

Bio-Analytical Laboratories' Executive Summary

Permittee: Magnolia Wastewater System
Columbia Road
Magnolia, AR

Project #: X8582

Outfall: 001 (treated domestic wastewater)

Permit #: AR0043613/ AFIN 14-00059

Contact: Tracie Love

Test Dates: December 13 - 20, 2022

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 (100.0%), enter a "1"; otherwise, enter a "0" for Parameter TLP6C - 0 (**Pass**).
2. If the NOEC for growth is less than the critical dilution (80.0%), enter a "1"; otherwise, enter a "0" for Parameter TGP6C- 0 (**Pass**).
3. Report the NOEC value for survival, Parameter TOP6C - 100.0%
4. Report the NOEC value for growth, Parameter TPP6C - 100.0%
5. Report the largest % coefficient of variation between the control and the critical dilution, Parameter TQP6C -18.33%.
6. PMSD Biomass = 23.35% (12.0 – 30.0%)- moderate precision, acceptable for passing test



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THE RESULTS OF A CHRONIC DEFINITIVE TOXICITY TEST FOR OUTFALL 001

AT

MAGNOLIA WASTEWATER SYSTEM
Magnolia, Arkansas

NPDES #AR0043613
AFIN: 14-00059

EPA Method 1000.0

Project X8582

Test Dates: December 13 - 20, 2022

Report Date: January 4, 2023

Prepared for:

Tracie Love
Magnolia Wastewater System
P.O. Box 666
Magnolia, AR 71753

Prepared by:

Ginger Briggs
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1.0 Introduction

Bio-Analytical Laboratories (BAL), Doyline, Louisiana conducted a chronic definitive toxicity test for Outfall 001 at the wastewater plant serving the city of Magnolia, 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 organism. Toxicity is defined as a statistically significant difference at the 95 percent confidence level between the survival and 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” 22nd Edition (APHA 2012) and BAL’s standard operating procedure.

2.2 Test Organisms

The fathead minnows were raised in-house at test temperature 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

Soft reconstituted water, made per method guidelines, was used as the dilution water and the control for the 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 tests were 100.0, 80.0, 56.0, 42.0 and 32.0 percent effluent and a control. The lethal and sub-lethal critical dilution was 100.0 and 80.0 percent effluent, respectively. 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 composite samples of Outfall 001 were collected by city personnel on December 12, 14 and 16, 2022, at 0800 hours. Upon collection and completion of each composite, the samples were packed in ice and delivered the same day to the laboratory. The temperature upon arrival each of the effluent samples was 6.8, 1.8 and 1.0° 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 $25\pm1^{\circ}$ Celsius. Total residual chlorine levels were measured in milligrams/Liter (mg/L) with a test strip and recorded if present. Total ammonia levels were measured in mg/L using a test strip. In the minnow test, each sample was treated with an 18-watt ultraviolet light (UV) at a rate of 113 ml/minute, with an extra 100 percent dilution set up with the treated portion. This was to document any toxicity that may be due to pathogen interference. Dissolved oxygen (4500-O G) and pH (4500-H+ B) measurements were measured in mg/L and standard units, respectively, on the control and each concentration at test initiation, at test renewal and at test termination. Conductivity (2510 B) measurements in umhos/cm were also taken at test initiation and at each renewal. Alkalinity (2320 B) and hardness (2340 C) levels were measured in mg/L as CaCO₃ 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^R heated liquid circulator to keep a constant temperature of $25\pm1^{\circ}$ 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

Survival data was analyzed using Steel's Many-One Rank Test, a nonparametric test comparing concentration data to control data. Growth (biomass) data was analyzed using Dunnett's Test, a parametric test comparing concentration data to control data. Other test 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, 100.0 percent survival occurred in the control and in the 100.0 percent critical dilution. The average weight gained per minnow in the control and in the 80.0 percent critical dilution was 0.780 and 0.740 milligram (mg), respectively. The NOEC for survival and growth in this test was 100.0 percent effluent ($p=.05$).

Table 1: Results of the Chronic Definitive Fathead Minnow Test

Percent Effluent	Percent Survival	Sig.*	Mean Dry Weight (mg)	Sig.*
Control	100.0		0.780	
32.0	90.0		0.820	
42.0	98.0		0.980	
56.0	100.0		0.930	
80.0	98.0		0.740	
100.0	100.0		0.830	
100.0 UV	95.0		0.860	

*significant when compared to the control ($p=.05$). +Test validity based on mean dry weight per surviving larvae in the control. NOEC value based on mean dry weight per the number of larvae at the start of the test.

The monthly chronic reference toxicant tests demonstrated that the test organisms were within the acceptable sensitivity levels. 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 from the wastewater plant serving the city of Magnolia, Arkansas, on December 12, 14 and 16, 2022, were not found to be lethally toxic to the *Pimephales promelas* test organisms in the 100.0 percent critical dilution after seven days exposure ($p=.05$). Sublethal effects (i.e., low growth) were not noted in the test ($p=.05$).

5.0 References

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

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



Bio-Analytical Laboratories

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NELAP/NELAP 01975, ADEQ 88-0630, TCEQ T104704278

Company: City of Magnolia	Phone: (870) 234-2955	Analysis:		Laboratory Use Only: Project Number: X8582
Address: P.O. Box 666, Magnolia, AR 71753	Fax: (870) 234-2203			Temp. upon arrival: <i>6-8</i>
Permit #: AR0043613/AFIN 14-00059	Purchase Order:	Therm #: <i>29</i>	Color: <i>clear</i>	
Sampler's Signature/Printed Name/Affiliation: <i>Jewell Morris</i>				
Date Start Date End	Time Start Time End	C G	# and type of container	Chronic mirmic Acute minnow(fresh/marine)
<i>12/10/22 - 12/10/22</i>	<i>8:00 - 8:00</i>	<i>X</i>	<i>8 half gallons</i>	<i>Acute Daphnia species</i>
			<i>001</i>	<i>Acute Daphnia species</i>
				<i>Feecal Coliform</i>
				<i>Acute Ceriodaphnia</i>
				<i>Acute Mysisid</i>
				<i>Acute Ceriodaphnia</i>
				<i>Chronic mirmicow</i>
				<i>Chronic Ceriodaphnia</i>
Relinquished by/Affiliation: <i>Jewell Morris</i>	Date: <i>12/10/22</i>	Time: <i>8:48A</i>	Received by/Affiliation: <i>Denise Lee</i>	Date: <i>12/12/22</i>
Relinquished by/Affiliation: <i>Denise Lee</i>	Date: <i>12/12/22</i>	Time: <i>11:34</i>	Received by/Affiliation: <i>Heidi Mabefelt</i>	Date: <i>12/12/22</i>
Relinquished by/Affiliation: <i></i>	Date: <i></i>	Time: <i></i>	Received by/Affiliation: <i></i>	Date: <i></i>
Method of Shipment: Comments:				

