Bio-Aquatic Testing, Inc.

TCEQ TNI Accredited

City of Mena
WWTP
OUTFALL 001

Chronic Biomonitoring Report

87081

Ceriodaphnia dubia
Pimephales promelas

August 08, 2023

Approved by _____
Lab director

Bio-Aquatic Testing, Inc. • *2501 Mayes Rd. Ste. 100* • *Carrollton, Texas* • *75006*

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***HAND-WRITTEN RAW DATA TABLES ARE AVAILABLE UPON REQUEST**

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TOXICITY TEST REPORT - Chronic

Client:	Mena, City of	Sample:	001
Facility:	WWTP	Laboratory Number:	87081
Permit No.	AR0036692	Date:	August 08, 2023

Ceriodaphnia dubia **passed** survival and reproduction testing requirements. *Pimephales promelas* **passed** survival and growth testing requirements.

SAMPLE COLLECTION:

Composite effluent samples from the City of Mena, WWTP, were received on August 08, 2023, August 10, 2023, and August 12, 2023. Effluent samples were collected from Outfall 001 by facility personnel.

The effluent samples were analyzed for total residual chlorine using the Hanna Ion Specific Meter #711 and contained <0.10 mg/L, <0.10 mg/L, and <0.10 mg/L, respectively. Effluent and laboratory dilution water pH, temperature, and dissolved oxygen data were collected daily.

TEST PROCEDURES:

EPA METHOD: 1002

Ceriodaphnia dubia

The seven-day (three brood) Chronic *Ceriodaphnia dubia* survival and reproduction test was initiated at 16:08 hours on August 08, 2023. Five effluent concentrations of 32%, 45%, 56%, 80%, and 100% were prepared using synthetic water as dilution water. The test was set up with 30mL plastic cups containing 15mL of test solution or control dilution water. Each effluent concentration or control dilution water included ten replicate cups with one organism in each cup. The control was conducted concurrently with the test. Test organisms were less than 24-hour old laboratory cultured neonates. Neonates were introduced into the test solutions using a blocking design. The test was renewed daily with newly prepared solutions. Food consisting of a half-milliliter suspension of the green algae, *Selenastrum capricornutum,* and YTC was added to the test solutions each day. The test proceeded for seven days or until 60% of the females in the control had three broods. Data on survival and number of young produced per female were collected daily. The test ended at 15:11 hours on August 16, 2023. Survival and reproduction data were statistically (p=0.05) analyzed according to EPA procedures to determine the Lowest Observable Effect Concentration (LOEC) and the No Observable Effect Concentration (NOEC).

SURVIVAL:

Ceriodaphnia dubia

Fisher's Exact test on *Ceriodaphnia dubia* survival test data demonstrated no statistically significant differences between the control and any of the effluent concentrations tested.

LOEC: Not Calculable (Q)
NOEC: 100% Effluent

REPRODUCTION:

Ceriodaphnia dubia

The *Ceriodaphnia dubia* reproduction data were normally distributed at the alpha level of 0.01 (13.277) using the Chi-square test for normality. Reproduction data were shown not to be homogeneous using Bartlett's test at the alpha level of 0.01 (15.09) without data transformations. Using the Steel's Many-One Rank Test on *Ceriodaphnia dubia* reproduction data demonstrated no statistically significant differences between the control and any of the effluent concentrations tested.

LOEC: Not Calculable (Q)
NOEC: 100% Effluent

TEST PROCEDURES:

EPA METHOD: 1000

Pimephales promelas

The seven-day Chronic *Pimephales promelas* survival and growth test was initiated at 16:39 hours on August 08, 2023. Five effluent concentrations of 32%, 45%, 56%, 80%, and 100% were prepared using synthetic water as dilution water. The test was set up with 450mL plastic cups containing 250mL of test solution as test chambers. Each concentration consisted of five replicate chambers containing eight organisms each, giving a total of 40 (forty) per treatment. The control test was conducted concurrently with the test. Test organisms were laboratory-cultured *Pimephales promelas* larvae less than 24-hours old. The number of surviving larvae and water quality parameters in the old test solutions were recorded after each 24-hour period. The test was renewed daily with fresh solutions. Surviving larvae in each test chamber were fed freshly hatched brine shrimp two times per day. The test proceeded for seven days.

At the end of the test, all organisms were sacrificed, dried, and weighed. Data on surviving organisms and water quality were collected. The test ended at 14:38 hours on August 15, 2023. Survival and growth (weight) were statistically (p=0.05) analyzed according to EPA procedures to determine the Lowest Observable Effect Concentration (LOEC) and the No Observable Effect Concentration (NOEC).

SURVIVAL:

Pimephales promelas

The non-parametric Steel's Many-One Rank test performed on *Pimephales promelas* survival data demonstrated no statistically significant differences between the control and any of the effluent concentrations tested.

LOEC: Not Calculable (Q)
NOEC: 100% Effluent

GROWTH:

Pimephales promelas

The *Pimephales promelas* growth data were normally distributed at the alpha level of 0.01 (0.900) using Shapiro Wilk's test for normality. Growth data were shown to be homogeneous using Bartlett's test at the alpha level of 0.01 (15.09) without data transformations. Using ANOVA and Dunnett's test on *Pimephales promelas* growth data demonstrated no statistically significant differences between the control and any of the effluent concentrations tested.

LOEC: Not Calculable (Q)
NOEC: 100% Effluent

BIO-AQUATIC TESTING, INC.

TOXICITY TEST

Chronic ***Ceriodaphnia dubia***

Client: Mena, City of WWTP

Lab ID: 87081

Permit Number: NPDES AR0036692

Sample Type: Composite

Outfall Name: 001

Receiving Water Name: Prairie Creek

Test Temperature (oC): 25 ± 1

Photo Period: 16 hours light, 8 hours dark

Dilution Water: synthetic

Begin Date: 8/8/2023

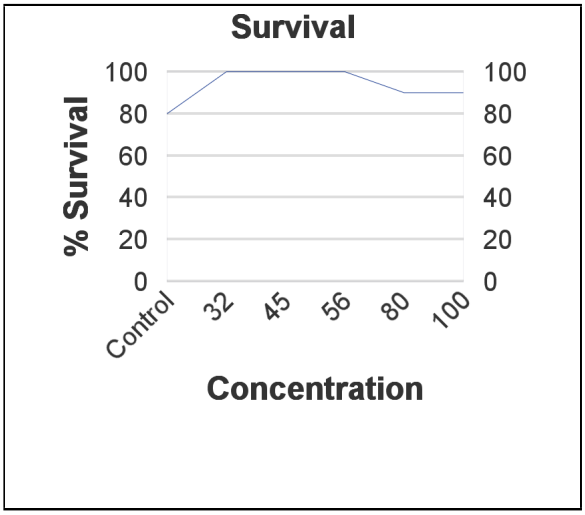
End Date: 8/16/2023

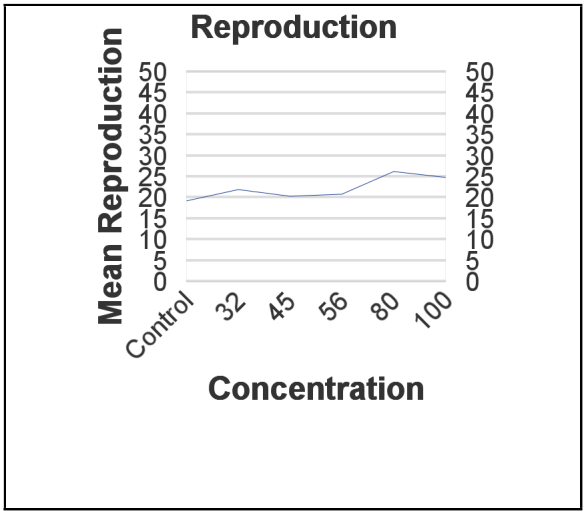
Test Start Time: 16:08 **Test End Time:** 15:11

SURVIVAL AND REPRODUCTION TABLE

FEMALE #	Control	32	%	45	%	56	%	80	%	100	%
1	20	26		23		25		35		20	
2	D- 10	25		21		22		21		23	
3	19	20		19		30		24		29	
4	20	20		18		17		32		20	
5	22	22		16		14		29		D- 0	
6	13	27		22		17		D- 16		26	
7	17	19		23		18		25		28	
8	D- 0	17		21		17		18		27	
9	19	18		18		17		22		29	
10	24	25		22		31		30		22	
Surv.Mean	19.2	21.9		20.3		20.8		26.2		24.8	
C.V%	17.1	16.4		11.8		28.6		21.4		14.8	
Total Mean	16.4	21.9		20.3		20.8		25.2		22.4	
Var	10.785	12.988		5.788		35.511		31.444		13.611	
Std.Dev.	3.284	3.604		2.406		5.959		5.607		3.689	
Max	24	27		23		31		35		29	
Min	13	17		16		14		18		20	

Concentration Response Relationships





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Survival and Reproduction

Control

Date	1	2	3	4	5	6	7	8	9	10
8/9	A	A	A	A	A	A	A	A	A	A
8/10	A	A	A	A	A	A	A	A	A	A
8/11	A	A	A	A	A	A	3	D	A	A
8/12	3	3	3	4	4	3	A	D	4	A
8/13	6	7	5	6	A	1	A	D	4	5
8/14	A	D	11	A	9	9	3	D	A	A
	9	10	19	10	13	13	6	0	8	5
8/15	A	D	A	A	A	A	A	D	11	9
	9	10	19	10	13	13	6	0	19	14
8/16	11	D	A	10	9	A	11	D	A	10
	20	10	19	20	22	13	17	0	19	24

Mean: 19.20 **CV%** 17.10
Var. 10.79 **Max** 24
Std.Dev. 3.28 **Min** 13

32

Date	1	2	3	4	5	6	7	8	9	10
8/9	A	A	A	A	A	A	A	A	A	A
8/10	A	A	A	A	A	A	A	A	A	A
8/11	5	4	3	3	4	A	4	A	3	A
8/12	6	A	A	A	A	A	A	A	A	A
8/13	A	10	8	10	A	4	7	6	A	5
8/14	A	11	9	A	8	13	8	A	9	11
	11	25	20	13	12	17	19	6	12	16
8/15	A	A	A	7	A	A	A	A	6	9
	11	25	20	20	12	17	19	6	18	25
8/16	15	A	A	A	10	10	A	11	A	A
	26	25	20	20	22	27	19	17	18	25

