

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

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 #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 μ g/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°C

- 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.	рН	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

^aAs CaCO3

^bTotal residual chlorine

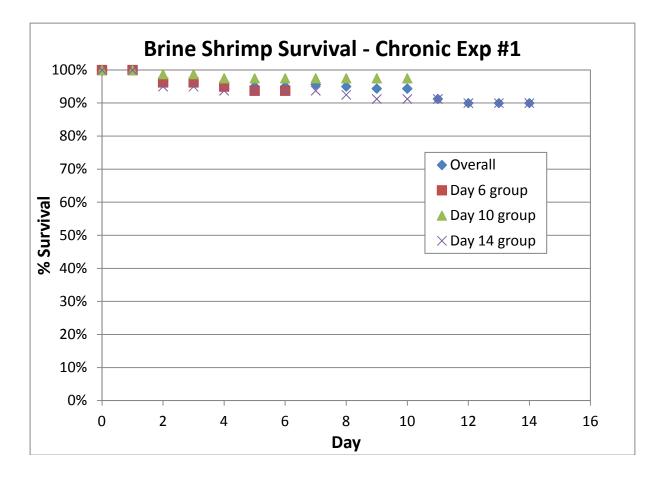
Test activities:

- 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.

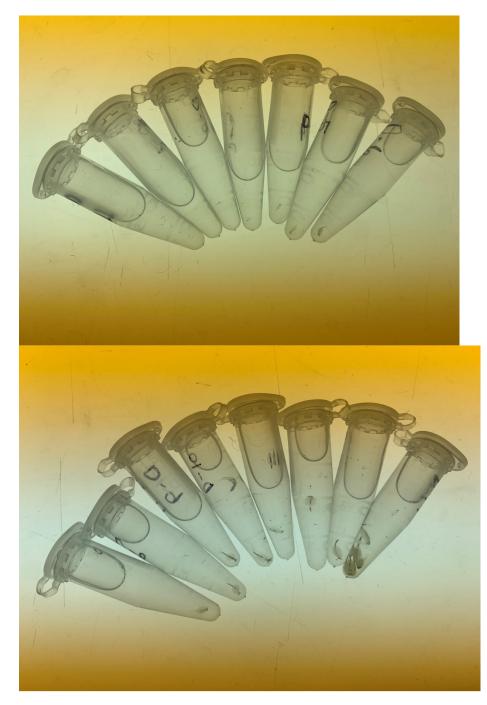
Mr. Bittner January 8, 2019 Page 3





Mr. Bittner January 8, 2019 Page 4

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

Mr. Bittner January 8, 2019 Page 5

- Growth was consistent throughout the test, but it is possible that the organisms are food limited with this guantity 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, Billel

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

17001-474-037

Attachment

cc: David Pillard, TRE

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



January 10, 2020

Mr. Christopher Bittner Standards Coordinator Utah Dept. of Environmental Quality 195 N 1950 W Salt Lake City, UT 84116 Dr. Gary Belovsky Environ. Res. Center & Dept. Biol Sci. University of Notre Dame Notre Dame, IN 46556

Subject: Results of 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 μ g/L Chla. Solutions were gently aerated.

Characterization of Recon Water

Sample No.	рН	Hard. (mg/L) ^a	Alk. (mg/L) ^a	Spec. Cond. (μS/cm)	TRC (mg/L) [♭]	NH₃-N (mg/L)	Salinity (ppt)
RW#13742	NR	NM	NM	NR	NM	NM	121

^aAs CaCO3

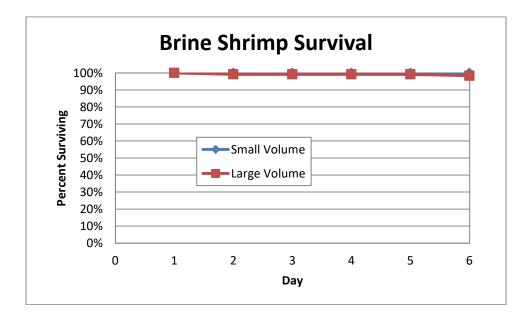
^bTotal residual chlorine

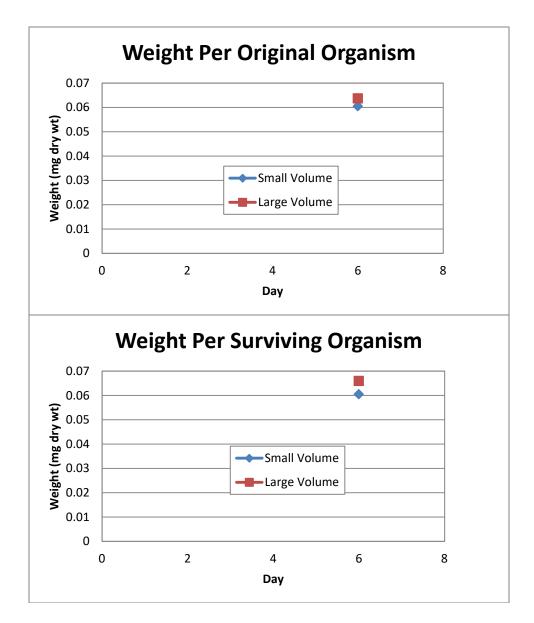
Test activities:

- 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.

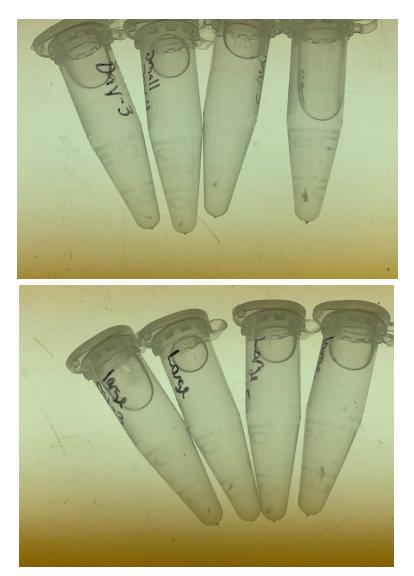




TRE

Mr. Bittner January 10, 2020 Page 4

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 \geq 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

TRE

Mr. Bittner January 10, 2020 Page 5

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,

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

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

17001-474-039

Attachment

cc: David Pillard, TRE



January 6, 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 #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 145μ g/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 μ g/L Chla. Solutions were gently aerated.

Characterization of Recon Water

Sample No.	рН	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							

^aAs CaCO3

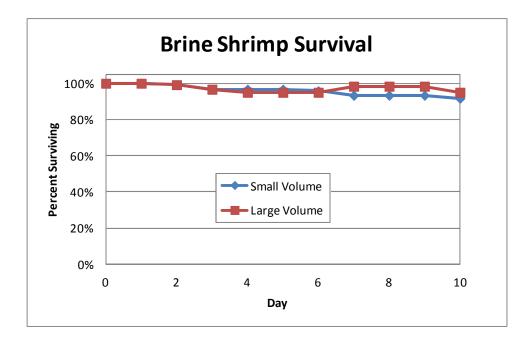
^bTotal residual chlorine

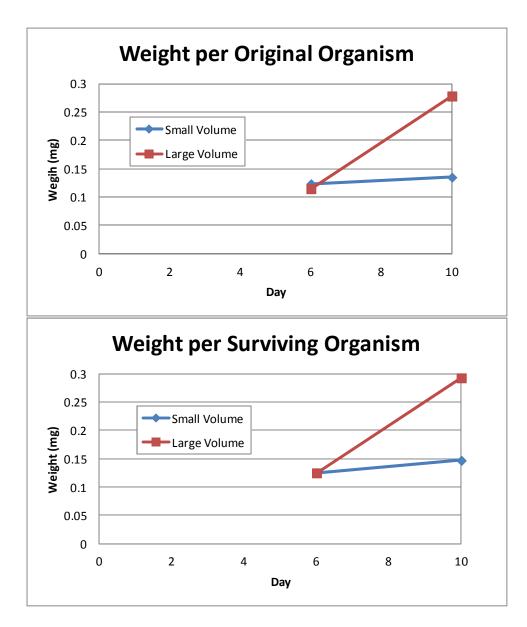
Test activities:

- 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

Mr. Bittner December 31, 2019 Page 4

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,

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

14001-474-041

Attachment

cc: David Pillard, TRE

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



January 10, 2020

Mr. Christopher Bittner Standards Coordinator Utah Dept. of Environmental Quality 195 N 1950 W Salt Lake City, UT 84116 Dr. Gary Belovsky Environ. Res. Center & Dept. Biol Sci. University of Notre Dame Notre Dame, IN 46556

Subject: Results of 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 μ g/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

Mr. Bittner January 10, 2020 Page 2

- 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 μ g/L Chla. Solutions were gently aerated.

Characterization of Recon Water

Sample No.	рН	Hard. (mg/L) ^a	Alk. (mg/L) ^a	Spec. Cond. (μS/cm)	TRC (mg/L) ^b	NH₃-N (mg/L)	Salinity (ppt)
RW#13772	7.8	NM	NM	131,600	NM	NM	120

^aAs CaCO3

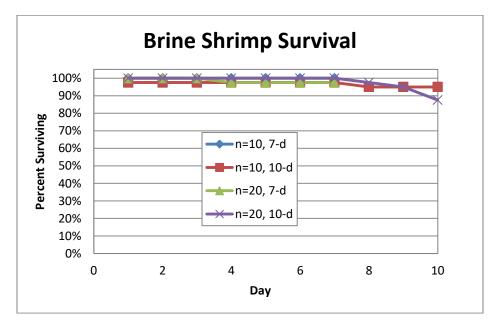
^bTotal residual chlorine

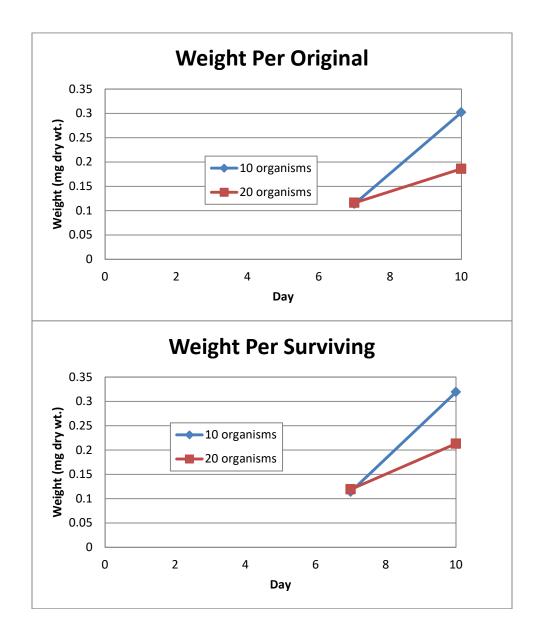
Test activities:

- 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.





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

TRE

Mr. Bittner January 10, 2020 Page 4

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.

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

17001-474-043

Attachment

cc: David Pillard, TRE

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

Page 1 of QA Form No. 051 **Revision 5** Effective 02/14 NGU) TOXICITY DATA PACKAGE COVER SHEET Test Type: Chronic Project Number: 17001-474-E Test Substance: Experiment Species: Artemia franciscana Dilution Water: rGSL Organism Lot or Batch Number: 111219 Aae: USh (48 hr) Concurrent Control Water: NA RE Supplier: Date and Time Test Began: 11/24/12 @ 13/0 whyles Q) 1350 Date and Time Test Ended: Protocol Number: Investigator(s): **Background Information** pH control?: Yes No Type of Test: Static-Renewal (Daily) If yes, give % CO₂: NA Test Temperature: Env. Chmbr/Bath #: 25 20 ± 1 °C 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: <u>Test Initiation</u> Alkalinity: Test Initiation NH₃: <u>Test Initiation</u> TRC: Test Initiation pH: <u>Daily</u> Conductivity: <u>Daily</u> 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: IC₂₅: to Hist. 95% Control Limits: Method for Determining Ref. Tox. Value: Linear Interpolation to 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: Date: WI44

Page 2 of _____ QA Form No. 014 Revision 1 Effective 02/14 QA MM V[5]20

TEST SUBSTANCE USAGE LOG

Project Number:

17001-474-Exp

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number				
	From:	From:	From:	From:
Test Substance Collection	@	@	@	@
Date and Time	То:	То:	То:	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 11/18/15/14 11/15/19 11/18/14/11 11/16/19 11/20/14 11/17/19 11/20/14			

Preparation of Test Solutions

Test Substance	Test Substance	Dilution Water	Total Volume	Test Substance	Dilution Water	Total Volume	Test Substance	Dilution Water	Total Volume
Conc.	Volume	Volume	(ml)	Volume	Volume	(ml)	Volume	Volume	(ml)
(% Effluent)	<u>(ml)</u>	(ml)		(ml)	(ml)		(ml)	(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	AG 1114	n As	11/22/19						
Initials / Date	AS INIS	In loe	11/23/19						
Initials / Date	CP 11/16	0/19							
Initials / Date	CP 11/1	7/19							
Initials / Date	Rex "112	8 (15)							
Initials / Date	AS WA	10							
Initials / Date	As ille								
Initials / Date	A 11/2	114							

Page 3 of ____ QA Form No. 060 Revision 3 Effective 02/14 TOL MAN J & W

BRINE SHRIMP (Artemia franciscana) CHRONIC BIOLOGICAL DATA

Project Number:

<u>17001-474-Exp</u>

7D 20 7D 10	Test Replicate A B A B A B B	Day 0 20 20 10 10 20 20	Day 1 20 20 10 10 10 20 20 20	Day 2 20 20 10 10 10	Day 3 20 20 10 10	Day 4 19 20 10 (0	Day 5 19 20 10 10	ring Orga Day 6 19 Jo 10	Day 7 19 20 10	Remarks
7D 20 7D 10	A B A B	20 20 10 10 20	20 20 10 10 20	2 20 20 10 10	3 20 20 10 10	4 19 20 10	5 19 20 10	6 19 Jo 10	7 19 20 10	Remarks
7D 10	B A B A	20 10 10 20	20 10 10 20	20 10 10	20 10 10	20 10	20 10	20 10	20 10	
7D 10	A B A	10 10 20	ιο ιο 20	10 10	(0 10	10	10	10	10	
	B	10	ι0 20	10	ID					
	B	10	ι0 20	10	ID					
	A	20	2 0			(0	10	10		
100.20				20*				10	10	
				20*						
100 20	В	20	20		20*	<u>}</u> ₀⊀	2010	ze	20	* 1 weak ors
10D 20			<i>av</i>	20	20	20	20*	20*	20*	* I ment org
	A	10	9	9	9	9	9	9	9	
10D 10	В	10	10	1D	10	W	10	10	10	
. <u></u>	Date:	ulun_	nlistig	11/16/19	11/17/19	illising	11/19/19	11/2019	112419	
	Time:	1350	1690	1510	1525	1435	1400	1445	1400	
	Initials:	MB	<i>I</i> ₽5	CP	CP	P	A	An	M	,
	Test	Day	Day	Day	Day	Day	Day	Day		
%Conc. R	eplicate	8	9	10	11	12	13	14		Remarks
	A	19	18	17	\leq	\sim				
10D 20	В	20	20*	18*	\leq	\leq	\leq	\leq		* I weak ong
	Α	9	9	9	\land	\angle	\angle	\nearrow		
10D 10	В	10	10	10	\sim	\geq	\angle	\angle		
						\angle				
	Date:	11/2/15	11/23/19	ubalis						
	Time:	1055	1450	1310						
l	Initials:	Az	CP	8						

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Page 4 of _____ QA Form No. 058 Revision 4 Effective 02/14

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.	<u> </u>
pH		-7-S	7.9	7.9	7.8	77	7.4	7.8	7.8	Finzs	
D.O. (mg/L)	,			2.2	5.2	7.8 4.9		Hig .	4.9	[
Temp. (°C)		5.0 20	5. <u>2</u> 20	20	10		5.0 20		20	17	
Cond. (µS/cm)		131 600	141000	133300	 	90 20		20		L79 15	
Hard. (mg/L)		151 000	141000	(55500	150,500	50100	139700	134000	134500	<u> </u>	
Alk. (mg/L)										たか	
TRC (mg/L)											
NH_3 (mg/L)								<u> </u>		72 HA7	
Conc.:			<u> </u>		L <u></u>	<u></u>	<u> </u>	<u> </u>		<u>ר'דעי</u>	· · · · · · · · · · · · · · · · · · ·
pH	<u>L</u>	·	<u> </u>								
D.O. (mg/L)							-				
Temp. (°C)											
Cond. (µS/cm)		·								-	
Hard. (mg/L)											
Alk. (mg/L)											
TRC (mg/L)											
NH ₃ (mg/L)											
Conc.:											
рН											
D.O. (mg/L)										· · · · · · · · · · · · · · · · · · ·	
Temp. (°C)						<u> </u>					
Cond. (µS/cm)											
Conc.:											
pН											
D.O. (mg/L)											
Temp. (°C)											
Cond. (µS/cm)											
	Date:	11/14/19	11/15/19	11/16/19	11/17/19	11/17/9	1119 12	11/2019	11/2015		
	Time:		1545	1455			1340	1 1	1400		
	Initials:		M	CP	CP	nex	5	m	AS		

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.

0 cp 11/16/19 E

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Och man 1/8/20

CHRONIC CHEMICAL DATA (FINAL)

Project Number:		1-474-Ex								
Test Species:	Artemi	a francis	cana							
%	Day	Day	Day	Day	Day	Day		Day	Motor #	Remarks
70	1	2 Day	3	4	5	6	Day 7	Day 8	weter #	Remarks
Conc.: 7D 20							140000		All Conc.	* conductivity ((s
рН	7.9	8.0	7.9	7.9	7.8	7.8	78		Fhaz	
D.O. (mg/L)	4.g	07.75.1	4.9	5.0	4.9	45	4.8		17	
Temp (°C)	20	20	20	21	19	19	20		L-37	
Conc.: 7D 10							132900	\square		
pН	8.0	8,D	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	Uco			
Temp (°C)	19	20	20	91	19	19	20			
Conc.: 10D 20										
рН	7.9	0,8	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	10	21	19	iq	20	20		
Conc.: 10D 10										* conductivity
рН	\$.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.)	4.4	4.5	4.3		
Temp (°C)	19	20	10	91	19	lg	20	20		
Dat		1416/19	11/17/19		11/19/19	11/20/19		11/22/19		
Tim				1515	1400	1445 Az	1400	1050 Az		
Initial		CP -	Cl	py	AS		100	N)		
%	Day	Day							Meter #	Remarks
	9	10							· ·	
Conc.: 10D 20	<u>-</u> 	129200								* conductivity (15)
pH							 			
D.O. (mg/L)	4. 6									
Temp (°C)	D 90 21		<u> </u>			<u> </u>		 		
Conc.: 10D 10	=	129,400								
рН	7.7	7.7								
D.O. (mg/L)	4.5	Ц.З								
Temp (°C)	2021	-			<u> </u>		<u> </u>			[]
Dat いろら Tim Initial	=: 11/22/19	4124/12				ļ		ļ	<u> </u>	
Initial	s: CP	BUD N								
		<u> </u>			<u> </u>			<u> </u>	J	F

Oct 11/16/19 E 3 of for AB 11/23/19 WP

CHRONIC CHEMICAL DATA (INITIAL)

Project Number:

17001-474-Exp

Test Species: Artemia franciscana

%		Day	Day	Day	Day	Dav	Day	1	Meter #	Remarks
		Day 8	Day 9	Day 10	11	Day 12	13			rtomarto
Conc.:	rGSL							 	All	
			70	$ \rightarrow $				 	Conc.	
pH		7.9	7.9		$\langle \rangle$		\leq	 ·		
D.O. (mg/L)		5.0	5.1			\leq	<	 		
Temp. (°C)		<u> </u>	20	$\langle \rangle$	\leq		<	 		
Cond. (µS/cm))	133000	132900		\leq		\leq	 		
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm))									
Conc.:										
рН										
D.O. (mg/L)								 		
Temp. (°C)										
Cond. (µS/cm))							 		
Conc.:										
рН								 		
D.O. (mg/L)										
Temp. (°C)								 		
Cond. (µS/cm))							 		
Conc.:						-				
pН										·····
D.O. (mg/L)								 		
Temp. (°C)								 		
Cond. (µS/cm)								 		
Hard. (mg/L)								 		
Alk. (mg/L)								 		anna ann ann ann ann ann ann ann ann an
TRC (ma/L)			<u> </u>					 		
TRC (mg/L) NH₃ (mg/L)								 		
	Date:	11/22/1	11/23/19				I	 	1	
· · · · · · · · · · · · · · · · · · ·		1035	1515					 		
	Initials:	1035 A	1910 CP							
lote: Hardness		1						 		

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.

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DAILY TOXICITY TEST LOG

Project Numb	er: 17001-474-Exp			
Test Species		· · · · · · · · · · · · · · · · · · ·		
	///com/a //alloioda/la			
General Comments	Random Chart:시스Min/Max Thermomete	r# M15	Feeding 145 ug/l Chla upon renewal	Initials/Date
Test Day 0	Test Solution Mixed at: ৭३১১ Test Organisms Added at: ৭১১৩		Fed @ _ໄ ງງແບ	AB 10/14/15
Test Day 1	Real Time: ነኅ °C Min-Max Range: ሉ -2	N °C	Fed @ 1555	123 1115/17
Test Day 2	Real Time: 20 °C Min-Max Range: ၂၅	- 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: ເງ °C Min-Max Range: ເງ	-91 ℃	Fed @ 12/35	Ry 11/13/12
Test Day 5	Real Time: ۱۹ ଂC Min-Max Range: ୲૧ - 2	.) °C	Fed @ 1350	A3 11/17/17
Test Day 6	Real Time: 19 °C Min-Max Range: 19-2 20 ag cups Vishbly more clear than 10 on tinted green		Fed @ [ા] ધા૦	11 (20)(q
Test Day 7	Real Time: _{2\} °C Min-Max Range: 2\-2 Þ		Fed @ 1350 1045 ₍₃₎	A3 11/21/17
Test Day 8	Real Time: շ০ °C Min-Max Range: լզ ֊ Ծ	ג∽ °C	Fed @ 1045	M3 11/22/14

003 11/20/19E

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DAILY TOXICITY TEST LOG

.

Project Num	oer:	1700)1-474-E>				······································	
Test Species	:	Artem	ia francis	scana				
General Comments							Feeding 145 ug/l Chla upon renewal	Initials/Date
Test Day 9	Real Time:	19	°C	Min-Max R	ange: /9-2	I°C	Fed @ (510	CP 11/23/19
Test Day 10	Real Time:	١٦	°C	Min-Max Ra	ange _{in -} ,	°C	None	Bg- 11/24/17

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TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Projec	Project Number: 17001-474 expt+4	17001	hth-	expthy	Test Substance	ance:				Comments:			
Species:	šs:				Analyst Tare: A.C.	'e: A.C	Analyst Gross:	sross: AH		Analytical Balance ID: Dried in Oven # 3 from Date: 1/0/19.me: 133<	ance ID: # 3 from D	ate: 11/20/19.	
Date/T	151 م مارا المالية المالية Date/Time of Tare Wt:	Wt.: III	1961	316	Date/Time	Date/Time of Gross Wt.:		5			1 2 2 2 2 2 3 3 3 3 3 3 3 3 3 3 3 3 3 3	ate: Werky T	me: 08 b
Boat	Treatment Rep.	t Rep.	Length		Weight Type (Circle): Wet	Wet Blot Dry	Dry Dry (> 100°C)	0-10-C	AFDW (>500°C)		Lot or Batch Number:	Number:	1112-15
					Tare Gross Weight (g) Weight (g)	Net Weight (g)	`ź	No. 6 Orig Organis	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	No. of Surv. Organisms	Mean Wt. per Surviving Organism	Mean Wt. per Treatment (mg) (Surviving)
	FD 100m	4		1.13145	1.13145 1,132,20,00107	0,00107					10	(bu)	
	JD 1000	e		1.14539	1.14539 1.14662 0.0022	0.0022					2.		
	Th see	A		1,13310	113310 1.135330,0023	0,00273					19		
	70 90m	£		1.13926	1.13926 1.14167 0.0024	0,00241					R		
									ę				
Blank				1.13496 1.1 349A	1,13494								
Range													
Mean													
Test Solu	Test Solution Volume:		~				Loading Rate:						
	Add in weight loss of blank boat, if appropriate.	f blank 2/	boat, if app	ropriate.									
,		đ											

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┷.

l					TEST	ORGANISI	n Lengths	s. WEIGHT	TEST ORGANISM LENGTHS. WEIGHTS, AND I DADING	UNIC.		Page of QA Form No.0 Revision 6 Effective 02/14	Page of QA Form No.010 Revision 6 Effective 02/14
Proiec	Project Number R		UTU-	PHUX MEN-1000	<u> </u>						6	84 NW	18/20
		2	5		I lest Substance:	tance:				Comments:			
Species	is:				Analyst Tare:	re: At	Analyst G	Analyst Gross: ∦₩		Analytical Balance ID:	nce ID: # 3, from D	T older 11-010	
Date/T	Date/Time of Tare Wt.:	\exists	<u>31/bz</u> ,	4 @ 13c) Date/Time	of Gross Wt.	29/10 @ 130 Date/Time of Gross Wt.: 11/29/19 @	Sell				to Date: 1/28/19 Time: 08/10	ime: <u>1335</u> ime: <u>0810</u>
Boat	Treatment Rep.		Length		Weight Type (Circle):	Wet Blot Dr	Dry (>100'C)		AFDW (>500°C)		I of or Batch Number		5/211
					Tare Gross Weight (g) Weight (g)	Net Weight (g)	Net	sms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	No. of Surv. Organisms	אס ייבו	Mean Wt. per Treatment (mg) (Surviving)
	10ers Icha	4		1.14671	1.14(571 1.14972	0.00301					0	(mg)	
	10cm looley			1.14481	1.14785	0.00304					- -		
	JOORIAL	Ł		11321)		01-200.0					0		
	Dontech			1.12969	1.12969 1.13345 0.00376	0.00376		-					
	,										0		
		+											
		+											
												•	
Blank		+		1-14671 1.14671		0.0000							
Range		_											
Mean													
Test Solu	Test Solution Volume:					ľ	Loading Rate:						
Add in w	Add in weight loss of blank boat, if appropriate	olank boa	at, if appr	opriate.									
3 octor 10 SMO	3 00 01						 -						

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QA Form No. 010a Revision 1 Effective 02/14 As 243/11 Charlen 1/8/20

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number:

17001-474

Artemia franciscana

Species:

Treatment Rep	Rep	Length Units:		Ĩ		Net Weight Net Weight No of Orig. (g) (g) Organisms	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)
Dav 7 10	۲a		1.13145	1.13252	0.00107	0.00107	10	0.107	0.1145	10	0.107	0.1145
Org				1, 14001	0.00122	0.00122	<u>0</u>	0.122		0	0.122	
	⊲		1 13310	1 13533		0 0003		0	0 1160	Ç	0 417	
Day 7 20			1.13926	1.14167		0.00241	20	0.121	0.1100	20	0 121	0.1108
Org												
			1.14671	1.14972		0.00301	10	0.301	0.3025	6	0.334	0.3192
Day 10 10 Org	m		1.14481	1.14785	0.00304	0.00304	10	0.304		10	0.304	
	∢		1.13212	1.13582	0.00370	0.00370	20	0.185	0.1865	17	0.218	0.2133
Day 10 20 Org	m		1.12969	1.13345	0.00376	0.00376	20	0.188		18	0.209	
Blank /d	╋		1.13496	1.13497	0.00001							
Blank 10d			1.14671	1.14671	0.00000							
Project Number	nber:		17001-474			Species:	Artemia franciscana	Inciscana				

Summary Statistics f	ics for Su	s for Survival Data	:	:		
	Zİ		Max	<u>Mean</u>	<u>0</u> 2	<u><</u>
	2	0.0	1.0	1.0000	0.0000	0.000%
	2	0.0	1.0	0.9750	0.0354	3.626%
	2	0.0	1.0	0.9500	0.0707	7.443%
	7	0.0	0.9	0.8750	0.0354	4.041%

QA Form No. 010a Revision 1 Effective 02/14 Ar 12/3/19 DA vav 1/8/00

	<u>, </u>	9.263%	5.486%	0.701%	1.137%
	<u>S</u>	0.0106	0.0064	0.0021	0.0021
original)	<u>Mean</u>	0.1145	0.1160	0.3025	0.1865
(dry wt per	Max Me	0.122	0.121	0.304	0.188
for Growth Data (0.111		
stics for Gr	ZI	2	7	7	7
Summary Statistics	<u>Treatment</u>	Day 7 10 Org	Day 7 20 Org	Day 10 10 Org	Day 10 20 Org

	<u>.<</u>	9.263%	1.862%	6.744%	2.904%
organism)	<u>SD</u>	0.0106	0.0022	0.0215	0.0062
()		0.1145	0.1189	0.3192	0.2133
(dry wt per	Max	0.122	0.121	0.334	0.218
s for Growth Data	Min	0.107	0.117	0.304	0.209
stics for G	ZI	ы	2	7	7
Summary Statistic	<u>Treatment</u>	Day 7 10 Org	Day 7 20 Org	Day 10 10 Org	Day 10 20 Org



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 Chla 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 Chla
- 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 μ g/L Chl*a*. Solutions were gently aerated.

Characterization of Recon Water

Sample No.	рН	Hard. (mg/L) ^a	Alk. (mg/L) ^a	Spec. Cond. (μS/cm)	TRC (mg/L) ^ь	NH₃-N (mg/L)	Salinity (ppt)
RW#13804	7.9	NM	NM	139,800	NM	NM	120

^aAs CaCO3

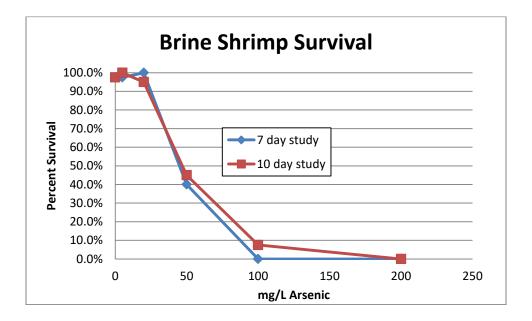
^bTotal residual chlorine

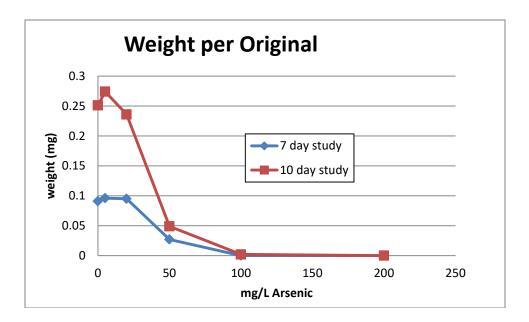
Test activities:

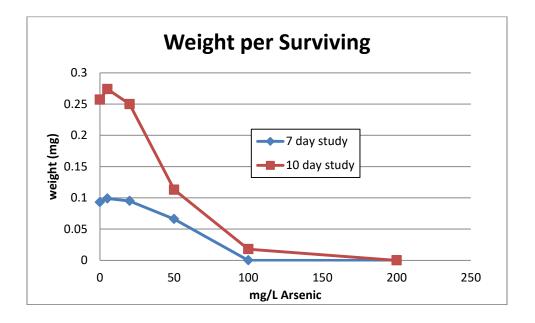
- Biological observations (primarily survival) taken daily.
- Chemistries taken on renewal days (i.e., pH, dissolved oxygen, and temperature).
- Conductivity was measured at test termination or when there was 0% survival in that treatment.
- Dry weights were determined at test termination.
- Arsenic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

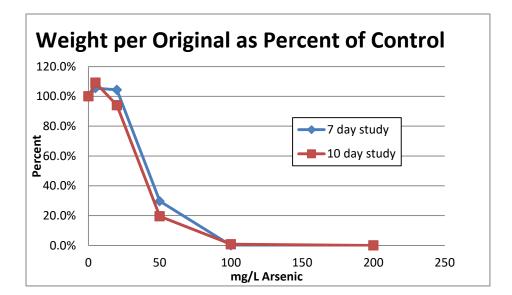
Results:

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









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 \geq 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.

