

April 24, 2020

Mr. Christopher Bittner
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Dr. Gary Belovsky
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Subject: Results of Analytical Data for Experiment #10

Mr. Bittner/ Dr. Belovsky:

Below is a summary of the analytical data for the short-term chronic brine shrimp experiment initiated on March 25, 2020. Total arsenic samples were collected in new solutions at test initiation and on day 6. Total arsenic samples were also collected in old solutions at test termination or when there was complete mortality in a treatment.

Characterization of Recon Water

Sample No.	pH	Hard. (mg/L) ^a	Alk. (mg/L) ^a	Spec. Cond. (µS/cm)	TRC (mg/L) ^b	NH ₃ -N (mg/L)	Salinity (ppt)
RW#13876	7.9	NM	NM	141,000	NM	NM	120

^aAs CaCO₃

^bTotal residual chlorine

Results of Arsenic Analysis

Nominal Value (mg/L)	Total Arsenic (mg/L)			Mean Value	Percent of Nominal
	Day 0 New Solution	Day 6 New Solution	Old Solution (Day Measured)		
0	0.11 U	0.11 U	0.11 U (7)	0.11 U	---
5	4.15	4.22	4.29 (7)	4.22	84%
20	17.6	15.1	15.3 (7)	16.0	80%
50	42.2	41.1	41.6 (7)	41.6	83%
100	83.1	--	79.4 (3)	81.2	81%
200	164	--	165 (1)	164	82%

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

Average measured arsenic concentrations are then used to recalculate test endpoints on a measured basis.

Test Endpoints

Basis	Survival NOEC	Survival IC20	Growth NOEC	Growth IC20
Nominal	20	31.0 (29.6-38.7)	20	24.0 (2.48-30.1)
Measured	16.0	25.4 (23.0-29.2)	16.0	19.4 (6.17-24.7)

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
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14001-474-058

Attachment

cc: David Pillard, TRE



Rami B. Naddy, Ph.D.
Manager / Environmental Toxicologist
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CETIS Analytical Report

Report Date: 24 Apr-20 16:01 (p 1 of 2)
 Test Code: 474-exp10 | 09-3915-2779

Mysidopsis 96-h Acute Survival Test

TRE Environmental Strategies

Analysis ID: 19-8914-7364	Endpoint: 4d Survival Rate	CETIS Version: CETISv1.8.7
Analyzed: 24 Apr-20 16:01	Analysis: Untrimmed Spearman-Kärber	Official Results: Yes
Batch ID: 13-5729-9412	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 25 Mar-20 15:00	Protocol: EPA/821/R-02-013 (2002)	Diluent: rGSL
Ending Date: 01 Apr-20 15:25	Species: Artemia franciscana	Brine: Crystal Sea
Duration: 7d 0h	Source: In-House Culture	Age: 48h
Sample ID: 04-7522-1036	Code: 1C534C2C	Client: Notre Dame
Sample Date: 25 Mar-20 15:00	Material: Arsenic	Project: Special Studies
Receive Date: 25 Mar-20 15:00	Source: Discharge Monitoring Report	
Sample Age: NA	Station: Effluent	

Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	0.00%	1.601	0.02626	39.95	35.4	45.08

4d Survival Rate Summary

C-mg/L	Control Type	Count	Mean	Min	Max	Calculated Variate(A/B)					
						Std Err	Std Dev	CV%	%Effect	A	B
0	Dilution Water	4	1	1	1	0	0	0.0%	0.0%	40	40
4.15		4	1	1	1	0	0	0.0%	0.0%	40	40
17.6		4	1	1	1	0	0	0.0%	0.0%	40	40
42.2		4	0.5	0.3	0.7	0.09129	0.1826	36.5%	50.0%	20	40
81.25		4	0	0	0	0	0		100.0%	0	40
164.5		4	0	0	0	0	0		100.0%	0	40

4d Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
4.15		1	1	1	1
17.6		1	1	1	1
42.2		0.3	0.6	0.7	0.4
81.25		0	0	0	0
164.5		0	0	0	0

4d 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
4.15		10/10	10/10	10/10	10/10
17.6		10/10	10/10	10/10	10/10
42.2		3/10	6/10	7/10	4/10
81.25		0/10	0/10	0/10	0/10
164.5		0/10	0/10	0/10	0/10

CETIS Analytical Report

Report Date: 21 Apr-20 10:16 (p 1 of 2)

Test Code: 474-exp10 | 09-3915-2779

Bare shrimp (1)

Fathead Minnow 7-d Larval Survival and Growth Test

TRE Environmental Strategies

Analysis ID: 19-0805-9569	Endpoint: 7d Survival Rate	CETIS Version: CETISv1.8.7
Analyzed: 21 Apr-20 10:16	Analysis: Parametric-Control vs Treatments	Official Results: Yes
Batch ID: 13-5729-9412	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 25 Mar-20 15:00	Protocol: EPA/821/R-02-013 (2002)	Diluent: rGSL
Ending Date: 01 Apr-20 15:25	Species: Artemia franciscana	Brine: Crystal Sea
Duration: 7d 0h	Source: In-House Culture	Age: 48h
Sample ID: 04-7522-1036	Code: 1C534C2C	Client: Notre Dame
Sample Date: 25 Mar-20 15:00	Material: Arsenic	Project: Special Studies
Receive Date: 25 Mar-20 15:00	Source: Discharge Monitoring Report	
Sample Age: NA	Station: Effluent	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	7.88%	16	41.63	25.81	

Dunnett Multiple Comparison Test

Control	vs C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water	4.22	-0.7441	2.29	0.125	6	0.9321	CDF	Non-Significant Effect
	16	-0.7441	2.29	0.125	6	0.9321	CDF	Non-Significant Effect
	41.63*	11.64	2.29	0.125	6	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.329014	0.4430048	3	73.89	<0.0001	Significant Effect
Error	0.0719444	0.005995367	12			
Total	1.400959		15			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	3.64	5.95	0.0448	Equal Variances
Variances	Levene Equality of Variance	7.667	5.95	0.0040	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8588	0.841	0.0184	Normal Distribution

7d Survival Rate Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	0.975	0.8954	1	1	0.9	1	0.025	5.13%	0.0%
4.22		4	1	1	1	1	1	1	0	0.0%	-2.56%
16		4	1	1	1	1	1	1	0	0.0%	-2.56%
41.63		4	0.45	0.2446	0.6554	0.45	0.3	0.6	0.06455	28.7%	53.8%
81.25		4	0	0	0	0	0	0	0		100.0%
164.5		4	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.371	1.242	1.501	1.412	1.249	1.412	0.04074	5.94%	0.0%
4.22		4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	-2.97%
16		4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	-2.97%
41.63		4	0.734	0.5244	0.9435	0.7351	0.5796	0.8861	0.06584	17.9%	46.5%
81.25		4	0.1588	0.1588	0.1588	0.1588	0.1588	0.1588	0	0.0%	88.4%
164.5		4	0.1588	0.1588	0.1588	0.1588	0.1588	0.1588	0	0.0%	88.4%

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CETIS Analytical Report

Report Date: 21 Apr-20 10:16 (p 2 of 2)

Test Code: 474-exp10 | 09-3915-2779

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Fathead Minnow 7-d Larval Survival and Growth Test

TRE Environmental Strategies

Analysis ID: 19-0805-9569
Analyzed: 21 Apr-20 10:16

Endpoint: 7d Survival Rate
Analysis: Parametric-Control vs Treatments

CETIS Version: CETISv1.8.7
Official Results: Yes

7d Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	0.9
4.22		1	1	1	1
16		1	1	1	1
41.63		0.3	0.5	0.6	0.4
81.25		0	0	0	0
164.5		0	0	0	0

