Bio-Aquatic Testing, Inc.

TCEQ TNI Accredited

City of Mena
WWTP
OUTFALL 001

Chronic Biomonitoring Report

86882

Ceriodaphnia dubia
Pimephales promelas

May 09, 2023

Approved by: Joshua Reed

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

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Unless otherwise noted in the body of the report, all data reported in this document are in compliance with current TNI standards and apply only to the samples referenced within. This report document may not be edited or reproduced in part or in full by any other entity, unless Bio-Aquatic Testing, Inc. issues written approval.

***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:	86882
Permit No.	AR0036692	Date:	May 09, 2023

Ceriodaphnia dubia **passed** survival and reproduction testing requirements. *Pimephales promelas* **passed** survival and growth testing requirements when the Performance Control (PCON) was used for statistical analysis. The True Control (TCON) was invalid because it did not meet the minimum test-acceptability criteria. Synthetic water will be used as dilution water for the remainder of the permit cycle.

SAMPLE COLLECTION:

Composite effluent samples from the City of Mena, WWTP, were received on May 08, 2023, May 10, 2023, and May 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:

Ceriodaphnia dubia

EPA METHOD: 1002

The seven-day (three brood) Chronic *Ceriodaphnia dubia* survival and reproduction test was initiated at 11:55 hours on May 09, 2023. Five effluent concentrations of 32%, 45%, 56%, 80% and 100% were prepared using receiving water (Prairie Creek) 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 11:02 hours on May 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 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, *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 14:12 hours on May 09, 2023. Five effluent concentrations of 32%, 45%, 56%, 80% and 100% were prepared using receiving water (Prairie Creek) 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 12:45 hours on May 16, 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

ANOVA and Dunnett's test performed on *Pimephales promelas* survival data demonstrated no statistically significant differences between the Performance Control (PCON) and the 31%, 42% and 56% effluent concentrations tested. The True Control (TCON) was invalid because it did not meet the minimum test-acceptability criteria.

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 a statistically significant difference between the Performance Control (PCON) and the 80% effluent concentration tested. The True Control (TCON) was invalid because it did not meet the minimum test-acceptability criteria.

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

BIO-AQUATIC TESTING, INC.

TOXICITY TEST

Chronic *Ceriodaphnia dubia*

Client: Mena, City of WWTP

Permit Number: NPDES AR0036692

Sample Type: Composite

Outfall Name: 001

Receiving Water Name: Prairie Creek

Lab ID: 86882

Test Temperature (oC): 25 ± 1

Photo Period: 16 Hours Light, 8 Hours Dark

Begin Date: 5/9/2023

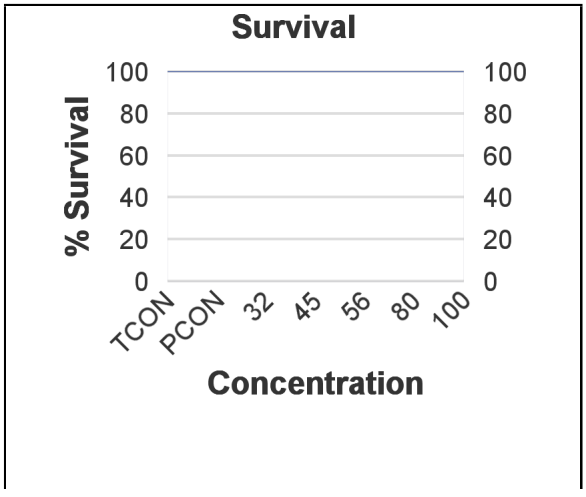
End Date: 5/16/2023

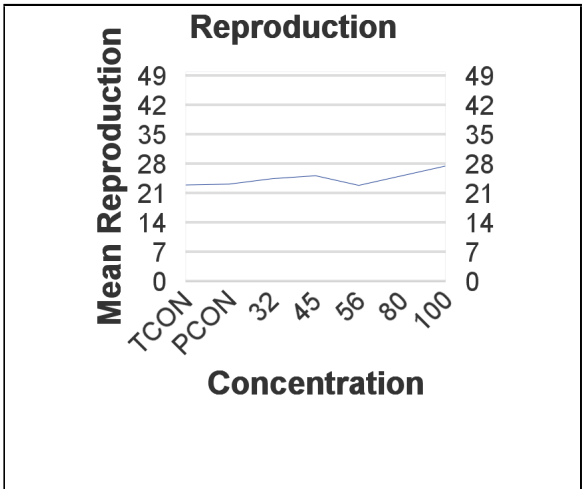
Test Start Time: 11:55 **Test End Time:** 11:02