Mr. Bittner January 14, 2020 Page 5

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,

Aul 12:

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

14001-474-046, 047

Attachment

cc: David Pillard, TRE

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

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DA NAS 1/11/20 **DATA PACKAGE COVER SHEET** TOXICITY Test Type: Chronic Project Number: 17001-474-Exp Test Substance: Arsenic (Na2HAsO4) Species: Artemia franciscana Organism Lot or Batch Number: **Dilution Water:** rGSL 121719 Age: 48 hr) Concurrent Control Water: NA Supplier: Date and Time Test Ended: Date and Time Test Began: 12/19/19 @ 1500 12126115 @ 1455 192 Investigator(s): Protocol Number: **Background Information** pH control?: Yes No Type of Test: Static-Renewal (Daily) If yes, give % CO₂: 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 TRC: Test Initiation Alkalinity: Test Initiation NH₃: 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: IC₂₅: to -Hist. 95% Control Limits: Method for Determining Ref. Tox. Value: Linear Interpolation to 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: Date:

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DA NAN 1/0/20

TEST SUBSTANCE USAGE LOG

17001-474-Exp

	Sample 1 🕖	Sample 2	Sample 3	Sample 4
Test Substance Number	As seto v			
	From: Store	From:	From:	From:
Test Substance Collection	@	@	@	@
Date and Time	То:	То:	То:	То:
	@	@	@	@
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	12/20/19 12/22/19 12/20/19 12/22/19 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 Spiki	ng 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 12/1	9/19							
Initials / Date	h pl	eoly							
Initials / Date	CP 121	21/19							
Initials / Date		2413							
Initials / Date		5/19							
Initials / Date	As op	+/19	<u></u>						<u></u>
Initials / Date	EN V2	Inlig							
Initials / Date				1			<u> </u>		

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Artemia franciscana CHRONIC BIOLOGICAL DATA

Project Number: ____1

17001-474-Exp

						Number	of Surviv	/ing Orga	nisms	To Sulvivol
mg/L	Test Replicate	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Remarks
0	Α	10	10	10	10	10	iO	10	9	92.5
	В	10	10	10	6	16	10	10	10	
	С	10	10	10	(0	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
5	А	10	10	10	10	10	W	10	10	97.5
	В	10	10	10	Ь	10	10	10	10	(U)
	С	10	10	9	9	9	9	9	9	
	D	10	ιο	10	10	10	10	10	10	
20	A	lo	6	10	io	10	10	10	10	100
	В	10	10	10	lo	10	10	10	W	
	С	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
50	А	10	5	4	4	4	4	4	4	40%
	В	W	3	2	2	2	2	2	à	
	с	10	6	6	6	(<i>φ</i>	6	6	4	
	D	_ 10	7	4	4	.4	ч	4	4	
100	А	16	ス	D	[]	$\overline{\Lambda}$	\backslash	\backslash		nl.
	В	10	0				\square			
	с	10	0					\Box		
	D	Ю	1	0						
200	A	10	Ø	١			\mathbf{N}	$\overline{\mathbb{N}}$		Oly
	В	10	0	$\left \right\rangle$		$ \rangle$		\square		
	с	10	Ø							
	D	10	0							
	Α									
	В									
	c			N						
	D									
	Date:	17115/18	W20 m	12/21/19	12/22/15	12 hazilis	12/24/19	12/25/19	IZDEIN	
	Time:	1500	1540	1450	B15	1450	1500	1100	1455	
	Initials:	m/5	hs	CP	6	~	ß	E.	4	

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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					<u> </u>		0		All Conc.	
рН	7.9	8.0	7.9	7.9	8.0	7.9	7.9		FAD8	
D.O. (mg/L)	5,2	5.3	5.3	5.5	5.2	51	5.5		170	
Temp. (°C)	20	20	20	30	20	20	20		1.2%	
Cond. (µS/cm)	139800	139800	145600	138500		138100	146700		15	
Hard. (mg/L)									Titr	
Alk. (mg/L)									Tim	
TRC (mg/L)									22	·
NH ₃ (mg/L)									411	ppt: 120
Conc.: 5										
рН	7.9	8.0	7.9	7.9	30	7.9	8.0			
D.O. (mg/L)	5.1	4.9	5.3	5.4	53	5.1	5.5			
Temp. (°C)	*	×	*	×	X	*	*			
Cond. (µS/cm)	139,200	137900	145200	138000	0	001751	141.400			
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH ₃ (mg/L)										
Conc.: 20										
рН	79	7.9	7.9	7.9	80	7.9	7.9			
D.O. (mg/L)	5.1	4.9	5.3	5.4	5.3	5.1	5.5	-		
Temp. (°C)	TX	*	*	*	*	*	¥			
Cond. (µS/cm)	139,400	137600	145300	137100	G	136200	141700			
Conc.: 50										
рН	7.9	8.(7.9	7.9	8.0	7.9	7.9			
D.O. (mg/L)	5.1	4.9	5.3	5.4	5.4	50	5.5			
Temp. (°C)	*	_*_	*	A	*	*	¥			
Cond. (µS/cm)	138,300	136200	145000	136,000	Ø	135300	141760			
Date:	12115115	12/26/9	12/21/19	P)23)14	12123/15	abula	12/25/19			í
Time:	1456	1515	1425	1200	1445	1450	1100			
Initials: Note: Hardness, alkalinit	R	AB	CP		R	M	re			

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.

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CHRONIC CHEMICAL DATA (INITIAL)

Project Number:

17001-474-Exp

Test Species: Artemia franciscana

%	Day	Day	Dav	Dav	Dav	Dav	Day	Dav	Meter #	Remarks
	0	1	Day 2	Day 3	Day 4	Day 5	6	Day 7		Remarks
Conc.: 100									All Conc.	
pН	7.9	8.0	1							
D.O. (mg/L)	5.1		\square							
Temp. (°C)	*	4.z	\square							
Cond. (µS/cm)	139,300						-			
Conc.:										
pН										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)						-				
Conc.:										
рН										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.:										
рН										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)				_			<u>,</u>			
Conc.: 200										
рН	7.9									
D.O. (mg/L)	5.2									
Temp. (°C)	5.) T							-		
Cond. (µS/cm)	136,600									
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L) NH₃ (mg/L)										
NH_3 (mg/L)										
Date	ialklix	12/20/17	18/21/19							
Time		1515	1425							
Initials	C P	AS	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.

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Effective 02/14

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CHRONIC CHEMICAL DATA (FINAL)

Project Numb	er:	1700	1-474-E	хр							
Test Species:		Artemia	a francis	scana							
0/											
%		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)
рН		8.1	8.0	80	80	8,0	8.0	81		FM28	
D.O. (mg/L)		5.4	5.4	3.7	5.0	4.9	5.1	6.1		n	
Temp (°C)		20	20	20	20	20	20	20		64	
Conc.:	5										
pH		8.0	8.0	80	8.0	% ,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	30	20	20	20	19			
Conc.:	20										
рН		8.1	8.0	8.0	80	80	8.0	8.1			
D.O. (mg/L)		5.4	5.3	5.5	5.1	4.9	5.2	59			
Temp (°C)		20	20	20	20	20	20	20			
Conc.:	50							129,00			(15)
рН		4.0	8.0	80	8.0	8.0	8.0	8.1			
D.O. (mg/L)		5.5	5.3	5.8	5.4	5.0	5.2	6.4			
Temp (°C)		20	20	20	06	20	20	90			
Conc.:	100		46 000	\mathbf{N}	\backslash	1	$\left[\right]$	\backslash			
pН		8,0	8.0				$\left \right\rangle$		ý		
D.O. (mg/L)		5.5	5.4								
Temp (°C)		20	20		$\langle \rangle$						· · · · · · · · · · · · · · · · · · ·
Conc.:	200	142700	$\mathbf{\Lambda}$	\mathbf{A}	$\int dt$	$\left(\right) \left(\right)$	< <u> </u>				* conductivity
рН		9,0					\square				
D.O. (mg/L)		5.5									
Temp (°C)		20	900								
Conc.:											
рН											
D.O. (mg/L)											
Temp (°C)											
	Date:	12/20/19	12/21/19	plant	10/23/14	12/24/19	12/25/19	Block			
	Time:	1550	1455	1325		1505	100	1540			
	Initials:	prs 1	CP	RS	n	Ars	EL.	R			

1) CP 12/21/19 E

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DAILY TOXICITY TEST LOG

Project Numb	ber: 17001-474-Exp)		
Test Species	Artemia francisc	ana		
General			Feeding	Initials/Date
Comments			145 ug/l Chla	
	Random Chart: <u>N</u>	Min/Max Thermometer # M-15	upon renewal	
Test Day 0	Test Solution Mixed at: 110 Test Organisms Added at: 150>		Fed @ 1110	R5
	Spiked C			12/Filly
Test Day 1	Real Time: 20 °C	Min-Max Range: 19-21 °C	Fed @ 1055	ßъ
	Spihea @ 1055			12 Wolcy .
Test Day 2	Real Time: ାୁମ୍ °C	Min-Max Range: / 9 - 2 (°C	Fed @ 1045	СГР
	Spiked e 1045			12/21/19
Test Day 3	Real Time: \૧ °C	Min-Max Range:) (ー み ぃ °C	Fed @ 0540	R\$
	Spiked @ 0940			6199115
Test Day 4	Real Time: ႑ဂ °C	Min-Max Range: ເຈົ.ລ ວ °C	Fed @ 10415	Ray
	Spille 1045			19/22/11
Test Day 5	Real Time: ₍₉ °C	Min-Max Range: ເຮຼວບ °C	Fed @ 1\20	Aa.

1212-14 Spited @ 1120 Real Time: 19 °C Min-Max Range: 19 - 20 °C Test Day 6 Fed @0740 Ee. Spilled COT40 Real Time: 19 °C 12/25/19 Min-Max Range: 19-20 Test Day 7 °C Fed @ R Non 121261A Test Day 8 Real Time: °C Min-Max Range: °C Fed @

			Y	-			.					Page of QA Form No.010 Revision 6	of No.010
N					TEST ORG	ORGANISA	A LENGTHS	, WEIGHT	ANISM LENGTHS, WEIGHTS, AND LOADING	DING	þ	Effective 02/14 AG 1/6/20 722 vev 1/8/20	02/14 18/20
Project	Number: [10	-HTH-	Project Number: 1001-474- exp (d7) Test Substance:	Test Subst		Arsenic (NazHASO4)	(HASO4)		Comments:	د ا		
Species: A		rcis	franciscone	-	Analyst Tare:	e: تا	Analyst G	Analyst Gross: AF/W^N	N	Analytical Balan Dried in Oven #	Ice ID: Vort	₩\ tte: <u> 2/26/1</u> 9Tin	ne: 1540
Date/Tir	Date/Time of Tare Wt.: い2/26/14 のいつい	Vt: 12	124/190	1120	Date/Time (of Gross Wt.:	Date/Time of Gross Wt.: \2/28/\여ው \\\ 0	0111		to Date: 12/2014Time: 1035	to Da	tte: \ <u>2,/28/</u> 9Ti	me: <u>1035</u>
Boat	Treatment	Rep.	Length		Weight Type (Circle):	Wet Blot Dry	Dry Dry (>100°C)		AFDW (>500°C)		Lot or Batch	Lot or Batch Number: 121719	914
2			Ollis.	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	Mean Wt. per Treatment (mg) (Surviving)
	0	A		1.16536	800191.1	0.00072					σ	(Rui)	
		θ		1.13819	1.13898	Pt000.0					0		
		J		1.14734	52841.1	10.000-1					0		
		٩		1.14048	1.14155	F0100.0					10		
	5	4		1.18277	1,18314	0.00087					10		
		£		1,17587	1.17587 1.17677	0.00090					01 24		
		ა		1.15584	1.5.994	6.00093					گم	-	
		0		1.15707	1.15806	0,00099					01		
	2	æ		1.15384	hth:21.1	0,00090					õ		
		£		1.15846	1.15932	0,000%					10		
		J		1,13895	1.14000 0.00 000 1	2,00 l05					10		
		0		1.15365	1.15469 0.00084	H8000.0					0		
Blank				1-13876	1.13872	-0.000W							
Range													
Mean													
Test Solu	Test Solution Volume:	••					Loading Rate:						
Add in w	Add in weight loss of blank boat, if appropriate	blank	boat, if ap	propriate.									
	0.100						•						

•

OAF12/28/19 E

<u>.</u>

					١							Page of QA Form No.010 Revision 6	of No.010 6
					TEST (ORGANISA	A LENGTHS	, WEIGHT	TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING	DING	·	Ellective UZ/14 An (10ho BUL NBN V8	48/20
Project	Project Number: 17000 - 474 - Exp (47)	1-100	174 - 61	(L) 4X	Test Substance:		Arsenic (Naz HAsQ.)	1AsQJ)		Comments:			
Species: A.		franciscana	ष		Analyst Tare:	ū	Analyst G	Analyst Gross: AF/USA	R	Analytical Balance ID: Sort#) Dried in Oven # 3 from Date:	rce ID: Sart	D: Sart#) from Date: ^{12/24/14} Time: 1540	ne: 1540
Date/Tir	Date/Time of Tare Wt.: 12/26/19	Vt.: 12/	26/12 @	@ 1120	Date/Time	of Gross Wt.:	Date/Time of Gross Wt.: 12/28/19 (© 1110	01110			2 0	Date: 12/28/9 Time: 10.55	me: 10.35
Boat	Treatment	Rep.	Length	Weight Type (Circle):		Wet Blot Dry	Dry Dry (>100°C)		AFDW (>500°C)		Lot or Batch	Lot or BatchNumber: 121-19	91419
			2	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 (ma)	Mean Wt. per Treatment (mg) (Surviving)
	R	¢.		1.11457	ht+11,11	-£10000°0						T	
		Ъ		1.16643	P0000.0 25001.1	0.00009						1	
		J		1.12376	1.12411	G£000+0						0)	
		9		1-12289	1,12318	0.00029						T	
		-							-				
							.0						
Blank													
Range													
Mean													
Test Solu	Test Solution Volume:	_					Loading Rate:						
Add in w	Add in weight loss of blank boat, if appropriate.	blank I	ooat, if app	oropriate.					* *				

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TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 14001-474 7 day Arsenic

Species: Artemia franciscana

								Mean Wt./	Mean Wt./	-	Mean Wt./	Mean Wt./
		Length	Tare	Gross	Net Weight Net Weight	Adjusted Net Weight	No of Orig.	Original Organism	Treatment (mg)	Number of Surv.	Surviving Organism	I reatment (mg)
Treatment F	Rep	Units:	Weight (g)	Weight (g)		(g)	Organisms	(mg)	(Original)	Organisms	(mg)	(Surviving)
	A		1.16536	1.16608	0.00072	0.00076	10	0.076	0.0913	6	0.084	0.0934
, Ç	m		1.13819	1.13898	62000.0	0.00083	10	0.083		10	0.083	
ר קטר בייטר	0		1.14734	1.14825	0.00091	0.00095	10	0.095		10	0.095	ų.
1	Δ		1.14048	1.14155	0.00107	0.00111	10	0.111		10	0.111	
	∢		1.18227	1.18314		0.00091	10	0.091	0.0963	10	0.091	0.0989
	m		1.17587	1.17677	06000.0	0.00094	10	0.094		10	0.094	
∟ л/6ш с	ပ		1.15584	1.15677	0.00093	0.00097	10	260.0		6	0.108	
L	Δ		1.15707	1.15806	0.00099	0.00103	10	0.103		10	0.103	
	∢		1.15384	1.15474	06000.0	0.00094	10	0.094	0.0953	10	0.094	0.0953
	в		1.15846	1.15932	0.00086	06000.0	10	060.0		10	060.0	
	ပ		1.13895	1.14000		0.00109	10	0.109		10	0.109	
1			1.15385	1.15469	0.00084	0.00088	10	0.088		10	0.088	
	A		1.11457	1.11474	0.00017	0.00021	10	0.021	0.0265	4	0.053	0.0663
	۵		1.16643	1.16652	60000.0	0.00013	10	0.013		2	0.065	
	ပ		1.12376	1.12411		0.00039	10	0.039		9	0.065	
L	۵		1.12289	1.12318	0.00029	0.00033	10	0.033		4	0.083	
	A		0.00000	0.00000		0.00000	10	0.000	0.0000	Э	1	
100 2001	В		0.00000	0.00000		0.00000	10	0000		0	L	
	с U		0.00000	0.00		0.00000	10	0.000		0	1	
	Δ		0.00000	0.00000	0.00000	0.00000	10	0.000		0	I	
	A		0.00000	0.00000		0.00000	10	0.000	0.0000		1	#DIV/0!
	В		0.00000	0.00000		0.00000		0000		0	1	
	ပ		0.00000	0.00000		0.00000	10	0.000		0	T	
	D		0.00000	0.00000	0.00000	0.00000	10	0.000		0	t	
Blank			1.13876	1.13872	-0.00004							

Project Number: 14001-474 7 day Arsenic

Species: Artemia franciscana

		00 5.128%		33 40.825%		i0//IC# 0(53 16.797%	51 5.323%		17 44.169%	•••	;0//\IC# 0(
S	0.0500	0.0500	0.0000	0.1633	0.000	0.0000		SD	0.0153	0.0051	0.009	0.0117	0.0000	0.0000	
<u>Mean</u>				0.4000		0.0000	er original)	Mean	0.0913	0.0963	0.0953	0.0265	0.0000	0.0000	
-	1.0	1.0	1.0	0.6	0.0	0.0	ia (drv wt pe	Max	0.111	0.103	0.109	0.039	0.000	0.00	
Summary Statistics for Survival Data Treatment N <u>Min</u>	0.9	0.0	1.0	0.2	0.0	0.0	Growth Dat	Min	0.076	0.091	0.088	0.013	0.000	0.000	
tistics for <u>N</u>	4	4	4	4	4	4	listics for	Z	4	4	4	4	4	4	
ummary Stat Freatment	rGSL	5 mg/L	20 mg/L	50 mg/L	00 mg/L	200 mg/L	Summary Statistics for Growth Data (dry wt per original)	Freatment	rGSL	5 mg/L	20 mg/L	50 mg/L	100 mg/L	200 mg/L	

I

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

						#DIV/0	
2	SD						
-	<u>Mean</u>						
	Max	0.111	0.108	0.109	0.083	0.000	0.000
OLOWIN DALA	Min	0.083	0.091	0.088	0.053	0.000	0.000
5	Z	4	4	4	4	0	0
ounning orduouco	Treatment	rGSL	5 mg/L	20 mg/L	50 mg/L	100 mg/L	200 mg/L

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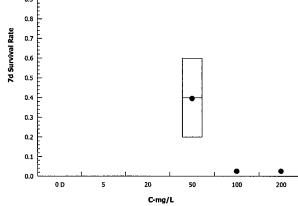
Arten										lest	Code:		4/4-/010	5-2577-039
Fathead Minne	ow 7-	d Larval S	urviva	and Growth	Test							TRE Envi	ronmenta	I Strategies
Analysis ID: Analyzed:)460-2271 Ian-20 9:07				vival Rat rametric-		vs T	reatments		IS Versio ial Resul		.8.7	
Batch ID:	18-0	895-7427		Test Type:	Growth	-Surviva	l (7d)			Anal	vst: La	ab Tech		
Start Date:		Dec-19 15:0	0			21/R-02-	• •	02)		Dilu	-	SSL		
Ending Date:	26 E	Dec-19 14:5	55			a francis				Brin	e: C	rystal Sea		
Duration:	7d			Source:	n-Hou	se Cultu	re			Age:	48	3h		
Sample ID:	03-3	624-0124		Code:	140A9	DFC				Clier	nt: U	niverity of Noti	e Dame	
Sample Date:	19 E	Dec-19 15:0	0	Material:	Arseni	5				Proj	ect: S	pecial Studies		
Receive Date:	19 E	Dec-19 15:0	0	Source:	researe	ch								
Sample Age:	NA			Station:										
Data Transfor	m		Zeta	Alt Hy	р Ті	rials	Seed			PMSD	NOEL	LOEL	TOEL	TU
Angular (Corre	cted)		NA	C > T	N	A	NA			10.7%	20 /	50	31.62	
Steel Many-Or	ne Ra	ink Sum Te	est							<u> </u>	/			
Control	vs	C-mg/L		Test St	tat C	ritical	Ties	DF	P-Value	P-Type	Decisio	on(α:5%)		
Dilution Water		5		18	1()	2	6	0.7500	Asymp	Non-Sig	nificant Effect		
		20		20	10)	1	6	0.9096	Asymp	Non-Sig	nificant Effect		
		50*		10	10)	0	6	0.0276	Asymp	Signific	ant Effect		
ANOVA Table														
Source		Sum Squ	ares	Mean S	Square	•	DF		F Stat	P-Value	Decisio	on(α:5%)		
Between		1.495771		0.4985	902		3		46.32	<0.0001	Signific	ant Effect		
Error		0.129159	5	0.0107	6329		12		_					
Total		1.62493		••••••			15							
Distributional	Test	S												
Attribute		Test				est Stat	Critic	al	P-Value	Decision				
Variances			-	uality of Varia		082	5.95		0.3939	Equal Var				
Variances			• •	of Variance		793	5.95		0.2021	Equal Var				
Distribution				Normality	0.	8358	0.841		0.0085	Non-norm	al Distribu			
7d Survival Ra		•	-											
			Cour			5% LCL	95% l	JCL	Median	Min	Max	Std Err	CV%	%Effect
D 5	Dilut	on Water	4 4	0.975 0.975		8954 8954	1 1		1 1	0.9 0.9	1 1	0.025 0.025	5.13% 5.13%	0.0% 0.0%
20			4	1	1	0904	1		1	0.9 1	1	0.025	0.0%	-2.56%
50			4	0.4		1402	0.659	8	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 (Corr	ected	l) Transfor	med S	ummary										
C-mg/L	Con	rol Type	Cour	nt Mean	9	5% LCL	95% l	JCL	Median	Min	Мах	Std Err	CV%	%Effect
0	Dilut	ion Water	4	1.371		242	1.501		1.412	1.249	1.412	0.04074	5.94%	0.0%
5			4	1.371		242	1.501		1.412	1.249	1.412	0.04074	5.94%	0.0%
20			4	1.412		412	1.412		1.412	1.412	1.412	0	0.0%	-2.97%
50			4	0.6798		4052	0.954		0.6847	0.4636	0.8861	0.08628	25.4%	50.4%
100			4	0.1588		1588	0.158		0.1588	0.1588	0.1588	0	0.0%	88.4%
200			4	0.1588	0.	1588	0.158	8 .	0.1588	0.1588	0.1588	0	0.0%	88.4%

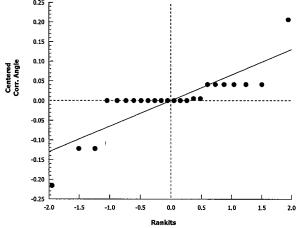
VE/20 QA: New

Analyst:

-.

Analyzed: 7d Survival Rat C-mg/L C	15-0460-2271 06 Jan-20 9:07			Test			
Analyzed: 7d Survival Rat C-mg/L C 0 E	06 Jan-20 9:07						TRE Environmental Strategies
C-mg/L C	to Dotail	4	-	'd Survival Ra Ionparametric	ate c-Control vs Treatments	CETIS Version: Official Results:	CETISv1.8.7 Yes
0 C	le Delan						
	Control Type	Rep 1	Rep 2	Rep 3	Rep 4		
5	Dilution Water	0.9	1	1	1		
-		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 (Correc	cted) Transfori	ned De	tail				
C-mg/L C	Control Type	Rep 1	Rep 2	Rep 3	Rep 4		
0 C	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	7 0.4636	0.8861	0.6847		
100		0.1588	3 0.1588	0.1588	0.1588		
200		0.1588	3 0.1588	0.1588	0.1588		
7d Survival Rat	te Binomials				· · ·		
C-mg/L C	Control Type	Rep 1	Rep 2	Rep 3	Rep 4		
0 C	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							· · · · · · · · · · · · · · · · · · ·
1.0 0.9		•			0.25		•





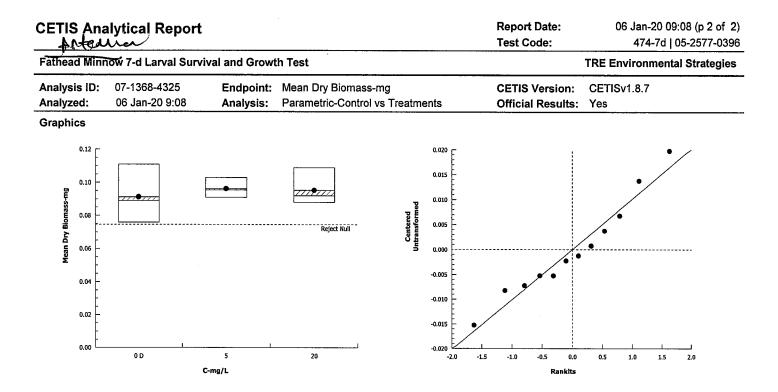
(

1/8/20 QA: 1/21 Analyst: AB

> .

CETIS Anal	lytical Repo	rt							ort Date: t Code:			08 (p 1 of 2 5-2577-039
	7-d Larval Su	ırvival	and Growt	h Te	st		• • • • • •			TRE Envi	ronmenta	I Strategies
Analysis ID: Analyzed:	07-1368-4325 06 Jan-20 9:08		Endpoint: Analysis:		an Dry Biom ametric-Con		tments		IS Version		8.7	
Batch ID:	18-0895-7427		Test Type:	Gro	wth-Surviva	l (7d)		Ana	lyst: La	b Tech		••••
Start Date:	19 Dec-19 15:00	כ	Protocol:	EPA	V821/R-02-0	013 (2002)		Dilu	ent: rG	SL		
Ending Date:	26 Dec-19 14:5	5	Species:	Arte	emia franciso	cana		Brir	ne: Cr	ystal Sea		
Duration:	7d		Source:	In-H	louse Cultur	e		Age	: 48	h		
Sample ID:	03-3624-0124		Code:	140	A9DFC			Clie	nt: Un	iverity of Notr	e Dame	
Sample Date:	19 Dec-19 15:00	כ	Material:	Arse	enic			Project: Special Studies				
Receive Date:	19 Dec-19 15:00	כ	Source:	rese	earch				_			
Sample Age:	NA		Station:									
Data Transform	n	Zeta	Alt H	ур	Trials	Seed		PMSD	NOEL	LOEL	TOEL	τu
Untransformed		NA	C > T		NA	NA		18.3%	20	>20	NA	
Dunnett Multip	ole Comparison	Test										• • • • • • •
Control	vs C-mg/L		Test	Stat	Critical	MSD DF	P-Value	P-Type	Decisio	n(α:5%)		
Dilution Water	5		-0.653	33	2.18	0.017 6	0.8676	CDF	Non-Sig	nificant Effect		
	20		-0.522	27	2.18	0.017 6	0.8368	CDF	-	nificant Effect		
ANOVA Table												
Source	Sum Squa	res	Mean	Squ	are	DF	F Stat	P-Value	Decisio	n(α:5%)		
Between	5.600005E	-05	2.800	002E	-05	2	0.239	0.7922	Non-Sig	nificant Effect		
Error	0.0010542	5	0.000	1171	389	9	_					
Total	0.0011102	5				11						
Distributional	Tests											
Attribute	Test				Test Stat	Critical	P-Value	Decision	ι(α:1%)			
Variances	Bartlett Ed	quality	of Variance		2.771	9.21	0.2502	Equal Va	riances			
Distribution	Shapiro-V	Vilk W	Normality		0.9577	0.802	0.7505	Normal D	istribution			
Mean Dry Bior	nass-mg Summ	ary										
C-mg/L	Control Type	Coun	t Mean	1	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	0.091	25	0.06686	0.1156	0.089	0.076	0.111	0.007663	16.8%	0.0%
5		4	0.096	25	0.0881	0.1044	0.0955	0.091	0.103	0.002562	5.32%	-5.48%
20		4	0.095	25	0.08013	0.1104	0.092	0.088	0.109	0.00475	9.97%	-4.38%
Mean Dry Bior	nass-mg Detail											
C-mg/L	Control Type	Rep 1	Rep 2	2	Rep 3	Rep 4			No. 10			
0	Dilution Water	0.076	0.083		0.095	0.111						
_		0.091	0.094		0.097	0.103						
5		0.031	0.004		0.001	0.105						

Analyst: M QA: WW



18)00 Analyst: Ag QA: 15

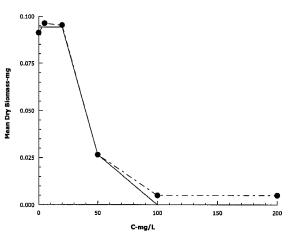
AA_	たい	-les	-						T	est Code:			474-7d 0	5-2577-03
Fathea	d Minna	🛪 7-d Larval S	urvival	and Growt	h Test			······				TRE Env	ironmenta	
Analysi		01-6014-3215		Endpoint:		•	mass-mg		-	ETIS Vers		CETISv	1.8.7	
Analyze	ea:	06 Jan-20 9:08	5	Analysis:	Linear	nterpo	lation (ICPII	N)	0	fficial Res	sults:	Yes		
Batch I	D:	18-0895-7427		Test Type:	Growth	-Surviv	/al (7d)		Α	nalyst:	Lab T	ech		
Start D	ate:	19 Dec-19 15:0	00	Protocol:	EPA/82	21/R-02	2-013 (2002))	D	iluent:	rGSL			
Ending	Date:	26 Dec-19 14:5	55	Species:	Artemia	a franci	iscana		В	rine:	Cryst	al Sea		
Duratio	n:	7d		Source:	In-Hous	se Cult	ure		A	ge:	48h			
Sample	D:	03-3624-0124	N.	Code:	140A9I	OFC			С	lient:	Unive	rity of No	tre Dame	
Sample	Date:	19 Dec-19 15:0	00	Material:	Arsenic	;			P	roject:	Speci	ial Studies	6	
Receive	e Date:	19 Dec-19 15:0	00	Source:	researc	:h								
Sample	e Age:	NA		Station:										
Linear	Interpo	lation Options												
X Trans	form	Y Transform	n	Seed	Resam	ples	Exp 95%	% CL Met	hod					
Linear	/	Linear		268245	200		Yes	Two	-Point Int	erpolation				
Point E	stimate	s												
Level	mg/L	95% LCL	95% I	JCL										
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	and the second designed in the second designed and the										
IC40	36.69	32	40.78											
IC50	40.87	35.87	46.11		···· .						<u>.</u>			
Mean D	ry Bion	nass-mg Sumn	nary				Ca	alculated Va	ariate				-	
C-mg/L		ontrol Type	Coun	t Mean	Mi	n	Max	Std Err	Std De	v CV%		%Effect		
0	Di	lution Water	4	0.091	25 0.0	076	0.111	0.007663	0.0153	3 16.89	6	0.0%		
5			4	0.096		091	0.103	0.002562				-5.48%		J
20			4	0.095		088	0.109	0.00475	0.0095			-4.38%		
50			4	0.026		013	0.039	0.005852		44.29		71.0%		
100			4	0	0		0	0	0			100.0%		
200			4	0	0		0	0	0			100.0%		
		nass-mg Detail												
C-mg/L		ontrol Type	Rep 1			ep 3	Rep 4							
0	Di	lution Water	0.076			095	0.111							
5			0.091			097	0.103							
20			0.094			109	0.088							
50			0.021)39	0.033							
100			0	0	0		0							
200			0	0	0		0							
1														

|w|8 in QA:__

Analyst: M

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CETIS Ana	alytical Report			Report Date: Test Code:	06 Jan-20 09:08 (p 2 of 2) 474-7d 05-2577-0396
Fathead Minr	now 7-d Larval Surv	ival and Grow	th 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



18/20 Analyst: M QA:

Page 1 of _____ QA Form No. 051 Revision 5 Effective 02/14

					Effective U2	1
		TOXICITYON	TA PACKAGE COVER	SHEET	Oh nh	w 1/11/20
Test Type:	Chronic	(pd)	Project Numbe	r:	<u>17001-474-E</u>	<u>xp</u>
Test Substance:	Arsenic (N	a2HAsO4)	Species:	Artemia franc	viscana	
Dilution Water:	rGSL		Organism Lot	t or Batch Numb	per:	12/719
Concurrent Control Water:	NA		Age	<u>(48 hr)</u>	Supplier:	TRE
Date and Time Test Began:	12/19/19	@ 1510	Date and Tim	e Test Ended:	12/29/19 0	1415
Protocol Number:			Investigator(s): A3 BOTH	PIENICF	
Background Information				1 1-		
Type of Test:	Static-Ren	ewal (Daily)	pH control?: If yes, give %	Yes	No	
Test Temperature:	<u>20 ±</u> 1 °C		Env. Chmbr/Bath #: _25	-	NA Test C	hmbrs: <u>147-ml cups</u>
Photoperiod:	<u> 16 h light :</u>	- <u>8 h dark</u>	Light intensity		<u>50-100 ft-c.</u>	1111013. <u>147-IIII Cups</u>
Test Solution Vol.:		50 ml	_ Replicates per		<u>00-100 n-c.</u> 4	
Length of Test:	10 days	_	Organisms pe		10	
Type of Food and Quantity pe	r Chamber:	145 ug/L Chla	- Feeding Frequ	Jency:	Initiation and	Renwals
Test Substance Characteriza	ation Param	eters and Freq	uency:			
Hardness: <u>Test Initiation</u>	Alkalinity:	Test Initiation	NH ₃ : <u>Test Initiation</u>	TRC: <u>Test Ini</u>	tiation	
pH: <u>Daily</u>	Conductivit	y: <u>Daily</u>				
Test Concentrations (Volume:	Volume):	rGSL, 5, 20, 50	, 100, and 200 mg/L as A	<u>S</u>		
Agency Summary Sheet(s)?:	<u> </u>	None				· · · · · · · · · · · · · · · · · · ·
Reference Toxicant Data:	Test Dates:		to		IC ₂₅ :	\sim
Hist. 95% Control Limits:	<u> </u>	to	Method for Determining F	Ref. Tox. Value:	Linear Interpo	plation
Special Procedures and Con	-1-1					
Organisms hatched 2 days prid						
			se with 100 ug/e Chia			
				. <u></u>		
Appropriate correction factors						
Appropriate correction factors Study Director Initials: Δ	nave been a	t (* 1	peratures recorded in this of	data package		
L/MS_		Date: 12 [9	(9			

