
Alk. (mg/L)		/		/	/	/	/	/		
TRC (mg/L)		/		/	/	/	/	/		
NH <sub>3</sub> (mg/L)		/		/	/	/	/	/		
Conc.:	20									
pH	7.7	/	8.0	/	/	/	/	/		
D.O. (mg/L)	4.9	/	5.0	/	/	/	/	/		
Temp. (°C)	20	/	20	/	/	/	/	/		
Cond. (µS/cm)	134,800	/	130,600	/	/	/	/	/		
Hard. (mg/L)		/		/	/	/	/	/		
Alk. (mg/L)		/		/	/	/	/	/		
TRC (mg/L)		/		/	/	/	/	/		
NH <sub>3</sub> (mg/L)		/		/	/	/	/	/		
Conc.:	50									
pH	7.7	/	8.0	/	/	/	/	/		
D.O. (mg/L)	4.9	/	5.0	/	/	/	/	/		
Temp. (°C)	20	/	20	/	/	/	/	/		
Cond. (µS/cm)	133,800	/	130,000	/	/	/	/	/		
Date:	6/17/20		6/19/20							
Time:	1450		1330							
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: JAP 6/23/20

Project Number:	17001-474-088
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.: 100									All Conc.	
pH	7.8	/	7.9	/	/	/	/	/		
D.O. (mg/L)	4.8	/	4.9	/	/	/	/	/		
Temp. (°C)	20	/	20	/	/	/	/	/		
Cond. (µS/cm)	133,000	/	131,000	/	/	/	/	/		
Conc.:		/		/	/	/	/	/		
pH		/		/	/	/	/	/		
D.O. (mg/L)		/		/	/	/	/	/		
Temp. (°C)		/		/	/	/	/	/		
Cond. (µS/cm)		/		/	/	/	/	/		
Conc.:		/		/	/	/	/	/		
pH		/		/	/	/	/	/		
D.O. (mg/L)		/		/	/	/	/	/		
Temp. (°C)		/		/	/	/	/	/		
Cond. (µS/cm)		/		/	/	/	/	/		
Conc.: 200		/		/	/	/	/	/		
pH	7.8	/	7.8	/	/	/	/	/		
D.O. (mg/L)	4.8	/	5.0	/	/	/	/	/		
Temp. (°C)	20	/	20	/	/	/	/	/		
Cond. (µS/cm)	132,500	/	131,400	/	/	/	/	/		
Hard. (mg/L)		/		/	/	/	/	/		
Alk. (mg/L)		/		/	/	/	/	/		
TRC (mg/L)		/		/	/	/	/	/		
NH <sub>3</sub> (mg/L)		/		/	/	/	/	/		
Date:	6/17/20		6/19/20							
Time:	1450		1330							
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.

QA: ~~DP~~ 6/23/20

CHRONIC CHEMICAL DATA (FINAL)

