

TOXICITY TESTING RESULTS

BROOKS MANUFACTURING CO.

BELLINGHAM, WASHINGTON

ANNUAL WHOLE EFFLUENT TOXICITY TESTING: OCTOBER 2023

Prepared for

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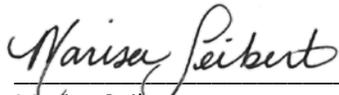
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NELAP, ORELAP ID 4165

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APPENDIX

Appendix A:	Laboratory Documents
Appendix B:	Chain-of-Custody and Sample Receipt Forms

ACRONYMS AND ABBREVIATIONS

ACEC	Acute critical effluent concentration
CCEC	Chronic critical effluent concentration
EPA	Environmental Protection Agency
LC ₂₅ /EC ₂₅	Lethal/ Effect Concentration to 25% of test population
LC ₅₀ /EC ₅₀	Lethal/Effect Concentration that results in a 50% reduction in survival
LOEL	Lowest Observed Effect Level
mg/L	Milligrams per liter
NPDES	National Pollutant Discharge Elimination System
NOEL	No Observed Effect Level
QM	Quality manual
µg/L	Micrograms per liter
SOP	Standard operation procedure
TU _c	Chronic toxic units
WDOE	Washington Department of Ecology
WET	Whole Effluent Toxicity

1. EXECUTIVE SUMMARY

EcoAnalysts conducted Whole Effluent Toxicity (WET) testing on three 24-hour composite effluent samples collected by Brooks Manufacturing Co. personnel as part of the effluent characterization. The objective of this program is to assess the potential toxicity of the effluent to aquatic organisms following procedures defined under the facility’s National Pollutant Discharge Elimination System (NPDES) permit.

Statistically significant reduction in biological effect was detected at the acute critical effluent concentration (ACEC) and the chronic critical effluent concentration (CCEC), both 100% effluent, for the *Pimephales promelas* biomass endpoint only (Table 1-1). The sample exceeds the defined permit requirements (Table 1-2), though this result may be considered anomalous. If WDOE agrees, this conclusion may result in the need to perform one additional chronic test with fathead minnows. See section 3.6 for more information.

Table 1-1. Toxicity Test Results Summary.

Test		NOEL (%)	LOEL (%)	LC ₂₅ /EC ₂₅ (%)	LC ₅₀ /EC ₅₀ (%)	TU
Acute	Water Flea (<i>Ceriodaphnia dubia</i>) 48-hour Survival	100	>100	>100	>100	1
	Fathead Minnow (<i>Pimephales promelas</i>) 96-hour Survival	100	>100	>100	>100	1
	Rainbow Trout (<i>Oncorhynchus mykiss</i>) 96-hour Survival	100	>100	>100	>100	1
Chronic	Water Flea (<i>Ceriodaphnia dubia</i>) 7-day Survival	100	>100	>100	>100	1
	Water Flea (<i>Ceriodaphnia dubia</i>) 7-day Reproduction	100	>100	>100	>100	1
	Fathead Minnow (<i>Pimephales promelas</i>) 7-day Survival	100	>100	>100	>100	1
	Fathead Minnow (<i>Pimephales promelas</i>) 7-day Biomass	<6.25	6.25	>100	>100	<1
	Green Alga (<i>Pseudokirchneriella subcapitata</i> , formerly known as <i>Selenastrum capricornutum</i>) 96-hour Growth	100	>100	>100	>100	<1

NOEL = No Observed Effect Level

LOEL = Lowest Observed Effect Level

LC₂₅/EC₂₅ = Lethal/Effect Concentration to 25% of test population

LC₅₀/EC₅₀ = Lethal/Effect Concentration to 50% of test population

TU = 100/NOEL (acute/chronic survival), 100/EC₂₅ (7-day reproduction, growth, and biomass).

Table 1-2. Permit Compliance Results.

Permit Requirement	<i>The Permittee must: Conduct yearly chronic toxicity testing on the final effluent starting in September 2023 after the treatment solution is switched from PCP to DCOI. Permittee must conduct acute and chronic toxicity testing using a series of dilutions which includes the ACEC that equals 100% effluent and the CCEC of 100% effluent.</i>
Result	A statistically significant reduction in biological effect was detected at the acute critical effluent concentration (ACEC) and the chronic critical effluent concentration (CCEC), both 100% effluent for the <i>Pimephales promelas</i> biomass endpoint.

2. METHODS

The samples analyzed for toxicity were tested using criteria outlined in the Washington Department of Ecology’s (WDOE) Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria (WDOE WQ-R-95-80 (2016)). These criteria are further defined through the Environmental Protection Agency’s (EPA) most recently promulgated effluent guidance documents outlined in Section 4.

Bioassay testing for this project consisted of three acute and three chronic bioassays. The tests conducted in support of this project are summarized in Table 2-1.

Table 2-1. Biological Testing Performed.

Test Type	Test Descriptor	Species	Method
Acute	48-hour Survival	<i>Ceriodaphnia dubia</i> Water Flea	WDOE WQ-R-95-80 (2016); EPA-821-R-02-012 Method 2000.0; SOP TOX004.11
	96-hour Survival	<i>Pimephales promelas</i> Fathead Minnow	WDOE WQ-R-95-80 (2016); EPA-821-R-02-012 Method 2000.0; SOP TOX017.10
	96-hour Survival	<i>Oncorhynchus mykiss</i> Rainbow Trout	WDOE WQ-R-95-80 (2016); EPA-821-R-02-012 Method 2019.0; SOP TOX016.08
Chronic	7-Day Survival and Reproduction	<i>Ceriodaphnia dubia</i> Water Flea	WDOE WQ-R-95-80 (2016); EPA-821-R-02-013; Test Method 1002.0; SOP TOX003.11
	7-Day Survival and Growth	<i>Pimephales promelas</i> Fathead Minnow	WDOE WQ-R-95-80 (2016); EPA-821-R-02-013; Test Method 1000.0; SOP TOX018.11
	96-Hour Growth	<i>Raphidocelis subcapitata</i> (formerly <i>Selenastrum capricornutum</i>) Green Alga	WDOE WQ-R-95-80 (2016); EPA-821-R-02-013; Test Method 1003.0; SOP TOX023.01

2.1 Sample Collection

Brooks Manufacturing Co. personnel collected samples on October 23, 25, 27, 2023. The samples were delivered by overnight courier (FedEx) and received at the EcoAnalysts Port Gamble laboratory on the day following collection. Sample temperatures upon receipt ranged from 0.0 – 5.5°C.

2.1.1 Sample Receipt Discussion

Sample temperatures upon receipt were within the recommended temperature range of 0 - 6°C. Additional sample conditions are summarized in Table 2-2. The effluent sample was held in a walk-in cold room at 4 ± 2 °C in the dark until utilized for testing.

Table 2-2. Sample Conditions Upon Receipt.

Sample	Outfall 1		
Laboratory ID	P231024.01	P231026.04	P231028.03
Date/Time Sampled	10/23/23; 1430	10/25/23; 0645	10/27/23; 1015
Date/Time Received	10/24/23; 1030	10/26/23; 1045	10/28/23; 1228
Time Between Sampling and Receipt	20 hours	28 hours	26 hours, 13 minutes
Test Dissolved Oxygen (mg/L) Recommended: >4.0 mg/L	10.0	10.4	12.6
Test Temperature (°C) Upon Receipt Ideal: 4°C or <1 hour from sample: 0 – 20°C <4 hours from sample: 0 – 12°C ≥4 hours from sample: 0 – 6°C	0.3 – 5.5	0.1	0.0
Test pH (units) Recommended: 6 – 9	7.0	7.0	7.3
Test Conductivity (µS/cm)	715	160	201
Hardness (mg/L CaCO ₃)	70	43	62
Alkalinity (mg/L CaCO ₃)	54	48	44
Total Chlorine (mg/L)	0.02	0.01	0.03
Total Ammonia (mg/L)	0.108	0.0430	0.00

2.2 Water for Bioassay Testing

Freshwater diluent used in this study was prepared using the EPA method for standard, synthetic moderately hard, reconstituted water, using the reagent grade chemicals (USEPA 2002), or, for the rainbow trout test, carbon-filtered tap water. The diluent used for the *Raphidocelis subcapitata* was nutrient-enriched moderately-hard synthetic water (USEPA 2002). Extensive testing on a variety of test species has shown that there is no significant potential for toxicity or bioaccumulation of contaminants from this water supply. Chemical analysis of this water source is conducted and reviewed on an annual basis.

2.3 Quality Assurance/Quality Control

The quality assurance objectives for toxicity testing conducted by the testing laboratory are detailed in the laboratory's quality manual (QM) as well as the method specific guidance documents (USEPA 2002; WDOE 2016). The methods employed in every phase of the toxicity testing program are detailed in the EcoAnalysts Standard Operating Practices (SOP). All EcoAnalysts staff members receive regular, documented training in all SOPs and test methods. Finally, all data collected and produced because of these analyses were recorded on approved data sheets.

2.4 Data Management and Analysis

Endpoint data were calculated for each replicate and the mean value and standard deviation were determined for each test treatment. All hand-entered data were reviewed for data entry errors, which were corrected prior to summary calculations. A minimum of 10% of all calculations and data sorting were reviewed for errors. Review counts were conducted on any apparent outliers.

Statistical comparisons were made according to the EPA guidance. Statistical comparisons were performed using CETIS™ software (CETIS 2022).

3. RESULTS

The results of the effluent testing are presented in this section. Statistical comparisons and laboratory documents are provided in Appendix A. Chain-of-custody and sample receipt logs are provided in Appendix B.

3.1 Water Flea (*Ceriodaphnia dubia*) Acute Test Results

The acute toxicity test with *C. dubia* was initiated on October 26, 2023, and met the survival acceptability criterion of $\geq 90\%$ with a mean control survival of 100%. Mean survival and statistical results are summarized in Table 3-1. The test conditions are summarized in Table 3-2.

Concentrations of 6.25, 12.5, 25, 50, and 100% effluent were prepared utilizing laboratory water. This concentration series includes the ACEC of 100%. The second sample (received 10/26/23) was used for test initiation.

Water quality parameters were within the acceptable limits throughout the duration of the 48-hour static test, with the exception of temperature on Day 1. The temperature of the stock was measured between 21.5 – 21.8°C. The room temperature was lowered, and the test remained within the recommended temperature range for the remainder of the test.

The LC₅₀ for the copper chloride reference-toxicant test was 10.4 µg Cu/L for survival. These results were within two standard deviations of the laboratory mean for survival and reproduction (Table 3-2). This indicates that the organisms are of a similar sensitivity to those previously tested at the EcoAnalysts laboratory.

Table 3-1. Endpoint Summary for the *Ceriodaphnia dubia* Acute Test

Conc. (%)	Outfall 1				
	Mean Survival (%)	Standard Deviation	NOEL (%)	LOEL (%)	LC ₅₀ Value (%)
Control (0)	100	0	100	>100	>100
6.25	100	0			
12.5	100	0			
25	100	0			
50	100	0			
100 ¹	100	0			

¹Acute Critical Effluent Concentration (ACEC).

Table 3-2. Test Condition Summary for Ceriodaphnia dubia Acute Test

Test Duration / Type		48-hour / Static
Species		<i>Ceriodaphnia dubia</i>
Supplier		Aquatic BioSystems
Date acquired		10/26/23
Test Dates		10/26/23 – 10/28/23
Age at test initiation (Recommended: < 24 hours)		< 24 hours
Samples used:		Outfall 1; P231026.04
Sample Holding Time at Initiation: Recommended: <36 hours; Not to exceed 72 hours		36 hours
Test Procedures		WDOE WQ-R-95-80 (2016); EPA-821-R-02-012 Method 2000.0; SOP TOX004.11
Test location		EcoAnalysts; Port Gamble, WA
Control water / Diluent		Cerio Reconstituted Freshwater (CRFW)
Test Lighting		16 hour light / 8 hour dark
Test Chamber		50-mL Plastic Chamber
Exposure volume		25 mL
Replicates/treatment		4
Concentration/treatment		6.25, 12.5, 25, 50, and 100%
Organisms/replicate		5
Feeding		None
Test solution renewal		None
Test Dissolved Oxygen (Recommended: ≥ 2.0 mg/L)		7.6 – 10.0 mg/L
Test Temperature (Recommended: 20 ± 1°C)		20.5 – 21.8 °C
Test Conductivity		165 – 517 µS/cm
Test pH (Range not specified) Targeted Range: 6 – 9 units		7.2 – 8.0 units
Quality Assurance		
Control performance standards Survival (Recommended): ≥ 90%		100%; meets acceptability criterion
Power Standard: ≤29% (Survival)		0%; meets criterion
Reference Toxicant Date		11/8/23
Survival	Reference Toxicant LC ₅₀	10.4 µg Cu/L
	Laboratory Mean LC ₅₀ ; Range LC ₅₀ (±2 SD)	10.98 µg Cu/L (3.6 – 33.1 µg Cu/L)
Deviations from Test Protocol		Temperature

3.2 Fathead Minnow (*Pimephales promelas*) Acute Test Results

The acute toxicity test with *P. promelas* was initiated on October 26, 2023. The test met the control acceptability criteria of $\geq 90\%$ mean survival with a mean control survival of 100%. Mean survival and statistical results are summarized in Table 3-3. The test conditions are summarized in Table 3-4.

Concentrations of 6.25, 12.5, 25, 50, and 100% effluent were prepared utilizing reconstituted fresh water. This concentration series includes the acute critical effluent concentration (ACEC) of 100%.

Water quality parameters were within the acceptable limits throughout the duration of the 96-hour static-renewal test.

The LC₅₀ for the copper chloride reference-toxicant test was 218.1 µg/L copper chloride. These results were within two standard deviations of the laboratory mean for survival (Table 3-4). This indicates that the organisms obtained from this supplier were of similar sensitivity to those previously tested at the EcoAnalysts laboratory.

Table 3-3. Endpoint Summary for the *Pimephales promelas* Acute Test

Conc. (%)	Effluent-Comp				
	Mean Survival (%)	Standard Deviation	NOEL (%)	LOEL (%)	LC ₅₀ Value (%)
Control (0)	100	0	100	>100	>100
1.8	100	0			
4 ¹	100	0			
10	100	0			
30	100	0			
100	95	5.8			

¹Acute Critical Effluent Concentration (ACEC).

NOEL = No Observed Effect Level

LOEL = Lowest Observed Effect Level

LC₅₀/EC₅₀ = Lethal/Effect Concentration to 50% of test population

Table 3-4. Test Condition Summary for *Pimephales promelas* Acute Test

Test Duration / Type		96-hour / Static-Renewal
Species		<i>Pimephales promelas</i>
Supplier		Aquatic Biosystems
Date acquired		10/24/23
Test Dates		10/26/23-10/30/23
Age at test initiation (Recommended: 1 - 14 days)		3 Days
Samples used:		Outfall 1; P231026.04
Sample Holding Time at Initiation: Recommended: <36 hours; Not to exceed 72 hours		36 hours
Test Procedures		WDOE WQ-R-95-80 (2016); EPA-821-R-02-012, Test Method 2000.0; SOP TOX017.10
Test location		EcoAnalysts Port Gamble Laboratory
Control water / Diluent		Reconstituted Fresh Water (RFW)
Test Lighting		16 hour light / 8 hour dark
Test Chamber		12 oz. Plastic Chamber
Exposure volume		250 mL
Replicates/treatment		4
Concentration/treatment		6.25, 12.5, 25, 50, 100%
Organisms/replicate		10
Feeding		0.2 mL concentrated <i>Artemia</i> nauplii, two hours prior to renewal
Test solution renewal		Day 2
Test Dissolved Oxygen (Recommended: ≥4.0 mg/L)		6.5 – 11.6 mg/L
Test Temperature (Recommended: 20 ± 1°C)		19.7 – 21.4°C
Test Conductivity (Recommended: NA)		165 – 486 µS/cm
Test pH (Range not specified) Targeted Range: 6 – 9 units		7.3 – 8.0 units
Quality Assurance		
Control performance standards Survival (Recommended): ≥ 90%		100%; meets acceptability criterion
Power Standard: ≤29% (Survival)		5%; meets criterion
Reference Toxicant Date		10/20/23
Survival	Reference Toxicant LC ₅₀	218.1 µg/L copper
	Laboratory Mean LC ₅₀ ; Range LC ₅₀ (±2 SD)	67.97 (13.4 – 345 µg/L copper)
Deviations from Test Protocol		None

3.3 Rainbow Trout (*Oncorhynchus mykiss*) Acute Test Results

The acute toxicity test with *O. mykiss* was initiated on October 24, 2023 and was validated by 100% survival in the laboratory control. Mean survival is summarized in Table 3-5. The test conditions are summarized in Table 3-6.

Concentrations of 6.25, 12.5, 25, 50, and 100% effluent were prepared utilizing laboratory water. This concentration series includes the acute critical effluent concentration (ACEC) of 100%. The initial sample (received 10/24/23) was used for test initiation and test solution renewal.

Water quality parameters were within the acceptable limits throughout the duration of the 96-hour static-renewal test.

The LC₅₀ for the sodium dodecyl sulfate (SDS) reference-toxicant test was 2.87 mg/L SDS. These results were within two standard deviations of the laboratory mean for survival (Table 3-6). This indicates that the organisms obtained from this supplier were of similar sensitivity to those previously tested at the EcoAnalysts laboratory.

Table 3-5. Results Summary for *Oncorhynchus mykiss* Acute Test.

Sample ID	Conc. (%)	Mean Survival (%)	Standard Deviation	NOEL (%)	LOEL (%)	LC ₅₀ Value
Outfall 1	Control (0)	100	0	100	>100	>100%
	6.25	100	0			
	12.5	100	0			
	25	100	0			
	50	100	0			
	100 ¹	100	0			

¹ Acute Critical Effluent Concentration (ACEC)

Table 3-6. Test Condition Summary for *Oncorhynchus mykiss* Acute Test.

Test Duration / Type	96-Hour; Static-Renewal	
Species	<i>Oncorhynchus mykiss</i>	
Supplier	Thomas Fish Co.	
Date acquired	10/20/23	
Test Dates	10/24/23 – 10/28/23	
Age at test initiation Recommended: 15 -30 days old	Actual: 15 days old	
Sample(s) used:	Outfall 1; P231024.01	
Holding Time at Initiation: Recommended: <36 hours	27 hours	
Test Procedures	WDOE WQ-R-95-80 (2016); EPA-821-R-02-012 Method 2019.0; SOP TOX016.08	
Test location	EcoAnalysts; Port Gamble, WA	
Control water / Diluent	Carbon Filtered Tap Water (CFTW)	
Test Lighting	16 hour light / 8 hour dark	
Test Chamber	8-L Chamber	
Exposure volume	4 L	
Replicates/treatment	4	
Concentration/treatment	6.25, 12.5, 25, 50, and 100%	
Organisms/replicate	10	
Feeding	None	
Test solution renewal	Day 2	
Test Water Quality		
Test Dissolved Oxygen	Recommended: ≥ 6.0 mg/L	Actual: 8.6 – 11.4 mg/L
Test Temperature	Recommended: 12 ± 1°C	Actual: 10.7 – 13.4 °C
Test pH	Recommended: 6 – 9	Actual: 7.2 – 9.0
Test Conductivity	Recommended: NA	Actual: 194 – 760 µS/cm
Quality Assurance		
Control performance standard	Recommended: ≥ 90% survival	Actual: 100%, Pass
Power Standard: ≤29% (Survival)	0%; meets criterion	
Reference Toxicant Date	10/24/23	
Reference Toxicant LC ₅₀	2.87 mg/L sodium dodecyl sulfate	
Laboratory Mean LC ₅₀	4.49 mg/L sodium dodecyl sulfate	
Acceptable Range LC ₅₀ (± sd)	2.65 – 7.61 mg/L sodium dodecyl sulfate	
Deviations from Test Protocol	None	

3.4 Water Flea (*Ceriodaphnia dubia*) Chronic Test Results

The chronic toxicity test with *C. dubia* was initiated on October 24, 2023 and was validated by 90% mean survival, a mean reproduction of 18.1 neonates per survivor, and 100% of the surviving female adults producing three broods of offspring, meeting the criteria of $\geq 80\%$ survival, ≥ 15 neonates per survivor, and $\geq 60\%$ producing 3 broods. The percent minimum significant difference (PMSD) of 50.8% for reproduction was above the acceptable range of 13 to 47%. As effects in the treatments did not exceed 11%, this deviation did not result in a false negative and thus did not affect the results. Mean survival and reproduction endpoints are summarized in Table 3-7. The statistical results are summarized in Table 3-8 and the test conditions are summarized in Table 3-9.

Concentrations of 6.25, 12.5, 25, 50, and 100% effluent were prepared utilizing laboratory water. This concentration series includes the CCEC of 100%. The initial sample (received 10/24/23) was used for test initiation and the second and third samples (received 10/26/23 and 10/28/23) were used for test solution renewals.

Water quality parameters were within the acceptable limits throughout the duration of the 7-day static-renewal test.

The LC₅₀ for the copper chloride reference-toxicant test was 82.5 µg Cu/L for survival and 70.2 µg Cu/L for reproduction. These results were within two standard deviations of the laboratory mean for survival and reproduction (Table 3-9). This indicates that the organisms are of a similar sensitivity to those previously tested at the EcoAnalysts laboratory.

Table 3-7. Endpoint Summary for the *Ceriodaphnia dubia* Chronic Test

Conc. (%)	Outfall 1	
	Mean Survival (%)	Mean Reproduction (# neonates/initial count)
Control (0)	90	16.3
6.25	90	16.9
12.5	90	21.8
25	80	17.0
50	80	14.6
100 ^{1,2}	90	15.0

¹ Chronic Critical Effluent Concentration (CCEC). ² Acute Critical Effluent Concentration (ACEC).

Table 3-8. Statistical Results Summary for *Ceriodaphnia dubia* Chronic Tests

Endpoint	Outfall 1	
	Survival	Reproduction
NOEL (%)	100	100
LOEL (%)	>100	>100
LC ₂₅ / EC ₂₅ (%)	>100	>100
LC ₅₀ / EC ₅₀ (%)	>100	>100
TU _c ¹	1	1

¹TU_c (chronic toxic units) = 100/NOEL (7-day survival), 100/EC₂₅ (7-day reproduction).

Table 3-9. Test Condition Summary for *Ceriodaphnia dubia* Chronic Test

Test Duration / Type		7-Day / Static-Renewal
Species		<i>Ceriodaphnia dubia</i>
Supplier		Aquatic Biosystems
Test Dates		10/24/23 – 11/01/23
Age at test initiation (Recommended: <24 hours, within an 8-hour age range)		<24 hours
Samples used:		Outfall 1; P231024.01, P231026.04, P231028.03
Sample Holding Time at Initiation: Recommended: <36 hours; Not to exceed 72 hours		26 hours
Test Procedures		WDOE WQ-R-95-80 (2016); EPA-821-R-02-013; Test Method 1002.0; SOP TOX003.11
Test location		EcoAnalysts; Port Gamble, WA
Control water / Diluent		Cerio Reconstituted Freshwater (CRFW)
Test Lighting		16 hour light / 8 hour dark
Test Chamber		30-mL Plastic Chamber
Exposure volume		15 mL
Replicates/treatment		10
Concentration/treatment		6.25, 12.5, 25, 50, and 100%
Organisms/replicate		1
Feeding		0.1 mL YCT and <i>Selenastrum</i> daily
Test solution renewal		Daily (Days 1-7)
Test Dissolved Oxygen (Recommended: ≥ 2.0 mg/L)		8.0 – 9.8 mg/L
Test Temperature (Recommended: 25 ± 1°C)		23.6 – 24.9 °C
Test Conductivity Recommended: NA		165 – 743 µS/cm
Test pH (Range not specified) Targeted Range: 6 – 9 units		7.3 – 8.3 units
Quality Assurance		
Control performance standards Survival (Recommended): ≥ 80%		90%; meets acceptability criterion
Reproduction (Recommended): ≥ 15 neonates (average) per survivor, ≥60% surviving females must have at least 3 broods, PMSD ≤47%		18.1 neonates per survivor; meets acceptability criterion 100% w/3 broods; meets acceptability criterion PMSD 50.8%; does not meet acceptability criterion
Power Standard: ≤39% (Mean reproduction per female at test initiation)		8%; meets criterion
Reference Toxicant Date		10/10/23
Survival	Reference Toxicant LC ₅₀	82.5 µg Cu/L
	Laboratory Mean LC ₅₀ ; Range LC ₅₀ (±2 SD)	58.6 µg Cu/L (31.3 – 110 µg Cu/L)
Reproduction	Reference Toxicant LC ₅₀	70.2 µg Cu/L
	Laboratory Mean LC ₅₀ ; Range LC ₅₀ (±2 SD)	48.7 µg Cu/L (24.3 – 97.8 µg Cu/L)
Deviations from Test Protocol		PMSD

3.5 Fathead Minnow (*Pimephales promelas*) Chronic Test Results

The chronic toxicity test with *P. promelas* was initiated on October 24, 2023. The test met the control acceptability criteria of $\geq 80\%$ mean survival with a mean control survival of 100%. The control treatment had a mean dry weight of 1.278 mg, meeting the recommended growth criterion of ≥ 0.25 mg mean dry weight.

The PMSD for growth was 10.4%, below the recommended range of 12 to 30%, which may indicate the potential for a false positive. Effects were seen at all concentrations, but the dose response was flat, the growth in all concentrations was good (above control acceptability criterion) and the control organisms performed exceptionally well. This dose response fits criterion 2 in WDOE WQ-R-95-80 (2016) for an anomalous test result. Per WDOE WQ-R-95-80 (2016) statistics were performed at an alpha of 0.01 in addition to the standard alpha of 0.05 but did not result in a change of statistical significance. No outliers were confirmed statistically. It may be necessary to repeat the test with a new sample.

Mean survival and growth endpoints are summarized in Table 3-10. The statistical results are summarized in Table 3-11 and the test conditions are summarized in Table 3-12.

Concentrations of 6.25, 12.5, 25, 50, and 100% effluent were prepared utilizing laboratory water. This concentration series includes the CCEC of 100%. The initial sample (received 10/24/23) was used for test initiation and the second and third samples (received 10/26/23 and 10/28/23) were used for test solution renewals.

Water quality parameters were within the acceptable limits throughout the duration of the 7-day static-renewal test, with the exception of dissolved oxygen in Replicate 1 of the 50% and 100% concentration. On Day 5, the dissolved oxygen was measured at 3.7 mg/L and 3.5 mg/L respectively. Gentle aeration was added to the entire test and remained within acceptable for the duration of the test.

The LC₅₀ for the copper chloride reference-toxicant test was 94.5 $\mu\text{g Cu/L}$ for survival and 48.3 $\mu\text{g Cu/L}$ for mean dry biomass. These results were within two standard deviations of the laboratory mean (Table 3-12). This indicates that the organisms obtained from this supplier were of similar sensitivity than those previously tested at the EcoAnalysts laboratory.

Table 3-10. Endpoint Summary for the *Pimephales promelas* Chronic Test

Conc. (%)	Outfall 1		
	Mean Survival (%)	Mean Growth (mg) ¹	Mean Biomass (mg) ²
Control (0)	100	1.278	1.278
6.25	100	1.059	1.059
12.5	100	0.930	0.930
25	97.5	1.091	1.064
50	100	1.007	1.007
100 ^{3,4}	100	1.076	1.076

¹ Average weight (mg) per survivor.

² Average weight (mg) per original number of animals stocked (Biomass).

³ Chronic Critical Effluent Concentration (CCEC).

⁴ Acute Critical Effluent Concentration (ACEC).

Table 3-11. Statistical Results Summary for *Pimephales promelas* Chronic Tests

Endpoint	Outfall 1		
	Survival	Growth	Biomass
NOEL (%)	100	<6.25	<6.25
LOEL (%)	>100	6.25	6.25
LC ₂₅ / EC ₂₅ (%)	>100	>100	>100
LC ₅₀ / EC ₅₀ (%)	>100	>100	>100
TU _c ¹	1	<1	<1

¹TU_c (chronic toxic units) = 100/NOEL (7-day survival), 100/EC₂₅ (7-day growth and biomass).

Table 3-12. Test Condition Summary for *Pimephales promelas* Chronic Test

Test Duration / Type		7-Day / Static-Renewal
Species		<i>Pimephales promelas</i>
Supplier		Aquatic Biosystems
Date acquired		10/24/23
Test Dates		10/24/23 – 10/31/23
Age at test initiation (Recommended: <24 hours preferred; <48 hours if shipped)		1 Day
Samples used:		Outfall 1; P231024.01, P231026.04, P231028.03
Sample Holding Time at Initiation: Recommended: <36 hours; Not to exceed 72 hours		26 hours
Test Procedures		WDOE WQ-R-95-80; EPA-821-R-02-013, Test Method 1000.0; SOP TOX018.11
Test location		EcoAnalysts; Port Gamble, WA
Control water / Diluent		Reconstituted Freshwater (RFW)
Test Lighting		16 hour light / 8 hour dark
Test Chamber		20 oz. Plastic Chamber
Exposure volume		250 mL
Replicates/treatment		4
Concentration/treatment		6.25, 12.5, 25, 50, and 100%
Organisms/replicate		10
Feeding		1500 <i>Artemia</i> nauplii twice daily, days 1 - 6
Test solution renewal		Daily (Days 1 - 6)
Test Dissolved Oxygen (Recommended: ≥ 4.0 mg/L)		3.5 – 11.3 mg/L
Test Temperature (Recommended: 25 ± 1°C)		24.0 – 25.8 °C
Test Conductivity Recommended: NA		164 – 776 µS/cm
Test pH (Range not specified) Targeted Range: 6 – 9 units		6.9 – 8.2 units
Quality Assurance		
Control performance standards Survival (Recommended): ≥ 80%		100%; meets acceptability criterion
Growth (Recommended): ≥ 0.25 mg; PMSD (Recommended): 12 to 30%		1.278 mg; meets acceptability criterion, 10.4% PMSD; below acceptability criterion
Power Standard: ≤39% (Mean Growth per Survivor)		16%; meets criterion
Reference Toxicant Date		10/17/23
Survival	Reference Toxicant LC ₅₀	94.5 µg Cu/L
	Laboratory Mean LC ₅₀ ; Range LC ₅₀ (±2 SD)	77.9 µg Cu/L (54.5 – 111 µg Cu/L)
Mean Dry Biomass	Reference Toxicant LC ₅₀	48.3 µg Cu/L
	Laboratory Mean LC ₅₀ ; Range LC ₅₀ (±2 SD)	43.2 µg Cu/L (31.2 – 59.7 µg Cu/L)
	PMSD (≤30% recommended)	12.0%
Deviations from Test Protocol		PMSD for growth, dissolved oxygen

3.6 Green Alga (*Raphidocelis subcapitata*) Chronic Test Results

The chronic toxicity test with *R. subcapitata* was initiated on October 26, 2023, and was validated by 2,737,143 mean cell density and a coefficient of variation for chlorophyll content of 15.1%, meeting the acceptability requirements of $\geq 1,000,00$ cells/mL and $\leq 20\%$ CV. Mean cell density endpoints are summarized in Table 3-13. The statistical results are summarized in Table 3-14 and the test conditions are summarized in Table 3-15.

Concentrations of 6.25, 12.5, 25, 50, and 100% effluent were prepared utilizing laboratory water. This concentration series includes the CCEC and ACEC, both 100%.

Water quality parameters were within the acceptable limits throughout the duration of the 96-hour static test.

The EC₅₀ for the copper chloride reference-toxicant test was 90.6 µg Cu/L. These results were within two standard deviations of the laboratory mean (Table 3-15). This indicates that the test organisms were of similar sensitivity to those previously tested at the EcoAnalysts laboratory.

Table 3-13. Endpoint Summary for the *Raphidocelis subcapitata* Chronic Test

Conc. (%)	Outfall 1
	Mean Chlorophyll A (µg/L)
Control	1538
6.25	1542
12.5	1592
25	1729
50	1939
100 ^{1,2}	1926

¹ Chronic Critical Effluent Concentration (CCEC).

² Acute Critical Effluent Concentration (ACEC).

Table 3-14. Statistical Results Summary for *Raphidocelis subcapitata* Chronic Test

Endpoint	Outfall 1
	Mean Chlorophyll A (µg/L)
NOEL (%)	100
LOEL (%)	>100
LC ₂₅ / EC ₂₅ (%)	>100
LC ₅₀ / EC ₅₀ (%)	>100
TU _c ¹	<1

¹TU_c (chronic toxic units) = 100/EC₂₅ (96-hour cell density).

