Bio-Aquatic Testing, Inc.

TCEQ TNI Accredited

City of Mena
WWTP
OUTFALL 001

Chronic Biomonitoring Report

87082

Ceriodaphnia dubia
Pimephales promelas

December 05, 2023

Approved by: Joshua Reed
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**

BIO-AQUATIC TESTING, INC.
2501 Mayes Road, Suite 100
Carrollton, Texas 75006
Tel: (972) 242-7750
Fax: (972) 242-7749

TOXICITY TEST REPORT - Chronic

Client:	Mena, City of	Sample:	001
Facility:	WWTP	Laboratory Number:	87082
Permit No.	AR0036692	Date:	December 05, 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 December 05, 2023, December 07, 2023, and December 09, 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 12:37 hours on December 05, 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 10:37 hours on December 13, 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 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 (with Bonferroni adjustment as appropriate for Sub-Lethality) 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 15:39 hours on December 05, 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 15:10 hours on December 12, 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: 87082

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: 12/5/2023

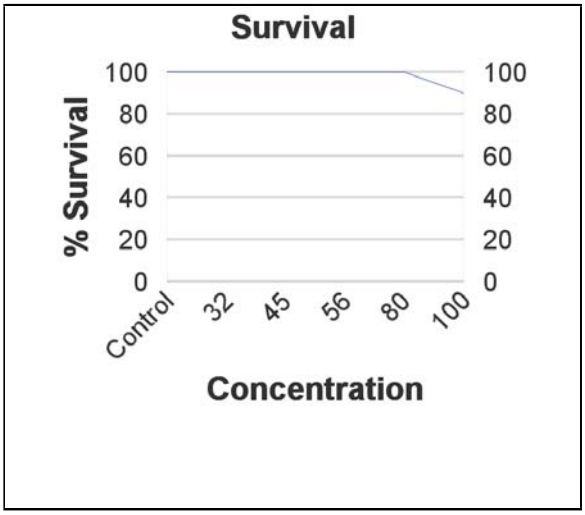
End Date: 12/13/2023

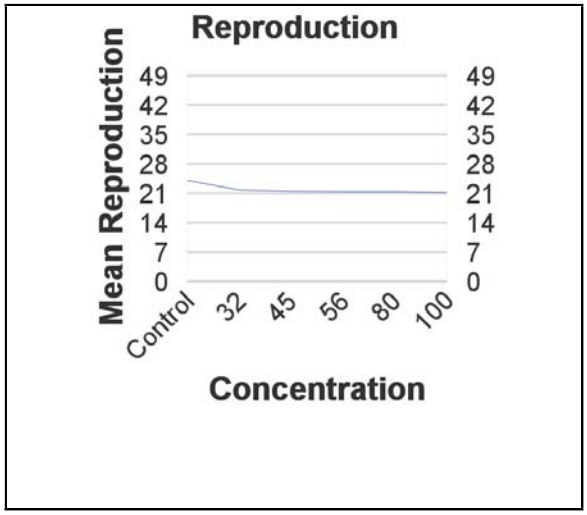
Test Start Time: 12:37 **Test End Time:** 10:37

SURVIVAL AND REPRODUCTION TABLE

FEMALE #	Control	32	%	45	%	56	%	80	%	100	%
1	21	25		26		10		E		19	
2	24	21		23		19		27		25	
3	28	24		17		28		21		24	
4	24	19		16		21		17		D- 3	
5	20	20		20		24		24		19	
6	26	20		17		18		19		19	
7	19	20		16		18		17		20	
8	21	25		24		23		20		18	
9	38	23		24		25		23		18	
10	20	22		33		29		26		30	
Surv.Mean	24.1	21.9		21.6		21.5		21.5		21.3	
C.V%	23.5	10.1		25.3		26		17.1		19.3	
Total Mean	24.1	21.9		21.6		21.5		21.6		19.5	
Var	32.322	4.988		30.044		31.388		13.527		17	
Std.Dev.	5.685	2.233		5.481		5.602		3.678		4.123	
Max	38	25		33		29		27		30	
Min	19	19		16		10		17		18	

Concentration Response Relationships





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Survival and Reproduction

Control

Date	1	2	3	4	5	6	7	8	9	10
12/6	A	A	A	A	A	A	A	A	A	A
12/7	A	A	A	A	A	A	A	A	A	A
12/8	A	A	A	A	A	A	A	A	A	A
12/9	2	1	2	3	3	5	3	1	A	4
12/10	A	A	6	A	A	7	6	6	9	4
12/11	8	9	A	8	5	A	A	A	A	A
	10	10	8	11	8	12	9	7	9	8
12/12	A	A	A	13	12	14	10	A	17	12
	10	10	8	24	20	26	19	7	26	20
12/13	11	14	20	A	A	A	A	14	12	A
	21	24	28	24	20	26	19	21	38	20

Mean: 24.10 **CV%** 23.50
Var. 32.32 **Max** 38
Std.Dev. 5.69 **Min** 19

32

Date	1	2	3	4	5	6	7	8	9	10
12/6	A	A	A	A	A	A	A	A	A	A
12/7	A	A	A	A	A	A	A	A	A	A
12/8	A	A	A	A	A	A	A	A	A	A
12/9	2	4	3	2	1	3	2	4	3	1
12/10	A	4	A	A	7	6	6	7	4	5
12/11	14	A	7	3	A	A	A	A	A	A
	16	8	10	5	8	9	8	11	7	6
12/12	A	13	14	A	A	11	12	14	A	A
	16	21	24	5	8	20	20	25	7	6
12/13	9	A	A	14	12	A	A	A	16	16
	25	21	24	19	20	20	20	25	23	22

Mean: 21.90 **CV%** 10.10
Var. 4.99 **Max** 25
Std.Dev. 2.23 **Min** 19

45

Date	1	2	3	4	5	6	7	8	9	10
12/6	A	A	A	A	A	A	A	A	A	A
12/7	A	A	A	A	A	A	A	A	A	A
12/8	A	A	A	A	A	A	A	A	A	A
12/9	1	3	2	3	1	3	2	2	A	A
12/10	6	5	2	A	A	A	A	A	5	1
12/11	A	A	A	A	5	A	3	8	A	A
	7	8	4	3	6	3	5	10	5	1
12/12	A	15	13	13	14	13	A	A	13	13
	7	23	17	16	20	16	5	10	18	14
12/13	19	A	A	A	A	1	11	14	6	19
	26	23	17	16	20	17	16	24	24	33

Mean: 21.60 **CV%** 25.30
Var. 30.04 **Max** 33
Std.Dev. 5.48 **Min** 16

56

Date	1	2	3	4	5	6	7	8	9	10
12/6	A	A	A	A	A	A	A	A	A	A
12/7	A	A	A	A	A	A	A	A	A	A
12/8	A	A	A	A	A	A	A	A	A	A
12/9	A	5	A	2	A	1	3	4	1	A
12/10	3	3	5	A	3	A	5	7	5	A
12/11	2	A	10	7	6	5	A	A	A	A
	5	8	15	9	9	6	8	11	6	0
12/12	A	11	A	A	A	12	10	12	A	13
	5	19	15	9	9	18	18	23	6	13
12/13	5	A	13	12	15	A	A	A	19	16
	10	19	28	21	24	18	18	23	25	29

