

Bio-Analytical Laboratories' Executive Summary

Permittee: Nashville Public Works
Nashville, AR 71852

Project #: X8744

Outfall: 001 (treated municipal wastewater)

Permit #: AR0021776/ AFIN #31-00036

Contact: Ana-Lab Corporation
4720 Viking Drive, Ste A
Bossier City, LA 71111

Test Dates: April 11 – 18, 2023

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 – 32.23%
6. Chronic WET Limit, DMR CODE 51710, - 78.0%
7. PMSD Reproduction-38.65%(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 –30.65%.
6. Chronic WET Limit, DMR CODE 51714, - 78.0%
7. PMSD Biomass-30.16% (12.0 – 30.0%)-moderate precision, acceptable for passing test.

This report contains a total of 54 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 X8744

Test Dates: April 11 - 18, 2023

Report Date: May 2, 2023

Prepared for:

Ana-Lab Corporation
4720 Viking Drive, Ste A
Bossier City, LA 71111

Prepared by:

Ginger Briggs
Bio-Analytical Laboratories
P.O. Box 527
Doyline, LA 71023
ADEQ #88-0630

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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 April 9, 12 and 14, 2023, at 1913, 0405 and 0620 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 Ana-Lab Corporation, personnel. The sample temperature upon arrival of each sample was 1.9, -0.1 and 1.8⁰ 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 data was analyzed using Steel's Many-One Rank Test, and growth (biomass) 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. Ninety percent survival occurred in the control and 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 24.5 and 22.1, 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	90.0		27.0	24.5	
25.0	100.0		22.0	22.0	
33.0	100.0		22.4	22.4	
44.0	90.0		26.3	23.7	
59.0	90.0		27.4	25.7	
78.0	90.0		24.0	22.1	

*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, 90.0 percent survival occurred in the control and 87.5 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 0.478 and 0.418 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	90.0		0.478	
25.0	90.0		0.305	
33.0	95.0		0.473	
44.0	97.5		0.445	
59.0	85.0		0.365	
78.0	87.5		0.418	

*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 April 9, 12 and 14, 2023, 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

SUBCONTRACT CHAIN OF CUSTODY

Printed 04/10/2023 Page 1 of 1

Bio-Analytical Laboratories
 Ginger Briggs
 PO Box 527
 Doyline, LA 71023-

NASH-L
116

Lab Number X8744/C24795
 PO Number _____ BAL3

Temperature upon arrival: 19°C

Thermometer #: 29

Tech: NA

Date: 4/10/23

24 Hr Comp Quarterly

Bio Monitoring Qtr

odor clear
 TAT no odor

client provides their auto-sampler

Matrix: Non-Potable Water

Sample Collection Start

Date: 4-9-23 Time: 0615

Sampler Printed Name: Kevin Funderburk

Sampler Affiliation: NASH

Sampler Signature: _____

Sample Collection Stop

Date: 4-9-23 Time: 1913

Sampler Printed Name: Kevin Funderburk

Sampler Affiliation: NASH

Sampler Signature: _____

Samples Radioactive? Samples Contains Dioxin? Samples Biological Hazard?

Polyethylene Quart

Short HoldSubc^{CCD} Chronic Ceriodaphnia dubia (SUB) Subcontract CAS: BAL3 (1.00 days)
 Short HoldSubc^{CPR} Chronic Pimephales promelas Subcontract CAS: BAL3 (1.00 days)

Ambient Conditions/Comments

Date Time	Relinquished	Date Time	Received
<u>4/10/23</u>	Printed Name: <u>STACY COWGILL</u> Signature: <u>[Signature]</u> Affiliation: <u>ANA-LAB</u>	<u>4/10/23</u> <u>1500</u>	Printed Name: <u>[Signature]</u> Signature: <u>[Signature]</u> Affiliation: <u>BA</u>
	Printed Name Signature Affiliation		Printed Name Signature Affiliation
	Printed Name Signature Affiliation		Printed Name Signature Affiliation
	Printed Name Signature Affiliation		Printed Name Signature Affiliation

Sample Received on Ice? Yes No Method of Shipment: UPS Bus FedEx Lone Star Hand Delivered Other
 Cooler/Sample Secure? Yes No If Shipped: Tracking Number & Temp - See Attached Hand Delivered to Region []

The accredited column designates accreditation by A - A2LA, N - NELAC, or z - not listed under scope of accreditation. Unless otherwise specified, ANA -LAB shall provide these ordered services pursuant to our Standard Terms & Conditions Agreement (available for download from the welcome page at <http://www.ana-lab.com>).
 Ana -Lab personnel collect samples as specified by Ana -Lab SOP # 000323.

Comments



Ark-La-Miss Region: 4720 Viking Dr. Suite A Bossier City LA 71111





Testing the Limits of Science and Service

SUBCONTRACT CHAIN OF CUSTODY

Printed 04/12/2023 Page 1 of 1

Bio-Analytical Laboratories
 Ginger Briggs
 PO Box 527
 Doyline, LA 71023-

NASH-L
116

Lab Number X8744 | C24819

PO Number _____ BAL3

Bio Monitoring Qtr

24 Hr Comp Quarterly

client provides their auto-sampler
 Matrix: Non-Potable Water

TAT _____
 Date: _____
 Tech: _____
 Thermometer #: _____
 Temperature upon arrival: _____

Sample Collection Start
 Date: 4-11-23 Time: 0615
 Sampler Printed Name: Kevin Funderburk
 Sampler Affiliation: NASH
 Sampler Signature: _____

Sample Collection Stop
 Date: 4/12/23 Time: 0405
 Sampler Printed Name: Kevin Funderburk
 Sampler Affiliation: NASH
 Sampler Signature: _____

Samples Radioactive? Samples Contains Dioxin? Samples Biological Hazard?

1 Polyethylene Quart

Short HoldSubcCCD Chronic Ceriodaphnia dubia (SUB) Subcontract CAS: BAL3 (1.00 days)
 Short HoldSubcCPR Chronic Pinephales promelas Subcontract CAS: BAL3 (1.00 days)

Ambient Conditions/Comments

Date Time		Relinquished	Date Time	Received	
<u>4/12/23</u>	Printed Name	Affiliation	<u>4/11/23</u>	Printed Name	Affiliation
	<u>STACY COWGILL</u>	<u>ANA-LAB</u>		<u>Kevin Funderburk</u>	<u>NASH</u>
	Signature		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	

Sample Received on Ice? Yes No Method of Shipment: UPS Bus FedEx Lone Star Hand Delivered Other
 Cooler/Sample Secure? Yes No If Shipped: Tracking Number & Temp - See Attached Hand Delivered to Region []

The accredited column designates accreditation by A - A2LA, N - NELAC, or Z - not listed under scope of accreditation. Unless otherwise specified, ANA -LAB shall provide these ordered services pursuant to our Standard Terms & Conditions Agreement (available for download from the welcome page at <http://www.ana-lab.com>). Ana -Lab personnel collect samples as specified by Ana -Lab SOP# 000323.

Comments

Temperature upon arrival: -0.1 °C
 Thermometer #: 29
elb 4/12/23



Ark-La-Miss Region: 4720 Viking Dr. Suite A Bossier City LA 71111



SUBCONTRACT CHAIN OF CUSTODY

Printed 04/12/2023 Page 1 of 1
 Lab Number X8744/C24829
 PO Number _____ BAL3

Bio-Analytical Laboratories
 Ginger Briggs
 PO Box 527
 Doyline, LA 71023-

NASH-L
116

Bio Monitoring Qtr
Clear NO ODOOR 1.8 #29 4/14/23

24 Hr Comp Quarterly

client provides their auto-sampler
 Matrix: Non-Potable Water

TAT _____

Sample Collection Start

Date: 4/13/23 Time: 0730

Sample Collection Stop

Date: 4/14/23 Time: 0620

Sampler Printed Name: STACY COWGILL ANA-LAB

Sampler Printed Name: Kevin Funderburk

Sampler Affiliation: Kevin Funderburk

Sampler Affiliation: NASH

Sampler Signature: NASH

Sampler Signature: _____

Samples Radioactive? Samples Contains Dioxin? Samples Biological Hazard?

1 Polyethylene Quart

Short HoldSubc^{CCD} Chronic Ceriodaphnia dubia (SUB) Subcontract CAS: BAL3 (1.00 days)
 Short HoldSubc^{CPR} Chronic Pimephales promelas Subcontract CAS: BAL3 (1.00 days)

Ambient Conditions/Comments

Date Time		Relinquished	Date Time	Received
<i>Stacy</i>	Printed Name	Affiliation	1450	Printed Name
	Signature	ANA-LAB		Signature
	Printed Name	Affiliation		Printed Name
	Signature			Signature
	Printed Name	Affiliation		Printed Name
	Signature			Signature
	Printed Name	Affiliation		Printed Name
	Signature			Signature

Sample Received on Ice? Yes No Method of Shipment: UPS Bus FedEx Lone Star Hand Delivered Other
 Cooler/Sample Secure? Yes No If Shipped: Tracking Number & Temp - See Attached Hand Delivered to Region []

The accredited column designates accreditation by A - A2LA, N - NELAC, or z - not listed under scope of accreditation. Unless otherwise specified, ANA -LAB shall provide these ordered services pursuant to our Standard Terms & Conditions Agreement (available for download from the welcome page at <http://www.ana-lab.com>).
 Ana-Lab personnel collect samples as specified by Ana-Lab SOP# 000323.

Comments



Ark-La-Miss Region: 4720 Viking Dr. Suite A Bossier City LA 71111

**APPENDIX B
RAW DATA SHEETS**

BIO-ANALYTICAL LABORATORIES CERIODAPHNIA DUBIA SURVIVAL AND REPRODUCTION TEST

Project# X8744 Date start: 4/11/23 Date end: 4/18/23

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 Temperature(°C) 25+1° Technicians: EGB/EDW/AM/PM

Adults isolated: Date 4/11/23 Time: 2330

Neonates collected: Date 4/11/23 Time: 0700 Board: V21mH
 Dissolved Oxygen Meter: Model YSI550 Serial #02F0741 AH
 pH Meter: Model Orion 230A+ Serial #015253
 Conductivity Meter: Model YSI EC300A Serial #JC02714

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
0.8.5/112.6/1cm	0.4/127.4/98.3/1cm	0.	0.
1.9.4/113.5/1mu	1.4/168.0/96.8/1mu		1.
2.9.8/113.7/1cm	2.4/188.1/98.2/1cm		2.
3.10.1/120.3/1cm	3.4/188.3/100.1/1cm		3.
4.9.9/1106.9/1mu	4.4/168.4/99.6/1mu		4.
5.9.4/1110.1/1mu	5.4/168.1/96.4/1mu		5.
6.10.1/119.4/1cm	6.4/168.4/99.3/1cm		6.
7.	7.		7.

Total Residual Chlorine (mg/L)/Tech	Dechlorinated? Amount?/Tech	Ammonia (NH3) (mg/L)/Tech	BAL Sample # Date in Use
1. 20.5/1cm	1. NO/1cm	1. 0.25/1cm	1. C24795 4/11/23
2. 20.5/1cm	2. NO/1cm	2. 20.5/1cm	2. C24819 4/13/23
3. 20.5/1cm	3. NO/1cm	3. 20.5/1cm	3. C24829 4/15/23