SURVIVAL AND REPRODUCTION TABLE

Replicate	TCON	PCON	32%	45%	56%	80%	100 %	
1	17	22	29	24	24	21	24	
2	21	25	19	27	23	17	29	
3	19	24	24	29	17	19	26	
4	33	15	29	23	23	27	20	
5	21	28	21	33	25	28	30	
6	24	24	22	22	19	30	25	
7	24	E	23	29	22	24	20	
8	19	27	26	27	24	25	24	
9	26	26	24	20	21	27	39	
10	26	18	28	18	31	34	38	
Surv. Mean	23	23.2	24.5	25.2	22.9	25.2	27.5	
C.V%	20.2	18.3	14	18.3	16.3	20.4	24.1	
Total Mean	23.0	23.2	24.5	25.2	22.9	25.2	27.5	?
Var	21.777	18.194	11.833	21.288	14.1	26.622	44.055	
stddev	4.666	4.265	3.439	4.613	3.754	5.159	6.637	
Max	33	28	29	33	31	34	39	
Min	17	15	19	18	17	17	20	

Concentration Response Relationships





BIO-AQUATIC TESTING, INC.

Survival and Reproduction

TCON

Date	1	2	3	4	5	6	7	8	9	10
5/10	A	A	A	A	A	A	A	A	A	A
5/11	A	A	A	A	A	A	A	A	A	A
5/12	A	4	5	A	A	3	A	4	A	5
5/13	8	8	10	8	10	A	7	9	9	10
5/14	A	A	A	A	A	10	A	A	A	A
5/15	9	A	A	11	A	A	4	A	5	A
	17	12	15	19	10	13	11	13	14	15
5/16	A	9	4	14	11	11	13	6	12	11
	17	21	19	33	21	24	24	19	26	26
5/17										

Mean:	23.00	**Coefficient of variation:**	20.20
Var.	21.777	**Max**	33
Std.Dev.	4.666	**Min**	17

PCON

Date	1	2	3	4	5	6	7	8	9	10
5/10	A	A	A	A	A	A	A	A	A	A
5/11	A	A	A	A	A	A	A	A	A	A
5/12	4	4	4	A	A	3	A	4	A	A
5/13	7	A	A	9	A	A	E	A	11	A
5/14	A	7	9	6	9	6	E	12	A	10
5/15	A	A	A	A	5	A	E	A	15	8
	11	11	13	15	14	9		16	26	18
5/16	11	14	11	A	14	15	E	11	A	A
	22	25	24	15	28	24		27	26	18
5/17										

Mean:	23.2	**Coefficient of variation:**	18.30
Var.	18.194	**Max**	28
Std.Dev.	4.265	**Min**	15

32

Date	1	2	3	4	5	6	7	8	9	10
5/10	A	A	A	A	A	A	A	A	A	A
5/11	A	A	A	A	A	A	A	A	A	A
5/12	4	A	A	3	4	5	A	A	A	3
5/13	A	A	A	9	8	10	A	A	10	8
5/14	10	7	8	A	9	7	8	6	A	A
5/15	7	4	7	17	A	A	8	9	A	17
	21	11	15	29	21	22	16	15	10	28
5/16	8	8	9	A	A	A	7	11	14	A
	29	19	24	29	21	22	23	26	24	28
5/17										

Mean:	24.5	**Coefficient of variation:**	14.00
Var.	11.833	**Max**	29
Std.Dev.	3.439	**Min**	19

45

Date	1	2	3	4	5	6	7	8	9	10
5/10	A	A	A	A	A	A	A	A	A	A
5/11	A	A	A	A	A	A	A	A	A	A
5/12	A	A	4	A	A	3	4	5	A	3
5/13	A	8	A	7	7	9	A	10	A	A
5/14	2	A	A	A	A	A	A	A	A	6
5/15	14	3	7	6	13	A	13	12	8	9
	16	11	11	13	20	12	17	27	8	18
5/16	8	16	18	10	13	10	12	A	12	A
	24	27	29	23	33	22	29	27	20	18
5/17										

Mean:	25.2	**Coefficient of variation:**	18.30
Var.	21.288	**Max**	33
Std.Dev.	4.613	**Min**	18