Mean: 21.50 **CV%** 26.00
Var. 31.39 **Max** 29
Std.Dev. 5.60 **Min** 10

80

Date	1	2	3	4	5	6	7	8	9	10
12/6	A	A	A	A	A	A	A	A	A	A
12/7	A	A	A	A	A	A	A	A	A	A
12/8	A	A	A	A	A	A	A	A	A	A
12/9	3	2	3	4	A	A	A	6	3	1
12/10	E	4	A	2	2	A	5	A	6	4
12/11	E	A	5	A	3	8	A	A	A	A
		6	8	6	5	8	5	6	9	5
12/12	E	A	13	11	19	11	12	12	14	A
		6	21	17	24	19	17	18	23	5
12/13	E	21	A	A	A	A	A	2	A	21
		27	21	17	24	19	17	20	23	26

Mean: 21.50 **CV%** 17.10
Var. 13.53 **Max** 27
Std.Dev. 3.68 **Min** 17

100

Date	1	2	3	4	5	6	7	8	9	10
12/6	A	A	A	A	A	A	A	A	A	A
12/7	A	A	A	A	A	A	A	A	A	A
12/8	A	A	A	A	A	A	A	A	A	A
12/9	1	5	5	3	6	1	3	3	3	4
12/10	A	7	7	D	A	A	3	3	4	A
12/11	8	A	A	D	5	4	A	A	A	A
	9	12	12	3	11	5	6	6	7	4
12/12	A	13	12	D	8	A	14	12	11	8
	9	25	24	3	19	5	20	18	18	12
12/13	10	A	A	D	A	14	A	A	A	18
	19	25	24	3	19	19	20	18	18	30

Mean: 21.30 **CV%** 19.30
Var. 17.00 **Max** 30
Std.Dev. 4.12 **Min** 18

BIO-AQUATIC TESTING, INC.

Chronic CERIODAPHNIA DUBIA SURVIVAL AND REPRODUCTION

Client: **Mena, City of - WWTP** Lab ID: **87082** Culture No.: Bio112825E

TEST INSTRUCTIONS: AFIN 57-00423

ORGANISMS ADDED: Date: 12-5-23 Time: 1237 Technician: SB

Photo Period 16hr Light/8hr dark

RANDOMIZATION: SC-10 21

Dilution: Control

DATE/TIME/ TECHNICIAN	1	2	3	4	5	6	7	8	9	10
24Hr 12-06-23 MW 1348	A	—	—	—	—	—	—	—	—	A
48Hr 12-7-23 SB 1100	A	—	—	—	—	—	—	—	—	A
72Hr 12-08-23 MW 1228	A	—	—	—	—	—	—	—	—	A
96Hr 12-9-23 CC 1334	2	1	2	3	3	5	3	1	2	4
5 days 12-10-23 MH 1010	5	5	6	A	A	7	6	6	7	4
6 days 12-11-23 MH 1310	3	4	A	8	5	A	A	A	A2	A
7 days 12-12-23 MW 1131	A	A	12	13	12	14	10	10	15	12
8 days 12-13-23 MH 1039	11	13	8	A	A	A	A	4	12	A

Dilution: 32 %

	1	2	3	4	5	6	7	8	9	10
24Hr	A	—	—	—	—	—	—	—	—	A
48Hr	A	—	—	—	—	—	—	—	—	A
72Hr	A	—	—	—	—	—	—	—	—	A
96Hr	2	4	3	2	1	3	2	4	3	1
5 days	7	4	A1	A	7	6	6	7	4	5
6 days	7	A	6	3	A	A	A	A	A	A
7 days	A	13	14	8, 10	11	12	14	12	12	12
8 days	9	A	A	6	A2	A	A	A	4	4

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: **87082** 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	A									A
96Hr	1	3	2	3	1	3	2	2	A	A
5 days	6	5	A_2	A	A	A	A_2	7	5	A_1
6 days	A	A	A	A	5	A	A_1	A_1	A	A
7 days	12	6	13	13	14	13	10	11	13	13
8 days	17	A	A	A	A	A_1	A_1	3	6	9

Dilution: 56 %

	1	2	3	4	5	6	7	8	9	10
24Hr	A									A
48Hr	A									A
72Hr	A									A
96Hr	A	5	A	2	2	1	3	4	1	A
5 days	3	3	5	A	A_1	A	5	7	5	A
6 days	A_2	A	10	7	6	5	A	A	A	A
7 days	A	11	A	7	13	12	10	12	12	13
8 days	14	A	13	5	A_2	A	A	A	17	16

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: **87082** 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	A									A
96Hr	3	2	3	4	1	A	A	0	3	1
5 days	A*	4	A	A_2	A_1	A	5	A	6	4
6 days		A	5	A	3	8	A	A	A	A
7 days		13	13	11	9	11	12	12	M	M
8 days		8	A	A	A	A	A	A_2	A	7

*Empty MH

Dilution: 100 %

	1	2	3	4	5	6	7	8	9	10
24Hr	A									A
48Hr	A									A
72Hr	A									A
96Hr	1	5	5	3	0	1	3	3	3	4
5 days	5	7	7	D	4	A	3	3	4	A
6 days	3	A	A		A_1	4	A	A	A	A
7 days	A	13	12		8	12	M	12	M	8
8 days	10	A	A		A	A_2	A	A	A	18

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: **87082** Culture No.:

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	25.0	25.4	25.2	25.2	25.0	25.4	25.1	25.5	25.4	25.5	25.1	25.4	25.3	25.1
32														
45														
56														
80														
100														
TIME/DATE TECH	12-5-23 SB 1237	12-06 MW	-23 1348	12-7- SB	23 1100	12-08 MW	-23 1228	12-9- CL	23 1347	12-10- MH	23 1010	12-11- MH	23 1310	12-12-23 MW 1131
IR GUN ID #	021	012		021		012		021		012		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