Comments: Wild organisms found; filtered through 60µ net on 4/13/23

Project# X 8744 Client Nasville

Test started: Date 4/11/03 Time 1225

Test ended: Date 4/13/03 Time 1750

Sample ID 007

Date/Tech: Day 0 4/11/03 11/2/23 4/13/23 4/14/23 4/15/23 4/16/23 4/17/23 4/18/23 4/19/23 4/20/23 4/21/23 4/22/23 4/23/23 4/24/23 4/25/23 4/26/23 4/27/23 4/28/23 4/29/23 4/30/23 5/1/23 5/2/23 5/3/23 5/4/23 5/5/23 5/6/23 5/7/23 5/8/23 5/9/23 5/10/23 5/11/23 5/12/23 5/13/23 5/14/23 5/15/23 5/16/23 5/17/23 5/18/23 5/19/23 5/20/23 5/21/23 5/22/23 5/23/23 5/24/23 5/25/23 5/26/23 5/27/23 5/28/23 5/29/23 5/30/23 5/31/23 6/1/23 6/2/23 6/3/23 6/4/23 6/5/23 6/6/23 6/7/23 6/8/23 6/9/23 6/10/23 6/11/23 6/12/23 6/13/23 6/14/23 6/15/23 6/16/23 6/17/23 6/18/23 6/19/23 6/20/23 6/21/23 6/22/23 6/23/23 6/24/23 6/25/23 6/26/23 6/27/23 6/28/23 6/29/23 6/30/23 7/1/23 7/2/23 7/3/23 7/4/23 7/5/23 7/6/23 7/7/23 7/8/23 7/9/23 7/10/23 7/11/23 7/12/23 7/13/23 7/14/23 7/15/23 7/16/23 7/17/23 7/18/23 7/19/23 7/20/23 7/21/23 7/22/23 7/23/23 7/24/23 7/25/23 7/26/23 7/27/23 7/28/23 7/29/23 7/30/23 7/31/23 8/1/23 8/2/23 8/3/23 8/4/23 8/5/23 8/6/23 8/7/23 8/8/23 8/9/23 8/10/23 8/11/23 8/12/23 8/13/23 8/14/23 8/15/23 8/16/23 8/17/23 8/18/23 8/19/23 8/20/23 8/21/23 8/22/23 8/23/23 8/24/23 8/25/23 8/26/23 8/27/23 8/28/23 8/29/23 8/30/23 8/31/23 9/1/23 9/2/23 9/3/23 9/4/23 9/5/23 9/6/23 9/7/23 9/8/23 9/9/23 9/10/23 9/11/23 9/12/23 9/13/23 9/14/23 9/15/23 9/16/23 9/17/23 9/18/23 9/19/23 9/20/23 9/21/23 9/22/23 9/23/23 9/24/23 9/25/23 9/26/23 9/27/23 9/28/23 9/29/23 9/30/23 10/1/23 10/2/23 10/3/23 10/4/23 10/5/23 10/6/23 10/7/23 10/8/23 10/9/23 10/10/23 10/11/23 10/12/23 10/13/23 10/14/23 10/15/23 10/16/23 10/17/23 10/18/23 10/19/23 10/20/23 10/21/23 10/22/23 10/23/23 10/24/23 10/25/23 10/26/23 10/27/23 10/28/23 10/29/23 10/30/23 10/31/23 11/1/23 11/2/23 11/3/23 11/4/23 11/5/23 11/6/23 11/7/23 11/8/23 11/9/23 11/10/23 11/11/23 11/12/23 11/13/23 11/14/23 11/15/23 11/16/23 11/17/23 11/18/23 11/19/23 11/20/23 11/21/23 11/22/23 11/23/23 11/24/23 11/25/23 11/26/23 11/27/23 11/28/23 11/29/23 11/30/23 12/1/23 12/2/23 12/3/23 12/4/23 12/5/23 12/6/23 12/7/23 12/8/23 12/9/23 12/10/23 12/11/23 12/12/23 12/13/23 12/14/23 12/15/23 12/16/23 12/17/23 12/18/23 12/19/23 12/20/23 12/21/23 12/22/23 12/23/23 12/24/23 12/25/23 12/26/23 12/27/23 12/28/23 12/29/23 12/30/23 12/31/23

Time: Day 0 1225 11807 21420 31245 41400 51211 61140 71850 8

Temp. (°C): Day 0 25.0 124.4 224.3 324.3 425.0 525.6 624.4 724.7 8

Conc	Day	1	2	3	4	5	6	7	8	9	10	Number of Live
0 M4	1	0										10
	2	0										10
	3	0										10
	4	113	112	113	112	112	111	114	112	113	114	10
	5	0										10
	6	2110	218	217	0	X	218	218	2110	217	0	9
	7	3116	3112	3128	215	↑	2318	2322	3116	2316	218	9
	8											
25.0	1	0										10
	2	0										10
	3	0										10
	4	113	113	112	112	111	114	112	113	112	112	10
	5	0										10
	6	2112	2110	2114	2112	2112	219	2117	211	2112	2111	10
	7	3112	0	316	0	318	311	318	2112	3114	3115	10
	8											
33.0	1	0										10
	2	0										10
	3	0										10
	4	111	112	113	114	113	114	113	112	114	113	10
	5	0										10
	6	219	2115	2113	2114	218	218	2110	2112	218	211	10
	7	3111	319	3111	3112	319	218	3114	0	3114	219	10
	8											
44.0	1	0										10
	2	0	X									9
	3	0		0								9
	4	112		114	113	113	112	114	113	112	114	9
	5	0										9
	6	212		2111	2112	2111	2114	2115	215	2111	2111	9
	7	0		3118	3116	3114	316	3120	2112	3116	3116	9
	8											
59.0	1	0										10
	2	0										10
	3	0										10
	4	113	112	113	113	112	114	115	112	113	114	10
	5	0										10
	6	2113	2111	2117	217	216	2111	2113	2111	2113	2110	10
	7	3116	3114	3114	X	0	3114	318	3112	3120	3116	9
	8											
78.0	1	0										10
	2	0										10
	3	0										10
	4	114	114	113	112	114	115	112	113	112	112	10
	5	0										10
	6	211	2113	218	2112	2111	X	2113	2110	2114	2112	9
	7	23126	3112	0	319	3114	↑	318	319	318	3110	9
	8											

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 (CHR CHEM Rev.4.0)

Project# X8744 Client Nashville Organism C. dubia

Date	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
	4/11/23 5384	4/12	4/13/23	4/14/23	4/15	4/16	4/17	4/18	
Concentration: <u>0mH</u>									
Temperature (°C)	22.4	23.6 22.9	23.1 23.0	22.4 24.1	23.4 22.0	24.1 22.9	24.5 22.9	23.9	
pH	7.5	7.1 7.1	7.1 7.3	7.2 7.4	7.5 7.3	7.1 7.3	6.8 7.5	4.6	
DO (mg/l)	7.1	8.4 8.4	8.3 7.6	7.5 7.5	8.0 8.2	8.4 7.9	8.3 8.1	8.1	
Cond (umhos/cm)	283	280	293	273	307	242	242		
Concentration: <u>25.0%</u>									
Temperature (°C)	22.4	23.6 22.2	23.1 23.3	22.4 24.0	23.4 22.5	24.1 23.4	24.5 22.9	23.9	
pH	7.7	7.3 7.4	7.1 7.8	7.5 7.4	7.6 7.6	7.3 7.6	7.2 7.5	7.4	
DO (mg/l)	7.0	8.4 8.3	8.2 7.6	8.4 7.5	8.0 8.1	8.0 8.0	8.3 8.0	9.0	
Cond (umhos/cm)	263	258	252	270	272	238	238		
Concentration: <u>33.0%</u>									
Temperature (°C)	23.0	23.7 22.2	23.3 23.5	22.4 24.3	23.4 22.7	24.1 23.4	24.7 22.9	24.1	
pH	7.7	7.4 7.5	7.1 7.7	7.5 7.3	7.5 7.7	7.4 7.6	7.3 7.5	7.5	
DO (mg/l)	7.0	8.4 8.2	8.3 7.6	8.4 7.4	8.0 8.0	8.2 8.0	8.5 8.0	8.9	
Cond (umhos/cm)	255	250	239	245	284	237	236		
Prerenewal Tech Initials/Time		1811 AM	1424 AM	1240 MU	1200 MU	1211 MU	1645 PM	1850 PM	
Postrenewal Tech Initials/Time		1122 MU	1050	1050	1053 MU	1047 MU	1025 PM		

Control Alkalinity (mg/L as CaCO₃) _____ Control Hardness (mg/L as CaCO₃) _____

ID# 5384 Result 56 Date Tested 4/14/23 ID# 6384 Result 116 Date Tested 4/14/23
 ID# _____ Result _____ Date Tested _____ ID# _____ Result _____ Date Tested _____
 ID# _____ Result _____ Date Tested _____ ID# _____ Result _____ Date Tested _____

Sample Alkalinity (mg/L as CaCO₃) _____ Sample Hardness (mg/L as CaCO₃) _____

ID# C24795 Result 36 Date Tested 4/14/23 ID# C24795 Result 56 Date Tested 4/14/23
 ID# C24819 Result 32 Date Tested 4/14/23 ID# C24819 Result 64 Date Tested 4/14/23
 ID# C24829 Result 100 Date Tested 4/20/23 ID# C24829 Result 56 Date Tested 4/20/23

0 AM 4/21/23

BIO-ANALYTICAL LABORATORIES CHRONIC WATER QUALITY DATA (CHR CHEM Rev.4.0)

Project# X8744 Client Nashville Organism C. dubia

Date	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Concentration: <u>44.0%</u> * 2 a * 4 10/23 MV									
Temperature (°C)	<u>23.1</u>	<u>23.9</u> <u>22.3</u>	<u>23.4</u> <u>23.6</u>	<u>23.1</u> <u>24.1</u>	<u>23.3</u> <u>22.7</u>	<u>24.1</u> <u>23.5</u>	<u>24.8</u> <u>22.9</u>	<u>24.1</u>	
pH	<u>7.7</u>	<u>7.4</u> <u>7.6</u>	<u>7.1</u> <u>7.7</u>	<u>7.3</u> <u>7.4</u>	<u>7.5</u> <u>7.6</u>	<u>7.4</u> <u>7.6</u>	<u>7.3</u> <u>7.4</u>	<u>7.5</u>	
DO (mg/l)	<u>6.8</u>	<u>8.4</u> <u>7.1</u>	<u>8.4</u> <u>7.4</u>	<u>8.0</u> <u>7.6</u>	<u>8.1</u> <u>8.0</u>	<u>8.4</u> <u>7.8</u>	<u>8.2</u> <u>8.1</u>	<u>9.0</u>	
Cond (umhos/cm)	<u>205</u>	<u>228</u>	<u>240</u>	<u>233</u>	<u>275</u>	<u>236</u>	<u>234</u>		
Concentration: <u>59.0%</u>									
Temperature (°C)	<u>23.2</u>	<u>23.9</u> <u>22.3</u>	<u>23.4</u> <u>23.7</u>	<u>22.7</u> <u>24.5</u>	<u>23.3</u> <u>22.7</u>	<u>24.1</u> <u>23.6</u>	<u>25.1</u> <u>23.0</u>	<u>24.2</u>	
pH	<u>7.6</u>	<u>7.4</u> <u>7.5</u>	<u>7.2</u> <u>7.6</u>	<u>7.3</u> <u>7.4</u>	<u>7.5</u> <u>7.6</u>	<u>7.4</u> <u>7.6</u>	<u>7.2</u> <u>7.4</u>	<u>7.5</u>	
DO (mg/l)	<u>6.9</u>	<u>8.5</u> <u>7.9</u>	<u>8.5</u> <u>7.7</u>	<u>8.4</u> <u>7.6</u>	<u>8.0</u> <u>8.1</u>	<u>8.4</u> <u>7.9</u>	<u>8.3</u> <u>8.1</u>	<u>8.9</u>	
Cond (umhos/cm)	<u>227</u>	<u>227</u>	<u>232</u>	<u>223</u>	<u>258</u>	<u>225</u>	<u>232</u>		
Concentration: <u>78.0%</u>									
Temperature (°C)	<u>23.4</u>	<u>24.1</u> <u>22.7</u>	<u>23.7</u> <u>23.8</u>	<u>22.9</u> <u>24.6</u>	<u>23.4</u> <u>22.7</u>	<u>24.1</u> <u>22.5</u>	<u>25.4</u> <u>23.2</u>	<u>24.4</u>	
pH	<u>7.6</u>	<u>7.4</u> <u>7.5</u>	<u>7.2</u> <u>7.7</u>	<u>7.3</u> <u>7.4</u>	<u>7.5</u> <u>7.6</u>	<u>7.4</u> <u>7.5</u>	<u>7.3</u> <u>7.4</u>	<u>7.4</u>	
DO (mg/l)	<u>6.8</u>	<u>8.5</u> <u>8.1</u>	<u>8.5</u> <u>7.5</u>	<u>8.4</u> <u>7.6</u>	<u>8.0</u> <u>8.0</u>	<u>8.5</u> <u>7.7</u>	<u>8.6</u> <u>8.2</u>	<u>8.9</u>	
Cond (umhos/cm)	<u>207</u>	<u>199</u>	<u>225</u>	<u>220</u>	<u>245</u>	<u>233</u>	<u>229</u>		
Prerenewal Tech Initials/Time		<u>1811 AM</u>	<u>1424 AL</u>	<u>1246 MV</u>	<u>1100 MV</u>	<u>1211 PM</u>	<u>1645 PM</u>	<u>1850 PM</u>	
Postrenewal Tech Initials/Time		<u>1122 MV</u>	<u>1050 MV</u>	<u>1050 MV</u>	<u>1053 MV</u>	<u>1053 MV</u>	<u>1025 PM</u>		

24/10/23 MV

Control Alkalinity (mg/L as CaCO₃)

Control Hardness (mg/L as CaCO₃)

ID# _____	Result _____	Date Tested _____	ID# _____	Result _____	Date Tested _____
ID# _____	Result _____	Date Tested _____	ID# _____	Result _____	Date Tested _____
ID# _____	Result _____	Date Tested _____	ID# _____	Result _____	Date Tested _____

Sample Alkalinity (mg/L as CaCO₃)

Sample Hardness (mg/L as CaCO₃)