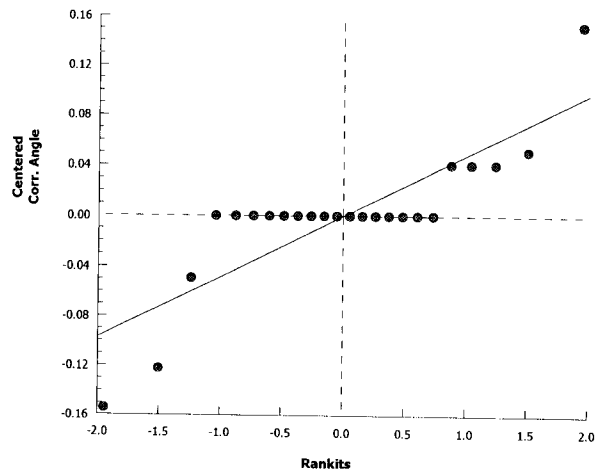
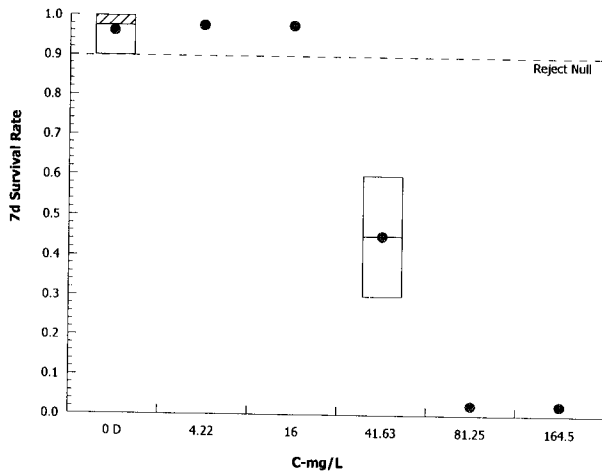
Angular (Corrected) Transformed Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.412	1.412	1.412	1.249
4.22		1.412	1.412	1.412	1.412
16		1.412	1.412	1.412	1.412
41.63		0.5796	0.7854	0.8861	0.6847
81.25		0.1588	0.1588	0.1588	0.1588
164.5		0.1588	0.1588	0.1588	0.1588

7d Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	10/10	10/10	10/10	9/10
4.22		10/10	10/10	10/10	10/10
16		10/10	10/10	10/10	10/10
41.63		3/10	5/10	6/10	4/10
81.25		0/10	0/10	0/10	0/10
164.5		0/10	0/10	0/10	0/10

Graphics



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CETIS Analytical Report

Report Date: 21 Apr-20 09:31 (p 1 of 2)

Test Code: 474-exp10 | 09-3915-2779

BRW shrimp

Flathead Mitten 7-d Larval Survival and Growth Test

TRE Environmental Strategies

Analysis ID: 04-9609-3362	Endpoint: 7d Survival Rate	CETIS Version: CETISv1.8.7
Analyzed: 21 Apr-20 9:31	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 13-5729-9412	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 25 Mar-20 15:00	Protocol: EPA/821/R-02-013 (2002)	Diluent: rGSL
Ending Date: 01 Apr-20 15:25	Species: Artemia franciscana	Brine: Crystal Sea
Duration: 7d 0h	Source: In-House Culture	Age: 48h
Sample ID: 04-7522-1036	Code: 1C534C2C	Client: Notre Dame
Sample Date: 25 Mar-20 15:00	Material: Arsenic	Project: Special Studies
Receive Date: 25 Mar-20 15:00	Source: Discharge Monitoring Report	
Sample Age: NA	Station: Effluent	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1152224	200	Yes	Two-Point Interpolation

Point Estimates

Level	mg/L	95% LCL	95% UCL
LC5	18.35	17.76	19.3
LC10	20.69	19.52	22.59
LC15	23.04	21.28	25.89
LC20	25.38	23.04	29.18
LC25	27.73	24.81	32.48
LC40	34.77	30.09	42.37
LC50	39.46	33.61	50.13

7d Survival Rate Summary

Calculated Variate(A/B)

C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Dilution Water	4	0.975	0.9	1	0.025	0.05	5.13%	0.0%	39	40
4.22		4	1	1	1	0	0	0.0%	-2.56%	40	40
16		4	1	1	1	0	0	0.0%	-2.56%	40	40
41.63		4	0.45	0.3	0.6	0.06455	0.1291	28.7%	53.8%	18	40
81.25		4	0	0	0	0	0		100.0%	0	40
164.5		4	0	0	0	0	0		100.0%	0	40

7d Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	0.9
4.22		1	1	1	1
16		1	1	1	1
41.63		0.3	0.5	0.6	0.4
81.25		0	0	0	0
164.5		0	0	0	0

7d 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	9/10
4.22		10/10	10/10	10/10	10/10
16		10/10	10/10	10/10	10/10
41.63		3/10	5/10	6/10	4/10
81.25		0/10	0/10	0/10	0/10
164.5		0/10	0/10	0/10	0/10

BRW 4/22/20 CF

CETIS Analytical Report

DBW

Report Date: 21 Apr-20 09:31 (p 2 of 2)

Test Code: 474-exp10 | 09-3915-2779

Fathead Minnow 7-d Larval Survival and Growth Test

TRE Environmental Strategies

Analysis ID: 04-9609-3362

Endpoint: 7d Survival Rate

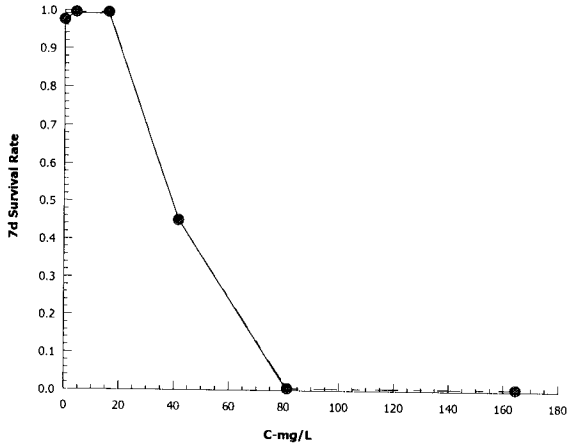
CETIS Version: CETISv1.8.7

Analyzed: 21 Apr-20 9:31

Analysis: Linear Interpolation (ICPIN)

Official Results: Yes

Graphics



DBW 4/22/20 CF

CETIS Analytical Report

Report Date: 21 Apr-20 10:16 (p 1 of 2)
 Test Code: 474-exp10 | 09-3915-2779

DBone summary

Fathead Minnow 7-d Larval Survival and Growth Test

TRE Environmental Strategies

Analysis ID: 00-4233-6562	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv1.8.7
Analyzed: 21 Apr-20 10:15	Analysis: Nonparametric-Control vs Treatments	Official Results: Yes
Batch ID: 13-5729-9412	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 25 Mar-20 15:00	Protocol: EPA/821/R-02-013 (2002)	Diluent: rGSL
Ending Date: 01 Apr-20 15:25	Species: Artemia franciscana	Brine: Crystal Sea
Duration: 7d 0h	Source: In-House Culture	Age: 48h
Sample ID: 04-7522-1036	Code: 1C534C2C	Client: Notre Dame
Sample Date: 25 Mar-20 15:00	Material: Arsenic	Project: Special Studies
Receive Date: 25 Mar-20 15:00	Source: Discharge Monitoring Report	
Sample Age: NA	Station: Effluent	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	19.8%	16	41.63	25.81	

Steel Many-One Rank Sum Test

Control	vs C-mg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water	4.22	14	10	0	6	0.2626	Asymp	Non-Significant Effect
	16	14	10	0	6	0.2626	Asymp	Non-Significant Effect
	41.63*	10	10	0	6	0.0276	Asymp	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.09445569	0.03148523	3	38.44	<0.0001	Significant Effect
Error	0.009828749	0.0008190624	12			
Total	0.1042844		15			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	9.149	11.3	0.0274	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.834	0.841	0.0080	Non-normal Distribution

Mean Dry Biomass-mg Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	0.234	0.1506	0.3174	0.25	0.158	0.278	0.02619	22.4%	0.0%
4.22		4	0.2237	0.1993	0.2482	0.2275	0.202	0.238	0.007685	6.87%	4.38%
16		4	0.209	0.191	0.227	0.211	0.194	0.22	0.005672	5.43%	10.7%
41.63		4	0.046	0.02542	0.06658	0.0505	0.027	0.056	0.006468	28.1%	80.3%
81.25		4	0	0	0	0	0	0	0		100.0%
164.5		4	0	0	0	0	0	0	0		100.0%

Mean Dry Biomass-mg Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.252	0.248	0.278	0.158
4.22		0.238	0.202	0.226	0.229
16		0.22	0.194	0.215	0.207
41.63		0.027	0.056	0.05	0.051
81.25		0	0	0	0
164.5		0	0	0	0