Mean: 21.90 **CV%** 16.40
Var. 12.99 **Max** 27
Std.Dev. 3.60 **Min** 17

45

Date	1	2	3	4	5	6	7	8	9	10
8/9	A	A	A	A	A	A	A	A	A	A
8/10	A	A	A	A	A	A	A	A	A	A
8/11	5	3	A	4	4	5	5	5	3	6
8/12	A	A	A	A	3	A	4	A	5	A
8/13	A	A	A	A	A	A	A	A	A	A
8/14	10	9	A	A	A	9	A	A	A	A
	15	12	0	4	7	14	9	5	8	6
8/15	A	A	11	9	A	A	A	11	A	5
	15	12	11	13	7	14	9	16	8	11
8/16	8	9	8	5	9	8	14	5	10	11
	23	21	19	18	16	22	23	21	18	22

Mean: 20.30 **CV%** 11.80
Var. 5.79 **Max** 23
Std.Dev. 2.41 **Min** 16

56

Date	1	2	3	4	5	6	7	8	9	10
8/9	A	A	A	A	A	A	A	A	A	A
8/10	A	A	A	A	A	A	A	A	A	A
8/11	6	5	A	A	A	3	5	3	3	5
8/12	6	5	7	4	3	A	A	A	3	A
8/13	A	A	A	4	A	6	6	1	A	A
8/14	A	12	10	A	A	8	7	13	A	13
	12	22	17	8	3	17	18	17	6	18
8/15	A	A	A	9	A	A	A	A	A	13
	12	22	17	17	3	17	18	17	6	31
8/16	13	A	13	A	11	A	A	A	11	A
	25	22	30	17	14	17	18	17	17	31

Mean: 20.80 **CV%** 28.60
Var. 35.51 **Max** 31
Std.Dev. 5.96 **Min** 14

80

Date	1	2	3	4	5	6	7	8	9	10
8/9	A	A	A	A	A	A	A	A	A	A
8/10	A	A	A	A	A	A	A	A	A	A
8/11	6	4	A	A	A	3	3	A	5	A
8/12	A	4	2	6	A	4	A	1	7	7
8/13	A	A	7	A	3	9	A	A	A	A
8/14	A	13	A	12	A	D	12	6	10	11
	6	21	9	18	3	16	15	7	22	18
8/15	14	A	A	A	12	D	10	A	A	12
	20	21	9	18	15	16	25	7	22	30
8/16	15	A	15	14	14	D	A	11	A	A
	35	21	24	32	29	16	25	18	22	30

Mean: 26.20 **CV%** 21.40
Var. 31.44 **Max** 35
Std.Dev. 5.61 **Min** 18

100

Date	1	2	3	4	5	6	7	8	9	10
8/9	A	A	A	A	A	A	A	A	A	A
8/10	A	A	A	A	A	A	A	A	A	A
8/11	5	4	A	A	D	A	A	A	A	3
8/12	5	4	7	4	D	2	7	7	7	6
8/13	10	A	A	6	D	A	A	A	A	A
8/14	A	15	10	10	D	12	7	10	11	13
	20	23	17	20	0	14	14	17	18	22
8/15	A	A	12	A	D	A	14	10	11	A
	20	23	29	20	0	14	28	27	29	22
8/16	A	A	A	A	D	12	A	A	A	A
	20	23	29	20	0	26	28	27	29	22

Mean: 24.80 **CV%** 14.80
Var. 13.61 **Max** 29
Std.Dev. 3.69 **Min** 20

BIO-AQUATIC TESTING, INC.

Chronic CERIODAPHNIA DUBIA SURVIVAL AND REPRODUCTION

Client: **Mena, City of - WWTP** Lab ID: **87081** Culture No.: Bio080823-38°

TEST INSTRUCTIONS: AFIN 57-00423

ORGANISMS ADDED: Date: 8-8-23 Time: 1608 Technician: CL

Photo Period 16hr Light/8hr dark

RANDOMIZATION: SC-10 15

Dilution: Control

	DATE/TIME/ TECHNICIAN	1	2	3	4	5	6	7	8	9	10
24Hr	8-9-23 MH 1600	A	—	—	—	—	—	—	—	—	A
48Hr	8-10-23 MH 1415	A	—	—	—	—	—	—	—	—	A
72Hr	8-11-23 BM 540	A	—	—	—	—	A	3	D	A	A
96Hr	8-12-23 1747 JM	3	3	3	4	4	3	A		4	4
5 days	8-13-23 CL 1454	6	7	5	6	1	1	A		4	1
6 days	8-14-23 SB 1413	A	D	11	A	8	9	3		A	A
7 days	8-15-23 MN 1059	A		A	A	A	A	A		11	9
8 days	8-16-23 MN 1511	11		A	10	9	A	11		A	10

Dilution: 32 %

	1	2	3	4	5	6	7	8	9	10
24Hr	A	—	—	—	—	—	—	—	—	A
48Hr	A	—	—	—	—	—	—	—	—	A
72Hr	5	4	3	3	4	A	4	A	3	A
96Hr	6	6	3	5	A	A	A	A	A	4
5 days	A	2	5	5	2	4	7	6	A	1
6 days	A	11	9	A	6	13	8	A	9	11
7 days	A	8	A	7	A	A	A	A	6	9
8 days	15	A	11	A	10	10	6	11	A	A

Code: Cells in numbered columns indicate daily survival and reproduction: "A" means adult alive and no young produced, a number means adult alive and that number of young produced, "D" followed by a zero means adult dead and no young produced, "D" followed by a number means adult dead and that number of young produced. "E" indicates toss out due to experimenter error. Lined through spaces preceded by a number or letter represent the same number. Lined spaces without a preceding number or letter indicate unused or not applicable spaces.

BIO-AQUATIC TESTING, INC.

Chronic CERIODAPHNIA DUBIA SURVIVAL AND REPRODUCTION

Client: **Mena, City of - WWTP** Lab ID: **87081** Culture No.:

TEST INSTRUCTIONS: AFIN 57-00423

Dilution: 45 %

	1	2	3	4	5	6	7	8	9	10
24Hr	A	—	—	—	—	—	—	—	—	A
48Hr	A	—	—	—	—	—	—	—	—	A
72Hr	5	3	A	4	4	5	5	5	3	0
96Hr	A	—	A	A	3	A	4	A	5	A
5 days	A	—	—	—	—	—	—	—	—	A
6 days	10	9	A	A	A	9	A	A	A	A
7 days	A	A	11	9	A	A	A	11	A	5
8 days	8	9	8	5	9	8	14	5	10	11

Dilution: 56 %

	1	2	3	4	5	6	7	8	9	10
24Hr	A	—	—	—	—	—	—	—	—	A
48Hr	A	—	—	—	—	—	—	—	—	A
72Hr	6	5	4	A	A	3	5	3	3	5
96Hr	6	5	3	4	3	A	A	A	3	A
5 days	A	A	A	4	A	6	5	1	A	A
6 days	A	12	10	A	A	8	7	13	A	B
7 days	A	A	A	9	A	A	A	A	A	13
8 days	13	A	13	A	11	0	15	A	11	A

Code: Cells in numbered columns indicate daily survival and reproduction: "A" means adult alive and no young produced, a number means adult alive and that number of young produced, "D" followed by a zero means adult dead and no young produced, "D" followed by a number means adult dead and that number of young produced. "E" indicates toss out due to experimenter error. Lined through spaces preceded by a number or letter represent the same number. Lined spaces without a preceding number or letter indicate unused or not applicable spaces.

BIO-AQUATIC TESTING, INC.

Chronic CERIODAPHNIA DUBIA SURVIVAL AND REPRODUCTION

Client: **Mena, City of - WWTP** Lab ID: **87081** Culture No.:

TEST INSTRUCTIONS: AFIN 57-00423

Dilution: 80 %

	1	2	3	4	5	6	7	8	9	10
24Hr	A	—	—	—	—	—	—	—	—	A
48Hr	A	—	—	—	—	—	—	—	—	A
72Hr	6	4	A	A	A	3	3	A	5	4
96Hr	A	4	2/A	6	A	4	A	1/A	7	3
5 days	A	A	7	4	3	9	A	—	—	A
6 days	9	B	A	8	A	D	12	6	10	11
7 days	5	12	A	A	12		10	8	14	12
8 days	15	A	15	2/12	14		A	3	A	A

Dilution: 100 %

	1	2	3	4	5	6	7	8	9	10
24Hr	A		—	—	—	—	—	—	—	A
48Hr	A	—	—	—	—	—	—	—	—	A
72Hr	5	4	A	A	D	A	A	A	A	3
96Hr	5	4	1/6	4		2/A	2/5	7	2/5	6
5 days	10	A	A	6		A	—	—	—	A
6 days	A	15	10	10		12	7	10	11	B
7 days	12	5	12	A		A	14	10	11	A
8 days	A	A	A	A		12	A	A	A	A

Code: Cells in numbered columns indicate daily survival and reproduction: "A" means adult alive and no young produced, a number means adult alive and that number of young produced, "D" followed by a zero means adult dead and no young produced, "D" followed by a number means adult dead and that number of young produced. "E" indicates toss out due to experimenter error. Lined through spaces preceded by a number or letter represent the same number. Lined spaces without a preceding number or letter indicate unused or not applicable spaces.

BIO-AQUATIC TESTING, INC.

Chror CERIODAPHNIA DUBIA SURVIVAL AND REPRODUCTION

Client: Mena, City (- WWTP Lab ID: **87081** Culture No.:

TEST INSTRUCTIONS: AFI 7-00423

Test Temperatures

	0Hr new	24Hr old	24Hr new	48Hr old	48Hr new	72Hr old	72Hr new	96Hr old	96Hr new	5 days old	5 days new	6 days old	6 days new	7 days old
Control	25.1	25.5	25.0	25.6	25.2	25.2	25.2	25.1	25.9	25.1	25.5	25.0	25.3	25.2
32														
45														
56														
80														
100														
TIME/DATE TECH	8-8-23 CG 1008	8-9-23 MH	1600	8-10-23 MH	1415	8-11-23 [illegible]	1210	8-12-23 1750	[illegible]	8-13-23 CG	1501	8-14-23 SM	1413	8-15-23 MW 1059
IR GUN ID #	021	012		021		021		021		021		021		012

Lined through spaces preceded by a number represent the same number. Lined spaces without a preceding number indicate unused or not applicable spaces.

