UTAH DEPARTMENT *of*
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Chronic Bioassay Method Development Reports for Great Salt Lake Brine Shrimp Nauplii

Prepared for and compiled by
the Utah Division of Water Quality
August 4, 2020

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January 8, 2020

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Subject: Results of Short-term Chronic Brine Shrimp Experiment #1

Mr. Bittner:

Below is a summary of the short-term chronic brine shrimp experiment initiated on September 26, 2019. The purpose of this experiment was to investigate three different test durations (6, 10, and 14 days) for use in a short-term chronic test method using *Artemia franciscana* as well as observe the life stage transitions of the organisms.

Three different treatments were tested,

- 6 day test with 50 ml,
- 10 day test with 50 ml, and
- 14 day test with 50 ml.

An additional replicate was run with sacrificial organisms removed each day and preserved to document the life stages of the organisms. The ratio of food volume to test media volume was consistent among treatments, that is, the estimated concentration of *Dunaliella* was 145 µg/L Chla in all containers. The test durations were selected to address what test duration is sufficient to determine differences in survival and growth. The test volume was lower than in acute toxicity tests with *A. franciscana* to help conserve algae (i.e., could we use a smaller volume of media-food [at same ratio] and still achieve adequate survival and growth over the test duration).

Species: *Artemia franciscana*

Test type:

- Test duration(s): 6, 10, and 14 days
- Test type: static-renewal (solutions and food renewed every 48 hours)
- Algae: *Dunaliella viridis*
- Algae concentration: 145 µg/L Chla
- Temperature: 20℃

- Test volume(s): 50 ml
- Replicates: 4
- Organisms/Rep: 20
- Test media: 120 ppt rGSL media (per Notre Dame recipe)

Pretest conditions: <24-h old *A. franciscana* were hatched out in ~29 ppt artificial seawater (Crystal Seasalt) and ~200 organisms were placed in 120 ppt rGSL water and fed *Dunaliella virdis* at a density of 100 µg/L Chla. 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#13742	NR	NM	NM	NR	NM	NM	121

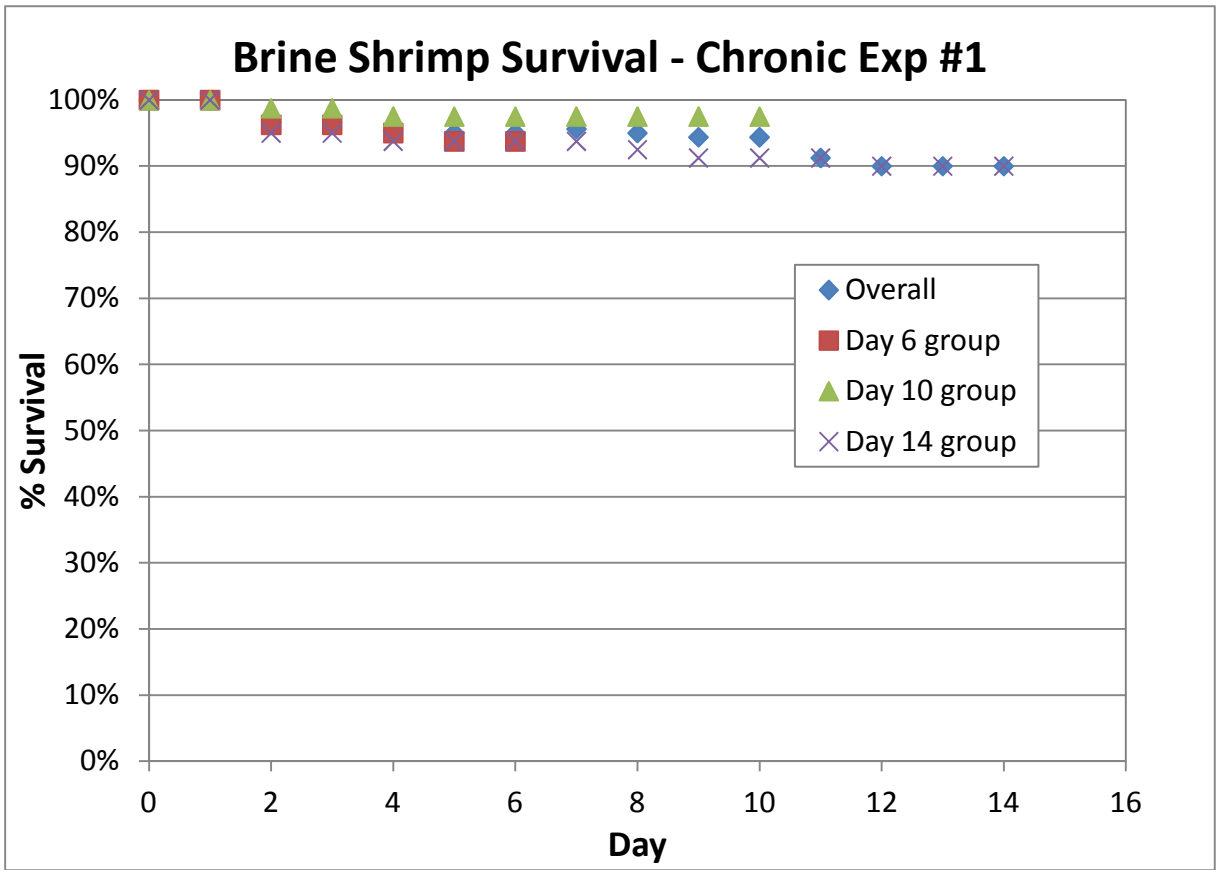
[a]As CaCO3
[b]Total residual chlorine

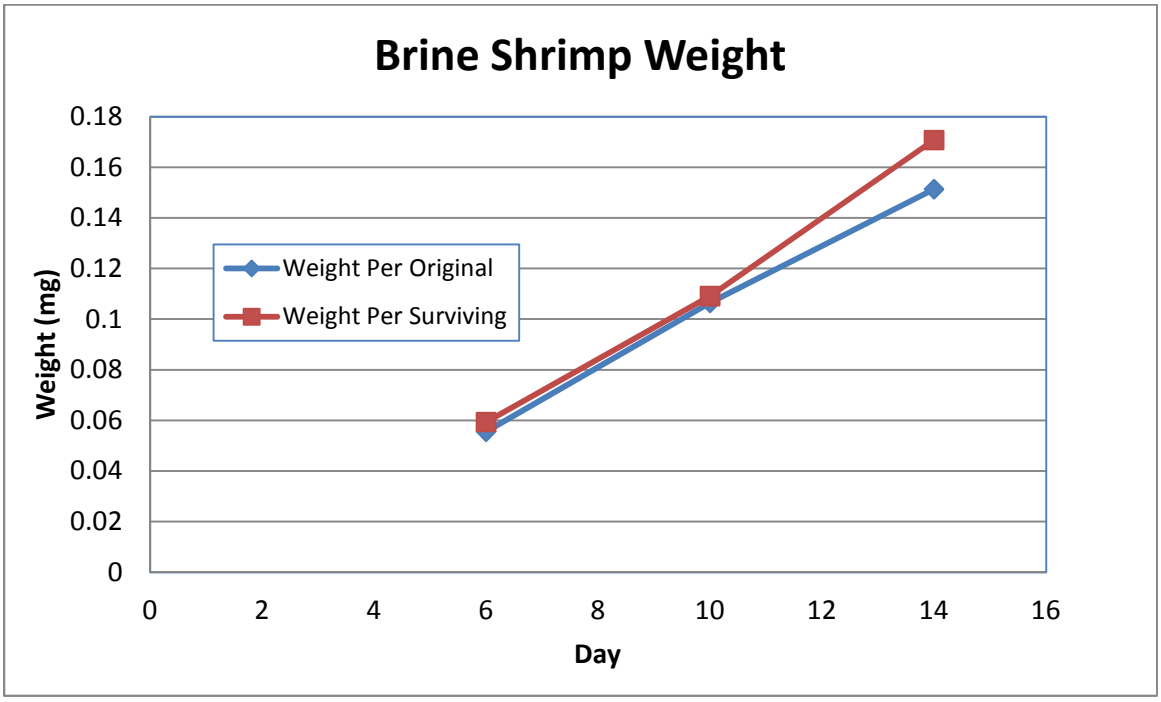
Test activities:

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

Results:

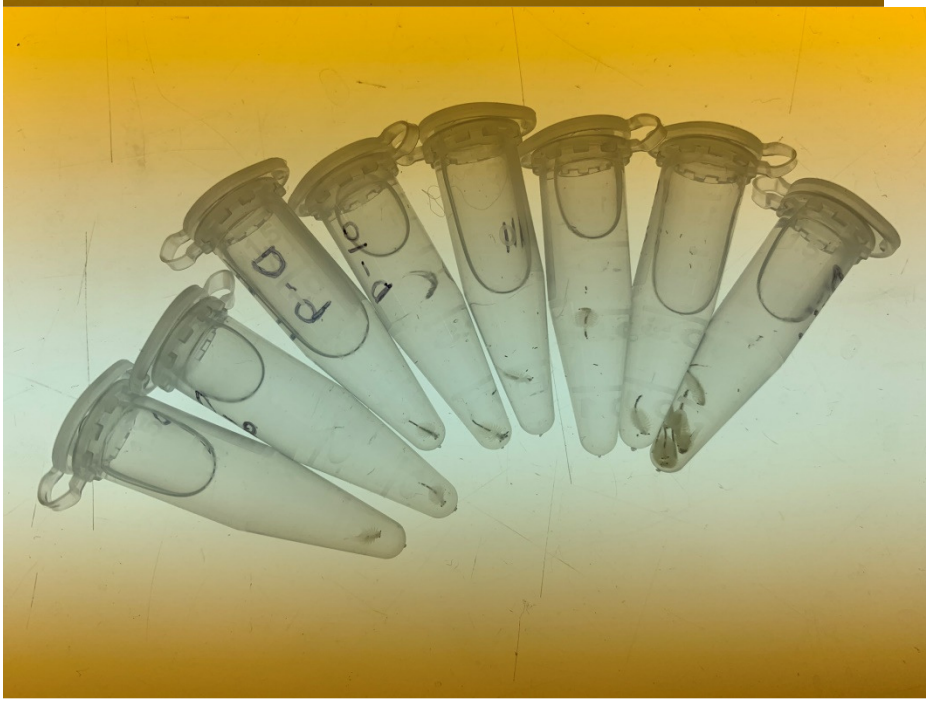
The survival and average dry weights for the brine shrimp are illustrated in the following figures.


Brine Shrimp Survival - Chronic Exp #1
% Survival
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
2
4
6
8
10
12
14
16
Day
Overall
Day 6 group
Day 10 group
Day 14 group


Brine Shrimp Weight
Weight (mg)
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
2
4
6
8
10
12
14
16
Day
Weight Per Original
Weight Per Surviving

TRE

Organisms were preserved daily and presented in the following photographs. Photograph 1 is Day 0-6 and photograph 2 is Day 7-14.



Summary and findings:

- Organism survival was ≥ 90% for all test durations.
- Beginning on day 6, the water was cleared of algae before the next renewal period.

- Growth was consistent throughout the test, but it is possible that the organisms are food limited with this quantity of *Dunaliella*.
- Organisms appear to transition from the nauplii to the juvenile stage between test days 3 and 4 and remain in the juvenile stage for the duration of the study.

Based on these results, survival was very good in both test designs; however, the organisms in the day 10 and day 14 treatments may be food limited after day 6 for this volume of water and this food concentration. This factor will be addressed in later experiments as we investigate study duration.

We greatly appreciate the opportunity to complete this study for you. Please do not hesitate to call if you have any questions regarding this study.

Sincerely,

Amanda Bidlack
Project Specialist / QA Officer
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17001-474-037

Attachment

cc: David Pillard, TRE

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January 10, 2020

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Subject: Results of Short-term Chronic Brine Shrimp Experiment #2

Mr. Bittner:

Below is a summary of the short-term chronic brine shrimp experiment initiated on October 17, 2019. The purpose of this experiment was to investigate the effect of increased food at two different volumes (50 ml and 150 ml) on the short-term chronic test method using *Artemia franciscana*.

Two different treatments were tested:

- 6 day test with 50 ml media, and
- 6 day test with 150 ml media.

An additional replicate was run with sacrificial organisms removed each day and preserved to document the life stages of the organisms. The ratio of food volume to test media volume was consistent between treatments, that is, the estimated concentration of *Dunaliella* was 435 µg/L Chla in all containers (this concentration of food was considered *ad libitum*).

Species: *Artemia franciscana*

Test type:

- Test duration: 6 days
- Test type: static-renewal (solutions and food renewed every 48 hours)
- Algae: *Dunaliella viridis*
- Algae concentration: 435 µg/L Chla
- Temperature: 20°C
- Test volume(s): 50 and 150 ml
- Replicates: 3
- Organisms/Rep: 20
- Test media: 120 ppt rGSL media (per Notre Dame recipe)

Pretest conditions: <24-h old *A. franciscana* were hatched out in ~29 ppt artificial seawater (Crystal Sea Marine Mix) and ~200 organisms were placed in 120 ppt rGSL water and fed *Dunaliella viridis* at a density of 100 µg/L Chla. 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#13742	NR	NM	NM	NR	NM	NM	121

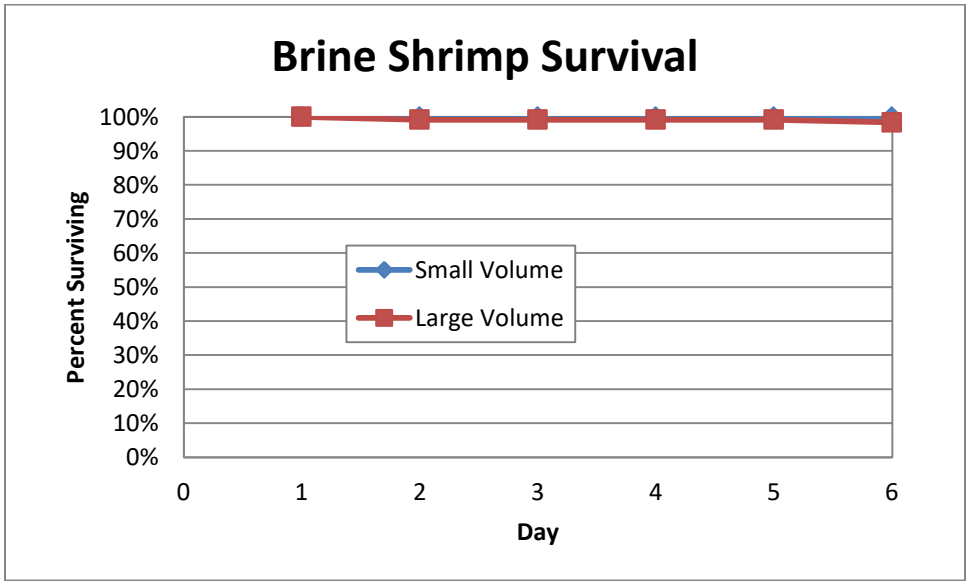
[a]As CaCO3
[b]Total residual chlorine

Test activities:

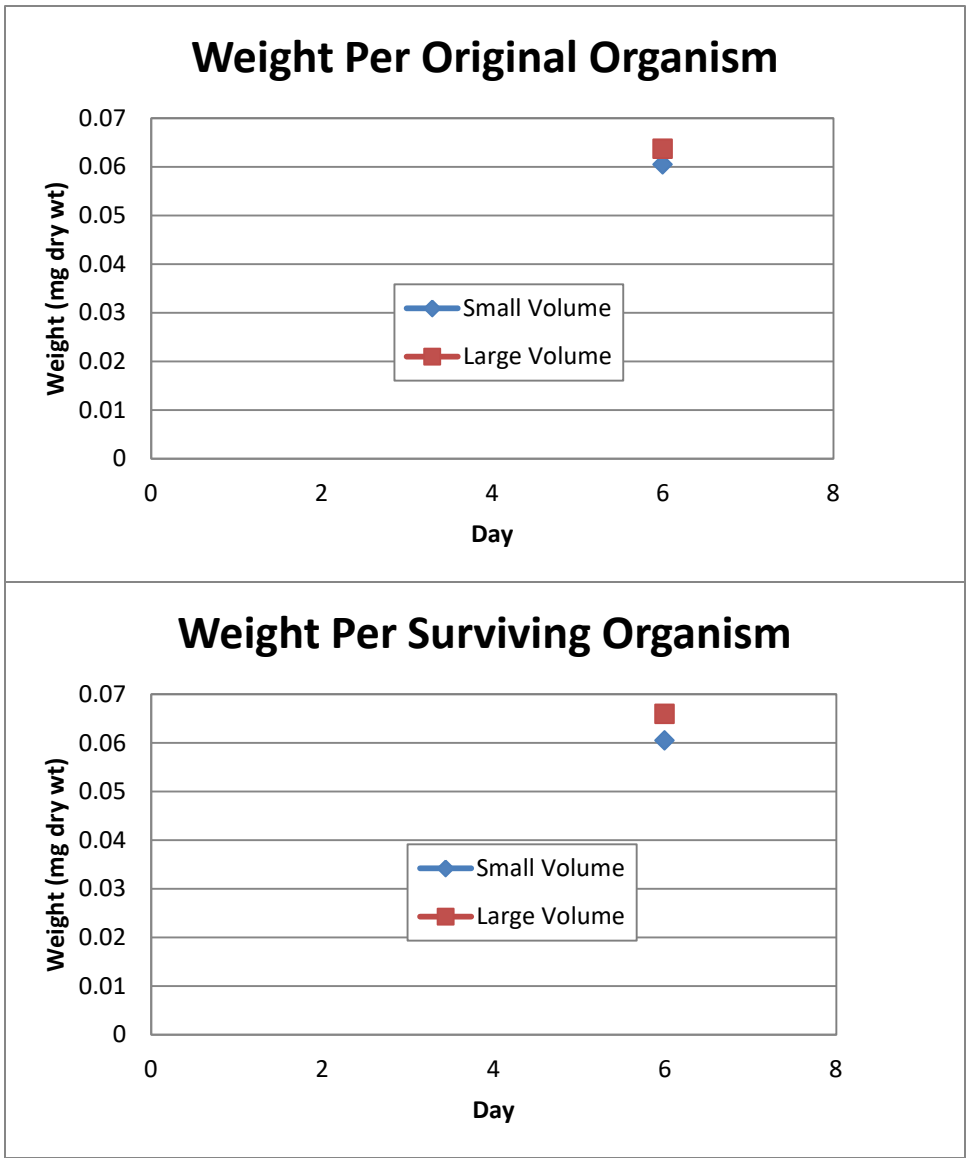
- Biological observations (primarily survival) taken daily.
- Chemistries were measured on renewal days (i.e., pH, dissolved oxygen, and temperature).
- Conductivity was measured at test termination or when there was 0% survival in that treatment.
- Dry weights were determined at test termination.

Results:

The survival and average dry weights for the brine shrimp are illustrated in the following figures.

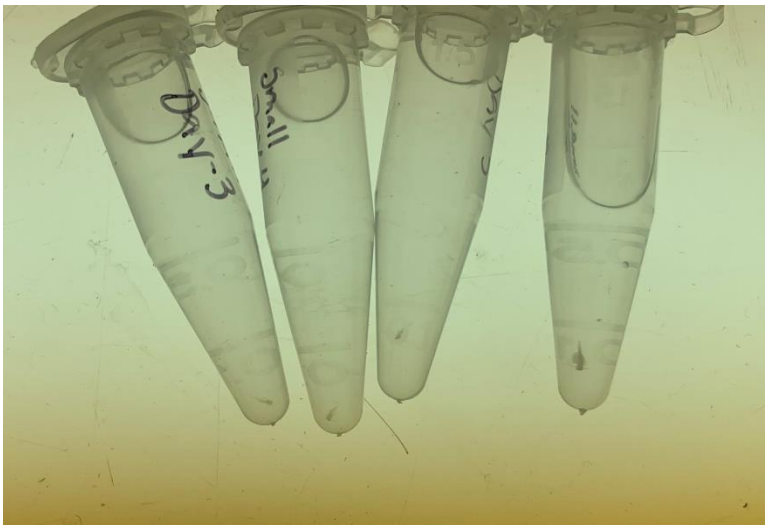


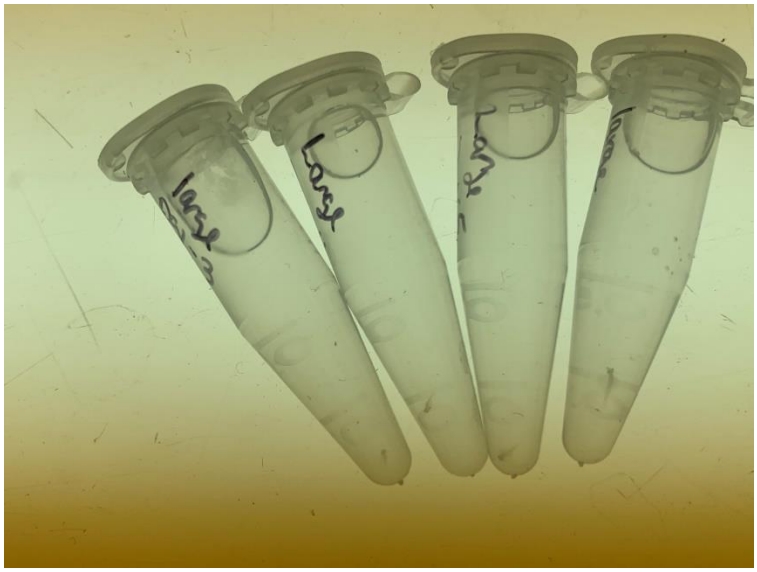
TRE


Weight Per Original Organism
Weight (mg dry wt)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
2
4
6
8
Day
Small Volume
Large Volume
Weight Per Surviving Organism
Weight (mg dry wt)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
2
4
6
8
Day
Small Volume
Large Volume

TRE

Organisms were preserved daily and are presented in the following photographs. Photograph 1 is Small Volume, Day 3-6, and photograph 2 is Large Volume, Day 3-6.





Summary and findings:

- Organism survival was ≥ 90% for both test treatments.
- *Dunaliella* was visibly present (definite green color) in the water for all test treatments through the duration of the test.
- There was no observable difference in organism response between the two volumes at this food concentration for this test length.
- A comparison of the results of this test with data from Study #1 (TRE report 17001-474-037) suggests that through day 6, organism growth was not food limited in the previous study.
- Organisms appear to transition from the nauplii to the juvenile stage between test days 3 and 4 and remain in the juvenile stage for the duration of the study. This was consistent

in both treatments (this was also consistent with results from Study #1, TRE study 17001-474-037).

Based on these results, survival was very good in both test designs. Increased *Dunaliella* availability did not change growth or life cycle transitions through 6 days of testing.

We greatly appreciate the opportunity to complete this study for you. Please do not hesitate to call if you have any questions regarding this study.

Sincerely,

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17001-474-039

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January 6, 2020

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Subject: Results of Short-term Chronic Brine Shrimp Experiment #3

Mr. Bittner:

Below is a summary of the short-term chronic brine shrimp experiment initiated on November 1, 2019. The purpose of this experiment was to investigate two different test durations (6 vs 10 days) as well as two different test volumes (small and large) on the short-term chronic test method using *Artemia franciscana*.

Four different treatments were tested,

- 6 day test with 50 ml,
- 6 day test with 150 ml,
- 10 day test with 50 ml, and
- 10 day test with 150 ml.

The ratio of food volume to test media volume was consistent between treatments, that is, the estimated concentration of *Dunaliella* was 145µg/L Chla in all containers. The test durations were selected to address what test duration is sufficient to pick up differences in survival and growth in later definitive toxicity tests. The test volume was varied to help conserve algae (i.e., could we use a smaller volume of media-food [since at same ratio] and still achieve adequate survival and growth over the test duration).

Species: *Artemia franciscana*

Test type:

- Test duration: 6 and 10 days
- Test type: static-renewal (solutions and food renewed every 48 hours)
- Algae: *Dunaliella virdis*
- Algae concentration: 145 µg/L Chla
- Temperature: 20℃
- Test volume(s): 50 and 150 ml

- Replicates: 3
- Organisms/Rep: 20
- Test media: 120 ppt rGSL media (per Notre Dame recipe)

Pretest conditions: <24-h old *A. franciscana* were hatched out in ~29 ppt artificial seawater (Crystal Seasalt) and ~200 organisms were placed in 120 ppt rGSL water and fed *Dunaliella virdis* at a density of 100 µg/L Chla. 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#13772	8.1	NM	NM	139,400	NM	NM	120

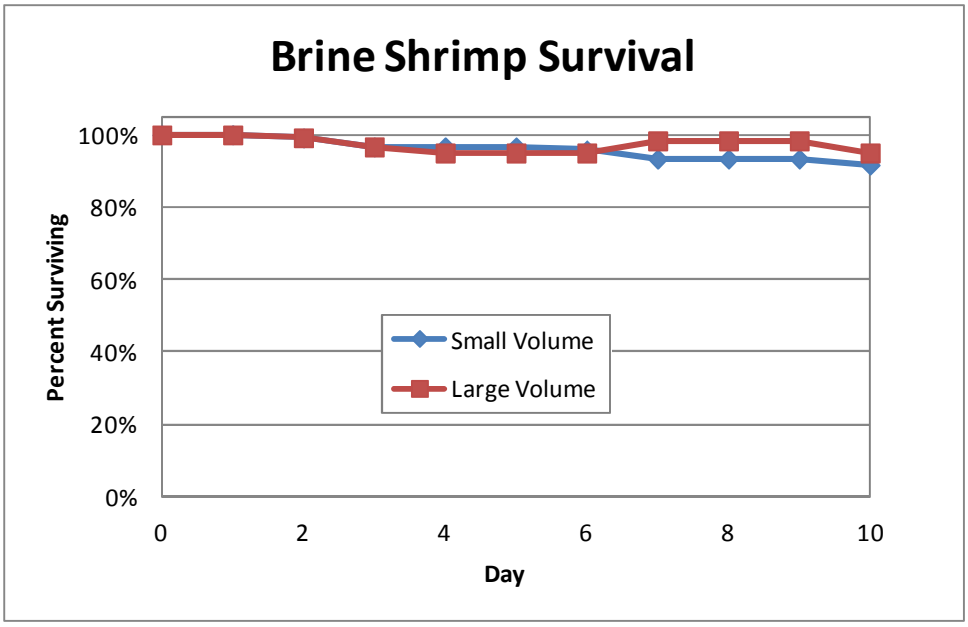
[a]As CaCO3
[b]Total residual chlorine

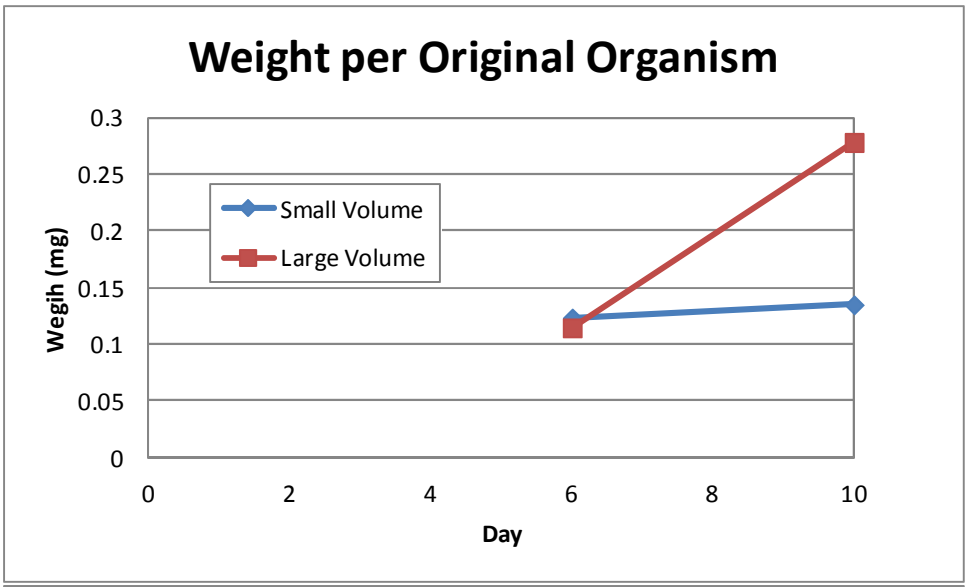
Test activities:

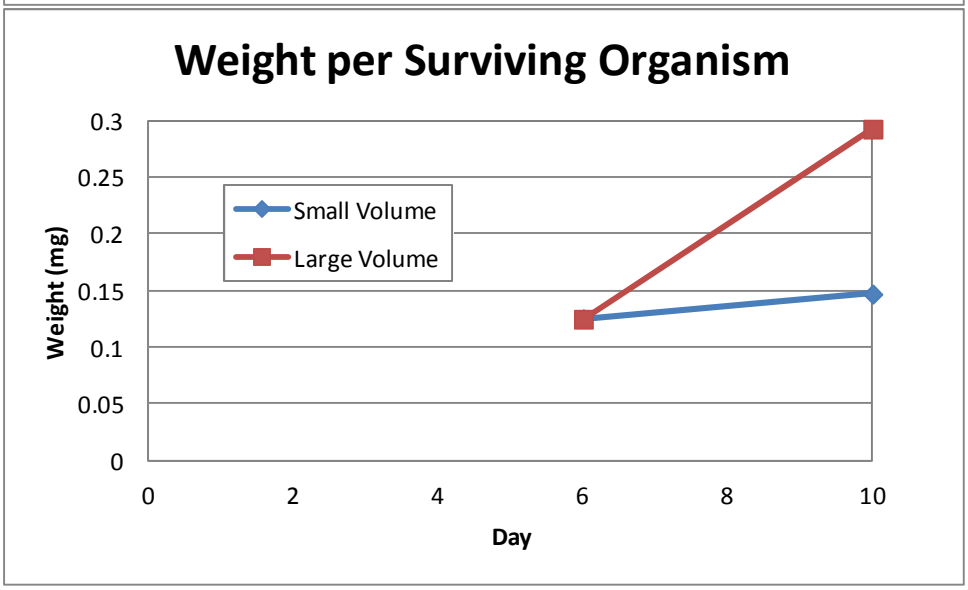
- Biological observations (primarily survival) taken daily.
- Chemistries take on renewal days (i.e., pH, dissolved oxygen, and temperature). Conductivity was measured at test termination or when there was 0% survival in that treatment.
- Dry weights were determined at test termination.

Results:

The survival and average dry weights for the brine shrimp are illustrated in the following figures.







Summary and findings:

- Organism survival was ≥ 90% in both the small and large test volumes for both test durations.
- On day 6, the weights between the two volumes were similar, both on a per original and a per surviving basis.
- Beginning on day 6, the small volume treatment water was cleared of algae before the next renewal period.
- On day 10, the weights of the organisms in the large volume treatment were significantly higher than those in the small volume treatment.

Based on these results, survival was very good in both test designs; however, the organisms in

the small volume treatment appeared to be food limited after day 6. The large volume treatments do not appear to be food limited, indication the concentration of available food (algae) is less important than the total load which is available to the shrimp as the scavenge throughout the container. This suggests that increased food in the small chambers, through either more frequent renewals or increased food density would allow for more growth. However, one variable that we want to be wary of, and not be a confounding variable in the test design, is food concentration; as changes in food density during a test could affect toxicant bioavailability. This factor will be addressed in later experiments.

We greatly appreciate the opportunity to complete this study for you. Please do not hesitate to call if you have any questions regarding this study.

Sincerely,

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14001-474-041

Attachment

cc: David Pillard, TRE

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January 10, 2020

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Subject: Results of Short-term Chronic Brine Shrimp Experiment #4

Mr. Bittner:

Below is a summary of the short-term chronic brine shrimp experiment initiated on November 14, 2019. The purpose of this experiment was to investigate two different test durations (7 vs 10 days) as well as the number of organisms in each test vessel (10 vs 20) on the short-term chronic test method using *Artemia franciscana* (brine shrimp). While 20 organisms were used in the acute test methods previously conducted, the rationale for reducing the number of test organisms in each vessel for the development of a short-term chronic method is multifold: 1) more similar to other WET methods, 2) easier to count 10 actively swimming organisms (compared to 20), 3) most importantly, it potentially reduces the food burden needed in each test vessel.

Four different treatments were tested:

- 7 day test with 10 organisms,
- 7 day test with 20 organisms,
- 10 day test with 10 organisms, and
- 10 day test with 20 organisms.

The ratio of food volume to test media volume was consistent among treatments, that is, the estimated concentration of *Dunaliella* was 145 µg/L Chla in all containers. The test durations were selected to address what test duration is sufficient to detect differences in survival and growth in later short-term chronic toxicity tests. The test volume was consistent at 50 ml.

Species: *Artemia franciscana*

Test type:

- Test duration(s): 7 and 10 days
- Test type: static-renewal (solutions and food renewed daily)
- Algae: *Dunaliella viridis*

- Algae concentration: 145 µg/L Chla
- Temperature: 20°C
- Test volume(s): 50 ml
- Replicates: 2
- Organisms/Rep: 10 or 20
- Test media: 120 ppt rGSL media (per Notre Dame recipe)

Pretest conditions: <24-h old *A. franciscana* were hatched out in ~29 ppt artificial seawater (Crystal Sea Marine Mix) and ~200 organisms were placed in 120 ppt rGSL water and fed *Dunaliella viridis* at a density of 100 µg/L Chla. 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#13772	7.8	NM	NM	131,600	NM	NM	120

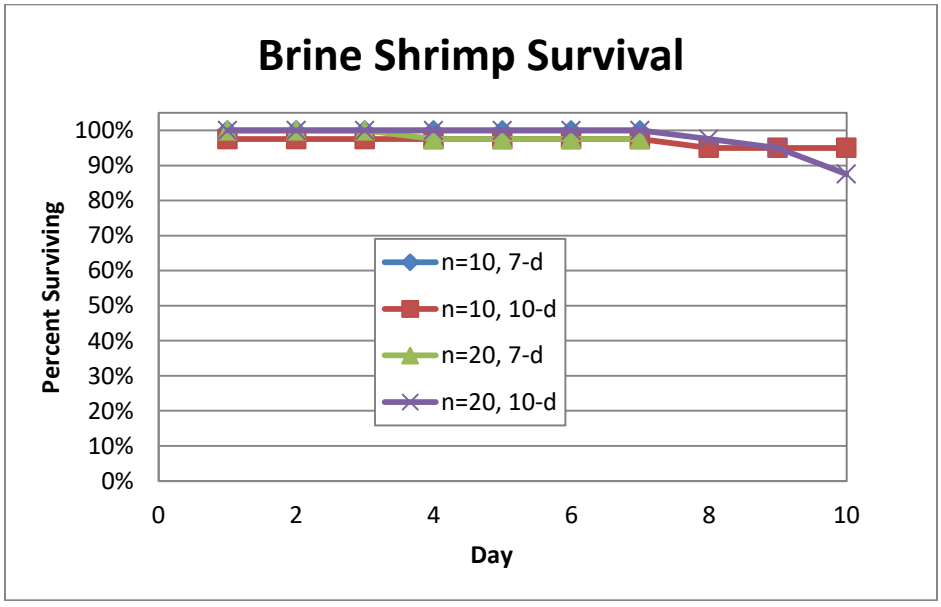
[a]As CaCO3
[b]Total residual chlorine

Test activities:

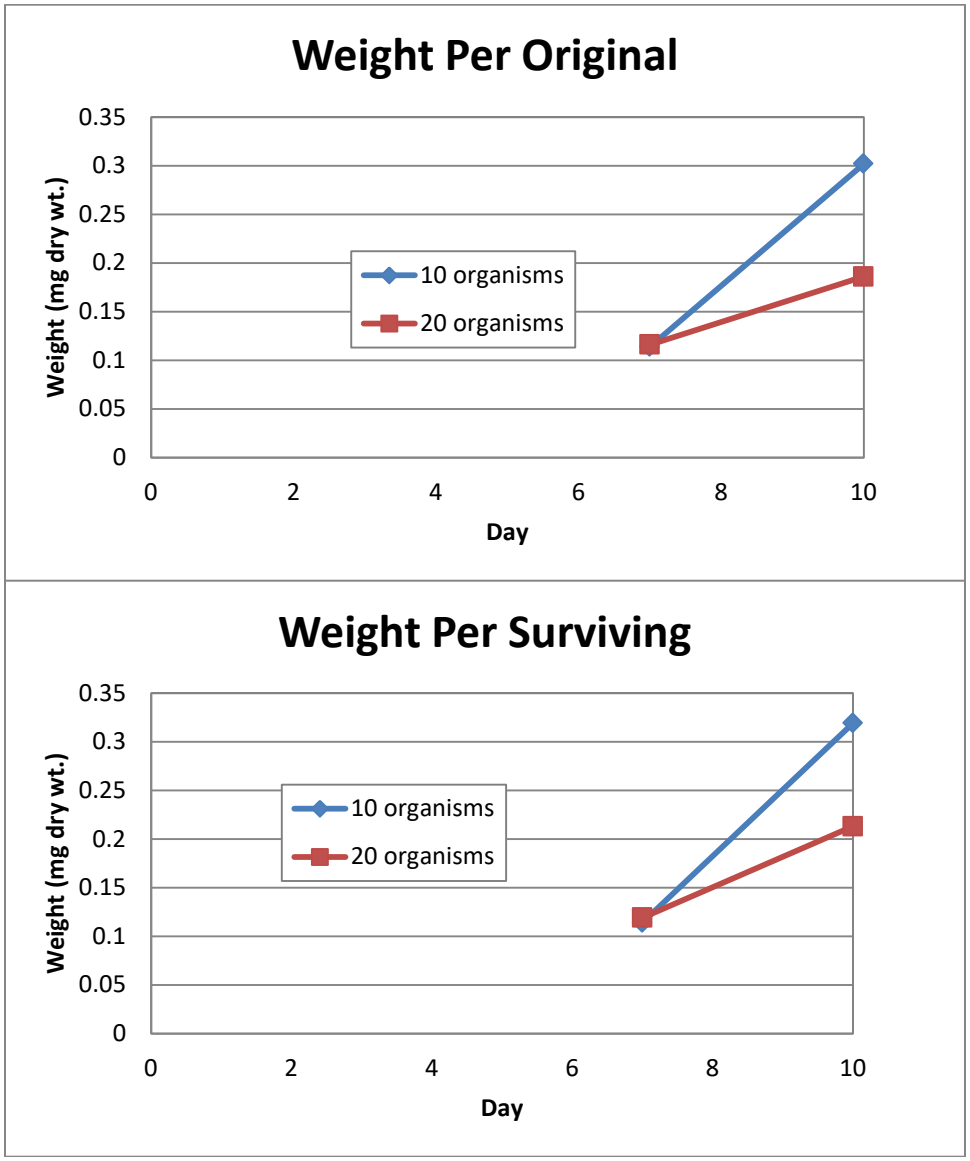
- Biological observations (primarily survival) taken daily.
- Chemistries taken on renewal days (i.e., pH, dissolved oxygen, and temperature).
- Conductivity was measured at test termination or when there was 0% survival in that treatment.
- Dry weights were determined at test termination.

Results:

The survival and average dry weights for the brine shrimp are illustrated in the following figures.