Lab ID: 87082

Permit Number: NPDES AR0036692

Test Temperature (oC): 25 ± 1

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

Photo Period: 16 Hours Light 8 Hours Dark

Receiving Water Name: Prairie Creek

Test Start Time: 15:39 Test End Time: 15:10

Begin Date: 12/5/2023

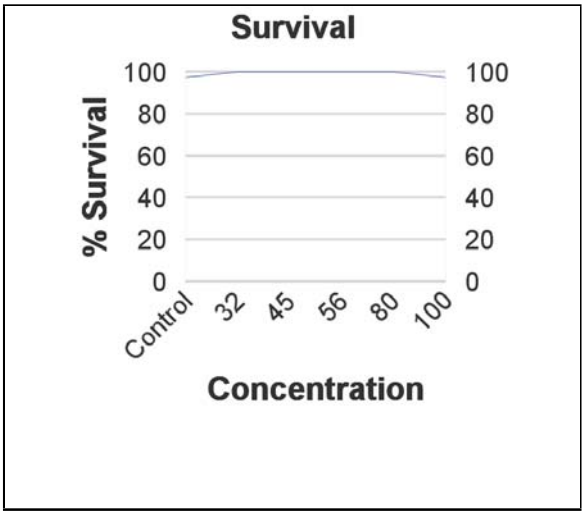
End Date: 12/12/2023

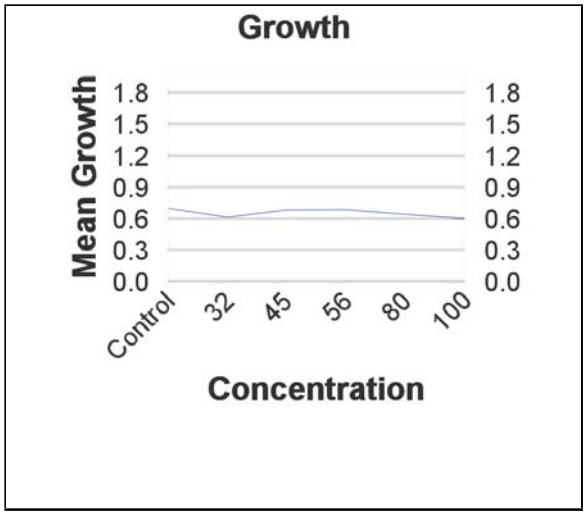
SURVIVAL

Effluent Concentration		Number Of Alive 12/5	12/6	12/7	12/8	12/9	12/10	12/11	12/12	Avg% Surv.
Control	A	8	8	8	8	8	8	8	8	97.5%
	B	8	8	8	7	7	7	7	7	
	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	
32	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	
45	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	
56	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	

Effluent Concentration		Number Of Alive								Avg% Surv.
		12/5	12/6	12/7	12/8	12/9	12/10	12/11	12/12	
80	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	
100	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	7	7	7	7	7	
	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: **87082**

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

TEST INSTRUCTIONS: AFIN 57-00423

Culture No. : PT-23-339B Photo Period: 16hr light, 8hr dark **RANDOMIZATION:** SC-5 1

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	12-5-23 1539 JC	8					8					8					8				
24Hr	12-6-23 1044 JC	8					8					8					8				
48Hr	12-7-23 925 JC	8					8					8					8				
72Hr	12-8-23 0828 AR	8	7	8			8					8					8				
96Hr	12-9-23 0854 AR	8	7	8			8					8					8				
5 days	12-10-23 0917 AR	8	7	8			8					8					8				
6 days	12-11-23 0858 AR	8	7	8			8					8					8				
7 days	12-12-23 1910 MW	7	7	7			7					7					7				

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					8														
72Hr	8					8			7	8										
96Hr	8					8			7	8										
5 days	8					8			7	8										
6 days	8					8			7	8										
7 days	7					7			7	8										

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BIO-AQUATIC TESTING, INC.

Chronic | Pimephales promelas SURVIVAL | Lab ID: **87082**

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	~~25.3~~	25.4	~~25.3~~	~~25.6~~	~~25.5~~	25.3	24.1	25.0	24.1	25.3	24.2	25.2	24.1	~~25.2~~
32														
45														
56														
80														
100														
TIME/DATE TECH	12-5-23 1539 JC		12-6-23 1044 JC		12-7-23 925 JC		12-8-23 0828 AR		12-9-23 0854 AR		12-10-23 0917 AR		12-11-23 0858 AR	12-12-23 1300
IR GUN ID #	020		020		020		020		020		020		020	020

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: 87082**

Permit Number: AR0036692

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

Receiving Water Name: Prairie Creek

Synthetic

	ON	SN	Wt.	Avg.	SN Avg.
A	8	8	5.121	0.640	0.640
B	8	7	5.270	0.659	0.753
C	8	8	5.389	0.674	0.674
D	8	8	5.879	0.735	0.735
E	8	8	6.319	0.790	0.790

Mean	C.V. %
0.699	8.8

SN Mean	SN C.V. %
0.718	8.4

32

	ON	Wt.	Avg.
A	8	4.163	0.520
B	8	4.895	0.612
C	8	4.992	0.624
D	8	5.085	0.636
E	8	5.408	0.676

Mean	C.V. %
0.614	9.4

45

	ON	Wt.	Avg.
A	8	4.726	0.591
B	8	5.315	0.664
C	8	5.637	0.705
D	8	5.762	0.720
E	8	5.898	0.737

Mean	C.V. %
0.683	8.5

56

	ON	Wt.	Avg.
A	8	5.948	0.744
B	8	4.484	0.561
C	8	5.516	0.690
D	8	5.553	0.694
E	8	5.946	0.743

Mean	C.V. %
0.686	10.9

80

	ON	Wt.	Avg.
A	8	4.668	0.584
B	8	4.870	0.609
C	8	4.655	0.582
D	8	5.977	0.747
E	8	5.502	0.688

Mean	C.V. %
0.642	11.4

100

	ON	Wt.	Avg.
A	8	4.814	0.602
B	8	4.657	0.582
C	8	4.008	0.501
D	8	5.088	0.636
E	8	5.509	0.689

Mean	C.V. %
0.602	11.5

	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: **87082**

Client: Mena, City of - WWTP

Balance: Radwag BAL-007

Begin Date: 12/5/2023 End Date: 12/12/2023 Organism: Pimephales promelas

Analyst: LAH

Weigh Date: 12/16/23

Date/Time placed in Oven: 12/12/23 / 1545

Date/Time removed from Oven: 12/13/23 / 1545

Control

	Qty.	Wt.
A	8	5.121
B	7	5.270
C	8	5.389
D		5.879
E		6.319

32 %

	Qty.	Wt.
A	8	4.163
B		4.895
C		4.922
D		5.085
E		5.408

45 %

	Qty.	Wt.
A	8	4.726
B		5.315
C		5.637
D		5.762
E		5.898

56 %

	Qty.	Wt.
A	8	5.948
B		4.484
C		5.516
D		5.553
E		5.946

80 %

	Qty.	Wt.
A	8	4.668
B		4.870
C		4.655
D		5.977
E		5.502

100 %

	Qty.	Wt.
A	8	4.814
B		4.657
C		4.008
D	7	5.088
E	8	5.509

	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 repro
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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	3.953	14.278	22.538	14.278	3.953
OBSERVED	2	18	23	13	3

Calculated Chi-Square goodness of fit test statistic = 2.2888
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

cerio repro
File: 87082.cdr Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 11.15

Bartlett's test using average degrees of freedom
Calculated B2 statistic = 11.12
Based on average replicate size of 8.83

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.
Data PASS B2 homogeneity test at 0.01 level. Continue analysis.

cerio repro
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	107.086	21.417	0.791

Within (Error)	53	1435.422	27.083
Total	58	1542.508	

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

cerio repro
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BONFERRONI t-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	con	24.100	24.100		
2	32	21.900	21.900	0.945	
3	45	21.600	21.600	1.074	
4	56	21.500	21.500	1.117	
5	80	21.556	21.556	1.064	
6	100	19.500	19.500	1.976	

Bonferroni t table value = 2.40 (1 Tailed Value, P=0.05, df=50,5)

cerio repro
File: 87082.cdr Transform: NO TRANSFORMATION

BONFERRONI t-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	5.593	23.2	2.200
3	45	10	5.593	23.2	2.500
4	56	10	5.593	23.2	2.600
5	80	9	5.747	23.8	2.544
6	100	10	5.593	23.2	4.600

fathead growth
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Shapiro - Wilk's test for normality

D = 0.105

W = 0.972

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: 87082.ppg Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 0.47

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: 87082.ppg Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.042	0.008	1.937
Within (Error)	24	0.105	0.004	
Total	29	0.147		

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

fathead growth
File: 87082.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	0.700	0.700		
2	32	0.614	0.614	2.056	
3	45	0.683	0.683	0.387	
4	56	0.686	0.686	0.316	
5	80	0.642	0.642	1.377	
6	100	0.602	0.602	2.333	

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

fathead growth
File: 87082.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	0.099	14.1	0.086
3	45	5	0.099	14.1	0.016
4	56	5	0.099	14.1	0.013
5	80	5	0.099	14.1	0.058
6	100	5	0.099	14.1	0.098

Bio-Aquatic Testing, Inc.