Chronic *Pimephales promelas*

Client: Mena, City of WWTP

Permit Number: NPDES AR0036692

Outfall Name: 001 **Sample Type:** Composite

Receiving Water Name: Prairie Creek

Lab ID: 87081

Test Temperature (oC): 25 ± 1

Photo Period: 16 Hours Light 8 Hours Dark

Test Start Time: 16:39 Test End Time: 14:38

Begin Date: 8/8/2023

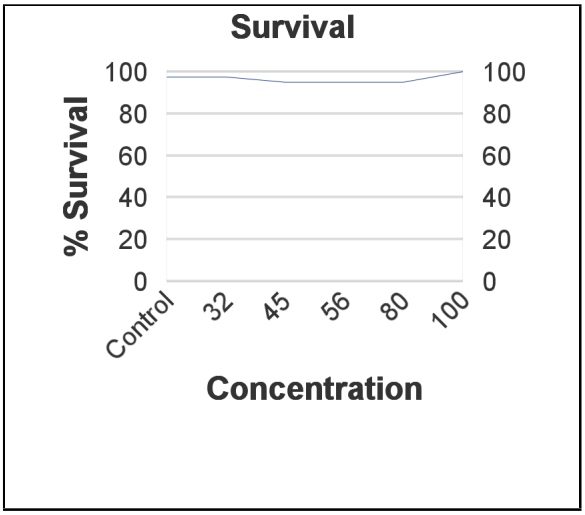
End Date: 8/15/2023

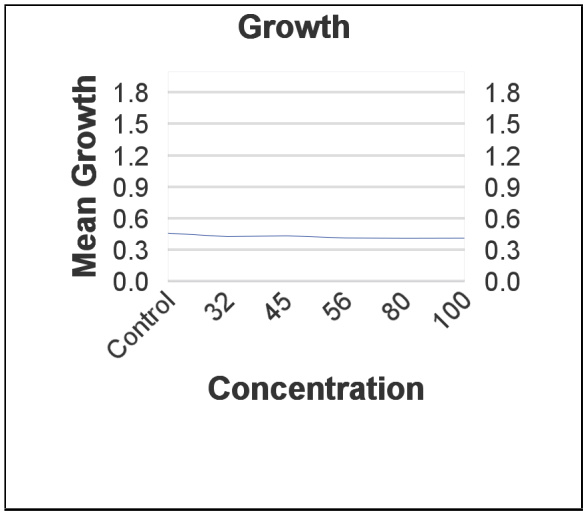
SURVIVAL

Effluent Concentration		Number Of Alive 8/8	8/9	8/10	8/11	8/12	8/13	8/14	8/15	Avg% Surv.
Control	A	8	8	8	8	8	8	8	8	97.5%
	B	8	8	8	8	8	8	8	8	
	C	8	8	8	8	8	8	8	8	
	D	8	8	8	8	8	7	7	7	
	E	8	8	8	8	8	8	8	8	
32	A	8	8	8	8	8	8	8	8	97.5%
	B	8	8	8	8	8	8	8	8	
	C	8	8	8	8	8	8	8	8	
	D	8	8	8	8	8	8	8	8	
	E	8	8	8	8	8	7	7	7	
45	A	8	8	8	8	8	8	7	7	95.0%
	B	8	8	8	8	8	8	8	8	
	C	8	8	8	8	8	8	8	8	
	D	8	8	8	8	8	8	8	8	
	E	8	8	7	7	7	7	7	7	
56	A	8	8	8	8	8	8	8	8	95.0%
	B	8	8	8	8	8	8	8	8	
	C	8	8	8	8	8	8	8	7	
	D	8	8	8	8	8	7	7	7	
	E	8	8	8	8	8	8	8	8	

Effluent Concentration		Number Of Alive 8/8	8/9	8/10	8/11	8/12	8/13	8/14	8/15	Avg% Surv.
80	A	8	8	8	7	7	7	7	7	95.0%
	B	8	8	8	8	8	8	8	8	
	C	8	8	8	8	8	8	8	8	
	D	8	8	8	8	8	8	8	8	
	E	8	8	7	7	7	7	7	7	
100	A	8	8	8	8	8	8	8	8	100.0%
	B	8	8	8	8	8	8	8	8	
	C	8	8	8	8	8	8	8	8	
	D	8	8	8	8	8	8	8	8	
	E	8	8	8	8	8	8	8	8	
	A									
	B									
	C									
	D									
	E									

Concentration Response Relationships





BIO-AQUATIC TESTING, INC.

Chronic Pimephales promelas SURVIVAL Lab ID: **87081**

Client: **Mena, City of** Facility **WWTP** Outfall:001 Sample Type: Composite

TEST INSTRUCTIONS: AFIN 57-00423

Culture No. : PI-23-219C Photo Period: 16hr light, 8hr dark **RANDOMIZATION:** **SC-5 3**

Dilution: Control 32 45 56

	DATE/TIME/ TECHNICIAN	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
0Hr	8-8-23 1639 JC	8	—	—	—	—	8	—	—	—	—	8	—	—	—	—	8	—	—	—	—
24Hr	8-9-23 SDT 0740	8	—	—	—	—	8	—	—	—	—	8	—	—	—	—	8	—	—	—	—
48Hr	8-10-23 SDT 0700	8	—	—	—	—	8	—	—	—	—	8	—	—	—	7	8	—	—	—	—
72Hr	8-11-23 SDT 0730	8	—	—	—	—	8	—	—	—	—	8	—	—	—	7	8	—	—	—	—
96Hr	8-12-23 SDT 0720	8	—	—	—	—	8	—	—	—	—	8	—	—	—	7	8	—	—	—	—
5 days	8-13-23 1147 AR	8	—	—	7	8	8	—	—	—	7	8	—	—	—	7	8	—	—	7	8
6 days	8-14-23 1001 SG	8	—	—	7	8	8	—	—	—	7	7	8	—	—	7	8	—	—	7	8
7 days	8-15-23 SDT 1438	8	8	8	7	8	8	8	8	8	7	7	8	8	8	7	8	8	7	7	8

Dilution: 80 100

	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
0Hr	8	—	—	—		8	—	—	—	—										
24Hr	8	—	—	—	—	8	—	—	—	—										
48Hr	8	—	—	—	7	8	—	—	—	—										
72Hr	7	8	8	8	7	8	—	—	—	—										
96Hr	7	8	—	—	7	8	—	—	—	—										
5 days	7	8	—	—	7	8	—	—	—	—										
6 days	7	8	—	—	7	8	—	—	—	—										
7 days	7	8	—	—	7	8	—	—	—	—										

Lined through spaces preceded by a number represent the same number. Lined spaces without a preceding number indicate unused or not applicable sp

BIO-AQUATIC TESTING, INC.

Chronic | Pimephales promelas SURVIVAL | Lab ID: **87081**

Client: **Mena, City of** | Facility **WWTP** | Outfall:001 | Sample Type: Composite

TEST INSTRUCTIONS: AFIN 57-00423

Test Temperatures

	0Hr	24Hr		48Hr		72Hr		96Hr		5 days		6 days		7 days
	new	old	new	old	new	old	new	old	new	old	new	old	new	old
Control	25.4	27.1	25.3	25.0	25.4	26.2	25.8	23.9	25.2	25.8	25.6	25.4	25.4	26.2
32														
45														
56														
80														
100														
TIME/DATE TECH	8-8-23 1639 JC	8-9-23 SDT	0740	8-10-23 SDT	0700	8-11-23 SDT	0730	8-12-23 SDT	0720	8-13-23 1147	VAR	8-14-23 1001	SG	8-15-23 SDT 1430
IR GUN ID #	020	024		024		024		024		024		024		024

Lined through spaces preceded by a number represent the same number. Lined spaces without a preceding number indicate unused or not applicable spaces.

2nd Organism **BIO-AQUATIC TESTING, INC.** **TOXICITY TEST**

Chronic *Pimephales promelas*

Client: Mena, City of WWTP **Lab ID: 87081**

Permit Number: AR0036692

Sample Type: Composite **Outfall Name:** 001

Receiving Water Name: Prairie Creek

Synthetic

	ON	SN	Wt.	Avg.	SN Avg.
A	8	8	4.973	0.622	0.622
B	8	8	3.514	0.439	0.439
C	8	8	3.578	0.447	0.447
D	8	7	4.255	0.532	0.608
E	8	8	4.071	0.509	0.509

Mean	C.V. %
0.510	14.5

SN Mean	SN C.V. %
0.525	16.5

32

	ON	Wt.	Avg.
A	8	3.542	0.443
B	8	3.491	0.436
C	8	3.434	0.429
D	8	3.656	0.457
E	8	2.954	0.369

Mean	C.V. %
0.427	7.9

45

	ON	Wt.	Avg.
A	8	3.277	0.410
B	8	3.664	0.458
C	8	3.636	0.455
D	8	3.790	0.474
E	8	3.014	0.377

Mean	C.V. %
0.435	9.2

56

	ON	Wt.	Avg.
A	8	3.702	0.463
B	8	3.049	0.381
C	8	2.382	0.298
D	8	3.210	0.401
E	8	4.212	0.527

Mean	C.V. %
0.414	20.9

80

	ON	Wt.	Avg.
A	8	2.785	0.348
B	8	2.700	0.338
C	8	3.218	0.402
D	8	3.147	0.393
E	8	2.562	0.320

Mean	C.V. %
0.360	9.9

100

	ON	Wt.	Avg.
A	8	3.283	0.410
B	8	3.322	0.415
C	8	3.440	0.430
D	8	3.479	0.435
E	8	2.945	0.368

Mean	C.V. %
0.412	6.4

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

Mean	C.V. %

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

Mean	C.V. %

Note: ON stands for original number per replicate, while SN refers to the number surviving after test completion.

BIO-AQUATIC TESTING, INC. TOXICITY TEST

Chronic **Pimephales promelas** Lab ID: **87081**

Client: Mena, City of - WWTP

Balance: Radwag BAL-007

Begin Date: 8/8/2023 End Date: 8/15/2023 Organism: Pimephales promelas

Analyst: BR

Weigh Date: 08/19/23

Date/Time placed in Oven: 08/15/23 1402

Date/Time removed from Oven: 08/16/23 1420

Control

	Qty.	Wt.
A	8	3.973
B	8	3.514
C	8	3.578
D	7	3.255
E	8	4.071

32 %

	Qty.	Wt.
A	8	3.542
B	|	3.491
C	|	3.434
D	|	3.656
E	7	2.954

45 %

	Qty.	Wt.
A	7	3.277
B	8	3.664
C	8	3.636
D	8	3.790
E	7	3.014

56 %

	Qty.	Wt.
A	8	3.702
B	8	3.049
C	7	2.382
D	7	3.210
E	8	4.212

80 %

	Qty.	Wt.
A	7	2.785
B	8	3.700
C	|	3.218
D	|	4.147
E	7	2.562

100 %

	Qty.	Wt.
A	8	3.283
B	|	3.322
C	|	3.440
D	|	3.479
E	|	2.945

	Qty.	Wt.
A		
B		
C		
D		
E		

	Qty.	Wt.
A		
B		
C		
D		
E		

	Qty.	Wt.
A		
B		
C		
D		
E		

Lined through spaces preceded by a number represent the same number. Lined spaces without a preceding number indicate unused or not applicable spaces.

