

## Bio-Analytical Laboratories' Executive Summary

**Permittee:** Nashville Public Works  
Nashville, AR 71852

**Project #:** X8909

**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:** August 22 - 29, 2023

**Test Type:** Chronic Static Renewal Survival and Growth Test using  
*Pimephales promelas* (EPA Method 1000.0)

### Results:

#### 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 -14.26%.
6. Chronic WET Limit, DMR CODE 51714, - 78.0%
7. PMSD Biomass-16.75% (12.0 – 30.0%)-moderate precision, acceptable for passing test.

This report contains a total of 33 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 A CHRONIC DEFINITIVE TOXICITY TEST FOR OUTFALL 001**

**AT**

**NASHVILLE PUBLIC WORKS  
Nashville, Arkansas**

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

**EPA Method 1000.0**

**Project X8909**

**Test Dates: August 22 - 29, 2023**

**Report Date: September 21, 2023**

**Prepared for:**

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

**Prepared by:**

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## **1.0 Introduction**

Bio-Analytical Laboratories (BAL), Doyline, Louisiana conducted a chronic definitive toxicity test for Outfall 001 at Nashville Public Works, Nashville, Arkansas. The test organism used was the fathead minnow, *Pimephales promelas*. The purpose of this study is to determine if appropriately dilute effluent samples adversely affect the survival and growth of the test organisms. Toxicity is defined as a statistically significant difference at the 95 percent confidence level between the survival and 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 and 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, 22<sup>nd</sup> Edition” (APHA 2012), and BAL’s standard operating procedure.

### **2.2 Test Organisms**

The fathead minnows were obtained from Aquatic Biosystems, Fort Collins, Colorado (ABS) and were less than 48 hours old at test initiation and hatched within the same 24-hour period. 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 test. 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 test (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 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 August 21, 23 and 25, 2023, at 2230, 0715 and 0554 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, 1.1 and 2.1<sup>0</sup> 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±1<sup>0</sup> 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 CaCO<sub>3</sub> on the control and the undiluted effluent samples.

## 2.7 Monitoring of the Test

The fathead minnow test was run in a circulating waterbath, using a Remcor<sup>R</sup> heated liquid circulator to keep a constant temperature of 25±1<sup>0</sup> Celsius. A data-logger was used to monitor diurnal test temperature. Test temperature was recorded at the beginning of the day, after test renewal and at the end of the day. Light cycle and intensity were recorded twice a month.

## 2.8 Data Analysis

Fathead minnow survival data was analyzed using Steel's Many-One Rank Test, a nonparametric test comparing concentration data to control data. 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 fathead minnow test results can be found in Table 1. After seven days of exposure, 97.5 percent survival occurred in the control and 100.0 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 0.580 and 0.658 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	97.5		0.580	
25.0	100.0		0.645	
33.0	100.0		0.668	
44.0	100.0		0.645	
59.0	100.0		0.650	
78.0	100.0		0.658	

\*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 August 21, 23 and 25, 2023, from Nashville Public Works, Nashville, Arkansas, were not found to be lethally toxic to 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) were not noted in the 78.0 percent critical dilution (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. 22<sup>nd</sup> Edition.

**APPENDIX A**  
**CHAIN-OF-CUSTODY DOCUMENTS**



X8909 / C25156

Temperature upon arrival: 1.8<sup>°C</sup>  
 Thermometer #: 29  
 Tech: EDW  
 Date: 8/21/23

color: clear  
 odor: none

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

Date 8.21.23 Time 1152

Sampler Printed Name KEVIN FUNDERBURY  
 Sampler Signature CLIENT  
 Sampler Affiliation NASH

Please analyze the following samples:

Sample Identification	Client Code	Date	Time	Testing Required- FCSC
Bio Monitoring	NASH	8.20.23	0900-2230	Chronic ceriodaphnia dubia
		8.21.23	0900-2230	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	
8.21.23	1516	Printed Name <u>Alexis Hrescat</u>	Affiliation <u>SPL</u>	Printed Name <u>Alexis Mitchell</u>	Affiliation
		Signature <u>[Signature]</u>		Signature <u>Alexis Mitchell</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	

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

Temperature upon arrival: 1.1 °C  
 Thermometer #: 29  
 Tech: AM  
 Date: 8/23/23

clear; no odor

Date 8.23.23 Time 0015  
 Sampler Printed Name KEVIN FUNDER BURK  
 Sampler Signature CLIENT  
 Sampler Affiliation NASH

Please analyze the following samples:

Sample Identification	Client Code	Date	Time	Testing Required- FCSC
Bio Monitoring	NASH	8.22.23 8.23.23	0715- 0015	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	
8.23.23	1556	Printed Name <u>MARK HASCH</u>	Affiliation <u>SPL</u>	Printed Name <u>Curtis D. Brupp</u>	Affiliation <u>BAL</u>
		Signature <u>[Signature]</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	

X8909/C25486

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

Temperature upon arrival: 2.10°C  
 Thermometer #: 29  
 Tech: EUB  
 Date: 8/25/23

Date 8.25.23 Time 0554  
 Sampler Printed Name KEVIN FUNDERBURK  
 Sampler Signature CLIENT  
 Sampler Affiliation NASH

clear, no odor EUB 8/25/23

Please analyze the following samples:

Sample Identification	Client Code	Date	Time	Testing Required- FCSC
Bio Monitoring	NASH	8-24-23	0615-	Chronic ceriodaphnia dubia
		8-25-23	0554	Chronic pimephales promelas

EUB 8/25/23

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	
		Printed Name	Affiliation	Printed Name	Affiliation
8.25.23	1410	Mark Hirsch	SPL	Kevin Funderburk	BAL
		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	

**APPENDIX B  
RAW DATA SHEETS**

BIO-ANALYTICAL LABORATORIES  
 PIMEPHALES PROMELAS SURVIVAL AND GROWTH DATA SHEET

Project# X8909 Date started: 8/22/23 Date ended 8/29/23

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 organism age: < 48 hours Vendor/ID# ABS 1233