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TEST SUBSTANCE USAGE LOG

Project Number:

<u>17001-474-Exp</u>

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number				Oample 4
	From:	From:	From:	From:
Test Substance Collection	@	@	@	@
Date and Time	То:	То:	To:	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#	MA 1	NA		
Date(s) Used	12/21/19 12/25/ 12/21/19 12/25/ 12/21/19 12/25/ 12/21/19 12/25/	a 12/28/19		

Preparation of Test Solutions

Test	Dilution	Total	Test	Dilution	Total	Test	Dilution	Total
Substance	Water	Volume	Substance	Water	Volume	Substance	Water	Volume
Volume	Volume	(mi)	Volume	Volume	(ml)	Volume	Volume	(ml)
(ml)	(ml)		(ml)	(ml)		(ml)		(,
250	0	250						
0.33	249.67	250						
1.34	248.66	250	See Spiki	ng Sheet				
3.34	246.66	250						
6.68.55	243.32	250						
13.37	236.63	250						
275.07	1224.93	1500						
ps 12/11	9		EN 12/27	119				
AS VA	9 (2							
CR 12/2	4/19							
Re aloa	117	11 A.						
0 * *						<u> </u>		
EN 12/2	s/(g							
	6/19					<u> </u>		
	Substance Volume (ml) 250 0.33 1.34 3.34 $6.98.55$ 13.37 275.07 Λ_3 Λ_3 275.07 Λ_3 L_2 L_2 L_2 L_2 L_2 L_2 Λ_3 L_2	Substance Water Volume Volume (ml) (ml) 250 0 0.33 249.67 1.34 248.66 3.34 246.66 0.33 249.67 1.34 248.66 3.34 246.66 $0.68.55$ 243.32 13.37 236.63 275.07 1224.93 Λ_3 $\nu \nu 0 0 11$ Λ_3 $\nu 20 (L) 19$ ℓ_{X} $\eta \partial \partial 11$ μ_{X} $\eta \partial \partial 11$ μ_{X} $\eta \partial \partial 11$ μ_{X} $\eta \partial \partial 11$ μ_{X} $\eta \partial \partial 11$ μ_{X} $\eta \partial \partial \partial 0 $ EN <	Substance Water Volume Volume Volume (mi) (mi) (mi) 250 0 250 0.33 249.67 250 1.34 248.66 250 3.34 246.66 250 0.33 249.67 250 1.34 248.66 250 3.34 246.66 250 0.33 249.67 250 1.34 248.66 250 0.334 246.66 250 0.334 246.63 250 13.37 236.63 250 13.37 236.63 250 275.07 1224.93 1500 A_3 ν ν ν A_3 ν ν ν A_3 ν ν ν A_3 ν ν ν A_4 ν ν ν A_4 ν <td>Substance Water Volume Substance Volume Volume (ml) Substance (ml) (ml) (ml) (ml) 250 0 250 (ml) 0.33 249.67 250 (ml) 1.34 248.66 250 See Spikin 3.34 246.66 250 (ml) 668.55 243.32 250 (ml) 13.37 236.63 250 (ml) 275.07 1224.93 1500 (ml) Λ_3 $\nu \nu_0 (\mu)$ EN $12/27$ Λ_3 $\nu 20 (\mu)$ (ml) (ml) Λ_3 $\nu 20 (\mu)$ EN $12/27$ Λ_3 $\nu 20 (\mu)$ (ml) (ml) (ml) Λ_3 $\nu 20 (\mu)$ EN $12/27$ (ml) Λ_4 $\nu 20 (\mu)$ EN $12/27$ (ml) Λ_4 $\nu 20 (\mu)$ EN $12/27 19$ (ml)</td> <td>Substance Water Volume Substance Water Volume Volume (ml) Substance Water (ml) (ml) (ml) (ml) (ml) 250 0 250 (ml) (ml) 0.33 249.67 250 (ml) (ml) 1.34 248.66 250 See Spiking Sheet 3.34 246.66 250 (ml) (ml) 6.66.55 243.32 250 (ml) (ml) 13.37 236.63 250 (ml) (ml) 275.07 1224.93 1500 (ml) (ml) Λ_3 12 m (m) En 12 / 7 / 10 Λ_3 12 m (m) En 12 / 7 / 10 Λ_3 12 m (m) En 12 / 7 / 10 Λ_3 12 m (m) En 12 / 7 / 10 Λ_3 12 m (m) En 12 / 7 / 10 Λ_4 10 / 3 / 1.1 En En 12 / 7 / 10 Λ_4 12 / 7 / 10 En 12 / 7 / 10 En</td> <td>Substance Water Volume Substance Water Volume Volume (ml) (ml) (ml) (ml) (ml) (ml) (ml) (ml) (ml) (ml) (ml) 250 0 250 </td> <td>Substance Water Volume Substance Volume (ml) Image: Substance Substance Volume (ml) Image: Substance Substance Volume (ml) Image: Substance Volume Image: Sub</td> <td>Substance Water Volume Substance Water Volume Water Volume (ml) Substance Water Volume (ml) (</td>	Substance Water Volume Substance Volume Volume (ml) Substance (ml) (ml) (ml) (ml) 250 0 250 (ml) 0.33 249.67 250 (ml) 1.34 248.66 250 See Spikin 3.34 246.66 250 (ml) 668.55 243.32 250 (ml) 13.37 236.63 250 (ml) 275.07 1224.93 1500 (ml) Λ_3 $\nu \nu_0 (\mu)$ EN $12/27$ Λ_3 $\nu 20 (\mu)$ (ml) (ml) Λ_3 $\nu 20 (\mu)$ EN $12/27$ Λ_3 $\nu 20 (\mu)$ (ml) (ml) (ml) Λ_3 $\nu 20 (\mu)$ EN $12/27$ (ml) Λ_4 $\nu 20 (\mu)$ EN $12/27$ (ml) Λ_4 $\nu 20 (\mu)$ EN $12/27 19$ (ml)	Substance Water Volume Substance Water Volume Volume (ml) Substance Water (ml) (ml) (ml) (ml) (ml) 250 0 250 (ml) (ml) 0.33 249.67 250 (ml) (ml) 1.34 248.66 250 See Spiking Sheet 3.34 246.66 250 (ml) (ml) 6.66.55 243.32 250 (ml) (ml) 13.37 236.63 250 (ml) (ml) 275.07 1224.93 1500 (ml) (ml) Λ_3 12 m (m) En 12 / 7 / 10 Λ_3 12 m (m) En 12 / 7 / 10 Λ_3 12 m (m) En 12 / 7 / 10 Λ_3 12 m (m) En 12 / 7 / 10 Λ_3 12 m (m) En 12 / 7 / 10 Λ_4 10 / 3 / 1.1 En En 12 / 7 / 10 Λ_4 12 / 7 / 10 En 12 / 7 / 10 En	Substance Water Volume Substance Water Volume Volume (ml) (ml) (ml) (ml) (ml) (ml) (ml) (ml) (ml) (ml) (ml) 250 0 250	Substance Water Volume Substance Volume (ml) Image: Substance Substance Volume (ml) Image: Substance Substance Volume (ml) Image: Substance Volume Image: Sub	Substance Water Volume Water Volume (ml) Substance Water Volume (ml) (

() EN 12/24/19 6

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Artemia franciscana CHRONIC BIOLOGICAL DATA

Project Number:

<u>17001-474-Exp</u>

				· ·							<u>- 79</u>
						Numb	er of Surv	viving Ord	anisms		Lobar Van
mg/L	Test Replicate	Day 0	Day 1	Day	Day	Day	Day	Day	Day		
0	A	10		10	3	4	5	6	7	Rema	arks
	B	10	10	10	10	<u>10</u>	10		10	* I weak on	0
	C	I	10	10	10	10	- 10	10	10	~	
		10	10	10		10		1.0	10	* I were org	
5	A	10	T	10	10	+	61	0	10		
	В	10	<u> </u>	10	10	10	10	10	10		1097
	C	10	10	10		10	10	10	10		
	D	10	10		10	10	10	0	10		
20	A	io		10		10	W	0	10		
	B	10	10	10	10	10	10 10×	9	9	I weak over	94.5
	C		+	10	+lo	10	10 × 10	10*	10*	A West org	
	D	10 10	10	10	10	10	10	10	10		
50	A	10	4					10	10		
	В	10	5	4	4	4	4	4	4		47.5
	C	10	5	5	5	5	5	5	5		
	D	10	5	5	5	5	5	5	5	· · · · · · · · · · · · · · · · · · ·	
100	A	10	3	2			5	5	6		
	В	10	<u> </u>	Ő		1		1)		- 25
	c	10	0	$\frac{0}{0}$							
	D	10	2	2	9	2					
200	A	10	0		2	<u> </u>	2	2	2		
	A B	10	0	∖		$ \land -$					5/2
	C D	10	0	+ -	$\left \cdot \right\rangle$			<u> </u>	-		
	D	10	0	+ +	\vdash	\vdash	$\overline{-}$	+	-		
	A			<u>├</u>		<u> </u>		<u> </u>			
	B										
	C										
	D										
		atialei	12/20/19	12/21/19	Obilie	1	ha ha ha	10h-lin	value 1		
· <u> </u>	Time:	1510	12019	1550	1350		12/24/19				
	Initials:	Bir	AS	HR	1350 Pd	1455 As	1455 En	1105 Er	1255		
		1.101			5	<u></u>	EN	U"	EN		~

04 440 1/1/20 2 d

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Artemia franciscana CHRONIC BIOLOGICAL DATA

1

Project Number:

<u> 17001-474-Exp</u>

	T				 Number	of Survi	iving Orga	anisme	
mg/L	Test Replicate	Day 8	Day 9	Day 10					Remarks a
0	A	10	10	9			<u> </u>		
	В	10	10	10					7 E.S. 10
	С	10	10	10			-		
	D	10	10	10			-		
5	A	10	10	10			<u> </u>	<u>+</u>	00
	В	10	10	10					100
	с	10	0	10					
	<u>D</u>	10	10	10					
20	A	9	9	8			1		956
	В	10*	10*	10*					* weak oig (tiny) 70 6
	с	10	10	10					
	D	[])	10	10					
50	A	4	4*	4*					* 1 weak ors 1157
	В	55	5	5					40 6
	С		5*	5					* I meak org
	D	6	5*	4					* 1 meak org * 1 weak org
100	Α	1	1	1					17
	В	-							
	C	~							
	D	2	2	2					
200	А	1	\backslash	\backslash					07
	В	1		$\left[\right]$					
	С	-		$\left \right\rangle$					
	D								
	А								
	В								
	С				X				
	D								
	Date:	12/27/19	12/28/19	12/29/19					
		1315	1440	1415	 				
	Initials:	EN	CP	CP					

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CHRONIC CHEMICAL DATA (INITIAL)

QL NO 1/11/20

Project Number:

J

17001-474-Exp

Test Species: Artemia franciscana

%	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day	Meter #	Remarks
Conc.: 0			<u> </u>		<u> </u>	<u> </u>		<u> </u>	All	
	\$.0	8.0	0		a				Conc.	
	5.4		8.0	7.9	8.0	7.9	7.9	7.9	Fm28	
D.O. (mg/L)	20	5.2	7.0	5.2	5.2	5.3	5.5	5.7	n	
Temp. (°C)		20	20	30	20	20	26	20	181	
Cond. (µS/cm)	150400	159300	1741800	136300	۵ (139000	140200	141700	15	
Hard. (mg/L)			<u> </u>							
Alk. (mg/L)		· · · · ·								
TRC (mg/L) NH₃ (mg/L)			<u> </u>	-			<u> </u>			
	1	<u> </u>	<u> </u>	 	<u> </u>	<u> </u>				
Conc.: 5			00		ļ			<u> </u>		
pH	7.8	7.9	8.0	7.9	\$,0	7.9	7.9	7.9		
D.O. (mg/L)	5,3	5.1	69	52	9.3	5.1	5.4	5.7		
Temp. (°C)	*	¥	×	<u>x</u>	×	*	*	`X		
Cond. (µS/cm)	140400	137100	130300	13670	Á	138400	1391700	140000		
Hard. (mg/L)	 			<u> </u>		<u> </u>				
Alk. (mg/L)										
TRC (mg/L) NH ₃ (mg/L)										
Conc.: 20										
рН	7.8	7.9	8.0	7.9	7.9	7.9	7.9	7.9		
D.O. (mg/L)	5.3	5,2	U.7	5.2	5.4	5.1	5.4	5.7		
Temp. (°C)	*	×	*	×	×	¥	×	¥		
Cond. (µS/cm)	139600	137100	137100	137600	Â	138100	137200	139500		
Conc.: 50										
pH	7.9	9.0	8.0	7.9	4.0	7.9	7.9	7.9		
D.O. (mg/L)	5.2	5.(6.6	5.3	5.4	5.1	5.2	56		······································
Temp. (°C)	*	×	*	☆	×	¥	¥	×		
Cond. (µS/cm)	134900	137500	137800	137,700	6	137700	135406	139400		
Date:	12/19/17			BDDIN		12/24/19	12/25/19			
Time:		1555		1335	MUD	1450	NOO	1250		
Initials: Note: Hardness, alkalinit		ks	HR	B	m	EN	Er	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.

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A meter errol

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CHRONIC CHEMICAL DATA (INITIAL)

Project Number:

17001-474-Exp

Test Species: Artemia franciscana

%		Day	Day	Day	Day	Day	Day	Day	Day	Meter #	Remarks
Conc.:		0	1	2	3	4	5	6	7		
Conc	100									All	
pH		7.9	8.0	8.0	7.9	4,0	7.9	7.9	7.9	Conc.	
D.O. (mg/L)		5.2	5.1	<u>U.U</u>	5.4	3.6	5.2	-	1.9 5.8		
Temp. (°C)		X	×	X	-*	¥	×	<u>5,3</u> ¥	*		
Cond. (µS/cm)		137200	1367	13790	137500	<u>м</u>		1339100	1201100		
Conc.:								1.55100			
рН				<u> </u>	<u> </u>					-	
D.O. (mg/L)											
Temp. (°C)							-				
Cond. (µS/cm)											
Conc.:							1				
pH											
D.O. (mg/L)								<u> </u>			
Temp. (°C)											
Cond. (µS/cm)											
Conc.:											
pH											
D.O. (mg/L)	_						·····				
Temp. (°C)											
Cond. (µS/cm)											
Conc.:	200										
pH		7.9			\backslash			-			
D.O. (mg/L)		5.2									
Temp. (°C)		Uget *	Þ								
Cond. (µS/cm)		137300	/								
Hard. (mg/L)						\					
Alk. (mg/L)				`							
TRC (mg/L) NH₃ (mg/L)											
	Date:	2 Alu	12/20/4	12/21/19	BIDIE	12/22/19	12/2W/10	rinsly	npilia		
	Time:	1505	1555	1555	1225	1440	1450		1250		
Ir	nitials:	M	AB	HR.	1335	₿B.	EN	K D	K.		
Note: Hardness, a	alkalinity	/ TRC a	DO NH3 d	ata annoa	ring on th			<u> </u>			

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.

OAB blains

@A3 wpoline

Page 4 of _____ QA Form No. 058 Revision 4 Effective 02/14

CHRONIC CHEMICAL DATA (INITIAL)

Project Number:

17001-474-Exp

Test Species: Artemia franciscana

%	Day O & B	Day	Day 0 210	Day 3	Day 4	Day 5	Day 6	Day	Meter #	Remarks
Conc.: 0			- 710		. 4		0	7	All	<u> </u>
рН	7.9	8.0							Conc. FM28	
D.O. (mg/L) 5.7	-								17	
Temp. (°C)	20	20			-				1-6	
Cond. (µS/cm)	134000	140000							15	
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L) NH₃ (mg/L)										
Conc.: 5	<u> </u>									
рН	7.9	8.0								
	134200									
Temp. (°C)	×	*								
Cond. (µS/cm)	134800	135500								
Hard. (mg/L)										·····
Alk. (mg/L)										
TRC (mg/L)										
NH ₃ (mg/L)						-				
Conc.: 20										
рН	7.9	8.0								
D.O. (mg/L)	5.3	5.9								
Temp. (°C)	¥	*								
Cond. (µS/cm)	134200	135200								
Conc.: 50										
рН	7.9	8.0								
D.O. (mg/L)	5.3	5.9								
Temp. (°C)	*	*								
Cond. (µS/cm)	134106	135200								
Date:	12/27/19	12/28/19							<u> </u>	
Time:	1310	1420								
Initials: Note: Hardness, alkalini		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.

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Page 5 of _____ QA Form No. 058 Revision 4 Effective 02/14

CHRONIC CHEMICAL DATA (INITIAL)

er is ilupo

Project Number:

17001-474-Exp

Test Species: Artemia franciscana

%		Day 8	Day	<u> </u>						Meter #	Remarks
Conc.:		<u> </u>	9	<u> </u>	<u></u>		<u> </u>				
	100									All Conc.	
pН		7.9	8.0		<u> </u>						
D.O. (mg/L)		5.3	6.0		†			-			
Temp. (°C)		¥	*				<u> </u>	1			
Cond. (µS/cm)		1333900	133800						<u> </u>		
Conc.:								<u> </u>			
pH					<u> </u>						
D.O. (mg/L)											
Temp. (°C)											
Cond. (µS/cm)								<u> </u>			
Conc.:											
pH											
D.O. (mg/L)											
Temp. (°C)											
Cond. (µS/cm)											
Conc.:											
pH											
D.O. (mg/L)											
Temp. (°C)											
Cond. (µS/cm)				_							
Conc.:	200	\mathbf{i}	<u> </u>		_						
pH			\rightarrow								
D.O. (mg/L)		_	\rightarrow								
Temp. (°C)			$- \downarrow \downarrow$								
Cond. (µS/cm)		-	$ \rightarrow $								
Hard. (mg/L)		Y									
Alk. (mg/L)											
TRC (mg/L) NH₃ (mg/L)											
	Date:		2/28/19								
	Time:		1420								
Ir Note: Hardness, a	nitials:	TRC	CP	10 or							

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.

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CHRONIC CHEMICAL DATA (FINAL)

^{6+ ~~ 1/11/20}

Test Species:		01-474-8 1110 11-474-8								
	Arten		iscana	<u>, and the second secon</u>						
%	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter #	Remarks
Conc.: 0			(T		<u>+</u>		All Conc.	* conductivity
pH	9,0	8.0	80	8.0	8.0	8.0	8.0	7.9	Fm28	conductivity
D.O. (mg/L)	5.1	58	5.8	4.8	5.0		-	4.7	17	
Temp (°C)	20	21	21	20	20	5.3 HO 19	20	20	LG	
Conc.: 5							<u> </u>	+		
рН	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	46		
Temp (°C)	20	224		20	20	20	20	20		· · · · · · · · · · · · · · · · · · ·
Conc.: 20						<u> </u>		1		
рН	8.0	8.0	80	8,0	8.0	8.0	8.0	7.9		<u> </u>
D.O. (mg/L)	4.9	5.7	5.8	4.9	5.3	5.6	5.0	4.6		
Temp (°C)	go	21	31	20	20	20	20	20		
Conc.: 50										
pH	8.0	8.0	8.0	90	8.0	8.0	8.0	8.0		
D.O. (mg/L)	5.0	<i>Q</i> .1	61	4.50	5.4	5.6	5.1	4.8		
Temp (°C)	20	224	31	20	20	20	20	20		
Conc.: 100										
oH	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0		
D.O. (mg/L)	5.0	U.3	6.4	4.8	5.6		5.3	49		
Temp (°C)	20	22*	21	20	20	20	20	20		
Conc.: 200	141500	Λ	1							^c conductivity
»Н	9.0	\square	\square							
D.O. (mg/L)	5.0									
emp (°C)	20									X
Conc.:										
H										
D.O. (mg/L)										
emp (°C)										
Date	12/20/19	12/21/19	12/20/19	12/23/19	12/04/19	12/25/14	12/26/19	12/27/19		
Time:	1625	1520	1410	MSS	1510		1255	1333		
Initials: EN V225(19	AB	HR	PS	AG	EN	EN	EN	EN		

Project Number:

17001-474-Exp

▲ All peps checked

Page __ of __ QA Form No. 059 Revision 3 Effective 02/14

CHRONIC CHEMICAL DATA (FINAL)

Desired				EWICAL	DATA	(FINAL)		
Project Number:	170	01-474-Exp						
Test Species:	Artem	ia franciscana		 				
%		T <u>_</u>	<u> </u>	 				
28	Day 9	Day 10					Meter #	Remarks
Conc.: 0	<u> </u>	133500					All Conc.	* conductivity (いち)
pH	7.9	7.9					FM28	
D.O. (mg/L)	4.8	4.8					17	
Temp (°C)	19	18					L-6	
Conc.: 5								
рН	7.9	7.9						
D.O. (mg/L)	4.7	4.7						
Temp (°C)	19	18						
Conc.: 20								
рН	7.9	7.9						
D.O. (mg/L)	4.7	4.8						
Temp (°C)	19	18						_
Conc.: 50								
рН	8.1	8.1						
D.O. (mg/L)	5.3	6.2						
Temp (°C)	19	18						
Conc.: 100		129000						
рН	8.1	8.2						
D.O. (mg/L)	5.3	6.9						
Temp (°C)	19	18						
Conc.: 200		1						* conductivity
pH	$\left \right\rangle$							
D.O. (mg/L)								
Temp (°C)				 				
Conc.:				 <u>Г</u>			<u>+</u>	
pH								
D.O. (mg/L)								,
Temp (°C)				 				
Date:	12/28/19	12/29/19						
Time:	1420	1535						
Initials:	CP	CP		 				

Page 7 of ____ QA Form No. 055 Revision 3 Effective 02/14 at Yw Yu(20

DAILY TOXICITY TEST LOG

Project Num	per: 17001-474-			
Test Species				
General Comments	Random Chart: P.	Min/Max Thermometer # ᡨ~\ऽ	Feeding 145 ug/l Chla	Initials/Date
Test Day 0	Test Solution Mixed at: 10-5 Test Organisms Added at: 1 Spiked & 112-5		upon renewal Fed @ ໂເວ 5	873 12/19/11
Test Day 1	Real Time: 20 °C Sp:Kedel115	Min-Max Range: 19-21 °C	Fed @ 1115	13 12/20/14
Test Day 2	Real Time: 19°C Spikele 1100	Min-Max Range: 19-21 °C	Fed @ //00	HP 12/21/10
Test Day 3	Real Time: 19 °C Spikel @ 1000	Min-Max Range: ルムーラン °C	Fed @ (coc)	12/21/19 Pz 19/23/17
Test Day 4	Real Time: 19 °C Spited @ 1635	Min-Max Range: ເຮົາຂບ °C	Fed @ 1635	A3 12123/4
Test Day 5	Real Time: 19 °C Spiked @1120	Min-Max Range: 18 - 20 °C	Fed @ 1\20	EN Iztulla
Test Day 6	Real Time: 19 °C	Min-Max Range: ໂ9∼2ø °C	Fed @ OTSS	EN 12/25/19
	Real Time: 19 °C Spika ⁽ @0925	Min-Max Range: \9 ~20 °C	Fed @0935	EN 12/24/19
Test Day 8	Real Time: 19 °C spiked @0925	Min-Max Range: (& ~ 2∂ °C	Fed @ 09 2\$	EN 12/2 9 /19

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DAILY TOXICITY TEST LOG

Project Num	ber	170	01-474-	Evp			
Test Species	S:	Artem	ia franc	Liscana		 	
General Comments	Random Cha					 Feeding 145 ug/l Chla	Initials/Date
Test Day 9	Real Time:		°C	Min/Max Thermometer #		 upon renewal	
loor Day o	Spiked (19 5011 G		Min-Max Range: 19 - 20	°C	Fed @ //00	cf 12/28/19
							12/28/19
Test Day 10	Real Time:	18	°C	Min-Max Range: <i>17 - 19</i>	°C	 None	Cf 12/29/19
						Net	

	1				TEST ORG		A LENGTHS	, WEIGHT	ANISM LENGTHS, WEIGHTS, AND LOADING	DING		Page of QA Form No.010 Revision 6 Effective 02/14 של אייר ייאליעם	of No.010 6 02/14 1/\$/20
Project	Number:	-100	a-hth	Project Number: 1400 - 474 - Exp No day Test Substance:	Test Substa	ance: Ansenic	enic			Comments:	(. =	
Species:	: Avteria	8	Pronci Scena		Analyst Tare:	2	Analyst Gross: EN		MB/Jan	Analytical Balance ID: Sart# Dried in Oven # <u>3</u> from Date: <u>12</u>	nce ID: S <i>er</i> # <u>3</u> from Dɛ	D: Sar t # 1 from Date: <u>[2/24/</u> nTime: <u>1535</u>	me: 1535
Date/Tir	Date/Time of Tare Wt.: \2/29/19 (2) العاد المراحي المراحي المراحي المراحي المراحي المراحي المراحي الم	M.: 12	129/19 (<u>e</u>]1355	Date/Time of Gr	of Gross Wt.:	055 Wt: 12/31/19 0 1050		1/2/20 B		to Da	Date: <u>\2/3////</u> Time: _	me: <u>1015</u>
Boat	Treatment	Rep.	Length	Weight Type (Circle):		Wet Blot [Blot Dry (Dry (>100°C)	0°C) AFD	AFDW (>500°C)		Lot or Batch Number:		ia1719
Ś					Tare Gross Weight (g) 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	Mean Wt. per Treatment (mg) (Surviving)
	ଚ	Ą		1.12584	1.12801	0.00a17					6.	6	
	0	f		1.15262	1.15534	0,00272					0)		
	٥	J		1.15189	1,15432	0.00243			-		10		
	0	ç		1.17387	17600.0 822/1.1	17600.0					10		
	ς	Ą		1.11710	1.11985	0.00275					10		
	S	-		1.12638	1.12898	0.00000					10		
	v	2		1.13438	1.13734	0,00296					10		
	ৎ	0		1.17574 1.17839		0,00265					10		
	30	Ł		1.13768	1.13991 6.00223	0.00223					8		
	ଡ୍ଟ	Ð		1.16951	26100,0 14171.1	0,00196					01		
	ģ	J		1-1106-24	1.16897	0.00273					10		
	9e	9		1.14325	1.14576 0.0025	0.00251					0/		
Blank				1.17270	1,17278 +0.0008	t 6.00008							
Range													
Mean								-					
Test Solu	Test Solution Volume:						Loading Rate:						
'Add in w () €√	Add in weight loss of blank boat, if appropriate $\bigcirc \mathbb{C}^{J} \ 1/2/2^{O} \ \mathbb{C}$	blank	boat, if ap	propriate.									

٠

					TEST (ORGANISA	A LENGTHS	, WEIGHT	TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING	DING		Page of QA Form No.010 Revision 6 Effective 02/14 Ø4 wev 1/6/20	of No.010 6 02/14 1 / K/20
Project	Project Number: 17001 - 474-60 Way	001-	0-hth	e liver	Test Substance:	N	Ansenic	,		Comments:			
Species:	: Anternix	_	Fren iscena	2	Analyst Tare:	re: CP	Analyst G	Analyst Gross: EN H	HB/	Analytical Balance ID: Sout + 4/	nce ID: Sourt	-#~1 ate: \2/20/16/Tin	me: 1635
Date/Tir		VL: 12,	29/19 6	2 1355	Date/Time	of Gross Wt.	の Date/Time of Gross Wt.: 12/21/14 © 10らら	_	112/20 0050		С С	to Date: 12/3/[4 Time: 1015	ime: 1015
Boat	Treatment	Rep.	Length	Weight Type (Circle):	je (Circle):	Wet Blot Dry	Dry Dry (>100°D)	00°O) AFD	AFDW (>500°C)		Lot or Raton Number:		12/19
o Z			Cuits:	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 (ma)	Mean Wt. per Treatment (mg) (Surviving)
	8	Ł		1.16283	1,16338	0,00055					H		
	56	8		1.16922	1	1.16963 0.00041					S		
	50	J		1.12550		1.12588 0.00038					S		
	Ŗ	Ģ		1.17218	29000.0 08861,1 81261.1	6.00062					7		
	(00	Ķ		1,16443	1.16 444	1,0000,0					/		
	ß	a	i	1.13757	1.13762	0.00005					2		
Blank													
Range													
Mean													
Test Solu	Test Solution Volume:	·.					Loading Rate:						
TAdd in w	Add in weight loss of blank boat, if appropriate. $(0E^{J} + 1/2)^{20} = 0$	f blank	boat, if ap	propriate.									