Method of Shipment: Lab Bus Fed Ex DHL UPS Client Other Tracking # _____

Comments: _____



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NELAP/PELAP 01975, ADEQ 88-0630, TCEQ T104704278

Company: City of Magnolia	Phone: (870) 234-2955	Analysis:		Laboratory Use Only: Project Number: 8382
Address: P.O. Box 666, Magnolia, AR 71753	Fax: (870) 234-2203			Temp. upon arrival: 85° Therm #: 29
Permit #: AR0043613/AFIN 14-00059	Purchase Order:			Color: Clear
Sampler's Signature/Printed Name/Affiliation: <i>Jeanne Love / Tracer Love / mws</i>				
Date Start Date End	Time Start Time End	C G	# and type of container	Chronic Cetropophnia
12/13/22 - 12/14/22	8:00 - 8:00	X	8 half gallons	Acute mimnow(fresh/marine)
			001	Acute Daphnia species
				Acute Ceriodaphnia
				Fecal Coliform
				Acute Mysid
				Acute Ceriodaphnia
				Chronic mimnow
				Acute Cetropophnia
Relinquished by/Affiliation: <i>Jeanne Love / mws</i>	Date: 12/14/22	Time: 8:47A	Received by/Affiliation: <i>Jeanne Love</i>	Date: 12/14/22
Relinquished by/Affiliation: <i>Jeanne Love</i>	Date: 12/14/22	Time: 12:16P	Received by/Affiliation: <i>Karen Mitchell</i>	Date: 12/14/22
Relinquished by/Affiliation:	Date:	Time:	Received by/Affiliation:	Date:
Method of Shipment:	Lab	Bus	Fed Ex	DHL
Comments:	UPS	Client	Other	Tracking #



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NELAP/LLAP 01975, ADEQ 88-0630, TCEQ T104704278

Company: City of Magnolia		Phone: (870) 234-2955		Analysis:		Laboratory Use Only: Project Number: X8582	
Address: P.O. Box 666, Magnolia, AR 71753		Fax: (870) 234-2203		Chronic Ceriodaphnia		Temp. upon arrival: 10 Therm #: 29	
Permit #: AR0043613/AFIN 14-00059		Purchase Order:		Acute minnow		Color: clear	
Sampler's Signature/Printed Name/Affiliation: <i>Jewell Trever Lowe / MWS</i>				Acute minnow(fresh/marine)		Odor: none	
Date Start Date End	Time Start Time End	C	G	# and type of container	Sample Identification	Lab Control Number: <i>CAN130</i>	Tech: <i>EDW</i>
12/15/22 - 12/16/22	8:00 - 8:30A	X		8 half gallons	001	X	ICE
Relinquished by/Affiliation: <i>Jewell Trever Lowe / MWS</i>		Date:	Time:	Received by/Affiliation: <i>Drew Lee</i>		Date:	Time:
Relinquished by/Affiliation: <i>Drew Lee</i>		<i>12/16/22</i>	<i>8:30A</i>	<i>Drew Lee</i>		<i>12/16/22</i>	<i>8:38A</i>
Relinquished by/Affiliation: <i>Drew Lee</i>		Date:	Time:	Received by/Affiliation: <i>Emily Moore</i>		Date:	Time:
		<i>12/16/22</i>	<i>12:07P</i>	<i>Emily Moore</i>		<i>12/16/22</i>	<i>12:07</i>
Method of Shipment: <i>Lab</i>		Date:	Time:	Received by/Affiliation:		Date:	Time:
Comments:							
Tracking #: _____							

APPENDIX B
RAW DATA SHEETS

BIO-ANALYTICAL LABORATORIES
PIMEPHALES PROMELAS SURVIVAL AND GROWTH DATA SHEET

Project# X8582 Date started: 11/13/22 Date ended 12/10/22

Client/Contact MAGN/Magnolia Waste Water
Address P.O. Box 666 Magnolia AR 71753
NPDES# AR0043613 AFIN14-00059

Sample Description 001 Dilution Water Soft Reconstituted
Test Temperature($^{\circ}\text{C}$) 25+1⁰ Celsius Technicians EGB/EDW/AM/PM
Test organism age 24 hrs Vendor/ID# BAL 121222

Day	Feeding Times			
	Technician/Time/Amount (per replicate)	AM	NOON	PM
0				
1	<u>PM 10815/0.1mL</u>	<u>PM 1500/0.1mL</u>		<u>AM 11840/0.1mL</u>
2	<u>PM 10845/0.1mL</u>	<u>PM 1400/0.1mL</u>		<u>AM 11800/0.1mL</u>
3	<u>AM 10830/0.10mL</u>	<u>AM 11223/0.1mL</u>		<u>AM 11725/0.1mL</u>
4	<u>EDW 10900/0.20mL</u>	<u>EDW 11220/0.1mL</u>		<u>EDW 11720/0.10mL</u>
5	<u>AM 10910/0.2mL</u>			<u>EDW 11520/0.20mL</u>
6	<u>PM 10825/0.1mL</u>			<u>EDW 11450/0.20mL</u>
				<u>PM 11130/0.2mL</u>

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

Effluent 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
0.9.2 110.31.141	0.4 18/831/100.41.141	0.	0.
1.8.6 102.71.141	1.4 16/9/95.0.141	1.	1.
2.7.9 93.11.141	2. No/AM	2.	2.
3.8.1 94.31.141	3. No/AM	3.	3.
4.8.0 97.4% EDW	4. No/EDW	4.	4.
5.8.0 98.1% EDW	5. No/EDW	5.	5.
6.9.1 108.31.141	6. 4/12/8.2/98.91.141	6.	6.
Total Residual Chlorine (mg/L) / Tech	Dechlorinated? Amount? / Tech	Ammonia (NH3) (mg/L) / Tech	BAL Sample # Date in use
1. 20.5 / AM	1. No / AM	1. 20.5 / AM	1. C24083 12/13/22
2. 20.5 / AM	2. No / AM	2. 1.0 / AM	2. C24103 12/13/22
3. 20.5 / EDW	3. No / EDW	3. 1.0 / EDW	3. C24130 12/17/22

Comments:

BIO-ANALYTICAL LABORATORIES 7-DAY CHRONIC MINNOW SURVIVAL DATA - EPA 1000, OECD 210
 Project# X8582
 Client City of magnolia Sample ID 001 Test started: Date 12/12/12 Time 1530
 Date/Tech: Day 0 12/13/12 PM 12/14/12 PM 2 12/15/12 PM 3 12/16/12 PM 4 12/17/12 PM 5 12/18/12 PM 6 12/19/12 PM 7 12/20/12 PM
 Time: Day 0 1530 1 1540 2 1144 3 1020 4 0955 5 1108 6 1230 7 1515
 Temp (°C) Day 0 21.4 1 26.4 2 25.9 3 25.1 4 26.1 5 26.3 6 26.8 7 26.7

