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TOXICITY LABORATORY & CONSULTING

Results of Toxicity Testing for the City of Snoqualmie Wastewater Treatment and Water Reclamation Facility

❖ Monitoring Period: February 2024

Prepared for: City of Snoqualmie
Wastewater Treatment and
Water Reclamation Facility
38190 SE Sterns Road
Snoqualmie, WA 98065

Prepared by: Enthalpy Analytical
4340 Vandever Ave
San Diego, California 92120

Submitted: March 14, 2024

Data Quality Assurance:

- Enthalpy Analytical is accredited in accordance with NELAP by the State of Oregon Environmental Laboratory Accreditation Program (ORELAP ID 4053). It is also certified by the State of California Department of Health Services Environmental Laboratory Accreditation Program (Certificate No. 1802) and the State of Washington Department of Ecology (Lab ID C552).
- All data have been reviewed and verified.
- All test results have met minimum test acceptability criteria under their respective EPA protocols, unless otherwise noted in this report.
- All test results have met internal Quality Assurance Program requirements.

Results verified by: _____

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Introduction

Toxicity tests were conducted using effluent collected from the City of Snoqualmie Wastewater Treatment and Water Reclamation Facility in February of 2024 to satisfy monitoring requirements. Acute and chronic bioassays were conducted using the fathead minnow *Pimephales promelas*. Acute bioassay testing was conducted with the water flea *Ceriodaphnia dubia*. Testing was conducted in order to meet National Pollutant Discharge Elimination System (NPDES) permit biomonitoring requirements for permit number WA0022403. Testing was performed at Enthalpy Analytical located in San Diego, California between February 20 and 27, 2024.

Materials and Methods

Effluent samples were collected into low-density polyethylene cubitainers. The cubitainers were packed in a cooler containing ice and shipped to Enthalpy via overnight delivery service. Appropriate chain-of-custody (COC) procedures were employed during sample collection and transport. Upon arrival at Enthalpy, coolers were opened, samples inspected, and the contents verified against information on the COC form. Receipt temperature was measured and recorded on the COC form. Standard water quality parameters were measured and recorded on a sample check-in sheet. Samples were stored at 4°C in the dark until used for testing. Standard water quality measurements conducted upon sample receipt are summarized in Table 1.

Table 1. Sample Information

Parameter	Effluent Samples		
Lab Log-In No.	24-0225	24-0238	24-0246
Collection Date and Time	2/19/2024, 13:00	2/21/2024, 10:00	2/22/2024, 10:00
Receipt Date and Time	2/20/2024, 09:20	2/22/2024, 09:50	2/23/2024, 09:30
Receipt Temperature (°C)	1.2	4.0	2.5
Dissolved Oxygen (mg/L)	10.6	11.0	11.2
pH	7.30	7.21	7.28
Conductivity (µS/cm)	464	445	465
Hardness (mg/L CaCO ₃)	138	106	144
Alkalinity (mg/L CaCO ₃)	100	94	89
Total Chlorine (mg/L)	<0.02	<0.02	<0.02
Total Ammonia (mg/L as N)	ND	ND	ND

ND = not detected

Test Methods

The chronic fathead minnow test was conducted according to procedures presented by USEPA (2002b) and the methods are summarized in Table 2. The acute toxicity tests were conducted according to procedures presented by USEPA (2002a) and the methods are summarized in Tables 3 and 4.

Table 2. Summary of Methods for the Chronic Fathead Minnow Toxicity Test

Test Period	2/20/2024, 14:00 to 2/27/2024 12:20
Test Organism	<i>Pimephales promelas</i> (fathead minnow)
Test Organism Source; Age at Initiation	Aquatic BioSystems, Fort Collins, CO; 1-day post-hatch
Test Duration	7 days
Feeding	<i>Artemia</i> nauplii, three times daily
Test Chamber	500 mL plastic cup
Test Solution Volume	250 mL
Test Temperature	25 ± 1°C
Control/Dilution Water	Diluted mineral water (per EPA protocol)
Test Concentrations (% Sample)	100, 41.7, 25, 12.5, 2.81, 0 (laboratory control).
Number of Replicates	4
Number of Organisms/Replicate	10
Photoperiod	16 hours light/8 hours dark
Test Protocol	EPA-821-R-02-013 (chronic) , WDOE 2016
Test Acceptability Criteria	Mean control survival ≥ 80%, mean control dry biomass ≥ 0.25 mg/org, Percent Minimum Significant Difference (PMSD) recommended range for biomass: 12 – 30
Statistical Analysis Software	CETIS™ v.2.1.4.11
Reference Toxicant	Copper chloride

Table 3. Summary of Methods for the 96-hour Fathead Minnow Acute Survival Test

Test Period	2/22/2024, 15:00 to 2/26/2024, 15:35
Test Organism	<i>Pimephales promelas</i> (fathead minnow)
Test Organism Source; Age	Aquatic BioSystems, Fort Collins, CO; 6 days post-hatch
Test Duration	96 ± 2 hours
Feeding	<i>Artemia</i> nauplii during holding time and 2 hours prior to test solution renewal
Test Chamber	500 mL plastic cup
Test Solution Volume	250 mL
Test Temperature	20 ± 1°C
Dilution/Control Water	Diluted mineral water (per EPA protocol)
Test Concentrations (% sample)	100, 41.7, 25, 12.5, 6.25 and 0 (laboratory control)
Number of Organisms/Chamber	10
Number of Replicates/Concentration	4
Photoperiod	16 hours light/8 hours dark
Test Protocol	EPA-821-R-02-012 , WDOE 2016
Test Acceptability Criterion for Controls	≥ 90% mean survival
Statistical Analysis Software	CETIS™ v2.1.4.11
Reference Toxicant	Copper chloride

Table 4. Summary of Methods for the 48-hour Water Flea Acute Survival Test

Test Period	2/22/2024, 15:00 to 2/24/2024, 14:50
Test Organism	<i>Ceriodaphnia dubia</i> (water flea)
Test Organism Source, Age	In-house culture, < 24 hours
Test Duration	48 ± 2 hours
Feeding	50:50 mixture Yeast-Trout Chow-Cerophyl (YTC): algal suspension during organism holding. No feeding during test.
Test Chamber	30 mL plastic cup
Test Solution Volume	15 mL
Test Temperature	20 ± 1°C
Dilution/Control Water	Diluted mineral water (per EPA protocol)
Test Concentrations (% Sample)	100, 41.7, 25, 12.5, 6.25, 0 (laboratory control)
Number of Organisms/Chamber	5
Number of Replicates	4
Photoperiod	16 hours light/8 hours dark
Test Protocols	EPA/821/R-02/012, WDOE 2016
Test Acceptability Criterion for Controls	≥ 90% mean survival
Statistical Analysis Software	CETIS™ v.2.1.4.11
Reference Toxicant	Copper chloride

Results

There were no acute or chronic statistically significant effects detected in any effluent concentration tested for both species, resulting in a no observed effect concentration (NOEC) of 100 percent for all tests. Additionally, no statistically significant effects were observed in the acute critical effluent concentration (ACEC) of 41.7 percent effluent and the chronic critical effluent concentration (CCEC) of 2.81 percent effluent. Both acute tests met the acute statistical power standard of 29 percent with a zero percent difference at the ACEC concentration of 41.7 percent effluent. The chronic fathead minnow test also met the chronic statistical power standard of 39 percent with a ten percent difference at the CCEC effluent concentration of 2.81 percent. The acute and chronic statistical power standards are defined in WAC 173-205-020 (WAC 1994).

Statistical results for the chronic toxicity fathead minnow test are summarized in Table 5 and the statistical results for the acute toxicity tests are summarized in Table 6. Detailed chronic and acute test results are summarized in Table 7 and 8 respectively. Individual statistical summaries for all tests including laboratory bench data sheets, and copies of the sample receipt information and COC forms are provided in Appendices A through C.

Table 5. Summary of Chronic Toxicity Statistical Results

Species and Endpoint	NOEC (% effluent)	EC ₅₀ (% effluent)
Fathead Minnow		
Survival	100	> 100
Growth (biomass)	100	> 100

NOEC = No Observed Effect Concentration; the highest concentration at which no effect is observed.

EC₅₀ = Median effect concentration; the effluent concentration estimated to produce an adverse effect to 50 percent of the test organisms.

Table 6. Summary of Acute Toxicity Statistical Results

Species and Endpoint	NOEC (% effluent)	EC ₅₀ (% effluent)
Water Flea		
48hr Survival	100	> 100
Fathead Minnow		
96hr Survival	100	> 100

NOEC = No Observed Effect Concentration; the highest concentration at which no effect is observed.

EC₅₀ = Median effect concentration; the effluent concentration estimated to produce an adverse effect to 50 percent of the test organisms.

Table 7. Summary of Chronic Toxicity Test Results

Test Concentration (% effluent)	Fathead Minnow	
	Mean Survival (%)	Mean Biomass (mg)
Lab Control	100	0.459
2.81	90.0	0.458
12.5	100	0.489
25	95.0	0.493
41.7	97.5	0.524
100	95.0	0.542

Table 8. Summary of Acute Toxicity Results

Test Concentration (% effluent)	Water Flea	Fathead Minnow
	Mean 48hr Survival (%)	Mean 96hr Survival (%)
Lab Control	100	100
6.25	100	97.5
12.5	100	100
25	100	100
14.7	100	100
100	95.0	97.5

Quality Assurance

Samples were received in good condition and within the recommended temperature range according to WDOE, 2016. All tests were initiated within the required 36-hour holding time. Mean control responses for all reported tests met minimum test acceptability criteria. In addition to the chronic fathead minnow test, a chronic water flea test was started on February 20, 2024, per the permit requirements. The chronic water flea test, however, did not meet test acceptability criteria, and is therefore not included in this report. The chronic water flea test will be completed by a different laboratory.

Statistical analyses followed standard USEPA flowchart selections and dose response relationships were reviewed to ensure the validity of the data. Based on the dose responses observed during testing, the statistical results are deemed reliable. Minor QA/QC issues that were not likely to have any bearing on results are noted on test data sheets. A list of laboratory qualifier codes used on bench data sheets is provided in Appendix D.

Reference Toxicant Test

The monthly reference toxicant tests for both species met minimum test acceptability requirements. The calculated median effect values for all endpoints were within two standard deviations of the historical means, indicating typical organism sensitivity to copper for our laboratory. Reference toxicant test results, including control chart coefficients of variation (CV), are summarized in Table 9 and presented in full in Appendix E.

Table 9. Reference Toxicant Test Results

Species and Endpoint	EC ₅₀ (µg/L copper)	Historical Mean ± 2 SD (µg/L copper)	CV (%)
Fathead minnow (chronic)			
Survival	42.3	108 ± 111.1	51.4
Growth (biomass)	31.8	97.8 ± 94.9	48.5
Fathead minnow (acute)			
96hr Survival	68.6	68.9 ± 74.4	54.0
Water flea (acute)			
48hr Survival	23.8	17.4 ± 12.9	37.0

EC₅₀ = Median effect concentration; the effluent concentration estimated to produce an adverse effect to 50 percent of the test organisms.

Historical Mean ± 2 SD = the mean EC₅₀ value from 20 previous reference toxicant tests conducted at Enthalpy, ± two standard deviations (SD).

CV = coefficient of variation

References

- Tidepool Scientific Software. 2000-2022. CETIS Comprehensive Environmental Toxicity Information System Software, Version 2.1.4.11.
- USEPA. 2002a. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition. EPA-821-R-02-012.
- USEPA. 2002b. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Fourth Edition. United States Environmental Protection Agency Office of Water, Washington DC. EPA/821/R-02/013.
- WDOE. 2016. Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria. Washington State Department of Ecology. Water Quality Program. Publication number: WQ-R-95-80, Revised June 2016.

Appendix A
Statistical Summaries and Raw Bench Sheets

Fathead Minnow Chronic Test

CETIS Summary Report

Report Date: 07 Mar-24 10:40 (p 1 of 1)
 Test Code/ID: 2402-S131 / 06-2506-5608

Fathead Minnow 7-d Larval Survival and Growth Test

Nautilus Environmental (CA)

Batch ID: 13-2898-8633	Test Type: Growth-Survival (7d)	Analyst:
Start Date: 20 Feb-24 14:00	Protocol: EPA/821/R-02-013 (2002)	Diluent: Diluted Mineral Water (8:2)
Ending Date: 27 Feb-24 12:20	Species: Pimephales promelas	Brine: Not Applicable
Test Length: 6d 22h	Taxon:	Source: Aquatic Biosystems, CO Age: 1d

Sample ID: 08-7875-6123	Code: 24-0225	Project:
Sample Date: 19 Feb-24 13:00	Material: POTW Effluent	Source: Snoqualmie WWTP (WA0022403)
Receipt Date: 20 Feb-24 09:20	CAS (PC):	Station: Effluent
Sample Age: 25h (1.2 °C)	Client: City of Snoqualmie	

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	TU	S
12-0373-6332	7d Survival Rate	Steel Many-One Rank Sum Test		100	>100	---	9.93%	1	1
10-4379-5238	Mean Dry Biomass-mg	Dunnett Multiple Comparison Test		100	>100	---	13.9%	1	1

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits			Decision
				Lower	Upper	Overlap	
12-0373-6332	7d Survival Rate	Control Resp	1	0.8	<<	Yes	Passes Criteria
10-4379-5238	Mean Dry Biomass-mg	Control Resp	0.459	0.25	<<	Yes	Passes Criteria
10-4379-5238	Mean Dry Biomass-mg	PMSD	0.139	0.12	0.3	Yes	Passes Criteria

7d Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LC	4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
2.81		4	0.900	0.770	1.030	0.800	1.000	0.041	0.082	9.07%	10.00%
12.5		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
25		4	0.950	0.858	1.040	0.900	1.000	0.029	0.058	6.08%	5.00%
41.7		4	0.975	0.895	1.050	0.900	1.000	0.025	0.050	5.13%	2.50%
100		4	0.950	0.791	1.110	0.800	1.000	0.050	0.100	10.53%	5.00%

Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LC	4	0.459	0.434	0.483	0.445	0.472	0.00765	0.0153	3.34%	0.00%
2.81		4	0.458	0.401	0.515	0.414	0.496	0.0178	0.0355	7.76%	0.16%
12.5		4	0.489	0.425	0.553	0.435	0.531	0.0201	0.0402	8.23%	-6.54%
25		4	0.493	0.443	0.544	0.452	0.52	0.0158	0.0316	6.39%	-7.57%
41.7		4	0.524	0.48	0.567	0.503	0.562	0.0137	0.0274	5.23%	-14.17%
100		4	0.542	0.448	0.637	0.456	0.59	0.0296	0.0592	10.92%	-18.26%

7d Survival Rate Detail

MD5: 708A437CD146A51D19BBD95185B6F099

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	LC	1.000	1.000	1.000	1.000
2.81		0.800	0.900	0.900	1.000
12.5		1.000	1.000	1.000	1.000
25		0.900	1.000	0.900	1.000
41.7		1.000	1.000	1.000	0.900
100		0.800	1.000	1.000	1.000

Mean Dry Biomass-mg Detail

MD5: C350AB82A02E8CA52C8ACFE791C39D4C

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	LC	0.472	0.472	0.445	0.446
2.81		0.414	0.447	0.496	0.475
12.5		0.531	0.435	0.487	0.502
25		0.486	0.52	0.452	0.516
41.7		0.562	0.503	0.505	0.525
100		0.456	0.558	0.59	0.566

CETIS Analytical Report

Report Date: 07 Mar-24 10:40 (p 1 of 3)
Test Code/ID: 2402-S131 / 06-2506-5608

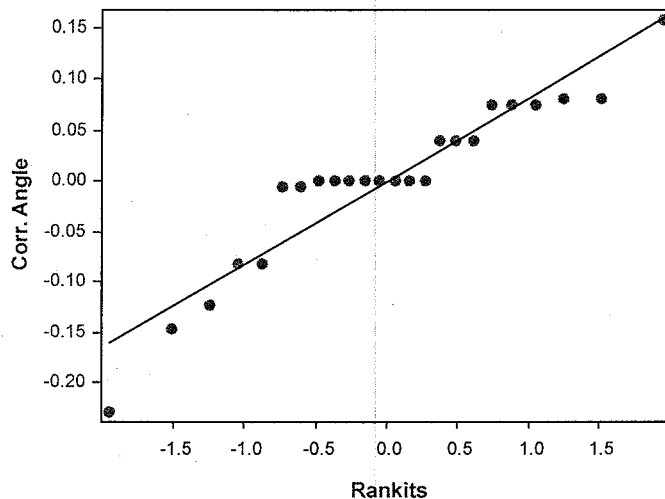
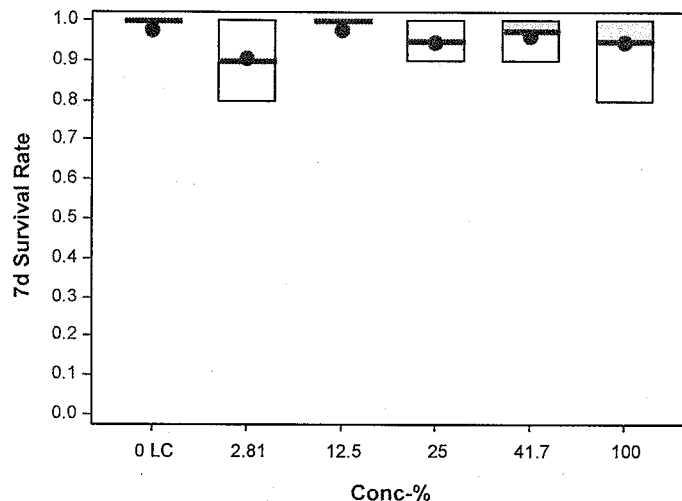
Fathead Minnow 7-d Larval Survival and Growth Test										Nautilus Environmental (CA)	
Analysis ID: 12-0373-6332		Endpoint: 7d Survival Rate					CETIS Version:		CETISv2.1.4		
Analyzed: 07 Mar-24 10:40		Analysis: Nonparametric-Control vs Treatments					Status Level:		1		
Edit Date: 07 Mar-24 10:13		MD5 Hash: 708A437CD146A51D19BBD95185B6F099					Editor ID:		000-502-715-6		
Data Transform		Alt Hyp			NOEL		LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)		C > T			100		>100	---	1	0.0993	9.93%
Steel Many-One Rank Sum Test											
Control	vs	Conc-%	df	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)		
Lab Control		2.81	6	12	10	1	CDF	0.1424	Non-Significant Effect		
		12.5	6	18	10	1	CDF	0.8333	Non-Significant Effect		
		25	6	14	10	1	CDF	0.3451	Non-Significant Effect		
		41.7	6	16	10	1	CDF	0.6105	Non-Significant Effect		
		100	6	16	10	1	CDF	0.6105	Non-Significant Effect		
ANOVA Table											
Source		Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α:5%)		
Between		0.0713546		0.0142709		5	1.58	0.2165	Non-Significant Effect		
Error		0.16277		0.0090428		18					
Total		0.234125				23					
ANOVA Assumptions Tests											
Attribute	Test					Test Stat	Critical	P-Value	Decision(α:1%)		
Variance	Bartlett Equality of Variance Test								Indeterminate		
Distribution	Shapiro-Wilk W Normality Test					0.906	0.884	0.0282	Normal Distribution		
7d Survival Rate Summary											
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
2.81		4	0.900	0.770	1.000	0.900	0.800	1.000	0.041	9.07%	10.00%
12.5		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
25		4	0.950	0.858	1.000	0.950	0.900	1.000	0.029	6.08%	5.00%
41.7		4	0.975	0.895	1.000	1.000	0.900	1.000	0.025	5.13%	2.50%
100		4	0.950	0.791	1.000	1.000	0.800	1.000	0.050	10.53%	5.00%
Angular (Corrected) Transformed Summary											
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	4	1.410	1.410	1.410	1.410	1.410	1.410	0.000	0.00%	0.00%
2.81		4	1.250	1.060	1.450	1.250	1.110	1.410	0.062	9.93%	11.17%
12.5		4	1.410	1.410	1.410	1.410	1.410	1.410	0.000	0.00%	0.00%
25		4	1.330	1.180	1.480	1.330	1.250	1.410	0.047	7.07%	5.77%
41.7		4	1.370	1.240	1.500	1.410	1.250	1.410	0.041	5.94%	2.89%
100		4	1.340	1.090	1.580	1.410	1.110	1.410	0.076	11.41%	5.40%

CETIS Analytical Report

Report Date: 07 Mar-24 10:40 (p 2 of 3)
Test Code/ID: 2402-S131 / 06-2506-5608

Fathead Minnow 7-d Larval Survival and Growth Test			Nautilus Environmental (CA)
Analysis ID: 12-0373-6332	Endpoint: 7d Survival Rate	CETIS Version: CETISv2.1.4	
Analyzed: 07 Mar-24 10:40	Analysis: Nonparametric-Control vs Treatments	Status Level: 1	
Edit Date: 07 Mar-24 10:13	MD5 Hash: 708A437CD146A51D19BBD95185B6F099	Editor ID: 000-502-715-6	

Graphics



CETIS Analytical Report

Report Date: 07 Mar-24 10:40 (p 3 of 3)
Test Code/ID: 2402-S131 / 06-2506-5608

Fathead Minnow 7-d Larval Survival and Growth Test				Nautilus Environmental (CA)	
Analysis ID: 10-4379-5238		Endpoint: Mean Dry Biomass-mg		CETIS Version: CETISv2.1.4	
Analyzed: 07 Mar-24 10:40		Analysis: Parametric-Control vs Treatments		Status Level: 1	
Edit Date: 07 Mar-24 10:13		MD5 Hash: C350AB82A02E8CA52C8ACFE791C39D4		Editor ID: 000-502-715-6	

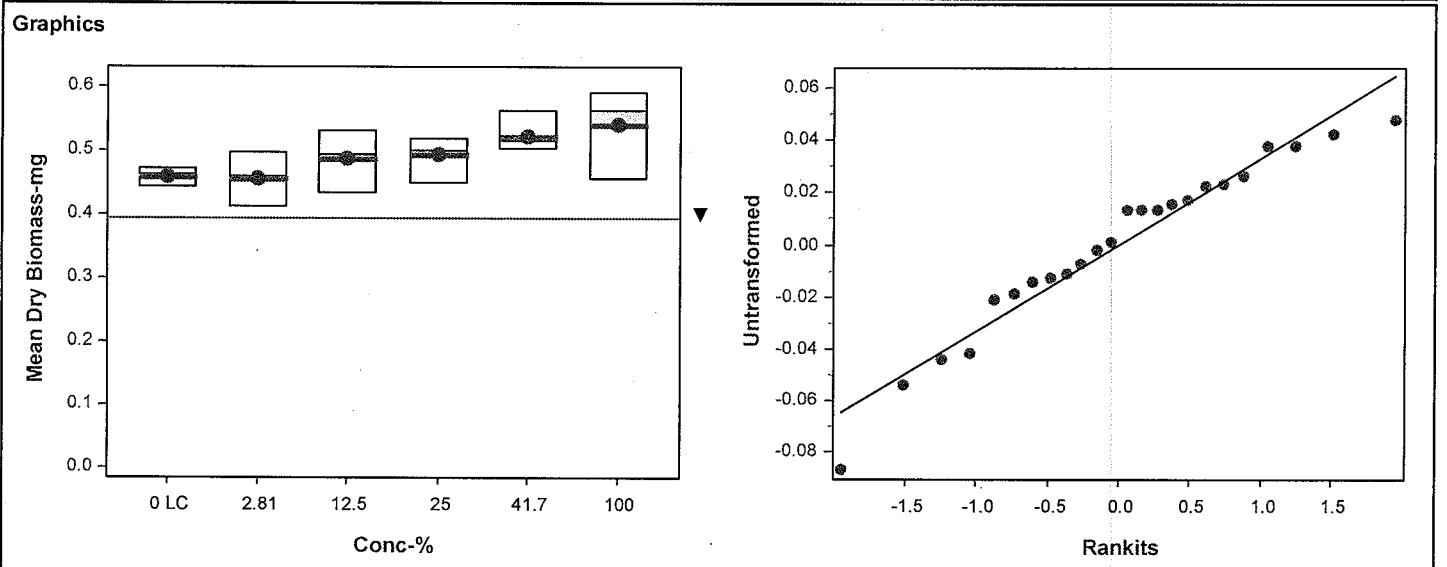
Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Untransformed	C > T	100	>100	---	1	0.0636	13.86%

Dunnett Multiple Comparison Test									
Control	vs	Conc-%	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Lab Control		2.81	6	0.0284	2.41	0.0636	CDF	0.8248	Non-Significant Effect
		12.5	6	-1.14	2.41	0.0636	CDF	0.9881	Non-Significant Effect
		25	6	-1.32	2.41	0.0636	CDF	0.9929	Non-Significant Effect
		41.7	6	-2.46	2.41	0.0636	CDF	0.9998	Non-Significant Effect
		100	6	-3.17	2.41	0.0636	CDF	1.0000	Non-Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0232137	0.0046427	5	3.33	0.0265	Significant Effect
Error	0.0251102	0.0013950	18			
Total	0.0483239		23			

ANOVA Assumptions Tests						
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)	
Variance	Bartlett Equality of Variance Test	4.71	15.1	0.4528	Equal Variances	
Distribution	Shapiro-Wilk W Normality Test	0.947	0.884	0.2295	Normal Distribution	

Mean Dry Biomass-mg Summary											
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	4	0.459	0.434	0.483	0.463	0.445	0.472	0.00765	3.34%	0.00%
2.81		4	0.458	0.401	0.515	0.461	0.414	0.496	0.0178	7.76%	0.16%
12.5		4	0.489	0.425	0.553	0.494	0.435	0.531	0.0201	8.23%	-6.54%
25		4	0.493	0.443	0.544	0.501	0.452	0.52	0.0158	6.39%	-7.57%
41.7		4	0.524	0.48	0.567	0.515	0.503	0.562	0.0137	5.23%	-14.17%
100		4	0.542	0.448	0.637	0.562	0.456	0.59	0.0296	10.92%	-18.26%



