

Bio-Analytical Laboratories' Executive Summary

Permittee: Nashville Public Works
Nashville, AR 71852

Project #: X9060

Outfall: 001 (treated municipal wastewater)

Permit #: AR0021776/ AFIN #31-00036

Contact: Southern Petroleum Laboratories
4720 Viking Drive, Ste A
Bossier City, LA 71111

Test Dates: January 8 – 16, 2024

Test Type: Chronic Static Renewal Survival and Reproduction Test using
Ceriodaphnia dubia (EPA Method 1002.0)
Chronic Static Renewal Survival and Growth Test using
Pimephales promelas (EPA Method 1000.0)

Results:

For *Ceriodaphnia dubia*:

1. If the NOEC for survival is less than the critical dilution (78.0%), enter a "1"; otherwise, enter a "0" for Parameter TLP3B - 0 -**Pass**
2. If the NOEC for reproduction is less than the critical dilution, enter a "1"; otherwise, enter a "0" for Parameter TGP3B - 0 - **Pass**
3. Report the NOEC value for survival, Parameter TOP3B - 78.0%.
4. Report the NOEC value for reproduction, Parameter TPP3B - 78.0%.
5. Report the largest % coefficient of variation between the control and the critical dilution, Parameter TQP3B – 27.93%
6. Chronic WET Limit, DMR CODE 51710, - 78.0%
7. PMSD Reproduction-37.34%(13.0 – 47.0%) moderate precision, acceptable for passing test.

For *Pimephales promelas*:

1. If the NOEC for survival is less than the critical dilution (78.0%), enter a "1"; otherwise, enter a "0" for Parameter TLP6C - 0 -**Pass**
2. If the NOEC for growth is less than the critical dilution, enter a "1"; otherwise, enter a "0" for Parameter TGP6C - 0 -**Pass**
3. Report the NOEC value for survival, Parameter TOP6C - 78.0%.
4. Report the NOEC value for growth, Parameter TPP6C - 78.0%.
5. Report the largest % coefficient of variation between the control and the critical dilution, Parameter TQP6C –24.45%.
6. Chronic WET Limit, DMR CODE 51714, - 78.0%
7. PMSD Biomass-30.0% (12.0 – 30.0%)-moderate precision, acceptable for passing test.

This report contains a total of 53 pages, including this page. The results contained within pertain only to the samples listed on the chain of custody documents in Appendix A. The information meets the standards set forth by ADEQ. The chemical data in this report is for monitoring purposes only and should not be reported on discharge monitoring reports.



Bio-Analytical Laboratories

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**THE RESULTS OF TWO CHRONIC
DEFINITIVE TOXICITY TESTS
FOR OUTFALL 001**

AT

**NASHVILLE PUBLIC WORKS
Nashville, Arkansas**

**NPDES #AR0021776
AFIN #31-00036**

EPA Methods 1000.0 and 1002.0

Project X9060

Test Dates: January 8 – 16, 2024

Report Date: January 25, 2024

Prepared for:

Southern Petroleum Laboratories
4720 Viking Drive, Ste A
Bossier City, LA 71111

Prepared by:

Ginger Briggs
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1.0 Introduction

Bio-Analytical Laboratories (BAL), Doyline, Louisiana conducted two chronic definitive toxicity tests for Outfall 001 at Nashville Public Works, Nashville, Arkansas. The test organisms used were the cladoceran, *Ceriodaphnia dubia*, and the fathead minnow, *Pimephales promelas*. The purpose of this study is to determine if appropriately dilute effluent samples adversely affect the survival, reproduction and/or growth of the test organisms. Toxicity is defined as a statistically significant difference at the 95 percent confidence level between the survival, reproduction and/or growth of the test organism in the critical dilution (the effluent concentration representative of the proportion of effluent in the receiving water during critical low flow or critical mixing conditions) compared to the survival, reproduction and/or growth of the test organism in the control. The test endpoint is the No-Observed-Effect-Concentration (NOEC), the highest effluent concentration that is not significantly different from the control.

2.0 Methods and Materials

2.1 Test Methods

All methods followed were according to the latest edition of “Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms” (EPA-821-R-02-013), “Standard Methods for The Examination of Water and Wastewater, 22nd Edition” (APHA 2012), and BAL’s standard operating procedure.

2.2 Test Organisms

The *Ceriodaphnia dubia* test organisms were cultured in-house at test temperature and dilution water hardness and were less than 24 hours old at test initiation. The neonates were released within the same 8-hour period. The fathead minnows were also raised in-house at test temperature and dilution water hardness and were less than 24 hours old at test initiation. Monthly chronic reference toxicant tests were conducted in order to document organism sensitivity and demonstration of capability.

2.3 Dilution Water

Moderately hard reconstituted water, made per method guidelines, was used as the dilution water and the control for the toxicity tests. Conductivity (SM 2510 B) and pH (SM 4500-H+ B) measurements, in umhos/cm and standard units, respectively, were checked on each lot of water prior to use to confirm water hardness.

2.4 Test Concentrations

The test concentrations used in the chronic toxicity tests (for a 3.5 MGD design flow) were 78.0, 59.0, 44.0, 33.0 and 25.0 percent effluent and a reconstituted water control. The critical dilution was 78.0 percent effluent. The *Ceriodaphnia* test was conducted using 10 replicates of one animal each for a total of 10 animals per concentration. The fathead minnow test was conducted using five replicates of eight animals each for a total of 40 animals per concentration.

2.5 Sample Collection

Three 24-hour composite samples of Outfall 001 were collected by Nashville Public Works personnel on January 7, 9 and 23, 2024, at 2100, 2236 and 2109 hours, respectively. Upon collection and completion of each composite, the samples were packed in ice and delivered to the laboratory the day of collection by Southern Petroleum Laboratories (SPL) personnel. The sample temperature upon arrival of each sample was 1.8, 1.0 and 2.2⁰ Celsius, respectively.

2.6 Sample Preparation

Upon arrival, the samples were logged in, given an identification number, and refrigerated unless needed. Prior to use, the samples were warmed to 25±1⁰ Celsius. Total residual chlorine levels were measured in milligrams/Liter (mg/L) using a test strip and recorded if present. Total ammonia levels were measured in mg/L using a test strip. Dissolved oxygen (SM 4500-0 G) and pH (SM 4500-H+ B) measurements, in mg/L and standard units, respectively, were measured on the control and each concentration at test initiation, at test renewal and at test termination. Conductivity (SM 2510 B) measurements, in umhos/cm, were also taken at test initiation and at each renewal. Alkalinity (SM 2320 B) and hardness (SM 2340 C) levels were measured in mg/L as CaCO₃ on the control and the undiluted effluent samples.

2.7 Monitoring of the Tests

The cladoceran test was run in a Precision^R dual-programmable, illuminated incubator at a temperature of 25±1⁰ Celsius. The fathead minnow test was run in a circulating waterbath, using a Remcor^R heated liquid circulator to keep a constant temperature of 25±1⁰ Celsius. Data-loggers were used to monitor diurnal test temperature. Test temperatures were recorded at the beginning of the day, after test renewal and at the end of the day. Light cycles and intensities were recorded twice a month.

2.8 Data Analysis

Ceriodaphnia dubia survival data was analyzed using Fisher's Exact Test, an equality test comparing concentration data to control data. Reproduction data was analyzed using Steel's Many-One Rank Test, a nonparametric test comparing concentration data to control data. Fathead minnow survival and growth data was analyzed using Dunnett's Test, a parametric test. Other endpoints were obtained by approved EPA methods of analysis.

3.0 Results and Discussion

The results of the *Ceriodaphnia dubia* test can be found in Table 1. Eighty percent survival occurred in the control and 90.0 percent survival occurred in the 78.0 percent critical dilution after seven days of exposure. The average number of neonates per female after three broods in the control and in the critical dilution was 15.9 and 17.9 respectively. The No-Observed-Effect-Concentration (NOEC) for survival and reproduction in this test was 78.0 percent effluent (p=.05).

Table 1: Results of the Chronic Definitive *Ceriodaphnia dubia* Test

Percent Effluent	Percent Survival	Sig.*	Mean # Neonates-Surviving	Mean # Neonates -Total	Sig.*
Control	80.0		17.4	15.9	
25.0	100.0		14.8	14.8	
33.0	80.0		16.3	13.9	
44.0	90.0		17.2	15.8	
59.0	90.0		14.6	13.3	
78.0	90.0		19.9	17.9	

*significant when compared to the control (p=.05). Test validity based on mean number of neonates per surviving female. NOEC value based on total mean number of neonates.

The fathead minnow test results can be found in Table 2. After seven days of exposure, 92.5 percent survival occurred in the control and 97.5 percent survival occurred in the 78.0 percent critical dilution. The average weight gained in the control and in the 78.0 percent critical dilution was 0.333 and 0.313 milligram (mg), respectively. The NOEC for survival and growth (biomass) was 78.0 percent effluent (p=.05).

Table 2: Results of the Chronic Definitive Fathead Minnow Test

Percent Effluent	Percent Survival	Sig.*	Mean Dry Weight (mg)	Sig.*
Control	92.5		0.333	
25.0	87.5		0.305	
33.0	87.5		0.350	
44.0	92.5		0.255	
59.0	95.0		0.265	
78.0	97.5		0.313	

*significant when compared to the control (p=.05).

The most recent reference toxicant tests showed the test organisms to be within the respective sensitivity range. The graphs of the results of the chronic reference toxicant tests can be found in Appendix D- Quality Assurance Charts.

4.0 Conclusions

The three composite samples of Outfall 001 collected on January 7, 9 and 11, 2024, from Nashville Public Works, Nashville, Arkansas, were not found to be lethally toxic to the *Ceriodaphnia dubia* test organisms nor the *Pimephales promelas* test organisms in the 78.0 percent critical dilution after seven days of exposure (p=.05). Sublethal effects (i.e., lack of growth or reproduction) were not noted in the 78.0 percent critical dilution in either test (p=.05).

5.0 References

- EPA, 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Fourth Edition. EPA-821-R-02-013, Office of Water.
- EPA, 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System. EPA-833-R-00-003, Office of Wastewater Management.
- EPA, 2000. Method Guidance and Recommendations for Whole Effluent (WET) Testing. EPA-821-B-00-04, Office of Water
- APHA, 2012. Standard Methods for The Examination of Water and Wastewater. 22nd Edition.

APPENDIX A
CHAIN-OF-CUSTODY DOCUMENTS

Bio-Analytical Laboratories
 3240 Spurgeon Rd
 Doyline, LA 71023
 (318)7452772

Temperature upon arrival: 1.8°C
 Thermometer #: 29
 Tech: EAB
 Date: 1/8/24

Date 1-8-24 Time 1230
 Sampler Printed Name KEVIN FUNDENBURG
 Sampler Signature CLIENT
 Sampler Affiliation NASH

Clear in color ^{sub}
 No odor 1/8/24

Please analyze the following samples:

Sample Identification	Client Code	Date	Time	Testing Required- FCSC
Bio Monitoring	NASH	1-7-24	0930-2100	Chronic ceriodaphnia dubia Chronic pimephales promelas

Requires RUSH status (24 hr Turn Around Time) Requires E-mail as soon as completed
 (skeeter@ana-lab.com)

If you have any questions about the testing, contact Skeeter Ludewig at (903)984-0551.

Date	Time	Relinquished		Received	
1-8-24	1530	Printed Name MARK HIRSCH	Affiliation SAL	Printed Name D. J. Burpp	Affiliation BAC
		Signature <i>Mark Hirsch</i>		Signature	
		Printed Name	Affiliation	Printed Name	Affiliation
		Signature		Signature	
		Printed Name	Affiliation	Printed Name	Affiliation
		Signature		Signature	
		Printed Name	Affiliation	Printed Name	Affiliation
		Signature		Signature	

X9060
 C26138

Bio-Analytical Laboratories
 3240 Spurgeon Rd
 Doyline, LA 71023
 (318)7452772

Temperature upon arrival: 1,0°C
 Thermometer #: 29
 Tech: EGB
 Date: 1/10/24

Date 1-10-24 Time 1100

Sampler Printed Name KEVIN FUNDERBORK

Color: Clear
 Odor: none

Sampler Signature CLIENT

Sampler Affiliation NASH

Please analyze the following samples:

Sample Identification	Client Code	Date	Time	Testing Required- FCSC
Bio Monitoring	NASH	1.9.24	0930-- 2236	Chronic ceriodaphnia dubia Chronic pimephales promelas

Requires RUSH status (24 hr Turn Around Time) Requires E-mail as soon as completed
 (skeeter@ana-lab.com)

If you have any questions about the testing, contact Skeeter Ludewig at (903)984-0551.