FRESH WATER TEST SETUP FORM

Client: Mena, City of

Permit AR0036692

Facility: WWTP

Lab Number 87082

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	12/05/23	08:25	12/03/23	12/04/23	08:00	08:00
2	12/07/23	08:45	12/05/23	12/06/23	08:00	08:00
3	12/09/23	08:15	12/07/23	12/08/23	08:00	08:00

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	127	53
2	127	53
3	127	53

Start Sx #	1	Date:	12/5/2023
Renew Sx #	1	Date:	12/6/2023
Renew Sx #	1	Date:	12/7/2023
Renew Sx #	2	Date:	12/8/2023
Renew Sx #	3/2	Date:	12/9/2023
Renew Sx #	3	Date:	12/10/2023
Renew Sx #	3	Date:	12/11/2023

Test Start Date: 12/5/2023

Test End Date: 12/12/2023

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:** 87082

Facility: WWTP **Outfall:** 001

Dilution Water(s): Synthetic Lab **Test Date:** December 5, 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	12/5/23	08:25	<0.10	N/A	1.5	JP	3.3
2	12/7/23	08:45	<0.10	N/A	1.7	JP	3.7
3	12/9/23	08:15	<0.10	N/A	1.7	JP	3.8

[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	6.6	11.0	43	22	237	JP
2	8.3	9.6	24	16	272	JP
3	7.4	9.4	40	26	154	JP

DAILY RENEWAL CONDUCTIVITY**

Date		Sample #	Values are at Highest Dilution		Analyst
			Specific Conductivity as umhos/cm	Salinity (ppt)	
12/5	Lab H2O		378	0.2	GS
12/6	Lab H2O		391	0.2	TM/JC
12/7	Lab H2O		375	0.2	MM
12/8	Lab H2O		389	0.2	AR/M
12/9	Lab H2O		379	0.2	AR
12/10	Lab H2O		361	0.2	CK
12/11	Lab H2O		390	0.2	JR
12/5	OUTFALL*	1	173	0.1	GS
12/6	OUTFALL*	1	182	0.1	TM/JC
12/7	OUTFALL*	1	173	0.1	MM
12/8	OUTFALL*	2	284	0.2	AR/M
12/9	OUTFALL*	3/2	277	0.2	AR
12/10	OUTFALL*	3	180	0.1	CK
12/11	OUTFALL*	3	166	0.1	JR

**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: 87082

Facility: WWTP

Outfall: 001

Dilution Water(s): Synthetic Lab

Test Begin Date: December 5, 2023

NR indicates that the test is non-renewal.

					Concentration							
ANALYST	DATE	TIME	SX#	UNIT	Control	32	45	56	80	100		
GS	12/5	Start	1	pH	7.9	8.0	8.0	8.0	8.0	8.0		
		25 ± 1		DO (mg/L)	8.6	8.5	8.5	8.5	8.6	8.6		
TM/JC	12/6	24 Hr	1	pH	7.9	7.8	7.8	7.8	7.8	7.6		
		25 ± 1		DO (mg/L)	8.8	8.7	8.7	8.6	8.6	8.4		
		Renew	1	pH	8.1	8.0	8.0	7.7	7.7	7.5		
				DO (mg/L)	8.3	8.3	8.3	8.7	8.7	8.8		
MM	12/7	48 Hr	1	pH	8.0	8.0	8.0	7.9	7.9	7.9		
		25 ± 1		DO (mg/L)	8.7	8.7	8.7	8.7	8.7	8.6		
		Renew	1	pH	7.6	7.7	7.7	7.7	7.7	7.6		
				DO (mg/L)	8.3	8.4	8.4	8.4	8.5	8.6		
AR/MM	12/8	72 Hr	1	pH	7.8	7.8	7.8	7.7	7.7	7.7		
		25 ± 1		DO (mg/L)	7.8	7.9	7.9	7.9	7.9	7.9		
		Renew	2	pH	7.7	7.7	7.7	7.6	7.6	7.5		
				DO (mg/L)	7.9	7.9	7.9	7.9	7.9	8.3		
AR	12/9	96 Hr	2	pH	8.6	8.5	8.3	8.2	8.1	8.0		
		25 ± 1		DO (mg/L)	8.8	8.4	7.6	7.6	7.2	7.4		
		Renew	3/2	pH	7.7	7.8	7.8	7.7	7.7	7.6		
				DO (mg/L)	8.0	8.0	8.0	8.4	8.4	8.6		
CK	12/10	120 Hr	3/2	pH	8.0	8.0	8.0	8.0	8.0	8.0		
		25 ± 1		DO (mg/L)	8.1	8.1	8.2	8.2	8.2	8.2		
		Renew	3	pH	7.4	7.5	7.5	7.5	7.6	7.6		
				DO (mg/L)	8.2	8.2	8.2	8.3	8.4	8.6		
AR	12/11	144 Hr	3	pH	8.0	7.9	7.9	7.9	7.9	7.8		
		25 ± 1		DO (mg/L)	8.3	8.3	8.3	8.3	8.3	8.4		
		Renew	3	pH	7.7	7.8	7.8	7.7	7.7	7.6		
				DO (mg/L)	8.6	8.5	8.5	8.8	8.8	9.1		
JR	12/12	168 Hr	3	pH	7.9	7.9	7.9	7.8	7.8	7.8		
		25 ± 1		DO (mg/L)	8.5	8.5	8.5	8.5	8.0	8.3		

BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen

Chronic **Pimephales promelas**

Client: Mena, City of **Lab Number:** 87082

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

Outfall: 001 **Test Begin Date:** December 5, 2023

NR indicates that the test is non-renewal.