56

Date	1	2	3	4	5	6	7	8	9	10
5/10	A	A	A	A	A	A	A	A	A	A
5/11	A	A	A	A	A	A	A	A	A	A
5/12	A	A	A	A	A	A	A	A	A	A
5/13	A	5	A	9	6	8	4	4	7	A
5/14	6	A	8	5	8	A	A	A	A	8
5/15	11	8	9	A	11	A	10	9	8	8
	17	13	17	14	25	8	14	13	15	16
5/16	7	10	A	9	A	11	8	11	6	15
	24	23	17	23	25	19	22	24	21	31
5/17										

Mean:	22.9	**Coefficient of variation:**	16.30
Var.	14.100	**Max**	31

80

Date	1	2	3	4	5	6	7	8	9	10
5/10	A	A	A	A	A	A	A	A	A	A
5/11	A	A	A	A	A	A	A	A	A	A
5/12	A	A	A	A	A	A	A	A	A	A
5/13	A	A	A	4	A	9	5	8	7	9
5/14	7	8	10	7	8	A	A	A	9	8
5/15	14	A	A	A	11	9	11	3	A	A
	21	8	10	11	19	18	16	11	16	17
5/16	A	9	9	16	9	12	8	14	11	17
	21	17	19	27	28	30	24	25	27	34
5/17										

Mean:	25.2	**Coefficient of variation:**	20.4
Var.	26.622	**Max**	34

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Survival and Reproduction

	100									
Date	1	2	3	4	5	6	7	8	9	10
5/10	A	A	A	A	A	A	A	A	A	A
5/11	A	A	A	A	A	A	A	A	A	A
5/12	A	A	A	A	A	A	A	A	A	A
5/13	9	7	4	11	9	10	9	11	8	9
5/14	A	A	A	A	A	A	A	A	A	A
5/15	5	11	10	9	17	15	11	A	15	14
	14	18	14	20	26	25	20	11	23	23
5/16	10	11	12	A	4	A	A	13	16	15
	24	29	26	20	30	25	20	24	39	38
5/17										

Mean:	27.5	**Coefficient of variation:**	24.10
Var.	44.055	**Max**	39
Std.Dev.	6.637	**Min**	20

Date	1	2	3	4	5	6	7	8	9	10
5/10										
5/11										
5/12										
5/13										
5/14										
5/15										
5/16										
5/17										

Mean:		**Coefficient of variation:**	
Var.		**Max**	
Std.Dev.		**Min**	

BIO-AQUATIC TESTING, INC.

Chronic CERIODAPHNIA DUBIA SURVIVAL AND REPRODUCTION

Client: **Mena, City of - WWTP** Lab ID: **86882** Culture No.: B10050223E

TEST INSTRUCTIONS:

ORGANISMS ADDED: Date: 05-09-23 Time: 1155 Technician: MW

Photo Period 16hr Light/8hr dark

RANDOMIZATION: RS-10 3

Dilution: TCON

	DATE/TIME/ TECHNICIAN	1	2	3	4	5	6	7	8	9	10
24Hr	5-10-23 MH 1430	A	—	—	—	—	—	—	—	—	A
48Hr	05-11-23 MW 1100	A	—	—	—	—	—	—	—	—	A
72Hr	5-12-23 DT 1435	A	4	5	A	A	3	A	4	A	5
96Hr	05-13-23 MW 1210	8	8	10	8	10	A	7	9	9	10
5 days	5-14-23 DT 1314	A	A	A	A	A	10	A	A	A	A
6 days	5-15-23 MH 1520	9	A	A	11	4	5	4	A	5	A
7 days	5-16-23 DT 1102	A	9	4	14	7	6	13	6	12	11
8 days											

Dilution: PCON

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

E* = empty-sac

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: **86882** Culture No.:

TEST INSTRUCTIONS:

Dilution: 32 %

	1	2	3	4	5	6	7	8	9	10
24Hr	A	—	—	—	—	—	—	—	—	A
48Hr	A	—	—	—	—	—	—	—	—	A
72Hr	4	A	A	3	4	5	A	A	A	3
96Hr	A	A	A	9	8	10	A	A	10	8
5 days	10	7	8	8	9	7	8	6	A	A
6 days	7	4	7	9	15	12	8	9	6	17
7 days	8	8	9	10	13	10	7	11	8	8
8 days										

Dilution: 45 %

	1	2	3	4	5	6	7	8	9	10
24Hr	A	—	—	—	—	—	—	—	—	A
48Hr	A	—	—	—	—	—	—	—	—	A
72Hr	A	A	4	A	A	3	A	5	A	3
96Hr	A	8	A	7	7	9	A	10	A	A
5 days	5/A	—	—	—	—	—	—	—	A	6
6 days	14	3	7	6	13	A	13	12	8	9
7 days	8	16	18	10	13	10	12	11	12	A
8 days										