Project Number:	17001-474-088
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				125700	/	/	/	/	All Conc.	* conductivity (15)
pH		8.0	8.0	8.1	8.0	/	/	/	/	Fm27	
D.O. (mg/L)		5.0	5.1	5.0	4.7	/	/	/	/	17	
Temp (°C)		23 <sup>Δ</sup>	22 <sup>Δ</sup>	20	22 <sup>Δ</sup>	/	/	/	/	LL	
Conc.:	5				126300	/	/	/	/		* conductivity (15)
pH		8.0	8.0	8.0	8.0	/	/	/	/		
D.O. (mg/L)		5.0	5.1	4.8	4.0	/	/	/	/		
Temp (°C)		23 <sup>Δ</sup>	22 <sup>Δ</sup>	20	22 <sup>Δ</sup>	/	/	/	/		
Conc.:	20				127900	/	/	/	/		* conductivity (15)
pH		8.0	8.0	8.0	8.0	/	/	/	/		
D.O. (mg/L)		5.0	5.1	4.8	4.5	/	/	/	/		
Temp (°C)		23 <sup>Δ</sup>	22 <sup>Δ</sup>	20	22 <sup>Δ</sup>	/	/	/	/		
Conc.:	50				128800	/	/	/	/		* conductivity (15)
pH		7.9	7.9	8.0	7.9	/	/	/	/		
D.O. (mg/L)		4.9	5.1	4.8	4.7	/	/	/	/		
Temp (°C)		23 <sup>Δ</sup>	22 <sup>Δ</sup>	20	22 <sup>Δ</sup>	/	/	/	/		
Conc.:	100				128000	/	/	/	/		* conductivity (15)
pH		8.0	8.0	8.0	8.0	/	/	/	/		
D.O. (mg/L)		4.9	5.2	4.8	5.0	/	/	/	/		
Temp (°C)		23 <sup>Δ</sup>	22 <sup>Δ</sup>	20	21	/	/	/	/		
Conc.:	200			133,700	/	/	/	/	/		* conductivity
pH		8.0	8.0	7.9	/	/	/	/	/		
D.O. (mg/L)		5.0	5.3	5.0	/	/	/	/	/		
Temp (°C)		23 <sup>Δ</sup>	22 <sup>Δ</sup>	20	/	/	/	/	/		
Conc.:					/	/	/	/	/		
pH					/	/	/	/	/		
D.O. (mg/L)					/	/	/	/	/		
Temp (°C)					/	/	/	/	/		
Date:		6/18/20	6/19/20	6/20/20	6/21/20						
Time:		0935	1420	1030	1440						
Initials:		AB	CP	CP	HR						

<sup>Δ</sup> checked all reps

QA: DAP 6/23/20

DAILY TOXICITY TEST LOG

Project Number:	17001-474-088
Test Species:	<i>Artemia franciscana</i>

General Comments	Feeding	Initials/Date
Random Chart: <u>D</u> Min/Max Thermometer # <del>M-15</del> <sup>L-290</sup>	72.5 ug/l Chla 0.3 ml YTC	
Test Day 0 Test Solution Mixed at: 1115 Test Organisms Added at: 1500	Fed @ 1115	CP 6/17/20
Test Day 1 Real Time: 23 °C Min-Max Range: 20-23 °C  * Bath # 2	None	AS 6/18/20
Test Day 2 Real Time: 22 °C Min-Max Range: 20-23 °C  * Bath # 2	Fed @ 1045 HR	CP 6/19/20
Test Day 3 Real Time: 21 °C Min-Max Range: 21-22 °C  * Bath # 2	NONE	CP 6/20/20
Test Day 4 Real Time: 22 °C Min-Max Range: 21-22 °C  * Bath # 2	NONE	HR 6/21/20

① CP 6/19/20 E

**CETIS Analytical Report**  
*Brine shrimp*

Report Date: 22 Jun-20 14:33 (p 1 of 2)  
 Test Code: 474-088 | 05-4297-1032

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

TRE Environmental Strategies

Analysis ID: 10-7219-9317	Endpoint: 96h Survival Rate	CETIS Version: CETISv1.8.7
Analyzed: 22 Jun-20 14:33	Analysis: Trimmed Spearman-Kärber	Official Results: Yes
Batch ID: 14-2120-0216	Test Type: Survival (96h)	Analyst: Lab Tech
Start Date: 17 Jun-20 15:00	Protocol: EPA/821/R-02-012 (2002)	Diluent: rGSL
Ending Date: 21 Jun-20 14:45	Species: Artemia franciscana	Brine: Crystal Sea
Duration: 96h	Source: In-House Culture	Age: 48h
Sample ID: 10-1115-6922	Code: 3C4507BA	Client: Notre Dame
Sample Date: 17 Jun-20 11:15	Material: Arsenic	Project: Special Studies
Receive Date: 17 Jun-20 11:15	Source: Discharge Monitoring Report	
Sample Age: 4h	Station:	

**Trimmed Spearman-Kärber Estimates**

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	1.25%	1.797	0.02955	62.71	54.73	71.85

**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	1	1	1	0	0	0.0%	0.0%	40	40
5		4	0.975	0.9	1	0.025	0.05	5.13%	2.5%	39	40
20		4	1	1	1	0	0	0.0%	0.0%	40	40
50		4	0.75	0.6	0.9	0.06455	0.1291	17.2%	25.0%	30	40
100		4	0.125	0.1	0.2	0.025	0.05	40.0%	87.5%	5	40
200		4	0	0	0	0	0	100.0%	100.0%	0	40