APPENDIX A

STATISTICS SUMMARY

Both the lethal and sub-lethal endpoints were statistically calculated according to their respective EPA guidelines. The Chronic Freshwater organisms were calculated according to EPA-821-R-02-013, October 2002 Fourth Edition. The Chronic Marine and Estuarine organisms were calculated according to EPA-821-R-02-014, October 2002 Third Edition. The Acute Freshwater and Marine organisms were calculated according to EPA-821-R-02-012, October 2002 Fifth Edition. The fertilization organisms were calculated according to EPA-600-R-95-136 or EPA-600-R-12-022, dependent upon the species. Listed below are the basic principles of these guidelines. If you would like a copy of the raw statistical calculations for your test then please contact us.

The chronic and acute *Pimephales promelas* and *Menidia beryllina* survival data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts (parametric). If the data fails Shapiro Wilks Test or Bartlett's Test then Steels Many One Test (non-parametric) is used. The chronic *Pimephales promelas* and *Menidia beryllina* growth data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes one of these tests then the data is run through ANOVA and Dunnetts. If the data fails Shipiro Wilks Test and Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The chronic *Mysidopsis bahia* survival data is analyzed using Chi-square test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Chi-square test or Bartlett's Test then Steels Many One Test is used. *Mysidopsis bahia* growth data is analyzed using Chi-square test and Bartlett's Test. If the data passes one of these tests then the data is run through ANOVA and Dunnetts. If the data fails Chi-square test and Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The acute *Mysidopsis bahia* survival data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Shipiro Wilks Test or Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The chronic *Ceriodaphnia dubia* survival data are analyzed using the Fisher's Exact Test. The chronic *Ceriodaphnia dubia* reproduction and are analyzed using the Chi-square test and Bartlett Test. If the data passes one of these tests then the data is run through ANOVA and Dunnetts. If the data fails Chi-square test and Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The acute *Daphnia pulex* and *Ceriodaphnia dubia* survival data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Shapiro Wilks Test or Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The fertilization data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Shapiro Wilks Test or Bartlett's Test then Steels Many One Test is used. Point estimation or TST methodology may also be used.

cerio reproduction
File: 87081.cdr Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.020	14.520	22.920	14.520	4.020
OBSERVED	3	18	16	20	3

Calculated Chi-Square goodness of fit test statistic = 5.5092
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

cerio reproduction
File: 87081.cdr Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 15.25

Table Chi-square value = 15.09 (alpha = 0.01, df = 5)
Table Chi-square value = 11.07 (alpha = 0.05, df = 5)

Data FAIL B1 homogeneity test at 0.01 level. Try another transformation.

cerio reproduction
File: 87081.cdr Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	419.333	83.867	2.321
Within (Error)	54	1951.000	36.130	
Total	59	2370.333		

Critical F value = 2.45 (0.05,5,40)
Since F < Critical F FAIL TO REJECT Ho: All equal

cerio reproduction
File: 87081.cdr Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	con	16.400	16.400		
2	32	21.900	21.900	-2.046	
3	45	20.300	20.300	-1.451	
4	56	20.800	20.800	-1.637	
5	80	25.200	25.200	-3.274	
6	100	22.400	22.400	-2.232	

Dunnett table value = 2.31 (1 Tailed Value, P=0.05, df=40,5)

cerio reproduction
File: 87081.cdr Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	con	10			
2	32	10	6.210	37.9	-5.500
3	45	10	6.210	37.9	-3.900
4	56	10	6.210	37.9	-4.400
5	80	10	6.210	37.9	-8.800
6	100	10	6.210	37.9	-6.000

cerio reproduction
File: 87081.cdr Transform: NO TRANSFORMATION

STEEL'S MANY-ONE RANK TEST - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG

1	con	16.400			
2	32	21.900	130.00	75.00	10.00
3	45	20.300	122.00	75.00	10.00
4	56	20.800	114.50	75.00	10.00
5	80	25.200	138.00	75.00	10.00
6	100	22.400	137.00	75.00	10.00

Critical values use k = 5, are 1 tailed, and alpha = 0.05

fathead survival
File: 87081.pps Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 5.200

W = 0.794

Critical W (P = 0.05) (n = 30) = 0.927
Critical W (P = 0.01) (n = 30) = 0.900

Data FAIL normality test. Try another transformation.

Warning - The first three homogeneity tests are sensitive to non-normal data and should not be performed.

fathead survival
File: 87081.pps Transform: NO TRANSFORMATION

Hartley's test for homogeneity of variance
Bartlett's test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

fathead survival
File: 87081.pps Transform: NO TRANSFORMATION

STEEL'S MANY-ONE RANK TEST - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	con	7.800				
2	32	7.800	27.50	16.00	5.00	
3	45	7.600	25.00	16.00	5.00	
4	56	7.600	25.00	16.00	5.00	
5	80	7.600	25.00	16.00	5.00	
6	100	8.000	30.00	16.00	5.00	

Critical values use k = 5, are 1 tailed, and alpha = 0.05

fathead growth
File: 87081.ppg Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.077

W = 0.980

Critical W (P = 0.05) (n = 30) = 0.927
Critical W (P = 0.01) (n = 30) = 0.900

Data PASS normality test at P=0.01 level. Continue analysis.

fathead growth
File: 87081.ppg Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 8.30

Table Chi-square value = 15.09 (alpha = 0.01, df = 5)
Table Chi-square value = 11.07 (alpha = 0.05, df = 5)

Data PASS B1 homogeneity test at 0.01 level. Continue analysis.

fathead growth
File: 87081.ppg Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.009	0.002	0.565
Within (Error)	24	0.077	0.003	
Total	29	0.087		

Critical F value = 2.62 (0.05,5,24)
Since F < Critical F FAIL TO REJECT Ho: All equal

fathead growth
File: 87081.ppg Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	con	0.460	0.460		
2	32	0.427	0.427	0.919	
3	45	0.435	0.435	0.696	
4	56	0.414	0.414	1.275	
5	80	0.410	0.410	1.381	
6	100	0.412	0.412	1.342	

Dunnett table value = 2.36 (1 Tailed Value, P=0.05, df=24,5)

fathead growth
File: 87081.ppg Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	con	5			
2	32	5	0.085	18.4	0.033

3	45	5	0.085	18.4	0.025
4	56	5	0.085	18.4	0.046
5	80	5	0.085	18.4	0.050
6	100	5	0.085	18.4	0.048

Bio-Aquatic Testing, Inc.

FRESH WATER TEST SETUP FORM

Client: Mena, City of

Permit AR0036692

Facility: WWTP

Lab Number 87081

Outfall Name: 001

Number of samples 3

Dilution Water: Synthetic Lab

Receiving Water Name: Prairie Creek

Dechlorinate Sample: No

Sx #	Rcvd Date	Rcvd Time	Sampling Dates		Sampling Times	
			Begin Date	End Date	Start	End
1	08/08/23	10:00	08/06/23	08/07/23	08:00	08:00
2	08/10/23	08:30	08/08/23	08/09/23	08:30	08:30
3	08/12/23	08:20	08/10/23	08/11/23	08:30	08:30

Type of Test(s)

Ceriodaphnia dubia	Chronic
Pimephales promelas	Chronic

Dilution Water

Sample #	Hardness As mg/L $CaCO_3$	Alkalinity as mg/L $CaCO_3$
1	156	54
2	142	60
3	142	60

Start Sx #	1	Date:	8/8/2023
Renew Sx #	1	Date:	8/9/2023
Renew Sx #	2/1	Date:	8/10/2023
Renew Sx #	2	Date:	8/11/2023
Renew Sx #	3/2	Date:	8/12/2023
Renew Sx #	3	Date:	8/13/2023
Renew Sx #	3	Date:	8/14/2023

Test Start Date: 8/8/2023

Test End Date: 8/15/2023

Ceriodaphnia dubia Test Set Up: 10 Reps & 1 Organisms per Rep

Pimephales Test Set Up: 5 Reps & 8 Organism per Rep

Concentrations: 32 45 56 80 100 %

Test Chemistry on these dilutions: 32 45 56 80 100

Samples received by: ○ Express Delivery ○ UPS Next Day ○ via Air Cargo ○ DHL ○ Federal Express ○ the Client ◉ Bio-Aquatic personnel

Other:

BIO-AQUATIC TESTING, INC.

Hardness, Alkalinity, Residual Chlorine, Specific Conductivity, and Salinity Analysis Data

Client: Mena, City of **Lab ID:** 87081

Facility: WWTP **Outfall:** 001

Dilution Water(s): Synthetic Lab **Test Date:** August 8, 2023

EFFLUENT PARAMETERS

Effluent Sample #	Received		Residual Cl_2 (mg/L)	DeChlor (ml/L)[1]	Ammonia (mg/L)	Analyst Initials	Temp. Received
	Date	Time					
1	8/8/23	10:00	<0.10	N/A	<0.25	JR	5.6
2	8/10/23	08:30	<0.10	N/A	<0.25	JP	3.4
3	8/12/23	08:20	<0.10	N/A	<0.25	JP	3.3

[1]**Dechlorination Reagent:** 0.025 N Sodium Thiosulfate

Effluent Sample #	pH	DO (mg/L)	Hardness (mg/L $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)	Conductivity (umhos/cm)	Analyst Initials
1	7.5	8.3	24	26	86	JR
2	6.3	6.7	35	1	52	JP
3	7.4	8.3	30	27	73	JP

DAILY RENEWAL CONDUCTIVITY**

Date		Sample #	Values are at Highest Dilution		Analyst
			Specific Conductivity as umhos/cm	Salinity (ppt)	
8/8	Lab H2O		359	0.2	GS
8/9	Lab H2O		394	0.2	JC
8/10	Lab H2O		403	0.2	JC/SG
8/11	Lab H2O		415	0.2	LC/MM
8/12	Lab H2O		410	0.2	JC
8/13	Lab H2O		417	0.2	JC/SG
8/14	Lab H2O		390	0.2	GS
8/8	OUTFALL*	1	58	0.1	GS
8/9	OUTFALL*	1	72	0.1	JC
8/10	OUTFALL*	2/1	67	0.1	JC/SG
8/11	OUTFALL*	2	58	0.1	LC/MM
8/12	OUTFALL*	3/2	77	0.1	JC
8/13	OUTFALL*	3	65	0.1	JC/SG
8/14	OUTFALL*	3	60	0.1	GS

**Conductivity is taken on the highest remaining effluent concentration used for test renewal, not necessarily 100%

Analysis Methods: Chlorine: Hanna Colorimeter #HI711, Ammonia: Hanna Colorimeter #HI733, Hardness: Hanna Photometer #HI96735, Alkalinity: Hanna Colorimeter #HI775, pH, DO, Conductivity: Thermo Versa Star Benchtop Meter

BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen

Chronic **Ceriodaphnia dubia**

Client: Mena, City of

Lab ID: 87081

Facility: WWTP

Outfall: 001

Dilution Water(s): Synthetic Lab

Test Begin Date: August 8, 2023

NR indicates that the test is non-renewal.