Client: City of Snoqualmie

Test Species: P. promelas

Sample ID: Effluent

Start Date/Time: 2/20/24 1400

Test No's.: 2402-5131

End Date/Time: 2/27/24 1220

Concentration (%)	Rep.	Rand #	Test Day / No. Organisms Alive								Percent Survival
			0	1	2	3	4	5	6	7	
Lab Control	a	4	10	10	10	10	10	10	10	10	100
	b	10	10	10	10	10	10	10	10	10	100
	c	5	10	10	10	10	10	10	10	10	100
	d	9	10	10	10	10	10	10	10	10	100
2.81	a	23	10	9	8	8	8	8	8	8	80
	b	18	10	10	10	10	10	10	10	9	90
	c	3	10	9	9	9	9	9	9	9	90
	d	6	10	10	10	10	10	10	10	10	100
12.5	a	19	10	10	10	10	10	10	10	10	100
	b	14	10	10	10	10	10	10	10	10	100
	c	2	10	10	10	10	10	10	10	10	100
	d	17	10	10	10	10	10	10	10	10	100
25	a	20	10	10	10	10	9	9	9	9	90
	b	11	10	10	10	10	10	10	10	10	100
	c	7	10	9	9	9	9	9	9	9	90
	d	22	10	10	10	10	10	10	10	10	100
41.7	a	8	10	10	10	10	10	10	10	10	100
	b	15	10	10	10	10	10	10	10	10	100
	c	1	10	10	10	10	10	10	10	10	100
	d	21	10	9	9	9	9	9	9	9	90
100	a	13	10	10	10	10	10	10	8	8	80
	b	16	10	10	10	10	10	10	10	10	100
	c	24	10	10	10	10	10	10	10	10	100
	d	12	10	10	10	10	10	10	10	10	100

Rand # QC: MU

Tech Initials

MU MU MU WF WF GM RT GM

Initial Count QC'd by: GM

Time

1400 1025 1205 1420 1255 1205 1415 1220

Initiated by: MU

Time Fed (day): 0 1 2 3 4 5 6

morning:	—	0925	0940	0840	0840	0930	0830
midday:	—	1305	1230	1230	1155	1240	1135
evening:	11030	1605	1615	1635	1625	1620	1605

Comments:

Drying Oven Info

Tare wt. Initials/Date: AD 02/20/24

Date/Time in: 2/27/24 1240

Date/Time out: 2/28/24 1045

Temp (°C): 67.0

QC Check: CM 3/6/24

Final Review: 20 3/14/24

Freshwater Chronic Bioassay

DF-010

Larval Fish Weights

Client: City of SnoqualmieTest Species: Pimephales promelasSample ID: EffluentStart Date/Time: 02/20/24 1400Test No.: 2402-S131End Date/Time: 2/27/24 1220Initial # Fish: 10

Conc. (^a <u>ug/L</u>) %	Rep.	pan weight (mg)	pan + fish weight (mg)	organism weight (mg)
Lab Control	a	34.54	39.26	4.72
	b	34.29	39.01	4.72
	c	32.88	37.33	4.45
	d	31.84	36.30	4.46
2.81	a	32.97	37.11	4.14
	b	33.34	37.81	4.47
	c	31.01	35.97	4.96
	d	32.58	37.33	4.75
12.5	a	32.47	37.78	5.31
	b	32.12	36.47	4.35
	c	32.47	37.34	4.87
	d	30.74	35.76	5.02
25	a	32.99	37.85	4.86
	b	29.05	34.25	5.20
	c	30.30	34.82	4.52
	d	31.06	36.22	5.16
^a 41.7 <u>41.7</u>	a	30.44	36.06	5.62
	b	30.11	35.14	5.03
	c	32.54	37.59	5.05
	d	31.05	36.30	5.25
100	a	29.95	34.51	4.56
	b	31.92	37.50	5.58
	c	30.42	36.32	5.90
	d	30.51	36.17	5.66
	a			0.00
	b			0.00
	c			0.00
	d			0.00
Tech Initials:		AD	CHR	
Date/Time:		02/26/24 1235	2/29/24 1045	

QC Check: CM 3/6/24Final Review: Bo 3/14/24

Freshwater Chronic Bioassay

DF-008

Water Quality Measurements

Client: City of Snoqualmie

Sample ID: Effluent

Test No's: 2402-5131

Test Species: *P. promelas*

Start Date/Time: 2/20/24 1400

End Date/Time: 2/27/24 1220

Concentration	Lab Control							
Day	0	1	2	3	4	5	6	7
Initial								
pH	8.14	8.06	8.10	8.00	8.25	8.16	8.03	
DO (mg/L)	7.7	8.3	8.2	8.1	8.1	8.2	7.8	
Cond. (µmhos/cm)	200	200	195	202	194	191	205	
Temp (°C)	26.0	24.1	24.1	24.0	24.0	24.0	24.2	
Final								
pH		8.09	8.11	7.85	7.45	7.80	7.92	7.80
DO (mg/L)		7.6	7.5	7.1	7.3	7.0	7.2	7.2
Temp (°C)		24.7	24.4	24.8	24.4	24.9	24.8	25.6

Concentration	2.81%							
Day	0	1	2	3	4	5	6	7
Initial								
pH	8.11	8.05	8.20	7.99	8.20	8.12	8.05	
DO (mg/L)	7.7	8.3	8.2	8.1	8.1	8.2	7.8	
Cond. (µmhos/cm)	211	211	209	209	208	205	214	
Temp (°C)	26.0	24.1	24.0	24.0	24.0	24.0	24.0	
Final								
pH		8.10	8.04	7.83	7.93	7.82	7.89	7.81
DO (mg/L)		7.0	6.8	6.8	7.0	6.8	6.8	6.6
Temp (°C)		25.1	25.2	24.9	25.0	25.0	24.9	25.7

Concentration	12.5%							
Day	0	1	2	3	4	5	6	7
Initial								
pH	8.02	7.98	8.17	7.88	8.09	8.00	8.03	
DO (mg/L)	7.7	8.2	8.3	8.1	8.1	8.2	7.8	
Cond. (µmhos/cm)	232	230	223	230	230	225	237	
Temp (°C)	26.0	24.2	24.1	24.1	24.0	24.0	24.1	
Final								
pH		7.98	8.00	7.66	7.86	7.71	7.84	7.79
DO (mg/L)		6.7	6.7	6.0	6.7	6.3	6.4	6.5
Temp (°C)		25.0	24.9	25.2	24.9	25.2	25.4	25.6

Environmental Chamber: A

Animal Source/Date Received: ABS / 2/20/24

Animal Age at Initiation: 1d

Animal Acclimation Qualifiers (circle all that apply): Q22 / Q23 / Q24 / none

Sample Log-in Numbers: A: 24-0225 B: 24-0238

C: 24-0246

Concentration	25%							
Day	0	1	2	3	4	5	6	7
Initial								
pH	7.35	7.39	7.98	7.73	7.92	7.85	7.95	
DO (mg/L)	7.9	8.3	8.2	8.1	8.2	8.3	8.0	
Cond. (µmhos/cm)	269	263	255	268	263	258	278	
Temp (°C)	26.0	24.2	24.2	24.1	24.1	24.0	24.3	
Final								
pH		8.02	8.04	7.80	7.87	7.78	7.81	7.75
DO (mg/L)		6.3	6.9	6.3	6.8	6.4	6.3	6.4
Temp (°C)		24.9	24.8	25.3	25.0	25.0	25.4	25.4

Concentration	41.7%							
Day	0	1	2	3	4	5	6	7
Initial								
pH	7.70	7.80	7.81	7.62	7.76	7.70	7.79	
DO (mg/L)	8.0	8.4	8.3	8.3	8.3	8.4	8.1	
Cond. (µmhos/cm)	308	302	293	308	309	302	324	
Temp (°C)	26.0	24.2	24.2	24.1	24.1	24.0	24.2	
Final								
pH		8.06	8.00	7.74	7.77	7.69	7.72	7.70
DO (mg/L)		6.8	6.8	6.2	6.4	6.1	6.0	6.2
Temp (°C)		24.4	24.6	24.5	24.9	25.2	25.5	25.1

Concentration	100%							
Day	0	1	2	3	4	5	6	7
Initial								
pH	7.39	7.53	7.45	7.34	7.44	7.39	7.56	
DO (mg/L)	8.8	8.6	8.4	8.5	8.6	8.7	8.3	
Cond. (µmhos/cm)	453	450	436	464	462	450	482	
Temp (°C)	24.2	24.3	24.5	24.2	24.0	24.0	24.3	
Final								
pH		7.94	7.88	7.60	7.57	7.46	7.53	7.48
DO (mg/L)		6.8	6.4	5.6	5.6	5.1	5.5	5.5
Temp (°C)		24.9	24.8	25.0	24.9	25.2	25.2	25.6

		0	1	2	3	4	5	6	7
Analysts:	Initial:	GM	MU	MU	WF	WF	GM	RT	
	Final:		MU	MU	WF	WF	GM	RT	MU
Dilutions made by:		LM	LM	LM	LM	LM	LM	HH	
Sample Used (A, B, C):		A	A	B	C	C	C	C	

Comments: @ 2/20/24 @ 2/21/24 @ 2/22/24

QC Check: CM 3/6/24

Final Review: BO 3/14/24

Fathead Minnow Acute Test

CETIS Summary Report

Report Date: 07 Mar-24 10:06 (p 1 of 1)
 Test Code/ID: 2402-S132 / 01-1095-4253

Fathead Minnow 96-h Acute Survival Test

Nautilus Environmental (CA)

Batch ID: 10-8952-8083	Test Type: Survival (96h)	Analyst:
Start Date: 22 Feb-24 15:00	Protocol: EPA/821/R-02-012 (2002)	Diluent: Diluted Mineral Water (8:2)
Ending Date: 26 Feb-24 15:35	Species: Pimephales promelas	Brine: Not Applicable
Test Length: 4d 1h	Taxon:	Source: Aquatic Biosystems, CO Age: 6d

Sample ID: 12-4802-3202	Code: 24-0238	Project:
Sample Date: 21 Feb-24 10:00	Material: POTW Effluent	Source: Snoqualmie WWTP (WA0022403)
Receipt Date: 22 Feb-24 09:50	CAS (PC):	Station: Effluent
Sample Age: 29h (4 °C)	Client: City of Snoqualmie	

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	TU	S
11-4191-5583	96h Survival Rate	Steel Many-One Rank Sum Test	100	>100	---	5.6%	1	1

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
11-4191-5583	96h Survival Rate	Control Resp	1	0.9	<<	Yes	Passes Criteria

96h Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LC	4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
6.25		4	0.975	0.895	1.050	0.900	1.000	0.025	0.050	5.13%	2.50%
12.5		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
25		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
41.7		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
100		4	0.975	0.895	1.050	0.900	1.000	0.025	0.050	5.13%	2.50%

96h Survival Rate Detail

MD5: 948DCF2C3756BC2A575B4F0784C1F62C

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	LC	1.000	1.000	1.000	1.000
6.25		1.000	1.000	1.000	0.900
12.5		1.000	1.000	1.000	1.000
25		1.000	1.000	1.000	1.000
41.7		1.000	1.000	1.000	1.000
100		0.900	1.000	1.000	1.000

CETIS Analytical Report

Report Date: 07 Mar-24 10:06 (p 1 of 2)
Test Code/ID: 2402-S132 / 01-1095-4253

Fathead Minnow 96-h Acute Survival Test				Nautilus Environmental (CA)	
Analysis ID: 11-4191-5583		Endpoint: 96h Survival Rate		CETIS Version: CETISv2.1.4	
Analyzed: 07 Mar-24 10:06		Analysis: Nonparametric-Control vs Treatments		Status Level: 1	
Edit Date: 07 Mar-24 9:32		MD5 Hash: 948DCF2C3756BC2A575B4F0784C1F62C		Editor ID: 000-502-715-6	

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	100	>100	---	1	0.056	5.60%

Steel Many-One Rank Sum Test									
Control	vs	Conc-%	df	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)
Lab Control		6.25	6	16	10	1	CDF	0.6105	Non-Significant Effect
		12.5	6	18	10	1	CDF	0.8333	Non-Significant Effect
		25	6	18	10	1	CDF	0.8333	Non-Significant Effect
		41.7	6	18	10	1	CDF	0.8333	Non-Significant Effect
		100	6	16	10	1	CDF	0.6105	Non-Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0088531	0.0017706	5	0.8	0.5640	Non-Significant Effect
Error	0.039839	0.0022133	18			
Total	0.0486921		23			

ANOVA Assumptions Tests						
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)	
Variance	Bartlett Equality of Variance Test				Indeterminate	
Distribution	Shapiro-Wilk W Normality Test	0.615	0.884	<1.0E-05	Non-Normal Distribution	

96h Survival Rate Summary											
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
6.25		4	0.975	0.895	1.000	1.000	0.900	1.000	0.025	5.13%	2.50%
12.5		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
25		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
41.7		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
100		4	0.975	0.895	1.000	1.000	0.900	1.000	0.025	5.13%	2.50%

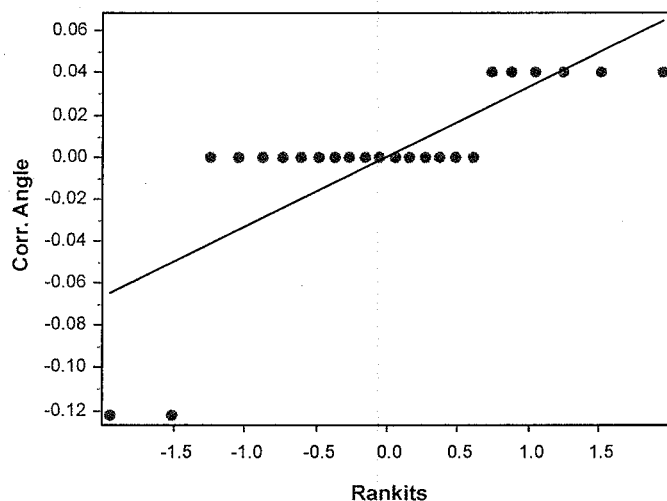
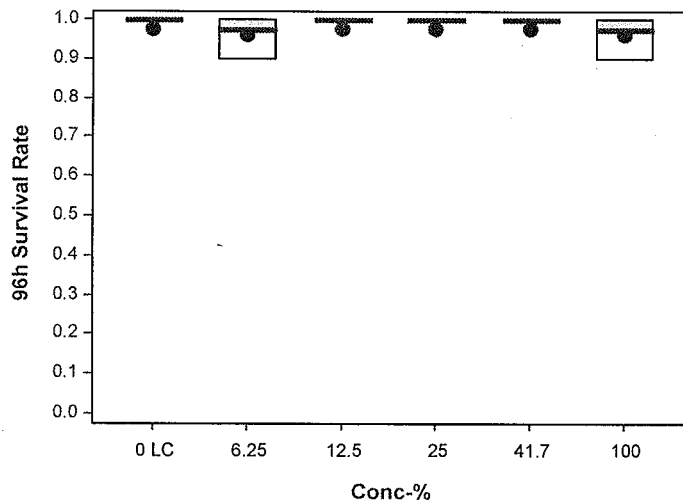
Angular (Corrected) Transformed Summary											
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	4	1.410	1.410	1.410	1.410	1.410	1.410	0.000	0.00%	0.00%
6.25		4	1.370	1.240	1.500	1.410	1.250	1.410	0.041	5.94%	2.89%
12.5		4	1.410	1.410	1.410	1.410	1.410	1.410	0.000	0.00%	0.00%
25		4	1.410	1.410	1.410	1.410	1.410	1.410	0.000	0.00%	0.00%
41.7		4	1.410	1.410	1.410	1.410	1.410	1.410	0.000	0.00%	0.00%
100		4	1.370	1.240	1.500	1.410	1.250	1.410	0.041	5.94%	2.89%

CETIS Analytical Report

Report Date: 07 Mar-24 10:06 (p 2 of 2)
Test Code/ID: 2402-S132 / 01-1095-4253

Fathead Minnow 96-h Acute Survival Test			Nautilus Environmental (CA)
Analysis ID: 11-4191-5583	Endpoint: 96h Survival Rate	CETIS Version: CETISv2.1.4	
Analyzed: 07 Mar-24 10:06	Analysis: Nonparametric-Control vs Treatments	Status Level: 1	
Edit Date: 07 Mar-24 9:32	MD5 Hash: 948DCF2C3756BC2A575B4F0784C1F62C	Editor ID: 000-502-715-6	

Graphics



96-hour Freshwater Acute Bioassay
Static-Renewal Conditions
DF-006

Water Quality Measurements
& Test Organism Survival

Client: City of Snoqualmie
Sample ID: Effluent
Sample Log-In: 24-0238
Test No.: 2402-5132

Test Species: P. promelas
Start Date/Time: 2/22/24 13:50
End Date/Time: 2/26/24 1535

Tech Initials				
0	24	48	72	96
WF	RT	WF	LM	PT
MF	WF	WF	GM	MM
LM		LM		

Counts: WF RT WF LM PT
Readings: MF WF WF GM MM

Dilutions made by: LM LM

Concentration (%)	RAND #	Number of Live Organisms					pH (units)					Dissolved Oxygen (mg/L)					Conductivity (µmhos/cm)					Temperature (°C)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	1	10	10	10	10	10	8.2	8.0	7.9	7.9	7.9	8.7	8.1	9.0	8.1	8.6	199	198	203	201	209	20.0	20.5	19.1	19.7	19.6
	13	10	10	10	10	10			7.9					8.7					208					19.0		
	20	10	10	10	10	10																				
	2	10	10	10	10	10																				
6.25	4	10	10	10	10	10	8.0	8.0	7.9	7.8	7.9	8.8	8.0	9.0	7.8	8.3	205	215	219	218	232	19.9	20.3	19.1	19.6	19.6
	7	10	10	10	10	10			8.1					8.7					233					18.8		
	17	10	10	10	10	10																	21			
	3	10	10	10	10	9																				
12.5	16	10	10	10	10	10	7.9	7.9	7.8	7.8	7.9	8.9	8.1	9.0	7.8	8.2	222	233	235	233	250	19.9	20.6	19.1	19.7	19.8
	19	10	10	10	10	10			7.9					8.5					243					19.1		
	21	10	10	10	10	10																				
	15	10	10	10	10	10																				
25	8	10	10	10	10	10	7.8	7.9	7.7	7.7	7.8	8.9	8.1	9.0	7.6	8.0	253	260	266	263	278	19.7	20.4	19.0	19.7	19.8
	24	10	10	10	10	10			7.9					8.5					275					18.9		
	22	10	10	10	10	10																	21			
	10	10	10	10	10	10																				
41.7 42	5	10	10	10	10	10	7.6	7.9	7.6	7.7	7.8	9.0	8.0	9.0	7.5	7.9	292	296	306	304	320	19.5	20.5	19.1	19.8	19.9
	11	10	10	10	10	10			7.9					8.3					311					19.1		
	18	10	10	10	10	10																				
	6	10	10	10	10	10																				
100	12	10	10	10	9	9	7.3	7.7	7.3	7.4	7.6	9.0	7.6	9.0	7.2	7.4	430	433	459	452	472	19.0	20.4	19.9	19.7	19.8
	23	10	10	10	10	10			7.8					8.0					460					19.0		
	14	10	10	10	10	10																				
	9	10	10	10	10	10																				

Rand # QC: WF
Initial Counts QC'd by: MF
Initiated by: WF

Environmental Chamber: C

Animal Source/Date Received: ABS/2/22/24

Age at Initiation: 5 days 6 days

Animal Acclimation Qualifiers (circle all that apply):

Q22 / Q23 / Q24 / none

Comments:

i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal Q23 8.6m 2/10/24

Organisms fed prior to initiation, circle one (y) / n) (B) Q18 m 2/22/24

QC Check:

LM 3/6/24

Q18 2/23/24

Q18 WF 2/24/24

Final Review: Bo 3/14/24

Enthalpy Analytical, 4340 Vandever Avenue, San Diego, CA 92120.

Feeding Times				
0	24	48	72	96
AM: --	--	<u>Q24</u>	--	--
PM: --	--	--	--	--

Water Flea Acute Test

CETIS Summary Report

Report Date: 07 Mar-24 10:02 (p 1 of 1)
 Test Code/ID: 2402-S134 / 16-6699-6433

Ceriodaphnia 48-h Acute Survival Test

Nautilus Environmental (CA)

Batch ID: 04-7535-1273	Test Type: Survival (48h)	Analyst:
Start Date: 22 Feb-24 15:00	Protocol: EPA/821/R-02-012 (2002)	Diluent: Diluted Mineral Water (8:2)
Ending Date: 24 Feb-24 14:50	Species: Ceriodaphnia dubia	Brine: Not Applicable
Test Length: 48h	Taxon:	Source: In-House Culture Age: <24

Sample ID: 01-7440-6428	Code: 24-0238	Project:
Sample Date: 21 Feb-24 10:00	Material: POTW Effluent	Source: Snoqualmie WWTP (WA0022403)
Receipt Date: 22 Feb-24 09:50	CAS (PC):	Station: Effluent
Sample Age: 29h (4 °C)	Client: City of Snoqualmie	

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	TU	S
07-2649-7184	48h Survival Rate	Steel Many-One Rank Sum Test	100	>100	---	9.2%	1	1

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
07-2649-7184	48h Survival Rate	Control Resp	1	0.9	<<	Yes	Passes Criteria

48h Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LC	4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
6.25		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
12.5		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
25		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
41.7		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
100		4	0.950	0.791	1.110	0.800	1.000	0.050	0.100	10.53%	5.00%

48h Survival Rate Detail

MD5: AD4EF308D1E22DC79AF92159E5D2E75E

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	LC	1.000	1.000	1.000	1.000
6.25		1.000	1.000	1.000	1.000
12.5		1.000	1.000	1.000	1.000
25		1.000	1.000	1.000	1.000
41.7		1.000	1.000	1.000	1.000
100		1.000	0.800	1.000	1.000

CETIS Analytical Report

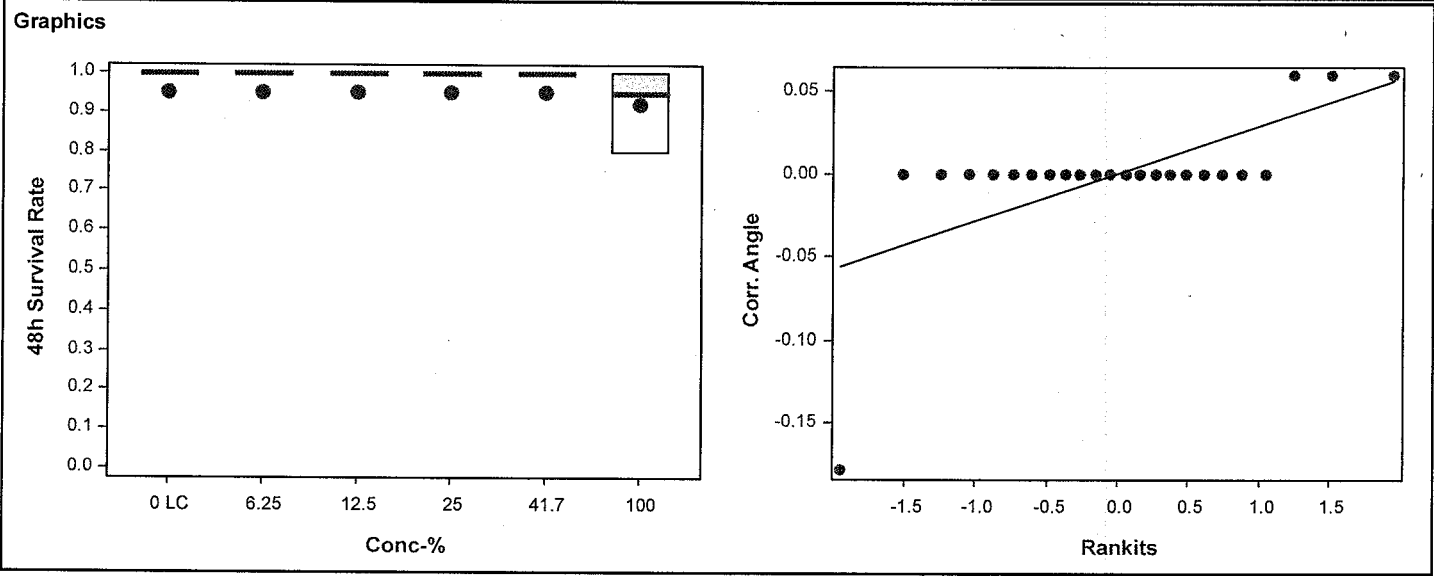
Report Date: 07 Mar-24 10:02 (p 1 of 2)
Test Code/ID: 2402-S134 / 16-6699-6433

Ceriodaphnia 48-h Acute Survival Test								Nautilus Environmental (CA)			
Analysis ID: 07-2649-7184		Endpoint: 48h Survival Rate			CETIS Version: CETISv2.1.4		CETISv2.1.4				
Analyzed: 07 Mar-24 10:02		Analysis: Nonparametric-Control vs Treatments			Status Level: 1		1				
Edit Date: 07 Mar-24 10:01		MD5 Hash: AD4EF308D1E22DC79AF92159E5D2E75E			Editor ID: 000-502-715-6		000-502-715-6				
Data Transform		Alt Hyp			NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD	
Angular (Corrected)		C > T			100	>100	---	1	0.092	9.20%	
Steel Many-One Rank Sum Test											
Control	vs	Conc-%	df	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)		
Lab Control		6.25	6	18	10	1	CDF	0.8333	Non-Significant Effect		
		12.5	6	18	10	1	CDF	0.8333	Non-Significant Effect		
		25	6	18	10	1	CDF	0.8333	Non-Significant Effect		
		41.7	6	18	10	1	CDF	0.8333	Non-Significant Effect		
		100	6	16	10	1	CDF	0.6105	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α:5%)			
Between	0.0118142		0.0023628		5	1	0.4457	Non-Significant Effect			
Error	0.0425309		0.0023628		18						
Total	0.0543451				23						
ANOVA Assumptions Tests											
Attribute	Test				Test Stat	Critical	P-Value	Decision(α:1%)			
Variance	Bartlett Equality of Variance Test							Indeterminate			
Distribution	Shapiro-Wilk W Normality Test				0.463	0.884	<1.0E-05	Non-Normal Distribution			
48h Survival Rate Summary											
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
6.25		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
12.5		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
25		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
41.7		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
100		4	0.950	0.791	1.000	1.000	0.800	1.000	0.050	10.53%	5.00%
Angular (Corrected) Transformed Summary											
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	4	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%
6.25		4	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%
12.5		4	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%
25		4	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%
41.7		4	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%
100		4	1.290	1.100	1.480	1.350	1.110	1.350	0.060	9.26%	4.43%

CETIS Analytical Report

Report Date: 07 Mar-24 10:02 (p 2 of 2)
Test Code/ID: 2402-S134 / 16-6699-6433

Ceriodaphnia 48-h Acute Survival Test			Nautilus Environmental (CA)	
Analysis ID: 07-2649-7184	Endpoint: 48h Survival Rate	CETIS Version: CETISv2.1.4		
Analyzed: 07 Mar-24 10:02	Analysis: Nonparametric-Control vs Treatments	Status Level: 1		
Edit Date: 07 Mar-24 10:01	MD5 Hash: AD4EF308D1E22DC79AF92159E5D2E75E	Editor ID: 000-502-715-6		



48-hour Freshwater Acute Bioassay
Static-Renewal Conditions
DF-020

Water Quality Measurements
& Test Organism Survival

Client: City of Snoqualmie
Test Species: *C. dubia*
Sample ID: Effluent
Sample Log-In: 24-0238
Test No.: 2402 - 5134
Start Date/Time: 2/22/24 1500
End Date/Time: 2/24/24 1450
Counts: LM HH VL
Readings: VC WF VL
Dilutions made by: LM

Tech Initials: 0 24 48

Concentration (%)	RAND #	Number of Live Organisms	pH (units)	Dissolved Oxygen (mg/L)	Conductivity (umhos/cm)	Temperature (°C)
Lab Control	1	5	8.12	8.6	198	20.5
	13	5	8.20	8.7	222	20.3
	20	5				
	2	5				
6.25	4	5	8.02	8.5	198	20.0
	7	5	8.21		228	20.3
	17	5				
	3	5				
12.5	16	5	7.92	8.7	228	20.5
	19	5	8.19		256	20.3
	21	5				
	15	5				
25	8	5	7.81	8.5	253	20.7
	24	5	8.5		279	20.3
	22	5				
	10	5				
41.7	5	5	7.43	8.5	298	20.0
	11	5	8.14		334	20.3
	18	5				
	6	5				
100	12	5	7.28	8.5	421	20.0
	23	5			470	20.3
	14	5				
	9	5				

Animal Source/Date Received: Internal N/A
Initial Count QC: 15
Rand # QC: 15
Environmental Chamber: 7
Age at Initiation: 224 hrs
Animal Acclimation Qualifiers (circle all that apply): Q22 / Q23 / Q24 / none
Comments: Organisms fed prior to initiation, circle one: ()
QC Check: (M 3/14/24)
Enthalpy Analytical, 4340 Vandever Avenue, San Diego, CA 92120

Final Review: 3/14/24

Appendix B
Sample Check-in Sheets

Enthalpy Analytical
4340 Vandever Avenue
San Diego, CA 92120

Client: City of Snoqualmie
Sample ID: 2A24, 22124, 22224
Test ID No(s): 2402-S131 to S134

NORTHWEST CLIENTS
Sample Check-In Information
DC-005

Sample (A, B, C):	A	B	C	
Log-in No. (24-xxxx):	0225	0238	0246	
Sample Collection Date & Time:	2/19/24 1300	2/21/24 1000	2/22/24 1000	
Sample Receipt Date & Time:	2/20/24 0920	2/22/24 0950	2/23/24 0930	
Number of Containers & Container Type:	1x10L cubi	1x10L cubi	1x10L cubi	
Approx. Total Volume Received (L):	~10	10	10	
Check-in Temperature (°C)	1.2	4.0	2.5	
Temperature OK? ¹	(Y) N	(Y) N	(Y) N	Y N
DO (mg/L)	10.6	11.0	11.2	
pH (units)	7.30	7.21	7.28	
Conductivity (µS/cm)	464	445	465	
Salinity (ppt)	0.2	0.2	0.2	
Alkalinity (mg/L) ²	100	94	89	
Hardness (mg/L) ^{2,3}	138	106	144	
Total Chlorine (mg/L)	10.02	10.02	10.02	
Technician Initials	mm	WF	AD	

Chronic Fathead, water
Plea, Acute Fathead, water Plea @

Test Performed: _____ Control/Dilution Water: 8:2 Lab SW / Lab ART Other: _____
Alkalinity: 89 Hardness or Salinity: 90
Additional Control? Y N = _____ Alkalinity: _____ Hardness or Salinity: _____

Test Performed: _____ Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: _____
Alkalinity: _____ Hardness or Salinity: _____
Additional Control? Y N = _____ Alkalinity: _____ Hardness or Salinity: _____

Test Performed: _____ Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: _____
Alkalinity: _____ Hardness or Salinity: _____
Additional Control? Y N = _____ Alkalinity: _____ Hardness or Salinity: _____

Notes: ¹ Temperature of sample should be 0-6°C at receipt.