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: **86882** Culture No.:

TEST INSTRUCTIONS:

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	9	6	8	4	4	7	A
5 days	6	A	8	5	8	A	—	—	A	9/6
6 days	11	8	9	A	11	A	10	9	8	8
7 days	7	10	A	9	A	11	8	11	6	15
8 days										

Dilution: 80 %

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

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: **86882** Culture No.:

TEST INSTRUCTIONS:

Dilution: 100 %

	1	2	3	4	5	6	7	8	9	10
24Hr	A	—	—	—	—	—	—	—	—	A
48Hr	A	—	—	—	—	—	—	—	—	—
72Hr	A	—	—	—	—	—	—	—	—	A
96Hr	9	7	4	11	9	10	9	11	8	9
5 days	A	—	—	—	—	—	—	—	—	A
6 days	5	11	10	9	17	15	11	A	15	14
7 days	10	11	12	A	4	(12)	A	13	16	15
8 days										

Dilution:

	1	2	3	4	5	6	7	8	9	10
24Hr										
48Hr										
72Hr										
96Hr										
5 days										
6 days										
7 days										
8 days										

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: **86882** Culture No.:

TEST INSTRUCTIONS:

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
TCON	21.3	25.6	24.9	25.2	15.0	25.6	22.6	25.2	15.0	25.6	25.1	25.5	25.4	25.6
PCON	25.2						21.6				21.6			
32	24.8										15.0			
45														
56														
80														
100														
TIME/DATE TECH	5-9-23 MW 1155	5-10	-23 MH 1430	05-11	-23 MW 1100	5-12-	23 DT 1435	05-13	-23 MW 1210	5.14-	23 DT 1314	5-15-	23 MH 1520	5-16-23 DT 1102
IR GUN ID #	012	012		012		021		012		021		021		021 DT

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: 86882

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: 14:12 Test End Time: 12:45