TRE



Summary and findings:

- Organism survival was ≥ 90% for all tests except the 20 organism 10 day treatment, which had 87.5% survival (this is higher than the standard chronic testing limit of 80%).
- Beginning on day 6, the test cups with 20 organisms were a lighter green color than the test cups with 10 organisms. This suggests that, although the 20 organism treatment had not exhausted the supply of algae, it was using a greater portion and may have created food limited conditions.
- On day 7, the brine shrimp weights between the two organism-number treatments were similar, both on a per original and a per surviving basis.
- On day 10, the weights of the organisms in the 10 organism treatment were significantly

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higher than those in the 20 organism treatment, suggesting that food limitation was occurring with the higher number of organisms.

Based on these results, organism survival was very good in both test designs, with average survival in all treatments ≥87.5%. Based on the weight differences, the 20 organism treatment appears to be food-limited after day 6. The 10 organism treatment does not appear to be food limited at day 10. As one of our concerns is the maintaince of adequate food levels in the test vessels between renewals, so as to not affect potential bioavailability of any potential toxicants, the reduction in the number of organisms per replicate may be important in reducing variability between tests.

The daily renewal test design using 10 organisms per test vessel and the 7-day study duration is more consistent with other USEPA chronic tests (e.g., fathead minnow, sheepshead minnow). However, an additional consideration is the higher growth rate of *Artemia* between days 7 and 10 may allow for better resolution of differences and subsequent detection of toxicity. This factor will be addressed in later experiments.

We greatly appreciate the opportunity to complete this study for you. Please do not hesitate to call if you have any questions.

Sincerely,

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17001-474-043

Attachment

cc: David Pillard, TRE

QA MSW 1/8/20

TOXICITY DATA PACKAGE COVER SHEET

Test Type: Chronic

Project Number: 17001-474-Exp (043)

Test Substance: Experiment

Species: *Artemia franciscana*

Dilution Water: rGSL

Organism Lot or Batch Number: 111219

Concurrent Control Water: NA

Age: 48 hr (48 hr)

Supplier: TRE

Date and Time Test Began: 11/14/19 @ 1350

Date and Time Test Ended: 11/24/19 @ 1310

Protocol Number: ____

Investigator(s): AS | BS | CP

Background Information

pH control?: Yes **No**

Type of Test: Static-Renewal (Daily)

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: 2

Length of Test: Varies

Organisms per Replicate: 20/10

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 7 day 20 org, 7 day 10 org, 10 day 20 org, 10 day 10 org

Agency Summary Sheet(s)?: None

Reference Toxicant Data: Test Dates: ____ to ____	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 100 ug/L Chla
Take pictures with scope on Day 3, 4, 7 and 10
Appropriate correction factors have been applied to all temperatures recorded in this data package
Study Director Initials: AS Date: 11/14/19

TEST SUBSTANCE USAGE LOG

Project Number: 17001-474-Exp

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number				
Test Substance Collection Date and Time	From: @ To: @	From: @ To: @	From: @ To: @	From: @ To: @
Sample Type (Grab or Comp)				
Date Test Substance Received				
Dilution Water Number RW# or TRE#, circle one	13772			
Concurrent Control Water RW#	NA			
Date(s) Used	11/14/19 11/15/19 11/16/19 11/17/19	11/18/19 11/22/19 11/19/19 11/23/19 11/20/19 11/21/19		

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)
7D 20	0	100	100						
7D 10	0	100	100						
10D 20	0	100	100						
10D 10	0	100	100						
	0	400	400						

Initials / Date	AS 11/14/19	AS 11/22/19		
Initials / Date	AS 11/15/19	CP 11/23/19		
Initials / Date	CP 11/16/19			
Initials / Date	CP 11/17/19			
Initials / Date	CP 11/18/19			
Initials / Date	AS 11/19/19			
Initials / Date	AS 11/20/19			
Initials / Date	AS 11/21/19			

BRINE SHRIMP (*Artemia franciscana*)
CHRONIC BIOLOGICAL DATA

Project Number: 17001-474-Exp

%Conc.	Test Replicate	Number of Surviving Organisms Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Remarks
7D 20	A	20	20	20	20	19	19	19	19	
	B	20	20	20	20	20	20	20	20	
7D 10	A	10	10	10	10	10	10	10	10	
	B	10	10	10	10	10	10	10	10	
10D 20	A	20	20	20*	20*	20*	20*	20	20	* 1 weak org
	B	20	20	20	20	20	20*	20*	20*	* 1 weak org
10D 10	A	10	9	9	9	9	9	9	9	
	B	10	10	10	10	10	10	10	10	
Date:		11/14/19	11/15/19	11/16/19	11/17/19	11/18/19	11/19/19	11/20/19	11/21/19	
Time:		1350	1600	1510	1525	1435	1400	1445	1400	
Initials:		AB/BF	AB	CP	CP	BF	AB	AB	AB	

%Conc.	Test Replicate	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14		Remarks
10D 20	A	19	18	17						
	B	20	20*	18*						* 1 weak org
10D 10	A	9	9	9						
	B	10	10	10						
Date:		11/22/19	11/23/19	11/24/19						
Time:		1055	1450	1310						
Initials:		AB	CP	BF						

DAB 11/19/19 E

CHRONIC CHEMICAL DATA (INITIAL)

Project Number: 17001-474-Exp

Test Species: ***Artemia franciscana***

%	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter #	Remarks
Conc.: rGSL									All Conc.	
pH	7.8	7.9	7.9	7.8	7.8	7.9	7.8	7.8	Fm26	
D.O. (mg/L)	5.0	5.2	~~5.2~~	5.2	4.9	5.0	4.9	4.9	17	
Temp. (°C)	20	20	20	20	20	20	20	20	L29	
Cond. (µS/cm)	131600	141000	133300	136300	136100	135700	134000	134500	15	
Hard. (mg/L)									Titr	
Alk. (mg/L)									Titr	
TRC (mg/L)									22	
NH_3 (mg/L)									HA7	
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Date:	11/14/19	11/15/19	11/16/19	11/17/19	11/17/19	11/19/19	11/20/19	11/21/19		
Time:	1340	1545	1455	1450	1515	1340	1420	1400		
Initials:	AB	AB	CP	CP	CP	AB	AB	AB		

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.

① CP 11/16/19 E

CHRONIC CHEMICAL DATA (FINAL)

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

%		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter #	Remarks
Conc.:	7D 20							140000		All Conc.	* conductivity (15)
pH		7.9	8.0	7.9	7.9	7.8	7.8	7.8		FM38	
D.O. (mg/L)		4.9	①~~7.75~~ 5.1	4.9	5.0	4.9	4.5	4.8		17	
Temp (°C)		20	20	20	21	19	19	20		L-37	
Conc.:	7D 10							132900			
pH		8.0	8.0	7.9	7.9	7.9	7.9	7.9			
D.O. (mg/L)		5.0	5.1	5.0	5.1	5.1	4.4	4.8			
Temp (°C)		19	20	20	21	19	19	20			
Conc.:	10D 20										
pH		7.9	8.0	7.9	7.9	7.9	7.8	7.8	7.7		
D.O. (mg/L)		4.9	5.1	5.1	5.0	5.1	4.3	4.2	4.5		
Temp (°C)		20	20	20	21	19	19	20	20		
Conc.:	10D 10										* conductivity
pH		8.0	8.0	8.0	7.9	7.9	7.9	7.8	7.7		
D.O. (mg/L)		5.0	5.1	5.1	5.2	5.1	4.4	4.5	4.3		
Temp (°C)		19	20	20	21	19	19	20	20		
Date:		11/15/19	11/16/19	11/17/19	11/18/19	11/19/19	11/20/19	11/21/19	11/22/19		
Time:		1605	1525	1455	1515	1400	1445	1400	1050		
Initials:		MS	CP	CP	RJ	AB	AB	AB	AB		

%		Day 9	Day 10							Meter #	Remarks
Conc.:	10D 20		129200								* conductivity (15)
pH		②~~7.80~~ 7.6	7.6								
D.O. (mg/L)		4.6	4.4								
Temp (°C)		②~~20~~ 21	20								
Conc.:	10D 10		129400								
pH		7.7	7.7								
D.O. (mg/L)		4.5	4.3								
Temp (°C)		②~~20~~ 21	20								
Date:		~~11/23/19~~	11/24/19								
1515 Time:		~~1555~~	1340								
Initials:		CP	AB								

① CP 11/16/19 E

② CP for AB 11/23/19 WP

QA NEW 1/8/20

CHRONIC CHEMICAL DATA (INITIAL)

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

%	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13			Meter #	Remarks
Conc.: rGSL									All Conc.	
pH	7.9	7.9								
D.O. (mg/L)	5.0	5.1								
Temp. (°C)	20	20								
Cond. (µS/cm)	133000	132900								
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.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Date:	11/22/19	11/23/19								
Time:	1035	1515								
Initials:	[illegible]	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 12W 1/8/20

DAILY TOXICITY TEST LOG

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

General Comments			Feeding 145 ug/l Chla upon renewal	Initials/Date
	Random Chart: NA	Min/Max Thermometer # M15		
Test Day 0	Test Solution Mixed at: 1335 Test Organisms Added at: 1350		Fed @ 1340	AB 11/14/19
Test Day 1	Real Time: 19 °C	Min-Max Range: 19-21 °C	Fed @ 1555	AB 11/15/19
Test Day 2	Real Time: 20 °C	Min-Max Range: 19-21 °C	Fed @ 1520	CP 11/16/19
Test Day 3	Real Time: 19 °C	Min-Max Range: 19-21 °C	Fed @ 1530	CP 11/17/19
Test Day 4	Real Time: 19 °C	Min-Max Range: 19-21 °C	Fed @ 1330	RG 11/18/19
Test Day 5	Real Time: 19 °C	Min-Max Range: 19-21 °C	Fed @ 1350	AB 11/19/19
Test Day 6	Real Time: 19 °C 20 org cups visibly more clear than 10 orgs but still tinted green	Min-Max Range: 19-21 °C	Fed @ 1410	AB 11/20/19
Test Day 7	Real Time: 21 °C D	Min-Max Range: 21-24 °C	Fed @ 1350 ~~1045~~ ①	AB 11/21/19
Test Day 8	Real Time: 20 °C D	Min-Max Range: 19-23 °C	Fed @ 1045	AB 11/22/19

① BB 11/20/19 E

DAILY TOXICITY TEST LOG

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

General Comments				Feeding 145 ug/l Chla upon renewal	Initials/Date
Test Day 9	Real Time: 19 °C	Min-Max Range: 19-21 °C		Fed @ 1510	CP 11/23/19
Test Day 10	Real Time: 19 °C	Min-Max Range: 19-21 °C		None	BJ 11/24/19

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 17001-474 exp#4	Test Substance:	Comments:
Species:	Analyst Tare: AB Analyst Gross: AH	Analytical Balance ID: Dried in Oven # 3 from Date: 11/20/19 Time: 1335 to Date: May 19 Time: 0830
Date/Time of Tare Wt.: 11/21/19 @ 1316	Date/Time of Gross Wt.: 11/21/19	

Weight Type (Circle): Wet Blot Dry Dry (>~~100°C~~ 60-90°C) AFDW (>500°C)

Lot or Batch Number: 111219

Boat No.	Treatment	Rep.	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)[1]	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	No. of Surv. Organisms	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)
	7D 100%	A		1.13145	1.13252	0.00107					10		
	7D 100%	B		1.14539	1.14661	0.0022					10		
	7D 20%	A		1.13310	1.13533	0.00223					19		
	7D 20%	B		1.13926	1.14167	0.00241					20		
Blank				1.13496	1.13497								
Range													
Mean													

Test Solution Volume: Loading Rate:

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

DB3 1/10/20 E

DH MW 1/8/20

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 1900-47-4 exp#4	Test Substance:	Comments:
Species:	Analyst Tare: AH Analyst Gross: HM	Analytical Balance ID:
Date/Time of Tare Wt.: 11/29/19 @ 1130	Date/Time of Gross Wt.: 11/29/19 @ 1135	Dried in Oven # 3 from Date: 11/24/19 Time: 1335 to Date: 11/28/19 Time: 0810

Weight Type (Circle): Wet Blot Dry Dry (60-90°C) (>100°C) AFDW (>500°C) Lot or (Batch) Number: 111219

Boat No.	Treatment	Rep.	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)[1]	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	No. of Surv. Organisms	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)
	10 org/10day	A		1.14671	1.14972	0.00301					9		
	10 org/10day	B		1.14481	1.14785	0.00304					10		
	20 org/10day	A		1.13212	1.13582	0.00370					17		
	20 org/10day	B		1.12969	1.13345	0.00376					18		
Blank				1.14671	1.14671	0.00000							
Range													
Mean													

Test Solution Volume: Loading Rate:

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

DH MW 1/10/20

QA Form No. 010a
Revision 1
Effective 02/14

AB 12/3/19
QA review 1/8/20

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 17001-474 Species: Artemia franciscana

Treatment	Rep	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)	No of Orig. Organisms	Mean Wt./ Original Organism (mg)	Mean Wt./ Treatment (mg) (Original)	Number of Surv. Organisms	Mean Wt./ Surviving Organism (mg)	Mean Wt./ Treatment (mg) (Surviving)
Day 7 10 Org	A		1.13145	1.13252	0.00107	0.00107	10	0.107	0.1145	10	0.107	0.1145
	B		1.14539	1.14661	0.00122	0.00122	10	0.122		10	0.122	
Day 7 20 Org	A		1.13310	1.13533	0.00223	0.00223	20	0.111	0.1160	19	0.117	0.1189
	B		1.13926	1.14167	0.00241	0.00241	20	0.121		20	0.121	
Day 10 10 Org	A		1.14671	1.14972	0.00301	0.00301	10	0.301	0.3025	9	0.334	0.3192
	B		1.14481	1.14785	0.00304	0.00304	10	0.304		10	0.304	
Day 10 20 Org	A		1.13212	1.13582	0.00370	0.00370	20	0.185	0.1865	17	0.218	0.2133
	B		1.12969	1.13345	0.00376	0.00376	20	0.188		18	0.209	
Blank 7d			1.13496	1.13497	0.00001							
Blank 10d			1.14671	1.14671	0.00000							

Project Number: 17001-474 Species: Artemia franciscana

Summary Statistics for Survival Data

Treatment	N	Min	Max	Mean	SD	C.V.
Day 7 10 Org	2	0.0	1.0	1.0000	0.0000	0.000%
Day 7 20 Org	2	0.0	1.0	0.9750	0.0354	3.626%
Day 10 10 Org	2	0.9	1.0	0.9500	0.0707	7.443%
Day 10 20 Org	2	0.9	0.9	0.8750	0.0354	4.041%

AS 12/3/19
DA NAN 1/8/20

Summary Statistics for Growth Data (dry wt per original)

Treatment	N	Min	Max	Mean	SD	C.V.
Day 7 10 Org	2	0.107	0.122	0.1145	0.0106	9.263%
Day 7 20 Org	2	0.111	0.121	0.1160	0.0064	5.486%
Day 10 10 Org	2	0.301	0.304	0.3025	0.0021	0.701%
Day 10 20 Org	2	0.185	0.188	0.1865	0.0021	1.137%

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

Treatment	N	Min	Max	Mean	SD	C.V.
Day 7 10 Org	2	0.107	0.122	0.1145	0.0106	9.263%
Day 7 20 Org	2	0.117	0.121	0.1189	0.0022	1.862%
Day 10 10 Org	2	0.304	0.334	0.3192	0.0215	6.744%
Day 10 20 Org	2	0.209	0.218	0.2133	0.0062	2.904%

TRE Environmental Strategies, LLC
100 Racquette Drive, Unit A, Fort Collins, Colorado, 80524
T 970.416.0916 F 970.490.2963

January 14, 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 Short-term Chronic Brine Shrimp Experiment #5

Mr. Bittner:

Below is a summary of the short-term chronic brine shrimp experiment initiated on December 19, 2019. The purpose of this experiment was to investigate whether two different test durations (7 vs 10 days) would affect the IC25 obtained with arsenic on the short-term chronic test method using *Artemia franciscana*.

Along with a control, five different arsenic concentrations were tested,

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

The ratio of food volume to test media volume was consistent between treatments, that is, the estimated concentration of *Dunaliella* was 145µg/L Chl*a* in all containers. The test durations were selected to address whether two different test durations would result in a similar IC25 values with arsenic. The results of these studies will help determine the experimental design of the definitive short-term chronic toxicity tests. The test volume was consistent at 50 ml.

Species: *Artemia franciscana*

Test type:

- Test duration: 7 and 10 days
- Test type: static-renewal (solutions and food renewed daily)
- Algae: *Dunaliella viridis*
- Algae concentration: 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)

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 100 µg/L Chl*a*. 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#13804	7.9	NM	NM	139,800	NM	NM	120

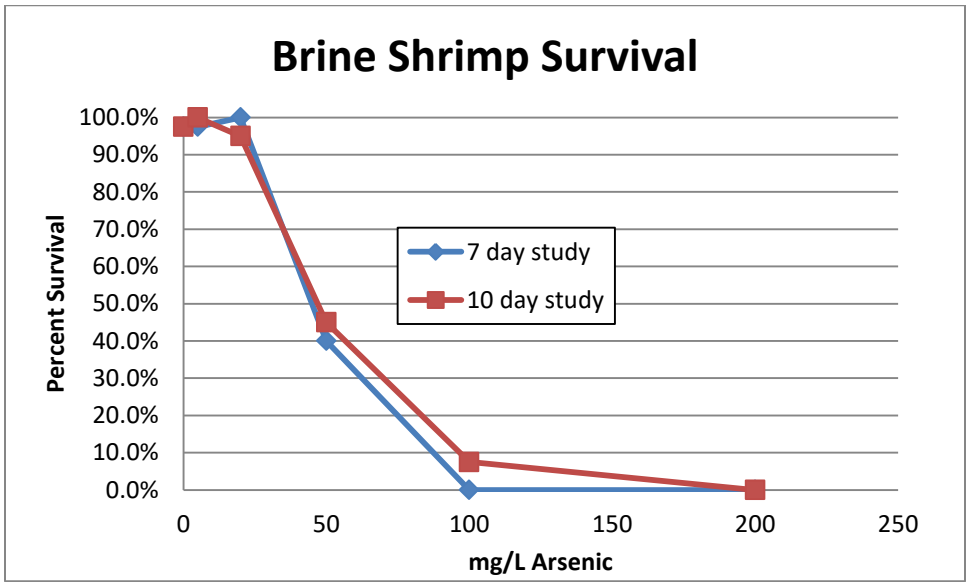
[a]As CaCO3
[b]Total residual chlorine

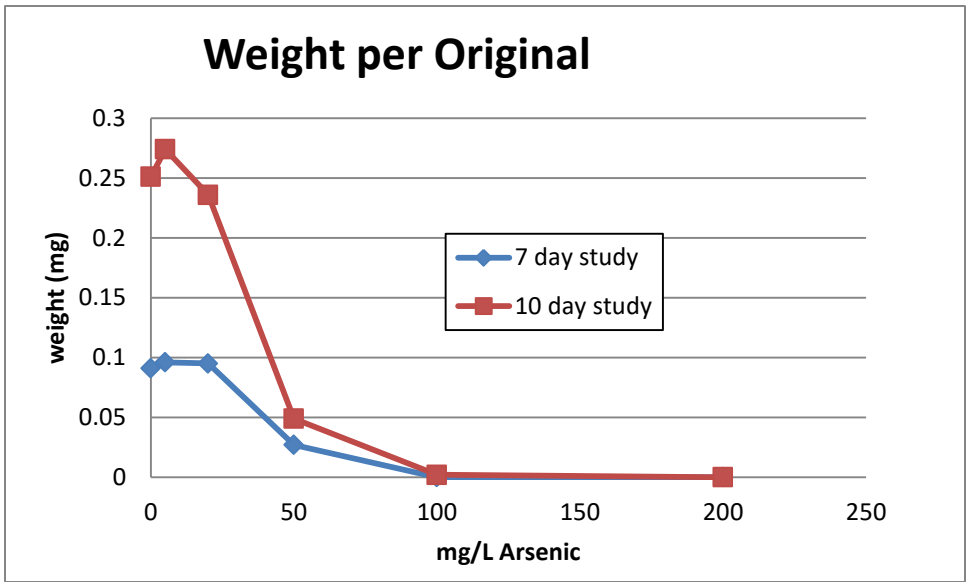
Test activities:

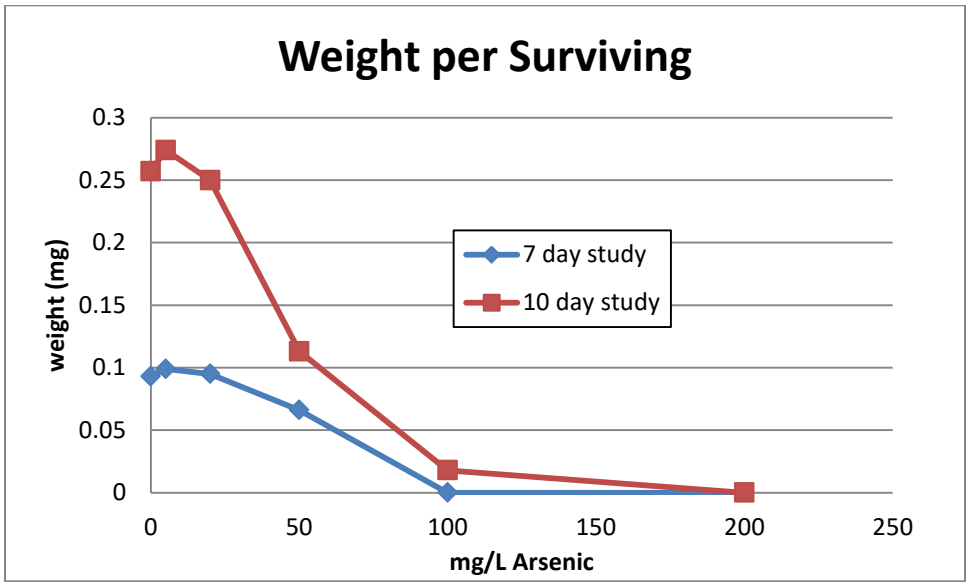
- Biological observations (primarily survival) taken daily.
- Chemistries taken on renewal days (i.e., pH, dissolved oxygen, and temperature).
- Conductivity was measured at test termination or when there was 0% survival in that treatment.
- Dry weights were determined at test termination.
- Arsenic was added to 1220 rGSL media containing food and allowed to equilibrate for 3 hours prior to use in the toxicity tests.

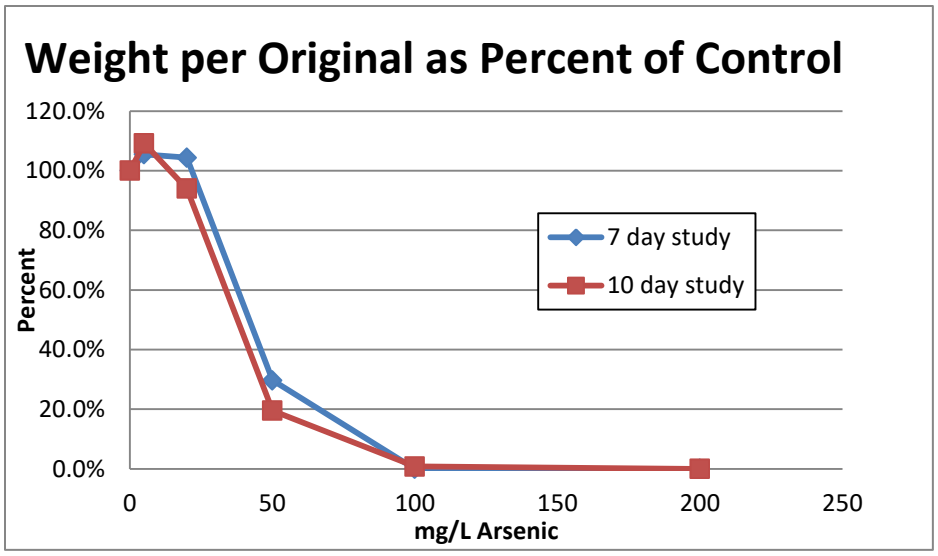
Results:

The survival and average dry weights for the brine shrimp are illustrated in the following figures.




Weight per Original
0.3
0.25
0.2
0.15
0.1
0.05
0
weight (mg)
0
50
100
150
200
250
mg/L Arsenic
7 day study
10 day study


Weight per Surviving
0.3
0.25
0.2
0.15
0.1
0.05
0
weight (mg)
0
50
100
150
200
250
mg/L Arsenic
7 day study
10 day study



Test Endpoints

Study	Survival NOEC (mg As/L)	Survival LOEC (mg As/L)	Growth NOEC (mg As/L)	Growth LOEC (mg As/L)	Growth IC25 (mg As/L)
7 Day	20	50	20	50	30.43 (25.72-32.91)
10 Day	20	50	20	50	26.26 (19.22-31.09)

Summary and findings:

- Organism survival was ≥ 90% for both controls.
- The effect of arsenic on brine shrimp survival was similar for both test durations.
- The growth IC25 values for each test were similar and fell within the confidence limits of the other test.

Based on these results, both 7 and 10 day durations appear to be adequate for detecting a survival and growth effect. Additional testing will help confirm this.

We greatly appreciate the opportunity to complete these studies for you. Please do not hesitate to call if you have any questions regarding this study.

Sincerely,

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

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

14001-474-046, 047

Attachment

cc: David Pillard, TRE

QA NW 1/11/20

TOXICITY DATA PACKAGE COVER SHEET

Test Type: Chronic 7d

Project Number: 17001-474-Exp

Test Substance: Arsenic (Na2HAsO4)

Species: *Artemia franciscana*

Dilution Water: rGSL

Organism Lot or Batch Number: 121719

Concurrent Control Water: NA

Age: 48hr (48 hr)

Supplier: TRE

Date and Time Test Began: 12/19/19 @ 1500

Date and Time Test Ended: 12/26/19 @ 1455

Protocol Number: NA

Investigator(s): BS/AS/CP/EN/EE

Background Information

pH control?: Yes **No**

Type of Test: Static-Renewal (Daily)

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: 7 days

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, 5, 20, 50, 100, and 200 mg/L as As

Agency Summary Sheet(s)?: None

Reference Toxicant Data:	Test Dates: ~	to ~	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 100 ug/L Chla
Appropriate correction factors have been applied to all temperatures recorded in this data package
Study Director Initials: AS Date: 12/19/19

ZDA MW 1/10/20

TEST SUBSTANCE USAGE LOG

Project Number: 17001-474-Exp

	Sample 1 @	Sample 2	Sample 3	Sample 4
Test Substance Number	As Spec Stock			
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	13804			
Concurrent Control Water RW#				
Date(s) Used	12/19/19 | 12/23/19			
	12/20/19 | 12/24/19			
	12/21/19 | 12/25/19			
	12/22/19 |			

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	250	0	250						
5	0.33	249.67	250						
20	1.34	248.66	250	See Spiking Sheet					
50	3.34	246.66	250						
100	6.68	243.32	250						
200	13.37	236.63	250						
	275.07	1224.93	1500						

Initials / Date	AB 12/19/19		
Initials / Date	MS 12/20/19		
Initials / Date	CP 12/21/19		
Initials / Date	EJ 12/22/19		
Initials / Date	EJ 12/23/19		
Initials / Date	AB 12/24/19		
Initials / Date	EN 12/24/19		
Initials / Date			

DMW 1/10/20 E

QA Form No. 060
Revision 3
Effective 02/14

QA mgw 1/11/20

Artemia franciscana
CHRONIC BIOLOGICAL DATA

Project Number: 17001-474-Exp

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
										20 survived
0	A	10	10	10	10	10	10	10	9	97.5
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
5	A	10	10	10	10	10	10	10	10	97.5
	B	10	10	10	10	10	10	10	10	
	C	10	10	9	9	9	9	9	9	
	D	10	10	10	10	10	10	10	10	
20	A	10	10	10	10	10	10	10	10	100
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
50	A	10	5	4	4	4	4	4	4	40%
	B	10	3	2	2	2	2	2	2	
	C	10	6	6	6	6	6	6	6	
	D	10	7	4	4	4	4	4	4	
100	A	10	2	0						0%
	B	10	0	—						
	C	10	0	—						
	D	10	1	0						
200	A	10	0							0%
	B	10	0							
	C	10	0							
	D	10	0							
	A									
	B									
	C									
	D									
Date:		12/16/19	12/20/19	12/21/19	12/22/19	12/23/19	12/24/19	12/25/19	12/26/19	
Time:		1500	1540	1450	1315	1450	1500	1100	1455	
Initials:		BM	MS	OP	BF	BF	MS	EE	BF	

Dr new 1/11/20

CHRONIC CHEMICAL DATA (INITIAL)

Project Number:	17001-474-Exp
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	7.9	7.9	8.0	7.9	7.9		FM08	
D.O. (mg/L)	5.2	5.3	5.3	5.5	5.2	5.1	5.5		17	
Temp. (°C)	20	20	20	20	20	20	20		L296	
Cond. (µS/cm)	139800	139800	145600	138500	*	138100	146700		15	
Hard. (mg/L)									Titr	
Alk. (mg/L)									Titr	
TRC (mg/L)									22	
NH_3 (mg/L)									4/11	ppt: 120
Conc.: 5										
pH	7.9	8.0	7.9	7.9	8.0	7.9	8.0			
D.O. (mg/L)	5.1	4.9	5.3	5.4	5.3	5.1	5.5			
Temp. (°C)	*	*	*	*	*	*	*			
Cond. (µS/cm)	139,200	137900	145200	138000	⊙	137700	141400			
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Conc.: 20										
pH	7.9	7.9	7.9	7.9	8.0	7.9	7.9			
D.O. (mg/L)	5.1	4.9	5.3	5.4	5.3	5.1	5.5			
Temp. (°C)	*	*	*	*	*	*	*			
Cond. (µS/cm)	139,400	137600	145300	137100	⊙	136200	141700			
Conc.: 50										
pH	7.9	8.1	7.9	7.9	8.0	7.9	7.9			
D.O. (mg/L)	5.1	4.9	5.3	5.4	5.4	5.0	5.5			
Temp. (°C)	*	*	*	*	*	*	*			
Cond. (µS/cm)	138,300	136200	145000	136,000	⊙	135300	141700			
Date:	12/18/19	12/20/19	12/21/19	12/23/19	12/23/19	12/24/19	12/25/19			
Time:	1450	1515	1425	1300	1445	1450	1100			
Initials:	BF	NB	CP	BF	BF	AM	EE			

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.

⊙ BF 12/19/19

* meter error

[illegible] 1/11/20

CHRONIC CHEMICAL DATA (INITIAL)

Project Number: 17001-474-Exp

Test Species: *Artemia franciscana*

%	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter #	Remarks
Conc.: 100									All Conc.	
pH	7.9	8.0								
D.O. (mg/L)	5.1	4.8								
Temp. (°C)	☆	*								
Cond. (µS/cm)	139,300	135400								
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.: 200										
pH	7.9									
D.O. (mg/L)	5.2									
Temp. (°C)	☆									
Cond. (µS/cm)	136,600									
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Date:	12/19/19	12/20/19	12/21/19							
Time:	1450	1515	1425							
Initials:	[illegible]	AB	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 now 1/11/20

CHRONIC CHEMICAL DATA (FINAL)

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

%		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter #	Remarks
Conc.:	0							132,200		All Conc.	* conductivity (15)
pH		8.1	8.0	8.0	8.0	8.0	8.0	8.1		FM28	
D.O. (mg/L)		5.4	5.4	5.7	5.0	4.9	5.1	6.1		17	
Temp (°C)		20	20	20	20	20	20	20		44	
Conc.:	5										
pH		8.0	8.0	8.0	8.0	8.0	8.0	8.0			
D.O. (mg/L)		5.5	5.3	5.5	5.0	4.9	5.1	6.0			
Temp (°C)		20	20	20	20	20	20	19			
Conc.:	20										
pH		8.1	8.0	8.0	8.0	8.0	8.0	8.1			
D.O. (mg/L)		5.4	5.3	5.5	5.1	4.9	5.2	5.9			
Temp (°C)		20	20	20	20	20	20	20			
Conc.:	50							129,000			(15)
pH		8.0	8.0	8.0	8.0	8.0	8.0	8.1			
D.O. (mg/L)		5.5	5.3	5.8	5.4	5.6	5.2	6.4			
Temp (°C)		20	20	20	20	20	20	20			
Conc.:	100		~~46000~~								
pH		8.0	8.0								
D.O. (mg/L)		5.5	5.4								
Temp (°C)		20	20								
Conc.:	200	142700									* conductivity
pH		8.0									
D.O. (mg/L)		5.5									
Temp (°C)		20	20①								
Conc.:											
pH											
D.O. (mg/L)											
Temp (°C)											
Date:		12/20/19	12/21/19	12/22/19	12/23/19	12/24/19	12/25/19	12/26/19			
Time:		1550	1455	1325	1500	1505	1100	1540			
Initials:		AM	CP	BF	BF	AM	EE	BF			

① CP 12/21/19 E

QA Form No. 055
Revision 3
Effective 02/14
QA MW 1/11/20

DAILY TOXICITY TEST LOG

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

General Comments			Feeding 145 ug/l Chla upon renewal	Initials/Date
	Random Chart: M	Min/Max Thermometer # M-15		
Test Day 0	Test Solution Mixed at: 1110 Test Organisms Added at: 1500 Spiked @ 1110		Fed @ 1110	BS 12/19/19
Test Day 1	Real Time: 20 °C	Min-Max Range: 19-21 °C	Fed @ 1055	AS 12/20/19
	Spiked @ 1055			
Test Day 2	Real Time: 19 °C	Min-Max Range: 19-21 °C	Fed @ 1045	CP 12/21/19
	Spiked @ 1045			
Test Day 3	Real Time: 19 °C	Min-Max Range: 19-20 °C	Fed @ 0940	BS 12/22/19
	Spiked @ 0940			
Test Day 4	Real Time: 19 °C	Min-Max Range: 18-20 °C	Fed @ 1045	RS 12/23/19
	Spiked @ 1045			
Test Day 5	Real Time: 19 °C	Min-Max Range: 18-20 °C	Fed @ 1120	AS 12/24/19
	Spiked @ 1120			
Test Day 6	Real Time: 19 °C	Min-Max Range: 19-20 °C	Fed @ 0740	EE 12/25/19
	Spiked @ 0740			
Test Day 7	Real Time: 19 °C	Min-Max Range: 19-20 °C	Fed @ None	BS 12/26/19
Test Day 8	Real Time: °C	Min-Max Range: °C	Fed @	

QA Form No.010
Revision 6
Effective 02/14
AG 1/6/20
TDA nan 1/8/20

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 17001-474-exp(d7)	Test Substance: Arsenic (Na_2HAsO_4)	Comments:
Species: A. franciscana	Analyst Tare: EN Analyst Gross: AF/NW	Analytical Balance ID: Sart #1
Date/Time of Tare Wt.: 12/26/19@1120	Date/Time of Gross Wt.: 12/28/19@1110	Dried in Oven # 3 from Date: 12/26/19 Time: 1540 to Date: 12/28/19 Time: 1035

Weight Type (Circle): Wet Blot Dry Dry (>100°C) AFDW (>500°C) Lot or Batch Number: 121719

Boat No.	Treatment	Rep.	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)[1]	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	No. of Surv. Organisms	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)
	0	A		1.16536	1.16608	0.00072					9		
		B		1.13819	1.13898	0.00079					10		
		C		1.14734	1.14825	0.00091					10		
		D		1.14048	1.14155	0.00107					10		
	5	A		1.18217	1.18314	0.00087					10		
		B		1.17587	1.17677	0.00090					~~10~~ 10		
		C		1.15584	1.15~~800~~ 1077	0.00093					9		
		D		1.15707	1.15806	0.00099					10		
	20	A		1.15384	1.15474	0.00090					10		
		B		1.15846	1.15932	0.00086					10		
		C		1.13895	1.14000	0.00105					10		
		D		1.15385	1.15469	0.00084					10		
Blank				1.13876	1.13872	-0.00004							
Range													
Mean													

Test Solution Volume: Loading Rate:

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

① AF 12/28/19 E

AB 1/6/20
BH WBN 1/8/20

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 17001-474-Exp (d7)	Test Substance: Arsenic (Na_2HAsO_4)	Comments: Analytical Balance ID: Sart#1
Species: A. franciscana	Analyst Tare: EN Analyst Gross: AF/WBN	Dried in Oven # 3 from Date: 12/26/19 Time: 1540
Date/Time of Tare Wt.: 12/26/19 @ 1120	Date/Time of Gross Wt.: 12/28/19 @ 1110	to Date: 12/28/19 Time: 1035

Weight Type (Circle): Wet Blot Dry Dry (>100°C) AFDW (>500°C)

Lot or Batch Number: 121719

Boat No.	Treatment	Rep.	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)[1]	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	No. of Surv. Organisms	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)
	Sa	A		1.11457	1.11474	0.00017						4	
		B		1.16643	1.16652	0.00009						2	
		C		1.12376	1.12411	0.00035						6	
		D		1.12289	1.12318	0.00029						4	
Blank													
Range													
Mean													

Test Solution Volume:

Loading Rate:

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

QA Form No. 010a
Revision 1
Effective 02/14
AS 1/6/20
[handwritten initials] 1/8/20

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 14001-474 7 day Arsenic   Species: Artemia franciscana

Treatment	Rep	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)	No of Orig. Organisms	Mean Wt./ Original Organism (mg)	Mean Wt./ Treatment (mg) (Original)	Number of Surv. Organisms	Mean Wt./ Surviving Organism (mg)	Mean Wt./ Treatment (mg) (Surviving)
rGSL	A		1.16536	1.16608	0.00072	0.00076	10	0.076	0.0913	9	0.084	0.0934
	B		1.13819	1.13898	0.00079	0.00083	10	0.083		10	0.083	
	C		1.14734	1.14825	0.00091	0.00095	10	0.095		10	0.095	
	D		1.14048	1.14155	0.00107	0.00111	10	0.111		10	0.111	
5 mg/L	A		1.18227	1.18314	0.00087	0.00091	10	0.091	0.0963	10	0.091	0.0989
	B		1.17587	1.17677	0.00090	0.00094	10	0.094		10	0.094	
	C		1.15584	1.15677	0.00093	0.00097	10	0.097		9	0.108	
	D		1.15707	1.15806	0.00099	0.00103	10	0.103		10	0.103	
20 mg/L	A		1.15384	1.15474	0.00090	0.00094	10	0.094	0.0953	10	0.094	0.0953
	B		1.15846	1.15932	0.00086	0.00090	10	0.090		10	0.090	
	C		1.13895	1.14000	0.00105	0.00109	10	0.109		10	0.109	
	D		1.15385	1.15469	0.00084	0.00088	10	0.088		10	0.088	
50 mg/L	A		1.11457	1.11474	0.00017	0.00021	10	0.021	0.0265	4	0.053	0.0663
	B		1.16643	1.16652	0.00009	0.00013	10	0.013		2	0.065	
	C		1.12376	1.12411	0.00035	0.00039	10	0.039		6	0.065	
	D		1.12289	1.12318	0.00029	0.00033	10	0.033		4	0.083	
100 mg/L	A		0.00000	0.00000	0.00000	0.00000	10	0.000	0.0000	0	-	#DIV/0!
	B		0.00000	0.00000	0.00000	0.00000	10	0.000		0	-	
	C		0.00000	0.00000	0.00000	0.00000	10	0.000		0	-	
	D		0.00000	0.00000	0.00000	0.00000	10	0.000		0	-	
200 mg/L	A		0.00000	0.00000	0.00000	0.00000	10	0.000	0.0000	0	-	#DIV/0!
	B		0.00000	0.00000	0.00000	0.00000	10	0.000		0	-	
	C		0.00000	0.00000	0.00000	0.00000	10	0.000		0	-	
	D		0.00000	0.00000	0.00000	0.00000	10	0.000		0	-	
Blank			1.13876	1.13872	-0.00004							

AS 1/6/20
SH NEW 1/8/20

Project Number: 14001-474 7 day Arsenic Species: Artemia franciscana

Summary Statistics for Survival Data

Treatment	N	Min	Max	Mean	SD	C.V.
rGSL	4	0.9	1.0	0.9750	0.0500	5.128%
5 mg/L	4	0.9	1.0	0.9750	0.0500	5.128%
20 mg/L	4	1.0	1.0	1.0000	0.0000	0.000%
50 mg/L	4	0.2	0.6	0.4000	0.1633	40.825%
100 mg/L	4	0.0	0.0	0.0000	0.0000	#DIV/0!
200 mg/L	4	0.0	0.0	0.0000	0.0000	#DIV/0!