ANALYST	DATE	TIME	SX#	UNIT	Concentration							
					Control	32	45	56	80	100		
GS	12/5	Start	1	pH	7.9	8.0	8.0	8.0	8.0	8.0		
		25 ± 1		DO (mg/L)	8.6	8.5	8.5	8.5	8.6	8.6		
TM/JC	12/6	24 Hr	1	pH	7.5	7.6	7.6	7.6	7.6	7.5		
		25 ± 1		DO (mg/L)	8.4	8.5	8.5	8.4	8.4	8.4		
		Renew	1	pH	8.1	8.0	8.0	7.7	7.7	7.5		
				DO (mg/L)	8.3	8.3	8.3	8.7	8.7	8.8		
MM	12/7	48 Hr	1	pH	7.6	7.6	7.6	7.6	7.6	7.6		
		25 ± 1		DO (mg/L)	8.5	8.5	8.4	8.3	8.3	8.2		
		Renew	1	pH	7.6	7.7	7.7	7.7	7.7	7.6		
				DO (mg/L)	8.3	8.4	8.4	8.4	8.5	8.6		
AR/MM	12/8	72 Hr	1	pH	7.3	7.4	7.4	7.4	7.4	7.3		
		25 ± 1		DO (mg/L)	7.9	7.7	7.7	7.6	7.6	7.5		
		Renew	2	pH	7.7	7.7	7.7	7.6	7.6	7.5		
				DO (mg/L)	7.9	7.9	7.9	7.9	7.9	8.3		
AR	12/9	96 Hr	2	pH	7.3	7.5	7.5	7.5	7.5	7.4		
		25 ± 1		DO (mg/L)	8.3	8.1	8.1	8.0	8.0	8.0		
		Renew	3/2	pH	7.7	7.8	7.8	7.7	7.7	7.6		
				DO (mg/L)	8.0	8.0	8.0	8.4	8.4	8.6		
CK	12/10	120 Hr	3/2	pH	7.2	7.3	7.4	7.4	7.5	7.5		
		25 ± 1		DO (mg/L)	8.4	8.2	8.1	8.1	8.1	8.0		
		Renew	3	pH	7.4	7.5	7.5	7.5	7.6	7.6		
				DO (mg/L)	8.2	8.2	8.2	8.3	8.4	8.6		
AR	12/11	144 Hr	3	pH	7.6	7.6	7.6	7.5	7.5	7.5		
		25 ± 1		DO (mg/L)	9.1	8.9	8.9	8.9	8.9	8.8		
		Renew	3	pH	7.7	7.8	7.8	7.7	7.7	7.6		
				DO (mg/L)	8.6	8.5	8.5	8.8	8.8	9.1		
JR	12/12	168 Hr	3	pH	8.1	8.1	8.1	8.1	8.2	8.2		
		25 ± 1		DO (mg/L)	8.5	8.4	8.3	8.3	8.3	8.3		

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.

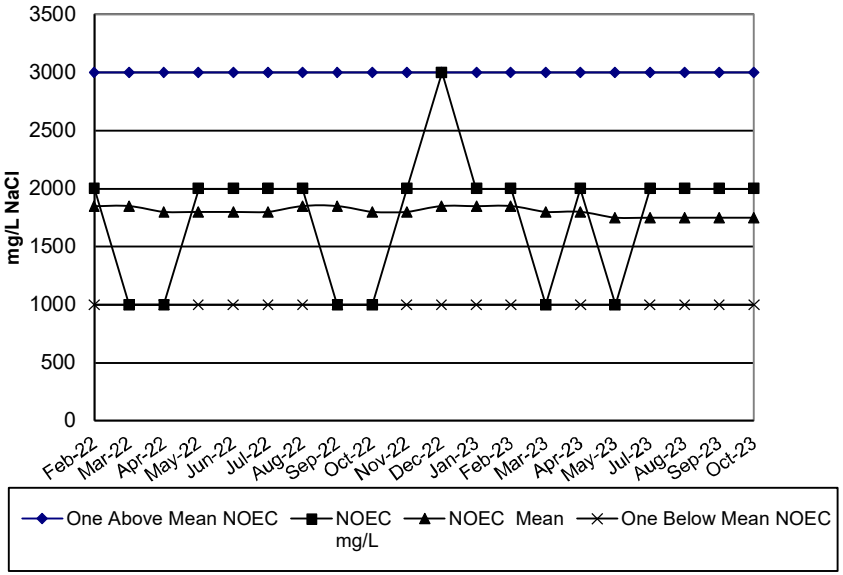
CHRONIC REFERENCE TOXICANT TEST RESULTS

DILUTION WATER:	Standard Synthetic Freshwater						
CHEMICAL:	Sodium Chloride						
DURATION:	3-Brood Chronic						
TEST NUMBER:	343						
PROJECT NUMBER:	88426						
START DATE:	10/31/2023						
START TIME:	16:30						
TOTAL NUMBER EXPOSED:	10 organisms per concentration						
CONCENTRATIONS (mg/L):	CON	250	500	1000	2000	3000	4000
NUMBER DEAD PER CONCENTRATION:	1	1	1	0	2	10	10
TEST METHODS:	As listed in EPA-821-R-02-013						
STATISTICAL METHODS:	SURVIVAL: Fisher's Exact Test REPRODUCTION: ANOVA-Dunnetts w/Bonf. Adj.						

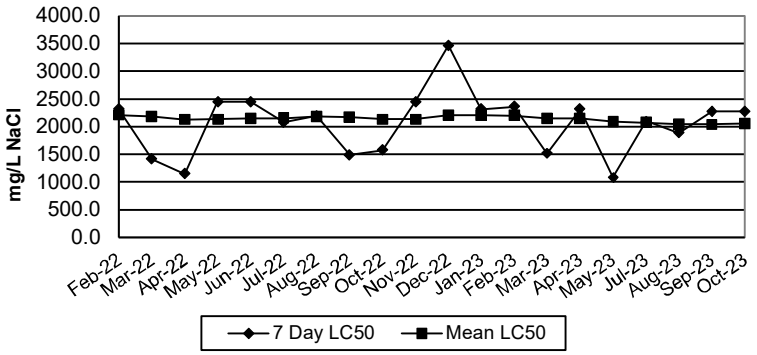
NOEC FOR SURVIVAL:	2000	mg/L
LOEC FOR SURVIVAL:	3000	mg/L
NOEC FOR REPRODUCTION:	1000	mg/L
LOEC FOR REPRODUCTION:	2000	mg/L

PMSD: 31.9

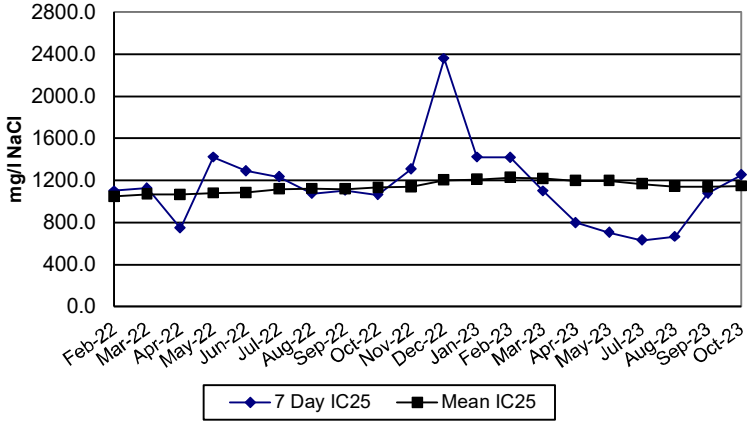
Ceriodaphnia Chronic Survival Control Chart