Begin Date: 5/9/2023

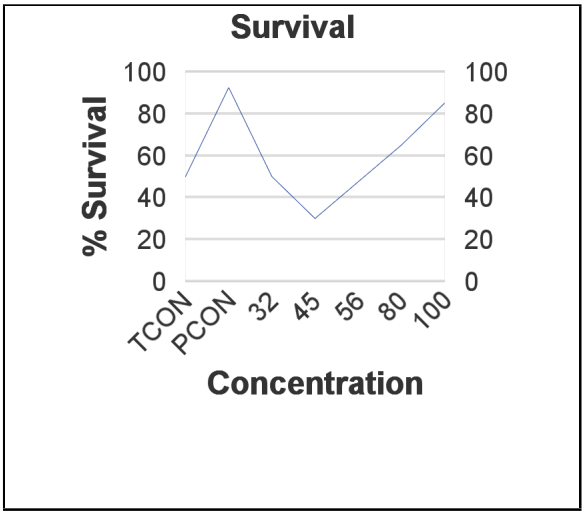
End Date: 5/16/2023

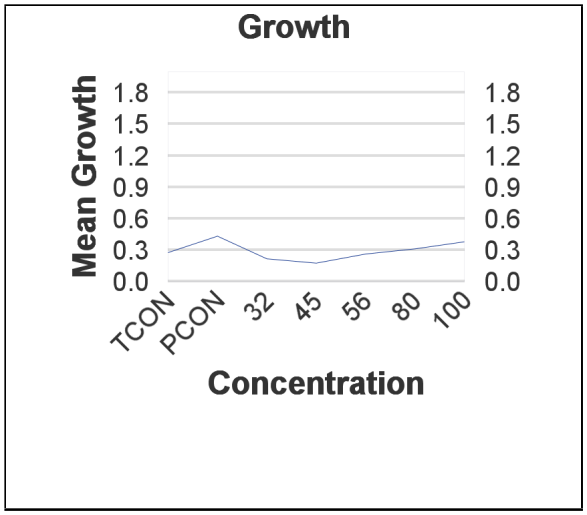
SURVIVAL

Effluent Concentration		Number Of Alive 5/9	5/10	5/11	5/12	5/13	5/14	5/15	5/16	Avg% Surv.
TCON	A	8	8	8	8	5	5	5	5	50.0%
	B	8	8	8	8	6	6	6	6	
	C	8	8	8	8	8	8	6	5	
	D	8	8	8	6	0	0	0	0	
	E	8	8	4	4	4	4	4	4	
PCON	A	8	8	8	7	7	7	7	7	92.5%
	B	8	8	8	8	8	8	8	8	
	C	8	8	8	8	8	8	7	7	
	D	8	8	8	8	8	8	7	7	
	E	8	8	8	8	8	8	8	8	
32	A	8	8	8	7	7	7	7	7	50.0%
	B	8	8	8	8	8	7	7	7	
	C	8	8	8	8	8	4	4	4	
	D	8	8	8	6	2	2	2	2	
	E	8	8	8	8	3	0	0	0	
45	A	8	8	8	8	8	6	5	4	30.0%
	B	8	8	8	8	7	6	5	5	
	C	8	8	8	6	4	3	3	2	
	D	8	8	8	6	2	1	1	1	
	E	8	8	8	5	4	2	0	0	

Effluent Concentration		Number Of Alive 5/9	5/10	5/11	5/12	5/13	5/14	5/15	5/16	Avg% Surv.
56	A	8	8	8	7	2	1	1	1	47.5%
	B	8	8	8	8	7	5	5	5	
	C	8	8	8	8	8	6	6	6	
	D	8	8	8	6	6	6	6	6	
	E	8	8	8	4	2	1	1	1	
80	A	8	8	8	5	4	4	4	4	65.0%
	B	8	8	8	3	7	6	6	6	
	C	8	8	8	8	7	6	6	6	
	D	8	8	8	7	7	7	7	7	
	E	8	8	7	7	6	5	3	3	
100	A	8	8	8	7	7	7	7	7	85.0%
	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	7	5	4	4	4	

Concentration Response Relationships





BIO-AQUATIC TESTING, INC.

Chronic Pimephales promelas SURVIVAL Lab ID: **86882**

Client: **Mena, City of** Facility **WWTP** Outfall:001

Sample Type: Composite

TEST INSTRUCTIONS:

Culture No. : _____ Photo Period: 16hr light, 8hr dark **RANDOMIZATION:** **RS-5 1**

Dilution: TCON

DATE/TIME/ TECHNICIAN	A	B	C	D	E
0Hr 5/9/23 14:12 ZC	8	—	—	—	—
24Hr 5/10/23 1110 RR	8	—	—	—	—
48Hr 5/11/23 1140 RR	8	—	—	—	4_4
72Hr 5/12/23 1155 MV	8	—	—	6_2	4
96Hr 5/13/23 0830 AR	5_3	6_2	8	0_6	4
5 days 5-14-23 0913 AR	5	6	8	|	4
6 days 5/15/23 0906 AR	5	6	6_2	|	4
7 days 5/16/23 1245 MV	5	6	5_1	|	4

Dilution: PCON

	A	B	C	D	E
0Hr	8	—	—	—	—
24Hr	8	—	—	—	—
48Hr	8	—	—	—	—
72Hr	7_1	8	—	—	—
96Hr	7	8	—	—	—
5 days	7	8	—	—	—
6 days	7	8	7_1	7_1	8
7 days	7	7	7	7	8

Dilution: 32

	A	B	C	D	E
0Hr	8	—	—	—	—
24Hr	8	—	—	—	—
48Hr	8	—	—	—	—
72Hr	7_1	8	7	6_4	8
96Hr	7	8	8	2_4	3_5
5 days	7	7_1	4_4	2	0_3
6 days	7	7	4	2	|
7 days	7	7	4	2	|

Dilution: 45

	A	B	C	D	E
0Hr	8	—	—	—	—
24Hr	8	—	—	—	—
48Hr	8	—	—	—	—
72Hr	8	8	6	6_2	5_3
96Hr	8	7_1	4_2	2_4	4_1
5 days	6_2	6_1	3_1	1_1	2_2
6 days	5_1	5_1	3	1	0_2
7 days	4_1	5	2	1	1

Dilution: 56

	A	B	C	D	E
0Hr	8	—	—	—	—
24Hr	8	—	—	—	—
48Hr	8	—	—	—	—
72Hr	7_1	8	8	6_2	4_4
96Hr	2_5	7_1	8	6	2_2
5 days	1_1	5_2	6_2	6	1_1
6 days	1	5	6	6	1
7 days	1	5	6	6	1

Dilution: 80

	A	B	C	D	E
0Hr	8	—	—	—	—
24Hr	8	—	—	—	—
48Hr	8	—	—	—	7_1
72Hr	5_2	8	8	7_1	7
96Hr	4_1	7_1	7_1	7	6_1
5 days	4	6_1	6_1	7	5_1
6 days	4	6	6	7	3_2
7 days	4	6	6	7	3

Dilution: 100

	A	B	C	D	E
0Hr	8	—	—	—	—
24Hr	8	—	—	—	—
48Hr	8	—	—	—	—
72Hr	7	8	8	7	7
96Hr	7	8	8	7	5_2
5 days	7	8	8	7	4_1
6 days	7	8	8	7	4
7 days	7	8	8	7	4

Dilution: _____

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

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BIO-AQUATIC TESTING, INC.