Date	Time	Relinquished		Received	
1-10-24	1410	Printed Name <u>Mark Hansen</u>	Affiliation <u>SPL</u>	Printed Name <u>Carl Brugg</u>	Affiliation <u>BAL</u>
		Signature <u>Mark Hansen</u>		Signature	
		Printed Name	Affiliation	Printed Name	Affiliation
		Signature		Signature	
		Printed Name	Affiliation	Printed Name	Affiliation
		Signature		Signature	
		Printed Name	Affiliation	Printed Name	Affiliation
		Signature		Signature	

X9060 / C26150

Bio-Analytical Laboratories
 3240 Spurgeon Rd
 Doyline, LA 71023
 (318)7452772

Temperature upon arrival: 2.2°C
 Thermometer #: 29
 Tech: EUB
 Date: 1/12/24

Date 1-12-24 Time 1215

Sampler Printed Name KEVIN FUNDERBURK

Sampler Signature CLIENT

Sampler Affiliation NASH

Clear in color, EUB
 no odor 1/12/24

Please analyze the following samples:

Sample Identification	Client Code	Date	Time	Testing Required- FCSC
Bio Monitoring	NASH	1/11/24	0900 2109	Chronic ceriodaphnia dubia Chronic pimephales promelas

Requires RUSH status (24 hr Turn Around Time) Requires E-mail as soon as completed
 (skeeter@ana-lab.com)

If you have any questions about the testing, contact Skeeter Ludewig at (903)984-0551.

Date	Time	Relinquished		Received	
1-12-24	1433	Printed Name <u>Mark Hirsch</u>	Affiliation <u>SPI</u>	Printed Name <u>Kevin Funderburk</u>	Affiliation <u>EUB</u>
		Signature <u>[Signature]</u>		Signature <u>[Signature]</u>	
		Printed Name	Affiliation	Printed Name	Affiliation
		Signature		Signature	
		Printed Name	Affiliation	Printed Name	Affiliation
		Signature		Signature	
		Printed Name	Affiliation	Printed Name	Affiliation
		Signature		Signature	

**APPENDIX B
RAW DATA SHEETS**

BIO-ANALYTICAL LABORATORIES CERIODAPHNIA DUBIA SURVIVAL AND REPRODUCTION TEST

Project# X9060 Date start: 1/9/24 Date end: 1/14/24

Client/Contact: NASH/Nashville Public Works

Address: 426 North Main, Nashville, AR 71852

NPDES#: AR0021776/ AFIN 31-00036

Sample Description: 001 Dilution Water: MH RECONSTITUTED

Adults isolated: Date 1/8/24 Time: 2315

Neonates collected: Date 1/9/24 Time: 0645 Board: 24-1, 25 m#

Dissolved Oxygen Meter#: 2

pH Meter#: 3 Conductivity Meter#: 9

ORP Meter#: — Salinity Meter#: —

Effluent Initial D.O. (mg/L & %)/Tech	Aerate?/Minutes /Final D.O. (mg/L & %)/Tech	Receiving Water Initial D.O. (mg/L & %)/Tech	Aerate?/Minutes /Final D.O. (mg/L & %)/Tech
0. <u>8.2/99.9% / 50u</u>	0. <u>NO / 50u</u>	0. _____	0. _____
1. <u>9.3/112.6% / 50u</u>	1. <u>Y/6/8.5/100.1% / 50u</u>	1. _____	1. _____
2. <u>9.8/118.7% / 50u</u>	2. <u>Y/6/8.4/99.8% / 50u</u>	2. _____	2. _____
3. <u>9.9/118.9% / 50u</u>	3. <u>Y/6/8.2/99.2% / 50u</u>	3. _____	3. _____
4. <u>10.3/120.1% / 50u</u>	4. <u>Y/6/8.4/100.1% / 50u</u>	4. _____	4. _____
5. <u>8.5/99.9% / 50u</u>	5. <u>NO / 50u</u>	5. _____	5. _____
6. <u>10.9/130.7% / 50u</u>	6. <u>Y/6/8.4/99.2% / 50u</u>	6. _____	6. _____
7. _____	7. _____	7. _____	7. _____

Total Residual Chlorine (mg/L)/Tech

- 1. <0.5 / 50u
- 2. <0.5 / 50u
- 3. <0.5 / 50u

Dechlorinated? Amount?/Tech

- 1. NO / 50u
- 2. NO / 50u
- 3. NO / 50u

Ammonia (NH3) (mg/L)/Tech

- 1. <0.5 / 50u
- 2. <0.5 / 50u
- 3. <0.5 / 50u

BAL Sample # Date in use

- 1. C26127 1/9/24
- 2. C26138 1/11/24
- 3. C26150 1/13/24

Comments:

CERIODAPHNIA DUBIA SURVIVAL AND REPRODUCTION TEST-LIVE NEONATE PRODUCTION

Project# X9060 Client Nashville Public Works Sample ID 201
 Test started: Date 1/14/04 Time 1255 Test ended: Date 1/16/04 Time 1415
 Date/Tech: Day 0 1/14/04 1 1/15/04 2 1/16/04 3 1/17/04 4 1/18/04 5 1/19/04 6 1/20/04 7 1/21/04 8
 Time: Day 0 1255 1 1343 2 1435 3 1055 4 1235 5 1130 6 1300 7 1415 8
 Temp. (°C): Day 0 24.3 1 24.0 2 23.7 3 24.0 4 24.0 5 24.2 6 24.2 7 24.3 8

Conc	Day	1	2	3	4	5	6	7	8	9	10	Number of Live	
0 mH	1	0										10	
	2	0										10	
	3	0										10	
	4	1/2	0	1/3	1/1	1/3	1/3	1/2	1/3	1/2	1/2	10	
	5	0	1/5	0	0	0	0	0	0	0	0	10	
	6	X	2/7	2/4	2/7	2/6	2/6	0	0	0	2/5	2/6	9
	7	↑	3/10	3/14	3/10	3/7	3/8	2/10	2/11	3/10	3/10	3/10	8
	8												
25	1	0										10	
	2	0										10	
	3	0										10	
	4	1/2	1/3	1/3	1/1	0	0	1/2	0	1/2	1/3	10	
	5	0	0	0	1/2	1/3	1/5	0	1/3	0	0	10	
	6	0	2/6	0	2/8	2/6	0	0	0	2/9	0	10	
	7	2/6	3/8	2/15	3/8	3/10	2/8	2/9	2/12	2/9	2/10	2/10	10
	8												
33	1	0										10	
	2	0										10	
	3	0										10	
	4	1/2	1/3	1/2	1/4	1/3	1/3	1/2	1/3	1/2	1/3	10	
	5	0	0	0	0	0	0	0	0	0	0	10	
	6	2/5	2/6	2/5	2/8	2/6	2/6	2/5	2/6	X	2/6	2/6	9
	7	X	3/8	3/8	3/7	3/10	3/7	3/5	3/7	↑	3/7	3/7	8
	8												
44	1	0										10	
	2	0										10	
	3	0										10	
	4	1/3	1/2	1/3	1/4	1/3	1/2	1/3	1/3	1/2	1/1	10	
	5	X	0	0	0	0	0	0	0	0	1/2	9	
	6	↑	2/2	0	2/7	2/6	2/5	2/9	2/7	2/8	2/4	2/4	9
	7	↑	2/7	2/7	3/10	3/7	3/10	3/9	3/7	3/10	3/10	3/10	9
	8												
59	1	0										10	
	2	0										10	
	3	0										10	
	4	1/3	1/2	1/2	1/4	1/2	1/3	1/2	1/3	1/2	1/1	10	
	5	0	0	X	0	0	0	0	0	0	0	9	
	6	2/6	2/5	↑	2/8	2/4	2/6	0	2/7	2/4	2/7	2/7	9
	7	3/10	3/9	↑	2/6	2/8	3/7	2/8	3/5	3/7	3/6	3/6	9
	8												
78	1	0										10	
	2	0										9	
	3	0								X	0	9	
	4	1/3	1/3	1/4	1/3	1/2	1/2	1/3	1/2	↑	0	9	
	5	0	0	0	0	0	0	0	0	↑	0	9	
	6	2/1	2/12	0	2/7	2/5	2/4	2/6	2/5	↑	1/5	9	
	7	2/10	3/11	2/8	3/7	2/9	3/10	3/8	3/8	↑	0	9	
	8										2/17	9	

Key: X=dead adult, Xⁿ=adult had n neonates before death, M=male.
 B/N = Brood count/#neonates

BIO-ANALYTICAL LABORATORIES CHRONIC WATER QUALITY DATA

Project# X9060 Client Nashville Public Works Organism C. dubia

Date	Day 0 1/12/24 5580	Day 1 1/10/24	Day 2 1/11/24	Day 3 1/12/24	Day 4 1/13/24	Day 5 1/14/24	Day 6 1/15/24	Day 7 1/16/24	Day 8
Concentration:	0 mH 5583 5583								
Temperature (°C)	22.1	23.8 23.0	23.3 23.0	23.8 23.3	23.1 23.0	24.1 23.1	23.2 20.3	23.2	
pH	7.2	7.1 7.8	7.3 7.7	7.4 7.9	7.3 8.2	7.3 7.3	7.1	7.7	
DO (mg/l)	8.4	7.3 8.3	7.2 8.2	7.2 8.0	7.4 7.4	7.2 8.2	7.6 8.8	7.7	
Cond (umhos/cm)	330	322	334	330	332	330	369		
Concentration:	25								
Temperature (°C)	22.5	24.0 23.0	23.7 23.2	23.8 24.4	23.4 23.1	24.2 23.2	23.4 20.9	23.4	
pH	7.3	7.2 7.8	7.2 7.7	7.4 7.9	7.6 8.2	7.4 7.5	8.0 7.5	7.7	
DO (mg/l)	8.2	7.3 7.9	7.4 7.9	7.3 7.4	7.2 7.5	7.7 7.7	8.0 8.7	8.0	
Cond (umhos/cm)	327	328	340	332	323	329	355		
Concentration:	33								
Temperature (°C)	22.5	23.9 22.8	23.8 23.2	23.9 24.5	23.5 23.1	24.3 23.7	23.6 21.1	23.6	
pH	7.3	7.2 7.8	7.3 7.7	7.2 7.9	7.3 8.1	7.4 7.5	8.0 7.6	8.0	
DO (mg/l)	8.4	7.4 8.1	7.2 7.7	7.3 7.4	7.1 7.7	7.1 7.9	8.1 8.7	8.0	
Cond (umhos/cm)	326	319	334	334	320	328	349		
Prerenewal Tech Initials/Time		EDW 1343	EDW 1435	EDW 1055	EDW 1235	EDW 1130	1300 AN	EDW 1415	
Postrenewal Tech Initials/Time	EDW 1050	EDW 1110	EDW 1115	EDW 0815	EDW 1008	EDW 0945	1200 AN		

BIO-ANALYTICAL LABORATORIES CHRONIC WATER QUALITY DATA

Project# X9060 Client Nashville Public Works Organism C. dubia

Date	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Concentration: 440/10									
Temperature (°C)	22.8	23.1 23.7	23.3 23.3	23.9 25.0	23.5 23.6	23.9 23.9	23.5 21.9	23.5	
pH	7.3	7.2 7.8	7.2 7.7	7.3 7.9	7.4 8.0	7.5 7.5	8.2 7.6	8.0	
DO (mg/l)	8.4	7.4 8.2	7.3 8.2	7.4 7.5	7.2 7.9	7.4 8.1	8.0 8.4	8.0	
Cond (umhos/cm)	324	322	330	332	316	325	331		
Concentration: 590/10									
Temperature (°C)	23.0	23.8 23.1	23.7 23.4	23.8 25.2	23.6 23.0	24.6 23.9	23.7 21.9	23.5	
pH	7.3	7.1 7.8	7.3 7.7	7.4 7.8	7.0 7.9	7.3 7.4	8.2 7.6	8.1	
DO (mg/l)	8.4	7.3 8.1	7.4 8.1	7.5 7.6	7.0 7.9	7.1 8.1	8.2 8.4	8.1	
Cond (umhos/cm)	320	317	322	327	310	318	329		
Concentration: 780/10									
Temperature (°C)	23.1	24.0 23.1	23.8 23.7	24.0 25.2	23.5 23.1	24.7 24.4	23.8 22.4	23.4	
pH	7.3	7.3 7.7	7.2 7.6	7.3 7.8	7.1 7.9	7.2 7.3	8.1 7.6	8.1	
DO (mg/l)	8.5	7.8 8.5	7.3 8.4	7.4 7.9	8.0 8.2	7.6 8.4	7.7 8.3	8.0	
Cond (umhos/cm)	314	319	314	318	305	314	318		
Prerenewal Tech Initials/Time		EDW 1343	EDW 1435	EDW 1055	EDW 1235	EDW 1130	1300 Pn	EDW 1415	
Postrenewal Tech Initials/Time	EDW 1050	EDW 1110	EDW 1115	EDW 0985	EDW 1008	EDW 0985	1200 Pn		

BIO-ANALYTICAL LABORATORIES
CERIODAPHNIA DUBIA CHRONIC TEST
PARENTAL BLOCK ASSIGNMENT RANDOM NUMBER TEMPLATE #3
FOR 6 CONCENTRATION TEST