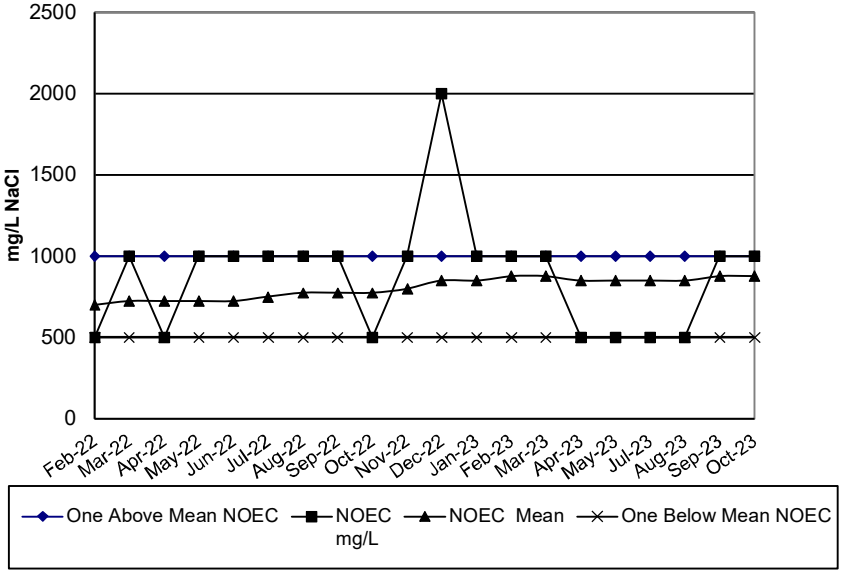
Ceriodaphnia 7-Day LC50



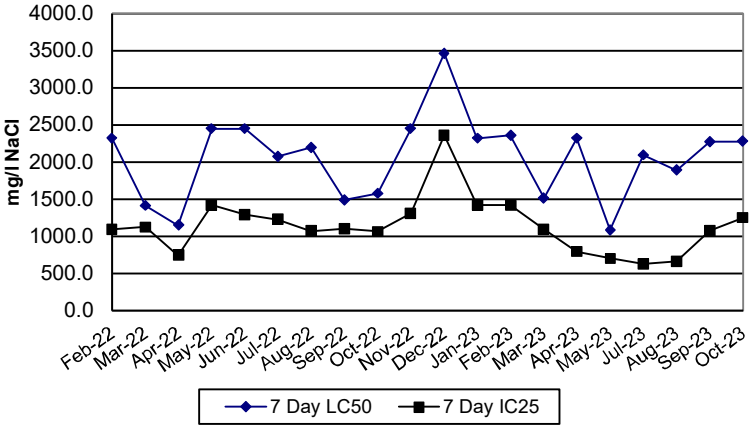
Ceriodaphnia 7-Day IC25



Ceriodaphnia Chronic Reproduction Control Chart



Ceriodaphnia 7-Day LC50 & IC25



Appendix B

Pimephales promelas

BIO-AQUATIC TESTING, INC.

Carrollton, TX

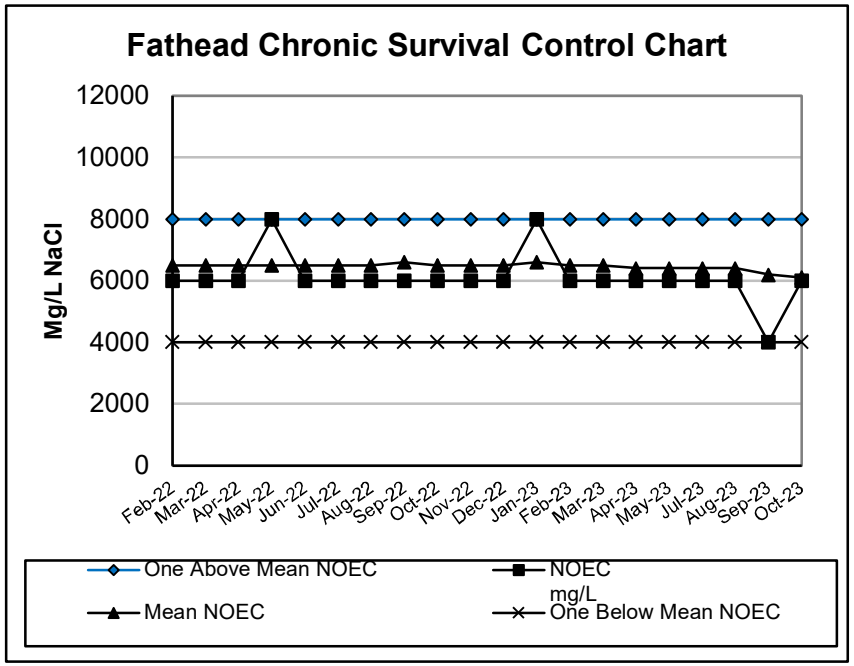
REFERENCE TOXICANTS

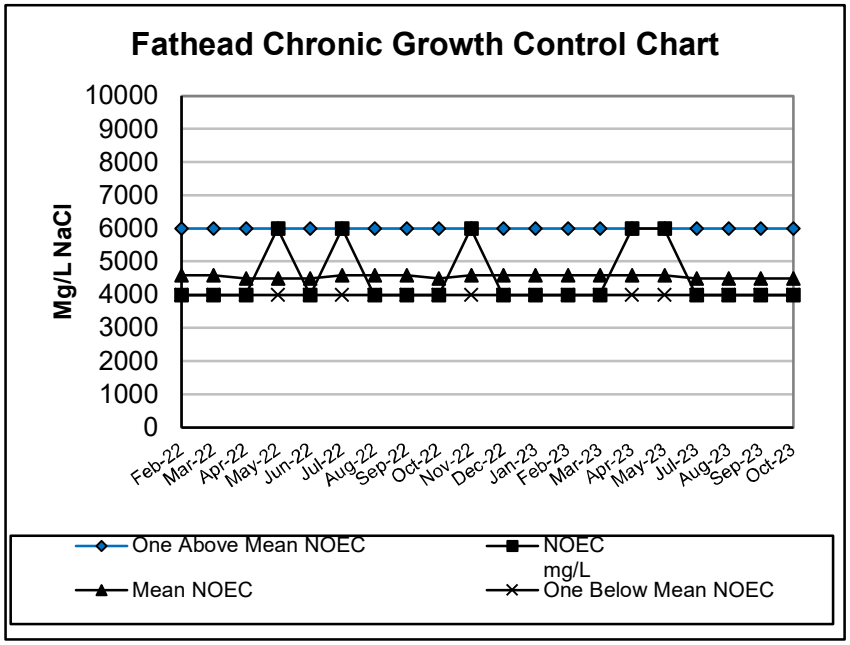
Bio-Aquatic Testing conducts reference toxicant testing monthly for organisms cultured in-house. For studies requiring purchased organisms, reference toxicant testing is performed simultaneously. Reference toxicant testing validates data and measures organism consistency. Only reagent grade chemicals 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.