Chronic Pimephales promelas SURVIVAL Lab ID: **86882**

Client: **Mena, City of** Facility **WWTP** Outfall:001

Sample Type: Composite

TEST INSTRUCTIONS:

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
TCON	25.2	25.0	24.9	25.0	24.9			25.1	24.9	25.2	24.7	24.9	24.6	
PCON														
32														
45														
56														
80														
100														
TIME/DATE TECH	5/9/23 1412 ZC	5/10/23 1110 RR		5/11/23 RR	1740	5/12/23 1155	MM	5/13/23 0930	AR	5-14-23 0913	AR	5/15/23 0906	AR	5/16/23 1209
IR GUN ID #	020	020		020		020		024		024		024		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: 86882**

Permit Number: AR0036692

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

Receiving Water Name: Prairie Creek

True Control

	ON	SN	Wt.	Avg.	SN Avg.
A	8	5	3.215	0.402	0.643
B	8	6	3.010	0.376	0.502
C	8	5	2.450	0.306	0.490
D	8	0	0	0.000	?
E	8	4	2.319	0.290	0.580

Mean	C.V. %
0.275	58.4

SN Mean	SN C.V. %
0.554	12.9

Performance Control

	ON	Wt.	Avg.
A	8	3.213	0.402
B	8	3.676	0.460
C	8	3.589	0.449
D	8	2.965	0.371
E	8	3.797	0.475

Mean	C.V. %
0.431	10.1

32

	ON	Wt.	Avg.
A	8	2.511	0.314
B	8	2.619	0.327
C	8	2.200	0.275
D	8	1.275	0.159
E	8	0.00	0.000

Mean	C.V. %
0.215	63.8

45

	ON	Wt.	Avg.
A	8	2.543	0.318
B	8	2.821	0.353
C	8	0.832	0.104
D	8	0.757	0.095
E	8	0.00	0.000

Mean	C.V. %
0.174	88.2

56

	ON	Wt.	Avg.
A	8	0.123	0.015
B	8	3.398	0.425
C	8	3.071	0.384
D	8	3.032	0.379
E	8	0.805	0.101

Mean	C.V. %
0.261	72.2

80

	ON	Wt.	Avg.
A	8	1.700	0.213
B	8	2.998	0.375
C	8	3.104	0.388
D	8	2.838	0.355
E	8	1.759	0.220

Mean	C.V. %
0.310	27.9

100

	ON	Wt.	Avg.
A	8	3.140	0.393
B	8	3.158	0.395
C	8	3.402	0.425
D	8	3.207	0.401
E	8	2.190	0.274

Mean	C.V. %
0.377	15.7

	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: 86882

Client: Mena, City of - WWTP

Balance: Radwag BAL-007

Begin Date: 5/9/2023 End Date: 5/16/2023 Organism: Pimephales promelas

Analyst: SDT

Weigh Date: 5/18/23

Date/Time placed in Oven: 5-16-23/1336

Date/Time removed from Oven: 5-17-23/1336

TCON

	Qty.	Wt.
A	5	3.215
B	6	3.010
C	5	2.450
D	0	0.000
E	4	2.319

PCON

	Qty.	Wt.
A	7	3.213
B	8	3.676
C	7	3.589
D	7	2.965
E	8	3.797

32 %

	Qty.	Wt.
A	7	2.511
B	7	2.619
C	4	2.200
D	2	1.275
E	0	0.000

45 %

	Qty.	Wt.
A	4	2.543
B	5	2.821
C	2	0.832
D	1	0.757
E	0	0.000

56 %

	Qty.	Wt.
A	1	0.123
B	5	3.398
C	6	3.071
D	6	3.032
E	1	0.805

80 %

	Qty.	Wt.
A	4	1.700
B	6	2.998
C	6	3.104
D	7	2.838
E	3	1.759

100 %

	Qty.	Wt.
A	7	3.140
B	8	3.158
C	8	3.402
D	7	3.207
E	4	2.190

	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
File: 86882.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	4	15	25	10	6