² mg/L as CaCO₃, ³ Measured for freshwater samples only, NA = Not Applicable

Additional Comments: @ Chronic water Plea test did not meet TAC. See @A
Section of Report B03/14/24

Sample Description:

A: colorless, clear, odorless, no debris
B: light yellow, clear, no odor, no debris
C: light yellow, slightly opaque, no odor, no debris

Subsamples for Additional Chemistry Required:

NH3 (always required)

Other _____

Tech Initials AMU BWF CLK/AD

COC Complete (Y/N)?

A Y B Y C Y

Filtration? Y N Initials: _____

Pore Size: _____

Organisms _____ or Debris _____

Salinity Adjustment? Y N

Test: _____ Source: _____ Target ppt: _____
Test: _____ Source: _____ Target ppt: _____
Test: _____ Source: _____ Target ppt: _____

pH Adjustment? Y N

	A	B	C
Initial pH:			
Amount of HCl added:			
Final pH:			

Cl₂ Adjustment? Y N

	A	B	C
Initial Free Cl ₂ :			
STS added:			
Final Free Cl ₂ :			

Sample Aeration? Y N

	A	B	C
Initial D.O.			
Duration & Rate			
Final D.O.			

QC Check: CM 3/4/24

Final Review: B03/14/24

DC-001

Client: City of Snoqualmie

Project: February 2024 monitoring

Test Type: Various

DI Blank: 0.0

Test Start Date: ~~2/13/2024~~ 2/20/24

Q18 B0
2/16/24

Analyst: CHR

Analysis Date: 2/29/24

N x 1.22

$$\text{Relative Percent Difference (RPD)} = \frac{[\text{sample}] \text{ (mg/L)} - [\text{sample duplicate}] \text{ (mg/L)}}{[\text{average ammonia}] \text{ (mg/L)}} \times 100$$

Acceptable Range: 0-20%

$$\text{Percent Recovery} = \frac{[\text{spiked sample}] \text{ (mg/L)} - [\text{sample}] \text{ (mg/L)}}{\text{nominal [spike] (mg/L)}} \times 100$$

Acceptable Range: 80-120%^b

Reagent 1

Reagent 2

Test Tubes

Comments: Q19 - CH2-2/29/24

Notes: ^aUnless otherwise noted, the last sample listed on the datasheet is used for duplicate and duplicate + spike QC check.

^b Acceptable range for % recovery applies only to the blank spike. Spike recoveries in samples may vary based on sample matrix and are for information only.

^c Calculation not performed due to one or both values below the method detection limit.

HACH Ammonia Nitrogen Test Kit, Test 'N Tube™ Vials. Method 10031. Method Detection Limit = 0.5 mg/L

QC Check: CM 3/6/24

Final Review: Bc 3/14/24

Appendix C
Chain-of-Custody Forms

Enthalpy Analytical - Environmental Toxicology

4340 Vandever Avenue
San Diego, CA 92120
Phone 858.587.7333
infoSD@enthalpy.com

Chain of Custody

Date _____ Page _____ of _____

Sample Collection By: <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> Report to: Company City of Snoqualmie Address 38190 SE Stearns RD City/State/Zip Snoqualmie, WA 98065 Contact Lyle Beach Phone 425-766-2590 Email lbeach@snoqualmiewa.gov </div> <div style="width: 45%;"> Invoice To: Same as Report to <input checked="" type="checkbox"/> Company Address P.O. Box 987 City/State/Zip Contact Tom Holmes Phone 425-766-1210 Email tholmes@snoqualmiewa.gov </div> </div>						ANALYSES REQUIRED Enthalpy Matrix Codes: G = Grab C = Composite FW = Freshwater SW = Seawater Sed = Sediment STRM = Stormwater GW = Groundwater WW = Wastewater O = Other (specify)				Receipt Temperature (°C)																																																																																			
<table border="1" style="width:100%; border-collapse: collapse;"> <thead> <tr> <th rowspan="2">SAMPLE ID</th> <th colspan="3">SAMPLE</th> <th>MATRIX CODE</th> <th colspan="2">Container</th> <th rowspan="2">COMMENTS</th> </tr> <tr> <th>Date</th> <th>Time</th> <th>Type (G or C)</th> <th>(FW, SW, Sed, STRM, GW, WW, O)</th> <th>Type</th> <th>Qty</th> </tr> </thead> <tbody> <tr> <td>21924</td> <td>2-19-24</td> <td>1pm</td> <td>C</td> <td>WW</td> <td>Cube</td> <td>1</td> <td></td> </tr> <tr><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td></tr> <tr><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td></tr> <tr><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td></tr> <tr><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td></tr> <tr><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td></tr> <tr><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td></tr> <tr><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td></tr> <tr><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td></tr> </tbody> </table>						SAMPLE ID	SAMPLE				MATRIX CODE	Container		COMMENTS	Date	Time	Type (G or C)	(FW, SW, Sed, STRM, GW, WW, O)	Type	Qty	21924	2-19-24	1pm	C	WW	Cube	1																																																																		Chronic Toxicity (G) Acute Toxicity (G)
SAMPLE ID	SAMPLE			MATRIX CODE	Container		COMMENTS																																																																																						
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PROJECT INFORMATION		SAMPLE RECEIPT		1) RELINQUISHED BY (CLIENT)		2) RECEIVED BY (COURIER)																																																																																							
Project Name:		Total No. of Containers 1		(Signature) _____ (Time) 2-19-24		(Signature) _____ (Time)																																																																																							
PO No.:		Received Good Condition? Y		(Printed Name) Lyle Beach (Date) 1:00p		(Printed Name) _____ (Date)																																																																																							
Shipped Via: FedEx		Matches Test Schedule? Y		(Company) City of Snoqualmie		(Company)																																																																																							
SPECIAL INSTRUCTIONS/COMMENTS: @Chronic fathead and water flea testing Acute fathead and water flea testing. Confirmed with project manager. CM 3/14/24				3) RELINQUISHED BY (COURIER)		4) RECEIVED BY (LABORATORY)																																																																																							
(Signature) _____ (Time)				(Signature) _____ (Time) 0920		(Signature)																																																																																							
(Printed Name) _____ (Date)				(Printed Name) Matt Kellogg (Date) 2/20/24		(Printed Name)																																																																																							
(Company)				(Company) EA SD		(Log-In #s) 24-0225																																																																																							

Additional costs may be required for sample disposal or storage. Payment net 30 unless otherwise contracted.
 Shaded areas are for lab use only
 Report turn-around-time varies depending on length of test; please inquire with your project manager.

<http://enthalpy.com/environmental-toxicology-2/>

4340 Vandever Avenue
San Diego, CA 92120
Phone 858.587.7333
infoSD@enthalpy.com

Date _____ Page _____ of _____

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Enthalpy Analytical - Environmental Toxicology

4340 Vandever Avenue
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Chain of Custody

Date _____ Page _____ of _____

Sample Collection By:							ANALYSES REQUIRED																													
Report to: Company City of Snoqualmie Address 38190 SE Stearns Road City/State/Zip Snoqualmie, WA 98065 Contact Lyle Beach Phone 425-888-4153 Email lbeach@snqualmie.wa.gov				Invoice To: Same as Report to <input type="checkbox"/> Company City of Snoqualmie Address P.O. Box 987 City/State/Zip Snoqualmie, WA 98065 Contact Tom Holmes Phone 425-766-1210 Email tholmes@snqualmie.wa.gov			<div style="display: flex; justify-content: space-between;"> <div style="writing-mode: vertical-rl; transform: rotate(180deg);"> Chronic Toxicity (C) Acute Toxicity (A) </div> <div> <table border="1" style="width:100%; border-collapse: collapse;"> <tr> <th colspan="2">Enthalpy Matrix Codes:</th> </tr> <tr><td>G</td><td>= Grab</td></tr> <tr><td>C</td><td>= Composite</td></tr> <tr><td>FW</td><td>= Freshwater</td></tr> <tr><td>SW</td><td>= Seawater</td></tr> <tr><td>Sed</td><td>= Sediment</td></tr> <tr><td>STRM</td><td>= Stormwater</td></tr> <tr><td>GW</td><td>= Groundwater</td></tr> <tr><td>WW</td><td>= Wastewater</td></tr> <tr><td>O</td><td>= Other (specify)</td></tr> </table> </div> </div>										Enthalpy Matrix Codes:		G	= Grab	C	= Composite	FW	= Freshwater	SW	= Seawater	Sed	= Sediment	STRM	= Stormwater	GW	= Groundwater	WW	= Wastewater	O	= Other (specify)
Enthalpy Matrix Codes:																																				
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SAMPLE ID	SAMPLE			MATRIX CODE	Container		COMMENTS																													
	Date	Time	Type (G or C)	(FW, SW, Sed, STRM, GW, WW, O)	Type	Qty																														
22224	2-22-24	10:00	C	WW	cube	1																														
1																																				
2																																				
3																																				
4																																				
5																																				
6																																				
7																																				
8																																				
9																																				
10																																				

PROJECT INFORMATION		SAMPLE RECEIPT		1) RELINQUISHED BY (CLIENT)		2) RECEIVED BY (COURIER)	
Project Name:		Total No. of Containers	1	(Signature)	(Time)	(Signature)	(Time)
PO No.:		Received Good Condition?	Y	(Printed Name)	(Date)	(Printed Name)	(Date)
Shipped Via:	Fedex	Matches Test Schedule?	Y	(Company)	(Date)	(Company)	(Date)

SPECIAL INSTRUCTIONS/COMMENTS:

ⓐ Chronic fathead and water flea testing.
 Acute fathead and water flea testing.
 Confirmed with project manager. CM 3/4/24

3) RELINQUISHED BY (COURIER)		4) RECEIVED BY (LABORATORY)	
(Signature)	(Time)	(Signature)	(Time)
(Printed Name)	(Date)	(Printed Name)	(Date)
(Company)	(Date)	(Company)	(Date)

Additional costs may be required for sample disposal or storage. Payment net 30 unless otherwise contracted.

Shaded areas are for lab use only

Report turn-around-time varies depending on length of test; please inquire with your project manager.

<http://enthalpy.com/environmental-toxicology-2/>

Appendix D
Qualifier Code Glossary

Glossary of Qualifier Codes

- Q1 - Temperature out of recommended range; corrective action taken and recorded in Test Temperature Correction Log
- Q2 - Temperature out of recommended range; no action taken, test terminated same day
- Q3 - Sample pH adjusted to within range of 6-9 with reagent grade NaOH or HCl, as needed
- Q4 - Test aerated; D.O. levels dropped below 4.0 mg/L
- Q5 - Test initiated with continuous aeration due to an anticipated drop in D.O.
- Q6 - Airline obstructed or fell out of replicate and replaced; drop in D.O. occurred
- Q7 - Salinity out of recommended range
- Q8 - Spilled test chamber/ Unable to recover test organism(s)
- Q9 - Inadequate sample volume remaining, partial renewal performed
- Q10 - Inadequate sample volume remaining, no renewal performed
- Q11 - Sample out of holding time; refer to QA section of report
- Q12 - Replicate(s) not initiated; excluded from data analysis
- Q13 - Survival counts not recorded due to poor visibility or heavy debris
- Q14 - D.O. percent saturation was checked and was $\leq 110\%$
- Q15 - Did not meet minimum test acceptability criteria. Refer to QA section of report.
- Q16 - Percent minimum significant difference (PMSD) was below the lower bound limit for acceptability. This indicates that statistics may be over-sensitive in detecting a difference from the control due to low variability in the data set. Test results were reviewed and reported in accordance with guidance found in EPA-833-R-00-003, 2000 unless otherwise specified.
- Q17 - Percent minimum significant difference (PMSD) was above the upper bound limit for acceptability. This indicates that statistics may be under-sensitive in detecting a difference from the control due to high variability in the data set. Test results were reviewed and reported in accordance with EPA-833-R-00-003, 2000 guidance unless otherwise specified.
- Q18 - Incorrect or illegible Entry
- Q19 - Miscalculation
- Q20 - PMSD criteria do not apply to the test of significant toxicity (TST) analysis
- Q21 - Other (provide reason in comments section)
- Q22 - Greater than 10% batch mortality observed upon receipt and/or in holding prior to test initiation. Organisms acclimated to test conditions at Enthalpy and ultimately deemed fit to use for testing.
- Q23 - Test organisms experienced a temperature shift greater than 3°C within 1 day or were received at a temperature greater than 3°C outside the recommended test temperature range and had minimal time to acclimate prior to test initiation. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate test(s). Organisms were ultimately deemed fit to use for testing.
- Q24 - Test organisms experienced a salinity shift greater than 3 ppt within 1 day or were received at a salinity greater than 3 ppt outside the recommended test salinity range and had minimal time to acclimate prior to test initiation. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate test(s). Organisms were ultimately deemed fit to use for testing.

Appendix E
Reference Toxicant Test Data

Fathead Minnow Chronic Test

CETIS Summary Report

Report Date: 26 Feb-24 13:22 (p 1 of 3)
Test Code/ID: 240213pprt / 07-2198-8906

Fathead Minnow 7-d Larval Survival and Growth Test

Nautilus Environmental (CA)

Batch ID: 04-5896-8881	Test Type: Growth-Survival (7d)	Analyst:
Start Date: 13 Feb-24 17:10	Protocol: EPA/821/R-02-013 (2002)	Diluent: Diluted Mineral Water (8:2)
Ending Date: 20 Feb-24 10:25	Species: Pimephales promelas	Brine: Not Applicable
Test Length: 6d 17h	Taxon:	Source: Aquatic Biosystems, CO Age: 1d

Sample ID: 05-0233-9835	Code: 240213pprt	Project:
Sample Date: 13 Feb-24	Material: Copper chloride	Source: Reference Toxicant
Receipt Date: 13 Feb-24	CAS (PC):	Station: Copper Chloride
Sample Age: 17h	Client: Internal	

Multiple Comparison Summary								
Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	S
05-5060-5582	7d Survival Rate	Dunnett Multiple Comparison Test	✓	<15	15	---	13.4%	1
16-2042-0583	96h Survival Rate	Dunnett Multiple Comparison Test		15	30	21.21	17.8%	1

Point Estimate Summary								
Analysis ID	Endpoint	Point Estimate Method	✓	Level	µg/L	95% LCL	95% UCL	S
04-7548-2074	7d Survival Rate	Trimmed Spearman-Kärber		EC50	42.3	29.6	60.6	1
02-5994-6806	96h Survival Rate	Trimmed Spearman-Kärber		EC50	114	84.3	155	1
19-5407-1803	Mean Dry Biomass-mg	Linear Interpolation (ICPIN)	✓	IC25	14.4	7.78	26.5	1
			✓	IC50	31.8	18.8	52.7	
17-3855-5138	Mean Dry Weight-mg	Linear Interpolation (ICPIN)		IC25	>240	---	---	1
				IC50	>240	---	---	

Test Acceptability				TAC Limits			
Analysis ID	Endpoint	Attribute	Test Stat	Lower	Upper	Overlap	Decision
04-7548-2074	7d Survival Rate	Control Resp	0.975	0.8	<<	Yes	Passes Criteria
05-5060-5582	7d Survival Rate	Control Resp	0.975	0.8	<<	Yes	Passes Criteria
19-5407-1803	Mean Dry Biomass-mg	Control Resp	0.56	0.25	<<	Yes	Passes Criteria

CETIS Summary Report

Report Date: 26 Feb-24 13:22 (p 2 of 3)
Test Code/ID: 240213pprt / 07-2198-8906

Fathead Minnow 7-d Larval Survival and Growth Test

Nautilus Environmental (CA)

7d Survival Rate Summary											
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LC	4	0.975	0.895	1.050	0.900	1.000	0.025	0.050	5.13%	0.00%
15		4	0.725	0.486	0.964	0.600	0.900	0.075	0.150	20.69%	25.64%
30		4	0.600	0.416	0.784	0.500	0.700	0.058	0.115	19.25%	38.46%
60		4	0.275	0.123	0.427	0.200	0.400	0.048	0.096	34.82%	71.79%
120		4	0.375	0.295	0.455	0.300	0.400	0.025	0.050	13.33%	61.54%
240		4	0.075	-0.077	0.227	0.000	0.200	0.048	0.096	127.66%	92.31%
96h Survival Rate Summary											
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LC	4	0.975	0.895	1.050	0.900	1.000	0.025	0.050	5.13%	0.00%
15		4	0.850	0.574	1.130	0.600	1.000	0.087	0.173	20.38%	12.82%
30		4	0.750	0.545	0.955	0.600	0.900	0.065	0.129	17.21%	23.08%
60		4	0.650	0.345	0.955	0.400	0.800	0.096	0.191	29.46%	33.33%
120		4	0.550	0.345	0.755	0.400	0.700	0.065	0.129	23.47%	43.59%
240		4	0.225	0.145	0.305	0.200	0.300	0.025	0.050	22.22%	76.92%
Mean Dry Biomass-mg Summary											
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LC	4	0.56	0.492	0.627	0.518	0.6	0.0212	0.0425	7.59%	0.00%
15		4	0.414	0.305	0.522	0.326	0.488	0.0341	0.0682	16.48%	26.09%
30		4	0.287	0.0977	0.477	0.177	0.399	0.0596	0.119	41.49%	48.61%
60		4	0.151	0.0221	0.279	0.072	0.241	0.0404	0.0809	53.64%	73.06%
120		4	0.162	0.0793	0.244	0.097	0.223	0.0259	0.0518	32.05%	71.09%
240		4	0.0365	-0.046	0.119	0	0.11	0.0259	0.0519	142.07%	93.48%
Mean Dry Weight-mg Summary											
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LC	4	0.576	0.471	0.68	0.518	0.658	0.0328	0.0657	11.40%	0.00%
15		4	0.576	0.476	0.676	0.542	0.67	0.0314	0.0629	10.92%	0.03%
30		4	0.463	0.288	0.639	0.354	0.57	0.0551	0.11	23.77%	19.54%
60		4	0.521	0.311	0.732	0.36	0.653	0.0663	0.133	25.41%	9.46%
120		4	0.425	0.268	0.581	0.323	0.557	0.0493	0.0985	23.21%	26.28%
240		2	0.455	-0.752	1.66	0.36	0.55	0.095	0.134	29.53%	21.00%

CETIS Summary Report

Report Date: 26 Feb-24 13:22 (p 3 of 3)
Test Code/ID: 240213ppt / 07-2198-8906

Fathead Minnow 7-d Larval Survival and Growth Test

Nautilus Environmental (CA)

7d Survival Rate Detail						MD5: 60B23AE580E7B185A528901E737061B9
Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	
0	LC	1.000	0.900	1.000	1.000	
15		0.800	0.900	0.600	0.600	
30		0.500	0.700	0.500	0.700	
60		0.300	0.200	0.400	0.200	
120		0.400	0.400	0.300	0.400	
240		0.000	0.000	0.200	0.100	
96h Survival Rate Detail						MD5: 4F9C2F1359FAC80686D7E9F908933DFF
Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	
0	LC	1.000	0.900	1.000	1.000	
15		0.900	1.000	0.600	0.900	
30		0.700	0.800	0.600	0.900	
60		0.800	0.600	0.800	0.400	
120		0.600	0.700	0.400	0.500	
240		0.200	0.300	0.200	0.200	
Mean Dry Biomass-mg Detail						MD5: CF04B580EEFF915B6959794C4AD00212
Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	
0	LC	0.518	0.592	0.528	0.6	
15		0.438	0.488	0.326	0.402	
30		0.177	0.399	0.192	0.382	
60		0.196	0.072	0.241	0.094	
120		0.171	0.223	0.097	0.156	
240		0	0	0.11	0.036	
Mean Dry Weight-mg Detail						MD5: 81BF2232D01A8BF9948B830F95743175
Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	
0	LC	0.518	0.658	0.528	0.6	
15		0.548	0.542	0.543	0.67	
30		0.354	0.57	0.384	0.546	
60		0.653	0.36	0.602	0.47	
120		0.427	0.557	0.323	0.39	
240		---	---	0.55	0.36	

CETIS Analytical Report

Report Date: 26 Feb-24 13:21 (p 1 of 4)
Test Code/ID: 240213ppt / 07-2198-8906

Fathead Minnow 7-d Larval Survival and Growth Test										Nautilus Environmental (CA)		
Analysis ID: 05-5060-5582			Endpoint: 7d Survival Rate				CETIS Version: CETISv2.1.4					
Analyzed: 26 Feb-24 13:21			Analysis: Parametric-Control vs Treatments				Status Level: 1					
Edit Date: 26 Feb-24 12:47			MD5 Hash: 83520B4C9EA56C0D9A22E5B928A71733				Editor ID: 000-502-715-6					
Data Transform		Alt Hyp		NOEL		LOEL		TOEL		Tox Units	MSDu	PMSD
Angular (Corrected)		C > T		<15		15		---		---	0.131	13.42%
Dunnett Multiple Comparison Test												
Control	vs	Conc-µg/L	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)			
Lab Control		15*	6	3.96	2.41	0.206	CDF	0.0020	Significant Effect			
		30*	6	5.63	2.41	0.206	CDF	8.1E-05	Significant Effect			
		60*	6	9.6	2.41	0.206	CDF	2.7E-05	Significant Effect			
		120*	6	8.31	2.41	0.206	CDF	2.7E-05	Significant Effect			
		240*	6	12.8	2.41	0.206	CDF	2.7E-05	Significant Effect			
ANOVA Table												
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α:5%)				
Between	2.98532		0.597063		5	40.6	<1.0E-05	Significant Effect				
Error	0.26459		0.0146994		18							
Total	3.24991				23							
ANOVA Assumptions Tests												
Attribute	Test				Test Stat	Critical	P-Value	Decision(α:1%)				
Variance	Bartlett Equality of Variance Test				4.29	15.1	0.5088	Equal Variances				
Distribution	Shapiro-Wilk W Normality Test				0.922	0.884	0.0644	Normal Distribution				
7d Survival Rate Summary												
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect	
0	LC	4	0.975	0.895	1.000	1.000	0.900	1.000	0.025	5.13%	0.00%	
15		4	0.725	0.486	0.964	0.667	0.600	0.900	0.075	20.69%	25.64%	
30		4	0.600	0.416	0.784	0.600	0.500	0.700	0.058	19.25%	38.46%	
60		4	0.275	0.123	0.427	0.233	0.200	0.400	0.048	34.82%	71.79%	
120		4	0.375	0.295	0.455	0.400	0.300	0.400	0.025	13.33%	61.54%	
240		4	0.075	0.000	0.227	0.033	0.000	0.200	0.048	127.66%	92.31%	
Angular (Corrected) Transformed Summary												
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect	
0	LC	4	1.370	1.240	1.500	1.410	1.250	1.410	0.041	5.94%	0.00%	
15		4	1.030	0.748	1.320	0.960	0.886	1.250	0.089	17.27%	24.74%	
30		4	0.888	0.699	1.080	0.888	0.785	0.991	0.059	13.37%	35.22%	
60		4	0.548	0.379	0.717	0.502	0.464	0.685	0.053	19.41%	60.04%	
120		4	0.658	0.575	0.742	0.685	0.580	0.685	0.026	7.98%	51.98%	
240		4	0.276	0.042	0.510	0.213	0.159	0.464	0.074	53.29%	79.89%	