Conc %	Rep.	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0 SOF2	1	8	8	8	8	8	8	8	8
	2	8	8	108	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
32.0	1	8	8	8	7	7	7	7	7
	2	8	8	8	8	8	8	8	8
	3	8	8	8	8	8	8	8	8
	4	8	8	8	8	8	8	8	7
	5	8	8	8	8	8	8	7	7
42.0	1	8	6	8	8	8	8	7	7
	2	8	8	8	8	8	8	8	8
	3	8	8	8	5	8	8	8	8
	4	8	8	8	8	8	8	8	8
	5	8	8	8	8	8	8	8	8
56.0	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	6	8	8	8	8	8	8
	5	8	6	8	8	8	8	8	8
80.0	1	8	8	2	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
100.0	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 7-DAY CHRONIC MINNOW SURVIVAL DATA - EPA 1000, OECD 210
 Project# X8382 Test started: Date 12/13/12 Time 1530
 Client City of Magnolia Sample ID001 Test ended: Date 12/19/12 Time 1515
 Date/Tech: Day 0 12/13/12 PM 12:44:19 1 12:44:19 2 12:44:22 3 12:44:22 4 12:44:22 5 12:44:22 6 12:44:22 7 12:44:22 AM
 Time: Day 0 1530 1 1540 2 1541 3 1520 4 0955 5 1108 6 1230 7 1515
 Temp (°C) Day 0 26.4 1 26.9 2 25.9 3 25.4 4 26.1 5 26.3 6 26.8 7 26.7

Conc. %	Rep.	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
100.0 OV	1	8	8	8	7	7	7	7	7
	2	8	8	8	8	8	8	8	8
	3	8	8	8	7	7	7	7	7
	4	8	8	8	8	8	8	8	8
	5	8	6	8	8	8	8	8	8
	1								
	2								
	3								
	4								
	5								
	1								
	2								
	3								
	4								
	5								
	1								
	2								
	3								
	4								
	5								
	1								
	2								
	3								
	4								
	5								
	1								
	2								
	3								
	4								
	5								

BIO-ANALYTICAL LABORATORIES MINNOW LARVAL GROWTH DATA SHEET (Minnow3 Rev 16 of 37)

Project#/Client Magnolia X8582Temp Start (°C) 80.5 Tech PM Date: 12/20/22 Time: 1515
Temp End (°C) 97.2 Tech PM Date: 12/21/22 Time: 0935

Conc. %	Replicate/ Pan number	Wt. of pan(g)/ Date <u>12/16/22</u> weighed: Tech: <u>PM</u>	Wt. of pan + larvae(g)/ Date <u>12/22/22</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 Soft	1 1	0.9723	0.9789				
	2 2	0.9797	0.9854				
	3 3	0.9166e	0.9739				
	4 4	0.9798	0.9846				
	5 5	0.9818	0.9884				
32	1 6	0.9825	0.9895				
	2 7	0.9743	0.9816				
	3 8	0.91627	0.9700				
	4 9	0.9812	0.9865				
	5 10	0.9819	0.9877				
42	1 11	0.9848	0.9932				
	2 12	0.9842	0.9929				
	3 13	0.91698	0.9759				
	4 14	0.971de	0.9857				
	5 15	0.973le	0.9805				
56	1 16	0.9723	0.9794				
	2 17	0.91643	0.9716				
	3 18	0.9733	0.9808				
	4 19	0.9756	0.9847				
	5 20	0.9710	0.9771				
80	1 21	0.9721	0.9773				
	2 22	1.0393	1.0457				
	3 23	1.0489	1.0563				
	4 24	1.0398	1.0444				
	5 25	1.0386	1.0445				
100	1 26	1.0354	1.0419				
	2 27	1.0463	1.0528				
	3 28	1.0367	1.0436				
	4 29	1.0329	1.0396				
	5 30	1.0340	1.0407				

* Test acceptance of control weight based on surviving larvae at end of test.

Calculated by: CETISCalculations checked by: EBB 12/28/22

BIO-ANALYTICAL LABORATORIES MINNOW LARVAL GROWTH DATA SHEET (Minnow3 Rev 2)

X8582

Project#/Client Magnolia Temp Start (°C) 80.5 Tech Pm Date: 12/15/22 Time: 1515
 Temp End (°C) 97.2 Tech Pm Date: 12/21/22 Time: 0935

Conc. %	Replicate/ Pan number	Wt. of pan(g)/ Date <u>12/15/22</u> weighed: Tech: <u>PM</u>	Wt. of pan + larvae(g)/ Date <u>12/22/22</u> weighed: Tech: <u>AN</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*
100 UV	1 31	1.0284	1.0343				
	2 32	1.0422	1.0488				
	3 33	1.0324	1.0392				
	4 34	1.0415	1.0485				
	5 35	1.0509	1.0588				
	1						
	2						
	3						
	4						
	5						
	1						
	2						
	3						
	4						
	5						
	1						
	2						
	3						
	4						
	5						
	1						
	2						
	3						
	4						
	5						
	1						
	2						
	3						
	4						
	5						

* Test acceptance of control weight based on surviving larvae at end of test.

Calculated by: CETISCalculations checked by: EJB 12/28/22

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

Project# X8582 Client City of Magnolia Organism P. promelas

Date	Day 0 12/13/22 5293	Day 1 12/14/22	Day 2 12/15/22	Day 3 12/16/22	Day 4 12/17/22	Day 5 12/18/22	Day 6 12/19/22	Day 7 12/20/22	Day 8
Concentration: 0.50%									
Temperature (°C)	26.1	25.8	25.3	24.6	24.1	23.1	26.2	26.2	
pH	7.6	6.6	6.5	7.3	7.4	7.3	6.5	6.4	
DO (mg/l)	8.1	7.2	5.9	6.2	7.3	7.2	6.2	6.3	
Cond (umhos/cm)	170	163	176	172	180	167	186	197	
Concentration: 32.0%									
Temperature (°C)	25.9	24.3	25.5	24.9	23.9	23.4	24.2	24.5	
pH	7.4	6.6	6.5	7.2	7.3	7.2	6.5	6.4	
DO (mg/l)	8.1	6.3	5.1	6.1	6.9	7.0	5.9	5.9	
Cond (umhos/cm)	274	268	209	203	208	188	191	247	
Concentration: 42.0%									
Temperature (°C)	25.8	26.2	25.5	25.1	24.1	24.1	26.6	26.5	
pH	7.3	6.5	6.6	7.1	7.4	7.2	6.5	6.4	
DO (mg/l)	8.1	6.3	5.3	6.3	6.9	7.0	5.9	6.1	
Cond (umhos/cm)	225	218	221	216	219	194	199	235	
Prerenewal Tech Initials/Time		1545 PM	N	N	EPW	80N	1235	1320	
Postrenewal Tech Initials/Time		1124 AM	0950 AM	0907 AM	0922 AM	EPW	EPW	1035 AM	

Control Alkalinity (mg/L as CaCO₃)

Control Hardness (mg/L as CaCO₃)