					Concentration							
ANALYST	DATE	TIME	SX#	UNIT	Control	32	45	56	80	100		
GS	8/8	Start	1	pH	7.7	7.7	7.8	7.8	7.9	7.9		
		25 ± 1		DO (mg/L)	8.1	8.1	8.1	8.1	8.1	8.1		
JC	8/9	24 Hr	1	pH	7.9	7.8	7.8	7.6	7.6	7.6		
		25 ± 1		DO (mg/L)	8.0	8.0	8.0	8.0	8.0	7.9		
		Renew	1	pH	7.9	7.8	7.8	7.7	7.7	7.4		
				DO (mg/L)	8.2	8.1	8.1	8.2	8.2	8.5		
JC/SG	8/10	48 Hr	1	pH	8.1	8.1	8.1	8.0	8.0	8.1		
		25 ± 1		DO (mg/L)	7.8	7.8	7.8	7.7	7.7	7.7		
		Renew	2/1	pH	7.8	7.8	7.8	7.8	7.8	7.7		
				DO (mg/L)	8.4	8.4	8.4	8.4	8.4	8.4		
LC/MM	8/11	72 Hr	2/1	pH	7.1	7.1	7.1	7.1	7.2	7.3		
		25 ± 1		DO (mg/L)	8.0	8.0	8.0	8.0	8.0	8.0		
		Renew	2	pH	7.8	7.9	7.9	7.8	8.2	8.1		
				DO (mg/L)	8.2	8.1	8.2	8.1	7.9	8.0		
JC	8/12	96 Hr	2	pH	7.8	7.9	7.9	7.8	7.9	7.9		
		25 ± 1		DO (mg/L)	8.3	8.2	8.1	7.3	8.2	7.9		
		Renew	3/2	pH	8.7	8.4	8.4	8.2	8.2	8.1		
				DO (mg/L)	8.3	8.2	8.2	8.3	8.3	8.3		
JC/SG	8/13	120 Hr	3/2	pH	8.5	8.4	8.4	8.4	8.4	8.5		
		25 ± 1		DO (mg/L)	7.7	7.7	7.7	7.8	7.8	7.8		
		Renew	3	pH	7.8	7.8	7.8	7.8	7.8	7.8		
				DO (mg/L)	8.4	8.3	8.3	8.3	8.3	8.4		
SG	8/14	144 Hr	3	pH	7.6	7.6	7.6	7.6	7.6	7.6		
		25 ± 1		DO (mg/L)	7.2	7.3	7.3	7.5	7.5	7.6		
		Renew	3	pH	7.7	7.8	7.8	7.7	7.7	7.6		
				DO (mg/L)	7.6	7.7	7.7	8.0	8.0	8.1		
GS	8/15	168 Hr	3	pH	7.7	7.7	7.7	7.7	7.7	7.7		
		25 ± 1		DO (mg/L)	8.1	8.0	8.0	7.9	7.9	7.8		

BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen

Chronic **Pimephales promelas**

Client: Mena, City of **Lab Number:** 87081

Facility: WWTP **Dilution Water(s):** Synthetic Lab

Outfall: 001 **Test Begin Date:** August 8, 2023

NR indicates that the test is non-renewal.

					Concentration							
ANALYST	DATE	TIME	SX#	UNIT	Control	32	45	56	80	100		
GS	8/8	Start	1	pH	7.7	7.7	7.8	7.8	7.9	7.9		
		25 ± 1		DO (mg/L)	8.1	8.1	8.1	8.1	8.1	8.1		
JC	8/9	24 Hr	1	pH	7.6	7.7	7.7	7.7	7.7	7.7		
		25 ± 1		DO (mg/L)	8.6	8.1	8.1	8.0	8.0	7.8		
		Renew	1	pH	7.9	7.8	7.8	7.7	7.7	7.4		
				DO (mg/L)	8.2	8.1	8.1	8.2	8.2	8.5		
JC/SG	8/10	48 Hr	1	pH	7.6	7.6	7.6	7.6	7.6	7.5		
		25 ± 1		DO (mg/L)	8.4	8.0	8.0	7.5	7.5	7.4		
		Renew	2/1	pH	7.8	7.8	7.8	7.8	7.8	7.7		
				DO (mg/L)	8.4	8.4	8.4	8.4	8.4	8.4		
LC/MM	8/11	72 Hr	2/1	pH	7.9	8.0	7.9	8.0	8.1	8.2		
		25 ± 1		DO (mg/L)	8.2	8.3	8.1	7.9	8.2	8.5		
		Renew	2	pH	7.8	7.9	7.9	7.8	8.2	8.1		
				DO (mg/L)	8.2	8.1	8.2	8.1	7.9	8.0		
JC	8/12	96 Hr	2	pH	7.7	7.7	7.7	7.7	7.7	7.7		
		25 ± 1		DO (mg/L)	8.3	8.0	8.0	7.8	7.8	7.5		
		Renew	3/2	pH	8.7	8.4	8.4	8.2	8.2	8.1		
				DO (mg/L)	8.3	8.2	8.2	8.3	8.3	8.3		
JC/SG	8/13	120 Hr	3/2	pH	7.4	7.5	7.5	7.5	7.5	7.6		
		25 ± 1		DO (mg/L)	8.6	8.0	8.0	7.7	7.7	7.4		
		Renew	3	pH	7.8	7.8	7.8	7.8	7.8	7.8		
				DO (mg/L)	8.4	8.3	8.3	8.3	8.3	8.4		
SG	8/14	144 Hr	3	pH	7.4	7.4	7.4	7.4	7.4	7.5		
		25 ± 1		DO (mg/L)	7.3	7.1	7.1	7.0	7.0	7.0		
		Renew	3	pH	7.7	7.8	7.8	7.7	7.7	7.6		
				DO (mg/L)	7.6	7.7	7.7	8.0	8.0	8.1		
GS	8/15	168 Hr	3	pH	7.7	7.7	7.6	7.6	7.6	7.6		
		25 ± 1		DO (mg/L)	8.2	8.0	7.9	7.9	7.5	7.5		

Appendix B

Ceriodaphnia dubia

BIO-AQUATIC TESTING, INC.

Carrollton, TX

REFERENCE TOXICANTS

Bio-Aquatic Testing conducts reference toxicant testing monthly for organisms cultured in-house. For studies requiring purchased organisms, reference toxicant testing is performed simultaneously. Reference toxicant testing validates data and measures organism consistency. Only reagent grade chemicals are used of the following choices: sodium laurel sulfate (SLS), copper sulfate, copper chloride, potassium chloride, and sodium chloride. Organism responses are tracked with control charts for each reference toxicant/organism combination. The data are examined for sensitivity trends and to determine if results are within EPA described limits.

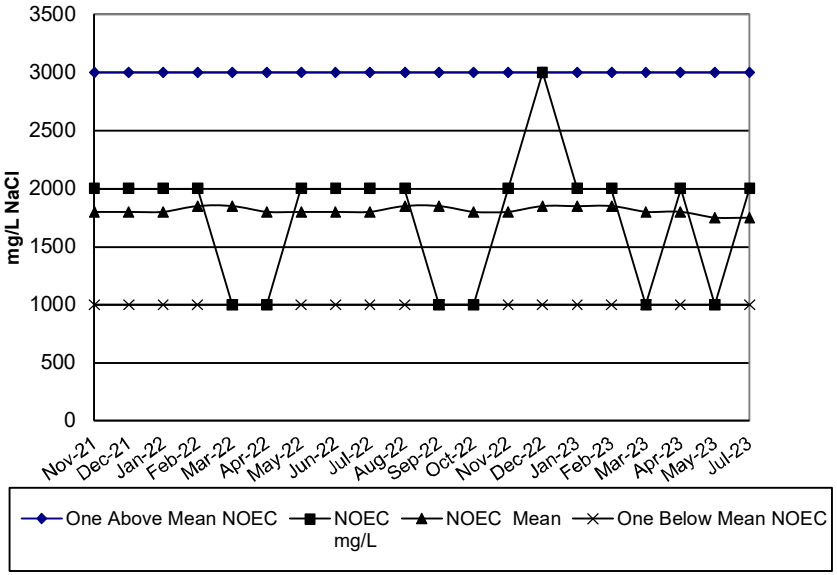
CHRONIC REFERENCE TOXICANT TEST RESULTS

DILUTION WATER:	Standard Synthetic Freshwater						
CHEMICAL:	Sodium Chloride						
DURATION:	3-Brood Chronic						
TEST NUMBER:	340						
PROJECT NUMBER:	87928						
START DATE:	7/26/2023						
START TIME:	15:52						
TOTAL NUMBER EXPOSED:	10 organisms per concentration						
CONCENTRATIONS (mg/L):	CON	250	500	1000	2000	3000	4000
NUMBER DEAD PER CONCENTRATION:	0	0	0	0	2	10	10
TEST METHODS:	As listed in EPA-821-R-02-013						
STATISTICAL METHODS:	SURVIVAL: Fisher's Exact Test REPRODUCTION: Wilcoxon's Rank-Sum Test						

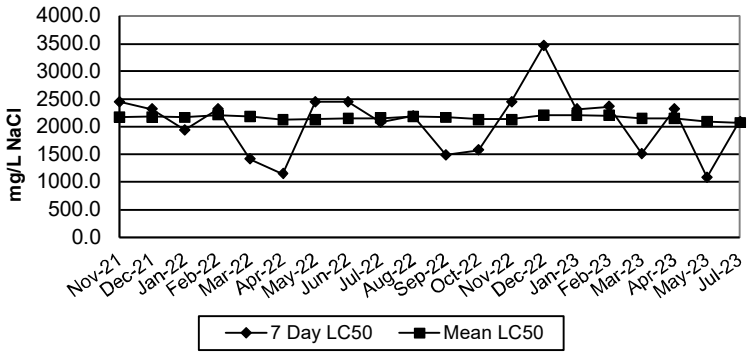
NOEC FOR SURVIVAL:	2000	mg/L
LOEC FOR SURVIVAL:	3000	mg/L
NOEC FOR REPRODUCTION:	500	mg/L
LOEC FOR REPRODUCTION:	1000	mg/L

PMSD: 24.4

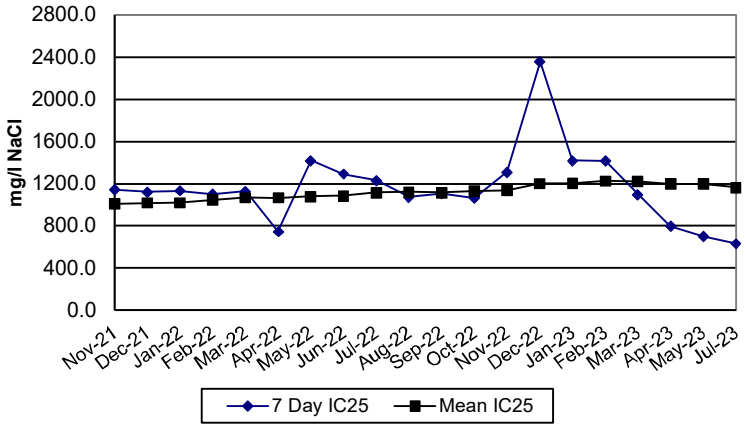
Ceriodaphnia Chronic Survival Control Chart