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CETIS Analytical Report

Report Date: 21 Apr-20 10:16 (p 2 of 2)

Test Code: 474-exp10 | 09-3915-2779

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

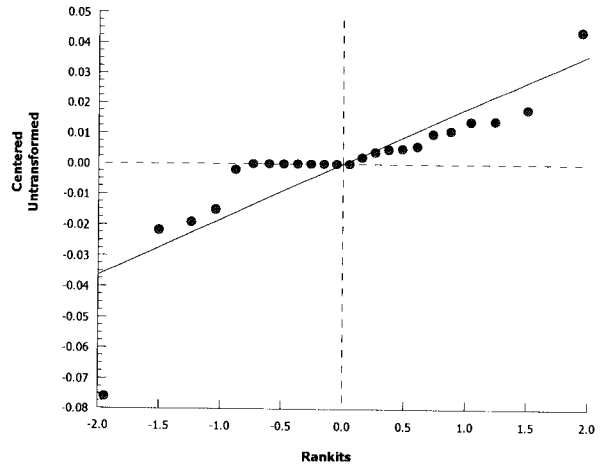
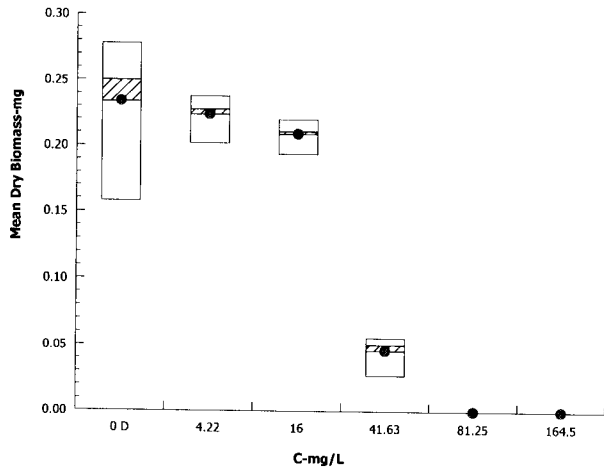
TRE Environmental Strategies

Analysis ID: 00-4233-6562
Analyzed: 21 Apr-20 10:15

Endpoint: Mean Dry Biomass-mg
Analysis: Nonparametric-Control vs Treatments

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



① new 4/22/20 CF

4/22/20

CETIS Analytical Report

Report Date: 21 Apr-20 09:31 (p 1 of 2)
 Test Code: 474-exp10 | 09-3915-2779

D. Brueschling

Pathead Minnow 7-d Larval Survival and Growth Test

TRE Environmental Strategies

Analysis ID: 19-2902-2621	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv1.8.7
Analyzed: 21 Apr-20 9:31	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 13-5729-9412	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 25 Mar-20 15:00	Protocol: EPA/821/R-02-013 (2002)	Diluent: rGSL
Ending Date: 01 Apr-20 15:25	Species: Artemia franciscana	Brine: Crystal Sea
Duration: 7d 0h	Source: In-House Culture	Age: 48h
Sample ID: 04-7522-1036	Code: 1C534C2C	Client: Notre Dame
Sample Date: 25 Mar-20 15:00	Material: Arsenic	Project: Special Studies
Receive Date: 25 Mar-20 15:00	Source: Discharge Monitoring Report	
Sample Age: NA	Station: Effluent	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	87420	200	Yes	Two-Point Interpolation

Point Estimates

Level	mg/L	95% LCL	95% UCL
IC5	5.378	N/A	25.06
IC10	14.72	N/A	22.14
IC15	17.59	N/A	23.11
IC20	19.43	6.172	24.72
IC25	21.27	13.73	26.31
IC40	26.79	21.01	31.15
IC50	30.47	25.83	34.59

Mean Dry Biomass-mg Summary

Calculated Variate

C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	0.234	0.158	0.278	0.02619	0.05238	22.4%	0.0%
4.22		4	0.2237	0.202	0.238	0.007685	0.01537	6.87%	4.38%
16		4	0.209	0.194	0.22	0.005672	0.01134	5.43%	10.7%
41.63		4	0.046	0.027	0.056	0.006468	0.01294	28.1%	80.3%
81.25		4	0	0	0	0	0		100.0%
164.5		4	0	0	0	0	0		100.0%

Mean Dry Biomass-mg Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.252	0.248	0.278	0.158
4.22		0.238	0.202	0.226	0.229
16		0.22	0.194	0.215	0.207
41.63		0.027	0.056	0.05	0.051
81.25		0	0	0	0
164.5		0	0	0	0

D. Brueschling 4/22/20 CP

4/22/20

CETIS Analytical Report

BARNE skimp

Report Date: 21 Apr-20 09:31 (p 2 of 2)

Test Code: 474-exp10 | 09-3915-2779

Fathead Minnow 7-d Larval Survival and Growth Test

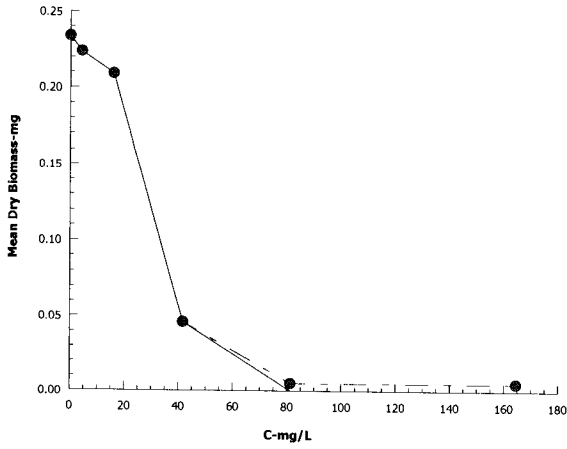
TRE Environmental Strategies

Analysis ID: 19-2902-2621
Analyzed: 21 Apr-20 9:31

Endpoint: Mean Dry Biomass-mg
Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



① use 4/22/20 CF

4/22/20

April 13, 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 #10

Mr. Bittner/ Dr. Belovsky:

Below is a summary of the short-term chronic brine shrimp experiment initiated on March 25, 2020. The purpose of this experiment was to investigate whether feeding an algae/YTC mix would affect the response of *Artemia franciscana* to arsenic in the short-term chronic test. Data from this 7-day study were compared to data from earlier 7-day and 10-day tests where brine shrimp were fed algae only.

Along with a control, an algae-only concurrent control and five different arsenic concentrations were tested:

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

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 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 0.33ml 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: <24-h old *A. franciscana* were hatched out in ~29 ppt artificial seawater

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

(Crystal Sea Marine Mix) and ~200 organisms were placed in 120 ppt rGSL water and fed *Dunaliella viridis* at a density of 100 µg/L Chla. Solutions were gently aerated.

Characterization of Recon Water

Sample No.	pH	Hard. (mg/L) ^a	Alk. (mg/L) ^a	Spec. Cond. (µS/cm)	TRC (mg/L) ^b	NH ₃ -N (mg/L)	Salinity (ppt)
RW#13876	7.9	NM	NM	141,000	NM	NM	120

^aAs CaCO₃

^bTotal residual chlorine

Test activities:

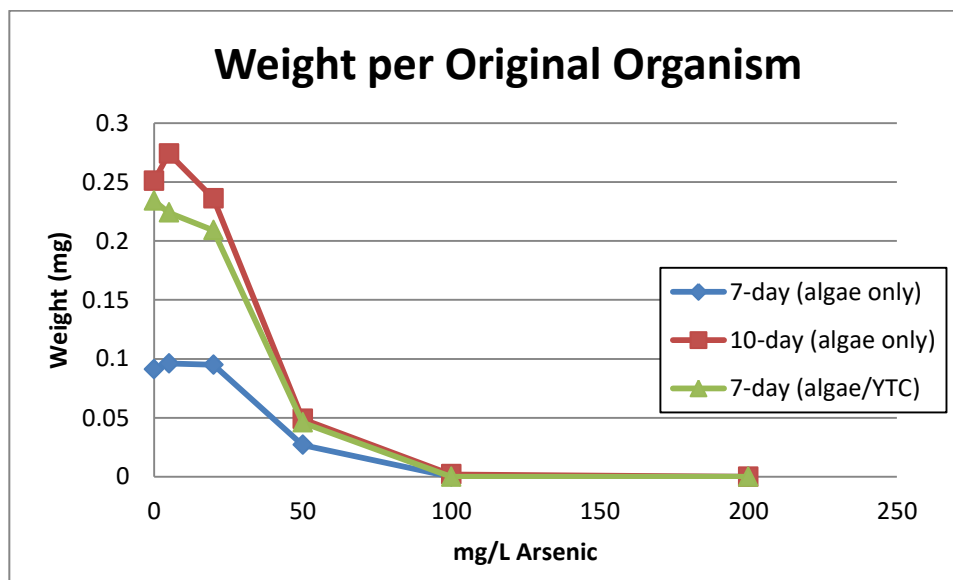
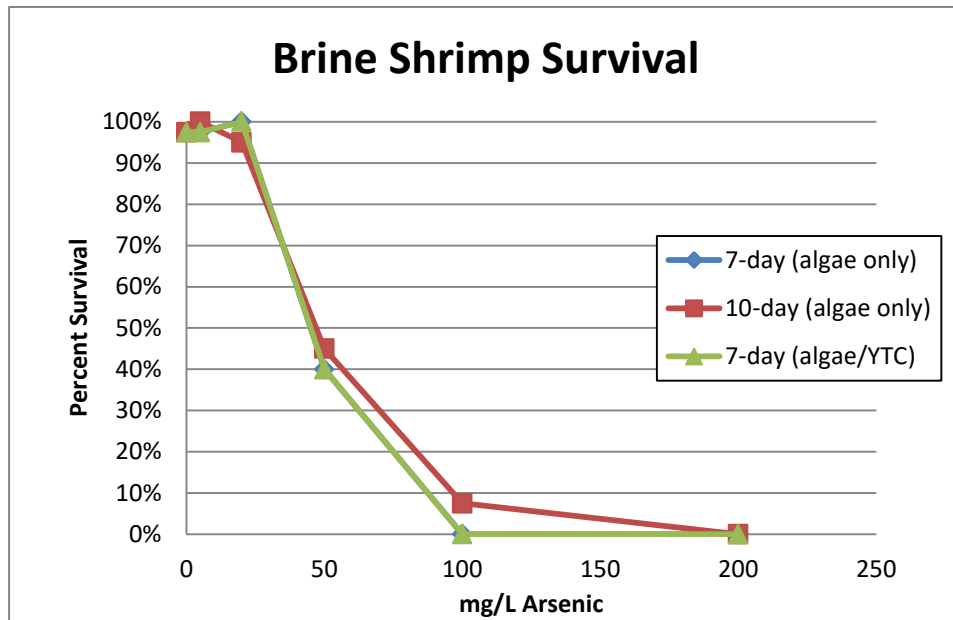
- Biological observations (primarily survival) taken daily.
- Chemistries taken on renewal days (i.e., pH, dissolved oxygen, and temperature).
- Conductivity was measured at test termination or when there was 0% survival in that treatment.
- Dry weights were determined at test termination.
- Arsenic was added to 120 rGSL media containing food and allowed to equilibrate for 3 hours prior to use in the toxicity tests.

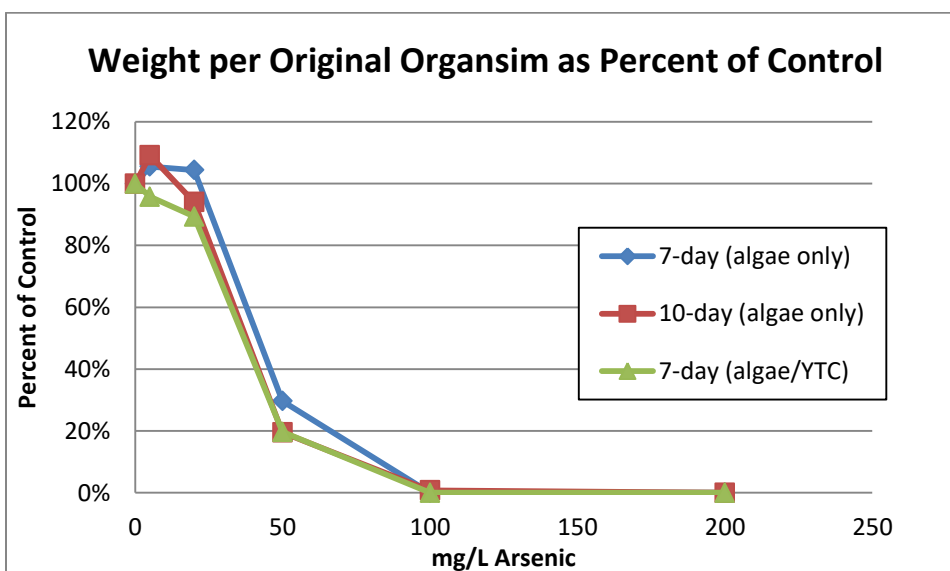
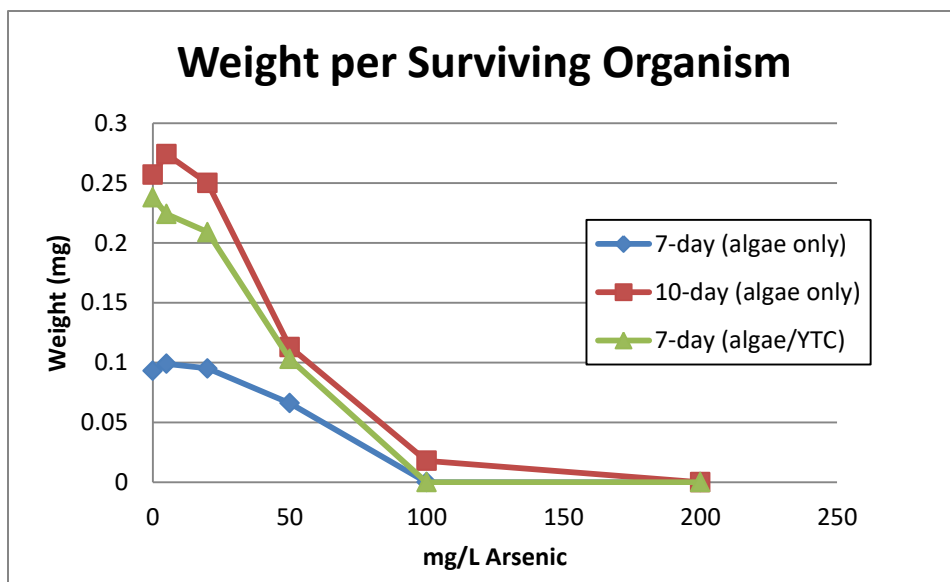
Results:

The results of the two control treatments (fed only *D. viridis* and fed a mixture of *D. viridis*/YTC) are below. Survival and growth (dry wt) were nearly identical between the two treatments.

Treatment	Survival	Weight Per Original Org	Weight Per Surviving Org
<i>D. viridis</i> only	97.5%	0.235 mg	0.241 mg
<i>D. viridis</i> / YTC	97.5%	0.234 mg	0.238 mg

The survival and average dry weights for the brine shrimp in the arsenic treatments are illustrated in the following figures and are compared to two previous arsenic studies.





Test Endpoints

Study	Test Endpoints (mg As/L, nominal)				
	Survival NOEC	Survival LOEC	Growth NOEC	Growth LOEC	Growth IC25
7-Day (Algae only, Exp 5)	20	50	20	50	30.43 (25.72-32.91)
10-Day (Algae only, Exp 5)	20	50	20	50	26.26 (19.22-31.09)
7-Day (YTC/Algae)	20	50	20	50	26.17 (18.02-31.89)

Summary and findings:

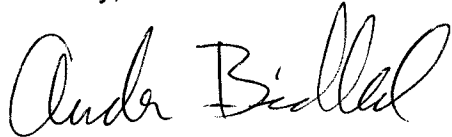
- Organism survival was $\geq 90\%$ for all controls.
- Growth was very similar between the algae-only treatment and the algae + YTC treatment.
- The effect of arsenic on brine shrimp survival was similar for both feeding regimes.
- The growth IC_{25} values for each test were similar and fell within the confidence limits of the previous tests (nominal basis).