ID# 5293	Result 36	Date Tested 12/10/22	ID# 5293	Result 44	Date Tested 12/10/22
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# C24083	Result 48	Date Tested 12/10/22	ID# C24083	Result 32.0	Date Tested 12/10/22
ID# C24103	Result 64	Date Tested 12/10/22	ID# C24103	Result 44.0	Date Tested 12/10/22
ID# C24130	Result 52	Date Tested 12/10/22	ID# C24130	Result 52.0	Date Tested 12/10/22

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

Project# X8582 Client City of Magnolia Organism P. promelas

Date	Day 0 12/13/22	Day 1 12/14/22	Day 2 12/15/22	Day 3 12/16/22	Day 4 12/17/22	Day 5 12/18/22	Day 6 12/19/22	Day 7 12/20/22	Day 8
Concentration:	56.0%								
Temperature (°C)	25.6	26.5 25.3	25.3	24.1	23.5	26.7	26.5		
pH	7.3	6.7 6.7	7.2	7.4	7.3	6.6	6.5		
DO (mg/l)	8.2	6.0 5.4	6.4	7.3	7.1	5.7	6.0		
Cond (umhos/cm)	244	236 240	234	230	203	207	273		
Concentration:	80.0%								
Temperature (°C)	25.3	26.3 25.3	25.5	23.9	24.8	26.7	26.5		
pH	7.3	6.8 6.8	7.2	7.3	7.2	6.6	6.6		
DO (mg/l)	8.3	6.1 5.4	6.3	6.5	6.8	5.1	5.9		
Cond (umhos/cm)	274	265 274	268	274	218	221	269		
Concentration:	100.0%								
Temperature (°C)	25.3	26.3 25.5	25.6	24.9	24.9	26.8	26.5		
pH	7.3	6.9 6.9	7.3	7.4	7.3	6.7	6.7		
DO (mg/l)	8.4	5.9 5.4	6.3	7.3	7.0	5.2	6.0		
Cond (umhos/cm)	300	291 307	298	290	234	236	290		
Prerenewal Tech Initials/Time		1545 PM	AM	AM	EPW	8W	1235 PM	1520 PM	
Postrenewal Tech Initials/Time	1126 AM	0950 AM	0907 AM	0922 AM	EDW	EW	1035 AM		

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 _____

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

Project# X8582 client City of Magnolia Organism P. Aromela s

Date	Day 0 12/13/22	Day 1 12/14/22	Day 2 12/15/22	Day 3 12/16/22	Day 4 12/17/22	Day 5 12/18/22	Day 6 12/19/22	Day 7	Day 8
Concentration: 100.0% UV									
Temperature (°C)	25.0	26.4	25.5	25.6	23.9	25.0	26.9	26.6	
pH	7.2	7.0	6.9	7.3	7.4	7.3	6.9	6.8	
DO (mg/l)	8.4	6.0	5.2	6.2	7.0	7.0	5.4	5.1	
Cond (umhos/cm)	299	287	298	302	301	273	230	322	
Concentration:									
Temperature (°C)									
pH									
DO (mg/l)									
Cond (umhos/cm)									
Concentration:									
Temperature (°C)									
pH									
DO (mg/l)									
Cond (umhos/cm)									
Prerenewal Tech Initials/Time	1545 PM	0W 1151	0W 1023	EPE 0955	EPM 1108	1235 PM	1520 PM		
Postrenewal Tech Initials/Time	1126 AM	0950 AM	0907 AM	0922 AM	EPM 0935	EPM 0940	1035 AM		

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: 12 Dec-22 09:23 (p 1 of 1)
Test Code/ID: 27857188 / 06-6305-6776

Fathead Minnow 7-d Larval Survival and Growth Test											Bio-Analytical Laboratories		
Start Date:	13 Dec-22 17:35	Species:	Pimephales promelas							Sample Code:	6446FF3B		
End Date:	20 Dec-22 16:30	Protocol:	EPA/821/R-02-013 (2002)							Sample Source:	AR0043613		
Sample Date:	12 Dec-22 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	To Weig	
32		5	1										
80		5	2										
42		1	3										
101		4	4										
80		3	5										
0	D	4	6										
100	✓	4	7										
56		1	8										
100		1	9										
42		2	10										
42		4	11										
56		2	12										
56		5	13										
80		2	14										
101		1	15										
80		1	16										
32		1	17										
100	■	3	18										
0	D	2	19										
0	D	5	20										
101		2	21										
32		4	22										
32		3	23										
42	✓	3	24										
32		2	25										
56		3	26										
56		4	27										
101		3	28										
100		2	29										
42		5	30										
100	✓	5	31										
101		5	32										
80		4	33										
0	D	1	34										
0	D	3	35										

APPENDIX C
STATISTICAL ANALYSIS

CETIS Analytical Report

Report Date: 26 Dec-22 14:53 (p 1 of 2)
Test Code/ID: 27857188 / 06-6305-6776

Fathead Minnow 7-d Larval Survival and Growth Test				Bio-Analytical Laboratories	
Analysis ID: 12-6395-2226	Endpoint: 7d Survival Rate	CETIS Version: CETISv1.9.7			
Analyzed: 26 Dec-22 14:53	Analysis: Nonparametric-Control vs Treatments	Status Level: 1			
Edit Date: 26 Dec-22 14:44	MD5 Hash: 9FF9432A4AE35CF86F0408B0595A84FC	Editor ID: 008-522-314-5			
Batch ID: 10-8977-6054	Test Type: Growth-Survival (7d)	Analyst:			
Start Date: 13 Dec-22 15:30	Protocol: EPA/821/R-02-013 (2002)	Diluent: Reconstituted Water			
Ending Date: 20 Dec-22 15:15	Species: Pimephales promelas	Brine:			
Test Length: 7d	Taxon: Actinopterygii	Source: In-House Culture		Age: <24	
Sample ID: 16-8237-4459	Code: X8582	Project: WET Monthly Compliance Test (DEC)			
Sample Date: 12 Dec-22 08:00	Material: POTW Effluent	Source: AR0043613			
Receipt Date: 12 Dec-22 11:13	CAS (PC):	Station: 001			
Sample Age: 32h (6.8 °C)	Client: Magnolia Wastewater System				

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	MSDu	PMSD
Angular (Corrected)	C > T	104	104	---	0.9901	0.087	8.72%

Steel Many-One Rank Sum Test								
Control	vs	Conc-%	Test Stat	Critical	Ties	DF	P-Type	P-Value
Dilution Water	32	20	16	1	8	CDF	0.2114	Non-Significant Effect
	42	25	16	1	8	CDF	0.6693	Non-Significant Effect
	56	28	16	1	8	CDF	0.8571	Non-Significant Effect
	80	25	16	1	8	CDF	0.6693	Non-Significant Effect
100	100	28	16	1	8	CDF	0.8571	Non-Significant Effect
100 UV	104	22	16	1	8	CDF	0.4265	Non-Significant Effect

Test Acceptability Criteria					
TAC Limits					
Attribute	Test Stat	Lower	Upper	Overlap	Decision
Control Resp	1	0.8	>>	Yes	Passes Criteria