Summary Statistics for Growth Data (dry wt per original)

Treatment	N	Min	Max	Mean	SD	C.V.
rGSL	4	0.076	0.111	0.0913	0.0153	16.797%
5 mg/L	4	0.091	0.103	0.0963	0.0051	5.323%
20 mg/L	4	0.088	0.109	0.0953	0.0095	9.974%
50 mg/L	4	0.013	0.039	0.0265	0.0117	44.169%
100 mg/L	4	0.000	0.000	0.0000	0.0000	#DIV/0!
200 mg/L	4	0.000	0.000	0.0000	0.0000	#DIV/0!

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

Treatment	N	Min	Max	Mean	SD	C.V.
rGSL	4	0.083	0.111	0.0934	0.0129	13.837%
5 mg/L	4	0.091	0.108	0.0989	0.0078	7.873%
20 mg/L	4	0.088	0.109	0.0953	0.0095	9.974%
50 mg/L	4	0.053	0.083	0.0663	0.0123	18.615%
100 mg/L	0	0.000	0.000	#DIV/0!	#DIV/0!	#DIV/0!
200 mg/L	0	0.000	0.000	#DIV/0!	#DIV/0!	#DIV/0!

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

TRE Environmental Strategies

Analysis ID:	15-0460-2271	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Jan-20 9:07	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	18-0895-7427	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Lab Tech
Start Date:	19 Dec-19 15:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	rGSL
Ending Date:	26 Dec-19 14:55	**Species:**	Artemia franciscana	**Brine:**	Crystal Sea
Duration:	7d	**Source:**	In-House Culture	**Age:**	48h
Sample ID:	03-3624-0124	**Code:**	140A9DFC	**Client:**	Univerity of Notre Dame
Sample Date:	19 Dec-19 15:00	**Material:**	Arsenic	**Project:**	Special Studies
Receive Date:	19 Dec-19 15:00	**Source:**	research		
Sample Age:	NA	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	10.7%	20	50	31.62	

Steel Many-One Rank Sum Test

Control	vs	C-mg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		5	18	10	2	6	0.7500	Asymp	Non-Significant Effect
		20	20	10	1	6	0.9096	Asymp	Non-Significant Effect
		50*	10	10	0	6	0.0276	Asymp	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.495771	0.4985902	3	46.32	<0.0001	Significant Effect
Error	0.1291595	0.01076329	12			
Total	1.62493		15			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1.082	5.95	0.3939	Equal Variances
Variances	Levene Equality of Variance	1.793	5.95	0.2021	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8358	0.841	0.0085	Non-normal Distribution

7d Survival Rate Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	0.975	0.8954	1	1	0.9	1	0.025	5.13%	0.0%
5		4	0.975	0.8954	1	1	0.9	1	0.025	5.13%	0.0%
20		4	1	1	1	1	1	1	0	0.0%	-2.56%
50		4	0.4	0.1402	0.6598	0.4	0.2	0.6	0.08165	40.8%	59.0%
100		4	0	0	0	0	0	0	0		100.0%
200		4	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.371	1.242	1.501	1.412	1.249	1.412	0.04074	5.94%	0.0%
5		4	1.371	1.242	1.501	1.412	1.249	1.412	0.04074	5.94%	0.0%
20		4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	-2.97%
50		4	0.6798	0.4052	0.9544	0.6847	0.4636	0.8861	0.08628	25.4%	50.4%
100		4	0.1588	0.1588	0.1588	0.1588	0.1588	0.1588	0	0.0%	88.4%
200		4	0.1588	0.1588	0.1588	0.1588	0.1588	0.1588	0	0.0%	88.4%

Analyst: _____ QA: _____ 1/6/20

Antenna

~~Fathead Minnow~~ 7-d Larval Survival and Growth Test — TRE Environmental Strategies

Analysis ID:	15-0460-2271	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Jan-20 9:07	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

7d Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9	1	1	1
5		1	1	0.9	1
20		1	1	1	1
50		0.4	0.2	0.6	0.4
100		0	0	0	0
200		0	0	0	0

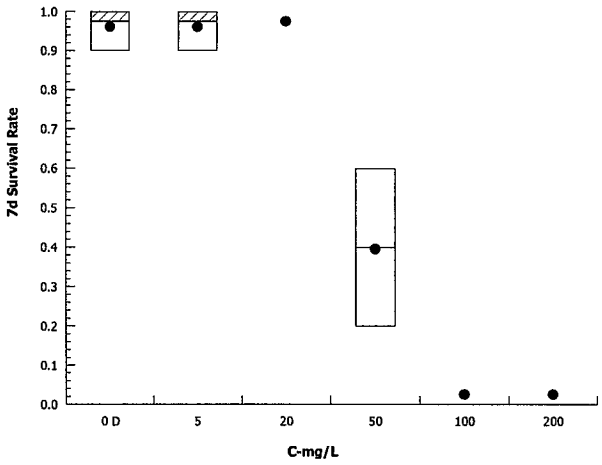
Angular (Corrected) Transformed Detail

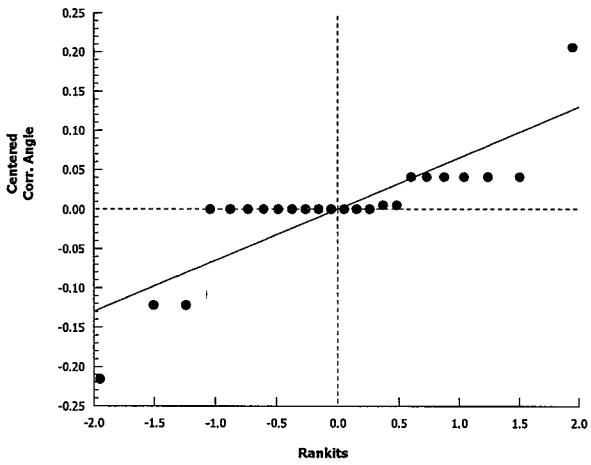
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.249	1.412	1.412	1.412
5		1.412	1.412	1.249	1.412
20		1.412	1.412	1.412	1.412
50		0.6847	0.4636	0.8861	0.6847
100		0.1588	0.1588	0.1588	0.1588
200		0.1588	0.1588	0.1588	0.1588

7d Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	9/10	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		4/10	2/10	6/10	4/10
100		0/10	0/10	0/10	0/10
200		0/10	0/10	0/10	0/10

Graphics





1/8/20

CETIS Analytical Report

Report Date: 06 Jan-20 09:08 (p 1 of 2)
Test Code: 474-7d | 05-2577-0396

Artemia

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

TRE Environmental Strategies

Analysis ID:	07-1368-4325	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Jan-20 9:08	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	18-0895-7427	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Lab Tech
Start Date:	19 Dec-19 15:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	rGSL
Ending Date:	26 Dec-19 14:55	**Species:**	Artemia franciscana	**Brine:**	Crystal Sea
Duration:	7d	**Source:**	In-House Culture	**Age:**	48h
Sample ID:	03-3624-0124	**Code:**	140A9DFC	**Client:**	Univerity of Notre Dame
Sample Date:	19 Dec-19 15:00	**Material:**	Arsenic	**Project:**	Special Studies
Receive Date:	19 Dec-19 15:00	**Source:**	research		
Sample Age:	NA	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	18.3%	20	>20	NA	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		5	-0.6533	2.18	0.017	6	0.8676	CDF	Non-Significant Effect
		20	-0.5227	2.18	0.017	6	0.8368	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5.600005E-05	2.800002E-05	2	0.239	0.7922	Non-Significant Effect
Error	0.00105425	0.0001171389	9			
Total	0.00111025		11			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.771	9.21	0.2502	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9577	0.802	0.7505	Normal Distribution

Mean Dry Biomass-mg Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	0.09125	0.06686	0.1156	0.089	0.076	0.111	0.007663	16.8%	0.0%
5		4	0.09625	0.0881	0.1044	0.0955	0.091	0.103	0.002562	5.32%	-5.48%
20		4	0.09525	0.08013	0.1104	0.092	0.088	0.109	0.00475	9.97%	-4.38%

Mean Dry Biomass-mg Detail

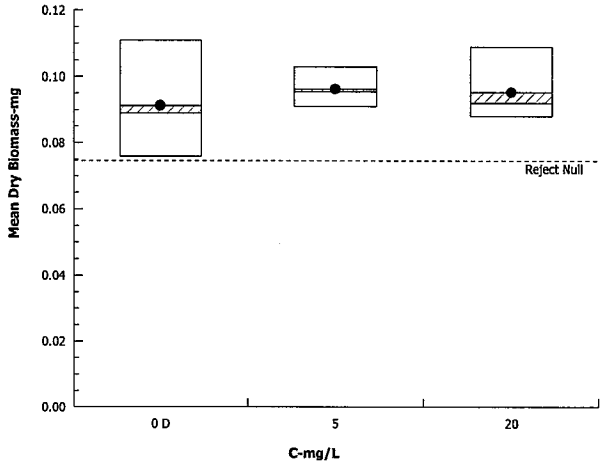
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.076	0.083	0.095	0.111
5		0.091	0.094	0.097	0.103
20		0.094	0.09	0.109	0.088

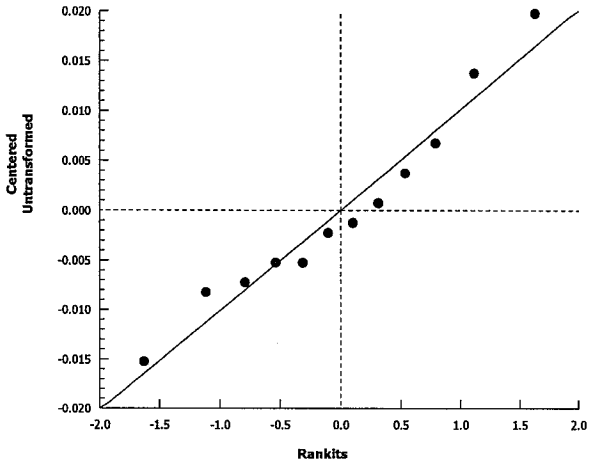
1/8/20

Fathead Minnow 7-d Larval Survival and Growth Test TRE Environmental Strategies

Analysis ID:	07-1368-4325	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Jan-20 9:08	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





1/8/20

~~Fathead Minnow~~ 7-d Larval Survival and Growth Test — TRE Environmental Strategies

Analysis ID:	01-6014-3215	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Jan-20 9:08	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Batch ID:	18-0895-7427	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Lab Tech
Start Date:	19 Dec-19 15:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	rGSL
Ending Date:	26 Dec-19 14:55	**Species:**	Artemia franciscana	**Brine:**	Crystal Sea
Duration:	7d	**Source:**	In-House Culture	**Age:**	48h

Sample ID:	03-3624-0124	**Code:**	140A9DFC	**Client:**	Univerity of Notre Dame
Sample Date:	19 Dec-19 15:00	**Material:**	Arsenic	**Project:**	Special Studies
Receive Date:	19 Dec-19 15:00	**Source:**	research		
Sample Age:	NA	**Station:**			

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	268245	200	Yes	Two-Point Interpolation

Point Estimates

Level	mg/L	95% LCL	95% UCL
IC5	22.09	N/A	22.58
IC10	24.17	18.27	25.16
IC15	26.26	20.8	27.75
IC20	28.35	23.34	30.33
IC25	30.43	25.72	32.91
IC40	36.69	32	40.78
IC50	40.87	35.87	46.11

Mean Dry Biomass-mg Summary

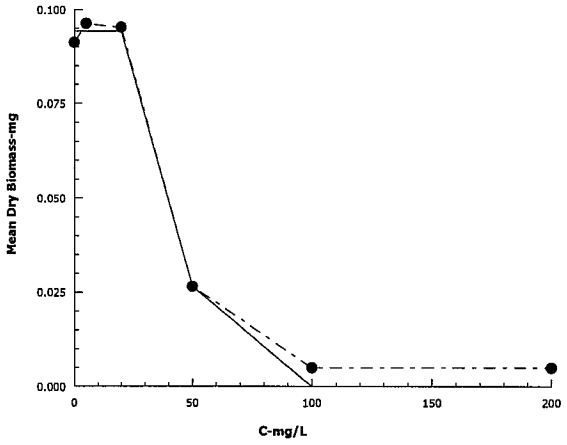
			Calculated Variate						
C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	0.09125	0.076	0.111	0.007663	0.01533	16.8%	0.0%
5		4	0.09625	0.091	0.103	0.002562	0.005123	5.32%	-5.48%
20		4	0.09525	0.088	0.109	0.00475	0.0095	9.97%	-4.38%
50		4	0.0265	0.013	0.039	0.005852	0.0117	44.2%	71.0%
100		4	0	0	0	0	0		100.0%
200		4	0	0	0	0	0		100.0%

Mean Dry Biomass-mg Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.076	0.083	0.095	0.111
5		0.091	0.094	0.097	0.103
20		0.094	0.09	0.109	0.088
50		0.021	0.013	0.039	0.033
100		0	0	0	0
200		0	0	0	0

Analysis ID:	01-6014-3215	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Jan-20 9:08	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



1/8/20

QA rev 1/11/20

TOXICITY DATA PACKAGE COVER SHEET

Test Type: Chronic (10d)

Project Number: 17001-474-Exp

Test Substance: Arsenic (Na2HAsO4)

Species: *Artemia franciscana*

Dilution Water: rGSL

Organism Lot or Batch Number: 121719

Concurrent Control Water: NA

Age: 48 hr (48 hr)

Supplier: TRE

Date and Time Test Began: 12/19/19 @ 1516

Date and Time Test Ended: 12/29/19 @ 1415

Protocol Number: —

Investigator(s): AB/BJ/HR/EN/CP

Background Information

Type of Test: Static-Renewal (Daily)

pH control?: Yes **No**

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: 10 days

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, 5, 20, 50, 100, and 200 mg/L as As

Agency Summary Sheet(s)?: None

Reference Toxicant Data: Test Dates: ~ to ~ 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 100 ug/L Chla

Appropriate correction factors have been applied to all temperatures recorded in this data package

Study Director Initials: AB Date: 12/19/19

Reviewed 1/11/20

TEST SUBSTANCE USAGE LOG

Project Number: 17001-474-Exp

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number				
Test Substance Collection Date and Time	From: @ To: @	From: @ To: @	From: @ To: @	From: @ To: @
Sample Type (Grab or Comp)				
Date Test Substance Received				
Dilution Water Number RW# or TRE#, circle one	13804/13818*	13818		
Concurrent Control Water RW#	NA	NA		
Date(s) Used	12/19/19	12/23/19	12/27/19	
	12/20/19	12/24/19	12/28/19	
	12/21/19	12/25/19*		
	12/22/19	12/26/19*		

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	250	0	250						
5	0.33	249.67	250						
20	1.34	248.66	250	See Spiking Sheet					
50	3.34	246.66	250						
100	6.68.55 ①	243.32	250						
200	13.37	236.63	250						
	275.07	1224.93	1500						

Initials / Date	AS 12/19/19	EN 12/27/19	
Initials / Date	AS 12/20/19	CP 12/28/19	
Initials / Date	CP 12/21/19		
Initials / Date	RG 12/22/19		
Initials / Date	RG 12/23/19		
Initials / Date	EN 12/24/19		
Initials / Date	EN 12/25/19		
Initials / Date	EN 12/26/19		

① EN 12/24/19 G

Artemia franciscana
CHRONIC BIOLOGICAL DATA

QA MW 1/8/20

Project Number: 17001-474-Exp

7d

mg/L	Test Replicate	Number of Surviving Organisms								Remarks
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
0	A	10	10	10	10	10	10	10	10	100
	B	10	10	10	10 *	10	10 *	10	10	* Weak org
	C	10	10	10	10	10	10 *	10	10	* Weak org
	D	10	10	10	10	10	10	10	10	
5	A	10	10	10	10	10	10	10	10	100
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
20	A	10	10	10	10	10	10	9	9	97.5
	B	10	10	10	10	10	10 *	10 *	10 *	* Weak org
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
50	A	10	4	4	4	4	4	4	4	47.5
	B	10	5	5	5	5	5	5	5	
	C	10	5	5	5	5	5	5	5	
	D	10	5	5	5	5	5	5	5	
100	A	10	3	2	1	1	1	1	1	7.5
	B	10	1	0	—	—	—	—	—	
	C	10	0	0	—	—	—	—	—	
	D	10	2	2	2	2	2	2	2	
200	A	10	0				—	—	—	0%
	B	10	0				—	—	—	
	C	10	0				—	—	—	
	D	10	0				—	—	—	
	A									
	B									
	C									
	D									
Date:		12/19/19	12/20/19	12/21/19	12/22/19	12/23/19	12/24/19	12/25/19	12/26/19	
Time:		1510	1620	1550	1350	1455	1455	1105	1255	
Initials:		BM	AS	HR	BM	AS	EN	EN	EN	

nw 1/8/20

Artemia franciscana
CHRONIC BIOLOGICAL DATA

10d survival

Project Number: 17001-474-Exp

mg/L	Test Replicate	Number of Surviving Organisms Day 8	Day 9	Day 10						Remarks
0	A	10	10	9						92.5 %
	B	10	10	10						
	C	10	10	10						
	D	10	10	10						
5	A	10	10	10						100
	B	10	10	10						
	C	10	10	10						
	D	10	10	10						
20	A	9	9	8						95 %
	B	10*	10*	10*						*1 weak org (tiny)
	C	10	10	10						
	D	10	10	10						
50	A	4	4*	4*						*1 weak org 45 %
	B	5	5	5						
	C	5	5*	5						*1 weak org
	D	5	5*	4						*1 weak org
100	A	1	1	1						7.5
	B	—	—	—						
	C	—	—	—						
	D	2	2	2						
200	A	—								0 %
	B	—								
	C	—								
	D	—								
	A									
	B									
	C									
	D									
Date:		12/27/19	12/28/19	12/29/19						
Time:		1315	1440	1415						
Initials:		EN	CP	CP						

QA nw 1/11/20

CHRONIC CHEMICAL DATA (INITIAL)

Project Number: 17001-474-Exp

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	8.0	8.0	8.0	7.9	8.0	7.9	7.9	7.9	FM28	
D.O. (mg/L)	5.4	5.2	7.01①	5.2	5.2	5.3	5.5	5.7	17	
Temp. (°C)	20	20	20	20	20	20	20	20	IR1	
Cond. (µS/cm)	138700	139300	136800	136300	Δ	139000	140200	141700	15	
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Conc.: 5										
pH	7.8	7.9	8.0	7.9	8.0	7.9	7.9	7.9		
D.O. (mg/L)	5.3	5.1	6.9	5.2	5.3	5.1	5.4	5.7		
Temp. (°C)	*	*	*	*	*	*	*	*		
Cond. (µS/cm)	140400	137100	136300	136700	Δ	138400	139700	140000		
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Conc.: 20										
pH	7.8	7.9	8.0	7.9	7.9	7.9	7.9	7.9		
D.O. (mg/L)	5.3	5.2	6.7	5.2	5.4	5.1	5.4	5.7		
Temp. (°C)	*	*	*	*	*	*	*	*		
Cond. (µS/cm)	139600	137100	137100	137600	Δ	138100	137200	139500		
Conc.: 50										
pH	7.9	8.0	8.0	7.9	8.0	7.9	7.9	7.9		
D.O. (mg/L)	5.2	5.1	6.6	5.3	5.4	5.1	5.2	5.6		
Temp. (°C)	*	*	*	*	*	*	*	*		
Cond. (µS/cm)	138900	137500	137800	137700	Δ	137700	135400	139400		
Date:	12/19/19	12/20/19	12/21/19	12/22/19	12/23/19	12/24/19	12/25/19	12/26/19		
Time:	1505	1555	1555	1335	1440	1450	1100	1250		
Initials:	AB	AB	HR	BP	AB	EN	EN	EN		

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

*Dilution/control water and effluent were brought to 25C prior to making the dilution series. The temperature of resulting effluent dilution is assumed to also be 25C.

①HR 12/21/19; E

Δ meter error

QA NPN 1/11/20

CHRONIC CHEMICAL DATA (INITIAL)

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

%	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter #	Remarks
Conc.: 100									All Conc.	
pH	7.9	8.0	8.0	7.9	8.0	7.9	7.9	7.9		
D.O. (mg/L)	5.2	5.1	6.6	5.4	5.6	5.2	5.3	5.8		
Temp. (°C)	*	*	*	*	*	*	*	*		
Cond. (µS/cm)	137200	1367	137900	137500	Δ	137300	133900	139100		
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.: 200										
pH	7.9									
D.O. (mg/L)	5.2									
Temp. (°C)	① ~~26.2~~ *	~~*~~ ②								
Cond. (µS/cm)	137300									
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Date:	12/19/19	12/20/19	12/21/19	12/22/19	12/23/19	12/24/19	12/25/19	12/26/19		
Time:	1505	1555	1555	1335	1440	1450	1100	1250		
Initials:	AB	AB	HR	PB	AB	EN	EN	EN		

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

*Dilution/control water and effluent were brought to 25C prior to making the dilution series. The temperature of resulting effluent dilution is assumed to also be 25C.

① AB 12/19/19 G
② AB 12/20/19 E

DA new 1/14/20

CHRONIC CHEMICAL DATA (INITIAL)

Project Number: 17001-474-Exp

Test Species: ***Artemia franciscana***

%	Day ① 8	Day ① 9	Day ① 10	Day 3	Day 4	Day 5	Day 6	Day 7	Meter #	Remarks
Conc.: 0									All Conc.	
pH	7.9	8.0							FM28	
D.O. (mg/L) 5.3	~~7.8~~ ①	6.0							17	
Temp. (°C)	20	20							L-6	
Cond. (µS/cm)	134000	140000							15	
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Conc.: 5										
pH	7.9	8.0								
D.O. (mg/L) 5.3	~~134200~~ ①	5.9								
Temp. (°C)	*	*								
Cond. (µS/cm)	134800	135500								
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Conc.: 20										
pH	7.9	8.0								
D.O. (mg/L)	5.3	5.9								
Temp. (°C)	*	*								
Cond. (µS/cm)	134200	135200								
Conc.: 50										
pH	7.9	8.0								
D.O. (mg/L)	5.3	5.9								
Temp. (°C)	*	*								
Cond. (µS/cm)	134100	135200								
Date:	12/27/19	12/28/19								
Time:	1310	1420								
Initials:	EN	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.

①EN 12/27/19

QA Form No. 058
Revision 4
Effective 02/14
[illegible] 1/11/20

CHRONIC CHEMICAL DATA (INITIAL)

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

%	Day 8	Day 9							Meter #	Remarks
Conc.: 100									All Conc.	
pH	7.9	8.0								
D.O. (mg/L)	5.3	6.0								
Temp. (°C)	*	*								
Cond. (µS/cm)	133900	133800								
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.: 200										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Date:		12/28/19								
Time:		1420								
Initials:		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.

EN 1/14/20

CHRONIC CHEMICAL DATA (FINAL)

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

%		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter #	Remarks
Conc.:	0									All Conc.	* conductivity
pH		8.0	8.0	8.0	8.0	8.0	8.0	8.0	7.9	FM28	
D.O. (mg/L)		5.1	5.8	5.8	4.8	5.0	5.3	5.1	4.7	17	
Temp (°C)		20	21	21	20	20	~~18~~ 19	20	20	L6	
Conc.:	5										
pH		8.0	8.0	8.0	8.0	8.0	8.0	8.0	7.9		
D.O. (mg/L)		5.0	5.5	6.0	4.8	5.2	5.5	5.0	4.6		
Temp (°C)		20	22Δ	21	20	20	20	20	20		
Conc.:	20										
pH		8.0	8.0	8.0	8.0	8.0	8.0	8.0	7.9		
D.O. (mg/L)		4.9	5.7	5.8	4.8	5.3	5.6	5.0	4.6		
Temp (°C)		20	21	21	20	20	20	20	20		
Conc.:	50										
pH		8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0		
D.O. (mg/L)		5.0	6.1	6.1	4.8	5.4	5.6	5.1	4.8		
Temp (°C)		20	22Δ	21	20	20	20	20	20		
Conc.:	100										
pH		8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0		
D.O. (mg/L)		5.0	6.3	6.4	4.8	5.6	5.6	5.3	4.9		
Temp (°C)		20	22Δ	21	20	20	20	20	20		
Conc.:	200	14150									* conductivity
pH		8.0									
D.O. (mg/L)		5.0									
Temp (°C)		20									
Conc.:											
pH											
D.O. (mg/L)											
Temp (°C)											
Date:		12/20/19	12/21/19	12/22/19	12/23/19	12/24/19	12/25/19	12/26/19	12/27/19		
Time:		1625	1520	1410	1455	1510	1100	1255	1335		
Initials:		AB	HR	RF	AB	EN	EN	EN	EN		

① EN 12/25/19

Δ All reps checked

DA NJO 1/14/20

CHRONIC CHEMICAL DATA (FINAL)

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

%		Day 9	Day 10							Meter #	Remarks
Conc.:	0		133500							All Conc.	* conductivity (15)
pH		7.9	7.9							FM28	
D.O. (mg/L)		4.8	4.8							17	
Temp (°C)		19	18							L-6	
Conc.:	5										
pH		7.9	7.9								
D.O. (mg/L)		4.7	4.7								
Temp (°C)		19	18								
Conc.:	20										
pH		7.9	7.9								
D.O. (mg/L)		4.7	4.8								
Temp (°C)		19	18								
Conc.:	50										
pH		8.1	8.1								
D.O. (mg/L)		5.3	6.2								
Temp (°C)		19	18								
Conc.:	100		129000								
pH		8.1	8.2								
D.O. (mg/L)		5.3	6.9								
Temp (°C)		19	18								
Conc.:	200										* conductivity
pH											
D.O. (mg/L)											
Temp (°C)											
Conc.:											
pH											
D.O. (mg/L)											
Temp (°C)											
Date:		12/28/19	12/29/19								
Time:		1420	1535								
Initials:		CP	CP								

AM MW 1/4/20

DAILY TOXICITY TEST LOG

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

General Comments			Feeding 145 ug/l Chla upon renewal	Initials/Date
	Random Chart: P1	Min/Max Thermometer # M-15		
Test Day 0	Test Solution Mixed at: 1125 Test Organisms Added at: 1510 Spiked @ 1125		Fed @ 1125	AM 12/19/19
Test Day 1	Real Time: 20 °C	Min-Max Range: 19-21 °C Spiked @ 1115	Fed @ 1115	AB 12/20/19
Test Day 2	Real Time: 19 °C	Min-Max Range: 19-21 °C Spiked @ 1100	Fed @ 1100	HR 12/21/19
Test Day 3	Real Time: 19 °C	Min-Max Range: 16-20 °C Spiked @ 1000	Fed @ 1000	RG 12/22/19
Test Day 4	Real Time: 19 °C	Min-Max Range: 18-20 °C Spiked @ 1635	Fed @ 1635	AB 12/23/19
Test Day 5	Real Time: 19 °C	Min-Max Range: 18-20 °C Spiked @ 1120	Fed @ 1120	EN 12/24/19
Test Day 6	Real Time: 19 °C	Min-Max Range: 19-20 °C Spiked @ 0755	Fed @ 0755	EN 12/25/19
Test Day 7	Real Time: 19 °C	Min-Max Range: 19-20 °C Spiked @ 0935	Fed @ 0935	EN 12/26/19
Test Day 8	Real Time: 19 °C	Min-Max Range: 18-20 °C Spiked @ 0925	Fed @ 0925	EN 12/29/19

[illegible] 1/11/20

DAILY TOXICITY TEST LOG

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

General Comments	Random Chart:___ Min/Max Thermometer #	Feeding 145 ug/l Chla upon renewal	Initials/Date
Test Day 9	Real Time: 19 °C Min-Max Range: 19 - 20 °C Spiked @ 1100	Fed @ 1100	cf 12/28/19
Test Day 10	Real Time: 18 °C Min-Max Range: 17 - 19 °C	None	cf 12/29/19

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 14001 - 474 - exp 10day | Test Substance: Arsenic | Comments: Analytical Balance ID: Sart #1
Species: Artemia franciscana | Analyst Tare: CP | Analyst Gross: ~~EN~~ ① MB/NAW | Dried in Oven # 3 from Date: 12/29/19 Time: 1535 to Date: 12/31/19 Time: 1015
Date/Time of Tare Wt.: 12/29/19 @ 1355 | Date/Time of Gross Wt.: ~~12/31/19 @ 1055~~ ① 1/2/20 @ 0950

Weight Type (Circle): Wet Blot Dry (Dry (>100°C)) AFDW (>500°C) | Lot or (Batch) Number: 12/17/19

Boat No.	Treatment	Rep.	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)[1]	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	No. of Surv. Organisms	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)
	0	A		1.12584	1.12801	0.00217					9		
	0	B		1.15262	1.15534	0.00272					10		
	0	C		1.15189	1.15432	0.00243					10		
	0	D		1.17387	1.17658	0.00271					10		
	5	A		1.11710	1.11985	0.00275					10		
	5	B		1.12638	1.12898	0.00260					10		
	5	C		1.13438	1.13734	0.00296					10		
	5	D		1.17574	1.17839	0.00265					10		
	20	A		1.13768	1.13991	0.00223					8		
	20	B		1.16951	1.17147	0.00196					10		
	20	C		1.16624	1.16897	0.00273					10		
	20	D		1.14325	1.14576	0.00251					10		
Blank				1.17270	1.17278	+0.00008							
Range													
Mean													

Test Solution Volume: | Loading Rate:

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

① EN 1/2/20 C

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 17001-474-CP Wday	Test Substance: Arsenic	Comments:
Species: Artemia Franciscana	Analyst Tare: CP Analyst Gross: EN MB/	Analytical Balance ID: Sart #1
Date/Time of Tare Wt.: 12/29/19 @ 1355	Date/Time of Gross Wt.: 12/31/19 @ 1055 ② 1/2/20 @ 0950	Dried in Oven # 3 from Date: 12/29/19 Time: 1535 to Date: 12/31/19 Time: 1015

Weight Type (Circle): Wet Blot Dry Dry (>100°C) AFDW (>500°C) Lot or Batch Number: 121719

Boat No.	Treatment	Rep.	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)[1]	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	No. of Surv. Organisms	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)
	50	A		1.16283	1.16338	0.00055					4		
	50	B		1.16922	1.16963	0.00041					5		
	50	C		1.12550	1.12588	0.00038					5		
	50	D		1.17218	1.17280	0.00062					4		
	100	A		1.16443	1.16444	0.00001					1		
	100	D		1.13757	1.13762	0.00005					2		
Blank													
Range													
Mean													

Test Solution Volume: Loading Rate:

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

DEN 1/2/20 ©

AG 1/6/20
QA rev 1/8/20

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 14001-474 10 day Arsenic Species: Artemia franciscana

Treatment	Rep	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)	No of Orig. Organisms	Mean Wt./ Original Organism (mg)	Mean Wt./ Treatment (mg) (Original)	Number of Surv. Organisms	Mean Wt./ Surviving Organism (mg)	Mean Wt./ Treatment (mg) (Surviving)
rGSL	A		1.12584	1.12801	0.00217	0.00217	10	0.217	0.2508	9	0.241	0.2568
	B		1.15262	1.15534	0.00272	0.00272	10	0.272		10	0.272	
	C		1.15189	1.15432	0.00243	0.00243	10	0.243		10	0.243	
	D		1.17387	1.17658	0.00271	0.00271	10	0.271		10	0.271	
5 mg/L	A		1.11710	1.11985	0.00275	0.00275	10	0.275	0.2740	10	0.275	0.2740
	B		1.12638	1.12898	0.00260	0.00260	10	0.260		10	0.260	
	C		1.13438	1.13734	0.00296	0.00296	10	0.296		10	0.296	
	D		1.17574	1.17839	0.00265	0.00265	10	0.265		10	0.265	
20 mg/L	A		1.13768	1.13991	0.00223	0.00223	10	0.223	0.2357	8	0.279	0.2497
	B		1.16951	1.17147	0.00196	0.00196	10	0.196		10	0.196	
	C		1.16624	1.16897	0.00273	0.00273	10	0.273		10	0.273	
	D		1.14325	1.14576	0.00251	0.00251	10	0.251		10	0.251	
50 mg/L	A		1.16283	1.16338	0.00055	0.00055	10	0.055	0.0490	4	0.138	0.1126
	B		1.16922	1.16963	0.00041	0.00041	10	0.041		5	0.082	
	C		1.12550	1.12588	0.00038	0.00038	10	0.038		5	0.076	
	D		1.17218	1.17280	0.00062	0.00062	10	0.062		4	0.155	
100 mg/L	A		1.16443	1.16444	0.00001	0.00001	10	0.001	0.0015	1	0.010	0.0175
	B (D)		1.13757	1.13762	0.00005	0.00005	10	0.005		2	0.025	
	C		0.00000	0.00000	0.00000	0.00000	10	0.000		0	-	
	D		0.00000	0.00000	0.00000	0.00000	10	0.000		0	-	
200 mg/L	A		0.00000	0.00000	0.00000	0.00000	10	0.000	0.0000	0	-	#DIV/0!
	B		0.00000	0.00000	0.00000	0.00000	10	0.000		0	-	
	C		0.00000	0.00000	0.00000	0.00000	10	0.000		0	-	
	D		0.00000	0.00000	0.00000	0.00000	10	0.000		0	-	
Blank			1.17270	1.17278	0.00008							

(D) AG 1/8/20 CP

AB 1/6/20
QA 1/8/20

Project Number: 14001-474 10 day Arsenic Species: Artemia franciscana

Summary Statistics for Survival Data

Treatment	N	Min	Max	Mean	SD	C.V.
rGSL	4	0.9	1.0	0.9750	0.0500	5.128%
5 mg/L	4	1.0	1.0	1.0000	0.0000	0.000%
20 mg/L	4	0.8	1.0	0.9500	0.1000	10.526%
50 mg/L	4	0.4	0.5	0.4500	0.0577	12.830%
100 mg/L	4	0.0	0.2	0.0750	0.0957	127.657%
200 mg/L	4	0.0	0.0	0.0000	0.0000	#DIV/0!

Summary Statistics for Growth Data (dry wt per original)

Treatment	N	Min	Max	Mean	SD	C.V.
rGSL	4	0.217	0.272	0.2508	0.0262	10.452%
5 mg/L	4	0.260	0.296	0.2740	0.0159	5.817%
20 mg/L	4	0.196	0.273	0.2357	0.0335	14.202%
50 mg/L	4	0.038	0.062	0.0490	0.0114	23.269%
100 mg/L	4	0.000	0.005	0.0015	0.0024	158.698%
200 mg/L	4	0.000	0.000	0.0000	0.0000	#DIV/0!

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

Treatment	N	Min	Max	Mean	SD	C.V.
rGSL	4	0.241	0.272	0.2568	0.0170	6.629%
5 mg/L	4	0.260	0.296	0.2740	0.0159	5.817%
20 mg/L	4	0.196	0.279	0.2497	0.0377	15.114%
50 mg/L	4	0.076	0.155	0.1126	0.0396	35.121%
100 mg/L	2	0.010	0.025	0.0175	0.0106	60.609%
200 mg/L	0	0.000	0.000	#DIV/0!	#DIV/0!	#DIV/0!