ID# _____	Result _____	Date Tested _____	ID# _____	Result _____	Date Tested _____
ID# _____	Result _____	Date Tested _____	ID# _____	Result _____	Date Tested _____
ID# _____	Result _____	Date Tested _____	ID# _____	Result _____	Date Tested _____

CETIS Test Data Worksheet

Report Date: 10 Apr-23 12:52 (p 1 of 2)
 Test Code/ID: E870CEB / 02-4373-1691

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

Start Date: 11 Apr-23 13:20 Species: Ceriodaphnia dubia Sample Code: 113BA4F0
 End Date: 18 Apr-23 12:10 Protocol: EPA/821/R-02-013 (2002) Sample Source: AR0021776
 Sample Date: 10 Apr-23 06:00 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	
0	D	2	1																			
33		6	2																			
59		2	3																			
25		10	4																			
25		9	5																			
44		5	6																			
0	D	4	7																			
25		4	8																			
0	D	10	9																			
59		4	10																			
0	D	9	11																			
25		3	12																			
78		10	13																			
33		1	14																			
0	D	6	15																			
44		9	16																			
44		6	17																			
78		2	18																			
0	D	8	19																			
33		4	20																			
59		5	21																			
59		7	22																			
25		5	23																			
59		8	24																			
44		10	25																			
78		4	26																			
59		6	27																			
25		7	28																			
59		10	29																			
78	/	6	30																			
78	/	9	31																			
78	/	8	32																			
33	/	7	33																			
78	/	5	34																			
25	/	6	35																			
59	/	3	36																			
59	/	9	37																			
44	/	7	38																			
25	/	1	39																			
44	/	3	40																			
33	/	3	41																			

CETIS Test Data Worksheet

Report Date: 10 Apr-23 12:52 (p 2 of 2)
 Test Code/ID: E870CEB / 02-4373-1691

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	
44		2	42																			
33		10	43																			
0	D	1	44																			
78		7	45																			
44		4	46																			
44		1	47																			
33		8	48																			
78		3	49																			
44		8	50																			
0	D	3	51																			
25		8	52																			
0	D	7	53																			
33		5	54																			
0	D	5	55																			
59		1	56																			
25		2	57																			
78		1	58																			
33		2	59																			
33		9	60																			

EDU
4/11/23

- Set #1
5,3,6,2,1,4 Parent# 1G
- Set #2
4,6,2,3,5,1 Parent# 1I
- Set #3
3,2,1,5,6,4 Parent# 1J
- Set #4
6,4,3,5,1,2 Parent# 2B
- Set #5
5,2,1,3,6,4 Parent# 3B
- Set #6
1,2,3,4,6,5 Parent# 1A
- Set #7
4,1,5,3,6,2 Parent# 1B
- Set #8
6,5,4,2,1,3 Parent# 1C
- Set #9
4,6,5,1,2,3 Parent# 1D
- Set #10
3,5,2,6,1,4 Parent# 2E

BIO-ANALYTICAL LABORATORIES
 PIMEPHALES PROMELAS SURVIVAL AND GROWTH DATA SHEET

Project# X8744 Date started: 4/11/23 Date ended 4/18/23

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 Temperature(°C) 25+1° Celsius Technicians EGB/EDW/AM/PM
 Test organism age <24hrs Vendor/ID# BAL041123

Day	Feeding Times		
	AM	NOON	PM
0		EDW/1210/0.20mL	EDW/2000/0.1mL
1	AM/0910/0.1mL	EDW/1240/0.10mL	AM/1855/0.1mL
2	AM/0855/0.1mL	EDW/1200/0.10mL	EDW/1830/0.10mL
3	EDW/0845/0.10mL	EDW/1140/0.10mL	EDW/1730/0.10mL
4	MV/0913/0.20mL		MV/1470/0.20mL
5	MV/0932/0.20mL		MV/1409/0.20mL
6	EDW/0900/0.10mL	EDW/1230/0.10mL	EDW/1800/0.10mL

Dissolved Oxygen Meter: Model YSI550 Serial #02F0741 AH
 pH Meter: Model Orion 230A+ Serial #015253
 Conductivity Meter: Model YSI EC300A Serial #JC02714

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
0.8.5/112.6/4/cm	0. y/12/7.4/98.3/1/cm	0. _____	0. _____
1.9.4/113.5/1/mv	1. y/16/2.0/96.8/1/mv	1. _____	1. _____
2.9.8/113.7/1/m	2. y/18/8.1/98.2/1/m	2. _____	2. _____
3.10.6/120.3/2/6av	3. y/12/8.3/100.1/2/5av	3. _____	3. _____
4.9.9/110.6/9/1/mv	4. y/16/2.4/99.6/1/mv	4. _____	4. _____
5.9.4/110.1/1/mv	5. y/16/3.1/96.4/1/mv	5. _____	5. _____
6.10.1/119.4/9/1/PM	6. y/16/8.4/99.3/9/1/PM	6. _____	6. _____

Total Residual Chlorine(mg/L)/Tech	Dechlorinated? Amount?/Tech	Ammonia(NH3) (mg/L)/Tech	BAL Sample # Date in use
1. 20.5/1/cm	1. NO/1/cm	1. 0.25/1/cm	1. C24795 4/11/23
2. 20.5/1/m	2. NO/1/m	2. 20.5/1/m	2. C24819 4/13/23
3. 20.5/1/mv	3. NO/1/mv	3. 20.5/1/mv	3. C24829 4/15/23

Comments: wild organisms found, filtered through 60µm net AM 4/13/23

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

Project# X8744

Test started: Date 4/11/23 Time 1210

Client Ms. Swille

Sample ID 001

Test ended: Date 4/18/23 Time 1100

Date/Tech: Day 0 4/11/23 M 1 4/12/23 M 2 4/13/23 M 3 4/14/23 M 4 4/15/23 M 5 4/15/23 M 6 4/17/23 M 7 4/18/23 PM

Time: Day 0 1210 1 1220 2 1153 3 1110 4 1137 5 1140 6 1230 7 1100

Temp (°C) Day 0 25.1 1 24.6 2 24.7 3 24.9 4 25.2 5 25.2 6 25.6 7 25.6

Conc. %	Rep.	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0 M/L	1	8	8	8	8	8	8	8	8
	2	8	8	8	8	8	8	7	7
	3	8	8	8	8	8	8	7	7
	4	8	8	8	8	8	8	8	8
	5	8	8	8	8	7	7	6	6
25.0	1	8	8	8	8	7	7	7	7
	2	8	8	8	8	6	6	5	5
	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
33.0	1	8	8	8	8	8	8	8	8
	2	8	8	8	8	8	8	7	7
	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
44.0	1	8	8	8	8	8	8	8	8
	2	8	8	8	8	8	8	8	8
	3	8	8	8	8	7	7	6	7
	4	8	8	8	8	8	8	8	8
	5	8	8	8	8	8	8	8	8
59.0	1	8	8	8	8	8	8	8	8
	2	8	8	8	8	8	8	7	7
	3	8	8	8	8	7	7	7	7
	4	8	8	8	8	8	8	8	8
	5	8	8	8	8	8	8	4	4
78.0	1	8	8	8	8	8	8	7	5
	2	8	8	8	8	8	8	8	8
	3	8	8	8	8	6	6	6	6
	4	8	8	8	8	8	8	8	8
	5	8	8	8	8	8	8	8	8

4 PM 4/17/23

Project#/Client X8744 Nashville Temp Start (°C) 70°C Tech PM Date: 4/18/23 Time: 1100
 Temp End (°C) 95.7 Tech AM Date: 4/19/23 Time: 0840

Conc.	Replicate/ Pan number	Wt. of pan(g)/ Date <u>4/14/23</u> weighed: Tech: <u>AM</u>	Wt. of pan + larvae(g)/ Date <u>4/19/23</u> weighed: Tech: <u>AM</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 1/8	1 61	0.7194	0.7233				
	2 62	0.7109	0.7164				
	3 63	0.7051	0.7089				
	4 64	0.6974	0.7005				
	5 65	0.7130	0.7158				
25%	1 66	0.7069	0.7098				
	2 67	0.7004	0.7025				
	3 68	0.7024	0.7053				
	4 69	0.7030	0.7055				
	5 70	0.7204	0.7222				
33%	1 71	0.7107	0.7148				
	2 72	0.7041	0.7075				
	3 73	0.7052	0.7089				
	4 74	0.7050	0.7083				
	5 75	0.7134	0.7178				
44%	1 76	0.7094	0.7126				
	2 77	0.6999	0.7031				
	3 78	0.7168	0.7204				
	4 79	0.7120	0.7161				
	5 80	0.6986	0.7023				
59%	1 81	0.7068	0.7098				
	2 82	0.7133	0.7171				
	3 83	0.7115	0.7142				
	4 84	0.7148	0.7184				
	5 85	0.7055	0.7070				
78%	1 86	0.7143	0.7169				
	2 87	0.7027	0.7071				
	3 88	0.7113	0.7137				
	4 89	0.7147	0.7175				
	5 90	0.7058	0.7103				

* Test acceptance of control weight based on surviving larvae at end of test.
 Calculated by: CETIS Calculations checked by: ELB 4/21/23

BIO-ANALYTICAL LABORATORIES CHRONIC WATER QUALITY DATA (CHR CHEM Rev.4.0)

Project# X8744 Client Nashville

Organism P. promelas

Date	Day 0 4/11/23 5384	Day 1 4/12 4112	Day 2 4/13/23 5390	Day 3 4/14/23 4113	Day 4 4/15 4115	Day 5 4/16 4116	Day 6 4/17 4117	Day 7 4/18 4118	Day 8
Concentration:	<u>0 mH</u>								
Temperature (°C)	<u>22.9</u>	<u>23.8</u>	<u>24.3</u>	<u>23.1</u>	<u>24.0</u>	<u>24.8</u>	<u>23.3</u>	<u>24.5</u>	
pH	<u>7.8</u>	<u>6.9</u>	<u>6.9</u>	<u>7.0</u>	<u>7.1</u>	<u>6.7</u>	<u>6.5</u>	<u>6.4</u>	
DO (mg/l)	<u>7.1</u>	<u>7.9</u>	<u>6.5</u>	<u>7.0</u>	<u>7.4</u>	<u>7.9</u>	<u>5.9</u>	<u>5.4</u>	
Cond (umhos/cm)	<u>281</u>	<u>275</u>	<u>254</u>	<u>243</u>	<u>260</u>	<u>263</u>	<u>259</u>		
Concentration:	<u>25.0%</u>								
Temperature (°C)	<u>22.9</u>	<u>24.2</u>	<u>24.3</u>	<u>24.1</u>	<u>23.8</u>	<u>24.8</u>	<u>23.3</u>	<u>24.7</u>	
pH	<u>7.8</u>	<u>7.0</u>	<u>6.8</u>	<u>7.2</u>	<u>6.8</u>	<u>7.0</u>	<u>6.7</u>	<u>6.6</u>	
DO (mg/l)	<u>7.0</u>	<u>7.4</u>	<u>5.9</u>	<u>7.0</u>	<u>6.2</u>	<u>7.4</u>	<u>6.3</u>	<u>5.3</u>	
Cond (umhos/cm)	<u>259</u>	<u>254</u>	<u>248</u>	<u>240</u>	<u>251</u>	<u>249</u>	<u>256</u>		
Concentration:	<u>33.0%</u>								
Temperature (°C)	<u>23.3</u>	<u>24.2</u>	<u>24.3</u>	<u>23.9</u>	<u>24.3</u>	<u>24.8</u>	<u>23.3</u>	<u>24.7</u>	
pH	<u>7.8</u>	<u>6.9</u>	<u>6.7</u>	<u>7.0</u>	<u>6.8</u>	<u>6.7</u>	<u>6.7</u>	<u>6.6</u>	
DO (mg/l)	<u>7.0</u>	<u>7.4</u>	<u>6.0</u>	<u>6.9</u>	<u>6.2</u>	<u>7.4</u>	<u>5.1</u>	<u>5.1</u>	
Cond (umhos/cm)	<u>251</u>	<u>243</u>	<u>240</u>	<u>233</u>	<u>248</u>	<u>257</u>	<u>248</u>		
Prerenewal Tech Initials/Time		<u>AS 1225</u>	<u>AL 1157</u>	<u>EDW 1110</u>	<u>MV 1138</u>	<u>MV 1140</u>	<u>PM 1235</u>	<u>PM 1105</u>	
Postrenewal Tech Initials/Time	<u>EM 1151</u>	<u>MV 1103</u>	<u>EDW 1040</u>	<u>EDW 1050</u>	<u>MV 1041</u>	<u>MV 1036</u>	<u>PM 1035</u>		

Control Alkalinity (mg/L as CaCO₃)

Control Hardness (mg/L as CaCO₃)

ID# 5384 Result 56 Date Tested 4/14/23 ID# 5384 Result 76 Date Tested 4/14/23
 ID# 5390 Result 64 Date Tested 4/14/23 ID# 5390 Result 244 Date Tested 4/14/23
 ID# _____ Result _____ Date Tested _____ ID# _____ Result _____ Date Tested _____