**96h Survival Rate Detail**

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
5		1	1	0.9	1
20		1	1	1	1
50		0.9	0.8	0.7	0.6
100		0.1	0.2	0.1	0.1
200		0	0	0	0

**96h 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
5		10/10	10/10	9/10	10/10
20		10/10	10/10	10/10	10/10
50		9/10	8/10	7/10	6/10
100		1/10	2/10	1/10	1/10
200		0/10	0/10	0/10	0/10

*① WSA 6/23/20 E*

CETIS Analytical Report

Report Date: 22 Jun-20 14:33 (p 2 of 2)

① Brine shrimp

Test Code: 474-088 | 05-4297-1032

Fathead Minnow 96-h Acute Survival Test

TRE Environmental Strategies

Analysis ID: 10-7219-9317

Endpoint: 96h Survival Rate

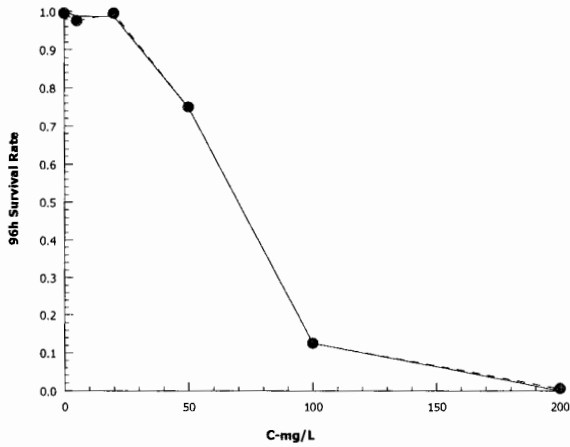
CETIS Version: CETISv1.8.7

Analyzed: 22 Jun-20 14:33

Analysis: Trimmed Spearman-Kärber

Official Results: Yes

Graphics



① ASD <sup>6</sup> 6/23/20 E

TOXICITY DATA PACKAGE COVER SHEET

QA: SEP 6/23/20

Test Type: Chronic Acute Project Number: 17001-474-089  
Test Substance: Arsenic (Na2HAsO4) Species: Artemia franciscana  
Dilution Water: rGSL Organism Lot or Batch Number: 061520  
Concurrent Control Water: NA Age: 48 hr (48 hr) Supplier: TPE  
Date and Time Test Began: 6/17/20 @ 1450 Date and Time Test Ended: 6/21/20 @ 1430  
Protocol Number: \_\_\_\_\_ Investigator(s): EM/CP/MS/HR/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, 5, 20, 50, 100, and 200 mg/L as As

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: MS Date: 6/16/20

MS 6/16/20 E



**TEST SUBSTANCE USAGE LOG**

Project Number: 17001-474-089

QA: DDP 6/23/20

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number	C17-021			
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	13953			
Concurrent Control Water RW#	NA			
Date(s) Used	6/17/20			
	6/19/20			

**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						
5	6	244	250						
20	25	225	250						
50	63	188	250						
100	125	125	250						
200	250	0	250						
	469	1031	1500						
Initials / Date	By 6/17/20 Mxw/BS								
Initials / Date	HR 6/19/20 "								
Initials / Date									
Initials / Date									
Initials / Date									
Initials / Date									
Initials / Date									
Initials / Date									