CETIS Analytical Report

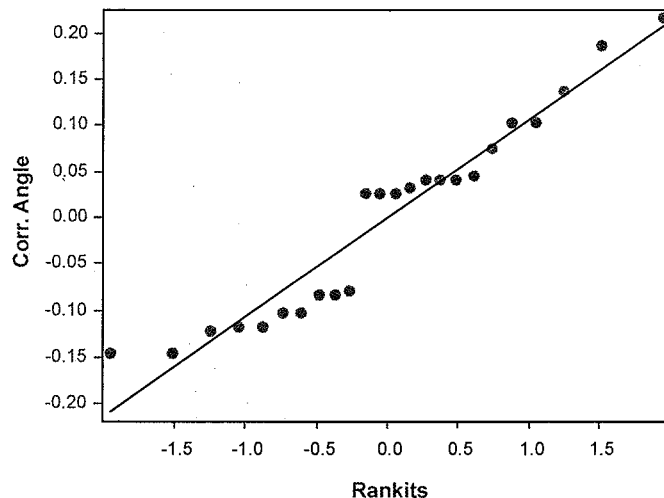
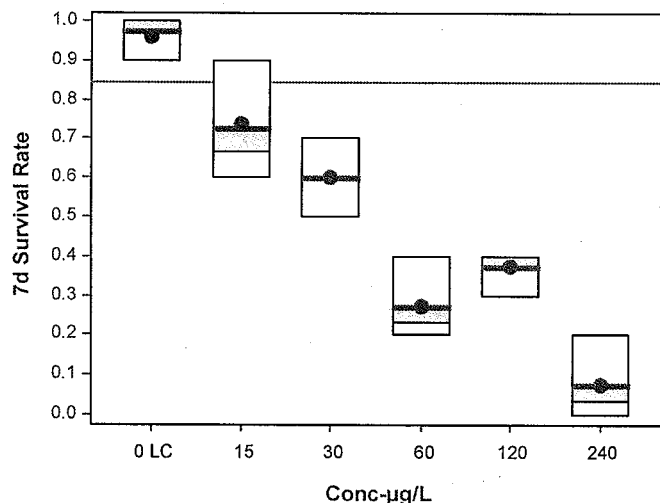
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Test Code/ID: 240213ppt / 07-2198-8906

Fathead Minnow 7-d Larval Survival and Growth Test

Nautilus Environmental (CA)

Analysis ID: 05-5060-5582 Endpoint: 7d Survival Rate CETIS Version: CETISv2.1.4
Analyzed: 26 Feb-24 13:21 Analysis: Parametric-Control vs Treatments Status Level: 1
Edit Date: 26 Feb-24 12:47 MD5 Hash: 83520B4C9EA56C0D9A22E5B928A71733 Editor ID: 000-502-715-6

Graphics



CETIS Analytical Report

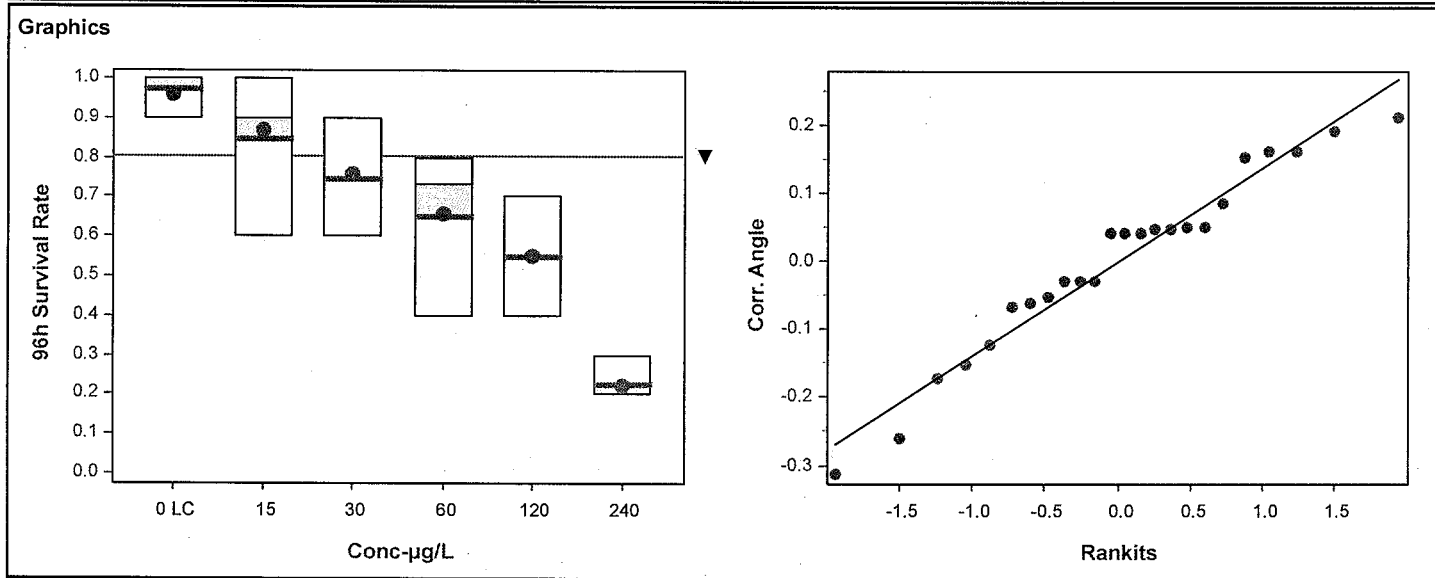
Report Date: 26 Feb-24 13:21 (p 3 of 4)
Test Code/ID: 240213pprt / 07-2198-8906

Fathead Minnow 7-d Larval Survival and Growth Test								Nautilus Environmental (CA)			
Analysis ID: 16-2042-0583		Endpoint: 96h Survival Rate			CETIS Version: CETISv2.1.4						
Analyzed: 26 Feb-24 13:21		Analysis: Parametric-Control vs Treatments			Status Level: 1						
Edit Date: 26 Feb-24 12:47		MD5 Hash: 12CB04CC6ED4B77AB25BD0F93C0E48A			Editor ID: 000-502-715-6						
Data Transform		Alt Hyp			NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD	
Angular (Corrected)		C > T			15	30	21.21	---	0.173	17.79%	
Dunnett Multiple Comparison Test											
Control	vs	Conc-µg/L	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)		
Lab Control		15	6	1.58	2.41	0.262	CDF	0.2029	Non-Significant Effect		
		30*	6	2.87	2.41	0.262	CDF	0.0200	Significant Effect		
		60*	6	3.9	2.41	0.262	CDF	0.0023	Significant Effect		
		120*	6	4.91	2.41	0.262	CDF	0.0003	Significant Effect		
		240*	6	8.07	2.41	0.262	CDF	2.7E-05	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α:5%)			
Between	1.86506		0.373011		5	15.7	<1.0E-05	Significant Effect			
Error	0.427076		0.0237264		18						
Total	2.29213				23						
ANOVA Assumptions Tests											
Attribute	Test				Test Stat	Critical	P-Value	Decision(α:1%)			
Variance	Bartlett Equality of Variance Test				5.92	15.1	0.3142	Equal Variances			
Distribution	Shapiro-Wilk W Normality Test				0.954	0.884	0.3376	Normal Distribution			
96h Survival Rate Summary											
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	4	0.975	0.895	1.000	1.000	0.900	1.000	0.025	5.13%	0.00%
15		4	0.850	0.574	1.000	0.900	0.600	1.000	0.087	20.38%	12.82%
30		4	0.750	0.545	0.955	0.750	0.600	0.900	0.065	17.21%	23.08%
60		4	0.650	0.345	0.955	0.733	0.400	0.800	0.096	29.46%	33.33%
120		4	0.550	0.345	0.755	0.550	0.400	0.700	0.065	23.47%	43.59%
240		4	0.225	0.145	0.305	0.200	0.200	0.300	0.025	22.22%	76.92%
Angular (Corrected) Transformed Summary											
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	4	1.370	1.240	1.500	1.410	1.250	1.410	0.041	5.94%	0.00%
15		4	1.200	0.845	1.550	1.250	0.886	1.410	0.111	18.54%	12.56%
30		4	1.060	0.810	1.310	1.050	0.886	1.250	0.078	14.73%	22.82%
60		4	0.946	0.623	1.270	1.030	0.685	1.110	0.102	21.47%	30.99%
120		4	0.837	0.627	1.050	0.836	0.685	0.991	0.066	15.74%	38.97%
240		4	0.493	0.400	0.585	0.464	0.464	0.580	0.029	11.77%	64.07%

CETIS Analytical Report

Report Date: 26 Feb-24 13:21 (p 4 of 4)
Test Code/ID: 240213ppt / 07-2198-8906

Fathead Minnow 7-d Larval Survival and Growth Test			Nautilus Environmental (CA)
Analysis ID: 16-2042-0583	Endpoint: 96h Survival Rate	CETIS Version: CETISv2.1.4	
Analyzed: 26 Feb-24 13:21	Analysis: Parametric-Control vs Treatments	Status Level: 1	
Edit Date: 26 Feb-24 12:47	MD5 Hash: 12CB04CC6ED4B77AB25BD0F93C0E48A	Editor ID: 000-502-715-6	



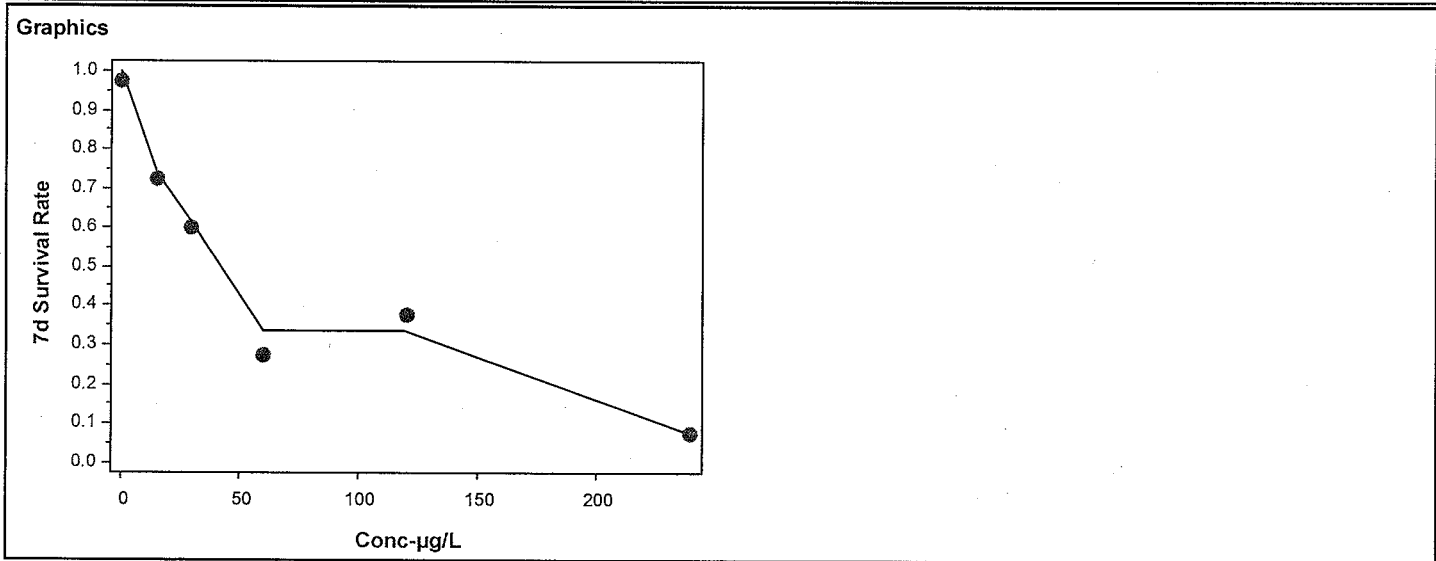
CETIS Analytical Report

Report Date: 26 Feb-24 13:22 (p 1 of 2)
Test Code/ID: 240213ppt / 07-2198-8906

Fathead Minnow 7-d Larval Survival and Growth Test				Nautilus Environmental (CA)	
Analysis ID: 04-7548-2074		Endpoint: 7d Survival Rate		CETIS Version: CETISv2.1.4	
Analyzed: 26 Feb-24 13:15		Analysis: Trimmed Spearman-Kärber		Status Level: 1	
Edit Date: 26 Feb-24 12:47		MD5 Hash: 83520B4C9EA56C0D9A22E5B928A71733		Editor ID: 000-502-715-6	

Trimmed Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.025	25.64%	1.63	0.0778	42.3	29.6	60.6

7d Survival Rate Summary			Calculated Variate(A/B)							Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	LC	4	0.975	1.000	0.900	1.000	5.13%	0.00%	39/40	0.975	0.00%
15		4	0.725	0.667	0.600	0.900	20.69%	25.64%	29/40	0.725	25.64%
30		4	0.600	0.600	0.500	0.700	19.25%	38.46%	24/40	0.600	38.46%
60		4	0.275	0.233	0.200	0.400	34.82%	71.79%	11/40	0.325	66.67%
120		4	0.375	0.400	0.300	0.400	13.33%	61.54%	15/40	0.325	66.67%
240		4	0.075	0.033	0.000	0.200	127.66%	92.31%	3/40	0.075	92.31%



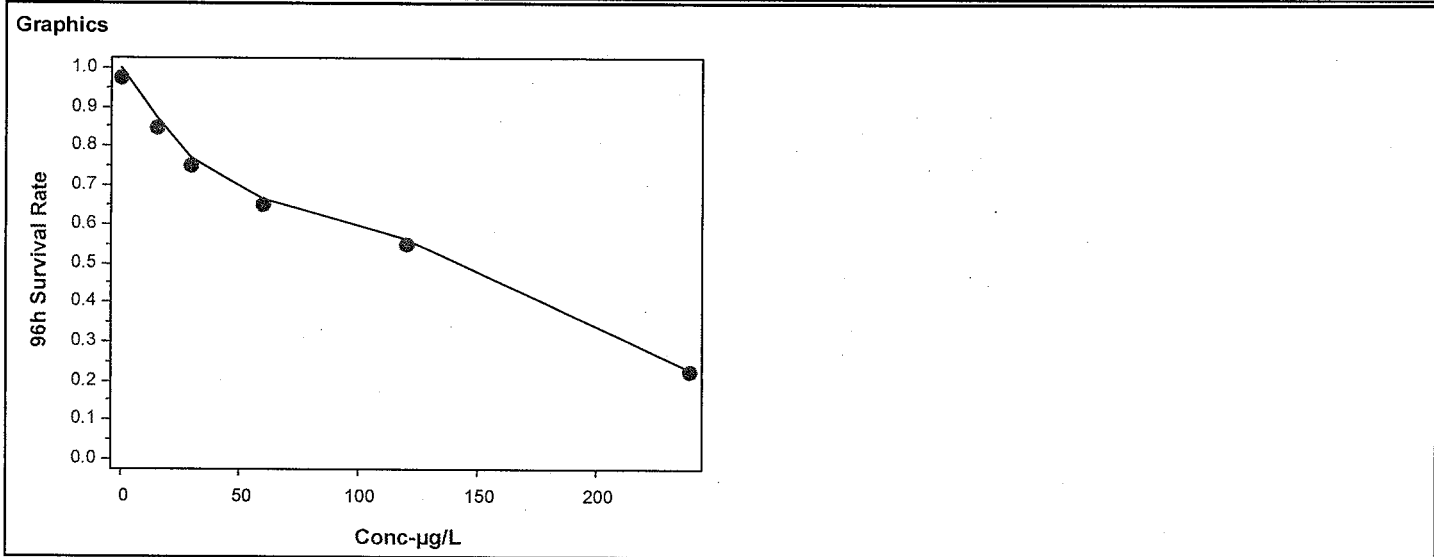
CETIS Analytical Report

Report Date: 26 Feb-24 13:22 (p 2 of 2)
Test Code/ID: 240213ppt / 07-2198-8906

Fathead Minnow 7-d Larval Survival and Growth Test				Nautilus Environmental (CA)	
Analysis ID: 02-5994-6806		Endpoint: 96h Survival Rate		CETIS Version: CETISv2.1.4	
Analyzed: 26 Feb-24 13:15		Analysis: Trimmed Spearman-Kärber		Status Level: 1	
Edit Date: 26 Feb-24 12:47		MD5 Hash: 12CB04CC6ED4B77AB25BD0F93C0E48A		Editor ID: 000-502-715-6	

Trimmed Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.025	23.08%	2.06	0.066	114	84.3	155

96h Survival Rate Summary			Calculated Variate(A/B)							Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	LC	4	0.975	1.000	0.900	1.000	5.13%	0.00%	39/40	0.975	0.00%
15		4	0.850	0.900	0.600	1.000	20.38%	12.82%	34/40	0.850	12.82%
30		4	0.750	0.750	0.600	0.900	17.21%	23.08%	30/40	0.750	23.08%
60		4	0.650	0.733	0.400	0.800	29.46%	33.33%	26/40	0.650	33.33%
120		4	0.550	0.550	0.400	0.700	23.47%	43.59%	22/40	0.550	43.59%
240		4	0.225	0.200	0.200	0.300	22.22%	76.92%	9/40	0.225	76.92%



CETIS Analytical Report

Report Date: 26 Feb-24 13:21 (p 1 of 2)
 Test Code/ID: 240213ppt / 07-2198-8906

Fathead Minnow 7-d Larval Survival and Growth Test				Nautilus Environmental (CA)	
Analysis ID:	19-5407-1803	Endpoint:	Mean Dry Biomass-mg	CETIS Version:	CETISv2.1.4
Analyzed:	26 Feb-24 13:15	Analysis:	Linear Interpolation (ICPIN)	Status Level:	1
Edit Date:	26 Feb-24 12:47	MD5 Hash:	17461483F0EC07A8727DDEE070F6ABED	Editor ID:	000-502-715-6

Linear Interpolation Options

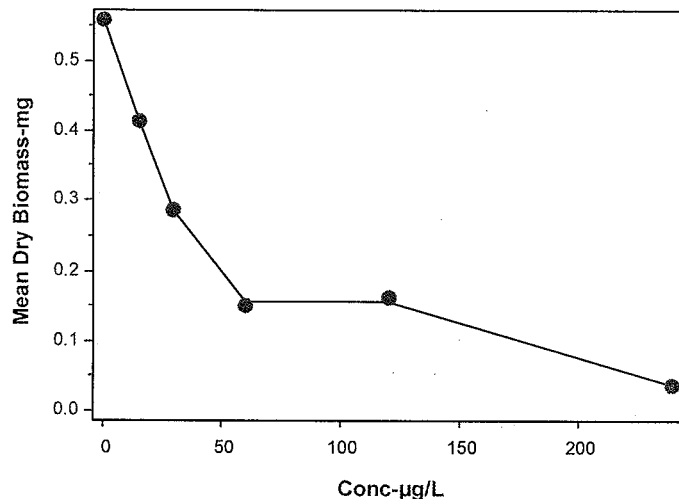
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1512167	1000	Yes	Two-Point Interpolation

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC25	14.4	7.78	26.5
IC50	31.8	18.8	52.7

Mean Dry Biomass-mg Summary			Calculated Variate						Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Median	Min	Max	CV%	%Effect	Mean	%Effect
0	LC	4	0.56	0.56	0.518	0.6	7.59%	0.00%	0.56	0.00%
15		4	0.414	0.42	0.326	0.488	16.48%	26.09%	0.414	26.07%
30		4	0.287	0.287	0.177	0.399	41.49%	48.61%	0.287	48.75%
60		4	0.151	0.145	0.072	0.241	53.64%	73.06%	0.156	72.14%
120		4	0.162	0.163	0.097	0.223	32.05%	71.09%	0.156	72.14%
240		4	0.0365	0.012	0	0.11	142.07%	93.48%	0.0365	93.48%

Graphics



CETIS Analytical Report

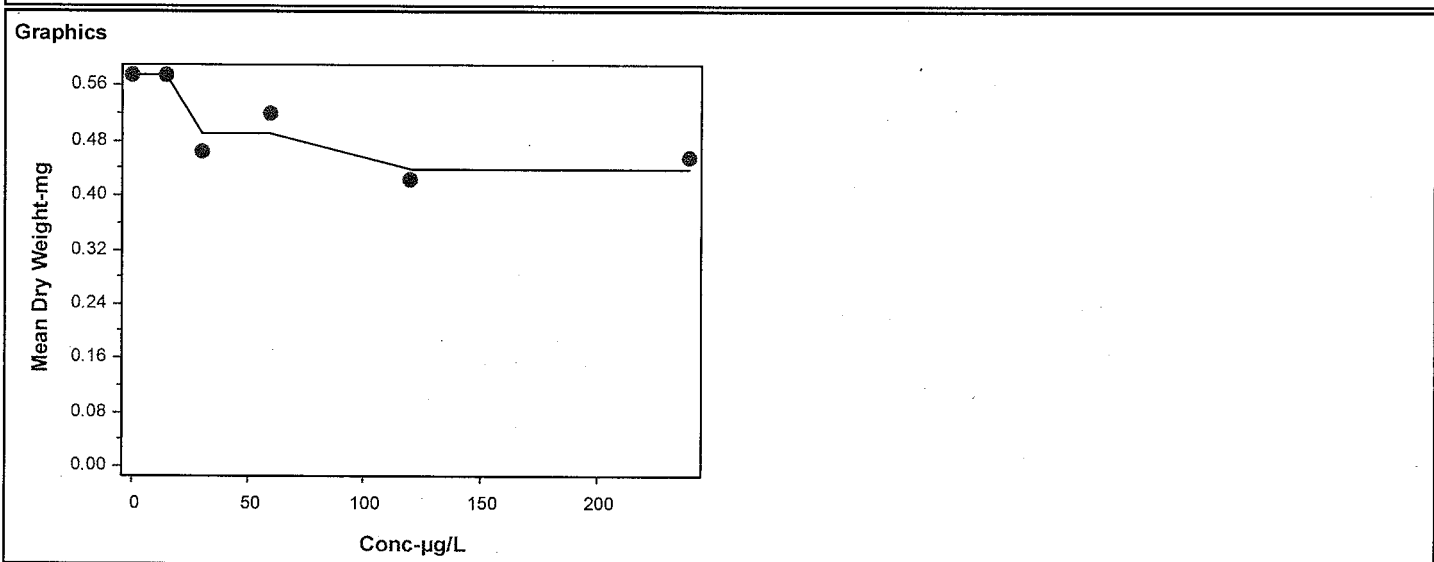
Report Date: 26 Feb-24 13:21 (p 2 of 2)
Test Code/ID: 240213ppt / 07-2198-8906

Fathead Minnow 7-d Larval Survival and Growth Test				Nautilus Environmental (CA)	
Analysis ID: 17-3855-5138		Endpoint: Mean Dry Weight-mg		CETIS Version: CETISv2.1.4	
Analyzed: 26 Feb-24 13:15		Analysis: Linear Interpolation (ICPIN)		Status Level: 1	
Edit Date: 26 Feb-24 12:47		MD5 Hash: 920FE52CB499A0BAE2D5B278406A9128		Editor ID: 000-502-715-6	

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1157149	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	µg/L	95% LCL	95% UCL
IC25	>240	---	---
IC50	>240	---	---

Mean Dry Weight-mg Summary			Calculated Variate						Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Median	Min	Max	CV%	%Effect	Mean	%Effect
0	LC	4	0.576	0.564	0.518	0.658	11.40%	0.00%	0.576	0.00%
15		4	0.576	0.545	0.542	0.67	10.92%	0.03%	0.576	0.00%
30		4	0.463	0.465	0.354	0.57	23.77%	19.54%	0.492	14.58%
60		4	0.521	0.536	0.36	0.653	25.41%	9.46%	0.492	14.58%
120		4	0.425	0.409	0.323	0.557	23.21%	26.28%	0.44	23.61%
240		2	0.455	0.455	0.36	0.55	29.53%	21.00%	0.44	23.61%

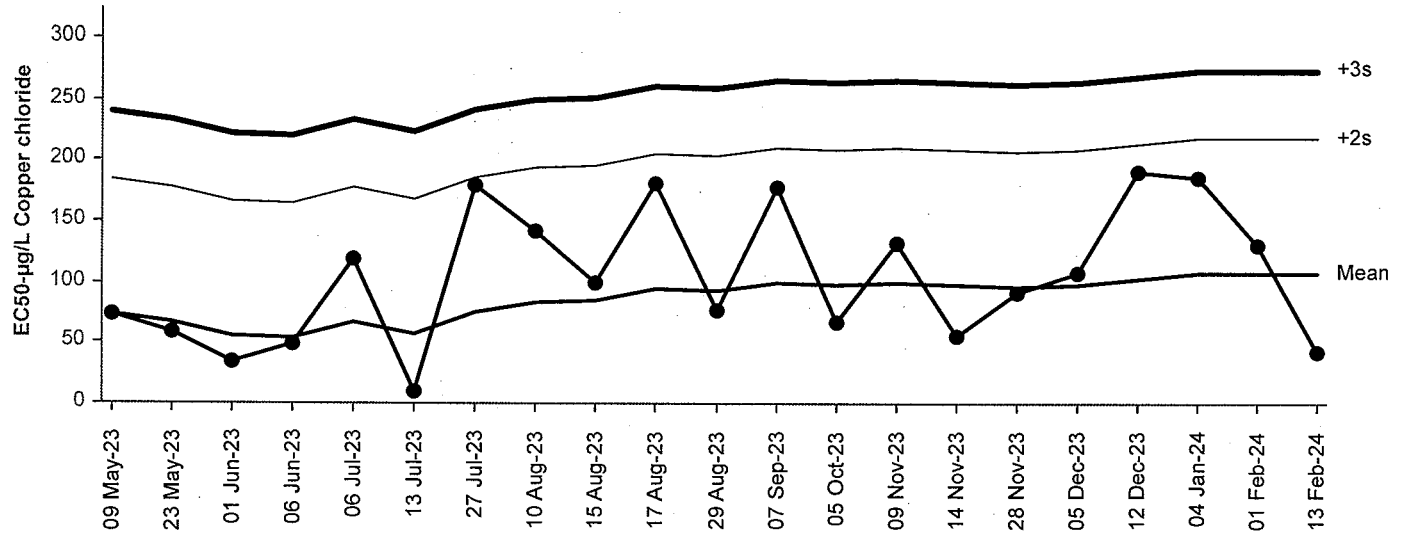


CETIS QC Plot

Report Date: 26 Feb-24 13:16 (1 of 1)

Fathead Minnow 7-d Larval Survival and Growth Test

Nautilus Environmental (CA)

Test Type: Growth-Survival (7d)
Protocol: EPA/821/R-02-013 (2002)Organism: Pimephales promelas
Endpoint: 7d Survival RateMaterial: Copper chloride
Source: Reference Toxicant-REFFathead Minnow 7-d Larval Survival and Growth Test
7d Survival Rate Endpoint