Calculated Chi-Square goodness of fit test statistic = 2.5870
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

cerio repro
File: 86882.cdr Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 4.85

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.

cerio repro
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	145.083	29.017	1.246
Within (Error)	54	1257.100	23.280	
Total	59	1402.183		

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

cerio repro
File: 86882.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	tcon	23.000	23.000		
2	32	24.500	24.500	-0.695	
3	45	25.200	25.200	-1.020	
4	56	22.900	22.900	0.046	
5	80	25.200	25.200	-1.020	
6	100	27.500	27.500	-2.085	

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

cerio repro
File: 86882.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	tcon	10			
2	32	10	4.984	21.7	-1.500
3	45	10	4.984	21.7	-2.200
4	56	10	4.984	21.7	0.100
5	80	10	4.984	21.7	-2.200
6	100	10	4.984	21.7	-4.500

fathead survival
File: 86882.pps Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 104.800

W = 0.959

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 survival
File: 86882.pps Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 8.93

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 survival
File: 86882.pps Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	91.067	18.213	4.171
Within (Error)	24	104.800	4.367	
Total	29	195.867		

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

fathead survival
File: 86882.pps 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	pcon	7.400	7.400		
2	32	4.000	4.000	2.573	*
3	42	2.400	2.400	3.783	*
4	56	3.800	3.800	2.724	*
5	80	5.200	5.200	1.665	
6	100	6.800	6.800	0.454	

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

fathead survival
File: 86882.pps 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	pcon	5			
2	32	5	3.119	42.1	3.400
3	42	5	3.119	42.1	5.000
4	56	5	3.119	42.1	3.600
5	80	5	3.119	42.1	2.200
6	100	5	3.119	42.1	0.600

fathead growth
File: 86882.ppg Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.051

W = 0.877

Critical W (P = 0.05) (n = 15) = 0.881
Critical W (P = 0.01) (n = 15) = 0.835

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

fathead growth
File: 86882.ppg Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 1.68

Table Chi-square value = 9.21 (alpha = 0.01, df = 2)
Table Chi-square value = 5.99 (alpha = 0.05, df = 2)

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

fathead growth
File: 86882.ppg Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	2	0.037	0.018	4.301
Within (Error)	12	0.051	0.004	
Total	14	0.088		

Critical F value = 3.89 (0.05,2,12)
Since F > Critical F REJECT Ho: All equal

fathead growth
File: 86882.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	pcon	0.431	0.431		
2	80	0.310	0.310	2.927	*
3	100	0.378	0.378	1.299	

Dunnett table value = 2.11 (1 Tailed Value, P=0.05, df=12,2)

fathead growth
File: 86882.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	pcon	5			
2	80	5	0.087	20.3	0.121
3	100	5	0.087	20.3	0.054

Bio-Aquatic Testing, Inc.

FRESH WATER TEST SETUP FORM

Client: Mena, City of

Permit AR0036692

Facility: WWTP

Lab Number 86882

Outfall Name: 001

Number of samples 3

Dilution Water: Receiving Stream

Receiving Water Name: Prairie Creek

Dechlorinate Sample: No

Sx #	Rcvd Date	Rcvd Time	Sampling Dates		Sampling Times	
			Begin Date	End Date	Start	End
1	05/08/23	10:00	05/07/23	05/08/23	08:00	08:00
2	05/10/23	10:00	05/09/23	05/10/23	08:00	08:00
3	05/12/23	10:00	05/11/23	05/12/23	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	32	10
2	30	18
3	33	14

Start Sx #	1	Date:	5/9/2023
Renew Sx #	1	Date:	5/10/2023
Renew Sx #	1	Date:	5/11/2023
Renew Sx #	2	Date:	5/12/2023
Renew Sx #	3/2	Date:	5/13/2023
Renew Sx #	3	Date:	5/14/2023
Renew Sx #	3	Date:	5/15/2023

Test Start Date: 5/9/2023

Test End Date: 5/16/2023

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:** 86882

Facility: WWTP **Outfall:** 001

Dilution Water(s): Receiving Stream **Test Date:** May 9, 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	5/8/23	10:00	<0.10	N/A	<0.25	JP	4.0
2	5/10/23	10:00	<0.10	N/A	<0.25	JP	3.8
3	5/12/23	10:00	<0.10	N/A	<0.25	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	10.6	45	15	106	JP
2	7.4	10.4	40	11	129	JP
3	6.3	10.4	49	8	102	JP