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

QA: DAP 6/23/20

Project Number: 17001-474-089

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	/	/	/	100
	B	10	10	10	10	10	/	/	/	
	C	10	10	② 10	10	10	/	/	/	
	D	10	10	10	10	10	/	/	/	
5	A	10	10	10	10	10	/	/	/	97.5
	B	10	10	10	10	10	/	/	/	
	C	10	10	10	10	10	/	/	/	
	D	10	10	9	9	9	/	/	/	
20	A	10	10	10	10	10	/	/	/	95
	B	10	9	9	9	9	/	/	/	
	C	10	10	10	10	10	/	/	/	
	D	10	10	10	10	9	/	/	/	
50	A	10	6	6	6	6	/	/	/	77.5
	B	10	10	10	10	9	/	/	/	
	C	10	10	8	8	7	/	/	/	
	D	10	10	9	9	9	/	/	/	
100	A	10	6	1	1	1	/	/	/	12.5
	B	10	7	4	2	1	/	/	/	
	C	10	8 <sup>9</sup>	4	4	1	/	/	/	
	D	10	① 6 <sup>9</sup>	3	3	2	/	/	/	
200	A	10	0	—	—	—	/	/	/	0
	B	10	1	1	1	0	/	/	/	
	C	10	② 1	0	—	—	/	/	/	
	D	10	2	0	—	—	/	/	/	
	A						/	/	/	
	B						/	/	/	
	C						/	/	/	
	D						/	/	/	
Date:		6/17/20	6/18/20	6/19/20	6/20/20	6/21/20				
Time:		1450	1005	1625	1100	1430				
Initials:		EN/CP	AS	CP	CP	ES/HR				

① AS 6/18/20 up

② CP 6/19/20 E

QA: ~~AP~~ 6/27/20

CHRONIC CHEMICAL DATA (INITIAL)

Project Number:	17001-474-089
Test Species: <i>Artemia franciscana</i>	

mg/L	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter #	Remarks
Conc.: 0									All Conc.	
pH	7.8	/	8.0	/	/	/	/	/	FM27	
D.O. (mg/L)	5.0	/	5.1	/	/	/	/	/	17	
Temp. (°C)	20	/	20	/	/	/	/	/	IR1	
Cond. (µS/cm)	134200	/	130,800	/	/	/	/	/	15	
Hard. (mg/L)	/	/	/	/	/	/	/	/		
Alk. (mg/L)	/	/	/	/	/	/	/	/		
TRC (mg/L)	/	/	/	/	/	/	/	/		
NH <sub>3</sub> (mg/L)	/	/	/	/	/	/	/	/		
Conc.: 5										
pH	7.8	/	8.0	/	/	/	/	/		
D.O. (mg/L)	5.0	/	5.0	/	/	/	/	/		
Temp. (°C)	20	/	20	/	/	/	/	/		
Cond. (µS/cm)	135400	/	131,400	/	/	/	/	/		
Hard. (mg/L)	/	/	/	/	/	/	/	/		
Alk. (mg/L)	/	/	/	/	/	/	/	/		
TRC (mg/L)	/	/	/	/	/	/	/	/		
NH <sub>3</sub> (mg/L)	/	/	/	/	/	/	/	/		
Conc.: 20										
pH	7.8	/	8.0	/	/	/	/	/		
D.O. (mg/L)	5.0	/	5.0	/	/	/	/	/		
Temp. (°C)	20	/	20	/	/	/	/	/		
Cond. (µS/cm)	134800	/	132,000	/	/	/	/	/		
Hard. (mg/L)	/	/	/	/	/	/	/	/		
Alk. (mg/L)	/	/	/	/	/	/	/	/		
TRC (mg/L)	/	/	/	/	/	/	/	/		
NH <sub>3</sub> (mg/L)	/	/	/	/	/	/	/	/		
Conc.: 50										
pH	7.8	/	8.0	/	/	/	/	/		
D.O. (mg/L)	5.0	/	5.0	/	/	/	/	/		
Temp. (°C)	20	/	20	/	/	/	/	/		
Cond. (µS/cm)	134700	/	132,200	/	/	/	/	/		
Hard. (mg/L)	/	/	/	/	/	/	/	/		
Alk. (mg/L)	/	/	/	/	/	/	/	/		
TRC (mg/L)	/	/	/	/	/	/	/	/		
NH <sub>3</sub> (mg/L)	/	/	/	/	/	/	/	/		
Date:	6/17/20		6/19/20							
Time:	1440		1610							
Initials:	EN		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: ~~DD~~ 6/23/20