Based on these results, feeding an algae/YTC mixture does not appear to affect arsenic toxicity when compared to feeding with just algae. Based on nominal arsenic concentrations, statistical endpoints were nearly identical in all tests.

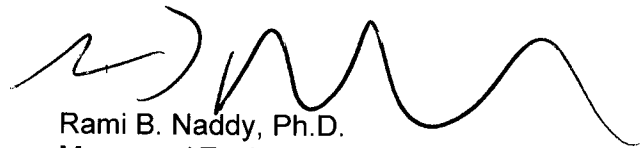
Analytical samples from each treatment have been collected and sent in for arsenic measurement. We will provide a summary of those results separately.

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,



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14001-474-058

Attachment

cc: David Pillard, TRE

TRE

SA NW 4/8/20
--OST

TOXICITY DATA PACKAGE COVER SHEET

Test Type: Chronic Project Number: 17001-474-Exp
Test Substance: Arsenic (Na₂HAsO₄) Species: Artemia franciscana
Dilution Water: rGSL Organism Lot or Batch Number: 032320
Concurrent Control Water: NA Age: 48HR (48 hr) Supplier: TRE
Date and Time Test Began: 3/25/20 @ 1500 Date and Time Test Ended: 4/1/20 @ 1525
Protocol Number: _____ Investigator(s): HR/CP/ARTEN

Background Information

Type of Test: Static-Renewal (Daily) pH control?: Yes No
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: 72.5 ug/L Chla/ 0.33 ml Y Feeding Frequency: Initiation and Renewals

Test Substance Characterization Parameters and Frequency:

Hardness: Test Initiation Alkalinity: Test Initiation NH₃: Test Initiation TRC: Test Initiation
pH: Daily Conductivity: Daily

Test Concentrations (Volume:Volume): D. viridis, rGSL, 5, 20, 50, 100, and 200 mg/L as As

Agency Summary Sheet(s)?: None

Reference Toxicant Data: Test Dates: NA to NA IC₂₅: _____
Hist. 95% Control Limits: _____ to _____ Method for Determining Ref. Tox. Value: Linear Interpolation

Special Procedures and Considerations:
Organisms hatched 2 days prior to initiation and held in rGSL with 100 ug/L Chla
Treatment one- Fed *D. viridis* 145 ug/L Chla

Appropriate correction factors have been applied to all temperatures recorded in this data package
Study Director Initials: W3V Date: 3/25/20

Artemia franciscana
CHRONIC BIOLOGICAL DATA

ea new 4/8/20

Project Number: ⁰⁵⁸ 17001-474-Exp

mg/L	Test Replicate	Number of Surviving Organisms								Remarks	
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7		
D. viridis	A	10	10	10	10	10	10	10	9	97.5	
	B	10	10	10	10	10	10	10	10		
	C	10	10	10	10	10	10	10	10		
	D	10	10	10	10	10	10	10	10		
0	A	10	10	10	10	10	10	10	10	97.5	
	B	10	10	10	10	10	10	10	10		
	C	10	10	10	10	10	10	10	10		
	D	10	10	10	10	10	10	9	9		
5	A	10	10	10	10	10	10	10	10	100	
	B	10	10	10	10	10	10	10	10		
	C	10	10	10	10	10	10	10	10		
	D	10	10	10	10	10	10	10	10		
20	A	10	10	10	10	10	10	10	10	100	
	B	10	10	10	10	10	10	10	10		
	C	10	10	10	10	10	10	10	10		
	D	10	10	10	10	10	10	10	10		
50	A	10	9	3	3	3	3	3	3	45	
	B	10	9	7	6	6	6	6	5		
	C	10	9*	7	7	7	7	7	6		* 1 weak org
	D	10	8	4	4	4	4	4	4		
100	A	10	0	—	—	—	—	—	—	0	
	B	10	3	1	0	—	—	—	—		
	C	10	0	—	—	—	—	—	—		
	D	10	1	0	—	—	—	—	—		
200	A	10	0	—	—	—	—	—	—	0	
	B	10	1	0	—	—	—	—	—		
	C	10	0	—	—	—	—	—	—		
	D	10	0	—	—	—	—	—	—		
Date:	3/25/20	3/26/20	3/27/20	3/28/20	3/29/20	3/30/20	3/31/20	4/1/20			
Time:	1500	1335	1340	1445	1355	1445	1515	1525			
Initials:	CP/M	CP	CP	CP	BY	EN	CP	CP			

CHRONIC CHEMICAL DATA (INITIAL)

DA in 4/8/20

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.:	D. viridis									All Conc.	
pH		8.0	8.0	8.0	8.0	7.9	7.8	8.0		FM27	
D.O. (mg/L)		5.1	5.1	5.1	5.2	5.3	5.1	5.1		17	
Temp. (°C)		20	20	20	20	20	20	20		IR1	
Cond. (µS/cm)		141000	141000	139000	139000	139500	203800	137600		15	
Hard. (mg/L)											
Alk. (mg/L)											
TRC (mg/L)											
NH ₃ (mg/L)											
Conc.:	0										
pH		7.9	8.0	8.0	7.9	7.9	7.8	8.0			
D.O. (mg/L)		5.1	5.2	5.2	5.2	5.3	5.1	5.2			
Temp. (°C)		20	20	20	20	20	20	20			
Cond. (µS/cm)		141000	141000	140000	136000	139,200	186,200	137300			Salinity = 120
Hard. (mg/L)											
Alk. (mg/L)											
TRC (mg/L)											
NH ₃ (mg/L)											
Conc.:	5										
pH		7.9	8.0	8.0	7.9	7.9	7.8	8.0			
D.O. (mg/L)		5.1	5.1	5.1	5.2	5.1	5.1	5.3			
Temp. (°C)		20	20	20	20	20	20	20			
Cond. (µS/cm)		141000	141000	140000	134000	138,900	223,400	137000			
Conc.:	20										
pH		7.9	8.0	8.0	7.9	7.9	7.8	7.9			
D.O. (mg/L)		5.2	5.2	5.3	5.1	5.1	5.1	5.3			
Temp. (°C)		20	20	20	20	20	20	20			
Cond. (µS/cm)		141000	141000	140000	135000	138,600	221,600	137300			
Date:		3/25/20	3/26/20	3/27/20	3/28/20	3/29/20	3/30/20	3/31/20			
Time:		1445	1320	1325	1435	1345	1430	1500			
Initials:		CP	CP	CP	CP	BJ	EN	CP			

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

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

058 CHRONIC CHEMICAL DATA (INITIAL)

QA run 4/8/20

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.: 50									All Conc.	
pH	7.9	8.0	8.0	7.9	7.9	7.8	7.9			
D.O. (mg/L)	5.2	5.1	5.2	5.2	5.2	5.1	5.4			
Temp. (°C)	20	20	20	20	20	20	20			
Cond. (µS/cm)	141000	141000	140000	136000	138100	222200	136800			
Conc.: 100										
pH	7.9	7.9	8.0	/	/	/				
D.O. (mg/L)	5.1	5.2	5.2	/	/	/				
Temp. (°C)	20	20	20							
Cond. (µS/cm)	141000	140000	141000							
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.: 200										
pH	7.9	7.9	/	/	/	/				
D.O. (mg/L)	5.2	5.3	/	/	/	/				
Temp. (°C)	20	20								
Cond. (µS/cm)	141000	141000	/	/	/	/				
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH ₃ (mg/L)										
Date:	3/25/20	3/26/20	3/27/20	3/28/20	3/29/20	3/30/20	3/31/20			
Time:	1445	1320	1325	1435	1345	1430	1500			
Initials:	CP	CP	CP	CP	RY	EN	CP			

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

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

058

CHRONIC CHEMICAL DATA (FINAL)