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision($\alpha:5\%$)
Between	0.0823153	0.0137192	6	2.1	0.0805	Non-Significant Effect
Error	0.17981	0.0064218	28			
Total	0.262125		34			

ANOVA Assumptions Tests						
Attribute	Test	Test Stat	Critical	P-Value	Decision($\alpha:1\%$)	
Variance	Bartlett Equality of Variance Test				Indeterminate	
Distribution	Shapiro-Wilk W Normality Test	0.87	0.91	0.0008	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	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00%	0.00%
32		5	0.90	0.77	1.00	0.88	0.75	1.00	0.05	11.62%	10.00%
42		5	0.98	0.91	1.00	1.00	0.88	1.00	0.03	5.73%	2.50%
56		5	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00%	0.00%
80		5	0.98	0.91	1.00	1.00	0.88	1.00	0.03	5.73%	2.50%
100		5	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00%	0.00%
100 UV		5	0.95	0.86	1.00	1.00	0.88	1.00	0.03	7.21%	5.00%

Angular (Corrected) Transformed Summary											
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	5	1.40	1.40	1.40	1.40	1.40	1.40	0.00	0.00%	0.00%
32		5	1.30	1.10	1.40	1.20	1.00	1.40	0.07	11.68%	10.24%
42		5	1.40	1.30	1.50	1.40	1.20	1.40	0.04	6.06%	2.64%
56		5	1.40	1.40	1.40	1.40	1.40	1.40	0.00	0.00%	0.00%
80		5	1.40	1.30	1.50	1.40	1.20	1.40	0.04	6.06%	2.64%
100		5	1.40	1.40	1.40	1.40	1.40	1.40	0.00	0.00%	0.00%
100 UV		5	1.30	1.20	1.40	1.40	1.20	1.40	0.05	7.62%	5.27%

CETIS Analytical Report

Report Date: 26 Dec-22 14:53 (p 2 of 2)
Test Code/ID: 27857188 / 06-6305-6776

Fathead Minnow 7-d Larval Survival and Growth Test

Bio-Analytical Laboratories

Analysis ID: 12-6395-2226 Endpoint: 7d Survival Rate
Analyzed: 26 Dec-22 14:53 Analysis: Nonparametric-Control vs Treatments
Edit Date: 26 Dec-22 14:44 MD5 Hash: 9FF9432A4AE35CF86F0408B0595A84FC

CETIS Version: CETISv1.9.7
Status Level: 1
Editor ID: 008-522-314-5

7d Survival Rate Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.00	1.00	1.00	1.00	1.00
32		0.88	1.00	1.00	0.75	0.88
42		0.88	1.00	1.00	1.00	1.00
56		1.00	1.00	1.00	1.00	1.00
80		1.00	0.88	1.00	1.00	1.00
100	(*)	1.00	1.00	1.00	1.00	1.00
-101- 100UV		0.88	1.00	0.88	1.00	1.00

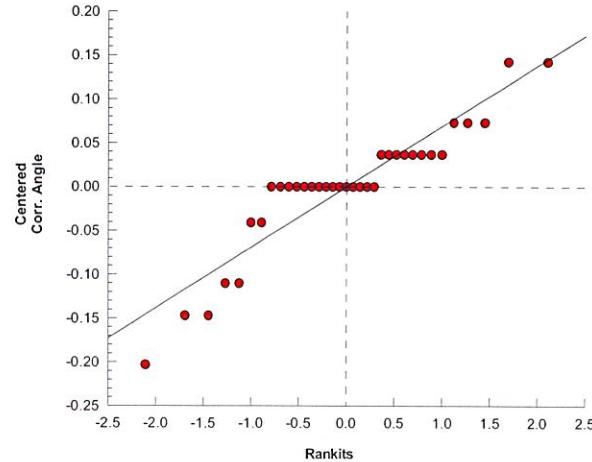
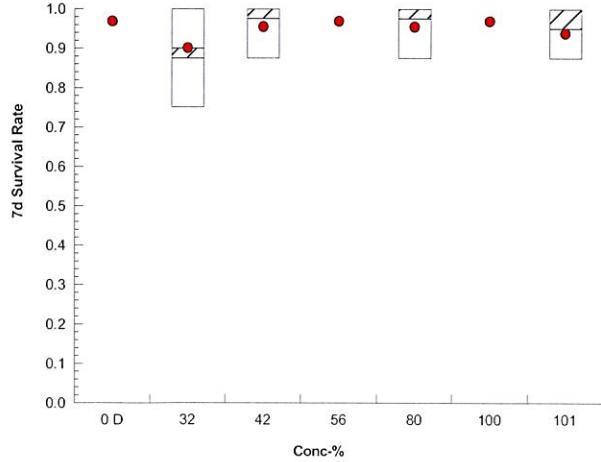
Angular (Corrected) Transformed Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.40	1.40	1.40	1.40	1.40
32		1.20	1.40	1.40	1.00	1.20
42		1.20	1.40	1.40	1.40	1.40
56		1.40	1.40	1.40	1.40	1.40
80		1.40	1.20	1.40	1.40	1.40
100	(*)	1.40	1.40	1.40	1.40	1.40
-101- 100UV		1.20	1.40	1.20	1.40	1.40

7d Survival Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	8/8	8/8	8/8	8/8	8/8
32		7/8	8/8	8/8	6/8	7/8
42		7/8	8/8	8/8	8/8	8/8
56		8/8	8/8	8/8	8/8	8/8
80		8/8	7/8	8/8	8/8	8/8
100	(*)	8/8	8/8	8/8	8/8	8/8
-101- 100UV		7/8	8/8	7/8	8/8	8/8

Graphics



CETIS Analytical Report

Report Date: 26 Dec-22 14:54 (p 1 of 2)
Test Code/ID: 27857188 / 06-6305-6776

Fathead Minnow 7-d Larval Survival and Growth Test				Bio-Analytical Laboratories					
Analysis ID: 10-8295-2841	Endpoint: Mean Dry Biomass-mg			CETIS Version: CETISv1.9.7					
Analyzed: 26 Dec-22 14:53	Analysis: Parametric-Control vs Treatments			Status Level: 1					
Edit Date: 26 Dec-22 14:44	MD5 Hash: 4A8E0E313ED3401F2DBFBEE77C63ED47			Editor ID: 008-522-314-5					
Batch ID: 10-8977-6054	Test Type: Growth-Survival (7d)			Analyst:					
Start Date: 13 Dec-22 15:30	Protocol: EPA/821/R-02-013 (2002)			Diluent: Reconstituted Water					
Ending Date: 20 Dec-22 15:15	Species: Pimephales promelas			Brine:					
Test Length: 7d	Taxon: Actinopterygii			Source: In-House Culture	Age: <24				
Sample ID: 16-8237-4459	Code: X8582			Project: WET Monthly Compliance Test (DEC)					
Sample Date: 12 Dec-22 08:00	Material: POTW Effluent			Source: AR0043613					
Receipt Date: 12 Dec-22 11:13	CAS (PC):			Station: 001					
Sample Age: 32h (6.8 °C)	Client: Magnolia Wastewater System								
Data Transform	Alt Hyp			NOEL	LOEL	TOEL	TU	MSDu	PMSD
Untransformed	C > T			101	101	---	0.9901	0.18	23.35%