 $\begin{array}{c} \text{Page} & \text{of} \\ \text{QA Form No. 010a} \\ \text{Revision 1} \\ \text{Revision 1} \\ \text{Effective 02/14} \\ \text{As} & \text{i} \left| \boldsymbol{c} \left(\boldsymbol{2} \boldsymbol{z} \right) \right| \\ \text{As} & \text{i} \left| \boldsymbol{c} \left(\boldsymbol{2} \boldsymbol{z} \right) \right| \\ \text{OM vs. } \left| \boldsymbol{j} \left(\boldsymbol{z} \right) \right| \\ \end{array}$

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 14001-474 10 day Arsenic

Artemia franciscana

Species:

Mean Wt./ Treatment (mg) (Surviving)	0.2568					0.2740				0.2497				0.1126					0.0175				#DIV/0					
Mean Wt./ Mean Wt./ Mean Wt./ Mean Wt./ Meanism (mg) (mg)	0.241	0.272	0.243	0.271	*	0.275	0.260	0.296	0.265	0.279	0.196	0.273	0.251	0.138	0.082	0.076	0.155	1	0.010	0.025	-	I	1		-	I		
Number of Surv. Organisms	თ	10	10	0		10	10	10	10	ω	10	10	10	4	5	5	4		-	2	0	0	 0	0	0	0		
Mean Wt./ Treatment (mg) (Original)	0.2508					0.2740				0.2357				0.0490					0.0015				0.0000					
Mean Wt./ Original Organism (mg)	0.217	0.272	0.243	0.271		0.275	0.260	0.296	0.265	0.223	0.196	0.273	0.251	0.055	0.041	0.038	0.062		0.001	0.005	0.000	0.000	 0.000	0.000	0.000	0.000		
No of Orig. Organisms	10			10			10		10	10	10	10	10	10	10	10	10		10	10	10	10	10	10	10	10		
Adjusted Net Weight (g)	0.00217			0.00271					0.00265	0.00223	0.00196	0.00273	0.00251	0.00055	0.00041	0.00038	0.00062		0.00001	0.00005	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000		
Net Weight (9)	0.00217	0.00272	0.00243	0.00271		0.00275	0.00260	0.00296	0.00265	0.00223	0.00196	0.00273	0.00251	0.00055	0.00041	0.00038	0.00062		0.00001	0.00005	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00008	
Gross Weight (g)	1.12801	1.15534	1.15432	1.17658		1.11985	1.12898	1.13734	1.17839	1.13991	1.17147	1.16897	1.14576	1.16338	1.16963	1.12588	1.17280		1.16444	1.13762	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	1.17278	cp
Tare Weight (g)	1.12584	1.15262	1.15189	1.17387		1.11710	1.12638	1.13438	1.17574	1.13768	1.16951	1.16624	1.14325	1.16283	1.16922	1.12550	1.17218		1.16443	1.13757	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	1.17270	·/ 20</td
Length Units:																				A								A BAN (I)
Rep	۲	ш	ပ	ò		∢	ш	ပ	۵	A	ß	ပ	۵	∢	m	ပ	۵	- -	- 1	- Y	ပ	۵	A	മ	ပ	Ω		
Treatment		rgsl	 				5 ma/l	0 119/L			20 ma/l				50 ma/l				E	100 mo/l				000 ma/l			Blank	

Project Number: 14001-474 10 day Arsenic

Species: Artemia franciscana

Summary Statistics for Survival Data	tics for Su	rvival Data				
<u>Treatment</u>	ZI	Min	Max	<u>Mean</u>	S	> 0
rgsl	4	0.0	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	i0//IQ#
Summary Statistics for Growth Data (dry wt per original)	tics for Gro	owth Data (d	irv wt per	original)		

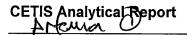
Summary Statistics for Growth Data (dry wt per original) Treatment N Min May Mean

<u>, </u>	10.452%	5.817%	14.202%	23.269%	158.698%	i0//IC#	
S	0.0262	0.0159	0.0335	0.0114	0.0024	0.0000	
Mean	0.2508	0.2740	0.2357	0.0490	0.0015	0.0000	
Max	0.272	0.296	0.273	0.062	0.005	0.000	
<u>Min</u>	0.217	0.260	0.196	0.038	0.000	0.000	
Z	4	4	4	4	4	4	
Treatment	rGSL	5 mg/L	20 mg/L	50 mg/L	100 mg/L	200 mg/L	

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

				B	(
<u>Treatment</u>	Z	<u>Min</u>	Max	<u>Mean</u>	<u>S</u>	<u>,<</u>
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	7	0.010	0.025	0.0175	0.0106	60.609%
200 mg/L	0	0.000	0.000	i0//IC#	i0//IC#	#DIV/0

Page of _____ QA Form No. 010a Revision 1 Effective 02/14 אין ילע Q1/8/1 mm 40



7d Jon

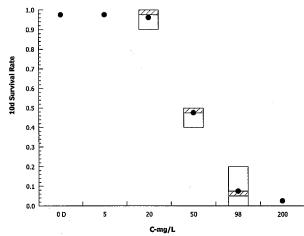
Report Date: Test Code: 06 Jan-20 09:16 (p 1 of 2) 474-10d | 03-1077-5012

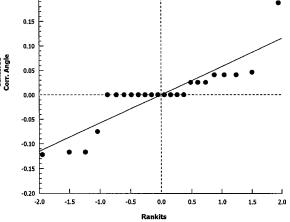
Fathead Minn	ww 7-d Larval	Surviva	I and Growth	Test		•••			TRE Env	ironmental	Strategie
Analysis ID:	-		Endpoint: 10d Survival Ra				CETIS Version:		on: CETISv1	CETISv1.8.7	
Analyzed:	06 Jan-20 9:1	6	Analysis: N	lonparametric	-Control v	s Treatments	Offi	cial Resu	lts: Yes		
Batch ID:	02-7744-5107		Test Type: 0	Growth-Surviva	al (10d)		Ana	lyst: L	ab Tech		
Start Date:			Protocol: E				Diluent: rGSL				
Ending Date:	29 Dec-19 14:	15	Species: A	Artemia francis	cana		Brir	ne: C	rystal Sea		
Duration:	9d 23h		Source: In	n-House Cultu	re		Age		8h		
Sample ID:	01-9148-7305		Code: E	369DD49			Clie	ent: U	Iniverity of Not	re Dame	
Sample Date:	19 Dec-19 15:	10	Material: A	rsenic			Pro	ject: S	pecial Studies	;	
Receive Date:	; 19 Dec-19 15:	10	Source: re	esearch				-			
Sample Age:	NA		Station:						\square		
Data Transfor	m	Zeta	Ait Hyp) Trials	Seed		PMSD	NOEL	LOEL	TOEL	τυ
Angular (Corre	cted)	NA	C > T	NA	NA		8.15%	20	50	31.62	
Steel Many-O	ne Rank Sum 1	est						$\overline{\checkmark}$			
Control	vs C-mg/L		Test St	at Critical	Ties	DF P-Value	P-Type	Decisio	on(α:5%)		
Dilution Water	5		18	10	1	6 0.8000	Asymp		gnificant Effec	t	
	20		16	10	1	6 0.5661	Asymp	Non-Si	- gnificant Effec	t	
	50*		10	10		6 0.0350	Asymp	Signific	ant Effect		
	98*		10	10	0	6 0.0350	Asymp	Signific	ant Effect		
ANOVA Table											
Source	Sum Sq		Mean S		DF	F Stat	P-Value	Decisio	οn(α:5%)		
Between	4.194818		1.04870		4	170.4	<0.0001	Signific	ant Effect		
Error	0.092307		0.00615	3808	15						
Total	4.287125)			19						
Distributional	Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision	ι(α:1%)			
Variances Mod Levene Equality of Variance				4.89	0.0409	Equal Va	riances				
Variances Levene Equality of Variance Distribution Shapiro-Wilk W Normality			8.429	4.89	0.0009	Unequal	Variances				
Distribution	Shapiro-	Wilk W	Normality	0.8394	0.866	0.0036	Non-norn	nal Distrib	ution		
	Rate Summary										
	Control Type	Cour		95% LCL	95% UC	L Median	Min	Max	Std Err	CV%	%Effect
	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%
98 200		4	0.075	0	0.2273	0.05	0	0.2	0.04787	128.0%	92.5%
		4	0	0	0	0	0	0	0		100.0%
	ected) Transfo	1	-								
	Control Type	Cour		95% LCL	95% UC	·	Min	Max	Std Err	CV%	%Effect
	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%
98 200		4 4	0.2757 0.1588	0.0419 0.1588	0.5096 0.1588	0.2403 0.1588	0.1588 0.1588	0.4636 0.1588	0.07348 0	53.3% 0.0%	80.5% 88.8%

Consu 1/8/20 E

naiyst: As QA: New

L MCJ	hi	ort						Report Date: Test Code:	06 Jan-20 09:16 (p 2 of 2) 474-10d 03-1077-5012
	Tow 7-d Larval Su	urvival an	d Growth T	est		-			TRE Environmental Strategies
Analysis ID: Analyzed:	13-4473-4001 06 Jan-20 9:16		•	d Survival R onparametric		Trea	tments	CETIS Version: Official Results:	CETISv1.8.7 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 (Cor	rected) Transforr	ned Detai	il			(•	
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						·			
1.0							0.20		
Ē	• •						Ē		•
0.9							0.15		A.
0.8 - e E							0.10	9 1 1	
10d Survival Rate						g e	Ē		
20.6						Centered Corr. Angle	0.05		
D 0.5						రి స్ర్	0.00		
97 0.3 E			Z 💞				5.00		



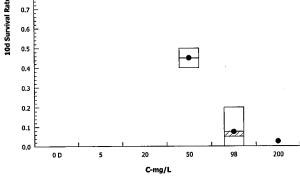


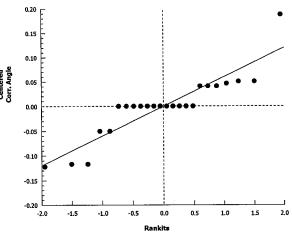
18/10 Analyst: 03 QA: NW

prience	lytical Rep ເມີດ	ort					-	ort Date: t Code:		Jan-20 11:07 (p 1 of 2) 474-10d 03-1077-5012	
Eathead Minno	ow 7-d Larval S	urviva	and Growth	Fest					TRE Env	ironmenta	Strategies
Analysis ID:	01-6294-2172		Endpoint: 1	Dd Survival R	ate		CET	IS Version	: CETISv	1.8.7	
Analyzed:	08 Jan-20 11:0)7	Analysis: P	arametric-Co	ntrol vs Trea	atments	Offic	cial Result	s: Yes		
Batch ID:	02-7744-5107		Test Type: G	rowth-Surviva	al (10d)		Ana	lyst: La	b Tech		
Start Date:	19 Dec-19 15:1	0		PA/821/R-02			Dilu	-			
Ending Date:	29 Dec-19 14:1	5	Species: A	rtemia francis	scana		Brin	e: Cr	ystal Sea		
Duration:	9d 23h		Source: In	-House Cultu	ire		Age		-		
Sample ID:	01-9148-7305		Code: B	69DD49			Clie	nt: Un	iverity of Not	re Dame	
Sample Date:	19 Dec-19 15:1	0	Material: A	rsenic			Proj	ect: Sp	ecial Studies	5	
Receive Date:	19 Dec-19 15:1	0	Source: re	search				~			
Sample Age:	NA		Station:					Δ			
Data Transforr	n	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Angular (Correc	cted)	NA	C > T	NA	NA		8.25%	20	50	31.62	
Dunnett Multip	ole Comparisor	n Test									
Control	vs C-mg/L		Test Sta	t Critical	MSD DF	P-Value	P-Type	Decisior	n(α:5%)		
Dilution Water	5		0	2.36	0.133 6	0.8000	CDF		nificant Effec	t	
	20		0.7246	2.36	0.133 6	0.5038	CDF	•	nificant Effec		
	50*		12.04	2.36	0.133 6	<0.0001	CDF	Significa	nt Effect		
	98*		20.21	2.36	0.133 6	<0.0001	CDF	Significa	nt Effect		
ANOVA Table											
Source	Sum Squ	ares	Mean So	quare	DF	F Stat	P-Value	Decisior	n(α:5%)		
Between	4.254439		1.06361		4	168.2	<0.0001	Significa	nt Effect		
Error	0.0948411	7	0.006322	2745	15	_					
Total	4.34928				19						
Distributional	Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision	(α:1%)			
Variances			ality of Variand		4.89	0.0220	Equal Va				
Variances			of Variance	9.484	4.89	0.0005	Unequal V				
Distribution	Shapiro-\	Vilk W	Normality	0.8771	0.866	0.0157	Normal D	istribution			
10d Survival R	ate Summary										
	Control Type	Coun		95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
	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%
98 200		4 4	0.075	0	0.2273	0.05	0	0.2 ·	0.04787	128.0%	92.5%
			0	0	0	0	0	0	0		100.0%
	ected) Transfor		•								
	Control Type	Coun		95% LCL			Min	Max	Std Err	CV%	%Effect
	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 50		4	1.371	1.242	1.501	1.412	1.249	1.412	0.04074	5.94%	2.89%
50 98		4	0.7351	0.6426	0.8276	0.7351	0.6847	0.7854	0.02906	7.91%	47.9%
98 200		4 4	0.2757 0.1588	0.0419 0.1588	0.5096 0.1588	0.2403 0.1588	0.1588	0.4636	0.07348	53.3%	80.5%
				11 1 1 1 1 1 1 1			0.1588	0.1588	0	0.0%	88.8%

Analyst: AB QA: MW

	alytical Repo	ort						Report Date: Test Code:	08 Jan-20 11:07 (p 2 of 2 474-10d 03-1077-5012
	10w7=d Larval S	urvival	and Growth	Test					TRE Environmental Strategies
Analysis ID: Analyzed:	01-6294-2172 08 Jan-20 11:0		•	0d Survival R Parametric-Co		tmen	ts	CETIS Version: Official Results:	CETISv1.8.7 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 (Cor	rected) Transfor	med De	etail						
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		0.684	0.7854	0.7854	0.6847				
98		0.321	8 0.1588	0.1588	0.4636				
200		0.158	0.1588	0.1588	0.1588				· · · · · · · · · · · · · · · · · · ·
10d Survival	Rate Binomials								
C-mg/L	Control Type	Rep 1	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									
1.0 E	• •	77					0.20 E	1	•
0.9				Reject Nu	ñ		0.15		
0.8 9							0.10		
d Survival Rate]	Centered Corr. Angle	0.05		
10.6 L					1	our.			

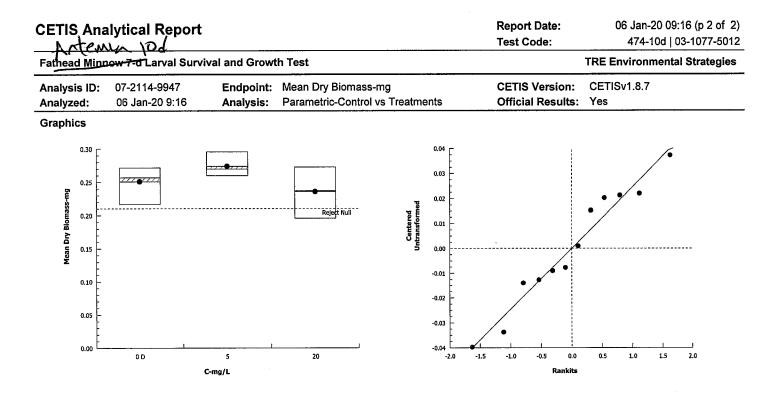




411/20 QA: NAN Analyst: M

	ren 10d				· · · · · · · · · · · · · · · · · · ·		105	t Code:		+/4-100	03-1077-50
Eathead Minir	fow 7 d Larval	Surviva	I and Growt	h Test					TRE Env	ironmenta	al Strategie
Analysis ID:	07-2114-9947		Endpoint:	Mean Dry Bior	nass-mg		CE	TIS Versio	on: CETISv1	.8.7	
Analyzed:	06 Jan-20 9:1	6	Analysis:	Parametric-Co	ntrol vs Trea	atments	Off	icial Resu			
Batch ID:	02-7744-5107		Test Type:	Growth-Surviva	al (10d)		Ana	alyst: L	.ab Tech		
Start Date:	19 Dec-19 15:		Protocol:	EPA/821/R-02			Dilu	Jent: r	GSL		
Ending Date:		15	Species:	Artemia francis	scana		Bri	ne: C	Crystal Sea		
Duration:	9d 23h		Source:	In-House Cultu	ire		Age	e: 4	8h		
Sample ID:	01-9148-7305		Code:	B69DD49			Clie	ent: i	Iniverity of Not	re Dame	
Sample Date:	19 Dec-19 15:	10	Material:	Arsenic					special Studies		
Receive Date	: 19 Dec-19 15:	10	Source:	research				J 000. C			
Sample Age:	NA		Station:					\cap			
Data Transfor	m	Zeta	Alt H	yp Trials	Seed	· · · · · · · · · · · · · · · · · · ·	PMSD		LOEL	TOEL	
Untransformed	1	NA	C > T		NA		16.1%	20	>20	NA	TU
Dunnett Multi	ple Compariso	n Toet						\vdash			· · · · · · · · · · · · · · · · · · ·
Control		111651						\smile			
Dilution Water	vs C-mg/L		Test S		•••••••••••••••••••••••••••••••••••••••	P-Value	P-Type		on(α:5%)		
Dilution water	5 20		-1.254		0.040 6	0.9549	CDF		gnificant Effect		
			0.8092	2 2.18	0.040 6	0.3375	CDF	Non-Sig	gnificant Effect		
ANOVA Table											
Source	Sum Squ		Mean	Square	DF	F Stat	P-Value	Decisio	on(α:5%)		
Between	0.002971		0.0014		2	2.162	0.1711	Non-Sig	gnificant Effect		
Error Total	0.006185		0.0006	6872776	9						
	0.009156	999			11						
Distributional	Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision	(α:1%)			
Variances		•	of Variance	1.324	9.21	0.5158	Equal Va	riances			
Distribution	Shapiro-	Wilk W	Normality	0.9513	0.802	0.6565	Normal D	istribution			
Mean Dry Bio	nass-mg Sumr	nary									
C-mg/L	Control Type	Coun	t Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
)	Dilution Water	4	0.2508	3 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		-9.27%
20		4	0.2358	3 0.1825	0.289	0.237	0.196	0.273	0.01674	14.2%	5.98%
lean Dry Bior	nass-mg Detail								- <u></u> ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	•					
)	Dilution Water	0.217	the second s	0.243	0.271						
5		0.275		0.296	0.265						

Analyst: Analyst: QA: NW



1/8/20 Analyst: A QA:

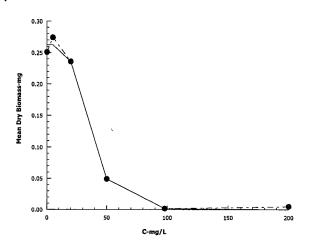
	ren	- 10d						Test	Code:	4	74-10d 03-1077-5012
Fathea	d Minna	w7-d Larvai S	urvival an	d Growt	h Test					TRE Envi	ronmental Strategies
Analys Analyz		20-4719-6220 06 Jan-20 9:16		dpoint: alysis:	Mean Dry Bi Linear Interp	omass-mg olation (ICPIN)		S Version ial Results		.8.7
Batch I	<u>.</u>	02-7744-5107	Tor	t Type:	Growth-Surv	ival (10d)		Ameli			
Start D		19 Dec-19 15:1		tocol:		02-013 (2002)		Analy		Tech	
Ending		29 Dec-19 14:1		cies:	Artemia fran	. ,		Dilue			
Duratio		9d 23h		irce:	In-House Cu			Brine		stal Sea	
					III-I IOUSE CU			Age:	481	l 	
Sample	D:	01-9148-7305	Co	de:	B69DD49			Clien	t: Uni	verity of Noti	e Dame
Sample	e Date:	19 Dec-19 15:1	0 Ma	terial:	Arsenic			Proje	ct: Spe	ecial Studies	
Receiv	e Date:	19 Dec-19 15:1	0 So i	irce:	research						
Sample	Age:	NA	Sta	tion:							
Linear	Interpo	lation Options		-							
X Trans	sform	Y Transform	n See	d	Resamples	Exp 95%	CL Meth	od			
Linear		Linear	143	681	200	Yes	Two-F	Point Interpo	lation		
Point E	stimate	S				·			·		
Level	mg/L	95% LCL	95% UCL								
IC5	12.39	6.105	27.35								<u>,</u>
IC10	19.78	7.275	25.93				1	.^			
IC15	22.05	11.49	27.61		May	12at t	n not l	oth			
IC20	24.15	16.05	29.39		JORY	want t			Tr.	1	
IC25	26.26	19.22	31.09	_	• 1		A	W a	102	E -	
IC40	32.58	27.41	36.47								
IC50	36.8	32.68	40.12								
Mean D	ry Bion	nass-mg Summ	ary			Cal	culated Var	iate			
C-mg/L		ontrol Type	Count	Mean	Min	Мах	Std Err	Std Dev	CV%	%Effect	
0	Di	ilution Water	4	0.250		0.272	0.0131	0.02621	10.5%	0.0%	
5			4	0.274		0.296	0.007969	0.01594	5.82%	-9.27%	
20			4	0.235		0.273	0.01674	0.03348	14.2%	5.98%	
50			4	0.049		0.062	0.005701	0.0114	23.3%	80.5%	
98 200			4 4	0.001 0		0.005	0.00119	0.00238	159.0%	99.4%	
			4		0	0	0	0		100.0%	
	-	nass-mg Detail									
C-mg/L		ontrol Type	Rep 1	Rep 2		Rep 4					
<u> </u>	Di	lution Water	0.217	0.272	0.243	0.271					
			0.275	0.26	0.296	0.265					
5			0.223	0.196	0.273	0.251					
5 20											
0 5 20 50			0.055	0.041	0.038	0.062					
5 20			0.055 0.001	0.041 0	0.038 0	0.062 0.005					

1/8/20 Analyst: AS QA: New

P

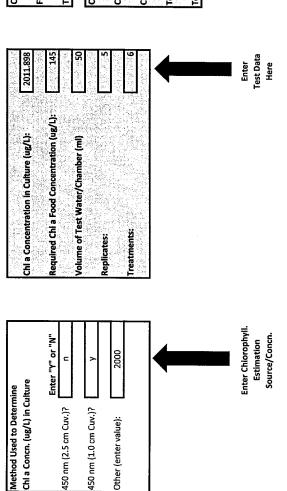
CETIS Ana	alytical Report			Report Date: Test Code:	06 Jan-20 09:17 (p 2 of 2) 474-10d 03-1077-5012
	Tow-7-d Larval Surv	ival and Growt	h Test		TRE Environmental Strategies
Analysis ID:	20-4719-6220	Endpoint:	Mean Dry Biomass-mg	CETIS Version:	CETISv1.8.7
Analyzed:	06 Jan-20 9:16	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

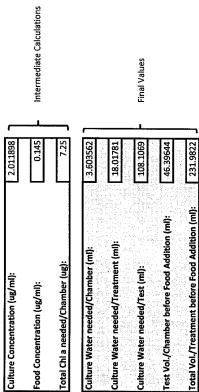
Graphics



Analyst: AB QA: NW

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





NOTE: Change values only in cells outlined inGREEN; do not change cells outlined inRED; these are equations

Page __ of __

ج طرامهمار Brine Shrimp Acate Studies

Nov 2017

		Volume needed:	n = 4 vol 50 ml rens	200		new analytical	0 ml - TR 200 0 ml - diss	200 ml		old analytical	0 ml - TR	0 ml - diss								
15.5755 g Na2HAsO4 * 7H20 / L H2O	0.25	dilution series	E 30/ 0/ conitor of vol	0.3% % Spike OI VOI						TOTAL VOL	3.0 L	0.86 gallons								
	ment (L)	Total	Vol(L)	0.25 0.25	0.25	0.25	0.25	0.25	1.50			Total	Vol(L)	0.25	0.25	0.25	0.25	0.25	0.25	1.5
3,740.00 mg/L As =	Volume per treatment (L) _	Stock	(ml)	13.37 6.68	3.34	1.34	0.33	0.00	25.07			Stock	(ml)	13.37	6.68	3.34	1.34	0.33	0.00	25.07
I	Volu	Conc.	ng/L	zuu,uuu 100,000	50,000	20,000	5,000	0			t Renewal	Conc.	ng/L	200,000	100,000	50,000	20,000	5,000	0	
Primary stock @	·		Trtmnt	مىم	4	ო	7	+	Total		Conc. at Test Renewal		Trtmnt	9	ŝ	4	с	2	1	Total

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



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 10⁶ cells per chamber (from Brix et. al., 2003)
- Treatment 2: 4.72 x 10⁶ cells per chamber (half of the Chla concentration as measured by spectrophotometric absorbance as *Dunaliella* in previous studies)
- Treatment 3: 9.46 x 10⁶ cells per chamber (same Chl*a* concentration as measured by spectrophotometric absorbance as *Dunaliella* in previous studies)
- Treatment 4: 18.9 x 10⁶ 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 μ g/L Chl*a* estimated with absorbance. Solutions were gently aerated.

Characterization of Recon Water

Sample No.	рН	Hard. (mg/L) ^a	Alk. (mg/L) ^ª	Spec. Cond. (μS/cm)	TRC (mg/L) ^b	NH₃-N (mg/L)	Salinity (ppt)
RW#13818	7.9	NM	NM	138,100	NM	NM	118

^aAs CaCO3

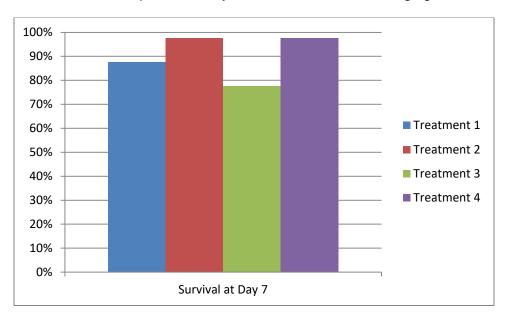
^bTotal residual chlorine

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:

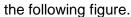
The survival of the brine shrimp in this study is illustrated in the following figure.

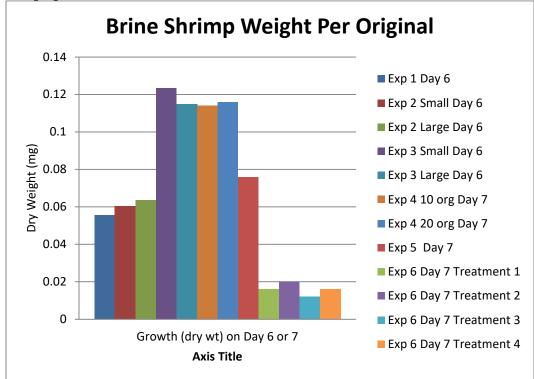


While survival in three of the four treatments met the 80% control perferomance threshold, the relative growth of these threatments compared to the earlier experiments is telling, as shown in

TRE

Mr. Bittner February 19, 2020 Page 3





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.

Mr. Bittner February 19, 2020 Page 4

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 <u>bidlackac.tre@gmail.com</u>

14001-474-051

Attachment

cc: David Pillard, TRE

Rami B. Naddy, Ph.D.

Manager / Environmental Toxicologist naddyrb.tre@gmail.com

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TOXICITY DATA PACKAGE COVER SHEET

Test Type:	Chronic		Project Number:	1	17001-474-Exp
Test Substance:	Platymonas		Species:	Artemia francis	scana
Dilution Water:	rGSL		Organism Lot o	r Batch Numbe	er:
Concurrent Control Water:	NA		Age: 47h (2	<u>18 hr)</u>	Supplier: TPE
Date and Time Test Began:	116120	@ 1445	Date and Time	Test Ended:	1/23/20 @ 1500
Protocol Number:			Investigator(s):	Botev/H	Plante
Background Information			pH control?:	Yes	No
Type of Test:	Static-Rene	wal (Daily)	If yes, give % C		NA
Test Temperature:	20 ± 1 °C		Env. Chmbr/Bath #: 25	_	Test Chmbrs: <u>147-ml cups</u>
Photoperiod:	<u> 16 h light : 8</u>	<u>h dark</u>	Light intensity:		<u>50-100 ft-c.</u>
Test Solution Vol.:	5	i0 ml	Replicates per	Treatment:	4
Length of Test:	7 days	-	Organisms per	Replicate:	10
Type of Food and Quantity pe	r Chamber:	Varies	Feeding Freque	ency:	1 x daily
Test Substance Characteriz	ation Param	eters and Frequ	iency:		
Hardness: <u>Test Initiation</u>	Alkalinity:	Test Initiation	NH ₃ : <u>Test Initiation</u>	TRC: <u>Test Ini</u>	tiation_
pH: <u>Daily</u>	Conductivity	/: <u>Daily</u>			
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 F	Ref. Tox. Value	: _Linear Interpolation
Special Procedures and Co	noiderationa			~~~	·
Organisms hatched 2 days pr			SL with 100 ug/L Chla	· · · · · · · · · · · · · · · · · · ·	
Treatment 1: 1.0 x 10^6 cell p					
Treatment 2: 4.72 x 10^6 cell					
Treatment 3: 9.46 x 10^6 cell	per chamber	(9.05 mL per ch	amber) Same absorb	ance as g	previous
Treatment 4: 18.9 x 10^6 cell					sieutous
Appropriate correction factors	have been a	pplied to all tem	peratures recorded in this	data package	
Study Director Initials:		Date: 1/14	20		

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TEST SUBSTANCE USAGE LOG

'n

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number				
	From:	From:	From:	From:
Test Substance Collection	@	@	@	@
Date and Time	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	11/20/20 1/17/20 11/20/20 1/17/20 11/20/20 1/18/20 11/20/20 1/18/20			

Project Number:

17001-474-Exp

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					n	
3	0	250	250						
4	0	250	250						
									· · · · · ·
L	0	1000	1000						
Initials / Date		16/20 N	i real BS						
Initials / Date	EN 1/1	7/20 "	"						
Initials / Date		8/20 "	11						
Initials / Date	CP 1/1	9/20 4	u						
Initials / Date	E 1/2	0/20 "	21						
Initials / Date	EN i/ul		ι,						
Initials / Date	CP 1/22	to a	и						
Initials / Date									

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Artemia franciscana CHRONIC BIOLOGICAL DATA

Project Number: ____ 17001-474-Exp

	Test	Day	Day	Day			r of Survi			
mg/L	Replicate		1 1	2	Day 3	Day 4	Day 5	Day 6	Day 7	Remarks
1	A	10	10	10	10	10	ιŨ	10	01-10	g1.5
	В	10	10	10	10	10	8	8	8	
	С	6	10	10	9	٩	9	9	9	
	D	Ŵ	10	10	8	8	8	8	8	
2	A	io .	10	9	9	9	9	9	9	92.5
	В	io	10	10	10	ID	10	10	10	
	С	lo	10	10	10	10	10	10	10	
	D	lo	io	10	10	10	10	10	10	
3	A	10	10	10	10	10	10	10	9	71.5
	В	10	10	10	10	10	9	9	io	
	С	10	10	10	10	10	10	10	8	
	D	10	9+	C	9	9	q	9	8	tlorg NF
4	А	io	10	10	10	10	10	10	10	<u><u>f</u>1.r</u>
	В	10	10	10	10	10	10	9+	9	+ long NF
	С	10	10	$\overline{10}$	10	10	10	10	6	
	D	10	10	ίŎ	10	10	10	10	10	
	А									
	В									
	С							<u> </u>		
	D							<u> </u>		
	A						I			
	В									
	С									
	D					<u>.</u>	<u> </u>			
	A					<u> </u>				
	В									
	c									
	<u>0</u>									
		1/16/20	117/20	1/18/20	1/19/20	1/20/20	1/21/20	1/22/20	1/201	
	Time:		1456	1545	1645	1005	1445	1455	1500	
	Initials:	PREN		HP	CP	re	1995 EN			
	initialo.				<u> </u>			CP	Eu	

0 22 1/23 20jE

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CHRONIC CHEMICAL DATA (INITIAL)

Project Number:

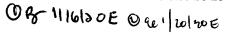
17001-474-Exp

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.	
pН		7.9	78	8.1	8.D	8.0	7.8	7.9		F428	
D.O. (mg/L)	4.9	Tio	4.8	54	5.3	4.9	4.9	5.0		17	
Temp. (°C)		20	20	20	20		20	20		L-38	
Cond. (µS/cm)		138,100		1411100	139700	20 151200 **	136600	140600		IS	* Meter error
Hard. (mg/L)										Titk.	
Alk. (mg/L)										Titp.	
TRC (mg/L)					1					22	
NH₃ (mg/L)		pot:11	6							HA1	
Conc.:	2										
pН											
D.O. (mg/L)											
Temp. (°C)											· · · · · · · · · · · · · · · · · · ·
Cond. (µS/cm)											
Hard. (mg/L)											
Alk. (mg/L)											
TRC (mg/L)											
NH₃ (mg/L)						AND 11					·
Conc.:	3										
pH											
D.O. (mg/L)						······					
Temp. (°C)									· · · · · · · · ·		
Cond. (µS/cm)											
Conc.:	4									İ da	
рН											
D.O. (mg/L)											
Temp. (°C)											
Cond. (µS/cm)											
	Date:	1116/19	1/17/20	1/18/20	1/19/20	120/00	1/21/20	1/22/20			
	Time:	1435	1465	1535	1635	1610	1430	1445			
	nitials:	Pó	EN	HP	CP	re	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.