Project Number:	17001-474-089
Test Species:	<i>Artemia franciscana</i>

mg/L	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter #	Remarks
Conc.: 100									All Conc.	
pH	7.8	/	7.9	/	/	/	/	/		
D.O. (mg/L)	5.0	/	5.0	/	/	/	/	/		
Temp. (°C)	20	/	20	/	/	/	/	/		
Cond. (µS/cm)	134600	/	132,600	/	/	/	/	/		
Conc.: 200										
pH	7.8	/	7.8	/	/	/	/	/		
D.O. (mg/L)	5.0	/	5.0	/	/	/	/	/		
Temp. (°C)	20	/	20	/	/	/	/	/		
Cond. (µS/cm)	133700	/	133,500	/	/	/	/	/		
Hard. (mg/L)	/	/	/	/	/	/	/	/		
Alk. (mg/L)	/	/	/	/	/	/	/	/		
TRC (mg/L)	/	/	/	/	/	/	/	/		
NH <sub>3</sub> (mg/L)	/	/	/	/	/	/	/	/		
Date:	6/17/20		6/19/20							
Time:	1440		1610							
Initials:	EN		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: DMP 6/23/20

Project Number:	17001-474-089
Test Species:	<i>Artemia franciscana</i>

mg/L	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter #	Remarks
Conc.: 0					/	/	/	/	All Conc.	* conductivity
pH	8.2	8.3	8.2	8.0	/	/	/	/	Fm27	124900
D.O. (mg/L)	5.4	5.3	5.4	5.0	/	/	/	/	17	
Temp (°C)	22°	21	20	22°	/	/	/	/	LL	
Conc.: 5					/	/	/	/		* conductivity
pH	8.1	8.2	8.2	8.0	/	/	/	/		125300
D.O. (mg/L)	5.3	5.3	5.4	4.9	/	/	/	/		
Temp (°C)	22°	21	20	22°	/	/	/	/		
Conc.: 20					/	/	/	/		* conductivity
pH	8.1	8.2	8.1	8.0	/	/	/	/		127000
D.O. (mg/L)	5.3	5.4	5.4	4.9	/	/	/	/		
Temp (°C)	22°	21	20	22°	/	/	/	/		
Conc.: 50					/	/	/	/		* conductivity
pH	8.1	8.2	8.1	8.0	/	/	/	/		126500
D.O. (mg/L)	5.3	5.4	5.5	4.9	/	/	/	/		
Temp (°C)	22°	21	20	22°	/	/	/	/		
Conc.: 100					/	/	/	/		* conductivity
pH	8.1	8.2	8.1	8.0	/	/	/	/		127100
D.O. (mg/L)	5.4	5.4	5.6	5.3	/	/	/	/		
Temp (°C)	22°	21	20	22°	/	/	/	/		
Conc.: 200					/	/	/	/		* conductivity
pH	8.1	8.2	8.0	8.0	/	/	/	/		127100
D.O. (mg/L)	5.4	5.5	6.0	5.5	/	/	/	/		
Temp (°C)	22°	21	20	22	/	/	/	/		
Conc.:					/	/	/	/		
pH					/	/	/	/		
D.O. (mg/L)					/	/	/	/		
Temp (°C)					/	/	/	/		
Date:	6/18/20	6/19/20	6/20/20	6/21/20						
Time:	1015	1650	1130	1435						
Initials:	M	CP	CP	ES/HR						

checked all refs

DAILY TOXICITY TEST LOG

QA: DAW 6/23/20

Project Number:	17001-474-089
Test Species:	<i>Artemia franciscana</i>

General Comments		Feeding 145 ug/l Chla	Initials/Date
	Random Chart: <u>D</u> Min/Max Thermometer # 1		
Test Day 0	Test Solution Mixed at: 1105 Test Organisms Added at: 1450	Fed @ 1105	EN 6/17/20
Test Day 1	Real Time: 23 °C Min-Max Range: 20-23 °C  * Bath # 2	None	AS 6/18/20
Test Day 2	Real Time: 22 °C Min-Max Range: 20 - 23 °C  * Bath # 2	Fed @ 1220 HR	CP 6/19/20
Test Day 3	Real Time: 21 °C Min-Max Range: 21 - 22 °C  * Bath # 2	NONE	CP 6/20/20
Test Day 4	Real Time: 22 °C Min-Max Range: 21-22 °C  * Bath # 2	none	ES/HR 6/21/20