Dunnett Multiple Comparison Test

Control	vs	Conc-%	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision($\alpha:5\%$)
Dilution Water	32	-0.57	2.4	0.18	8	CDF	0.9592	Non-Significant Effect	
	42	-2.7	2.4	0.18	8	CDF	1.0000	Non-Significant Effect	
	56	-2	2.4	0.18	8	CDF	0.9996	Non-Significant Effect	
	80	0.5	2.4	0.18	8	CDF	0.6808	Non-Significant Effect	
	100	-0.76	2.4	0.18	8	CDF	0.9759	Non-Significant Effect	
	104	-1.1	2.4	0.18	8	CDF	0.9898	Non-Significant Effect	

Test Acceptability Criteria

Attribute	Test Stat	Lower	Upper	Overlap	Decision
Control Resp	0.78	0.25	>>	Yes	Passes Criteria
PMSD	0.23	0.12	0.3	Yes	Passes Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision($\alpha:5\%$)
Between	0.212434	0.0354057	6	2.5	0.0456	Significant Effect
Error	0.395568	0.0141274	28			
Total	0.608002		34			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision($\alpha:1\%$)
Variance	Bartlett Equality of Variance Test	11	17	0.0989	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.98	0.91	0.8094	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	0.78	0.62	0.93	0.83	0.6	0.91	0.054	15.60%	0.00%
32		5	0.82	0.67	0.96	0.88	0.66	0.91	0.052	14.20%	-5.48%
42		5	0.98	0.78	1.2	1.1	0.76	1.1	0.072	16.32%	-26.45%
56		5	0.93	0.76	1.1	0.91	0.76	1.1	0.061	14.59%	-19.68%
80		5	0.74	0.57	0.91	0.74	0.57	0.93	0.06	18.33%	4.84%
100		5	0.83	0.81	0.86	0.84	0.81	0.86	0.0094	2.51%	-7.42%
104		5	0.86	0.74	0.97	0.85	0.74	0.99	0.04	10.57%	-10.32%

CETIS Analytical Report

Report Date: 26 Dec-22 14:54 (p 2 of 2)
Test Code/ID: 27857188 / 06-6305-6776

Fathead Minnow 7-d Larval Survival and Growth Test

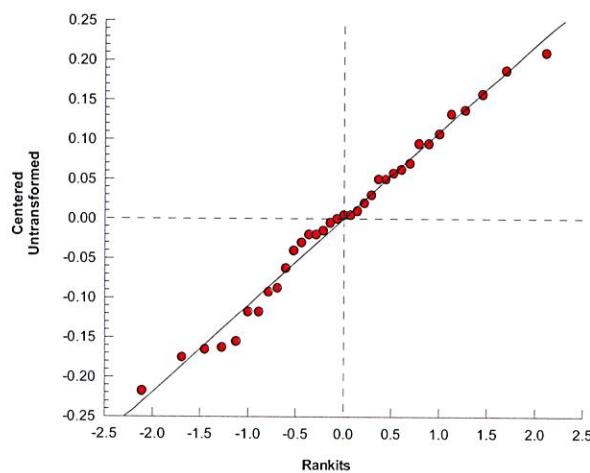
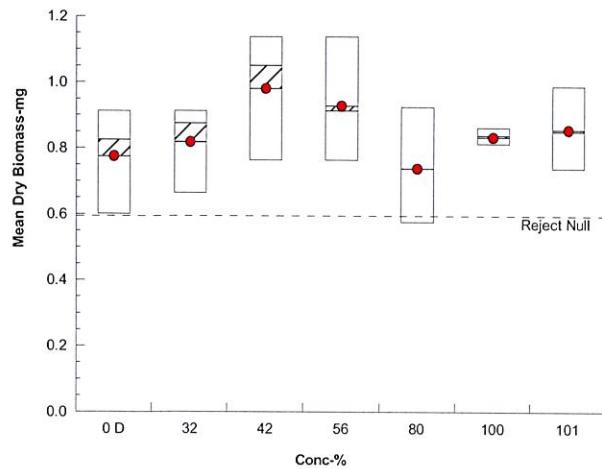
Bio-Analytical Laboratories

Analysis ID: 10-8295-2841 Endpoint: Mean Dry Biomass-mg CETIS Version: CETISv1.9.7
Analyzed: 26 Dec-22 14:53 Analysis: Parametric-Control vs Treatments Status Level: 1
Edit Date: 26 Dec-22 14:44 MD5 Hash: 4A8E0E313ED3401F2DBFBEE77C63ED47 Editor ID: 008-522-314-5

Mean Dry Biomass-mg Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	0.83	0.71	0.91	0.6	0.83
32		0.88	0.91	0.91	0.66	0.72
42		1.1	1.1	0.76	1.1	0.86
56		0.89	0.91	0.94	1.1	0.76
80		0.65	0.8	0.93	0.57	0.74
100		0.81	0.81	0.86	0.84	0.84
101	100 UV	0.74	0.83	0.85	0.88	0.99

Graphics



CETIS Analytical Report

Report Date: 26 Dec-22 14:54 (p 1 of 2)
Test Code/ID: 27857188 / 06-6305-6776

Fathead Minnow 7-d Larval Survival and Growth Test				Bio-Analytical Laboratories
Analysis ID: 19-1244-0862	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv1.9.7		
Analyzed: 26 Dec-22 14:54	Analysis: Linear Interpolation (ICPIN)	Status Level: 1		
Edit Date: 26 Dec-22 14:44	MD5 Hash: 4A8E0E313ED3401F2DBFBEE77C63ED47	Editor ID: 008-522-314-5		
Batch ID: 10-8977-6054	Test Type: Growth-Survival (7d)	Analyst:		
Start Date: 13 Dec-22 15:30	Protocol: EPA/821/R-02-013 (2002)	Diluent: Reconstituted Water		
Ending Date: 20 Dec-22 15:15	Species: Pimephales promelas	Brine:		
Test Length: 7d	Taxon: Actinopterygii	Source: In-House Culture	Age: <24	
Sample ID: 16-8237-4459	Code: X8582	Project: WET Monthly Compliance Test (DEC)		
Sample Date: 12 Dec-22 08:00	Material: POTW Effluent	Source: AR0043613		
Receipt Date: 12 Dec-22 11:13	CAS (PC):	Station: 001		
Sample Age: 32h (6.8 °C)	Client: Magnolia Wastewater System			

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	750956	1000	Yes	Two-Point Interpolation

Test Acceptability Criteria

TAC Limits					
Attribute	Test Stat	Lower	Upper	Overlap	Decision
Control Resp	0.78	0.25	>>	Yes	Passes Criteria