Day	Feeding Times		
	Technician/Time/Amount (per replicate)		
	AM	NOON	PM
0			
1	PM/0920/0.1ml	PM/1245/0.1ml	AM/11830/0.2ml
2	PM/1055/0.1ml	PM/1245/0.1ml	AM/11825/0.1ml
3	PM/1091/0.1ml	PM/1245/0.1ml	PM/11845/0.1ml
4	SPW/1055/0.20ml	PM/1300/0.1ml	PM/11725/0.1ml
5	SPW/0900/0.20ml		SPW/1555/0.20ml
6	PM/0920/0.10ml	SPW/1333/0.10ml	SPW/1600/0.20ml SPW/1750/0.10ml

Dissolved Oxygen Meter #: 2  
 pH Meter#: 3  
 ORP Meter#: \_\_\_\_\_ Conductivity Meter#: 8  
 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. 9.7/114.0% / dm	0. 4/12/8.1/97.8% / dm	0. _____	0. _____
1. 9.7/117.6% / dm	1. 4/6/8.2/97.7% / dm	1. _____	1. _____
2. 9.5/114.2% / dm	2. 4/6/8.2/99.9% / dm	2. _____	2. _____
3. 9.0/108.0% / dm	3. 4/6/7.8/94.7% / dm	3. _____	3. _____
4. 8.1/99.7% / SPW	4. NO / SPW	4. _____	4. _____
5. 8.9/110.2% / SPW	5. 4/6/7.9/95.6% / SPW	5. _____	5. _____
6. 8.9/100.4% / SPW	6. NO / SPW	6. _____	6. _____

Total Residual Chlorine (mg/L)/Tech	Dechlorinated? Amount?/Tech	Ammonia (NH3) (mg/L)/Tech	BAL Sample # Date in use
1. <0.5 / dm	1. No / dm	1. <0.5 / dm	1. C25456 8/22/23
2. <0.5 / dm	2. No / dm	2. <0.5 / dm	2. C25475 8/24/23
3. <0.5 / SPW	3. NO / SPW	3. <0.5 / SPW	3. C25486 8/26/23

Comments:

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

Project# 18909

Test started: Date 8/21/03 Time 1350

Client Nashville

Sample ID 001

Test ended: Date 8/21/03 Time 1300

Date/Tech: Day 0 8/21/03 1 8/22/03 2 8/23/03 3 8/24/03 4 8/25/03 5 8/26/03 6 8/27/03 7 8/28/03 8 8/29/03

Time: Day 0 1350 1 1100 2 1140 3 1555 4 1206 5 1208 6 1332 7 1300

Temp (°C) Day 0 25.0 1 25.0 2 26.1 3 24.2 4 23.0 5 23.0 6 23.5 7 24.6

Conc.	Rep.	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0 MH	1	8	8	8	8	8	8	8	8
	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	8	8	8
25%	1	8	8	8	8	8	8	8	8
	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
33%	1	8	8	8	8	8	8	8	8
	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
44%	1	8	8	8	8	8	8	8	8
	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
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	8	8	8
	4	8	8	8	8	8	8	8	8
	5	8	8	8	8	8	8	8	8
78%	1	8	8	8	8	8	8	8	8
	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

BIO-ANALYTICAL LABORATORIES MINNOW LARVAL GROWTH DATA SHEET (Minnow3 Rev Page 1 of 33)

Project#/Client X8909 Nashville Temp Start (°C) 76.0 Tech PM Date: 8/29/23 Time: 1300  
Temp End (°C) 25.5 Tech MV Date: 8/30/23 Time: 1000

Conc. %	Replicate/ Pan number	Wt. of pan(g)/ Date weighed: Tech:	Wt. of pan + larvae(g)/ Date weighed: Tech:	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%	1 131	1.0887 Date: 8/25/23 Tech: AM	1.0744 Date: 8/30/23 Tech: MV	7/30/23 MV			
	2 132	1.0725	1.0773				
	3 133	1.0814	1.0855				
	4 134	1.0930	1.0971				
	5 135	1.0832	1.0877				
25%	1 136	1.0790	1.0849				
	2 137	1.0985	1.1038				
	3 138	1.0980	1.1023				
	4 139	1.0852	1.0877				
	5 140	1.0976	1.1032				
33%	1 141	1.0924	1.0981				
	2 142	1.0964	1.1014				
	3 143	1.0652	1.0701				
	4 144	1.1142	1.1192				
	5 145	1.0947	1.1008				
44%	1 146	1.0888	1.0935				
	2 147	1.1024	1.1078				
	3 148	1.0986	1.1041				
	4 149	1.0873	1.0929				
	5 150	1.0746	1.0792				
59%	1 151	1.0774	1.0826				
	2 152	1.0761	1.0812				
	3 153	1.0989	1.1042				
	4 154	1.0970	1.1027				
	5 155	1.1116	1.1163				
78%	1 156	1.1017	1.1070				
	2 157	1.0853	1.0900				
	3 158	1.0997	1.1049				
	4 159	1.0919	1.0975				
	5 160	1.0912	1.0967				

\* Test acceptance of control weight based on surviving larvae at end of test.  
Calculated by: CETIS Calculations checked by: EBB 9/6/23