Sample Alkalinity (mg/L as CaCO₃)

Sample Hardness (mg/L as CaCO₃)

ID# C24793 Result 36 Date Tested 4/14/23 ID# C24795 Result 56 Date Tested 4/14/23
 ID# C24819 Result 32 Date Tested 4/14/23 ID# C24819 Result 64 Date Tested 4/15/23
 ID# C24829 Result 60 Date Tested 4/20/23 ID# C24829 Result 56 Date Tested 4/20/23

BIO-ANALYTICAL LABORATORIES CHRONIC WATER QUALITY DATA (CHR CHEM Rev.4.0)

Project# X8744 Client Nashville Organism P. promelas

Date	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Concentration: <u>44.0%</u>									
Temperature (°C)	23.4	24.3 22.6	24.4 24.0	23.6 23.9	24.4 23.6	24.3 24.0	23.2 23.6	24.7	
pH	7.7	7.0 7.5	6.7 7.7	7.5 7.4	6.8 7.5	7.0 7.4	6.6 7.5	6.6	
DO (mg/l)	6.8	7.1 8.1	6.0 7.5	8.0 7.3	8.8 8.1	7.1 7.9	5.2 8.0	5.1	
Cond (umhos/cm)	241	236	239	240	243	244	254		
Concentration: <u>59.0%</u>									
Temperature (°C)	23.5	24.3 22.6	24.4 24.2	24.1 24.1	24.5 23.6	24.8 23.4	23.1 23.7	24.7	
pH	7.7	6.9 7.5	6.7 7.7	7.5 7.4	6.8 7.5	6.7 7.6	6.6 7.5	6.6	
DO (mg/l)	6.9	7.2 8.1	6.1 7.5	7.3 7.3	6.2 8.6	7.2 7.9	3.5 7.9	5.2	
Cond (umhos/cm)	227	218	228	230	238	253	244		
Concentration: <u>78.0%</u>									
Temperature (°C)	24.0	24.4 22.8	24.5 24.4	23.9 23.9	24.5 23.4	24.8 24.0	23.0 23.9	24.7	
pH	7.6	6.9 7.3	6.6 7.7	7.8 7.4	6.7 7.5	6.9 7.6	6.6 8.0	6.6	
DO (mg/l)	6.9	7.3 7.9	5.9 7.5	6.1 4.6	6.2 8.2	7.2 7.7	5.3 8.0	5.0	
Cond (umhos/cm)	205	205	220	223	228	236	237		
Prerenewal Tech Initials/Time		1224 M	1157 M	820 1110	1138 MU	1140 MU	1235 PM	1105 PM	
Postrenewal Tech Initials/Time	1041 1151	1103 MU	820 1040	820 1050	1041 MU	1034 MU	1035 PM		

Control Alkalinity (mg/L as CaCO₃)

Control Hardness (mg/L as CaCO₃)

ID#	Result	Date Tested	ID#	Result	Date Tested
ID#	Result	Date Tested	ID#	Result	Date Tested
ID#	Result	Date Tested	ID#	Result	Date Tested

Sample Alkalinity (mg/L as CaCO₃)

Sample Hardness (mg/L as CaCO₃)

ID#	Result	Date Tested	ID#	Result	Date Tested
ID#	Result	Date Tested	ID#	Result	Date Tested
ID#	Result	Date Tested	ID#	Result	Date Tested

CETIS Test Data Worksheet

Report Date: 10 Apr-23 12:53 (p 1 of 1)
 Test Code/ID: 5E40592 / 00-9882-9714

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

Start Date: 11 Apr-23 15:15 Species: Pimephales promelas Sample Code: 9102FFC
 End Date: 18 Apr-23 13:50 Protocol: EPA/821/R-02-013 (2002) Sample Source: AR0021776
 Sample Date: 10 Apr-23 08:00 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
25		4	1												
0	D	5	2												
78		1	3												
44		5	4												
0	D	1	5												
25		1	6												
59		2	7												
25		2	8												
33		1	9												
78		2	10												
44		2	11												
44		4	12												
0	D	3	13												
33		4	14												
33		3	15												
44		1	16												
0	D	4	17												
59		5	18												
59		4	19												
44		3	20												
33		2	21												
59		3	22												
78		3	23												
59		1	24												
0	D	2	25												
78		4	26												
25		3	27												
33		5	28												
25		5	29												
78		5	30												

APPENDIX C
STATISTICAL ANALYSES

CETIS Analytical Report

Report Date: 21 Apr-23 10:08 (p 1 of 2)
Test Code/ID: E870CEB / 02-4373-1691

Ceriodaphnia 7-d Survival and Reproduction Test			Bio-Analytical Laboratories		
Analysis ID: 16-3011-7700	Endpoint: 7d Survival Rate	CETIS Version: CETISv2.1.4			
Analyzed: 21 Apr-23 10:08	Analysis: STP 2xK Contingency Tables	Status Level: 1			
Edit Date: 21 Apr-23 9:51	MD5 Hash: 6D27C722A37F24AD487C5B34A221C117	Editor ID: 008-522-314-5			
Batch ID: 21-2809-0947	Test Type: Reproduction-Survival (2-8d)	Analyst: Lab Tech			
Start Date: 11 Apr-23 12:25	Protocol: EPA/821/R-02-013 (2002)	Diluent: Reconstituted Water			
Ending Date: 18 Apr-23 18:50	Species: Ceriodaphnia dubia	Brine:			
Test Length: 7d 6h	Taxon: Branchiopoda	Source: In-House Culture	Age: <24		
Sample ID: 02-8912-1520	Code: X8744	Project: WET Monthly Compliance Test (APR)			
Sample Date: 09 Apr-23 06:15	Material: POTW Effluent	Source: AR0021776 (AR0021776)			
Receipt Date: 10 Apr-23 15:00	CAS (PC):	Station: 001			
Sample Age: 54h (1.9 °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	1.0000	Exact	1.0000	Non-Significant Effect
		44	0.7632	Exact	1.0000	Non-Significant Effect
		59	0.7632	Exact	1.0000	Non-Significant Effect
		78	0.7632	Exact	1.0000	Non-Significant Effect

7d Survival Rate Frequencies

Conc-%	Code	NR	R	NR + R	Prop NR	Prop R	%Effect
0	D	9	1	10	0.9000	0.1000	0.00%
25		10	0	10	1.0000	0.0000	-11.11%
33		10	0	10	1.0000	0.0000	-11.11%
44		9	1	10	0.9000	0.1000	0.00%
59		9	1	10	0.9000	0.1000	0.00%
78		9	1	10	0.9000	0.1000	0.00%

7d Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	10	0.9000	0.6738	1.0000	1.0000	0.0000	1.0000	0.1000	35.14%	0.00%
25		10	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	-11.11%
33		10	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	-11.11%
44		10	0.9000	0.6738	1.0000	1.0000	0.0000	1.0000	0.1000	35.14%	0.00%
59		10	0.9000	0.6738	1.0000	1.0000	0.0000	1.0000	0.1000	35.14%	0.00%
78		10	0.9000	0.6738	1.0000	1.0000	0.0000	1.0000	0.1000	35.14%	0.00%

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	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000
25		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
33		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
44		1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
59		1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
78		1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000

EW
4/21/23

CETIS Analytical Report

Report Date: 21 Apr-23 10:08 (p 2 of 2)
 Test Code/ID: E870CEB / 02-4373-1691

Ceriodaphnia 7-d Survival and Reproduction Test

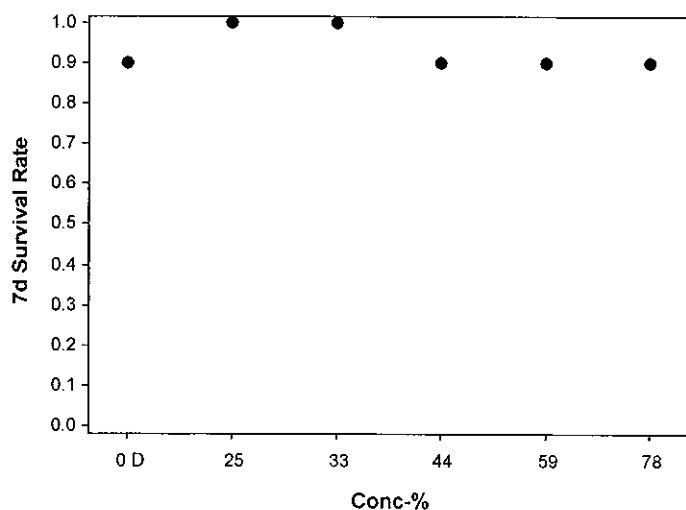
Bio-Analytical Laboratories

Analysis ID: 16-3011-7700 Endpoint: 7d Survival Rate CETIS Version: CETISv2.1.4
 Analyzed: 21 Apr-23 10:08 Analysis: STP 2xK Contingency Tables Status Level: 1
 Edit Date: 21 Apr-23 9:51 MD5 Hash: 6D27C722A37F24AD487C5B34A221C117 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	1/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1	1/1
25		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
33		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
44		1/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
59		1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1
78		1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1

Graphics



ELB
 4/21/23

CETIS Analytical Report

Report Date: 02 May-23 15:18 (p 1 of 2)
Test Code/ID: E870CEB / 02-4373-1691

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

Analysis ID: 02-4371-9734	Endpoint: Reproduction	CETIS Version: CETISv2.1.4
Analyzed: 02 May-23 15:18	Analysis: Nonparametric-Multiple Comparison	Status Level: 1
Edit Date: 02 May-23 15:17	MD5 Hash: C77EEF58EF1A58B9155CC8DA56B925E6	Editor ID: 008-522-314-5
Batch ID: 21-2809-0947	Test Type: Reproduction-Survival (2-8d)	Analyst: Lab Tech
Start Date: 11 Apr-23 12:25	Protocol: EPA/821/R-02-013 (2002)	Diluent: Reconstituted Water
Ending Date: 18 Apr-23 18:50	Species: Ceriodaphnia dubia	Brine:
Test Length: 7d 6h	Taxon: Branchiopoda	Source: In-House Culture Age: <24
Sample ID: 02-8912-1520	Code: X8744	Project: WET Monthly Compliance Test (APR)
Sample Date: 09 Apr-23 06:15	Material: POTW Effluent	Source: AR0021776 (AR0021776)
Receipt Date: 10 Apr-23 15:00	CAS (PC):	Station: 001
Sample Age: 54h (1.9 °C)	Client: Nashville Public Works	

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Untransformed	C > T	78	>78	---	1.3	8.53	31.59%

Wilcoxon/Bonferroni Adj Test

Control	vs	Conc-%	df	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)
Dilution Water		25	17	81.5	---	3	Exact	0.3432	Non-Significant Effect
		33	17	82	---	1	Exact	0.3740	Non-Significant Effect
		44	16	86.5	---	3	Exact	1.0000	Non-Significant Effect
		59	16	86	---	4	Exact	1.0000	Non-Significant Effect
		78	16	76.5	---	2	Exact	1.0000	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	269.36	53.872	5	0.9502	0.4571	Non-Significant Effect
Error	2834.62	56.6924	50			
Total	3103.98		55			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	4.877	15.09	0.4310	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9334	0.9426	0.0041	Non-Normal Distribution

Reproduction Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	9	27	20.31	33.69	28	12	38	2.901	32.23%	0.00%
25		10	22	17.82	26.18	23	13	28	1.85	26.59%	18.52%
33		10	22.4	18.28	26.52	24.33	13	30	1.821	25.71%	17.04%
44		9	26.33	18.57	34.1	29	4	39	3.367	38.35%	2.47%
59		9	27.44	21.17	33.71	29	8	36	2.719	29.72%	-1.65%
78		9	24	19.5	28.5	24	11	31	1.951	24.38%	11.11%

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	29	22	38	17	27	34	28	36	12	
25		27	13	22	14	21	24	27	16	28	28
33		21	26	27	30	20	20	27	14	26	13
44		4	33	31	28	22	39	20	29	31	
59		32	27	34	8	29	26	25	36	30	
78		31	29	11	23	29	23	22	24	24	