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

TRE Environmental Strategies

Analysis ID:	13-4473-4001	**Endpoint:**	10d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Jan-20 9:16	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	02-7744-5107	**Test Type:**	Growth-Survival (10d)	**Analyst:**	Lab Tech
Start Date:	19 Dec-19 15:10	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	rGSL
Ending Date:	29 Dec-19 14:15	**Species:**	Artemia franciscana	**Brine:**	Crystal Sea
Duration:	9d 23h	**Source:**	In-House Culture	**Age:**	48h
Sample ID:	01-9148-7305	**Code:**	B69DD49	**Client:**	Univerity of Notre Dame
Sample Date:	19 Dec-19 15:10	**Material:**	Arsenic	**Project:**	Special Studies
Receive Date:	19 Dec-19 15:10	**Source:**	research		
Sample Age:	NA	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	8.15%	20	50	31.62	

Steel Many-One Rank Sum Test

Control	vs	C-mg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		5	18	10	1	6	0.8000	Asymp	Non-Significant Effect
		20	16	10	1	6	0.5661	Asymp	Non-Significant Effect
		50*	10	10	0	6	0.0350	Asymp	Significant Effect
		98*	10	10	0	6	0.0350	Asymp	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	4.194818	1.048705	4	170.4	<0.0001	Significant Effect
Error	0.09230711	0.006153808	15			
Total	4.287125		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	3.267	4.89	0.0409	Equal Variances
Variances	Levene Equality of Variance	8.429	4.89	0.0009	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8394	0.866	0.0036	Non-normal Distribution

10d Survival Rate Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1	1	1	1	1	1	0	0.0%	0.0%
5		4	1	1	1	1	1	1	0	0.0%	0.0%
20		4	0.975	0.8954	1	1	0.9	1	0.025	5.13%	2.5%
50		4	0.475	0.3954	0.5546	0.5	0.4	0.5	0.025	10.5%	52.5%
98		4	0.075	0	0.2273	0.05	0	0.2	0.04787	128.0%	92.5%
200		4	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
5		4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
20		4	1.371	1.242	1.501	1.412	1.249	1.412	0.04074	5.94%	2.89%
50		4	0.7602	0.6801	0.8403	0.7854	0.6847	0.7854	0.02517	6.62%	46.2%
98		4	0.2757	0.0419	0.5096	0.2403	0.1588	0.4636	0.07348	53.3%	80.5%
200		4	0.1588	0.1588	0.1588	0.1588	0.1588	0.1588	0	0.0%	88.8%

1/8/20

1/8/20

~~Fathead Minnow~~ 7-d Larval Survival and Growth Test — TRE Environmental Strategies

Analysis ID:	13-4473-4001	**Endpoint:**	10d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Jan-20 9:16	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

10d 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	1
20		0.9	1	1	1
50		0.4	0.5	0.5	0.5
98		0.1	0	0	0.2
200		0	0	0	0

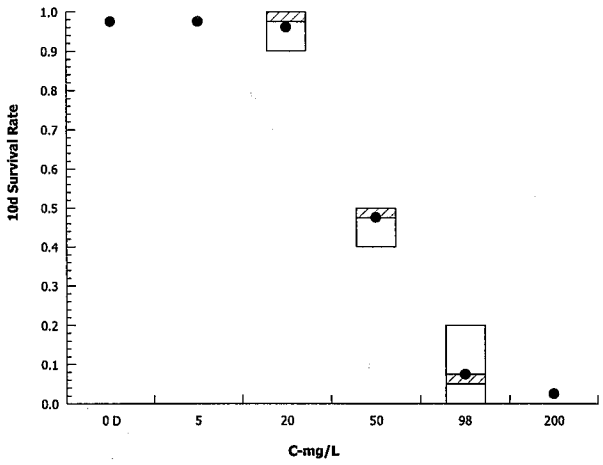
Angular (Corrected) Transformed Detail

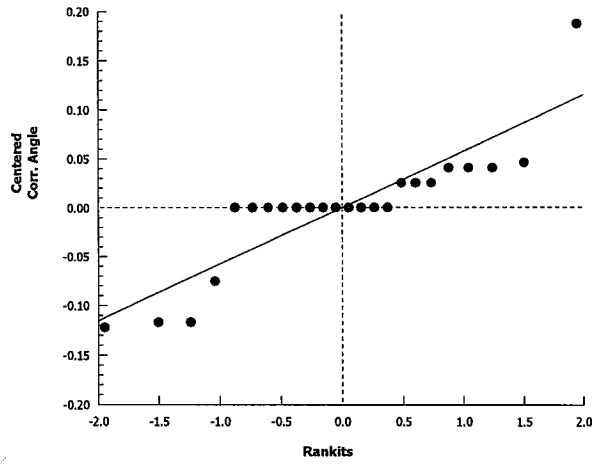
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.412	1.412	1.412	1.412
5		1.412	1.412	1.412	1.412
20		1.249	1.412	1.412	1.412
50		0.6847	0.7854	0.7854	0.7854
98		0.3218	0.1588	0.1588	0.4636
200		0.1588	0.1588	0.1588	0.1588

10d 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	10/10
20		9/10	10/10	10/10	10/10
50		4/10	5/10	5/10	5/10
98		1/10	0/10	0/10	2/10
200		0/10	0/10	0/10	0/10

Graphics





4/8/20

CETIS Analytical Report

artemia 10d

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

TRE Environmental Strategies

Analysis ID:	01-6294-2172	**Endpoint:**	10d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	08 Jan-20 11:07	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	02-7744-5107	**Test Type:**	Growth-Survival (10d)	**Analyst:**	Lab Tech
Start Date:	19 Dec-19 15:10	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	rGSL
Ending Date:	29 Dec-19 14:15	**Species:**	Artemia franciscana	**Brine:**	Crystal Sea
Duration:	9d 23h	**Source:**	In-House Culture	**Age:**	48h
Sample ID:	01-9148-7305	**Code:**	B69DD49	**Client:**	Univerity of Notre Dame
Sample Date:	19 Dec-19 15:10	**Material:**	Arsenic	**Project:**	Special Studies
Receive Date:	19 Dec-19 15:10	**Source:**	research		
Sample Age:	NA	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	8.25%	20	50	31.62	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		5	0	2.36	0.133	6	0.8000	CDF	Non-Significant Effect
		20	0.7246	2.36	0.133	6	0.5038	CDF	Non-Significant Effect
		50*	12.04	2.36	0.133	6	<0.0001	CDF	Significant Effect
		98*	20.21	2.36	0.133	6	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	4.254439	1.06361	4	168.2	<0.0001	Significant Effect
Error	0.09484117	0.006322745	15			
Total	4.34928		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	3.948	4.89	0.0220	Equal Variances
Variances	Levene Equality of Variance	9.484	4.89	0.0005	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8771	0.866	0.0157	Normal Distribution

10d Survival Rate Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1	1	1	1	1	1	0	0.0%	0.0%
5		4	1	1	1	1	1	1	0	0.0%	0.0%
20		4	0.975	0.8954	1	1	0.9	1	0.025	5.13%	2.5%
50		4	0.45	0.3581	0.5419	0.45	0.4	0.5	0.02887	12.8%	55.0%
98		4	0.075	0	0.2273	0.05	0	0.2	0.04787	128.0%	92.5%
200		4	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
5		4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
20		4	1.371	1.242	1.501	1.412	1.249	1.412	0.04074	5.94%	2.89%
50		4	0.7351	0.6426	0.8276	0.7351	0.6847	0.7854	0.02906	7.91%	47.9%
98		4	0.2757	0.0419	0.5096	0.2403	0.1588	0.4636	0.07348	53.3%	80.5%
200		4	0.1588	0.1588	0.1588	0.1588	0.1588	0.1588	0	0.0%	88.8%

1/11/20

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

TRE Environmental Strategies

Analysis ID:	01-6294-2172	**Endpoint:**	10d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	08 Jan-20 11:07	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

10d 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	1
20		0.9	1	1	1
50		0.4	0.5	0.5	0.4
98		0.1	0	0	0.2
200		0	0	0	0

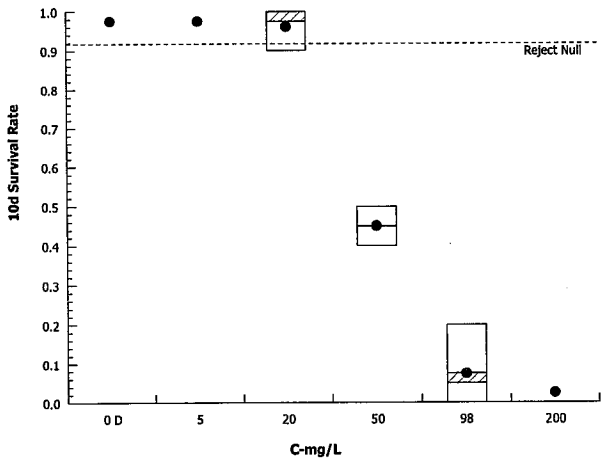
Angular (Corrected) Transformed Detail

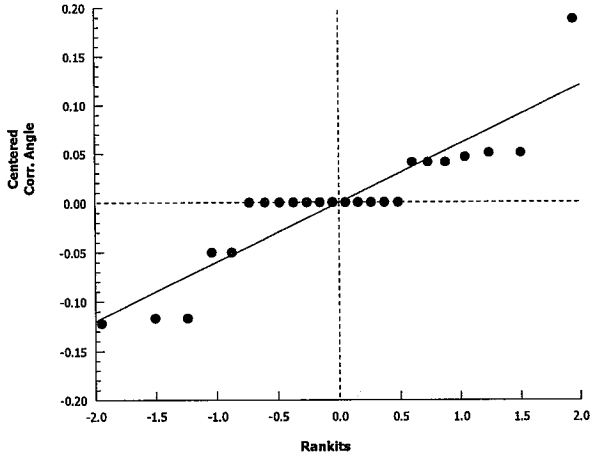
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.412	1.412	1.412	1.412
5		1.412	1.412	1.412	1.412
20		1.249	1.412	1.412	1.412
50		0.6847	0.7854	0.7854	0.6847
98		0.3218	0.1588	0.1588	0.4636
200		0.1588	0.1588	0.1588	0.1588

10d 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	10/10
20		9/10	10/10	10/10	10/10
50		4/10	5/10	5/10	4/10
98		1/10	0/10	0/10	2/10
200		0/10	0/10	0/10	0/10

Graphics





CETIS Analytical Report

Artemia 10d

Report Date: 06 Jan-20 09:16 (p 1 of 2)
Test Code: 474-10d | 03-1077-5012

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

TRE Environmental Strategies

Analysis ID:	07-2114-9947	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Jan-20 9:16	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	02-7744-5107	**Test Type:**	Growth-Survival (10d)	**Analyst:**	Lab Tech
Start Date:	19 Dec-19 15:10	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	rGSL
Ending Date:	29 Dec-19 14:15	**Species:**	Artemia franciscana	**Brine:**	Crystal Sea
Duration:	9d 23h	**Source:**	In-House Culture	**Age:**	48h
Sample ID:	01-9148-7305	**Code:**	B69DD49	**Client:**	Univerity of Notre Dame
Sample Date:	19 Dec-19 15:10	**Material:**	Arsenic	**Project:**	Special Studies
Receive Date:	19 Dec-19 15:10	**Source:**	research		
Sample Age:	NA	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	16.1%	20	>20	NA	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		5	-1.254	2.18	0.040	6	0.9549	CDF	Non-Significant Effect
		20	0.8092	2.18	0.040	6	0.3375	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0029715	0.00148575	2	2.162	0.1711	Non-Significant Effect
Error	0.006185499	0.0006872776	9			
Total	0.009156999		11			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.324	9.21	0.5158	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9513	0.802	0.6565	Normal Distribution

Mean Dry Biomass-mg Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	0.2508	0.209	0.2925	0.257	0.217	0.272	0.0131	10.5%	0.0%
5		4	0.274	0.2486	0.2994	0.27	0.26	0.296	0.007969	5.82%	-9.27%
20		4	0.2358	0.1825	0.289	0.237	0.196	0.273	0.01674	14.2%	5.98%

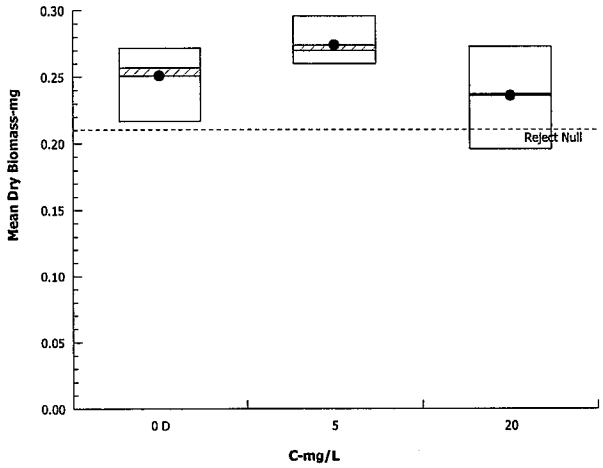
Mean Dry Biomass-mg Detail

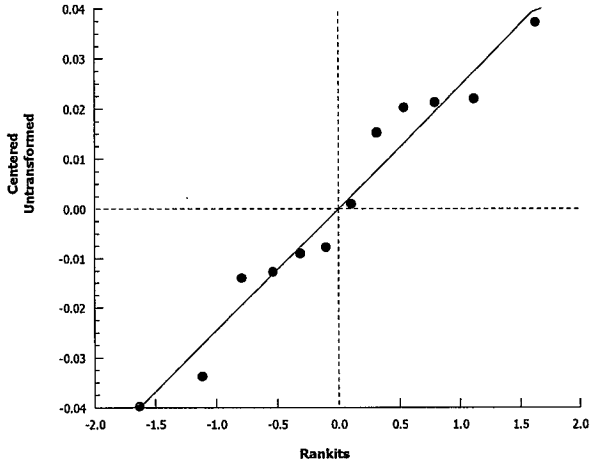
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.217	0.272	0.243	0.271
5		0.275	0.26	0.296	0.265
20		0.223	0.196	0.273	0.251

1/8/20

Analysis ID:	07-2114-9947	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Jan-20 9:16	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

TRE Environmental Strategies

Analysis ID:	20-4719-6220	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Jan-20 9:16	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	02-7744-5107	**Test Type:**	Growth-Survival (10d)	**Analyst:**	Lab Tech
Start Date:	19 Dec-19 15:10	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	rGSL
Ending Date:	29 Dec-19 14:15	**Species:**	Artemia franciscana	**Brine:**	Crystal Sea
Duration:	9d 23h	**Source:**	In-House Culture	**Age:**	48h
Sample ID:	01-9148-7305	**Code:**	B69DD49	**Client:**	Univerity of Notre Dame
Sample Date:	19 Dec-19 15:10	**Material:**	Arsenic	**Project:**	Special Studies
Receive Date:	19 Dec-19 15:10	**Source:**	research		
Sample Age:	NA	**Station:**			

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	143681	200	Yes	Two-Point Interpolation

Point Estimates

Level	mg/L	95% LCL	95% UCL
IC5	12.39	6.105	27.35
IC10	19.78	7.275	25.93
IC15	22.05	11.49	27.61
IC20	24.15	16.05	29.39
IC25	26.26	19.22	31.09
IC40	32.58	27.41	36.47
IC50	36.8	32.68	40.12

may want to rpt both IC20 & IC25

Mean Dry Biomass-mg Summary

			Calculated Variate						
C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	0.2508	0.217	0.272	0.0131	0.02621	10.5%	0.0%
5		4	0.274	0.26	0.296	0.007969	0.01594	5.82%	-9.27%
20		4	0.2358	0.196	0.273	0.01674	0.03348	14.2%	5.98%
50		4	0.049	0.038	0.062	0.005701	0.0114	23.3%	80.5%
98		4	0.0015	0	0.005	0.00119	0.00238	159.0%	99.4%
200		4	0	0	0	0	0		100.0%

Mean Dry Biomass-mg Detail

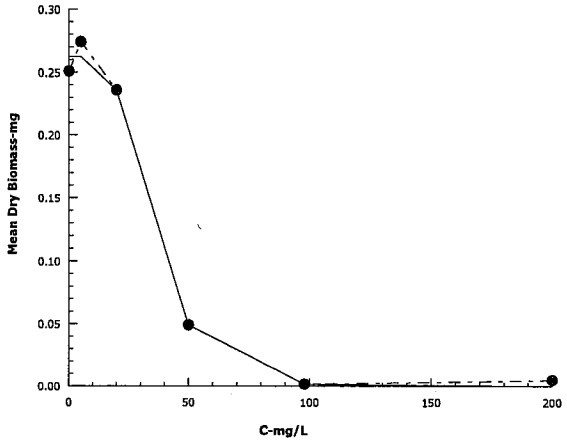
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.217	0.272	0.243	0.271
5		0.275	0.26	0.296	0.265
20		0.223	0.196	0.273	0.251
50		0.055	0.041	0.038	0.062
98		0.001	0	0	0.005
200		0	0	0	0

1/8/20

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test **TRE Environmental Strategies**

Analysis ID:	20-4719-6220	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Jan-20 9:16	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



Analyst: AS QA: NW 1/8/20

Calculation of Volume of *D. viridis* Culture Media Required for Feeding

Method Used to Determine Chl a Concn. (ug/L) in Culture	Enter "Y" or "N"
450 nm (2.5 cm Cuv.)?	n
450 nm (1.0 cm Cuv.)?	y
Other (enter value):	2000

Enter Chlorophyll. Estimation Source/Concn.

Chl a Concentration in Culture (ug/L):	2011.898
Required Chl a Food Concentration (ug/L):	145
Volume of Test Water/Chamber (ml)	50
Replicates:	5
Treatments:	6

Enter Test Data Here

Culture Concentration (ug/ml):	2.011898	Intermediate Calculations
Food Concentration (ug/ml):	0.145	
Total Chl a needed/Chamber (ug):	7.25	
Culture Water needed/Chamber (ml):	3.603562	Final Values
Culture Water needed/Treatment (ml):	18.01781	
Culture Water needed/Test (ml):	108.1069	
Test Vol./Chamber before Food Addition (ml):	46.39644	
Total Vol./Treatment before Food Addition (ml):	231.9822	

NOTE: Change values only in cells outlined in GREEN; do not change cells outlined in RED; these are equations

St
dilution

Brine Shrimp ~~Acute~~ Studies

Nov 2017

Primary stock @ 3,740.00 mg/L As = 15.5755 g Na2HAsO4 * 7H2O / L H2O

Volume per treatment (L) 0.25

Trtmnt	Conc. ug/L	Stock **(ml)**	Total Vol(L)
6	200,000	13.37	0.25
5	100,000	6.68	0.25
4	50,000	3.34	0.25
3	20,000	1.34	0.25
2	5,000	0.33	0.25
1	0	0.00	0.25
Total		25.07	1.50

_____ dilution series

5.3% % spike of vol

TOTAL VOL
3.0 L
0.86 gallons

Volume needed:

n = 4
vol 50 ml reps
200

new analytical
0 ml - TR
200 0 ml - diss

200 ml

old analytical
0 ml - TR
0 ml - diss

Conc. at Test Renewal

Trtmnt	Conc. ug/L	Stock (ml)	Total Vol(L)
6	200,000	13.37	0.25
5	100,000	6.68	0.25
4	50,000	3.34	0.25
3	20,000	1.34	0.25
2	5,000	0.33	0.25
1	0	0.00	0.25
Total		25.07	1.5

Analytical Volume: 100 ml per concentration (plus duplicates) at test initiation, new dissolved
100 ml per concentration (plus duplicates) at test renewal, old dissolved
100 ml per concentration (plus duplicates) at test renewal, old total recoverable

TRE Environmental Strategies, LLC
100 Racquette Drive, Unit A, Fort Collins, Colorado, 80524
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February 19, 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 Short-term Chronic Brine Shrimp Experiment #6

Mr. Bittner/ Dr. Belovsky:

Below is a summary of the short-term chronic brine shrimp experiment initiated on January 16, 2020. The purpose of this experiment was to investigate whether a marine algae, *Platymonas* sp. could be used as an alternative food source during short term chronic testing with *Artemia franciscana* (brine shrimp). The results of these studies will help determine if *Platymonas* sp. can be substituted for *Dunaliella viridis* as the food source during the test. *Platymonas* sp. grows at a faster rate, so it would be a simpler food source to maintain for testing.

Four different algal cell concentrations were tested:

- Treatment 1: 1.0 x 10^6 cells per chamber (from Brix et. al., 2003)
- Treatment 2: 4.72 x 10^6 cells per chamber (half of the Chl*a* concentration as measured by spectrophotometric absorbance as *Dunaliella* in previous studies)
- Treatment 3: 9.46 x 10^6 cells per chamber (same Chl*a* concentration as measured by spectrophotometric absorbance as *Dunaliella* in previous studies)
- Treatment 4: 18.9 x 10^6 cells per chamber (same cell density as *Dunaliella* in previous studies)

The test duration was 7 days. The test volume was consistent at 50 ml and test solutions were renewed daily.

Species: *Artemia franciscana*

Test type:

- Test duration: 7 days
- Test type: static-renewal (solutions and food renewed daily)
- Algae: *Platymonas* sp. (obtained from Carolina Biological)
- Algae concentration: various
- Temperature: 20°C
- Test volume(s): 50 ml
- Replicates: 4

- Organisms/Rep: 10
- Test media: 120 ppt rGSL media (per Notre Dame recipe)

Pretest conditions: *A. franciscana* cysts were hatched out in ~29 ppt artificial seawater (Crystal Sea Marine Mix) and ~200 organisms were placed in 120 ppt rGSL water and fed *Platymonas* sp. at an approximate density of 100 µg/L Chl*a* estimated with absorbance. 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#13818	7.9	NM	NM	138,100	NM	NM	118

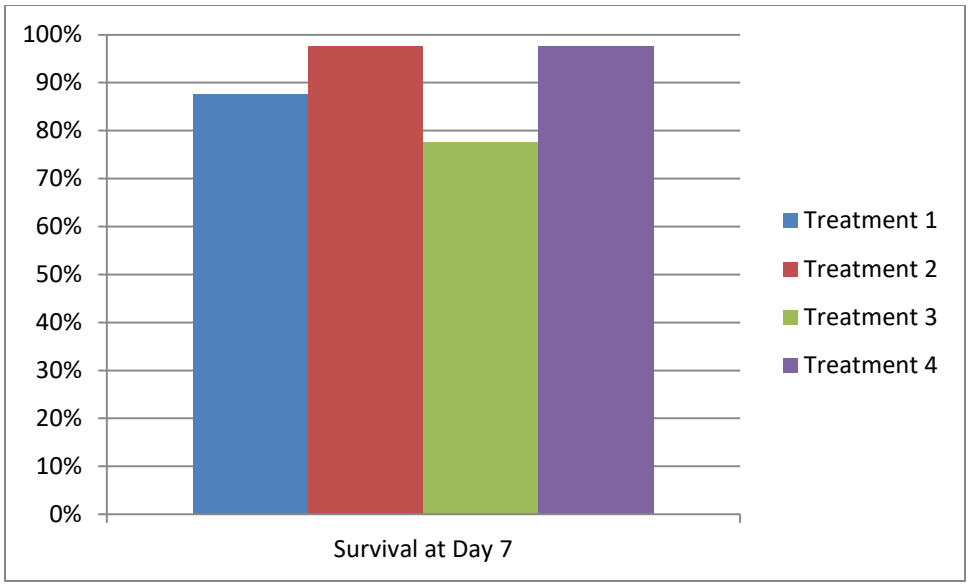
[a]As CaCO3
[b]Total residual chlorine

Test activities:

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

Results:

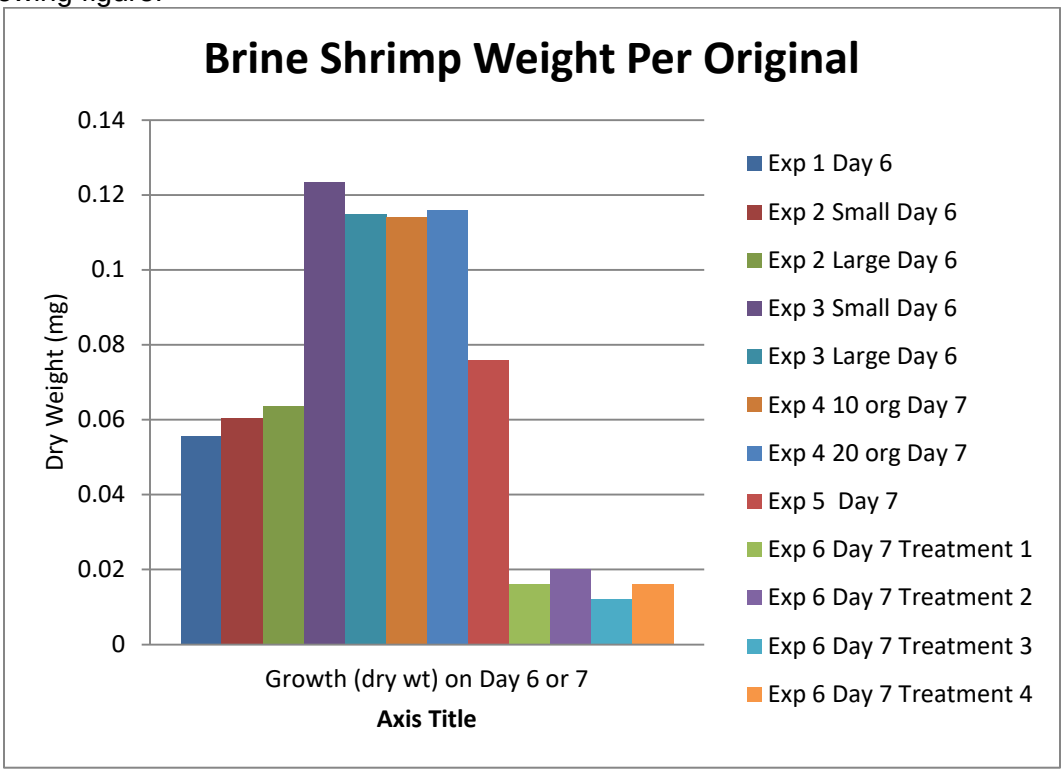
The survival of the brine shrimp in this study is illustrated in the following figure.



While survival in three of the four treatments met the 80% control perferomance threshold, the relative growth of these threatments compared to the earlier experiments is telling, as shown in

TRE

the following figure.



Summary and findings:

- Organism survival was between 77.5% and 97.5% for all treatments.
- The 7 day growth for the brine shrimp was substantially lower than in any studies where *Dunaliella viridis* was the sole food source.

Based on these results, *Platymonas* sp. was a poorer food source for *A. franciscana* than *D. viridis*. Both survival and weight were reduced compared to the previous experiments. Organisms still transitioned from nauplii to juvenile between test days 3 and 4, but appeared to have little growth after the transition stage. Based on visual observation, the *Platymonas* sp. appears to die in the rGSL water and settle out of solution, limiting its availability to the brine shrimp. *Platymonas* sp. does not appear to be a viable alternative food source under these testing conditions.

TRE

We greatly appreciate the opportunity to complete this study for you. Please do not hesitate to call if you have any questions regarding this study.

Sincerely,

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

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

14001-474-051

Attachment

cc: David Pillard, TRE

TOXICITY DATA PACKAGE COVER SHEET

Test Type: Chronic

Project Number: 17001-474-Exp

Test Substance: Platymonas

Species: *Artemia franciscana*

Dilution Water: rGSL

Organism Lot or Batch Number: _____

Concurrent Control Water: NA

Age: 48hr (48 hr)

Supplier: TRE

Date and Time Test Began: 1/16/20 @ 1445

Date and Time Test Ended: 1/23/20 @ 1500

Protocol Number: _____

Investigator(s): BJ/EU/HR/CP/EE

Background Information

pH control?: Yes **No**

Type of Test: Static-Renewal (Daily)

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: 7 days

Organisms per Replicate: 10

Type of Food and Quantity per Chamber: Varies

Feeding Frequency: 1 x daily

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): See Below

Agency Summary Sheet(s)?: None

Reference Toxicant Data: Test Dates: _____ to _____	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 100 ug/L Chla
Treatment 1: 1.0 x 10^6 cell per chamber (1.04 mL per chamber)
Treatment 2: 4.72 x 10^6 cell per chamber (4.52 mL per chamber)
Treatment 3: 9.46 x 10^6 cell per chamber (9.05 mL per chamber) same absorbance as previous
Treatment 4: 18.9 x 10^6 cell per chamber (18.1 mL per chamber) same cell density as previous
Appropriate correction factors have been applied to all temperatures recorded in this data package
Study Director Initials: AB Date: 1/14/20

TEST SUBSTANCE USAGE LOG

Project Number: 17001-474-Exp

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number				
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	13818			
Concurrent Control Water RW#	NA			
Date(s) Used	1/16/20 1/20/20			
	1/17/20 1/21/20			
	1/18/20 1/22/20			
	1/19/20			

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)
1	0	250	250						
2	0	250	250						
3	0	250	250						
4	0	250	250						
	0	1000	1000						

Initials / Date	BR 1/16/20 Mixed BS		
Initials / Date	EN 1/17/20 " "		
Initials / Date	HR 1/18/20 " "		
Initials / Date	CP 1/19/20 " "		
Initials / Date	EA 1/20/20 " "		
Initials / Date	EN 1/21/20 " "		
Initials / Date	CP 1/22/20 " "		
Initials / Date			

Artemia franciscana
CHRONIC BIOLOGICAL DATA

Project Number: 17001-474-Exp

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
1	A	10	10	10	10	10	10	10	~~9~~ 10	97.5
	B	10	10	10	10	10	8	8	8	
	C	10	10	10	9	9	9	9	9	
	D	10	10	10	8	8	8	8	8	
2	A	10	10	9	9	9	9	9	9	92.5
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
3	A	10	10	10	10	10	10	10	9	77.5
	B	10	10	10	10	10	9	9	9	
	C	10	10	10	10	10	10	10	8	
	D	10	9 +	9	9	9	9	9	8	+1 org NF
4	A	10	10	10	10	10	10	10	10	97.5
	B	10	10	10	10	10	10	9+	9	+1 org NF
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
	A									
	B									
	C									
	D									
	A									
	B									
	C									
	D									
	A									
	B									
	C									
	D									
Date:		1/16/20	1/17/20	1/18/20	1/19/20	1/20/20	1/21/20	1/22/20	1/23/20	
Time:		1445	1455	1545	1645	1605	1445	1455	1500	
Initials:		PB/EN	EN	HR	CP	ee	EN	CP	ee	

① ee 1/23/20;E

CHRONIC CHEMICAL DATA (INITIAL)

Project Number: 17001-474-Exp

Test Species: ***Artemia franciscana***

%	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter #	Remarks
Conc.: 1									All Conc.	
pH	7.9	7.8	8.1	8.0	8.0	7.8	7.9		FH28	
D.O. (mg/L) 4.9	~~7.0~~	4.8	5.4	5.3	4.9	4.9	5.0		17	
Temp. (°C)	20	20	20	20	20	20	20		L-38	
Cond. (µS/cm)	138,100	136900	141000	139700	~~151200~~ ⊗	136600	140600		15	~~* meter error ⊗~~
Hard. (mg/L)									Titr.	
Alk. (mg/L)									Titr.	
TRC (mg/L)									22	
NH_3 (mg/L)	Pot: 118								HA1	
Conc.: 2										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Conc.: 3										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.: 4										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Date:	1/16/19	1/17/20	1/18/20	1/19/20	1/20/20	1/21/20	1/22/20			
Time:	1435	1455	1535	1635	1610	1430	1445			
Initials:	BF	EN	HR	CP	EE	EN	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.

① BF 1/16/20 E ② EE 1/20/20 E

CHRONIC CHEMICAL DATA (FINAL)

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

%	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter #	Remarks
Conc.: 1							1379000		All Conc.	* conductivity
pH	7.8	7.7	8.0	8.0	7.8	7.8	7.8		FM21	
D.O. (mg/L)	7.2	5.2	5.3	4.7	4.8	4.9	4.9		17	
Temp (°C)	20	19	19	19	20	20	20		L-37	
Conc.: 2							127300			* conductivity
pH	7.8	7.7	8.0	8.1	7.8	7.9	7.9			
D.O. (mg/L)	7.2	5.1	5.1	4.7	4.8	4.8	4.7			
Temp (°C)	20	19	19	19	20	20	20			
Conc.: 3							126300			* conductivity
pH	7.8	7.7	8.0	8.0	7.8	7.8	7.9			
D.O. (mg/L)	7.0	4.9	4.9	4.7	4.6	4.6	4.9			
Temp (°C)	20	20	19	19	20	20	20			
Conc.: 4							125700			* conductivity
pH	7.8	7.7	8.0	8.0	7.8	7.8	7.9			
D.O. (mg/L)	6.9	5.0	4.9	4.7	4.6	4.5	5.1			
Temp (°C)	20	19	19	19	20	20	20			
Conc.:										
pH										
D.O. (mg/L)										
Temp (°C)										
Conc.:										
pH										
D.O. (mg/L)										
Temp (°C)										
Conc.:										
pH										
D.O. (mg/L)										
Temp (°C)										
Date:	1/17/20	1/18/20	1/19/20	1/20/20	1/21/20	1/22/20	1/23/20			
Time:	1500	1540	1705	1610	1445	1520	1545			
Initials:	EN	HR	CP	EE	EN	CP	EE			

DAILY TOXICITY TEST LOG

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

General Comments			Feeding	Initials/Date
	Random Chart: JJ	Min/Max Thermometer # M-15		
Test Day 0	Test Solution Mixed at: 1430 Test Organisms Added at: 1445		Fed @1440	BR 1116/20
Test Day 1	Real Time: 18 °C	Min-Max Range: 17-20 °C	Fed @1445	EN 1/17/20
Test Day 2	Real Time: 19 °C	Min-Max Range: 17-21 °C	Fed @1525	HR 1/18/20
Test Day 3	Real Time: 19 °C	Min-Max Range: 19-20 °C	Fed @ 1640	CP 1/19/20
Test Day 4	Real Time: 18 °C	Min-Max Range: 18-20 °C	Fed @ 1550	EE 1/20/20
Test Day 5	Real Time: 20 °C	Min-Max Range: 18-20 °C	Fed @1435	EN 1/21/20
Test Day 6	Real Time: 20 °C	Min-Max Range: 18-21 °C	Fed @ 1445	CP 1/22/20
Test Day 7	Real Time: 20 °C	Min-Max Range: 18-21 °C	Fed @ NONE	EE 1/23/20
Test Day 8	Real Time: °C	Min-Max Range: °C	Fed @	

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 17001-474-Exp	Test Substance: Platymonas	Comments:
Species: Artemia franciscana	Analyst Tare: MB/ DF Analyst Gross: CP	Analytical Balance ID: Sart #1
Date/Time of Tare Wt.: 1/23/20 @ 1125	Date/Time of Gross Wt.: 1/26/20 @ 1635	Dried in Oven # 3 from Date: 1/23/20 Time: 1545 to Date: 1/25/20 Time: 0805

Boat No.	Treatment	Rep.	Length Units:	Weight Type (Circle): Wet Blot Dry Dry (>100°C) AFDW (>500°C)							Lot or Batch Number:		
				Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)[1]	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	No. of Surv. Organisms	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)
	1	A		1.12982	1.12991						10		
		B		1.13233	1.13240						8		
		C		1.14630	1.14643						9		
		D		1.12223	1.12259						8		
	2	A		1.12647	1.12668						9		
		B		1.14211	1.14229						10		
		C		1.14156	1.14176						10		
		D		1.12570	1.12593						10		
	3	A		1.13963	1.13981						9		
		B		1.13438	1.13444						6		
		C		1.11715	1.11728						8		
		D		1.13943	1.13955						8		
Blank				1.13379	1.13379								
Range													
Mean													

Test Solution Volume:	Loading Rate:

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

QA Form No.010
Revision 6
Effective 02/14

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 17001-474-Exp	Test Substance: Platymonas	Comments:
Species: Artemia franciscana	Analyst Tare: MB/ [illegible] Analyst Gross: CP	Analytical Balance ID: Sart #1
Date/Time of Tare Wt.: 1/23/20 @ 1130	Date/Time of Gross Wt.: 1/26/20 @ 1635	Dried in Oven # 3 from Date: 1/23/20 Time: 1545 to Date: 1/25/20 Time: 0805

Weight Type (Circle): Wet Blot Dry Dry (>100°C) AFDW (>500°C)

Boat No.	Treatment	Rep.	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)[1]	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	Lot or Batch Number: No. of Surv. Organisms	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)
	4	A		1.14150	1.14157						10		
		B		1.14762	1.14800						9		
		C		1.12595	1.12605						10		
		D		1.13040	1.13049						10		
Blank													
Range													
Mean													

Test Solution Volume: Loading Rate:

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

QA Form No. 010a
Revision 1
Effective 02/14

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 14001-474 7 day Playtmonas Species: Artemia franciscana

Treatment	Rep	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)	No of Orig. Organisms	Mean Wt./ Original Organism (mg)	Mean Wt./ Treatment (mg) (Original)	Number of Surv. Organisms	Mean Wt./ Surviving Organism (mg)	Mean Wt./ Treatment (mg) (Surviving)
1	A		1.12982	1.12991	0.00009	0.00009	10	0.009	0.0162	10	0.009	0.0193
	B		1.13233	1.13240	0.00007	0.00007	10	0.007		8	0.009	
	C		1.14630	1.14643	0.00013	0.00013	10	0.013		9	0.014	
	D		1.12223	1.12259	0.00036	0.00036	10	0.036		8	0.045	
2	A		1.12647	1.12668	0.00021	0.00021	10	0.021	0.0205	9	0.023	0.0211
	B		1.14211	1.14229	0.00018	0.00018	10	0.018		10	0.018	
	C		1.14156	1.14176	0.00020	0.00020	10	0.020		10	0.020	
	D		1.12570	1.12593	0.00023	0.00023	10	0.023		10	0.023	
3	A		1.13963	1.13981	0.00018	0.00018	10	0.018	0.0123	9	0.020	0.0153
	B		1.13438	1.13444	0.00006	0.00006	10	0.006		6	0.010	
	C		1.11715	1.11728	0.00013	0.00013	10	0.013		8	0.016	
	D		1.13943	1.13955	0.00012	0.00012	10	0.012		8	0.015	
4	A		1.14150	1.14157	0.00007	0.00007	10	0.007	0.0160	10	0.007	0.0171
	B		1.14762	1.14800	0.00038	0.00038	10	0.038		9	0.042	
	C		1.12595	1.12605	0.00010	0.00010	10	0.010		10	0.010	
	D		1.13040	1.13049	0.00009	0.00009	10	0.009		10	0.009	
Blank			1.13379	1.13379	0.00000							

Project Number: 14001-474 7 day Playtmonas Species: Artemia franciscana

Summary Statistics for Survival Data

Treatment	N	Min	Max	Mean	SD	C.V.
1	4	0.8	1.0	0.8750	0.0957	10.942%
200.00%	4	0.9	1.0	0.9750	0.0500	5.128%
300.0%	4	0.6	0.9	0.7750	0.1258	16.236%
400%	4	0.9	1.0	0.9750	0.0500	5.128%
0.0%	0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
0%	0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Summary Statistics for Growth Data (dry wt per original)

Treatment	N	Min	Max	Mean	SD	C.V.
1	4	0.007	0.036	0.0162	0.0134	82.467%
200.00%	4	0.018	0.023	0.0205	0.0021	10.154%
300.0%	4	0.006	0.018	0.0123	0.0049	40.199%
400%	4	0.007	0.038	0.0160	0.0147	91.998%
0.0%	0	0.000	0.000	0.0000	#DIV/0!	#DIV/0!
0%	0	0.000	0.000	0.0000	#DIV/0!	#DIV/0!

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

Treatment	N	Min	Max	Mean	SD	C.V.
1	4	0.009	0.045	0.0193	0.0173	89.823%
200.00%	4	0.018	0.023	0.0211	0.0025	12.067%
300.0%	4	0.010	0.020	0.0153	0.0041	26.972%
400%	4	0.007	0.042	0.0171	0.0168	98.643%
0.0%	0	0.000	0.000	0.0000	#DIV/0!	#DIV/0!
0%	0	0.000	0.000	0.0000	#DIV/0!	#DIV/0!