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC10	>100	---	---	<0.9901	---	---
IC15	>100	---	---	<0.9901	---	---
IC20	>100	---	---	<0.9901	---	---
IC25	>100	---	---	<0.9901	---	---
IC40	>100	---	---	<0.9901	---	---
IC50	>100	---	---	<0.9901	---	---

Mean Dry Biomass-mg Summary			Calculated Variate					Isotonic Variate		
Conc-%	Code	Count	Mean	Median	Min	Max	CV%	%Effect	Mean	%Effect
0	D	5	0.78	0.83	0.6	0.91	15.60%	0.00%	0.88	0.00%
32		5	0.82	0.88	0.66	0.91	14.20%	-5.48%	0.88	0.00%
42		5	0.98	1.1	0.76	1.1	16.32%	-26.45%	0.88	0.00%
56		5	0.93	0.91	0.76	1.1	14.59%	-19.68%	0.88	0.00%
80		5	0.74	0.74	0.57	0.93	18.33%	4.84%	0.81	7.62%
100	(+)	5	0.83	0.84	0.81	0.86	2.51%	-7.42%	0.81	7.62%
101	100 uV	5	0.86	0.85	0.74	0.99	10.57%	-10.32%	0.81	7.62%

Mean Dry Biomass-mg Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	0.83	0.71	0.91	0.6	0.83
32		0.88	0.91	0.91	0.66	0.72
42		1.1	1.1	0.76	1.1	0.86
56		0.89	0.91	0.94	1.1	0.76
80		0.65	0.8	0.93	0.57	0.74
100	(+)	0.81	0.81	0.86	0.84	0.84
101	100 uV	0.74	0.83	0.85	0.88	0.99

CETIS Analytical Report

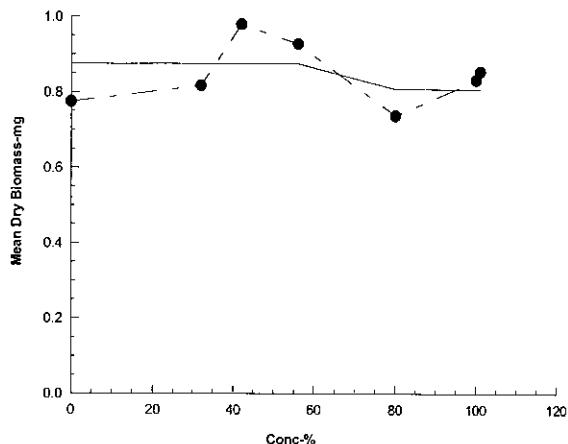
Report Date: 26 Dec-22 14:54 (p 2 of 2)
Test Code/ID: 27857188 / 06-6305-6776

Fathead Minnow 7-d Larval Survival and Growth Test

Bio-Analytical Laboratories

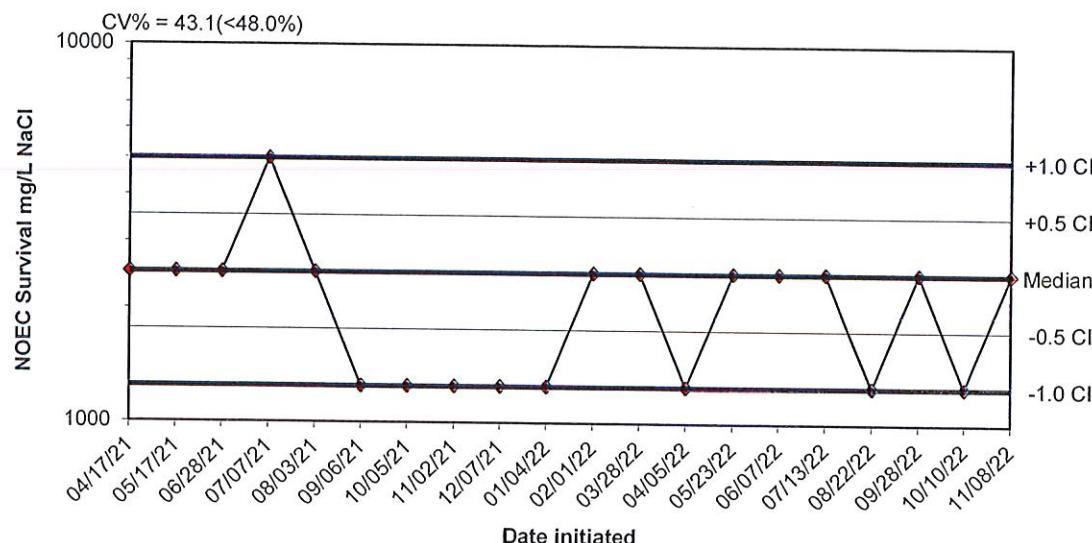
Analysis ID: 19-1244-0862 Endpoint: Mean Dry Biomass-mg CETIS Version: CETISv1.9.7
Analyzed: 26 Dec-22 14:54 Analysis: Linear Interpolation (ICPIN) Status Level: 1
Edit Date: 26 Dec-22 14:44 MD5 Hash: 4A8E0E313ED3401F2DBFBEE77C63ED47 Editor ID: 008-522-314-5