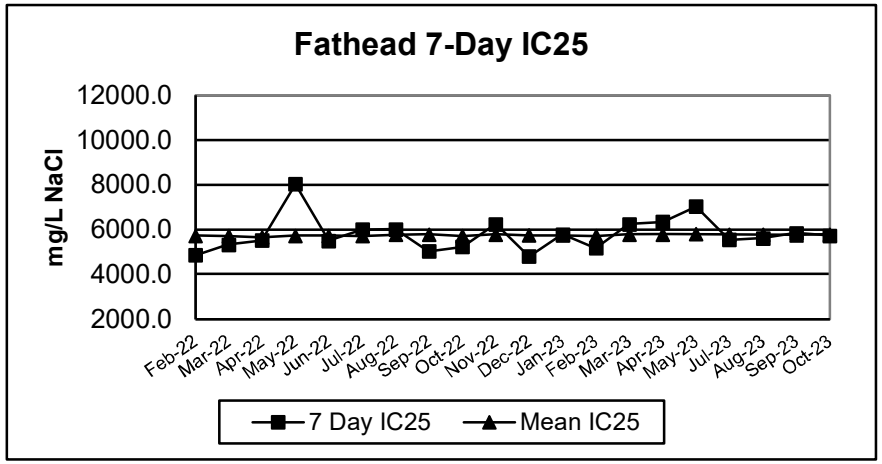
CHRONIC REFERENCE TOXICANT TEST RESULTS

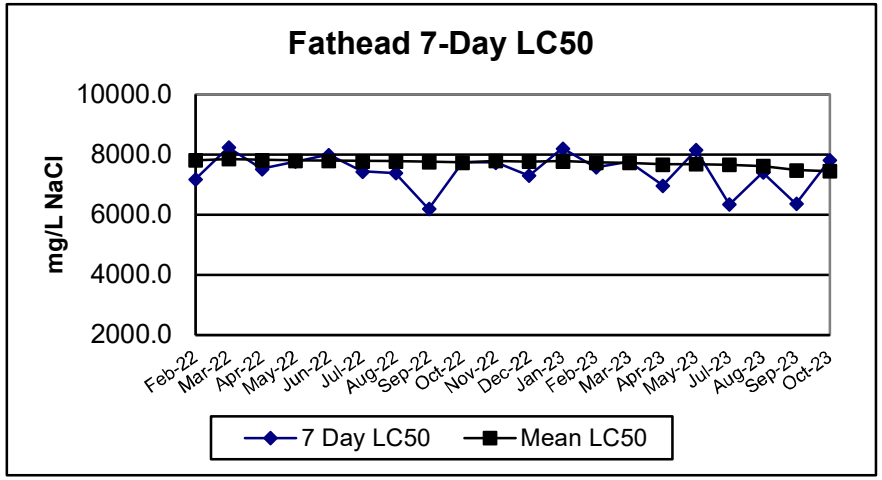
DILUTION WATER:	Standard Synthetic Freshwater						
CHEMICAL:	Sodium Chloride						
DURATION:	7 Days						
TEST NUMBER:	383						
PROJECT NUMBER:	88431						
START DATE:	10/31/2023						
START TIME:	17:10						
TOTAL NUMBER EXPOSED:	40 organisms per concentration						
CONCENTRATIONS (mg/L):	CON	2000	4000	6000	8000	10000	12000
NUMBER DEAD PER CONCENTRATION:	3	0	0	5	17	39	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						

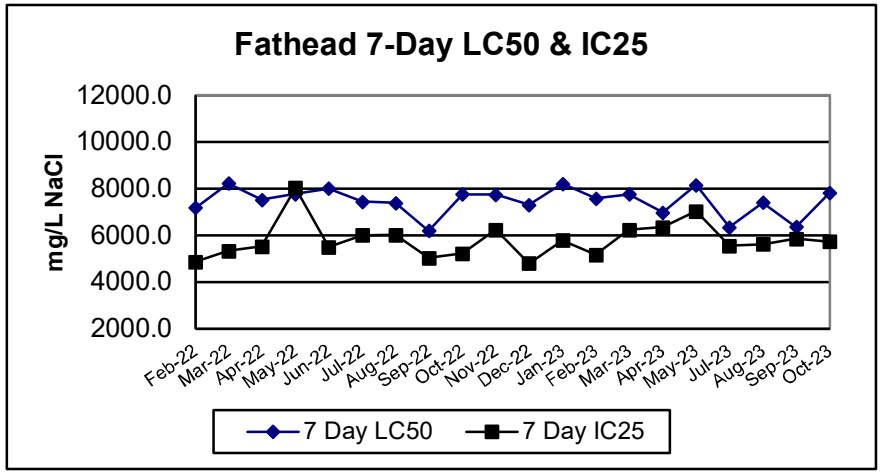
PMSD: 14.9


Fathead Chronic Survival Control Chart
Mg/L NaCl
12000
10000
8000
6000
4000
2000
0
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 Aug-23 Sep-23 Oct-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
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 Aug-23 Sep-23 Oct-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
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 Aug-23 Sep-23 Oct-23
7 Day IC25
Mean IC25


Fathead 7-Day LC50
mg/L NaCl
10000.0
8000.0
6000.0
4000.0
2000.0
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 Aug-23 Sep-23 Oct-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
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 Aug-23 Sep-23 Oct-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., 1991. Technical Support Document For Water Quality-Based Toxics Control, U.S. Environmental Protection Agency, EPA-505-2-90-001.

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CHAIN-OF-CUSTODY SHEETS

Appendix D

Report Date 01/04/2024 Revision 0

33 of 41

BIO-AQUATIC TESTING, INC.

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

CHAIN OF CUSTODY

☐ Bio Only: No Sample Left

Lab Id : 87082

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

Sample No: 87082 – ____

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

Concentration: 32 45 56 80 100

To Ship the 1st Sample on: 12/4/2023

(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: 4th 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	30Dec23	4Dec23	0400	0800	Comp	M Spencer	1
2									
3									

D.

	Relinquished By:	Date	Time	Received By:	Date	Time
1	K Hopper	4Dec23	1000	Rance B Bow	4Dec23	1000
2	Kmerdanoff	12/4/23	1730	Jmy Payne	12-5-23	0825

Bio-Aquatic Lab ID: 87082

Bio-Aquatic Sample Login

BAT sample personnel: ● Yes ○ No

Dechlorinate Sample: ○ Yes ● No

Dilution Water: ○ Receiving Stream ● Synthetic Lab

Date: 12-5-23 Time: 1013 By: JP Temperature: 3.3 (C) IR#: 002

Chlorine: <0.1 mg/l	Ammonia: 1.5 mg/l	Int. Sal\Cond: 237 ppt/uS	Adj. Salinity ppt		
pH: 6.6	Hardness: 43 mg/l (LR)	Other			
DO: 11.0 mg/l	Alkalinity: 22 mg/l (OK)	Condition: colormetric NH3, Cl2			

BIO-AQUATIC TESTING, INC.

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

CHAIN OF CUSTODY

☐ Bio Only: No Sample Left

Lab Id : 87082

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

Sample No: 87082 –

Revision 2 Effective Date 9/25/2017

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

P.O. No:

Report Date 01/04/2024 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

Concentration: 32 45 56 80 100

To Ship the 1st Sample on: 12/4/2023

(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: 4th 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	5Dec23	6Dec23	0900	0900	Comp		1
2									
3									

D.

	Relinquished By:	Date	Time	Received By:	Date	Time
1	L. Hopper	6Dec23	1000	Rance / BBow	6Dec23	1000
2	Rance Parrott	12/6/23	1200	Jmy Payne	12.7.23	0845
3						

Bio-Aquatic Lab ID: 87082

Bio-Aquatic Sample Login

BAT sample personnel: ● Yes ○ No

Dechlorinate Sample: ○ Yes ◉ No

Dilution Water: ○ Receiving Stream ◉ Synthetic Lab

Date: 12.7.23	Time: 1414	By: JP	Temperature: 3.7 (C)	IR#: 002			
Chlorine: <0.1 mg/l	Ammonia: 1.7 mg/l	Int. Sal\Cond: 272 ppt/uS	Adj. Salinity ppt				
pH: 8.3	Hardness: 24 mg/l (LR)	Other					
DO: 4.6 mg/l	Alkalinity: 16 mg/l (OK)	Condition: colormetric NH_3, Cl_2					

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

CHAIN OF COUSTODY

☐ Bio Only: No Sample Left

Lab Id : 87082

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

Sample No: 87082 - ____

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: 12/4/2023

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: 4th 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 I	E	8 Dec 23	8 Dec 23	8:00	8:00	comp	L. Hopper L. Hopper	1
2									
3									

D.