Set #1

2,4,1,6,3,5 Parent# 1F 25 m#

Set #2

3,5,1,2,4,6 Parent# 1G 25 m#

Set #3

3,6,2,1,5,4 Parent# 1I 25 m#

Set #4

2,4,6,1,5,3 Parent# 25 m# 3C

Set #5

6,2,5,3,1,4 Parent# 3D 25 m#

Set #6

1,3,6,5,2,4 Parent# 3F 24-1 m#

Set #7

1,2,5,6,3,4 Parent# 3G 24-1 m#

Set #8

1,6,5,3,2,4 Parent# 4C 24-1 m#

Set #9

1,6,2,4,5,3 Parent# 5D 24-1 m#

Set #10

2,1,5,3,6,4 Parent# 6E 24-1 m#

SPW
1/9/24

CETIS Test Data Worksheet

Report Date: 08 Jan-24 09:08 (p 1 of 2)
 Test Code/ID: 655DDBD3 / 17-0064-9939

Ceriodaphnia 7-d Survival and Reproduction Test				Bio-Analytical Laboratories			
Start Date: 09 Jan-24	Species: Ceriodaphnia dubia	Sample Code: 55E3129A					
End Date: 16 Jan-24	Protocol: EPA/821/R-02-013 (2002)	Sample Source: AR0021776					
Sample Date: 08 Jan-24	Material: POTW Effluent	Sample Station: 001					

Conc-%	Code	Rep	Pos	# Exposed	1d Surv	2d Surv	3d Surv	4d Surv	5d Surv	6d Surv	7d Surv	8d Surv	2d Neo	3d Neo	4d Neo	5d Neo	6d Neo	7d Neo	8d Neo	Male	Notes	
33		6	1																			
59		8	2																			
33		5	3																			
25		8	4																			
44		9	5																			
33		9	6																			
33		3	7																			
44		6	8																			
59		7	9																			
44		8	10																			
78		3	11																			
25		3	12																			
59		1	13																			
25		2	14																			
0	D	7	15																			
59		2	16																			
33		10	17																			
44		3	18																			
78		4	19																			
25		5	20																			
33		2	21																			
0	D	9	22																			
33		8	23																			
44		10	24																			
44		1	25																			
0	D	8	26																			
25		4	27																			
78		8	28																			
59		10	29																			
44		4	30																			
25		9	31																			
78		1	32																			
44		5	33																			
59		9	34																			
0	D	2	35																			
78		5	36																			
0	D	6	37																			
25		1	38																			
25		7	39																			
33		4	40																			
33		1	41																			

CETIS Test Data Worksheet

Report Date: 08 Jan-24 09:08 (p 2 of 2)
 Test Code/ID: 655DDBD3 / 17-0064-9939

Conc-%	Code	Rep	Pos	# Exposed	1d Surv	2d Surv	3d Surv	4d Surv	5d Surv	6d Surv	7d Surv	8d Surv	2d Neo	3d Neo	4d Neo	5d Neo	6d Neo	7d Neo	8d Neo	Male	Notes	
0	D	5	42																			
25		10	43																			
0	D	10	44																			
0	D	1	45																			
59		5	46																			
78		6	47																			
78		7	48																			
59		6	49																			
44		7	50																			
59		4	51																			
78		2	52																			
78		9	53																			
44		2	54																			
59		3	55																			
33		7	56																			
0	D	4	57																			
0	D	3	58																			
25		6	59																			
78		10	60																			

BIO-ANALYTICAL LABORATORIES
PIMEPHALES PROMELAS SURVIVAL AND GROWTH DATA SHEET

Project# X9060 Date started: 1/8/24 Date ended 1/15/24

Client/Contact: NASH/Nashville Public Works

Address: 426 North Main, Nashville, AR 71852

NPDES# AR0021776/AFIN 31-00036

Sample Description: 001 Dilution Water: MH Reconstituted
Test organism age: <24 hours Vendor/ID# BAL 010824

Feeding Times

Day	Technician/Time/Amount (per replicate)		
	AM	NOON	PM
0			
1	EDW/0910/0.10ml	EDW/1210/0.10ml	EDW/1750/0.20ml
2	EDW/1015/0.10ml	EDW/1330/0.10ml	EDW/1802/0.10ml
3	EDW/0910/0.10ml	EDW/1305/0.10ml	EDW/1735/0.10ml
4	EDW/0740/0.10ml	EDW/1105/0.10ml	EDW/1845/0.10ml
5	EDW/0920/0.20ml		EDW/1815/0.1ml
6	EDW/0830/0.20ml		EDW/1555/0.20ml
			EDW/1420/0.20ml

Dissolved Oxygen Meter #: 2
pH Meter #: 3 Conductivity Meter #: 9
ORP Meter #: Salinity Meter #:

Effluent Initial DO (mg/L & %)/Tech	Aerate?/Minutes /Final DO (mg/L & %)/Tech	Receiving Water Initial DO (mg/L & %)/Tech	Aerate?/Minutes /Final DO (mg/L & %)/Tech
0. <u>8.3/99.6%</u> / EDW	0. <u>NO</u> / EDW	0. <u> </u>	0. <u> </u>
1. <u>8.2/99.9%</u> / EDW	1. <u>NO</u> / EDW	1. <u> </u>	1. <u> </u>
2. <u>9.3/112.6%</u> / EDW	2. <u>X/6/85/100.1%</u> / EDW	2. <u> </u>	2. <u> </u>
3. <u>9.8/118.7%</u> / EDW	3. <u>X/6/84/99.8%</u> / EDW	3. <u> </u>	3. <u> </u>
4. <u>9.9/118.9%</u> / EDW	4. <u>X/6/82/99.2%</u> / EDW	4. <u> </u>	4. <u> </u>
5. <u>10.3/120.1%</u> / EDW	5. <u>X/6/84/100.1%</u> / EDW	5. <u> </u>	5. <u> </u>
6. <u>8.5/99.9%</u> / EDW	6. <u>NO</u> / EDW	6. <u> </u>	6. <u> </u>
<u>Total Residual Chlorine (mg/L) / Tech</u>	<u>Dechlorinated? Amount?/Tech</u>	<u>Ammonia (NH3) (mg/L) /Tech</u>	<u>BAL Sample # Date in use</u>
1. <u><0.5</u> / EDW	1. <u>NO</u> / EDW	1. <u><0.5</u> / EDW	1. <u>C26127 1/8/24</u>
2. <u><0.5</u> / EDW	2. <u>NO</u> / EDW	2. <u><0.5</u> / EDW	2. <u>C26138 1/11/24</u>
3. <u><0.5</u> / EDW	3. <u>NO</u> / EDW	3. <u><0.5</u> / EDW	3. <u>C26150 1/13/24</u>

Comments:

BIO-ANALYTICAL LABORATORIES 7-DAY CHRONIC MINNOW SURVIVAL DATA- EPA 1000, OECD 210

Project# X9060 Test started: Date 1/8/04 Time 1720

Client Nashville P.W. Sample ID 001 Test ended: Date 1/8/04 Time 1615

Date/Tech: Day 0 1/8/04/son 1 1/9/04/son 2 1/10/04/son 3 1/11/04/son 4 1/12/04/son 5 1/13/04/son 6 1/14/04/son 7 1/15/04/son
 Time: Day 0 1720 1 1200 2 1225 3 1305 4 0835 5 1045 6 1010 7 1615
 Temp (°C) Day 0 25.0 1 24.8 2 24.3 3 24.9 4 24.7 5 24.0 6 24.2 7 24.8

0/0 Conc.	Rep.	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0 mtl	1	8	8	8	8	8	8	8	8
	2	8	8	8	6	6	6	6	6
	3	8	8	8	8	8	8	8	8
	4	8	8	8	8	8	8	8	7
	5	8	8	8	8	8	8	8	8
25	1	8	8	8	8	8	8	8	7
	2	8	8	8	8	8	8	8	7
	3	8	8	8	8	8	8	8	8
	4	8	8	8	8	8	8	8	7
	5	8	8	8	7	7	6	6	6
33	1	8	8	8	8	8	8	8	8
	2	8	8	8	8	8	7	7	6
	3	8	8	8	8	8	8	8	8
	4	8	8	8	8	8	8	8	8
	5	8	8	8	6	6	5	5	5
44	1	8	8	8	8	8	8	8	7
	2	8	8	8	8	8	8	8	7
	3	8	8	8	8	8	8	8	8
	4	8	8	8	8	8	8	8	8
	5	8	8	8	8	8	7	7	7
59	1	8	8	8	8	8	8	8	8
	2	8	8	8	8	8	8	8	8
	3	8	8	8	8	8	7	7	7
	4	8	8	8	8	8	8	8	8
	5	8	8	8	8	8	7	7	7
78	1	8	8	8	8	8	7	8.7	7
	2	8	8	8	8	8	8	8	8
	3	8	8	8	8	8	8	8	8
	4	8	8	8	8	8	8	8	8
	5	8	8	8	8	8	8	8	8

8.80/1/11/04

Project#/Client X9060 Temp Start (°C) 12.1 Tech EDM Date: 1/31/24 Time: 1615
Nashville Temp End (°C) 17.5 Tech EB Date: 1/31/24 Time: 0815

Conc. %	Replicate/ Pan number	Wt. of pan(g)/ Date: <u>1/11/23</u> Tech: <u>MV</u>	Wt. of pan + larvae(g)/ Date: <u>1/17/24</u> Tech: <u>PM</u>	Total wt. of larvae (g)	Original # of larvae at test initiation	Mean Dry wt. of larvae (mg)	Mean Dry wt. - surviving larvae (mg) Control Only*
0 50F	1	156	1.0422	1.0445			
	2	157	1.0510	1.0600			
	3	158	1.0484	1.0516			
	4	159	1.0330	1.0359			
	5	160	1.0391	1.0408			
25	1	161	1.0490	1.0515			
	2	162	1.0594	1.0612			
	3	163	1.0541	1.0568			
	4	164	1.0441	1.0469			
	5	165	1.0558	1.0582			
33	1	166	1.0509	1.0541			
	2	167	1.0548	1.0574			
	3	168	1.0409	1.0442			
	4	169	1.0389	1.0412			
	5	170	1.0354	1.0380			
44	1	171	1.0430	1.0455			
	2	172	1.0403	1.0424			
	3	173	1.0137	1.0154			
	4	174	1.0431	1.0451			
	5	175	1.0648	1.0667			
59	1	176	1.0659	1.0675			
	2	177	1.0558	1.0580			
	3	178	1.0548	1.0575			
	4	179	1.0239	1.0250			
	5	180	1.0648	1.0678			
78	1	181	1.0707	1.0725			
	2	182	1.0726	1.0751			
	3	183	1.0500	1.0522			
	4	184	1.0671	1.0700			
	5	185	1.0768	1.0799			

* Test acceptance of control weight based on surviving larvae at end of test.
 Calculated by: CE 115 Calculations checked by: EB 1/22/24

BIO-ANALYTICAL LABORATORIES CHRONIC WATER QUALITY DATA

Project# X9060 Client Nashville Public Works Organism P. promelas

Date	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
	1/8/24 5582	1/9/24	1/10/24	1/11/24	1/12/24	1/13/24	1/14/24	1/15/24	
Concentration:	0 mH		5586						
Temperature (°C)	24.1	24.0 23.4	24.1 23.4	24.6 23.0	23.9 24.4	23.4 23.1	23.9 23.2	24.1	
pH	7.4	7.0 7.0	7.8 7.4	7.3 7.5	7.4 7.2	8.1 7.3	7.9 7.3	7.4	
DO (mg/l)	8.0	7.0 8.1	7.2 8.2	7.3 8.1	7.4 7.9	7.4 8.2	7.3 8.3	7.6	
Cond (umhos/cm)	320	325	322	320	325	333	338		
Concentration:	250/0								
Temperature (°C)	23.9	24.1 23.5	23.9 23.0	24.8 23.3	24.1 25.1	23.5 23.0	24.1 23.2	23.5	
pH	7.4	7.3 7.1	7.2 7.9	7.4 7.6	7.2 7.3	7.4 8.2	7.5 7.4	7.5	
DO (mg/l)	7.8	7.2 7.9	7.0 8.1	7.3 8.1	7.4 7.5	7.3 8.0	7.4 8.0	7.5	
Cond (umhos/cm)	321	320	321	342	340	326	330		
Concentration:	330/0								
Temperature (°C)	23.8	23.9 23.5	24.0 23.0	24.9 23.3	23.9 25.4	23.7 23.0	24.2 23.9	24.1	
pH	7.7	7.2 7.1	7.3 7.9	7.3 7.5	7.2 7.1	7.4 8.1	7.3 7.2	7.4	
DO (mg/l)	8.2	7.0 8.1	7.0 8.3	7.4 8.2	7.4 7.5	7.3 8.1	7.6 8.2	7.6	
Cond (umhos/cm)	320	319	321	338	337	323	329		
Prerenewal Tech Initials/Time		EPW 1200	EPW 1225	EPW 1305	EPW 0835	EPW 1045	EPW 1010	EPW 1615	
Postrenewal Tech Initials/Time	EPW 1650	EPW 1050	EPW 1110	EPW 1115	EPW 0815	EPW 1008	EPW 0915		