EGB
5/2/23

CETIS Analytical Report

Report Date: 02 May-23 15:19 (p 1 of 2)
Test Code/ID: E870CEB / 02-4373-1691

Ceriodaphnia 7-d Survival and Reproduction Test

Bio-Analytical Laboratories

Analysis ID: 03-0080-6731	Endpoint: Reproduction	CETIS Version: CETISv2.1.4
Analyzed: 02 May-23 15:19	Analysis: Nonparametric-Control vs Treatments	Status Level: 1
Edit Date: 02 May-23 15:17	MD5 Hash: 80F4CB5DE7B22EEF5BC1FBA1B76C1872	Editor ID: 008-522-314-5
Batch ID: 21-2809-0947	Test Type: Reproduction-Survival (2-8d)	Analyst: Lab Tech
Start Date: 11 Apr-23 12:25	Protocol: EPA/821/R-02-013 (2002)	Diluent: Reconstituted Water
Ending Date: 18 Apr-23 18:50	Species: Ceriodaphnia dubia	Brine:
Test Length: 7d 6h	Taxon: Branchiopoda	Source: In-House Culture Age: <24
Sample ID: 02-8912-1520	Code: X8744	Project: WET Monthly Compliance Test (APR)
Sample Date: 09 Apr-23 06:15	Material: POTW Effluent	Source: AR0021776 (AR0021776)
Receipt Date: 10 Apr-23 15:00	CAS (PC):	Station: 001
Sample Age: 54h (1.9 °C)	Client: Nashville Public Works	

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Untransformed	C > T	78	>78	---	1.3	9.469	38.65%

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	91.5	75	3	CDF	0.4046	Non-Significant Effect
		33	18	92	75	1	CDF	0.4218	Non-Significant Effect
		44	18	105.5	75	3	CDF	0.8444	Non-Significant Effect
		59	18	106	75	4	CDF	0.8549	Non-Significant Effect
		78	18	96.5	75	2	CDF	0.5806	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	112.4	22.48	5	0.2628	0.9314	Non-Significant Effect
Error	4620	85.5556	54			
Total	4732.4		59			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	8.856	15.09	0.1149	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9235	0.9459	0.0011	Non-Normal Distribution

Reproduction Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	10	24.5	16.35	32.65	27.5	2	38	3.603	46.51%	0.00%
25		10	22	17.82	26.18	23	13	28	1.85	26.59%	10.20%
33		10	22.4	18.28	26.52	24.33	13	30	1.821	25.71%	8.57%
44		10	23.7	14.65	32.75	28.5	0	39	4	53.37%	3.27%
59		10	25.7	18.93	32.47	28	8	36	2.993	36.82%	-4.90%
78		10	22.1	16.26	27.94	23.5	5	31	2.58	36.91%	9.80%

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	29	22	38	17	2	27	34	28	36	12
25		27	13	22	14	21	24	27	16	28	28
33		21	26	27	30	20	20	27	14	26	13
44		4	0	33	31	28	22	39	20	29	31
59		32	27	34	10	8	29	26	25	36	30
78		31	29	11	23	29	5	23	22	24	24

EVB
5/12/23

CETIS Analytical Report

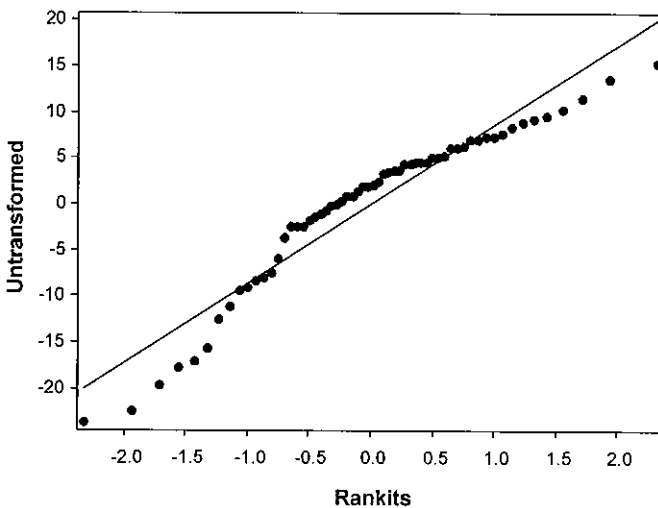
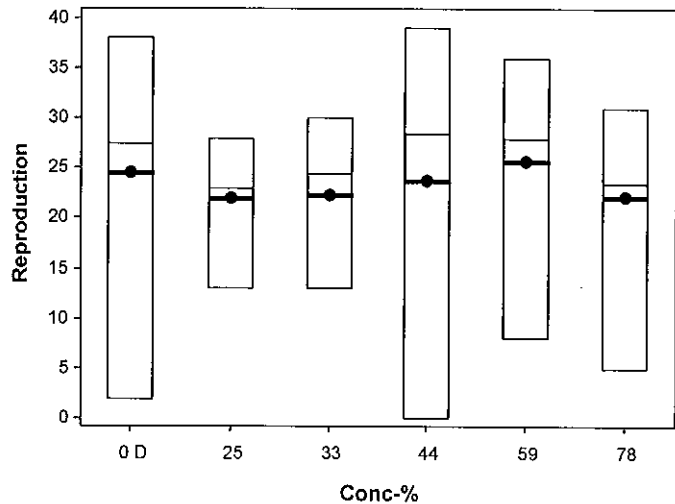
Report Date: 02 May-23 15:19 (p 2 of 2)
Test Code/ID: E870CEB / 02-4373-1691

Ceriodaphnia 7-d Survival and Reproduction Test

Bio-Analytical Laboratories

Analysis ID: 03-0080-6731 Endpoint: Reproduction CETIS Version: CETISv2.1.4
Analyzed: 02 May-23 15:19 Analysis: Nonparametric-Control vs Treatments Status Level: 1
Edit Date: 02 May-23 15:17 MD5 Hash: 80F4CB5DE7B22EEF5BC1FBA1B76C1872 Editor ID: 008-522-314-5

Graphics



EVB
5/2/23

CETIS Analytical Report

Report Date: 02 May-23 15:20 (p 1 of 2)
Test Code/ID: E870CEB / 02-4373-1691

Ceriodaphnia 7-d Survival and Reproduction Test

Bio-Analytical Laboratories

Analysis ID: 20-1909-8854	Endpoint: Reproduction	CETIS Version: CETISv2.1.4
Analyzed: 02 May-23 15:20	Analysis: Linear Interpolation (ICPIN)	Status Level: 1
Edit Date: 02 May-23 15:17	MD5 Hash: 80F4CB5DE7B22EEF5BC1FBA1B76C1872	Editor ID: 008-522-314-5
Batch ID: 21-2809-0947	Test Type: Reproduction-Survival (2-8d)	Analyst: Lab Tech
Start Date: 11 Apr-23 12:25	Protocol: EPA/821/R-02-013 (2002)	Diluent: Reconstituted Water
Ending Date: 18 Apr-23 18:50	Species: Ceriodaphnia dubia	Brine:
Test Length: 7d 6h	Taxon: Branchiopoda	Source: In-House Culture Age: <24
Sample ID: 02-8912-1520	Code: X8744	Project: WET Monthly Compliance Test (APR)
Sample Date: 09 Apr-23 06:15	Material: POTW Effluent	Source: AR0021776 (AR0021776)
Receipt Date: 10 Apr-23 15:00	CAS (PC):	Station: 001
Sample Age: 54h (1.9 °C)	Client: Nashville Public Works	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1193134	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	24.5	27.5	2	38	46.51%	0.00%	24.5	0.00%
25		10	22	23	13	28	26.59%	10.20%	23.45	4.29%
33		10	22.4	24.33	13	30	25.71%	8.57%	23.45	4.29%
44		10	23.7	28.5	0	39	53.37%	3.27%	23.45	4.29%
59		10	25.7	28	8	36	36.82%	-4.90%	23.45	4.29%
78		10	22.1	23.5	5	31	36.91%	9.80%	22.1	9.80%

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	29	22	38	17	2	27	34	28	36	12
25		27	13	22	14	21	24	27	16	28	28
33		21	26	27	30	20	20	27	14	26	13
44		4	0	33	31	28	22	39	20	29	31
59		32	27	34	10	8	29	26	25	36	30
78		31	29	11	23	29	5	23	22	24	24

Handwritten: 5/12/23

CETIS Analytical Report

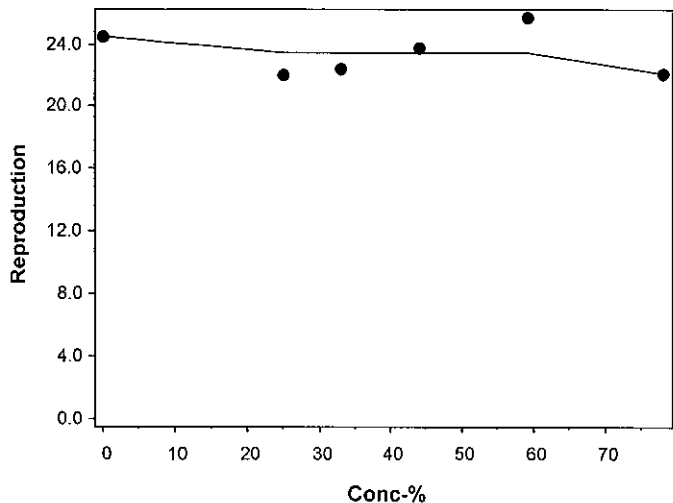
Report Date: 02 May-23 15:20 (p 2 of 2)
Test Code/ID: E870CEB / 02-4373-1691

Ceriodaphnia 7-d Survival and Reproduction Test

Bio-Analytical Laboratories

Analysis ID: 20-1909-8854	Endpoint: Reproduction	CETIS Version: CETISv2.1.4
Analyzed: 02 May-23 15:20	Analysis: Linear Interpolation (ICPIN)	Status Level: 1
Edit Date: 02 May-23 15:17	MD5 Hash: 80F4CB5DE7B22EEF5BC1FBA1B76C1872	Editor ID: 008-522-314-5

Graphics



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5/2/23

CETIS Analytical Report

Report Date: 21 Apr-23 10:39 (p 1 of 2)
Test Code/ID: 5E40592 / 00-9882-9714

Fathead Minnow 7-d Larval Survival and Growth Test

Bio-Analytical Laboratories

Analysis ID: 09-2680-2492	Endpoint: 7d Survival Rate	CETIS Version: CETISv2.1.4
Analyzed: 21 Apr-23 10:39	Analysis: Nonparametric-Control vs Treatments	Status Level: 1
Edit Date: 21 Apr-23 10:23	MD5 Hash: ECF6E605249FF10ACB6F7A7DBAD6F16F	Editor ID: 008-522-314-5
Batch ID: 01-8947-8712	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 11 Apr-23 12:10	Protocol: EPA/821/R-02-013 (2002)	Diluent: Reconstituted Water
Ending Date: 18 Apr-23 11:00	Species: Pimephales promelas	Brine:
Test Length: 6d 23h	Taxon: Actinopterygii	Source: In-House Culture Age: <24
Sample ID: 01-5205-5804	Code: X8744	Project: WET Monthly Compliance Test (MAR)
Sample Date: 09 Apr-23 06:15	Material: POTW Effluent	Source: AR0021776 (AR0021776)
Receipt Date: 10 Apr-23 15:00	CAS (PC):	Station: 001
Sample Age: 54h (1.9 °C)	Client: Nashville Public Works	

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	78	>78	---	1.3	0.2109	23.44%

Steel Many-One Rank Sum Test

Control	vs	Conc-%	df	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)
Dilution Water		25	8	29	16	3	CDF	0.9104	Non-Significant Effect
		33	8	31	16	3	CDF	0.9676	Non-Significant Effect
		44	8	33	16	3	CDF	0.9907	Non-Significant Effect
		59	8	27	16	3	CDF	0.8003	Non-Significant Effect
		78	8	27.5	16	3	CDF	0.8333	Non-Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits		Overlap	Decision
		Lower	Upper		
Control Resp	0.9	0.8	<<	Yes	Passes Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0867817	0.0173563	5	0.5267	0.7538	Non-Significant Effect
Error	0.790894	0.0329539	24			
Total	0.877676		29			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	6.538	15.09	0.2573	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9002	0.9031	0.0085	Non-Normal Distribution

7d Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	5	0.9000	0.7701	1.0000	0.8750	0.7500	1.0000	0.0468	11.62%	0.00%
25		5	0.9000	0.6976	1.0000	1.0000	0.6250	1.0000	0.0729	18.11%	0.00%
33		5	0.9500	0.8650	1.0000	1.0000	0.8750	1.0000	0.0306	7.21%	-5.56%
44		5	0.9750	0.9056	1.0000	1.0000	0.8750	1.0000	0.0250	5.73%	-8.33%
59		5	0.8500	0.5950	1.0000	0.8750	0.5000	1.0000	0.0919	24.16%	5.56%
78		5	0.8750	0.6555	1.0000	1.0000	0.6250	1.0000	0.0791	20.20%	2.78%

Angular (Corrected) Transformed Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	5	1.2500	1.0690	1.4320	1.2090	1.0470	1.3930	0.0653	11.68%	0.00%
25		5	1.2600	0.9989	1.5210	1.3930	0.9117	1.3930	0.0941	16.69%	-0.77%
33		5	1.3200	1.1950	1.4450	1.3930	1.2090	1.3930	0.0450	7.62%	-5.53%
44		5	1.3560	1.2540	1.4580	1.3930	1.2090	1.3930	0.0367	6.06%	-8.47%
59		5	1.1980	0.8898	1.5060	1.2090	0.7854	1.3930	0.1110	20.73%	4.19%
78		5	1.2280	0.9401	1.5150	1.3930	0.9117	1.3930	0.1036	18.86%	1.82%