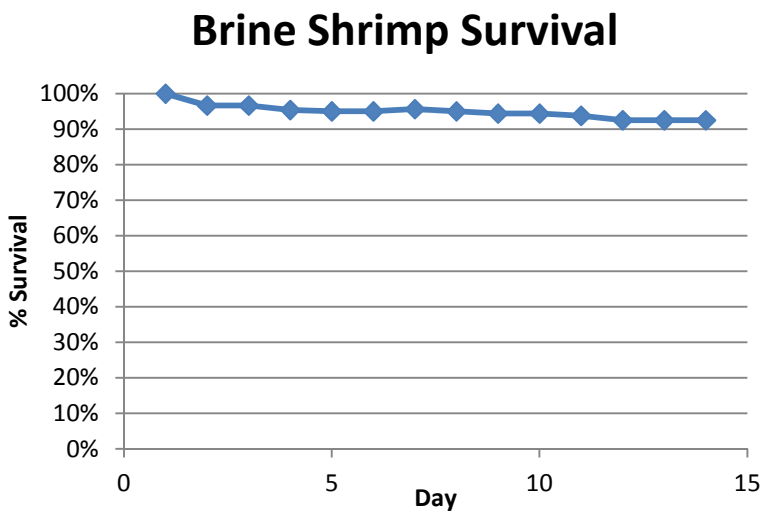
Artemia franciscana (Brine Shrimp) Short Term Chronic Test Development

TRE Environmental Strategies

Experiment 1

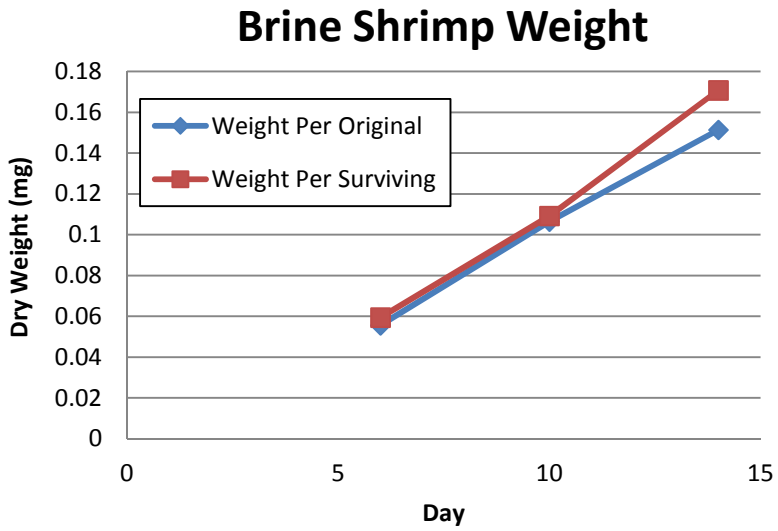
Test Design

- Three test durations:
  - 6 day, 10 day, 14 day
- Solution volume: 50 ml
- Fed *D. viridis*, conc 145 µg/L Chl*a*
- Renewed and fed every 48h
- 20 organisms per replicate
- 4 Replicates per treatment
- Media: 120ppt rGSL



Conclusions

- Water cleared of food after 24 hours on Day 6 and later
- Organisms transitioned from nauplii to juveniles between Days 3 and 4



Experiment 2

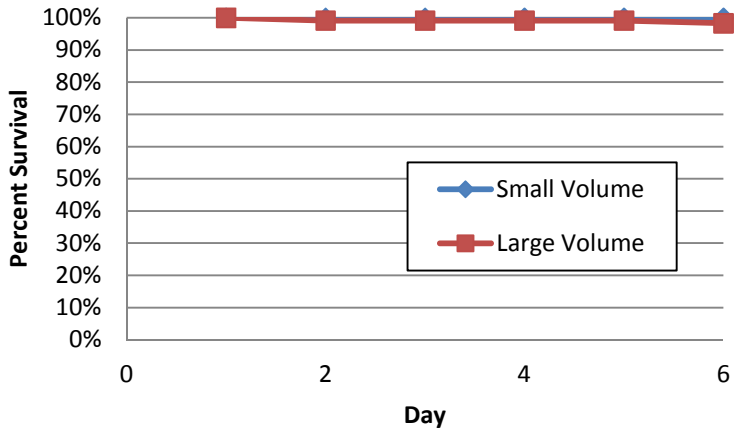
Test Design

- Test duration: 6 day
- Solution volume
  - 50 ml and 150 ml
- Fed *D. viridis*, conc 435 µg/L Chl*a* (ad libitum)
- Renewed and fed every 48h
- 20 organisms per replicate
- 3 Replicates per treatment
- Media: 120ppt rGSL

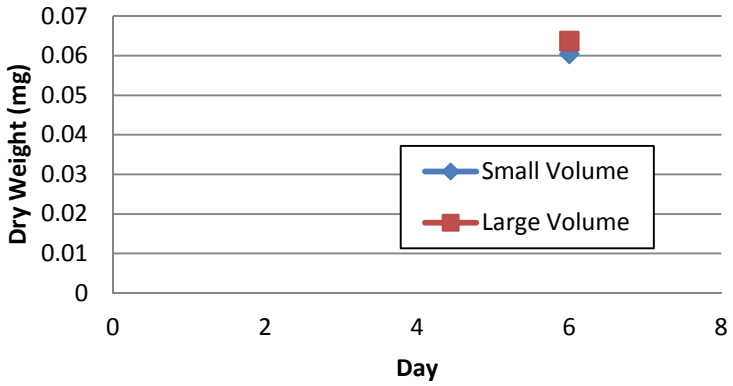
Conclusions

- Water remained tinted green throughout test
- Organisms transitioned from nauplii to juveniles between Days 3 and 4
- Growth was similar to Experiment 1 Day 6 growth

Brine Shrimp Survival



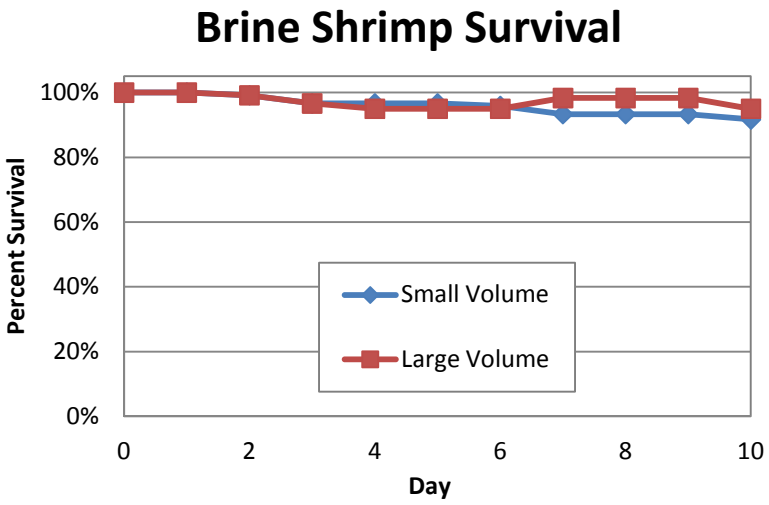
Weight Per Original Organism



Experiment 3

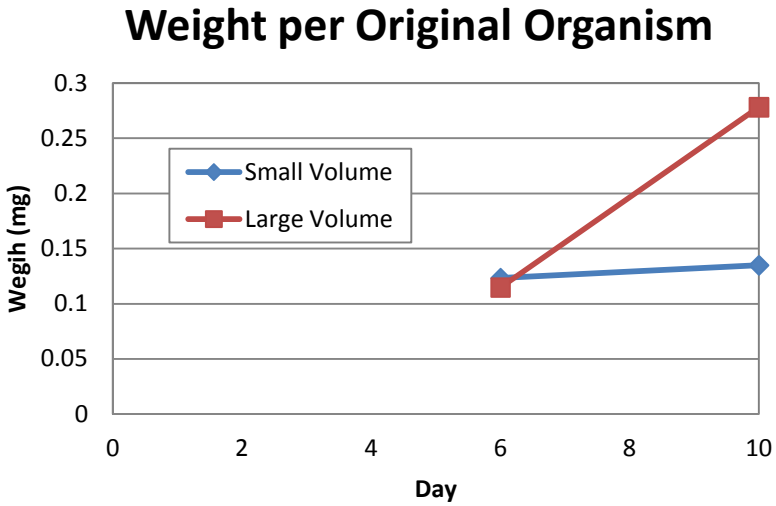
Test Design

- Test duration: 6 days, 10 days
- Solution volume: 50 ml and 150 ml
- Fed *D. viridis*, conc 145 µg/L Chl*a*
- Renewed and fed every 48h
- 20 organisms per replicate
- 3 Replicates per treatment
- Media: 120ppt rGSL



Conclusions

- 150 ml vol.: Water remained tinted green throughout test
- 50 ml vol.: Water cleared of food after 24 hours on Day 6 and later
- Organisms transitioned from nauplii to juveniles between Days 3 and 4
- 50 ml vol treatment appeared to be food limited after day 6



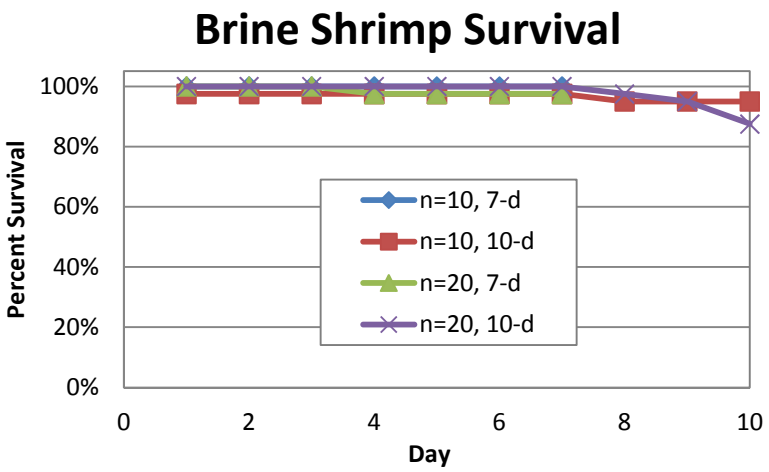
Experiment 4

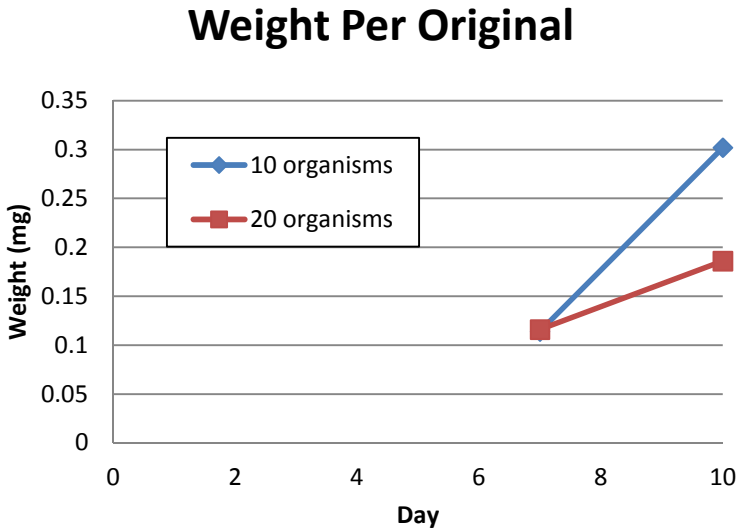
Test Design

- Test duration: 7 days, 10 days
- Solution volume: 50 ml and 150 ml
- Fed *D. viridis*, conc 145 µg/L Chl*a*
- Renewed and fed daily
- # of organisms per rep: 10 and 20
- 2 Replicates per treatment
- Media: 120ppt rGSL

Conclusions

- 10 orgs/cup: Water remained tinted green throughout test
- 20 orgs/cup: After day 6, test solution was a lighter green
- Organisms transitioned from nauplii to juveniles between Days 3 and 4
- 20 orgs/cup treatment appeared to be food limited starting ~day 6

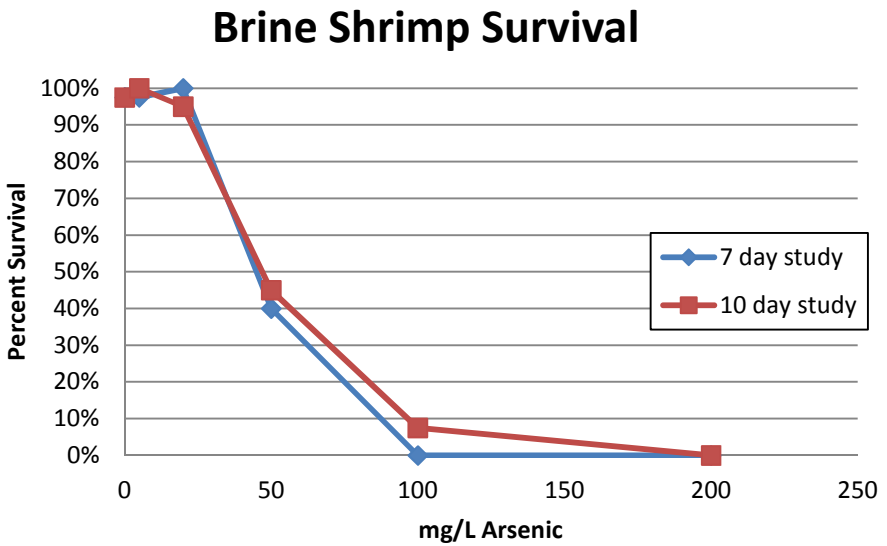




Experiment 5

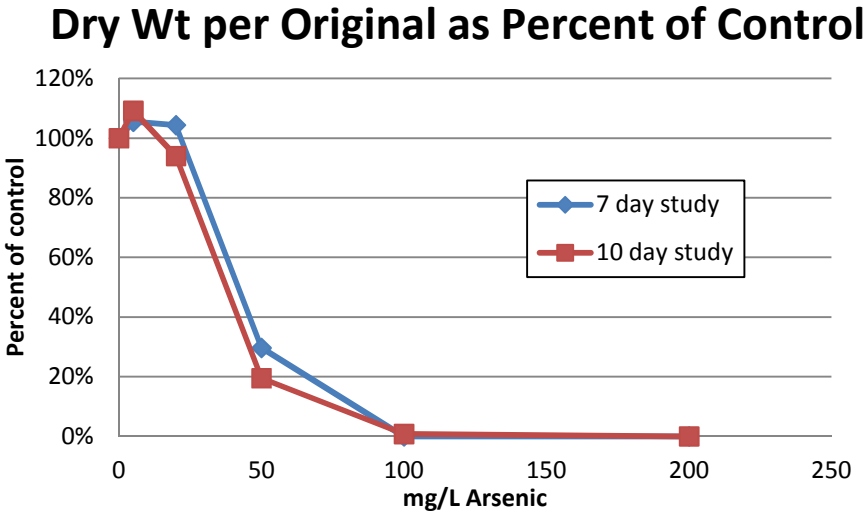
Test Design

- Test duration: 7 days, 10 days
- 50 ml volume
- Fed *D. viridis*, conc 145 µg/L Chl*a*
- Renewed and fed daily
- 4 Replicates per treatment
- Media: 120ppt rGSL
- Tested Control, and 5, 20, 50, 100, and 200 mg/L Arsenic



Conclusions

- Survival and growth NOEC and LOEC values were the same for both test durations
  - 7-day: 20 mg/L
  - 10 day: 20 mg/L
- IC25 values were similar for both test durations
  - 7 day: 30.4 (25.7-32.9)
  - 10 day: 26.3 (19.2-31.1)
- Organisms transitioned from nauplii to juveniles between Days 3 and 4



Experiment 6

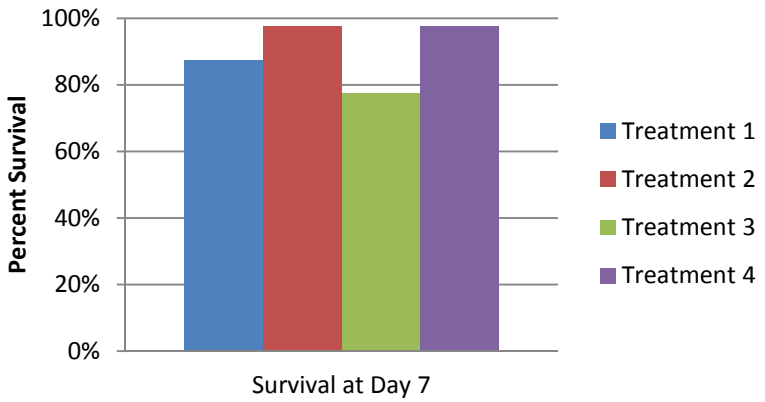
Test Design

- Test duration: 7 days
- Solution volume: 50 ml
- Fed *Platymonas* sp.,
  - Treatment 1: 1.0 x 10^6 cells per chamber
  - Treatment 2: 4.72 x 10^6 cells per chamber
  - Treatment 3: 9.46 x 10^6 cells per chamber
  - Treatment 4: 18.9 x 10^6 cells per chamber
- Renewed and fed daily
- 4 Replicates per treatment
- Media: 120ppt rGSL

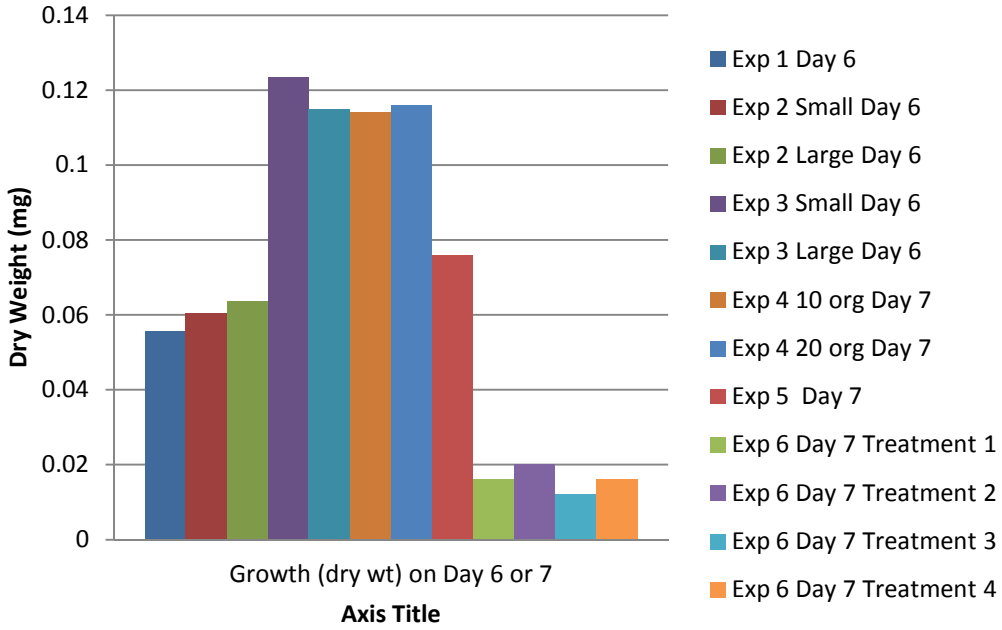
Conclusions

- Similar results among all 4 treatments
- *Platymonas* sp. doesn't appear to be an adequate food source
- Organisms transitioned from nauplii to juveniles between Days 3 and 4

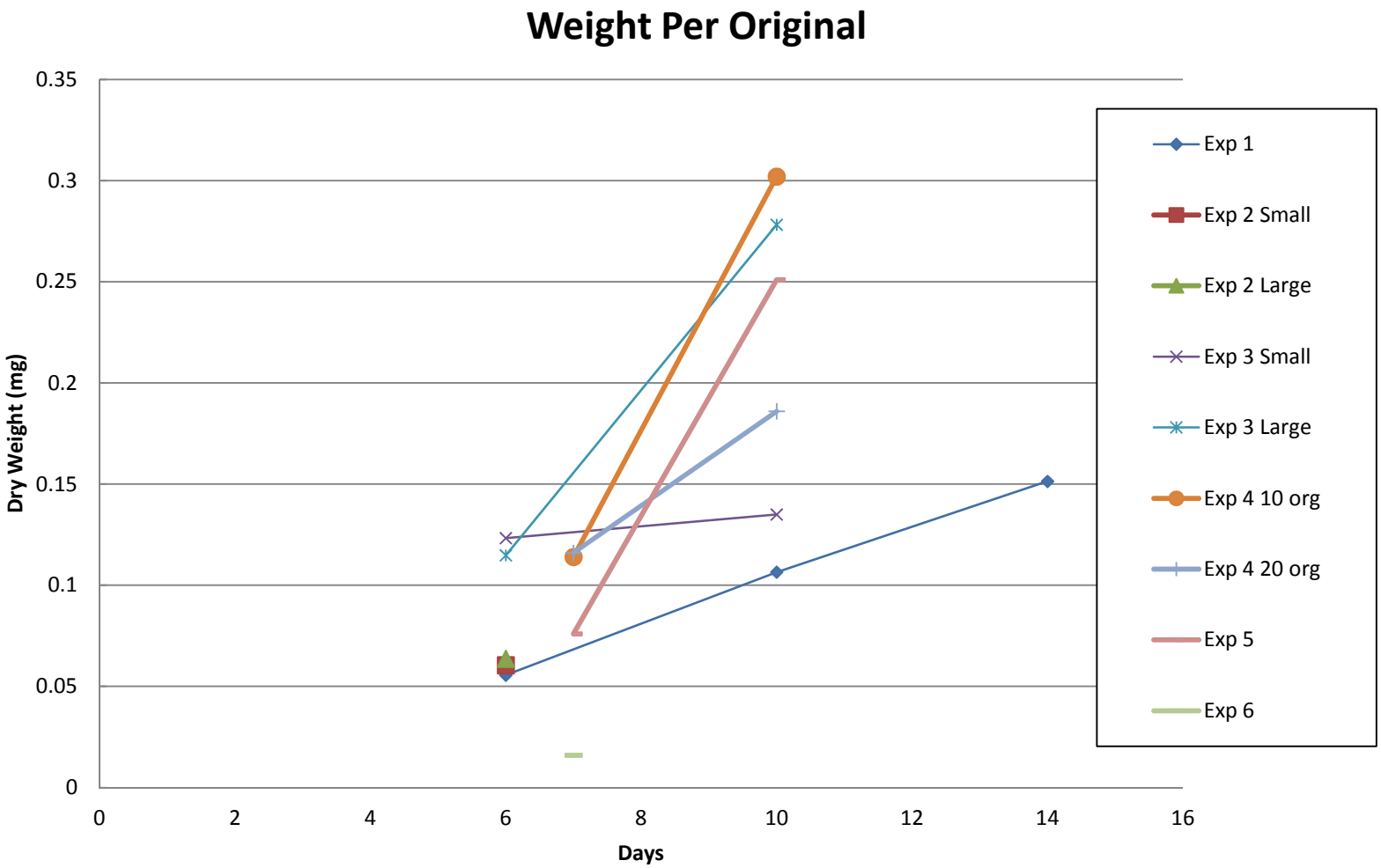
Brine Shrimp Survival



Brine Shrimp Weight Per Original



Growth Across All Experiments



Proposed Experimental Design

- # organisms per test chamber: 10
- Test solution volume: 50 ml
- Food type / conc.: *D. viridis*, conc 145 µg/L Chl*a*
- Solution renewal frequency: daily
- Test duration: 7 days
- Endpoints: survival (≥80%) and growth (>0.05 mg dry wt)

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March 3, 2020

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Subject: Results of Short-term Chronic Brine Shrimp Experiment #7

Mr. Bittner/ Dr. Belovsky:

Below is a summary of the short-term chronic brine shrimp experiment initiated on February 12, 2020. The purpose of this experiment was to investigate whether the marine alga, *Platymonas* sp. could be used as a supplemental food source during short term chronic testing with *Artemia franciscana* (brine shrimp). From our previous experiment, we concluded that *Platymonas* sp. was not an adequate food source for *A. franciscana* on its own.

The results of these studies will help determine if *Dunaliella viridis* can be supplemented with *Platymonas* sp. as the food source during the test. *Platymonas* sp. grows at a faster rate, so it would be an easier food source to maintain for testing.

Three different algal cell concentrations were tested:

- Treatment 1: *D. viridis* (145 µg/L Chl*a*)
- Treatment 2: *D. viridis* (72.5 µg/L Chl*a*)/ *Platymonas* sp. (72.5 µg/L Chl*a*)
- Treatment 3: *D. viridis* (72.5 µg/L Chl*a*, 50% of the normal density)

The test duration was 7 days. The test volume was consistent at 50 ml and test solutions were renewed daily.

Species: *Artemia franciscana*

Test type:

- Test duration: 7 days
- Test type: static-renewal (solutions and food renewed daily)
- Algae: *D. viridis, Platymonas* sp. (obtained from Carolina Biological)
- Algae concentration: various (see above)
- Temperature: 20°C
- Test volume(s): 50 ml
- Replicates: 3
- Organisms/Rep: 10
- Test media: 120 ppt rGSL media (per Notre Dame recipe)

Pretest conditions: *A. franciscana* cysts were hatched out in ~29 ppt artificial seawater (Crystal Sea Marine Mix) and ~200 organisms were placed in 120 ppt rGSL water and fed *D. viridis* at an approximate density of 100 µg/L Chl*a* estimated with absorbance. 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#13852	7.9	NM	NM	140,200	NM	NM	122

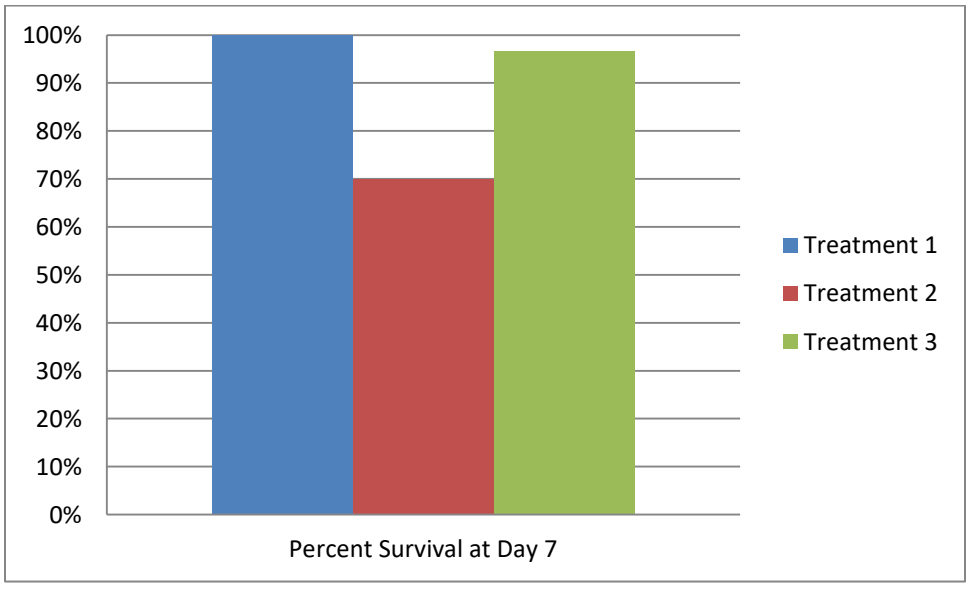
[a]As CaCO3
[b]Total residual chlorine

Test activities:

- Biological observations (primarily survival) taken daily.
- Chemistries taken on renewal days (i.e., pH, dissolved oxygen, and temperature).
- Conductivity was measured at test termination or when there was 0% survival in that treatment.
- Dry weights (±0.01 mg) were determined at test termination.

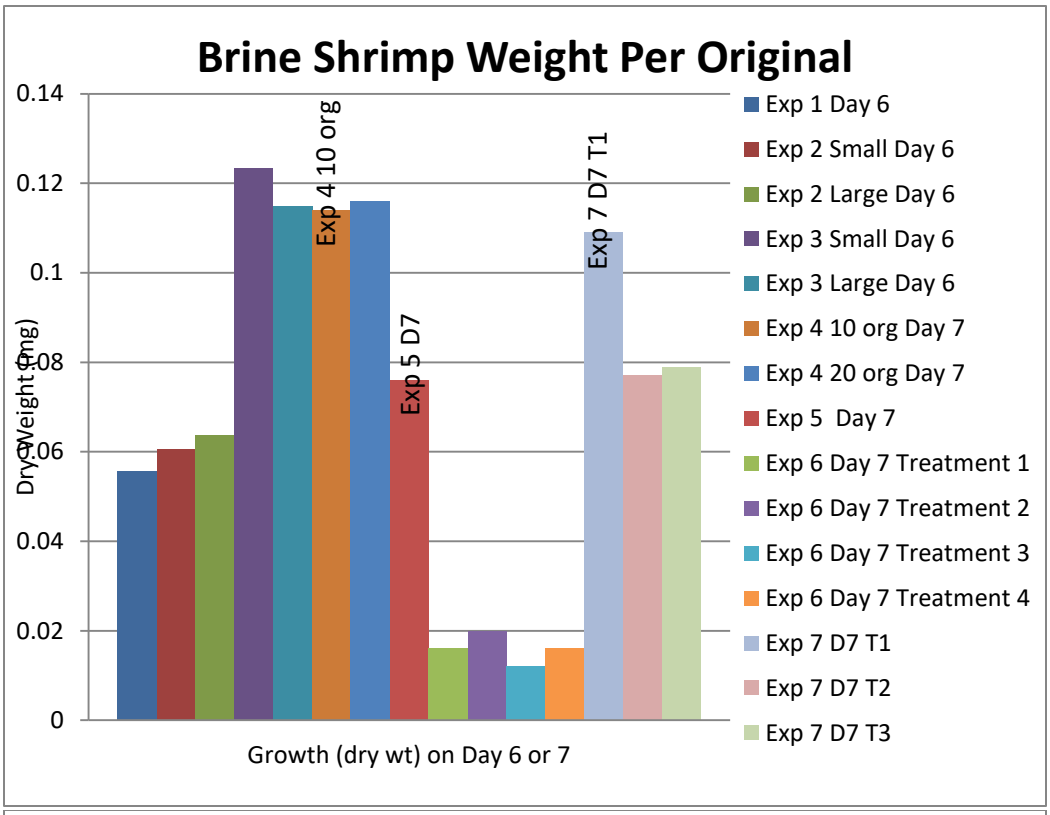
Results:

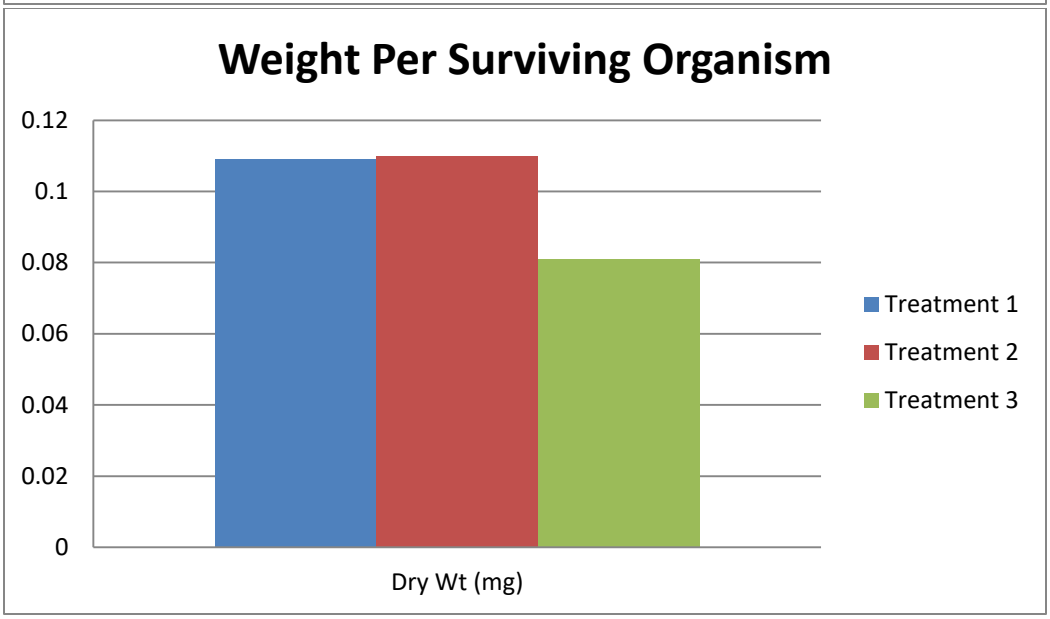
The survival of the brine shrimp in this study is illustrated in the following figure.



Survival in the two *D. viridis* treatments met the 80% control performace threshold, survival in the mixed treatment did not.

TRE





Summary and findings:

- Organism survival was between 70% and 100% for all treatments, with the mixed feeding treatment doing the worst and it was less than the target 80% survival threshold.
- The 7 day growth for the brine shrimp in the mixed food and 50% *D. viridis* treatments were similar on a per original basis. Both were lower than the full *D. viridis* treatment.

TRE

- The mixed food treatment was comparable to the full *D.* viridis treatment for dry weight on a per surviving basis.
- All organisms in this study still transitioned from nauplii to juvenile between test days 3 and 4.
- Based on visual observation, the *Platymonas* sp. appears to die in the rGSL water and settle out of solution, which was probably limiting its availability to the brine shrimp.

Based on these results, supplemental *Platymonas* sp. proved to be a poor food source for *A. franciscana* compared to *D. viridis* alone, as brine shrimp survival and weight in the treatment with *Platymonas* sp. were both reduced. *Platymonas* sp. does not appear to be a viable food source alone or as a supplement under the proposed testing conditions.

We greatly appreciate the opportunity to complete this study for you. Please do not hesitate to call if you have any questions regarding this study.

Sincerely,

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

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

14001-474-053

Attachment

cc: David Pillard, TRE

TOXICITY DATA PACKAGE COVER SHEET

Test Type: Chronic

Project Number: 17001-474-~~Exp~~ 053

Test Substance: Platymonas/Duniellia

Species: *Artemia franciscana*

Dilution Water: rGSL

Organism Lot or (Batch) Number: 021020

Concurrent Control Water: NA

Age: 48hr (48 hr)

Supplier: ORE

Date and Time Test Began: 2/12/20 @ 1615

Date and Time Test Ended: 2/19/20 @ 1515

Protocol Number: ____

Investigator(s): AB/CP/EE/EN

Background Information

pH control?: Yes **No**

Type of Test: Static-Renewal (Daily)

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: 3

Length of Test: 7 days

Organisms per Replicate: 10

Type of Food and Quantity per Chamber: Varies

Feeding Frequency: 1 x daily

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): See Below

Agency Summary Sheet(s)?: None

Reference Toxicant Data: Test Dates: NA to NA IC_{25}: ——

Hist. 95% Control Limits: NA to NA Method for Determining Ref. Tox. Value: Linear Interpolation

Special Procedures and Considerations:

Organisms hatched 2 days prior to initiation and held in rGSL with 100 ug/L Chla

Treatment 1: Feed 2.7mL D. viridis

Treatment 2: Feed 1.35mL D. viridis and 4.5 mL platymonas

Treatment 3: Feed 1.35mL D. viridis

Appropriate correction factors have been applied to all temperatures recorded in this data package

Study Director Initials: AB Date: 2/12/20

QA ver 2/18/20

TEST SUBSTANCE USAGE LOG

Project Number: 17001-474-Exp

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number				
Test Substance Collection Date and Time	From: @ To: @	From: @ To: @	From: @ To: @	From: @ To: @
Sample Type (Grab or Comp)				
Date Test Substance Received				
Dilution Water Number RW# or TRE#, circle one	13552			
Concurrent Control Water RW#	NA			
Date(s) Used	2/12/20 – 2/16/20 2/13/20 – 2/17/20 2/14/20 – 2/18/20 2/15/20			

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)
1	0	① ~~250~~ 170	250						
2	0	① ~~250~~ 170	250						
3	0	① ~~250~~ 170	250						
	0	750	750						

Initials / Date	AS 2/12/20		
Initials / Date	AS 2/13/20		
Initials / Date	AS 2/14/20		
Initials / Date	CP 2/15/20		
Initials / Date	CP 2/16/20		
Initials / Date	EE 2/17/20		
Initials / Date	EN 2/18/20		
Initials / Date			

① AS 2/12/20 2

Artemia franciscana
CHRONIC BIOLOGICAL DATA

QA RDW 2/28/20

Project Number: 17001-474-Exp

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
1	A	10	10	10	10	10	10	10	10	100
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D									
2	A	10	9	8	8*	7	7	7	7	* 1 weak 70
	B	10	9	7	7	7	7	7	7	
	C	10	10	9	8	8	8	8	0+	+ 2/18/20 TE
	D									
3	A	10	10	10	10	10	10	10	10	96.7
	B	10	10	9	9	9	9	9	9	
	C	10	10	10	10	10	10	10	10	
	D									
	A									
	B									
	C									
	D									
	A									
	B									
	C									
	D									
	A									
	B									
	C									
	D									
	A									
	B									
	C									
	D									
Date:		2/12/20	2/13/20	2/14/20	2/15/20	2/16/20	2/17/20	2/18/20	2/19/20	
Time:		1615	1130	1530	1550	1030	1520	1540	1515	
Initials:		AB/CP	AB	AB	CP	CP	EQ	EN	EQ	

CHRONIC CHEMICAL DATA (INITIAL)

QA NW 2/26/20

Project Number: 17001-474-Exp

Test Species: ***Artemia franciscana***

%	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter #	Remarks
Conc.: rGSL									All Conc.	
pH	7.9	7.9	7.9	8.0	8.0	7.8	7.8		FM28	
D.O. (mg/L)	5.1	5.3	5.2	5.1	5.0	~~8.5~~ 5.1 ①	7.6		17	
Temp. (°C)	20	20	20	20	20	20	20		L39	
Cond. (µS/cm)	140200	146400	137900	134800	139600	149400	137100		15	
Hard. (mg/L)									Titr	
Alk. (mg/L)									Titr	
TRC (mg/L)									22	
NH_3 (mg/L)									HA1	ppt: 122
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Date:	2/12/20	2/13/20	2/14/20	2/15/20	2/16/20	2/17/20	2/18/20			
Time:	1600	1115	1515	1530	1015	1520	1530			
Initials:	AB	AB	AB	CP	CP	EE	EN			

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

*Dilution/control water and effluent were brought to 25C prior to making the dilution series. The temperature of resulting effluent dilution is assumed to also be 25C.