CP 4/1/20

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

%		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter #	Remarks
Conc.:	D. viridis								130700	All Conc.	* conductivity
pH		8.0	8.1	8.0	7.9	7.9	7.8	7.9		FM27	
D.O. (mg/L)		5.9	5.1	5.2	5.2	5.4	4.9	4.8		17	
Temp (°C)		20	20	20	19	20	20	21		L-6	
Conc.:	0								129200		* conductivity
pH		7.9	8.1	7.9	7.9	7.9	7.8	7.8			
D.O. (mg/L)		5.6	4.9	5.0	4.8	5.2	4.6	4.6			
Temp (°C)		20	21	20	19	20	20	21			
Conc.:	5								128600		* conductivity
pH		7.9	8.0	7.9	7.9	7.8	7.8	7.8			
D.O. (mg/L)		5.5	5.0	5.0	4.8	4.9	4.7	4.5			
Temp (°C)		20	21	20	19	20	20	21			
Conc.:	20								128300		* conductivity
pH		8.0	8.0	8.0	7.9	7.8	7.8	7.8			
D.O. (mg/L)		5.4	5.1	5.0	4.8	4.9	4.7	4.5			
Temp (°C)		20	21	20	19	20	20	21			
Conc.:	50								127600		* conductivity
pH		8.0	8.0	7.9	7.9	7.8	7.8	7.9			
D.O. (mg/L)		5.7	5.1	5.0	4.9	5.1	5.1	4.6			
Temp (°C)		20	21	20	19	20	20	21			
Conc.:	100			133000							* conductivity
pH		8.0	8.0	8.0							
D.O. (mg/L)		5.6	5.2	5.4							
Temp (°C)		20	21	20							
Conc.:	200		138000								* conductivity
pH		8.0	8.0								
D.O. (mg/L)		5.4	5.3								
Temp (°C)		20	21								
Date:		3/26/20	3/27/20	3/28/20	3/29/20	3/30/20	3/31/20	4/1/20			
Time:		1355	1400	1500	1355	1440	1540	1545			
Initials:		CP	CP	CP	PF	EN	CP	CP			

CP 4/1/20 E

BA new 4/8/20

- 556 DAILY TOXICITY TEST LOG

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

General Comments		Feeding 72.5 ug/l Chla 0.33ml YTC	Initials/Date
	Random Chart: "D" Min/Max Thermometer # M-15		
Test Day 0	Test Solution Mixed at: 1435 Test Organisms Added at: 1500	Fed @ 1130 R	CP 3/25/20
Test Day 1	Real Time: 18 °C Min-Max Range: 18-20 °C	Fed @ 1035	CP 3/26/20
Test Day 2	Real Time: 19 °C Min-Max Range: 18-21 °C	Fed @ 1055	CP 3/27/20
Test Day 3	Real Time: 19 °C Min-Max Range: 19-19 °C	Fed @ 1135 CP	CP 3/28/20
Test Day 4	Real Time: 20 °C Min-Max Range: 17-20 °C	Fed @ 1030 R	3/29/20 R
Test Day 5	Real Time: 19 °C Min-Max Range: 18-21 °C	Fed @ 1130 R	EN 3/30/20
Test Day 6	Real Time: 19 °C Min-Max Range: 18-21 °C	Fed @ 1150	CP 3/31/20
Test Day 7	Real Time: 19 °C Min-Max Range: 18-21 °C	Fed @ NONE	CP 4/1/20

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Project Number: 14001-474-2056

Species: Artemia franciscana

QA: ASD 4/15/20

Treatment	Rep	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)	No of Orig. Organisms	Mean Wt./ Original Organism (mg)	Mean Wt./ Treatment (mg) (Original)	Number of Surv. Organisms	Mean Wt./ Surviving Organism (mg)	Mean Wt./ Treatment (mg) (Surviving)
rGSL algae only	A		1.12424	1.12645	0.00221	0.00223	10	0.223	0.2350	9	0.248	0.2412
	B		1.14313	1.14581	0.00268	0.00270	10	0.270		10	0.270	
	C		1.12878	1.13089	0.00211	0.00213	10	0.213		10	0.213	
	D		1.14131	1.14363	0.00232	0.00234	10	0.234		10	0.234	
rGSL	A		1.13593	1.13843	0.00250	0.00252	10	0.252	0.2340	10	0.252	0.2384
	B		1.13146	1.13392	0.00246	0.00248	10	0.248		10	0.248	
	C		1.12059	1.12335	0.00276	0.00278	10	0.278		10	0.278	
	D		1.13712	1.13868	0.00156	0.00158	10	0.158		9	0.176	
5 mg/L	A		1.13440	1.13676	0.00236	0.00238	10	0.238	0.2238	10	0.238	0.2238
	B		1.13115	1.13315	0.00200	0.00202	10	0.202		10	0.202	
	C		1.13508	1.13732	0.00224	0.00226	10	0.226		10	0.226	
	D		1.13942	1.14169	0.00227	0.00229	10	0.229		10	0.229	
20 mg/L	A		1.13798	1.14016	0.00218	0.00220	10	0.220	0.2090	10	0.220	0.2090
	B		1.13780	1.13972	0.00192	0.00194	10	0.194		10	0.194	
	C		1.11500	1.11713	0.00213	0.00215	10	0.215		10	0.215	
	D		1.13955	1.14160	0.00205	0.00207	10	0.207		10	0.207	
50 mg/L	A		1.13976	1.14001	0.00025	0.00027	10	0.027	0.0460	3	0.090	0.1032
	B		1.11762	1.11816	0.00054	0.00056	10	0.056		5	0.112	
	C		1.14765	1.14813	0.00048	0.00050	10	0.050		6	0.083	
	D		1.13105	1.13154	0.00049	0.00051	10	0.051		4	0.128	
100 mg/L	A		0.00000	0.00000	0.00000	0.00000	10	0.000	0.0000	0	-	#DIV/0!
	B		0.00000	0.00000	0.00000	0.00000	10	0.000		0	-	
	C		0.00000	0.00000	0.00000	0.00000	10	0.000		0	-	
	D		0.00000	0.00000	0.00000	0.00000	10	0.000		0	-	

As of 4/1/14

A	0.00000	0.00000	0.00000	0.00000	0.00000	10	0.000	0.0000	0	#DIV/0!
B	0.00000	0.00000	0.00000	0.00000	0.00000	10	0.000	0.0000	0	
C	0.00000	0.00000	0.00000	0.00000	0.00000	10	0.000	0.0000	0	
D	0.00000	0.00000	0.00000	0.00000	0.00000	10	0.000	0.0000	0	
Blank	1.13002	1.13000	-0.00002							

Project Number: 14001-474

Species: Artemia franciscana

Summary Statistics for Survival Data

Treatment	N	Min	Max	Mean	SD	C.V.
rGSL	4	0.9	1.0	0.9750	0.0500	5.128%
5 mg/L	4	1.0	1.0	1.0000	0.0000	0.000%
20 mg/L	4	1.0	1.0	1.0000	0.0000	0.000%
50 mg/L	4	0.3	0.6	0.4500	0.1291	28.689%
100 mg/L	4	0.0	0.0	0.0000	0.0000	#DIV/0!
200 mg/L	4	0.0	0.0	0.0000	0.0000	#DIV/0!

Summary Statistics for Growth Data (dry wt per original)

Treatment	N	Min	Max	Mean	SD	C.V.
rGSL	4	0.158	0.278	0.2340	0.0524	22.386%
5 mg/L	4	0.202	0.238	0.2238	0.0154	6.869%
20 mg/L	4	0.194	0.220	0.2090	0.0113	5.427%
50 mg/L	4	0.027	0.056	0.0460	0.0129	28.121%
100 mg/L	4	0.000	0.000	0.0000	0.0000	#DIV/0!
200 mg/L	4	0.000	0.000	0.0000	0.0000	#DIV/0!

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

Treatment	N	Min	Max	Mean	SD	C.V.
rGSL	4	0.176	0.278	0.2384	0.0439	18.436%
5 mg/L	4	0.202	0.238	0.2238	0.0154	6.869%
20 mg/L	4	0.194	0.220	0.2090	0.0113	5.427%
50 mg/L	4	0.083	0.128	0.1032	0.0203	19.674%
100 mg/L	0	0.000	0.000	#DIV/0!	#DIV/0!	#DIV/0!
200 mg/L	0	0.000	0.000	#DIV/0!	#DIV/0!	#DIV/0!