Table 3-15. Test Condition Summary for *Raphidocelis subcapitata* Chronic Test

Test Duration / Type		96-hour / Static
Species		<i>Raphidocelis subcapitata</i> (formerly <i>Selenastrum capricornutum</i>)
Supplier		Aquatic BioSystems
Test Dates		10/26/23 – 10/30/23
Age at test initiation (Recommended: 4 – 7 days)		6 days
Samples used:		Outfall 1; P231026.04
Sample Holding Time at Initiation: Recommended: <36 hours; Not to exceed 72 hours		34 hours
Test Procedures		WDOE WQ-R-95-80; EPA-821-R-02-013, Test Method 1003.0, SOP TOX023.01
Test location		EcoAnalysts; Port Gamble, WA
Control water / Diluent		Nutrient-enriched reconstituted fresh water
Test Lighting (Recommended: Continuous, 400 ± 40 ft-c)		Continuous; 400 – 430 ft-c
Test Chamber		125-mL flask
Exposure volume		50 mL
Replicates/treatment		4
Concentration/treatment		6.25, 12.5, 25, 50, and 100%
Organisms/replicate (Recommended 10,000 cells/mL ± 50%)		9444.4 cells/mL
Test solution renewal		None
Test Dissolved Oxygen Recommended: NA		7.2 – 12.8 mg/L
Test Temperature (Recommended: 25 ± 1°C)		23.7 – 25.8 °C
Test Conductivity Recommended: NA		239 – 617 µS/cm
Test pH (Range not specified)		7.3 – 10.4 units
Quality Assurance		
Control performance standards Average of 1,000,000 cells/mL at end of test with variability not exceeding 20% coefficient of variation.		2,737,143 cells/mL and 15.1% CV (Chlorophyll a); meets acceptability criteria
Power Standard: ≤39% (Mean Chlorophyll a)		-25%; meets criterion
Reference Toxicant Date		10/26/23
Cell Density	Reference Toxicant LC ₅₀	90.6 µg Cu/L
	Laboratory Mean LC ₅₀ ; Range LC ₅₀ (±2 SD)	61.2 µg Cu/L (20.8 – 180 µg Cu/L)
Deviations from Test Protocol		None

4. REFERENCES

- CETIS. 2022 CETIS™ Comprehensive Environmental Toxicity Information System User's Guide. Tidepool Scientific Software. McKinleyville, CA.
- Lee, D.R. 1980. Reference toxicants in quality control of aquatic bioassays: Aquatic invertebrate bioassays. In Buikema AL Jr, Cairns J Jr, eds, *Proceedings*, 2nd Annual Symposium on Aquatic Toxicology. STP 715. American Society for Testing and Materials, Philadelphia, PA, pp 188–199.
- USEPA. 2002. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition. EPA-821-R-02-013.
- USEPA. 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition. EPA-821-R-02-012.
- 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

LABORATORY DOCUMENTS

APPENDIX A.1

***CERIODAPHNIA DUBIA* (WATER FLEA) 48-HOUR SURVIVAL TEST**

STATISTICAL COMPARISONS AND LABORATORY DATA SHEETS

CETIS Summary Report

Report Date: 27 Nov-23 10:30 (p 1 of 1)
 Test Code/ID: P231026.04 / 17-2108-0589

Ceriodaphnia 48-h Acute Survival Test

EcoAnalysts

Batch ID: 08-7489-5657	Test Type: Survival (48h)	Analyst: Marisa Seibert
Start Date: 26 Oct-23 18:29	Protocol: EPA/821/R-02-012 (2002)	Diluent: Cerio Reconstituted Fresh Water
Ending Date: 28 Oct-23 16:33	Species: Ceriodaphnia dubia	Brine: Not Applicable
Test Length: 46h	Taxon: Branchiopoda	Source: Aquatic Biosystems, CO Age: <1d
Sample ID: 18-5870-4233	Code: P231026.04	Project: Toxicity Testing
Sample Date: 25 Oct-23 06:45	Material: Stormwater	Source: Brooks Manufacturing Co. (WA003080)
Receipt Date: 26 Oct-23 10:45	CAS (PC):	Station: Outfall 1
Sample Age: 36h	Client: Brooks Manufacturing Co.	

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	TU	S
03-2338-6621	48h Proportion Survived	Steel Many-One Rank Sum Test	100	>100	---	---	1	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	%	95% LCL	95% UCL	TU	S
13-7939-4922	48h Proportion Survived	Linear Interpolation (ICPIN)	EC15	>100	---	---	<1	1
			EC20	>100	---	---	<1	
			EC25	>100	---	---	<1	
			EC40	>100	---	---	<1	
			EC50	>100	---	---	<1	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
03-2338-6621	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
13-7939-4922	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria

48h Proportion Survived Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
6.25		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
12.5		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
25		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
50		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
100		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%

48h Proportion Survived Detail

MD5: 68E117461239090AA7E1427F0F536296

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
6.25		1.0000	1.0000	1.0000	1.0000
12.5		1.0000	1.0000	1.0000	1.0000
25		1.0000	1.0000	1.0000	1.0000
50		1.0000	1.0000	1.0000	1.0000
100		1.0000	1.0000	1.0000	1.0000

48h Proportion Survived Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	5/5	5/5	5/5	5/5
6.25		5/5	5/5	5/5	5/5
12.5		5/5	5/5	5/5	5/5
25		5/5	5/5	5/5	5/5
50		5/5	5/5	5/5	5/5
100		5/5	5/5	5/5	5/5

CETIS Test Data Worksheet

Report Date: 27 Nov-23 10:30 (p 1 of 1)
 Test Code/ID: P231026.04 / 17-2108-0589

Ceriodaphnia 48-h Acute Survival Test **EcoAnalysts**

Start Date: 26 Oct-23 18:29 Species: Ceriodaphnia dubia Sample Code: P231026.04
 End Date: 28 Oct-23 16:33 Protocol: EPA/821/R-02-012 (2002) Sample Source: Brooks Manufacturing Co.
 Sample Date: 25 Oct-23 06:45 Material: Stormwater Sample Station: Outfall 1

Conc-%	Code	Rep	Pos	# Exposed	Survival 24h	Survival 48h	Notes
0	D	1	17	5	5	5	
0	D	2	8	5	5	5	
0	D	3	9	5	5	5	
0	D	4	19	5	5	5	
6.25		1	14	5	5	5	
6.25		2	13	5	5	5	
6.25		3	6	5	5	5	
6.25		4	7	5	5	5	
12.5		1	18	5	5	5	
12.5		2	11	5	5	5	
12.5		3	4	5	5	5	
12.5		4	24	5	5	5	
25		1	20	5	5	5	
25		2	22	5	5	5	
25		3	15	5	5	5	
25		4	3	5	5	5	
50		1	10	5	5	5	
50		2	1	5	5	5	
50		3	12	5	5	5	
50		4	16	5	5	5	
100		1	21	5	5	5	
100		2	23	5	5	5	
100		3	5	5	5	5	
100		4	2	5	5	5	

GENERAL

Client	Brooks Manufacturing Co.
Project	Toxicity Testing
Project Number	PG1731
Project Manager	M. Seibert
Date Sample Received	10/26/2023
Test type	48-Hour Acute Toxicity with Cerio
Matrix	Liquid
Test Acceptability	≥ 90% average survival in control
Test Start Date	10/26/23
Test Species	Ceriodaphnia dubia
Organism Batch (Brood Board #)	ABS102623
Organism Acquired	① In-House Culture 10/26/23
Organism Acclimation	NA
Organism Age	< 24 hrs
Test Protocol	TOX 004
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Test Location	Bath 2
Water Description	cerio reconstituted freshwater
Organisms per Replicate	5
Test Chamber Size	2 oz
Exposure Volume	25 mL
Feeding Information	None
Test Dissolved Oxygen	> 2.0
Test Temperature	20 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	2.0	
Temp	19	21
Conductivity		
pH	6	9

TEST START TIME/INIT:	① 1741 1829 JI
TEST END TIME/INIT:	1633 NL

CLIENT SAMPLE ID	LAB ID
Outfall 001	P231026.04

Concentrations	
1	Control
2	6.25%
3	12.5%
4	25%
5	50%
6	100%
7	.
8	.
9	.

① IE J110/26/23, MS 11/27

CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	10/26/23	PROTOCOL	TOX 004
PROJECT	Toxicity Testing	TEST START DATE	10/26/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall 001	TEST END DATE	10/28/23	SPECIES	<i>Ceriodaphnia dubia</i>
LAB SAMPLE ID	P231026.04	MATRIX	Liquid	NO. OF ORGANISMS	5

48-Hour Acute Toxicity with Cerio

	Concentration (%)	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH
Day 0	Control	> 2.0 ① 8.4 8.5	19 - 21 ① 21.8 20.9	6 - 9 ① 854 419	① 7.9 7.2
Stock	6.25%	8.6	20.0	341	8.0
Date 10/26/23	12.5%	8.6	20.9	451	7.9
Time 1744	25%	9.0	20.9	313	7.8
Tech J1	50%	9.2	20.6	260	7.8
Meter # 8/9	100%	10.0	20.5	165	7.3
	.				
	.				
	.				
Day 1	Control	8.5	21.8 ②	445	7.5
Stock	6.25%	8.3	21.6 ②	352	7.9
Date 10/27/23	12.5%	8.5	21.5 ②	457	7.6
Time 1505	25%	8.5	21.6 ②	313	7.6
Tech J1	50%	8.4	21.5 ②	262	7.6
Meter # 9/8	100%	7.6	21.4 ②	167	7.5
	.				
	.				
	.				
Day 2	Control	8.9	20.9	517	7.8
Stock	6.25%	8.7	20.8	416	7.8
Date 10/28/29	12.5%	8.7	20.7	462	7.8
Time 1415	25%	8.7	20.8	314	7.9
Tech NL	50%	8.6	20.8	264	7.7
Meter # 9	100%	8.4	20.7	168	7.8
	.				
	.				
	.				

① cond. high, replaced sample B and measured new J1 10/26/23
 ② Km. temp decreased by 0.5°C. MK 10/27

CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	10/26/23
PROJECT	Toxicity Testing	TEST START DATE	10/26/23
CLIENT SAMPLE ID	Outfall 001	TEST END DATE	10/28/23
LAB SAMPLE ID	P231026.04	MATRIX	Liquid
PROTOCOL	TOX 004	SPECIES	<i>Ceriodaphnia dubia</i>
PROJECT MANAGER	M. Seibert	NO. OF ORGANISMS	5

Abbreviation Key:

NB = No Body
 FB = Found Body
 ST = Stranded

48-Hour Acute Toxicity with Cerio

Concentration (%)	REP.	Day 1		Day 2	
		Date	10/27/23	Date	10/28/23
		Time	1449	Time	1633
		Tech	JL	Tech	NL
		Alive	Dead	Alive	Dead
Control	1	0 to 5	0	5	0
	2	0 to 5	0	5	0
	3	0 to 5	0	5	0
	4	0 to 5	0	5	0
6.25%	1	0 to 5	0	5	0
	2	0 to 5	0	5	0
	3	0 to 5	0	5	0
	4	0 to 5	0	5	0
12.5%	1	5	0	5	0
	2	5	0	5	0
	3	5	0	5	0
	4	5	0	5	0
25%	1	5	0	5	0
	2	5	0	5	0
	3	5	0	5	0
	4	5	0	5	0
50%	1	5	0	5	0
	2	5	0	5	0
	3	5	0	5	0
	4	5	0	5	0
100%	1	5	0	5	0
	2	5	0	5	0
	3	5	0	5	0
	4	5	0	5	0

01E J110/27/23

CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	10/26/23	PROTOCOL	TOX 004
PROJECT	Toxicity Testing	TEST START DATE	10/26/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall 001	TEST END DATE	10/28/23	SPECIES	<i>Ceriodaphnia dubia</i>
LAB SAMPLE ID	P231026.04	MATRIX	Liquid	NO. OF ORGANISMS	5

48-Hour Acute Toxicity with Cerio

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)	Diluent Type	CRFW
0	0%	0	0.0	200.0	CRFW	0
	6.25%	0	12.5	200.0		
	12.5%	0	25	200.0		
	25%	0	50	200.0		
	50%	0	100	200.0		
	100%	0	200	200.0		
		#VALUE!	#VALUE!	0		
		#VALUE!	#VALUE!	0		
		#VALUE!	#VALUE!	0		

Test Dilution Prep

Date	Balance ID	Sample ID (P#)	Water Batch ID	Initials
10/26	7	P231026.04	CRFW102023.02	UL

① 1E 12.5.23 MAM

Ceriodaphnia dubia Acute Survival
Acute Power Standard Calculation

Replicate	Number Surviving				Mean
	1	2	3	4	
ACEC (100)	5	5	5	5	5
Control	5	5	5	5	5

Control Mean - ACEC Mean

0

Difference Divided by Control Mean

0

Express as %

0%

≤29% meets the power standard

Pass

ORGANISM RECEIPT LOG

Date: 10/26/23		Time: 1020		Batch No. ABS 102623.01			
Organism: Ceriodaphnia dubia							
Source / Supplier: Aquatic Bio Systems							
No. Ordered: 260		No. Received: 280		Source Batch: Collection date, <u>hatch date</u> , etc.): 10/25/23			
Condition of Organisms: Good				Approximate Size or Age: (Days from hatch, life stage, size class, etc.): < 24 hours			
Shipper: Fedex				B of L (Tracking No.): 1374 9602 7173			
Condition of Container: Good				Received By: LG			
Container	D.O. (mg/L)	Temp. (°C)	Cond. or Sal. (Include Units)	pH (Units)	# Dead	% Dead*	Tech. (Initials)
1	8.4	20.8	458	7.6	—	—	LG
2	8.4	21.8	456	7.6	—	—	LG
<small>*if >10% contact lab manager</small>							
Notes:							

1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524



Toll Free: 800/331-5916
Tel: 970/484-5091 Fax: 970/484-2514

ORGANISM HISTORY

DATE: 10/25/2023

SPECIES: Ceriodaphnia dubia

AGE: < 24 hour

LIFE STAGE: Neonate

HATCH DATE: 10/25/2023

BEGAN FEEDING: Immediately

FOOD: YTC, Raphidocelis subcapitata*

Water Chemistry Record:

	Current	Range
TEMPERATURE:	<u>25°C</u>	<u>22-25°C</u>
SALINITY/CONDUCTIVITY:	<u>--</u>	<u>--</u>
TOTAL HARDNESS (as CaCO ₃):	<u>120 mg/l</u>	<u>80-120 mg/l</u>
TOTAL ALKALINITY (as CaCO ₃):	<u>100 mg/l</u>	<u>80-105 mg/l</u>
pH:	<u>7.80</u>	<u>7.70-8.20</u>

Comments: * Formerly known as *Psuedokirschneriella subcapitata* and *Selenastrum capricornutum*



Facility Supervisor

Reference Toxicant 96-h Acute Survival Test

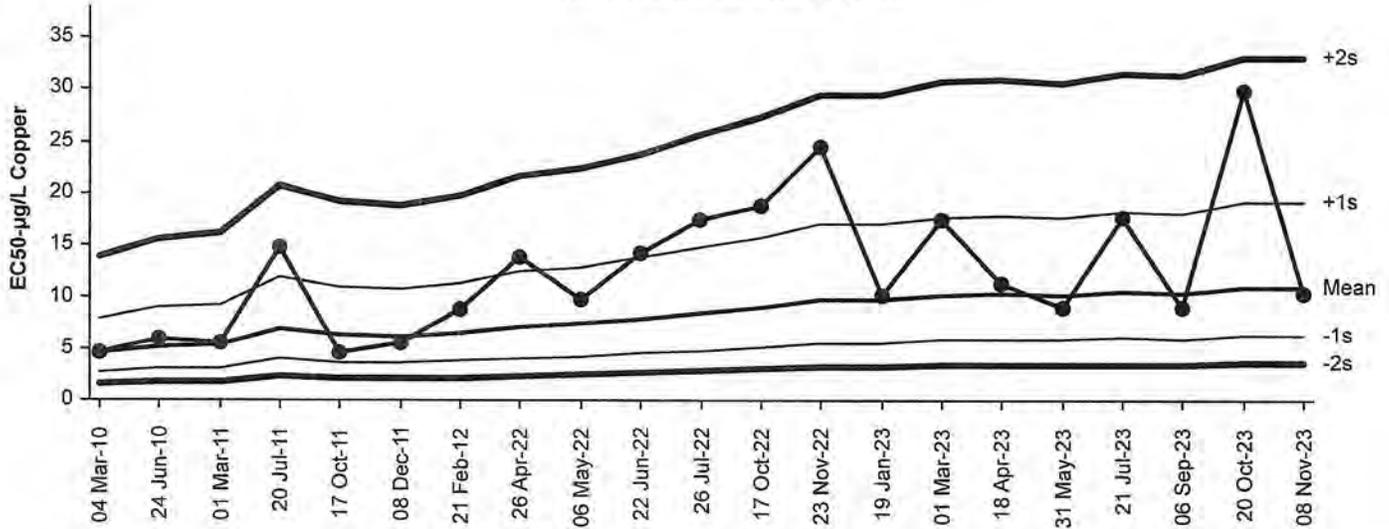
All Matching Labs

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

Organism: Ceriodaphnia dubia
 Endpoint: 48h Proportion Survived

Material: Copper
 Source: Reference Toxicant-REF

Reference Toxicant 96-h Acute Survival Test
 48h Proportion Survived Endpoint



Lognormal Cumulative Mean Plot

Mean: 10.98 Count: 20 -1s Warning Limit: 6.32 -2s Action Limit: 3.64
 Sigma: NA CV: 59.70% +1s Warning Limit: 19.1 +2s Action Limit: 33.1

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2010	Mar	4	18:30	4.547	-6.432	-1.596	(-)		17-9932-3229	16-8467-1882	NewFields
2		Jun	24	14:30	5.865	-5.114	-1.136	(-)		14-1367-8392	17-3151-8157	NewFields
3	2011	Mar	1	16:40	5.652	-5.327	-1.202	(-)		15-8174-1428	07-0371-9940	NewFields
4		Jul	20	16:00	14.82	3.839	0.543			01-3932-6175	10-6710-8541	NewFields
5		Oct	17	16:30	4.547	-6.432	-1.596	(-)		04-2838-6055	21-2111-7859	NewFields
6		Dec	8	15:15	5.557	-5.422	-1.233	(-)		18-6695-3500	09-8943-4292	NewFields
7	2012	Feb	21	15:30	8.792	-2.187	-0.4022			06-6891-7621	04-6404-9101	NewFields
8	2022	Apr	26	15:42	13.87	2.89	0.4232			04-2772-2301	08-3645-8168	EcoAnalysts
9		May	6	11:41	9.747	-1.232	-0.2156			14-6079-5366	04-6122-5474	EcoAnalysts
10		Jun	22	14:30	14.12	3.138	0.4553			03-9516-4140	08-3775-0354	EcoAnalysts
11		Jul	26	15:53	17.4	6.423	0.8341			16-5122-0831	19-4717-7084	EcoAnalysts
12		Oct	17	16:02	18.74	7.758	0.9679			05-1042-5237	15-6629-8376	EcoAnalysts
13		Nov	23	12:55	24.51	13.53	1.454	(+)		15-9762-0016	10-4592-3835	EcoAnalysts
14	2023	Jan	19	16:29	10.09	-0.8884	-0.1528			13-5960-2628	20-1620-3751	EcoAnalysts
15		Mar	1	14:04	17.51	6.533	0.8455			00-8438-6701	09-7672-3683	EcoAnalysts
16		Apr	18	13:22	11.24	0.2566	0.04184			02-7026-4991	14-9856-8438	EcoAnalysts
17		May	31	12:02	9.043	-1.936	-0.3514			18-4592-8734	05-9583-5084	EcoAnalysts
18		Jul	21	12:40	17.57	6.59	0.8514			07-9537-4421	04-3808-5603	EcoAnalysts
19		Sep	6	14:30	9.094	-1.885	-0.3411			15-6638-1111	03-7989-0521	EcoAnalysts
20		Oct	20	16:02	29.96	18.98	1.818	(+)		19-0887-6353	06-4184-1225	EcoAnalysts
21		Nov	8	9:21	10.43	-0.5521	-0.09344			18-3891-8742	01-7885-8441	EcoAnalysts

CETIS Summary Report

Report Date: 01 Dec-23 10:13 (p 1 of 1)
 Test Code/ID: P220110.128 / 18-3891-8742

Reference Toxicant 96-h Acute Survival Test

EcoAnalysts

Batch ID: 02-8282-4626	Test Type: Survival (96h)	Analyst: Marisa Seibert
Start Date: 08 Nov-23 09:21	Protocol: EPA/821/R-02-012 (2002)	Diluent: Cerio Reconstituted Fresh Water
Ending Date: 10 Nov-23 08:51	Species: Ceriodaphnia dubia	Brine: Not Applicable
Test Length: 48h	Taxon: Branchiopoda	Source: In-House Culture
		Age: <1d
Sample ID: 07-4850-6229	Code: P220110.128	Project: Reference Toxicant
Sample Date: 10 Jan-22	Material: Copper	Source: Reference Toxicant
Receipt Date: 10 Jan-22	CAS (PC):	Station: P220110.128
Sample Age: 667d 9h	Client: Internal Lab	

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
12-6410-0668	48h Proportion Survived	Steel Many-One Rank Sum Test	6	12	8.485	25.8%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
01-7885-8441	48h Proportion Survived	Linear Interpolation (ICPIN)	EC15	7.109	6.557	7.76	1
			EC20	7.516	6.749	8.436	
			EC25	7.944	6.944	9.162	
			EC40	9.36	7.547	11.67	
			EC50	10.43	7.962	13.64	

48h Proportion Survived Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9000	0.7163	1.0840	0.8000	1.0000	0.0577	0.1155	12.83%	0.00%
3		4	0.9500	0.7909	1.1090	0.8000	1.0000	0.0500	0.1000	10.53%	-5.56%
6		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-11.11%
12		4	0.3500	-0.0505	0.7504	0.0000	0.6000	0.1258	0.2517	71.90%	61.11%
24		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%
48		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

48h Proportion Survived Detail

MD5: 61327B6280E00976971107DB8BA6B73E

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.8000	1.0000	1.0000	0.8000
3		1.0000	1.0000	0.8000	1.0000
6		1.0000	1.0000	1.0000	1.0000
12		0.6000	0.4000	0.0000	0.4000
24		0.0000	0.0000	0.0000	0.0000
48		0.0000	0.0000	0.0000	0.0000

48h Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	4/5	5/5	5/5	4/5
3		5/5	5/5	4/5	5/5
6		5/5	5/5	5/5	5/5
12		3/5	2/5	0/5	2/5
24		0/5	0/5	0/5	0/5
48		0/5	0/5	0/5	0/5

CETIS Test Data Worksheet

Report Date: 01 Dec-23 10:13 (p 1 of 1)
 Test Code/ID: P220110.128 / 18-3891-8742

Reference Toxicant 96-h Acute Survival Test				EcoAnalysts	
Start Date: 08 Nov-23 09:21	Species: Ceriodaphnia dubia		Sample Code: P220110.128		
End Date: 10 Nov-23 08:51	Protocol: EPA/821/R-02-012 (2002)		Sample Source: Reference Toxicant		
Sample Date: 10 Jan-22	Material: Copper		Sample Station: P220110.128		

Conc-µg/L	Code	Rep	Pos	# Exposed	Survival 24h	Survival 48h	Survival 72h	Survival 96h	Notes
0	D	1	10	5		4			
0	D	2	2	5		5			
0	D	3	8	5		5			
0	D	4	24	5		4			
3		1	5	5		5			
3		2	12	5		5			
3		3	13	5		4			
3		4	17	5		5			
6		1	9	5		5			
6		2	20	5		5			
6		3	11	5		5			
6		4	21	5		5			
12		1	23	5		3			
12		2	14	5		2			
12		3	7	5		0			
12		4	4	5		2			
24		1	22	5		0			
24		2	18	5		0			
24		3	19	5		0			
24		4	3	5		0			
48		1	15	5		0			
48		2	6	5		0			
48		3	16	5		0			
48		4	1	5		0			

48-Hour *Ceriodaphnia dubia* Reference Toxicant Test

Test ID: P220110 128		Replicates: 4		Study Director: M Seibert		Location: Bath 2	
Dilution Water Batch: CRFW110223-02		Organism Batch: BBG103123.01		Associated Test(s): Various		No. of Organisms: 5	
Chamber Size/Type: 50 mL deli cup				Exposure Volume: 25 mL			
Toxicant: Copper Chloride		Lot No: MKCK7155		Date Prepared: 11/8/23		Initials: LG	
Stock Conc.: 400 mg/L Cu		Quantity of Copper Stock: Target: 0.048 mL Actual: 48 µL		Quantity of Diluent: Target: 400 mL Actual: 400 mL			
Highest Test Conc.: 48 µg/L							
Serial Dilute by 1/2 to obtain subsequent test concentrations.							
0 Hours		Date: 11/8/23		WQ Time: 0929		Start Time: 0921	
						Initials: LG	
STOCK							
Meter #		Control	3 µg/L	6 µg/L	12 µg/L	24 µg/L	48 µg/L
D.O. (mg/L) > 2.0		9 8.5	8.5	8.5	8.6	8.6	8.6
Temperature (°C) 19-21		9 20.5	20.7	20.8	20.9	20.9	21.0
Conductivity (µS/cm)		9 345	310	310	310	310	311
pH 6-9		9 7.5	7.6	7.7	7.8	7.8	7.8
24 Hours		Date: 11/09/23		Time: 1610		Initials: SR	
Meter #		Control	3 µg/L	6 µg/L	12 µg/L	24 µg/L	48 µg/L
No. Alive Rep 1		5	5	5	4 (1)	3 (2)	0 (5)
No. Alive Rep 2		5	5	5	2 (3)	0 (5)	0 (5)
No. Alive Rep 3		5	5	5	0 (5)	0 (5)	0 (5)
No. Alive Rep 4		5	5	5	4 (1)	0 (5)	0 (5)
48 Hours		Date: 11/10/23		WQ Time/Init: 0835 NL		End Time/Init: 0851 NL	
WQ Surrogate							
Meter #		Control	3 µg/L	6 µg/L	12 µg/L	24 µg/L	48 µg/L
D.O. (mg/L) > 2.0		7 8.8	8.9	9.0	9.0	9.0	9.0
Temperature (°C) 19-21		7 19.8	19.7	19.8	19.7	19.6	19.5
Conductivity (µS/cm)		7 358	317	317	315	314	315
pH 6-9		7 7.8	8.0	8.1	8.1	8.1	8.1
		Control	3 µg/L	6 µg/L	12 µg/L	24 µg/L	48 µg/L
No. Alive Rep 1		4(1)	5	5	3(1)	0(2, 1NB)	/
No. Alive Rep 2		5	5	5	2		
No. Alive Rep 3		5	4(1)	5	—		
No. Alive Rep 4		4(1)	5	5	2(2)		

Control Acceptability Criteria:
 ≥ 90 % Control Survival (48 Hour)

1	16
2	4
3	2
4	9
5	3
6	13
7	24
8	5
9	14
10	15
11	1
12	7
13	11
14	6
15	18
16	8
17	20
18	22
19	19
20	21
21	10
22	17
23	12
24	23

P220110.128

APPENDIX A.2

***PIMEPHALES PROMELAS* (FATHEAD MINNOW) 96-HOUR SURVIVAL TEST**

STATISTICAL COMPARISONS AND LABORATORY DATA SHEETS

CETIS Summary Report

Report Date: 06 Dec-23 10:00 (p 1 of 1)
 Test Code/ID: P231026.04P.p. / 03-7559-1247

Fathead Minnow 96-h Acute Survival Test

EcoAnalysts

Batch ID: 20-2306-2039	Test Type: Survival (96h)	Analyst: Marisa Seibert
Start Date: 26 Oct-23 18:35	Protocol: EPA/821/R-02-012 (2002)	Diluent: Reconstituted Water
Ending Date: 30 Oct-23 18:25	Species: Pimephales promelas	Brine: Not Applicable
Test Length: 96h	Taxon: Actinopterygii	Source: Aquatic Biosystems, CO Age: 3d
Sample ID: 18-5302-2499	Code: P231026.04P.p.	Project: Toxicity Testing
Sample Date: 25 Oct-23 06:45	Material: Stormwater	Source: Brooks Manufacturing Co. (WA003080)
Receipt Date: 26 Oct-23 10:45	CAS (PC):	Station: Outfall 1
Sample Age: 36h	Client: Brooks Manufacturing Co.	

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	TU	S
09-9019-1869	96h Proportion Survived	Steel Many-One Rank Sum Test	100	>100	---	4.94%	1	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	%	95% LCL	95% UCL	TU	S
18-3699-2044	96h Proportion Survived	Linear Interpolation (ICPIN)	EC15	>100	---	---	<1	1
			EC20	>100	---	---	<1	
			EC25	>100	---	---	<1	
			EC40	>100	---	---	<1	
			EC50	>100	---	---	<1	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
09-9019-1869	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
18-3699-2044	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria

96h Proportion Survived Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
6.25		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
12.5		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
25		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
50		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
100		4	0.9500	0.8581	1.0420	0.9000	1.0000	0.0289	0.0577	6.08%	5.00%

96h Proportion Survived Detail

MD5: C7139C808A8C74EAAF8016ABD68CD9E8

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
6.25		1.0000	1.0000	1.0000	1.0000
12.5		1.0000	1.0000	1.0000	1.0000
25		1.0000	1.0000	1.0000	1.0000
50		1.0000	1.0000	1.0000	1.0000
100		1.0000	1.0000	0.9000	0.9000

96h Proportion Survived Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	10/10	10/10	10/10	10/10
6.25		10/10	10/10	10/10	10/10
12.5		10/10	10/10	10/10	10/10
25		10/10	10/10	10/10	10/10
50		10/10	10/10	10/10	10/10
100		10/10	10/10	9/10	9/10

CETIS Test Data Worksheet

Report Date: 27 Nov-23 10:49 (p 1 of 1)
 Test Code/ID: P231026.04P.p. / 03-7559-1247

Fathead Minnow 96-h Acute Survival Test

EcoAnalysts

Start Date: 26 Oct-23 18:35 Species: Pimephales promelas Sample Code: P231026.04P.p.
 End Date: 30 Oct-23 18:25 Protocol: EPA/821/R-02-012 (2002) Sample Source: Brooks Manufacturing Co.
 Sample Date: 25 Oct-23 06:45 Material: Stormwater Sample Station: Outfall 1

Conc-%	Code	Rep	Pos	# Exposed	Survival 24h	Survival 48h	Survival 72h	Survival 96h	Notes
0	D	1	24	10	10	10	10	10	
0	D	2	2	10	10	10	10	10	
0	D	3	23	10	10	10	10	10	
0	D	4	1	10	10	10	10	10	
6.25		1	8	10	10	10	10	10	
6.25		2	17	10	10	10	10	10	
6.25		3	20	10	10	10	10	10	
6.25		4	5	10	10	10	10	10	
12.5		1	3	10	10	10	10	10	
12.5		2	12	10	10	10	10	10	
12.5		3	9	10	10	10	10	10	
12.5		4	22	10	10	10	10	10	
25		1	15	10	10	10	10	10	
25		2	6	10	10	10	10	10	
25		3	21	10	10	10	10	10	
25		4	7	10	10	10	10	10	
50		1	13	10	10	10	10	10	
50		2	16	10	10	10	10	10	
50		3	14	10	10	10	10	10	
50		4	19	10	10	10	10	10	
100		1	11	10	10	10	10	10	
100		2	10	10	10	10	10	10	
100		3	4	10	10	9	9	9	
100		4	18	10	9	9	9	9	

GENERAL

Client	Brooks Manufacturing Co.
Project	Toxicity Testing
Project Number	PG1913
Project Manager	M. Seibert
Date Sample Received	10/26/2023
Test type	96-Hour Acute Toxicity with FHM
Matrix	Liquid
Test Acceptability	≥ 90% average survival of control
Test Start Date	10/26/23
Test Species	Pimephales promelas
Organism Batch	ABS102423.04
Organism Acquired	10/24/2023
Organism Acclimation	2
Organism Age	3 1/2 days ①
Test Protocol	Tox 017
Regional Protocol	WDOE WQ-R-95-80
Test Location	
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Water Description	moderately hard fresh water
Organisms per Replicate	10
Test Chamber Size	12 oz
Exposure Volume	250 mL
Feeding Information	0.2 mL <i>Artemia nauplii</i> 2 hrs prior to renewal (96-hour test)
Test Dissolved Oxygen	> 4.0
Test Temperature	20 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	4.0	
Temp	19	21
Conductivity		
pH	6	9

TEST START TIME/INIT:	1835 MATH/DM
TEST END TIME/INIT:	1825 RG

CLIENT SAMPLE ID	LAB ID
Outfall 001	P231026.04
Outfall 1	P231078.03

Concentrations	
1	Control
2	6.25%
3	12.5%
4	25%
5	50%
6	100%
7	.
8	.
9	.