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

Project# X8909 Client Nashville Organism P. promelas

Date	Day 0 8/22/23 5489	Day 1 8/23/23	Day 2 8/24/23	Day 3 8/25/23	Day 4 8/26/23	Day 5 8/27/23	Day 6 8/28/23	Day 7 8/29/23	Day 8
Concentration:	<u>0 MH</u>								
Temperature (°C)	24.1	24.0	23.8	24.2	24.1	23.1	23.1	23.7	
pH	7.1	6.7	6.3	6.5	7.0	7.3	7.2	6.5	
DO (mg/l)	7.7	6.3	5.2	4.5	6.4	6.2	6.3	5.4	
Cond (umhos/cm)	300	295	325	335	330	340	320		
Concentration:	<u>25%</u>								
Temperature (°C)	24.2	23.9	23.9	24.1	23.4	23.2	23.5	23.6	
pH	7.4	6.7	6.4	6.7	6.9	6.0	7.0	6.9	
DO (mg/l)	7.5	6.3	5.3	4.9	5.3	6.0	6.8	5.6	
Cond (umhos/cm)	365	380	375	390	385	395	390		
Concentration:	<u>33%</u>								
Temperature (°C)	24.4	23.9	23.9	24.1	23.0	22.9	23.2	23.5	
pH	7.4	6.8	6.7	6.8	7.2	7.0	6.9	6.9	
DO (mg/l)	7.5	6.3	5.0	4.8	6.2	5.4	6.0	5.4	
Cond (umhos/cm)	385	395	390	405	410	420	425		
Prerenewal Tech Initials/Time		SEM 1027	1740	1600	EDV 1206	EDW 1208	SPC 1332	1305	
Postrenewal Tech Initials/Time	SEM 1150	1325	1020	1030	SEM 0937	EDW 1010	SEM 1145		

Control Alkalinity (mg/L as CaCO<sub>3</sub>)

Control Hardness (mg/L as CaCO<sub>3</sub>)

ID# 5489 Result 56.0 Date Tested 8/23/23 ID# 5489 Result 92.0 Date Tested 8/23/23  
 ID# 5493 Result 48.0 Date Tested 8/31/23 ID# 5493 Result 92.0 Date Tested 8/31/23  
 ID# \_\_\_\_\_ Result \_\_\_\_\_ Date Tested \_\_\_\_\_ ID# \_\_\_\_\_ Result \_\_\_\_\_ Date Tested \_\_\_\_\_

Sample Alkalinity (mg/L as CaCO<sub>3</sub>)

Sample Hardness (mg/L as CaCO<sub>3</sub>)

ID# C25456 Result 88.0 Date Tested 8/31/23 ID# C25456 Result 124.0 Date Tested 9/5/23  
 ID# C25475 Result 76.0 Date Tested 8/31/23 ID# C25475 Result 124.0 Date Tested 9/5/23  
 ID# C25486 Result 84.0 Date Tested 8/31/23 ID# C25486 Result 148.0 Date Tested 9/5/23



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

Project# X8909 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>441.</u>									
Temperature (°C)	<u>24.4</u>	<u>23.9</u> <u>25.0</u>	<u>23.9</u> <u>24.9</u>	<u>24.0</u> <u>24.7</u>	<u>23.1</u> <u>25.4</u>	<u>23.8</u> <u>24.4</u>	<u>24.0</u> <u>25.1</u>	<u>23.3</u>	
pH	<u>7.2</u>	<u>6.8</u> <u>7.7</u>	<u>6.8</u> <u>7.4</u>	<u>6.9</u> <u>7.5</u>	<u>6.9</u> <u>7.6</u>	<u>6.3</u> <u>7.9</u>	<u>7.0</u> <u>7.7</u>	<u>7.0</u>	
DO (mg/l)	<u>7.3</u>	<u>5.9</u> <u>7.1</u>	<u>5.0</u> <u>7.8</u>	<u>4.6</u> <u>7.5</u>	<u>5.4</u> <u>7.4</u>	<u>6.0</u> <u>7.5</u>	<u>6.5</u> <u>7.6</u>	<u>5.2</u>	
Cond (umhos/cm)	<u>405</u>	<u>420</u>	<u>410</u>	<del>335</del> <u>425</u>	<u>430</u>	<u>450</u>	<u>455</u>		
Concentration: <u>591.</u>									
Temperature (°C)	<u>24.5</u>	<u>23.9</u> <u>23.0</u>	<u>23.9</u> <u>25.0</u>	<u>24.0</u> <u>24.7</u>	<u>23.2</u> <u>25.5</u>	<u>22.9</u> <u>24.4</u>	<u>23.2</u> <u>25.0</u>	<u>23.0</u>	
pH	<u>7.3</u>	<u>6.8</u> <u>7.3</u>	<u>6.8</u> <u>7.4</u>	<u>6.9</u> <u>7.5</u>	<u>7.1</u> <u>7.6</u>	<u>7.3</u> <u>7.9</u>	<u>7.5</u> <u>7.6</u>	<u>7.0</u>	
DO (mg/l)	<u>7.4</u>	<u>6.2</u> <u>7.7</u>	<u>5.3</u> <u>8.7.8</u>	<u>5.1</u> <u>7.5</u>	<u>6.3</u> <u>7.6</u>	<u>5.4</u> <u>7.4</u>	<u>6.0</u> <u>7.5</u>	<u>5.5</u>	
Cond (umhos/cm)	<u>450</u>	<u>450</u>	<u>440</u>	<del>390</del> <u>460</u>	<u>455</u>	<u>480</u>	<u>490</u>		
Concentration: <u>781.</u>									
Temperature (°C)	<u>24.6</u>	<u>23.9</u> <u>23.0</u>	<u>23.9</u> <u>25.0</u>	<u>23.9</u> <u>24.8</u>	<u>23.6</u> <u>25.6</u>	<u>22.8</u> <u>25.6</u>	<u>23.0</u> <u>24.7</u>	<u>22.9</u>	
pH	<u>7.4</u>	<u>6.8</u> <u>7.3</u>	<u>6.9</u> <u>7.4</u>	<u>6.9</u> <u>7.5</u>	<u>7.2</u> <u>7.5</u>	<u>7.2</u> <u>7.7</u>	<u>7.0</u> <u>7.7</u>	<u>7.0</u>	
DO (mg/l)	<u>7.4</u>	<u>5.9</u> <u>7.1</u>	<u>5.0</u> <u>7.9</u>	<u>4.8</u> <u>7.6</u>	<u>6.2</u> <u>7.4</u>	<u>5.4</u> <u>7.5</u>	<u>5.8</u> <u>7.9</u>	<u>5.4</u>	
Cond (umhos/cm)	<u>490</u>	<u>490</u>	<u>485</u>	<del>4050</del> <u>4500</u>	<u>510</u>	<u>535</u>	<u>600</u>		
Prerenewal Tech Initials/Time		<u>60M</u> <u>1602</u>	<u>1740</u> <u>PM</u>	<u>1600</u> <u>PM</u>	<u>80M</u> <u>206</u>	<u>80M</u> <u>1208</u>	<u>80M</u> <u>1332</u>	<u>1305</u> <u>PM</u>	
Postrenewal Tech Initials/Time	<u>80M</u> <u>1150</u>	<u>1325</u> <u>AM</u>	<u>1020</u> <u>PM</u>	<u>1030</u> <u>PM</u>	<u>80M</u> <u>0937</u>	<u>80M</u> <u>1010</u>	<u>80M</u> <u>1145</u>		