Cumulative Mean Plot

Mean: 108.1 Count: 20 -2s Warning Limit: -2.99 -3s Action Limit: -58.5
Sigma: 55.55 CV: 51.40% +2s Warning Limit: 219 +3s Action Limit: 275

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2023	May	9	14:30	73.4	-34.7	-0.6246			17-0374-9686	18-2424-4316
2			23	16:45	58.84	-49.26	-0.8867			14-2465-1061	17-5820-5358
3		Jun	1	15:20	33.73	-74.37	-1.339			17-6692-1441	18-5975-0028
4			6	14:30	48.65	-59.45	-1.07			01-4119-8216	15-0962-2339
5		Jul	6	16:05	118.4	10.33	0.1859			00-1199-7404	05-4589-8956
6			13	18:10	10.36	-97.74	-1.759			15-1359-4935	20-1590-9019
7			27	15:35	179.8	71.7	1.291			15-2532-4879	06-3122-3197
8		Aug	10	17:00	142.7	34.6	0.6229			18-3587-1709	05-8679-9386
9			15	16:20	100.1	-8.022	-0.1444			06-1941-3784	04-4151-2765
10			17	15:35	181.1	73	1.314			17-1726-6494	13-3957-8394
11			29	15:40	77.22	-30.88	-0.5559			02-7061-6597	08-3695-9368
12		Sep	7	18:05	177.7	69.63	1.253			13-0699-6225	20-7758-4048
13		Oct	5	15:50	66.33	-41.77	-0.752			08-2577-9038	14-5898-0931
14		Nov	9	15:00	132.4	24.32	0.4377			05-1906-7363	06-5903-1683
15			14	16:00	55.98	-52.12	-0.9382			01-8571-2337	16-1673-5874
16			28	14:20	91.52	-16.58	-0.2985			17-5429-6828	03-6960-2958
17		Dec	5	16:00	107	-1.112	-0.02002			07-5058-6143	20-3082-0319
18			12	14:50	190.5	82.39	1.483			07-2534-8193	07-9693-4841
19	2024	Jan	4	16:20	186.5	78.43	1.412			01-3179-2737	08-1973-7377
20		Feb	1	16:50	130	21.89	0.3941			05-9064-3399	14-4596-4600
21			13	17:10	42.35	-65.75	-1.184			07-2198-8906	04-7548-2074

Fathead Minnow 7-d Larval Survival and Growth Test

Nautilus Environmental (CA)

Test Type: Growth-Survival (7d)

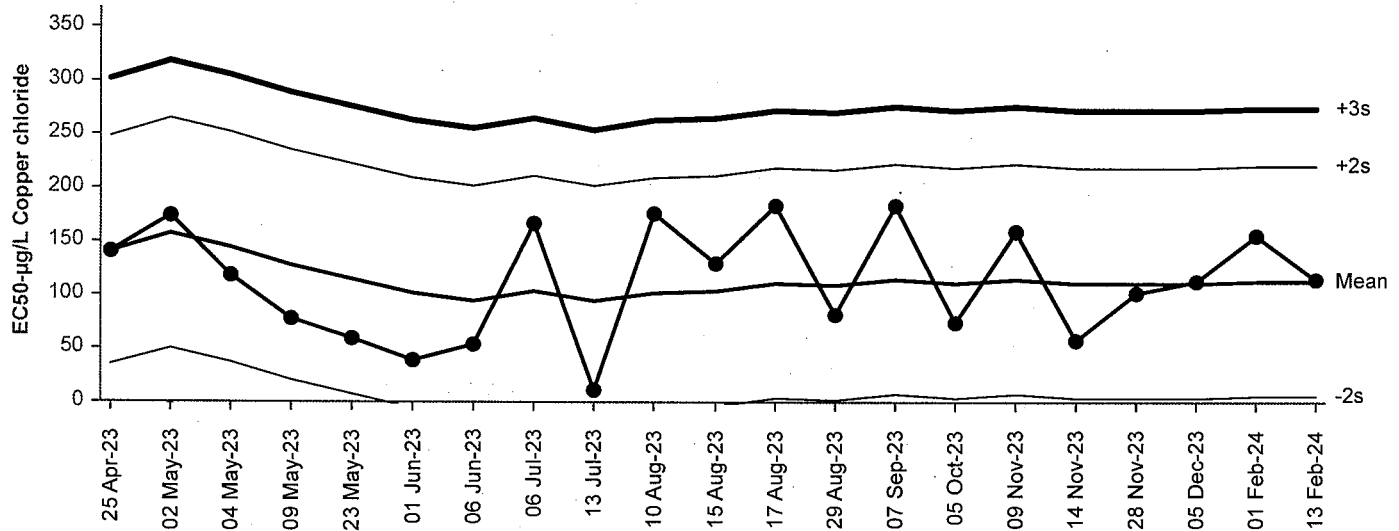
Organism: Pimephales promelas

Material: Copper chloride

Protocol: EPA/821/R-02-013 (2002)

Endpoint: 96h Survival Rate

Source: Reference Toxicant-REF

Fathead Minnow 7-d Larval Survival and Growth Test
96h Survival Rate Endpoint

Cumulative Mean Plot

Mean: 113.2 Count: 20 -2s Warning Limit: 5.71 -3s Action Limit: -48
 Sigma: 53.76 CV: 47.50% +2s Warning Limit: 221 +3s Action Limit: 275

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2023	Apr	25	17:00	142	28.76	0.5349			01-1934-3614	01-3046-7651
2		May	2	14:45	174.3	61.09	1.136			15-8099-3820	06-7277-5434
3			4	17:35	119.1	5.858	0.109			21-1157-0000	13-5059-2451
4			9	14:30	78.12	-35.08	-0.6526			17-0374-9686	04-9661-2068
5			23	16:45	59.86	-53.34	-0.9921			14-2465-1061	00-9740-4974
6		Jun	1	15:20	39.66	-73.54	-1.368			17-6692-1441	05-3207-8732
7			6	14:30	54.23	-58.97	-1.097			01-4119-8216	16-0333-2330
8		Jul	6	16:05	167.2	54	1.005			00-1199-7404	08-1703-2255
9			13	18:10	10.96	-102.2	-1.902			15-1359-4935	10-3624-4179
10		Aug	10	17:00	176.4	63.17	1.175			18-3587-1709	01-5579-1799
11			15	16:20	129.3	16.11	0.2997			06-1941-3784	12-0379-9668
12			17	15:35	184.6	71.43	1.329			17-1726-6494	19-4387-3602
13			29	15:40	82.1	-31.1	-0.5785			02-7061-6597	06-2142-4101
14		Sep	7	18:05	184.1	70.92	1.319			13-0699-6225	15-1678-7378
15		Oct	5	15:50	75.16	-38.04	-0.7077			08-2577-9038	10-2798-6922
16		Nov	9	15:00	159.9	46.72	0.869			05-1906-7363	02-2806-7575
17			14	16:00	57.73	-55.47	-1.032			01-8571-2337	02-8913-8982
18			28	14:20	101.4	-11.76	-0.2188			17-5429-6828	10-5069-6899
19		Dec	5	16:00	113.1	-0.1167	-0.00217			07-5058-6143	12-6665-9623
20	2024	Feb	1	16:50	155.5	42.25	0.786			05-9064-3399	11-5693-5898
21			13	17:10	114.2	1.003	0.01866			07-2198-8906	02-5994-6806

Fathead Minnow 7-d Larval Survival and Growth Test

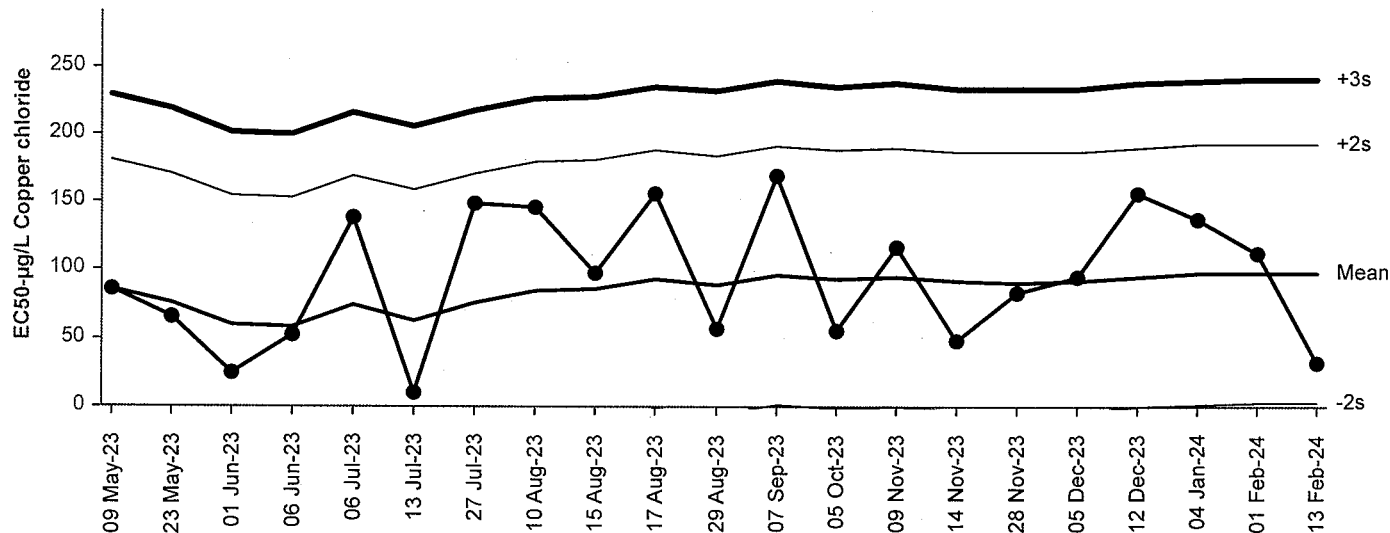
Nautilus Environmental (CA)

Test Type: Growth-Survival (7d)
Protocol: EPA/821/R-02-013 (2002)

Organism: Pimephales promelas
Endpoint: Mean Dry Biomass-mg

Material: Copper chloride
Source: Reference Toxicant-REF

Fathead Minnow 7-d Larval Survival and Growth Test
Mean Dry Biomass-mg Endpoint



Cumulative Mean Plot

Mean: 97.8 Count: 20 -2s Warning Limit: 2.88 -3s Action Limit: -44.6
Sigma: 47.46 CV: 48.50% +2s Warning Limit: 193 +3s Action Limit: 240

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2023	May	9	14:30	86.26	-11.54	-0.2431			17-0374-9686	14-8787-4182
2			23	16:45	66.02	-31.78	-0.6696			14-2465-1061	00-0991-1748
3		Jun	1	15:20	25.47	-72.33	-1.524			17-6692-1441	04-1893-7128
4			6	14:30	52.92	-44.88	-0.9455			01-4119-8216	21-3838-9951
5		Jul	6	16:05	139	41.2	0.868			00-1199-7404	03-6110-5464
6			13	18:10	10.3	-87.5	-1.844			15-1359-4935	06-1664-2359
7			27	15:35	148.9	51.12	1.077			15-2532-4879	02-8786-1271
8		Aug	10	17:00	145.5	47.73	1.006			18-3587-1709	11-1892-3870
9			15	16:20	97.09	-0.7121	-0.015			06-1941-3784	21-2666-6167
10			17	15:35	155.9	58.09	1.224			17-1726-6494	13-8989-7047
11			29	15:40	56.86	-40.94	-0.8626			02-7061-6597	12-8044-8072
12		Sep	7	18:05	169.2	71.38	1.504			13-0699-6225	20-6031-2987
13		Oct	5	15:50	54.84	-42.96	-0.9052			08-2577-9038	19-0774-0960
14		Nov	9	15:00	117.2	19.4	0.4088			05-1906-7363	03-0796-0999
15			14	16:00	48.59	-49.21	-1.037			01-8571-2337	18-6973-5078
16			28	14:20	82.95	-14.85	-0.3129			17-5429-6828	10-8204-0231
17		Dec	5	16:00	94.11	-3.694	-0.07782			07-5058-6143	03-4641-1945
18			12	14:50	156.3	58.53	1.233			07-2534-8193	14-3078-8228
19	2024	Jan	4	16:20	136.8	38.97	0.8211			01-3179-2737	00-5038-6538
20		Feb	1	16:50	111.8	14	0.2951			05-9064-3399	12-7267-8647
21			13	17:10	31.77	-66.03	-1.391			07-2198-8906	19-5407-1803

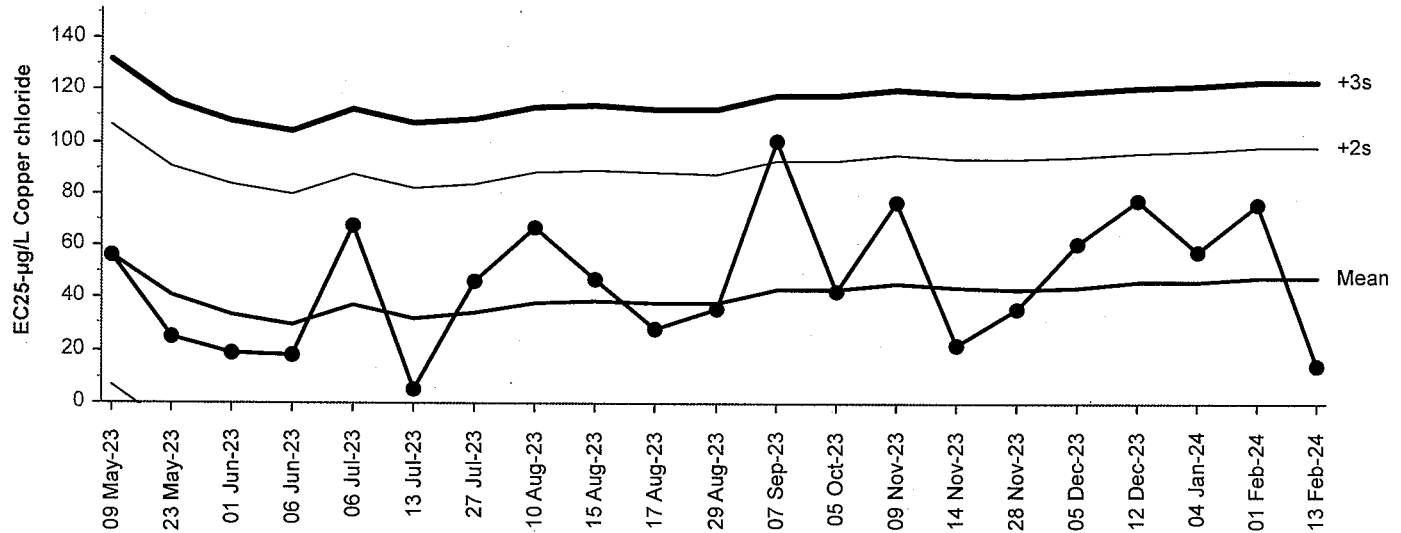
Fathead Minnow 7-d Larval Survival and Growth Test

Nautilus Environmental (CA)

Test Type: Growth-Survival (7d)
Protocol: EPA/821/R-02-013 (2002)

Organism: Pimephales promelas
Endpoint: Mean Dry Biomass-mg

Material: Copper chloride
Source: Reference Toxicant-REF

Fathead Minnow 7-d Larval Survival and Growth Test
Mean Dry Biomass-mg Endpoint

Cumulative Mean Plot

Mean: 48.28 Count: 20 -2s Warning Limit: -1.74 -3s Action Limit: -26.7
Sigma: 25.01 CV: 51.80% +2s Warning Limit: 98.3 +3s Action Limit: 123

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2023	May	9	14:30	56.52	8.238	0.3294			17-0374-9686	14-8787-4182
2			23	16:45	25.13	-23.15	-0.9257			14-2465-1061	00-0991-1748
3		Jun	1	15:20	18.68	-29.6	-1.184			17-6692-1441	04-1893-7128
4			6	14:30	18.24	-30.04	-1.201			01-4119-8216	21-3838-9951
5		Jul	6	16:05	68.09	19.81	0.792			00-1199-7404	03-6110-5464
6			13	18:10	5.149	-43.13	-1.725			15-1359-4935	06-1664-2359
7			27	15:35	46.45	-1.828	-0.07309			15-2532-4879	02-8786-1271
8		Aug	10	17:00	66.68	18.4	0.7357			18-3587-1709	11-1892-3870
9			15	16:20	47.29	-0.9877	-0.03949			06-1941-3784	21-2666-6167
10			17	15:35	27.95	-20.33	-0.8129			17-1726-6494	13-8989-7047
11			29	15:40	36.08	-12.2	-0.488			02-7061-6597	12-8044-8072
12		Sep	7	18:05	100.9	52.62	2.104	(+)		13-0699-6225	20-6031-2987
13		Oct	5	15:50	42.42	-5.861	-0.2343			08-2577-9038	19-0774-0960
14		Nov	9	15:00	76.75	28.47	1.138			05-1906-7363	03-0796-0999
15			14	16:00	21.71	-26.57	-1.062			01-8571-2337	18-6973-5078
16			28	14:20	35.44	-12.84	-0.5135			17-5429-6828	10-8204-0231
17		Dec	5	16:00	61.16	12.88	0.515			07-5058-6143	03-4641-1945
18			12	14:50	77.48	29.2	1.168			07-2534-8193	14-3078-8228
19	2024	Jan	4	16:20	57.71	9.434	0.3772			01-3179-2737	00-5038-6538
20		Feb	1	16:50	75.8	27.52	1.101			05-9064-3399	12-7267-8647
21			13	17:10	14.37	-33.91	-1.356			07-2198-8906	19-5407-1803

Client: Internal

Test Species: P. promelas

Sample ID: CuCl₂

Start Date/Time: 2/13/24 1710

Test No.: 240213 p014

End Date/Time: 2/20/24 1025

Concentration (µg/L)	Rep.	Rand #	Test Day / No. Organisms Alive								Percent Survival
			0	1	2	3	4	5	6	7	
Lab Control	a	4	10	10	10	10	10	10	10	10	100
	b	10	10	9	9	9	9	9	9	9	90
	c	5	10	10	10	10	10	10	10	10	100
	d	9	10	10	10	10	10	10	10	10	100
15	a	23	10	10	10	10	9	9	8	8	80
	b	18	10	10	10	10	10	9	9	9	90
	c	3	10	9	8	8	6	6	6	6	60
	d	6	10	10	9	9	9	8	6	6	60
30	a	19	10	10	9	9	7	6	6	5	50
	b	14	10	10	10	10	8	8	7	7	70
	c	2	10	10	10	10	6	6	5	5	50
	d	17	10	10	9	9	9	8	8	7	70
60	a	20	10	10	9	9	8	5	3	3	30
	b	11	10	10	10	10	6	6	2	2	20
	c	7	10	10	8	8	8	7	4	4	40
	d	22	10	9	7	7	4	2	2	2	20
120	a	8	10	10	9	9	6	5	4	4	40
	b	15	10	9	9	9	7	6	4	4	40
	c	1	10	9	5	5	4	4	4	3	30
	d	21	10	9	8	7	5	4	4	4	40
240	a	13	10	8	6	3	2	1	0	-	0
	b	16	10	8	5	4	3	1	0	-	0
	c	24	10	7	4	4	2	2	2	2	20
	d	12	10	5	3	2	2	2	1	1	10

Rand # QC: MK

Tech Initials: mm

Initial Count QC'd by: WF

Time

Initiated by: mm

<u>mm</u>	<u>GM</u>	<u>GM</u>	<u>GM</u>	<u>WF</u>	<u>MK</u>	<u>HH</u>	<u>GM</u>
<u>1710</u>	<u>1055</u>	<u>1130</u>	<u>1250</u>	<u>1150</u>	<u>1320</u>	<u>1255</u>	<u>1025</u>

Time Fed (day): 0 1 2 3 4 5 6

morning:	<u>—</u>	<u>0835</u>	<u>0820</u>	<u>0815</u>	<u>0900</u>	<u>0825</u>	<u>0810</u>
midday:	<u>—</u>	<u>1150</u>	<u>1230</u>	<u>1320</u>	<u>1210</u>	<u>1125</u>	<u>1325</u>
evening:	<u>1715</u>	<u>1530</u>	<u>1530</u>	<u>1620</u>	<u>1525</u>	<u>1635</u>	<u>1625</u>

Comments: QA 186m 2/15/24

Drying Oven Info

Tare wt. Initials/Date: AD 1503 02/19/24

Date/Time in: 2/20/24 1035

Date/Time out: 2/22/24 0945

Temp (°C): 68.0

QC Check: CM 2/20/24

Final Review: BO 2/22/24

Freshwater Chronic Bioassay
DF-010

Larval Fish Weights

Client: Internal Test Species: Pimephales promelas
 Sample ID: CuCl2 Start Date/Time: 2/13/24 1710
 Test No.: 240213pprt End Date/Time: 2/20/24 1025
 Initial # Fish: 10

Conc. (<u> </u> µg/L <u> </u>)	Rep.	pan weight (mg)	pan + fish weight (mg)	organism weight (mg)
Lab Control	a	31.56	36.74	5.18
	b	31.96	37.88	5.92
	c	30.44	35.72	5.28
	d	31.36	37.36	6.00
15	a	29.18	33.56	4.38
	b	30.80	35.68	4.88
	c	31.29	34.55	3.26
	d	30.04	34.06	4.02
30	a	31.22	32.99	1.77
	b	30.77	34.76	3.99
	c	31.03	32.95	1.92
	d	31.12	34.94	3.82
60	a	33.49	35.45	1.96
	b	29.94	30.66	0.72
	c	32.01	34.42	2.41
	d	27.57	28.51	0.94
120	a	32.81	34.52	1.71
	b	34.36	36.59	2.23
	c	31.26	32.23	0.97
	d	31.42	32.98	1.56
240	a	0.00	0.00	0.00
	b	0.00	0.00	0.00
	c	31.34	32.44	1.10
	d	29.90	30.26	0.36
	a			0.00
	b			0.00
	c			0.00
	d			0.00

Tech Initials:	AD	FM
Date/Time:	2/19/24 1503	2/22/24 0945

QC Check: CM 2/20/24
 Final Review: BO 2/22/24

Client: Internal

Sample ID: CuCl₂

Test No: 240213 ppr4

Test Species: *P. promelas*

Start Date/Time: 2/13/24 1710

End Date/Time: 2/20/24 1025

Concentration	Lab Control							
Day	0	1	2	3	4	5	6	7
pH	8.16	8.17	8.10	8.01	8.13	8.07	8.12	
DO (mg/L)	8.1	7.8	7.7	7.1	8.1	8.1	8.0	
Cond. (µmhos/cm)	196	192	197	194	193	194	197	
Temp (°C)	24.3	24.2	24.0	24.8	24.2	24.5	24.9	
pH		8.04	7.84	7.75	7.35	7.30	7.92	
DO (mg/L)		7.3	6.7	6.6	6.6	6.5	6.9	
Temp (°C)		25.2	25.7	25.3	25.3	25.1	25.3	

Concentration	15 µg/L							
Day	0	1	2	3	4	5	6	7
pH	8.14	8.15	8.10	8.02	8.15	8.10	8.10	
DO (mg/L)	8.0	7.8	7.7	7.2	8.0	8.0	7.8	
Cond. (µmhos/cm)	195	192	197	194	193	194	193	
Temp (°C)	24.5	24.1	24.2	24.8	24.1	24.5	25.6	
pH		8.07	7.80	7.67	7.30	7.70	7.86	
DO (mg/L)		7.3	6.5	6.3	6.3	6.2	6.7	
Temp (°C)		25.5	26.0	25.5	25.6	25.6	25.4	

Concentration	30 µg/L							
Day	0	1	2	3	4	5	6	7
pH	8.12	8.15	8.15	8.02	8.15	8.10	8.10	
DO (mg/L)	7.9	7.8	7.7	7.1	8.0	8.0	7.8	
Cond. (µmhos/cm)	195	191	195	194	193	194	193	
Temp (°C)	24.4	24.0	24.2	24.9	24.1	24.5	25.7	
pH		8.10	7.90	7.67	7.30	7.71	7.89	
DO (mg/L)		7.3	6.6	6.2	6.2	6.2	6.3	
Temp (°C)		25.4	26.0	25.6	25.6	25.7	25.6	

Environmental Chamber: A

Animal Source/Date Received: ABS

Animal Age at Initiation: 1 day

Animal Acclimation Qualifiers (circle all that apply): Q22 / Q23 / Q24 / none

Cu Stock Concentration (µg/L): 90,000

Comments:

QC Check:

Enthalpy Analytical, 4340 Vandever Avenue, San Diego, CA 92120.