	Relinquished By:	Date	Time	Received By:	Date	Time
1	S. Wyman	8 Dec 23	10:30	Rance / B Bow	8 Dec. 23	10:30
2	RanceLarrott	12/8/23	1630	Amy Payne	12.9.23	0815
3						

Bio-Aquatic Sample Login

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

Date: 12.9.23	Time: 0925	By: JP	Temperature: 3.8 (C) IR#: 002		
Chlorine: <0.1 mg/l	Ammonia: 1.7 mg/l	Int. Sal\Cond: 154 ppt/uS	Adj. Salinity ppt		
pH: 7.4	Hardness: 40 mg/l (LR)	Other			
DO: 9.4 mg/l	Alkalinity: 26 mg/l (OK)	Condition: colormetric ~~Cl2~~ NH3			

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:	12/3/2023 @ 08:00	TO:	12/4/2023 @ 08:00
	FROM:	12/5/2023 @ 08:00	TO:	12/6/2023 @ 08:00
	FROM:	12/7/2023 @ 08:00	TO:	12/8/2023 @ 08:00

Test Initiation: Time: 12:37 Date: 12/5/2023

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	21	25	26	10	E	19
B	24	21	23	19	27	25
C	28	24	17	28	21	24
D	24	19	16	21	17	D- 3
E	20	20	20	24	24	19
F	26	20	17	18	19	19
G	19	20	16	18	17	20
H	21	25	24	23	20	18
I	38	23	24	25	23	18
J	20	22	33	29	26	30
Surv. MEAN	24.1	21.9	21.6	21.5	21.5	21.3
Total MEAN	24.1	21.9	21.6	21.5	21.6	19.5
CV % [1]	23.5	10.1	25.3	26	17.1	19.3
PMSD	Acceptable Range 47 or Less					23.2 %

[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. (E) anomalous value, spilled cup, or technician error.

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	100.0	100.0	100.0	100.0	100.0	100.0
48 HOURS	100.0	100.0	100.0	100.0	100.0	100.0
7-DAY	100.0	100.0	100.0	100.0	100.0	90.0

1. DUNNETT'S PROCEDURE OR STEEL'S MANY-ONE RANK TEST OR WILCOXON RANK SUM TEST
(with Bonferroni adjustment as appropriate for Sub-Lethality)
Is the mean number of young produced per adult significantly less (p=0.05) than the number of young per adult in the control for the % effluent corresponding to significant non-lethal effects?

CRITICAL DILUTION (100) : _____YES _____X_____NO

*If you report NO, enter a '0' on the DMR form for Parameter **TWP3B**, 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 less (p=0.05) than the control's survival for the % effluent corresponding to lethality?

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. NOEC Survival = 100 % Effluent (Parameter TOP3B)

b. LOEC Survival = Q* % Effluent (Parameter TXP3B)

c. NOEC Reproduction = 100 % Effluent (Parameter TPP3B)

d. LOEC Reproduction = Q* % Effluent (Parameter TYP3B)

Q* refers to a value that is not calculable

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:	12/3/2023 @ 08:00	TO:	12/4/2023 @ 08:00
	FROM:	12/5/2023 @ 08:00	TO:	12/6/2023 @ 08:00
	FROM:	12/7/2023 @ 08:00	TO:	12/8/2023 @ 08:00

Test Initiation: Time: 15:39 Date: 12/5/2023

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%	0.640	0.659	0.674	0.735	0.790	0.699	8.8
32 %	0.520	0.612	0.624	0.636	0.676	0.614	9.4
45 %	0.591	0.664	0.705	0.720	0.737	0.683	8.5
56 %	0.744	0.561	0.690	0.694	0.743	0.686	10.9
80 %	0.584	0.609	0.582	0.747	0.688	0.642	11.4
100 %	0.602	0.582	0.501	0.636	0.689	0.602	11.5
PMSD	Acceptable Range 30 or Less					14.1 %	

[1] Coefficient of Variation = (standard deviation/mean) x 100) ?= cannot be calculated due to 100% mortality or lab exception

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	87.5	100	100	100	100	100	97.5	5.7
32 %	100	100	100	100	100	100	100	100	0.0
45 %	100	100	100	100	100	100	100	100	0.0
56 %	100	100	100	100	100	100	100	100	0.0
80 %	100	100	100	100	100	100	100	100	0.0
100 %	100	100	100	87.5	100	100	100	97.5	5.7

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 OR WILCOXON RANK SUM TEST (with Bonferroni adjustment as appropriate for Sub-Lethality)

Is the mean dry weight at 7 days significantly less (p=0.05) than the control's mean dry weight for the % effluent corresponding to significant non-lethal effects?

CRITICAL DILUTION (100) : _____YES X NO

*If you report NO, enter a '0' on the DMR form for Parameter **TWP6C**, 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 OR WILCOXON RANK SUM TEST (as appropriate for Lethality) Is the survival at 7 days significantly less (p=0.05) than the control's survival for % effluent corresponding to lethality?

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:

			For DMR Form:
a.	NOEC Survival =	100 % Effluent	(Parameter TOP6C)
b.	LOEC Survival =	Q* % Effluent	(Parameter TXP6C)
c.	NOEC Growth =	100 % Effluent	(Parameter TPP6C)
d.	LOEC Growth =	Q* % Effluent	(Parameter TYP6C)

Q* refers to a value that is not calculable

City of Mena WWTF
NPDES Permit No. AR0036692
Biomonitoring Reporting
Test Date: 12/05/2023

Ceriodaphnia dubia	**Parameter**	**Response**
A. If the NOEC for survival is less than the critical dilution, enter a "1"; otherwise, enter a "0".	TLP3B	0
B. Report the NOEC value for Survival	TOP3B	100
C. Report the NOEC value for Reproduction	TPP3B	100
D. If the NOEC for reproduction is less than the critical dilution, enter a "1", otherwise, enter a "0"	TGP3B	0
E. Report the higher (critical dilution or control) Coefficient of Variation (CV%)	TQP3B	23.5
Report Parameter No. 22414 (lowest NOEC value) for Ceriodaphnia dubia		100

Pimephales promelas	**Parameter**	**Response**
A. If the NOEC for survival is less than the critical dilution, enter a "1"; otherwise, enter a "0".	TLP6C	0
B. Report the NOEC value for Survival	TOP6C	100
C. Report the NOEC value for Growth	TPP6C	100
D. If the NOEC for growth is less than the critical dilution, enter a "1", otherwise, enter a "0"	TGP6C	0
E. Report the higher (critical dilution or control) Coefficient of Variation (CV%)	TQP6C	11.5
Report Parameter No. 22414 (lowest NOEC value) for Pimephales promelas		100