Control Alkalinity (mg/L as CaCO<sub>3</sub>)

Control Hardness (mg/L as CaCO<sub>3</sub>)

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 CaCO<sub>3</sub>)

Sample Hardness (mg/L as CaCO<sub>3</sub>)

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: 21 Aug-23 09:56 (p 1 of 1)  
 Test Code/ID: B36EBE5 / 01-8814-8709

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

Start Date: 22 Aug-23 09:56    Species: Pimephales promelas    Sample Code: 1F3F2EAC  
 End Date: 29 Aug-23 09:56    Protocol: EPA/821/R-02-013 (2002)    Sample Source: AR0021776  
 Sample Date: 21 Aug-23 09:56    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
59		5	1												
0	D	1	2												
44		1	3												
59		3	4												
59		4	5												
44		2	6												
25		3	7												
25		4	8												
25		1	9												
78		4	10												
33		2	11												
33		4	12												
0	D	4	13												
59		1	14												
59		2	15												
33		3	16												
33		1	17												
44		4	18												
0	D	3	19												
78		1	20												
0	D	2	21												
44		5	22												
78		2	23												
33		5	24												
25		2	25												
44		3	26												
78		5	27												
78		3	28												
25		5	29												
0	D	5	30												

**APPENDIX C**  
**STATISTICAL ANALYSES**

# CETIS Analytical Report

Report Date: 05 Sep-23 10:35 (p 1 of 2)  
Test Code/ID: B36EBE5 / 01-8814-8709

## Fathead Minnow 7-d Larval Survival and Growth Test

Bio-Analytical Laboratories

Analysis ID: 10-8649-6054	Endpoint: 7d Survival Rate	CETIS Version: CETIS v2.1.5
Analyzed: 05 Sep-23 10:35	Analysis: Nonparametric-Control vs Treatments	Status Level: 1
Edit Date: 05 Sep-23 10:28	MD5 Hash: 7941F3B4BE2B240B24BC43B1B94571A2	Editor ID: 008-522-314-5
Batch ID: 07-1081-0053	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 22 Aug-23 13:50	Protocol: EPA/821/R-02-013 (2002)	Diluent: Receiving Water
Ending Date: 29 Aug-23 13:00	Species: Pimephales promelas	Brine:
Test Length: 6d 23h	Taxon: Actinopterygii	Source: Aquatic Biosystems, CO Age: <48
Sample ID: 05-2423-4412	Code: X8909	Project: WET Monthly Compliance Test (AUG)
Sample Date: 21 Aug-23 09:00	Material: POTW Effluent	Source: AR0021776 (AR0021776)
Receipt Date: 21 Aug-23 15:16	CAS (PC):	Station: 001
Sample Age: 29h (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	0.04336	4.45%

### 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	30	16	1	CDF	0.9446	Non-Significant Effect
		33	8	30	16	1	CDF	0.9446	Non-Significant Effect
		44	8	30	16	1	CDF	0.9446	Non-Significant Effect
		59	8	30	16	1	CDF	0.9446	Non-Significant Effect
		78	8	30	16	1	CDF	0.9446	Non-Significant Effect

### Test Acceptability Criteria

Attribute	Test Stat	TAC Limits		Overlap	Decision
		Lower	Upper		
Control Resp	0.975	0.8	>>	Yes	Passes Criteria

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0056216	0.0011243	5	1	0.4389	Non-Significant Effect
Error	0.0269838	0.0011243	24			
Total	0.0326054		29			

### ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test				Indeterminate
Distribution	Shapiro-Wilk W Normality Test	0.4063	0.9031	<1.0E-05	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	0.9750	0.9056	1.0000	1.0000	0.8750	1.0000	0.0250	5.73%	0.00%
25		5	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	-2.56%
33		5	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	-2.56%
44		5	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	-2.56%
59		5	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	-2.56%
78		5	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	-2.56%

### Angular (Corrected) Transformed Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	5	1.3560	1.2540	1.4580	1.3930	1.2090	1.3930	0.0367	6.06%	0.00%
25		5	1.3930	1.3930	1.3930	1.3930	1.3930	1.3930	0.0000	0.00%	-2.71%
33		5	1.3930	1.3930	1.3930	1.3930	1.3930	1.3930	0.0000	0.00%	-2.71%
44		5	1.3930	1.3930	1.3930	1.3930	1.3930	1.3930	0.0000	0.00%	-2.71%
59		5	1.3930	1.3930	1.3930	1.3930	1.3930	1.3930	0.0000	0.00%	-2.71%
78		5	1.3930	1.3930	1.3930	1.3930	1.3930	1.3930	0.0000	0.00%	-2.71%