EUB
4/21/23

CETIS Analytical Report

Report Date: 21 Apr-23 10:39 (p 2 of 2)
 Test Code/ID: 5E40592 / 00-9882-9714

Fathead Minnow 7-d Larval Survival and Growth Test

Bio-Analytical Laboratories

Analysis ID: 09-2680-2492 Endpoint: 7d Survival Rate CETIS Version: CETISv2.1.4
 Analyzed: 21 Apr-23 10:39 Analysis: Nonparametric-Control vs Treatments Status Level: 1
 Edit Date: 21 Apr-23 10:23 MD5 Hash: ECF6E605249FF10ACB6F7A7DBAD6F16F 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	0.8750	0.8750	1.0000	0.7500
25		0.8750	0.6250	1.0000	1.0000	1.0000
33		1.0000	0.8750	1.0000	0.8750	1.0000
44		1.0000	1.0000	0.8750	1.0000	1.0000
59		1.0000	0.8750	0.8750	1.0000	0.5000
78		0.6250	1.0000	0.7500	1.0000	1.0000

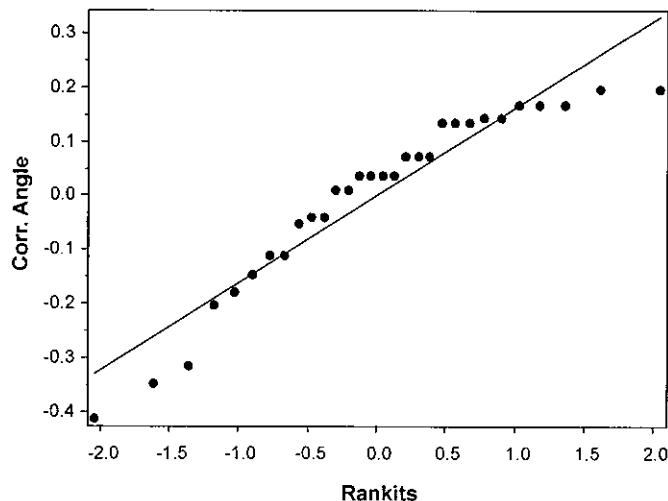
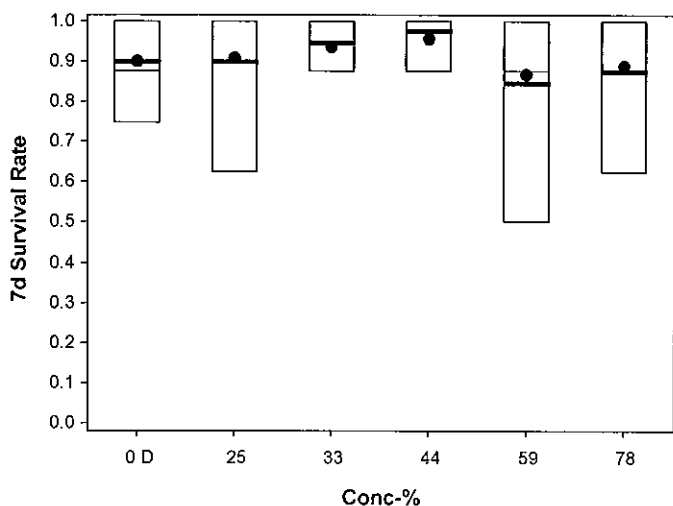
Angular (Corrected) Transformed Detail

Conc.-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.3930	1.2090	1.2090	1.3930	1.0470
25		1.2090	0.9117	1.3930	1.3930	1.3930
33		1.3930	1.2090	1.3930	1.2090	1.3930
44		1.3930	1.3930	1.2090	1.3930	1.3930
59		1.3930	1.2090	1.2090	1.3930	0.7854
78		0.9117	1.3930	1.0470	1.3930	1.3930

7d Survival Rate Binomials

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

Graphics



EUB
4/21/23

CETIS Analytical Report

Report Date: 25 Apr-23 13:22 (p 1 of 2)
Test Code/ID: 5E40592 / 00-9882-9714

Fathead Minnow 7-d Larval Survival and Growth Test

Bio-Analytical Laboratories

Analysis ID: 14-6473-0669	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv2.1.4
Analyzed: 25 Apr-23 13:22	Analysis: Parametric-Control vs Treatments	Status Level: 1
Edit Date: 25 Apr-23 13:20	MD5 Hash: 60F3276B70A85D847942660A39936A5A	Editor ID: 008-522-314-5
Batch ID: 01-8947-8712	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 11 Apr-23 12:10	Protocol: EPA/821/R-02-013 (2002)	Diluent: Reconstituted Water
Ending Date: 18 Apr-23 11:00	Species: Pimephales promelas	Brine:
Test Length: 6d 23h	Taxon: Actinopterygii	Source: In-House Culture Age: <24
Sample ID: 01-5205-5804	Code: X8744	Project: WET Monthly Compliance Test (MAR)
Sample Date: 09 Apr-23 06:15	Material: POTW Effluent	Source: AR0021776 (AR0021776)
Receipt Date: 10 Apr-23 15:00	CAS (PC):	Station: 001
Sample Age: 54h (1.9 °C)	Client: Nashville Public Works	

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Untransformed	C > T	78	>78	---	1.3	0.144	30.16%

Dunnett Multiple Comparison Test

Control	vs	Conc-%	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		25*	8	2.829	2.362	0.144	CDF	0.0187	Significant Effect
		33	8	0.082	2.362	0.144	CDF	0.8080	Non-Significant Effect
		44	8	0.533	2.362	0.144	CDF	0.6325	Non-Significant Effect
		59	8	1.845	2.362	0.144	CDF	0.1302	Non-Significant Effect
		78	8	0.9839	2.362	0.144	CDF	0.4271	Non-Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits			Decision
		Lower	Upper	Overlap	
Control Resp	0.4775	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	0.113548	0.0227096	5	2.443	0.0633	Non-Significant Effect
Error	0.223123	0.0092968	24			
Total	0.336671		29			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	6.721	15.09	0.2422	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9897	0.9031	0.9893	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	0.4775	0.3149	0.6401	0.475	0.35	0.6875	0.05855	27.42%	0.00%
25		5	0.305	0.2293	0.3807	0.3125	0.225	0.3625	0.02727	19.99%	36.13%
33		5	0.4725	0.4002	0.5448	0.4625	0.4125	0.55	0.02604	12.32%	1.05%
44		5	0.445	0.3863	0.5037	0.45	0.4	0.5125	0.02114	10.62%	6.81%
59		5	0.365	0.2239	0.5061	0.375	0.1875	0.475	0.05084	31.14%	23.56%
78		5	0.4175	0.2586	0.5764	0.35	0.3	0.5625	0.05723	30.65%	12.57%

Mean Dry Biomass-mg Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	0.4875	0.6875	0.475	0.3875	0.35
25		0.3625	0.2625	0.3625	0.3125	0.225
33		0.5125	0.425	0.4625	0.4125	0.55
44		0.4	0.4	0.45	0.5125	0.4625
59		0.375	0.475	0.3375	0.45	0.1875
78		0.325	0.55	0.3	0.35	0.5625

EBB
4/25/23

CETIS Analytical Report

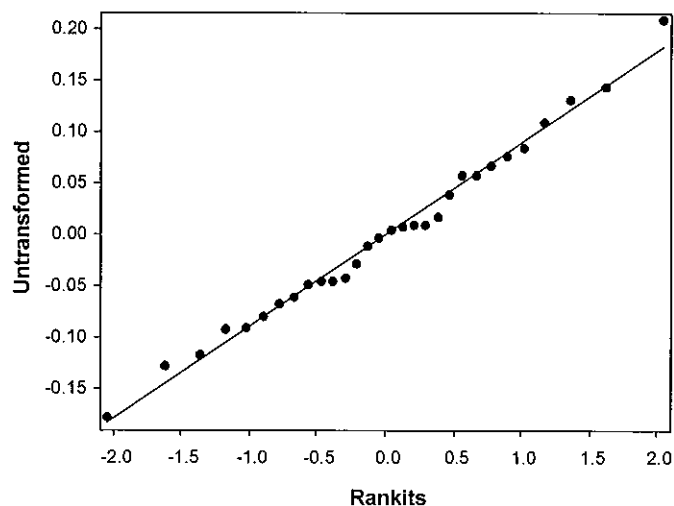
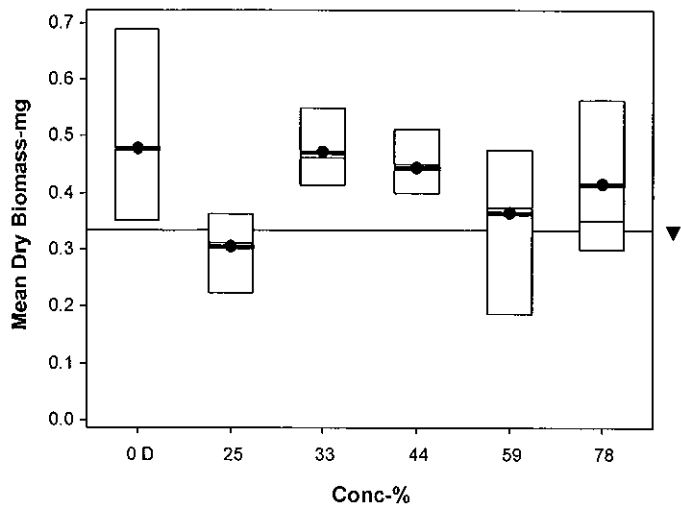
Report Date: 25 Apr-23 13:22 (p 2 of 2)
Test Code/ID: 5E40592 / 00-9882-9714

Fathead Minnow 7-d Larval Survival and Growth Test

Bio-Analytical Laboratories

Analysis ID: 14-6473-0669	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv2.1.4
Analyzed: 25 Apr-23 13:22	Analysis: Parametric-Control vs Treatments	Status Level: 1
Edit Date: 25 Apr-23 13:20	MD5 Hash: 60F3276B70A85D847942660A39936A5A	Editor ID: 008-522-314-5

Graphics



EVB
4/25/23

CETIS Analytical Report

Report Date: 25 Apr-23 13:22 (p 1 of 2)
Test Code/ID: 5E40592 / 00-9882-9714

Fathead Minnow 7-d Larval Survival and Growth Test

Bio-Analytical Laboratories

Analysis ID: 17-4128-5553	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv2.1.4
Analyzed: 25 Apr-23 13:22	Analysis: Linear Interpolation (ICPIN)	Status Level: 1
Edit Date: 25 Apr-23 13:20	MD5 Hash: 60F3276B70A85D847942660A39936A5A	Editor ID: 008-522-314-5
Batch ID: 01-8947-8712	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 11 Apr-23 12:10	Protocol: EPA/821/R-02-013 (2002)	Diluent: Reconstituted Water
Ending Date: 18 Apr-23 11:00	Species: Pimephales promelas	Brine:
Test Length: 6d 23h	Taxon: Actinopterygii	Source: In-House Culture Age: <24
Sample ID: 01-5205-5804	Code: X8744	Project: WET Monthly Compliance Test (MAR)
Sample Date: 09 Apr-23 06:15	Material: POTW Effluent	Source: AR0021776 (AR0021776)
Receipt Date: 10 Apr-23 15:00	CAS (PC):	Station: 001
Sample Age: 54h (1.9 °C)	Client: Nashville Public Works	

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1302426	1000	Yes	Two-Point Interpolation

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits		Overlap	Decision
		Lower	Upper		
Control Resp	0.4775	0.25	<<	Yes	Passes Criteria

Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
IC15	45.5	---	---	2.2	---	---
IC20	>78	---	---	<1.3	---	---
IC25	>78	---	---	<1.3	---	---
IC40	>78	---	---	<1.3	---	---
IC50	>78	---	---	<1.3	---	---

Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Mean	Median	Calculated Variate				Isotonic Variate	
					Min	Max	CV%	%Effect	Mean	%Effect
0	D	5	0.4775	0.475	0.35	0.6875	27.42%	0.00%	0.4775	0.00%
25		5	0.305	0.3125	0.225	0.3625	19.99%	36.13%	0.4075	14.66%
33		5	0.4725	0.4625	0.4125	0.55	12.32%	1.05%	0.4075	14.66%
44		5	0.445	0.45	0.4	0.5125	10.62%	6.81%	0.4075	14.66%
59		5	0.365	0.375	0.1875	0.475	31.14%	23.56%	0.3913	18.05%
78		5	0.4175	0.35	0.3	0.5625	30.65%	12.57%	0.3913	18.05%