Concentration	60 µg/L							
Day	0	1	2	3	4	5	6	7
pH	8.12	8.14	8.15	8.02	8.14	8.09	8.09	
DO (mg/L)	7.8	7.8	7.7	7.1	7.7	8.0	7.8	
Cond. (µmhos/cm)	195	191	195	194	193	193	198	
Temp (°C)	24.4	24.0	24.1	25.0	24.2	24.5	25.6	
pH		8.10	7.92	7.79	7.81	7.79	8.00	
DO (mg/L)		7.3	6.7	6.4	6.3	6.3	6.7	
Temp (°C)		25.4	26.0	25.3	25.4	25.6	25.2	

Concentration	120 µg/L							
Day	0	1	2	3	4	5	6	7
pH	8.11	8.10	8.13	8.01	8.14	8.09	8.08	
DO (mg/L)	7.9	7.8	7.7	7.0	7.9	7.9	7.8	
Cond. (µmhos/cm)	195	191	195	194	193	193	193	
Temp (°C)	24.4	24.0	24.2	24.9	24.0	24.4	25.3	
pH		8.06	7.92	7.73	7.82	7.84	7.96	
DO (mg/L)		7.2	6.7	6.2	6.3	6.4	6.7	
Temp (°C)		25.3	25.8	25.5	25.6	25.4	25.4	

Concentration	240 µg/L							
Day	0	1	2	3	4	5	6	7
pH	8.09	8.09	8.10	8.01	8.07	8.06	8.06	
DO (mg/L)	7.8	7.8	7.8	7.0	8.0	7.9	7.8	
Cond. (µmhos/cm)	195	191	195	193	192	191	193	
Temp (°C)	24.4	24.0	24.2	24.9	24.0	24.3	24.8	
pH		8.05	7.93	7.79	7.92	7.88	8.03	
DO (mg/L)		7.2	6.7	6.4	6.5	6.6	6.9	
Temp (°C)		25.4	25.9	25.5	25.4	25.5	25.4	

Analysts:	Initial:	0	1	2	3	4	5	6	7
		GM	GM	HH	GM	MM	MM	MM	—
	Final:		GM	HH	Ⓟ	MM	MM	MM	MM
Dilutions made by:		GM	LM	LM	GM	WF	MM	HH	—
High conc. made (µg/L):		240	240	240	240	240	240	240	—
Vol. Cu stock added (mL):		5.3	5.3	5.3	5.3	5.3	5.3	5.3	—
Added to Final Volume = 2000 mL									

Final Review:

Fathead Minnow Acute Test

CETIS Summary Report

Report Date: 07 Mar-24 09:18 (p 1 of 2)
Test Code/ID: 240222ppra / 01-0897-6283

Fathead Minnow 96-h Acute Survival Test Nautilus Environmental (CA)

Batch ID: 12-8591-4584	Test Type: Survival (96h)	Analyst:
Start Date: 22 Feb-24 15:15	Protocol: EPA/821/R-02-012 (2002)	Diluent: Diluted Mineral Water (8:2)
Ending Date: 26 Feb-24 15:40	Species: Pimephales promelas	Brine: Not Applicable
Test Length: 4d 0h	Taxon:	Source: Aquatic Biosystems, CO Age: 6d

Sample ID: 21-4593-2308	Code: 240222ppra	Project:
Sample Date: 22 Feb-24	Material: Copper chloride	Source: Reference Toxicant
Receipt Date: 22 Feb-24	CAS (PC):	Station: Copper Chloride
Sample Age: 15h	Client: Internal	

Multiple Comparison Summary							
Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD
07-3174-6445	48h Survival Rate	Steel Many-One Rank Sum Test	60	120	84.85	16.3%	1
12-7413-7791	96h Survival Rate	Steel Many-One Rank Sum Test	✓ 30	60	42.43	14.7%	1

Point Estimate Summary							
Analysis ID	Endpoint	Point Estimate Method	✓	Level	µg/L	95% LCL	95% UCL
11-2537-0098	48h Survival Rate	Trimmed Spearman-Kärber	EC50	144	90.8	229	1
16-1209-7268	96h Survival Rate	Trimmed Spearman-Kärber	✓ EC50	68.6	53.4	88	1

Test Acceptability							
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
12-7413-7791	96h Survival Rate	Control Resp	1	0.9	<<	Yes	Passes Criteria
16-1209-7268	96h Survival Rate	Control Resp	1	0.9	<<	Yes	Passes Criteria

48h Survival Rate Summary											
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LC	4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
15		4	0.975	0.895	1.050	0.900	1.000	0.025	0.050	5.13%	2.50%
30		4	0.875	0.723	1.030	0.800	1.000	0.048	0.096	10.94%	12.50%
60		4	0.800	0.509	1.090	0.600	1.000	0.091	0.183	22.82%	20.00%
120		4	0.550	0.274	0.826	0.300	0.700	0.087	0.173	31.49%	45.00%
240		4	0.350	0.145	0.555	0.200	0.500	0.065	0.129	36.89%	65.00%

96h Survival Rate Summary											
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LC	4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
15		4	0.875	0.723	1.030	0.800	1.000	0.048	0.096	10.94%	12.50%
30		4	0.825	0.625	1.030	0.700	1.000	0.063	0.126	15.25%	17.50%
60		4	0.450	0.358	0.542	0.400	0.500	0.029	0.058	12.83%	55.00%
120		4	0.350	0.074	0.626	0.100	0.500	0.087	0.173	49.49%	65.00%
240		4	0.175	0.023	0.327	0.100	0.300	0.048	0.096	54.71%	82.50%

CETIS Summary Report

Report Date:
Test Code/ID:

07 Mar-24 09:18 (p 2 of 2)
240222ppra / 01-0897-6283

Fathead Minnow 96-h Acute Survival Test

Nautilus Environmental (CA)

48h Survival Rate Detail						MD5: 8C6024D02801D1E7BE7D722434EDC802
Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	
0	LC	1.000	1.000	1.000	1.000	
15		1.000	0.900	1.000	1.000	
30		0.800	0.800	0.900	1.000	
60		1.000	0.900	0.600	0.700	
120		0.700	0.600	0.300	0.600	
240		0.200	0.500	0.300	0.400	
96h Survival Rate Detail						MD5: 721B72B2585719EAC225FBED97A4E051
Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	
0	LC	1.000	1.000	1.000	1.000	
15		1.000	0.800	0.900	0.800	
30		0.800	0.700	0.800	1.000	
60		0.500	0.400	0.400	0.500	
120		0.500	0.400	0.100	0.400	
240		0.100	0.300	0.100	0.200	

CETIS Analytical Report

Report Date: 07 Mar-24 09:18 (p 1 of 4)
Test Code/ID: 240222ppra / 01-0897-6283

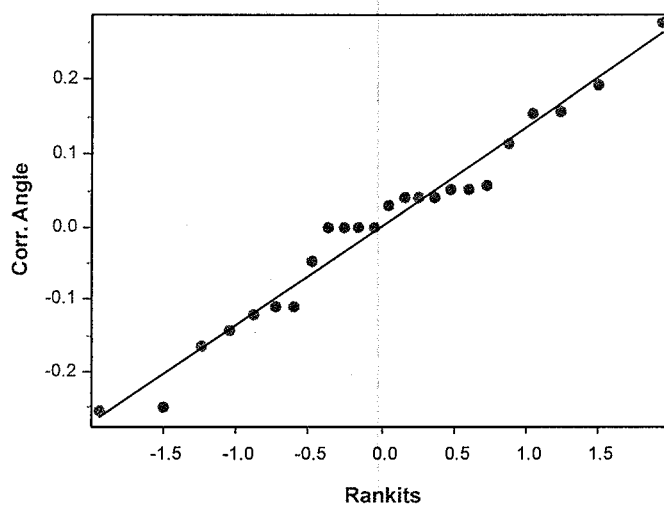
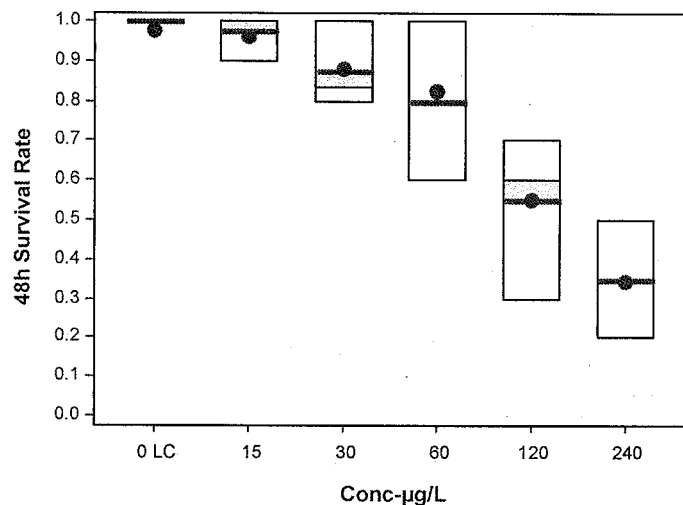
Fathead Minnow 96-h Acute Survival Test									Nautilus Environmental (CA)			
Analysis ID: 07-3174-6445			Endpoint: 48h Survival Rate				CETIS Version:		CETISv2.1.4			
Analyzed: 07 Mar-24 9:17			Analysis: Nonparametric-Control vs Treatments				Status Level:		1			
Edit Date: 07 Mar-24 9:15			MD5 Hash: 8C6024D02801D1E7BE7D722434EDC802				Editor ID:		000-502-715-6			
Data Transform		Alt Hyp			NOEL		LOEL	TOEL	Tox Units	MSDu	PMSD	
Angular (Corrected)		C > T			60		120	84.85	---	0.163	16.25%	
Steel Many-One Rank Sum Test												
Control	vs	Conc-µg/L	df	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)			
Lab Control		15	6	16	10	1	CDF	0.6105	Non-Significant Effect			
		30	6	12	10	1	CDF	0.1424	Non-Significant Effect			
		60	6	12	10	1	CDF	0.1424	Non-Significant Effect			
		120*	6	10	10	0	CDF	0.0417	Significant Effect			
		240*	6	10	10	0	CDF	0.0417	Significant Effect			
ANOVA Table												
Source		Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α:5%)			
Between		1.9142		0.382839		5	16.9	<1.0E-05	Significant Effect			
Error		0.407646		0.022647		18						
Total		2.32184				23						
ANOVA Assumptions Tests												
Attribute		Test				Test Stat	Critical	P-Value	Decision(α:1%)			
Variance		Bartlett Equality of Variance Test							Indeterminate			
Distribution		Shapiro-Wilk W Normality Test				0.968	0.884	0.6238	Normal Distribution			
48h Survival Rate Summary												
Conc-µg/L		Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0		LC	4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
15			4	0.975	0.895	1.000	1.000	0.900	1.000	0.025	5.13%	2.50%
30			4	0.875	0.723	1.000	0.833	0.800	1.000	0.048	10.94%	12.50%
60			4	0.800	0.509	1.000	0.800	0.600	1.000	0.091	22.82%	20.00%
120			4	0.550	0.274	0.826	0.600	0.300	0.700	0.087	31.49%	45.00%
240			4	0.350	0.145	0.555	0.350	0.200	0.500	0.065	36.89%	65.00%
Angular (Corrected) Transformed Summary												
Conc-µg/L		Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0		LC	4	1.410	1.410	1.410	1.410	1.410	1.410	0.000	0.00%	0.00%
15			4	1.370	1.240	1.500	1.410	1.250	1.410	0.041	5.94%	2.89%
30			4	1.220	0.988	1.450	1.150	1.110	1.410	0.073	11.91%	13.68%
60			4	1.130	0.753	1.520	1.120	0.886	1.410	0.120	21.13%	19.65%
120			4	0.836	0.553	1.120	0.886	0.580	0.991	0.089	21.27%	40.81%
240			4	0.628	0.408	0.848	0.632	0.464	0.785	0.069	22.00%	55.50%

CETIS Analytical Report

Report Date: 07 Mar-24 09:18 (p 2 of 4)
Test Code/ID: 240222ppra / 01-0897-6283

Fathead Minnow 96-h Acute Survival Test			Nautilus Environmental (CA)
Analysis ID: 07-3174-6445	Endpoint: 48h Survival Rate	CETIS Version: CETISv2.1.4	
Analyzed: 07 Mar-24 9:17	Analysis: Nonparametric-Control vs Treatments	Status Level: 1	
Edit Date: 07 Mar-24 9:15	MD5 Hash: 8C6024D02801D1E7BE7D722434EDC802	Editor ID: 000-502-715-6	

Graphics



CETIS Analytical Report

Report Date: 07 Mar-24 09:18 (p 3 of 4)
Test Code/ID: 240222ppra / 01-0897-6283

Fathead Minnow 96-h Acute Survival Test										Nautilus Environmental (CA)			
Analysis ID: 12-7413-7791			Endpoint: 96h Survival Rate					CETIS Version:		CETISv2.1.4			
Analyzed: 07 Mar-24 9:17			Analysis: Nonparametric-Control vs Treatments					Status Level:		1			
Edit Date: 07 Mar-24 9:15			MD5 Hash: 721B72B2585719EAC225FBED97A4E051					Editor ID:		000-502-715-6			
Data Transform			Alt Hyp					NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)			C > T					30	60	42.43	---	0.147	14.70%
Steel Many-One Rank Sum Test													
Control	vs	Conc-µg/L	df	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)				
Lab Control		15	6	12	10	1	CDF	0.1424	Non-Significant Effect				
		30	6	12	10	1	CDF	0.1424	Non-Significant Effect				
		60*	6	10	10	0	CDF	0.0417	Significant Effect				
		120*	6	10	10	0	CDF	0.0417	Significant Effect				
		240*	6	10	10	0	CDF	0.0417	Significant Effect				
ANOVA Table													
Source	Sum Squares			Mean Square		DF	F Stat	P-Value	Decision(α:5%)				
Between	3.03621			0.607242		5	31.9	<1.0E-05	Significant Effect				
Error	0.342163			0.0190091		18							
Total	3.37838					23							
ANOVA Assumptions Tests													
Attribute	Test					Test Stat	Critical	P-Value	Decision(α:1%)				
Variance	Bartlett Equality of Variance Test								Indeterminate				
Distribution	Shapiro-Wilk W Normality Test					0.973	0.884	0.7479	Normal Distribution				
96h Survival Rate Summary													
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect		
0	LC	4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%		
15		4	0.875	0.723	1.000	0.833	0.800	1.000	0.048	10.94%	12.50%		
30		4	0.825	0.625	1.000	0.800	0.700	1.000	0.063	15.25%	17.50%		
60		4	0.450	0.358	0.542	0.450	0.400	0.500	0.029	12.83%	55.00%		
120		4	0.350	0.074	0.626	0.400	0.100	0.500	0.087	49.49%	65.00%		
240		4	0.175	0.023	0.327	0.133	0.100	0.300	0.048	54.71%	82.50%		
Angular (Corrected) Transformed Summary													
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect		
0	LC	4	1.410	1.410	1.410	1.410	1.410	1.410	0.000	0.00%	0.00%		
15		4	1.220	0.988	1.450	1.150	1.110	1.410	0.073	11.91%	13.68%		
30		4	1.150	0.868	1.440	1.110	0.991	1.410	0.090	15.62%	18.25%		
60		4	0.735	0.643	0.828	0.735	0.685	0.785	0.029	7.91%	47.94%		
120		4	0.619	0.295	0.944	0.685	0.322	0.785	0.102	32.93%	56.15%		
240		4	0.422	0.223	0.620	0.369	0.322	0.580	0.062	29.58%	70.14%		

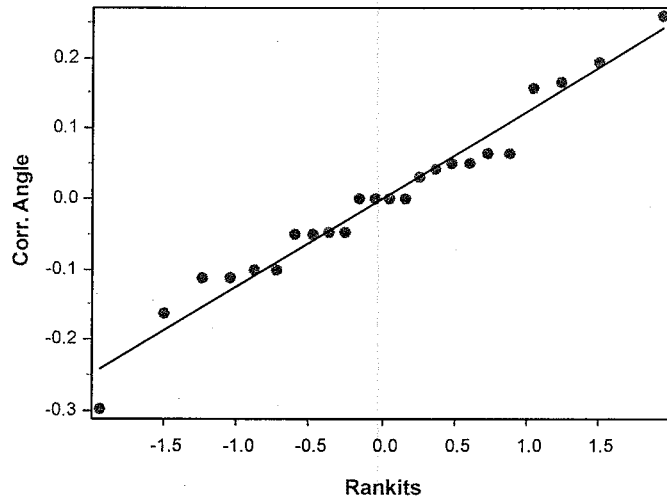
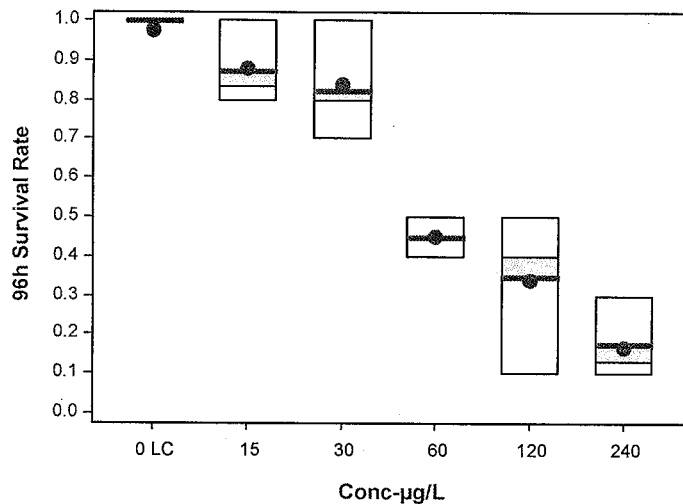
CETIS Analytical Report

Report Date:
Test Code/ID:

07 Mar-24 09:18 (p 4 of 4)
240222ppra / 01-0897-6283

Fathead Minnow 96-h Acute Survival Test			Nautilus Environmental (CA)
Analysis ID: 12-7413-7791	Endpoint: 96h Survival Rate	CETIS Version: CETISv2.1.4	
Analyzed: 07 Mar-24 9:17	Analysis: Nonparametric-Control vs Treatments	Status Level: 1	
Edit Date: 07 Mar-24 9:15	MD5 Hash: 721B72B2585719EAC225FBED97A4E051	Editor ID: 000-502-715-6	

Graphics



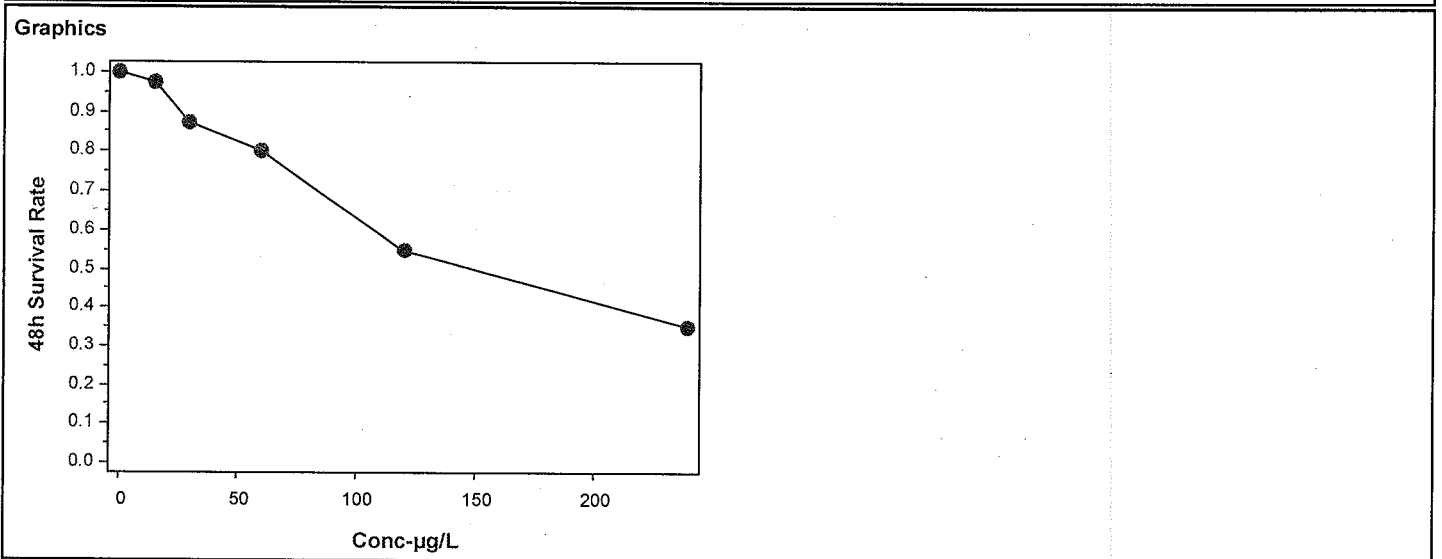
CETIS Analytical Report

Report Date: 07 Mar-24 09:18 (p 1 of 2)
Test Code/ID: 240222ppra / 01-0897-6283

Fathead Minnow 96-h Acute Survival Test				Nautilus Environmental (CA)	
Analysis ID: 11-2537-0098		Endpoint: 48h Survival Rate		CETIS Version: CETISv2.1.4	
Analyzed: 07 Mar-24 9:17		Analysis: Trimmed Spearman-Kärber		Status Level: 1	
Edit Date: 07 Mar-24 9:15		MD5 Hash: 8C6024D02801D1E7BE7D722434EDC802		Editor ID: 000-502-715-6	

Trimmed Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	35.00%	2.16	0.101	144	90.8	229

48h Survival Rate Summary			Calculated Variate(A/B)						Isotonic Variate		
Conc-µg/L	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	LC	4	1.000	1.000	1.000	1.000	0.00%	0.00%	40/40	1.000	0.00%
15		4	0.975	1.000	0.900	1.000	5.13%	2.50%	39/40	0.975	2.50%
30		4	0.875	0.833	0.800	1.000	10.94%	12.50%	35/40	0.875	12.50%
60		4	0.800	0.800	0.600	1.000	22.82%	20.00%	32/40	0.800	20.00%
120		4	0.550	0.600	0.300	0.700	31.49%	45.00%	22/40	0.550	45.00%
240		4	0.350	0.350	0.200	0.500	36.89%	65.00%	14/40	0.350	65.00%

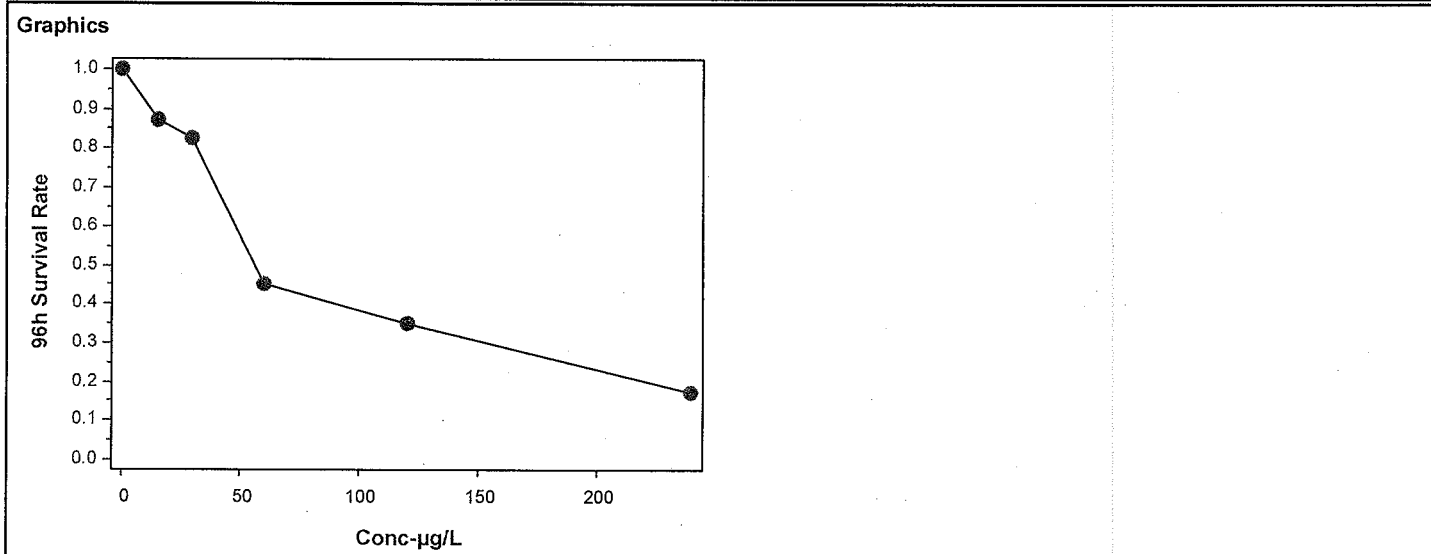


CETIS Analytical Report

Report Date: 07 Mar-24 09:18 (p 2 of 2)
Test Code/ID: 240222ppra / 01-0897-6283

Fathead Minnow 96-h Acute Survival Test				Nautilus Environmental (CA)	
Analysis ID: 16-1209-7268		Endpoint: 96h Survival Rate		CETIS Version: CETISv2.1.4	
Analyzed: 07 Mar-24 9:17		Analysis: Trimmed Spearman-Kärber		Status Level: 1	
Edit Date: 07 Mar-24 9:15		MD5 Hash: 721B72B2585719EAC225FBED97A4E051		Editor ID: 000-502-715-6	

Trimmed Spearman-Kärber Estimates											
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL				
Control Threshold	0	17.50%	1.84	0.0542	68.6	53.4	88				
96h Survival Rate Summary			Calculated Variate(A/B)							Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	LC	4	1.000	1.000	1.000	1.000	0.00%	0.00%	40/40	1.000	0.00%
15		4	0.875	0.833	0.800	1.000	10.94%	12.50%	35/40	0.875	12.50%
30		4	0.825	0.800	0.700	1.000	15.25%	17.50%	33/40	0.825	17.50%
60		4	0.450	0.450	0.400	0.500	12.83%	55.00%	18/40	0.450	55.00%
120		4	0.350	0.400	0.100	0.500	49.49%	65.00%	14/40	0.350	65.00%
240		4	0.175	0.133	0.100	0.300	54.71%	82.50%	7/40	0.175	82.50%



Fathead Minnow 96-h Acute Survival Test

Nautilus Environmental (CA)

Test Type: Survival (96h)

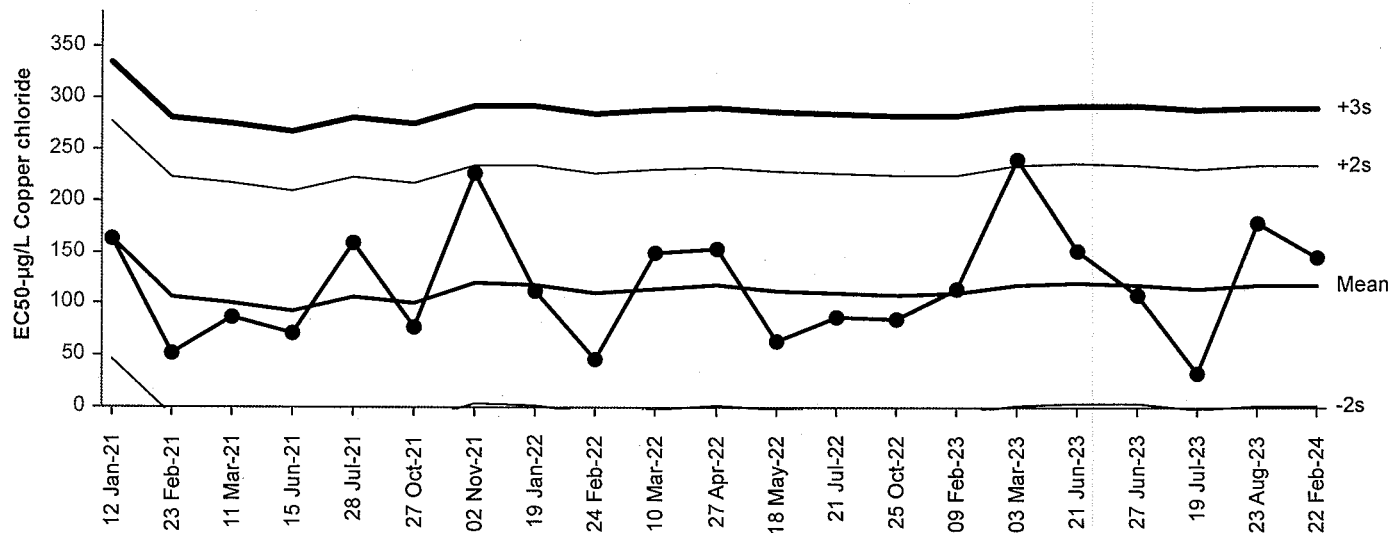
Organism: Pimephales promelas

Material: Copper chloride

Protocol: EPA/821/R-02-012 (2002)