**CETIS Analytical Report**

*Brine Shrimp*

Report Date: 22 Jun-20 14:40 (p 1 of 3)

Test Code: 474-089 | 12-4146-2524

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

TRE Environmental Strategies

<b>Analysis ID:</b> 18-2349-2333	<b>Endpoint:</b> 96h Survival Rate	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 22 Jun-20 14:39	<b>Analysis:</b> Linear Regression (MLE)	<b>Official Results:</b> Yes
<b>Batch ID:</b> 16-8842-7942	<b>Test Type:</b> Survival (96h)	<b>Analyst:</b> Lab Tech
<b>Start Date:</b> 17 Jun-20 14:50	<b>Protocol:</b> EPA/821/R-02-012 (2002)	<b>Diluent:</b> rGSL
<b>Ending Date:</b> 21 Jun-20 14:30	<b>Species:</b> Artemia franciscana	<b>Brine:</b> Crystal Sea
<b>Duration:</b> 96h	<b>Source:</b> In-House Culture	<b>Age:</b> 48h
<b>Sample ID:</b> 17-2241-3862	<b>Code:</b> 66A9F326	<b>Client:</b> Notre Dame
<b>Sample Date:</b> 17 Jun-20 11:25	<b>Material:</b> Arsenic	<b>Project:</b> Special Studies
<b>Receive Date:</b> 21 Jun-20 14:30	<b>Source:</b> Discharge Monitoring Report	
<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%)
12	-50.45	108.1	110.4	1.825	0.1527	0.917	2.52	3.16	0.0905	Non-Significant Lack of Fit ✓

**Point Estimates**

Level	mg/L	95% LCL	95% UCL
LC5	37.46	26.55	45.26
LC10	42.57	31.92	50.17
LC15	46.4	36.08	53.88
LC20	49.69	39.71	57.1
LC25	52.7	43.05	60.1
LC40	61.11	52.32	68.99
LC50	66.8	58.33	75.59

**Regression Parameters**

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
Threshold	0.0248	0.01425	-0.00314	0.05274	1.74	0.0965	Non-Significant Parameter
Slope	6.547	1.108	4.376	8.718	5.911	<0.0001	Significant Parameter
Intercept	-11.95	2.049	-15.96	-7.931	-5.83	<0.0001	Significant Parameter

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	168.6177	168.6177	1	256	<0.0001	Significant
Lack of Fit	4.091077	1.363692	3	2.52	0.0905	Non-Significant
Pure Error	9.738869	0.541048	18			
Residual	13.82995	0.658569	21			

**Residual Analysis**

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Goodness-of-Fit	Pearson Chi-Sq GOF	13.83	32.67	0.8768	Non-Significant Heterogeneity
	Likelihood Ratio GOF	13.2	32.67	0.9014	Non-Significant Heterogeneity
Variances	Mod Levene Equality of Variance	3.452	2.773	0.0232	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8441	0.9169	0.0017	Non-normal Distribution
	Anderson-Darling A2 Normality	1.681	2.492	<0.0001	Non-normal Distribution

*QADP 6/23/20*

**CETIS Analytical Report**

*Brine Shrimp*

Report Date: 22 Jun-20 14:40 (p 2 of 3)

Test Code: 474-089 | 12-4146-2524

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

TRE Environmental Strategies

Analysis ID: 18-2349-2333      Endpoint: 96h Survival Rate      CETIS Version: CETISv1.8.7  
 Analyzed: 22 Jun-20 14:39      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	1	1	1	0	0	0.0%	0.0%	40	40	
5		4	0.975	0.9	1	0.025	0.05	5.13%	2.5%	39	40	
20		4	0.95	0.9	1	0.02887	0.05773	6.08%	5.0%	38	40	
50		4	0.775	0.6	0.9	0.075	0.15	19.4%	22.5%	31	40	
100		4	0.125	0.1	0.2	0.025	0.05	40.0%	87.5%	5	40	
200		4	0	0	0	0	0	100.0%	0	0	40	