BIO-ANALYTICAL LABORATORIES CHRONIC WATER QUALITY DATA

Project# X9060 Client Nashville Public Works Organism P. promelas

Date	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Concentration: <u>44%</u>									
Temperature (°C)	23.7	23.9 23.6	24.0 23.1	24.6 23.6	24.5 25.8	23.9 23.2	24.1 24.1	23.9	
pH	7.3	7.2 7.2	7.1 7.8	7.4 7.5	7.5 7.3	7.4 8.0	7.5 7.2	7.9	
DO (mg/l)	8.1	7.1 8.2	7.3 8.3	7.6 8.3	7.7 7.4	7.3 8.1	7.4 8.1	7.6	
Cond (umhos/cm)	319	321	322	331	336	320	325		
Concentration: <u>59%</u>									
Temperature (°C)	23.7	24.1 23.6	24.1 23.2	24.5 23.7	24.6 26.0	24.1 23.2	23.9 24.2	24.1	
pH	7.3	7.2 7.2	7.3 7.8	7.4 7.5	7.3 7.3	7.3 7.6	7.3 7.2	7.5	
DO (mg/l)	8.1	7.4 8.5	7.3 8.4	7.3 8.3	7.4 7.6	7.3 7.7	7.4 8.3	7.5	
Cond (umhos/cm)	315	316	319	323	330	313	311		
Concentration: <u>78%</u>									
Temperature (°C)	23.4	23.9 23.8	23.9 23.6	24.6 24.0	24.5 26.1	23.9 23.9	24.1 24.6	24.1	
pH	7.3	7.0 7.2	7.4 7.7	7.3 7.4	7.5 7.4	7.4 7.9	7.5 7.2	7.6	
DO (mg/l)	8.2	7.4 8.1	7.6 8.2	7.2 8.1	7.4 7.9	7.2 8.2	7.3 8.5	7.5	
Cond (umhos/cm)	310	316	318	314	323	308	316		
Prerenewal Tech Initials/Time		EDW 1200	EDW 1225	EDW 1305	EDW 0835	EDW 1045	EDW 1010	EDW 1615	
Postrenewal Tech Initials/Time	EDW 1650	EDW 1050	EDW 1110	EDW 1115	EDW 0815	EDW 1008	EDW 0915		

CETIS Test Data Worksheet

Report Date: 08 Jan-24 09:10 (p 1 of 1)
 Test Code/ID: 686A8C45 / 17-5181-3189

Fathead Minnow 7-d Larval Survival and Growth Test Bio-Analytical Laboratories

Start Date: 08 Jan-24	Species: Pimephales promelas	Sample Code: 2C7CA977
End Date: 15 Jan-24	Protocol: EPA/821/R-02-013 (2002)	Sample Source: AR0021776
Sample Date: 08 Jan-24	Material: POTW Effluent	Sample Station: 001

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
78		3	1												
59		2	2												
33		1	3												
• 33		2	4												
44		5	5												
44		2	6												
33		5	7												
• 59		3	8												
0	D	4	9												
59		1	10												
0	D	5	11												
78		1	12												
• 78		5	13												
25		4	14												
33		4	15												
0	D	1	16												
78		4	17												
44		1	18												
0	D	3	19												
59		5	20												
• 25		5	21												
• 25		1	22												
44		4	23												
• 78		2	24												
25		3	25												
44		3	26												
25		2	27												
59		4	28												
0	D	2	29												
33		3	30												

EUB
1/8/24

Control Water ID#	Total Alkalinity	Date Tested	Total Hardness	Date Tested
5580	60.0	1/4/24	84.0	1/4/24
5582	68.0	1/11/24	104.0	1/11/24
5583	60.0		96.0	
5586	68.0	1/18/24	96.0	1/18/24
Sample ID#	Total Alkalinity	Date Tested	Total Hardness	Date Tested
C210127	104.0	1/11/24	108.0	1/11/24
C210138	88.0		100.0	
C210150	40.0	1/18/24	88.0	1/18/24
Test Blank ID#	Total Alkalinity	Date Tested	Total Hardness	Date Tested

Results are in mg/L CaCO3

APPENDIX C
STATISTICAL ANALYSES

CETIS Analytical Report

Report Date: 18 Jan-24 15:16 (p 1 of 2)
Test Code/ID: 655DDBD3 / 17-0064-9939

Ceriodaphnia 7-d Survival and Reproduction Test			Bio-Analytical Laboratories		
Analysis ID: 19-6839-5551	Endpoint: 7d Survival Rate	CETIS Version: CETIS v2.1.5			
Analyzed: 18 Jan-24 15:16	Analysis: STP 2xK Contingency Tables	Status Level: 1			
Edit Date: 18 Jan-24 15:07	MD5 Hash: DC0C7BD062145A6D81F87ABA3ABF5E1	Editor ID: 008-522-314-5			
Batch ID: 13-9909-8442	Test Type: Reproduction-Survival (2-8d)	Analyst: Lab Tech			
Start Date: 09 Jan-24 12:55	Protocol: EPA/821/R-02-013 (2002)	Diluent: Reconstituted Water			
Ending Date: 16 Jan-24 14:15	Species: Ceriodaphnia dubia	Brine:			
Test Length: 7d 1h	Taxon: Branchiopoda	Source: In-House Culture	Age: <24		
Sample ID: 14-4094-4794	Code: X9060	Project: WET Monthly Compliance Test (JAN)			
Sample Date: 07 Jan-24 21:00	Material: POTW Effluent	Source: AR0021776 (AR0021776)			
Receipt Date: 08 Jan-24 15:30	CAS (PC):	Station: 001			
Sample Age: 40h (1.8 °C)	Client: Nashville Public Works				

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units
Untransformed	C > T	78	>78	---	1.3

Fisher Exact/Bonferroni-Holm Test

Control	vs	Conc-%	Test Stat	P-Type	P-Value	Decision(α:5%)
Dilution Water		25	1.0000	Exact	1.0000	Non-Significant Effect
		33	0.7090	Exact	1.0000	Non-Significant Effect
		44	0.8947	Exact	1.0000	Non-Significant Effect
		59	0.8947	Exact	1.0000	Non-Significant Effect
		78	0.8947	Exact	1.0000	Non-Significant Effect

7d Survival Rate Frequencies

Conc-%	Code	NR	R	NR + R	Prop NR	Prop R	%Effect
0	D	8	2	10	0.8000	0.2000	0.00%
25		10	0	10	1.0000	0.0000	-25.00%
33		8	2	10	0.8000	0.2000	0.00%
44		9	1	10	0.9000	0.1000	-12.50%
59		9	1	10	0.9000	0.1000	-12.50%
78		9	1	10	0.9000	0.1000	-12.50%

7d Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	10	0.8000	0.4984	1.0000	1.0000	0.0000	1.0000	0.1333	52.70%	0.00%
25		10	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	-25.00%
33		10	0.8000	0.4984	1.0000	1.0000	0.0000	1.0000	0.1333	52.70%	0.00%
44		10	0.9000	0.6738	1.0000	1.0000	0.0000	1.0000	0.1000	35.14%	-12.50%
59		10	0.9000	0.6738	1.0000	1.0000	0.0000	1.0000	0.1000	35.14%	-12.50%
78		10	0.9000	0.6738	1.0000	1.0000	0.0000	1.0000	0.1000	35.14%	-12.50%

7d Survival Rate Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	D	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000
25		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
33		0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000
44		0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
59		1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
78		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000

ELB
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CETIS Analytical Report

Report Date: 18 Jan-24 15:16 (p 2 of 2)
 Test Code/ID: 655DDBD3 / 17-0064-9939

Ceriodaphnia 7-d Survival and Reproduction Test

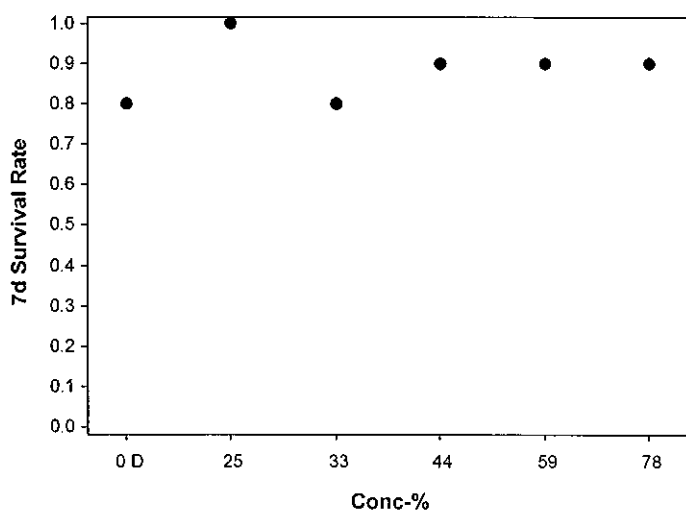
Bio-Analytical Laboratories

Analysis ID: 19-6839-5551 Endpoint: 7d Survival Rate CETIS Version: CETIS v2.1.5
 Analyzed: 18 Jan-24 15:16 Analysis: STP 2xK Contingency Tables Status Level: 1
 Edit Date: 18 Jan-24 15:07 MD5 Hash: DC0C7BD062145A6D81F87ABA3ABF5E1 Editor ID: 008-522-314-5

7d Survival Rate 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	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1
25		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
33		0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1
44		0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
59		1/1	1/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
78		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1

Graphics



EUB
1/22/24

CETIS Analytical Report

Report Date: 18 Jan-24 15:17 (p 1 of 2)
Test Code/ID: 655DDBD3 / 17-0064-9939

Ceriodaphnia 7-d Survival and Reproduction Test **SURVIVING** Bio-Analytical Laboratories

Analysis ID: 07-5207-3570	Endpoint: Reproduction	CETIS Version: CETIS v2.1.5
Analyzed: 18 Jan-24 15:16	Analysis: Parametric-Multiple Comparison	Status Level: 1
Edit Date: 18 Jan-24 15:07	MD5 Hash: 7D3FE59BA7F129AF8D3EC2D8E1187796	Editor ID: 008-522-314-5
Batch ID: 13-9909-8442	Test Type: Reproduction-Survival (2-8d)	Analyst: Lab Tech
Start Date: 09 Jan-24 12:55	Protocol: EPA/821/R-02-013 (2002)	Diluent: Reconstituted Water
Ending Date: 16 Jan-24 14:15	Species: Ceriodaphnia dubia	Brine:
Test Length: 7d 1h	Taxon: Branchiopoda	Source: In-House Culture Age: <24
Sample ID: 14-4094-4794	Code: X9060	Project: WET Monthly Compliance Test (JAN)
Sample Date: 07 Jan-24 21:00	Material: POTW Effluent	Source: AR0021776 (AR0021776)
Receipt Date: 08 Jan-24 15:30	CAS (PC):	Station: 001
Sample Age: 40h (1.8 °C)	Client: Nashville Public Works	

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Untransformed	C > T	78	>78	---	1.3	4.476	25.76%

Bonferroni Adj t Test

Control	vs	Conc-%	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		25	16	1.419	2.408	4.37	CDF	0.4061	Non-Significant Effect
		33	14	0.5882	2.408	4.606	CDF	1.0000	Non-Significant Effect
		44	15	0.0822	2.408	4.476	CDF	1.0000	Non-Significant Effect
		59	15	1.517	2.408	4.476	CDF	0.3399	Non-Significant Effect
		78	15	-1.353	2.408	4.476	CDF	1.0000	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	176.547	35.3094	5	2.413	0.0500	Significant Effect
Error	687.642	14.6307	47			
Total	864.189		52			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	8.955	15.09	0.1109	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9765	0.9398	0.3769	Normal Distribution

Reproduction Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	8	17.38	14.28	20.47	17	12	23	1.308	21.30%	0.00%
25		10	14.8	12.21	17.39	15	8	19	1.143	24.42%	14.82%
33		8	16.25	14.37	18.13	16	12	19	0.7962	13.86%	6.47%
44		9	17.22	13.88	20.57	17	10	22	1.451	25.27%	0.88%
59		9	14.56	12.83	16.28	15	10	18	0.7474	15.40%	16.23%
78		9	19.89	15.62	24.16	21	12	27	1.852	27.93%	-14.47%

Reproduction Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	D	22	23	18	16	17	12	14	17		
25		8	17	18	19	19	13	11	15	15	13
33		17	15	19	19	16	12	16	16		
44		11	10	21	16	17	21	17	20	22	
59		15	16	18	14	16	10	15	13	14	
78		14	26	12	27	16	16	21	25	22	