Endpoint: 48h Survival Rate

Source: Reference Toxicant-REF

Fathead Minnow 96-h Acute Survival Test
48h Survival Rate Endpoint

Cumulative Mean Plot

Mean: 117.8

Count: 20

-2s Warning Limit: 2.07

-3s Action Limit: -55.8

Sigma: 57.87

CV: 49.10%

+2s Warning Limit: 234

+3s Action Limit: 291

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2021	Jan	12	16:10	162	44.17	0.7633			10-2818-5435	06-6635-0923
2		Feb	23	16:00	52.12	-65.68	-1.135			00-7897-7348	11-8791-1156
3		Mar	11	16:25	87.73	-30.07	-0.5196			20-0307-6488	09-7792-6619
4		Jun	15	17:10	72.41	-45.39	-0.7843			13-1662-1659	08-5606-9821
5		Jul	28	17:30	158.5	40.71	0.7034			04-8837-0734	01-0161-1237
6		Oct	27	17:55	76.85	-40.95	-0.7076			04-8454-9323	14-4414-7186
7		Nov	2	14:55	226.5	108.7	1.879			02-5381-8973	16-7936-7486
8	2022	Jan	19	14:00	112	-5.836	-0.1008			20-2049-9334	01-6000-4303
9		Feb	24	16:10	46.11	-71.69	-1.239			17-0760-7068	20-2870-9446
10		Mar	10	15:55	148.3	30.51	0.5272			12-1339-4334	05-8313-0752
11		Apr	27	15:10	152.4	34.62	0.5983			04-5378-2545	12-0148-2591
12		May	18	16:55	63.25	-54.55	-0.9427			18-5661-4183	10-1564-5343
13		Jul	21	17:45	87.7	-30.1	-0.5201			07-1587-3363	01-9531-4154
14		Oct	25	15:50	84.92	-32.88	-0.5682			06-6314-9915	16-5489-8506
15	2023	Feb	9	16:50	114.7	-3.139	-0.05424			06-2469-6093	06-5193-3336
16		Mar	3	16:35	240	122.2	2.112	(+)		05-5862-3435	09-2232-2619
17		Jun	21	14:55	151.5	33.66	0.5816			03-3580-9094	02-6448-3283
18			27	14:30	107.7	-10.12	-0.1748			11-9788-2598	20-7987-6598
19		Jul	19	16:55	33.27	-84.53	-1.461			12-1198-2690	14-4220-8343
20		Aug	23	14:10	178.3	60.52	1.046			16-5077-3459	06-6997-4897
21	2024	Feb	22	15:15	144.4	26.56	0.459			01-0897-6283	11-2537-0098

CETIS QC Plot

Report Date: 07 Mar-24 09:18 (1 of 1)

Fathead Minnow 96-h Acute Survival Test

Nautilus Environmental (CA)

Test Type: Survival (96h)

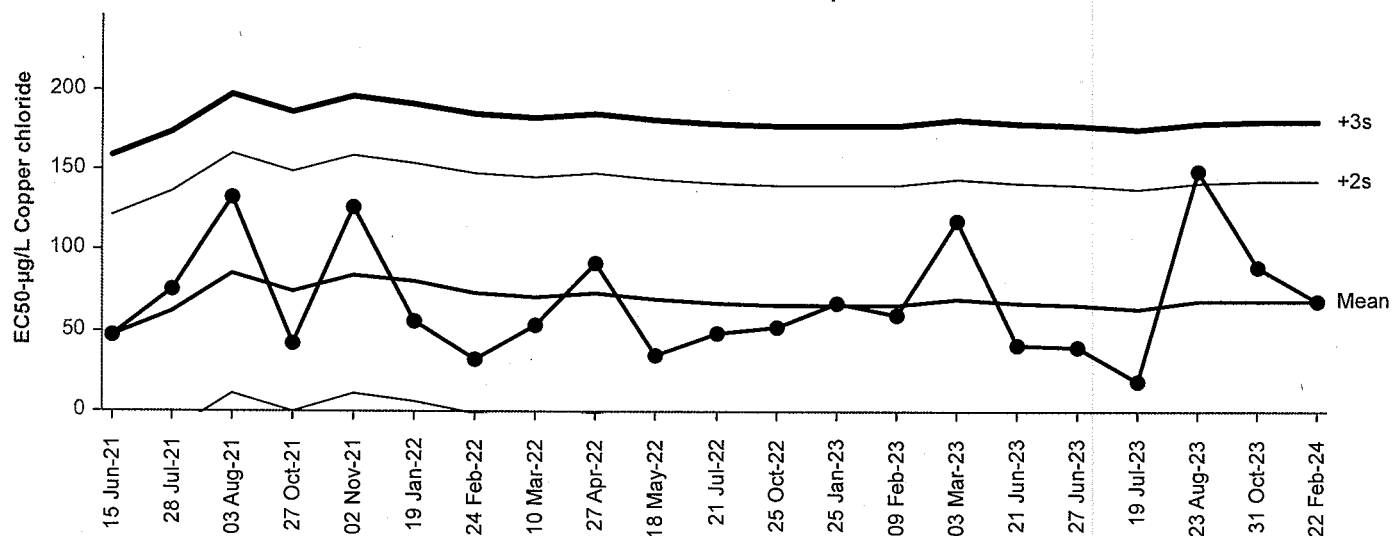
Organism: Pimephales promelas

Material: Copper chloride

Protocol: EPA/821/R-02-012 (2002)

Endpoint: 96h Survival Rate

Source: Reference Toxicant-REF

Fathead Minnow 96-h Acute Survival Test
96h Survival Rate Endpoint

Cumulative Mean Plot

Mean: 68.89

Count: 20

-2s Warning Limit: -5.51

-3s Action Limit: -42.7

Sigma: 37.2

CV: 54.00%

+2s Warning Limit: 143

+3s Action Limit: 180

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2021	Jun	15	17:10	47.63	-21.26	-0.5716			13-1662-1659	03-5455-1927
2		Jul	28	17:30	75.92	7.027	0.1889			04-8837-0734	00-2418-4039
3		Aug	3	15:30	133.2	64.29	1.728			01-5905-1678	06-9846-1307
4		Oct	27	17:55	41.65	-27.24	-0.7323			04-8454-9323	21-2291-3266
5		Nov	2	14:55	126.6	57.68	1.551			02-5381-8973	13-3748-9296
6	2022	Jan	19	14:00	56.14	-12.75	-0.3426			20-2049-9334	07-9545-0483
7		Feb	24	16:10	32.6	-36.29	-0.9754			17-0760-7068	11-1555-4113
8		Mar	10	15:55	54.03	-14.86	-0.3995			12-1339-4334	02-2933-1085
9		Apr	27	15:10	91.63	22.74	0.6112			04-5378-2545	07-8420-2882
10		May	18	16:55	35.22	-33.67	-0.905			18-5661-4183	07-3447-2353
11		Jul	21	17:45	48.45	-20.44	-0.5496			07-1587-3363	06-2880-7627
12		Oct	25	15:50	52.4	-16.49	-0.4434			06-6314-9915	03-2187-1829
13	2023	Jan	25	18:01	67.41	-1.479	-0.03975			19-0784-2205	08-9150-6242
14		Feb	9	16:50	59.42	-9.469	-0.2546			06-2469-6093	00-8217-4012
15		Mar	3	16:35	118.4	49.54	1.332			05-5862-3435	03-5254-4793
16		Jun	21	14:55	40.38	-28.51	-0.7665			03-3580-9094	00-4894-4439
17			27	14:30	39.27	-29.62	-0.7961			11-9788-2598	16-4880-2585
18		Jul	19	16:55	19.16	-49.73	-1.337			12-1198-2690	17-1336-5643
19		Aug	23	14:10	149	80.13	2.154	(+)		16-5077-3459	12-7429-9907
20		Oct	31	16:30	89.22	20.33	0.5464			14-5248-4726	19-3257-9871
21	2024	Feb	22	15:15	68.56	-0.3346	-0.009			01-0897-6283	16-1209-7268

96-hour Freshwater Acute Bioassay
Static-Renewal Conditions
DF-006

Water Quality Measurements
& Test Organism Survival

Client: Internal
Sample ID: CuCl₂
Test No.: 240222 ppra

Test Species: P. promelas
Start Date/Time: 2/22/24 1515
Renewal Date/Time: 2/24/24 1450
End Date/Time: 2/26/24 1540

Tech Initials				
0	24	48	72	96
Count: MK	RT	WF	LM	RT
Reading: MK	WF	WF	LM	MK
Dilutions made by: LM	-	LM	-	-
High conc. made (µg/L): 240	-	240	-	-
Vol. Cu stock added (mL): 5.3	-	5.3	-	-
Final Volume (mL): 2000	-	2000	-	-

Cu stock concentration (µg/L): 90,000

Concentration µg/L	RAND #	Number of Live Organisms					pH (units)					Dissolved Oxygen (mg/L)					Conductivity (µmhos/cm)					Temperature (°C)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	19	10	10	10	10	10	7.9	8.04	8.02	7.99	8.00	8.3	8.0	8.8	8.0	7.9	191	203	203	209	221	19.9	21.4	19.2	20.7	20.3
	4	10	10	10	10	10			8.05					8.4					218					19.9		
	21	10	10	10	10	10																				
	24	10	10	10	10	10																				
15	11	10	10	10	10	10	8.01	8.09	8.09	8.03	8.07	8.3	8.1	8.7	8.1	8.1	191	203	187	188	220	19.9	21.1	19.2	20.7	20.3
	13	10	10	9	8	8			8.23					8.5					217					19.7		
	20	10	10	10	10	9																				
	10	10	10	10	9	8																				
30	18	10	10	8	8	8	8.03	8.11	8.06	8.10	8.13	8.7	8.1	8.9	8.3	8.2	191	206	203	210	230	20.0	21.4	19.3	20.3	20.4
	3	10	10	8	8	7			8.18					8.4					221					20.0		
	8	10	9	9	9	8																				
	15	10	10	10	10	10																				
60	2	10	10	10	8	5	8.06	8.06	8.06	8.02	8.06	8.3	8.0	8.9	8.1	8.1	191	222	203	207	225	19.9	21.4	19.3	20.4	20.6
	14	10	10	9	5	4			8.09					8.2					230					20.1		
	22	10	8	6	4	4																				
	16	10	9	7	5	5																				
120	12	10	10	7	5	5	8.08	8.11	8.11	8.06	8.09	8.9	8.2	8.9	8.1	8.2	191	203	202	203	220	20.0	21.3	19.3	20.5	20.6
	6	10	9	6	5	4			8.19					8.2					214					20.1		
	1	10	8	3	2	1																				
	23	10	9	6	5	4																				
240	9	10	4	2	1	1	8.06	8.12	8.10	8.06	8.07	8.9	8.2	8.9	8.2	8.2	191	201	203	202	210	19.9	21.2	19.2	20.6	20.6
	5	10	8	5	3	3			8.17					8.3					211					20.1		
	7	10	8	3	1	1																				
	17	10	7	4	3	2																				

Rand # QC: WF
Counts QC'd by: 25030 WF
Initiated by: MK

Environmental Chamber: C

Animal Source/Date Received: ABS/ 2/22/24

Age at Initiation: 5 days

Animal Acclimation Qualifiers (circle all that apply):

Q22 ☐ Q23 ☒ Q24 ☐ none ☒

Comments:

i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal

Organisms fed prior to initiation, circle one (y n) y

2/23/24

2/24/24

QC Check: CM 3/6/24

Final Review: BO 3/14/24

Water Flea Acute Test

CETIS Summary Report

Report Date: 06 Mar-24 16:16 (p 1 of 2)
Test Code/ID: 240222cdra / 01-3884-2391

Ceriodaphnia 96-h Acute Survival Test

Nautilus Environmental (CA)

Batch ID: 18-4907-8800	Test Type: Survival (96h)	Analyst:	
Start Date: 22 Feb-24 15:50	Protocol: EPA/821/R-02-012 (2002)	Diluent: Diluted Mineral Water (8:2)	
Ending Date: 26 Feb-24 15:20	Species: Ceriodaphnia dubia	Brine: Not Applicable	
Test Length: 96h	Taxon:	Source: In-House Culture	Age: <24 <i>W</i>

Sample ID: 02-3067-5417	Code: 240222cdra	Project:	
Sample Date: 22 Feb-24	Material: Copper chloride	Source: Reference Toxicant	
Receipt Date: 22 Feb-24	CAS (PC):	Station: Copper Chloride	
Sample Age: 16h	Client: Internal		

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	S
06-5742-3071	48h Survival Rate	Steel Many-One Rank Sum Test	✓	20	40	28.28	28.4%	1
04-9755-7963	96h Survival Rate	Steel Many-One Rank Sum Test	✓	20	40	28.28	25.7%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	µg/L	95% LCL	95% UCL	S
20-8623-2542	48h Survival Rate	Spearman-Kärber		EC50	23.8	20.8	27.2	1
04-2542-0939	96h Survival Rate	Spearman-Kärber	✓	EC50	23	19.9	26.5	1

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits			Decision
				Lower	Upper	Overlap	
06-5742-3071	48h Survival Rate	Control Resp	1	0.9	<<	Yes	Passes Criteria
20-8623-2542	48h Survival Rate	Control Resp	1	0.9	<<	Yes	Passes Criteria
04-2542-0939	96h Survival Rate	Control Resp	1	0.9	<<	Yes	Passes Criteria
04-9755-7963	96h Survival Rate	Control Resp	1	0.9	<<	Yes	Passes Criteria

48h Survival Rate Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LC	4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
5		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
10		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
20		4	0.750	0.148	1.350	0.200	1.000	0.189	0.379	50.48%	25.00%
40		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%
80		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%

96h Survival Rate Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LC	4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
5		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
10		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
20		4	0.700	0.149	1.250	0.200	1.000	0.173	0.346	49.49%	30.00%
40		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%
80		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%

CETIS Summary Report

Report Date:

06 Mar-24 16:16 (p 2 of 2)

Test Code/ID:

240222cdra / 01-3884-2391

Ceriodaphnia 96-h Acute Survival Test

Nautilus Environmental (CA)

48h Survival Rate Detail

MD5: 79D08418228AB36D9D892BFC58E3EC4D

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	LC	1.000	1.000	1.000	1.000
5		1.000	1.000	1.000	1.000
10		1.000	1.000	1.000	1.000
20		0.200	1.000	0.800	1.000
40		0.000	0.000	0.000	0.000
80		0.000	0.000	0.000	0.000

96h Survival Rate Detail

MD5: 048B4C3C3DAFFE90F5F3CC608673A007

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	LC	1.000	1.000	1.000	1.000
5		1.000	1.000	1.000	1.000
10		1.000	1.000	1.000	1.000
20		0.200	0.800	0.800	1.000
40		0.000	0.000	0.000	0.000
80		0.000	0.000	0.000	0.000

CETIS Analytical Report

Report Date:

06 Mar-24 16:16 (p 1 of 4)

Test Code/ID:

240222cdra / 01-3884-2391

Ceriodaphnia 96-h Acute Survival Test										Nautilus Environmental (CA)				
Analysis ID: 06-5742-3071			Endpoint: 48h Survival Rate					CETIS Version:		CETISv2.1.4				
Analyzed: 06 Mar-24 16:15			Analysis: Nonparametric-Control vs Treatments					Status Level:		1				
Edit Date: 06 Mar-24 16:14			MD5 Hash: 79D08418228AB36D9D892BFC58E3EC4D					Editor ID:		000-502-715-6				
Data Transform			Alt Hyp			NOEL		LOEL		TOEL		Tox Units	MSDu	PMSD
Angular (Corrected)			C > T			20		40		28.28		---	0.284	28.43%
Steel Many-One Rank Sum Test														
Control		vs	Conc-µg/L		df	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)			
Lab Control		5	6	18	10	1	CDF	0.7500	Non-Significant Effect					
		10	6	18	10	1	CDF	0.7500	Non-Significant Effect					
		20	6	14	10	1	CDF	0.2626	Non-Significant Effect					
ANOVA Table														
Source		Sum Squares			Mean Square			DF	F Stat	P-Value	Decision(α:5%)			
Between		0.235103			0.0783677			3	1.81	0.1995	Non-Significant Effect			
Error		0.520518			0.0433765			12						
Total		0.755621						15						
ANOVA Assumptions Tests														
Attribute		Test					Test Stat	Critical	P-Value	Decision(α:1%)				
Variance		Bartlett Equality of Variance Test								Indeterminate				
Distribution		Shapiro-Wilk W Normality Test					0.578	0.841	1.0E-05	Non-Normal Distribution				
48h Survival Rate Summary														
Conc-µg/L		Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect		
0		LC	4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%		
5			4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%		
10			4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%		
20			4	0.750	0.148	1.000	0.933	0.200	1.000	0.189	50.48%	25.00%		
40			4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%		
80			4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%		
Angular (Corrected) Transformed Summary														
Conc-µg/L		Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect		
0		LC	4	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%		
5			4	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%		
10			4	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%		
20			4	1.070	0.403	1.730	1.270	0.464	1.350	0.208	39.10%	20.81%		
40			4	0.226	0.225	0.226	0.226	0.226	0.226	0.000	0.00%	83.24%		
80			4	0.226	0.225	0.226	0.226	0.226	0.226	0.000	0.00%	83.24%		

CETIS Analytical Report

Report Date:

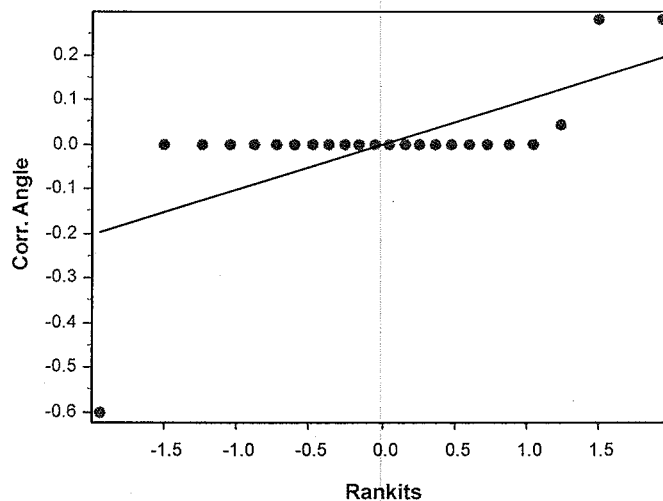
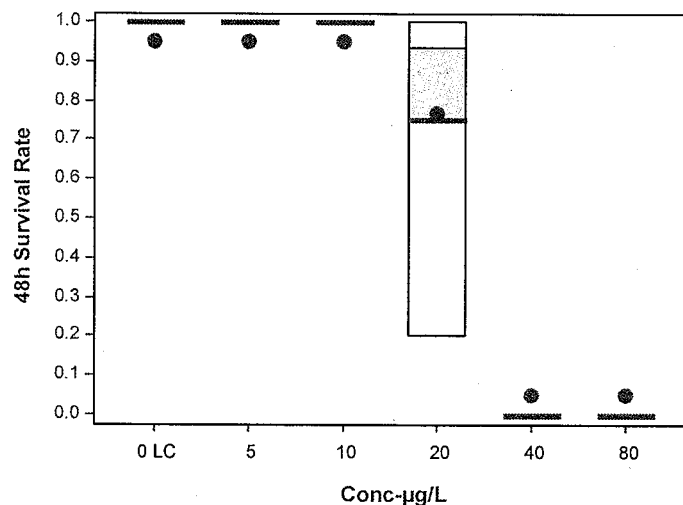
06 Mar-24 16:16 (p 2 of 4)

Test Code/ID:

240222cdra / 01-3884-2391

Ceriodaphnia 96-h Acute Survival Test			Nautilus Environmental (CA)
Analysis ID: 06-5742-3071	Endpoint: 48h Survival Rate	CETIS Version:	CETISv2.1.4
Analyzed: 06 Mar-24 16:15	Analysis: Nonparametric-Control vs Treatments	Status Level:	1
Edit Date: 06 Mar-24 16:14	MD5 Hash: 79D08418228AB36D9D892BFC58E3EC4D	Editor ID:	000-502-715-6

Graphics



CETIS Analytical Report

Report Date:

06 Mar-24 16:16 (p 3 of 4)

Test Code/ID:

240222cdra / 01-3884-2391

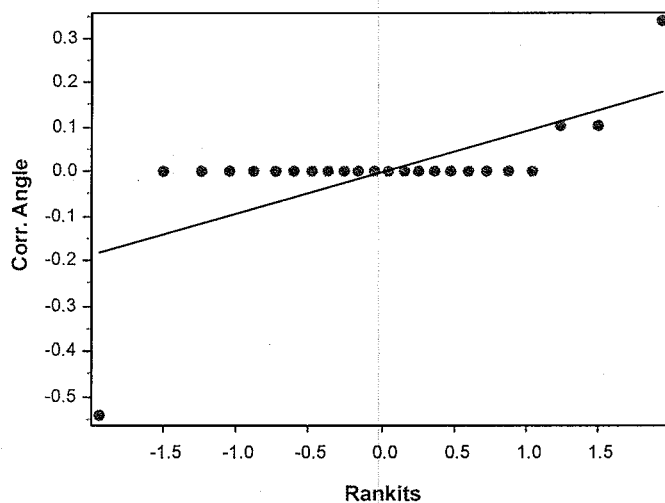
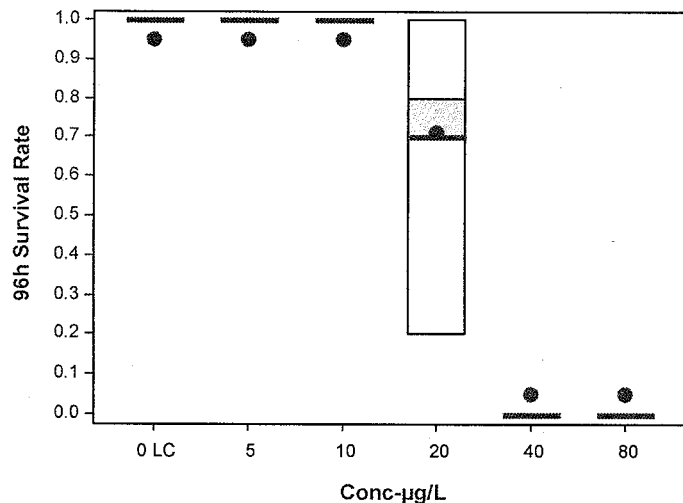
Ceriodaphnia 96-h Acute Survival Test										Nautilus Environmental (CA)				
Analysis ID: 04-9755-7963			Endpoint: 96h Survival Rate					CETIS Version:		CETISv2.1.4				
Analyzed: 06 Mar-24 16:15			Analysis: Nonparametric-Control vs Treatments					Status Level:		1				
Edit Date: 06 Mar-24 16:14			MD5 Hash: 048B4C3C3DAFFE90F5F3CC608673A007					Editor ID:		000-502-715-6				
Data Transform		Alt Hyp				NOEL		LOEL		TOEL		Tox Units	MSDu	PMSD
Angular (Corrected)		C > T				20		40		28.28		---	0.257	25.70%
Steel Many-One Rank Sum Test														
Control		vs	Conc-µg/L		df	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)			
Lab Control		5	6	18	10	1	CDF	0.7500	Non-Significant Effect					
		10	6	18	10	1	CDF	0.7500	Non-Significant Effect					
		20	6	12	10	1	CDF	0.1003	Non-Significant Effect					
ANOVA Table														
Source		Sum Squares			Mean Square			DF	F Stat	P-Value	Decision(α:5%)			
Between		0.345732			0.115244			3	3.22	0.0614	Non-Significant Effect			
Error		0.429721			0.0358101			12						
Total		0.775453						15						
ANOVA Assumptions Tests														
Attribute		Test					Test Stat	Critical	P-Value	Decision(α:1%)				
Variance		Bartlett Equality of Variance Test								Indeterminate				
Distribution		Shapiro-Wilk W Normality Test					0.593	0.841	1.4E-05	Non-Normal Distribution				
96h Survival Rate Summary														
Conc-µg/L		Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect		
0		LC	4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%		
5			4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%		
10			4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%		
20			4	0.700	0.149	1.000	0.800	0.200	1.000	0.173	49.49%	30.00%		
40			4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%		
80			4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%		
Angular (Corrected) Transformed Summary														
Conc-µg/L		Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect		
0		LC	4	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%		
5			4	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%		
10			4	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%		
20			4	1.010	0.404	1.610	1.110	0.464	1.350	0.189	37.63%	25.23%		
40			4	0.226	0.225	0.226	0.226	0.226	0.226	0.000	0.00%	83.24%		
80			4	0.226	0.225	0.226	0.226	0.226	0.226	0.000	0.00%	83.24%		

CETIS Analytical Report

Report Date: 06 Mar-24 16:16 (p 4 of 4)
Test Code/ID: 240222cdra / 01-3884-2391

Ceriodaphnia 96-h Acute Survival Test			Nautilus Environmental (CA)
Analysis ID: 04-9755-7963	Endpoint: 96h Survival Rate	CETIS Version: CETISv2.1.4	
Analyzed: 06 Mar-24 16:15	Analysis: Nonparametric-Control vs Treatments	Status Level: 1	
Edit Date: 06 Mar-24 16:14	MD5 Hash: 048B4C3C3DAFFE90F5F3CC608673A007	Editor ID: 000-502-715-6	