Food Batch ID
281729

① IE-MS 12/6

V.3 CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	10/26/23	PROTOCOL	Tox 017
PROJECT	Toxicity Testing	TEST START DATE	10/26/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall 001	TEST END DATE	10/30/23	SPECIES	<i>Pimephales promelas</i>
LAB SAMPLE ID	P231026.04	MATRIX	Liquid	NO. OF ORGANISMS	10

96-Hour Acute Toxicity with FHM

	Concentration (%)	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH
Day 0	Control	8.3	21.4	372	7.9
Stock	6.25%	8.5	21.4	321	7.9
Date 10/26/23	12.5%	8.7	20.9	① 310.5-311	7.9
Time 1903	25%	9.1	20.4	291	7.88-06①
Tech J1	50%	9.5	20.5	249	7.6
Meter # 8	100%	10.3	21.1	165.	7.3
Day 1	Control	① 8.4 8.3	21.0	486	7.9
Rep 1	6.25%	8.5	21.3	330	7.9
Date 10/27/23	12.5%	8.3	21.2	319	7.9
Time 1400	25%	8.2	21.7	① 298 299	7.8
Tech TT	50%	7.9	② 258 21.0	258	7.4
Meter # 8	100%	4.5	21.4	173	7.4
Day 2	Control	8.1	20.7	383	7.7
Rep 2	6.25%	8.2	20.9	336	7.7
Date 10.28.23	12.5%	8.0	20.7	325	7.8
Time 1400	25%	7.8	20.7	305	7.7
Tech MARK	50%	7.4	20.6	262	7.6
Meter # 8	100%	7.0	20.8	180	7.5
Day 2	Control	8.5	21.4	333	7.9
Renewal Stock	6.25%	8.7	21.0	323	8.0
Date 10.29.23	12.5%	9.1	19.9	294	7.8
Time 1412	25%	9.3	20.8	314	7.9
Tech MARK	50%	9.9	19.8	254	7.7
Meter # 8	100%	11.6	20.0	173	7.5

① KJ 110/26/23, TT 10/27/23, TT 10/27/23, TT 10/27/23

V.3 CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	10/26/23	PROTOCOL	Tox 017
PROJECT	Toxicity Testing	TEST START DATE	10/26/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall 001	TEST END DATE	10/30/23	SPECIES	<i>Pimephales promelas</i>
LAB SAMPLE ID	P231026.04	MATRIX	Liquid	NO. OF ORGANISMS	10

96-Hour Acute Toxicity with FHM

	Concentration (%)	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH
		> 4.0	19 - 21		6 - 9
Day 3	Control	8.4	19.7	354	7.8
Rep 3 10/29/23	6.25%	8.4	19.7	333	7.8
Date	12.5%	8.4 8.3 8.3	19.8	303 319	7.8 7.8
Time 1119	25%	8.5 8.4	19.8	262 303	7.8 7.8
Tech T	50%	8.5 8.4	19.9	262	7.8
Meter # 7	100%	8.4	20.0	178 179	7.8
Day 4	Control	8.3	20.8	364	7.8
Rep 4	6.25%	8.1	21.2	332	7.9
Date 10/30/23	12.5%	8.3	20.9	322	7.9
Time 1815	25%	8.2	21.0	301	7.9
Tech RL	50%	8.2	21.0	259	7.9
Meter # 8	100%	8.2	21.0	179	7.9

① T 10/29/23

v.3 CLIENT	Brooks Manufacturing Co.
PROJECT	Toxicity Testing
CLIENT SAMPLE ID	Outfall 001
LAB SAMPLE ID	P231026.04
PROTOCOL	Tox 017
PROJECT MANAGER	M. Seibert

DATE RECEIVED	10/26/23
TEST START DATE	10/26/23
TEST END DATE	10/30/23
MATRIX	Liquid
SPECIES	<i>Pimephales promelas</i>
NO. OF ORGANISMS	10

Abbreviation Key:

- NB = No Body
- FB = Found Body
- ST = Stranded

96-Hour Acute Toxicity with FHM

Concentration (%)	REP	Day 1		Day 2		Day 3		Day 4	
		Date	10/27/23	Date	10.28.23	Date	10/29/23	Date	10/30/23
		Time	1430	Time	1436	Time	1151	Time	1825
		Tech	TT	Tech	MARH	Tech	TT	Tech	RE
		Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
Control	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
6.25%	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
12.5%	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
25%	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
50%	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
100%	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	9	1	9	0	9	0
	4	9	1	9	0	9	0	9	0
Feed (Time/Init.)									
0.2 mL Artemia nauplii 2 hrs prior to renewal (96-hour test)				NL 0900					

CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	10/26/23	PROTOCOL	Tox 017
PROJECT	Toxicity Testing	TEST START DATE	10/26/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall 001	TEST END DATE	10/30/23	SPECIES	<i>Pimephales promelas</i>
LAB SAMPLE ID	P231026.04	MATRIX	Liquid	NO. OF ORGANISMS	10

96-Hour Acute Toxicity with FHM

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)	Diluent Type	RFW
0	0%	0	1000.0	1000		
	6.25%	62.5	937.5	1000		
	12.5%	125	875.0	1000		
	25%	250	750.0	1000		
	50%	500	500.0	1000		
	100%	1000	0.0	1000		
	.	#VALUE!	#VALUE!	1000		
	.	#VALUE!	#VALUE!	1000		

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)
2	0%	0	1000.0	1000
	6.25%	62.5	937.5	1000
	12.5%	125	875.0	1000
	25%	250	750.0	1000
	50%	500	500.0	1000
	100%	1000	0.0	1000
	.	#VALUE!	#VALUE!	1000
	.	#VALUE!	#VALUE!	1000

Test Dilution Prep

Date	Balance ID	Sample ID (P#)	Water Batch ID	Initials
10/26	7	P231026.04	RFW102023.02	JL
10.28.23	7	P231026.04	RFW102023.02	MARH

① P231028.03

① Not enough sample waited for renewal sample for chronic test to arrive 10.28.23 MARCH

POWER STANDARD CALCULATIONS

Fathead Minnow Acute Survival
Acute Power Standard Calculation

	Number Surviving				
Replicate	1	2	3	4	Mean
ACEC (100)	10	10	9	9	9.5
Control	10	10	10	10	10

Control Mean - ACEC Mean

0.5

Difference Divided by Control Mean

0.05

Express as %

5%

≤29% meets the power standard

Pass

ORGANISM RECEIPT LOG

Date: 10/24/23		Time: 1200		Batch No. ABS102423.04			
Organism: <i>Pimephales promelas</i>							
Source / Supplier: Aquatic BioSystems							
No. Ordered: <u> </u>		No. Received: 1320		Source Batch: 10/23/23 Collection date, hatch date, etc.):			
Condition of Organisms: Good			Approximate Size or Age: (Days from hatch, life stage, size class, etc.): ① hatch date 1 day old				
Shipper: UPS			B of L (Tracking No.)				
Condition of Container: GOOD			Received By: DM/JI				
Container	D.O. (mg/L)	Temp. (°C)	Cond. or Sal. (Include Units)	pH (Units)	# Dead	% Dead*	Tech. (Initials)
1	22.1	20.6	① 433 ① 431.4 us/cm	① 7.7 ① 7.69	① 5	① 0.38	J1
2	21.1	20.6	432	7.7	① 2	① 0.15	J1
3	① 21.0	20.6	431	7.7	① 8	① 0.62	J1
4	24.8	20.6	428	7.7	① 4	① 0.31	J1
						cumulative dead = 1.4%	
*if >10% contact lab manager							
Notes:							

016 10/24/23 DM 10/23/23

ive, Suite C
orado 80524



Toll Free: 800/331-5916
Tel: 970/484-5091 Fax: 970/484-2514

ORGANISM HISTORY

DATE: 10/23/2023

SPECIES: *Pimephales promelas*

AGE: N/A

LIFE STAGE: Embryo

HATCH DATE: 10/23/2023

BEGAN FEEDING: N/A

FOOD: N/A

Water Chemistry Record:

	Current	Range
TEMPERATURE:	<u>24°C</u>	<u>22-25°C</u>
SALINITY/CONDUCTIVITY:	<u>--</u>	<u>--</u>
TOTAL HARDNESS (as CaCO ₃):	<u>120 mg/l</u>	<u>80-120 mg/l</u>
TOTAL ALKALINITY (as CaCO ₃):	<u>95 mg/l</u>	<u>80-105 mg/l</u>
pH:	<u>7.84</u>	<u>7.70-8.20</u>

Comments:

Facility Supervisor

Reference Toxicant 96-h Acute Survival Test

All Matching Labs

Test Type: Survival

Organism: Pimephales promelas

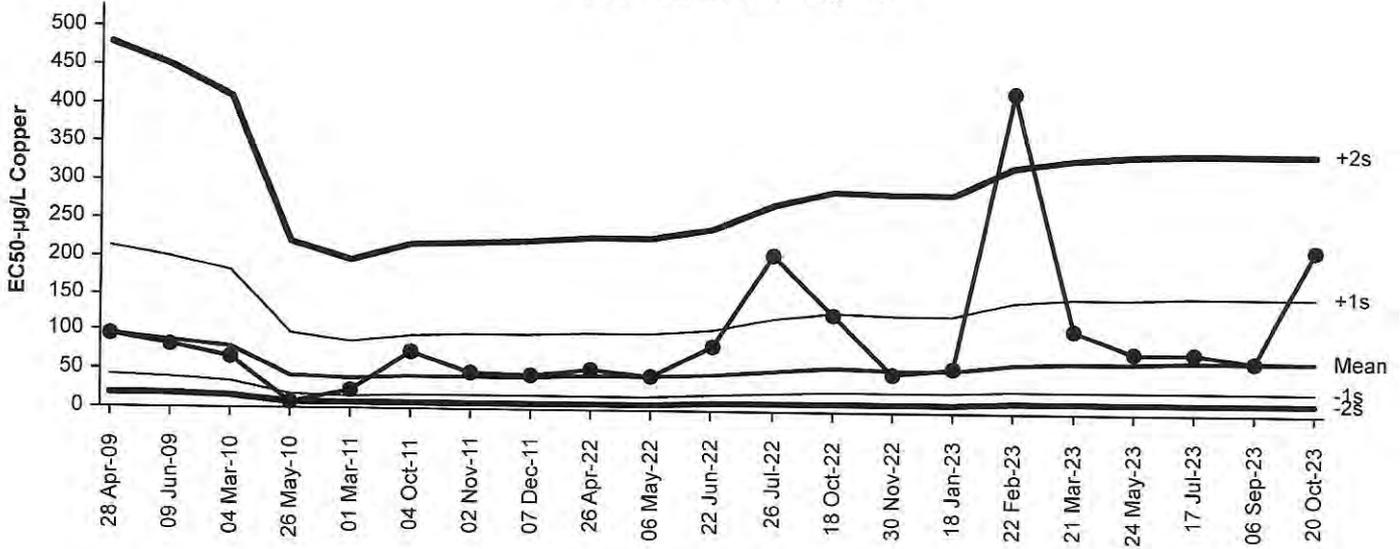
Material: Copper

Protocol: All Protocols

Endpoint: Proportion Survived

Source: Reference Toxicant-REF

Reference Toxicant 96-h Acute Survival Test
Proportion Survived Endpoint



Lognormal Cumulative Mean Plot

Mean: 67.97 Count: 20 -1s Warning Limit: 30.2 -2s Action Limit: 13.4
 Sigma: NA CV: 96.60% +1s Warning Limit: 153 +2s Action Limit: 345

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2009	Apr	28	15:00	94.48	26.51	0.4057			06-3696-9951	18-3878-0128	NewFields
2		Jun	9	15:30	83.43	15.46	0.2525			02-7456-5363	03-1147-5047	NewFields
3	2010	Mar	4	18:15	66.99	-0.9796	-0.01788			15-8908-6559	17-4197-4976	NewFields
4		May	26	16:00	6.716	-61.25	-2.851	(-)	(-)	06-0415-5799	15-4018-4612	NewFields
5	2011	Mar	1	16:25	24.16	-43.8	-1.274	(-)		17-3642-8280	19-0000-9582	NewFields
6		Oct	4	15:45	74.09	6.123	0.1063			03-6423-8219	13-1123-2117	NewFields
7		Nov	2	16:45	47.43	-20.53	-0.443			05-5334-1475	20-1165-4806	NewFields
8		Dec	7	16:40	45.34	-22.62	-0.4986			07-6665-7177	09-3475-8342	NewFields
9	2022	Apr	26	15:21	53.88	-14.09	-0.2861			15-2473-8981	21-2244-0369	EcoAnalysts
10		May	6	15:10	45.34	-22.62	-0.4986			15-4436-5345	06-4737-7601	EcoAnalysts
11		Jun	22	14:00	85.27	17.3	0.2794			09-2484-6701	03-6339-0355	EcoAnalysts
12		Jul	26	16:15	207.9	139.9	1.377	(+)		19-9606-8541	14-4105-6380	EcoAnalysts
13		Oct	18	15:33	126.6	58.6	0.7659			00-1244-9651	13-0675-8972	EcoAnalysts
14		Nov	30	17:05	50.09	-17.88	-0.3759			05-6217-6706	05-6087-1351	EcoAnalysts
15	2023	Jan	18	15:48	59.46	-8.505	-0.1647			17-0281-5837	00-7848-0629	EcoAnalysts
16		Feb	22	14:21	423.7	355.7	2.254	(+)	(+)	19-9432-7182	17-7365-6004	EcoAnalysts
17		Mar	21	14:05	110	42	0.5927			04-0968-3570	09-3250-3169	EcoAnalysts
18		May	24	14:19	79.72	11.76	0.1966			20-8461-2086	00-7824-5483	EcoAnalysts
19		Jul	17	16:28	78.89	10.93	0.1837			18-7140-7619	00-8079-6291	EcoAnalysts
20		Sep	6	15:55	68.38	0.41	0.007409			10-4912-0113	18-0428-8216	EcoAnalysts
21		Oct	20	18:22	218.1	150.2	1.436	(+)		07-3619-2172	09-4739-7103	EcoAnalysts

CETIS Summary Report

Report Date: 25 Oct-23 10:48 (p 1 of 1)
 Test Code/ID: P220110.121 / 07-3619-2172

Reference Toxicant 96-h Acute Survival Test

EcoAnalysts

Batch ID: 10-8790-9404	Test Type: Survival	Analyst: Michelle Knowlen
Start Date: 20 Oct-23 18:22	Protocol: EPA/821/R-02-012 (2002)	Diluent: Reconstituted Water
Ending Date: 24 Oct-23 16:40	Species: Pimephales promelas	Brine: Not Applicable
Test Length: 94h	Taxon: Actinopterygii	Source: Aquatic Biosystems, CO Age: 11d
Sample ID: 14-8572-0812	Code: P220110.121	Project: Reference Toxicant
Sample Date: 10 Jan-22	Material: Copper	Source: Reference Toxicant
Receipt Date: 10 Jan-22	CAS (PC):	Station: P220110.121
Sample Age: 648d 18h	Client: Internal Lab	

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
06-2743-3045	Proportion Survived	Dunnett Multiple Comparison Test	25	50	35.36	15.8%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
09-4739-7103	Proportion Survived	Linear Interpolation (ICPIN)	EC15	43.82	26.21	66.15	1
			EC20	52.6	28.33	70.76	
			EC25	59.5	32.71	80.48	
			EC40	86.01	58.11	197.8	
			EC50	218.1	15.9	261.6	

Proportion Survived Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	0.00%
25		4	0.9500	0.8581	1.0420	0.9000	1.0000	0.0289	0.0577	6.08%	2.56%
50		4	0.8000	0.5750	1.0250	0.6000	0.9000	0.0707	0.1414	17.68%	17.95%
100		4	0.5000	0.2095	0.7905	0.3000	0.7000	0.0913	0.1826	36.51%	48.72%
200		4	0.5500	0.4581	0.6419	0.5000	0.6000	0.0289	0.0577	10.50%	43.59%
400		4	0.2250	-0.0137	0.4637	0.1000	0.4000	0.0750	0.1500	66.67%	76.92%

Proportion Survived Detail

MD5: DC687ED71F7E2A00955487566D86531B

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	0.9000
25		1.0000	0.9000	1.0000	0.9000
50		0.9000	0.8000	0.9000	0.6000
100		0.7000	0.4000	0.6000	0.3000
200		0.6000	0.5000	0.5000	0.6000
400		0.1000	0.1000	0.4000	0.3000

Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	10/10	10/10	10/10	9/10
25		10/10	9/10	10/10	9/10
50		9/10	8/10	9/10	6/10
100		7/10	4/10	6/10	3/10
200		6/10	5/10	5/10	6/10
400		1/10	1/10	4/10	3/10

CETIS Test Data Worksheet

Report Date: 25 Oct-23 10:47 (p 1 of 1)
 Test Code/ID: P220110.121 / 07-3619-2172

Reference Toxicant 96-h Acute Survival Test

EcoAnalysts

Start Date: 20 Oct-23 18:22 Species: Pimephales promelas Sample Code: P220110.121
 End Date: 24 Oct-23 16:40 Protocol: EPA/821/R-02-012 (2002) Sample Source: Reference Toxicant
 Sample Date: 10 Jan-22 Material: Copper Sample Station: P220110.121

Conc-µg/L	Code	Rep	Pos	# Exposed	# Survived	Notes
0	D	1	15	10	10	
0	D	2	6	10	10	
0	D	3	16	10	10	
0	D	4	22	10	9	
25		1	23	10	10	
25		2	14	10	9	
25		3	11	10	10	
25		4	21	10	9	
50		1	3	10	9	
50		2	19	10	8	
50		3	12	10	9	
50		4	7	10	6	
100		1	4	10	7	
100		2	5	10	4	
100		3	13	10	6	
100		4	2	10	3	
200		1	1	10	6	
200		2	20	10	5	
200		3	10	10	5	
200		4	9	10	6	
400		1	24	10	1	
400		2	8	10	1	
400		3	18	10	4	
400		4	17	10	3	

96-Hour FHM CuCl₂ Reference Toxicant Test

Toxicant:	Copper Chloride
Ref Tox ID:	P220110-121
Lot #:	MKCK7155
Protocol:	TOX017
Replicates:	4

Date Test Started:	10/20/2023
Date Test Ended:	10/24/2023
Matrix:	Liquid
Species:	<i>Pimephales promelas</i>
No. of Org. per Chamber:	10

	Conc. (µg/L)	Meter #:	DO (mg/L) (>4.0)	Meter #:	Temp (°C) (20±1°C)	Meter #:	Conductivity (µS/cm)	Meter #:	pH (6 - 9)
Day 0 (Stock)	Control	8	8.3	8	20.3	8	334	8	7.9
Date: 10/20/23	25		8.4		20.2		333		8.0
Time: 1555	50		8.5		20.2		334		8.0
Technician: MK	100		8.4 8.5		20.1		334		8.0
	200		8.6		20.0		336		8.0
	400		8.6		20.1		336		8.0
			Day 1		Day 2		Day 3		Day 4
Temperature (OLD)			19.8		20.0		20.2		
Temperature (NEW)					20.2				
Feed: 0.2mL Artemia (Time/Init.)					0820 TN				
Day 4	Control	7	8.1	7	20.5	7	345	7	7.7
Date: 10/24/23	25		8.1		20.6		342		7.8
Time: 1530	50		8.1		20.6		340		7.8
Replicate No.: 4	100		8.1		20.6		342		7.8
Technician: MK	200		8.1		20.6		344		7.8
	400		8.0		20.6		343		7.8

Dilution Preparation

Stock Concentration: 400 mg/L Cu		Highest Conc.: 400 µg/L	
Serial Dilute by ½ to obtain subsequent test concentrations.			
Test Initiation	Quantity of Copper Stock:	Quantity of Diluent:	
Day 0 Target:	2mL	2,000mL	
Day 0 Actual:	2mL	2,000mL	
Renewal	Quantity of Copper Stock:	Quantity of Diluent:	
Target (400 µg/L):	2mL	2,000mL	
Target (200 µg/L):	1mL	2,000mL	
[If complete mortality in higher conc.]:			
Day 2 Actual:	2mL	2,000mL	

Start Time:	1822 MK MS
End Time:	1640 MK
Test Acceptability:	≥90% survival in control

⊙ MK. MK 10/20.

Test Location:	BATH 2
Dilution Water Batch:	RFW101423.01
Renewal Water Batch:	RFW 102023.01
Supplier:	Aquatic Biosystems
Organism Batch:	ABS102023.01
Chamber Size/Type:	12 oz cup
Exposure Volume:	250 mL

96-Hour FHM Ammonia Reference Toxicant Test

Toxicant:	Copper Chloride
Ref Tox ID:	P220110.121
Species:	<i>Pimephales promelas</i>

Date Test Started:	10/20/2023
Date Test Ended:	10/24/2023

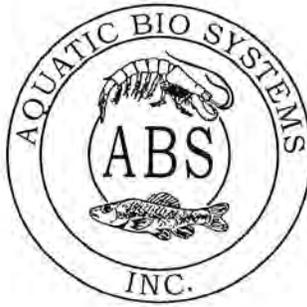
Concentration (µg/L)	Rep	Day 1		Day 2		Day 3		Day 4	
		# Alive	# Dead						
		Date: 10/21/23 Time: 1233		Date: 10/22/23 Time: 1307		Date: 10/23/23 Time: 1840		Date: 10/24/23 Time: 1640	
Control	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	9	1
25	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	9	1	9	0
	3	10	0	9 ¹⁰	0	10	0	10	0
	4	10	0	9	1	9	0	9	0
50	1	10	0	10	0	9	1	9	0
	2	10	0	10	0	9	1	8	1
	3	10	0	10	0	9	1	9	0
	4	10	0	9	1	8	1	6	2
100	1	10	0	9	1	8	1	7	1
	2	10	0	10	0	4	6	4	0
	3	10	0	8	2	7	1	6	1
	4	10	0	8	2	5	3	3	2
200	1	10	0	7	3	6	1	6	0
	2	9	1	8	1	6	2	5	1
	3	10	0	8	2	5	3	5	0
	4	9	1	9	0	6	3	6	0
400	1	5	5	1	4	1	0	1	0
	2	7	3	4	3	2	2	1	1
	3	7	3	5	2	5	0	4	1
	4	10	0	7	3	5	2	3	2
INITIALS:		NL		RE		NG		ML	

Ⓢ I E RE 10/22

ORGANISM RECEIPT LOG

Date: 10/20/23		Time: 1105		Batch No. ABS102023.01			
Organism: Pimephales promelas							
Source / Supplier: Aquatic Biosystems							
No. Ordered: 870		No. Received: 955		Source Batch: Collection date, hatch date, etc.): hatch: 10/9/23			
Condition of Organisms: Good			Approximate Size or Age: (Days from hatch, life stage, size class, etc.): 11 days				
Shipper: UPS			B of L (Tracking No.) 1ZF4673R0193848558				
Condition of Container: Good			Received By: M. Seibert				
Container	D.O. (mg/L)	Temp. (°C)	Cond. or Sal. (Include Units)	pH (Units)	# Dead	% Dead*	Tech. (Initials)
1	10.3	20.0	765 μ S/cm	7.1	2	—	MS
2	19.2	25.8	748 μ S/cm	7.2	15	<10%	MS
*if >10% contact lab manager							
Notes:							

1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524



Toll Free: 800/331-5916
Tel: 970/484-5091 Fax: 970/484-2514

ORGANISM HISTORY

DATE: 10/19/2023

SPECIES: *Pimephales promelas*

AGE: 10 day

LIFE STAGE: Larvae

HATCH DATE: 10/9/2023

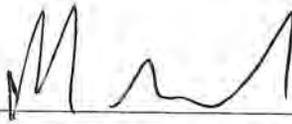
BEGAN FEEDING: 10/10/2023

FOOD: *Artemia* sp.

Water Chemistry Record:

	Current	Range
TEMPERATURE:	<u>24°C</u>	<u>22-25°C</u>
SALINITY/CONDUCTIVITY:	<u>--</u>	<u>--</u>
TOTAL HARDNESS (as CaCO ₃):	<u>120 mg/l</u>	<u>80-120 mg/l</u>
TOTAL ALKALINITY (as CaCO ₃):	<u>95 mg/l</u>	<u>80-105 mg/l</u>
pH:	<u>7.84</u>	<u>7.70-8.20</u>

Comments:



Facility Supervisor

APPENDIX A.3

***ONCORHYNCHUS MYKISS* (RAINBOW TROUT) 96-HOUR SURVIVAL TEST**

STATISTICAL COMPARISONS AND LABORATORY DATA SHEETS

CETIS Summary Report

Report Date: 27 Nov-23 10:36 (p 1 of 1)
 Test Code/ID: P231024.01 / 05-8477-6891

Fish 96-h Acute Survival Test

EcoAnalysts

Batch ID: 20-2576-0938	Test Type: Survival (96h)	Analyst: Marisa Seibert
Start Date: 24 Oct-23 17:44	Protocol: EPA/821/R-02-012 (2002)	Diluent: Carbon Filtered Municipal Water
Ending Date: 28 Oct-23 16:28	Species: Oncorhynchus mykiss	Brine: Not Applicable
Test Length: 95h	Taxon: Actinopterygii	Source: Thomas Fish Co. Age: 15d
Sample ID: 14-0838-4993	Code: P231024.01	Project: Toxicity Testing
Sample Date: 23 Oct-23 14:30	Material: Stormwater	Source: Brooks Manufacturing Co. (WA003080)
Receipt Date: 24 Oct-23 10:30	CAS (PC):	Station: Outfall 1
Sample Age: 27h	Client: Brooks Manufacturing Co.	

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	TU	S
12-6129-8962	96h Proportion Survived	Steel Many-One Rank Sum Test	100	>100	---	---	1	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	%	95% LCL	95% UCL	TU	S
12-0888-3476	96h Proportion Survived	Linear Interpolation (ICPIN)	EC15	>100	---	---	<1	1
			EC20	>100	---	---	<1	
			EC25	>100	---	---	<1	
			EC40	>100	---	---	<1	
			EC50	>100	---	---	<1	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits			Decision
				Lower	Upper	Overlap	
12-0888-3476	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
12-6129-8962	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria

96h Proportion Survived Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
6.25		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
12.5		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
25		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
50		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
100		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%

96h Proportion Survived Detail

MD5: 68E117461239090AA7E1427F0F536296

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
6.25		1.0000	1.0000	1.0000	1.0000
12.5		1.0000	1.0000	1.0000	1.0000
25		1.0000	1.0000	1.0000	1.0000
50		1.0000	1.0000	1.0000	1.0000
100		1.0000	1.0000	1.0000	1.0000

96h Proportion Survived Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	10/10	10/10	10/10	10/10
6.25		10/10	10/10	10/10	10/10
12.5		10/10	10/10	10/10	10/10
25		10/10	10/10	10/10	10/10
50		10/10	10/10	10/10	10/10
100		10/10	10/10	10/10	10/10

CETIS Test Data Worksheet

Report Date: 27 Nov-23 10:36 (p 1 of 1)
 Test Code/ID: P231024.01 / 05-8477-6891

Fish 96-h Acute Survival Test				EcoAnalysts					
Start Date:	24 Oct-23 17:44	Species:	Oncorhynchus mykiss	Sample Code:	P231024.01				
End Date:	28 Oct-23 16:28	Protocol:	EPA/821/R-02-012 (2002)	Sample Source:	Brooks Manufacturing Co.				
Sample Date:	23 Oct-23 14:30	Material:	Stormwater	Sample Station:	Outfall 1				

Conc-%	Code	Rep	Pos	# Exposed	Survival 24h	Survival 48h	Survival 72h	Survival 96h	Notes
0	D	1	9	10	10	10	10	10	
0	D	2	18	10	10	10	10	10	
0	D	3	12	10	10	10	10	10	
0	D	4	8	10	10	10	10	10	
6.25		1	15	10	10	10	10	10	
6.25		2	21	10	10	10	10	10	
6.25		3	6	10	10	10	10	10	
6.25		4	1	10	10	10	10	10	
12.5		1	22	10	10	10	10	10	
12.5		2	3	10	10	10	10	10	
12.5		3	10	10	10	10	10	10	
12.5		4	11	10	10	10	10	10	
25		1	20	10	10	10	10	10	
25		2	19	10	10	10	10	10	
25		3	16	10	10	10	10	10	
25		4	2	10	10	10	10	10	
50		1	5	10	10	10	10	10	
50		2	17	10	10	10	10	10	
50		3	7	10	10	10	10	10	
50		4	24	10	10	10	10	10	
100		1	23	10	10	10	10	10	
100		2	14	10	10	10	10	10	
100		3	4	10	10	10	10	10	
100		4	13	10	10	10	10	10	

Version V.2

GENERAL

Client	Brooks Manufacturing Co.
Project	Toxicity Testing
Project Number	PG1913
Project Manager	M. Seibert
Date Sample Received	10/24/2023
Test type	96-Hour Acute Toxicity with RBT
Matrix	Liquid
Test Acceptability	≥ 90% average survival of control
Test Start Date	10/24/23
Test Species	<i>Oncorhynchus mykiss</i>
Organism Batch	① TC102023 TF 102023.01
Organism Acquired	10/20/2023
Organism Acclimation	4
Organism Age	15 days psw
Test Protocol	Tox 016
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Test Location	Bath 4
Water Description	moderately hard fresh water
Organisms per Replicate	10
Test Chamber Size	5000 mL minimum
Exposure Volume	4000 mL minimum
Test Dissolved Oxygen	> 6.0
Test Temperature	12 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	6.0	
Temp	10.5	13.4
Conductivity		
pH	6	9

TEST START TIME/INIT:	1744 DM/LG
TEST END TIME/INIT:	1628 MADA

CLIENT SAMPLE ID	LAB ID
Outfall 1	P231024.01

Concentrations	
1	Control
2	6.25%
3	12.5%
4	25%
5	50%
6	100%
7	.
8	.
9	.

Copy and Past VALUES

Treatment	Rep	Chamber
Control	1	10
Control	2	7
Control	3	22
Control	4	21
6.25%	1	16
6.25%	2	24
6.25%	3	2
6.25%	4	20
12.5%	1	12
12.5%	2	6
12.5%	3	4
12.5%	4	1
25%	1	8
25%	2	9
25%	3	17
25%	4	19
50%	1	15
50%	2	14
50%	3	13
50%	4	23
100%	1	18
100%	2	3
100%	3	11
100%	4	5
.	1	

① IE-LG 10/24

CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	10/24/23	PROTOCOL	Tox 016
PROJECT	Toxicity Testing	TEST START DATE	10/24/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall 1	TEST END DATE	10/28/23	SPECIES	<i>Oncorhynchus mykiss</i>
LAB SAMPLE ID	P231024.01	MATRIX	Liquid	NO. OF ORGANISMS	10

96-Hour Acute Toxicity with RBT

	Concentration (%)	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH
Day 0	Control	10.2	11.8	215	8.4
Stock	6.25%	9.9	11.7	249	8.3
Date 10/24/23	12.5%	10.2	11.4	283	8.3
Time 1608	25%	10.2	11.9	352	8.1
Tech VG	50%	10.3	11.5	487	7.8
Meter # 7	100%	10.5	10.7	755	7.4
Day 1	Control	10.7	12.8	214	8.2
Rep 1	6.25%	10.7	12.8	246	8.3
Date 10/25/23	12.5%	10.7	12.7	271	8.2
Time 2522	25%	10.7	12.8	346	8.2
Tech SR	50%	10.7	12.6	480	8.2
Meter # 8	100%	10.7	12.7	745	8.0
Day 2	Control	9.6	12.5	219	7.9
Rep 2	6.25%	10.2	11.8	251	8.0
Date 10/26	12.5%	10.3	12.2	285	8.1
Time 1255	25%	10.1	11.8	353	8.0
Tech JL	50%	10.2	12.2	488	8.0
Meter # 7	100%	10.3	12.0	756	7.9
Day 2	Control	10.2	12.8	194	9.0
Renewal Stock	6.25%	8.96	13.4	229	9.0
Date 10/26	12.5%	9.2	13.4	264	8.9
Time 1400	25%	9.4	12.8	334	8.9
Tech JL	50%	10.0	11.5	477	8.4
Meter # 7	100%	11.4	11.0	760	7.2
Day 3	Control	10.4	11.5	210	8.2
Rep 3	6.25%	10.6	11.7	237	8.2
Date 10/27	12.5%	10.7	11.7	271	8.3
Time 0915	25%	10.6	11.4	339	8.2
Tech TT	50%	10.6	11.6	476	8.1
Meter # 8	100%	10.7	11.6	749	8.0
Day 4	Control	10.9	11.5	204	8.2
Rep 4	6.25%	11.0	11.5	239	8.2
Date 10.28.23	12.5%	10.6	11.5	274	7.9
Time 1053	25%	11.0	11.6	342	8.1
Tech MACH	50%	10.9	11.8	479	8.1
Meter # 8	100%	10.9	11.7	751	7.8

① WNN-MS 10/26
 ② WNC-10/25, 10/28, 25 MACH

v2

CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	10/24/23	PROTOCOL	Tox 016
PROJECT	Toxicity Testing	TEST START DATE	10/24/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall 1	TEST END DATE	10/28/23	SPECIES	<i>Oncorhynchus mykiss</i>
LAB SAMPLE ID	P231024.01	MATRIX	Liquid	NO. OF ORGANISMS	10

96-Hour Acute Toxicity with RBT

Abbreviation Key:
 NB = No Body
 FB = Found Body
 ST = Stranded

Day 1		Day 2		Day 3		Day 4	
Date	20/25/23	Date	10/26/23	Date	10/27/23	Date	10/28/23
Time	2657	Time	1410	Time	0935	Time	1628
Tech	SL	Tech	JL/M	Tech	TT	Tech	MANA

Concentration (%)	REP	Day 1		Day 2		Day 3		Day 4	
		Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
Control	1	20	0	10	0	10	0	10	0
	2	20	0	10	0	10	0	10	0
	3	20	0	10	0	10	0	10	0
	4	20	0	10	0	10	0	10	0
6.25%	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	20	0	10	0	10	0	10	0
12.5%	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	20	0	10	0	10	0	10	0
25%	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	20	0	10	0	10	0	10	0
50%	1	20	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
100%	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	20	0	10	0	10	0	10	0

CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	10/24/23	PROTOCOL	Tox 016
PROJECT	Toxicity Testing	TEST START DATE	10/24/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall 1	TEST END DATE	10/28/23	SPECIES	<i>Oncorhynchus mykiss</i>
LAB SAMPLE ID	P231024.01	MATRIX	Liquid	NO. OF ORGANISMS	10

96-Hour Acute Toxicity with RBT

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)	Diluent Type	RFW
0	0%	0	16000.0	16000		
	6.25%	1000	15000.0	16000		
	12.5%	2000	14000.0	16000		
	25%	4000	12000.0	16000		
	50%	8000	8000.0	16000		
	100%	16000	0.0	16000		

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)
2	0%	0	12000.0	12000
	6.25%	750	11250.0	12000
	12.5%	1500	10500.0	12000
	25%	3000	9000.0	12000
	50%	6000	6000.0	12000
	100%	12000	0.0	12000

Test Dilution Prep

Date	Balance ID	Sample ID (P#)	Water Batch ID	Initials
10/24/23	#7	P231024.01	CFTW102223.01	LG
10/26	NA	P231024.01	CFTW102623.01	MS