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*9/10/23*

**CETIS Analytical Report**

Report Date: 05 Sep-23 10:35 (p 2 of 2)  
 Test Code/ID: B36EBE5 / 01-8814-8709

**Fathead Minnow 7-d Larval Survival and Growth Test**

Bio-Analytical Laboratories

Analysis ID: 10-8649-6054      Endpoint: 7d Survival Rate      CETIS Version: CETIS v2.1.5  
 Analyzed: 05 Sep-23 10:35      Analysis: Nonparametric-Control vs Treatments      Status Level: 1  
 Edit Date: 05 Sep-23 10:28      MD5 Hash: 7941F3B4BE2B240B24BC43B1B94571A2      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	1.0000	1.0000	1.0000
25		1.0000	1.0000	1.0000	1.0000	1.0000
33		1.0000	1.0000	1.0000	1.0000	1.0000
44		1.0000	1.0000	1.0000	1.0000	1.0000
59		1.0000	1.0000	1.0000	1.0000	1.0000
78		1.0000	1.0000	1.0000	1.0000	1.0000

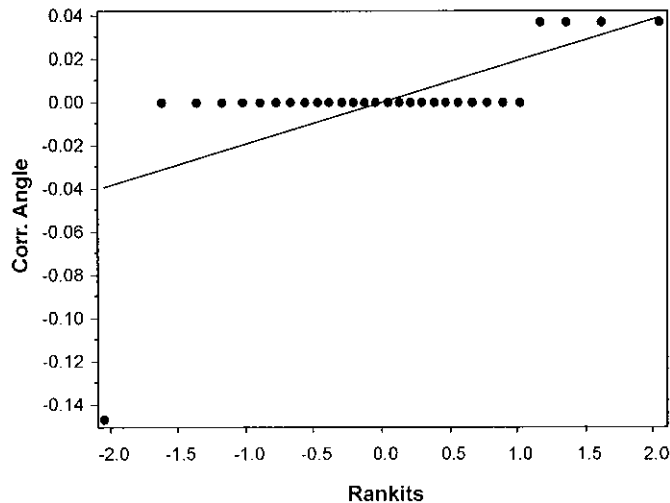
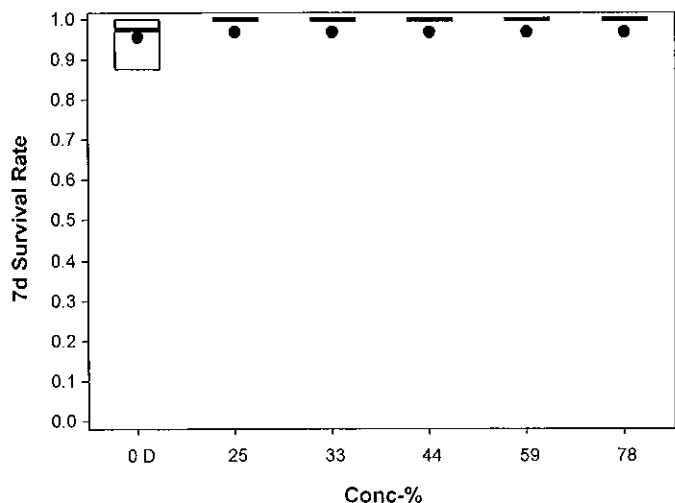
**Angular (Corrected) Transformed Detail**

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.3930	1.2090	1.3930	1.3930	1.3930
25		1.3930	1.3930	1.3930	1.3930	1.3930
33		1.3930	1.3930	1.3930	1.3930	1.3930
44		1.3930	1.3930	1.3930	1.3930	1.3930
59		1.3930	1.3930	1.3930	1.3930	1.3930
78		1.3930	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	7/8	8/8	8/8	8/8
25		8/8	8/8	8/8	8/8	8/8
33		8/8	8/8	8/8	8/8	8/8
44		8/8	8/8	8/8	8/8	8/8
59		8/8	8/8	8/8	8/8	8/8
78		8/8	8/8	8/8	8/8	8/8

**Graphics**



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 9/16/23

# CETIS Analytical Report

Report Date: 05 Sep-23 10:37 (p 1 of 2)  
Test Code/ID: B36EBE5 / 01-8814-8709

## Fathead Minnow 7-d Larval Survival and Growth Test

Bio-Analytical Laboratories

Analysis ID: 00-3355-4621	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETIS v2.1.5
Analyzed: 05 Sep-23 10:37	Analysis: Parametric-Control vs Treatments	Status Level: 1
Edit Date: 05 Sep-23 10:28	MD5 Hash: E15D7B9F9ECEA5518E4B82E38A4BB362	Editor ID: 008-522-314-5
Batch ID: 07-1081-0053	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 22 Aug-23 13:50	Protocol: EPA/821/R-02-013 (2002)	Diluent: Receiving Water
Ending Date: 29 Aug-23 13:00	Species: Pimephales promelas	Brine:
Test Length: 6d 23h	Taxon: Actinopterygii	Source: Aquatic Biosystems, CO Age: <48
Sample ID: 05-2423-4412	Code: X8909	Project: WET Monthly Compliance Test (AUG)
Sample Date: 21 Aug-23 09:00	Material: POTW Effluent	Source: AR0021776 (AR0021776)
Receipt Date: 21 Aug-23 15:16	CAS (PC):	Station: 001
Sample Age: 29h (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	0.09717	16.75%

### Dunnnett Multiple Comparison Test

Control	vs	Conc-%	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		25	8	-1.58	2.362	0.09717	CDF	0.9971	Non-Significant Effect
		33	8	-2.127	2.362	0.09717	CDF	0.9995	Non-Significant Effect
		44	8	-1.58	2.362	0.09717	CDF	0.9971	Non-Significant Effect
		59	8	-1.701	2.362	0.09717	CDF	0.9980	Non-Significant Effect
		78	8	-1.884	2.362	0.09717	CDF	0.9989	Non-Significant Effect

### Test Acceptability Criteria

Attribute	Test Stat	TAC Limits		Overlap	Decision
		Lower	Upper		
Control Resp	0.58	0.25	>>	Yes	Passes Criteria
PMSD	0.1675	0.12	0.3	Yes	Passes Criteria