Mean Dry Biomass-mg Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	0.4875	0.6875	0.475	0.3875	0.35
25		0.3625	0.2625	0.3625	0.3125	0.225
33		0.5125	0.425	0.4625	0.4125	0.55
44		0.4	0.4	0.45	0.5125	0.4625
59		0.375	0.475	0.3375	0.45	0.1875
78		0.325	0.55	0.3	0.35	0.5625

EUB
4/25/23

CETIS Analytical Report

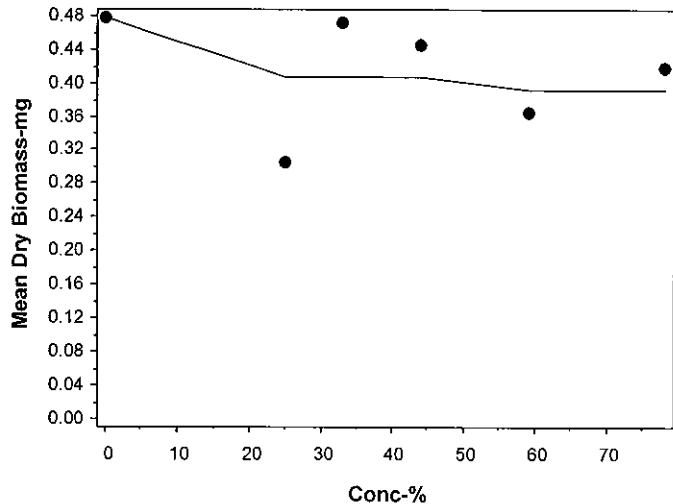
Report Date: 25 Apr-23 13:22 (p 2 of 2)
Test Code/ID: 5E40592 / 00-9882-9714

Fathead Minnow 7-d Larval Survival and Growth Test

Bio-Analytical Laboratories

Analysis ID: 17-4128-5553	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv2.1.4
Analyzed: 25 Apr-23 13:22	Analysis: Linear Interpolation (ICPIN)	Status Level: 1
Edit Date: 25 Apr-23 13:20	MD5 Hash: 60F3276B70A85D847942660A39936A5A	Editor ID: 008-522-314-5

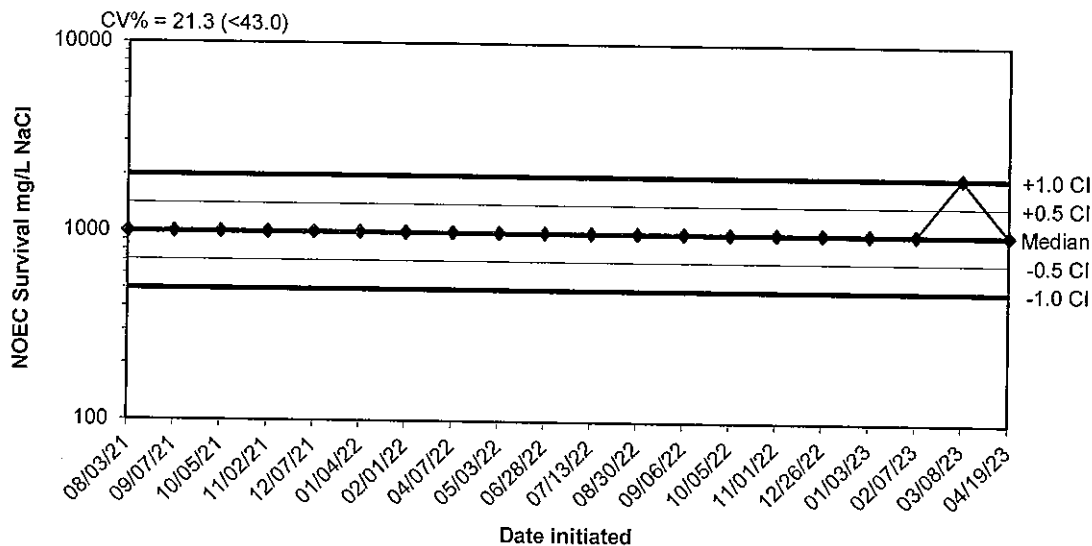
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EWB
4/25/23

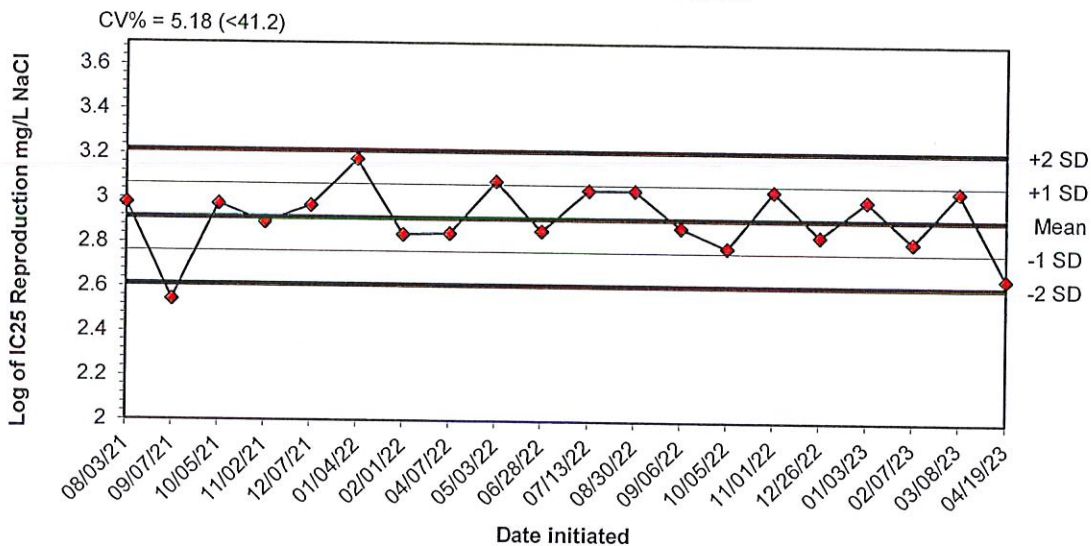
APPENDIX D
QUALITY ASSURANCE CHARTS

2023 CHRONIC REFERENCE TOXICANT TEST RESULTS FOR CERIODAPHNIA DUBIA IN MH WATER



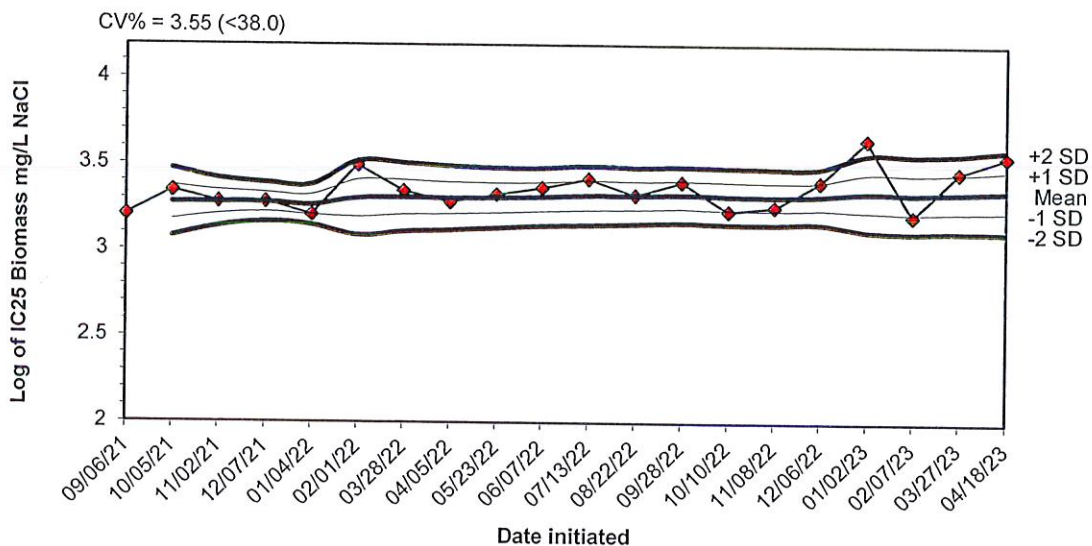
Dates	Values	Median	-0.5 CI	-1.0 CI	+0.5 CI	+1.0 CI
08/03/21	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
09/07/21	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
10/05/21	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
11/02/21	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
12/07/21	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
01/04/22	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
02/01/22	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
04/07/22	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
05/03/22	1000.0000	1000.0000	707.1068	500.0000	1414.2136	2000.0000
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

**2023 CHRONIC REFERENCE TOXICANT TEST RESULTS FOR
 CERIODAPHНИЯ DUBIA IN MH WATER**



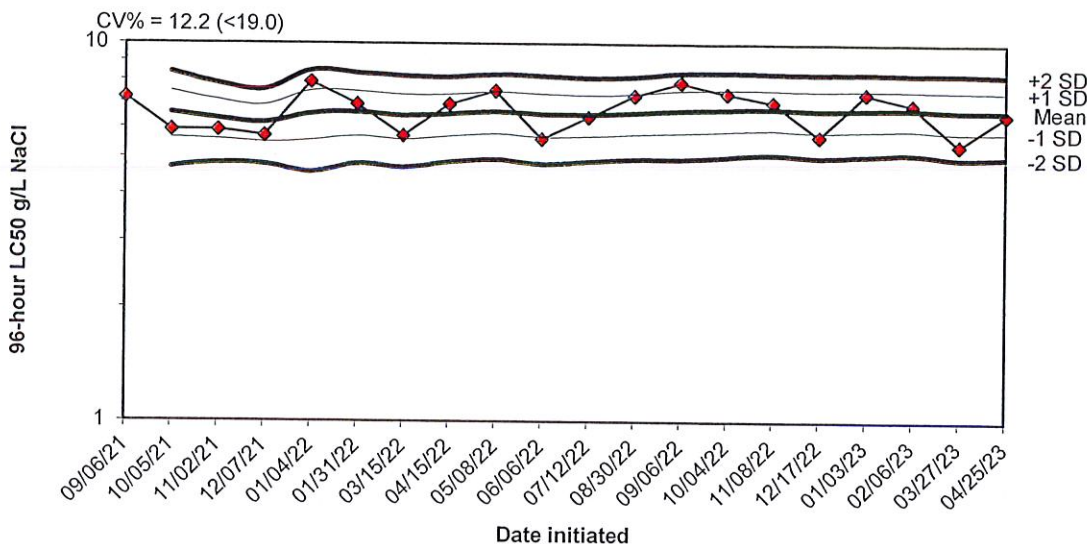
Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
08/03/21	2.9777	2.9140	2.7630	2.6120	3.0650	3.2160
09/07/21	2.5441	2.9140	2.7630	2.6120	3.0650	3.2160
10/05/21	2.9731	2.9140	2.7630	2.6120	3.0650	3.2160
11/02/21	2.8921	2.9140	2.7630	2.6120	3.0650	3.2160
12/07/21	2.9685	2.9140	2.7630	2.6120	3.0650	3.2160
01/04/22	3.1761	2.9140	2.7630	2.6120	3.0650	3.2160
02/01/22	2.8388	2.9140	2.7630	2.6120	3.0650	3.2160
04/07/22	2.8451	2.9140	2.7630	2.6120	3.0650	3.2160
05/03/22	3.0792	2.9140	2.7630	2.6120	3.0650	3.2160
06/28/22	2.8573	2.9140	2.7630	2.6120	3.0650	3.2160
07/13/22	3.0414	2.9140	2.7630	2.6120	3.0650	3.2160
08/30/22	3.0414	2.9140	2.7630	2.6120	3.0650	3.2160
09/06/22	2.8751	2.9140	2.7630	2.6120	3.0650	3.2160
10/05/22	2.7853	2.9140	2.7630	2.6120	3.0650	3.2160
11/01/22	3.0414	2.9140	2.7630	2.6120	3.0650	3.2160
12/26/22	2.8388	2.9140	2.7630	2.6120	3.0650	3.2160
01/03/23	3.0000	2.9140	2.7630	2.6120	3.0650	3.2160
02/07/23	2.8129	2.9140	2.7630	2.6120	3.0650	3.2160
03/08/23	3.0414	2.9140	2.7630	2.6120	3.0650	3.2160
04/19/23	2.6501	2.9140	2.7630	2.6120	3.0650	3.2160