Rainbow Trout Acute Survival
Acute Power Standard Calculation

Replicate	Number Surviving				Mean
	1	2	3	4	
ACEC (100)	10	10	10	10	10
Control	10	10	10	10	10

Control Mean - ACEC Mean

0

Difference Divided by Control Mean

0

Express as %

0%

≤29% meets the power standard

Pass

ORGANISM RECEIPT LOG

Date: 10/20/23	Time: 1145	Batch No. TF102023.01
--------------------------	----------------------	---------------------------------

Organism:
Oncorhynchus mykiss

Source / Supplier:
Thomas Fish Company

No. Ordered: 580	No. Received: 580	Source Batch: Collection date, hatch date, etc.): Hatch: 9/25/23
----------------------------	-----------------------------	---

Condition of Organisms:	Approximate Size or Age: (Days from hatch, life stage, size class, etc.): 11 days pse
--------------------------------	--

Shipper: FedEx	B of L (Tracking No.) 7738 0236 3539
--------------------------	--

Condition of Container: Good	Received By: DM
--	---------------------------

Container	D.O. (mg/L)	Temp. (°C)	Cond. or Sal. (Include Units)	pH (Units)	# Dead	% Dead*	Tech. (Initials)
1	31.3	10.8 11.1	359 us/cm	6.9	0	←	DM

*if >10% contact lab manager

Notes:

① MR-DM-10/20/23

Thomas Fish Company LLC

PO BOX 851
ANDERSON, CA 96007
thomasfishco@gmail.com
(530)378-1006

Packing Slip

BILL TO
Eco Analysts
4770 NE. View Dr.
Port Gamble, WA. 98364

SHIP TO
Eco Analysts
4770 NE. View Dr.
Port Gamble, WA. 98364

INVOICE 27058
DATE 10/19/2023

DATE	SERVICE	DESCRIPTION	QTY
10/19/2023	Rainbow Trout	Oncorhynchus mykiss--Reference- A FIELD GUIDE TO FRESHWATER FISHES, Lawrence Page / Brooks Burr--copyright 1991 pg. 54 (01/02/2023- Rob Thomas) Hatch Date: 9/25/23,swim up:10/9/23 PO.#Danny Mulligan CC	580

Reference Toxicant 96-h Acute Survival Test

EcoAnalysts

Test Type: Survival

Organism: Oncorhynchus mykiss

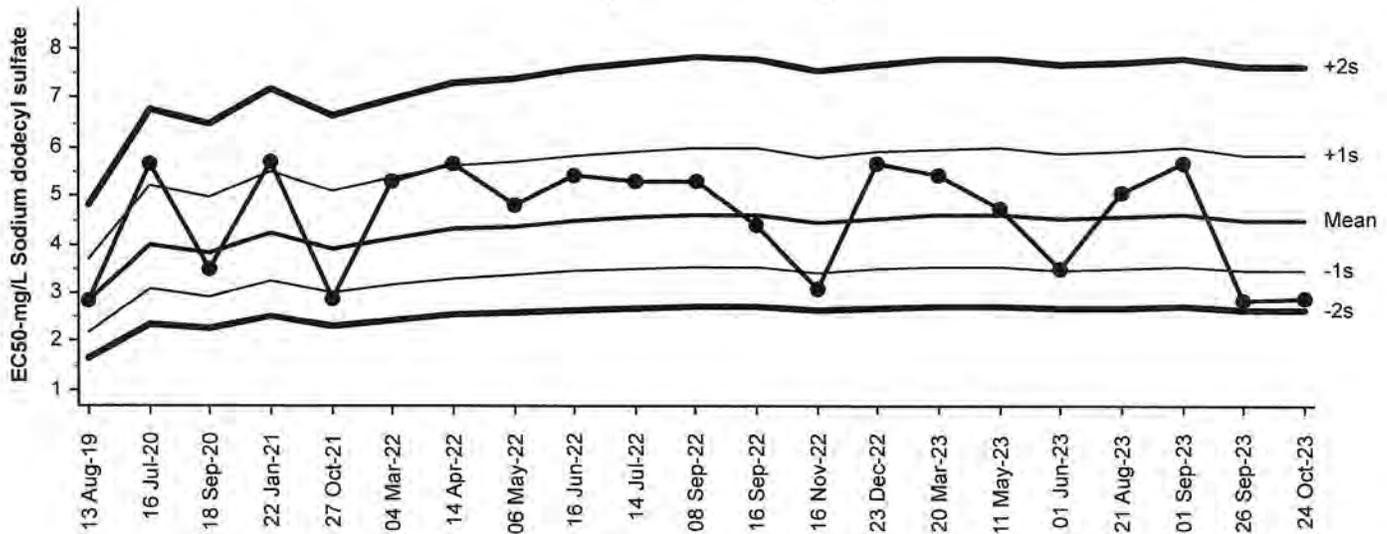
Material: Sodium dodecyl sulfate

Protocol: All Protocols

Endpoint: Proportion Survived

Source: Reference Toxicant-REF

Reference Toxicant 96-h Acute Survival Test
Proportion Survived Endpoint



Lognormal Cumulative Mean Plot

Mean: 4.486 Count: 20 -1s Warning Limit: 3.45 -2s Action Limit: 2.65
 Sigma: NA CV: 26.90% +1s Warning Limit: 5.84 +2s Action Limit: 7.61

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	Aug	13	16:30	2.828	-1.658	-1.747	(-)		03-4311-6703	07-0791-4983
2	2020	Jul	16	16:04	5.657	1.171	0.878			20-1757-7261	19-9982-7646
3		Sep	18	15:10	3.482	-1.004	-0.9592			15-4829-8908	17-1689-4163
4	2021	Jan	22	14:00	5.708	1.222	0.9122			15-7313-5934	01-7529-8552
5		Oct	27	14:37	2.895	-1.592	-1.659	(-)		04-5604-1349	05-8183-6236
6	2022	Mar	4	13:58	5.278	0.7919	0.6155			06-7531-6463	13-8864-0335
7		Apr	14	13:46	5.657	1.171	0.878			20-2335-4820	02-8010-1649
8		May	6	14:39	4.79	0.3038	0.2481			01-6715-1927	18-1382-9602
9		Jun	16	16:13	5.401	0.9152	0.703			00-0081-2959	10-6177-2221
10		Jul	14	14:42	5.298	0.8114	0.6295			10-0478-6162	17-5806-7323
11		Sep	8	16:20	5.278	0.7919	0.6155			05-2463-3891	02-1144-4269
12			16	14:57	4.387	-0.09887	-0.08438			09-5327-8659	07-5092-9157
13		Nov	16	16:03	3.102	-1.384	-1.397	(-)		04-3259-4483	20-8758-5143
14		Dec	23	15:22	5.657	1.171	0.878			13-6721-7759	13-1826-3156
15	2023	Mar	20	16:13	5.401	0.9152	0.703			06-0335-0896	11-6894-5418
16		May	11	16:18	4.702	0.216	0.1781			15-0059-3610	16-1659-7252
17		Jun	1	16:59	3.482	-1.004	-0.9592			15-3461-0414	06-5857-0458
18		Aug	21	14:27	5.04	0.5535	0.4405			02-1332-0780	01-1065-4617
19		Sep	1	16:25	5.657	1.171	0.878			15-2655-6815	06-5614-4747
20			26	15:42	2.828	-1.658	-1.747	(-)		19-2605-2960	19-3701-7978
21		Oct	24	18:03	2.873	-1.613	-1.687	(-)		11-6258-9594	16-1167-6267

CETIS Summary Report

Report Date: 05 Dec-23 12:08 (p 1 of 1)
 Test Code/ID: P230706.04 / 11-6258-9594

Reference Toxicant 96-h Acute Survival Test

EcoAnalysts

Batch ID: 19-7343-9842	Test Type: Survival	Analyst: Marisa Seibert
Start Date: 24 Oct-23 18:03	Protocol: EPA/821/R-02-012 (2002)	Diluent: Carbon Filtered Municipal Water
Ending Date: 28 Oct-23 16:36	Species: Oncorhynchus mykiss	Brine: Not Applicable
Test Length: 95h	Taxon: Actinopterygii	Source: Thomas Fish Co. Age: 15d
Sample ID: 06-4095-6301	Code: P230706.04	Project: Reference Toxicant
Sample Date: 06 Jul-23	Material: Sodium dodecyl sulfate	Source: Reference Toxicant
Receipt Date: 06 Jul-23	CAS (PC):	Station: P230706.04
Sample Age: 110d 18h	Client: Internal Lab	

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
14-3899-9154	Proportion Survived	Steel Many-One Rank Sum Test	2	4	2.828	---	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	mg/L	95% LCL	95% UCL	S
16-1167-6267	Proportion Survived	Linear Interpolation (ICPIN)	EC15	2.239	2.239	2.239	1
			EC20	2.323	2.323	2.323	
			EC25	2.409	2.409	2.409	
			EC40	2.68	2.68	2.68	
			EC50	2.873	2.873	2.873	

Proportion Survived Summary

Conc-mg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
1		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
2		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
4		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%
8		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%
16		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

Proportion Survived Detail

MD5: AC212028B9DA8111BC50B14FBD3AF6FB

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
1		1.0000	1.0000	1.0000	1.0000
2		1.0000	1.0000	1.0000	1.0000
4		0.0000	0.0000	0.0000	0.0000
8		0.0000	0.0000	0.0000	0.0000
16		0.0000	0.0000	0.0000	0.0000

Proportion Survived Binomials

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	10/10	10/10	10/10	10/10
1		10/10	10/10	10/10	10/10
2		10/10	10/10	10/10	10/10
4		0/10	0/10	0/10	0/10
8		0/10	0/10	0/10	0/10
16		0/10	0/10	0/10	0/10

CETIS Test Data Worksheet

Report Date: 05 Dec-23 12:08 (p 1 of 1)
 Test Code/ID: P230706.04 / 11-6258-9594

Reference Toxicant 96-h Acute Survival Test				EcoAnalysts	
Start Date:	24 Oct-23 18:03	Species:	Oncorhynchus mykiss	Sample Code:	P230706.04
End Date:	28 Oct-23 16:36	Protocol:	EPA/821/R-02-012 (2002)	Sample Source:	Reference Toxicant
Sample Date:	06 Jul-23	Material:	Sodium dodecyl sulfate	Sample Station:	P230706.04

Conc-mg/L	Code	Rep	Pos	# Exposed	# Survived	Notes
0	D	1	1	10	10	
0	D	2	6	10	10	
0	D	3	22	10	10	
0	D	4	24	10	10	
1		1	2	10	10	
1		2	14	10	10	
1		3	19	10	10	
1		4	4	10	10	
2		1	16	10	10	
2		2	8	10	10	
2		3	20	10	10	
2		4	11	10	10	
4		1	12	10	0	
4		2	23	10	0	
4		3	5	10	0	
4		4	21	10	0	
8		1	3	10	0	
8		2	15	10	0	
8		3	17	10	0	
8		4	18	10	0	
16		1	9	10	0	
16		2	10	10	0	
16		3	7	10	0	
16		4	13	10	0	

96-Hour Acute SDS Reference Toxicant Test
Oncorhynchus mykiss

Test ID:	P230706.04
Toxicant:	SDS
Stock Conc.	50,000mg/L
Stock Preparation:	2.5g SDS / 50 mL DI
Lot #:	22k1261002
Study Director:	M. Seibert

Version: 5

Date Test Started:	24 Oct 23
Date Test Ended:	28 Oct 23
Matrix:	Liquid
Species:	<i>Oncorhynchus mykiss</i>
# Organisms/Chamber:	10

	Conc. (mg/L)	DO ≥ 6.0 (mg/L)	Temp 12 ± 1 (°C)	Cond. (µS/cm)	pH	Hardness mg/L CaCO ₃	Alkalinity mg/L CaCO ₃
Day 0 (0 hours)	Control	9.8	12.4	214	8.4	84	100
	1	12.0	12.2	201	8.4		
Date: 10/24/23	2	10.0	12.3	201	8.4		
Time: 1733	4	9.9	12.5	202	8.4		
Rep #: 1	8	9.9	12.9	202	8.4		
Technician: UG	16	9.7	12.2	204	8.4		
24 hours	Control	10.7	22.8	215	8.3		
	1	10.6	22.7	219	8.3		
Date: 10/25/23	2	10.6	22.7	200	8.2		
Time: 2549	4	10.5	22.7	202	8.2		
Rep #: 1	8	10.6	22.8	204	8.3		
Technician: SR	16	10.6	22.2	206	8.3		
48 hours	Control	10.1	12.9	217	8.2		
	1	10.1	12.0	204	8.1		
Date: 10/26	2	10.2	12.4	204	8.2		
Time: 1310	4	10.4	12.0	207	8.2		
Rep #: 2	8						
Technician: JL	16						
48 hours	Control	9.8	13.3	193	9.0		
Renewal Stock	1	9.8	12.7	203	8.7		
Date: 10/26	2	9.3	13.0	194	9.0		
Time: 1510	4	9.5	13.1	194	9.0		
Rep #: NA	8						
Technician: JL	16						
72 hours	Control	10.57	12.1	208	7.8		
	1	10.5	11.9	202	8.0		
Date: 10/27	2	10.3	12.3	196	7.8		
Time: 0850	4						
Rep #: 3	8						
Technician: TT	16						
96 hours	Control	10.8	11.7	205	8.1		
Meter # 8	1	10.9	11.7	204	8.2		
Date: 10/28/23	2	11.0	11.4	199	8.2		
Time: 1046	4						
Rep #: 4	8						
Technician: MARK	16						

Spiking Calculations

Target Concentrations:	Quantity of Stock: Target: (mL)	Quantity of Diluent: Target:
1 mg/L	0.1	5 L
2 mg/L	0.2	5 L
4 mg/L	0.4	5 L
8 mg/L	0.8	5 L
16mg/L	1.6	5 L

Prepared by: SR

Start Time:	1803	Initials:	UG/SR/10 ^M
End Time:	1636		
Supplier:	THOMAS FISH COMPANY		
Organism Batch:	TR02023.01	Age:	15 days PSM
Test Location:	Bath 4		

Test Acceptability: ≥ 90% Survival in Control

OPE - SR 10/25/23

Renewal

0.24mL 12L
0.48mL 12L
0.96mL 12L

JL

96-Hour Acute SDS Reference Toxicant Test
Oncorhynchus mykiss

Test ID:	P230706.04
Toxicant:	SDS
Stock Conc.	50,000mg/L
Stock Preparation:	2.5g SDS / 50 mL DI
Lot #:	22K126002
Study Director:	M. Seibert

Version: 5

Date Test Started:	24 Oct 23
Date Test Ended:	28 Oct 23
Matrix:	Liquid
Species:	<i>Oncorhynchus mykiss</i>
# Organisms/Chamber:	10

Survival		24 Hours		48 Hours		72 Hours		96 Hours	
		Date: 10/25/23		Date: 10/26		Date: 10/27/23		Date: 10/28/23	
		Time: 1620		Time: 1535		Time: 8109		Time: 1636	
Conc.	Rep	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead
Control	1	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
1	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
2	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
4	1	10	0	0	10	—	—	—	—
	2	7	3	0	7	—	—	—	—
	3	5	4, 1NB	0	5	—	—	—	—
	4	0	10	—	—	—	—	—	—

**96-Hour Acute SDS Reference Toxicant Test
Oncorhynchus mykiss**

8	1	0	8-210 ¹⁰						
	2	0	10						
	3	0	10						
	4	0	10						
16	1	0	10						
	2	0	10						
	3	0	10						
	4	0	10						
INITIALS:		SR		UL					

① IE-SR 10/25/23

1	22
2	11
3	13
4	9
<hr/>	
5	4
6	23
7	3
8	15
<hr/>	
9	6
10	5
11	24
12	2
<hr/>	
13	7
14	17
15	12
16	20
<hr/>	
17	18
18	14
19	8
20	16
<hr/>	
21	21
22	1
23	10
24	19
<hr/>	

RBT
SDS RT

Test ID: P230706.04

APPENDIX A.4

***CERIODAPHNIA DUBIA* (WATER FLEA) 7-DAY SURVIVAL AND REPRODUCTION TEST**

STATISTICAL COMPARISONS AND LABORATORY DATA SHEETS

CETIS Summary Report

Report Date: 27 Nov-23 11:30 (p 1 of 2)
 Test Code/ID: P231024.01C.du. / 14-9684-1984

Ceriodaphnia 7-d Survival and Reproduction Test

EcoAnalysts

Batch ID: 10-3320-7767	Test Type: Reproduction-Survival (6-8d)	Analyst: Marisa Seibert
Start Date: 24 Oct-23 16:25	Protocol: EPA/821/R-02-013 (2002)	Diluent: Cerio Reconstituted Fresh Water
Ending Date: 01 Nov-23 16:12	Species: Ceriodaphnia dubia	Brine: Not Applicable
Test Length: 8d	Taxon: Branchiopoda	Source: Aquatic Biosystems, CO Age: <1d
Sample ID: 20-4892-7174	Code: P231024.01C.du.	Project: Toxicity Testing
Sample Date: 23 Oct-23 14:30	Material: Stormwater	Source: Brooks Manufacturing Co. (WA003080)
Receipt Date: 24 Oct-23 10:30	CAS (PC):	Station: Outfall 1
Sample Age: 26h	Client: Brooks Manufacturing Co.	

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	TU	S
19-0625-6625	8d Proportion Survived	Fisher Exact/Bonferroni-Holm Test	100	>100	---	---	1	1
14-4527-6982	8d Reproduction	Wilcoxon/Bonferroni Adj Test	100	>100	---	50.8%	1	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	%	95% LCL	95% UCL	TU	S
11-6897-9861	8d Proportion Survived	Linear Interpolation (ICPIN)	✓ EC15	>100	---	---	<1	1
			✓ EC20	>100	---	---	<1	
			✓ EC25	>100	---	---	<1	
			✓ EC40	>100	---	---	<1	
			✓ EC50	>100	---	---	<1	
17-5671-0434	8d Reproduction	Linear Interpolation (ICPIN)	✓ IC15	39.01	17.71	---	2.6	1
			✓ IC20	>100	---	---	<1	
			✓ IC25	>100	---	---	<1	
			✓ IC40	>100	---	---	<1	
			✓ IC50	>100	---	---	<1	

8d Proportion Survived Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	10	0.9000	0.6738	1.1260	0.0000	1.0000	0.1000	0.3162	35.14%	0.00%
6.25		10	0.9000	0.6738	1.1260	0.0000	1.0000	0.1000	0.3162	35.14%	0.00%
12.5		10	0.9000	0.6738	1.1260	0.0000	1.0000	0.1000	0.3162	35.14%	0.00%
25		10	0.8000	0.4984	1.1020	0.0000	1.0000	0.1333	0.4216	52.70%	11.11%
50		10	0.8000	0.4984	1.1020	0.0000	1.0000	0.1333	0.4216	52.70%	11.11%
100		10	0.9000	0.6738	1.1260	0.0000	1.0000	0.1000	0.3162	35.14%	0.00%

8d Reproduction Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	9	16.33	10.39	22.27	0	27	2.577	7.73	47.33%	0.00%
6.25		10	16.9	11.86	21.94	0	25	2.228	7.047	41.70%	-3.47%
12.5		10	21.8	15.88	27.72	0	29	2.615	8.27	37.94%	-33.47%
25		9	17	12.28	21.72	7	24	2.048	6.144	36.14%	-4.08%
50		10	14.6	7.295	21.9	0	26	3.229	10.21	69.94%	10.61%
100		10	15	12	18	5	19	1.325	4.19	27.93%	8.16%

CETIS Summary Report

Report Date: 27 Nov-23 11:30 (p 2 of 2)
 Test Code/ID: P231024.01C.du. / 14-9684-1984

Ceriodaphnia 7-d Survival and Reproduction Test

EcoAnalysts

8d Proportion Survived Detail

MD5: E77D7AEC8A37C69FC228B78A31814BE5

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	D	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000
6.25		0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
12.5		1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
25		1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000
50		1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000
100		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000

8d Reproduction Detail

MD5: 85AA65BF70ABCF0E0D2B91DEA717B5A4

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	D	14	16	24	—	12	0	27	19	19	16
6.25		0	20	15	13	17	25	20	21	23	15
12.5		0	25	21	25	25	29	20	21	29	23
25		12	18	19	7	22	20	22	9	—	24
50		19	14	0	26	2	18	20	22	25	0
100		17	19	12	13	16	15	5	19	17	17

8d Proportion Survived Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	D	1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1
6.25		0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
12.5		1/1	1/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
25		1/1	1/1	1/1	0/1	1/1	1/1	1/1	0/1	1/1	1/1
50		1/1	1/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1
100		1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1

CETIS Test Data Worksheet

Report Date: 27 Nov-23 11:27 (p 1 of 2)
 Test Code/ID: P231024.01C.du. / 14-9684-1984

Ceriodaphnia 7-d Survival and Reproduction Test

EcoAnalysts

Start Date: 24 Oct-23 16:25 Species: Ceriodaphnia dubia Sample Code: P231024.01C.du.
 End Date: 01 Nov-23 16:12 Protocol: EPA/821/R-02-013 (2002) Sample Source: Brooks Manufacturing Co.
 Sample Date: 23 Oct-23 14:30 Material: Stormwater Sample Station: Outfall 1

Conc-%	Code	Rep	Pos	# Exposed	1d Survival	2d Survival	3d Survival	4d Survival	5d Survival	6d Survival	7d Survival	8d Survival	6d Neonates	7d Neonates	8d Neonates	Male	Notes
0	D	1	5	1	1	1	1	1	1	1	1	1			14	0	
0	D	2	37	1	1	1	1	1	1	1	1	1			16	0	
0	D	3	39	1	1	1	1	1	1	1	1	1			24	0	
0	D	4	20	1	1	1	1	1	1	1	1	1			0	-1	
0	D	5	44	1	1	1	1	1	1	1	1	1			12	0	
0	D	6	14	1	1	0	0	0	0	0	0	0			0	0	
0	D	7	34	1	1	1	1	1	1	1	1	1			27	0	
0	D	8	31	1	1	1	1	1	1	1	1	1			19	0	
0	D	9	40	1	1	1	1	1	1	1	1	1			19	0	
0	D	10	52	1	1	1	1	1	1	1	1	1			16	0	
6.25		1	4	1	1	1	0	0	0	0	0	0			0	0	
6.25		2	27	1	1	1	1	1	1	1	1	1			20	0	
6.25		3	22	1	1	1	1	1	1	1	1	1			15	0	
6.25		4	60	1	1	1	1	1	1	1	1	1			13	0	
6.25		5	18	1	1	1	1	1	1	1	1	1			17	0	
6.25		6	56	1	1	1	1	1	1	1	1	1			25	0	
6.25		7	15	1	1	1	1	1	1	1	1	1			20	0	
6.25		8	24	1	1	1	1	1	1	1	1	1			21	0	
6.25		9	8	1	1	1	1	1	1	1	1	1			23	0	
6.25		10	50	1	1	1	1	1	1	1	1	1			15	0	
12.5		1	59	1	1	1	1	1	1	1	1	1			0	0	
12.5		2	47	1	1	1	1	1	1	1	1	1			25	0	
12.5		3	38	1	1	1	1	1	1	1	1	0			21	0	
12.5		4	7	1	1	1	1	1	1	1	1	1			25	0	
12.5		5	28	1	1	1	1	1	1	1	1	1			25	0	
12.5		6	30	1	1	1	1	1	1	1	1	1			29	0	
12.5		7	46	1	1	1	1	1	1	1	1	1			20	0	
12.5		8	1	1	1	1	1	1	1	1	1	1			21	0	
12.5		9	6	1	1	1	1	1	1	1	1	1			29	0	
12.5		10	19	1	1	1	1	1	1	1	1	1			23	0	
25		1	29	1	1	1	1	1	1	1	1	1			12	0	
25		2	2	1	1	1	1	1	1	1	1	1			18	0	
25		3	54	1	1	1	1	1	1	1	1	1			19	0	
25		4	33	1	1	1	1	1	0	0	0	0			7	0	
25		5	42	1	1	1	1	1	1	1	1	1			22	0	
25		6	10	1	1	1	1	1	1	1	1	1			20	0	
25		7	11	1	1	1	1	1	1	1	1	1			22	0	
25		8	51	1	1	1	1	1	1	1	0	0			9	0	
25		9	26	1	1	1	1	1	1	1	1	1			0	-1	
25		10	53	1	1	1	1	1	1	1	1	1			24	0	

CETIS Test Data Worksheet

Report Date: 27 Nov-23 11:27 (p 2 of 2)

Test Code/ID: P231024.01C.du. / 14-9684-1984

Conc-%	Code	Rep	Pos	# Exposed	1d Survival	2d Survival	3d Survival	4d Survival	5d Survival	6d Survival	7d Survival	8d Survival	6d Neonates	7d Neonates	8d Neonates	Male	Notes
50		1	21	1	1	1	1	1	1	1	1	1			19	0	
50		2	58	1	1	1	1	1	1	1	1	1			14	0	
50		3	48	1	1	0	0	0	0	0	0	0			0	0	
50		4	36	1	1	1	1	1	1	1	1	1			26	0	
50		5	12	1	1	1	1	1	1	1	1	1			2	0	
50		6	23	1	1	1	1	1	1	1	1	1			18	0	
50		7	55	1	1	1	1	1	1	1	1	1			20	0	
50		8	45	1	1	1	1	1	1	1	1	1			22	0	
50		9	9	1	1	1	1	1	1	1	1	1			25	0	
50		10	57	1	1	1	1	1	1	0	0	0			0	0	
100		1	35	1	1	1	1	1	1	1	1	1			17	0	
100		2	32	1	1	1	1	1	1	1	1	1			19	0	
100		3	16	1	1	1	1	1	1	1	1	1			12	0	
100		4	17	1	1	1	1	1	1	1	1	1			13	0	
100		5	3	1	1	1	1	1	1	1	1	1			16	0	
100		6	49	1	1	1	1	1	1	1	1	1			15	0	
100		7	25	1	1	1	1	1	0	0	0	0			5	0	
100		8	13	1	1	1	1	1	1	1	1	1			19	0	
100		9	43	1	1	1	1	1	1	1	1	1			17	0	
100		10	41	1	1	1	1	1	1	1	1	1			17	0	

Version V.4

GENERAL

Client	Brooks Manufacturing Co.
Project	Toxicity Testing
Project Number	PG1913
Project Manager	M. Seibert
Date Sample Received	10/24/2023
Test type	7 Day Chronic Toxicity with Cerio
Matrix	Liquid
Test Acceptability	≥ 80% average survival of control ≥ 60% of surviving control females ≥ 3 broods Controls average ≥ 15 neonates/survivor ≥ 80% surviving controls = female
Test Start Date	10/24/23
Test Species	Ceriodaphnia dubia
Organism Batch (Brood Board #)	ABS102423.02
Organism Acquired	① in-house culture 10/24/23
Organism Acclimation	N/A
Organism Age	<24 hrs
Test Protocol	TOX003
Test Location	Temp Control Room
Light Intensity	
Light Cycle	16L:8D
Water Description	cerio reconstituted freshwater
Organisms per Replicate	1
Test Chamber Size	1 oz
Exposure Volume	15 mL
Feeding Information	100 µL Raphidocelis & 100 µL YCT suspension daily
Test Dissolved Oxygen	> 4.0
Test Temperature	25 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	4.0	
Temp	23.5	26.4
Conductivity		
pH	6.0	9.0

TEST START TIME/INIT: 1625 OL
TEST END TIME/INIT: 1622 SR

CLIENT SAMPLE ID	LAB ID
Outfall 1	P231024.01
0.1% Fall 1	P23020.04
0.1% Fall 1	P231028.03

Concentrations	
1	Control
2	6.25%
3	12.5%
4	25.0%
5	50.0%
6	100.0%
7	
8	
9	

(Measure and record, should be 5(±100 fr-)

Food Batch ID
See WQ sheets

Copy and Past VALUES

Treatment	Rep	Chamber
Control	1	8
Control	2	17
Control	3	19
Control	4	15
Control	5	39
Control	6	38
Control	7	53
Control	8	40
Control	9	9
Control	10	34
6.25%	1	59
6.25%	2	43
6.25%	3	25
6.25%	4	1
6.25%	5	22
6.25%	6	58
6.25%	7	51
6.25%	8	49
6.25%	9	20
6.25%	10	23
12.5%	1	26
12.5%	2	7
12.5%	3	48
12.5%	4	45
12.5%	5	13
12.5%	6	52
12.5%	7	21
12.5%	8	32
12.5%	9	44
12.5%	10	47
25%	1	42
25%	2	36
25%	3	10
25%	4	2
25%	5	41
25%	6	11
25%	7	56
25%	8	54
25%	9	46
25%	10	60
50%	1	50
50%	2	35
50%	3	3
50%	4	24
50%	5	28
50%	6	16
50%	7	18
50%	8	57
50%	9	29
50%	10	4
100%	1	31
100%	2	6
100%	3	37
100%	4	33
100%	5	12
100%	6	5
100%	7	14
100%	8	27
100%	9	30
100%	10	55

*Test may be terminated on Day, 6, 7, or 8 when control reproduction acceptability criteria are met for non-Washington State tests(EPA 2002).

Must have Day 7 survival for Washington State tests (WDOE 2016).