CETIS Analytical Report

Report Date: 06 Apr-20 08:54 (p 1 of 2)
 Test Code: 474-exp | 03-4558-8240

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

Analysis ID: 12-6256-2136	Endpoint: 7d Survival Rate	CETIS Version: CETISv1.8.7
Analyzed: 06 Apr-20 8:53	Analysis: Parametric-Control vs Treatments	Official Results: Yes
Batch ID: 13-5729-9412	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 25 Mar-20 15:00	Protocol: EPA/821/R-02-013 (2002)	Diluent: rGSL
Ending Date: 01 Apr-20 15:25	Species: Artemia franciscana	Brine: Crystal Sea
Duration: 7d 0h	Source: In-House Culture	Age: 48h
Sample ID: 01-8576-9191	Code: B129CE7	Client: Notre Dame
Sample Date: 25 Mar-20 15:00	Material: Arsenic	Project: Special Studies
Receive Date: 25 Mar-20 15:00	Source: Discharge Monitoring Report	
Sample Age: NA	Station: Effluent	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	7.88%	20	50	31.62	

Dunnnett Multiple Comparison Test

Control	vs C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water	5	-0.7441	2.29	0.125	6	0.9321	CDF	Non-Significant Effect
	20	-0.7441	2.29	0.125	6	0.9321	CDF	Non-Significant Effect
	50*	11.64	2.29	0.125	6	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.329014	0.4430048	3	73.89	<0.0001	Significant Effect
Error	0.0719444	0.005995367	12			
Total	1.400959		15			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	3.64	5.95	0.0448	Equal Variances
Variances	Levene Equality of Variance	7.667	5.95	0.0040	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8588	0.841	0.0184	Normal Distribution

7d Survival Rate Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	0.975	0.8954	1	1	0.9	1	0.025	5.13%	0.0%
5		4	1	1	1	1	1	1	0	0.0%	-2.56%
20		4	1	1	1	1	1	1	0	0.0%	-2.56%
50		4	0.45	0.2446	0.6554	0.45	0.3	0.6	0.06455	28.7%	53.8%
100		4	0	0	0	0	0	0	0		100.0%
200		4	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.371	1.242	1.501	1.412	1.249	1.412	0.04074	5.94%	0.0%
5		4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	-2.97%
20		4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	-2.97%
50		4	0.734	0.5244	0.9435	0.7351	0.5796	0.8861	0.06584	17.9%	46.5%
100		4	0.1588	0.1588	0.1588	0.1588	0.1588	0.1588	0	0.0%	88.4%
200		4	0.1588	0.1588	0.1588	0.1588	0.1588	0.1588	0	0.0%	88.4%

CETIS Analytical Report

Report Date: 06 Apr-20 08:54 (p 2 of 2)
 Test Code: 474-exp | 03-4558-8240

Fathead Minnow 7-d Larval Survival and Growth Test

TRE Environmental Strategies

Analysis ID: 12-6256-2136 Endpoint: 7d Survival Rate CETIS Version: CETISv1.8.7
 Analyzed: 06 Apr-20 8:53 Analysis: Parametric-Control vs Treatments Official Results: Yes

7d Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9	1	1	1
5		1	1	1	1
20		1	1	1	1
50		0.3	0.5	0.6	0.4
100		0	0	0	0
200		0	0	0	0

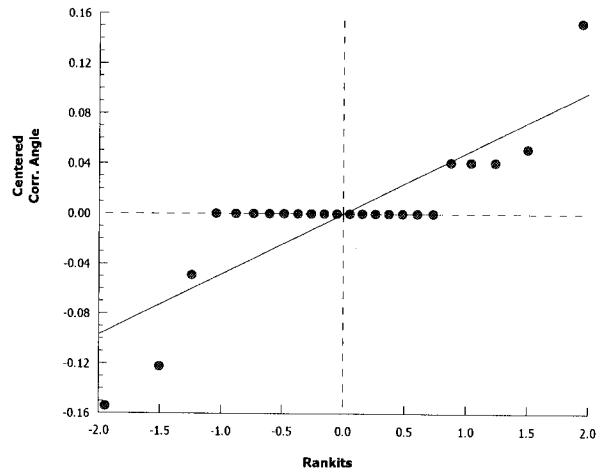
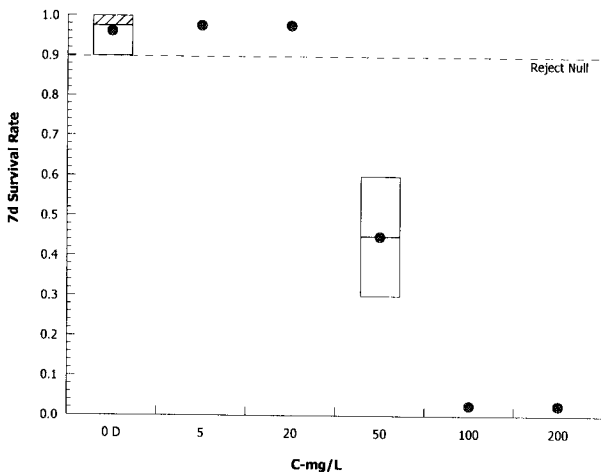
Angular (Corrected) Transformed Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.249	1.412	1.412	1.412
5		1.412	1.412	1.412	1.412
20		1.412	1.412	1.412	1.412
50		0.5796	0.7854	0.8861	0.6847
100		0.1588	0.1588	0.1588	0.1588
200		0.1588	0.1588	0.1588	0.1588

7d Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	9/10	10/10	10/10	10/10
0	Lab Water	10/10	10/10	10/10	9/10
5		10/10	10/10	10/10	10/10
20		10/10	10/10	10/10	10/10
50		3/10	5/10	6/10	4/10
100		0/10	0/10	0/10	0/10
200		0/10	0/10	0/10	0/10

Graphics



CETIS Analytical Report

Report Date: 06 Apr-20 08:54 (p 1 of 2)
 Test Code: 474-exp | 03-4558-8240

Fathead Minnow 7-d Larval Survival and Growth Test

TRE Environmental Strategies

Analysis ID: 13-7830-0865	Endpoint: 7d Survival Rate	CETIS Version: CETISv1.8.7
Analyzed: 06 Apr-20 8:54	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 13-5729-9412	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 25 Mar-20 15:00	Protocol: EPA/821/R-02-013 (2002)	Diluent: rGSL
Ending Date: 01 Apr-20 15:25	Species: Artemia franciscana	Brine: Crystal Sea
Duration: 7d 0h	Source: In-House Culture	Age: 48h
Sample ID: 01-8576-9191	Code: B129CE7	Client: Notre Dame
Sample Date: 25 Mar-20 15:00	Material: Arsenic	Project: Special Studies
Receive Date: 25 Mar-20 15:00	Source: Discharge Monitoring Report	
Sample Age: NA	Station: Effluent	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1392438	200	Yes	Two-Point Interpolation

Point Estimates

Level	mg/L	95% LCL	95% UCL
LC5	22.75	21.92	23.74
LC10	25.49	23.84	27.48
LC15	28.24	25.77	31.22
LC20	30.98	27.69	34.96
LC25	33.73	29.61	38.7
LC40	41.97	35.38	49.93
LC50	47.46	39.22	59.4

7d Survival Rate Summary

Calculated Variate(A/B)

C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Dilution Water	4	0.975	0.9	1	0.025	0.05	5.13%	0.0%	39	40
5		4	1	1	1	0	0	0.0%	-2.56%	40	40
20		4	1	1	1	0	0	0.0%	-2.56%	40	40
50		4	0.45	0.3	0.6	0.06455	0.1291	28.7%	53.8%	18	40
100		4	0	0	0	0	0		100.0%	0	40
200		4	0	0	0	0	0		100.0%	0	40

7d Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9	1	1	1
5		1	1	1	1
20		1	1	1	1
50		0.3	0.5	0.6	0.4
100		0	0	0	0
200		0	0	0	0

7d Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	9/10	10/10	10/10	10/10
0	Lab Water	10/10	10/10	10/10	9/10
5		10/10	10/10	10/10	10/10
20		10/10	10/10	10/10	10/10
50		3/10	5/10	6/10	4/10
100		0/10	0/10	0/10	0/10
200		0/10	0/10	0/10	0/10

4/8/20

CETIS Analytical Report

Report Date: 06 Apr-20 08:54 (p 2 of 2)