Graphics



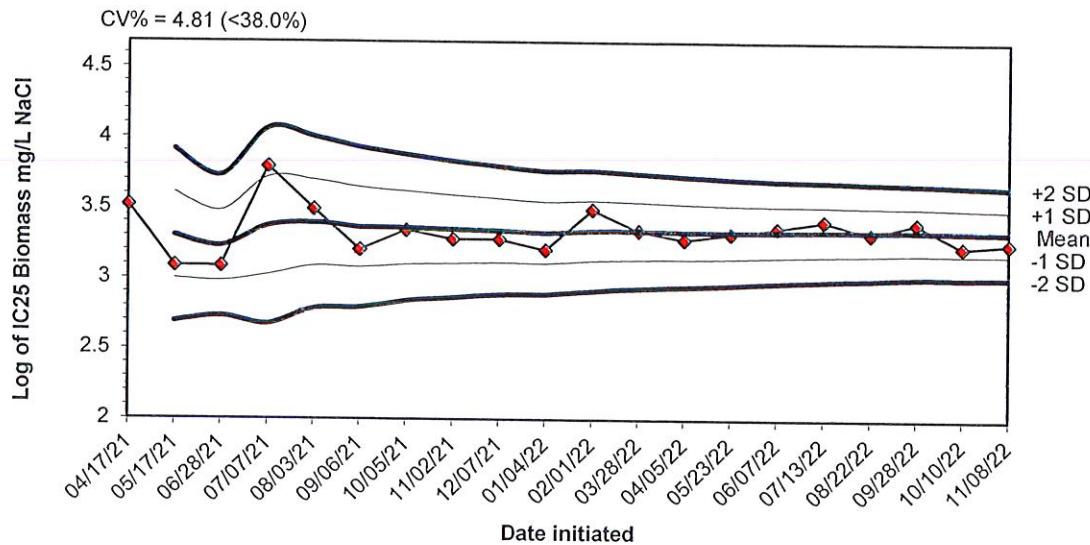
APPENDIX D
QUALITY ASSURANCE CHARTS

CHRONIC REFERENCE TOXICANT TEST RESULTS FOR PIMEPHALES
PROMELAS IN MH WATER



Dates	Values	Median	-0.5 CI	-1.0 CI	+0.5 CI	+1.0 CI
04/17/21	2500.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
05/17/21	2500.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
06/28/21	2500.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
07/07/21	5000.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
08/03/21	2500.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
09/06/21	1250.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
10/05/21	1250.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
11/02/21	1250.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
12/07/21	1250.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
01/04/22	1250.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
02/01/22	2500.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
03/28/22	2500.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
04/05/22	1250.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
05/23/22	2500.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
06/07/22	2500.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
07/13/22	2500.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
08/22/22	1250.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
09/28/22	2500.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
10/10/22	1250.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000
11/08/22	2500.0000	2500.0000	1767.7670	1250.0000	3535.5339	5000.0000

CHRONIC REFERENCE TOXICANT TEST RESULTS FOR PIMEPHALES
PROMELAS IN MH WATER



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
04/17/21	3.5199					
05/17/21	3.0878	3.3039	2.9983	2.6927	3.6094	3.9150
06/28/21	3.0854	3.2310	2.9809	2.7307	3.4812	3.7314
07/07/21	3.7924	3.3714	3.0242	2.6771	3.7185	4.0657
08/03/21	3.4914	3.3954	3.0900	2.7846	3.7008	4.0061
09/06/21	3.2041	3.3635	3.0794	2.7953	3.6476	3.9317
10/05/21	3.3424	3.3605	3.1010	2.8416	3.6199	3.8794
11/02/21	3.2788	3.3503	3.1083	2.8664	3.5922	3.8342
12/07/21	3.2788	3.3423	3.1148	2.8872	3.5699	3.7975
01/04/22	3.2041	3.3285	3.1096	2.8906	3.5475	3.7664
02/01/22	3.4914	3.3433	3.1299	2.9164	3.5568	3.7702
03/28/22	3.3424	3.3432	3.1397	2.9362	3.5467	3.7503
04/05/22	3.2788	3.3383	3.1426	2.9469	3.5339	3.7296
05/23/22	3.3222	3.3371	3.1491	2.9611	3.5252	3.7132
06/07/22	3.3617	3.3388	3.1575	2.9761	3.5201	3.7014
07/13/22	3.4150	3.3435	3.1673	2.9911	3.5197	3.6959
08/22/22	3.3222	3.3423	3.1716	3.0009	3.5130	3.6836
09/28/22	3.3979	3.3454	3.1793	3.0132	3.5115	3.6776
10/10/22	3.2304	3.3393	3.1758	3.0122	3.5029	3.6664
11/08/22	3.2553	3.3351	3.1748	3.0145	3.4954	3.6557

**APPENDIX E
AGENCY FORMS**

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

Permittee: Magnolia Wastewater System

NPDES No.: AR0043613

AFIN: 14-00059

Time	Date	Time	Date
Composite 1 Collected from: 0800	12/11/22 To	0800	12/12/22
Composite 2 Collected from: 0800	12/13/22 To	0800	12/14/22
Composite 3 Collected from: 0800	12/15/22 To	0800	12/16/22

Test initiated:	1530	am/pm	12/13/22	Date
Test terminated:	1515	am/pm	12/20/22	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	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.00
32.0	88.0	100.0	100.0	75.0	88.0	100.0	100.0	90.0	11.68
42.0	88.0	100.0	100.0	100.0	100.0	100.0	100.0	98.0	6.06
56.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.00
80.0	100.0	88.0	100.0	100.0	100.0	100.0	100.0	98.0	6.06
100.0	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	0.830	0.710	0.910	0.600	0.830	0.780	15.60
32.0	0.880	0.910	0.910	0.660	0.720	0.820	14.20
42.0	1.100	1.100	0.760	1.100	0.860	0.980	16.32
56.0	0.890	0.910	0.940	1.100	0.760	0.930	14.59
80.0	0.650	0.800	0.930	0.570	0.740	0.740	18.33
100.0	0.810	0.810	0.860	0.840	0.840	0.830	2.51

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

PMSD = 23.35 %

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

1. Dunnett's Procedure or Steel's 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 (100.0%) | YES | X | NO |
| b) $\frac{1}{2}$ 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 (100.0%) | YES | X | NO |
| b) $\frac{1}{2}$ 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 and circle lowest number:

- | | |
|-------------------|-----------------|
| a.) NOEC survival | 100.0% effluent |
| b.) NOEC growth | 100.0% effluent |

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

Permittee:	Magnolia Wastewater System	Sample #1 Collected:	Date: 12/12/2022 Time: 800
NPDES#:	AR0043613/AFN 14-00059	Sample #2 Collected:	Date: 12/14/2022 Time: 800
Contact:	Tracie Love	Sample #3 Collected:	Date: 12/16/2022 Time: 800
Analysts:	Ware, Mitchell, Miller	Test Begin:	Date: 12/13/2022 Time: 1530
Dilution:	0%	Dilution:	56.0%
Day:	1	2	3
T (°C)	26.4	25.9	25.4
DO Initial	7.2	5.9	5.2
DO Final	7.3	7.4	7.2
pH Initial	6.6	6.5	7.3
pH Final	6.9	6.8	7.5
Conductivity	163.0	170.0	172.0
Alkalinity	36.0		
Hardness	44.0		
Chlorine	<0.5		
Dilution:	32.0%	Dilution:	80.0%
Day:	1	2	3
T (°C)	26.4	25.9	25.4
DO Initial	6.3	5.1	6.1
DO Final	7.4	7.5	7.4
pH Initial	6.6	6.5	7.2
pH Final	7.1	6.8	7.2
Conductivity	208.0	204.0	203.0
Alkalinity			
Hardness			
Chlorine			
Dilution:	42.0%	Dilution:	100.0%
Day:	1	2	3
T (°C)	26.4	25.9	25.4
DO Initial	6.3	5.3	6.3
DO Final	7.5	7.6	7.5
pH Initial	6.5	6.6	7.1
pH Final	7.2	6.8	7.1
Alkalinity			
Hardness			
Conductivity	218.0	221.0	216.0
Chlorine			
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: City of Magnolia

Project#: X8582

Chain of Custody Documents Checked by: Endre 12/27/22
Technician/Date

Raw Data Documents Checked by: Endre 12/27/22
Technician/Date

Statistical Analysis Package Checked by: ECB 12/28/22
Quality Manager/Date

Quality Control Data Checked by: ECB 1/6/23
Quality Manager/Date

Report Checked by: ECB 1/6/23
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.

Endre Brupp, BS 1/6/23
Quality Manager Date

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Report Rev. 3.0