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0240411	0.0048082	5	1.136	0.3685	Non-Significant Effect
Error	0.101565	0.0042319	24			
Total	0.125606		29			

### ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	2.681	15.09	0.7490	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9849	0.9031	0.4110	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	0.58	0.4773	0.6827	0.5625	0.5125	0.7125	0.037	14.26%	0.00%
25		5	0.645	0.5435	0.7465	0.6625	0.5375	0.7375	0.03657	12.68%	-11.21%
33		5	0.6675	0.5849	0.7501	0.625	0.6125	0.7625	0.02974	9.96%	-15.09%
44		5	0.645	0.5717	0.7183	0.675	0.575	0.7	0.0264	9.15%	-11.21%
59		5	0.65	0.594	0.706	0.65	0.5875	0.7125	0.02015	6.93%	-12.07%
78		5	0.6575	0.6031	0.7119	0.6625	0.5875	0.7	0.01961	6.67%	-13.36%

### Mean Dry Biomass-mg Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	0.7125	0.6	0.5125	0.5125	0.5625
25		0.7375	0.6625	0.5375	0.5875	0.7
33		0.7125	0.625	0.6125	0.625	0.7625
44		0.5875	0.675	0.6875	0.7	0.575
59		0.65	0.6375	0.6625	0.7125	0.5875
78		0.6625	0.5875	0.65	0.7	0.6875

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9/16/23

# CETIS Analytical Report

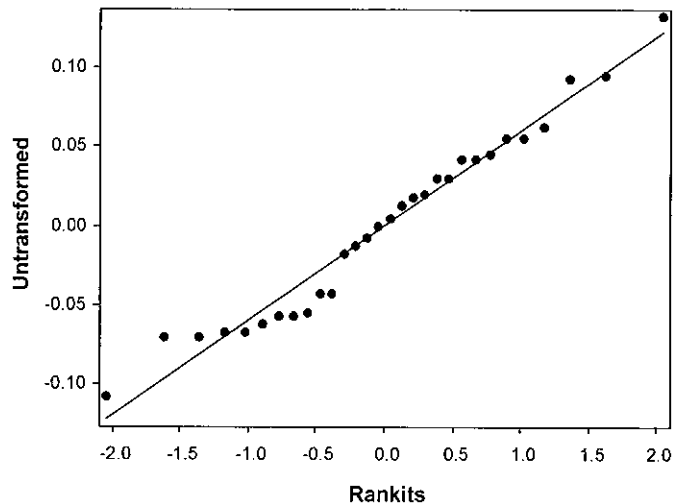
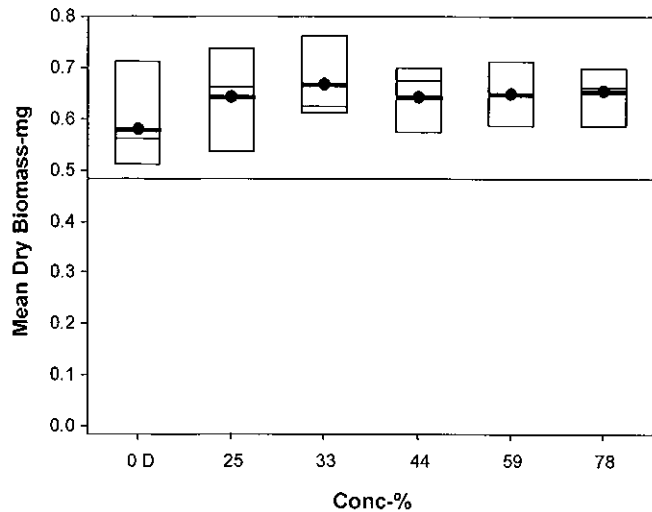
Report Date: 05 Sep-23 10:37 (p 2 of 2)  
Test Code/ID: B36EBE5 / 01-8814-8709

## Fathead Minnow 7-d Larval Survival and Growth Test

Bio-Analytical Laboratories

Analysis ID: 00-3355-4621	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETIS v2.1.5
Analyzed: 05 Sep-23 10:37	Analysis: Parametric-Control vs Treatments	Status Level: 1
Edit Date: 05 Sep-23 10:28	MD5 Hash: E15D7B9F9ECEA5518E4B82E38A4BB362	Editor ID: 008-522-314-5

### Graphics



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*9/6/23*

**CETIS Analytical Report**

Report Date: 05 Sep-23 10:37 (p 1 of 2)  
Test Code/ID: B36EBE5 / 01-8814-8709

**Fathead Minnow 7-d Larval Survival and Growth Test**

Bio-Analytical Laboratories

Analysis ID: 13-6386-6736	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETIS v2.1.5
Analyzed: 05 Sep-23 10:37	Analysis: Linear Interpolation (ICPIN)	Status Level: 1
Edit Date: 05 Sep-23 10:28	MD5 Hash: E15D7B9F9ECEA5518E4B82E38A4BB362	Editor ID: 008-522-314-5
Batch ID: 07-1081-0053	Test Type: Growth-Survival (7d)	Analyst: Lab Tech
Start Date: 22 Aug-23 13:50	Protocol: EPA/821/R-02-013 (2002)	Diluent: Receiving Water
Ending Date: 29 Aug-23 13:00	Species: Pimephales promelas	Brine:
Test Length: 6d 23h	Taxon: Actinopterygii	Source: Aquatic Biosystems, CO Age: <48
Sample ID: 05-2423-4412	Code: X8909	Project: WET Monthly Compliance Test (AUG)
Sample Date: 21 Aug-23 09:00	Material: POTW Effluent	Source: AR0021776 (AR0021776)
Receipt Date: 21 Aug-23 15:16	CAS (PC):	Station: 001
Sample Age: 29h (1.8 °C)	Client: Nashville Public Works	

**Linear Interpolation Options**

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1699297	1000	Yes	Two-Point Interpolation