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CHRONIC CHEMICAL DATA (FINAL)

Project Number:
Test Species:

17001-474-Exp Artemia franciscana

%					<u> </u>		T				
%		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter #	Remarks
Conc.:	1							1379000		All Conc	* conductivity
pН		7.8	77	8.0	0.0	78	7.8	7.8		FM21	/
D.O. (mg/L)		7.2	52	53	4.7	4.8	4.9	4.9		17	
Temp (°C)		20	19	19	19	20	20	20		L-37	
Conc.:	2				<u> </u>	1		127300			* conductivity
pН		7.8	7.7	R.O	8.)	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	W	20	20	· · · · · · · · · · · · · · · · · · ·		
Conc.:	3					Γ		126300			* conductivity
рН		78	77	8.0	80	7.8	7.8	7.9			-
D.O. (mg/L)		7.0	49	49	4.7	4.6	4.6	4.9			
Temp (°C)		20	20	19	19	20	20	20			a 1880a h
Conc.:	4							125700			* conductivity
рН		78	77	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	W	20	20			
Conc.:											
рН				_							
D.O. (mg/L)											
Temp (°C)											
Conc.:											
рН											
D.O. (mg/L)											
Temp (°C)											
Conc.:											
pH											
D.O. (mg/L)											
Temp (°C)											
	Date:	VITho	1/18/20	1/19/20	Topo	1/2/120	1/22/20	123/20			
		1550	1540	1705	1610	1445	1520	1545			
	Initials:		HP	CP	Ee	EN	CP	æ			

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DAILY TOXICITY TEST LOG

· · · · · · · · · · · · · · · · · · ·		OXICITY TEST LOG
Project Number:	17001-474-Exp	
Test Species:	Artemia franciscana	

General			Feeding	Initials/Date
Comments	Random Chart: 55 Min/Max Thermom			
Test Day 0	Random Chart: <u>35</u> Min/Max Thermom Test Solution Mixed at: <u>1930</u> Test Organisms Added at: 1945	eter # M-15	Fed @ <i>เฯน</i> :ั <i>ง</i>	Ar 1116/200
Test Day 1	Real Time: 🖗 °C Min-Max Range: 1	7.70 °C	Fed @ 1445	EN 1/17/20
Test Day 2	Real Time: 19°C Min-Max Range:	7-21℃	Fed @1525	HFR 1/18/20
Test Day 3	Real Time: (9 °C Min-Max Range:	19 - Zo °C	Fed @ 1640	CP 1/19/20
Test Day 4	Real Time: 18 °C Min-Max Range: 1	S - 20 °C	Fed @ 1550	<i>че</i> 1/20/20
Test Day 5	Real Time: 20 °C Min-Max Range: }{	3-20 °C	Fed @ 14355	EN 1/21/20
Test Day 6	Real Time: 20 °C Min-Max Range:	18 - ZI °C	Fed @ 1445	ср 1/22/20
Test Day 7	Real Time: 20 °C Min-Max Range: (8-21 °C	Fed@ NONE	Fe 1 23 70
Test Day 8	Real Time: °C Min-Max Range:	°C	Fed @	

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TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project	Project Number: 11001 - 474 - Exp	- 1001	474-6	(XP	Test Substance		Platymonas						
Species:	s: Artemia franciscana	t fran	ciscano		Analyst Tare: HB	E. HB/	Analvst Gross	ross: CP		Analytical Balar		(# 1	1001
Date/Ti	Date/Time of Tare Wt.: ${}^{\prime\prime}\lambda_{3}/_{\mathcal{AL}}$	4: 1/a	3/ac h	Q 1125	Date/Time c	Date/Time of Gross Wt.:	1 1			to Date: 11522 Time: 1242 to Date: 11522 Time: 0805		ate: <u>1/25/20</u> TI	me: (5 + 2) ime: 0805
Boat	Treatment Rep.		Length	Weight Type (Circle):	e (Circle):	Wet Blot Dry	Dry Dry (>100°C)	00°C) AFC	AFDW (>500°C)		Lot or Batch Number:	Number:	
			Curts:	Tare Gross Weight (g) 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	Mean Wt. per Treatment (mg) (Surviving)
		4		1.12982	1.12991						2	(6111)	
		ø		1.13233	1.13240						8		
		ა		1.14630 1.14643	1.14643						a		
		۵		1.12223	1.12259						8		
	7	- A		1.12647 1.12668	1.12668						6		
		В		i.14211	1.14229						0		
		ა		11/156	1.14176						0		
		۵		1. 125 70 1	1.12593						01		
	б	A		1.13963 1.13981	1.13981						a		
		ß		1,13438 1	1-13444						v		
		J		11715 1	1.11728						8		
		۵		1.13943	113955						¢		
Blank				1.13379 1	1.13379								
Range													
Mean													
Test Solu	Test Solution Volume:						Loading Rate:						
Add in w	Add in weight loss of blank boat, if appropriate.	blank b	oat, if appl	ropriate.									
							• • • •						

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Project	Project Number: 17001 - 474 - Exp	- 1001	- 474 - E	×0	Test Substance:		Platymonas						
Specie	Species: Artemia franciscana	a fn	anciscan	r r	Analyst Tare: $\mathcal{M}_{\mathcal{M}}$	e: HB/W	Analyst Gross:	sross: CP		Comments: Analytical Balance ID: Sart # 1 Dried in Oven # 3_from Date 1/23/to Time: 7545	nce ID: Sar # 3_from D	+ # ate://23/vo Ti	me. /545
Date/Ti	Date/Time of Tare Wt.: ^{1/}	$v_{t: l_{\hat{a}}}$		R 1130	Date/Time c	of Gross Wt.:	Date/Time of Gross Wt.: 1/2ん(20 0 16 35	2 1635			2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	ate: <u>12570</u> T	ime: 0805
Boat	Treatment Rep.	Rep.	Length		Weight Type (Circle): Wet	Wet Blot Dry	Dry Dry (>100°C)		AFDW (>500°C)		Lot or Batch Number:	Number:	
					Tare Gross Weight (g) 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	Mean Wt. per Treatment (mg) (Surviving)
	Ч	А		1.14150	1.14157						0	(811)	
		в		1.14762 1.14800	1.14800						σ		
		ა		1,12595 1.12605	1.12605						10		
		۵		1.13040	1-13049						0		
		ş											
Blank													
Range													
Mean													
Test Solu	Test Solution Volume:						Loading Rate:						
Add in w	Add in weight loss of blank boat, if appropriate.	blank b	ooat, if app	rropriate.									
							·						

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TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 14001-474 7 day Playtmonas

Species: Artemia franciscana

					Adjusted		Mean Wt./ Original	Mean Wt./ Treatment	Number of	Mean Wt./ Surviving	Mean Wt./ Treatment
Treatment R	Length Rep Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Net Weight Net Weight No of Orig. (g) (g) Organisms	No of Orig. Organisms	Organism (mg)	(mg) (Original)	Surv. Organisms	Organism (mg)	(mg) (Surviving)
	A	1.12982	1.12991	0.00009	0.00009	10	0.009		10	0.009	0.0193
	В	1.13233	1.13240		0.00007	10	0.007		8	0.009	
<u> </u>	U	1 14630	1.14643		0.00013	10	0.013		6	0.014	
	D	1.12223	1.12259	0.00036	0.00036	10	0.036		8	0.045	
<u>`</u>	A	1.12647	1.12668				0.021	0.0205	6	0.023	0.0211
<u> </u>	в	1 14211	1.14229				0.018		10	0.018	
	U	1.14156	1.14176		0.00020		0.020		10	0.020	
	0	1.12570	1.12593	0.00023	0.00023		0.023		10	0.023	
<u> </u>	A	1.13963	1.13981	0.00018	0.00018	10	0.018	0.0123	თ	0.020	0.0153
<u>۳</u>	В	1.13438	1.13444		0.00006	10	0.006		9	0.010	
	U	1.11715	1.11728		0.00013	10	0.013		8	0.016	
	Ω	1.13943	1.13955	0.00012	0.00012	10	0.012		ω	0.015	
<u> </u>	A	1.14150	1.14157	0.00007	0.00007	10	0.007	0.0160	10	0.007	0.0171
4	В	1.14762	1.14800	0.00038	0.00038	10	0.038		6	0.042	
	U	1.12595	1.12605	0.00010	0.00010	10	0.010		10	0.010	
		1.13040	1.13049	6000000	0.00009	10	0.009		10	0.009	
1			-								
										3	
							5				
Jucia		1 12270	1 12270								
DIGUL		1.100/8	1.133/3	0.0000							1

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Project Number: 14001-474 7 day Playtmonas

Artemia franciscana Species:

				16.236%			
	S	0.0957	0.0500	0.1258	0.0500	#DIV/0	i0//IC#
	Mean	0.8750	0.9750	0.7750	0.9750	i0//IC#	i0//I 0 #
ta	Max	1.0	1.0	0.0	1.0	i0//IC#	i0//IC#
Survival Da	Min	0.8	6 [.] 0	0.0	0.0	i0//IC#	i0//IC#
ics for	Z	4	4	4	4	0	0
Summary Statistics for Survival Data	<u>Treatment</u>	~	200.00%	300.0%	400%	0.0%	%0

Summary Statistics for Growth Data (dry wt per original) <u>Treatment</u> <u>Nam</u><u>Max</u><u>Mean</u>

					i0//IC# i0//IC#	
Mean	0.0162	0.0205	0.0123	0.0160	0.0000	0.0000
Max	0.036	0.023	0.018	0.038	0.000	0.000
Min	0.007	0.018	0.006	0.007	0.000	0.000
Z	4	ব	4	4	0	0
<u>reatment</u>	~	200.00%	300.0%	400%	0.0%	%0

Summary Statistics for Growth Data (dry wt per surviving organism) <u>Treatment</u> <u>Nin</u> <u>Max</u> <u>Mean</u> <u>SD</u>

•				0		
<u>Treatment</u>	Z	<u>Nin</u>	Max	<u>Mean</u>		
~	4	0.009	0.045	0.0193		
200.00%	4	0.018	0.023	0.0211		
300.0%	4	0.010	0.020	0.0153	0.0041	26.972%
400%	4	0.007	0.042	0.0171		
0.0%	0	0.000	0.000	0.0000		
%0	0	0.000	0.000	0.0000		

Artemia franciscana (Brine Shrimp) Short Term Chronic Test Development

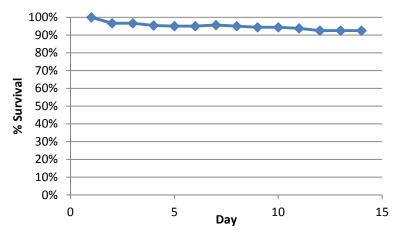


TRE Environmental Strategies

Test Design

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

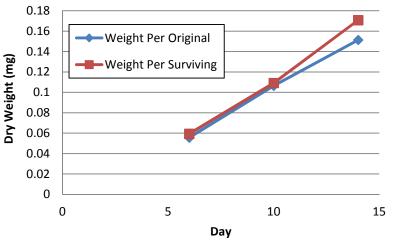
Brine Shrimp Survival



Brine Shrimp Weight

Conclusions

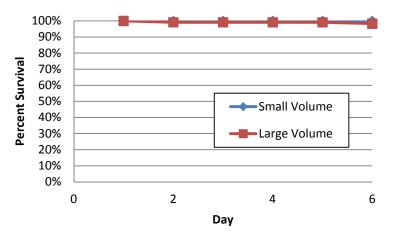
- Water cleared of food after 24 hours on Day 6 and later
- Organisms transitioned from nauplii to juveniles between Days 3 and 4



Test Design

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

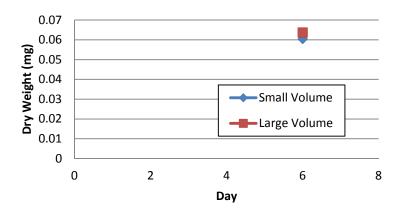
Brine Shrimp Survival



Weight Per Original Organism

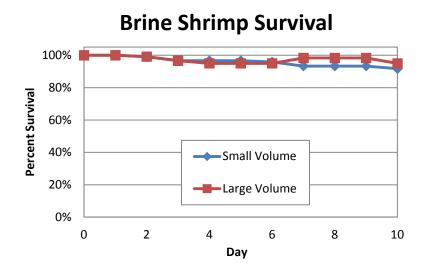
Conclusions

- 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



Test Design

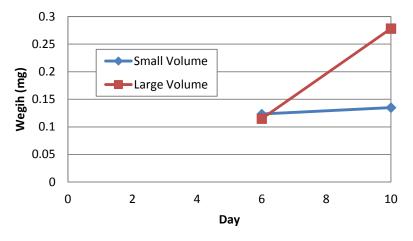
- Test duration: 6 days, 10 days
- Solution volume: 50 ml and 150 ml
- Fed *D. viridis*, conc 145 µg/L Chla
- Renewed and fed every 48h
- 20 organisms per replicate
- 3 Replicates per treatment
- Media: 120ppt rGSL



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

Weight per Original Organism



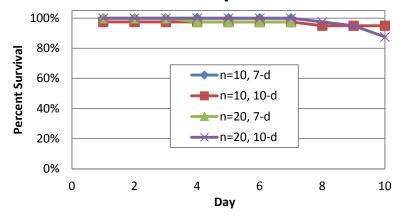
Test Design

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

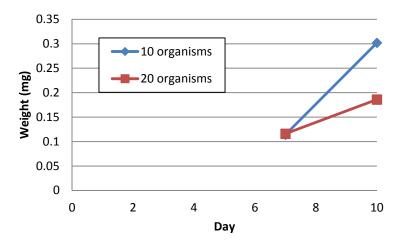
Conclusions

- 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

Brine Shrimp Survival



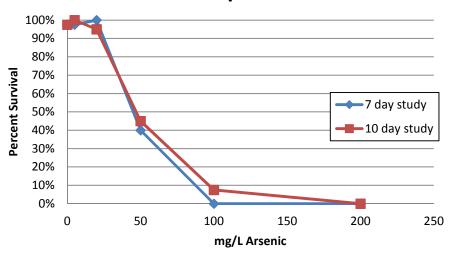
Weight Per Original



Test Design

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

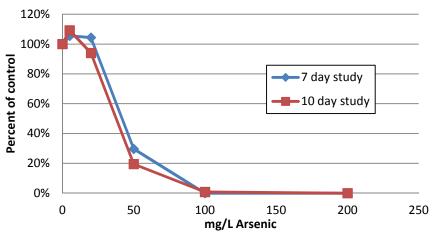
Brine Shrimp Survival



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

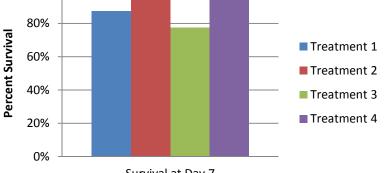
Dry Wt per Original as Percent of Control



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

Brine Shrimp Survival

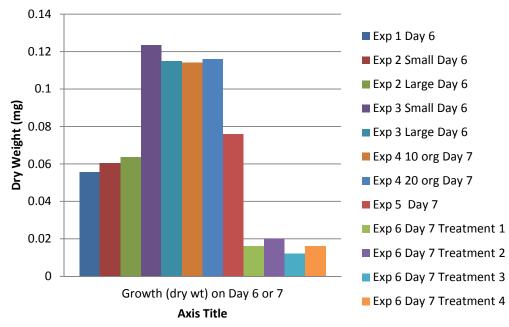


Survival at Day 7

Conclusions

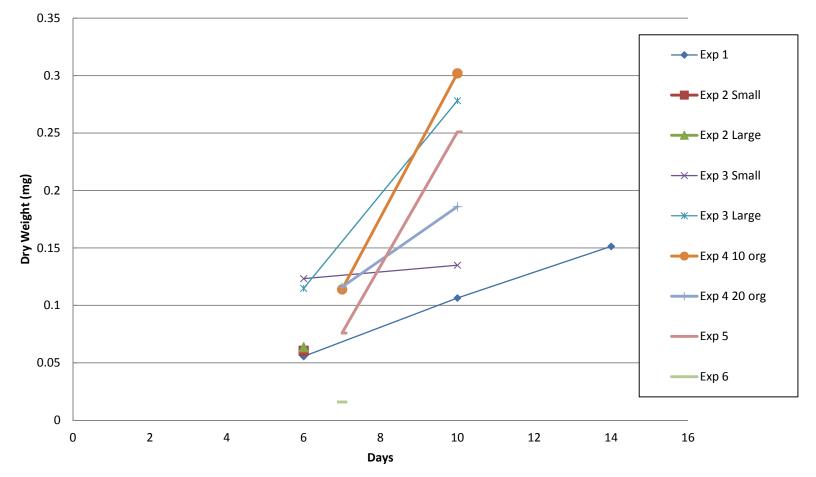
- Similar results among all 4 treatments
- *Platymonas* sp. doesn't appear to be an adequate food source
- Organisms transitioned from nauplii to juveniles between Days 3 and 4

Brine Shrimp 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 Chla
- Solution renewal frequency: daily
- Test duration: 7 days
- Endpoints: survival (≥80%) and growth (>0.05 mg dry wt)



March 3, 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 #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 Chla)
- Treatment 2: D. viridis (72.5 μg/L Chla)/ Platymonas sp. (72.5 μg/L Chla)
- Treatment 3: D. viridis (72.5 µg/L Chla, 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 Chla estimated with absorbance. Solutions were gently aerated.

Characterization of Recon Water

Sample No.	рН	Hard. (mg/L) ^a	Alk. (mg/L) ^a	Spec. Cond. (μS/cm)	TRC (mg/L) ^b	NH₃-N (mg/L)	Salinity (ppt)
RW#13852	7.9	NM	NM	140,200	NM	NM	122

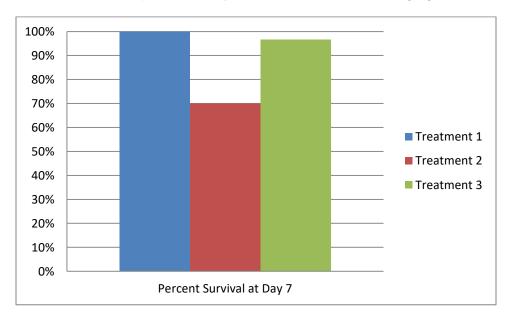
^aAs CaCO3 ^bTotal residual chlorine

Test activities:

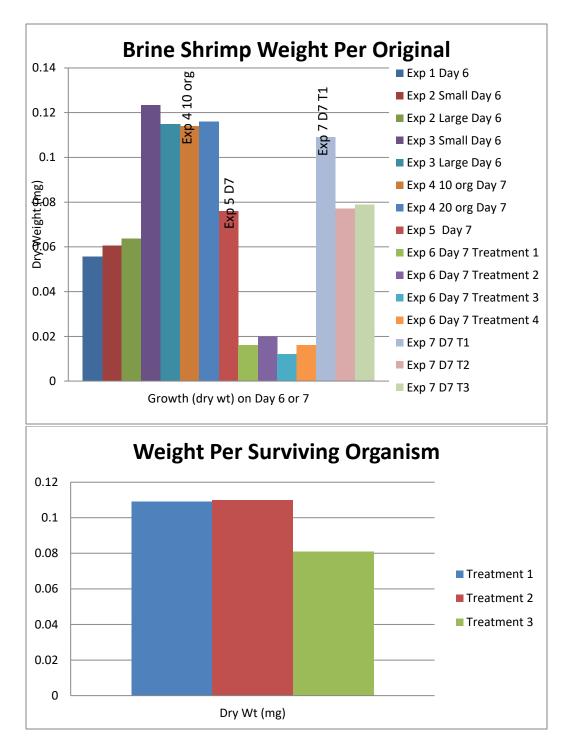
- Biological observations (primarily survival) taken daily.
- Chemistries taken on renewal days (i.e., pH, dissolved oxygen, and temperature).
- Conductivity was measured at test termination or when there was 0% survival in that treatment.
- Dry weights (±0.01 mg) were determined at test termination.

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.



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

Mr. Bittner March 3, 2020 Page 4

- 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 <u>bidlackac.tre@gmail.com</u>

14001-474-053

Attachment

cc: David Pillard, TRE

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

Page 1 of _____ QA Form No. 051 Revision 5 Effective 02/14 RP Nor Hud W

TOXICITY DATA PACKAGE COVER SHEET

Test Type:	Chronic	_ Project Number:	<i>სა</i> ა 17001-474-ნადე
Test Substance:	Platymonas/Duniellia	Species: <u>Artemia franciscana</u>	
Dilution Water:	rGSL	Organism Lot or Batch Numb	oer: 031020
Concurrent Control Water:	ΝΑ	Age: <u>48 hr</u>)	Supplier: TRE
Date and Time Test Began:	2/2/20 @ 1615	Date and Time Test Ended:	2/19/20 @ 1515
Protocol Number:	·	Investigator(s): 13 CP CE	EN
Background Information		,	
Type of Test:	Static-Renewal (Daily)	pH control?: Yes If yes, give % CO ₂ :	No NA
Test Temperature:	20 ± 1 °C	Env. Chmbr/Bath #: <u>25</u>	Test Chmbrs: <u>147-ml cups</u>
Photoperiod:	<u>16 h light : 8 h dark</u>	Light intensity:	<u>50-100 ft-c.</u>
Test Solution Vol.:	50 ml	Replicates per Treatment:	3
Length of Test:	7 days	Organisms per Replicate:	10
Type of Food and Quantity pe	r Chamber: Varies	Feeding Frequency:	1 x daily
Test Substance Characterization Parameters and Frequency:			
Hardness: <u>Test Initiation</u>	Alkalinity: <u>Test Initiation</u>	-	itiation
pH: <u>Daily</u>	Conductivity: <u>Daily</u>		
Test Concentrations (Volume:Volume): See Below			
Agency Summary Sheet(s)?:	None	_	
Reference Toxicant Data:	Test Dates:	to PA	
Hist. 95% Control Limits:	NA to NA	Method for Determining Ref. Tox. Value	
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. vindis			
Treatment 2: Feed 1.35mc D. visidis and 4.5 mc platymanas			
Treatment 3: Freech 1.35mc D visidis			
Appropriate correction factors have been applied to all temperatures recorded in this data package			
Study Director Initials: A Date: 2122			

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TEST SUBSTANCE USAGE LOG

tet non 2/28/20

17001-474-Exp

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number				
	From:	From:	From:	From:
Test Substance Collection	@	@	@	@
Date and Time	То:	To:	То:	То:
	@	@	@	@
Sample Type (Grab or Comp)				
Date Test Substance Received				
Dilution Water Number RW# or TRE#, circle one	13852			
Concurrent Control Water RW#	MA			
Date(s) Used	2/12/20 2/16/20 2/13/20 2/17/20 2/18/20 2/18/20 2/15/20			

Preparation of Test Solutions

Test	Test	Dilution	Total	Test	Dilution	Total	Test	Dilution	Total					
Substance	Substance	Water	Volume	Substance	Water	Volume	Substance	Water	Volume					
Conc.	Volume	Volume	(ml)	Volume	Volume	(ml)	Volume	Volume	(ml)					
(% Effluent)	(ml)	(ml)		(ml)	(ml)		(ml)	(ml)						
1	0	025090	250											
2	0	0 250 170	250											
3	0	0 250170	250											
				r										
	0	750	750											
Initials / Date	AB 212	ພ												
Initials / Date	AS 2/13	υ												
Initials / Date	AB 2/11/0													
Initials / Date	CP 2/15/	to												
Initials / Date	CP 2/16/	20												
Initials / Date	80 2/17/2	0												
Initials / Date	EN 2/18/20	>												
Initials / Date														

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Artemia franciscana CHRONIC BIOLOGICAL DATA

POW 2/28/20 QA

Project Number: _____17001-474-Exp

	1	1			_					
	Test	Devi				Numbe		ving Orga		Je SUMIUM
mg/L	Replicate	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Remarks
1	А	10	10	10	10	10	10	10	10	102
	В	10	10	10	10	10	10	10	10	
	С	10	10	10	10	10	10	10	10	
	D					1				
2	А	10	9	8	8*	1	7	7	17	* 1 weak 20
	В	10	9	7	7	7	1	7	7	10
	С	10	ID	9	8	8	8	8	0+	+ 2/18/20 TE
	D									
3	А	10	10	10	10	10	10	10	10	96.7
	В	10	10	9	9	9	9	9	9	
	С	lo	10	10	10	10	10	10	10	
	D					1				
	А				1		1		†	
	В						<u> </u>			
	С							1		
	D					······				
	А				† <u> </u>		<u> </u>	1	1	
	В								1	
	С				<u> </u>					
	D	<u> </u>	ļ		<u> </u>			<u> </u>		
	А				<u>† </u>					
	В							1		
	С									
	D									
Ī	А							<u> </u>		
	В									
	C		 							
	D									
	Date:	2/12/26	2/13/20	2/14/20	2/15/20	2/16/20	2/17/20	2/18/20	2/19/20	
	1	1615	1130	1530	1550	1030	1520	1540	1515	
		M/CP	AS	AB	CP	ĊP	le	EN	8	

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CHRONIC CHEMICAL DATA (INITIAL)

QN NON 2/28/20

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	
			2					10		Conc.	
pH		7.9	7.4	7.9	8.0	8.0	7.8	7.8		Fm28	······································
D.O. (mg/L)		5.1	5.3	<u> </u>	5.1	5.0	0881	76		17	
Temp. (°C)		20	20	20	20	20	20	20		639	
Cond. (µS/cm)	140200	146460	137900	134800	139600	149400	137.100		15	
Hard. (mg/L)										Tite	
Alk. (mg/L)										Titr	
TRC (mg/L)										22	
NH ₃ (mg/L)										492	ppt-122
Conc.:											
рН											
D.O. (mg/L)											
Temp. (°C)											
Cond. (µS/cm))										
Hard. (mg/L)									·		
Alk. (mg/L)											
TRC (mg/L)						·-··					
NH₃ (mg/L)											
Conc.:											
рН					·						<u> </u>
D.O. (mg/L)											
Temp. (°C)											Ma with the
Cond. (µS/cm)											
Conc.:				······································							
pH											
D.O. (mg/L)							<u>.</u>				
<u>Temp. (°C)</u>											
Cond. (µS/cm)											
		alula	21.12.1		2/16/20	2/11/2	2/10/20	2 40 1- 2			
······	Time:	2/12/20 1600			2/15/20	1015					
	Initials:		<u>1115</u> 1975	1515 A5	CP			1530 End			
Note: Hardness						CP	Ee_	22			

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.

0 & 2/ 17/20;E

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CHRONIC CHEMICAL DATA (FINAL)

Ex Nor 2/2×/20

		·			CHRU			DATA (F	<u>INAL)</u>		St ver 5/26/26
Project Number Test Species:	r:)1-474-E i a franc i								
rest species.		Artem	a franci	scana							
%		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter #	Remarks
Conc.:	1					ĺ		132900		All Conc.	* conductivity (15)
рН		g. v	8.0	8.D	8.0	80	7.8	7.9		Em25	
D.O. (mg/L)		5.0	5.5	5.1	5.2	750	7.4	4.7	-	17	
Temp (°C)		20	20	21	20	20	21	21		134	
Conc.:	2							129720			* conductivity
рН		7.9	7.9	8.0	8.0	7.9	7.8	7.9			and a second second second second second second second second second second second second second second second
D.O. (mg/L)		5.0	5.2	5.1	5.0	5.0	72	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.D	5.0	7.1	4.6			
Temp (°C)		20	20	21	20	19	21	21			
Conc.:											
рН											
D.O. (mg/L)											
Temp (°C)											an an an an an an an an an an an an an a
Conc.:											
рН											
D.O. (mg/L)	_										
Temp (°C)											
Conc.:											
pН											
D.O. (mg/L)											
Temp (°C)											
Conc.:											
рН											
D.O. (mg/L)											
Temp (°C)											
	Date:	2/12/20	Inbe	2/15/20	2/16/20	2/17/20	2/16/20	2/10/20			
	Time:		1535	1540	1040	1520	1540	1600			
Ir	nitials:	þ	AS	СР	Cl	Ee .	ŧn	æ			
	-	802/17/	20.5								

Oee2/17/20;E

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DAILY TOXICITY TEST LOG

at we 2/12/20

Project Number: Test Species: A

17001-474-Exp Artemia franciscana

General						Feeding	Initials/Date
Comments						, county	
	Random CI	hart:	Z	Min/Max Thermometer	# M-15		
Test Day 0	Test Solution			5		Fed @ _{າະປັບ}	Δ.
	Test Organ	isms Ac	ded at:)	15		1600	A3
							2/12/20
Test Day 1	Real Time:	14	°C	ر- ۱۹ Min-Max Range: ۱۹	۰°C	Fed @	A3
						1120	
							2/13/20
Test Day 2	Real Time:	19	°C	Min-Max Range: 19-2c) °C	Fed @ +5407	.43
						1570	12/14/20
							-2/15/20-
Test Day 3	Real Time:	20	°C	Min-Max Range: 19 -	21 °C	Fed @ 1540	CP
							1 1
							2/15/20
Test Day 4	Real Time:	20	°C	Min-Max Range: 19-	21 °C	Fed @ 1025	. 0
							CP 2/16/20
							2/16/20
Test Day 5	Real Time:	18	°C	Min-Max Range: 18~2	-3 °C	Fed @ 1500	
							20
							2/17/20
Test Day 6	Real Time:	20	°C	Min-Max Range: 20 - 2	<u>۰</u>	Fed @1530	
							En
							2/18/20
Test Day 7	Real Time:	20	°C	Min-Max Range: 70-7	22 °C	Fed @	
		-•			-	NONE	Se .
							2/19/20
Test Day 8	Real Time:		°C	Min-Max Range:	°C	Fed @	

() CP 2/15/20 WP

AND LOADING	
LENGTHS, WEIGHTS,	
TEST ORGANISM I	

Page of OA Form No.010 Revision 6 Effective 02/14

Project 1	Project Number: 17001-474-Exn	201-1	オヨリービス	0	Test Substance:	Ince: PPC/IT	Pertina (Platimona c Miniellia)	wo chini	ellin)	Comments:			
Species.	Species: Artemia franciscana	Far.	nciscar	20	Analyst Tare: ${\cal C}$	e: C	Analyst G	Analyst Gross: MB		Analytical Balance ID: Surt#/ Dried in Oven # 3 from Date: 1/14/26 Time: 1545	rce ID: Surt:	#1 ate: Med2e Til	me: 1545
Date/Tin	Date/Time of Tare Wt.: 2/19 / 20 @ 1325	rt: 2/	19 /20) 1328	Date/Time (Date/Time of Gross Wt.:	8	2 1120			to Da	ite:2-12420 TI	ime: <u>An</u>
Boat	Treatment Rep.	Rep.	Length	Weight Type (Circle):		Wet Blot Dry	IV	Dry (>100 :00 AFDW (>500°C))W (>500°C)		Lot or Batch	Lot or Batch Number: 031026	arose
ò			Contra-	Tare Weight (g)	Tare Gross Weight (g) 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 (mo)	Mean Wt. per Treatment (mg) (Surviving)
	_	А		1.12544 1.12649	1.12649	0.00100					10		
		æ		1.13836	1.13836 1.13946 0.0010	010000					10		
		J		1.14665	1-14665 1.14 778	0.00.113					01		
	2	٩		1.13632	1.13632 1.13724	0.000 42					2		
		Ŕ		1.13224	1.13224 1.13286	L J 000. O					7		
		ى		1.14899	١	1					0		
	~	٩		1.14450	1.14534	0.00054					0		
		8		1.11728 1.1180		0.00013					6		
		J		1.12908 1.13987		0.00079					10		
												•	
Blank				1.11512	1.11513								
Range													
Mean													
Test Solut	Test Solution Volume:						Loading Rate:					· ·	
Add in weight los の格 えばど É	Add in weight loss of blank boat, if appropriate. のめ オリジョ	blank t	ooat, if app	vropriate.							-		

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lyster ;	2/25/20
4C	GA NUN

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 14001-474 Exp 7

Species: Artemia franciscana

						Adinetad		Mean Wt./ Original	Mean Wt./ Treatment	Number of	Mean Wt./ Surviving	Mean Wt./ Treatment
Treatment F	Rep	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight Net Weight No of Orig. (g) Organisms	Net Weight (g)	No of Orig. Organisms	Organism (mg)	(Driginal)		Organism (mg)	(Surviving)
	A		1.12544	1.12649		0.00105	10	0.105			0.105	0.1093
 	В		1.13836	1.13946		0.00110		0.110		10	0.110	
_	ပ		1.14665	1.14778	0.00113	0.00113	10	0.113		10	0.113	
	<		1 10000	FULUF F			4		0110 0	r	101.0	0011 0
1_			1.13032	1.13/24		0.00092	10	0.092	0.07/0	_	0.131	0.1100
~	m		1.13224	1.13286	0.00062	0.00062	10	0.062		7	0.089	
1	U											
	۵											
	۲		1.14450	1.14534	0.00084	0.00084	10	0.084	0.0787	10	0.084	0.0814
~	В		1.11728	1.11801	0.00073	0.00073	10	0.073		6	0.081	
0	С		1.12908	1.12987	0.00079	0.00079	10	0.079		10	0.079	
	\rightarrow											
1	+											
	+											
	+											
	+											
	-											
	+											
[-											
	+											
Blank	_		1.11512	1.11513	0.00001							

Page of QA Form No. 010a Revision 1 Effective 02/14 y_1 $y_1 y_2/y_2$

inciscana		<u>C.V.</u> #DIV/0!	i0//IC#	i0///I0#		i0//IC#		<u>C <</u>	3.696%	27.550%	7.001%	;0//IC#	i0//IC#	i0//IC#		C.V.	3.696%	27.550%	3.085%	i0//IC#	:0//IC#	#DIV/0
Artemia franciscana		:i0//IC#	#DIV/0	#DIV/0		i0//IC#		SD	0.0040	0.0212	0.0055	i0//IC#	i0//I0#	#DIV/0i	organism)	SD	0.0040	0.0303	0.0025	i0//I U #	#DIV/0i	#DIV/0i
Species:		<u>Mean</u> #DIV/0!	i0//IC#	#DIV/0		i0//IC#	er original)	Mean	0.1093	0.0770	0.0787	0.0000	0.0000	0.0000	er surviving	Mean	0.1093	0.1100	0.0814	0.0000	0.0000	0.0000
	ta	<u>Max</u> #DIV/0!	;0//IC#	#DIV/0i #DIV/0i	i0///I0#	i0//IC#	a (dry wt pe	Max	0.113	0.092	0.084	0.000	000.0	0.000	a (dry wt pe	Max	0.113	0.131	0.084	0.000	0.000	0.000
2	Survival Da	<u>Min</u> #DIV/0!	#DIV/0	#DIV/0	#DIV/0	#DIV/0	Growth Dat	Min	0.105	0.062	0.073	0.000	0.000	0.000	Growth Dat:	Min	0.105	0.089	0.079	0.000	0.000	0.000
дXШ	s for (Z ∣ ო	2	ო	0	0	s for (Z	ო	2	ო	0	0	0	s for (Z	ო	2	ო	0	0	0
14001-474 Exp 7	Summary Statistics for Survival Data	<u>Treatment</u>	200.00%	300.0% n%	0.0%	%0	Summary Statistics for Growth Data (dry wt per original)	Treatment		200.00%	300.0%	%0	0.0%	%0	Summary Statistics for Growth Data (dry wt per surviving organism)	Treatment	-	200.00%	300.0%	%0	0.0%	%0
Project Number:	Ō						ũ								S	r—1						



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 Chla)
- Treatment 2: Yeast (15 mg/rep)
- Treatment 3: *D. viridis* (72.5 µg/L Chla, 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 Chla, 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 μ g/L Chl*a* estimated with absorbance. Solutions were gently aerated.