① EE 2/17/20; E

CHRONIC CHEMICAL DATA (FINAL) CA [illegible] 2/24/20

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

%		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter #	Remarks
Conc.:	1							132900		All Conc.	* conductivity (15)
pH		8.0	8.0	8.0	8.0	8.0	7.8	7.9		FM28	
D.O. (mg/L)		5.0	5.5	5.1	5.2	~~7.7~~ 5.0 ①	7.4	4.7		17	
Temp (°C)		20	20	21	20	20	21	21		L34	
Conc.:	2							129200			* conductivity
pH		7.9	7.9	8.0	8.0	7.9	7.8	7.9			
D.O. (mg/L)		5.0	5.2	5.1	5.0	5.0	7.2	4.8			
Temp (°C)		20	20	21	20	19	21	21			
Conc.:	3							128700			* conductivity
pH		7.9	7.9	8.0	8.0	7.9	7.8	7.9			
D.O. (mg/L)		5.0	5.2	5.1	5.0	5.0	7.1	4.6			
Temp (°C)		20	20	21	20	19	21	21			
Conc.:											
pH											
D.O. (mg/L)											
Temp (°C)											
Conc.:											
pH											
D.O. (mg/L)											
Temp (°C)											
Conc.:											
pH											
D.O. (mg/L)											
Temp (°C)											
Conc.:											
pH											
D.O. (mg/L)											
Temp (°C)											
	Date:	2/13/20	2/14/20	2/15/20	2/16/20	2/17/20	2/18/20	2/19/20			
	Time:	1130	1535	1540	1040	1520	1540	1600			
	Initials:	[illegible]	AB	CP	CP	[illegible]	[illegible]	[illegible]			

① [illegible] 2/17/20; E

QA NEW 2/12/20

DAILY TOXICITY TEST LOG

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

General Comments					Feeding	Initials/Date
	Random Chart: Z		Min/Max Thermometer # M-15			
Test Day 0	Test Solution Mixed at: 1555 Test Organisms Added at: 1615				Fed @ 1600	AB 2/12/20
Test Day 1	Real Time: 19	°C	Min-Max Range: 19-21	°C	Fed @ 1120	AB 2/13/20
Test Day 2	Real Time: 19	°C	Min-Max Range: 19-20	°C	Fed @ ~~1540~~ ① 1520	AB CP ①2/14/20 ~~2/15/20~~
Test Day 3	Real Time: 20	°C	Min-Max Range: 19-21	°C	Fed @ 1540	CP 2/15/20
Test Day 4	Real Time: 20	°C	Min-Max Range: 19-21	°C	Fed @ 1025	CP 2/16/20
Test Day 5	Real Time: 18	°C	Min-Max Range: 18-23	°C	Fed @ 1500	EE 2/17/20
Test Day 6	Real Time: 20	°C	Min-Max Range: 20-21	°C	Fed @ 1530	EN 2/18/20
Test Day 7	Real Time: 20	°C	Min-Max Range: 20-22	°C	Fed @ NONE	EE 2/19/20
Test Day 8	Real Time:	°C	Min-Max Range:	°C	Fed @	

① CP 2/15/20 WP

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 17001-474-Exp	Test Substance: Feeding (Platymonas/Dunaliella)	Comments: Analytical Balance ID: Sart#1 Dried in Oven # 3 from Date: 2/20/20 Time: 1545 to Date: 2/24/20 Time: MR
Species: Artemia franciscana	Analyst Tare: CP Analyst Gross: MB	
Date/Time of Tare Wt.: 2/19/20 @ 1325	Date/Time of Gross Wt.: 2/21/20 @ 1120	

Weight Type (Circle): Wet Blot Dry Dry (~~>100°C~~ 60-90) AFDW (>500°C) Lot or Batch Number: 02102e

Boat No.	Treatment	Rep.	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)[1]	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	No. of Surv. Organisms	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)
	1	A		1.12544	1.12649	0.00105					10		
		B		1.13836	1.13946	0.00110					10		
		C		1.14665	1.14778	0.00113					10		
	2	A		1.13632	1.13724	0.00092					7		
		B		1.13224	1.13286	0.00062					7		
		C		1.14899	–	–					0		
	3	A		1.14450	1.14534	0.00084					10		
		B		1.11728	1.11801	0.00073					9		
		C		1.12908	1.12987	0.00079					10		
Blank				1.11512	1.11513								
Range													
Mean													

Test Solution Volume:	Loading Rate:

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

OAS 3/3/20 E

AB 2/25/20
GA rec'd 2/25/20

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 14001-474 Exp 7 Species: Artemia franciscana

Treatment	Rep	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)	No of Orig. Organisms	Mean Wt./ Original Organism (mg)	Mean Wt./ Treatment (mg) (Original)	Number of Surv. Organisms	Mean Wt./ Surviving Organism (mg)	Mean Wt./ Treatment (mg) (Surviving)
1	A		1.12544	1.12649	0.00105	0.00105	10	0.105	0.1093	10	0.105	0.1093
	B		1.13836	1.13946	0.00110	0.00110	10	0.110		10	0.110	
	C		1.14665	1.14778	0.00113	0.00113	10	0.113		10	0.113	
	D											
2	A		1.13632	1.13724	0.00092	0.00092	10	0.092	0.0770	7	0.131	0.1100
	B		1.13224	1.13286	0.00062	0.00062	10	0.062		7	0.089	
	C											
	D											
3	A		1.14450	1.14534	0.00084	0.00084	10	0.084	0.0787	10	0.084	0.0814
	B		1.11728	1.11801	0.00073	0.00073	10	0.073		9	0.081	
	C		1.12908	1.12987	0.00079	0.00079	10	0.079		10	0.079	
	D											
Blank			1.11512	1.11513	0.00001							

Project Number: 14001-474 Exp 7 Species: Artemia franciscana

Summary Statistics for Survival Data

Treatment	N	Min	Max	Mean	SD	C.V.
1	3	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
200.00%	2	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
300.0%	3	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
0%	0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
0.0%	0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
0%	0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Summary Statistics for Growth Data (dry wt per original)

Treatment	N	Min	Max	Mean	SD	C.V.
1	3	0.105	0.113	0.1093	0.0040	3.696%
200.00%	2	0.062	0.092	0.0770	0.0212	27.550%
300.0%	3	0.073	0.084	0.0787	0.0055	7.001%
0%	0	0.000	0.000	0.0000	#DIV/0!	#DIV/0!
0.0%	0	0.000	0.000	0.0000	#DIV/0!	#DIV/0!
0%	0	0.000	0.000	0.0000	#DIV/0!	#DIV/0!

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

Treatment	N	Min	Max	Mean	SD	C.V.
1	3	0.105	0.113	0.1093	0.0040	3.696%
200.00%	2	0.089	0.131	0.1100	0.0303	27.550%
300.0%	3	0.079	0.084	0.0814	0.0025	3.085%
0%	0	0.000	0.000	0.0000	#DIV/0!	#DIV/0!
0.0%	0	0.000	0.000	0.0000	#DIV/0!	#DIV/0!
0%	0	0.000	0.000	0.0000	#DIV/0!	#DIV/0!

TRE Environmental Strategies, LLC
100 Racquette Drive, Unit A, Fort Collins, Colorado, 80524
T 970.416.0916 F 970.490.2963

March 16, 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 Short-term Chronic Brine Shrimp Experiment #8

Mr. Bittner/ Dr. Belovsky:

Below is a summary of the short-term chronic brine shrimp experiment initiated on February 25, 2020. The purpose of this experiment was to investigate whether yeast or YTC could be used as an alternative or supplemental food source during short term chronic testing with *Artemia franciscana* (brine shrimp).

The results of these studies will help determine if *Dunaliella viridis* can be replaced or supplemented with yeast or YTC as the food source during the test. Yeast can be purchased and made into slurry and YTC is prepared regularly for organism culture, so they would be an easier food source to maintain for testing.

Five different algal cell concentrations were tested:

- Treatment 1: *D. viridis* (145 µg/L Chl*a*)
- Treatment 2: Yeast (15 mg/rep)
- Treatment 3: *D. viridis* (72.5 µg/L Chl*a*, 50% of the normal density)/ Yeast (7.5 mg/rep)
- Treatment 4: Cerio YTC (1.2 mg/rep)
- Treatment 5: *D. viridis* (72.5 µg/L Chl*a*, 50% of the normal density)/ YTC (0.6 mg/rep)

The test duration was 7 days. The test volume was consistent at 50 ml and test solutions were renewed daily.

Species: *Artemia franciscana*

Test type:

- Test duration: 7 days
- Test type: static-renewal (solutions and food renewed daily)
- Algae: *D. viridis*
- Algae concentration: various (see above)
- Temperature: 20°C
- Test volume(s): 50 ml
- Replicates: 3

- Organisms/Rep: 10
- Test media: 120 ppt rGSL media (per Notre Dame recipe)

Pretest conditions: *A. franciscana* cysts were hatched out in ~29 ppt artificial seawater (Crystal Sea Marine Mix) and ~200 organisms were placed in 120 ppt rGSL water and fed *D. viridis* at an approximate density of 100 µg/L Chl*a* estimated with absorbance. 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#13852	7.7	NM	NM	157,300	NM	NM	122

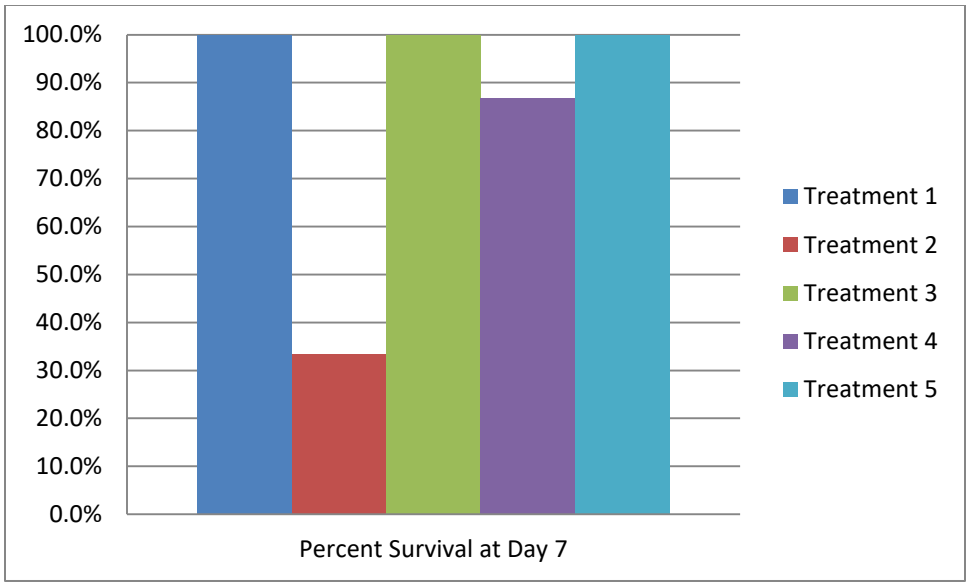
[a]As CaCO3
[b]Total residual chlorine

Test activities:

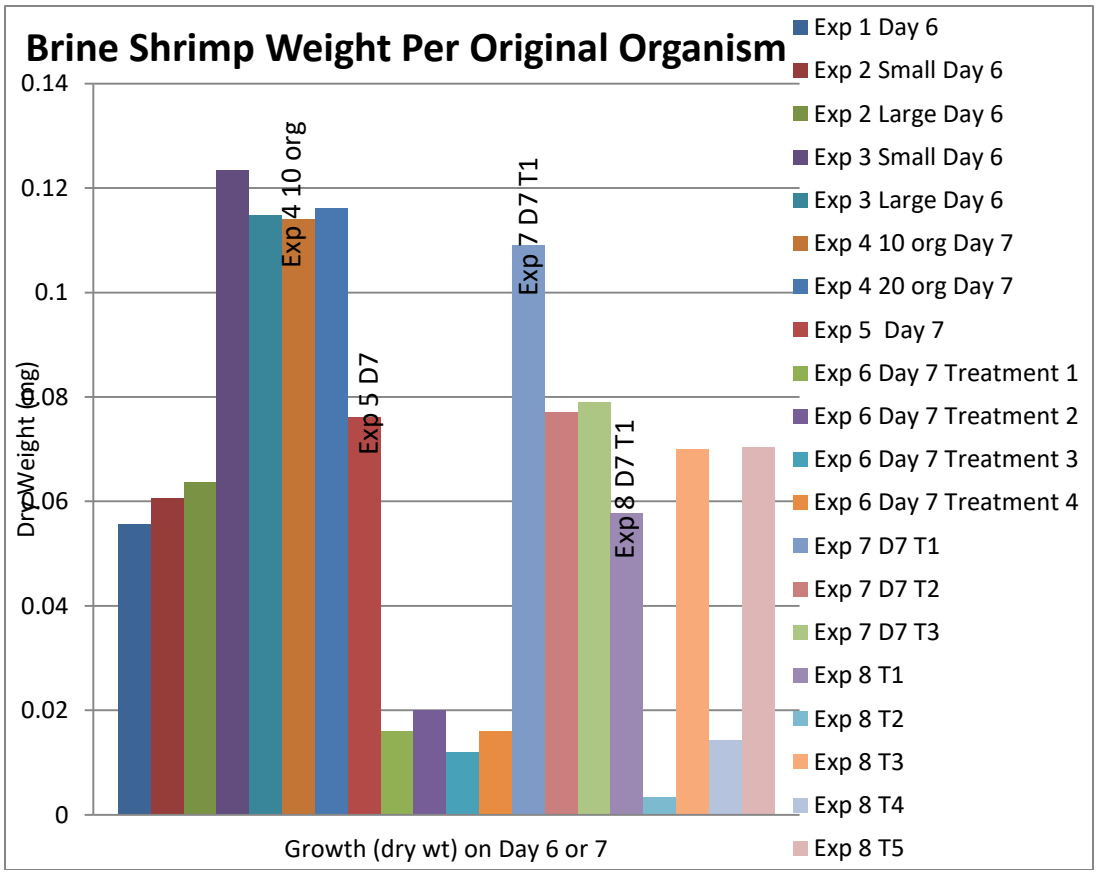
- Biological observations (primarily survival) taken daily.
- Chemistries taken on renewal days (i.e., pH, dissolved oxygen, and temperature).
- Conductivity was measured at test termination or when there was 0% survival in that treatment.
- Dry weights (±0.01 mg) were determined at test termination.

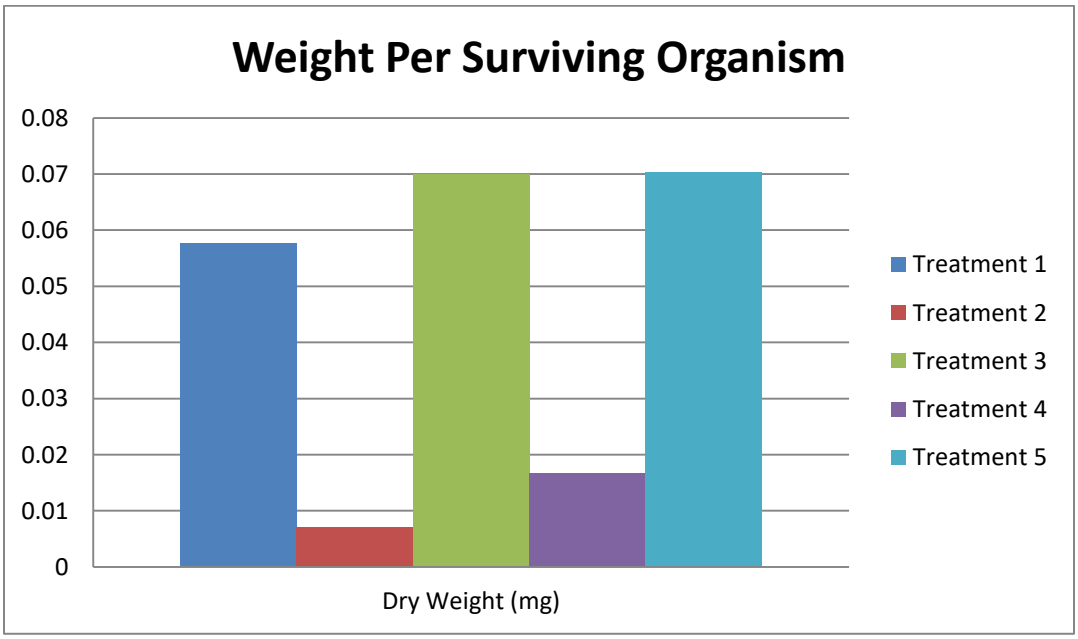
Results:

The survival of the brine shrimp in this study is illustrated in the following figure.



Survival in four of the treatments, 100% *D. viridis*, *D. viridis*/yeast mix, YTC, and *D. viridis*/YTC mix met the 80% control performace threshold, survival in the yeast treatment did not.





Summary and findings:

- Organism survival was between 33% and 100% for all treatments, with the yeast-fed treatment doing the worst, less than the target 80% survival threshold.

- The 7-day growth for the brine shrimp in the mixed food treatments were similar on both a per original and per survival basis. Both were higher than the full *D. viridis* treatment.
- The 7 day growth for the yeast and YTC treatments was considerably lower than the full *D. viridis* treatment.
- All organisms in this study still transitioned from nauplii to juvenile between test days 3 and 4.
- Based on visual observation, the yeast appears to settle out in the rGSL water, which was probably limiting its availability to the brine shrimp.

Based on these results, replacement yeast and YTC proved to be a poor food source for *A. franciscana* compared to *D. viridis* alone, as brine shrimp survival and weight in the treatments were both reduced and survival in the former did not meet the required test criterion of 80%. Supplementing the *D. viridis* with yeast or YTC appear to be a viable food source as both had higher growth than the *D. viridis* alone. Further experiments are being conducted to confirm these results.

We greatly appreciate the opportunity to complete this study for you. Please do not hesitate to call if you have any questions regarding this study.

Sincerely,

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

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

14001-474-054

Attachment

cc: David Pillard, TRE

QA Form No. 051
Revision 5
Effective 02/14

TOXICITY DATA PACKAGE COVER SHEET

QA: DLP 3/16/20

Test Type: Chronic
Project Number: 17001-474-054
Test Substance: Various
Species: *Artemia franciscana*
Dilution Water: rGSL
Organism Lot of (Batch) Number: 022320
Concurrent Control Water: NA
Age: 48hr (48 hr)
Supplier: TRE
Date and Time Test Began: 2/25/20 @ 1430
Date and Time Test Ended: 3/3/20 @ 1425
Protocol Number:
Investigator(s): AB | BG | EP

Background Information

pH control?: Yes **No**
If yes, give % CO_2: NA
Type of Test: Static-Renewal (Daily)
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: 3
Length of Test: 7 days
Organisms per Replicate: 10
Type of Food and Quantity per Chamber: Varies
Feeding Frequency: 1 x daily

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): See Below

Agency Summary Sheet(s)?: None

Reference Toxicant Data: Test Dates: ______ to ______		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 100 ug/L Chla
Treatment 1: *D. viridis* (145 ug/L Chla)- 2.6 ml
Treatment 2: Yeast (0.8ml ~~15.4~~ 18.9 g/L) ①
Treatment 3: 1/2 *D. viridis* (1.3 ml) 1/2 yeast (0.4 ml)
Treatment 4: Cerio YTC (0.66ml)
Treatment 5: 1/2 *D. viridis* (1.3 ml) 1/2 cerio YTC (0.33 ml)
Appropriate correction factors have been applied to all temperatures recorded in this data package
Study Director Initials: AB Date: 2/25/20

① AB 2/25/20 E

TEST SUBSTANCE USAGE LOG

Project Number: 17001-474-054

QA: DDA 3/16/20

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number				
Test Substance Collection Date and Time	From: @ To: @	From: @ To: @	From: @ To: @	From: @ To: @
Sample Type (Grab or Comp)				
Date Test Substance Received				
Dilution Water Number RW# or TRE#, circle one	13852			
Concurrent Control Water RW#	NA			
Date(s) Used	2/25/20 – 2/29/20 2/26/20 – 3/1/20 2/27/20 – 3/2/20 2/28/20			

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)
1	0	170	170						
2	0	170	170						
3	0	170	170						
4	0	170	170						
5	0	170	170						
	0	850	850						

Initials / Date	MB 2/25/20		
Initials / Date	CP 2/26/20		
Initials / Date	MB 2/27/20		
Initials / Date	CP 2/28/20		
Initials / Date	CP 2/29/20		
Initials / Date	CP 3/1/20		
Initials / Date	CP 3/2/20		
Initials / Date			

QA Form No. 060
Revision 3
Effective 02/14

Artemia franciscana
CHRONIC BIOLOGICAL DATA

QA: DAP 3/16/20

Project Number: 17001-474-054

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	
1	A	10	10	10	10	10	10	10	10	
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D								10	
2	A	10	10	10	10	10	10*	7	5	*2 weak, no significant growth
	B	10	10	9	9	9	9	7	4	no significant growth
	C	10	10	10	9	7	6	4*	1	no significant growth; *1 weak
	D									
3	A	10	10	10	10	10	10	10	10	
	B	10	10	10+	10	10	10	10	10	+removed extra org
	C	10	10+	10	10	10	10	10	10	+removed extra org
	D									
4	A	10	10	10	10	9	9*	9*	9*	no significant growth; *1 weak
	B	10	10	10	10	10	9	9	9	" "
	C	10	10	10	10	9	9	8	8	" "
	D									
5	A	10	10	10	10	10	10	10	10	
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D									
	A									
	B									
	C									
	D									
	A									
	B									
	C									
	D									
Date:		2/25/20	2/26/20	2/27/20	2/28/20	2/29/20	3/1/20	3/2/20	3/3/20	
Time:		1430	1435	1030	0950	1300	1030	1030	1425	
Initials:		AS/RF	CP	AS	CP	CP	CP	CP	RF	

QA Form No. 058
Revision 4
Effective 02/14

CHRONIC CHEMICAL DATA (INITIAL)

QA: DAP 3/16/20

Project Number: 17001-474-054

Test Species: ***Artemia franciscana***

%	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter #	Remarks
Conc.: rGSL									All Conc.	
pH	7.7	8.0	7.9	8.0	8.0	8.0	7.9		FM27	
D.O. (mg/L)	5.1	5.2	5.2	5.3	5.3	5.1	5.2		14	
Temp. (°C)	20	20	20	20	20	20	20		L38	
Cond. (µS/cm)	157300	141400	139300	139700	139900	141700	145900		14	
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Date:	2/25/20	2/26/20	2/27/20	2/28/20	2/29/20	3/1/20	3/2/20			
Time:	1415	1405	0955	0940	1255	1025	1025			
Initials:	AM	CP	AM	CP	CP	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: DAP 3/16/20

DAILY TOXICITY TEST LOG

Project Number:	17001-474-054
Test Species:	***Artemia franciscana***

General Comments	Random Chart: JJ Min/Max Thermometer # M-15	Feeding	Initials/Date
Test Day 0	Test Solution Mixed at: 1410 Test Organisms Added at: 1430	Fed @ 1420	AB 2/25/20
Test Day 1	Real Time: 19 °C Min-Max Range: 18-21 °C	Fed @ 1425	CP 2/26/20
Test Day 2	Real Time: 19 °C Min-Max Range: 19-20 °C Yeast appears to be settling out	Fed @ 1005	AB 2/27/20
Test Day 3	Real Time: 20 °C Min-Max Range: 18-21 °C	Fed @ 0935	CP 2/28/20
Test Day 4	Real Time: 20 °C Min-Max Range: 18-22 °C *No significant growth in 100% yeast and 100% YTC	Fed @ 1255	CP 2/29/20
Test Day 5	Real Time: 20 °C Min-Max Range: 18-22 °C * u u; very weak orgs in 100% yeast	Fed @ 1025	CP 3/1/20
Test Day 6	Real Time: 20 °C Min-Max Range: 18-21 °C * u u; u	Fed @ 1020	CP 3/2/20
Test Day 7	Real Time: 20 °C Min-Max Range: 18-22 °C @ 3 & 5 reps darker in color than 1 reps * u u; u	Fed @ Not	BY 3/3/20
Test Day 8	Real Time: °C Min-Max Range: °C	Fed @	

06 DAP 3/16/20 E

QA Form No.010
Revision 6
Effective 02/14

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

QA: DAP 3/16/20

Project Number: 17001-474-054	Test Substance: Various	Comments: Analytical Balance ID: Sart #1
Species: Artemia franciscana	Analyst Tare: MB Analyst Gross: MB	Dried in Oven # 3 from Date: 3/13/20 Time: 1535 to Date: 3/16/20 Time: 1030
Date/Time of Tare Wt.: 3/3/20 @ 1000	Date/Time of Gross Wt.: 3/6/20 @ 1115	

Weight Type (Circle): Wet Blot Dry (Dry (>100°C)) AFDW (>500°C) Lot or Batch Number: 022320

Boat No.	Treatment	Rep.	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)[1]	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	No. of Surv. Organisms	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)
	1	A		1.14692	1.14744	0.00052					10		
		B		1.13547	1.13607	0.00060					10		
		C		1.14688	1.14746	0.00058					10		
	2	A		1.14879	1.14886	0.00007					5		
		B		1.14032	1.14033	0.00001					4		
		C		1.13650	1.13650	0.00000					1		
	3	A		1.14683	1.14761	0.00078					10		
		B		1.15034	1.15105	0.00071					10		
		C		1.12851	1.12909	0.00058					10		
Blank				1.13816	1.13815	-0.00001							
Range													
Mean													

Test Solution Volume: Loading Rate:

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

QA Form No.010
Revision 6
Effective 02/14

QA: DMB 3/16/20

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 17001-474-054
Test Substance: Various
Species: Artemia franciscana
Analyst Tare: MB Analyst Gross: MB
Date/Time of Tare Wt.: 3/3/20 @ 1000
Date/Time of Gross Wt.: 3/6/20 @ 1115
Comments:
Analytical Balance ID: Sart #1
Dried in Oven # 3 from Date: 3/3/20 Time: 1535
to Date: 3/6/20 Time: 1030

Weight Type (Circle): Wet Blot Dry (Dry (>100°C)) AFDW (>500°C)
Lot or (Batch) Number: 02B20

Boat No.	Treatment	Rep.	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)[1]	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	No. of Surv. Organisms	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)
	4	A		1.13101	1.13115	0.00014					10 9		
		B		1.15247	1.15260	0.00013					9 9		
		C		1.13429	1.13442	0.00013					10 9		
	5	A		1.13462	1.13526	0.00064					9 10		
		B		1.14685	1.14751	0.00066					10		
		C		1.12853	1.12931	0.00078					10		
Blank													
Range													
Mean													

Test Solution Volume:
Loading Rate:

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

DMB 3/16/20

QA Form No. 010a
Revision 1
Effective 02/14

AB 3/6/20

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 14001-474 Exp 8 Species: Artemia franciscana

QA: DAP 3/16/20

Treatment	Rep	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)	No of Orig. Organisms	Mean Wt./ Original Organism (mg)	Mean Wt./ Treatment (mg) (Original)	Number of Surv. Organisms	Mean Wt./ Surviving Organism (mg)	Mean Wt./ Treatment (mg) (Surviving)
1	A		1.14692	1.14744	0.00052	0.00053	10	0.053	0.0577	10	0.053	0.0577
	B		1.13547	1.13607	0.00060	0.00061	10	0.061		10	0.061	
	C		1.14688	1.14746	0.00058	0.00059	10	0.059		10	0.059	
	D											
2	A		1.14879	1.14886	0.00007	0.00008	10	0.008	0.0033	5	0.016	0.0070
	B		1.14032	1.14033	0.00001	0.00002	10	0.002		4	0.005	
	C		1.13650	1.13650	0.00000	0.00000	10	0.000		1	0.000	
	D											
3	A		1.14683	1.14761	0.00078	0.00079	10	0.079	0.0700	10	0.079	0.0700
	B		1.15034	1.15105	0.00071	0.00072	10	0.072		10	0.072	
	C		1.12851	1.12909	0.00058	0.00059	10	0.059		10	0.059	
	D											
4	A		1.13101	1.13115	0.00014	0.00015	10	0.015	0.0143	9	0.017	0.0166
	B		1.15247	1.15260	0.00013	0.00014	10	0.014		9	0.016	
	C		1.13429	1.13442	0.00013	0.00014	10	0.014		8	0.018	
	D											
5	A		1.13462	1.13526	0.00064	0.00065	10	0.065	0.0703	10	0.065	0.0703
	B		1.14685	1.14751	0.00066	0.00067	10	0.067		10	0.067	
	C		1.12853	1.12931	0.00078	0.00079	10	0.079		10	0.079	
	D											
Blank			1.13816	1.13815	-0.00001							

AB 3/6/20

QA: DAP 3/16/20

Project Number: 14001-474 Exp 8 Species: Artemia franciscana

Summary Statistics for Survival Data

Treatment	N	Min	Max	Mean	SD	C.V.
1	3	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
200.00%	3	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
300.0%	3	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
400%	3	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
500.0%	3	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
0%	0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Summary Statistics for Growth Data (dry wt per original)

Treatment	N	Min	Max	Mean	SD	C.V.
1	3	0.053	0.061	0.0577	0.0042	7.220%
200.00%	3	0.000	0.008	0.0033	0.0042	124.900%
300.0%	3	0.059	0.079	0.0700	0.0101	14.498%
400%	3	0.014	0.015	0.0143	0.0006	4.028%
500.0%	3	0.065	0.079	0.0703	0.0076	10.766%
0%	0	0.000	0.000	0.0000	#DIV/0!	#DIV/0!

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

Treatment	N	Min	Max	Mean	SD	C.V.
1	3	0.053	0.061	0.0577	0.0042	7.220%
200.00%	3	0.000	0.016	0.0070	0.0082	116.934%
300.0%	3	0.059	0.079	0.0700	0.0101	14.498%
400%	3	0.016	0.018	0.0166	0.0010	5.886%
500.0%	3	0.065	0.079	0.0703	0.0076	10.766%
0%	0	0.000	0.000	0.0000	#DIV/0!	#DIV/0!

TRE Environmental Strategies, LLC
100 Racquette Drive, Unit A, Fort Collins, Colorado, 80524
T 970.416.0916 F 970.490.2963

April 7, 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

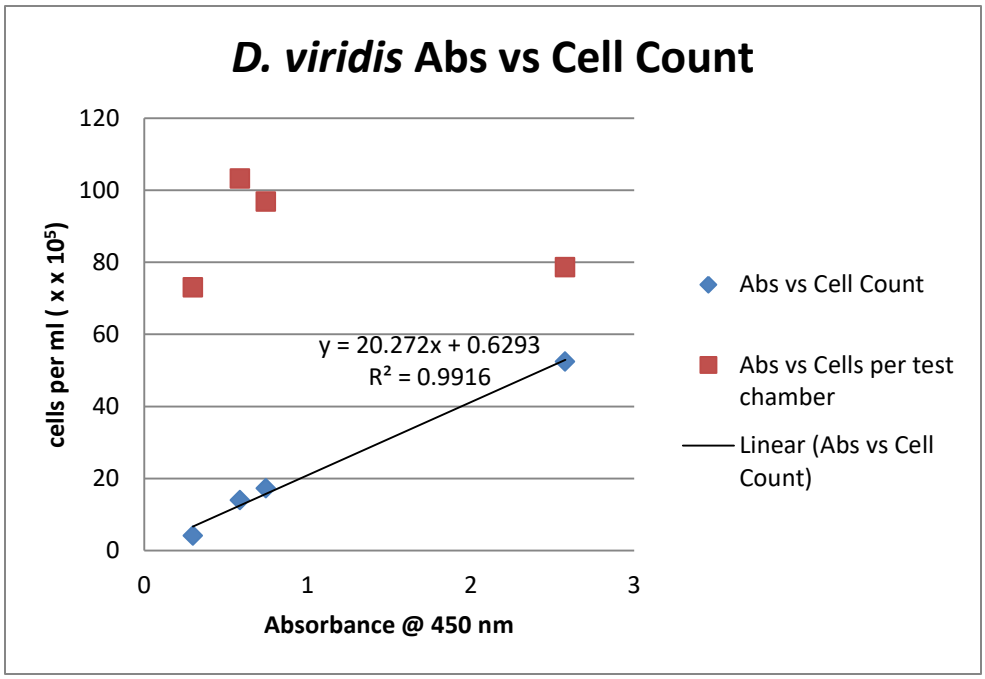
Subject: Algae Absorbance vs Cell Count

Mr. Bittner / Dr. Belovsky:

Dunaliella viridis has been the main source of food for brine shrimp chronic tests. The amount of *D. viridis* added to each test has been determined by spectrophotometric absorbance at 450 nm, which is then converted into approximate Chl*a* concentration to determine the volume of algae that is needed to feed each test chamber. This relationship was established using extensive fluorometric and spectrophotometric analyses during the acute studies with three metals. The purpose of this study was to determine the relationship between absorbance at 450 nm and cell density to determine if cell density could be used as a reliable quantitative metric for determining food density. Cell density is commonly used to prepare food for other WET methods and would allow for the amount of algae being fed to be more consistent across multiple laboratories.

Dunaliella viridis was collected from four different cultures. Absorbance was measured on each sample and the remaining volume was preserved with Lugol's solution. Preserved samples were counted with a hemocytometer under a compound microscope. Absorbance values were converted to approximate Chl*a* values to determine how much volume would be used in a hypothetical test. This value was multiplied by the cell density to obtain how many cells would be added to each test chamber. Values are shown in the table and graph below:

Abs	Count (x 10^5)	Volume in test	Cells added to test chamber
0.298	4.15	17.6	73.04 x 10^5
2.578	52.4	1.5	78.60 x 10^5
0.586	13.95	7.4	103.23 x 10^5
0.745	17.3	5.6	96.88 x 10^5



Cell density and absorbance demonstrated a strong, consistent relationship within the range studied, generating an r^2 value of 0.9916. This suggests that switching to a specified *D. viridis* cell count may be an acceptable method to help ensure consistent feeding during a WET test.

Sincerely,

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

David Pillard, Ph.D.
Principal/Senior Toxicologist
pillardda.tre@gmail.com

17001-474-059

Attachment

cc: Rami B. Naddy, TRE

TRE Environmental Strategies, LLC
100 Racquette Drive, Unit A, Fort Collins, Colorado, 80524
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March 30, 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 Short-term Chronic Brine Shrimp Experiment #9

Mr. Bittner / Dr. Belovsky:

Below is a summary of the short-term chronic brine shrimp experiment initiated on March 10, 2020. The purpose of this experiment was to verify the results from the previous experiment showing whether yeast or YTC[1] could be used as an alternative or supplemental food source during short term chronic testing with *Artemia franciscana* (brine shrimp).

The results of these studies will help determine if *Dunaliella viridis* can be replaced or supplemented with yeast or YTC as the food source during the test. Yeast can be purchased and made into slurry and YTC is prepared regularly for organism culture, so they would be an easier food source to maintain for testing, and are commonly used by other testing laboratories.

Five different treatments were tested (algal density was 50% in the supplemented treatments):

- Treatment 1: *D. viridis* (145 µg/L Chl*a*)
- Treatment 2: Yeast (7.5 mg/rep)
- Treatment 3: *D. viridis* (72.5 µg/L Chl*a*, 50% of the normal density)/ Yeast (7.5 mg/rep)
- Treatment 4: *D. viridis* (72.5 µg/L Chl*a*, 50% of the normal density)/ YTC (0.6 mg/rep)
- Treatment 5: *D. viridis* (72.5 µg/L Chl*a*, 50% of the normal density)/ YTC (1.2 mg/rep)

The test duration was 7 days. The test volume was consistent at 50 ml and test solutions were renewed daily.

Species: *Artemia franciscana*

Test type:

- Test duration: 7 days
- Test type: static-renewal (solutions and food renewed daily)
- Algae: *D. viridis*
- Algae concentration: 72.5 (supplemented treatments) or 145 µg/L Chl*a* (see above)
- Temperature: 20°C

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

- Test volume(s): 50 ml
- Replicates: 3
- Organisms/Rep: 10
- Test media: 120 ppt rGSL media (per Notre Dame recipe)

Pretest conditions: *A. franciscana* cysts were hatched out in ~29 ppt artificial seawater (Crystal Sea Marine Mix) and ~200 organisms were placed in 120 ppt rGSL water and fed *D. viridis* at an approximate density of 100 µg/L Chl*a* estimated with absorbance. 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#13852	7.8	NM	NM	144,800	NM	NM	122

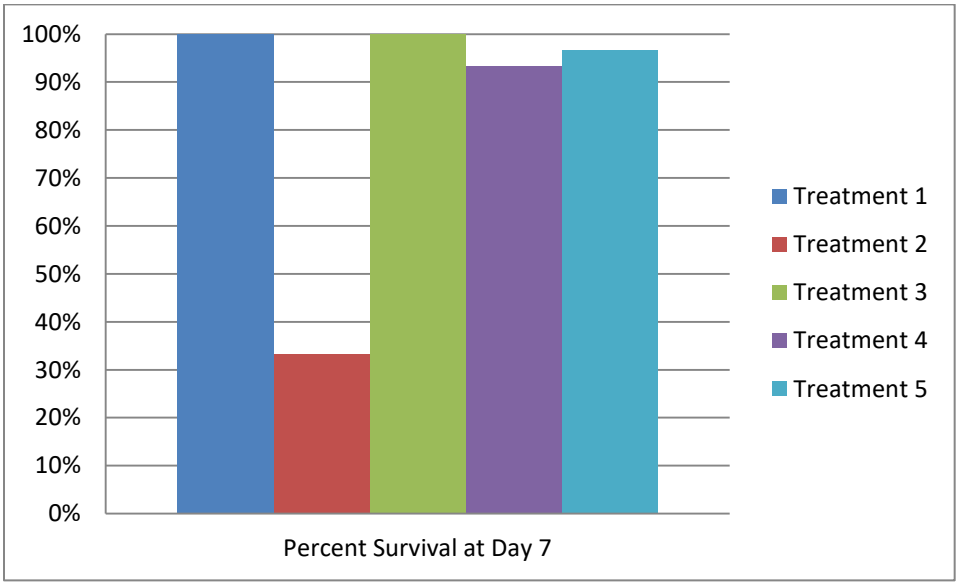
[a]As CaCO3
[b]Total residual chlorine

Test activities:

- Biological observations (primarily survival) taken daily.
- Chemistries taken on renewal days (i.e., pH, dissolved oxygen, and temperature).
- Conductivity was measured at test termination or when there was 0% survival in that treatment.
- Dry weights (±0.01 mg) were determined at test termination.

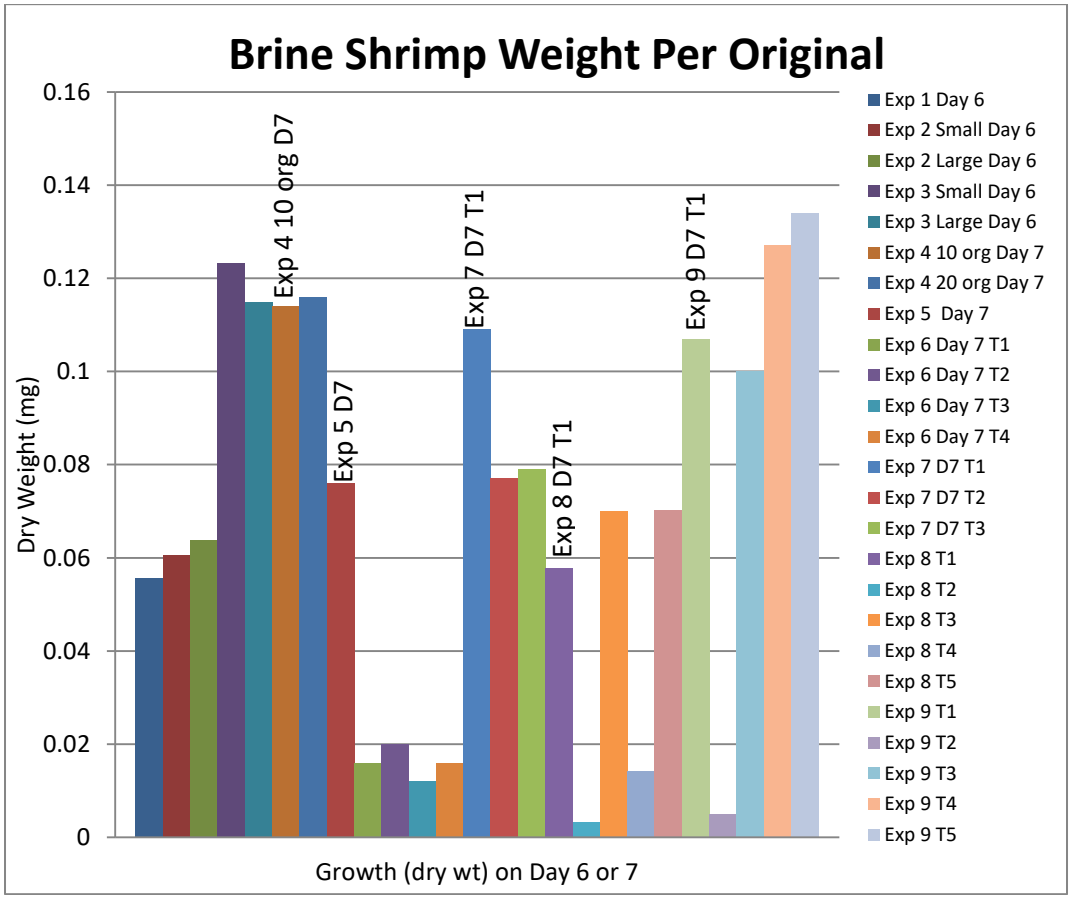
Results:

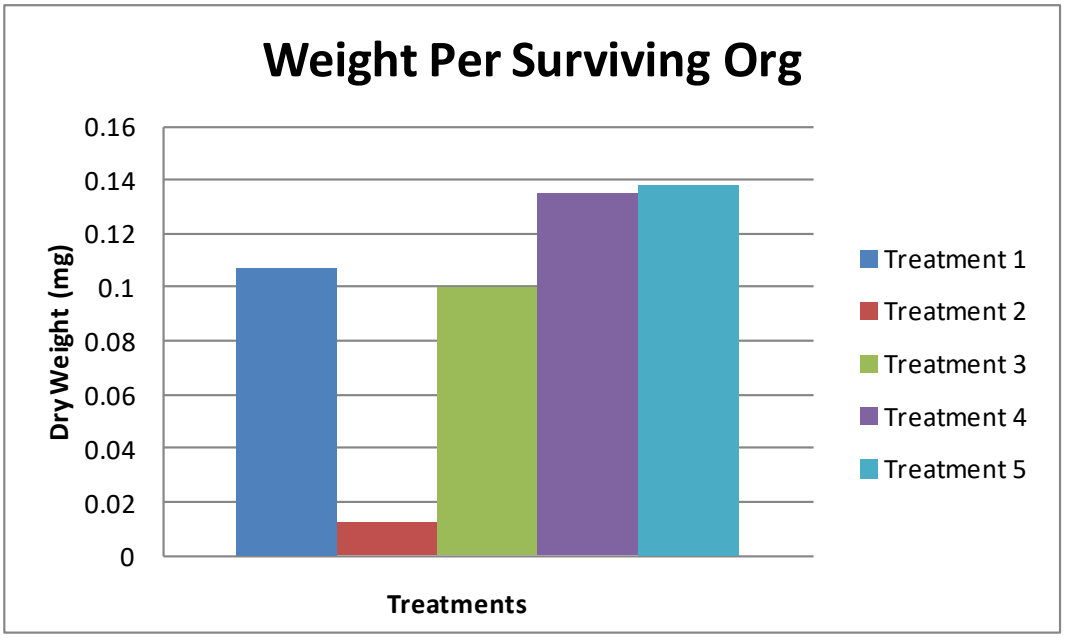
The survival of the brine shrimp in this study is illustrated in the following figure.