**All water quality parameters are measured on the "old" sample at test termination.

① 16.0L 10/24

CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	10/24/2023	PROTOCOL	TOX003
PROJECT	Toxicity Testing	TEST START DATE	10/24/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall 1	TEST END DATE	11/1/23	SPECIES	Ceriodaphnia dubia
LAB SAMPLE ID	P231024.01	MATRIX	Liquid	NO. OF ORGANISMS	1

7 Day Chronic Toxicity with Cerio

	Concentration (%)	DO (mg/L)	TEMP (°C)	pH	DO (mg/L)	Conductivity	pH	Renewal & Feeding Parameters
		(Old)	(New Day 0; Old Days 1-7)	(Old)	(New)	(New; Old at termination)	(New)	
Day 0	Control		23.6		8.3	334	7.8	100 µL Raphidocelis & 100 µL YCT suspension daily
	6.25%		23.6		8.4	363	7.9	Time
	12.5%		23.6		8.5	388	7.8	Tech
	25.0%		23.6		8.6	439	7.7	Feed Init
	50.0%		23.6		8.7	541	7.7	Sel Batch
	100.0%		23.6		9.0	740	7.7	YCT Batch
Day 1	Control	8.4	24.0	8.1	9.0	462	7.8	Time
	6.25%	8.5	24.9	8.1	8.9	365	8.0	Tech
	12.5%	8.4	24.9	8.0	9.0	386	7.9	Feed Init
	25.0%	8.3	24.9	8.0	9.0	441	7.8	Sel Batch
	50.0%	8.3	24.9	8.0	9.0	538	7.7	YCT Batch
	100.0%	8.1	24.9	7.9	9.1	742	7.4	
Day 2	Control	8.6	24.0	7.8	8.5	335	7.7	Time
	6.25%	8.6	24.0	7.9	8.5	362	7.8	Tech
	12.5%	8.7	24.0	8.0	8.6	387	7.8	Feed Init
	25.0%	8.5	24.0	8.0	8.7	438	7.8	Sel Batch
	50.0%	8.4	24.0	7.9	9.1	539	7.7	YCT Batch
	100.0%	8.1	24.0	7.8	9.8	743	7.5	
Day 3	Control	8.3	24.6	8.3	8.5	330	7.9	Time
	6.25%	8.4	24.6	8.2	8.6	326	7.8	Tech
	12.5%	8.5	24.6	8.2	8.7	316	7.8	Feed Init
	25.0%	8.5	24.6	8.2	8.7	295	7.8	Sel Batch
	50.0%	8.4	24.6	8.1	9.2	254	7.7	YCT Batch
	100.0%	8.4	24.6	8.1	9.6	165	7.65	
Day 4	Control	8.4	24.4	7.7	8.6	335	7.9	Time
	6.25%	8.4	24.4	7.8	8.5	328	7.9	Tech
	12.5%	8.4	24.4	7.8	8.7	317	7.9	Feed Init
	25.0%	8.3	24.4	7.8	8.8	295	7.9	Sel Batch
	50.0%	8.2	24.4	7.8	9.2	253	7.7	YCT Batch
	100.0%	8.0	24.4	7.9	9.8	165	7.6	
Day 5	Control	8.6	24.4	8.2	8.4	339	7.8	Time
	6.25%	8.5	24.4	8.2	8.4	331	7.8	Tech
	12.5%	8.3	24.4	8.1	8.5	321	7.8	Feed Init
	25.0%	8.2	24.4	8.1	8.5	299	7.8	Sel Batch
	50.0%	8.0	24.4	8.0	8.7	259	7.8	YCT Batch
	100.0%	8.1	24.4	8.0	9.3	180	7.8	
Day 6*	Control	8.4	24.8	7.9	8.6	343	7.9	Time
	6.25%	8.5	24.8	8.0	8.6	339	8.0-8.0	Tech
	12.5%	8.6	24.8	8.0	8.6	332	8.0	Feed Init
	25.0%	8.6	24.8	8.0	8.7	308	7.9	Sel Batch
	50.0%	8.5	24.8	8.0	8.9	269	7.8	YCT Batch
	100.0%	8.4	24.8	8.0	9.5	177	7.7	
Day 7*	Control	9.0	24.6	7.5	8.2	336	8.0	Time
	6.25%	8.7	24.6	7.7	8.0	330	8.2	Tech
	12.5%	8.5	24.6	7.8	8.3	326	8.0	Feed Init
	25.0%	8.4	24.6	7.8	8.5	299	7.9	Sel Batch
	50.0%	8.3	24.6	7.9	9.0	259	7.8	YCT Batch
	100.0%	8.2	24.6	7.9	9.5	177	7.7	
Day 8*	Control	8.1	24.7	8.0		341		
	6.25%	8.4	24.7	8.0		337		
	12.5%	8.5	24.7	8.1		328		
	25.0%	8.5	24.7	8.0		306		
	50.0%	8.1	24.7	8.0		263		
	100.0%	8.2	24.7	8.0		181		

① 16 J1 10/25/23 NL 10/27, 11/03/23
 ② used temp blank 10/25/23 J1, 10/26 JL, 11/01/23, NL 10/28
 - NL 10/29, TW 10/30
 ③ 1W 11/03/23

10/24/23

CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	#####	PROTOCOL	TOX003
PROJECT	Toxicity Testing	TEST START DATE	10/24/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall 1	TEST END DATE	11/1/23	SPECIES	Ceriodaphnia dubia
LAB SAMPLE ID	P231024.01	MATRIX	Liquid	NO. OF ORGANISMS	1

Key:
 E = No body found
 D = Dead body found
 A = Accident, animal removed from testing

7 Day Chronic Toxicity with Cerio

Day/Date/Time	Concentration %					Control					# of Adults	Initials	
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10			
1/													
2/													
3/	4							4		3			
4/		3	4		5		5		3				
5/	7	4	7		3		10	7	5	6			
6/	3							1		7			
7/			1		4		1		1				
8/		9	12				11	7	10				
Checked for Male (final day)				√ Male									
Reproduction Total	14	16	24		12		27	19	19	16			

Day/Date/Time	Concentration %					6.25%					# of Adults	Initials
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10		
1/												
2/												
3/		3	3	3	5		1	5	4	1		
4/						2	1			5		
5/		8	3	6	8	9	8	8	5			
6/			1	4	4		10	8	14	9		
7/						14						
8/		9	8									
Checked for Male (final day)												
Reproduction Total		20	15	13	17	25	20	21	23	15		

Day/Date/Time	Concentration %					12.5%					# of Adults	Initials
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10		
1/												
2/												
3/		2	4		5		4	3				
4/				6	7	9			6	3		
5/		9	6	6			5	9	9	9		
6/		14	11		2	10	11	9				
7/				13	11	10			14	11		
8/												
Checked for Male (final day)												
Reproduction Total		25	21	25	25	29	20	21	29	23		

v.4 CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	10/24/23 #####	PROTOCOL	TOX003
PROJECT	Toxicity Testing	TEST START DATE	10/24/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall 1	TEST END DATE	11/1/23	SPECIES	<i>Ceriodaphnia dubia</i>
LAB SAMPLE ID	P231024.01	MATRIX	Liquid	NO. OF ORGANISMS	1

Key:

Concentration % 25%

Day/Date/Time	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10	# of Adults	Initials
1/												
2/												
3/	2	3	3	1	5	1				3		
4/	5	5		2	1		6					
5/		1	7	4	9	6	5	5		8		
6/	5	9	9		7	13				13		
7/							11	4				
8/												
Checked for Male (final day)										√ Male		
Reproduction Total	12	18	19	7	22	20	22	9		24		

Concentration % 50%

Day/Date/Time	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10	# of Adults	Initials
1/												
2/												
3/	4	3		4	2	3	4	4				
4/	6	7				8	6		3			
5/		4		2				10	13			
6/	9			12		7	10	8				
7/				8					9			
8/												
Checked for Male (final day)												
Reproduction Total	19	14		26	2	18	20	22	25			

Concentration % 100%

Day/Date/Time	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10	# of Adults	Initials
1/												
2/												
3/	3		1	1	1		1	2	2	3		
4/	6	3	5	6		4	4	4				
5/		10	6	6	5	11		13	7	3		
6/	8	1			10				8	11		
7/		5										
8/												
Checked for Male (final day)												
Reproduction Total	17	19	12	13	16	15	5	19	17	17		

V.4 CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	10/24/23 #####	PROTOCOL	TOX003
PROJECT	Toxicity Testing	TEST START DATE	10/24/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall 1	TEST END DATE	11/1/23	SPECIES	Ceriodaphnia dubia
LAB SAMPLE ID	P231024.01	MATRIX	Liquid	NO. OF ORGANISMS	1

Key:
 E = No body found
 D = Dead body found
 A = Accident, animal removed from testing
 DN = Dead neonate

7 Day Chronic Toxicity with Cerio

Day/Date/Time	Concentration %						Control				# of Adults	Initials	
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10			
1/ 10/25/23 1414	0	0	0	0	0	0	0	0	0	0	0	10	JJ
2/ 10/26/23 1700	0	0	0	0	0	0	0	0	0	0	0	10	BS
3/ 10/27 1607	4	0	0	0	0	/	0	4	0	3	9	NL	
4/ 10/28 1211	0	3	4	0	5	/	5, 1DN	0	3	0	9	NL	
5/ 10/29 1304	7	4	7	0	3	/	10	7	5	6	9	NL	
6/ 10/30 1144	3	0	0	0	0	/	0	1	0	7	9	JJ	
7/ 10/31 2220	0	0	1	0	4	/	2	0	2	3	9	SR	
8/ 11/01 2622	7	9	12	0	9	/	11	7	10	0	9	SR	
Checked for Male (final day)				Male									
Reproduction Total													

Day/Date/Time	Concentration %						6.25%				# of Adults	Initials	
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10			
1/ 10/25/23 1414	0	0	0	0	0	0	0	0	0	0	0	10	JJ
2/ 10/26/23 1700	0	0	0	0	0	0	0	0	0	0	0	10	BS
3/ 10/27 1607	D, 0	3	3	3	5	0	1	5	4	1	9	NL	
4/ 10/28 1211	/	0	0	0	0	2	1	0	0	5	9	NL	
5/ 10/29 1304	/	8	3	6	8	9	8	8	5	0	9	NL	
6/ 10/30 1144	/	0	1	4	4	0	10	8	14	9	9	JJ	
7/ 10/31 2220	/	0	0	0	0	14	0	0	13	12	9	SR	
8/ 11/01 2622	/	9	8	9	12	12	14	13	0	9	9	SR	
Checked for Male (final day)													
Reproduction Total													

Day/Date/Time	Concentration %						12.5%				# of Adults	Initials	
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10			
1/ 10/25/23 1414	0	0	0	0	0	0	0	0	0	0	0	10	JJ
2/ 10/26/23 1700	0	0	0	0	0	0	0	0	0	0	0	10	BS
3/ 10/27 1607	0	2	4	0	5	0	4	3	0	0	10	NL	
4/ 10/28 1211	0	0	0	6	7	9	0	0	6	3, 1DN	10	NL	
5/ 10/29 1304	0	9	6	6	0	0	5	9	9	9	10	NL	
6/ 10/30 1144	0	14	11	0	2	10	11	9	0	0	10	JJ	
7/ 10/31 2220	0	0	0	13	11	10	0	0	14	12	10	SR	
8/ 11/01 2622	0	0	D, 10	13	11	10	14	13	12	0	9	SR	
Checked for Male (final day)	Female		-										
Reproduction Total													

① IE-MS 10/26, 11/01

10/24/23

V.4 CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	#####	PROTOCOL	TOX003
PROJECT	Toxicity Testing	TEST START DATE	10/24/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall 1	TEST END DATE	11/1/23	SPECIES	Ceriodaphnia dubia
LAB SAMPLE ID	P231024.01	MATRIX	Liquid	NO. OF ORGANISMS	1

Key:

Concentration % 25%

Day/Date/Time	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10	# of Adults	Initials
1/ 10/25/23 1414	0	0	0	0	0	0	0	0	0	0	10	J1
2/ 10/26/23 1700	0	0	0	0	0	0	0	0	0	0	10	N
3/ 10/27 1607	2	3	3	1	5	1	0	0	0	3	10	NL
4/ 10/28 1211	5	5	0	2	1	0	6	0	0	0	10	NL
5/ 10/29 1304	0	1	7	D, 4	9	6	5	5	0	8	9	NL
6/ 10/30 1144	5	9	9	/	7	13	0	0	0	13	9	J1
7/ 10/31 2220	24	20	0	/	0	0	11	D, 4	0	0	8	SR
8/ 11/01 2622	11	8	14	/	10	0	12	-	0	11	8	SR
Checked for Male (final day)								-	male			
Reproduction Total												

Concentration % 50%

Day/Date/Time	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10	# of Adults	Initials
1/ 10/25/23 1414	0	0	0	0	0	0	0	0	0	0	10	J1
2/ 10/26/23 1700	0	0	0	0	0	0	0	0	0	0	9	N
3/ 10/27 1607	4	3	/	4	2	3	4	4	0	0	9	NL
4/ 10/28 1211	6	7	/	0	0	8	6	0	3	0	9	NL
5/ 10/29 1304	0	4	/	2	0	0	0	10	13	0	9	NL
6/ 10/30 1144	9	1	/	12	0	7	10	8	0	D, 0	8	J1
7/ 10/31 2220	10	0	/	8	0	12	5	0	9	/	8	SR
8/ 11/01 2622	0	20	/	0	0	10	0	9	8	/	8	SR
Checked for Male (final day)			/									
Reproduction Total			/									

Concentration % 100%

Day/Date/Time	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10	# of Adults	Initials
1/ 10/25/23 1414	0	0	0	0	0	0	0	0	0	0	10	J1
2/ 10/26/23 1700	0	0	0	0	0	0	0	0	0	0	10	N
3/ 10/27 1607	3	0	1	1	1	0	1	2	2	3	10	NL
4/ 10/28 1211	6	3	5	6	0	4	4	4	0	0	10	NL
5/ 10/29 1304	0	10	6	10	5	11	D	13	7	3	9	NL
6/ 10/30 1144	8	0	0	0	10	0	/	0	8	11	9	J1
7/ 10/31 2220	6	1	0	20	0	0	/	5	0	0	9	SR
8/ 11/01 2622	9	5	D, 0	5	0	0	/	3	0	0	9	SR
Checked for Male (final day)			-									
Reproduction Total												

V.4 CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	10/24/2023	PROTOCOL	TOX003
PROJECT	Toxicity Testing	TEST START DATE	10/24/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall 1	TEST END DATE	11/1/23	SPECIES	Ceriodaphnia dubia
LAB SAMPLE ID	P231024.01	MATRIX	Liquid	NO. OF ORGANISMS	1

7 Day Chronic Toxicity with Cerio

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)	Diluent Type	CRFW
0	0.0%	0.0	200.0	200		
	6.25%	12.5	187.5	200		
	12.5%	25.0	175.0	200.0		
	25.0%	50.0	150.0	200.0		
	50.0%	100.0	100.0	200.0		
	100.0%	200.0	0.0	200.0		

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)
1 - 6	0.0%	0.0	200.0	200
	6.25%	12.5	187.5	200
	12.5%	25.0	175.0	200
	25.0%	50.0	150.0	200
	50.0%	100.0	100.0	200
	100.0%	200.0	0.0	200

Test Dilution Prep

Date	Balance ID	Sample ID (P#)	Water Batch ID	Initials
10/24/23	#7	P231024.01	CRFW102023.02	JL
10/25/23	7	P231024.01	CRFW102023.01	JL
10/26	7	P231026.01	CRFW102023.01	JL
10/27	7	P231026.04	CRFW102023.02	NL
10.28.23	7	P231026.04	CRFW102023.03	MATCH
10/29/23	7	P231028.01	CRFW102023.03	NL
10/30/23	7	P231028.03	CRFW102023.03	TW

① P231024.01. JL 10/26

10/31/23 7 P231028.03 CRFW102023.04 LG

Ceriodaphnia dubia Mean reproduction per Initial Female

Chronic Power Standard Calculation

	average reproduction/initial count										
Replicate	1	2	3	4	5	6	7	8	9	10	Mean
CCEC (100)	17	19	12	13	16	15	5	19	17	17	15
Control	14	16	24		12	0	27	19	19	16	16.3

Control Mean - CCEC Mean

1.33333333

Difference Divided by Control Mean

0.08163265

Express as %

8%

≤39% meets the power standard

Pass

ORGANISM RECEIPT LOG

Date: 10/24/23	Time: 1200	Batch No. ABS102423.02
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Organism:
Ceriodaphnia dubia

Source / Supplier:
Aquatic Biosystems

No. Ordered: —	No. Received: 80	Source Batch: Collection date, hatch date, etc.): 10/23/23
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Condition of Organisms: Good	Approximate Size or Age: (Days from hatch, life stage, size class, etc.): Neonates L24 hrs
--	---

Shipper: UPS	B of L (Tracking No.): 1Z F46 73R 01 9635 2168
------------------------	--

Condition of Container: Good	Received By: DM
--	---------------------------

Container	D.O. (mg/L)	Temp. (°C)	Cond. or Sal. (Include Units)	pH (Units)	# Dead	% Dead*	Tech. (Initials)
1	9.2	20.0	526 μ S/cm	8.0	—	—	DM/MS

*if >10% contact lab manager

Notes:

1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524



Toll Free: 800/331-5916
Tel: 970/484-5091 Fax: 970/484-2514

ORGANISM HISTORY

DATE: 10/23/2023

SPECIES: Ceriodaphnia dubia

AGE: < 24 hour

LIFE STAGE: Neonate

HATCH DATE: 10/23/2023

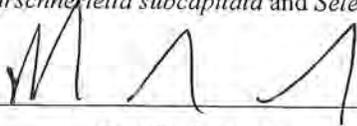
BEGAN FEEDING: Immediately

FOOD: YTC, Raphidocelis subcapitata*

Water Chemistry Record:

	Current	Range
TEMPERATURE:	<u>25°C</u>	<u>22-25°C</u>
SALINITY/CONDUCTIVITY:	<u>--</u>	<u>--</u>
TOTAL HARDNESS (as CaCO ₃):	<u>116 mg/l</u>	<u>80-120 mg/l</u>
TOTAL ALKALINITY (as CaCO ₃):	<u>105 mg/l</u>	<u>80-105 mg/l</u>
pH:	<u>7.77</u>	<u>7.70-8.20</u>

Comments: * Formerly known as *Psuedokirschneriella subcapitata* and *Selenastrum capricornutum*



Facility Supervisor

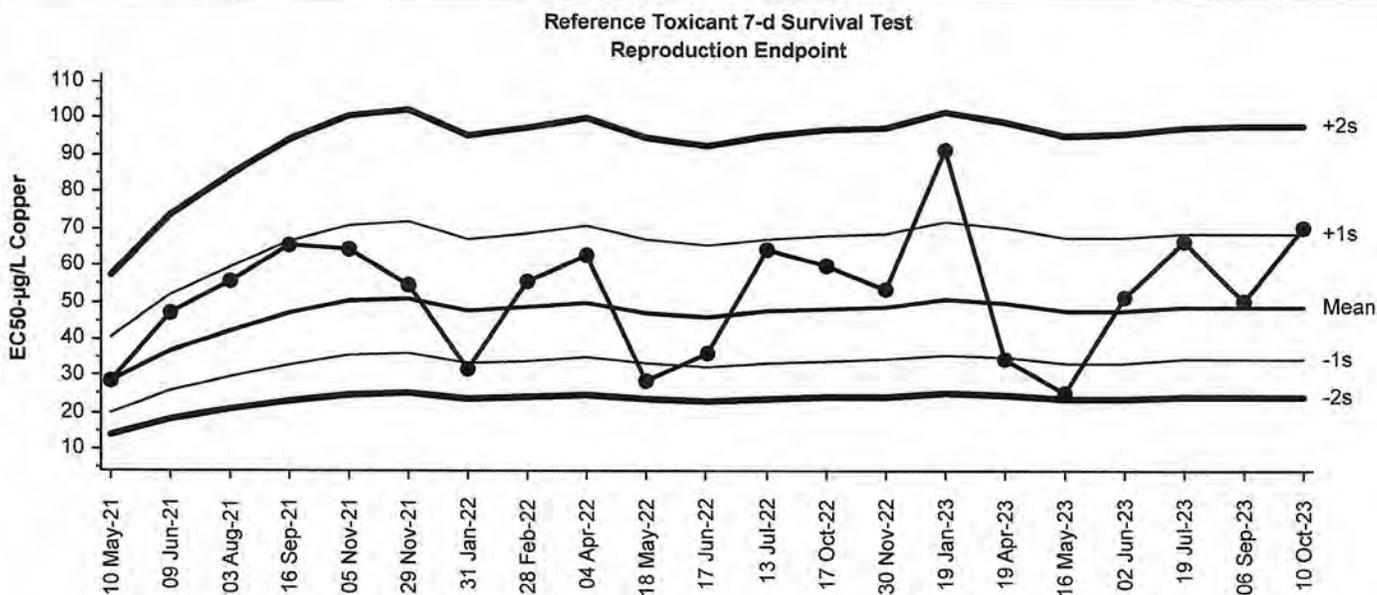
Reference Toxicant 7-d Survival Test

All Matching Labs

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

Organism: Ceriodaphnia dubia
Endpoint: Reproduction

Material: Copper
Source: Reference Toxicant-REF



Lognormal Cumulative Mean Plot

Mean: 48.72 Count: 20 -1s Warning Limit: 34.4 -2s Action Limit: 24.3
Sigma: NA CV: 35.90% +1s Warning Limit: 69 +2s Action Limit: 97.8

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2021	May	10	14:54	28.48	-20.24	-1.542	(-)		05-0048-4186	00-0988-1712	EcoAnalysts
2		Jun	9	8:20	46.95	-1.767	-0.1061			16-0582-4904	11-8804-9752	EcoAnalysts
3		Aug	3	15:16	55.94	7.223	0.3969			14-0345-0880	00-3961-3520	EcoAnalysts
4		Sep	16	15:38	65.57	16.85	0.853			17-5539-1702	06-4636-2651	EcoAnalysts
5		Nov	5	10:02	64.68	15.97	0.814			18-9584-4855	01-0859-7893	EcoAnalysts
6			29	14:02	54.88	6.163	0.342			20-9449-0952	12-3955-6346	EcoAnalysts
7	2022	Jan	31	14:16	31.6	-17.12	-1.243	(-)		20-2038-4587	04-2617-4620	EcoAnalysts
8		Feb	28	15:03	55.95	7.238	0.3977			18-3184-6460	00-7600-1755	EcoAnalysts
9		Apr	4	13:48	62.6	13.89	0.7201			11-4943-3707	14-5417-9325	EcoAnalysts
10		May	18	15:23	28.74	-19.98	-1.515	(-)		13-6332-6879	12-5887-6740	EcoAnalysts
11		Jun	17	9:00	36.21	-12.5	-0.8515			02-6779-6377	20-5319-6315	EcoAnalysts
12		Jul	13	8:11	64.42	15.7	0.8022			02-9880-3401	06-0663-0954	EcoAnalysts
13		Oct	17	15:23	59.94	11.22	0.5951			19-1787-8342	06-4585-6726	EcoAnalysts
14		Nov	30	15:16	53.5	4.787	0.2691			08-2048-3363	03-2308-5694	EcoAnalysts
15	2023	Jan	19	16:13	91.79	43.08	1.819	(+)		08-0633-0446	08-7830-6606	EcoAnalysts
16		Apr	19	15:50	34.43	-14.28	-0.9963			11-9575-2681	18-5912-3879	EcoAnalysts
17		May	16	14:20	25.36	-23.36	-1.875	(-)		14-9075-7583	06-1067-3008	EcoAnalysts
18		Jun	2	15:14	51.13	2.413	0.1388			04-5731-3549	13-0172-9868	EcoAnalysts
19		Jul	19	16:07	66.34	17.63	0.8866			06-9500-3423	19-3324-7583	EcoAnalysts
20		Sep	6	14:30	50.58	1.862	0.1077			03-3149-1934	10-4654-8842	EcoAnalysts
21		Oct	10	10:11	70.19	21.47	1.048	(+)		19-2038-7631	18-0570-8714	EcoAnalysts

CETIS Summary Report

Report Date: 05 Dec-23 13:54 (p 1 of 2)
 Test Code/ID: P220110.118 / 19-2038-7631

Reference Toxicant 7-d Survival Test

EcoAnalysts

Batch ID: 10-7702-7739	Test Type: Reproduction-Survival (7d)	Analyst: Julia Levengood
Start Date: 10 Oct-23 10:11	Protocol: EPA/821/R-02-013 (2002)	Diluent: Cerio Reconstituted Fresh Water
Ending Date: 17 Oct-23 10:31	Species: Ceriodaphnia dubia	Brine: Not Applicable
Test Length: 7d 0h	Taxon: Branchiopoda	Source: In-House Culture
		Age: <1d
Sample ID: 06-0637-0618	Code: P220110.118	Project: Reference Toxicant
Sample Date: 10 Jan-22	Material: Copper	Source: Reference Toxicant
Receipt Date: 10 Jan-22	CAS (PC):	Station: P220110.118
Sample Age: 638d 10h	Client: Internal Lab	

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
09-1698-4794	7d Proportion Survived	Fisher Exact/Bonferroni-Holm Test	72	144	101.8	---	1
06-8743-4057	Reproduction	Dunnett Multiple Comparison Test	✓ 36	72	50.91	29.3%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
00-3846-5749	7d Proportion Survived	Trimmed Spearman-Kärber	EC50	82.5	63.5	107.2	1
19-6530-6486	Reproduction	Linear Interpolation (ICPIN)	IC15	36.75	3.968	43.8	1
			IC20	40.33	7.477	48.23	
			IC25	44.25	17.75	54.85	
			IC40	58.39	43.97	78.42	
			✓ IC50	70.19	51.83	86.8	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits			Overlap	Decision
				Lower	Upper			
00-3846-5749	7d Proportion Survived	Control Resp	1	0.8	<<	Yes	Passes Criteria	
09-1698-4794	7d Proportion Survived	Control Resp	1	0.8	<<	Yes	Passes Criteria	
06-8743-4057	Reproduction	Control Resp	28.8	15	<<	Yes	Passes Criteria	
19-6530-6486	Reproduction	Control Resp	28.8	15	<<	Yes	Passes Criteria	
06-8743-4057	Reproduction	PMSD	0.293	0.13	0.47	Yes	Passes Criteria	

7d Proportion Survived Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	10	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
9		10	0.9000	0.6738	1.1260	0.0000	1.0000	0.1000	0.3162	35.14%	10.00%
18		10	0.9000	0.6738	1.1260	0.0000	1.0000	0.1000	0.3162	35.14%	10.00%
36		10	0.9000	0.6738	1.1260	0.0000	1.0000	0.1000	0.3162	35.14%	10.00%
72		10	0.7000	0.3544	1.0460	0.0000	1.0000	0.1528	0.4830	69.01%	30.00%
144		10	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

Reproduction Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	10	28.8	23.1	34.5	18	38	2.52	7.969	27.67%	0.00%
9		10	27	19.98	34.02	8	40	3.102	9.809	36.33%	6.25%
18		10	25.7	20.56	30.84	12	31	2.271	7.181	27.94%	10.76%
36		10	24.8	20.6	29	16	31	1.855	5.865	23.65%	13.89%
72		10	14	6.34	21.66	0	32	3.386	10.71	76.49%	51.39%
144		10	0	0	0	0	0	0	0	---	100.00%

CETIS Summary Report

Report Date: 05 Dec-23 13:54 (p 2 of 2)
 Test Code/ID: P220110.118 / 19-2038-7631

Reference Toxicant 7-d Survival Test

EcoAnalysts

7d Proportion Survived Detail

MD5: 3C87CADAAAFCAF187A6EC2389BCCF7

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	D	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
9		1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000
18		1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
36		1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000
72		1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	0.0000	1.0000	0.0000
144		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Reproduction Detail

MD5: 45696C0547A7E456892B256EF09E0229

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	D	18	20	20	21	38	34	32	34	35	36
9		18	34	40	31	30	8	35	31	17	26
18		16	19	31	12	31	31	29	30	30	28
36		17	19	26	23	29	16	31	25	31	31
72		13	18	10	26	32	0	23	0	7	11
144		0	0	0	0	0	0	0	0	0	0

7d Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	D	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
9		1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1
18		1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1
36		1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1
72		1/1	1/1	1/1	1/1	1/1	0/1	1/1	0/1	1/1	0/1
144		0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1

CETIS Test Data Worksheet

Report Date: 05 Dec-23 13:43 (p 1 of 2)
 Test Code/ID: P220110.118 / 19-2038-7631

Reference Toxicant 7-d Survival Test EcoAnalysts

Start Date: 10 Oct-23 10:11 Species: Ceriodaphnia dubia Sample Code: P220110.118
 End Date: 17 Oct-23 10:31 Protocol: EPA/821/R-02-013 (2002) Sample Source: Reference Toxicant
 Sample Date: 10 Jan-22 Material: Copper Sample Station: P220110.118

Conc-µg/L	Code	Rep	Pos	# Exposed	1d Survival	2d Survival	3d Survival	4d Survival	5d Survival	6d Survival	7d Survival	Neonates	Male	Notes
0	D	1	19	1							1	18	0	
0	D	2	2	1							1	20	0	
0	D	3	48	1							1	20	0	
0	D	4	10	1							1	21	0	
0	D	5	45	1							1	38	0	
0	D	6	55	1							1	34	0	
0	D	7	33	1							1	32	0	
0	D	8	34	1							1	34	0	
0	D	9	43	1							1	35	0	
0	D	10	59	1							1	36	0	
9		1	8	1							1	18	0	
9		2	5	1							1	34	0	
9		3	9	1							1	40	0	
9		4	40	1							1	31	0	
9		5	16	1							1	30	0	
9		6	18	1							0	8	0	
9		7	46	1							1	35	0	
9		8	47	1							1	31	0	
9		9	20	1							1	17	0	
9		10	32	1							1	26	0	
18		1	36	1							1	16	0	
18		2	14	1							1	19	0	
18		3	52	1							1	31	0	
18		4	50	1							0	12	0	
18		5	24	1							1	31	0	
18		6	13	1							1	31	0	
18		7	6	1							1	29	0	
18		8	57	1							1	30	0	
18		9	7	1							1	30	0	
18		10	21	1							1	28	0	
36		1	41	1							1	17	0	
36		2	23	1							1	19	0	
36		3	37	1							1	26	0	
36		4	1	1							1	23	0	
36		5	35	1							1	29	0	
36		6	22	1							0	16	0	
36		7	39	1							1	31	0	
36		8	30	1							1	25	0	
36		9	28	1							1	31	0	
36		10	29	1							1	31	0	

CETIS Test Data Worksheet

Report Date: 05 Dec-23 13:43 (p 2 of 2)
 Test Code/ID: P220110.118 / 19-2038-7631

Conc-µg/L	Code	Rep	Pos	# Exposed	1d Survival	2d Survival	3d Survival	4d Survival	5d Survival	6d Survival	7d Survival	Neonates	Male	Notes
72		1	49	1							1	13	0	
72		2	44	1							1	18	0	
72		3	58	1							1	10	0	
72		4	27	1							1	26	0	
72		5	3	1							1	32	0	
72		6	15	1							0	0	0	
72		7	38	1							1	23	0	
72		8	31	1							0	0	0	
72		9	60	1							1	7	0	
72		10	53	1							0	11	0	
144		1	26	1							0	0	0	
144		2	54	1							0	0	0	
144		3	11	1							0	0	0	
144		4	51	1							0	0	0	
144		5	42	1							0	0	0	
144		6	17	1							0	0	0	
144		7	12	1							0	0	0	
144		8	4	1							0	0	0	
144		9	25	1							0	0	0	
144		10	56	1							0	0	0	

7 Day Chronic Survival and Reproduction Test

Toxicant:	Copper Chloride
Ref Tox ID:	3220110.110
Lot #:	MK43155
Protocol:	TOX003
Replicates:	10

Date Test Started:	10/10/23
Date Test Ended:	10/17/23
Matrix:	Liquid
Species:	Ceriodaphnia dubia
No. of Org. per Chamber:	1

	Conc. (ppb)	Meter #:	DO (mg/L) (>4.0)	Meter #:	Temp (°C) (25±1°C)	Meter #:	Conductivity (µS/cm)	Meter #:	pH (6 - 9)	
Day 0 (Stock)	Control	9K	7.7	9/15	23.8	9	343	9	8.1	
Date: 10/10/23	9		8.0		24.1		332		8.0	
Time: 1000	18		7.9		24.2		355		8.1	
Technician: DM/LG	36		8.0		24.1		350		8.1	
Feed: LG (0.1ml Selenastrum, 0.1ml YCT/chamber)	72		7.5		23.5		346		8.0	
	144		7.9		23.7		331		8.1	
	Day 1		Day 2		Day 3		Day 4		Day 5	Day 6
Temperature (OLD)	24.3		24.6		24.3		24.3		24.2	24.4
Temperature (NEW)	23.6		23.5		23.8		23.6		23.9	23.8
Feeding: 0.1ml Selenastrum, 0.1ml YCT/chamber	J1		J1		SR		SR		LG	LG
Day 7	Control	8	8.2	8	23.9	8	358	8	8.0	
Date: 10/17/23	9		8.3	8	23.9		343		8.0	
Time: 1008	18		8.2	8	23.9		342		8.0	
Surrogate	36		8.2	8	23.9		344		8.0	
Technician: LG	72		8.1	8	23.9		345		8.0	
	144		-	8	23.4		-		-	

Dilution Preparation (Serial dilute by 50%)

CuCl ₂ Stock Solution:	Target Stock Solution Conc.	Volume of Stock Solution	Amt. of Toxicant
400,000 µg Cu/L	144 ppb	500 mL	0.180 mL
400,000 µg Cu/L	72 ppb	500 mL	0.090 mL

Day	Date/Init.	Water Batch	Highest Conc.	Day	Date/Init.	Water Batch	Highest Conc.
0	10/10 DM	CRFW101223.02	144 ppb	4	10/24/23 SR	CRFW101223.02	72 ppb
1	10/11 J1	CRFW092923.02	144 ppb	5	10/15 LG	CRFW101223.02	72 ppb
2	10/12 J1	CRFW092923.02	72 ppb	6	10/16 LG	CRFW101223.02	72 ppb
3	10/13/23 SR	CRFW101223.02	72 ppb				

Start Time:	1011 LG
End Time:	077 1031 LG
Test Acceptability:	>80% survival in control >60% of surviving adults have 3 broods or more Mean neonates per surviving adults ≥15
Light Range (ft-c)	77-95 fc

Test Location:	TEST ROOM 1
Dilution Water Batch:	CRFW092923.02
Supplier:	IN HOUSE CULTURE
Organism Batch:	BBG100323.02
Age:	<1 day
Chamber Size/Type:	30 mL
Exposure Volume:	15 mL

① IE-DM-10/10/23, J1 10/12/23, SR 10/23/23

② WL-LG 10/10

③ Temp blank used - LG 10/17

④ No dilution of this concentration

Survival Data

T.1

Control												
Day/Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10	No. of Adults	Initials
1/												
2/												
3/				3	5			5		4		
4/	5	5	7			6	6		5			
5/	13	15	13	2	12	12	12	11	15	11		
6/	0			16	21	16	14	18	15	21		
7/												
8/												
Totals	18	20	20	21	38	34	<u>32</u>	<u>34</u>	35	36		
Concentration: <u>9 ppb</u>												
Day/Date	Rep 11	Rep 12	Rep 13	Rep 14	Rep 15	Rep 16	Rep 17	Rep 18	Rep 19	Rep 20	No. of Adults	Initials
1/												
2/												
3/				2			3	4		1		
4/	5	4	5			4			5			
5/	13	14	14	10	13	4	13	11	12	10		
6/		16	21	19	17		19	16		15		
7/												
8/												
Totals	18	34	40	31	30	8	<u>35</u>	<u>31</u>	17	26		
Concentration: <u>18 ppb</u>												
Day/Date	Rep 21	Rep 22	Rep 23	Rep 24	Rep 25	Rep 26	Rep 27	Rep 28	Rep 29	Rep 30	No. of Adults	Initials
1/												
2/												
3/				5	3	2						
4/	3	6	5		1				5			
5/	13	13	10	7	10	13	11	10	12	10		
6/			16		17	16	18	20	13	18		
7/												
8/												
Totals	16	19	31	12	31	31	29	30	30	28		

A = Accident, animal removed from testing

D = Dead body found

E = Empty, no body found

x = 4th brood

TEST ACCEPTABILITY:

____ 80% survival in controls

____ 60% controls have 3 broods

____ Average 15 neonates in controls

Survival Data

T.1

Concentration: 36 ppb												
Day/Date	Rep 31	Rep 32	Rep 33	Rep 34	Rep 35	Rep 36	Rep 37	Rep 38	Rep 39	Rep 40	No. of Adults	Initials
1/												
2/												
3/				3	2		6			2		
4/	5	4	4			4			5			
5/	12	15	11	6	11	12	12	9	13	10		
6/			11	14	16		13	16	13	19		
7/												
8/												
Totals	17	19	26	23	29	16	31	25	31	31		

Concentration: 72 ppb												
Day/Date	Rep 41	Rep 42	Rep 43	Rep 44	Rep 45	Rep 46	Rep 47	Rep 48	Rep 49	Rep 50	No. of Adults	Initials
1/												
2/												
3/				4	4							
4/	3	5	3				4		2			
5/	10	13	7	9	12		8		5	11		
6/				13	16		11					
7/												
8/												
Totals	13	18	10	26	32	0	23	0	7	11		

Concentration: 144 ppb												
Day/Date	Rep 51	Rep 52	Rep 53	Rep 54	Rep 55	Rep 56	Rep 57	Rep 58	Rep 59	Rep 60	No. of Adults	Initials
1/												
2/												
3/												
4/												
5/												
6/												
7/												
8/												
Totals	0	0	0	0	0	0	0	0	0	0		