EBB
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CETIS Analytical Report

Report Date: 18 Jan-24 15:19 (p 1 of 2)
Test Code/ID: 655DDBD3 / 17-0064-9939

Ceriodaphnia 7-d Survival and Reproduction Test

Bio-Analytical Laboratories

Analysis ID: 07-1580-9196	Endpoint: Reproduction	CETIS Version: CETIS v2.1.5
Analyzed: 18 Jan-24 15:18	Analysis: Nonparametric-Control vs Treatments	Status Level: 1
Edit Date: 18 Jan-24 15:07	MD5 Hash: 70BBB1A664D1800DD054D4D76A38F4AC	Editor ID: 008-522-314-5
Batch ID: 13-9909-8442	Test Type: Reproduction-Survival (2-8d)	Analyst: Lab Tech
Start Date: 09 Jan-24 12:55	Protocol: EPA/821/R-02-013 (2002)	Diluent: Reconstituted Water
Ending Date: 16 Jan-24 14:15	Species: Ceriodaphnia dubia	Brine:
Test Length: 7d 1h	Taxon: Branchiopoda	Source: In-House Culture Age: <24
Sample ID: 14-4094-4794	Code: X9060	Project: WET Monthly Compliance Test (JAN)
Sample Date: 07 Jan-24 21:00	Material: POTW Effluent	Source: AR0021776 (AR0021776)
Receipt Date: 08 Jan-24 15:30	CAS (PC):	Station: 001
Sample Age: 40h (1.8 °C)	Client: Nashville Public Works	

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Untransformed	C > T	78	>78	---	1.3	5.937	37.34%

Steel Many-One Rank Sum Test

Control	vs	Conc.-%	df	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)
Dilution Water		25	18	95	75	2	CDF	0.5278	Non-Significant Effect
		33	18	92.5	75	4	CDF	0.4393	Non-Significant Effect
		44	18	104	75	3	CDF	0.8098	Non-Significant Effect
		59	18	83.5	75	4	CDF	0.1720	Non-Significant Effect
		78	18	112.5	75	4	CDF	0.9503	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	135.733	27.1467	5	0.8072	0.5496	Non-Significant Effect
Error	1816	33.6296	54			
Total	1951.73		59			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	6.543	15.09	0.2569	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9149	0.9459	0.0005	Non-Normal Distribution

Reproduction Summary

Conc.-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	10	15.9	11.7	20.1	17	2	23	1.859	36.97%	0.00%
25		10	14.8	12.21	17.39	15	8	19	1.143	24.42%	6.92%
33		10	13.9	9.99	17.81	16	2	19	1.729	39.32%	12.58%
44		10	15.8	11.44	20.16	17	3	22	1.925	38.53%	0.63%
59		10	13.3	10.08	16.52	14.5	2	18	1.422	33.82%	16.35%
78		10	17.9	12.05	23.75	18.5	0	27	2.588	45.72%	-12.58%

Reproduction Detail

Conc.-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	D	2	22	23	18	16	17	12	14	17	18
25		8	17	18	19	19	13	11	15	15	13
33		7	17	15	19	19	16	12	16	2	16
44		3	11	10	21	16	17	21	17	20	22
59		15	16	2	18	14	16	10	15	13	14
78		14	26	12	27	16	16	21	25	0	22

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1/22/24

CETIS Analytical Report

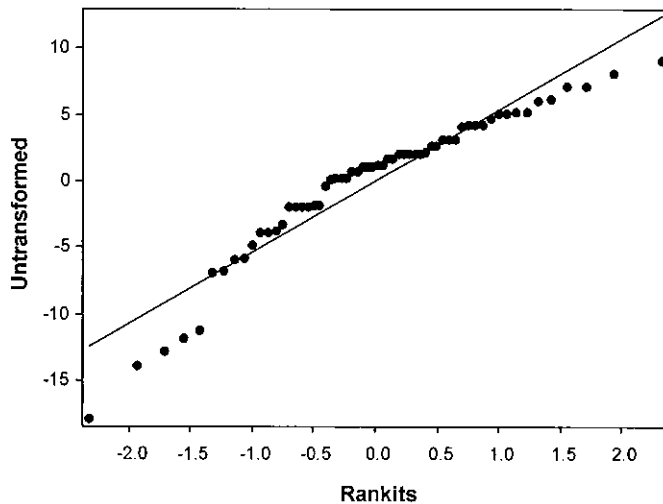
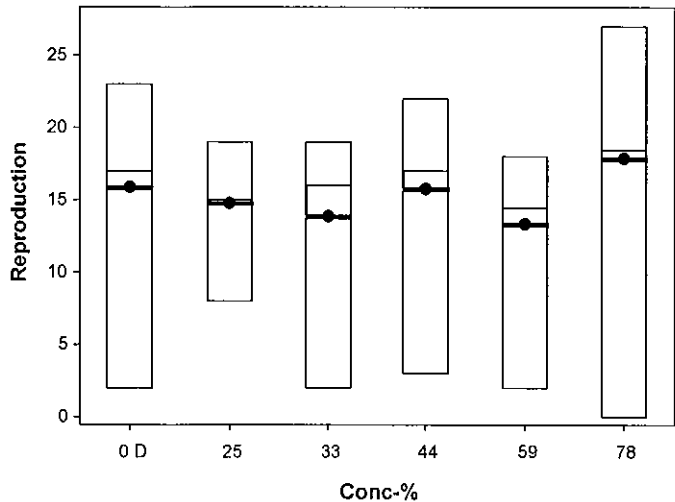
Report Date: 18 Jan-24 15:19 (p 2 of 2)
Test Code/ID: 655DDBD3 / 17-0064-9939

Ceriodaphnia 7-d Survival and Reproduction Test

Bio-Analytical Laboratories

Analysis ID: 07-1580-9196	Endpoint: Reproduction	CETIS Version: CETIS v2.1.5
Analyzed: 18 Jan-24 15:18	Analysis: Nonparametric-Control vs Treatments	Status Level: 1
Edit Date: 18 Jan-24 15:07	MD5 Hash: 70BBB1A664D1800DD054D4D76A38F4AC	Editor ID: 008-522-314-5

Graphics



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CETIS Analytical Report

Report Date: 18 Jan-24 15:19 (p 1 of 2)
Test Code/ID: 655DDBD3 / 17-0064-9939

Ceriodaphnia 7-d Survival and Reproduction Test

Bio-Analytical Laboratories

Analysis ID: 07-5056-7579	Endpoint: Reproduction	CETIS Version: CETIS v2.1.5
Analyzed: 18 Jan-24 15:19	Analysis: Linear Interpolation (ICPIN)	Status Level: 1
Edit Date: 18 Jan-24 15:07	MD5 Hash: 70BBB1A664D1800DD054D4D76A38F4AC	Editor ID: 008-522-314-5
Batch ID: 13-9909-8442	Test Type: Reproduction-Survival (2-8d)	Analyst: Lab Tech
Start Date: 09 Jan-24 12:55	Protocol: EPA/821/R-02-013 (2002)	Diluent: Reconstituted Water
Ending Date: 16 Jan-24 14:15	Species: Ceriodaphnia dubia	Brine:
Test Length: 7d 1h	Taxon: Branchiopoda	Source: In-House Culture Age: <24
Sample ID: 14-4094-4794	Code: X9060	Project: WET Monthly Compliance Test (JAN)
Sample Date: 07 Jan-24 21:00	Material: POTW Effluent	Source: AR0021776 (AR0021776)
Receipt Date: 08 Jan-24 15:30	CAS (PC):	Station: 001
Sample Age: 40h (1.8 °C)	Client: Nashville Public Works	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	644740	1000	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
IC15	>78	---	---	<1.3	---	---
IC20	>78	---	---	<1.3	---	---
IC25	>78	---	---	<1.3	---	---
IC40	>78	---	---	<1.3	---	---
IC50	>78	---	---	<1.3	---	---

Reproduction Summary

Conc-%	Code	Count	Calculated Variate						Isotonic Variate	
			Mean	Median	Min	Max	CV%	%Effect	Mean	%Effect
0	D	10	15.9	17	2	23	36.97%	0.00%	15.9	0.00%
25		10	14.8	15	8	19	24.42%	6.92%	15.14	4.78%
33		10	13.9	16	2	19	39.32%	12.58%	15.14	4.78%
44		10	15.8	17	3	22	38.53%	0.63%	15.14	4.78%
59		10	13.3	14.5	2	18	33.82%	16.35%	15.14	4.78%
78		10	17.9	18.5	0	27	45.72%	-12.58%	15.14	4.78%

Reproduction Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	D	2	22	23	18	16	17	12	14	17	18
25		8	17	18	19	19	13	11	15	15	13
33		7	17	15	19	19	16	12	16	2	16
44		3	11	10	21	16	17	21	17	20	22
59		15	16	2	18	14	16	10	15	13	14
78		14	26	12	27	16	16	21	25	0	22

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CETIS Analytical Report

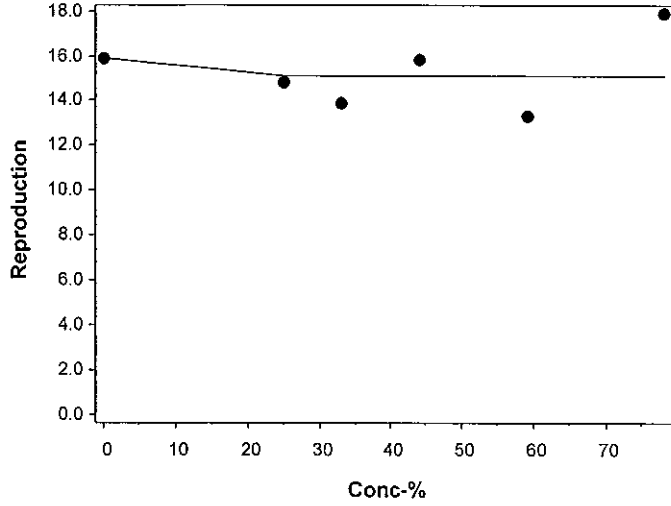
Report Date: 18 Jan-24 15:19 (p 2 of 2)
Test Code/ID: 655DDBD3 / 17-0064-9939

Ceriodaphnia 7-d Survival and Reproduction Test

Bio-Analytical Laboratories

Analysis ID: 07-5056-7579	Endpoint: Reproduction	CETIS Version: CETIS v2.1.5
Analyzed: 18 Jan-24 15:19	Analysis: Linear Interpolation (ICPIN)	Status Level: 1
Edit Date: 18 Jan-24 15:07	MD5 Hash: 70BBB1A664D1800DD054D4D76A38F4AC	Editor ID: 008-522-314-5

Graphics



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CETIS Analytical Report

Report Date: 22 Jan-24 10:13 (p 1 of 2)
Test Code/ID: 686A8C45 / 17-5181-3189

Fathead Minnow 7-d Larval Survival and Growth Test

Bio-Analytical Laboratories

Analysis ID: 20-7919-6960	Endpoint: 7d Survival Rate	CETIS Version: CETIS v2.1.5
Analyzed: 22 Jan-24 10:13	Analysis: Parametric-Control vs Treatments	Status Level: 1
Edit Date: 22 Jan-24 10:07	MD5 Hash: 6F49A20DF1359E9B37F8566A1C2D22D9	Editor ID: 008-522-314-5
Batch ID: 07-8899-1583	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 08 Jan-24 17:20	Protocol: EPA/821/R-02-013 (2002)	Diluent: Reconstituted Water
Ending Date: 15 Jan-24 16:15	Species: Pimephales promelas	Brine:
Test Length: 6d 23h	Taxon: Actinopterygii	Source: In-House Culture Age: <24
Sample ID: 07-4636-7351	Code: X9060	Project: WET Quarterly Compliance Test (1Q)
Sample Date: 07 Jan-24 21:00	Material: POTW Effluent	Source: AR0021776 (AR0021776)
Receipt Date: 08 Jan-24 15:30	CAS (PC):	Station: 001
Sample Age: 20h (1.8 °C)	Client: Nashville Public Works	

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	78	>78	---	1.3	0.1505	16.27%

Dunnnett Multiple Comparison Test

Control	vs	Conc-%	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		25	8	0.8216	2.362	0.2112	CDF	0.5010	Non-Significant Effect
		33	8	0.6659	2.362	0.2112	CDF	0.5726	Non-Significant Effect
		44	8	0.04793	2.362	0.2112	CDF	0.8188	Non-Significant Effect
		59	8	-0.3629	2.362	0.2112	CDF	0.9185	Non-Significant Effect
		78	8	-0.7737	2.362	0.2112	CDF	0.9694	Non-Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits			Decision
		Lower	Upper	Overlap	
Control Resp	0.925	0.8	>>	Yes	Passes Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0729313	0.0145863	5	0.7298	0.6080	Non-Significant Effect
Error	0.479647	0.0199853	24			
Total	0.552578		29			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	5.657	15.09	0.3411	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9455	0.9031	0.1277	Normal Distribution

7d Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	5	0.9250	0.7862	1.0000	1.0000	0.7500	1.0000	0.0500	12.09%	0.00%
25		5	0.8750	0.7653	0.9847	0.8750	0.7500	1.0000	0.0395	10.10%	5.41%
33		5	0.8750	0.6555	1.0000	1.0000	0.6250	1.0000	0.0791	20.20%	5.41%
44		5	0.9250	0.8400	1.0000	0.8750	0.8750	1.0000	0.0306	7.40%	0.00%
59		5	0.9500	0.8650	1.0000	1.0000	0.8750	1.0000	0.0306	7.21%	-2.70%
78		5	0.9750	0.9056	1.0000	1.0000	0.8750	1.0000	0.0250	5.73%	-5.41%