TRE

Survival in four of the treatments, 100% *D. viridis*, *D. viridis*/yeast mix, and *D. viridis*/YTC mixes met the 80% control performace threshold, survival in the yeast treatment did not.





TRE

Summary and findings:

- Organism survival was between 33% and 100% for all treatments,
- The yeast-fed treatment performed the worst and did not meet the target 80% survival threshold
    - This result is similar to what was observed in the prior experiment (see TRE report 14001-474-054)
    - Based on visual observation, the yeast appears to settle out in the rGSL water, which was probably limiting its availability to the brine shrimp.
- 7-day growth for the brine shrimp in the mixed food treatments had the following results
    - The yeast – *D. viridis* mixture had a 7% decrease compared to *D. viridis* alone
    - The first YTC – *D. viridis* mixture had a 15.7% increase compared to *D. viridis* alone
    - The second YTC – *D. viridis* mixture demonstrated an additional 5.2% increase over the first YTC - *D. viridis* mixture
- The 7 day growth for the yeast treatment was considerably lower than the full *D. viridis* treatment. Again, this was similar to the previous study (TRE report -054).
- All organisms in this study still transitioned from nauplii to juvenile between test days 3 and 4.

Based on these, and previous results, feeding with only yeast has proven to be a poor food source for *A. franciscana* compared to *D. viridis* alone, providing inadequate nutrition over the course of the test. Reducing the *D. viridis* concentration and supplementing with yeast or YTC appears to be a viable feeding option as organisms in these treatments had similar or higher growth than the *D. viridis* alone. There was not a significant increase in growth with the higher YTC amount, suggesting that the lower amount appears to be adequate.

With this study completed, we are proposing to set the feeding regime in the test method we are developing to a mixture of YTC – *D. viridis*. We recommend this for two reasons: 1) organism weight appeared to increase with the addition of a relatively small amount of YTC and 2) this will help reduce dependency of a relatively slow growing algal species / and thus facilitate testing (and for other potential laboratories in the future).

We greatly appreciate the opportunity to complete this study for you. Please do not hesitate to call if you have any questions regarding this study.

Sincerely,

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

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

14001-474-056

Attachment

cc: David Pillard, TRE

TRE

Summary and findings:

- Organism survival was between 33% and 100% for all treatments,
- The yeast-fed treatment performed the worst and did not meet the target 80% survival threshold
    - This result is similar to what was observed in the prior experiment (see TRE report 14001-474-054)
    - Based on visual observation, the yeast appears to settle out in the rGSL water, which was probably limiting its availability to the brine shrimp.
- 7-day growth for the brine shrimp in the mixed food treatments had the following results
    - The yeast – *D. viridis* mixture had a 7% decrease compared to *D. viridis* alone
    - The first YTC – *D. viridis* mixture had a 15.7% increase compared to *D. viridis* alone
    - The second YTC – *D. viridis* mixture demonstrated an additional 5.2% increase over the first YTC - *D. viridis* mixture
- The 7 day growth for the yeast treatment was considerably lower than the full *D. viridis* treatment. Again, this was similar to the previous study (TRE report -054).
- All organisms in this study still transitioned from nauplii to juvenile between test days 3 and 4.

Based on these, and previous results, feeding with only yeast has proven to be a poor food source for *A. franciscana* compared to *D. viridis* alone, providing inadequate nutrition over the course of the test. Reducing the *D. viridis* concentration and supplementing with yeast or YTC appears to be a viable feeding option as organisms in these treatments had similar or higher growth than the *D. viridis* alone. There was not a significant increase in growth with the higher YTC amount, suggesting that the lower amount appears to be adequate.

With this study completed, we are proposing to set the feeding regime in the test method we are developing to a mixture of YTC – *D. viridis*. We recommend this for two reasons: 1) organism weight appeared to increase with the addition of a relatively small amount of YTC and 2) this will help reduce dependency of a relatively slow growing algal species / and thus facilitate testing (and for other potential laboratories in the future).

We greatly appreciate the opportunity to complete this study for you. Please do not hesitate to call if you have any questions regarding this study.

Sincerely,

Amanda Bidlack
Project Specialist / QA Officer
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Rami B. Naddy, Ph.D.
Manager / Environmental Toxicologist
naddyrb.tre@gmail.com

14001-474-056

Attachment

cc: David Pillard, TRE

CDA NSV 3/26/20

TOXICITY DATA PACKAGE COVER SHEET

056

Test Type: Chronic

Project Number: 17001-474-~~054~~ ①

Test Substance: Various

Species: *Artemia franciscana*

Dilution Water: rGSL

Organism Lot or Batch Number: 036820

Concurrent Control Water: NA

Age: ~~48~~ (48 hr)

Supplier: TRE

Date and Time Test Began: 3/16/20 @ 1415

Date and Time Test Ended: 3/17/20 @ 1500

Protocol Number: ____

Investigator(s): BC/EN/EE/MS/CP/AS

Background Information

Type of Test: Static-Renewal (Daily)

pH control?: Yes **No**

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: 3

Length of Test: 7 days

Organisms per Replicate: 10

Type of Food and Quantity per Chamber: Varies

Feeding Frequency: 1 x daily

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): See Below

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 100 ug/L Chla
Treatment 1: *D. viridis* (145 ug/L Chla)- 2.6 ml
Treatment 2: Yeast (0.4ml 15.4g/L)
Treatment 3: 1/2 *D. viridis* (1.3 ml) 1/2 yeast (0.4 ml)
Treatment 4: 1/2 D. viridis (1.3 ml) 1/2 cerio YTC (0.33 ml)
Treatment 5: 1/2 *D. viridis* (1.3 ml) 1/2 cerio YTC (0.66 ml)
Appropriate correction factors have been applied to all temperatures recorded in this data package
Study Director Initials: AS Date: 3/10/20

① AS 3/26/20E

QA rw 3/26/20

TEST SUBSTANCE USAGE LOG

Project Number: 17001-474-05①6

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number				
Test Substance Collection Date and Time	From: @ To: @	From: @ To: @	From: @ To: @	From: @ To: @
Sample Type (Grab or Comp)				
Date Test Substance Received				
Dilution Water Number RW# or TRE#, circle one	13582 13876*			
Concurrent Control Water RW#	NA			
Date(s) Used	3/10/20 3/14/20* 3/11/20 3/15/20* 3/12/20 3/16/20* 3/13/20*			

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)
1	0	170	170						
2	0	170	170						
3	0	170	170						
4	0	170	170						
5	0	170	170						
	0	850	850						

Initials / Date	BS 3/10/20 Mixed/BS		
Initials / Date	EE 3/11/20 "		
Initials / Date	AS 3/12/20 "		
Initials / Date	CP 3/13/20 "		
Initials / Date	CP 3/14/20 "		
Initials / Date	CP 3/15/20 "		
Initials / Date	BS 3/16/20 "		
Initials / Date			

①RW 3/26/20

QA new 3/26/20

Artemia franciscana
CHRONIC BIOLOGICAL DATA

Project Number: 17001-474-054 ① 6

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)
1	A	10	10	10	10	10	10	10	10	100
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D ①	~~10~~	—	—	—	—	—	—	—	
2	A	10	10	10	10	7*	4*	1*	1	* very small, weak orgs 33.3
	B	10	10	9^	9	9	8*	5*	3	^ 1 NF; * " "
	C	10	10	10	9	9	9*	9*	6	* " "
	D	—	—	—	—	—	—	—	—	
3	A	10	10	10	10	10	10	10	10	100
	B	10	10	10 ¤	10+	10	10	10	10	¤ 2 extra org removed + removed 1 extra org
	C	10	10	10	—*	—	—	—	—	* no orgs; TE
	D	—	—	—	—	—	—	—	—	
4	A	10	10	10	10	10	10	10	10	93.3
	B	10	② ~~10~~ 9	8 ^②	8	8	8	8	8	~~^ 1 NF~~ ②
	C	10	~~9~~ 10	10	10	10	10	10	10	
	D	—	—	—	—	—	—	—	—	
5	A	10	9	9	9	9	9	9	9	96.7
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D	—	—	—	—	—	—	—	—	
	A									
	B									
	C									
	D	—								
	A									
	B									
	C									
	D									
	Date:	3/10/20	3/11/20	3/12/20	3/13/20	3/14/20	3/15/20	3/16/20	3/17/20	
	Time:	1415	1500	1520	1515	1045	1130	1335	③ ~~1630~~ 1500	
	Initials:	BJ/EN	ee	BJ	CP	CP	CP	BJ	ee	

① BJ 3/10/20 WP ② ee 3/12/20 E ③ ee 3/17/20 E

CHRONIC CHEMICAL DATA (INITIAL)

QA ww 3/26/20

Project Number: 17001-474-054

Test Species: ***Artemia franciscana***

%	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter #	Remarks
Conc.: rGSL									All Conc.	
pH	7.8	7.9	7.9	7.9	8.0	8.0	7.8		FM29	
D.O. (mg/L)	5.6	5.1	5.5	5.2	5.2	5.0	5.5		17	
Temp. (°C)	20	20	20	20	20	20	20		L-39	
Cond. (µS/cm)	144,800	137400	135200	137700	137700	138700	177300		15	
Hard. (mg/L)									Titr	
Alk. (mg/L)									Titr	
TRC (mg/L)									22	
NH_3 (mg/L)									HA1	
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Date:	3/10/20	3/11/20	3/12/20	3/13/20	3/14/20	3/15/20	3/16/20			
Time:	1410	1500	1515	1450	1030	1125	1336			
Initials:										

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.

DA nw 3/26/20

CHRONIC CHEMICAL DATA (FINAL)

Project Number:	17001-474-054
Test Species:	***Artemia franciscana***

%		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter #	Remarks
Conc.:	1							126800		All Conc.	* conductivity
pH		7.9	7.9	7.8	7.9	8.0	7.9	7.7		FM29	
D.O. (mg/L)		5.6	5.2	5.2	5.2	5.3	5.1	5.6		17	
Temp (°C)		20	20	21	19	19	19	21		L-37	
Conc.:	2							123800			* conductivity
pH		7.8	7.7	7.8	7.8	7.9	7.9	7.6			
D.O. (mg/L)		5.0	5.0	4.7	5.1	4.7	4.7	3.3			
Temp (°C)		20	20	22▲	19	19	19	21			
Conc.:	3							123900			* conductivity
pH		7.8	7.8	7.8	7.8	7.9	7.7	7.7			
D.O. (mg/L)		4.8	4.9	5.5	①5.4.8	4.1	3.7	3.1			
Temp (°C)		20	20	21	19	19	19	21			
Conc.:	4							122200			* conductivity
pH		7.9	7.9	7.8	7.9	8.0	7.8	7.6			
D.O. (mg/L)		5.1	4.9	5.0	5.1	5.0	4.1	3.7			
Temp (°C)		20	20	21	19	19	19	21			
Conc.:	5							121200			* conductivity
pH		7.9	7.8	7.8	7.8	8.0	7.8	7.6			
D.O. (mg/L)		5.1	4.9	4.8	4.7	5.1	4.1	4.1			
Temp (°C)		20	20	21	19	19	19	21			
Conc.:											
pH											
D.O. (mg/L)											
Temp (°C)											
Conc.:											
pH											
D.O. (mg/L)											
Temp (°C)											
Date:		3/11/20	3/12/20	3/13/20	3/14/20	3/15/20	3/16/20	3/17/20			
Time:		1500	1520	1550	1100	1150	1350	1530			
Initials:		ee	A	cp	cp	cp	RF	ee			

▲checked all reps
①cp 3/14/20 E

QA rev 3/26/20

DAILY TOXICITY TEST LOG

Project Number:	17001-474-054
Test Species:	***Artemia franciscana***

General Comments					Feeding	Initials/Date
	Random Chart: ~~M-150~~ D		Min/Max Thermometer #	M-15		
Test Day 0	Test Solution Mixed at: 1400 Test Organisms Added at: 1415				Fed @ 1405	BF 3/10/20
Test Day 1	Real Time: 20	°C	Min-Max Range: 18 - 21	°C	Fed @ 1430	ee 3/11/20
Test Day 2	Real Time: 20	°C	Min-Max Range: 20 - 22	°C	Fed @ 1515	AB 3/12/20
Test Day 3	Real Time: 20	°C	Min-Max Range: 19 - 22	°C	Fed @ 1510	cp 3/13/20
Test Day 4	Real Time: 19	°C	Min-Max Range: 19 - 21	°C	Fed @ 1035	cp 3/14/20
Test Day 5	Real Time: 19	°C	Min-Max Range: 18-21	°C	Fed @ 1120	cp 3/15/20
Test Day 6	Real Time: 19	°C	Min-Max Range: 19-21	°C	Fed @ 1330	BF 3/16/20
Test Day 7	Real Time: 19	°C	Min-Max Range: 19 - 21	°C	Fed @ NONE	ee 3/17/20
Test Day 8	Real Time:	°C	Min-Max Range:	°C	Fed @	

D BF 3/10/10 WP

QA Form No.010
Revision 7
Effective 01/20

QA rev 3/26/20

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 17001-474-054
Species: Artemia franciscana
Date/Time of Tare Wt.: 3/17/20 @1435

Test Substance:
Analyst Tare: SK Analyst Gross: AS
Date/Time of Gross Wt.: 3/20/20 @ 1600

Comments:
Analytical Balance ID: Sart #1
Dried in Oven # 3 from Date: 3/17/20 Time: 1556
to Date: 3/19/20 Time: 1140

Weight Type (Circle): Wet Blot Dry (Dry (60-90°C)) Dry (>100°C) AFDW (>500°C)

Boat No.	Treatment	Rep.	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)[1]	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	Lot or Batch Number: No. of Surv. Organisms	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)
	1	A		1.14181	1.14273	0.00092					10		
		B		1.13959	1.14068	0.00109					10		
		C		1.13960	1.14081	0.00121					10		
	2	A		1.11607	1.11607						1		
		B		1.13854	1.13859	0.00005					3		
		C		1.11517	1.11528	0.00011					6		
	3	A		1.14436	1.14531	0.00095					10		
		B		1.13805	1.13910	0.00105					10		
		~~C~~		~~1.13267~~									
Blank				1.14947	1.14950								
Range													
Mean													

Test Solution Volume:
Loading Rate:

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

QA Form No.010
Revision 7
Effective 01/20

DLW 3/26/20

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 17001-474-054	Test Substance:	Comments: Analytical Balance ID: Sart #1
Species: Artemia franciscana	Analyst Tare: SK Analyst Gross: AS	Dried in Oven # 3 from Date: 3/17/20 Time: 1510 to Date: 3/19/20 Time: 1140
Date/Time of Tare Wt.: 3/17/20 @1435	Date/Time of Gross Wt.: 3/20/20 @1600	

Weight Type (Circle): Wet Blot Dry (Dry (60-90°C)) Dry (>100°C) AFDW (>500°C)

Boat No.	Treatment	Rep.	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)[1]	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	Lot or Batch Number: No. of Surv. Organisms	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)
	4	A		1.14155	1.14300	0.00145					10		
		B		1.14008	1.14107	0.00099					8		
		C		1.13841	1.13977	0.00136					10		
		A		1.14172	1.14297	0.00125					9		
	5	B		1.13205	1.13334	0.00129					10		
		C		1.14951	1.15098	0.00147					10		
Blank													
Range													
Mean													

Test Solution Volume: Loading Rate:

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

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 14001-474 Exp 9 Species: Artemia franciscana QA nnn 3/26/20

Treatment	Rep	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)	No of Orig. Organisms	Mean Wt./ Original Organism (mg)	Mean Wt./ Treatment (mg) (Original)	Number of Surv. Organisms	Mean Wt./ Surviving Organism (mg)	Mean Wt./ Treatment (mg) (Surviving)
1	A		1.14181	1.14273	0.00092	0.00092	10	0.092	0.1073	10	0.092	0.1073
	B		1.13959	1.14068	0.00109	0.00109	10	0.109		10	0.109	
	C		1.13960	1.14081	0.00121	0.00121	10	0.121		10	0.121	
	D											
2	A		1.11607	1.11607	0.00000	0.00000	10	0.000	0.0053	1	0.000	0.0117
	B		1.13854	1.13859	0.00005	0.00005	10	0.005		3	0.017	
	C		1.11517	1.11528	0.00011	0.00011	10	0.011		6	0.018	
	D											
3	A		1.14436	1.14531	0.00095	0.00095	10	0.095	0.1000	10	0.095	0.1000
	B		1.13805	1.13910	0.00105	0.00105	10	0.105		10	0.105	
	C		0.00000	0.00000	0.00000	0.00000	0			0	-	
	D											
4	A		1.14155	1.14300	0.00145	0.00145	10	0.145	0.1267	10	0.145	0.1349
	B		1.14008	1.14107	0.00099	0.00099	10	0.099		8	0.124	
	C		1.13841	1.13977	0.00136	0.00136	10	0.136		10	0.136	
	D											
5	A		1.14172	1.14297	0.00125	0.00125	10	0.125	0.1337	9	0.139	0.1383
	B		1.13205	1.13334	0.00129	0.00129	10	0.129		10	0.129	
	C		1.14951	1.15098	0.00147	0.00147	10	0.147		10	0.147	
	D											
Blank			1.14947	1.14950	0.00003							

Project Number: 14001-474 Exp 9 Species: Artemia franciscana

QA nw 3/26/20

Summary Statistics for Survival Data

Treatment	N	Min	Max	Mean	SD	C.V.
1	3	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
200.00%	3	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
300.0%	3	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
400%	3	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
500.0%	3	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
0%	0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Summary Statistics for Growth Data (dry wt per original)

Treatment	N	Min	Max	Mean	SD	C.V.
1	3	0.092	0.121	0.1073	0.0146	13.576%
200.00%	3	0.000	0.011	0.0053	0.0055	103.267%
300.0%	2	0.095	0.105	0.1000	0.0071	7.071%
400%	3	0.099	0.145	0.1267	0.0244	19.247%
500.0%	3	0.125	0.147	0.1337	0.0117	8.767%
0%	0	0.000	0.000	0.0000	#DIV/0!	#DIV/0!

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

Treatment	N	Min	Max	Mean	SD	C.V.
1	3	0.092	0.121	0.1073	0.0146	13.576%
200.00%	3	0.000	0.018	0.0117	0.0101	86.897%
300.0%	2	0.095	0.105	0.1000	0.0071	7.071%
400%	3	0.124	0.145	0.1349	0.0107	7.906%
500.0%	3	0.129	0.147	0.1383	0.0090	6.518%
0%	0	0.000	0.000	0.0000	#DIV/0!	#DIV/0!

TRE Environmental Strategies, LLC
100 Racquette Drive, Unit A, Fort Collins, Colorado, 80524
T 970.416.0916 F 970.490.2963

April 20, 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 Short-term Chronic Brine Shrimp Experiment #11

Mr. Bittner/ Dr. Belovsky:

Below is a summary of the short-term chronic brine shrimp experiment initiated on April 1, 2020. The purpose of this experiment was to investigate whether the performance of *Artemia franciscana* controls was affected by salinity.

Along with a 120 ppt control and an algae-only concurrent control, three salinities were tested:

- Treatment 1: 25 ppt
- Treatment 2: 56 ppt
- Treatment 3: 88 ppt
- Treatment 4: 120 ppt
- Treatment 5: 120 ppt, fed algae only

The results of these studies will help determine the applicability of this short-term chronic toxicity test at lower salinities that may occur in the Great Salt Lake.

Species: *Artemia franciscana*

Test type:

- Test duration: 7 days
- Test type: static-renewal (solutions and food renewed daily)
- Algae: *Dunaliella viridis*
- Algae concentration: 145 µg/L Chl*a*, or 72.5 µg/L Chl*a* and 0.33 ml/ chamber YTC[1]
- Temperature: 20°C
- Test volume(s): 50 ml
- Replicates: 4
- Organisms/Rep: 10
- Test media: Various (see above)

[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 100 µg/L Chl*a*. 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#13888	8.0	NM	NM	135,400	NM	NM	120

[a]As CaCO3
[b]Total residual chlorine

Test activities:

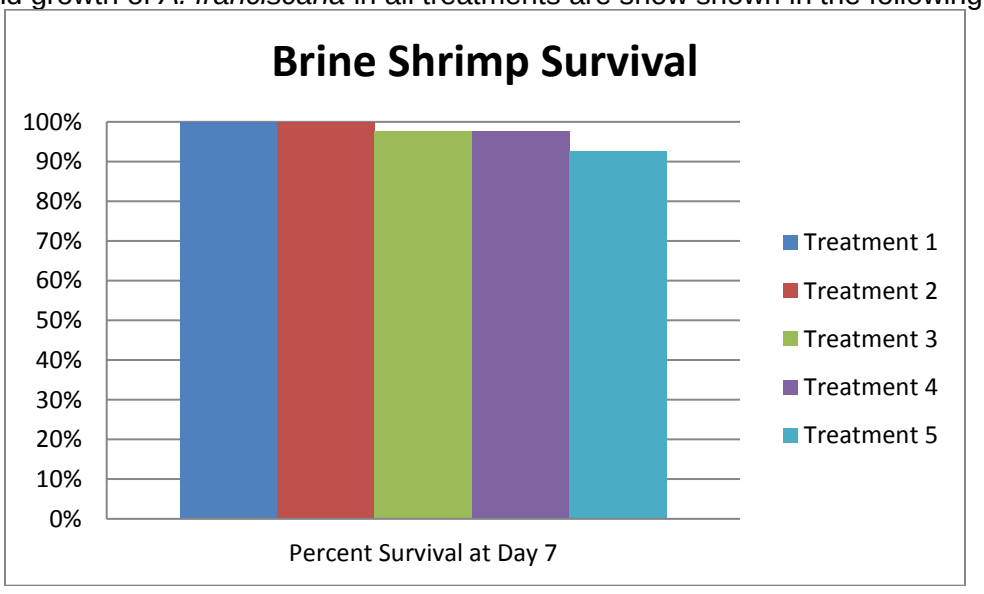
- Biological observations (primarily survival) taken daily.
- Chemistries taken on renewal days (i.e., pH, dissolved oxygen, and temperature).
- Salinity was measured daily in new solutions.
- Conductivity was measured at test termination or when there was 0% survival in that treatment.
- Dry weights were determined at test termination.

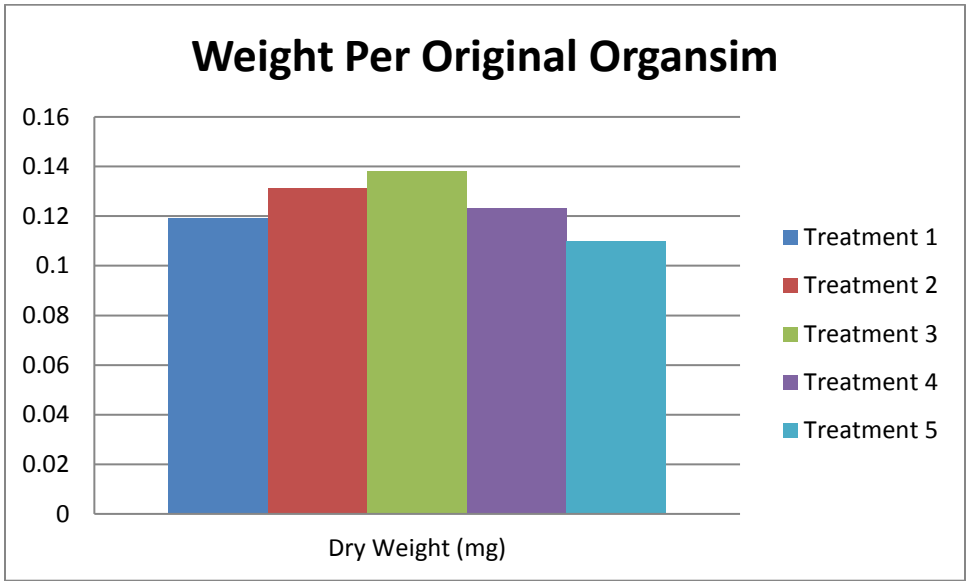
Results:

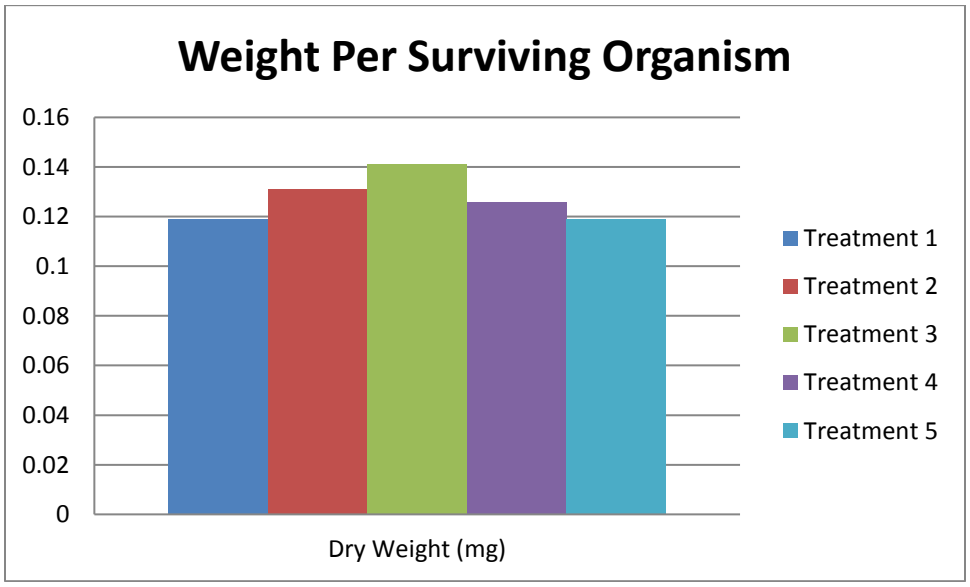
The results of the two control treatments (fed only *D. viridis* and fed a mixture of *D. viridis*/YTC) are shown in the following table. Survival and growth (dry wt) were similar between the two treatments.

Treatment	Survival	Weight Per Original Org	Weight Per Surviving Org
D. viridis/ YTC	97.5%	0.123 mg	0.126 mg
D. viridis only	92.5%	0.110 mg	0.119 mg

Survival and growth of *A. franciscana* in all treatments are show shown in the following figures.







Summary and findings:

- Organism survival was ≥ 90% for all treatments.
- Growth was very similar between the *D. viridis* -only treatment and the *D. viridis* + YTC treatment.
    - The *D. viridis* – YTC mixture had an 11% increase in growth compared to *D. viridis* alone
- Growth was similar across all salinities measured (13% difference from highest to

lowest). This suggests that the method is applicable across a range of salinities, from 25 – 120 ppt.

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

14001-474-060

Attachment

cc: David Pillard, TRE

TOXICITY DATA PACKAGE COVER SHEET

① 060

Test Type: Chronic

Project Number: 17001-474-~~Exp~~

Test Substance: Platymonas

Species: *Artemia franciscana*

Dilution Water: ~~rGSL~~ Milli-Q

Organism Lot or Batch Number: 033020

Concurrent Control Water: NA

Age: 48 Hr (48 hr)

Supplier: TRE

Date and Time Test Began: 4/1/20 @1355

Date and Time Test Ended: 4/8/20 @1340

Protocol Number: ____

Investigator(s): CP/AB/EN

Background Information

Type of Test: Static-Renewal (Daily)

pH control?: Yes **No**

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: 7 days

Organisms per Replicate: 10

Type of Food and Quantity per Chamber: D. viridis/ YTC

Feeding Frequency: 1 x daily

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): See Below

Agency Summary Sheet(s)?: None

Reference Toxicant Data: Test Dates: ~ to ~ 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 100 ug/L Chla

Treatment 1: 25 ppt Treatment 1-4: Algae 1.5 ml 0.33 ml YTC

Treatment 2: 56 ppt

Treatment 3: 88 ppt

Treatment 4: 120 ppt

Treatment 5: 120 ppt Algae only: 3.1 ml per cup

Appropriate correction factors have been applied to all temperatures recorded in this data package

Study Director Initials: AB Date: 4/1/20

① New 4/20/20 CF

QA rev 4/20/20

TEST SUBSTANCE USAGE LOG

Project Number: 17001-474-Exp

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number	13888			
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	② ~~13888~~			
Concurrent Control Water RW#	NA			
Date(s) Used	4/1/20 | 4/5/20			
	4/2/20 | 4/6/20			
	4/3/20 | 4/7/20			
	4/4/20			

rGS MQ

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)
25ppt	52	198	250						
56ppt	117	133	250						
88ppt	183	67	250						
120ppt	25 0	~~250~~ 0	250						
120 algae	25 0	~~250~~ 0	250						
	8	398							
	~~352~~ 0	~~898~~ 0	1250						

Initials / Date	CP 4/1/20 Mixed B.S.		
Initials / Date	CP 4/2/20 " "		
Initials / Date	CP 4/3/20 " "		
Initials / Date	CP 4/4/20 " "		
Initials / Date	RY 4/5/20 " "		
Initials / Date	EN 4/6/20 " "		
Initials / Date	CP 4/7/20 " "		
Initials / Date			

① MS 4/1/20 E ② EN 4/6/20 E

QA rev 4/24/20

Artemia franciscana
CHRONIC BIOLOGICAL DATA

Project Number: 17001-474-Exp

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)
25ppt	A	10	10	10	10	10	10	10	10	100
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
56ppt	A	10	10	10	10	10	10	10	10	100
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10*	10*	* 1 very small Bg.
88ppt	A	10	10	10	10	10	10	10	10	97.5
	B	10	10	10	9	9	9	9	9	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
120ppt	A	10	10	10	10	10	9	9	9	97.5
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
120 algae	A	10	10	10	10	10	①9 10	10	10	92.5
	B	10	10	10	10	10	9	9	8	
	C	10	10+	10	10	10	10	10	10	+ removed extra org
	D	10	10	10	10	10	10	10	9	
	A									
	B									
	C									
	D									
	A									
	B									
	C									
	D									
Date:		4/1/20	4/2/20	4/3/20	4/4/20	4/5/20	4/6/20	4/7/20	4/8/20	
Time:		1355	1355	1350	1100	1430	1445	1545	1340	
Initials:		CP/AT	CP	CP	CP	BJ	EN	CP	EN	

① EN 4/6/20 WP

QA NW 4/20/20

CHRONIC CHEMICAL DATA (INITIAL)

Project Number: 17001-474-Exp

Test Species: ***Artemia franciscana***

%	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter #	Remarks
Conc.: 25ppt									All Conc.	
pH	8.5	8.5	8.4	8.5	8.4	8.5	8.5		FM30	
D.O. (mg/L)	4.9	5.1	5.2	4.9	4.6	4.8	4.9		17	
Temp. (°C)	*	*	*	*	*	*	*		L-39	
Cond. (µS/cm)	40,400	41,200	40,900	41,500	40,500	41600	42,000		15	
Salinity (ppt)	25	25	25	25	25	25	25		1	
Conc.: 56ppt										
pH	8.3	8.4	8.2	8.4	8.3	8.3	8.3			
D.O. (mg/L)	5.1	5.2	5.3	5.1	4.7	5.0	5.1			
Temp. (°C)	*	*	*	*	*	x	*			
Cond. (µS/cm)	80,200	80,700	80,800	82,400	80,200	88100	83,200			
Salinity (ppt)	56	57	56	57	57	56	57			
Conc.: 88ppt										
pH	8.2	8.2	8.0	8.2	8.1	8.1	8.1			
D.O. (mg/L)	5.1	5.3	5.4	5.2	4.8	5.0	5.1			
Temp. (°C)	*	*	*	*	*	*	*			
Cond. (µS/cm)	113,900	115,100	115,000	117,600	115,100	126600	119,700			
Salinity (ppt)	88	89	88	89	89	87	88			
Conc.: 120ppt										
pH	8.0	8.0	7.8	8.0	7.9	7.9	8.0			
D.O. (mg/L)	5.2	5.2	5.3	5.2	4.9	5.0	5.0			
Temp. (°C)	20	20	20	20	20	20	20			
Cond. (µS/cm)	135,400	~~136,100~~ ①	136,800	~~138~~,200	135,200	150900	139,400			
Salinity (ppt)	120	120	121	121	121	120	121			
Date:	4/1/20	4/2/20	4/3/20	4/4/20	4/5/20	4/6/20	4/7/20			
Time:	1340	1325	1335	1035	1415	1415	1440			
Initials:	CP	CP	CP	CP	BS	EN	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 25°C prior to making the dilution series. The temperature of resulting effluent dilution is assumed to also be 25°C.