A = Accident, animal removed from testing D = Dead body found E = Empty, no body found x = 4th brood

TEST ACCEPTABILITY:

_____ 80% survival in controls _____ 60% controls have 3 broods _____ Average 15 neonates in controls

7 Day Chronic Survival and Reproduction Test

Toxicant: Copper Chloride
 Ref Tox ID: P22010:119

Date Test Started: 10/11/23
 Date Test Ended: 10/17/23

Species: Ceriodaphnia dubia

Control												
Day/Date	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	# of Adults	Tech
1/ 10/11/23	0	0	0	0	0	0	0	0	0	0	10	J1
2/ 10/12/23	0	0	0	0	0	0	0	0	0	0	10	J1
3/ 10/13/23	0	0	0	3	5	0	0	5	0	4	20	SR
4/ 10/14/23	5	5	7	0	0	6	6	0	5	0	20	SR
5/ 10/15	13	15	13	2	12	12	12	11	15	11	70	LG
6/ 10/16	0	0	0	16	21	16	14	18	15	21	10	LG
7/ 10/17	②	②	②	②	0	0	0	0	0	②	10	LG
8/												
Checked for Male (final day)												
Reproduction Total												

9 ppb												
Day/Date	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	# of Adults	Tech
1/ 10/11/23	0	0	0	0	0	0	0	0	0	0	10	J1
2/ 10/12/23	0	0	0	0	0	0	0	0	0	0	10	J1
3/ 10/13/23	0	0	0	2	0	0	3	4	0	2	20	SR
4/ 10/14/23	5	4	5	0	0	4	0	0	5	0	20	SR
5/ 10/15	13	14	14	10	13	4	13	11	12	10	10	LG
6/ 10/16	0	16	21	19	17	0, 0	19	16	0	15	9	LG
7/ 10/17	②	0	0	②	0	/	0	0	②	0	9	LG
8/												
Checked for Male (final day)												
Reproduction Total												

18 ppb												
Day/Date	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	# of Adults	Tech
1/ 10/11/23	0	0	0	0	0	0	0	0	0	0	10	J1
2/ 10/12/23	0	0	0	0	0	0	0	0	0	0	10	J1
3/ 10/13/23	0	0	0	5	3	2	0	0	0	0	20	SR
4/ 10/14/23	3	6	5	0	2	0	0	0	5	0	20	SR
5/ 10/15	13	13	10	0, 7	10, 2	13	11	10	12	10	9	LG
6/ 10/16	0	0	16	/	17	16, 3	18	20	13	18	9	LG
7/ 10/17	②	②	0	/	0	0	0	0	0	0	9	LG
8/												
Checked for Male (final day)												
Reproduction Total												

① DN = dead neonate - LG 10/15, LG 10/16
 ② Cerio met reproduction requirement on day 6 - LG 10/17
 05/14/15 Cerio Chronic CuCl RT

7 Day Chronic Survival and Reproduction Test

Toxicant: Copper Chloride
 Ref Tox ID: P220110.119

Date Test Started: 10/10/23
 Date Test Ended: 10/17/23

Species: Ceriodaphnia dubia

36 ppb

Day/Date	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	# of Adults	Tech
1/ 10/11/23	0	0	0	0	0	0	0	0	0	0	10	J1
2/ 10/12/23	0	0	0	0	0	0	0	0	0	0	10	J1
3/ 10/13/23	0	0	0	3	2	0	6	0	0	2	10	SR
4/ 10/14/23	5	4	4	0	0	4	0	0	5	0	10	SR
5/ 10/15	12	15	11	6	11	12	12	9	13	10	10	LG
6/ 10/16	0	0	11	14	16	0	13	16	13	19	10	LG
7/ 10/17	②	②	0	0	0	D②	0	0	0	0	9	LG
8/												
Checked for Male (final day)												
Reproduction Total												

72 ppb

Day/Date	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	# of Adults	Tech
1/ 10/11/23	0	0	0	0	0	0	0	D,0	0	0	9	J1
2/ 10/12/23	0	0	0	0	0	0,0	0		0	0	8	J1
3/ 10/13/23	0	0	0	4	4		0		0	0	8	SR
4/ 10/14/23	3	5	3	0	0		4		2	0	8	SR
5/ 10/15	10	13	7	9, DN	12		8		5	0, 11	7	LG
6/ 10/16	0	0	0	13, DN	16		11		0		7	LG
7/ 10/17	②	②	②	②	②		0		②		7	LG
8/												
Checked for Male (final day)												
Reproduction Total												

144 ppb

Day/Date	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	# of Adults	Tech
1/ 10/11/23	D,0	P,0	P,0	P,0	D,0	P,0	D,0	D,0	P,0	D,0	0	J1
2/ 10/12/23											0	SR
3/												
4/												
5/												
6/												
7/												
8/												
Checked for Male (final day)												
Reproduction Total												

① DN = dead neonate, LG 10/15, LG 10/16

APPENDIX A.5

***PIMEPHALES PROMELAS* (FATHEAD MINNOW) 7-DAY SURVIVAL AND GROWTH TEST**

STATISTICAL COMPARISONS AND LABORATORY DATA SHEETS

CETIS Summary Report

Report Date: 27 Nov-23 14:47 (p 1 of 3)
 Test Code/ID: P231024.01P.p. / 02-2576-0011

Fathead Minnow 7-d Larval Survival and Growth Test

EcoAnalysts

Batch ID: 05-1019-1604	Test Type: Growth-Survival (7d)	Analyst: Marisa Seibert
Start Date: 24 Oct-23 16:54	Protocol: EPA/821/R-02-013 (2002)	Diluent: Reconstituted Water
Ending Date: 31 Oct-23 15:21	Species: Pimephales promelas	Brine: Not Applicable
Test Length: 6d 22h	Taxon: Actinopterygii	Source: Aquatic Biosystems, CO Age: 1d
Sample ID: 14-2841-4560	Code: P231024.01P.p.	Project: Toxicity Testing
Sample Date: 23 Oct-23 14:30	Material: Stormwater	Source: Brooks Manufacturing Co. (WA003080)
Receipt Date: 24 Oct-23 10:30	CAS (PC):	Station: Outfall 1
Sample Age: 26h	Client: Brooks Manufacturing Co.	

Multiple Comparison Summary

α 0.05

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	TU	S
11-6107-8441	7d Proportion Survived	Steel Many-One Rank Sum Test	100	>100	---	4.57%	1	1
15-7035-4896	Mean Dry Biomass-mg	Dunnett Multiple Comparison Test	✓ <6.25	6.25	---	10.7%	>16	1
12-8911-3604	Mean Dry Weight-mg	Dunnett Multiple Comparison Test	✓ <6.25	6.25	---	10.4%	>16	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	%	95% LCL	95% UCL	TU	S
20-5279-5685	7d Proportion Survived	Linear Interpolation (ICPIN)	EC15	>100	---	---	<1	1
			EC20	>100	---	---	<1	
			✓ EC25	>100	---	---	<1	
			✓ EC40	>100	---	---	<1	
			✓ EC50	>100	---	---	<1	
03-8304-5531	Mean Dry Biomass-mg	Linear Interpolation (ICPIN)	✓ IC15	4.686	0.8091	---	21.3	1
			✓ IC20	11.81	---	---	8.5	
			✓ IC25	>100	---	---	<1	
			✓ IC40	>100	---	---	<1	
			✓ IC50	>100	---	---	<1	
18-2002-5218	Mean Dry Weight-mg	Linear Interpolation (ICPIN)	✓ IC15	4.686	1.071	---	21.3	1
			IC20	>100	---	---	<1	
			✓ IC25	>100	---	---	<1	
			✓ IC40	>100	---	---	<1	
			✓ IC50	>100	---	---	<1	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
11-6107-8441	7d Proportion Survived	Control Resp	1	0.8	<<	Yes	Passes Criteria
20-5279-5685	7d Proportion Survived	Control Resp	1	0.8	<<	Yes	Passes Criteria
03-8304-5531	Mean Dry Biomass-mg	Control Resp	1.278	0.25	<<	Yes	Passes Criteria
15-7035-4896	Mean Dry Biomass-mg	Control Resp	1.278	0.25	<<	Yes	Passes Criteria
15-7035-4896	Mean Dry Biomass-mg	PMSD	0.1072	0.12	0.3	Yes	Below Criteria

CETIS Summary Report

Report Date: 27 Nov-23 14:47 (p 2 of 3)
 Test Code/ID: P231024.01P.p. / 02-2576-0011

Fathead Minnow 7-d Larval Survival and Growth Test

EcoAnalysts

7d Proportion Survived Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
6.25		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
12.5		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
25		4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	2.50%
50		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
100		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%

Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.278	1.071	1.485	1.16	1.45	0.06493	0.1299	10.16%	0.00%
6.25		4	1.059	0.9742	1.145	0.982	1.1	0.0268	0.0536	5.06%	17.10%
12.5		4	0.9297	0.8276	1.032	0.834	0.971	0.03209	0.06418	6.90%	27.25%
25		4	1.064	0.9455	1.182	0.992	1.162	0.03714	0.07429	6.98%	16.76%
50		4	1.007	0.8689	1.145	0.917	1.124	0.04332	0.08664	8.61%	21.22%
100		4	1.076	1.004	1.147	1.016	1.118	0.02246	0.04492	4.18%	15.83%

Mean Dry Weight-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.278	1.071	1.485	1.16	1.45	0.06493	0.1299	10.16%	0.00%
6.25		4	1.059	0.9742	1.145	0.982	1.1	0.0268	0.0536	5.06%	17.10%
12.5		4	0.9297	0.8276	1.032	0.834	0.971	0.03209	0.06418	6.90%	27.25%
25		4	1.091	1	1.182	1.024	1.162	0.02865	0.0573	5.25%	14.61%
50		4	1.007	0.8689	1.145	0.917	1.124	0.04332	0.08664	8.61%	21.22%
100		4	1.076	1.004	1.147	1.016	1.118	0.02246	0.04492	4.18%	15.83%

7d Proportion Survived Detail

MD5: 29D08B498513AADB9865C2EF5C0B2E06

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
6.25		1.0000	1.0000	1.0000	1.0000
12.5		1.0000	1.0000	1.0000	1.0000
25		1.0000	0.9000	1.0000	1.0000
50		1.0000	1.0000	1.0000	1.0000
100		1.0000	1.0000	1.0000	1.0000

Mean Dry Biomass-mg Detail

MD5: 7AB0BACE042F3EF370D708C88A3BB319

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.16	1.304	1.45	1.198
6.25		1.066	0.982	1.09	1.1
12.5		0.834	0.971	0.958	0.956
25		1.077	0.992	1.024	1.162
50		0.98	1.124	1.006	0.917
100		1.068	1.101	1.118	1.016

Mean Dry Weight-mg Detail

MD5: BC71F1778E999EFAAD3643D3B0D5D4EA

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.16	1.304	1.45	1.198
6.25		1.066	0.982	1.09	1.1
12.5		0.834	0.971	0.958	0.956
25		1.077	1.102	1.024	1.162
50		0.98	1.124	1.006	0.917
100		1.068	1.101	1.118	1.016

CETIS Summary Report

Report Date: 27 Nov-23 14:47 (p 3 of 3)
Test Code/ID: P231024.01P.p. / 02-2576-0011

Fathead Minnow 7-d Larval Survival and Growth Test

EcoAnalysts

7d Proportion Survived Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	10/10	10/10	10/10	10/10
6.25		10/10	10/10	10/10	10/10
12.5		10/10	10/10	10/10	10/10
25		10/10	9/10	10/10	10/10
50		10/10	10/10	10/10	10/10
100		10/10	10/10	10/10	10/10

CETIS Summary Report

Report Date: 05 Dec-23 12:20 (p 1 of 1)
 Test Code/ID: P231024.01P.p. / 02-2576-0011

Fathead Minnow 7-d Larval Survival and Growth Test

EcoAnalysts

Batch ID: 05-1019-1604	Test Type: Growth-Survival (7d)	Analyst: Marisa Seibert
Start Date: 24 Oct-23 16:54	Protocol: EPA/821/R-02-013 (2002)	Diluent: Reconstituted Water
Ending Date: 31 Oct-23 15:21	Species: Pimephales promelas	Brine: Not Applicable
Test Length: 6d 22h	Taxon: Actinopterygii	Source: Aquatic Biosystems, CO Age: 1d
Sample ID: 14-2841-4560	Code: P231024.01P.p.	Project: Toxicity Testing
Sample Date: 23 Oct-23 14:30	Material: Stormwater	Source: Brooks Manufacturing Co. (WA003080)
Receipt Date: 24 Oct-23 10:30	CAS (PC):	Station: Outfall 1
Sample Age: 26h	Client: Brooks Manufacturing Co.	

Multiple Comparison Summary

$\alpha = 0.01$

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	TU	S
16-9299-4623	Mean Dry Biomass-mg	Dunnett Multiple Comparison Test	✓	<6.25	6.25	---	14.3%	>16	1
09-0124-6770	Mean Dry Weight-mg	Dunnett Multiple Comparison Test	✓	<6.25	6.25	---	13.9%	>16	1

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
16-9299-4623	Mean Dry Biomass-mg	Control Resp	1.278	0.25	<<	Yes	Passes Criteria
16-9299-4623	Mean Dry Biomass-mg	PMSD	0.1428	0.12	0.3	Yes	Passes Criteria

Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.278	1.071	1.485	1.16	1.45	0.06493	0.1299	10.16%	0.00%
6.25		4	1.059	0.9742	1.145	0.982	1.1	0.0268	0.0536	5.06%	17.10%
12.5		4	0.9297	0.8276	1.032	0.834	0.971	0.03209	0.06418	6.90%	27.25%
25		4	1.064	0.9455	1.182	0.992	1.162	0.03714	0.07429	6.98%	16.76%
50		4	1.007	0.8689	1.145	0.917	1.124	0.04332	0.08664	8.61%	21.22%
100		4	1.076	1.004	1.147	1.016	1.118	0.02246	0.04492	4.18%	15.83%

Mean Dry Weight-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.278	1.071	1.485	1.16	1.45	0.06493	0.1299	10.16%	0.00%
6.25		4	1.059	0.9742	1.145	0.982	1.1	0.0268	0.0536	5.06%	17.10%
12.5		4	0.9297	0.8276	1.032	0.834	0.971	0.03209	0.06418	6.90%	27.25%
25		4	1.091	1	1.182	1.024	1.162	0.02865	0.0573	5.25%	14.61%
50		4	1.007	0.8689	1.145	0.917	1.124	0.04332	0.08664	8.61%	21.22%
100		4	1.076	1.004	1.147	1.016	1.118	0.02246	0.04492	4.18%	15.83%

Mean Dry Biomass-mg Detail

MD5: 7AB0BACE042F3EF370D708C88A3BB319

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.16	1.304	1.45	1.198
6.25		1.066	0.982	1.09	1.1
12.5		0.834	0.971	0.958	0.956
25		1.077	0.992	1.024	1.162
50		0.98	1.124	1.006	0.917
100		1.068	1.101	1.118	1.016

Mean Dry Weight-mg Detail

MD5: BC71F1778E999EFAAD3643D3B0D5D4EA

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.16	1.304	1.45	1.198
6.25		1.066	0.982	1.09	1.1
12.5		0.834	0.971	0.958	0.956
25		1.077	1.102	1.024	1.162
50		0.98	1.124	1.006	0.917
100		1.068	1.101	1.118	1.016

CETIS Test Data Worksheet

Report Date: 27 Nov-23 14:46 (p 1 of 1)
 Test Code/ID: P231024.01P.p. / 02-2576-0011

Fathead Minnow 7-d Larval Survival and Growth Test										EcoAnalysts	
Start Date:	24 Oct-23 16:54	Species:	Pimephales promelas	Sample Code:	P231024.01P.p.						
End Date:	31 Oct-23 15:21	Protocol:	EPA/821/R-02-013 (2002)	Sample Source:	Brooks Manufacturing Co.						
Sample Date:	23 Oct-23 14:30	Material:	Stormwater	Sample Station:	Outfall 1						

Conc-%	Code	Rep	Pos	# Exposed	1d Survival	2d Survival	3d Survival	4d Survival	5d Survival	6d Survival	7d Survival	Weight-mg Total	Weight-mg Tare	Pan Count	Notes
0	D	1	21	10	10	10	10	10	10	10	10	71.67	60.07	10	
0	D	2	12	10	10	10	10	10	10	10	10	71.39	58.35	10	
0	D	3	16	10	10	10	10	10	10	10	10	77.37	62.87	10	
0	D	4	15	10	10	10	10	10	10	10	10	64.81	52.83	10	
6.25		1	10	10	10	10	10	10	10	10	10	72.02	61.36	10	
6.25		2	8	10	10	10	10	10	10	10	10	58.59	48.77	10	
6.25		3	23	10	10	10	10	10	10	10	10	72.43	61.53	10	
6.25		4	9	10	10	10	10	10	10	10	10	74.15	63.15	10	
12.5		1	7	10	10	10	10	10	10	10	10	62.21	53.87	10	
12.5		2	3	10	10	10	10	10	10	10	10	70.85	61.14	10	
12.5		3	1	10	10	10	10	10	10	10	10	67.94	58.36	10	
12.5		4	19	10	10	10	10	10	10	10	10	71.77	62.21	10	
25		1	5	10	10	10	10	10	10	10	10	73.32	62.55	10	
25		2	18	10	10	10	10	10	10	10	9	55.13	45.21	9	
25		3	14	10	10	10	10	10	10	10	10	70.62	60.38	10	
25		4	17	10	10	10	10	10	10	10	10	74.93	63.31	10	
50		1	2	10	10	10	10	10	10	10	10	69.8	60	10	
50		2	13	10	10	10	10	10	10	10	10	71.63	60.39	10	
50		3	6	10	10	10	10	10	10	10	10	71.72	61.66	10	
50		4	20	10	10	10	10	10	10	10	10	71.79	62.62	10	
100		1	4	10	10	10	10	10	10	10	10	73.22	62.54	10	
100		2	22	10	10	10	10	10	10	10	10	72.74	61.73	10	
100		3	24	10	10	10	10	10	10	10	10	74.18	63	10	
100		4	11	10	10	10	10	10	10	10	10	74.23	64.07	10	

GENERAL

Client	Brooks Manufacturing Co.
Project	Toxicity Testing
Project Number	PG1913
Project Manager	M. Seibert
Date Sample Received	10/24/2023
Test type	7 Day Chronic Toxicity with FHM
Matrix	Liquid
Test Acceptability	≥ 80% average survival of control Average dry weight is ≥ 0.25 mg per surviving organism in control
Test Start Date	10/24/23
Test Species	Pimephales promelas
Organism Batch	ABS102423.04
Organism Acquired	10/24/2023
Organism Acclimation	0
Organism Age	1 day
Test Protocol	Tox 018
Regional Protocol	WDOE WQ-R-95-80
Test Location	Temp Control Room
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Water Description	moderately hard fresh water
Organisms per Replicate	10
Test Chamber Size	20 oz
Exposure Volume	250 mL
Feeding Information	1500 nauplii/chamber twice daily
Test Dissolved Oxygen	> 4.0
Test Temperature	25 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	4.0	
Temp	24	26
Conductivity		
pH	6	9

TEST START TIME/INIT:	1654 RE/mg
TEST END TIME/INIT:	1522 SR

CLIENT SAMPLE ID	LAB ID
Outfall 1	P231024.01
OUTFALL 1	P231024.04
OUTFALL 1	P231028.03

Concentrations	
1	Control
2	6.25%
3	12.5%
4	25.0%
5	50.0%
6	100.0%
7	.
8	.
9	.

Food Batch ID
281729

Copy and Past VALUES

Treatment	Rep	Chamber
Control	1	7
Control	2	1
Control	3	9
Control	4	4
6.25%	1	6
6.25%	2	16
6.25%	3	12
6.25%	4	3
12.5%	1	11
12.5%	2	15
12.5%	3	8
12.5%	4	5
25%	1	17
25%	2	24
25%	3	20
25%	4	22
50%	1	13
50%	2	23
50%	3	10
50%	4	18
100%	1	14
100%	2	2
100%	3	21
100%	4	19
.	1	
.	2	
.	3	

v.4 CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	10/24/23	PROTOCOL	Tox 018
PROJECT	Toxicity Testing	TEST START DATE	10/24/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall 1	TEST END DATE	10/31/23	SPECIES	<i>Pimephales promelas</i>
LAB SAMPLE ID	P231024.01	MATRIX	Liquid	NO. OF ORGANISMS	10

7 Day Chronic Toxicity with FHM

	Concentration (%)	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH
Day 0 Stock Date 10/24/23 Time 1205 Tech JL Meter # 7 Feed 19	Control	8.5	24.0	334	8.0
	6.25%	8.4	24.6	360	7.9
	12.5%	8.6	24.0	386	7.8
	25%	9.0	24.3	439	7.7
	50%	9.6	24.0	544	7.4
	100%	11.3	24.0	759	7.1
	Day 1 Rep 1 Date 10/25/23 Time 1055 Tech DM Meter # 7	Control	6.1	24.0	367
6.25%		① 7.0 6.1	24.0	385	7.6
12.5%		6.1	24.1	412	7.5
25%		6.2	24.0	463	7.5
50%		6.3	24.0	565	7.5
100%		6.4	24.1	769	7.4
Day 1 Renewal Stock Date 10/25/23 Time 0110 Tech J1 Meter # 7		Control	8.2	24.1	340
	6.25%	8.4	24.2	361	8.1
	12.5%	8.4	24.4	369	8.0
	25%	8.7	24.4	437	7.8
	50%	9.3	24.3	537	7.6
	100%	10.2	24.8	741	7.2
	Day 2 Rep 2 Date 10/26 Time 1130 Tech JL Meter # 9	Control	4.5	24.6	359
6.25%		4.9	24.8	390	7.5
12.5%		4.9	24.9	413	7.4
25%		5.0	24.9	460	7.4
50%		5.2	24.7	566	7.4
100%		5.0	24.9	760	7.3
Day 2 Renewal Stock Date 10/26 Time 1145 Tech JL Meter # 9		Control	8.1	24.0	332
	6.25%	8.3	24.0	360	7.9
	12.5%	8.4	24.0	387	7.9
	25%	8.6	24.0	438	7.8
	50%	8.9	24.0	541	7.7
	100%	9.4	24.0	745	7.5

① MR-DM-10/24/23
② IE-DM-10/25/23

CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	10/24/23	PROTOCOL	Tox 018
PROJECT	Toxicity Testing	TEST START DATE	10/24/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall 1	TEST END DATE	10/31/23	SPECIES	<i>Pimephales promelas</i>
LAB SAMPLE ID	P231024.01	MATRIX	Liquid	NO. OF ORGANISMS	10

7 Day Chronic Toxicity with FHM

	Concentration (%)	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH
Day 3	Control	5.0	24.8	372	6.9
Rep 3	6.25%	5.0	24.8	395	7.2
Date 10/27/23	12.5%	4.9	24.9	417	7.3
Time 11:37	25%	5.2	24.8	466	7.3
Tech J1	50%	4.4	24.9	567	7.3
Meter # 9	100%	4.8	24.9	776	7.3
Day 3	Control	8.3	24.2	335	7.7
Renewal Stock	6.25%	8.4	24.2	326	7.8
Date 10/27/23	12.5%	8.5	24.2	315	7.8
Time 13:19	25%	8.6	24.0	294	7.8
Tech J1	50%	8.8	24.2	251	7.8
Meter # 9	100%	9.3	24.0	164	7.4
Day 4	Control	5.3	24.9	366	7.6
Rep 4	6.25%	5.2	25.1	355	7.6
Date 10/28/23	12.5%	5.1	25.0	345	7.5
Time 11:32	25%	4.5	24.9	331	7.5
Tech NL	50%	4.7	24.9	296	7.5
Meter # 9	100%	5.4	24.8	215	7.3
Day 4	Control	8.5	25.3	337	8.1
Renewal Stock	6.25%	8.5	25.0	325	8.1
Date 10/28/23	12.5%	8.6	24.5	315	8.1
Time 10:18	25%	8.7	24.5	294	8.0
Tech NL	50%	9.1	25.8	267	7.9
Meter # 9	100%	10.2	24.9	164	7.4
Day 5	Control	5.3	24.4	406	7.5
Rep 1	6.25%	4.5	24.8	387	7.4
Date 10/29/23	12.5%	4.3	24.7	384	7.3
Time 11:52	25%	4.1	24.7	358	7.3
Tech RE	50%	3.7	24.8	328	7.2
Meter # 8	100%	3.5	24.7	224	7.1
Day 5	Control	8.3	24.7	345	8.2
Renewal Stock	6.25%	8.5	24.4	324	8.1
Date 10/29/23	12.5%	8.6	24.6	314	8.1
Time 10:34	25%	9.0	24.1	295	7.9
Tech NL	50%	9.4	24.6	255	7.7
Meter # 8	100%	10.9	24.6	174	7.5

① DO low, added airtlines to all chambers - RE 10/29

CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	10/24/23	PROTOCOL	Tox 018
PROJECT	Toxicity Testing	TEST START DATE	10/24/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall 1	TEST END DATE	10/31/23	SPECIES	<i>Pimephales promelas</i>
LAB SAMPLE ID	P231024.01	MATRIX	Liquid	NO. OF ORGANISMS	10

7 Day Chronic Toxicity with FHM

	Concentration (%)	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH
		> 4.0	24 - 26		6 - 9
Day 6	Control	7.0	24.5	404	7.6
Rep 2	6.25%	7.8	24.9	399	7.8
Date 10/30/23	12.5%	7.7	25.0	381	7.9
Time 1104	25%	7.8	25.0	357	7.9
Tech JW	50%	7.8	24.9	377	7.8
Meter # 8	100%	7.8	25.1	210	8.0
Day 6	Control	8.6	24.8	373	7.8
Renewal Stock	6.25%	8.7	25.0	339	7.8
Date 10/30/23	12.5%	8.9	25.1	329	7.8
Time 1500	25%	9.0	24.6	307	7.8
Tech J1	50%	9.6	25.5	0246 264	7.6
Meter # 7	100%	11.1	24.7	176	7.4
Day 7	Control	8.1	24.9	391	8.0
Rep 3	6.25%	8.0	24.8	373	8.1
Date 10/31/23	12.5%	8.0	24.9	356	8.2
Time 0807	25%	7.9	24.5	341	8.1
Tech J1	50%	8.0	25.0 24.40	296	8.1
Meter # 8	100%	8.0	24.9	214	8.1

① IE J110/30/23

v.4 CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	10/24/23	PROTOCOL	Tox 018
PROJECT	Toxicity Testing	TEST START DATE	10/24/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall 1	TEST END DATE	10/31/23	SPECIES	<i>Pimephales promelas</i>
LAB SAMPLE ID	P231024.01	MATRIX	Liquid	NO. OF ORGANISMS	10

Abbreviation Key:
 NB = No Body
 FB = Found Body
 ST = Stranded

7 Day Chronic Toxicity with FHM

Concentration (%)	REP	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
		Date	Time	Date	Time	Date	Time	Date	Time	Date	Time	Date	Time	Date	Time
		Tech		Tech		Tech		Tech		Tech		Tech		Tech	
Control	1	10/25	1232	10/26	1511	10/27	1630	10/28	1302	10/29	1158	10/30	1728	10/31/23	1522
	2														
	3														
	4														
6.25%	1														
	2														
	3														
	4														
12.5%	1														
	2														
	3														
	4														
25%	1														
	2														
	3														
	4														
50%	1														
	2														
	3														
	4														
100%	1														
	2														
	3														
	4														
Feed (Init.)	AM	UG		JU		UG		NL		TT 0830		TT		NONE	
1500 nauplii/chamber twice daily	PM	NL		DM		NL		NL		NL		MS		NONE	

v.4

CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	10/24/23	PROTOCOL	Tox 018
PROJECT	Toxicity Testing	TEST START DATE	10/24/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall 1	TEST END DATE	10/31/23	SPECIES	<i>Pimephales promelas</i>
LAB SAMPLE ID	P231024.01	MATRIX	Liquid	NO. OF ORGANISMS	10

7 Day Chronic Toxicity with FHM

Concentration (%)	REP	Boat Number	Weight Empty Boat (mg)	Weight Boat & Animals (mg)	Pan Count
Control	1	1	60.07	71.67	10
	2	2	58.35	71.39	10
	3	3	62.87	77.37	10
	4	4	52.83	64.81	10
6.25%	1	5	61.36	72.02	10
	2	6	48.77	58.59	10
	3	7	61.53	72.43	10
	4	8	63.15	74.15	10
12.5%	1	9	53.87	62.21	10
	2	10	61.14	70.85	10
	3	11	58.36	67.94	10
	4	12	62.21	71.77	10
25%	1	13	62.55	73.32	10
	2	14	45.21	55.13	9
	3	15	60.38	70.62	10
	4	16	63.31	74.93	10
50%	1	17	60.00	69.80	10
	2	18	60.39	71.63	10
	3	19	61.66	71.72	10
	4	20	62.62	71.79	10
100%	1	21	62.54	73.22	10
	2	22	61.73	72.74	10
	3	23	63.00	74.18	10
	4	24	64.07	74.23	10

	Oven Event 1	Oven Event 2
Oven ID:	Beelzebub	Beelzebub
Date/Time/Initials In Oven:	10/29/23 1720M	10/31/23 1625 SR
Oven Temp °C:	60	99
Date/Time/Initials Out Oven into:	10/30/23 1825M	11/1/23 1430M
Date/Time/Initials Weighed:	10/31/23 1004RW	11/27/23 1339 TT
Balance ID:	3	3

X1 → 59.53 mg
 X2 → 62.53 mg

v.4 CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	10/24/23	PROTOCOL	Tox 018
PROJECT	Toxicity Testing	TEST START DATE	10/24/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall 1	TEST END DATE	10/31/23	SPECIES	<i>Pimephales promelas</i>
LAB SAMPLE ID	P231024.01	MATRIX	Liquid	NO. OF ORGANISMS	10

7 Day Chronic Toxicity with FHM

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)	Diluent Type	RFW
0	0%	0	1000.0	1000		
	6.25%	62.5	937.5	1000		
	12.5%	125	875.0	1000		
	25%	250	750.0	1000		
	50%	500	500.0	1000		
	100%	1000	0.0	1000		

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)
1 - 6	0%	0	1000.0	1000
	6.25%	62.5	937.5	1000
	12.5%	125	875.0	1000
	25%	250	750.0	1000
	50%	500	500.0	1000
	100%	1000	0.0	1000

Test Dilution Prep

Date	Balance ID	Sample ID (P#)	Water Batch ID	Initials
10/24/23	#7	P231024.01	RFW102023.01	JL
10/25/23	7	P231024.01	RFW102023.01	JL
10/26/23	7	P231024.01	RFW102023.02	JL
10/27/23	7	P231026.04	RFW102023.02	NL
10.28.23	7	P231026.04	RFW102023.02	MACH
10/29/23	7	P231028.03	RFW102023.02	NL
10/30/23	7	P231028.03	RFW102023.01	LG

POWER STANDARD CALCULATIONS

Fathead Minnow Chronic Survival

Chronic Power Standard Calculation

	Growth per survivor				
Replicate	1	2	3	4	Mean
CCEC (100)	1.068	1.101	1.118	1.016	1.07575
Control	1.16	1.304	1.45	1.198	1.278

Control Mean - CCEC Mean

0.20225

Difference Divided by Control Mean

0.1582509

Express as %

16%

≤39% meets the power standard

Pass

Fathead Minnow 7-d Larval Survival and Growth Test

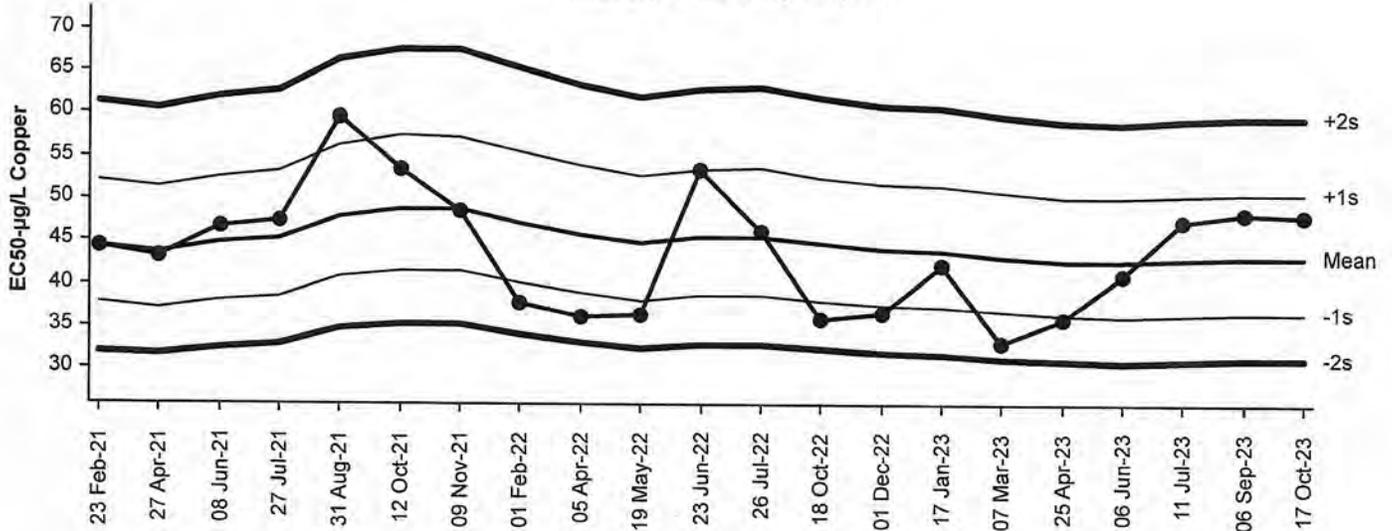
All Matching Labs

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

Organism: Pimephales promelas
 Endpoint: Mean Dry Biomass-mg

Material: Copper
 Source: Reference Toxicant-REF

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



Lognormal Cumulative Mean Plot

Mean: 43.16 Count: 20 -1s Warning Limit: 36.7 -2s Action Limit: 31.2
 Sigma: NA CV: 16.30% +1s Warning Limit: 50.8 +2s Action Limit: 59.7