Characterization of Recon Water

Sample No.	рН	Hard. (mg/L) ^a	Alk. (mg/L) ^a	Spec. Cond. (μS/cm)	TRC (mg/L) ^b	NH₃-N (mg/L)	Salinity (ppt)
RW#13852	7.7	NM	NM	157,300	NM	NM	122

^aAs CaCO3

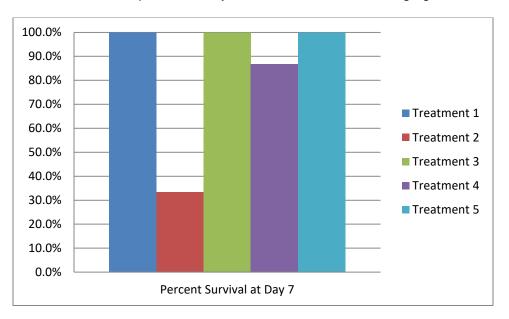
^bTotal residual chlorine

Test activities:

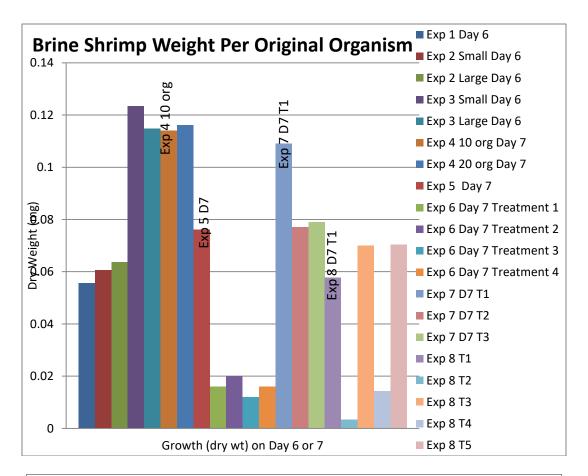
- Biological observations (primarily survival) taken daily.
- Chemistries taken on renewal days (i.e., pH, dissolved oxygen, and temperature).
- Conductivity was measured at test termination or when there was 0% survival in that treatment.
- Dry weights (±0.01 mg) were determined at test termination.

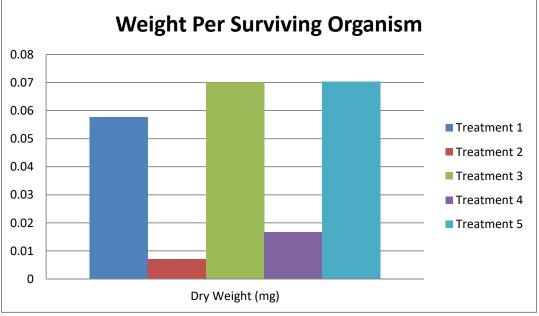
Results:

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,

Bidlad

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

14001-474-054

Attachment

cc: David Pillard, TRE

Dil A. Pellel for RBN

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

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TOXICITY DATA PACKAGE COVER SHEET

		TA PACKAGE COVER SHEET	QA: NHP 3/16/10
Test Type:	Chronic	Project Number:	17001-474-054
Test Substance:	Various	Species: <u>Artemia franc</u>	iscana
Dilution Water:	rGSL	Organism Lot of Batch Numb	per: 022320
Concurrent Control Water:	NA	Age: 48 hr)	Supplier: TRE
Date and Time Test Began:	2/25/20 @ 1420	Date and Time Test Ended:	313120 @ 1425
Protocol Number:		Investigator(s):	P
Background Information		. 0 +	
Type of Test:	Static-Renewal (Daily)	pH control?: <u>Yes</u> If yes, give % CO ₂ :	No NA
Test Temperature:	_20 ± 1 °C	Env. Chmbr/Bath #: _25	Test Chmbrs: <u>147-ml cups</u>
Photoperiod:	<u>16 h light : 8 h dark</u>	Light intensity:	<u>50-100 ft-c.</u>
Test Solution Vol.:	50 ml	Replicates per Treatment:	3
Length of Test:	7_days	Organisms per Replicate:	10
Type of Food and Quantity pe	r Chamber: Varies	_ Feeding Frequency:	1 x daily
Test Substance Characteriz	ation Parameters and Fred	liency	
Hardness: <u>Test Initiation</u>	Alkalinity: <u>Test Initiation</u>	NH ₃ : <u>Test Initiation</u> TRC: <u>Test Init</u>	iation
pH: <u>Daily</u>	Conductivity: <u>Daily</u>		
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:	
Special Procedures and Con			
Organisms hatched 2 days price	or to initiation and held in rG	SL with 100 ug/L Chla	
Treatment 1: <i>D. viridis</i> (145 ug	/L Chla)- 🔔. 🂪 ml		
Treatment 2: Yeast (0.8ml 15	ig/L)		
Treatment 3: 1/2 D. viridis ().	<u> </u>		
Treatment 4: Cerio YTC (0.66n			
Treatment 5: 1/2 D. viridis ()	プ ml) 1/2 cerio YTC (0.33)	ml)	
Appropriate correction factors I	have been applied to all temp	peratures recorded in this data package	
Study Director Initials:	Date: 2 25	20	
M16 2/25/20 B			

1 AB 2125/20 B

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TEST SUBSTANCE USAGE LOG

QA: DOP 3/16/20

Project Number:

17001-474-054

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number				Oample 4
	From:	From:	From:	From:
Test Substance Collection	@	@	@	@
Date and Time	То:	To:	To:	To:
	@	@	@	@
Sample Type (Grab or Comp)				
Date Test Substance Received				
Dilution Water Number RW# or TRE#, circle one	138 52			
Concurrent Control Water RW#	٨٨.			
	2/25/20 2/29/20			
Date(s) Used	2/26/20 3/1/20			
	2/28/20			

Preparation of Test Solutions

Test Substance	Test Substance	Dilution Water	Total Volume	Test Substance	Dilution Water	Totai Volume	Test Substance	Dilution Water	Total Volume
Conc.	Volume	Volume	(ml)	Volume	Volume	(ml)	Volume	Volume	(ml)
(% Effluent)	(ml)	(ml)		(ml)	(ml)		(ml)	(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	N3 2/5/20								
Initials / Date	CP 2/26	20					······································		
Initials / Date	13 2/27/2	20		<u> </u>					
Initials / Date	CP 2/28			· · · · · · · · · · · · · · · · · · ·			·		
Initials / Date	CP 2/29	120		·	- · · ·				
Initials / Date	CP 3/1/	20							
Initials / Date	CP 3/2/	20							
Initials / Date									
Initials / Date									

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Artemia franciscana CHRONIC BIOLOGICAL DATA

QA: DOP 3/16/20

Project Number: ____ 17001-474-054

						Numbo	r of Sund		niomo	
	Test	Day	Day	Day	Day	Day	Day	iving Orga	Day	
mg/L	Replicate	0	1	2	3	4	5	6	7	Remarks
1	A	10	10	10	10	10	10	10	10	
	В	10	10	10	10	10	10	10	10	
	С	10	ID	10	10	10	(0	10	10	
	D								10	
2	Α	10	10	10	10	10	10*	1	5	* 2 weak, no significant growth
	В	10	0	9	9	9	9	7	4	no significant growth
	с	10	0	10	9	7	6	4*		no significant growth, *1 weak
	D									, , ,
3	A	io	10	10	10	10	10	10	10	
	В	10	10	lu †	10	10	10	10	10	tremoved estra org
	С	10	10+	10	10	10	10	10	10	tremoved extra org
	D									
4	А	10	10	10	10	9	9*	9*	97	no significant growth. * I weak
	В	10	10	10	10	10	9	9	9	u u
	С	10	10	10	10	9	9	8	8	n n
	D	·							0	
5	А	10	10	10	10	10	10	10	10	
	В	10	10	10	10	10	10	10	10	
	С	lo	10	Ŵ	10	10	10	10	10	
	D							<u> </u>		
	А								1	
	В		<u> </u>			[
	С				<u> </u>					
	D						<u> </u>	+		
	A				<u> </u>					
	B			<u></u>						
	c									
	D									
	Date:	ababa	2/26/20	alasta	2/28/20	1/20/0-	3/1/20	3/2/20	2101	
	Time:	1430	/1435	1050	0950	1300	1030			
	Initials:	ASIN	r 1					1030	1425	
	initials:	(יו בייי	<u></u> 4	m_	Cl	cl	CP	CP	N	

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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.	
рН		רר	8,0	7.5	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		145	
Temp. (°C)		20	20	20	20	20	20	20		L 38	
Cond. (µS/cm)		157300	141400	13/300	139700	139900	141 700	145900	·······	18	
Hard. (mg/L)						-					
Alk. (mg/L)									,,,,,,		
TRC (mg/L) NH₃ (mg/L)											
Conc.:					L						
pН											··· ··· ··· ··· ··· ···
D.O. (mg/L)											
Temp. (°C)						<u>.</u>					· · · · · · · · · · · · · · · · · · ·
Cond. (µS/cm)											
Hard. (mg/L)											
Alk. (mg/L)									.		· · · · · · · · · · · · · · · · · · ·
TRC (mg/L) NH ₃ (mg/L)											
Conc.:										<u> </u>	
рН											
D.O. (mg/L)											
Temp. (°C)											
Cond. (µS/cm)											
Conc.:				<u> </u>							
р <u>Н</u>											1944 - 1944 - 1944 - 1944 - 1944 - 1944 - 1944 - 1944 - 1944 - 1944 - 1944 - 1944 - 1944 - 1944 - 1944 - 1944 -
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		0955	0940	1255	1025	1025			
Note: Hardness, a	nitials:	ß	CP	An	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.

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DAILY TOXICITY TEST LOG

QA: DAD 3/6/20

Project Number: Test Species:

17001-474-054 Artemia franciscana

General		Feeding	Initials/Date
Comments			
Test Day 0	Random Chart: Min/Max Thermometer # M-15		
Test Day 0	Test Solution Mixed at: เหน่ง Test Organisms Added at:เหวง	Fed @	AB
	Test Organishis Added al. 14 30	1-1-1	2/25/20
			0105100
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: 11-2* °C	Ead @	
•	Yeast appears to be settling out	Fed @ 1005	.Az
	ieus approis i - O		2/27/20
Test Day 0			
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: \8 - 22 °C	Fed @ 1255	
			CP
	*No significant growth in 100% yeart and 100% MTC		2/29/20
Test Day 5	Real Time: 20 °C Min-Max Range: 18-22 °C	Fed @ 1025	
		1025	CP
	* " " very weak orgs in 100% yeast		3/1/20
Test Day 6			5/1/20
. col Day o	Real Time: 20 °C Min-Max Range: 18 - 21 °C	Fed @ 1020	CP
	* "		CP 312120
			312120
Test Day 7	Real Time: ລວ °C Min-Max Range: ເຊິ-ລູ °C	Fed @ Nove	0
	• 3 + 5 mps down in Colon than Ings	Noce	Br
	★ ¹		313120
Fest Day 8	Real Time: °C Min-Max Range: °C	Fed @	
	3 1		
UDAF	3/16/20 E		

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Image: Transmit Analysis Transmit Anan Withete Analysis Transmit <t< th=""><th></th><th>1-474-</th><th>054</th><th>Test Substa</th><th></th><th>rious</th><th>011</th><th></th><th>Comments: Analytical Bala</th><th>nce ID: Sar</th><th>1 # 1</th><th>:</th></t<>		1-474-	054	Test Substa		rious	011		Comments: Analytical Bala	nce ID: Sar	1 # 1	:
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						JRGANISN	I LENGTHS	s, weight	I EST ORGANISM LENGTHS, WEIGHTS, AND LOADING	DING		QA: No	Q4: Nov 3/1120
Project	Project Number: 17001-474-054	-1001	474-C	वि	Test Substance:		Various			Comments:	1		
Specie.	Species: Artenia franciscana	a fra	nciscana	د	Analyst Tare:	2	Analyst Gross:	iross: MB		Analytical Balance ID: Sart # 1 Dried in Oven # \mathcal{J} from Date: $^3/3\Delta_{\Lambda}$ Time: 1535	nce ID: Sev # 3 from Da	+ # 1 ate: 3/3/2∧Ti	me: 1535
Date/Ti	Date/Time of Tare Wt.: $3/3/20$	M.: 3		020Q1 02		Date/Time of Gross Wt.: $^{3/_{6}/_{2O}}$	3/6/20	Ø 1115			2 2	ate: <u>3/6/20</u> T	ime: <u>/୦୫୦</u>
Boat	Treatment	Rep.	Length	Weight Type (Circle):	e (Circle):	Wet Blot Dry	Dry Dry (>100°C)	\square	AFDW (>500°C)		Lot or Batch Number:	1 1	03820
Š Z					Tare Gross Weight (g) Weight (g)	Net Weight (g)	Ž	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	No. of Surv. Organisms	t, bř	Mean Wt. per Treatment (mg) (Surviving)
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Page of _____ QA Form No. 010a Revision 1 Effective 02/14

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TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

07/91/2

Project Number		14001-474 Exp 8	8 0		Species:	Artemia fr	Artemia franciscana			OA: DAP	DAP 3/16/.
Rep	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Net Weight Net Weight (9) (9)	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)
∢		1.14692			0.00053	10	0.053	0.0577	10	0.053	0.0577
۵		1.13547			0.00061	10	0.061		10	0.061	
ပ		1.14688		0.00058	0.00059	10	0.059		10	0.059	
∣∢		1.14879	1.14886	0.00007	0.00008	10	0.008	0 0033	5	0.016	0.0070
മ		1.14032	1.14033				0.002		4	0.005	
U		1.13650	1.13650	0.00000		ŀ	000.0		-	000.0	
<		1 14683	1.14761		0.00079	10	0.079	0.0700	10	0.079	0.0700
മ		1.15034			0.00072	10	0.072		10	0.072	
ပ		1.12851	1.12909	0.00058	0.00059	10	0.059		10	0.059	
⊲		1 13101	1 12115	0.00014	0 00015	10	0.015	0.0142	c	710 0	0.0466
: m		1.15247	1.15260	0.00013	0.00014	10	0.014	2	ი ი	0.016	00000
ပ		1.13429	1.13442	0.00013	0.00014	10	0.014		ω	0.018	
∢		1.13462	1.13526	0.00064	0.00065	10	0.065	0.0703	10	0.065	0.0703
œ		1.14685	1.14751	0.00066	0.00067	10	0.067		10	0.067	
ပ		1.12853	1.12931	0.00078	0.00079	10	0.079		10	0.079	
								1			
		1.13816	1.13815	-0.00001							

Page of OA Form No. 010a Revision 1 Effective 02/14

CLIDIFORD : NO

nciscana			i0//IC#	#DIV/0	#DIV/0	#DIV/0	;0//IC#		<u>C.V.</u>	7.220%	124.900%	14.498%	4.028%	10.766%	i0//IC#		C.V.	7.220%	116.934%	14.498%	5.886%	10.766%	#DIV/0
Artemia franciscana	C		i0//IC#	#DIV/0!	#DIV/0i	i0//IC#	i0//IC#		<u>SD</u>	0.0042	0.0042	0.0101	0.0006	0.0076	i0//IC#	organism)	SD	0.0042	0.0082	0.0101	0.0010	0.0076	#DIV/0
Species:	neeM	#DIV/0i	i0//IC#	i0//IC#	i0//IC#	i0//IC#	i0//IC#	er original)	Mean	0.0577	0.0033	0.0700	0.0143	0.0703	0.0000	er surviving	Mean	0.0577	0.0070	0.0700	0.0166	0.0703	0.000
	ta May	#DIV/0	i0//IC#	#DIV/0	i0//IC#	#DIV/0	#DIV/0i	a (dry wt pe	Max	0.061	0.008	0.079	0.015	0.079	0.000	a (dry wt pe	Max	0.061	0.016	0.079	0.018	0.079	0.000
	S urvival Da Min	#DIV/0i	#DIV/0	i0//IC#	;0//IC#	i0//IC#	i0//IC#	Srowth Dat :	<u>Min</u>	0.053	0.000	0.059	0.014	0.065	0.000	srowth Data	<u>Min</u>	0.053	0.000	0.059	0.016	0.065	0.000
Exp 8	z for \$	ო	ო	ო	ო	ო	0	for 0	Z	ო	ო	ო	ო	ო	0	for G	Z	ო	ო	ო	ო	ო	0
14001-474 Exp 8	Summary Statistics for Survival Data Treatment N Min	1	200.00%	300.0%	400%	500.0%	%0	Summary Statistics for Growth Data (dry wt per original)	<u>Treatment</u>	~	200.00%	300.0%	400%	500.0%	%0	Summary Statistics for Growth Data (dry wt per surviving organism)	Treatment	~	200.00%	300.0%	400%	500.0%	%0
Project Number	S L	1						Ñ								S							



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

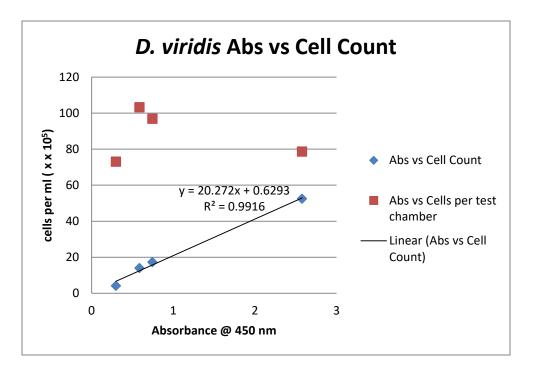
Subject: Algae Absorbance vs Cell Count

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 Chla 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 Chla 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,

la Billet

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

17001-474-059

Attachment

cc: Rami B. Naddy, TRE

D-1 1. Pillel

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



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 YTC¹ 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 Chla)
- Treatment 2: Yeast (7.5 mg/rep)
- Treatment 3: D. viridis (72.5 µg/L Chla, 50% of the normal density)/ Yeast (7.5 mg/rep)
- Treatment 4: D. viridis (72.5 µg/L Chla, 50% of the normal density)/ YTC (0.6 mg/rep)
- Treatment 5: *D. viridis* (72.5 µg/L Chla, 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 Chla (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 μ g/L Chl*a* estimated with absorbance. Solutions were gently aerated.

Characterization of Recon Water

Sample No.	рН	Hard. (mg/L) ^a	Alk. (mg/L) ^a	Spec. Cond. (μS/cm)	TRC (mg/L) [♭]	NH₃-N (mg/L)	Salinity (ppt)
RW#13852	7.8	NM	NM	144,800	NM	NM	122

^aAs CaCO3

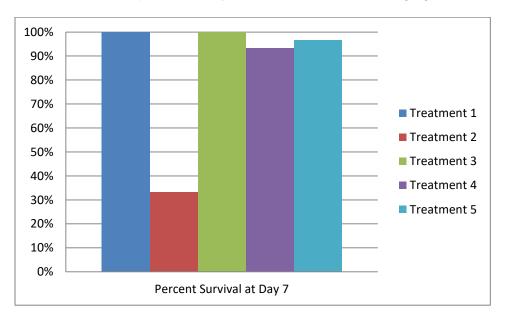
^bTotal residual chlorine

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.

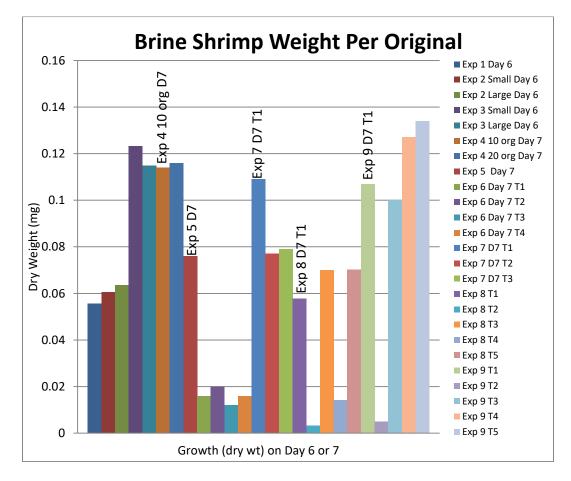
Results:

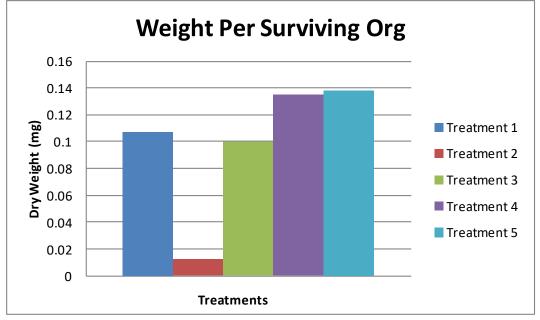
The survival of the brine shrimp in this study is illustrated in the following figure.



Mr. Bittner March 30, 2020 Page 3

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.







Mr. Bittner March 30, 2020 Page 4

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

14001-474-056

Attachment

cc: David Pillard, TRE

Rami B. Naddy, Ph.D. Manager / Environmental Toxicologist naddyrb.tre@gmail.com Mr. Bittner March 30, 2020 Page 4

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
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- 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 <u>bidlackac.tre@gmail.com</u>

14001-474-056

Attachment

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

cc: David Pillard, TRE

Page 1 of _____ QA Form No. 051 Revision 5 Effective 02/14

CBA NON 3/26/20

TOXICITY DATA PACKAGE COVER SHEET

			+56
Test Type:	Chronic	Project Number:	b) Ø <u>17001-474-054</u>
Test Substance:	Various	Species: <u>Artemia fran</u>	ciscana
Dilution Water:	rGSL	Organism Lot or Batch Num	ber: 03.082 0
Concurrent Control Water:	NA	Age: 48 hr)	Supplier: TRE
Date and Time Test Began:	3/10/20 @ 1415	Date and Time Test Ended:	3/17/20 @ 1500
Protocol Number:		Investigator(s): R/EN	eelm criAs
Background Information		, , , , , , , , , , , , , , , , , , ,] /
Type of Test:	Static-Renewal (Daily)	pH control?: <u>Yes</u> If yes, give % CO ₂ :	No
Test Temperature:	20 ± 1 °C	Env. Chmbr /Bath #: <u>25</u>	Test Chmbrs: <u>147-ml cups</u>
Photoperiod:	<u>16 h light : 8 h dark</u>	Light intensity:	<u>50-100 ft-c.</u>
Test Solution Vol.:	50 ml	Replicates per Treatment:	3
Length of Test:	7 days	- Organisms per Replicate:	10
Type of Food and Quantity pe	er Chamber: Varies	_ Feeding Frequency:	1 x daily
Test Substance Characteriz	ation Parameters and Free	liency:	
Hardness: <u>Test Initiation</u>		NH ₃ : <u>Test Initiation</u> TRC: <u>Test In</u>	itiation
pH: <u>Daily</u>	Conductivity: <u>Daily</u>	·	
Test Concentrations (Volume:	Volume): See Below		
Agency Summary Sheet(s)?:	None	_	······································
Reference Toxicant Data:	Test Dates: PA	to NA	IC ₂₅ :
Hist. 95% Control Limits:	to	Method for Determining Ref. Tox. Value	
Special Procedures and Cor	siderations:		
Organisms hatched 2 days pri	or to initiation and held in rGS	SL with 100 ug/L Chla	
Treatment 1: D. viridis (145 ug	g/L Chla)- 2.6 ml		
Treatment 2: Yeast (0.4ml 15.4			
Treatment 3: 1/2 D. viridis (3 ml) 1/2 yeast (0.4 ml)		
	.3 ml) 1/2 cerio YTC (0.33		
Treatment 5: 1/2 D. viridis (• 3 ml) 1/2 cerio YTC (0.66	ml)	
Appropriate correction factors		peratures recorded in this data package	
Study Director Initials:	Date:	20 ,	

OA3 3/20/20E

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DA NW 3/26/20

TEST	SUBSTANCE	USAGE	LOG
U,			

Project Number

	V,
er:	17001-474-05#

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number				
	From:	From:	From:	From:
Test Substance Collection	@	@	@	@
Date and Time	То:	To:	To:	То:
	@	@	@	@
Sample Type (Grab or Comp)				Ŭ
Date Test Substance Received				
Dilution Water Number	13582 113776*			
RW# or TRE#, circle one	100000			
Concurrent Control Water RW#	MA .		n ni <u>ni na na na na na na na na na na na na na </u>	
	3/10/20 3/14/20*			
Date(s) Used	3/11/20 3/15/204			
	3/12/20 31/6/20			
	3/13/201			

Preparation of Test Solutions

Test	Test	Dilution	Total	Test	Dilution	Total	Test	Dilution	Total
Substance	Substance	Water	Volume	Substance	Water	Volume	Substance	Water	Volume
Conc.	Volume	Volume	(ml)	Volume	Volume	(mi)	Volume	Volume	(ml)
(% Effluent)	(ml)	(ml)		(mi)	(ml)		(ml)	(ml)	()
1	0	170	170						······
2	0	170	170						
3	0	170	170						
4	0	170	170						
5	0	170	170						
	00	850	850						
Initials / Date	By 3/1) 120 M	ved RES		<u> </u>				
Initials / Date	22 3/11	1	ĨĨ .						
Initials / Date	AS 3/12	20 "	13	- m - m					
Initials / Date	CP 3/13	120 "	te	·····	<u> </u>				
Initials / Date	OP 3/14	120 "	í.	. <u></u>					
Initials / Date	CP 3/15	120 "	ч	· · · · · · · · · · · · · · · · · · ·					
Initials / Date	Br 3116	120 n	n	<u></u>			<u>-</u>	<u> </u>	
Initials / Date							······		

Onw 3/24/20 €

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Artemia franciscana CHRONIC BIOLOGICAL DATA

										Effective 02/14	
							nciscana OGICAL I	DATA		the new 3	126/20
Project I	Number:	1700	1-474-054	9 4 b							
	<u> </u>	1	<u></u>	<u> </u>	<u></u>	Number					
	Test	Day	Day	Day	Day	Day	r of Survi Day	Day	Day	To Juri	WN
mg/L	Replicate	0	1	2	3	4	5	6	7	Remarks	
1	A	10	10	10	10	10	10	10	ID		100
	В	10	10	10	10	10	10	10	10		
	C	10	10	10	10	10	10	(0	10		
2	A	10	10	10	10	7*	4*	1*	~1	* very small, weak orgs	33.3
	В	10	10	9*	9	9	8*	5*	3	SINF, * II U	
	с	6	10	10	9	9	9*	97	i\$	* 11 11	
	D	\geq									
3	A	10	10	10	10	10	(D	10	10		, 100
	В	10	16	10 "	10+	ID	10	Ŵ	10	+ removed 1 extra org	<u> </u>
·	с	10	10	10	*	-				* no orgs; TE	
	D							-			
4	А	10	10	10	10	10	10	10	10		75.3
	В	10	10709	g xa	8	8	18	8	8	AINFO	1/1/
	с	10	39,10	10	10	10	10	(0)	10		-44.
	D										
5	A	10	9	9	9	9	9	9	9		96.7
	В	10	16	10	10	10	10	10	10		/ - / /
	С	10	10	10	10	10	10	10	10		
	D										
	A										
	В										
	с					1					
	D	/									
	А				ſ	l l					
	В			······							
	С										
	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	12355	1500		
	Initials:	BOTEN		by	cl	cl	CP	Br	9) 90/		

Initials: B/EN 20 Br CP CP Fr Er OR 3/10/20 WP O223/12/20E 3/20/20E

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CHRONIC CHEMICAL DATA (INITIAL)

02A was 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.	
рН		7.8	7.9	7.9	7.9	8.D	8.0	7.8		Fudg	
D.O. (mg/L)		5.6	51	5.5	5.2	5.2	5.0	5.5		17	
Temp. (°C)	·	20	20	20	20	10	20	20		6-39	
Cond. (µS/cm))	144,860	137400	135200	197700	137700	138700	177300		15	· * * * · · · · · · · · · · · · · · · ·
Hard. (mg/L)										Tir	
Alk. (mg/L)										Tim	
TRC (mg/L)										99	
NH ₃ (mg/L)										HAL	2744 - 2747 - 2747 - 2747 - 2747 - 2747 - 2747 - 2747 - 2747 - 2747 - 2747 - 2747 - 2747 - 2747 - 2747 - 2747 -
Conc.:											
рН											
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)											anna anna anna an an
Temp. (°C)											
Cond. (µS/cm)											
	Date:	3110410	3/1/20	3/12/20	3/13/20	3/14/20	3/15/20	3/16/20			
	Time:		/ _	1515	1450	1030		1330			
Note: Hardness	nitials:		80	A,	cl	Cl	CP	Pr			

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.

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Dr new 3/26/20

CHRONIC CHEMICAL DATA (FINAL)

					CHRON	NIC CHE	MICAL	DATA (F	INAL)		
Project Numb	er:		1-474-0								
Test Species:		Artemi	a francis	scana					<u></u>		· · · · · · · · · · · · · · · · · · ·
%		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter #	Remarks
Conc.:	1							126800		All Conc.	* conductivity
pН		7.9	79	7.8	7.9	8.0	7.9	7.7		Fuda	-
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		6-37	
Conc.:	2							123800			* conductivity
pН		7.8	7.7	7.8	7.8	7.9	7.9	7.0			
D.O. (mg/L)		5.0	5.0	4.7	5.1	4.7	4.7	33			
Temp (°C)		20	20	22*	19	19	19	21			
Conc.:	3							1239100			* conductivity
рН		7.8	7.8	7.8	7.8	7.9	7.7	7.7			
D.O. (mg/L)		4.8	4.9	5.5	05.4.8	4.1	3.7	3.1			
Temp (°C)		20	20	21	19	19	19	21			
Conc.:	4							122200			* conductivity
рН		7.9	7.4	7.8	7.9	8.0	7.8	7.6			
D.O. (mg/L)		5.1	4.9	5.0	5.1	5.O	4.1	3.7			
Temp (°C)		20	20	21	19	19	19	21			
Conc.:	5							121200			* conductivity
рН		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	51	4.1	41			
Temp (°C)		20	20	21	19	19	19	21			
Conc.:											
рН		· · · · · · · · · · · · · · · · · · ·									
D.O. (mg/L)											
Temp (°C)											
Conc.:											
рН											
D.O. (mg/L)											
Temp (°C)											
	Date:	3/11/20	3/2/20	3/13/20	3/14/20	3/15/20	3116/20	3/17/20			
	Time:	1560	1520	1550	1100	1150	1350	1530			
	Initials:	Ee .	h	Cl	CP	GP	R	re			

Checked all reps € 3/14/20 E

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DAILY TOXICITY TEST LOG

Qx ner 3/26/20

General						Feeding	Initials/Date
Comments		1	D ~				
	Random Ch	nart: M	2150	Min/Max Thermometer #	M-TT		
Test Day 0	Test Solutio		v •	-		Fed @ 1405	R
	Test Organi	isms Ac	lded at:	415			
							3/1925
Test Day 1	Real Time:	20	°C	Min-Max Range: 18-2	<u> </u>	Fed @1430	
							re
							3/11/20
Test Day 2	Real Time:	20	°C	در روح Min-Max Range: عدر	°C	Fed @ isis	
				~		15.15	A3 3/12/20
							3/12/20
Test Day 3	Real Time:	20	°C	Min-Max Range: 19-22	°C	Fed @ 1510	- 1
							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: (ターフ)	°C	Fed @ 1330	01
		•					B
							3116/20
Test Day 7	Real Time:	19	°C	Min-Max Range: 19 - 2	°C	Fed @	
						NONE	Ee .
							3/17/20
Test Day 8	Real Time:	. <u> </u>	°C	Min-Max Range:	°C	Fed @	

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

OF 3/10/10 WP

	•			r	TEST O	IRGANISM	I LENGTHS	, WEIGHTS	TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING	DING		Page of QA Form No.010 Revision 7 Effective 01/20	Page of QA Form No.010 Revision 7 Effective 01/20 &A ver 3/20/20
Project Number:		- 100)	17001-474-054	054	Test Substance:	nce:				Comments:	(
Species: Artemia	rtemia		Franciscana		Analyst Tare:	зК	Analyst Gross:	oss: AS		Analytical Balance ID: Sart # 1 Dried in Oven # 3 from Date: 3/(1	nce ID: Sav t 3 from Da	D: Sart # 1 from Date: 3/17/20Time: 156	ne: 15 6
Date/Time of Tare Wt: \mathscr{B}^{IT}	of Tare V	/t.: 3/1		20 @ 1435	Date/Time o	0	3/20/20		0		to to	Date: <u>अपि/</u> रूTime: <u>गमर</u> ः	me: IHC
Boat T	Treatment	Rep.	Length	Weight Type (Circle):		Wet Blot D	Blot Dry Dry (60-90°C) Dry (>100°C)	°C) Dry (>1(AFDW (>500°C)	Lot or Batch Number:	Number:	
o Z			CUNITS:		Tare Gross Weight (g) 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)
	+	A		1.14181	LH273	U.ccc92					2		
		В		1.13959	139591,14665	0,00109					IC		
		ა		1.13960 1.14031	1,14031	0:00121					Q		
	7	4		1.11607 1.11VC7	i.IIVC7								
		Ø		1.13854	1.13854 1.13859	0, 6605					M		
		હ		1.11517	1.11525	CECON					٦		
	ю	۷		1.14436 1.14531		c . tto 95					2		
		Ø		1.13805	1,13910	501070					Q		
	1	3		1.13267									
Blank				[.14947	1.14950					-			
Range													
Mean													
Test Solution Volume:	on Volume	ä					Loading Rate:						
Add in we	ight loss o	of blank	boat, if ap	Add in weight loss of blank boat, if appropriate.									