① 136,100
CP 4/2/20 E

QA new 4/20/20

CHRONIC CHEMICAL DATA (FINAL)

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

%	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter #	Remarks
Conc.: 25ppt							45700		All Conc.	* conductivity 15
pH	7.8	7.7	7.8	7.6	7.4	7.3	7.3		FM30	
D.O. (mg/L)	4.8	4.9	4.7	4.7	4.95①	4.3	4.6		17	
Temp (°C)	20	19	20	21	20	20	20		L-36	
Conc.: 56ppt							84500			* conductivity
pH	8.0	8.0	8.0	7.7	7.7	7.7	7.5			
D.O. (mg/L)	4.8	5.0	4.9	4.8	4.4	4.4	4.5			
Temp (°C)	20	19	20	20	20	20	20			
Conc.: 88ppt							120800			* conductivity
pH	8.1	8.1	8.1	8.0	7.9	7.8	7.5			
D.O. (mg/L)	4.9	5.0	4.9	4.8	4.5	4.5	4.5			
Temp (°C)	20	19	20	21	20	20	20			
Conc.: 120ppt							131,100			* conductivity
pH	8.0	8.0	8.0	7.9	7.9	7.8	7.7			
D.O. (mg/L)	5.0	5.0	5.0	4.8	4.6	4.5	4.6			
Temp (°C)	20	19	20	21	20	20	20			
Conc.: 120 algae							130600			* conductivity
pH	8.0	8.0	8.1	8.0	7.9	7.9	7.7			
D.O. (mg/L)	5.2	5.2	5.1	5.2	4.7	4.6	4.8			
Temp (°C)	20	19	20	21	20	20	20			
Conc.:										
pH										
D.O. (mg/L)										
Temp (°C)										
Conc.:										
pH										
D.O. (mg/L)										
Temp (°C)										
Date:	4/2/20	4/3/20	4/4/20	4/5/20	4/6/20	4/7/20	4/8/20			
Time:	1415	1405	1120	1500	1505	1605	1340			
Initials:	CP	CP	CP	BF	EN	CP	EN			

①EN 4/6/20 E "4.5"

RA MW 4/20/20

DAILY TOXICITY TEST LOG

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

General Comments	Random Chart: K Min/Max Thermometer # M-15				Feeding	Initials/Date
Test Day 0	Test Solution Mixed at: 1335 Test Organisms Added at: 1355				Fed @ 1355	CP 4/1/20
Test Day 1	Real Time: 20	°C	Min-Max Range: 19-23	°C	Fed @ 1355	CP 4/2/20
Test Day 2	Real Time: 19	°C	Min-Max Range: 19-21	°C	Fed @ 1335	CP 4/3/20
Test Day 3	Real Time: 20	°C	Min-Max Range: 19-22	°C	Fed @ 1055	CP 4/4/20
Test Day 4	Real Time: 20	°C	Min-Max Range: 20-23	°C	Fed @ 1405	CP 4/5/20
Test Day 5	Real Time: 19	°C	Min-Max Range: 19-22	°C	Fed @1430	EN 4/6/20
Test Day 6	Real Time: 19	°C	Min-Max Range: 19-22	°C	Fed @1545	CP 4/7/20
Test Day 7	Real Time: 20	°C	Min-Max Range: 19-23	°C	Fed @ NONE	EN 4/8/20
Test Day 8	Real Time:	°C	Min-Max Range:	°C	Fed @	

QA new 4/20/20

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 17001-474-Exp	Test Substance: ~~Platymonas~~ Brine shrimp	Comments: Analytical Balance ID: Sart #1
Species: Artemia franciscana	Analyst Tare: EN Analyst Gross: AF	Dried in Oven # 3 from Date: 4/6/20 Time: 1410 to Date: 4/10/20 Time: 0630
Date/Time of Tare Wt.: 4/8/20 @1315	Date/Time of Gross Wt.: 4/10/20 @1040	

Weight Type (Circle): Wet Blot Dry (Dry (60-90°C)) Dry (>100°C) AFDW (>500°C) Lot or (Batch) Number: 033020

Boat No.	Treatment	Rep.	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)[1]	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	No. of Surv. Organisms	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)
	1 (25ppt)	A		1.14228	1.14360	0.00132					10		
		B		1.14422	1.14512	0.00090					10		
		C		1.13775	1.13913	0.00138					10		
		D		1.14425	1.14541	0.00116					10		
	2 (56ppt)	A		1.13572	1.13719	0.00147					10		
		B		1.14257	1.14379	0.00122					10		
		C		1.12977	1.13101	0.00124					10		
		D		1.12264	1.12396	0.00132					10		
	3 (88ppt)	A		1.12702	1.12859	0.00157					10		
		B		1.13551	1.13689	0.00138					9		
		C		1.14664	1.14787	0.00123					10		
		D		1.14016	1.14148	0.00132					10		
Blank				1.14026	1.14027	+0.00001							
Range													
Mean													

Test Solution Volume:	Loading Rate:

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

D new 4/20/20 CF

QA rev 4/20/20

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 17001-474-Exp	Test Substance: ~~Platymonas~~ Brine shrimp	Comments:
Species: Artemia franciscana	Analyst Tare: EN Analyst Gross: AF	Analytical Balance ID: Sart #1
Date/Time of Tare Wt.: 4/8/20@1315	Date/Time of Gross Wt.: 4/10/20 @1040	Dried in Oven # 3 from Date: 4/6/20 Time: 1410 to Date: 4/10/20 Time: 0630

Weight Type (Circle): Wet Blot Dry Dry (60-90°C) Dry (>100°C) AFDW (>500°C) Lot or Batch Number: 033020

Boat No.	Treatment	Rep.	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)[1]	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	No. of Surv. Organisms	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)
	4(120 ppt)	A		1.14322	1.14457	0.00135					9		
		B		1.12961	1.13088	0.00127					10		
		C		1.13120	1.13225	0.00105					10		
		D		1.13313	1.13437	0.00124					10		
	5(120 ppt)	A		1.13025	1.13168	0.00143					10		
		B		1.14867	1.14960	0.00093					8		
		C		1.11250	1.11353	0.00103					10		
		D		1.12886	1.12987	0.00101					9		
Blank													
Range													
Mean													

Test Solution Volume:	Loading Rate:

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

(2) rev 4/20/20 E

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 14001-474 Species: Artemia franciscana

DA mc 4/20/20

Treatment	Rep	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)	No of Orig. Organisms	Mean Wt./ Original Organism (mg)	Mean Wt./ Treatment (mg) (Original)	Number of Surv. Organisms	Mean Wt./ Surviving Organism (mg)	Mean Wt./ Treatment (mg) (Surviving)
25 ppt	A		1.14228	1.14360	0.00132	0.00132	10	0.132	0.1190	10	0.132	0.1190
	B		1.14422	1.14512	0.00090	0.00090	10	0.090		10	0.090	
	C		1.13775	1.13913	0.00138	0.00138	10	0.138		10	0.138	
	D		1.14425	1.14541	0.00116	0.00116	10	0.116		10	0.116	
56 ppt	A		1.13572	1.13719	0.00147	0.00147	10	0.147	0.1312	10	0.147	0.1312
	B		1.14257	1.14379	0.00122	0.00122	10	0.122		10	0.122	
	C		1.12977	1.13101	0.00124	0.00124	10	0.124		10	0.124	
	D		1.12264	1.12396	0.00132	0.00132	10	0.132		10	0.132	
88 ppt	A		1.12702	1.12859	0.00157	0.00157	10	0.157	0.1375	10	0.157	0.1413
	B		1.13551	1.13689	0.00138	0.00138	10	0.138		9	0.153	
	C		1.14664	1.14787	0.00123	0.00123	10	0.123		10	0.123	
	D		1.14016	1.14148	0.00132	0.00132	10	0.132		10	0.132	
120 ppt	A		1.14322	1.14457	0.00135	0.00135	10	0.135	0.1228	9	0.150	0.1265
	B		1.12961	1.13088	0.00127	0.00127	10	0.127		10	0.127	
	C		1.13120	1.13225	0.00105	0.00105	10	0.105		10	0.105	
	D		1.13313	1.13437	0.00124	0.00124	10	0.124		10	0.124	
120 ppt Algae only	A		1.13025	1.13168	0.00143	0.00143	10	0.143	0.1100	10	0.143	0.1186
	B		1.14867	1.14960	0.00093	0.00093	10	0.093		8	0.116	
	C		1.11250	1.11353	0.00103	0.00103	10	0.103		10	0.103	
	D		1.12886	1.12987	0.00101	0.00101	10	0.101		9	0.112	
Blank			1.14026	1.14027	0.00001							

Project Number: 14001-474 Species: Artemia franciscana

SD new 4/20/20

Summary Statistics for Survival Data

Treatment	N	Min	Max	Mean	SD	C.V.
25 ppt	4	1.0	1.0	1.0000	0.0000	0.000%
56 ppt	4	1.0	1.0	1.0000	0.0000	0.000%
88 ppt	4	0.9	1.0	0.9750	0.0500	5.128%
120 ppt	4	0.9	1.0	0.9750	0.0500	5.128%
120 ppt Algae only	4	0.8	1.0	0.9250	0.0957	10.351%

Summary Statistics for Growth Data (dry wt per original)

Treatment	N	Min	Max	Mean	SD	C.V.
25 ppt	4	0.090	0.138	0.1190	0.0214	18.023%
56 ppt	4	0.122	0.147	0.1312	0.0114	8.651%
88 ppt	4	0.123	0.157	0.1375	0.0144	10.464%
120 ppt	4	0.105	0.135	0.1228	0.0127	10.356%
120 ppt Algae only	4	0.093	0.143	0.1100	0.0224	20.382%

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

Treatment	N	Min	Max	Mean	SD	C.V.
25 ppt	4	0.090	0.138	0.1190	0.0214	18.023%
56 ppt	4	0.122	0.147	0.1312	0.0114	8.651%
88 ppt	4	0.123	0.157	0.1413	0.0165	11.645%
120 ppt	4	0.105	0.150	0.1265	0.0184	14.583%
120 ppt Algae only	4	0.103	0.143	0.1186	0.0172	14.479%

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May 6, 2020

Mr. Christopher Bittner
Standards Coordinator
Utah Dept. of Environmental Quality
195 N 1950 W
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Dr. Gary Belovsky
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University of Notre Dame
Notre Dame, IN 46556

Subject: Results of Short-term Chronic Brine Shrimp Experiment #12

Mr. Bittner/ Dr. Belovsky:

Below is a summary of the short-term chronic brine shrimp experiment initiated on April 22, 2020. The purpose of this experiment was to investigate whether the pre-test holding conditions would affect the performance of *Artemia franciscana* controls. The test conditions during the 7-day test period were all the same.

In previous studies, cysts were added to ~29 ppt artificial seawater (Crystal Sea Marine Mix). After 24 hours, approximately 200 newly hatched nauplii were isolated and held in 50 mL of gently aerating rGSL and fed one time a concentration of 100 µg/L Chl*a D. viridis* for the 48 hour pre-test period. In this study, five different pre-test treatments, all in 50 mL of rGSL, and all gently aerated, were examined:

- Treatment 1: 100 organisms, 100 µg/L Chl*a D. viridis*
- Treatment 2: 200 organisms, 100 µg/L Chl*a D. viridis*
- Treatment 3: 300 organisms, 100 µg/L Chl*a D. viridis*
- Treatment 4: 200 organisms, 72.5 µg/L Chl*a D. viridis* and 0.3 ml/ chamber YTC[1]
- Treatment 5: 200 organisms, 100 µg/L Chl*a D. viridis*, all organisms hatched within 8 hours of cysts being added to water

The results of these studies will help determine if pre-test holding conditions could impact variability in weight gain observed among studies or brine shrimp control response.

Species: *Artemia franciscana*

Test type:

- All conditions during the 7-day period were the same for all treatments
- Test duration: 7 days
- Test type: static-renewal (solutions and food renewed daily)
- Algae: *Dunaliella viridis*

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

- Algae/ Food concentration: 72.5 µg/L Chl*a D. viridis* and 0.3 ml/ chamber YTC[1]
- Temperature: 20°C
- Test volume(s): 50 ml
- Replicates: 4
- Organisms/Rep: 10
- Test media: 120 ppt rGSL media (per Notre Dame recipe)

Pretest conditions: Various (see above)

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#13888	8.0	NM	NM	135,800	NM	NM	124

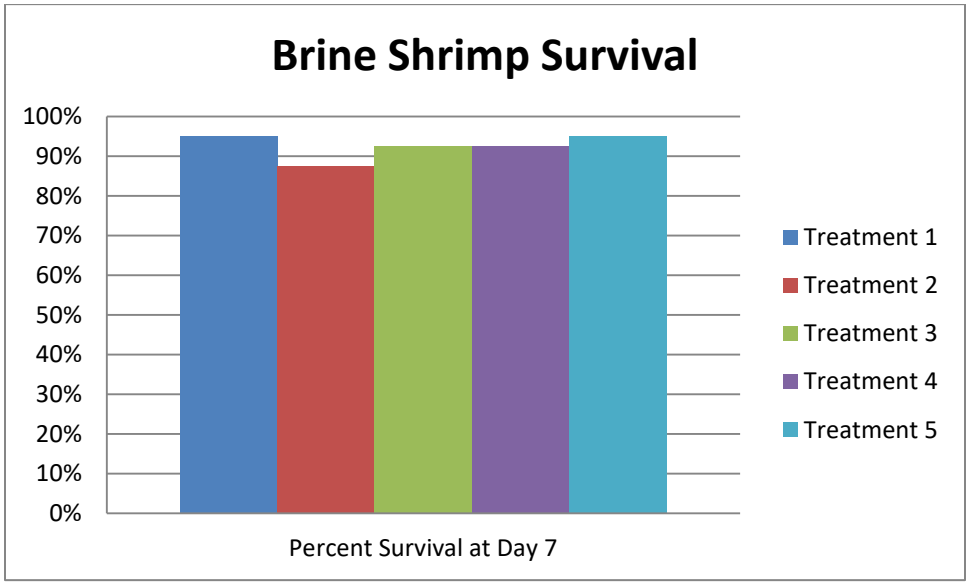
[a]As CaCO3
[b]Total residual chlorine

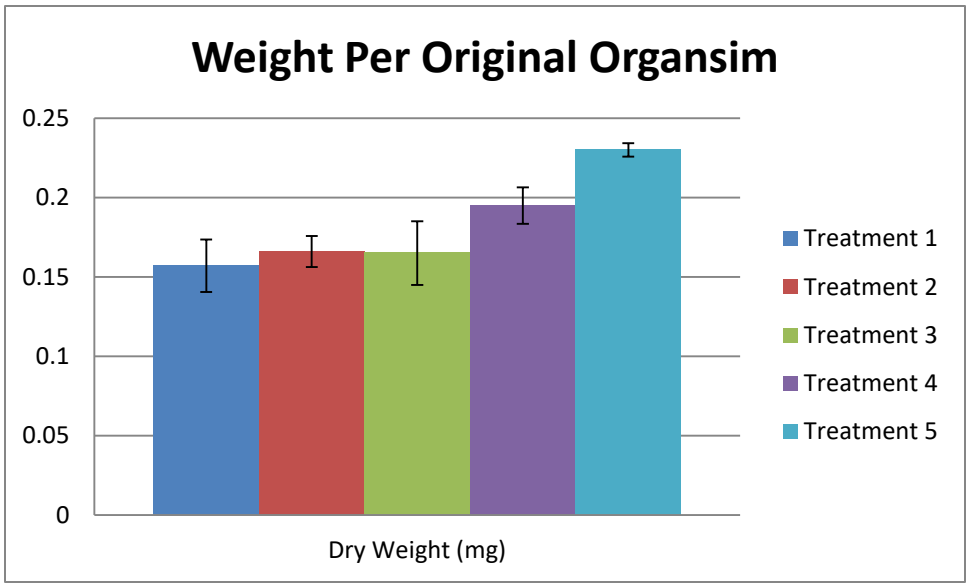
Test activities:

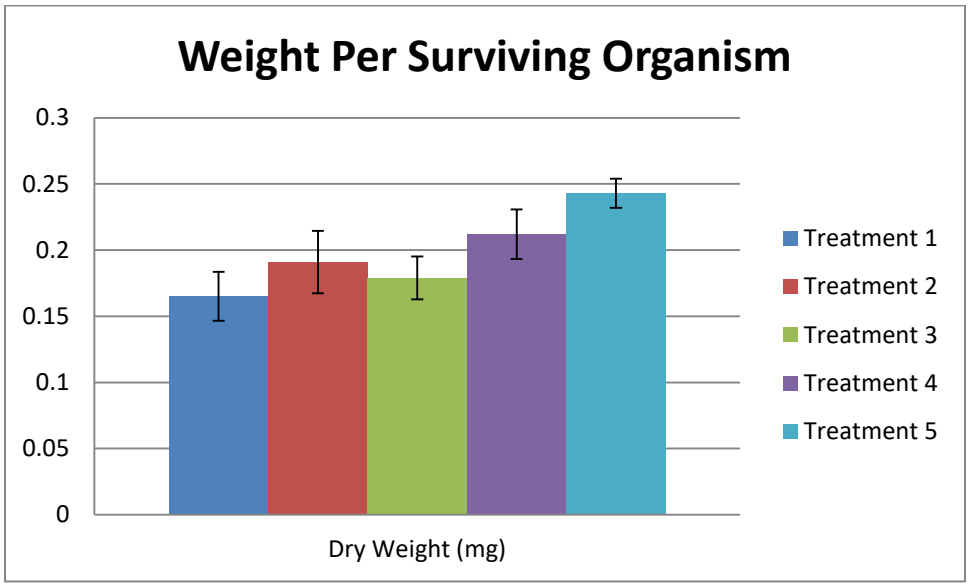
- Biological observations (primarily survival) taken daily.
- Chemistries taken on renewal days (i.e., pH, dissolved oxygen, and temperature).
- Conductivity was measured at test termination or when there was 0% survival in that treatment.
- Dry weights were determined at test termination.

Results:

Survival and growth of *A. franciscana* in all treatments are show shown in the following figures.







Summary and findings:

- Organism survival was ≥ 80% for all treatments.
- Growth was similar across treatments 1, 2, and 3. This suggests that, at densities between 100 and 300, organism density in pre-test holding does not impact test data.
- Treatment 4 growth was higher than treatments 1, 2, and 3, suggesting that the YTC/algae mixture feeding is acceptable for the pre-test holding.
    - This allows the pre-test and the test to be fed the same, simplifying feeding protocols and reducing volume of *D. viridis* required.

- Treatment 5 growth was higher than any of the other treatments and also demonstrates the lowest among-replicate variability of any of the treatments. This suggests that organism age within the <24 hour period may have contributed to variability seen among tests, and by more precisely controlling the age of the test organism, variability maybe reduced which could increase the utility of a WET test.
    - Organisms in this treatment are fed after being isolated, approximately 16 hours before the other treatments, which could also explain the higher growth.

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

14001-474-064

Attachment

cc: David Pillard, TRE

QA nw 5/5/20

TOXICITY DATA PACKAGE COVER SHEET

Test Type: Chronic

Project Number: 17001-474-064

Test Substance: Pre Test

Species: *Artemia franciscana*

Dilution Water: rGSL

Organism Lot or Batch Number: 042020

Concurrent Control Water: NA

Age: 48hr (48 hr)

Supplier: TRE

Date and Time Test Began: 4/22/20 @ 1440

Date and Time Test Ended: 4/29/20 @ 1435

Protocol Number: ____

Investigator(s): EN/CA/BS

Background Information

Type of Test: Static-Renewal (Daily)

pH control?: Yes **No**

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: 7 days

Organisms per Replicate: 10

Type of Food and Quantity per Chamber: D. viridis/ YTC

Feeding Frequency: 1 x daily

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): See Below

Agency Summary Sheet(s)?: None

Reference Toxicant Data: Test Dates: ~ to ~	IC_{25}: ~
Hist. 95% Control Limits: ~ to ~	Method for Determining Ref. Tox. Value: Linear Interpolation

Special Procedures and Considerations: Feed 0.5mL D. viridis/ 0.33 ml cerio YTC
Organisms hatched 2 days prior to initiation and held in rGSL with 100 ug/L Chla
Treatment 1: 100 org
Treatment 2: 200 org
Treatment 3: 300 org
Treatment 4: 200 org fed algae/ytc
Treatment 5: 200 org, 8 hour window
Appropriate correction factors have been applied to all temperatures recorded in this data package
Study Director Initials: AB Date: 4/22/20

QA new 5/5/20

TEST SUBSTANCE USAGE LOG

Project Number: 17001-474-064

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number				
Test Substance Collection Date and Time	From: @ To: @	From: @ To: @	From: @ To: @	From: @ To: @
Sample Type (Grab or Comp)				
Date Test Substance Received				
Dilution Water Number RW# or TRE#, circle one	13888/13919*			
Concurrent Control Water RW#	NA			
Date(s) Used	4/22/20 4/26/20 4/23/20 4/27/20* 4/24/20 4/28/20* 4/25/20			

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)
100 org	0	250	250						
200 org	0	250	250						
300 org	0	250	250						
Algae/ YTC	0	250	250						
8h	0	250	250						
	0	1250	1250						

Initials / Date	EN 4/22/20 mixed BS		
Initials / Date	AB 4/23/20 " "		
Initials / Date	~~CP~~ 1 CP 4/24/20 " "		
Initials / Date	CP 4/25/20 " "		
Initials / Date	AB 4/26/20 " "		
Initials / Date	EN 4/27/20 " "		
Initials / Date	CP 4/28/20 " "		
Initials / Date			

① CP 4/24/20 E

Artemia franciscana
CHRONIC BIOLOGICAL DATA

QA rev 5/5/20

Project Number: 17001-474-064

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
100 org	A	10	10	10	9-	9	9	9-	9-	-1 small org	95
	B	10	10	10	10	10	10	10	10		
	C	10	10	10	10	10	10	10	10		
	D	10	10	10	9	9	9	9	9		
200 org	A	10	10	10	10	10	10	9	9		87.5
	B	10	10	10	10*	9	9	8	8	* 1 weak org	
	C	10	10	10	10	10	10	10*	10*	* 1 weak org	
	D	10	9	8	8	8	8	8	8		
300 org	A	10	10	10	10	9	8	8	8		92.5
	B	10	10	10	10	10	10	10	10		
	C	10	10	10	10	10	10	10	10		
	D	10	9	9	9	9	9	9	9		
Algae/ YTC	A	10	10	10*	10	10	9	9	9	*1 weak org	92.5
	B	10	10	10	9	9	8	8	8		
	C	10	10	10	10	10	10	10	10		
	D	10	10	10	10	10	10	10	10		
8h	A	10	10	10	10*	9	9	9	9	* 1 weak org	95
	B	10	10	10	10	10	10	10	10		
	C	10	10	10	10	10	10	10	10		
	D	10	10	10	9	9	9	9	9		
	A										
	B										
	C										
	D										
	A										
	B										
	C										
	D										
Date:		4/22/20	4/23/20	4/24/20	4/25/20	4/26/20	4/27/20	4/28/20	4/29/20		
Time:		1440	0925	1200	1115	1040	1655	1750	1435		
Initials:		EN/CP	AS	AS	CP	AS	EN	CP	CP		

CHRONIC CHEMICAL DATA (INITIAL)

QA rev'd 5/5/20

Project Number:	17001-474-064
Test Species:	***Artemia franciscana***

%		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter #	Remarks
Conc.:	rGSL									All Conc.	
pH		7.9	8.0	8.0	8.0	7.7	7.9	7.9		FM30	
D.O. (mg/L)		5.2	5.1	5.1	5.2	5.2	5.1	5.0		17	
Temp. (°C)		20	20	20	20	20	20	20		L14	
Cond. (µS/cm)		135800	152300	135940	140,400	162600	309400	127,600		15	
Salinity (ppt)		124	—	—	—	—	—	—	—	1	
Conc.:											
pH											
D.O. (mg/L)											
Temp. (°C)											
Cond. (µS/cm)											
Salinity (ppt)											
Conc.:											
pH											
D.O. (mg/L)											
Temp. (°C)											
Cond. (µS/cm)											
Salinity (ppt)											
Conc.:											
pH											
D.O. (mg/L)											
Temp. (°C)											
Cond. (µS/cm)											
Salinity (ppt)											
Date:		4/22/20	4/23/20	4/24/20	4/25/20	4/26/20	4/27/20	4/28/20			
Time:		1435	0905	1140	1110	1020	1645	1740			
Initials:		EN	AB	AB	CP	AB	EN	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 on 5/5/20

CHRONIC CHEMICAL DATA (FINAL)

Project Number:	17001-474-064
Test Species:	***Artemia franciscana***

%	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter #	Remarks
Conc.: 100 org							130,200		All Conc.	* conductivity
pH	8.0	8.0	7.8	7.4	7.7	7.7	7.9		pH ① 30	
D.O. (mg/L)	5.1	4.9	4.8	5.2	4.6	4.8	5.0		17	
Temp (°C)	20	21	21	20	23Δ	22	21		L36	
Conc.: 200 org							131,200			* conductivity
pH	8.0	8.0	7.8	7.9	7.7	7.7	7.8			
D.O. (mg/L)	5.0	4.9	4.7	5.0	4.4	4.7	4.9			
Temp (°C)	20	21	21	20	24Δ	22	21			
Conc.: 300 org							130,800			* conductivity
pH	8.0	8.0	7.9	7.9	7.7	7.7	7.8			
D.O. (mg/L)	4.9	5.0	4.8	4.9	4.4	4.5	4.8			
Temp (°C)	20	21	21	20	24Δ	22	21			
Conc.: Algae/ YTC							130,500			* conductivity
pH	8.0	8.0	7.9	7.9	7.7	7.7	7.8			
D.O. (mg/L)	4.9	5.0	4.8	5.0	4.4	4.5	4.8			
Temp (°C)	20	21	21	20	24Δ	22	21			
Conc.: 8h							129,800 ②			* conductivity
pH	8.0	8.0	7.8	7.8	7.7	7.7	7.8			
D.O. (mg/L)	4.9	5.0	4.7	4.9	4.2	4.5	4.8			
Temp (°C)	20	21	21	20	24Δ	22	21			
Conc.:										
pH										
D.O. (mg/L)										
Temp (°C)										
Conc.:										
pH										
D.O. (mg/L)										
Temp (°C)										
Date:	4/23/20	4/24/20	4/25/20	4/26/20	4/27/20	4/28/20	4/29/20			
Time:	~~0950~~ 0930	1210	1135	1005	1650	1805	1525 ②			
Initials:	AB	AB	CP	AB	EJ	CP	CP			

① AB 4/23/20 E
② CP 4/29/20 E

Δ checked all reps

EDD sent 5/6/20

DAILY TOXICITY TEST LOG

Project Number:	17001-474-064
Test Species:	***Artemia franciscana***

General Comments	Random Chart: D Min/Max Thermometer # M15				Feeding	Initials/Date
Test Day 0	Test Solution Mixed at: 1425 Test Organisms Added at: 1440				Fed @ 1435	EN 4/22/20
Test Day 1	Real Time: 22	°C	Min-Max Range: 22-22	°C	Fed @ 0910	AS 4/23/20
Test Day 2	Real Time: 21	°C	Min-Max Range: 21-23	°C	Fed @ 1150	~~AS~~ CP (1) 4/24/20
Test Day 3	Real Time: 21	°C	Min-Max Range: 21-23	°C	Fed @ 1110	CP 4/25/20
Test Day 4	Real Time: 21	°C	Min-Max Range: 21-23	°C	Fed @ 1030	AS 4/26/20
Test Day 5	Real Time: 21	°C	Min-Max Range: 21-23	°C	Fed @ 1650	EN 4/27/20
Test Day 6	Real Time: 22	°C	Min-Max Range: 21-23	°C	Fed @ 1745	CP 4/28/20
Test Day 7	Real Time: 22	°C	Min-Max Range: 22-23	°C	Fed @ NONE	CP 4/29/20
Test Day 8	Real Time:	°C	Min-Max Range:	°C	Fed @	

(1) CP 4/24/20 E

[redacted]

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

QA [initials] 5/5/20

Project Number: 17001-474-064
Test Substance: Pre Test
Species: Artemia franciscana
Analyst Tare: RJW for JRW
Analyst Gross: SK
Date/Time of Tare Wt.: 4/29/20 ~1300
Date/Time of Gross Wt.: 5/4/20 @ 1240

Comments:
Analytical Balance ID: Sart #1
Dried in Oven # 3 from Date: 4/29/20 Time: 1525 to Date: 5/4/20 Time: 1040

Weight Type (Circle): Wet Blot Dry Dry (>100°C) AFDW (>500°C)

Lot or (Batch) Number: 042020

Boat No.	Treatment	Rep.	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)[1]	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	No. of Surv. Organisms	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)
	100 org	A		1.13713	1.13846	0.00133					9		
		B		1.13097	1.13245	0.00148					10		
		C		1.14710	1.14876	0.00166					10		
		D		1.13980	1.14148	0.00168					9		
	200 org	A		1.14377	1.14536	0.00159					9		
		B		1.12644	1.12796	0.00152					8		
		C		1.12081	1.12246	0.00165					10		
		D		1.11702	1.11877	0.00175					8		
	300 org	A		1.12409	1.12543	0.00134					8		
		B		1.14424	1.14586	0.00162					10		
		C		1.12077	1.12251	0.00174					10		
		D		1.13531	1.13710	0.00179					9		
Blank				1.12940	1.12937	-0.00003							
Range													
Mean													

Test Solution Volume:

Loading Rate:

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

QA Form No.010
Revision 6
Effective 02/14

QA wau 5/5/20

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 17001-474-064	Test Substance: Pre Test	Comments: Analytical Balance ID: Sart #1
Species: Artemia franciscana	Analyst Tare: SO for JH Analyst Gross: SK	Dried in Oven # 3 from Date: 4/29/20 Time: 1525 to Date: 5/4/20 Time: 1040
Date/Time of Tare Wt.: 4/29/20 ~1300	Date/Time of Gross Wt.: 5/4/20 @ 1240	

Weight Type (Circle): Wet Blot Dry Dry (>100°C) AFDW (>500°C) — Lot or (Batch) Number: 042020

Boat No.	Treatment	Rep.	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)[1]	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	No. of Surv. Organisms	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)
	Alg/YTC	A		1.12958	1.13163	0.00205					9		
		B		1.13241	1.13418	0.00177					8		
		C		1.14637	1.14829	0.00192					10		
		D		1.13747	1.13941	0.00194					10		
	8HR	A		1.12957	1.13183	0.00226					9		
		B		1.14755	1.14987	0.00232					10		
		C		1.12762	1.12991	0.00229					10		
		D		1.12731	1.12953	0.00222					9		
Blank													
Range													
Mean													

Test Solution Volume: Loading Rate:

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

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 14001-474 Species: Artemia franciscana

QA mw 5/5/20

Treatment	Rep	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)	No of Orig. Organisms	Mean Wt./ Original Organism (mg)	Mean Wt./ Treatment (mg) (Original)	Number of Surv. Organisms	Mean Wt./ Surviving Organism (mg)	Mean Wt./ Treatment (mg) (Surviving)
100 org	A		1.13713	1.13846	0.00133	0.00136	10	0.136	0.1568	9	0.151	0.1653
	B		1.13097	1.13245	0.00148	0.00151	10	0.151		10	0.151	
	C		1.14710	1.14876	0.00166	0.00169	10	0.169		10	0.169	
	D		1.13980	1.14148	0.00168	0.00171	10	0.171		9	0.190	
200 org	A		1.14377	1.14536	0.00159	0.00162	10	0.162	0.1657	9	0.180	0.1911
	B		1.12644	1.12796	0.00152	0.00155	10	0.155		8	0.194	
	C		1.12081	1.12246	0.00165	0.00168	10	0.168		10	0.168	
	D		1.11702	1.11877	0.00175	0.00178	10	0.178		8	0.223	
300 org	A		1.12409	1.12543	0.00134	0.00137	10	0.137	0.1652	8	0.171	0.1789
	B		1.14424	1.14586	0.00162	0.00165	10	0.165		10	0.165	
	C		1.12077	1.12251	0.00174	0.00177	10	0.177		10	0.177	
	D		1.13531	1.13710	0.00179	0.00182	10	0.182		9	0.202	
YTC/Algae	A		1.12958	1.13163	0.00205	0.00208	10	0.208	0.1950	9	0.231	0.2120
	B		1.13241	1.13418	0.00177	0.00180	10	0.180		8	0.225	
	C		1.14637	1.14829	0.00192	0.00195	10	0.195		10	0.195	
	D		1.13747	1.13941	0.00194	0.00197	10	0.197		10	0.197	
8 hour	A		1.12957	1.13183	0.00226	0.00229	10	0.229	0.2302	9	0.254	0.2429
	B		1.14755	1.14987	0.00232	0.00235	10	0.235		10	0.235	
	C		1.12762	1.12991	0.00229	0.00232	10	0.232		10	0.232	
	D		1.12731	1.12953	0.00222	0.00225	10	0.225		9	0.250	
Blank			1.12940	1.12937	-0.00003							

QA New 5/Chro

Project Number: 14001-474 Species: Artemia franciscana

Summary Statistics for Survival Data

Treatment	N	Min	Max	Mean	SD	C.V.
100 org	4	0.9	1.0	0.9500	0.0577	6.077%
200 org	4	0.8	1.0	0.8750	0.0957	10.942%
300 org	4	0.8	1.0	0.9250	0.0957	10.351%
YTC/Algae	4	0.8	1.0	0.9250	0.0957	10.351%
8 hour	4	0.9	1.0	0.9500	0.0577	6.077%

Summary Statistics for Growth Data (dry wt per original)

Treatment	N	Min	Max	Mean	SD	C.V.
100 org	4	0.136	0.171	0.1568	0.0165	10.526%
200 org	4	0.155	0.178	0.1657	0.0097	5.878%
300 org	4	0.137	0.182	0.1652	0.0201	12.187%
YTC/Algae	4	0.180	0.208	0.1950	0.0115	5.907%
8 hour	4	0.225	0.235	0.2302	0.0043	1.855%

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

Treatment	N	Min	Max	Mean	SD	C.V.
100 org	4	0.151	0.190	0.1653	0.0185	11.209%
200 org	4	0.168	0.223	0.1911	0.0235	12.274%
300 org	4	0.165	0.202	0.1789	0.0163	9.125%
YTC/Algae	4	0.195	0.231	0.2120	0.0187	8.816%
8 hour	4	0.232	0.254	0.2429	0.0110	4.541%

TRE Environmental Strategies, LLC
100 Racquette Drive, Unit A, Fort Collins, Colorado, 80524
T 970.416.0916 F 970.490.2963

TRE Environmental Strategies

April 7, 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: Algae Concentration

Mr. Bittner / Dr. Belovsky:

Dunaliella viridis has been the main source of food for brine shrimp chronic tests. The amount of *D. viridis* added to each test has been determined by spectrophotometric absorbance at 450 nm, which is then converted into approximate Chl*a* concentration to determine the volume of algae that is needed to feed each test chamber. The purpose of this study was to determine if *D. viridis* would survive centrifuging and re-suspending to allow batches to be concentrated to a specific cell density so that a consistent volume can be added during feeding. This is the typical process utilized in some other chronic WET tests.

Previous experimentation has shown that *D. viridis* cell count correlated with absorbance. Based on TRE's absorbance model, which was established using extensive fluorometric and spectrophotometric analyses during the acute studies with three metals, an absorbance of 3.75 would allow for an addition of 1 mL of *D. viridis* to provide 145 ug/L Chl*a*. This absorbance converts to a cell count of 76.6×10^5 cells/mL when it is entered in the formula derived in previous experimentation. By centrifuging and concentrating *D. viridis*, this concentration can be consistently achieved. This will also allow the volume fed to be reduced, limiting the dilution of the test substances.

A sample of *D. viridis* was centrifuged for twenty minutes at approximately 1,650 rpm. The supernatant was removed and the pellet was re-suspended in rGSL water. The supernatant was still tinged pale green, suggesting that a longer centrifugation period might provide for a higher rate of recovery. The re-suspended solution was examined by microscope immediately after centrifuging and after one hour. The *D. viridis* cells were intact and active during both examinations.

Sincerely,

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

David Pillard, Ph.D.
Principal/Senior Toxicologist
pillardda.tre@gmail.com

17001-474-062

Attachment

cc: Rami B. Naddy, TRE

TRE Environmental Strategies, LLC
100 Racquette Drive, Unit A, Fort Collins, Colorado, 80524
T 970.416.0916 F 970.490.2963

April 20, 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: CVs for weight in Short-term Chronic Brine Shrimp experiments

Mr. Bittner/ Dr. Belovsky:

Please find below a summary of the data collected from the controls (and other appropriate treatments) from short-term chronic brine shrimp tests conducted so far. Data include, average dry weights (mg), standard deviation (Std Dev.), number of replicates tested, and the coefficient of variations (CV). The treatments in bold designates those studies which use our current and proposed final experimental design (i.e., 10 organisms per 50 ml test solution, daily renewal, 4 replicates).

Data in the first table are those studies fed 145 µg/L Chla *Dunaliellia viridis*, whereas data in the second table represent those studies fed 72.5 µg/L Chla *D. viridis* and 0.6 mg YTC (yeast, trout chow, cereal leaves mixture, per EPA 2002).

Experiment	Treatment	Average dry wt (mg)	Std Dev.	# of Reps	C.V (%)
1	6d	0.0556	0.0047	4	8.38
	10d	0.1065	0.0152	4	14.24
	14d	0.1514	0.0051	4	3.34
2	50 ml	0.0605	0.0013	3	2.19
	150ml	0.0637	0.0008	3	1.2
3	50ml 6d	0.1233	0.0033	3	2.64
	150 ml 6d	0.1148	0.0106	3	9.20
	50 ml 10d	0.1350	0.0136	3	10.07
	150 ml 10d	0.2782	0.0191	3	6.87
4	10 org 7d	0.1145	0.0106	2	9.26
	20 org 7d	0.1160	0.0064	2	5.49
	10 org 10d	0.3025	0.0021	2	0.701
	20 org 10d	0.1865	0.0021	2	1.14
5	**Control 7d**	**0.0913**	**0.0153**	**4**	**16.80**
	Control 10d	0.2508	0.0262	4	10.45
7	*D viridis*	0.1093	0.0040	3	3.70
	1/2 *D. viridis*	0.0787	0.0055	3	7.00
8	*D. viridis*	0.0577	0.0042	3	7.22
9	*D. viridis*	0.1073	0.0146	3	13.58
10	***D. viridis***	**0.2350**	**0.0249**	**4**	**10.07**
11	**120 ppt algae**	**0.110**	**0.0224**	**4**	**20.38**

Experiment	Treatment	Average dry wt (mg)	Std Dev.	# of Reps	C.V (%)
8	*D. viridis* / YTC	0.0703	0.0076	3	10.766
9	*D. viridis* / YTC	0.1267	0.0244	3	19.247
10	**Control**	**0.2340**	**0.0524**	**4**	**22.386**
11	**Control**	**0.1228**	**0.0127**	**4**	**10.356**

Sincerely,

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

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

cc: David Pillard, TRE

TRE Environmental Strategies, LLC
100 Racquette Drive, Unit A, Fort Collins, Colorado, 80524
T 970.416.0916 F 970.490.2963

TRE Environmental Strategies

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

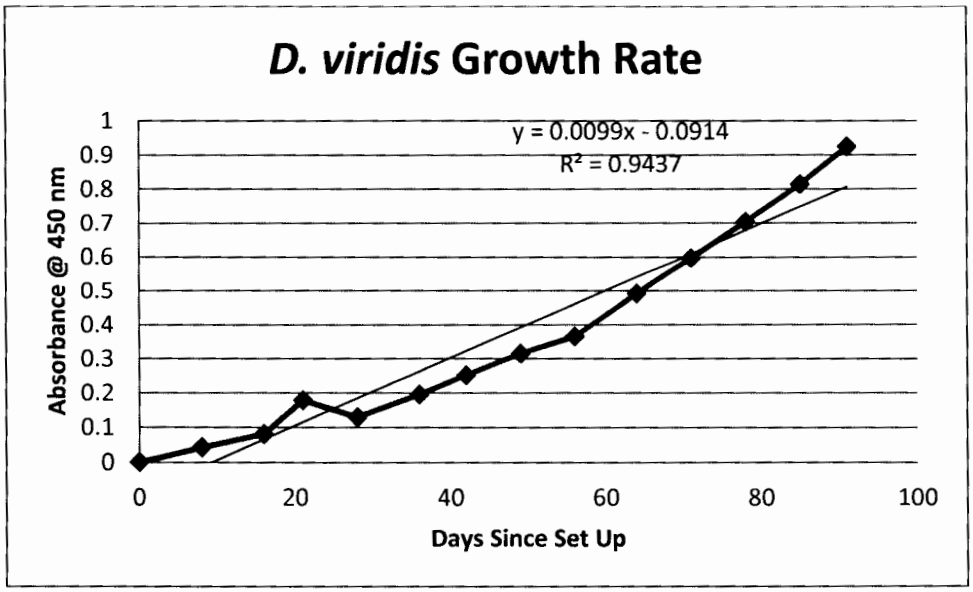
Subject: Algae Concentration

Mr. Bittner / Dr. Belovsky:

Dunaliella viridis has been the main source of food for brine shrimp chronic tests. The purpose of this study was to examine the growth rate of *D. viridis*. This is necessary for laboratories to be able to allow sufficient time for growth when scheduling WET tests.

On April 1, 2020. A 1 mL aliquot of *D. viridis* was collected from an established culture and added to 500 mL of brine (Notre Dame recipe) which was aerated gently. 1 mL of nutrient solution (Notre Dame recipe) was added weekly. Salinity was maintained at 120 ppt and solution volume was maintained at 500 mL. Temperature was maintained at 10°C and the photoperiod was 16h light: 8h dark. The absorbance of the culture was measured on this weekly and is presented in the following table and graph:

Date	Days Since Set Up	Absorbance
4/1/2020	0	0
4/9/2020	8	0.042
4/17/2020	16	0.081
4/22/2020	21	0.178
4/29/2020	28	0.129
5/7/2020	36	0.195
5/13/2020	42	0.252
5/21/2020	49	0.315
5/27/2020	56	0.365
6/4/2020	64	0.492
6/11/2020	71	0.597
6/18/2020	78	0.703
6/25/2020	85	0.814
7/1/2020	91	0.924



These data show that *D. viridis* can be successfully grown starting with a very small number of cells. Important considerations during culturing include consistent cold temperature, lighting, and regular nutrient additions to stimulate growth.

Sincerely,

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David Pillard, Ph.D.
Principal/Senior Toxicologist
pillardda.tre@gmail.com

17001-474-081

Attachment
cc: Rami B. Naddy, TRE

Algae Culturing

Rami Naddy <naddyrb.tre@gmail.com> Mon, Jan 20, 11:13 AM

to me

conversation between Amanda (TRE) and Shannon (ND) about D. viridis growth rate.

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Date: Mon, Jan 20, 2020 at 10:56 AM
Subject: Fwd: D. viridis culture
To: Rami Naddy <naddyrb.tre@gmail.com>

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They are getting feed 2ml of nutrient stock per liter once a week. The nutrient stock is 10% blue solution, 10% P/N solution and 80% RO water.

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Right now, I feed it once a week and add about 10% more media each week.

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Yeast (Fleishmans)

Shannon Jones <sjones32@nd.edu> Thu, Feb 20, 3:23 PM

to Rami, me

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Here's the basic protocol we were using for our latest yeast experiments, which were done in individual 400 ml jars. Let me know if you have any questions!

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Shannon Jones

Belovsky Lab Research Technician

Lab phone: 574-631-0949

096/098 Galvin Life Science Center

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Algae Culturing

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