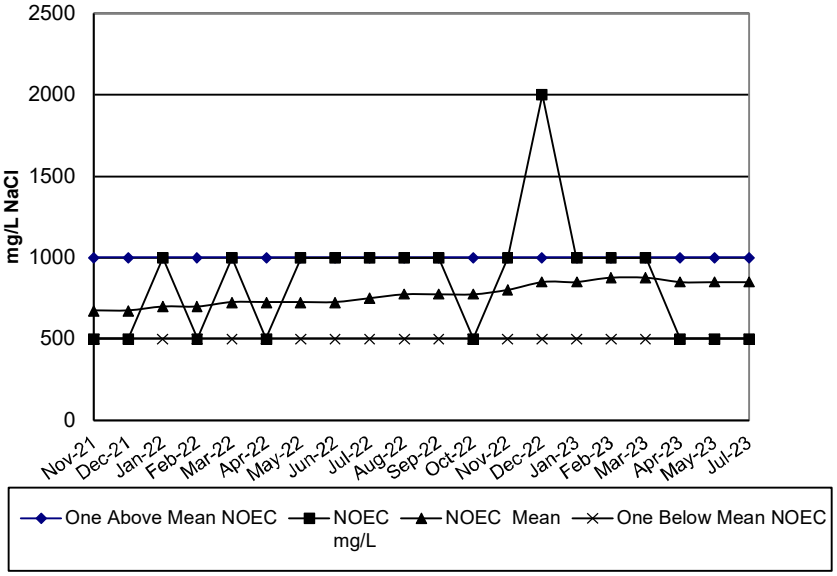
Ceriodaphnia 7-Day LC50



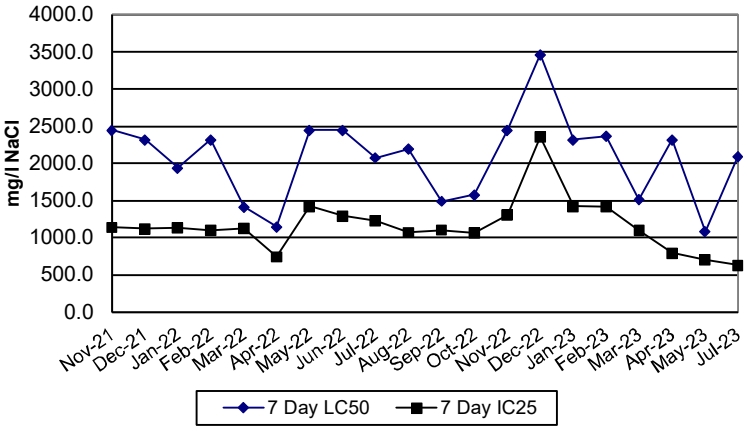
Ceriodaphnia 7-Day IC25



Ceriodaphnia Chronic Reproduction Control Chart



Ceriodaphnia 7-Day LC50 & IC25



Appendix B

Pimephales promelas

BIO-AQUATIC TESTING, INC.

Carrollton, TX

REFERENCE TOXICANTS

Bio-Aquatic Testing conducts reference toxicant testing monthly for organisms cultured in-house. For studies requiring purchased organisms, reference toxicant testing is performed simultaneously. Reference toxicant testing validates data and measures organism consistency. Only reagent grade chemicals are used of the following choices: sodium laurel sulfate (SLS), copper sulfate, copper chloride, potassium chloride, and sodium chloride. Organism responses are tracked with control charts for each reference toxicant/organism combination. The data are examined for sensitivity trends and to determine if results are within EPA described limits.

CHRONIC REFERENCE TOXICANT TEST RESULTS

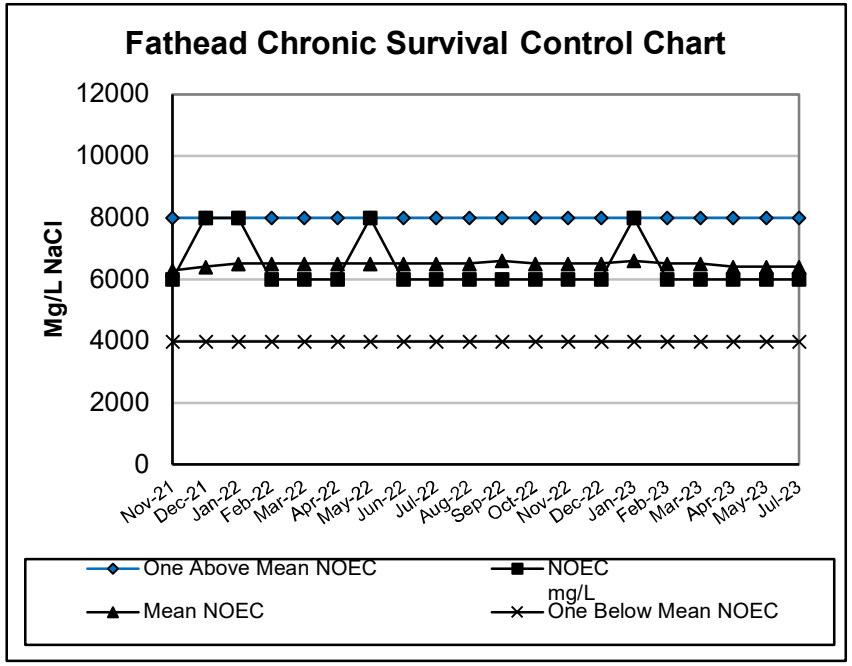
DILUTION WATER:	Standard Synthetic Freshwater
CHEMICAL:	Sodium Chloride
DURATION:	7 Days
TEST NUMBER:	380
PROJECT NUMBER:	87926
START DATE:	7/26/2023
START TIME:	11:30
TOTAL NUMBER EXPOSED:	40 organisms per concentration

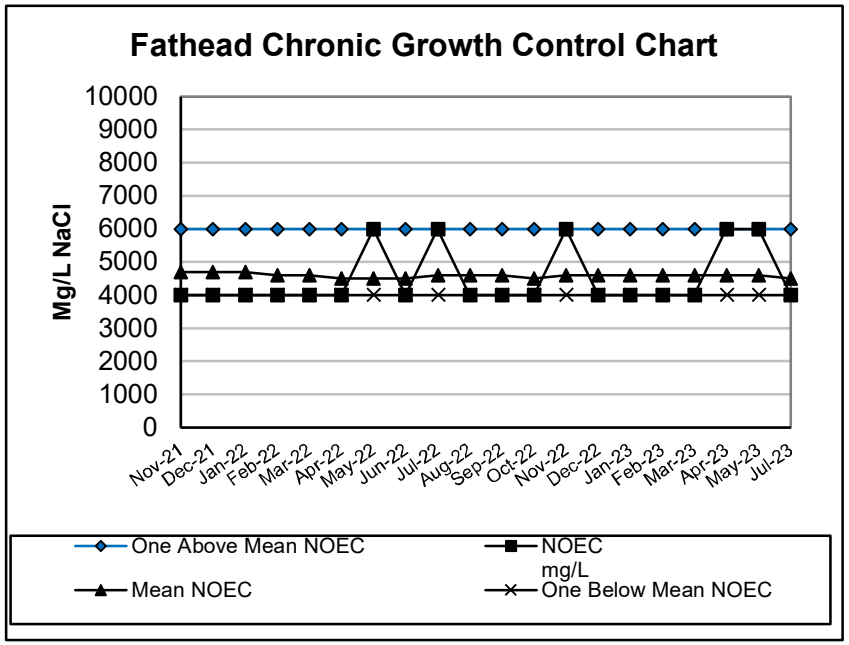
CONCENTRATIONS (mg/L):	CON	2000	4000	6000	8000	10000	12000
NUMBER DEAD PER CONCENTRATION:	5	4	7	14	34	40	40

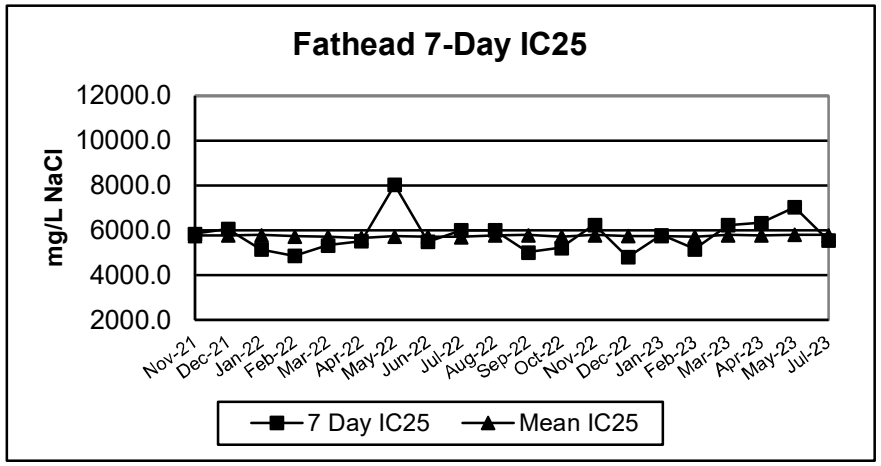
TEST METHODS:	As listed in EPA-821-R-02-013
STATISTICAL METHODS:	SURVIVAL: Steel's Many-One Rank Test GROWTH: ANOVA-Dunnetts

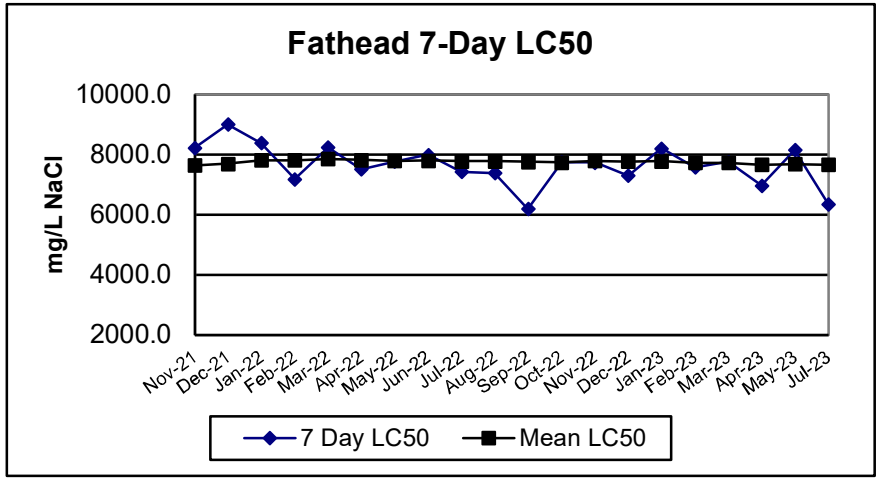
NOEC FOR SURVIVAL:	6000	mg/L
LOEC FOR SURVIVAL:	8000	mg/L
NOEC FOR GROWTH:	4000	mg/L
LOEC FOR GROWTH:	6000	mg/L