**Test Acceptability Criteria**

Attribute	Test Stat	TAC Limits		Overlap	Decision
		Lower	Upper		
Control Resp	0.58	0.25	>>	Yes	Passes Criteria

**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	---	---

**Mean Dry Biomass-mg Summary**

Conc-%	Code	Count	Calculated Variate						Isotonic Variate	
			Mean	Median	Min	Max	CV%	%Effect	Mean	%Effect
0	D	5	0.58	0.5625	0.5125	0.7125	14.26%	0.00%	0.6408	0.00%
25		5	0.645	0.6625	0.5375	0.7375	12.68%	-11.21%	0.6408	0.00%
33		5	0.6675	0.625	0.6125	0.7625	9.96%	-15.09%	0.6408	0.00%
44		5	0.645	0.675	0.575	0.7	9.15%	-11.21%	0.6408	0.00%
59		5	0.65	0.65	0.5875	0.7125	6.93%	-12.07%	0.6408	0.00%
78		5	0.6575	0.6625	0.5875	0.7	6.67%	-13.36%	0.6408	0.00%

**Mean Dry Biomass-mg Detail**

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	0.7125	0.6	0.5125	0.5125	0.5625
25		0.7375	0.6625	0.5375	0.5875	0.7
33		0.7125	0.625	0.6125	0.625	0.7625
44		0.5875	0.675	0.6875	0.7	0.575
59		0.65	0.6375	0.6625	0.7125	0.5875
78		0.6625	0.5875	0.65	0.7	0.6875

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# CETIS Analytical Report

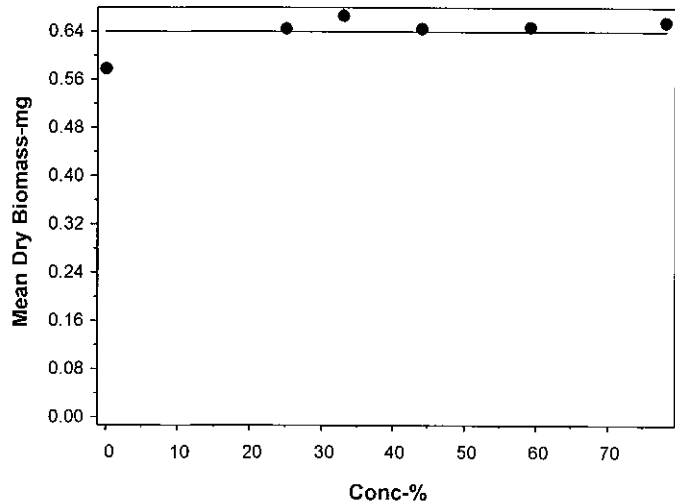
Report Date: 05 Sep-23 10:37 (p 2 of 2)  
Test Code/ID: B36EBE5 / 01-8814-8709

## Fathead Minnow 7-d Larval Survival and Growth Test

Bio-Analytical Laboratories

Analysis ID: 13-6386-6736	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETIS v2.1.5
Analyzed: 05 Sep-23 10:37	Analysis: Linear Interpolation (ICPIN)	Status Level: 1
Edit Date: 05 Sep-23 10:28	MD5 Hash: E15D7B9F9ECEA5518E4B82E38A4BB362	Editor ID: 008-522-314-5

### Graphics



*Handwritten signature:* EUP  
9/14/23

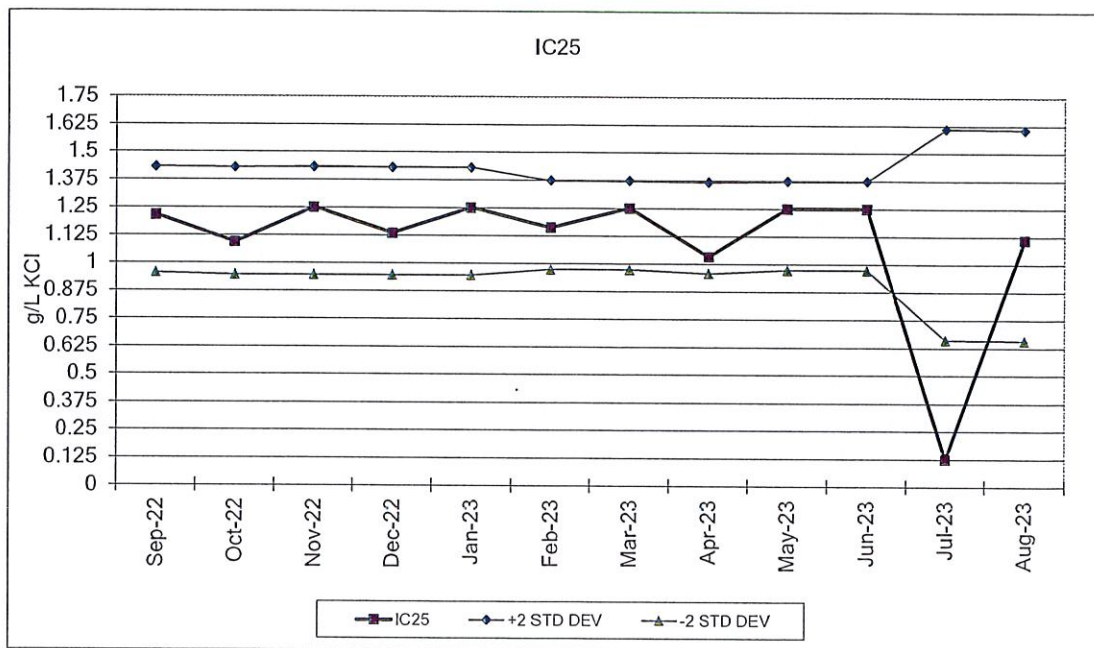
**APPENDIX D**  
**QUALITY ASSURANCE CHARTS**