96h Survival Rate Detail						
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	
0	Dilution Water	1	1	1	1	
5		1	1	1	0.9	
20		1	0.9	1	0.9	
50		0.6	0.9	0.7	0.9	
100		0.1	0.1	0.1	0.2	
200		0	0	0	0	

96h 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	
5		10/10	10/10	10/10	9/10	
20		10/10	9/10	10/10	9/10	
50		6/10	9/10	7/10	9/10	
100		1/10	1/10	1/10	2/10	
200		0/10	0/10	0/10	0/10	

*① DAP 6/23/20*



① *Brown Shrimp*

Fathead Minnow 96-h Acute Survival Test

TRE Environmental Strategies

Analysis ID: 18-2349-2333

Endpoint: 96h Survival Rate

CETIS Version: CETISv1.8.7

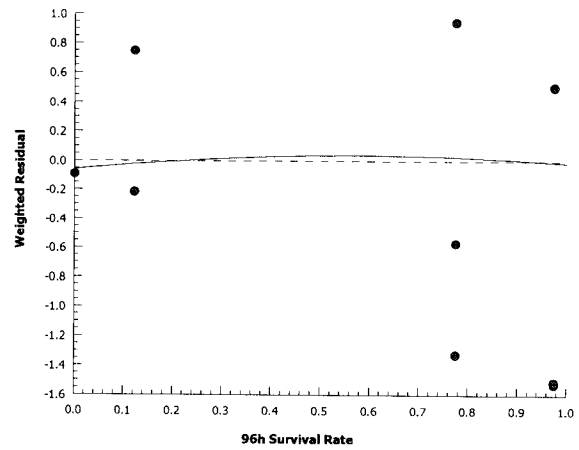
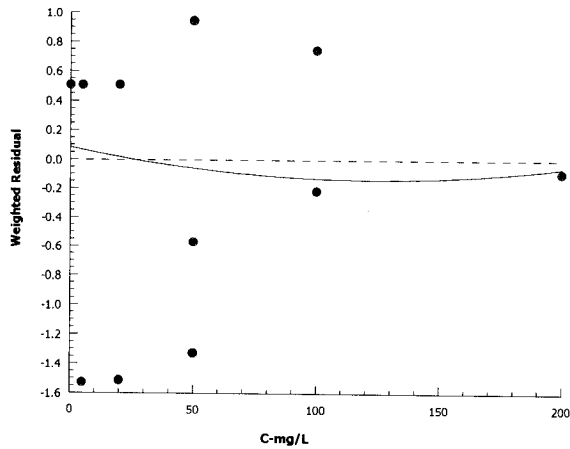
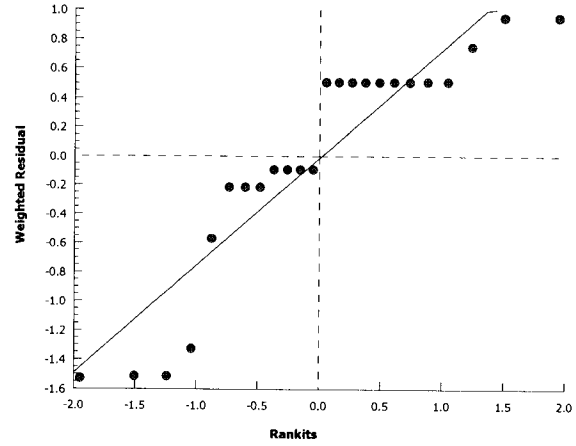
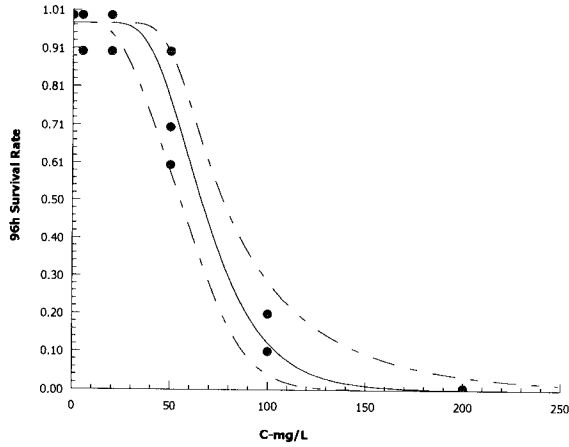
Analyzed: 22 Jun-20 14:39