Test Code: 474-exp | 03-4558-8240

Fathead Minnow 7-d Larval Survival and Growth Test

TRE Environmental Strategies

Analysis ID: 13-7830-0865

Endpoint: 7d Survival Rate

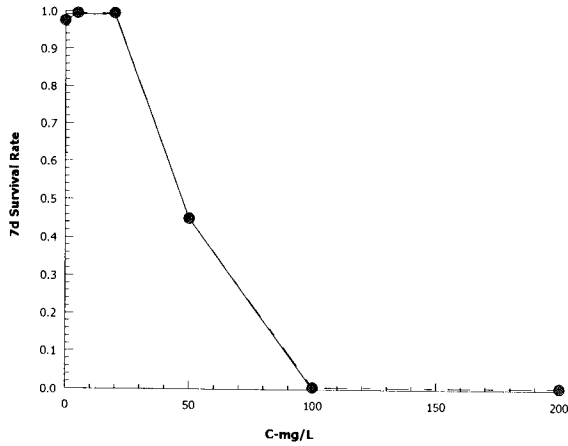
CETIS Version: CETISv1.8.7

Analyzed: 06 Apr-20 8:54

Analysis: Linear Interpolation (ICPIN)

Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 10 Apr-20 10:29 (p 1 of 2)
 Test Code: 474-exp | 03-4558-8240

Fathead Minnow 7-d Larval Survival and Growth Test

TRE Environmental Strategies

Analysis ID: 00-4158-9396	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv1.8.7
Analyzed: 10 Apr-20 10:29	Analysis: Nonparametric-Control vs Treatments	Official Results: Yes
Batch ID: 13-5729-9412	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 25 Mar-20 15:00	Protocol: EPA/821/R-02-013 (2002)	Diluent: rGSL
Ending Date: 01 Apr-20 15:25	Species: Artemia franciscana	Brine: Crystal Sea
Duration: 7d 0h	Source: In-House Culture	Age: 48h
Sample ID: 01-8576-9191	Code: B129CE7	Client: Notre Dame
Sample Date: 25 Mar-20 15:00	Material: Arsenic	Project: Special Studies
Receive Date: 25 Mar-20 15:00	Source: Discharge Monitoring Report	
Sample Age: NA	Station: Effluent	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	19.8%	20	50	31.62	

Steel Many-One Rank Sum Test

Control	vs	C-mg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α :5%)
Lab Water		5	14	10	0	6	0.2626	Asymp	Non-Significant Effect
		20	14	10	0	6	0.2626	Asymp	Non-Significant Effect
		50*	10	10	0	6	0.0276	Asymp	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α :5%)
Between	0.09445569	0.03148523	3	38.44	<0.0001	Significant Effect
Error	0.009828749	0.0008190624	12			
Total	0.1042844		15			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α :1%)
Variances	Bartlett Equality of Variance	9.149	11.3	0.0274	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.834	0.841	0.0080	Non-normal Distribution

Mean Dry Biomass-mg Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Water	4	0.234	0.1506	0.3174	0.25	0.158	0.278	0.02619	22.4%	0.0%
5		4	0.2237	0.1993	0.2482	0.2275	0.202	0.238	0.007685	6.87%	4.38%
20		4	0.209	0.191	0.227	0.211	0.194	0.22	0.005672	5.43%	10.7%
50		4	0.046	0.02542	0.06658	0.0505	0.027	0.056	0.006468	28.1%	80.3%
100		4	0	0	0	0	0	0	0		100.0%
200		4	0	0	0	0	0	0	0		100.0%

Mean Dry Biomass-mg Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water	0.252	0.248	0.278	0.158
5		0.238	0.202	0.226	0.229
20		0.22	0.194	0.215	0.207
50		0.027	0.056	0.05	0.051
100		0	0	0	0
200		0	0	0	0

Fathead Minnow 7-d Larval Survival and Growth Test

TRE Environmental Strategies

Analysis ID: 00-4158-9396

Endpoint: Mean Dry Biomass-mg

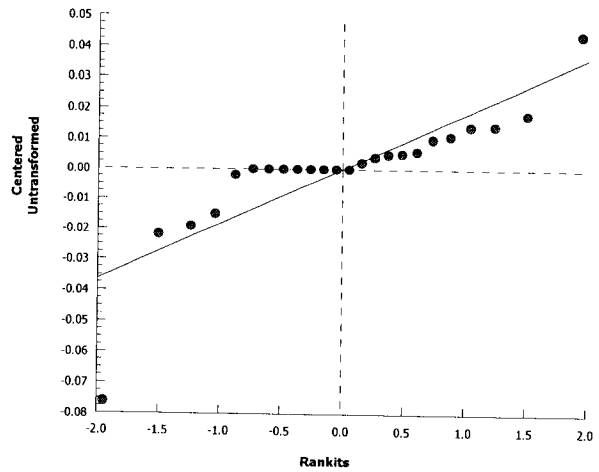
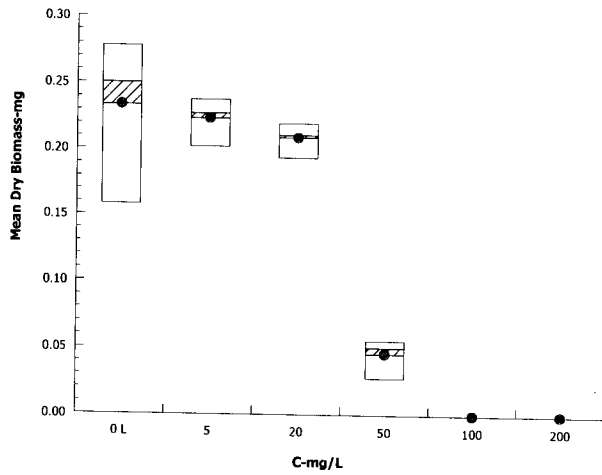
CETIS Version: CETISv1.8.7

Analyzed: 10 Apr-20 10:29

Analysis: Nonparametric-Control vs Treatments

Official Results: Yes

Graphics



4/10/20

CETIS Analytical Report

Report Date: 10 Apr-20 10:30 (p 1 of 2)
 Test Code: 474-exp | 03-4558-8240

Fathead Minnow 7-d Larval Survival and Growth Test

TRE Environmental Strategies

Analysis ID: 05-0372-0767	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv1.8.7
Analyzed: 10 Apr-20 10:30	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 13-5729-9412	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 25 Mar-20 15:00	Protocol: EPA/821/R-02-013 (2002)	Diluent: rGSL
Ending Date: 01 Apr-20 15:25	Species: Artemia franciscana	Brine: Crystal Sea
Duration: 7d 0h	Source: In-House Culture	Age: 48h
Sample ID: 01-8576-9191	Code: B129CE7	Client: Notre Dame
Sample Date: 25 Mar-20 15:00	Material: Arsenic	Project: Special Studies
Receive Date: 25 Mar-20 15:00	Source: Discharge Monitoring Report	
Sample Age: NA	Station: Effluent	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1452613	200	Yes	Two-Point Interpolation

Point Estimates

Level	mg/L	95% LCL	95% UCL
IC5	6.475	N/A	31.23
IC10	18.37	N/A	27.21
IC15	21.86	N/A	28.24
IC20	24.01	2.478	30.06
IC25	26.17	18.02	31.89
IC40	32.63	26.09	37.49
IC50	36.93	31.18	41.41

Mean Dry Biomass-mg Summary

Calculated Variate

C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water	4	0.234	0.158	0.278	0.02619	0.05238	22.4%	0.0%
5		4	0.2237	0.202	0.238	0.007685	0.01537	6.87%	4.38%
20		4	0.209	0.194	0.22	0.005672	0.01134	5.43%	10.7%
50		4	0.046	0.027	0.056	0.006468	0.01294	28.1%	80.3%
100		4	0	0	0	0	0		100.0%
200		4	0	0	0	0	0		100.0%

Mean Dry Biomass-mg Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water	0.252	0.248	0.278	0.158
5		0.238	0.202	0.226	0.229
20		0.22	0.194	0.215	0.207
50		0.027	0.056	0.05	0.051
100		0	0	0	0
200		0	0	0	0

CETIS Analytical Report

Report Date: 10 Apr-20 10:30 (p 2 of 2)
Test Code: 474-exp | 03-4558-8240

Fathead Minnow 7-d Larval Survival and Growth Test

TRE Environmental Strategies

Analysis ID: 05-0372-0767
Analyzed: 10 Apr-20 10:30

Endpoint: Mean Dry Biomass-mg
Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics