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



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

*Pimephales promelas*



Chronic 7 Day Survival Test Data

Date	NOEC (g/L KCl)	LOEC (g/L KCl)
Mar-23	0.50	1.0
Apr-23	0.50	1.0
May-23	0.50	1.0
Jun-23	0.50	1.0
Jul-23	0.50	1.0
Aug-23	0.50	1.0

IC 25 for Growth Test

Date	IC25 g/L KCl	95% Confidence (upper)	95% Confidence (lower)	Avg. IC25 g/L KCl	+2 STD DEV	-2 STD DEV
Mar-23	1.250	1.250	1.210	1.173	1.374	0.972
Apr-23	1.032	1.272	0.023	1.163	1.369	0.957
May-23	1.250	1.250	1.141	1.173	1.374	0.973
Jun-23	1.250	1.250	1.250	1.173	1.374	0.973
Jul-23	0.125	1.705	0.074	1.136	1.611	0.660
Aug-23	1.110	1.316	0.320	1.131	1.606	0.657

\*\*Current Test Dates: 8/3-10/2023

**APPENDIX E**  
**AGENCY FORMS**

**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	To	Time	Date
Composite 1 Collected from:	0900	08/20/23		2230	08/21/23
Composite 2 Collected from:	0715	08/22/23		0715	08/23/23
Composite 3 Collected from:	0645	08/24/23		0554	08/25/23
Test initiated:	1350	am/pm		08/22/23	date
Test terminated:	1300	am/pm		08/29/23	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	100.0	100.0	100.0	100.0	100.0	97.5	6.06
25.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.00
33.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.00
44.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.00
59.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.00
78.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.00

**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	0.713	0.600	0.513	0.513	0.563	0.580	14.26
25.0	0.738	0.663	0.538	0.588	0.700	0.645	12.68
33.0	0.713	0.625	0.613	0.625	0.763	0.668	9.96
44.0	0.588	0.675	0.688	0.700	0.575	0.645	9.15
59.0	0.650	0.638	0.663	0.713	0.588	0.650	6.93
78.0	0.663	0.588	0.650	0.700	0.688	0.658	6.67

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

PMSD: 16.75%

**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, Morado, Miller

Sample #1 Collected: 8/21/2023 Time: 2230  
Sample #2 Collected: 8/23/2023 Time: 715  
Sample #3 Collected: 8/25/2023 Time: 554  
Test Begin: 8/22/2023 Time: 1350  
Test End: 8/29/2023 Time: 1300

Dilution:	0%							44.0%						
Day:	1	2	3	4	5	6	7	1	2	3	4	5	6	7
T (°C)	25.0	26.1	24.2	23.0	23.0	23.5	24.6	25.0	26.1	24.2	23.0	23.0	23.5	24.6
DO Initial	6.3	5.2	4.5	6.4	6.2	6.3	5.4	5.9	5.0	4.6	5.4	6.0	6.5	5.2
DO Final	7.9	7.7	7.5	7.6	7.7	7.6	7.6	7.1	7.8	7.5	7.4	7.5	7.6	7.0
pH Initial	6.7	6.3	6.5	7.0	7.3	7.2	6.5	6.8	6.8	6.9	6.9	6.3	7.0	7.0
pH Final	7.6	7.4	7.2	7.4	7.4	7.5	7.5	7.7	7.4	7.5	7.6	7.9	7.7	7.7
Conductivity	295.0	325.0	335.0	390.0	340.0	320.0	320.0	420.0	410.0	425.0	430.0	450.0	455.0	455.0
Alkalinity	56.0	48.0												
Hardness	92.0	92.0												
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)	25.0	26.1	24.2	23.0	23.0	23.5	24.6	25.0	26.1	24.2	23.0	23.0	23.5	24.6
DO Initial	6.3	5.3	4.9	5.3	6.0	6.8	5.6	6.2	5.3	5.1	6.3	5.4	6.0	5.5
DO Final	7.2	7.2	7.5	7.6	7.3	7.6	7.6	7.7	7.8	7.5	7.6	7.4	7.5	7.0
pH Initial	6.7	6.4	6.7	6.9	6.0	7.0	6.9	6.8	6.8	6.9	7.1	7.3	7.5	7.0
pH Final	7.5	7.4	7.4	7.5	8.3	7.9	7.9	7.3	7.4	7.5	7.6	7.9	7.6	7.6
Conductivity	380.0	375.0	390.0	385.0	395.0	390.0	390.0	450.0	440.0	460.0	455.0	480.0	490.0	490.0
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)	25.0	26.1	24.2	23.0	23.0	23.5	24.6	25.0	26.1	24.2	23.0	23.0	23.5	24.6
DO Initial	6.3	5.0	4.8	6.2	5.4	6.0	5.4	5.9	5.0	4.8	6.2	5.4	5.8	5.4
DO Final	7.7	7.8	7.5	7.7	7.4	7.7	7.9	7.1	7.9	7.6	7.4	7.5	7.9	7.0
pH Initial	6.8	6.7	6.8	7.2	7.0	6.9	6.9	6.8	6.9	6.9	7.2	7.2	7.0	7.0
pH Final	7.4	7.4	7.5	7.5	8.1	8.1	8.1	7.3	7.4	7.5	7.5	7.7	7.7	7.7
Alkalinity								88.0	76.0		84.0			
Hardness								124.0	124.0		148.0			
Conductivity	395.0	390.0	405.0	410.0	420.0	425.0	425.0	490.0	485.0	500.0	510.0	535.0	600.0	600.0
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

Project#: X8909

Chain of Custody Documents Checked by: Alexis Mitchell 9/15/23  
Technician/Date

Raw Data Documents Checked by: Alexis Mitchell 9/15/23  
Technician/Date

Statistical Analysis Package Checked by: EOB 9/16/23  
Quality Manager/Date

Quality Control Data Checked by: EOB 9/21/23  
Quality Manager/Date

Report Checked by: EOB 9/21/23  
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.

Erin J. Burpp, BS  
Quality Manager

9/21/23  
Date

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