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	J			1	TEST C	RGANISA	A LENGTHS	S, WEIGHT	TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING	DING		Effective 01/20	sion / ctive 01/20 Sh.w. 3/26/20
Project	Project Number: 17001- 474- 054	-1001	474-0	54	Test Substance:	ince:				Comments:	(
Species	Species: Artenia franciscana	i fra	nciscan	A	Analyst Tare: δl	e: Sk	Analyst G	Analyst Gross: HS		Analytical Balance ID: Sart # Dried in Oven # 3 from Date: ¾	nce ID: Sar # 3 from Da	D: Sart # 1 from Date: <u>\$/17/2</u> 0 Time: 1 510	ne: 1 570
Date/Tir	Date/Time of Tare Wt.: $3/i7/w @ /435$	/t.: 3/i	3 02/L)	¥1435	Date/Time c	Date/Time of Gross Wt.:		3/20/20 @1000			ڭ بو ا	Date: <u>अंश्रभ्र</u> Time:	me: 11410
Boat	Treatment Rep.	Rep.	Length	Weight Type (Circle):		Wet Blot D	Blot Dry (Dry (60-90°C)) Dry (>100°C)	0°C) Dry (>1		AFDW (>500°C)	Lot or Batch Number:	Number:	
o Z			Units:		Tare Gross Weight (g) 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 (ma)	Mean Wt. per Treatment (mg) (Surviving)
	t	A		1. 14155 1-14300	1-14300	SHOD' O					0		
		9		1.14008 1.14 1c-7	1. 14 ICT	6,00099					X		
		ს		1.13841	1.13977						ال ا		
		A		1. 14172	1.14297	C : CC125					વ		
	2	Ø		1.13205	1.13334	C 150129					· 0		
		ა		(.1495)	1.15 095	C .66 14 7					0		
Blank													
Range													
Mean													
Test Sol	Test Solution Volume:						Loading Rate:						
Add in	Add in weight loss of blank boat, if appropriate.	of blank	t boat, if a	opropriate.									

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TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

02/72/2 mm 42

3/21/20	Wt:/ ment g) ving)	0.1073				0.0117				0.1000				0.1349					0.1303							٦
	Mean Wt./ Treatment (mg) (Surviving)																									
&A wend	Mean Wt./ Surviving Organism (mg)	0.092	0.109	0.121		0.000	0.017	0.018		260'0	0.105	I		0.145	0.124	0.136			U. 138	0.129	0.147					
¢	Number of Surv. Organisms	10	10	10		1	3	9		10	10	0		10	8	10		c	מ	10	10					
	Mean Wt./ Treatment (mg) (Original)	0.1073				0.0053				0.1000				0.1267					U. 1337							
anciscana	Mean Wt./ Original Organism (mg)	0.092	0.109	0.121		0.000	0.005	0.011		0.095	0.105			0.145	0.099	0.136			0.120	0.129	0.147					
Artemia franciscana	No of Orig. Organisms	10	10	10		10	10	10		10	10	0		10	10	10	1	4			10					
Species:	Net Weight Net Weight (g)	0.00092	0.00109	0.00121		0.00000	0.00005	0.00011		0.00095	0.00105	00000'0		0.00145	0.00099	0.00136		0.004.01	071 NN 120	0.00129	0.00147					
	Net Weight (9)	0.00092		0.00121				0.00011		0.00095	0.00105	0.00000				0.00136		0.004.01			0.00147					0.0003
6 0	Gross Weight (g)	1.14273	1.14068	1.14081		1.11607	1.13859	1.11528		1.14531	1.13910	0.00000		1.14300	1.14107	1.13977			1.1429/	1.13334	1.15098				4 4 4050	1.14950
14001-474 Exp 9	Tare Weight (g)	1.14181	1.13959	1.13960		1.11607	1.13854	1.11517		1.14436	1.13805	0.00000		1.14155	1.14008	1.13841		1 1 1 1 1 1	1.141/2	1.13205	1.14951				4 40047	1.14947
	Length Units:																									
umber	: Rep	A	ш	ပ		A	ш	ပ	Ω	 A	ш	ပ	۵	A	മ	ပ	۵	<	<	ш	ပ	Δ				
Project Number	Treatment		~	-			ç	٩			٣	ົ			~	t				ſ	>					blank

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ON un 3/20/20

Project Number:

ar. 14001-474 Exp 9	4 Exp	6		Species:	Artemia franciscana	anciscana
Summary Statistics for Survival Data	is for	Survival Da	ta			
<u>Treatment</u>	Z	Min	Max	Mean	SD	C.<.
₹	ო	#DIV/0	i0//IC#	#DIV/0i	i0//IC#	#DIV/0
200.00%	ო	i0//IC#	i0//IC#	i0//IC#	#DIV/0	i0//IC#
300.0%	ო	#DIV/0	i0//IC#	#DIV/0	i0//IC#	#DIV/0i
400%	ო	i0//IC#	i0//IC#	i0//IC#	#DIV/0!	i0//IC#
500.0%	ო	#DIV/0	i0//IC#	#DIV/0!	i0//I0#	#DIV/0i
%0	0	i0//IC#	i0//IC#	i0//IC#	i0//IC#	i0//IC#
Summary Statistics for Growth Data (dry wt per original)	s for	Growth Dat	a (dry wt pe	r original)		
Treatment	Z	<u>Min</u>	Max	Mean	<u>SD</u>	<u> </u>
	ო	0.092	0.121	0.1073	0.0146	13.576%
200.00%	ო	0.000	0.011	0.0053	0.0055	103.267%
300.0%	2	0.095	0.105	0.1000	0.0071	7.071%
400%	ო	0.099	0.145	0.1267	0.0244	19.247%
500.0%	ო	0.125	0.147	0.1337	0.0117	8.767%
%0	0	0.000	0.000	0.0000	i0//IC#	#DIV/0!
Summary Statistics for Growth Data (dry wt per surviving organism)	s for	Growth Dat	a (dry wt pe	r surviving	organism)	
Treatment	Z	Min	Max	Mean	SD	<u>C.V.</u>
~	ო	0.092	0.121	0.1073	0.0146	13.576%
200.00%	ო	0.000	0.018	0.0117	0.0101	86.897%
300.0%	2	0.095	0.105	0.1000	0.0071	7.071%
400%	ო	0.124	0.145	0.1349	0.0107	7.906%
500.0%	ო	0.129	0.147	0.1383	0.0090	6.518%
%0	0	0.000	0.000	0.0000	i0//IC#	;0//IC#



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 Chla, or 72.5 μg/L Chla and 0.33 ml/ chamber YTC¹
- Temperature: 20°C
- Test volume(s): 50 ml
- Replicates: 4
- Organisms/Rep: 10
- Test media: Various (see above)

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 μ g/L Chl*a*. Solutions were gently aerated.

Characterization of Recon Water

Sample No.	рН	Hard. (mg/L) ^a	Alk. (mg/L)ª	Spec. Cond. (μS/cm)	TRC (mg/L) ^ь	NH₃-N (mg/L)	Salinity (ppt)
RW#13888	8.0	NM	NM	135,400	NM	NM	120

^aAs CaCO3

^bTotal residual chlorine

Test activities:

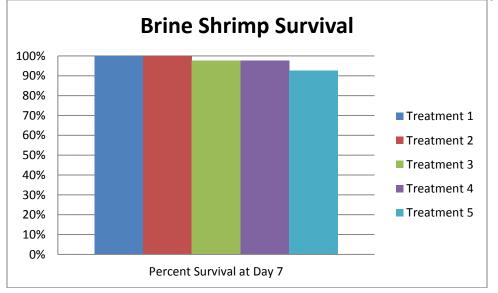
- Biological observations (primarily survival) taken daily.
- Chemistries taken on renewal days (i.e., pH, dissolved oxygen, and temperature).
- Salinity was measured daily in new solutions.
- Conductivity was measured at test termination or when there was 0% survival in that treatment.
- Dry weights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

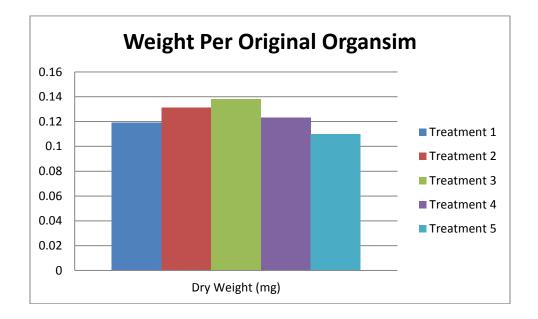
Results:

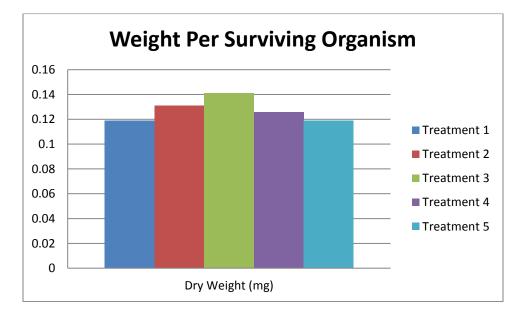
The results of the two control treatments (fed only *D. viridis* and fed a mixture of *D. viridis*/YTC) are shown in the following table. Survival and growth (dry wt) were similar between the two treatments.

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 \geq 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

Mr. Bittner / Dr. Belovsky April 20, 2020 Page 4

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

14001-474-060

Attachment

cc: David Pillard, TRE

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

Page 1 of _____ QA Form No. 051 Revision 5 Effective 02/14

				Liective t	12/14
		TOXICITY DA	TA PACKAGE COVER SHEET	â	
Test Type:	Chronic		Project Number:	<u>_ 17001-474</u>) <i>O</i> (#0 Ext
Test Substance:	Platymona	as	Species: <u>Artemia</u> fra	nciscana	
Dilution Water:	- rest Mi	illi-Q	Organism Lot or Batch Nu	mber:	033020
Concurrent Control Water:	NA		Age: 48 Hr (48 hr)	Supplier:	TRE
Date and Time Test Began:	4/1/20	@ (355	_ Date and Time Test Endeo		@ 1340
Protocol Number:			Investigator(s):	R/EN	
Background Information					
Type of Test:	Static-Ren	ewal (Daily)	pH control?: <u>Yes</u> If yes, give % CO ₂ :	No NA	
Test Temperature:	<u>20 ± 1 °C</u>	_	Env. Chmbr/Bath #: _25_	Test	Chmbrs: <u>147-ml cups</u>
Photoperiod:	<u>16 h light :</u>	<u>8 h dark</u>	Light intensity:	<u>50-100 ft-c</u>	
Test Solution Vol.:		50 ml	Replicates per Treatment:	4	-
Length of Test:	7 days		Organisms per Replicate:	10	
Type of Food and Quantity pe		<u>D. viridis/ YTC</u>		1 x daily	
Test Substance Characteriz	ation Paran	neters and Fred		<u> </u>	
Hardness: <u>Test Initiation</u>	Alkalinity:		NH ₃ : <u>Test Initiation</u> TRC: <u>Test I</u>	Initiation	
pH: <u>Daily</u>	Conductivit			muanon	
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. Valu		relation
Special Procedures and Cor]
Organisms hatched 2 days pri	or to initiatio				
	ment 1-4: Alo	gae 1,5 ml 0.	33 ml YTC	·	
Treatment 2: 56 ppt Treatment 3: 88 ppt					
Treatment 4: 120 ppt					
Treatment 5: 120 ppt Algae on	her 2 1-1-				
Appropriate correction factors	have been a	polied to all terr	peratures recorded in this data package		
Study Director Initials:		Date: 4120	seratures recorded in this data package)	

-

@ New 4/20/20 cf

Page 2 of _____ QA Form No. 014 Revision 1 Effective 02/14

TEST SUBSTANCE USAGE LOG

QA new 4/20/20

Project Number:

E

17001-474-Exp

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number	13898			
	From:	From:	From:	From:
Test Substance Collection	@	@	@	@
Date and Time	To:	To:	To:	To:
	@	@	@	@
Sample Type (Grab or Comp)				
Date Test Substance Received				
Dilution Water Number	Diagon	· · · · · · · · · · · · · · · · · · ·		
(RW#)or TRE#, circle one	13888			
Concurrent Control Water RW#	NA			
	4/1/20 415120			
Date(s) Used	4/2/20 4/6/20			·····
	43/204/7/20			
	4/4/20			·····

rust MQ

Preparation of Test Solutions

Test	Dilutio			loi rest 30	r			
				Dilution	Total	Test	Dilution	Total
	Water	Volume	Substance	Water	Volume	Substance	Water	Volume
Volume	Volume	(ml)	Volume	Volume	(ml)	Volume	Volume	(ml)
(ml)	(ml)		(ml)	(ml)		(ml)	(ml)	()
52	198	250					(////)	
117	133	250						
183	67	250						
250	25 0	250						
25 0	$\frac{0}{250}$	250						• <u> </u>
								<u> </u>
7	398							
152		1250						······
CP 4/1	/20 Mi,	led B.S.					<u> </u>	
CP 4/2	120	n n		<u>-</u>				
<u>CP</u> 4/3	3/20 1	r a		-			·······	
		4 4					<u> </u>	
er 4151	90 "	4						
						<u> </u>	· · · · ·	
		ч						
				<u> </u>	∦-			
	52 117 183 250 250 250 250 250 250 250 252 CP 4/1 CP 4/2 CP 4/2	Substance Water Volume Volume (ml) (ml) 52 198 117 133 183 67 25 250 25 250 25 250 25 250 25 250 25 250 25 250 25 250 25 250 25 250 25 250 25 250 25 250 25 250 25 250 25 298 CP 4/1/20 CP 4/2/20 CP 4/4/20 CP 4/4/20 EN 4/6/20	Substance Water Volume Volume Volume (ml) (ml) (ml) (ml) (ml) 52 198 250 117 133 250 117 133 250 117 133 250 250 250 250 250 250 250 250 250 250 250 250 250 250 250 250 250 250 250 250 250 250 252 298 1250 CP $4/1/20$ m CP $4/2/20$ m n CP $4/4/70$ m n CP $4/4/20$ m n CP $4/4/200$ m n CP $4/4/200$ m n CP $4/4/200$ m n EN $4/4/200$ 100 100	Substance Water Volume Itest Volume Volume (ml) Substance (ml) (ml) (ml) (ml) (ml) (ml) (ml) (ml) 52 198 250 117 133 250 117 133 250 117 133 250 117 133 250 250 250 250 250 250 250 250 250 250 250 250 250 250 250 250 250 250 250 250 250 250 250 250 250 252 298 1250 252 298 1250 CP $4/1/20$ n CP $4/2/20$ n CP $4/4/200$ n EN $4/4/200$ n	Substance Water Volume Substance Water Volume (ml) (ml) Volume Volume (ml) (ml) (ml) (ml) (ml) 52 198 250 (ml) (ml) 117 133 250 (ml) (ml) 183 67 250 (ml) (ml) 25 0 250 250 (ml) (ml) 25 0 250 250 (ml) (ml) $25 0$ 250 250 (ml) (ml) $25 0$ 250 250 (ml) (ml) $25 0$ 250 (ml) (ml) (ml) $25 0$ 250 (ml) (ml) (ml) $25 0$ 250 (ml) (ml) (ml) 252 2938 1250 (ml) (ml) 252 2938 1250 (ml) (ml) 26 $4/1/20$ (ml) (ml) (ml) 26 $4/4/700$ (ml) (ml) (ml) <td>Substance Water Volume Substance Water Volume Substance Water Volume (ml) (ml) (ml) (ml) (ml) (ml) (ml) (ml) 52 198 250 </td> <td>Substance Water Volume Substance Volume (ml) Substance Volume (ml) Substance Volume (ml) (ml)</td> <td>Substance VolumeWater VolumeVolume (ml)Substance VolumeWater VolumeVolume (ml)Test VolumeDilution VolumeTest VolumeDilution Water(ml)(ml)(ml)(ml)Volume (ml)(ml)Volume (ml)<</td>	Substance Water Volume Substance Water Volume Substance Water Volume (ml) (ml) (ml) (ml) (ml) (ml) (ml) (ml) 52 198 250	Substance Water Volume Substance Water Volume Substance Water Volume Substance Water Volume Substance Water Volume Substance Water Volume Substance Water Volume Substance Water Volume Substance Volume (ml) Substance Volume (ml) Substance Volume (ml) (ml)	Substance VolumeWater VolumeVolume (ml)Substance VolumeWater VolumeVolume (ml)Test VolumeDilution VolumeTest VolumeDilution Water(ml)(ml)(ml)(ml)Volume (ml)(ml)Volume (ml)<

ON 4/1/20 E (3EN 4/6/20 E

Page ___ of ___ QA Form No. 060 Revision 3 Effective 02/14

Artemia franciscana CHRONIC BIOLOGICAL DATA

apply my the

Project Number: _____17001-474-Exp

Project N	Number:	1700	<u>)1-474-Ex</u>	<u>p</u>						
	T					Nimela				
	Test	Day	Day	Day	Day	Day	Day	iving Orga Day	anisms Day	to SUNIU
mg/L	Replicate			2	3	4	5	6	7	Remarks
25ppt	A	10	10	10	10	10	10	10	10	60
	В	10	10	10	10	0	10	10	10	
	С	10	10	10	10	10	10	10	10	
	D	10	(0	10	ίð	10	10	10	10	
56ppt	A	10	0J	10	io	10	10	10	10	100
	В	10	lo	10	10	10	10	10	10	
	С	10	01	10	6	10	10	(0	10	
	D	10	(D	10	10	10	ID	10*	10*	* 1 very small B3.
88ppt	А	10	10	(0	10	10	10	10	10	97.5
	В	10	10	10	9	9	9	9	9	
	С	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
120ppt	А	10	10	10	10	10	9	9	9	97.5
	В	10	10	10	10	10	10	10	10	1013
_	С	10	10	10	10	10	10	10	10	
	D	(0	10	10	10	10	10	10	10	
120	А	10	10	10	10	ÍN	9110	10	10	92.5
algae	В	10	10	10	10	10	9	9	8	
	С	(0	10+	10	10	10	10	10	10	+ removed extra org
	D	10	10	10	10	10	10	10	9	
	A									
	В				<u> </u>	<u> </u>		<u> </u>		
	С					<u> </u>				
	D					<u> </u>		1		
	A				<u> </u>					
	В									
	С						<u> </u>	ł	·	
	D						<u> </u>			
	Date:	4/1/20	4/2/20	4/3/20	4/4/20	yelon	4/1/20	4/7/20	419/10	
	Time:	1355	13355	1350	1100	1430	1445		1340	
	Initials:	CP/A	CP	CP	CP	P7	EN	cl	1370 EN	
EN 41	6/20 WP	/								

()EN 4/6/20 WP

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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	Meter #	Remarks
Conc.:	25ppt	{	1			4		0	7	 All	
	Zoppi	<u> </u>								Conc.	
рН		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	35	25	25		1	
Conc.:	56ppt										
рН		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										
рН		8.2	8.2	8.D	8.2	8.)	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		126600	119,700			
Salinity (ppt)		88	89	88	89	89	87	88			
Conc.:	120ppt										
pH		6,8	8.0	7.8	8.0	7.9	7.9	8.0			
D.O. (mg/L)		5.2	5.2	5.3	5.2	49	50	5.0			
Temp. (°C)		20	20	20	20	90	20	20			
Cond. (µS/cm)		135,400	340,100,	136,800		135,200		139,400			
Salinity (ppt)		120	120	121	121		120	121			
	Date:	4/120	4/2/20	4/3/20	4/4/20		4/6/20	4/1/20			
	Time:	1340	1325	1335		1415	1415	1440			
	Initials:	CP	CP	CP	CP	R/	EN	CP			
lote: Hardness,							<u> </u>				

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.

136,100 cf 4/2/20 E

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CHRONIC CHEMICAL DATA (FINAL)

SA NUN 4/20/20

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

	Day 1	Day	Day	Day	Day	Day	Day	Day	Meter #	Remarks
OFeed	╬═╧═	+		<u> 4</u> 	1 5	6			<u> </u>	
Zoppt	$\frac{1}{2}$		+		<u> </u>				All Conc.	* conductivity 15
	┨─────				1.4					
		+		4.7					17	
		19	10	121	20	20			L-36	
56ppt			<u> </u>			ļ				* conductivity
<u> </u>					-	7.7				
	╢────		4.9		4.4	4.4	4.5			
	20	19	10	90	20	20	20			
88ppt	∦	<u> </u>	L				120,800			* conductivity
	8.1	8.1	8.1	80	79	7.8	7.5			
	4.9	5.0	49	4.8	45	4.5	4.5			
	20	19	20	31	20	20	20			
120ppt							131,100			* conductivity
	8.0	8.0	8.0	7.9	7.9	7.8	7.7			
	5.0	5.0	5.0	4.8	4.6	4.5	4.6			<u> </u>
	20	19	20	21		20	20			
20 algae	2						130600			* conductivity
	8.0	8.0	8.1	8.0	7.9	7.9	7.7			- Strauburry
	5.2	5.2	5.1		4.7	4.6	++			
	20	19	20	91		20	20			·····
						T				<u> </u>
					<u> </u>		╞───┤			
					İ — — —					
Date	4/2/20	4/3/20	4/4/20	41tho	NILLAN	4/7/2	4/0/10			
	1415	1405	1120			1600	1240			
		CP	CP	500	EN EN	CP	EN			·······
	120ppt 20 algae Date: Time:	1 25ppt 7.8 4.8 20 56ppt 8.0 4.8 20 88ppt 8.1 4.9 20 88ppt 8.1 4.9 20 120ppt 8.0 5.0 20 120ppt 8.0 5.0 20 20 20 20 20 20 20 20 20 2	1225ppt 7.8 7.7 7.8 7.7 4.8 4.9 20 14 20 14 $56ppt$ 8.0 8.0 $56ppt$ 8.0 8.0 8.0 8.0 8.0 $88ppt$ 20 19 $88ppt$ 20 19 $120ppt$ 8.0 8.0 $120ppt$ 8.0 8.0 20 algae 20 19 20 algae 8.0 8.0 20 algae 20 19 20 algae 10 10 20 algae 10 19 20 algae 10 19 20 algae 4.0 3.0 5.2 5.2 5.2 20 19 20 algae 4.0 10 10 $1120ppt$ 10 $120ppt$ 10 $120ppt$ 10 10 10 20 algae 10	1 2 3 25ppt 7.8 7.7 7.8 7.8 7.7 7.8 4.8 4.9 4.7 20 19 20 56ppt 8.0 8.0 8.0 8.0 8.0 9.0 19 20 88ppt 9.0 4.9 20 19 20 88ppt 5.0 4.9 20 19 20 120ppt 9.0 8.0 8.0 8.0 8.0 9.0 19 20 120ppt 9.0 19 120ppt 9.0 5.0 120ppt 9.0 8.0 8.0 8.0 8.1 120 19 20 20 algae 9.0 9.0 10 19 20 10 19 20 10 19 20 10 19 20 10 19 20 10 10	1 2 3 4 25ppt - - - 7.8 7.7 7.8 $\overline{7.6}$ 4.8 4.9 4.7 $\overline{4.7}$ 20 14 20 \overline{all} 56ppt - - \overline{all} 56ppt 8.0 $\overline{8.0}$ $\overline{8.0}$ $\overline{7.7}$ 4.8 5.0 4.9 $\overline{4.8}$ $\overline{200}$ $\overline{4.9}$ $\overline{4.8}$ 56ppt - 4.8 $\overline{5.0}$ $\overline{4.9}$ $\overline{4.8}$ $\overline{200}$ $\overline{300}$ 88ppt - - $\overline{8.0}$ $\overline{8.1}$ $\overline{8.1}$ $\overline{8.0}$ $\overline{3.1}$ 120ppt - - $\overline{200}$ $\overline{194}$ $\overline{200}$ $\overline{11.8}$ 120ppt - - - $\overline{200}$ $\overline{191}$ $\overline{200}$ $\overline{11.8}$ 20 algae - - - $\overline{5.2}$ $\overline{5.1}$ $\overline{5.3}$ $\overline{5.3}$ 20 algae - - - - $\overline{5.0}$ $\overline{5.0}$ $\overline{5.0}$ $\overline{5.0}$	1 2 3 4 5 25ppt 7.8 7.7 7.8 7.4 7.4 4.8 4.9 4.7 4.7 4.7 4.7 20 14 20 λ 1 20 56ppt 20 14 20 λ 1 20 56ppt 8.0 8.0 7.7 7.7 7.7 4.8 5.0 4.9 4.8 4.4 20 14 20 30 20 56ppt 20 14 20 31 20 4.8 5.0 4.9 4.8 4.4 20 14 10 30 20 88ppt 5.0 4.9 4.8 4.5 20 14 20 31 20 120ppt 20 8.0 8.0 8.0 7.7 7.9 5.0 6.0 5.0 4.8 4.0 20 20 algae 20 19 20 31 20	1 2 3 4 5 6 25ppt 7.8 7.7 7.8 7.4 1.4 7.3 7.8 7.7 7.8 7.4 1.4 7.3 4.8 4.9 4.7 4.7 4.7 4.7 4.7 4.7 4.8 4.9 4.7 4.7 4.7 4.7 4.7 4.7 10 10 10 20 31 20 20 56ppt 8.0 8.0 7.7 7.7 7.7 4.8 5.0 4.9 4.8 4.4 4.4 10 14 2.0 30 20 20 20 88ppt 8.1 8.1 8.1 8.1 8.1 9.0 7.9 7.8 20 19 20 21 20	1 2 3 4 5 6 7 25ppt - - - 46700 7.8 7.7 7.8 7.4 1.4 7.3 7.3 4.8 4.9 4.7 4.7 4.7 4.9 4.3 4.6 20 19 20 3.1 20 20 20 20 56ppt - - - 94600 94600 94600 94600 5.0 8.0 8.0 8.0 7.7 7.7 7.7 7.7 7.5 4.8 5.0 4.9 4.8 4.9 4.7 4.7 4.4 4.5 20 19 10 30 20 70 20 20 88ppt - 8.1 8.1 8.1 8.0 1.9 1.0 1.0 1.0 1.0 1.0 1.0 20 1.0 20 1.0 20 1.0 20 1.0 20 1.0 20 1.0 1.0 1.0 1.0 1.0 1.0	1 2 3 4 5 6 7 8 25ppt	1 2 3 4 5 6 7 80 Number of the second s

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DAILY TOXICITY TEST LOG

22 m 1/20/20

Project Number: Test Species:

17001-474-Exp Artemia franciscana

General					Feeding	Initials/Date
Comments		V				
Test Day 0		K	Min/Max Thermometer # A	N-15		
rest Day 0	Test Solution Mixe Test Organisms A				Fed @ 1355	- 0
		uueu at: I	355			CP
						4/1/20
Test Day 1	Real Time: 20	°C	Min-Max Range: 19 - 23	°C	Fed @ 1355	1
						cl 4/2/20
						4/2/20
Test Day 2	Real Time: 19	°C	Min Moy Dongo,			1/-100
···· /		C	Min-Max Range: 19 - 21	°C	Fed @ (335	CP
						4/3/20
Test Day 3	Real Time: 10	°C	Min-Max Range: 19-22	°C	Fed @ 1055	
						CP
						4/4/20
Test Day 4	Real Time: 30	°C	Min-Max Range: 20-33	°C	Fod @ yy	<u> </u>
			20. s)	Ŭ	Fed @ 1475	Rec
						er 415/20
Toot Dov 5	Deal Time 10					11290
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	
					GIJIS	CP 4/7/20
						4/7/20
Test Day 7	Real Time: 20	°C	Min May Parses 10 ar			
, .		C	Min-Max Range: 19-23	°C	Fed @	EN
					Noney	
						4/8/20
Fest Day 8	Real Time:	°C	Min-Max Range:	°C	Fed @	

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TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

orfarth me Ho

	ľ											-
		Length	Tare	Gross	Net Weight	Adjusted Net Weight Net Weight No of Orig	No of Oria	Mean Wt./ Original Organism	Mean Wt./ Treatment	Number of Surv	Mean Wt./ Surviving	Mean Wt./ Treatment
Treatment	Rep	Units:	Weight (g)	Weight (g)	(g)	(6)	Organisms	(mg)	(Original)	Organisms	(mg)	(Survivina)
	\triangleleft		1.14228	1.14360	0.00132	0.00132	10	0.132		10	0 132	0 1190
25 nnt	8		1.14422	1.14512	06000.0	0.00090	10	060.0		10	0000	
	ပ		1.13775	1.13913	0.00138	0.00138	10	0.138		10	0 138	
			1.14425	1.14541	0.00116	0.00116	10	0.116		10	0.116	
	-		1 13570	1 13710	0.00147	0.00147	4					
	m		1 14257	1 14379	0.00122	0.00147	26	0.14/	0.1312	01	0.147	0.1312
	0		1 12077	1 1 2 1 0 1	0.00 0	0.00	2 4	0.122		2	0.122	
1			1 1021.1		0.00124	0.00124	0	0.124		10	0.124	
	- - >		1.12204	1.12390	0.00132	0.00132	10	0.132		10	0.132	
	-			1								
_4-	ح 1		1.12702	1.12859	0.00157	0.00157	10	0.157	0.1375	10	0.157	0.1413
88 ppt –	n		1.13551	1.13689	0.00138	0.00138	10	0.138		თ	0.153	
	0		1.14664	1.14787	0.00123	0.00123	10	0.123		10	0.123	
			1.14016	1.14148	0.00132	0.00132	10	0.132		10	0.132	
	<											
	< (1.14322	1.1445/	0.00135	0.00135	10	0.135	0.1228	6	0.150	0.1265
120 ppt			1.12961	1.13088	0.00127	0.00127	10	0.127		10	0.127	
1_ :	ာ ၊		1.13120	1.13225	0.00105	0.00105	10	0.105		10	0.105	
			1.13313	1.13437	0.00124	0.00124	10	0.124		10	0.124	
	<		1.13025	1.13168	0.00143	0.00143	10	0.143	0.1100	10	0.143	0 1186
120 ppt			1.14867	1.14960	0.00093	0.00093	10	0.093		ω	0.116	
Algae only	၂ ၂		1.11250	1.11353	0.00103	0.00103	10	0.103		10	0 103	
			1.12886	1.12987	0.00101	0.00101	10	0.101		റ	0 112	
Blank			1 14026	1 14027	10000							

14001-474 Project Number:

Artemia franciscana Species:

0.0000 0.0000 0.0500 0.0500 S 0.0957 1.0000 0.9750 0.9750 1.0000 <u>Mean</u> 0.9250 1.0 0.1 1.0 Max Summary Statistics for Survival Data <u>Min</u> 1.0 0.9 0.8 Z| 4 4 4 4 4 25 ppt 56 ppt 88 ppt 120 ppt Treatment 120 ppt Algae only

<u>C.V.</u> 0.000% 0.000%

5.128% 5.128% 10.351%

<u>C.V.</u> 18.023% 8.651% 10.464% 10.356% 20.382% SD 0.0214 0.0114 0.0144 0.0127 0.0224 0.1190 Mean 0.1375 0.1228 0.1312 0.1100 Summary Statistics for Growth Data (dry wt per original) 0.138 0.147 0.157 0.135 0.143 Max Σ 0.090 0.123 0.105 0.122 0.093 Z 4 4 4 4 4 25 ppt 56 ppt 88 ppt 120 ppt **Treatment** 120 ppt Algae only

) | | 18.023% 8.651% 11.645% 14.583% 0.0214 0.0114 0.0165 0.0184 0.0172 SD Summary Statistics for Growth Data (dry wt per surviving organism) 0.1190 0.1312 0.1413 0.1265 0.1186 Mean 0.138 Max 0.147 0.157 0.150 0.143 <u>Min</u> 0.090 0.122 0.123 0.105 0.103 Z 4 4 4 4 4 88 ppt 25 ppt 56 ppt 120 ppt Treatment 120 ppt Algae only

14.479%

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Els rue



May 6, 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 #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 μ g/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 Chla D. viridis
- Treatment 2: 200 organisms, 100 µg/L Chla D. viridis
- Treatment 3: 300 organisms, 100 µg/L Chla D. viridis
- Treatment 4: 200 organisms, 72.5 μg/L Chla D. viridis and 0.3 ml/ chamber YTC¹
- Treatment 5: 200 organisms, 100 µg/L Chla 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

- Algae/ Food concentration: 72.5 μg/L Chla D. viridis and 0.3 ml/ chamber YTC¹
- 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.	рН	Hard. (mg/L) ^a	Alk. (mg/L) ^a	Spec. Cond. (μS/cm)	TRC (mg/L) ^b	NH₃-N (mg/L)	Salinity (ppt)
RW#13888	8.0	NM	NM	135,800	NM	NM	124

^aAs CaCO3

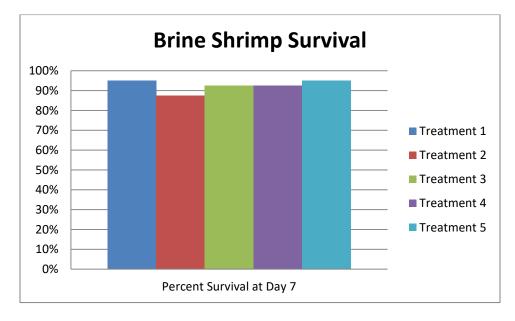
^bTotal residual chlorine

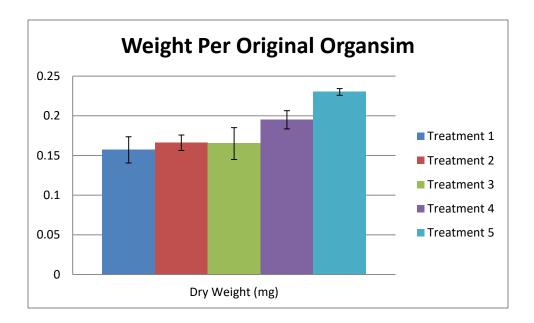
Test activities:

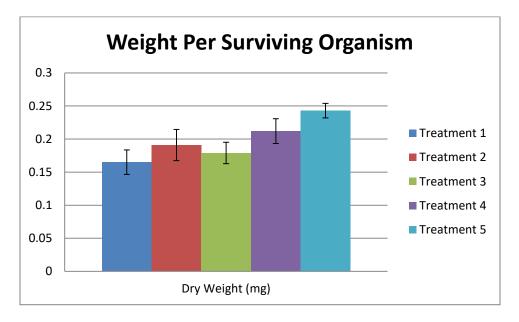
- Biological observations (primarily survival) taken daily.
- Chemistries taken on renewal days (i.e., pH, dissolved oxygen, and temperature).
- Conductivity was measured at test termination or when there was 0% survival in that treatment.
- Dry weights were determined at test termination.