Analysis: Linear Regression (MLE)

Official Results: Yes

Graphics

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



① DAP 6/23/20 E

**CETIS Analytical Report**

Report Date: 22 Jun-20 14:41 (p 1 of 2)

Test Code: 474-089 | 12-4146-2524

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

TRE Environmental Strategies

<b>Analysis ID:</b> 17-1204-9077	<b>Endpoint:</b> 96h Survival Rate	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 22 Jun-20 14:41	<b>Analysis:</b> Trimmed Spearman-Kärber	<b>Official Results:</b> Yes
<b>Batch ID:</b> 16-8842-7942	<b>Test Type:</b> Survival (96h)	<b>Analyst:</b> Lab Tech
<b>Start Date:</b> 17 Jun-20 14:50	<b>Protocol:</b> EPA/821/R-02-012 (2002)	<b>Diluent:</b> rGSL
<b>Ending Date:</b> 21 Jun-20 14:30	<b>Species:</b> Artemia franciscana	<b>Brine:</b> Crystal Sea
<b>Duration:</b> 96h	<b>Source:</b> In-House Culture	<b>Age:</b> 48h
<b>Sample ID:</b> 17-2241-3862	<b>Code:</b> 66A9F326	<b>Client:</b> Notre Dame
<b>Sample Date:</b> 17 Jun-20 11:25	<b>Material:</b> Arsenic	<b>Project:</b> Special Studies
<b>Receive Date:</b> 21 Jun-20 14:30	<b>Source:</b> Discharge Monitoring Report	
<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	2.50%	1.791	0.03527	61.84	52.57	72.75

**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	1	1	1	0	0	0.0%	0.0%	40	40
5		4	0.975	0.9	1	0.025	0.05	5.13%	2.5%	39	40
20		4	0.95	0.9	1	0.02887	0.05773	6.08%	5.0%	38	40
50		4	0.775	0.6	0.9	0.075	0.15	19.4%	22.5%	31	40
100		4	0.125	0.1	0.2	0.025	0.05	40.0%	87.5%	5	40
200		4	0	0	0	0	0	100.0%	100.0%	0	40

**96h Survival Rate Detail**

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
5		1	1	1	0.9
20		1	0.9	1	0.9
50		0.6	0.9	0.7	0.9
100		0.1	0.1	0.1	0.2
200		0	0	0	0

**96h 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
5		10/10	10/10	10/10	9/10
20		10/10	9/10	10/10	9/10
50		6/10	9/10	7/10	9/10
100		1/10	1/10	1/10	2/10
200		0/10	0/10	0/10	0/10

*QAP 6/23/20 E*

CETIS Analytical Report  
*Brine Shrimp*

Report Date: 22 Jun-20 14:41 (p 2 of 2)  
Test Code: 474-089 | 12-4146-2524

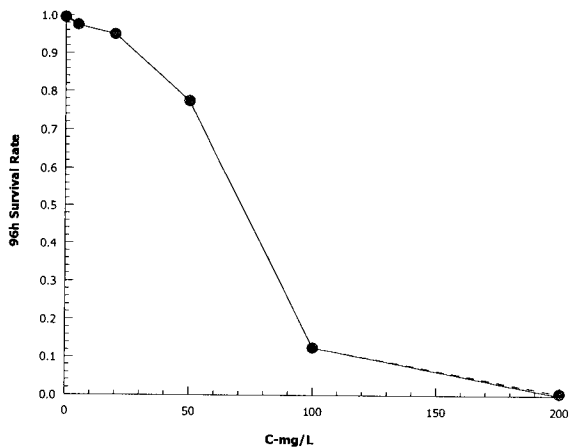
① Fathead Minnow 96-h Acute Survival Test

TRE Environmental Strategies

Analysis ID: 17-1204-9077      Endpoint: 96h Survival Rate  
Analyzed: 22 Jun-20 14:41      Analysis: Trimmed Spearman-Kärber

CETIS Version: CETISv1.8.7  
Official Results: Yes

Graphics



① DAD 6/23/20 E