Angular (Corrected) Transformed Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	5	1.2870	1.0940	1.4810	1.3930	1.0470	1.3930	0.0697	12.12%	0.00%
25		5	1.2140	1.0620	1.3660	1.2090	1.0470	1.3930	0.0548	10.09%	5.71%
33		5	1.2280	0.9401	1.5150	1.3930	0.9117	1.3930	0.1036	18.86%	4.63%
44		5	1.2830	1.1580	1.4080	1.2090	1.2090	1.3930	0.0450	7.84%	0.33%
59		5	1.3200	1.1950	1.4450	1.3930	1.2090	1.3930	0.0450	7.62%	-2.52%
78		5	1.3560	1.2540	1.4580	1.3930	1.2090	1.3930	0.0367	6.06%	-5.37%

ECB
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CETIS Analytical Report

Report Date: 22 Jan-24 10:13 (p 2 of 2)
 Test Code/ID: 686A8C45 / 17-5181-3189

Fathead Minnow 7-d Larval Survival and Growth Test

Bio-Analytical Laboratories

Analysis ID: 20-7919-6960 Endpoint: 7d Survival Rate CETIS Version: CETIS v2.1.5
 Analyzed: 22 Jan-24 10:13 Analysis: Parametric-Control vs Treatments Status Level: 1
 Edit Date: 22 Jan-24 10:07 MD5 Hash: 6F49A20DF1359E9B37F8566A1C2D22D9 Editor ID: 008-522-314-5

7d Survival Rate Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.0000	0.7500	1.0000	0.8750	1.0000
25		0.8750	0.8750	1.0000	0.8750	0.7500
33		1.0000	0.7500	1.0000	1.0000	0.6250
44		0.8750	0.8750	1.0000	1.0000	0.8750
59		1.0000	1.0000	0.8750	1.0000	0.8750
78		0.8750	1.0000	1.0000	1.0000	1.0000

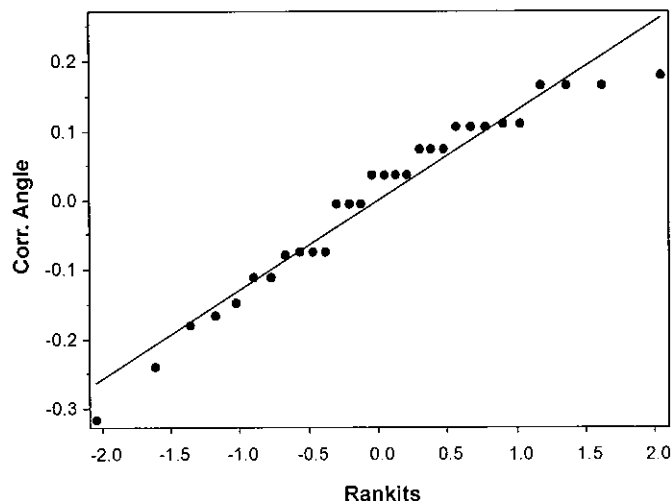
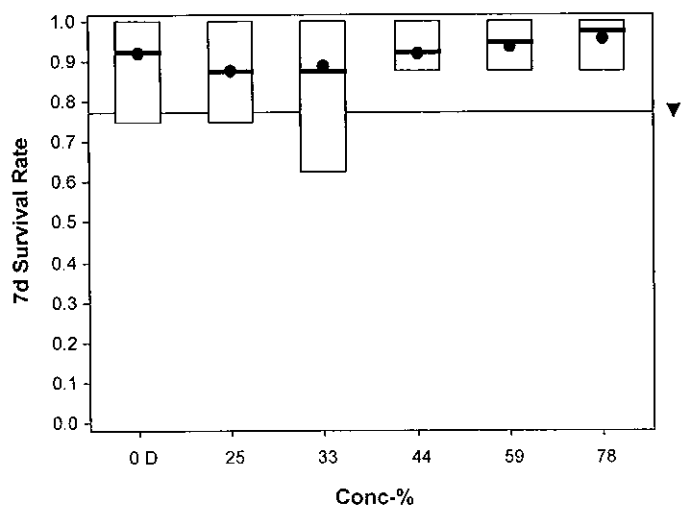
Angular (Corrected) Transformed Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.3930	1.0470	1.3930	1.2090	1.3930
25		1.2090	1.2090	1.3930	1.2090	1.0470
33		1.3930	1.0470	1.3930	1.3930	0.9117
44		1.2090	1.2090	1.3930	1.3930	1.2090
59		1.3930	1.3930	1.2090	1.3930	1.2090
78		1.2090	1.3930	1.3930	1.3930	1.3930

7d Survival Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	8/8	6/8	8/8	7/8	8/8
25		7/8	7/8	8/8	7/8	6/8
33		8/8	6/8	8/8	8/8	5/8
44		7/8	7/8	8/8	8/8	7/8
59		8/8	8/8	7/8	8/8	7/8
78		7/8	8/8	8/8	8/8	8/8

Graphics



EVB
1/22/24

CETIS Analytical Report

Report Date: 22 Jan-24 10:20 (p 1 of 2)
Test Code/ID: 686A8C45 / 17-5181-3189

Fathead Minnow 7-d Larval Survival and Growth Test

Bio-Analytical Laboratories

Analysis ID: 11-2995-3081	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETIS v2.1.5
Analyzed: 22 Jan-24 10:20	Analysis: Parametric-Control vs Treatments	Status Level: 1
Edit Date: 22 Jan-24 10:19	MD5 Hash: 13F6081C79BB5707131F0FBF18C70747	Editor ID: 008-522-314-5
Batch ID: 07-8899-1583	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 08 Jan-24 17:20	Protocol: EPA/821/R-02-013 (2002)	Diluent: Reconstituted Water
Ending Date: 15 Jan-24 16:15	Species: Pimephales promelas	Brine:
Test Length: 6d 23h	Taxon: Actinopterygii	Source: In-House Culture Age: <24
Sample ID: 07-4636-7351	Code: X9060	Project: WET Quarterly Compliance Test (1Q)
Sample Date: 07 Jan-24 21:00	Material: POTW Effluent	Source: AR0021776 (AR0021776)
Receipt Date: 08 Jan-24 15:30	CAS (PC):	Station: 001
Sample Age: 20h (1.8 °C)	Client: Nashville Public Works	

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Untransformed	C > T	78	>78	---	1.3	0.1003	30.16%

Dunnnett Multiple Comparison Test

Control	vs	Conc-%	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		25	8	0.6477	2.362	0.1003	CDF	0.5809	Non-Significant Effect
		33	8	-0.412	2.362	0.1003	CDF	0.9268	Non-Significant Effect
		44	8	1.825	2.362	0.1003	CDF	0.1346	Non-Significant Effect
		59	8	1.59	2.362	0.1003	CDF	0.1961	Non-Significant Effect
		78	8	0.471	2.362	0.1003	CDF	0.6595	Non-Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits			Decision
		Lower	Upper	Overlap	
Control Resp	0.3325	0.25	>>	Yes	Passes Criteria
PMSD	0.3016	0.12	0.3	Yes	Above Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0346044	0.0069209	5	1.535	0.2164	Non-Significant Effect
Error	0.108185	0.0045077	24			
Total	0.14279		29			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	4.328	15.09	0.5032	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9695	0.9031	0.5262	Normal Distribution

Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	5	0.3325	0.2316	0.4334	0.3625	0.2125	0.4	0.03635	24.45%	0.00%
25		5	0.305	0.2443	0.3657	0.3125	0.225	0.35	0.02187	16.03%	8.27%
33		5	0.35	0.2832	0.4168	0.325	0.2875	0.4125	0.02404	15.36%	-5.26%
44		5	0.255	0.209	0.301	0.25	0.2125	0.3125	0.01658	14.54%	23.31%
59		5	0.265	0.1441	0.3859	0.275	0.1375	0.375	0.04355	36.75%	20.30%
78		5	0.3125	0.2311	0.3939	0.3125	0.225	0.3875	0.02931	20.98%	6.02%

Mean Dry Biomass-mg Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	0.2875	0.4	0.4	0.3625	0.2125
25		0.3125	0.225	0.3375	0.35	0.3
33		0.4	0.325	0.4125	0.2875	0.325
44		0.3125	0.2625	0.2125	0.25	0.2375
59		0.2	0.275	0.3375	0.1375	0.375
78		0.225	0.3125	0.275	0.3625	0.3875

ECB
1/22/24

CETIS Analytical Report

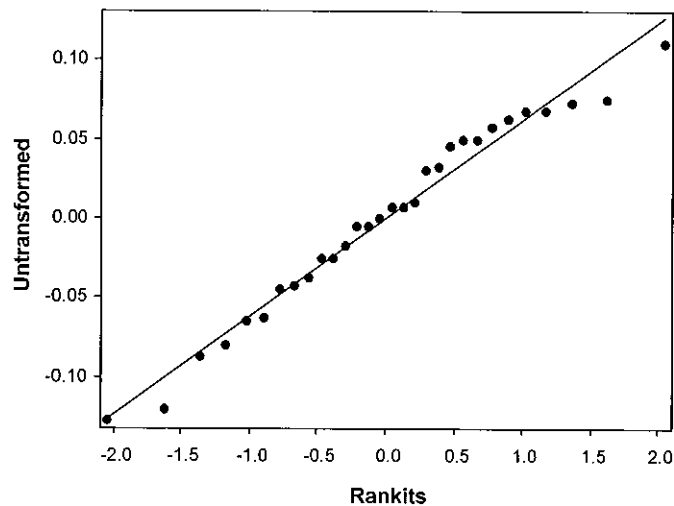
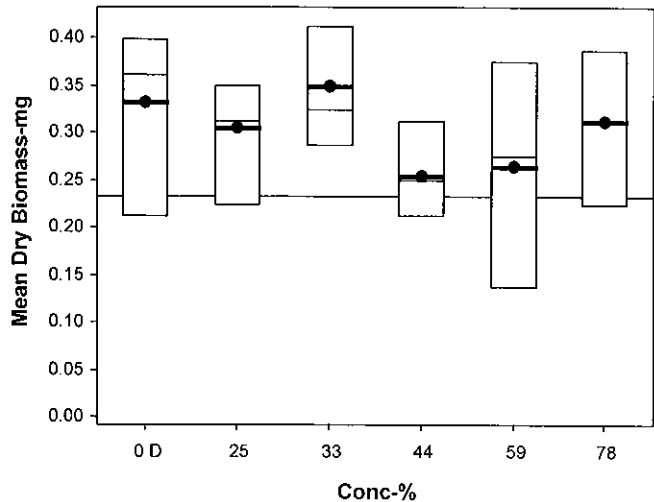
Report Date: 22 Jan-24 10:20 (p 2 of 2)
Test Code/ID: 686A8C45 / 17-5181-3189

Fathead Minnow 7-d Larval Survival and Growth Test

Bio-Analytical Laboratories

Analysis ID: 11-2995-3081	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETIS v2.1.5
Analyzed: 22 Jan-24 10:20	Analysis: Parametric-Control vs Treatments	Status Level: 1
Edit Date: 22 Jan-24 10:19	MD5 Hash: 13F6081C79BB5707131F0FBF18C70747	Editor ID: 008-522-314-5