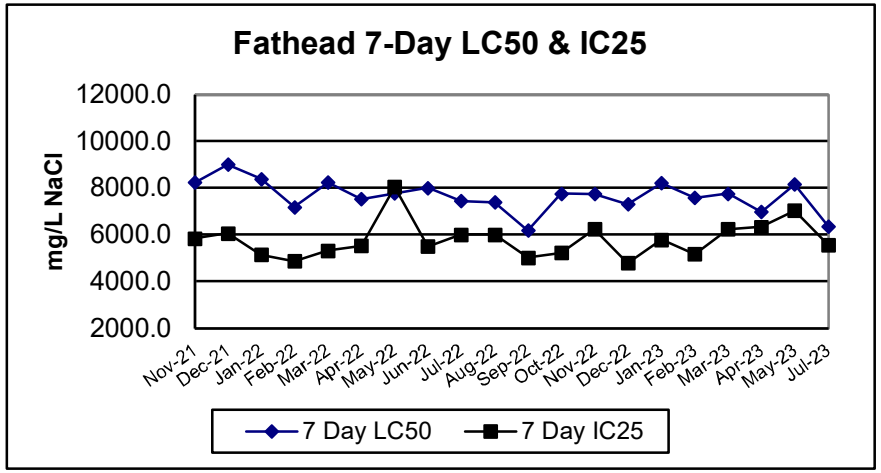
PMSD: 15.1


Fathead Chronic Survival Control Chart
Mg/L NaCl
12000
10000
8000
6000
4000
2000
0
Nov-21 Dec-21 Jan-22 Feb-22 Mar-22 Apr-22 May-22 Jun-22 Jul-22 Aug-22 Sep-22 Oct-22 Nov-22 Dec-22 Jan-23 Feb-23 Mar-23 Apr-23 May-23 Jul-23
One Above Mean NOEC
NOEC
Mean NOEC
One Below Mean NOEC
mg/L


Fathead Chronic Growth Control Chart
Mg/L NaCl
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Nov-21 Dec-21 Jan-22 Feb-22 Mar-22 Apr-22 May-22 Jun-22 Jul-22 Aug-22 Sep-22 Oct-22 Nov-22 Dec-22 Jan-23 Feb-23 Mar-23 Apr-23 May-23 Jul-23
One Above Mean NOEC
NOEC
Mean NOEC
One Below Mean NOEC
mg/L


Fathead 7-Day IC25
mg/L NaCl
12000.0
10000.0
8000.0
6000.0
4000.0
2000.0
Nov-21 Dec-21 Jan-22 Feb-22 Mar-22 Apr-22 May-22 Jun-22 Jul-22 Aug-22 Sep-22 Oct-22 Nov-22 Dec-22 Jan-23 Feb-23 Mar-23 Apr-23 May-23 Jul-23
7 Day IC25
Mean IC25


Fathead 7-Day LC50
mg/L NaCl
10000.0
8000.0
6000.0
4000.0
2000.0
Nov-21 Dec-21 Jan-22 Feb-22 Mar-22 Apr-22 May-22 Jun-22 Jul-22 Aug-22 Sep-22 Oct-22 Nov-22 Dec-22 Jan-23 Feb-23 Mar-23 Apr-23 May-23 Jul-23
7 Day LC50
Mean LC50


Fathead 7-Day LC50 & IC25
mg/L NaCl
12000.0
10000.0
8000.0
6000.0
4000.0
2000.0
Nov-21 Dec-21 Jan-22 Feb-22 Mar-22 Apr-22 May-22 Jun-22 Jul-22 Aug-22 Sep-22 Oct-22 Nov-22 Dec-22 Jan-23 Feb-23 Mar-23 Apr-23 May-23 Jul-23
7 Day LC50
7 Day IC25

APPENDIX C

LITERATURE REFERENCES

U.S.E.P.A., 2002. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To Freshwater Organisms (Fifth Edition) U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-821-R-02-012.

U.S.E.P.A., 2002. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents and Receiving Water To Marine And Estuarine Organisms (Third Edition) U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-821-R-02-014.

U.S.E.P.A., 2002. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To Freshwater Organisms (Fourth Edition) U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-821-R-02-013.

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U.S.E.P.A., 1995. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To West Coast Marine and Estuarine Organisms (First Edition) U.S. Environmental Protection Agency, EPA-600-R-95-136.

U.S.E.P.A., 2010. National Pollutant Discharge Elimination System Test of Significant Toxicity Technical Document, U.S. Environmental Protection Agency, Office of Wastewater, Washington D.C., EPA-833-R-10-004.

U.S.E.P.A., 1991. Technical Support Document For Water Quality-Based Toxics Control, U.S. Environmental Protection Agency, EPA-505-2-90-001.

Zarr, Jerrold, H., 1984. Biostatistical Analysis, (Second Edition). Prentice-Hall, Inc., Englewood Cliffs, N.J.

CHAIN-OF-CUSTODY SHEETS

Appendix D

HUTHER & ASSOCIATES
2501 MAYES RD., STE. 100
CARROLLTON, TX 75006
PH: 972-242-7750 FAX: 972-242-7749

CHAIN OF CUSTODY

☐ Huther Only: No Sample Left

Lab Id : 87081

Please Review & Complete Sections A, B, C, & D.

Sample No: 87081 – ____

Revision 2 Effective Date 9/25/2017

Check Sample No. : ____ First, ____ Second, or ____ Third.

P.O. No:

Client: Mena, City of
Facility: WWTP
Permit No: AR0036692
Outfall: 001
Client Contact: MIKE SPENCER
Client Phone: 479-234-2592

A. REVIEW SCHEDULED TEST(s):

Chronic	Ceriodaphnia dubia
Chronic	Pimephales promelas

To Ship the 1st Sample on: 8/7/2023

Concentration: 32 45 56 80 100

(For TX) Setup separate 24hr Acute Test? No

B. Use area below to make changes, if the Scheduled Test(s) in "A" are incorrect:

Freshwater Species					Saltwater Species	
C. dubia (water flea)	*D. pulex* (water flea)	*D. magna* (water flea)	*P. promelas* (minnow)	*Selenastrum* (green algae)	*M. beryllina* (minnow)	*Mysidopsis* (shrimp)
☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour

Notes: 3rd Qtr -CH

C.

Sample ID or Location: (Outfall No. or Name)	Sample Type: E = Effluent RS = Rec. Stream S = Sediment	Sample Date From	Sample Date To	Sample Time (military) From	Sample Time (military) To	Grab or Composite	Sampled By: (Sign and Print Name)	Number Of Containers Shipped
1 Outfall 1	Eff	6 Aug 23 ~~0800~~	7 Aug 23	0800	0800	Comp	[signature] M D Spencer	2
2								
3								

D.

	Relinquished By:	Date	Time	Received By:	Date	Time
1	[signature]	7 Aug 0930	0930	Lance BBow WWTP	7 Aug 23	0900
2	[signature]	8/7/23	1700			
3				[signature]	8-8-23	1000

Huther Sample Login

HA sample personnel: ○ Yes ● No
Dechlorinate Sample: ○ Yes ◉ No
Dilution Water: ○ Receiving Stream ◉ Synthetic Lab

Date: 8/8/23	Time: 1404	By: LTS	Temperature: 5-6 (C) IR#: 002
Chlorine: <0.1 mg/l	Ammonia: <0.25 mg/l	Int. Sal\Cond: 86. ppt/uS	Adj. Salinity ppt
pH: 7.5	Hardness: 24 mg/l (LR)	Other	
DO: 8.7 mg/l	Alkalinity: 26 mg/l (OK)	Condition: GOOD	

Report Date: 09/06/2023 Revision 0

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Bio-Aquatic Lab ID: 87081

HUTHER & ASSOCIATES

2501 MAYES RD., STE. 100
CARROLLTON, TX 75006
PH: 972-242-7750 FAX: 972-242-7749

CHAIN OF CUSTODY

☐ Huther Only: No Sample Left

Lab Id : 87081

Please Review & Complete Sections A, B, C, & D.

Sample No: 87081 – ____

Revision 2 Effective Date 9/26/2017

Check Sample No. : ____ First, ____ Second, or ____ Third.

P.O. No:

Client: Mena, City of

Facility: WWTP

Permit No: AR0036692

Outfall: 001

Client Contact: MIKE SPENCER

Client Phone: 479-234-2592

A. REVIEW SCHEDULED TEST(s):

Chronic	Ceriodaphnia dubia
Chronic	Pimephales promelas

Concentration: 32 45 56 80 100

To Ship the 1st Sample on: 8/7/2023

(For TX) Setup separate 24hr Acute Test? No

B. Use area below to make changes, if the Scheduled Test(s) in "A" are incorrect:

Freshwater Species					Saltwater Species	
C. dubia (water flea)	*D. pulex (water flea)*	*D. magna (water flea)*	*P. promelas (minnow)*	*Selenastrum (green algae)*	*M. beryllina (minnow)*	*Mysidopsis (shrimp)*
☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour

Notes: 3rd Qtr -CH

C.

	Sample ID or Location: (Outfall No. or Name)	Sample Type: E = Effluent RS = Rec. Stream S = Sediment	Sample Date From	Sample Date To	Sample Time (military) From	Sample Time (military) To	Grab or Composite	Sampled By: (Sign and Print Name)	Number Of Containers Shipped
1	Outfall001	E	9Aug	9Aug23	0830	0830	Comp	Mike Spencer	2
2									
3									

D.

	Relinquished By:	Date	Time	Received By:	Date	Time
1	Lindsey Hopper	9Aug23	1015	Rance Elbow	9Aug23	1015
2	Rance Parrott	8/9/23	1600	Tony Payne	8.10.23	0830
3						

Huther Sample Login

HA sample personnel: ● Yes ○ No

Dechlorinate Sample: ○ Yes ◉ No

Dilution Water: ○ Receiving Stream ◉ Synthetic Lab

Date: 8.10.23	Time: 1000	By: dp	Temperature: 3.4 (C)	IR#: 002			
Chlorine: <0.1 mg/l	Ammonia: <0.25 mg/l	Int. Sal\Cond: 52 ppt/uS	Adj. Salinity ppt				
pH: 6.3	Hardness: 35 mg/l (LR)	Other					
DO: 6.7 mg/l	Alkalinity: 1 mg/l (OK)	Condition: good					

HUTHER & ASSOCIATES
2501 MAYES RD., STE. 100
CARROLLTON, TX 75006
PH: 972-242-7750 FAX: 972-242-7749

CHAIN OF CUSTODY

☐ Huther Only: No Sample Left

Lab Id : 87081

Please Review & Complete Sections A, B, C, & D.