Results:

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







Summary and findings:

- Organism survival was \geq 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.

Mr. Bittner / Dr. Belovsky May 6, 2020 Page 4

- 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,

n Biller

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

14001-474-064

Attachment

cc: David Pillard, TRE

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

Page 1 of _____ QA Form No. 051 Revision 5 Effective 02/14 &A NM S/S/W

TOXICITY DATA PACKAGE COVER SHEET

Test Type:	Chronic	_ Project Number:	17001-474-064
Test Substance:	Pre Test	Species: <u>Artemia franc</u>	iscana
Dilution Water:	rGSL	Organism Lot or eatch Numb	er 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): ENCAN	5
Background Information		. 1	
Type of Test:	Static-Renewal (Daily)	pH control?: <u>Yes</u> If yes, give % CO ₂ :	No NA
Test Temperature:	_20 ± 1 °C	Env. Chmbr/Bath #: <u>25</u>	Test Chmbrs: <u>147-ml cups</u>
Photoperiod:	<u>16 h light : 8 h dark</u>	Light intensity:	<u>50-100 ft-c.</u>
Test Solution Vol.:	50 ml	Replicates per Treatment:	4
Length of Test:	7 days	Organisms per Replicate:	10
Type of Food and Quantity pe	r Chamber: <u>D. viridis/ YTC</u>		1 x daily
Test Substance Characteriza Hardness: <u>Test Initiation</u> pH: <u>Daily</u> Test Concentrations (Volume:	Alkalinity: <u>Test Initiation</u> Conductivity: <u>Daily</u>	uency: NH₃: <u>Test Initiation</u> TRC: <u>Test Init</u>	iation
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 Con	siderations:	Feed 0.5mL D. viridis/ 0.33 ml cerio YTC	
Organisms hatched 2 days pric			
Treatment 1: 100 org			
Treatment 2: 200 org			
Treatment 3: 300 org			
Treatment 4: 200 org fed algae			
Treatment 5: 200 org, 8 hour w Appropriate correction factors I		peratures recorded in this data package	
Study Director Initials:			

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TEST SUBSTANCE USAGE LOG

be new 5/5/20

17001-474-064

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number				
	From:	From:	From:	From:
Test Substance Collection	@	@	@	@
Date and Time	То:	То:	To:	То:
	@	@	@	@
Sample Type (Grab or Comp)				
Date Test Substance Received				
Dilution Water Number	13888/1391			
RW# or TRE#, circle one	150001911	1		
Concurrent Control Water RW#	NA			
	4/2/00 4/26/20			
Date(s) Used	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/	12/10 m	ixed BS						
Initials / Date		23/20 "	L.						······································
Initials / Date	EP 134/2	1/20	n n						
Initials / Date	CP 4/25	120	i n				····		
Initials / Date	A3 4 /20	، مد	(
Initials / Date		7/20 "	~ *					2	
Initials / Date	CP 4/25		ч				•		
Initials / Date									

Ocp 4/24/20 E

Page __ of __ QA Form No. 060 Revision 3 Effective 02/14

Artemia franciscana CHRONIC BIOLOGICAL DATA

@A non 5/5/20

Project Number: ____ 17001-474-064

		<u></u>					•				
	Test	Day	Day		Davi			iving Orga		10	SWUWAL
mg/L	Replicate		1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Rema	arks
100 org	A	10	10	lo	9-	9	9	9-	9-	-1 Small org	95
	В	10	10	10	10	10	10	10	10		
	с	10	10	10	10	10	10	10	10		<u></u> 1
	D	10	10	10	9	9	9	9	9		
200 org	А	10	10	10	10	10	01	9	9		87.5
	В	10	10	(0	10*	9	9	8	8	*1 weak org	
	С	10	10	10	10	10	10	10*	10*	* I weak org	
	D	10	9	8	8	g	8	8	8		
300 org	A	01	10	10	10	9	8	8	8		972.5
	B	10	10	10	10	10	10	10	10		
	C	10	10	(0	10	10	10	10	10		
	D	10	9	9	9	9	9	9	9		
Algae/	A	10	10	10*	10	10	9	9	9	*I weak org	92.5
YTC	В	10	10	10	9	q	8	8	8		
	С	10	10	10	10	10	10	10	10		
	D	10	10	10	10	10	10	10	10		
8h	А	10	10	10	10*	9	9	9	9	* 1 weak org	95
	В	10	10	رى	10	10	10	10	10		
	С	10	10	10	10	10	10	10	10		
	D	10	10	10	9	9	Q	9	9		
	А										
	В										
	с										
	D										
	Α							<u> </u>			
	В					······································					
	С						<u> </u>	1			
	D						1				
	Date:	4/22/20	4/23/20	4/24/20	4/25/20	466/20	4/27/20	4/20/20	4/29/20		
····.	Time:	144D	0425	1200	1115	1040	1655	1750	1435		
	Initials:	ENCP	An	AS	CP	An	EN	cr	СР		

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CHRONIC CHEMICAL DATA (INITIAL)

OA wer 5/2/20

Project Number:

17001-474-064

Test Species: Artemia franciscana

%		Day 0	Day	Day	Day	Day	Day	Day	Day	Meter #	Remarks
	<u> </u>		1	2	3	4	5	6	7		
Conc.:	rGSL									All Conc.	
рН		7.9	8.0	8.0	8.0	ר.ר	7.9	7.9		FM30	
D.O. (mg/L)		5.2	5.1	5-1	5.2	5.2	51	5.D		17	
Temp. (°C)		20	20	20	20	20	20	20		L14	
Cond. (µS/cm)		135800	152300	13540	140,400	112000	307400	127,600		15	
Salinity (ppt)		124								1	
Conc.:											
рН											
D.O. (mg/L)											
Temp. (°C)											
Cond. (µS/cm)											
Salinity (ppt)											
Conc.:											
pН									<u>-</u> -1		
D.O. (mg/L)											
Temp. (°C)											
Cond. (µS/cm)											
Salinity (ppt)											
Conc.:		I									
рН				1 1 1 2 1							
D.O. (mg/L)											
Temp. (°C)											
Cond. (µS/cm)											
Salinity (ppt)											
	Date:	4/22/22	4/23/20	4/24/20	4/25/20	4/20/20	4/27/20	4/28/20			
			0105	1140	1110	1020	1045	1740			
Note: Hardness	Initials:	EN	As	B	ср	M	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.

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CHRONIC CHEMICAL DATA (FINAL)

Q1 ~ slstu

<u> </u>					CHRUI			DATA (F	INAL)	-	
Project Numb			1-474-0								
Test Species:		Artemi	a franci	scana							
%		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
рH		8.0	8.0	7.8	7.4	7.7	7.7	7.9		pmp30	
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	23*	22	21		136	
Conc.:	200 org							131,200			* conductivity
pH		8.0	\$,D	7.8	7.9	7.7	7.7	7.8			
D.O. (mg/L)		5.0	નંતુ	4.7	5.0	4.4	4.7	4.9			
Temp (°C)	,	20	21	21	20	244	22	21			
Conc.:	300 org							130,800			* conductivity
pН		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	240	22	21			
Conc.:	Algae/	YTC						130,500			* conductivity
pН		\$,0	8.0	7.9	7.9	77	7.7	7.8			
D.O. (mg/L)		4.9	5.0	4.8	5.0	44	4.5	4.8			
Temp (°C)	_	20	21	21	20	240	22	21			
Conc.:	8h							1829,800			* conductivity
pН		8.0	8.0	7.8	7.8	7.7	7.7	7.8			
D.O. (mg/L)		4.5	5.0	4.7	4.9	4.2	4.5	4.8			NA
Temp (°C)		20	21	21	20	244	22	21			
Conc.:											
pH											
D.O. (mg/L)											
Temp (°C)											······································
Conc.:											and the second second second second second second second second second second second second second second second
рН									······································	-	7 .
D.O. (mg/L)											- 100 - 100
Temp (°C)											
	Date:	4 23/20	4/24/20	4/25/20	4/20/20	4/27/20	4/28/20	4/29/20			
	Time:	09.50	1210	1135	ioner	1650	1805	14525			
	Initials:	AS	AI	ĊP	13	Ę	CP	CP CP			
nh uladani						<u> </u>					

(1) | 23 | 20 €
(2) | 2 | 20 €

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DAILY TOXICITY TEST LOG

El va 5/2/20

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

General Comments					Feeding	Initials/Date
	Random Chart:)	Min/Max Thermometer # 🏠	115		
Test Day 0	Test Solution Mixe Test Organisms Ac	d at: 142	5		Fed @ 1435	EN
						4/22/20
Test Day 1	Real Time: 22	°C	Min-Max Range: אירק - כק	°C	Fed @ Ofio	83 41/23/2e
Test Day 2	Real Time: 21	°C	Min-Max Range:	°C	Fed @ 1150	
Test Day 3	Real Time: 🤈	°C	Min-Max Range: 2(- 23	°C	Fed @ 1110	CP 4/25/20
Test Day 4	Real Time: 21	°C	Min-Max Range: ឧ៶-२٦	°C	Fed @ ۱۵3 ن	A7 4/26/2c
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	ср 4/28/20
Test Day 7	Real Time: 22	°C	Min-Max Range: 22 - 23	°C	Fed @ NoNE	сР 4/29/20
Test Day 8	Real Time:	°C	Min-Max Range:	°C	Fed @	

Ocp 4/24/20 E

	I		١									Page of QA Form No.010 Revision 6 Effective 02/14	of No.010 6 02/14
						UKGANISI	M LENGTHS	s, weight	I ES I UKGANISM LENGTHS, WEIGHTS, AND LOADING	DING	C	ch un	or/2/5
Projec	Project Number:	1001	17001-474-064	064	Test Substance:	ance: Pre	Test			Comments:			
Species:		tia f	Artenia Franciscana	na	Analyst Tare:	20	Analyst Gross:	iross: SK		Analytical Balance ID: $Sart #$ Dried in Oven # 3 from Date: 4	nce ID: Sar 3 from D:	-t ≠ 1 ate: 4/16/1∧Tir	ne. 1525
Date/	Date/Time of Tare Wt.:	1	2/29/20	-1300	Date/Time	Date/Time of Gross Wt.:	5	\$ 1240			2 2 2	to Date: 5/4/20 Time: 1040	me: 1040
Boat	Treatment Rep.	t Rep.	Length	Weight Type (Circle):		Wet Blot Dry	Dry Dry (>100°C)		AFDW (>500°C)		Lot or Batch)Number:		070200
		·····	Single Contract of the second s	Tare Weight (g)	Tare Gross Weight (g) 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	Mean Wt. per Treatment (mg) (Surviving)
	100 org	A		1.13713	137131.13846	0.00133					6	(bu)	
		æ		i.13097	1.13245	0.0					0		
		3		014710	1.1471011.148760.00166	0.00166					0		
		۵		1,13980	1.139801. 14148 6.00168	6.00168					6		
	200 013	A		1,1437	1.14536	.145360.00159					σ		
		ø		1,12644	1.12-796 0.00152	0.00152					. ~		
		ა		1.12081	1.12246	.122460.0016S					01		
		٥		1,11707	1.11877 0.00175	0.00175					~		
	300 003	∢		1,12409	1.125430.00	0.00134					8		
		æ		1.1442411	145860.00162	20100					10		
		ა		1120711	12074 1.12251 0.00174	0.00174					10		
		۵		1.135311.13710 0.00	13710	0.00179					6		
Blank				1.179401	1.12937	-0.00003							
Range					,								
Mean													
Test Solu	Test Solution Volume:						Loading Rate:						
' Add in v	Add in weight loss of blank boat, if appropriate	f blank	boat, if app	ropriate.					· •				

÷.

	·			•	TEST ORG	ORGANISA	A LENGTHS	, WEIGHT	ANISM LENGTHS, WEIGHTS, AND LOADING	DING		Page of QA Form No.010 Revision 6 Effective 02/14	of No.010 6 02/14 s/()
Project	Project Number: 17001 - 474 - 06	17001 ma	- 474 -	- 064	Test Substance:	N Q	re Test	5		Comments: Analytical Balance ID: Satt $\# t$	nce ID: Sar	r # -	
Date/Ti	Date/Time of Tare Wt.:	Vt.: 4	1/20/2	4/29/20 × 300	Anaryst Lare: D Date/Time of Gro	ss /	JHAnalyst Gross: 0k 5/4/po @ 1240	3 1240		Dried in Oven #		from Date: <u>412</u> 11र्मा: ¹⁵²⁵ to Date: <u>इम्रिफ</u> Time: <u>154</u> 2	me: <u>/525</u> ime: <u>1040</u>
Boat	Treatment Rep.	Rep.	Length	Weight Type (Circle):		Wet Blot Dry	Dry Dry (>100°C)	00°C) AFC	AFDW (>500°C)		Lot of Batch Number:		Outrow
					Tare Gross Weight (g) 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	Mean Wt. per Treatment (mg) (Surviving)
	Alg / YTC	A		1.12958	1.13163	0.00205					6	(Biii)	
		B		1,13241	1,13241 1.13418	0.0					· ∞		
		ა		1,14637	146371.14829	0. 00192					q		
		Δ		1,13747	13747 1.13941 0.00194	0.00194					2 0		
	846	A .		1.12957	13183	0.00ZZb					6		
		Ю		11,14756	14987	0.00232					Oj		
		ა		1,17762	1.12991	12991 0.00229					0.		
		۵		112731	1.12953 0.00222	0.00222					0		
													· .
												*	
Blank													
Range													
Mean								<u>```</u>					
Test Solu	Test Solution Volume:						Loading Rate:						
Add in w	Add in weight loss of blank boat, if appropriate.	blank t	oat, if ap	propriate.					· · .				

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TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

alsls un vo

Project Number:	mber:		14001-4/4			-popodo						
		Length	Tare	Gross	Net Weight	Adjusted Net Weight Net Weight		Mean Wt./ Original Organism	Mean Wt./ Treatment (mg)	Number of Surv.	Mean Wt./ Surviving Organism	Mean Wt./ Treatment (mg)
Treatment	Rep	Units:	Weight (g)	Weight (g)	(g)	(g)	Organisms	(mg)	(Original)	Organisms	(mg)	(Surviving)
	A		1.13713	1.13846	0.00133	0.00136	10	0.136	0.1568		0.151	0.1653
	В		1.13097	1.13245	0.00148	0.00151	10	0.151		10	0.151	
	υ		1.14710	1.14876	0.00166	0.00169	10	0.169		10	0.169	
	Ω		1.13980	1.14148	0.00168	0.00171	10	0.171		6	0.190	
	A		1.14377	1.14536	0.00159	0.00162	10	0.162	0.1657	ი	0.180	0.1911
	В		1.12644	1.12796	0.00152	0.00155	10	0.155		8	0.194	
	с С		1.12081	1.12246	0.00165	0.00168	10	0.168		10	0.168	
	۵		1.11702	1.11877	0.00175	0.00178	10	0.178		8	0.223	
								-				
	۲		1.12409	1.12543	0.00134	0.00137	10	0.137	0.1652	ø	0.171	0.1789
300 Ora	В		1.14424	1.14586	0.00162	0.00165	10	0.165		10	0.165	
50000	ပ		1.12077	1.12251	0.00174	0.00177	10	0.177		10	0.177	
	Ω		1.13531	1.13710	0.00179	0.00182	10	0.182		6	0.202	
	A		1.12958	1.13163	0.00205	0.00208	10	0.208	0.1950	6	0.231	0.2120
	В		1.13241	1.13418	0.00177	0.00180	10	0.180		8	0.225	
	ပ		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	
	A		1.12957	1.13183	0.00226	0.00229	10	0.229	0.2302	6	0.254	0.2429
n n n n n n n n n n n n n n n n n n n	В		1.14755	1.14987	0.00232	0.00235	10	0.235		10	0.235	
	ပ		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		6	0.250	
Blank			1 12940	1.12937	-0.00003							

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

QA New 5/6/10

Species: Artemia franciscana

,

14001-474

Project Number:

	<u>C</u>	6.077%	10.942%	10.351%	10.351%	6.077%
	<u>S</u>	0.0577	0.0957	0.0957	0.0957	0.0577
	<u>Mean</u>	0.9500	0.8750	0.9250	0.9250	0.9500
	2		1.0			
ırvival Data	<u>Min</u>	0.0	0.8	0.8	0.8	0.0
stics for Su	Z	4	4	4	4	4
Summary Statis	<u>Treatment</u> <u>N</u> <u>Min</u>	100 org	200 org	300 org	YTC/Algae	8 hour

	<u>, <</u>	10.526%	5.878%	12.187%	5.907%	1.855%
	S	0.0165	0.0097	0.0201	0.0115	0.0043
· original)	Mean	0.1568	0.1657	0.1652	0.1950	0.2302
(dry wt per	Max	0.171	0.178	0.182	0.208	0.235
Srowth Data	<u>N Min Max</u>	0.136	0.155	0.137	0.180	0.225
stics for G	Z	4	4	4	4	4
Summary Statistics	Treatment	100 org	200 org	300 org	YTC/Algae	8 hour

rganism)	<u>SD</u>
surviving or	Mean
(dry wt per	Max
rowth Data	Min
tics for G	ZI
Summary Statist	Treatment

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



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 Chla 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 Chla. This absorbance converts to a cell count of 76.6 x 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 <u>bidlackac.tre@gmail.com</u>

17001-474-062

Attachment

R. P. P.

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

cc: Rami B. Naddy, TRE



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 μ g/L Chla *Dunaliellia viridis*, whereas data in the second table represent those studies fed 72.5 μ g/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

Mr. Bittner / Dr. Belovsky April 20, 2020 Page 3

Sincerely,

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

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

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



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

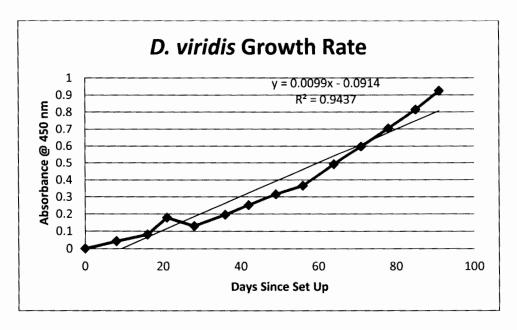
Subject: Algae Concentration

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,

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

17001-474-081

Attachment cc: Rami B. Naddy, TRE

Dil A. Pilland

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

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.

------ Forwarded message ------From: **Amanda Bidlack** <<u>bidlackac.tre@gmail.com</u>> Date: Mon, Jan 20, 2020 at 10:56 AM Subject: Fwd: D. viridis culture To: Rami Naddy <<u>naddyrb.tre@gmail.com</u>>

------ Forwarded message ------From: **Shannon Jones** <<u>sjones32@nd.edu</u>> Date: Tue, Dec 3, 2019 at 1:17 PM Subject: Re: D. viridis culture To: Amanda Bidlack <<u>bidlackac.tre@gmail.com</u>>

Hi Amanda,

We normally culture at 120 ppt. I'm looking into why that jug was at a lower salinity -I'd been relying heavily on undergrads while I worked on a massive experiment for the last month, and one of them may have adjusted the salinity in error.

D. viridis grows very slowly. I set up a 1 gallon jug generally two to three months before I want to use it (generally I set it up at 1-2ug/l chl a, and after 2 months it's around 200-250 ug/l chl a). It doesn't grow fast.

Sorry that that may bnot be the answer you're looking for, but feel free to ask me whatever questions you have.

Shannon

On Sun, Dec 1, 2019 at 9:51 PM Amanda Bidlack <<u>bidlackac.tre@gmail.com</u>> wrote: Hi,

I had a couple follow up questions. The jugs we recieved from you measured at 40ppt, is that where you normally culture? Also, how much do you expect the algae to grow in a week?

Thanks

On Tue, Nov 19, 2019 at 1:30 PM Amanda Bidlack <<u>bidlackac.tre@gmail.com</u>> wrote: Thanks for the help!

• What temperature are you keeping your cultures at?

We are culturing at about 10C. They are in a walk in cooler and the temperature is pretty stable between about 9 and 12 degrees.

• How much are you aerating them (ex, 1 bubble every second, constant stream, etc.)

They are aerating at a constant stream.

• When you feed, what are you feeding and how much?

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.

• What are you using for your media?

The media is 120 ppt brine made with moton's water softener salt and instant ocean.

• What size containers are you using to enclose your cultures? Are they relatively closed systems?

Most of the cultures are in glass 1 gallon jugs. The smaller volumes may be in flasks, we only have one of those going at the moment. The jugs have a lid which the airline passes through, and there is a small hole in the lid to allow air to escape, but it is pretty closed. I have attached a picture of the current set up.

• When you receive a shipment of D. viridis, how are you treating it? Are you decanting and getting it bubbling immediately?

Yes, when we receive them, we get them bubbling right away. Depending on volume, it would either go into a flask or a jug. Typically under a liter goes into a flask and over a liter goes into a jug.

• Do you ever start new cultures, transfer cultures, or split old cultures? Or are you just trying to progressively grow what you have?

Whenever the volume on a culture in a jug starts to get near the capacity (probably when it is about 3L), I split it off into two containers. If a container seems to be developing a build up on the sides, I transfer the culture into a new container. In general, we are just growing progressively.

• How are you determining how much to dilute the culture you use before feeding it to the animals? If you have 12 L but it's above 40ug/L chlorophyll, you should probably be diluting it.

Prior to when I took over the culturing, a series of chlorophyll extractions vs absorbance levels was measured to establish the relationship. Now, when we are feeding the animals, I measure the absorbance and plug it into that formula and it tells me how much to add to get the desired test concentration. For the 150ml containers, this has typically worked out to be around 7ml of algae per testing vessel per day.

Thank you for sending the 4L, that will be extremely helpful. I'll get it aerating when we get it.

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If you'd rather, we can discuss this on the phone. Shannon

On Fri, Nov 15, 2019 at 7:24 PM Amanda Bidlack <<u>bidlackac.tre@gmail.com</u>> wrote: Thanks.

We are trying to generate as much as possible right now. Currently we have about 12L and we will burn through that pretty quickly with the brine shrimp tests, so we are trying to stay ahead.

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

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Thanks, Amanda

Amanda Bidlack Quality Assurance Manager/ Project Specialist TRE Environmental Strategies, LLC T <u>970.416.0916</u> F 970.490.296 <u>bidlackac.tre@gmail.com</u>

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Yeast (Fleishmans)

Shannon Jones <sjones32@nd.edu>

Thu, Feb 20, 3:23 PM

to Rami, me

Hi Rami and Chris,

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!

Shannon

Brine shrimp should be fed a dissolved yeast solution once every two days.

- 1. Fill a 1L beaker with 500 ml RO water
- 2. Add yeast Stock solution = 1 g yeast in 500 ml RO

3. Add a stir bar to the beaker, and place it on a stir plate for at least 10 minutes, or until the yeast is thoroughly dissolved.

4. Add variable volumes of this mixture to 400 ml brine solution in each bottle:

Volume added to each bottle & mass equivalent

7.5 ml	=	10 mg
3.75 ml	=	7.5 mg
2.5 ml	=	5 mg
1.25 ml	=	2.5 mg
0.5 ml	=	1 mg
0.01 ml	=	20 ug

--

Shannon Jones

Belovsky Lab Research Technician

Lab phone: 574-631-0949

096/098 Galvin Life Science Center

University of Notre Dame

Notre Dame, IN 46556

Algae Culturing

Rami Naddy <naddyrb.tre@gmail.com>

Mon, Jan 20, 11:13 AM

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

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Hi Amanda,

We normally culture at 120 ppt. I'm looking into why that jug was at a lower salinity - I'd been relying heavily on undergrads while I worked on a massive experiment for the last month, and one of them may have adjusted the salinity in error.

D. viridis grows very slowly. I set up a 1 gallon jug generally two to three months before I want to use it (generally I set it up at 1-2ug/l chl a, and after 2 months it's around 200-250 ug/l chl a). It doesn't grow fast.

Sorry that that may bnot be the answer you're looking for, but feel free to ask me whatever questions you have.

Shannon

On Sun, Dec 1, 2019 at 9:51 PM Amanda Bidlack <<u>bidlackac.tre@gmail.com</u>> wrote:

Hi,

I had a couple follow up questions. The jugs we recieved from you measured at 40ppt, is that where you normally culture? Also, how much do you expect the algae to grow in a week?

Thanks

On Tue, Nov 19, 2019 at 1:30 PM Amanda Bidlack <<u>bidlackac.tre@gmail.com</u>> wrote:

Thanks for the help!

• What temperature are you keeping your cultures at?

We are culturing at about 10C. They are in a walk in cooler and the temperature is pretty stable between about 9 and 12 degrees.

• How much are you aerating them (ex, 1 bubble every second, constant stream, etc.)

They are aerating at a constant stream.

• When you feed, what are you feeding and how much?

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.

• What are you using for your media?

The media is 120 ppt brine made with moton's water softener salt and instant ocean.

• What size containers are you using to enclose your cultures? Are they relatively closed systems?

Most of the cultures are in glass 1 gallon jugs. The smaller volumes may be in flasks, we only have one of those going at the moment. The jugs have a lid which the airline passes through, and there is a small hole in the lid to allow air to escape, but it is pretty closed. I have attached a picture of the current set up.

• When you receive a shipment of D. viridis, how are you treating it? Are you decanting and getting it bubbling immediately?

Yes, when we receive them, we get them bubbling right away. Depending on volume, it would either go into a flask or a jug. Typically under a liter goes into a flask and over a liter goes into a jug.

• Do you ever start new cultures, transfer cultures, or split old cultures? Or are you just trying to progressively grow what you have?

Whenever the volume on a culture in a jug starts to get near the capacity (probably when it is about 3L), I split it off into two containers. If a container seems to be developing a build up on the sides, I transfer the culture into a new container. In general, we are just growing progressively.

• How are you determining how much to dilute the culture you use before feeding it to the animals? If you have 12 L but it's above 40ug/L chlorophyll, you should probably be diluting it.

Prior to when I took over the culturing, a series of chlorophyll extractions vs absorbance levels was measured to establish the relationship. Now, when we are feeding the animals, I measure the absorbance and plug it into that formula and it tells me how much to add to get the desired test concentration. For the 150ml containers, this has typically worked out to be around 7ml of algae per testing vessel per day.

Thank you for sending the 4L, that will be extremely helpful. I'll get it aerating when we get it.

On Tue, Nov 19, 2019 at 12:37 PM Shannon Jones <<u>sjones32@nd.edu</u>> wrote:

Hi again,

How are you determining how much to dilute the culture you use before feeding it to the animals? If you have 12 L but it's above 40ug/L chlorophyll, you should probably be diluting it.

Shannon

On Tue, Nov 19, 2019 at 9:45 AM Shannon Jones <<u>sjones32@nd.edu</u>> wrote:

Hi Amanda,

First of all, I'm going to overnight you ~4L of D. viridis today. It should arrive tomorrow, I'll give you the tracking number once it's ready to go out.

Gary and I spoke yesterday, and I have some questions for you.

- What temperature are you keeping your cultures at?
- How much are you aerating them (ex, 1 bubble every second, constant stream, etc.)
- When you feed, what are you feeding and how much?
- What are you using for your media?
- What size containers are you using to enclose your cultures? Are they relatively closed systems?
- When you receive a shipment of D. viridis, how are you treating it? Are you decanting and getting it bubbling immediately?
- Do you ever start new cultures, transfer cultures, or split old cultures? Or are you just trying to progressively grow what you have?

If you'd rather, we can discuss this on the phone. Shannon

On Fri, Nov 15, 2019 at 7:24 PM Amanda Bidlack <<u>bidlackac.tre@gmail.com</u>> wrote:

Thanks.

We are trying to generate as much as possible right now. Currently we have about 12L and we will burn through that pretty quickly with the brine shrimp tests, so we are trying to stay ahead.

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

On Fri, Nov 15, 2019 at 7:11 AM Shannon Jones <<u>sjones32@nd.edu</u>> wrote:

Hi,

Slow growth is fairly standard here as well. What volume are you trying to get to, and what rate are you using the culture at?

On Thu, Nov 14, 2019 at 5:22 PM Amanda Bidlack < <u>bidlackac.tre@gmail.com</u>> wrote:

Hello,

I am working on the D. viridis cultures for TRE. Our cultures have been maintaining well, but we are seeing very slow grow, so Rami suggested I reach out to you and see if you had any techniques to speed up the process. We were considering aeration with CO2 as a possibility.

Thanks,

Amanda

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Amanda Bidlack

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Yeast (Fleishmans)

Shannon Jones <sjones32@nd.edu>

Thu, Feb 20, 3:23 PM

to Rami, me

Hi Rami and Chris,

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!

Shannon

Brine shrimp should be fed a dissolved yeast solution once every two days.

1. Fill a 1L beaker with 500 ml RO water

2. Add yeast - Stock solution = 1 g yeast in 500 ml RO

3. Add a stir bar to the beaker, and place it on a stir plate for at least 10 minutes, or until the yeast is thoroughly dissolved.

4. Add variable volumes of this mixture to 400 ml brine solution in each bottle:

Volume added to each bottle & mass equivalent

7.5 ml = 10 mg 3.75 ml 7.5 mg = 2.5 ml = 5 mg 1.25 ml = 2.5 mg 0.5 ml = 1 mg 0.01 ml = 20 ug

Shannon Jones

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