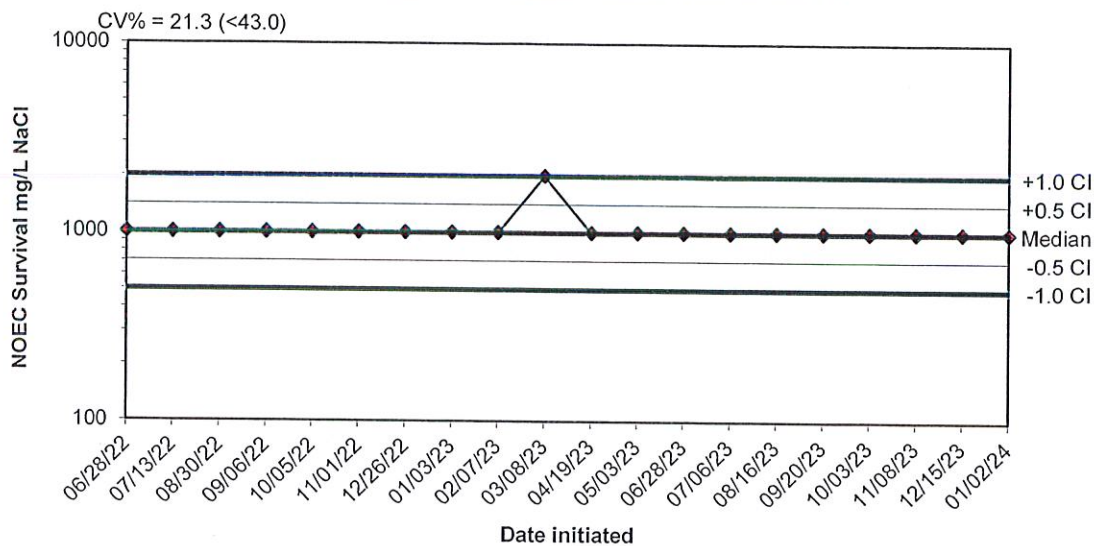
Graphics



EVB
1/22/24

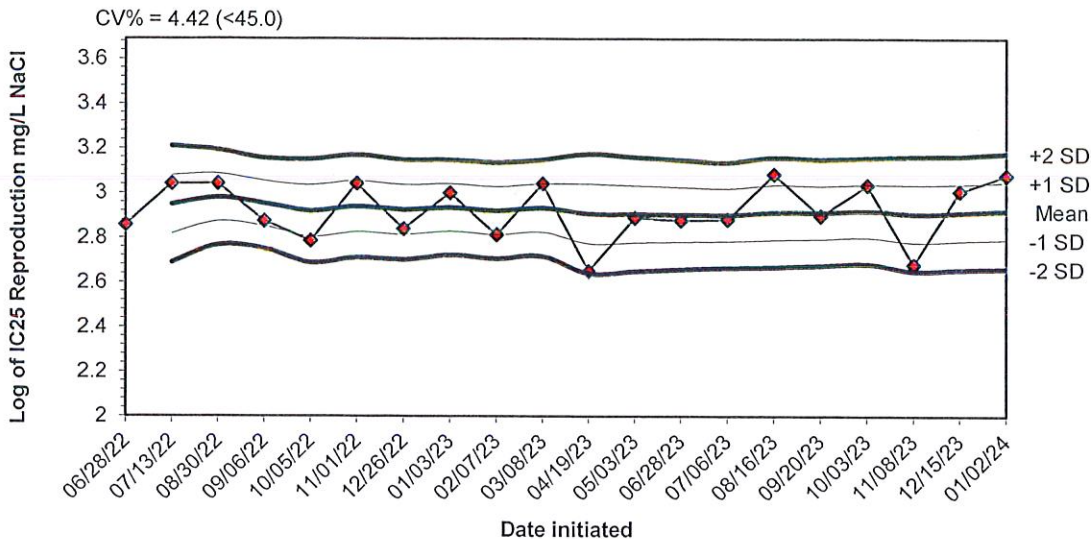
APPENDIX D
QUALITY ASSURANCE CHARTS

CHRONIC REFERENCE TOXICANT TEST RESULTS FOR CERIODAPHNIA DUBIA IN MODERATELY HARD WATER



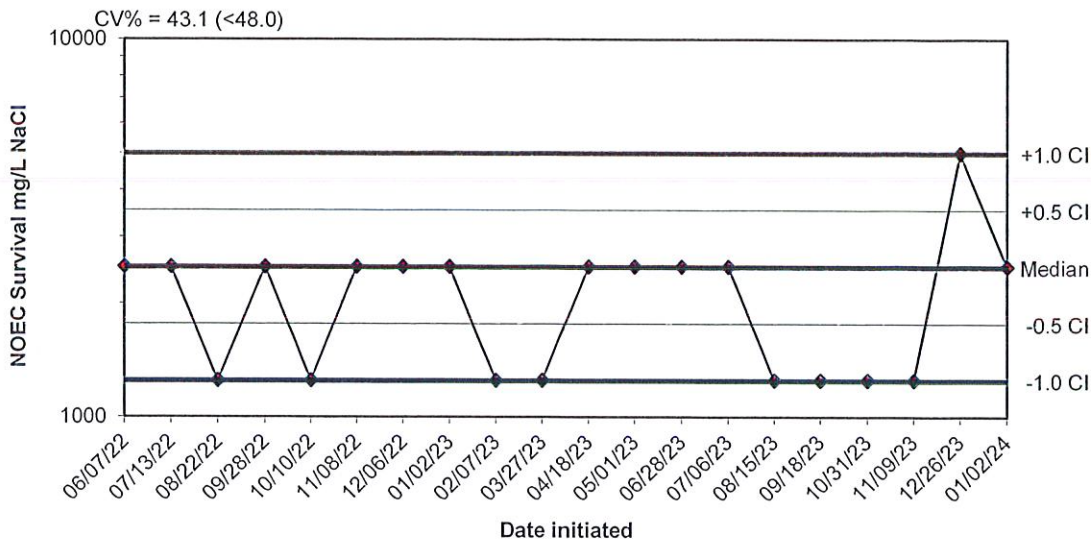
Dates	Values	Median	-0.5 CI	-1.0 CI	+0.5 CI	+1.0 CI
06/28/22	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
07/13/22	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
08/30/22	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
09/06/22	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
10/05/22	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
11/01/22	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
12/26/22	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
01/03/23	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
02/07/23	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
03/08/23	2000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
04/19/23	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
05/03/23	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
06/28/23	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
07/06/23	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
08/16/23	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
09/20/23	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
10/03/23	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
11/08/23	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
12/15/23	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
01/02/24	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000

**CHRONIC REFERENCE TOXICANT TEST RESULTS FOR
 CERIODAPHNIA DUBIA IN MODERATELY HARD WATER**



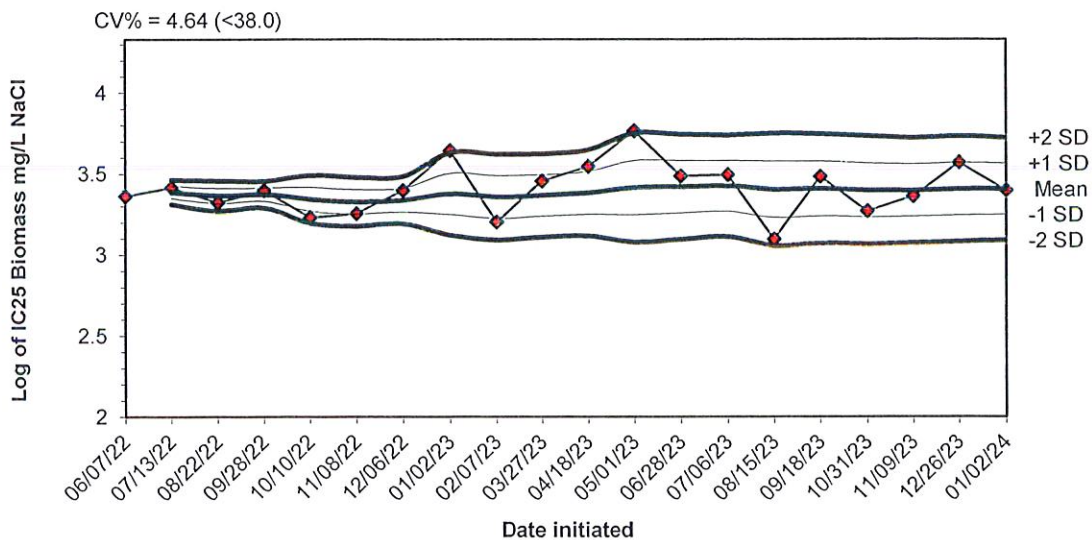
Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
06/28/22	2.8573					
07/13/22	3.0414	2.9494	2.8192	2.6891	3.0795	3.2097
08/30/22	3.0414	2.9800	2.8738	2.7675	3.0863	3.1926
09/06/22	2.8751	2.9538	2.8524	2.7510	3.0552	3.1566
10/05/22	2.7853	2.9201	2.8044	2.6887	3.0358	3.1515
11/01/22	3.0414	2.9403	2.8256	2.7109	3.0550	3.1698
12/26/22	2.8388	2.9258	2.8143	2.7028	3.0374	3.1489
01/03/23	3.0000	2.9351	2.8286	2.7220	3.0416	3.1482
02/07/23	2.8129	2.9215	2.8139	2.7062	3.0292	3.1368
03/08/23	3.0414	2.9335	2.8252	2.7168	3.0419	3.1502
04/19/23	2.6501	2.9077	2.7741	2.6404	3.0414	3.1751
05/03/23	2.8890	2.9062	2.7786	2.6511	3.0337	3.1613
06/28/23	2.8774	2.9040	2.7816	2.6592	3.0264	3.1487
07/06/23	2.8824	2.9024	2.7847	2.6670	3.0202	3.1379
08/16/23	3.0839	2.9145	2.7918	2.6690	3.0373	3.1600
09/20/23	2.8981	2.9135	2.7948	2.6762	3.0321	3.1508
10/03/23	3.0350	2.9206	2.8020	2.6834	3.0392	3.1578
11/08/23	2.6784	2.9072	2.7787	2.6503	3.0356	3.1641
12/15/23	3.0069	2.9124	2.7855	2.6586	3.0393	3.1662
01/02/24	3.0788	2.9208	2.7918	2.6627	3.0498	3.1788

**CHRONIC REFERENCE TOXICANT TEST RESULTS FOR PIMEPHALES
 PROMELAS IN MODERATELY HARD WATER**



Dates	Values	Median	-0.5 CI	-1.0 CI	+0.5 CI	+1.0 CI
06/07/22	2500.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
07/13/22	2500.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
08/22/22	1250.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
09/28/22	2500.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
10/10/22	1250.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
11/08/22	2500.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
12/06/22	2500.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
01/02/23	2500.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
02/07/23	1250.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
03/27/23	1250.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
04/18/23	2500.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
05/01/23	2500.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
06/28/23	2500.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
07/06/23	2500.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
08/15/23	1250.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
09/18/23	1250.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
10/31/23	1250.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
11/09/23	1250.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
12/26/23	5000.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
01/02/24	2500.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000

**CHRONIC REFERENCE TOXICANT TEST RESULTS FOR PIMEPHALES
 PROMELAS IN MODERATELY HARD WATER**



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
06/07/22	3.3617					
07/13/22	3.4150	3.3884	3.3507	3.3131	3.4260	3.4637
08/22/22	3.3222	3.3663	3.3198	3.2732	3.4129	3.4594
09/28/22	3.3979	3.3742	3.3331	3.2919	3.4154	3.4565
10/10/22	3.2304	3.3455	3.2719	3.1984	3.4190	3.4925
11/08/22	3.2553	3.3304	3.2551	3.1797	3.4058	3.4812
12/06/22	3.3979	3.3401	3.2667	3.1933	3.4134	3.4868
01/02/23	3.6435	3.3780	3.2510	3.1241	3.5050	3.6319
02/07/23	3.2041	3.3587	3.2265	3.0944	3.4908	3.6230
03/27/23	3.4550	3.3683	3.2400	3.1118	3.4966	3.6248
04/18/23	3.5469	3.3845	3.2515	3.1184	3.5176	3.6507
05/01/23	3.7654	3.4163	3.2484	3.0805	3.5842	3.7520
06/28/23	3.4878	3.4218	3.2598	3.0979	3.5837	3.7457
07/06/23	3.4949	3.4270	3.2702	3.1134	3.5838	3.7406
08/15/23	3.0973	3.4050	3.2316	3.0581	3.5785	3.7519
09/18/23	3.4823	3.4099	3.2412	3.0725	3.5785	3.7472
10/31/23	3.2704	3.4017	3.2349	3.0681	3.5684	3.7352
11/09/23	3.3632	3.3995	3.2375	3.0754	3.5616	3.7236
12/26/23	3.5712	3.4086	3.2462	3.0839	3.5709	3.7332
01/02/24	3.3981	3.4080	3.2500	3.0920	3.5661	3.7241

APPENDIX E
AGENCY FORMS

**SUMMARY REPORTING FORMS
 CHRONIC BIOMONITORING
Ceriodaphnia dubia Survival and Reproduction**

Permittee: Nashville Public Works

NPDES No.: AR0021776/ AFIN 31-00036

	Time	Date		Time	Date
Composite 1 Collected From:	0930	01/07/24	To	2100	01/07/24
Composite 2 Collected From:	0930	01/09/24	To	2236	01/09/24
Composite 3 Collected From:	0900	01/11/24	To	2109	01/11/24
Test initiated:	1255	am/pm		01/09/24	Date
Test terminated:	1415	am/pm		01/16/24	Date
Dilution water used:	Receiving		X	Reconstituted	

PERCENT SURVIVAL

Time of Reading	Percent Effluent					
	0	25.0	33.0	44.0	59.0	78.0
24h	100.0	100.0	100.0	100.0	100.0	100.0
48h	100.0	100.0	100.0	100.0	100.0	90.0
End of test	80.0	100.0	80.0	90.0	90.0	90.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ END OF TEST

Rep	0	25.0	33.0	44.0	59.0	78.0
A	D2	8	D7	D3	15	14
B	22	17	17	11	16	26
C	23	18	15	10	D2	12
D	18	19	19	21	18	27
E	16	19	19	16	14	16
F	17	13	16	17	16	16
G	12	11	12	21	10	21
H	14	15	16	17	15	25
I	17	15	D2	20	13	D
J	D18	13	16	22	14	22
Surv. Mean	17.4	14.8	16.3	17.2	14.6	19.9
Total Mean	15.9	14.8	13.9	15.8	13.3	17.9
CV%*	21.30	24.42	13.86	25.27	15.40	27.93