Sample No: 87081 – ____

Revision 2 Effective Date 9/25/2017

Check Sample No. : ____ First, ____ Second, or ____ Third.

P.O. No:

Report Date: 09/08/2023 Revision 0

Client: Mena, City of

Facility: WWTP

Permit No: AR0036692

Outfall: 001

Client Contact: MIKE SPENCER

Client Phone: 479-234-2592

A. REVIEW SCHEDULED TEST(s):

Chronic	Ceriodaphnia dubia
Chronic	Pimephales promelas

To Ship the 1st Sample on: 8/7/2023

Concentration: 32 45 56 80 100

(For TX) Setup separate 24hr Acute Test? No

B. Use area below to make changes, if the Scheduled Test(s) in "A" are incorrect:

Freshwater Species					Saltwater Species	
C. dubia (water flea)	*D. pulex (water flea)*	*D. magna (water flea)*	*P. promelas (minnow)*	*Selenastrum (green algae)*	*M. beryllina (minnow)*	*Mysidopsis (shrimp)*
☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour

Notes: 3rd Qtr -CH

C.

	Sample ID or Location: (Outfall No. or Name)	Sample Type: E = Effluent RS = Rec. Stream S = Sediment	Sample Date From	Sample Date To	Sample Time (military) From	Sample Time (military) To	Grab or Composite	Sampled By: (Sign and Print Name)	Number Of Containers Shipped
1	OUTFALL 1	E	10Aug23	11Aug23	0830	0830	Comp	MK Spencer	2
2									
3									

Bio-Aquatic Lab ID: 87081

D.

Relinquished By:	Date	Time	Received By:	Date	Time
Linden Hopper	11Aug23	1000	Rance / Bio-Aq OK	11Aug23	1050
Rance Parrott	8/11/23	1530	Jay Payne	8.12.23	0820

Huther Sample Login

HA sample personnel: ● Yes ○ No

Dechlorinate Sample: ○ Yes ◉ No

Dilution Water: ○ Receiving Stream ◉ Synthetic Lab

Date: 8-12-23 Time: 1158 By: JP Temperature: 3.3 (C) IR#: 002

Chlorine: <0.1 mg/l	Ammonia: <0.25 mg/l	Int. Sal\Cond: 73 ppt/uS	Adj. Salinity ppt		
pH: 7.4	Hardness: 30 mg/l (LR)	Other			
DO: 8.3 mg/l	Alkalinity: 27 mg/l (OK)	Condition: good			

REGULATORY AGENCY TABLES

Appendix E

Table 1 (Sheet 1 of 4)

BIOMONITORING REPORT

Ceriodaphnia dubia SURVIVAL AND REPRODUCTION TEST

Permittee: Mena, City of - WWTP

Permit No.: AR0036692

Outfall No.: 001

		Date/Time		Date/Time
Dates and times Composites were collected:	FROM:	8/6/2023 @ 08:00	TO:	8/7/2023@ 08:00
	FROM:	8/8/2023 @ 08:30	TO:	8/9/2023@ 08:30
	FROM:	8/10/2023 @ 08:30	TO:	8/11/2023@ 08:30

Test Initiation: Time: 16:08 Date: 8/8/2023

Dilution Water Used: [ ] Receiving Water [X] Synthetic Dilution Water

NUMBER OF YOUNG PRODUCED PER ADULT AT TEST TERMINATION

	EFFLUENT CONCENTRATION (%)					
REPLICATE	0%	32 %	45 %	56 %	80 %	100 %
A	20	26	23	25	35	20
B	D- 10	25	21	22	21	23
C	19	20	19	30	24	29
D	20	20	18	17	32	20
E	22	22	16	14	29	D- 0
F	13	27	22	17	D- 16	26
G	17	19	23	18	25	28
H	D- 0	17	21	17	18	27
I	19	18	18	17	22	29
J	24	25	22	31	30	22
Surv. MEAN	19.2	21.9	20.3	20.8	26.2	24.8
Total MEAN	16.4	21.9	20.3	20.8	25.2	22.4
CV % [1]	17.1	16.4	11.8	28.6	21.4	14.8
PMSD	Acceptable Range 47 or Less					37.9 %

[1] Coefficient of Variation = (standard deviation/mean) x 100) Calculations are based on young of the surviving females. Males are designated (M), and dead females are designated (D) along with the number of neonates released prior to death.

Table 1 (Sheet 2 of 4)

BIOMONITORING REPORT

Ceriodaphnia dubia SURVIVAL AND REPRODUCTION TEST

Permittee: Mena, City of - WWTP

Permit No.: AR0036692

Outfall No.: 001

PERCENT SURVIVAL

	EFFLUENT CONCENTRATION (%)					
TIme of Reading	0%	32 %	45 %	56 %	80 %	100 %
24 HOURS	100	100	100	100	100	100
48 HOURS	100	100	100	100	100	100
7-DAY	80	100	100	100	90	90

1. DUNNETT'S PROCEDURE OR STEEL'S MANY-ONE RANK TEST(with Bonferroni adjustment as appropriate for Sub-Lethality)

Is the mean number of young produced per adult significantly different (p=0.05) than the number of young per adult in the control for the low flow or critical dilution?

CRITICAL DILUTION (100 %) : _____YES _____X_____NO

*If you report NO, enter a '0' on the DMR form for Parameter **TGP3B**, other wise enter a '1'. This parameter is also referred to as the 7-DAY Ceriodaphnia Sub-Lethal Pass/Fail.*

2. FISHER'S EXACT TEST (as appropriate for Lethality)

Is the mean survival at test end significantly different (p=0.05) than the control's survival for the low flow or critical dilution?

CRITICAL DILUTION (100 %) : _____YES _____X_____NO

*If you report NO, enter a '0' on the DMR form for Parameter **TLP3B**, other wise enter a '1'. This parameter is also referred to as the 7-DAY Ceriodaphnia Lethal Pass/Fail.*

3. Enter the percent effluent corresponding to each NOEC/LOEC below:

a. NOEL Survival = _____100_____ % Effluent **(Parameter TOP3B)**

b. NOEL Reproduction = _____100_____ % Effluent **(Parameter TPP3B)**

Q* refers to a value that is not calculable

4. If you are required to report Parameter No. **TQP3B**, report the percent coefficient of variation value that is the highest between the control and the critical dilution (100 %), found in the reproduction table above for Ceriodaphnia dubia (= 17.1).

5. If you are required to report Parameter No. **TJP3B**, report the percent mortality in the critical dilution at the completion of the test for the Ceriodaphnia dubia (= 10).

Table 1 (Sheet 3 of 4)

BIOMONITORING REPORT

Pimephales promelas SURVIVAL AND GROWTH TEST

Permittee: Mena, City of - WWTP

Permit No.: AR0036692

Outfall No.: 001

		Date/Time		Date/Time
Dates and times Composites were collected:	FROM:	8/6/2023 @08:00	TO:	8/7/2023@ 08:00
	FROM:	8/8/2023 @08:30	TO:	8/9/2023@ 08:30
	FROM:	8/10/2023 @08:30	TO:	8/11/2023@ 08:30

Test Initiation: Time: 16:39 Date: 8/8/2023

Dilution Water Used: [ ] Receiving Water [X] Synthetic Dilution Water

DATA TABLE FOR GROWTH OF *Pimephales promelas*

Effluent Concentration	Average Dry Weight in milligrams (mg) per replicate					Mean Dry Weight (mg)	CV % [1]
	A	B	C	D	E		
0%	0.497	0.439	0.447	0.407	0.509	0.460	9.2
32 %	0.443	0.436	0.429	0.457	0.369	0.427	7.9
45 %	0.410	0.458	0.455	0.474	0.377	0.435	9.2
56 %	0.463	0.381	0.298	0.401	0.527	0.414	20.9
80 %	0.348	0.463	0.402	0.518	0.320	0.410	19.8
100 %	0.410	0.415	0.430	0.435	0.368	0.412	6.4
PMSD	Acceptable Range 30 or Less					18.4 %	

DATA TABLE FOR SURVIVAL OF *Pimephales promelas*

Effluent Concentration	Percent Survival per replicate					Average % Survival			CV % [1]
	A	B	C	D	E	24 Hours	48 Hours	7-Day	
0%	100	100	100	87.5	100	100	100	97.5	5.7
32 %	100	100	100	100	87.5	100	100	97.5	5.7
45 %	87.5	100	100	100	87.5	100	97.5	95	7.2
56 %	100	100	87.5	87.5	100	100	100	95	7.2
80 %	87.5	100	100	100	87.5	100	97.5	95	7.2
100 %	100	100	100	100	100	100	100	100	0.0

[1] Coefficient of Variation = (standard deviation/mean) x 100)

?= cannot be calculated due to 100% mortality or lab exception

Table 1 (Sheet 4 of 4)

BIOMONITORING REPORT

Pimephales promelas SURVIVAL AND GROWTH TEST

Permittee: Mena, City of - WWTP

Permit No.: AR0036692

Outfall No.: 001

1. DUNNETT'S PROCEDURE OR STEEL'S MANY-ONE RANK TEST
(with Bonferroni adjustment as appropriate for Sub-Lethality)

Is the mean dry weight at 7 days significantly different (p=0.05) than the control's mean dry weight for the low flow or critical dilution?

CRITICAL DILUTION (100 %) : _____YES X NO

*If you report NO, enter a '0' on the DMR form for Parameter **TGP6C,** other wise enter a '1'. This parameter is also referred to as the 7-DAY Pimephales Sub-Lethal Pass/Fail.*

2. DUNNETT'S PROCEDURE OR STEEL'S MANY-ONE RANK TEST (as appropriate for Lethality)

Is the mean survival at 7 days significantly different (p=0.05) than the control's survival for low flow or critical dilution?

CRITICAL DILUTION (100 %) : _____YES X NO

*If you report NO, enter a '0' on the DMR form for Parameter **TLP6C**, other wise enter a '1'. This parameter is also referred to as the 7-DAY Pimephales Lethal Pass/Fail.*

3. Enter the percent effluent corresponding to each NOEC/LOEC below:

a. NOEL Survival = 100 % Effluent **(Parameter TOP6C)**

b. NOELGrowth = 100 % Effluent **(Parameter TPP6C)**

Q* refers to a value that is not calculable

4. If you are required to report Parameter No. **TQP6C**, report the percent coefficient of variation value that is the highest between the control and the critical dilution, (100 %), found in the growth table above for Pimephales promelas (= 9.2).

5. If you are required to report Parameter No. **TJP6C**, report the percent mortality in the critical dilution at the completion of the test for the Pimephales promelas (= 0).