Graphics



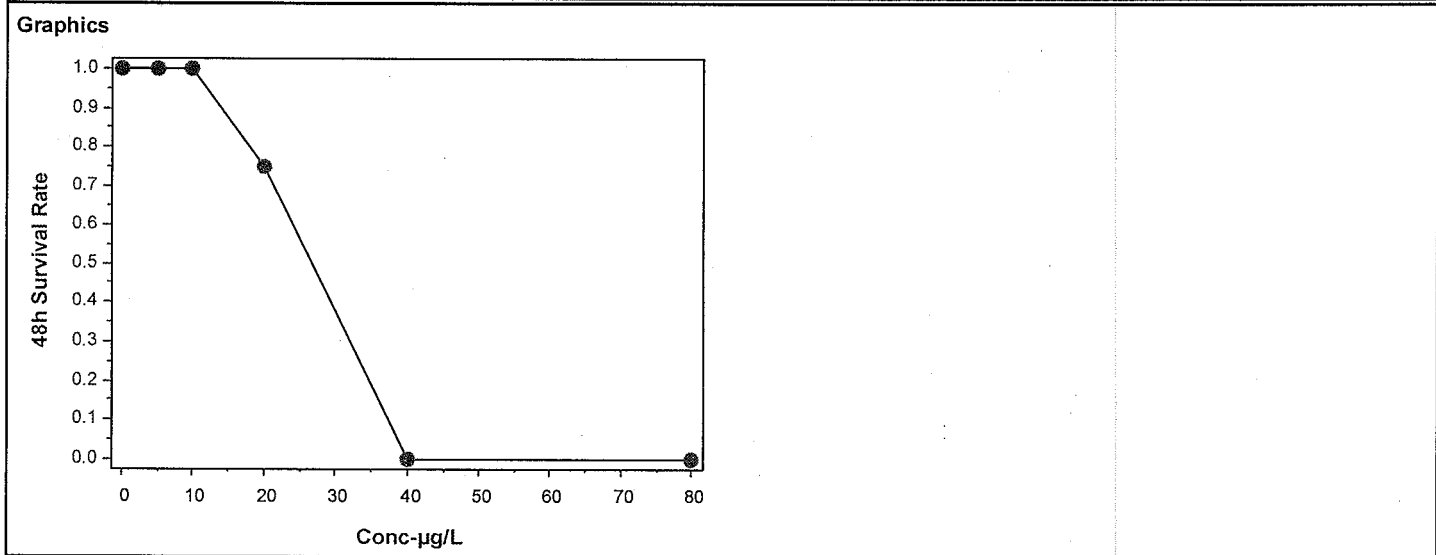
CETIS Analytical Report

Report Date: 06 Mar-24 16:16 (p 1 of 2)
Test Code/ID: 240222cdra / 01-3884-2391

Ceriodaphnia 96-h Acute Survival Test						Nautilus Environmental (CA)					
Analysis ID: 20-8623-2542	Endpoint: 48h Survival Rate				CETIS Version: CETISv2.1.4						
Analyzed: 06 Mar-24 16:15	Analysis: Untrimmed Spearman-Kärber				Status Level: 1						
Edit Date: 06 Mar-24 16:14	MD5 Hash: 79D08418228AB36D9D892BFC58E3EC4D				Editor ID: 000-502-715-6						

Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	0.00%	1.38	0.0291	23.8	20.8	27.2

48h Survival Rate Summary			Calculated Variate(A/B)							Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	LC	4	1.000	1.000	1.000	1.000	0.00%	0.00%	20/20	1.000	0.00%
5		4	1.000	1.000	1.000	1.000	0.00%	0.00%	20/20	1.000	0.00%
10		4	1.000	1.000	1.000	1.000	0.00%	0.00%	20/20	1.000	0.00%
20		4	0.750	0.933	0.200	1.000	50.48%	25.00%	15/20	0.750	25.00%
40		4	0.000	0.000	0.000	0.000	---	100.00%	0/20	0.000	100.00%
80		4	0.000	0.000	0.000	0.000	---	100.00%	0/20	0.000	100.00%



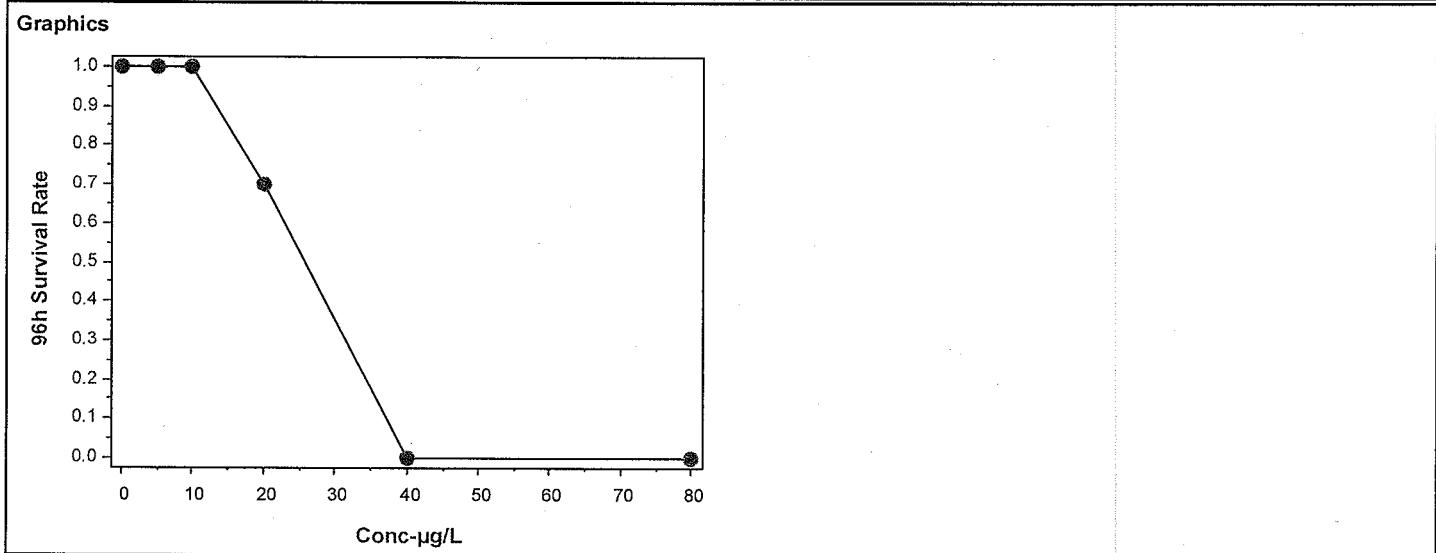
CETIS Analytical Report

Report Date: 06 Mar-24 16:16 (p 2 of 2)
Test Code/ID: 240222cdra / 01-3884-2391

Ceriodaphnia 96-h Acute Survival Test				Nautilus Environmental (CA)	
Analysis ID: 04-2542-0939		Endpoint: 96h Survival Rate		CETIS Version: CETISv2.1.4	
Analyzed: 06 Mar-24 16:15		Analysis: Untrimmed Spearman-Kärber		Status Level: 1	
Edit Date: 06 Mar-24 16:14		MD5 Hash: 048B4C3C3DAFFE90F5F3CC608673A007		Editor ID: 000-502-715-6	

Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	0.00%	1.36	0.0308	23	19.9	26.5

96h Survival Rate Summary			Calculated Variate(A/B)							Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	LC	4	1.000	1.000	1.000	1.000	0.00%	0.00%	20/20	1.000	0.00%
5		4	1.000	1.000	1.000	1.000	0.00%	0.00%	20/20	1.000	0.00%
10		4	1.000	1.000	1.000	1.000	0.00%	0.00%	20/20	1.000	0.00%
20		4	0.700	0.800	0.200	1.000	49.49%	30.00%	14/20	0.700	30.00%
40		4	0.000	0.000	0.000	0.000	---	100.00%	0/20	0.000	100.00%
80		4	0.000	0.000	0.000	0.000	---	100.00%	0/20	0.000	100.00%



CETIS QC Plot

Report Date: 06 Mar-24 16:16 (1 of 1)

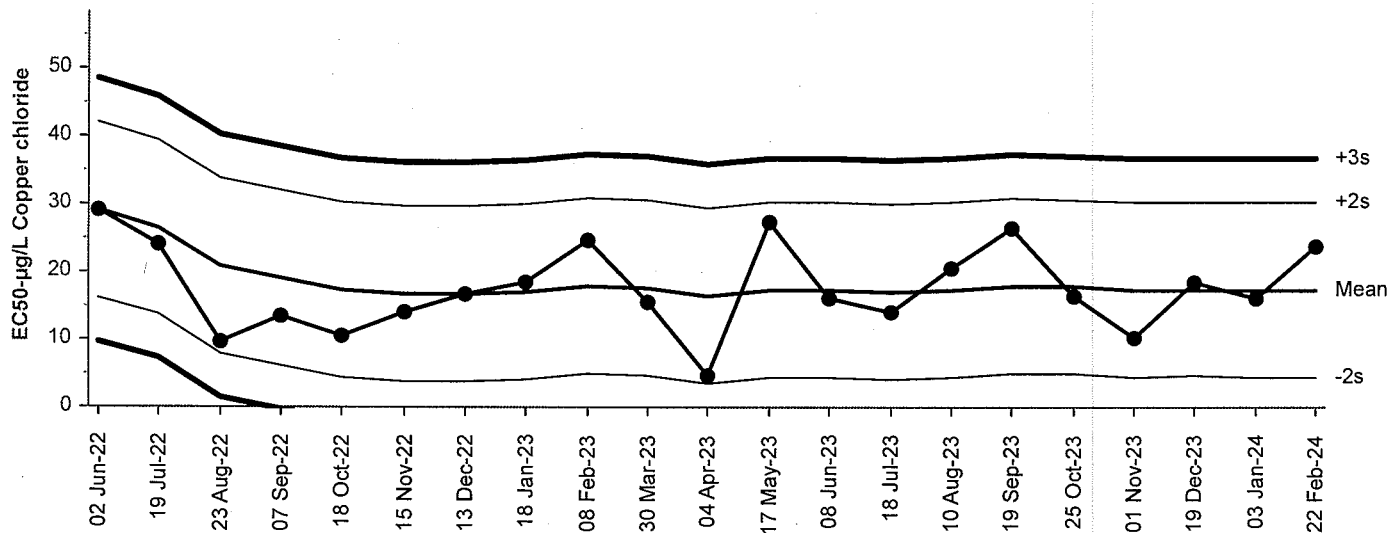
Ceriodaphnia 96-h Acute Survival Test

Nautilus Environmental (CA)

Test Type: Survival (96h)
Protocol: EPA/821/R-02-012 (2002)

Organism: Ceriodaphnia dubia
Endpoint: 48h Survival Rate

Material: Copper chloride
Source: Reference Toxicant-REF

Ceriodaphnia 96-h Acute Survival Test
48h Survival Rate Endpoint

Cumulative Mean Plot

Mean: 17.42 Count: 20 -2s Warning Limit: 4.52 -3s Action Limit: -1.93
Sigma: 6.447 CV: 37.00% +2s Warning Limit: 30.3 +3s Action Limit: 36.8

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2022	Jun	2	15:40	29.19	11.77	1.826			13-6497-5171	06-9942-1854
2		Jul	19	15:45	23.99	6.575	1.02			03-1124-9631	12-3859-6928
3		Aug	23	15:50	9.659	-7.761	-1.204			20-3988-3287	17-3739-4911
4		Sep	7	15:00	13.66	-3.76	-0.5832			01-7106-9553	21-1245-7931
5		Oct	18	15:25	10.52	-6.903	-1.071			00-3320-6805	10-3076-2017
6		Nov	15	15:05	14.14	-3.278	-0.5084			00-5461-1467	10-1769-9308
7		Dec	13	15:05	16.65	-0.7686	-0.1192			12-0086-6428	02-1199-7151
8	2023	Jan	18	13:55	18.66	1.241	0.1924			09-7538-0685	11-5700-2740
9		Feb	8	15:20	24.8	7.379	1.145			17-7377-6097	13-5479-5533
10		Mar	30	14:50	15.69	-1.728	-0.2681			00-8046-0950	12-2862-1113
11		Apr	4	15:20	4.75	-12.67	-1.965			07-0540-7813	03-2121-3277
12		May	17	15:30	27.32	9.901	1.536			04-0322-0654	02-8046-2726
13		Jun	8	15:35	16.25	-1.175	-0.1822			04-4576-9053	14-0223-6638
14		Jul	18	15:55	14.14	-3.278	-0.5084			09-9837-6211	04-2183-4412
15		Aug	10	16:00	20.71	3.285	0.5096			10-9457-1593	17-9736-0526
16		Sep	19	15:20	26.39	8.97	1.391			05-7527-0059	18-5992-4492
17		Oct	25	15:30	16.53	-0.891	-0.1382			12-6281-0744	05-3358-8558
18		Nov	1	14:45	10.35	-7.067	-1.096			11-4473-5077	01-6940-4248
19		Dec	19	15:15	18.66	1.241	0.1924			18-6862-1657	09-4880-9105
20	2024	Jan	3	14:30	16.25	-1.175	-0.1822			06-2149-8718	05-2352-2825
21		Feb	22	15:50	23.78	6.364	0.9871			01-3884-2391	20-8623-2542

CETIS QC Plot

Report Date: 06 Mar-24 16:16 (1 of 1)

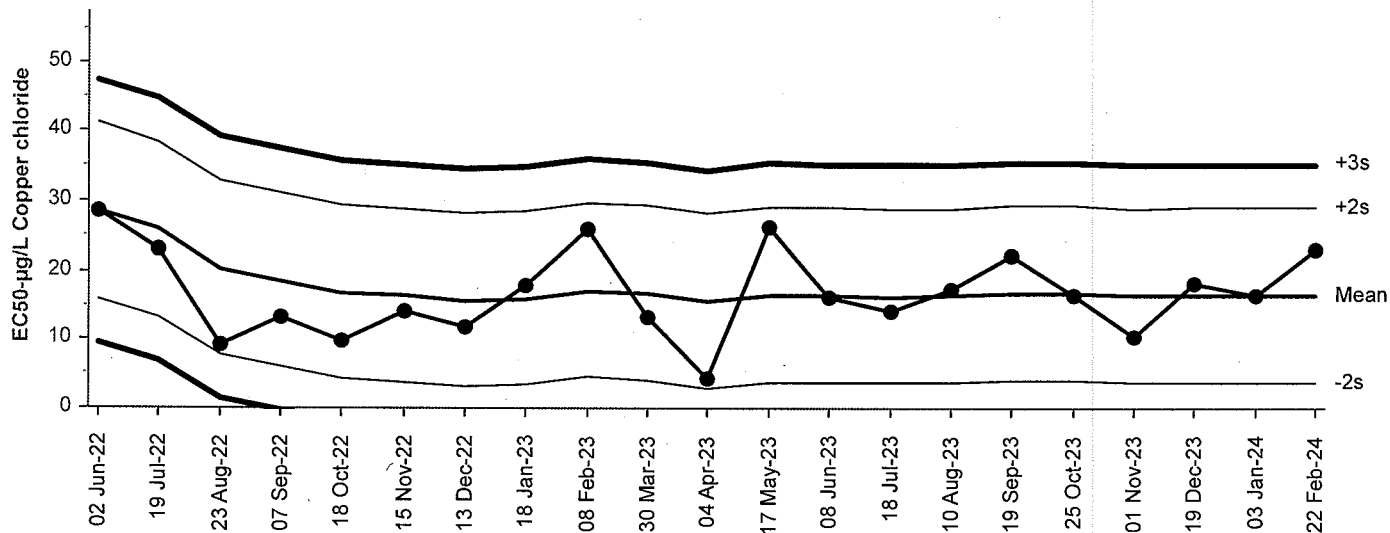
Ceriodaphnia 96-h Acute Survival Test

Nautilus Environmental (CA)

Test Type: Survival (96h)
Protocol: EPA/821/R-02-012 (2002)

Organism: Ceriodaphnia dubia
Endpoint: 96h Survival Rate

Material: Copper chloride
Source: Reference Toxicant-REF

Ceriodaphnia 96-h Acute Survival Test
96h Survival Rate Endpoint

Cumulative Mean Plot

Mean: 16.46 Count: 20 -2s Warning Limit: 3.87 -3s Action Limit: -2.43
Sigma: 6.298 CV: 38.30% +2s Warning Limit: 29.1 +3s Action Limit: 35.4

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2022	Jun	2	15:40	28.57	12.11	1.922			13-6497-5171	08-6278-4122
2		Jul	19	15:45	23.16	6.704	1.064			03-1124-9631	00-8098-2495
3		Aug	23	15:50	9.33	-7.13	-1.132			20-3988-3287	11-6761-6164
4		Sep	7	15:00	13.2	-3.265	-0.5184			01-7106-9553	05-0730-6008
5		Oct	18	15:25	9.862	-6.598	-1.048			00-3320-6805	04-6100-3813
6		Nov	15	15:05	14.14	-2.318	-0.368			00-5461-1467	19-6868-6561
7		Dec	13	15:05	11.7	-4.758	-0.7555			12-0086-6428	08-7195-5995
8	2023	Jan	18	13:55	18.03	1.565	0.2485			09-7538-0685	07-8161-1407
9		Feb	8	15:20	25.96	9.498	1.508			17-7377-6097	02-7722-6792
10		Mar	30	14:50	13.2	-3.265	-0.5184			00-8046-0950	18-8859-9281
11		Apr	4	15:20	4.318	-12.14	-1.928			07-0540-7813	08-4239-9426
12		May	17	15:30	26.39	9.93	1.577			04-0322-0654	15-8704-5627
13		Jun	8	15:35	16.25	-0.215	-0.03413			04-4576-9053	18-8894-4384
14		Jul	18	15:55	14.14	-2.318	-0.368			09-9837-6211	11-0291-4351
15		Aug	10	16:00	17.41	0.951	0.151			10-9457-1593	13-6631-9515
16		Sep	19	15:20	22.19	5.731	0.91			05-7527-0059	21-3108-9916
17		Oct	25	15:30	16.53	0.06901	0.01096			12-6281-0744	01-0251-4169
18		Nov	1	14:45	10.35	-6.107	-0.9697			11-4473-5077	14-7590-4791
19		Dec	19	15:15	18.26	1.797	0.2853			18-6862-1657	12-1996-9983
20	2024	Jan	3	14:30	16.32	-0.1371	-0.02177			06-2149-8718	18-3000-4863
21		Feb	22	15:50	22.97	6.514	1.034			01-3884-2391	04-2542-0939

96-hour Freshwater Acute Bioassay
Static-Renewal Conditions
DF-002

Water Quality Measurements
& Test Organism Survival

Client: Internal
Sample ID: CuCl₂
Test No.: 240222cd12a

Test Species: C. dubia
Start Date/Time: 2/22/24 1550
Renewal Date/Time: 2/24/24 1510
End Date/Time: 2/26/24 1520

Tech Initials					
0	24	48	72	96	
HM	HM	HM	HM	HM	
WF	WF	WF	WF	WF	
LM	LM	LM	LM	LM	
Counts:					
Readings:					
Dilutions made by:					
High conc. made (µg/L):					
Vol. Cu stock added (mL):					
Final Volume (mL):					

Cu stock concentration (µg/L): 9,200

Concentration µg/L	Rand #	Number of Live Organisms					pH (units)					Dissolved Oxygen (mg/L)					Conductivity (µmhos/cm)					Temperature (°C)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	6	5	5	5	5	5	8.0	8.22	7.94	8.13	8.16	8.7	8.3	9.0	8.9	8.7	197	203	195	203	222	20.9	21.2	19.9	20.5	20.3
	16	5	5	5	5	5			8.01					8.8					210					20.2		
	5	5	5	5	5	5																				
	15	5	5	5	5	5																				
5	24	5	5	5	5	5	8.0	8.22	8.01	8.12	8.20	8.8	8.4	9.0	8.0	8.7	197	201	196	203	236	20.9	21.3	19.3	19.7	20.3
	21	5	5	5	5	5			8.15					8.9					215					20.2		
	9	5	5	5	5	5																				
	1	5	5	5	5	5																				
10	2	5	5	5	5	5	8.11	8.21	7.94	8.12	8.27	8.9	8.4	9.0	8.9	8.7	197	209	195	197	230	20.2	21.1	19.9	19.7	20.3
	4	5	5	5	5	5			8.18					8.9					225					20.2		
	13	5	5	5	5	5																				
	12	5	5	5	5	5																				
20	22	5	5	5	5	5	8.09	8.21	7.98	8.23	8.26	8.8	8.5	9.0	8.8	8.7	197	204	195	197	230	20.9	21.1	19.9	19.7	20.3
	17	5	5	5	4	4			8.15					8.8					214					20.2		
	10	5	5	4	4	4																				
	20	5	5	5	5	5																				
40	23	5	0				8.09	8.21				8.8	8.4				197	197				20.9	21.5			
	11	5	0																							
	18	5	0																							
	8	5	0																							
80	19	5	0				8.11	8.23				8.8	8.6				197	231				20.5	20.4			
	7	5	0																							
	3	5	0																							
	14	5	0																							

Rand # QC: QC
Initial Count QC'd by: LC

Environmental Chamber: C
Animal Source/Date Received: Internal / NA Age at Initiation: < 24 hr

Comments:

i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal

Organisms fed prior to initiation, circle one (A) (B) (C) (D) (E) (F) (G) (H) (I) (J) (K) (L) (M) (N) (O) (P) (Q) (R) (S) (T) (U) (V) (W) (X) (Y) (Z) (AA) (AB) (AC) (AD) (AE) (AF) (AG) (AH) (AI) (AJ) (AK) (AL) (AM) (AN) (AO) (AP) (AQ) (AR) (AS) (AT) (AU) (AV) (AW) (AX) (AY) (AZ) (BA) (BB) (BC) (BD) (BE) (BF) (BG) (BH) (BI) (BJ) (BK) (BL) (BM) (BN) (BO) (BP) (BQ) (BR) (BS) (BT) (BU) (BV) (BW) (BX) (BY) (BZ) (CA) (CB) (CC) (CD) (CE) (CF) (CG) (CH) (CI) (CJ) (CK) (CL) (CM) (CN) (CO) (CP) (CQ) (CR) (CS) (CT) (CU) (CV) (CW) (CX) (CY) (CZ) (DA) (DB) (DC) (DD) (DE) (DF) (DG) (DH) (DI) (DJ) (DK) (DL) (DM) (DN) (DO) (DP) (DQ) (DR) (DS) (DT) (DU) (DV) (DW) (DX) (DY) (DZ) (EA) (EB) (EC) (ED) (EE) (EF) (EG) (EH) (EI) (EJ) (EK) (EL) (EM) (EN) (EO) (EP) (EQ) (ER) (ES) (ET) (EU) (EV) (EW) (EX) (EY) (EZ) (FA) (FB) (FC) (FD) (FE) (FF) (FG) (FH) (FI) (FJ) (FK) (FL) (FM) (FN) (FO) (FP) (FQ) (FR) (FS) (FT) (FU) (FV) (FW) (FX) (FY) (FZ) (GA) (GB) (GC) (GD) (GE) (GF) (GG) (GH) (GI) (GJ) (GK) (GL) (GM) (GN) (GO) (GP) (GQ) (GR) (GS) (GT) (GU) (GV) (GW) (GX) (GY) (GZ) (HA) (HB) (HC) (HD) (HE) (HF) (HG) (HH) (HI) (HJ) (HK) (HL) (HM) (HN) (HO) (HP) (HQ) (HR) (HS) (HT) (HU) (HV) (HW) (HX) (HY) (HZ) (IA) (IB) (IC) (ID) (IE) (IF) (IG) (IH) (II) (IJ) (IK) (IL) (IM) (IN) (IO) (IP) (IQ) (IR) (IS) (IT) (IU) (IV) (IW) (IX) (IY) (IZ) (JA) (JB) (JC) (JD) (JE) (JF) (JG) (JH) (JI) (JJ) (JK) (JL) (JM) (JN) (JO) (JP) (JQ) (JR) (JS) (JT) (JU) (JV) (JW) (JX) (JY) (JZ) (KA) (KB) (KC) (KD) (KE) (KF) (KG) (KH) (KI) (KJ) (KK) (KL) (KM) (KN) (KO) (KP) (KQ) (KR) (KS) (KT) (KU) (KV) (KW) (KX) (KY) (KZ) (LA) (LB) (LC) (LD) (LE) (LF) (LG) (LH) (LI) (LJ) (LK) (LL) (LM) (LN) (LO) (LP) (LQ) (LR) (LS) (LT) (LU) (LV) (LW) (LX) (LY) (LZ) (MA) (MB) (MC) (MD) (ME) (MF) (MG) (MH) (MI) (MJ) (MK) (ML) (MN) (MO) (MP) (MQ) (MR) (MS) (MT) (MU) (MV) (MW) (MX) (MY) (MZ) (NA) (NB) (NC) (ND) (NE) (NF) (NG) (NH) (NI) (NJ) (NK) (NL) (NM) (NN) (NO) (NP) (NQ) (NR) (NS) (NT) (NU) (NV) (NW) (NX) (NY) (NZ) (OA) (OB) (OC) (OD) (OE) (OF) (OG) (OH) (OI) (OJ) (OK) (OL) (OM) (ON) (OO) (OP) (OQ) (OR) (OS) (OT) (OU) (OV) (OW) (OX) (OY) (OZ) (PA) (PB) (PC) (PD) (PE) (PF) (PG) (PH) (PI) (PJ) (PK) (PL) (PM) (PN) (PO) (PP) (PQ) (PR) (PS) (PT) (PU) (PV) (PW) (PX) (PY) (PZ) (QA) (QB) (QC) (QD) (QE) (QF) (QG) (QH) (QI) (QJ) (QK) (QL) (QM) (QN) (QO) (QP) (QQ) (QR) (QS) (QT) (QU) (QV) (QW) (QX) (QY) (QZ) (RA) (RB) (RC) (RD) (RE) (RF) (RG) (RH) (RI) (RJ) (RK) (RL) (RM) (RN) (RO) (RP) (RQ) (RR) (RS) (RT) (RU) (RV) (RW) (RX) (RY) (RZ) (SA) (SB) (SC) (SD) (SE) (SF) (SG) (SH) (SI) (SJ) (SK) (SL) (SM) (SN) (SO) (SP) (SQ) (SR) (SS) (ST) (SU) (SV) (SW) (SX) (SY) (SZ) (TA) (TB) (TC) (TD) (TE) (TF) (TG) (TH) (TI) (TJ) (TK) (TL) (TM) (TN) (TO) (TP) (TQ) (TR) (TS) (TT) (TU) (TV) (TW) (TX) (TY) (TZ) (UA) (UB) (UC) (UD) (UE) (UF) (UG) (UH) (UI) (UJ) (UK) (UL) (UM) (UN) (UO) (UP) (UQ) (UR) (US) (UT) (UU) (UV) (UW) (UX) (UY) (UZ) (VA) (VB) (VC) (VD) (VE) (VF) (VG) (VH) (VI) (VJ) (VK) (VL) (VM) (VN) (VO) (VP) (VQ) (VR) (VS) (VT) (VU) (VV) (VW) (VX) (VY) (VZ) (WA) (WB) (WC) (WD) (WE) (WF) (WG) (WH) (WI) (WJ) (WK) (WL) (WM) (WN) (WO) (WP) (WQ) (WR) (WS) (WT) (WU) (WV) (WW) (WX) (WY) (WZ) (XA) (XB) (XC) (XD) (XE) (XF) (XG) (XH) (XI) (XJ) (XK) (XL) (XM) (XN) (XO) (XP) (XQ) (XR) (XS) (XT) (XU) (XV) (XW) (XX) (XY) (XZ) (YA) (YB) (YC) (YD) (YE) (YF) (YG) (YH) (YI) (YJ) (YK) (YL) (YM) (YN) (YO) (YP) (YQ) (YR) (YS) (YT) (YU) (YV) (YW) (YX) (YZ) (ZA) (ZB) (ZC) (ZD) (ZE) (ZF) (ZG) (ZH) (ZI) (ZJ) (ZK) (ZL) (ZM) (ZN) (ZO) (ZP) (ZQ) (ZR) (ZS) (ZT) (ZU) (ZV) (ZW) (ZX) (ZY) (ZZ)

QC Check: CM 3/6/24

Final Review: BO 3/14/24