*coefficient of variation = standard deviation x 100/mean. D=dead adult

PMSD =37.34%

Ceriodaphnia dubia
Survival and Reproduction (continued)

1. Fisher's Exact Test:

Is the mean survival at the end of the test significantly different ($p=.05$) than the control survival for the % effluent corresponding to (lethality):

- | | | | |
|---|-----|---|----|
| a) LOW FLOW OR CRITICAL DILUTION (78.0%): | YES | X | NO |
| b) ½ LOW FLOW DILUTION (NA%): | YES | | NO |

2. Dunnett's Procedure or Steel's Many-One Rank Test as appropriate:

Is the mean number of young produced per female significantly different ($p=.05$) than the control's number of young per female for the % effluent corresponding to (significant non-lethal effects):

- | | | | |
|---|-----|---|----|
| a) LOW FLOW OR CRITICAL DILUTION (78.0%): | YES | X | NO |
| b) ½ LOW FLOW DILUTION (NA%): | YES | | NO |

3. If you answered NO to 1. a) and 2. a) enter (0) otherwise enter (1): 0

4. If you answered NO to 1. b) and 2. b) enter (0) otherwise enter (1):

5. Enter response to item 3 on DMR Form, parameter #TEP3B.

6. Enter response to item 4 on DMR Form, parameter #TFP3B.

7. Enter percent effluent corresponding to each NOEC below:

- | | |
|-----------------------|-----------------|
| a) NOEC survival: | 78.0 % effluent |
| b) NOEC reproduction: | 78.0 % effluent |

Biomonitoring Form
Chronic Toxicity Summary Form for Ceriodaphnia dubia
Chemical Parameters Chart

Permittee: Nashville Public Works
NPDES#: ARO021776/ AFIN 31-00035
Contact: Kevin Funderburk
Analysts: Ware, Miller, Briggs

Sample #1 Collected: 1/7/2024 Time: 2100
Sample #2 Collected: 1/9/2024 Time: 2236
Sample #3 Collected: 1/11/2024 Time: 2109
Test Begin: 1/9/2024 Time: 1255
Test End: 1/16/2024 Time: 1415

Dilution: 0%							Dilution: 44.0%							Dilution: 59.0%							Dilution: 78.0%										
Day:	1	2	3	4	5	6	7	Day:	1	2	3	4	5	6	7	Day:	1	2	3	4	5	6	7	Day:	1	2	3	4	5	6	7
T (°C)	24.0	23.7	24.0	24.0	24.2	24.2	24.3	T (°C)	24.0	23.7	24.0	24.0	24.2	24.2	24.3	T (°C)	24.0	23.7	24.0	24.0	24.2	24.2	24.3	T (°C)	24.0	23.7	24.0	24.0	24.2	24.2	24.3
DO Initial	7.3	7.2	7.2	7.4	7.2	7.6	7.7	DO Initial	7.4	7.3	7.4	7.4	7.2	7.4	8.0	DO Initial	7.8	7.3	7.4	7.4	7.6	7.7	8.0	DO Initial	7.8	7.3	7.4	7.4	7.6	7.7	8.0
DO Final	8.3	8.2	8.0	7.4	8.2	8.8		DO Final	8.2	8.2	7.5	7.4	8.1	8.4		DO Final	8.5	8.4	7.9	8.2	8.4	8.3		DO Final	8.5	8.4	7.9	8.2	8.4	8.3	
pH Initial	7.1	7.3	7.4	7.3	7.3	7.1	7.7	pH Initial	7.2	7.2	7.3	7.4	7.5	8.2	8.0	pH Initial	7.3	7.2	7.3	7.1	7.2	8.1	8.1	pH Initial	7.3	7.2	7.3	7.1	7.2	8.1	8.1
pH Final	7.8	7.7	7.9	8.2	7.3	7.1		pH Final	7.8	7.7	7.9	8.0	7.5	7.6		pH Final	7.7	7.6	7.8	7.9	7.3	7.6		pH Final	7.7	7.6	7.8	7.9	7.3	7.6	
Conductivity	322.0	334.0	330.0	332.0	330.0	369.0		Conductivity	322.0	330.0	332.0	316.0	325.0	331.0		Conductivity	319.0	314.0	318.0	305.0	314.0	318.0		Conductivity	319.0	314.0	318.0	305.0	314.0	318.0	
Alkalinity	60.0			60.0	60.0			Alkalinity								Alkalinity	104.0	88.0		40.0				Alkalinity	104.0	88.0		40.0			
Hardness	84.0			96.0	96.0			Hardness								Hardness	108.0	100.0		88.0				Hardness	108.0	100.0		88.0			
Chlorine	<0.5			<0.5	<0.5			Chlorine								Chlorine	<0.5	<0.5		<0.5				Chlorine	<0.5	<0.5		<0.5			

Comments:

**SUMMARY REPORTING FORMS CHRONIC BIOMONITORING
 FATHEAD MINNOW LARVAE GROWTH AND SURVIVAL
 (*Pimephales promelas*)**

Permittee: Nashville Public Works

NPDES No.: AR0021776/ AFIN 31-00036

	Time	Date		Time	Date
Composite 1 Collected from:	0930	01/07/24	To	2100	01/07/24
Composite 2 Collected from:	0930	01/09/24	To	2236	01/09/24
Composite 3 Collected from:	0900	01/11/24	To	2109	01/11/24
Test initiated:	1720	am/pm		01/08/24	date
Test terminated:	1615	am/pm		01/15/24	date
Dilution water used:		Receiving	X	Reconstituted	

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in Replicate Chambers					Mean Percent Survival			CV%*
	A	B	C	D	E	24h	48h	7 days	
0	100.0	75.0	100.0	87.5	100.0	100.0	100.0	92.5	12.12
25.0	87.5	87.5	100.0	87.5	75.0	100.0	100.0	87.5	10.09
33.0	100.0	75.0	100.0	100.0	62.5	100.0	100.0	87.5	18.86
44.0	87.5	87.5	100.0	100.0	87.5	100.0	100.0	92.5	7.84
59.0	100.0	100.0	87.5	100.0	87.5	100.0	100.0	95.0	7.62
78.0	87.5	100.0	100.0	100.0	100.0	100.0	100.0	97.5	6.06

DATA TABLE FOR GROWTH

Effluent Conc. %	Average Dry Weight in milligrams in replicate chambers					Mean Dry Weight mg	CV*
	A	B	C	D	E		
0	0.288	0.400	0.400	0.363	0.213	0.333	24.45
25.0	0.313	0.225	0.338	0.350	0.300	0.305	16.03
33.0	0.400	0.325	0.413	0.288	0.325	0.350	15.36
44.0	0.313	0.263	0.213	0.250	0.238	0.255	14.54
59.0	0.200	0.275	0.338	0.138	0.375	0.265	36.75
78.0	0.225	0.313	0.275	0.363	0.388	0.313	20.98

*coefficient of variation = standard deviation x 100/mean.

PMSD: 30.0%

FATHEAD MINNOW LARVAE GROWTH AND SURVIVAL (cont)
(Pimephales promelas)

1. Dunnett's Procedure or Steels Many-One Rank Test as appropriate:

Is the mean survival at 7 days significantly different ($p=.05$) than the control survival for the % effluent corresponding to:

a) LOW FLOW OR CRITICAL DILUTION (78.0 %)	YES	X	NO
b) ½ LOW FLOW DILUTION (NA %)	YES		NO

2. Dunnett's Procedure (or appropriate test):

Is the mean dry weight (growth) at 7 days significantly different ($p=.05$) than the control's dry weight for the % effluent corresponding to (significant non-lethal effects):

a) LOW FLOW OR CRITICAL DILUTION (78.0 %)	YES	X	NO
b) ½ LOW FLOW DILUTION (NA %)	YES		NO

3. If you answered NO to 1. a) and 2. a) enter (0) otherwise enter (1): 0

4. If you answered NO to 1. b) and 2. b) enter (0) otherwise enter (1):

5. Enter response to item 3 on DMR Form, parameter #TEP6C.

6. Enter response to item 4 on DMR Form, parameter #TFP6C.

7. Enter percent effluent corresponding to each NOEC below:

a.) NOEC survival	78.0% effluent.
b.) NOEC growth	78.0% effluent.

Biomonitoring Form
 Chronic Toxicity Summary Form for Pimephales promelas
 Chemical Parameters Chart

Permittee: Nashville Public Works
 NPDES#: AR0021776/ AFIN 31-00036
 Contact: Kevin Funderburk
 Analysts: Ware

Sample #1 Collected: 1/7/2024 Time: 2100
 Sample #2 Collected: 1/9/2024 Time: 2236
 Sample #3 Collected: 1/11/2024 Time: 2109
 Test Begin: 1/8/2024 Time: 1720
 Test End: 1/15/2024 Time: 1615

Dilution:	0%							44.0%						
Day:	1	2	3	4	5	6	7	1	2	3	4	5	6	7
T (°C)	24.8	24.3	24.9	24.7	24.0	24.2	24.8	24.8	24.3	24.9	24.7	24.0	24.2	24.8
DO Initial	7.0	7.2	7.3	7.4	7.4	7.3	7.6	7.1	7.3	7.6	7.7	7.3	7.4	7.6
DO Final	8.1	8.2	8.1	7.9	8.2	8.3	8.1	8.2	8.3	8.3	7.4	8.1	8.1	7.5
pH Initial	7.3	7.4	7.5	7.2	7.3	7.3	7.4	7.2	7.1	7.4	7.5	7.4	7.5	7.4
pH Final	7.0	7.8	7.3	7.4	8.1	7.9	7.4	7.2	7.8	7.5	7.3	8.0	7.2	7.4
Conductivity	325.0	322.0	320.0	325.0	333.0	338.0	321.0	322.0	331.0	336.0	330.0	320.0	325.0	321.0
Alkalinity	68.0				68.0									
Hardness	104.0				96.0									
Chlorine	<0.5				<0.5									
Dilution:	25.0%							59.0%						
Day:	1	2	3	4	5	6	7	1	2	3	4	5	6	7
T (°C)	24.8	24.3	24.9	24.7	24.0	24.2	24.8	24.8	24.3	24.9	24.7	24.0	24.2	24.8
DO Initial	7.2	7.0	7.3	7.4	7.3	7.4	7.5	7.4	7.3	7.3	7.4	7.3	7.4	7.5
DO Final	7.9	8.1	8.1	7.5	8.0	8.0	8.3	8.5	8.4	8.3	7.6	7.7	8.3	8.3
pH Initial	7.3	7.2	7.4	7.2	7.4	7.5	7.5	7.2	7.3	7.4	7.3	7.3	7.3	7.5
pH Final	7.1	7.9	7.6	7.3	8.2	7.4	7.2	7.2	7.8	7.5	7.3	7.6	7.2	7.2
Conductivity	320.0	321.0	342.0	340.0	326.0	330.0	316.0	319.0	323.0	330.0	330.0	313.0	319.0	319.0
Alkalinity														
Hardness														
Chlorine														
Dilution:	33.0%							78.0%						
Day:	1	2	3	4	5	6	7	1	2	3	4	5	6	7
T (°C)	24.8	24.3	24.9	24.7	24.0	24.2	24.8	24.8	24.3	24.9	24.7	24.0	24.2	24.8
DO Initial	7.0	7.0	7.4	7.4	7.3	7.6	7.6	7.4	7.6	7.2	7.4	7.2	7.3	7.5
DO Final	8.1	8.3	8.2	7.5	8.1	8.2	8.1	8.1	8.2	8.1	7.9	8.2	8.5	7.6
pH Initial	7.2	7.3	7.3	7.2	7.4	7.3	7.4	7.2	7.4	7.3	7.5	7.4	7.5	7.6
pH Final	7.1	7.9	7.5	7.1	8.1	7.2	7.1	7.2	7.7	7.4	7.4	7.9	7.2	7.2
Conductivity	319.0	321.0	338.0	337.0	323.0	329.0	316.0	318.0	314.0	323.0	323.0	308.0	316.0	316.0
Alkalinity							104.0	88.0				40.0		
Hardness							108.0	100.0				88.0		
Chlorine							<0.5	<0.5				<0.5		

Comments:

APPENDIX F
REPORT QUALITY ASSURANCE FORM



Bio-Analytical Laboratories

3240 Spurgin Road
Post Office Box 527
Doyline, LA 71023

(318) 745-2772
1-800-259-1246
Fax: (318) 745-2773

REPORT QUALITY ASSURANCE FORM

Client: Nashville Public Works

Project#: X9060

Chain of Custody Documents Checked by: EOB 1/22/24
Technician/Date

Raw Data Documents Checked by: EOB 1/22/24
Technician/Date

Statistical Analysis Package Checked by: EOB 1/22/24
Quality Manager/Date

Quality Control Data Checked by: EOB 1/25/24
Quality Manager/Date

Report Checked by: EOB 1/24/24
Quality Manager/Date

I certify that this document was prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. The information contained in this document, to the best of my knowledge, is true, accurate and complete.

Quinn L. Bragg, BS
Quality Manager

1/24/24
Date

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