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2021	Feb	23	16:59	44.35	1.186	0.167			09-3420-3025	06-2566-6215	EcoAnalysts
2		Apr	27	15:40	43.17	0.009584	0.001369			07-0830-8066	03-0136-3973	EcoAnalysts
3		Jun	8	16:01	46.83	3.671	0.5029			03-8671-7305	21-0768-5743	EcoAnalysts
4		Jul	27	17:45	47.32	4.159	0.5668			01-2982-0188	08-4369-4205	EcoAnalysts
5		Aug	31	16:19	59.72	16.55	2	(+)	(+)	09-0380-1980	17-3633-2206	EcoAnalysts
6		Oct	12	16:55	53.44	10.27	1.315	(+)		04-8407-6728	10-0624-0919	EcoAnalysts
7		Nov	9	15:54	48.66	5.5	0.739			01-0755-2793	06-9860-6752	EcoAnalysts
8	2022	Feb	1	16:41	37.85	-5.315	-0.8097			05-6989-9230	14-7100-6767	EcoAnalysts
9		Apr	5	15:41	36.12	-7.046	-1.098	(-)		11-9451-4248	20-9598-0446	EcoAnalysts
10		May	19	15:40	36.38	-6.78	-1.053	(-)		11-3099-9123	08-6488-2174	EcoAnalysts
11		Jun	23	14:06	53.5	10.34	1.323	(+)		02-6119-7414	04-3846-1507	EcoAnalysts
12		Jul	26	16:17	46.32	3.16	0.4354			13-3210-7538	14-2553-7042	EcoAnalysts
13		Oct	18	15:45	35.91	-7.251	-1.133	(-)		07-4167-2780	04-4359-1052	EcoAnalysts
14		Dec	1	14:02	36.7	-6.466	-0.9998			01-0761-7752	11-7849-0980	EcoAnalysts
15	2023	Jan	17	16:25	42.18	-0.9832	-0.142			19-2130-3667	03-0734-5927	EcoAnalysts
16		Mar	7	16:58	33.13	-10.03	-1.63	(-)		20-3366-9862	16-4161-2641	EcoAnalysts
17		Apr	25	16:55	35.83	-7.331	-1.147	(-)		03-4046-6997	02-9036-2213	EcoAnalysts
18		Jun	6	14:58	41.14	-2.027	-0.2964			20-4127-6557	06-2769-6458	EcoAnalysts
19		Jul	11	16:46	47.52	4.352	0.5918			12-4002-8041	14-1656-7576	EcoAnalysts
20		Sep	6	15:40	48.44	5.272	0.71			14-1196-1394	03-3976-6763	EcoAnalysts
21		Oct	17	16:01	48.25	5.086	0.6863			10-3256-1326	00-0837-6578	EcoAnalysts

CETIS Summary Report

Report Date: 14 Nov-23 12:23 (p 1 of 2)
 Test Code/ID: P220110.119 / 10-3256-1326

Fathead Minnow 7-d Larval Survival and Growth Test

EcoAnalysts

Batch ID: 12-4863-6959	Test Type: Survival-Growth	Analyst: Julia Levengood
Start Date: 17 Oct-23 16:01	Protocol: EPA/821/R-02-013 (2002)	Diluent: Reconstituted Water
Ending Date: 24 Oct-23 16:05	Species: Pimephales promelas	Brine: Not Applicable
Test Length: 7d 0h	Taxon: Actinopterygii	Source: Aquatic Biosystems, CO
		Age: 1d
Sample ID: 01-3386-8512	Code: P220110.119	Project: Reference Toxicant
Sample Date: 10 Jan-22	Material: Copper	Source: Reference Toxicant
Receipt Date: 10 Jan-22	CAS (PC):	Station: P220110.119
Sample Age: 645d 16h	Client: Internal Lab	

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
10-8107-7173	Mean Dry Biomass-mg	Steel Many-One Rank Sum Test	✓ 14.0625	28.125	19.89	13.2%	1
10-1141-2705	Mean Dry Weight-mg	Bonferroni Adj t Test	28.125	56.25	39.77	12.0%	1
08-3746-8123	Proportion Survived	Steel Many-One Rank Sum Test	56.25	112.5	79.55	16.8%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
00-0837-6578	Mean Dry Biomass-mg	Linear Interpolation (ICPIN)	✓ IC15	24.65	18.65	32.35	1
			✓ IC20	28.82	21.17	33.44	
			✓ IC25	31.42	25.04	36.19	
			✓ IC40	40.67	32.7	48.24	
			✓ IC50	48.25	36.61	59.27	
18-1497-5252	Mean Dry Weight-mg	Linear Interpolation (ICPIN)	IC15	33.57	28.21	35.77	1
			IC20	35.91	30.84	38.58	
			IC25	38.4	33.58	41.6	
			IC40	46.93	41.18	52.23	
			IC50	53.63	46.38	67.28	
13-7882-4621	Proportion Survived	Trimmed Spearman-Kärber	EC50	94.47	79.93	111.6	1

Mean Dry Biomass-mg Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.101	1.036	1.166	1.064	1.151	0.02034	0.04069	3.70%	0.00%
14.0625		4	1.133	1.04	1.226	1.057	1.192	0.0293	0.0586	5.17%	-2.93%
28.125		4	0.9095	0.7717	1.047	0.783	0.977	0.0433	0.0866	9.52%	17.39%
56.25		4	0.458	0.2108	0.7052	0.225	0.54	0.07769	0.1554	33.93%	58.40%
112.5		4	0.1992	0.06564	0.3329	0.085	0.269	0.04198	0.08397	42.14%	81.90%
225		4	0.00575	-0.01255	0.02405	0	0.023	0.00575	0.0115	200.00%	99.48%

Mean Dry Weight-mg Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.131	1.045	1.216	1.064	1.191	0.02692	0.05385	4.76%	0.00%
14.0625		4	1.133	1.04	1.226	1.057	1.192	0.0293	0.0586	5.17%	-0.22%
28.125		4	1.111	0.9621	1.259	0.977	1.186	0.04673	0.09345	8.41%	1.77%
56.25		4	0.5254	0.3582	0.6926	0.375	0.6	0.05255	0.1051	20.00%	53.54%
112.5		4	0.3877	0.2907	0.4847	0.3133	0.4483	0.03048	0.06095	15.72%	65.71%
225		1	0.23			0.23	0.23	---	---	---	79.66%

Proportion Survived Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
14.0625		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
28.125		4	0.8250	0.6248	1.0250	0.7000	1.0000	0.0629	0.1258	15.25%	17.50%
56.25		4	0.8500	0.5744	1.1260	0.6000	1.0000	0.0866	0.1732	20.38%	15.00%
112.5		4	0.5250	0.1722	0.8778	0.2000	0.7000	0.1109	0.2217	42.24%	47.50%
225		4	0.0250	-0.0546	0.1046	0.0000	0.1000	0.0250	0.0500	200.00%	97.50%

CETIS Summary Report

Report Date: 14 Nov-23 12:23 (p 2 of 2)
 Test Code/ID: P220110.119 / 10-3256-1326

Fathead Minnow 7-d Larval Survival and Growth Test

EcoAnalysts

Mean Dry Biomass-mg Detail

MD5: B32A0E484EF29E067135518696681832

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.072	1.064	1.117	1.151
14.0625		1.121	1.163	1.192	1.057
28.125		0.783	0.949	0.929	0.977
56.25		0.54	0.225	0.536	0.531
112.5		0.269	0.255	0.085	0.188
225		0	0	0.023	0

Mean Dry Weight-mg Detail

MD5: 641B04B25D649C06449AC8884F987CE2

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.191	1.064	1.117	1.151
14.0625		1.121	1.163	1.192	1.057
28.125		1.119	1.186	1.161	0.977
56.25		0.6	0.375	0.5956	0.531
112.5		0.4483	0.3643	0.425	0.3133
225		---	---	0.23	---

Proportion Survived Detail

MD5: 4642F25F31F653A57F8EF393EAD72CFB

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
14.0625		1.0000	1.0000	1.0000	1.0000
28.125		0.7000	0.8000	0.8000	1.0000
56.25		0.9000	0.6000	0.9000	1.0000
112.5		0.6000	0.7000	0.2000	0.6000
225		0.0000	0.0000	0.1000	0.0000

Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	10/10	10/10	10/10	10/10
14.0625		10/10	10/10	10/10	10/10
28.125		7/10	8/10	8/10	10/10
56.25		9/10	6/10	9/10	10/10
112.5		6/10	7/10	2/10	6/10
225		0/10	0/10	1/10	0/10

CETIS Test Data Worksheet

Report Date: 14 Nov-23 12:23 (p 1 of 1)
 Test Code/ID: P220110.119 / 10-3256-1326

Fathead Minnow 7-d Larval Survival and Growth Test

EcoAnalysts

Start Date: 17 Oct-23 16:01 Species: Pimephales promelas Sample Code: P220110.119
 End Date: 24 Oct-23 16:05 Protocol: EPA/821/R-02-013 (2002) Sample Source: Reference Toxicant
 Sample Date: 10 Jan-22 Material: Copper Sample Station: P220110.119

Conc-µg/L	Code	Rep	Pos	# Exposed	# Survived	Weight-mg Total	Weight-mg Tare	Pan Count	Mean Length-mm	Notes
0	D	1	20	10	10	72.32	61.6	9		
0	D	2	6	10	10	63.63	52.99	10		
0	D	3	15	10	10	70.23	59.06	10		
0	D	4	23	10	10	76.13	64.62	10		
14.0625		1	8	10	10	66.8	55.59	10		
14.0625		2	3	10	10	68.51	56.88	10		
14.0625		3	13	10	10	68.71	56.79	10		
14.0625		4	12	10	10	65.63	55.06	10		
28.125		1	14	10	7	60.58	52.75	7		
28.125		2	22	10	8	66.77	57.28	8		
28.125		3	2	10	8	65.8	56.51	8		
28.125		4	9	10	10	60.64	50.87	10		
56.25		1	4	10	9	59.59	54.19	9		
56.25		2	17	10	6	57.22	54.97	6		
56.25		3	5	10	9	60.48	55.12	9		
56.25		4	10	10	10	56.54	51.23	10		
112.5		1	1	10	6	59.12	56.43	6		
112.5		2	18	10	7	49.23	46.68	7		
112.5		3	21	10	2	62.48	61.63	2		
112.5		4	11	10	6	60.54	58.66	6		
225		1	16	10	0	0	0	0		
225		2	7	10	0	0	0	0		
225		3	19	10	1	46.62	46.39	1		
225		4	24	10	0	0	0	0		

7 Day Chronic Survival and Growth Test

Toxicant:	Copper Chloride
Ref Tox ID:	P220110.119

Date Test Started:	10/17/2023
Date Test Ended:	10/24/2023

Species:	<i>Pimephales promelas</i>
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Concentration (µg/L)	Rep	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
		# Alive	# Dead												
Control	1	10	0	10	0	10	0	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0	10	0	10	0	10	0
14.0625	1	10	0	10	0	10	0	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0	10	0	10	0	10	0
28.125	1	10	0	10	0	10	0	9	1NB	9	0	9	0	7	2
	2	10	0	10	0	10	0	10	0	10	0	10	0	8	2
	3	10	0	10	0	10	0	10	0	10	0	10	0	8	2
	4	10	0	10	0	10	0	10	0	10	0	10	0	10	0
56.25	1	10	0	10	0	9	1	9	0	9	0	9	0	9	0
	2	10	0	9	1	9	0	9	0	7	2	6	1NB	6	0
	3	10	0	10	0	10	0	10	0	9	1	9	0	9	0
	4	10	0	10	0	10	0	10	0	10	0	10	0	10	0
112.5	1	10	0	10	0	9	1	8	1NB	8	0	6	2	6	0
	2	10	0	10	0	9	1	9	0	9	0	7	2	7	0
	3	10	0	5	5	4	1	3	1	3	0	2	1	2	0
	4	10	0	9	1	9	0	8	1	8	0	8	0	6	1+1NB
225	1	9	1	4	5	4	0	3	1NB	3	0	1	2	0	1
	2	8	2	5	3	5	0	2	3	2	0	2	0	0	2
	3	9	1	6	3	5	1	3	2	3	0	2	1	1	1
	4	9	1	5	4	4	0	4	0	3	1	2	1	0	2

Feed	AM	UG	UG	SR	SR	TW (1)	TW (1)	None
1500 nauplii/chamber	PM	SR	UL	TL	NL	UG (2)	UL (2)	None

D.I.E. - SR 10/29/23, TL (2) Chambers with 5 or less fish fed half. - SR 10/29/23
 - SR 10/20/23
 - UG 10/22
 - TW 10/22
 - TW 10/23

7 Day Chronic Survival and Growth Test

Toxicant:	Copper Chloride
Ref Tox ID:	P220110.119

Date Test Started:	10/17/2023
Date Test Ended:	10/24/2023
Species:	<i>Pimephales promelas</i>

Concentration (µg/L)	Replicate	Boat Number	Weight Empty Boat (mg)	Weight Boat & Animals (mg)	Pan Count
Control	1	1	61.60	72.32	9 (3)
	2	2	58.99 (1)	63.63	10
	3	3	59.06	70.23	10
	4	4	64.62	76.13	10
14.0625	1	5	55.59	66.80	10
	2	6	56.88	68.51	10
	3	7	56.79	68.71	10
	4	8	55.06	65.63	10
28.125	1	9	52.75	60.58	7
	2	10	57.28	66.77	8
	3	11	56.51	65.80	8
	4	12	50.87	60.64	10
56.25	1	13	54.19	59.59	9
	2	14	54.97	57.22	6
	3	15	55.12	60.48	9
	4	16	51.23	56.54	10
112.5	1	17	56.43	59.12	6
	2	18	46.68	49.23	7
	3	19	61.63	62.48	2
	4	20	58.66	60.54	6
225	1	21	45.68	—	0
	2	22	47.24	—	0
	3	23	46.39	46.62	1
	4	24	52.41	—	0

Date/Time in oven: [Init.]	10/24/23 0821	LG	10/24/23 1823	98 DM
Oven Temp: [Init.]	100°C	LG	98	
Date/Time removed from oven (and placed in dessicator): [Init.]	10/24/23 1314	TW	10/25/23 0841	J1
Weight date and time (removed from desiccator): [Init.]	10/24/23 1430	RE	10/30/23 1230	RE

(1) MR. RE 10/24
 (2) IE-DM - 10/24/23

Balance 3

(3) Pan count different Day 7 survival

7 Day Chronic Survival and Growth Test

Toxicant:	Copper Chloride
Ref Tox ID:	P220110.129
Lot #:	MKCK2155
Protocol:	TOX018
Replicates:	4

Date Test Started:	10/17/2023
Date Test Ended:	10/24/2023
Matrix:	Liquid
Species:	<i>Pimephales promelas</i>
No. of Org. per Chamber:	10

	Conc. (µg/L)	Meter #:	DO (mg/L) (>4.0)	Meter #:	Temp (°C) (25±1°C)	Meter #:	Conductivity (µS/cm)	Meter #:	pH (6 - 9)	
Day 0 (Stock)	Control	8	8.1	8	24.8	8	331	8	8.1	
Date: 10/17/23	14.0625		8.1		24.9		330		8.0	
Time: 1104	28.125		8.2		24.8		331		8.1	
Technician: LG	56.25		8.2		24.7		331		8.1	
Feed: JL	112.5		8.2		24.6		331		8.1	
(1500 nauplii/chamber)	225		8.2		24.9		332		8.1	
	Day 1		Day 2		Day 3		Day 4		Day 5	Day 6
Temperature (OLD)	24.8		24.5		24.7		24.7		0	24.7
Temperature (NEW)	24.7		24.3		24.2		23.6		0	24.6
Day 7	Control	7	4.7	7	24.1	7	346	7	7.4	
Date: 10/24/23	14.0625		5.5		24.1		349		7.5	
Time: 1142	28.125		5.3		24.1		344		7.4	
Replicate No.: 3	56.25		5.7		24.0		345		7.4	
Technician: JJ	112.5		6.7		24.2		338		7.6	
	225		7.5		24.0		339		7.7	

Dilution Preparation (Serial dilute by 50%)

CuCl ₂ *2H ₂ O Stock Solution:	Target Stock Solution Conc.	Volume of Stock Solution	Amt. of Toxicant
400,000 µg/L	225 µg/L	2000 g	1.125 g
400,000 µg/L	112.5 µg/L	2000 g	0.5625 g

Day	Date	Init.	Highest Conc.	Day	Date	Init.	Highest Conc.
0	10/17/23	SR	225 µg/L	4	10/21/23	TT	225 µg/L
1	10/18/23	LG	225 µg/L	5	10/22/23	TW	225 µg/L
2	10/19/23	TW	225 µg/L	6	10/23/23	JJ	225 µg/L
3	10/20/23	TT	225 µg/L				

Start Time:	1602 SR/MS/LG
End Time:	1605 RE
Test Location:	Test Room 1
Dilution Water Batch:	RFW101423.02

Supplier:	Aquatic Bio Systems	
Organism Batch:	ABS101723.02	Age: 1 day
Chamber Size/Type:	20 oz cup	
Exposure Volume:	250 mL	

① Not Recorded - NL 10/22/23

1	13
2	6
3	11
4	19
<hr/>	
5	12
6	22
7	20
8	15
<hr/>	
9	18
10	7
11	21
12	1
<hr/>	
13	17
14	3
15	9
16	5
<hr/>	
17	2
18	10
19	14
20	16
<hr/>	
21	24
22	23
23	8
24	4
<hr/>	

FHM C CuCl₂ RT

Test ID: P220110.119

10/17 - 10/24

ORGANISM RECEIPT LOG

Date: 10/17/23		Time: 1215		Batch No. ABS 10/17/23.02			
Organism: P. promelas							
Source / Supplier: Aquatic Biosystems							
No. Ordered: 870		No. Received: 955		Source Batch: Hatch: 10/16/23 Collection date, hatch date, etc.):			
Condition of Organisms: Good			Approximate Size or Age: 1 day (Days from hatch, life stage, size class, etc.):				
Shipper: UPS			B of L (Tracking No.): 1Z F4673R0196319650				
Condition of Container: Good			Received By: M. Seibert / L. Gardiner				
Container	D.O. (mg/L)	Temp. (°C)	Cond. or Sal. (Include Units)	pH (Units)	# Dead	% Dead*	Tech. (Initials)
1	20	22.2	754	7.4	5	0.80	LG
2	21.5	22.2	744	7.4	3	0.80	LG

*if >10% contact lab manager

Notes:

① 96 dead is total 90 dead for both containers^{SS} - LG 10/17
combined ↑

7/27/15

Organism Receipt Log v1.1

Page ___ of ___

1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524



Toll Free: 800/331-5916
Tel: 970/484-5091 Fax: 970/484-2514

ORGANISM HISTORY

DATE: 10/16/2023

SPECIES: *Pimephales promelas*

AGE: N/A

LIFE STAGE: Embryo

HATCH DATE: 10/16/2023

BEGAN FEEDING: N/A

FOOD: N/A

Water Chemistry Record:

	Current	Range
TEMPERATURE:	<u>24°C</u>	<u>22-25°C</u>
SALINITY/CONDUCTIVITY:	<u>--</u>	<u>--</u>
TOTAL HARDNESS (as CaCO ₃):	<u>120 mg/l</u>	<u>80-120 mg/l</u>
TOTAL ALKALINITY (as CaCO ₃):	<u>95 mg/l</u>	<u>80-105 mg/l</u>
pH:	<u>7.84</u>	<u>7.70-8.20</u>

Comments:

Facility Supervisor

APPENDIX A.6

***RAPHIDOCELIS SUBCAPITATA* (GREEN ALGA) 96-HOUR GROWTH TEST**

STATISTICAL COMPARISONS AND LABORATORY DATA SHEETS

CETIS Summary Report

Report Date: 27 Nov-23 12:25 (p 1 of 1)
 Test Code/ID: P231026.04R.s. / 18-2659-3334

Selenastrum Growth Test

EcoAnalysts

Batch ID: 01-6514-6856	Test Type: Cell Growth	Analyst: Marisa Seibert
Start Date: 26 Oct-23 17:00	Protocol: EPA/821/R-02-013 (2002)	Diluent: Algal Culture Media
Ending Date: 30 Oct-23 17:26	Species: Selenastrum capricornutum	Brine: Not Applicable
Test Length: 4d 0h	Taxon: Chlorophyta	Source: Aquatic Biosystems, CO Age: 6d
Sample ID: 09-2211-1789	Code: P231026.04R.s.	Project: Toxicity Testing
Sample Date: 25 Oct-23 06:45	Material: Stormwater	Source: Brooks Manufacturing Co. (WA003080)
Receipt Date: 26 Oct-23 10:45	CAS (PC):	Station: Outfall 1
Sample Age: 34h	Client: Brooks Manufacturing Co.	

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	TU	S
05-8902-6098	Chlorophyll a	Dunnett Multiple Comparison Test		100	>100	---	19.6%	1	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	%	95% LCL	95% UCL	TU	S
08-4417-4446	Chlorophyll a	Linear Interpolation (ICPIN)		IC15	>100	---	---	<1	1
				IC20	>100	---	---	<1	
				IC25	>100	---	---	<1	
				IC40	>100	---	---	<1	
				IC50	>100	---	---	<1	

Chlorophyll a Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1538	1168	1908	1298	1764	116.3	232.6	15.12%	0.00%
6.25		4	1542	1321	1762	1438	1746	69.33	138.7	8.99%	-0.24%
12.5		4	1592	1303	1880	1412	1756	90.64	181.3	11.39%	-3.48%
25		4	1729	1360	2097	1580	2074	115.8	231.7	13.40%	-12.40%
50		4	1939	1715	2163	1762	2106	70.44	140.9	7.27%	-26.07%
100		4	1926	1774	2079	1821	2050	47.83	95.65	4.97%	-25.26%

Chlorophyll a Detail

MD5: D7B33AF4E2CC95C6FC1A3126AE97233C

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1382	1709	1298	1764
6.25		1438	1487	1746	1496
12.5		1412	1459	1739	1756
25		1646	2074	1580	1615
50		2106	1926	1962	1762
100		1821	2050	1895	1940

CETIS Test Data Worksheet

Report Date: 27 Nov-23 12:25 (p 1 of 1)
 Test Code/ID: P231026.04R.s / 18-2659-3334

Selenastrum Growth Test				EcoAnalysts	
Start Date: 26 Oct-23 17:00	Species: Selenastrum capricornutum	Sample Code: P231026.04R.s.			
End Date: 30 Oct-23 17:26	Protocol: EPA/821/R-02-013 (2002)	Sample Source: Brooks Manufacturing Co.			
Sample Date: 25 Oct-23 06:45	Material: Stormwater	Sample Station: Outfall 1			

Conc-%	Code	Rep	Pos	Cell Density	Absorbance	Biomass	Chlorophyll a	Notes
0	D	1	12				1382	
0	D	2	14				1709	
0	D	3	1				1297.5	
0	D	4	4				1763.5	
6.25		1	8				1437.5	
6.25		2	11				1487	
6.25		3	13				1746	
6.25		4	16				1496	
12.5		1	6				1412	
12.5		2	23				1459	
12.5		3	2				1739	
12.5		4	17				1756	
25		1	9				1645.5	
25		2	5				2074	
25		3	15				1580.5	
25		4	24				1615	
50		1	7				2105.5	
50		2	22				1926.5	
50		3	19				1961.5	
50		4	10				1762.5	
100		1	18				1821	
100		2	21				2049.5	
100		3	3				1895	
100		4	20				1940.5	

GENERAL

Client	Brooks Manufacturing Co.
Project	Toxicity Testing
Project Number	PG1913
Project Manager	M. Seibert
Date Sample Received	10/26/2023
Test type	96-Hour Freshwater Algal Growth Bioassay
Matrix	Liquid
Test Acceptability	±1x10 ⁶ cells/mL in control @ 96 hrs
Test Start Date	10/26/23
Test Species	Raphidocelis subcapitata
Organism Batch	ABS102423.03
Organism Acquired	10/24/2023
Organism Acclimation	2
Organism Age	6 DAYS
Test Protocol	Tox 023
Regional Protocol	USEPA EPA-821-R-02-013
Test Location	Test Room 2
Light Intensity	360-440 foot candles
Light Cycle	Continuous
Water Description	Algal Reconstituted Moderately Hard Water
Organisms per Replicate	10,000 cells/mL ± 50%
Test Chamber Size	125 mL
Exposure Volume	50 mL
Feeding Information	None
Test Dissolved Oxygen	
Test Temperature	25 ± 1
Conductivity	
Test pH	

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO		
Temp	24	26
Conductivity		
pH		

TEST START TIME/INIT:	1700	DM
TEST END TIME/INIT:	1726	DM

CLIENT SAMPLE ID	LAB ID
Outfall 1	P231026.04

Actual Light Intensity
400-430 fC

Concentrations	
1	Control
2	6.25%
3	12.5%
4	25%
5	50%
6	100%
7	-
8	-
9	-

Copy and Past VALUES from Jar Randomizer Tab

Day 0			Day 1			Day 2			Day 3		
Treatment	Rep	Chamber									
Control	1	14	Control	1	4	Control	1	25	Control	1	3
Control	2	22	Control	2	10	Control	2	10	Control	2	21
Control	3	30	Control	3	6	Control	3	27	Control	3	6
Control	4	2	Control	4	11	Control	4	7	Control	4	24
Control	Surr	11	Control	Surr	5	Control	Surr	11	Control	Surr	14
6.25%	1	15	6.25%	1	22	6.25%	1	1	6.25%	1	20
6.25%	2	18	6.25%	2	19	6.25%	2	9	6.25%	2	16
6.25%	3	16	6.25%	3	23	6.25%	3	23	6.25%	3	9
6.25%	4	26	6.25%	4	20	6.25%	4	26	6.25%	4	22
6.25%	Surr	19	6.25%	Surr	17	6.25%	Surr	19	6.25%	Surr	10
12.5%	1	23	12.5%	1	1	12.5%	1	12	12.5%	1	5
12.5%	2	7	12.5%	2	25	12.5%	2	18	12.5%	2	4
12.5%	3	9	12.5%	3	30	12.5%	3	20	12.5%	3	12
12.5%	4	4	12.5%	4	14	12.5%	4	24	12.5%	4	27
12.5%	Surr	29	12.5%	Surr	12	12.5%	Surr	2	12.5%	Surr	2
25%	1	24	25%	1	3	25%	1	6	25%	1	8
25%	2	8	25%	2	28	25%	2	30	25%	2	11
25%	3	25	25%	3	9	25%	3	4	25%	3	26
25%	4	5	25%	4	13	25%	4	15	25%	4	15
25%	Surr	27	25%	Surr	21	25%	Surr	17	25%	Surr	7
50%	1	20	50%	1	18	50%	1	29	50%	1	23
50%	2	21	50%	2	16	50%	2	5	50%	2	28
50%	3	10	50%	3	7	50%	3	22	50%	3	13
50%	4	17	50%	4	2	50%	4	8	50%	4	1
50%	Surr	12	50%	Surr	29	50%	Surr	14	50%	Surr	29
100%	1	13	100%	1	8	100%	1	3	100%	1	25
100%	2	28	100%	2	15	100%	2	28	100%	2	17
100%	3	3	100%	3	26	100%	3	16	100%	3	19
100%	4	6	100%	4	27	100%	4	21	100%	4	18
100%	Surr	1	100%	Surr	24	100%	Surr	13	100%	Surr	30

v.2	CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	10/26/23	PROTOCOL	Tox 023
	PROJECT	Toxicity Testing	TEST START DATE	10/26/23	PROJECT MANAGER	M. Seibert
	CLIENT SAMPLE ID	Outfall 1	TEST END DATE	10/30/23	SPECIES	<i>Raphidocelis subcapitata</i>
	LAB SAMPLE ID	P231026.04	MATRIX	Liquid	NO. OF ORGANISMS	10,000 cells/mL ± 50%

96-Hour Freshwater Algal Growth Bioassay

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)	Diluent Type	ARFW
0	0%	0	400.0	400		
	6.25%	25	375.0	400		
	12.5%	50	350.0	400		
	25%	100	300.0	400		
	50%	200	200.0	400		
	100%	400	0.0	400		

Test Dilution Prep

Date	Balance ID	Sample ID (P#)	Water Batch ID	Initials
10/26/2023	5	P231026.04	ARFW102023.01	NL

V.2

CLIENT	Brooks Manufacturing Co.
PROJECT	Toxicity Testing
CLIENT SAMPLE ID	Outfall 1
LAB SAMPLE ID	P231026.04
PROTOCOL	Tox 023
PROJECT MANAGER	M. Seibert

DATE RECEIVED	10/26/23
TEST START DATE	10/26/23
TEST END DATE	10/30/23
MATRIX	Liquid
SPECIES	<i>Raphidocelis subcapitata</i>
NO. OF ORGANISMS	10,000 cells/mL ± 50%

96-Hour Freshwater Algal Growth Bioassay

Initial Cell Counts

	Algal Stock				Innoculum		
	Original Count	Method	Converted Count		Original Count	Method	Converted Count
Count 1	120	2	333.36		100	3	100
Count 2	117	2	325.026		114	3	114
Count 3	120	2	333.36		109	3	109
Count 4	111	2	308.358		111	3	111
Count 5	104	2	288.912	Inoculum Prep Calculation: (1,000,000 cells/mL ÷ algal stock cells/mL) X total volume of inoculum in mL = mL of algal stock needed	78	3	78
Count 6	107	2	297.246		102	3	102
Average			314.377				102.33333
Dilution Factor			10	Target Volume of Innoculum (ml)			1
Cells/mL Calculation	31437700			35	1023333.333		
				mL algal stock needed			
Date	10/26/23			1.113	10/26/23		
Time	1605				1643		
Technician	DM				DM		

Zero Time Counts

	Control			12.5%			50%		
	Original Count	Method	Converted Count	Original Count	Method	Converted Count	Original Count	Method	Converted Count
Count 1	0	3	0	1	3	1	1	3	1
Count 2	2	3	2	1	3	1	2	3	2
Count 3	1	3	1	0	3	0	1	3	1
Count 4	1	3	1	2	3	2	0	3	0
Count 5	1	3	1	0	3	0	0	3	0
Count 6	1	3	1	2	3	2	1	3	1
Average			1			1			0.8333333
Cells/mL Calculation	10000			10000			8333.333333		
Average Cells/mL	9444.444444								
Date	10/26/23			10/26/23			10/26/23		
Time	1713			1718			1724		
Technician	DM			DM			DM		

CLIENT	Brooks Manufacturing Co.	DATE RECEIVED	10/26/23	PROTOCOL	Tox 023
PROJECT	Toxicity Testing	TEST START DATE	10/26/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall 1	TEST END DATE	10/30/23	SPECIES	<i>Raphidocelis subcapitata</i>
LAB SAMPLE ID	P231026.04	MATRIX	Liquid	NO. OF ORGANISMS	10,000 cells/mL ± 50%

96-Hour Freshwater Algal Growth Bioassay

		Concentration (%)	DO (mg/L)	TEMP (°C) 24 - 26	CONDUCTIVITY (µS/cm)	pH	Hardness	Alkalinity
Day 0		Control	7.3	24.3	414	8.1	116	79
Stock		6.3%	7.4	25.3	430	7.7	130	70
Date	10/26/2023	12.5%	7.3	25.2	393	7.8		
Time	1549	25%	7.2	24.7	373	7.8	104	65
Tech	NL	50%	7.7	24.7	333	7.7		
Meter #	8	100%	9.1	25.3	248	7.3	160	54
Day 1		Control	8.9	25.5	417	8.2		
Surrogate		6.3%	8.7	25.8	410	8.2		
Date	10/27/2023	12.5%	8.7	25.5	401	8.2		
Time	1710	25%	8.8	25.7	382	8.1		
Tech	DM	50%	8.9	25.6	342	8.1		
Meter #	7/T22	100%	8.9	25.5	253	7.9		
Day 2		Control	8.9	23.7	524	8.3		
Surrogate		6.3%	8.9	24.7	408	8.5		
Date	10/28/2023	12.5%	8.8	24.6	398	8.4		
Time	1127	25%	8.7	24.5	378	8.4		
Tech	MARH	50%	8.6	24.2	339	8.3		
Meter #	8	100%	8.5	24.5	252	8.2		
Day 3		Control	9.3	25.3	617	9.7		
Surrogate		6.3%	9.3	25.2	415	9.8		
Date	10/29/2003	12.5%	9.3	25.4	393	9.7		
Time	1441	25%	9.3	25.3	377	9.7		
Tech	NL	50%	9.2	25.2	339	9.4		
Meter #	8/T22	100%	9.1	25.1	455	9.0		
Day 4		Control	9.2	25.3	387	9.9		
Surrogate		6.3%	11.1	25.2	430	10.4		
Date	10/30/2023	12.5%	12.5	25.5	387	10.0		
Time	1632	25%	12.8	25.2	357	10.0		
Tech	DM	50%	12.8	25.1	323	10.0		
Meter #	9	100%	11.9	25.3	239	9.4		