2023 CHRONIC REFERENCE TOXICANT TEST RESULTS FOR PIMEPHALES PROMELAS IN MH WATER



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
09/06/21	3.2041					
10/05/21	3.3424	3.2733	3.1755	3.0777	3.3711	3.4689
11/02/21	3.2788	3.2751	3.2059	3.1367	3.3443	3.4135
12/07/21	3.2788	3.2760	3.2195	3.1629	3.3326	3.3891
01/04/22	3.2041	3.2616	3.2030	3.1445	3.3202	3.3788
02/01/22	3.4914	3.2999	3.1925	3.0851	3.4074	3.5148
03/28/22	3.3424	3.3060	3.2066	3.1072	3.4054	3.5047
04/05/22	3.2788	3.3026	3.2101	3.1176	3.3951	3.4876
05/23/22	3.3222	3.3048	3.2180	3.1312	3.3916	3.4783
06/07/22	3.3617	3.3105	3.2267	3.1429	3.3942	3.4780
07/13/22	3.4150	3.3200	3.2345	3.1490	3.4055	3.4910
08/22/22	3.3222	3.3202	3.2386	3.1571	3.4017	3.4832
09/28/22	3.3979	3.3261	3.2452	3.1642	3.4071	3.4881
10/10/22	3.2304	3.3193	3.2374	3.1555	3.4012	3.4831
11/08/22	3.2553	3.3150	3.2344	3.1538	3.3957	3.4763
12/06/22	3.3979	3.3202	3.2396	3.1590	3.4008	3.4814
01/02/23	3.6435	3.3392	3.2286	3.1180	3.4499	3.5605
02/07/23	3.2041	3.3317	3.2198	3.1078	3.4437	3.5556
03/27/23	3.4550	3.3382	3.2258	3.1134	3.4506	3.5630
04/18/23	3.5469	3.3486	3.2297	3.1108	3.4676	3.5865

**2023 ACUTE REFERENCE TOXICANT TEST RESULTS FOR
 PIMEPHALES PROMELAS IN MH WATER**



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
09/06/21	7.2000					
10/05/21	5.9000	6.5500	5.6308	4.7115	7.4692	8.3885
11/02/21	5.9000	6.3333	5.5828	4.8322	7.0839	7.8344
12/07/21	5.7000	6.1750	5.4852	4.7954	6.8648	7.5546
01/04/22	7.9000	6.5200	5.5443	4.5686	7.4957	8.4714
01/31/22	6.9000	6.5833	5.6970	4.8106	7.4697	8.3561
03/15/22	5.7000	6.4571	5.5818	4.7065	7.3325	8.2078
04/15/22	6.9000	6.5125	5.6871	4.8617	7.3379	8.1633
05/08/22	7.5000	6.6222	5.7829	4.9436	7.4615	8.3008
06/06/22	5.6000	6.5200	5.6652	4.8104	7.3748	8.2296
07/12/22	6.4000	6.5091	5.6974	4.8856	7.3208	8.1326
08/30/22	7.3000	6.5750	5.7681	4.9611	7.3819	8.1889
09/06/22	7.9000	6.6769	5.8214	4.9659	7.5324	8.3880
10/04/22	7.4000	6.7286	5.8842	5.0398	7.5729	8.4173
11/08/22	7.0000	6.7467	5.9300	5.1133	7.5633	8.3800
12/17/22	5.7000	6.6813	5.8500	5.0188	7.5125	8.3437
01/03/23	7.4000	6.7235	5.9000	5.0765	7.5470	8.3705
02/06/23	6.9000	6.7333	5.9333	5.1333	7.5333	8.3333
03/27/23	5.4000	6.6632	5.8277	4.9922	7.4986	8.3341
04/25/23	6.5000	6.6550	5.8410	5.0270	7.4690	8.2830

**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	0615	04/09/23	To	1913	04/09/23
Composite 2 Collected From	0615	04/11/23	To	0405	04/12/23
Composite 3 Collected From	0730	04/13/23	To	0620	04/14/23
Test initiated:	1225	am/pm		04/11/23	Date
Test terminated:	1850	am/pm		04/18/23	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	90.0	100.0	100.0
End of test	90.0	100.0	100.0	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	29	27	21	4	32	31
B	22	13	26	D	27	29
C	38	22	27	33	34	11
D	17	14	30	31	D10	23
E	D2	21	20	28	8	29
F	27	24	20	22	29	D5
G	34	27	27	39	26	23
H	28	16	14	20	25	22
I	36	28	26	29	36	24
J	12	28	13	31	30	24
Surv. Mean	27.0	22.0	22.4	26.3	27.4	24.0
Total Mean	24.5	22.0	22.4	23.7	25.7	22.1
CV%*	32.23	26.59	25.71	38.35	29.72	24.38

*coefficient of variation = standard deviation x 100/mean. D=dead adult
 PMSD = 38.65%

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#: AR0021776/ AFIN 31-00036
Contact: Larry Dunaway
Analysts: Ware, Mitchell, Miller, Valle

Sample #1 Collected: Date: 4/9/2023 Time: 1913
Sample #2 Collected: Date: 4/12/2023 Time: 405
Sample #3 Collected: Date: 4/14/2023 Time: 620
Test Begin: Date: 4/11/2023 Time: 1225
Test End: Date: 4/18/2023 Time: 1850

Dilution:	0%							44.0%						
Day:	1	2	3	4	5	6	7	1	2	3	4	5	6	7
T (°C)	24.4	24.3	24.3	25.0	25.6	24.4	24.7	24.4	24.3	24.3	25.0	25.6	24.4	24.7
DO Initial	8.4	8.3	8.5	8.0	8.4	8.3	8.1	8.4	8.4	8.0	8.1	8.4	8.2	9.0
DO Final	8.4	7.6	7.5	8.2	7.9	8.1	6.6	7.1	7.4	7.6	8.0	7.8	8.1	
pH Initial	7.1	7.1	7.2	7.5	7.1	6.9	6.6	7.4	7.1	7.3	7.5	7.4	7.3	7.5
pH Final	7.1	7.3	7.4	7.3	7.3	7.5		7.6	7.7	7.4	7.6	7.6	7.4	
Conductivity	280.0	293.0	273.0	309.0	242.0			228.0	240.0	233.0	275.0	236.0	234.0	
Alkalinity	56.0													
Hardness	116.0													
Chlorine	<0.5													
Dilution:	25.0%							59.0%						
Day:	1	2	3	4	5	6	7	1	2	3	4	5	6	7
T (°C)	24.4	24.3	24.3	25.0	25.6	24.4	24.7	24.4	24.3	24.3	25.0	25.6	24.4	24.7
DO Initial	8.4	8.2	8.4	8.0	8.0	8.3	9.0	8.5	8.5	8.4	8.0	8.4	8.3	8.9
DO Final	8.3	7.6	7.5	8.1	8.0	8.0		7.9	7.7	7.6	8.1	7.9	8.1	
pH Initial	7.3	7.1	7.5	7.6	7.3	7.2	7.4	7.4	7.2	7.3	7.5	7.4	7.2	7.5
pH Final	7.4	7.8	7.4	7.6	7.6	7.5		7.5	7.6	7.4	7.6	7.6	7.4	
Conductivity	258.0	252.0	270.0	292.0	238.0	238.0		227.0	232.0	223.0	258.0	225.0	232.0	
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)	24.4	24.3	24.3	25.0	25.6	24.4	24.7	24.4	24.3	24.3	25.0	25.6	24.4	24.7
DO Initial	8.6	8.3	8.4	8.0	8.2	8.5	8.9	8.5	8.5	8.4	8.0	8.5	8.6	8.9
DO Final	8.2	7.6	7.4	8.0	8.0	8.0		8.1	7.5	7.6	8.0	7.9	8.2	
pH Initial	7.4	7.1	7.5	7.5	7.4	7.3	7.5	7.4	7.2	7.3	7.5	7.4	7.3	7.4
pH Final	7.5	7.7	7.3	7.7	7.6	7.5		7.5	7.7	7.4	7.6	7.5	7.4	
Conductivity								199.0	225.0	220.0	245.0	233.0	229.0	
Alkalinity								36.0	32.0		60.0			
Hardness								56.0	64.0		56.0			
Chlorine								<0.5	<0.5		<0.5			

Comments: Alkalinity and hardness conducted on 100% effluent.

**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:	0615	04/09/23	To	1913	04/09/23
Composite 2 Collected from:	0615	04/11/23	To	0405	04/12/23
Composite 3 Collected from:	0730	04/13/23	To	0620	04/14/23
Test initiated:	1210	am/pm		04/11/23	date
Test terminated:	1100	am/pm		04/18/23	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	87.5	87.5	100.0	75.0	100.0	100.0	90.0	11.68
25.0	87.5	62.5	100.0	100.0	100.0	100.0	100.0	90.0	16.69
33.0	100.0	87.5	100.0	87.5	100.0	100.0	100.0	95.0	7.62
44.0	100.0	100.0	87.5	100.0	100.0	100.0	100.0	97.5	6.06
59.0	100.0	87.5	87.5	100.0	50.0	100.0	100.0	85.0	20.73
78.0	62.5	100.0	75.0	100.0	100.0	100.0	100.0	87.5	18.86

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	0.488	0.688	0.475	0.388	0.350	0.478	27.42
25.0	0.363	0.263	0.363	0.313	0.225	0.305	19.99
33.0	0.513	0.425	0.463	0.413	0.550	0.473	12.32
44.0	0.400	0.400	0.450	0.513	0.463	0.445	10.62
59.0	0.375	0.475	0.338	0.450	0.188	0.365	31.14
78.0	0.325	0.550	0.300	0.350	0.563	0.418	30.65

*coefficient of variation = standard deviation x 100/mean.
 PMSD: 30.16%

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: Larry Dunaway
Analysts: Ware, Mitchell, Valle, Miller, Morado

Sample #1 Collected: 4/9/2023 Time: 1913
Sample #2 Collected: 4/12/2023 Time: 405
Sample #3 Collected: 4/14/2023 Time: 620
Test Begin: 4/11/2023 Time: 1210
Test End: 4/18/2023 Time: 1100

Dilution:	0%						
Day:	1	2	3	4	5	6	7
T (°C)	24.6	24.7	24.5	25.2	25.2	23.6	25.6
DO Initial	7.9	6.5	7.0	7.4	7.9	5.9	5.4
DO Final	8.2	7.7	7.7	8.0	7.8	7.9	
pH Initial	6.9	6.9	7.0	7.1	6.9	6.5	6.4
pH Final	7.6	7.7	7.6	7.6	7.7	7.6	
Conductivity	275.0	254.0	243.0	260.0	263.0	259.0	
Alkalinity	56.0	64.0					
Hardness	76.0	244.0					
Chlorine	<0.5	<0.5					
Dilution:	25.0%						
Day:	1	2	3	4	5	6	7
T (°C)	24.6	24.7	24.5	25.2	25.2	23.6	25.6
DO Initial	7.4	5.9	7.0	6.2	7.4	6.3	5.3
DO Final	8.2	7.6	7.5	8.1	7.9	8.0	
pH Initial	7.0	6.8	7.2	6.8	7.0	6.7	6.6
pH Final	7.6	7.7	7.4	7.6	7.7	7.6	
Conductivity	254.0	248.0	240.0	251.0	249.0	256.0	
Alkalinity							
Hardness							
Chlorine							
Dilution:	59.0%						
Day:	1	2	3	4	5	6	7
T (°C)	24.6	24.7	24.5	25.2	25.2	23.6	25.6
DO Initial	7.2	6.1	7.3	6.2	7.2	3.5	5.2
DO Final	8.1	7.5	7.3	8.6	7.9	7.9	
pH Initial	6.9	6.7	7.5	6.8	6.9	6.6	6.6
pH Final	7.5	7.7	7.4	7.5	7.6	7.5	
Conductivity	218.0	228.0	230.0	238.0	253.0	244.0	
Alkalinity							
Hardness							
Chlorine							
Dilution:	78.0%						
Day:	1	2	3	4	5	6	7
T (°C)	24.6	24.7	24.5	25.2	25.2	23.6	25.6
DO Initial	7.3	5.9	6.9	6.2	7.2	5.3	5.0
DO Final	7.9	7.5	7.6	8.2	7.9	8.0	
pH Initial	6.9	6.6	7.2	6.7	6.9	6.6	6.6
pH Final	7.3	7.7	7.4	7.5	7.6	7.5	
Alkalinity	36.0	32.0		60.0			
Hardness	56.0	64.0		56.0			
Conductivity	205.0	220.0	223.0	228.0	236.0	237.0	
Chlorine	<0.5	<0.5		<0.5			

Comments: Alkalinity and hardness conducted on 100% effluent

APPENDIX F
REPORT QUALITY ASSURANCE FORM



Bio-Analytical Laboratories

3240 Spurgin Road
Post Office Box 527
Doyline, LA 71023

(318) 745-2772
1-800-269-1246
Fax: (318) 745-2773

REPORT QUALITY ASSURANCE FORM

Client: Nashville

Project#: X8744

Chain of Custody Documents Checked by: Alexis Mitchell 4/21/23
Technician/Date

Raw Data Documents Checked by: Alexis Mitchell 4/21/23
Technician/Date

Statistical Analysis Package Checked by: EOB 4/25/23 | 5/2/23
Quality Manager/Date

Quality Control Data Checked by: EOB 5/4/23
Quality Manager/Date

Report Checked by: EOB 5/4/23
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.

Paul Beapp, BS
Quality Manager

5/4/23
Date

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