CLIENT	Brooks Manufacturing Co.
PROJECT	Toxicity Testing
CLIENT SAMPLE ID	Outfall 1
LAB SAMPLE ID	P231026.04
PROTOCOL	Tox 023
PROJECT MANAGER	M. Seibert

DATE RECEIVED	10/26/23
TEST START DATE	10/26/23
TEST END DATE	10/30/23
MATRIX	Liquid
SPECIES	<i>Raphidocelis subcapitata</i>
NO. OF ORGANISMS	10,000 cells/mL ± 50%

96-Hour Freshwater Algal Growth Bioassay

Tech:	DM	Chlorophyll a (µg/L)	Chlorophyll a (µg/L)	Chlorophyll a (µg/L)
Date:	10/30/2023			
Time:	1726	Reading 1	Reading 2	Average
Fluorometer Serial #	808245			
Concentration (%)	REP.			
Control	1	1395	1369	1382
	2	1700	1718	1709
	3	1272	1323	1297.5
	4	1773	1754	1763.5
6.3%	1	1432	1443	1437.5
	2	1473	1501	1487
	3	1741	1751	1746
	4	1481	1511	1496
12.5%	1	1397	1427	1412
	2	1449	1469	1459
	3	1762	1716	1739
	4	1747	1765	1756
25%	1	1609	1682	1645.5
	2	2042	2106	2074
	3	1564	1597	1580.5
	4	1646	1584	1615
50%	1	2060	2151	2105.5
	2	1937	1916	1926.5
	3	1929	1994	1961.5
	4	1736	1789	1762.5
100%	1	1820	1822	1821
	2	2023	2076	2049.5
	3	1894	1896	1895
	4	1935	1946	1940.5

v.2 CLIENT	Brooks Manufacturing Co.
PROJECT	Toxicity Testing
CLIENT SAMPLE ID	Outfall 1
LAB SAMPLE ID	P231026.04
PROTOCOL	Tox 023
PROJECT MANAGER	M. Seibert

DATE RECEIVED	10/26/23
TEST START DATE	10/26/23
TEST END DATE	10/30/23
MATRIX	Liquid
SPECIES	<i>Raphidocelis subcapitata</i>
NO. OF ORGANISMS	10,000 cells/mL ± 50%

96-Hour Freshwater Algal Growth Bioassay

Final Control Cell Counts

Replicate	1			2			3			4		
	Original Count	Method	Converted Count									
Count 1	101	2	280.578	106	2	294.468	103	2	286.134	103	2	286.134
Count 2	100	2	277.8	209	3	209	100	2	277.8	100	2	277.8
Average			279.189			251.734			281.967			281.967
Dilution Factor			1			1			1			1
Cells/mL Calculation	2791890			2517340			2819670			2819670		
Average Cells/mL	2737142.5											
Date	10/30/23			10/30/23			10/30/23			10/30/23		
Time	1727			1735			1740			1748		
Technician	DM			DM			DM			DM		

Mean cell density of at least 1x10⁶ cells/mL Yes

Green Alga Growth

Chronic Power Standard Calculation

	average cell density (cells/mL)				
Replicate	1	2	3	4	Mean
CCEC (100)	1821	2050	1895	1940	1926.5
Control	1382	1709	1298	1764	1538.25

Control Mean - CCEC Mean

-388.25

Difference Divided by Control Mean

-0.2523972

Express as %

-25%

≤39% meets the power standard

Pass

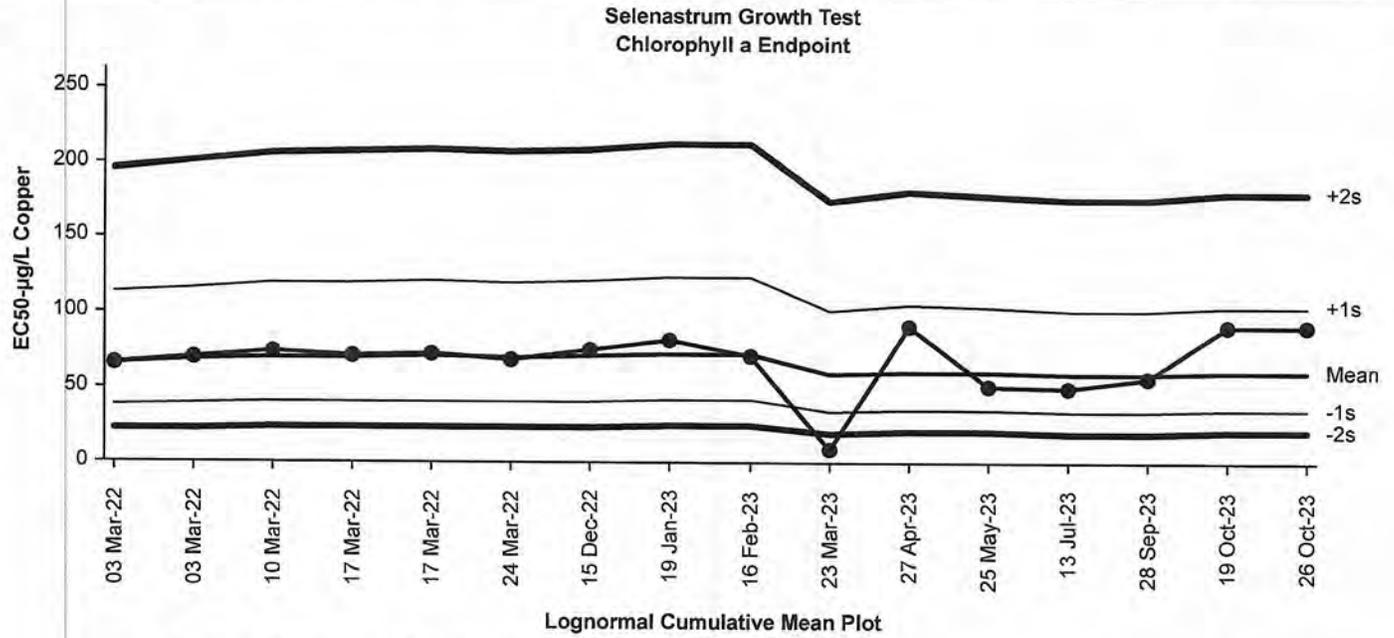
Selenastrum Growth Test

All Matching Labs

Test Type: Cell Growth
 Protocol: EPA/821/R-02-013 (2002)

Organism: Raphidocelis subcapitata
 Endpoint: Chlorophyll a

Material: Copper
 Source: Reference Toxicant-REF



Lognormal Cumulative Mean Plot

Mean: 61.19 Count: 15 -1s Warning Limit: 35.7 -2s Action Limit: 20.8
 Sigma: NA CV: 58.20% +1s Warning Limit: 105 +2s Action Limit: 180

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2022	Mar	3	16:12	66.46	5.269	0.153			00-5875-8623	08-9079-1230	EcoAnalysts
2			3	16:46	69.72	8.527	0.2416			04-9086-6783	14-2837-9844	EcoAnalysts
3			10	12:55	74.29	13.1	0.3592			05-9474-5080	13-3537-6983	EcoAnalysts
4			17	10:57	71.2	10.01	0.2806			15-0797-5903	10-9074-6856	EcoAnalysts
5			17	13:17	72.51	11.32	0.3143			18-1218-2467	19-0941-0363	EcoAnalysts
6			24	12:12	68.39	7.195	0.2059			08-7862-3403	19-7867-8588	EcoAnalysts
7		Dec	15	11:43	75.47	14.28	0.3885			17-4041-5652	05-5480-8309	EcoAnalysts
8	2023	Jan	19	14:37	81.77	20.58	0.5369			02-4986-8435	18-6151-1600	EcoAnalysts
9		Feb	16	13:43	71.16	9.972	0.2796			13-8956-4100	08-1120-2877	EcoAnalysts
10		Mar	23	15:32	9.623	-51.57	-3.426	(-)	(-)	09-7715-2704	01-8809-8490	EcoAnalysts
11		Apr	27	15:13	90.82	29.63	0.7314			12-3527-2769	06-2880-1295	EcoAnalysts
12		May	25	13:53	51.88	-9.313	-0.3058			09-5129-5584	13-5943-0906	EcoAnalysts
13		Jul	13	12:53	50.08	-11.11	-0.3712			18-7962-6742	07-6569-6799	EcoAnalysts
14		Sep	28	12:05	57.24	-3.953	-0.1237			16-8637-6763	00-3655-8784	EcoAnalysts
15		Oct	19	15:43	90.74	29.55	0.7298			01-7865-5906	01-0488-8686	EcoAnalysts
16			26	9:58	90.58	29.39	0.7265			18-6423-1375	05-0268-1183	EcoAnalysts

CETIS Summary Report

Report Date: 17 Nov-23 12:34 (p 1 of 1)
 Test Code/ID: P220110.123 / 18-6423-1375

Selenastrum Growth Test

EcoAnalysts

Batch ID: 12-4187-1909	Test Type: Cell Growth	Analyst: Marisa Seibert
Start Date: 26 Oct-23 09:58	Protocol: EPA/821/R-02-013 (2002)	Diluent: Algal Culture Media
Ending Date: 30 Oct-23 10:18	Species: Raphidocelis subcapitata	Brine: Not Applicable
Test Length: 4d 0h	Taxon: Chlorophyta	Source: Aquatic Biosystems, CO Age: 6d
Sample ID: 20-9835-3644	Code: P220110.123	Project: Reference Toxicant
Sample Date: 10 Jan-22	Material: Copper	Source: Reference Toxicant
Receipt Date: 10 Jan-22	CAS (PC):	Station: P220110.123
Sample Age: 654d 10h	Client: Internal Lab	

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
11-7798-7151	Chlorophyll a	Steel Many-One Rank Sum Test	64	128	90.51	14.6%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
05-0268-1183	Chlorophyll a	Linear Interpolation (ICPIN)	IC15	70.55	62.28	71.52	1
			IC20	73.12	65.03	74.07	
			IC25	75.78	67.91	76.72	
			IC40	84.34	77.29	85.21	
			IC50	90.58	84.24	91.38	

Chlorophyll a Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1574	1196	1952	1372	1856	118.8	237.6	15.10%	0.00%
16		4	1432	1197	1667	1303	1612	73.94	147.9	10.33%	9.01%
32		4	1617	1370	1865	1491	1838	77.79	155.6	9.62%	-2.76%
64		4	1607	1476	1737	1508	1704	41.07	82.14	5.11%	-2.09%
128		4	22.22	17.37	27.07	18.57	25.88	1.524	3.049	13.72%	98.59%
256		4	8.864	6.839	10.89	7.169	10.12	0.6365	1.273	14.36%	99.44%

Chlorophyll a Detail

MD5: E2EC64D9AB21378175AB4E0EE74C92D9

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1382	1372	1684	1856
16		1320	1303	1493	1612
32		1527	1491	1614	1838
64		1508	1584	1630	1704
128		22.98	18.57	25.88	21.45
256		7.169	9.472	10.12	8.691

CETIS Test Data Worksheet

Report Date: 22 Nov-23 15:50 (p 1 of 1)
Test Code/ID: P220110.123 / 18-6423-1375

Selenastrum Growth Test				EcoAnalysts	
Start Date: 26 Oct-23 09:58	Species: Raphidocelis subcapitata	Sample Code: P220110.123			
End Date: 30 Oct-23 10:18	Protocol: EPA/821/R-02-013 (2002)	Sample Source: Reference Toxicant			
Sample Date: 10 Jan-22	Material: Copper	Sample Station: P220110.123			

Conc-µg/L	Code	Rep	Pos	Cell Density	Absorbance	Biomass	Chlorophyll a	Notes
0	D	1	18				1382	
0	D	2	21				1372.5	
0	D	3	9				1684	
0	D	4	14				1856.5	
16		1	3				1319.5	
16		2	11				1303	
16		3	6				1493	
16		4	15				1612.5	
32		1	24				1527	
32		2	17				1491	
32		3	12				1613.5	
32		4	19				1837.5	
64		1	16				1508.5	
64		2	22				1584	
64		3	5				1629.5	
64		4	7				1704.5	
128		1	4				22.98	
128		2	13				18.565	
128		3	8				25.875	
128		4	20				21.45	
256		1	10				7.1695	
256		2	23				9.472	
256		3	2				10.125	
256		4	1				8.691	

GENERAL

Client	Internal
Associated Test	Various
Compound	Copper Chloride
Toxicant	Copper
Test Type	Reference Toxicant
Test Type	96-Hour Freshwater Algal Growth Bioassay
Matrix	Liquid
Test Acceptability	$\geq 1 \times 10^8$ cells/mL in control @ 96 hrs
Test Start Date	10/26/23
Test Species	Raphidocelis subcapitata
Organism Batch	ABS102523.03
Organism Acquired	10/24/2023
Organism Acclimation	2
Organism Age	6 days
Test Protocol	Tox 023
Regional Protocol	USEPA EPA-821-R-02-013
Test Location	Test Room 2
Light Intensity	360-440 foot candles
Light Cycle	Continuous
Water Description	Algal Reconstituted Moderately Hard Water
Organisms per Replicate	10,000 cells/mL \pm 50%
Test Chamber Size	125 mL
Exposure Volume	50 mL
Feeding Information	None
Test Dissolved Oxygen	
Test Temperature	25 \pm 1
Conductivity	
Test pH	

Note: Input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO		
Temp	24	26
Conductivity		
pH		

TEST START TIME/INIT:	958	NL
TEST END TIME/INIT:	1018	DM

REFERENCE TOXICANT TEST ID	Lot #
P220110.123	MKCK7155

Actual Light Intensity
400-430 FC

Concentrations

1	Control
2	16 ug/L
3	32 ug/L
4	64 ug/L
5	128 ug/L
6	256 ug/L
7	-
8	-
9	-

Copy and Past VALUES from Jar Randomizer Tab

Day 0			Day 1			Day 2			Day 3		
Treatment	Rep	Chamber									
Control	1	15	Control	1	21	Control	1	14	Control	1	21
Control	2	12	Control	2	8	Control	2	1	Control	2	7
Control	3	24	Control	3	18	Control	3	11	Control	3	23
Control	4	17	Control	4	19	Control	4	6	Control	4	9
16 ug/L	1	4	16 ug/L	1	14	16 ug/L	1	12	16 ug/L	1	4
16 ug/L	2	3	16 ug/L	2	23	16 ug/L	2	3	16 ug/L	2	18
16 ug/L	3	19	16 ug/L	3	6	16 ug/L	3	9	16 ug/L	3	24
16 ug/L	4	22	16 ug/L	4	20	16 ug/L	4	2	16 ug/L	4	14
32 ug/L	1	13	32 ug/L	1	22	32 ug/L	1	18	32 ug/L	1	3
32 ug/L	2	7	32 ug/L	2	15	32 ug/L	2	17	32 ug/L	2	17
32 ug/L	3	8	32 ug/L	3	9	32 ug/L	3	23	32 ug/L	3	19
32 ug/L	4	11	32 ug/L	4	12	32 ug/L	4	22	32 ug/L	4	2
64 ug/L	1	18	64 ug/L	1	17	64 ug/L	1	20	64 ug/L	1	12
64 ug/L	2	2	64 ug/L	2	3	64 ug/L	2	7	64 ug/L	2	6
64 ug/L	3	16	64 ug/L	3	7	64 ug/L	3	19	64 ug/L	3	5
64 ug/L	4	23	64 ug/L	4	10	64 ug/L	4	15	64 ug/L	4	11
128 ug/L	1	14	128 ug/L	1	4	128 ug/L	1	13	128 ug/L	1	16
128 ug/L	2	1	128 ug/L	2	13	128 ug/L	2	24	128 ug/L	2	22
128 ug/L	3	6	128 ug/L	3	1	128 ug/L	3	8	128 ug/L	3	13
128 ug/L	4	9	128 ug/L	4	24	128 ug/L	4	16	128 ug/L	4	20
256 ug/L	1	10	256 ug/L	1	11	256 ug/L	1	21	256 ug/L	1	10
256 ug/L	2	20	256 ug/L	2	16	256 ug/L	2	10	256 ug/L	2	15
256 ug/L	3	5	256 ug/L	3	5	256 ug/L	3	5	256 ug/L	3	8
256 ug/L	4	21	256 ug/L	4	7	256 ug/L	4	4	256 ug/L	4	1
.	1	.	.	1	.	.	1	.	.	1	.
.	2	.	.	2	.	.	2	.	.	2	.

v.2

CLIENT	Internal
PROJECT	Various
REFERENCE TOXICANT TEST ID	P220110.123
LOT NUMBER	MKCK7155
PROTOCOL	Tox 023
PROJECT MANAGER	Copper

TEST TYPE	Reference Toxicant
TEST START DATE	10/26/23
TEST END DATE	10/30/23
MATRIX	Liquid
SPECIES	<i>Raphidocelis subcapitata</i>
NO. OF ORGANISMS	10,000 cells/mL ± 50%

96-Hour Freshwater Algal Growth Bioassay

Initial Cell Counts

	Algal Stock			Inoculum Prep Calculation: (1,000,000 cells/mL + algal stock cells/mL) X total volume of inoculum in mL = mL of algal stock needed	Innoculum		
	Original Count	Method	Converted Count		Original Count	Method	Converted Count
Count 1	111	2	308.358		103	3	103
Count 2	106	2	294.468		96	3	96
Count 3	117	2	325.026		110	3	110
Count 4	97	2	269.466		102	3	102
Count 5	113	2	313.914		108	3	108
Count 6	91	2	252.798		99	3	99
Average			294.005				103
Dilution Factor			10	Target Volume of Innoculum (ml)			1
Cells/mL Calculation	29400500		35	mL algal stock needed	1030000		
Date	26-Oct		1.190		10/26/2023		
Time	841				919		
Technician	NL				NL		

Zero Time Counts

	Control			32 ug/L			128 ug/L		
	Original Count	Method	Converted Count	Original Count	Method	Converted Count	Original Count	Method	Converted Count
Count 1	1	3	1	1	3	1	2	3	2
Count 2	0	3	0	1	3	1	0	3	0
Count 3	0	3	0	1	3	1	1	3	1
Count 4	2	3	2	2	3	2	1	3	1
Count 5	1	3	1	2	3	2	1	3	1
Count 6	1	3	1	0	3	0	1	3	1
Average			0.8333333			1.1666667			1
Cells/mL Calculation	8333.333333			11666.66667			10000		
Average Cells/mL	10000								
Date	10/26/2023			10/26/2023			10/26/2023		
Time	1004			1011			1016		
Technician	NL			NL			NL		

V.2 CLIENT	Internal	TEST TYPE	Reference Toxicant	PROTOCOL	Tox 023
ASSOCIATED TEST	Various	TEST START DATE	10/26/23	TOXICANT	Copper
REFERENCE TOXICANT TEST ID	P220110.123	TEST END DATE	10/30/23	SPECIES	<i>Raphidocelis subcapitata</i>
LOT NUMBER	MKCK7155	MATRIX	Liquid	NO. OF ORGANISMS	10,000 cells/mL ± 50%

96-Hour Freshwater Algal Growth Bioassay

Day of Test	Highest Concentration	Vol. Stock Added (mL)	Vol. Diluent Added (mL)	Diluent Type	ARFW
0	256	0.512	799.5	Target Volume (ml)	800

Serial dilute by 1/2 to obtain lower concentrations

Test Dilution Prep

Date	Balance ID	Water Batch ID	Initials	Concentration of Stock	Units
10/26/2023	5	ARFW102023.01	NL	400000	ug/L

v.2 CLIENT	Internal	TEST TYPE	Reference Toxicant	PROTOCOL	Tox 023
PROJECT	Various	TEST START DATE	10/26/23	TOXICANT	Copper
REFERENCE TOXICANT TEST ID	P220110.123	TEST END DATE	10/30/23	SPECIES	<i>Raphidocelis subcapitata</i>
LOT NUMBER	MKCK7155	MATRIX	Liquid	NO. OF ORGANISMS	10,000 cells/mL ± 50%

96-Hour Freshwater Algal Growth Bioassay

		DO	TEMP (°C)	CONDUCTIVITY	pH	
		(mg/L)	24 - 26	(µS/cm)	-	
Concentration (%)						
Day 0		Control	7.3	24.3	414	8.1
Stock		16 ug/L	7.3	24.3	415	8.1
Date	10/26/2023	32 ug/L				
Time	948	64 ug/L	7.4	24.3	417	8.1
Tech	NL	128 ug/L				
Meter #	8/T22	256 ug/L	7.2	24.3	419	8.1
Day 4		Control	8.8	24.8	439	10.5
Surrogate		16 ug/L	8.5	24.6	392	10.4
Date	10/30/2023	32 ug/L				
Time	959	64 ug/L	8.8	25.0	416	9.9
Tech	DM	128 ug/L				
Meter #	7	256 ug/L	8.5	25.0	426	8.7

CLIENT	Internal
PROJECT	Various
REFERENCE TOXICANT TEST ID	P220110.123
LOT NUMBER	MKCK7155
PROTOCOL	Tox 023
TOXICANT	Copper

TEST TYPE	Reference Toxicant
TEST START DATE	10/26/23
TEST END DATE	10/30/23
MATRIX	Liquid
SPECIES	<i>Raphidocelis subcapitata</i>
NO. OF ORGANISMS	10,000 cells/mL ± 50%

96-Hour Freshwater Algal Growth Bioassay

Tech:	DM	Chlorophyll a (µg/L)	Chlorophyll a (µg/L)	Chlorophyll a (µg/L)
Date:	10/30/2023			
Time:	1020	Reading 1	Reading 2	Average
Fluorometer Serial #	808245			
Concentration (%)	REP			
Control	1	1368	1396	1382
	2	1404	1341	1372.5
	3	1675	1693	1684
	4	1880	1833	1856.5
16 ug/L	1	1303	1336	1319.5
	2	1306	1300	1303
	3	1488	1498	1493
	4	1594	1631	1612.5
32 ug/L	1	1508	1546	1527
	2	1505	1477	1491
	3	1611	1616	1613.5
	4	1869	1806	1837.5
64 ug/L	1	1551	1466	1508.5
	2	1573	1595	1584
	3	1622	1637	1629.5
	4	1709	1700	1704.5
128 ug/L	1	23.28	22.68	22.98
	2	18.35	18.78	18.565
	3	25.75	26.0	25.875
	4	21.23	21.67	21.45
256 ug/L	1	7.112	7.227	7.1695
	2	9.61	9.334	9.472
	3	10.08	10.17	10.125
	4	8.469	8.913	8.691

v.2

CLIENT	Internal
PROJECT	Various
REFERENCE TOXICANT TEST ID	P220110.123
LOT NUMBER	MKCK7155
PROTOCOL	Tox 023
TOXICANT	Copper

TEST TYPE	Reference Toxicant
TEST START DATE	10/26/23
TEST END DATE	10/30/23
MATRIX	Liquid
SPECIES	<i>Raphidocelis subcapitata</i>
NO. OF ORGANISMS	10,000 cells/mL ± 50%

96-Hour Freshwater Algal Growth Bioassay

Final Control Cell Counts

Replicate	1			2			3			4		
	Original Count	Method	Converted Count									
Count 1	126	2	350.028	197	3	197	133	2	369.474	98	2	272.244
Count 2	119	2	330.582	73	2	202.794	137	2	380.586	102	2	283.356
Average			340.305			199.897			375.03			277.8
Dilution Factor			1			1			1			1
Cells/mL Calculation	3403050			1998970			3750300			2778000		
Average Cells/mL	2982580											
Date	10/30/23			10/30/23			10/30/23			10/30/23		
Time	1018			1025			1035			1049		
Technician	DM			DM			DM			DM		

Mean cell density of at least 1x10⁶ cells/mL Yes

SELENASTRUM RECEIPT LOG

Date: 10/29/23	Time: 1200	Batch No. ABS102423.03
Organism: Raphidocelis subcapitata		
Source / Supplier: Aquatic Biosystems		
Supplier Batch Number: "Sel Test"	Shipper/B of L (Tracking No.): 12 FAB 73R 01 9708 3957	
Age of Batch: 4 days	Received By: DM	
Inoculation Water Batch: NA	Inoculation date/Initials: 10/20/23	
Cell Density Count: 31,437,700 cells/mL		
Notes:		

1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524



Toll Free: 800/331-5916
Tel: 970/484-5091 Fax: 970/484-2514

Algae Preparation History

DATE: 10/23/2023

SPECIES: Raphidocelis subcapitata*

INOCULATION DATE: 10/20/2023

HARVEST DATE: 10/23/2023

CONCENTRATION DATE: N/A

CELL COUNT (/ml): None taken

Comments:

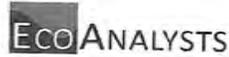
* Formerly known as *Psuedokirschneriella subcapitata* and *Selenastrum capricornutum*

Supervisor

APPENDIX B

CHAIN-OF-CUSTODY AND SAMPLE RECEIPT FORMS

CHAIN OF CUSTODY



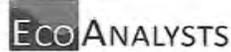
EcoAnalysts, Inc.
4770 NE View Dr., Port Gamble, WA. 98364
Tel: (360) 297-6040

Destination: EcoAnalysts Inc.	Sample Originator (Organization): Brooks Manufacturing Co.	Report Results To: Kevin Moseley	Phone:
Destination Contact: Marisa Seibert	PERSON WHO COLLECTED SAMPLE: Kevin Moseley	Contact Name:	Fax:
Date: 10/23/23	Address: 2120 Pacific St. Bellingham, WA 98229	Address:	Email:
Turn-Around-Time: NA	Phone: 360-733-1700	Invoicing To:	
Project Name: Toxicity Testing	Fax:		
Contract/PO:	E-mail: kmoseley@brooksmfg.com	Comments or Special Instructions:	

No.	Sample ID	Secondary ID: Replicate, X of Y, etc.	Matrix	Volume/Mass	Date	Time	WET Testing	Analyses:			Invoicing To:	
								Preservation	Sample Temp Upon Receipt	LAB ID		
1	Outfall 1		L	20L	10/23	1470	X					
2	Outfall 1		L	20L	"	"	X					Ice 0.6°C P231024.01a
3	Outfall 1		L	20L	"	"	X					Ice 0.3°C P231024.01b
4	Outfall 1		L	10L	"	"	X					Ice 5.5°C P231024.01c
5												Ice 4.5°C P231024.01d
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												

Relinquished by:		Received by:		Relinquished by:		Received by:		Matrix Codes FW = Fresh Water SB = Salt & Brackish Water SS = Soil & Sediment TS = Tissue
Print Name: Kevin Moseley	Signature:	Print Name: Marisa Seibert	Signature:	Print Name:	Signature:	Print Name:	Signature:	
Affiliation: Technical Director	Date/Time: 10/23/23 1530	Affiliation: EcoAnalysts	Date/Time: 10/24/23 1030	Affiliation:	Date/Time:	Affiliation:	Date/Time:	

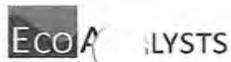
CHAIN OF CUSTODY



EcoAnalysts, Inc.
4770 NE View Dr., Port Gamble, WA. 98364
Tel: (360) 297-6040

Destination: EcoAnalysts Inc.		Sample Originator (Organization): Brooks Manufacturing Co.					Report Results To: Kevin Moseley			Phone:			
Destination Contact: Marisa Seibert		PERSON WHO COLLECTED SAMPLE: Kevin Moseley					Contact Name:			Fax:			
Date: 10/23/23		Address: 2120 Pacific St. Bellingham, WA 98229					Address:			Email:			
Turn-Around-Time: NA		Phone: 360-733-1700					Analyses:			Invoicing To:			
Project Name: Toxicity Testing		Fax:								Comments or Special Instructions:			
Contract/PO:		E-mail: kmoseley@brooksmfg.com					WET Testing			Preservation		Sample Temp Upon Receipt	LAB ID
No.	Sample ID	Secondary ID: Replicate, X of Y, etc.	Matrix	Volume/Mass	Date	Time				Preservation	Sample Temp Upon Receipt	LAB ID	
1	Outfall 1		L	900 10L	10/25/23	6:45 AM	X	Ice	0.1°C	P231026.04			
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
12													
13													
14													
15													
16													
17													
18													
19													
20													
Relinquished by:		Received by:					Relinquished by:			Received by:		Matrix Codes	
Print Name: Kevin Moseley		Print Name: Nicole Lundgren					Print Name:			Print Name:		FW = Fresh Water	
Signature: <i>Kevin Moseley</i>		Signature: <i>Nicole Lundgren</i>					Signature:			Signature:		SB = Salt & Brackish Water	
Affiliation: Technical Director		Affiliation: ECOA					Affiliation:			Affiliation:		SS = Soil & Sediment	
Date/Time: 10/25/23 0710		Date/Time: 10/26/23 1045					Date/Time:			Date/Time:		TS = Tissue	

CHAIN OF CUSTODY



EcoAnalysts, Inc.
4770 NE View Dr., Port Blaine, WA. 98364
Tel: (360) 297-6040

Destination: EcoAnalysts Inc.		Sample Originator (Organization): Brooks Manufacturing Co.				Report Results To: Kevin Moseley				Phone:	
Destination Contact: Marisa Seibert		PERSON WHO COLLECTED SAMPLE: Kevin Moseley				Contact Name:				Fax:	
Date: 10/23/23		Address: 2120 Pacific St. Bellingham, WA 98229				Address:				Email:	
Turn-Around-Time: NA		Phone: 360-733-1700				Analyses:				Invoicing To:	
Project Name: Toxicity Testing		Fax:								Comments or Special Instructions:	
Contract/PO:		E-mail: kmoseley@brooksmfg.com									

No.	Sample ID	Secondary ID: Replicate, X of Y, etc.	Matrix	Volume/Mass	Date	Time	WET Testing	Analyses				Preservation	Sample Temp Upon Receipt	LAB ID
1	Outfall 1		L	10L	10/27/23	10:15	X					Ice	0.0	P231628.03
2														
3														
4														
5														
6														
7														
8														
9														
10														
11														
12														
13														
14														
15														
16														
17														
18														
19														
20														

Relinquished by:		Received by:		Relinquished by:		Received by:		Matrix Codes <small>FW = Fresh Water SB = Salt & Brackish Water SS = Soil & Sediment TS = Tissue</small>
Print Name: Kevin Moseley		Print Name: <i>Maryann Rempel-Hester</i>		Print Name:		Print Name:		
Signature:		Signature: <i>[Signature]</i>		Signature:		Signature:		
Affiliation: Technical Director		Affiliation: <i>EcoAnalysts</i>		Affiliation:		Affiliation:		
Date/Time: <i>10/27/23 1015</i>		Date/Time: <i>10/28/23 12:28</i>		Date/Time:		Date/Time:		

SAMPLE RECEIPT

Client:	Client ID:	Lab ID:	Renewals:	
Brooks Manufacturing Co.	Duffall	P231024.01	P231026.04	P231028.03
Project:				
Toxicity Testing				
Date/Time Received:	10/24/23 1030	10/26/23 1045	10/28/23 1228	
Airbill #:	129x874515702536	129x87450171164	129x8745447265	5636
Shipper Tracking Information Kept for Records: (Y/N/NA)	N	N	N	
Collection Date/Time:	10/23/23 1430	10/25/23 0645	10/27/23 1015	
Sample Holding Time (must be ≤36 hours at test initiation)	Y	✓		
Condition of Shipping Container:	Good	Good	Good	
Type and Capacity of Sample Container:	10L + 20L cubis	10L cubi	10L cubi	
Total Sample Volume (L):	70L	~9L (cubi)	~10L	
Condition of Sampling Container:	Good	Good	Good	
Sample Container Appropriate: (Y/N)	Y	Y	Y	
Custody Seals Intact: (Intact/Broken/Not Present)	Not present	NP	Not Present	
Frozen Wet or Blue Ice Present During Shipment/Transport: (Y/N)	Y	Y	Y	
Sampler's Name Present on COC Form: (Print Name/Not Present)	Kevin Moseley	Kevin Moseley	Kevin Moseley	
Color:	pale yellow	Pale yellow	Pale yellow	

TAKE THE FOLLOWING MEASUREMENTS UPON ARRIVAL

LAB ID	Meter #	Temp. (°C) * (0-6°C)	Meter #	Dissolved Oxygen (mg/L)	Meter #	pH	Meter #	Cond. (µS/cm)	Meter #	Sal. (ppt)	Hardness (mg CaCO ₃ /L)	Alkalinity (mg CaCO ₃ /L)	Total Chlorine (mg/L)	Total NH ₃ (mg/L)	Tech
P231024.01	TA	②	7	10.0	7	7.0	7	715	7	0.3	70	54	0.02	③ 0.108	MS/OL
P231026.04	TS	0.1	8	10.4	8	7.0	8	160	8	0.108	48	48	0.01	③ 0.0430	TW/NL DM
P231028.03	TS	0.0	7	12.6	7	7.3	7	201	7	NA	62	44	0.03	③ 0.00	MMH

*Notify project manager or study director of temperatures above 6°C or ≥36 hours holding time. Client must be notified ASAP.

If there are sample receipt problems, complete the following:

Reason for unacceptability:	
Name of Client Contact:	Contacted by:
Client Response and/or Action to be Taken:	Date Action Taken:

① IE-MS 10/24 11/02/24 P231024.01 a = 0.6°C
 ② temp. of each cubi: P231024-01 b = 0.3°C
 P231026-01 c = 5.5°C
 P231028-01 d = 4.5°C
 MS 10/24
 ③ sample preserved for later analysis - MS 10/24, NL 10/24, V.1, MMH 10/24
 ④ IE, TW 10/26, DM-10/26/23
 ⑤ Ran on 10/26-6g 10/26/23