DAILY RENEWAL CONDUCTIVITY**

Date		Sample #	Values are at Highest Dilution		Analyst
			Specific Conductivity as umhos/cm	Salinity (ppt)	
5/9	TCON		76	0.1	GS
5/10	TCON		46	0.1	IC
5/11	TCON		69	0.1	RR
5/12	TCON		66	0.1	MM
5/13	TCON		82	0.1	IC/JC
5/14	TCON		46	0.1	AR/IC
5/15	TCON		48	0.1	JR/GS
5/9	OUTFALL*	1	114	0.1	GS
5/10	OUTFALL*	1	110	0.1	IC
5/11	OUTFALL*	1	113	0.1	RR
5/12	OUTFALL*	2	137	0.1	MM
5/13	OUTFALL*	3/2	145	0.1	IC/JC
5/14	OUTFALL*	3	107	0.1	AR/IC
5/15	OUTFALL*	3	108	0.1	JR/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: 86882

Facility: WWTP

Dilution Water(s): Receiving Stream

Outfall: 001

Test Begin Date: May 9, 2023

NR indicates that the test is non-renewal.

					Concentration							
ANALYST	DATE	TIME	SX#	UNIT	TCON	32	45	56	80	100		
GS	5/9	Start	1	pH	8.1	7.8	7.6	7.6	7.3	7.3		
		25 ± 1		DO (mg/L)	8.4	8.3	8.4	8.4	8.4	8.4		
IC	5/10	24 Hr	1	pH	8.2	8.2	8.2	8.0	8.0	7.7		
		25 ± 1		DO (mg/L)	8.0	7.9	7.9	8.0	8.0	8.0		
		Renew	1	pH	6.9	6.8	6.8	6.6	6.6	6.5		
				DO (mg/L)	8.2	8.3	8.3	8.5	8.5	8.6		
RR	5/11	48 Hr	1	pH	8.1	7.9	7.8	7.7	7.6	7.6		
		25 ± 1		DO (mg/L)	7.7	7.7	7.9	7.9	7.9	7.9		
		Renew	1	pH	8.0	7.8	7.5	7.4	7.2	7.2		
				DO (mg/L)	8.0	8.3	8.5	8.6	8.6	8.6		
MM	5/12	72 Hr	1	pH	8.0	7.8	7.7	7.5	7.5	7.4		
		25 ± 1		DO (mg/L)	8.1	8.1	8.1	8.0	8.0	7.9		
		Renew	2	pH	7.1	6.9	6.9	6.9	6.9	6.8		
				DO (mg/L)	8.3	8.2	8.4	8.5	8.5	8.6		
IC/JC	5/13	96 Hr	2	pH	7.7	7.5	7.5	7.3	7.3	7.2		
		25 ± 1		DO (mg/L)	7.9	7.9	7.9	7.9	7.9	7.8		
		Renew	3/2	pH	7.1	7.0	7.0	6.8	6.8	6.7		
				DO (mg/L)	8.4	8.6	8.8	9.1	9.1	9.2		
AR/IC	5/14	120 Hr	3/2	pH	7.6	7.4	7.4	7.2	7.2	7.1		
		25 ± 1		DO (mg/L)	7.9	7.9	7.9	7.9	7.9	7.9		
		Renew	3	pH	6.3	6.2	6.2	6.2	6.2	6.2		
				DO (mg/L)	8.8	9.0	9.0	9.2	9.2	9.4		
AR/IC	5/15	144 Hr	3	pH	8.1	7.8	7.8	7.5	7.5	7.3		
		25 ± 1		DO (mg/L)	8.0	8.0	8.0	8.0	8.0	8.0		
		Renew	3	pH	6.6	6.5	6.5	6.5	6.5	6.4		
				DO (mg/L)	8.7	8.9	8.9	9.0	9.0	9.2		
JR/GS	5/16	168 Hr	3	pH	7.5	7.4	7.3	7.3	7.2	7.1		
		25 ± 1		DO (mg/L)	7.6	7.5	7.5	7.6	7.6	7.7		

BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen

Chronic **Pimephales promelas**

Client: Mena, City of
Facility: WWTP
Outfall: 001

Lab Number: 86882
Dilution Water(s): Receiving Stream
Test Begin Date: May 9, 2023

NR indicates that the test is non-renewal.

ANALYST	DATE	TIME	SX#	UNIT	Concentration							
					TCON	32	45	56	80	100		
GS	5/9	Start	1	pH	8.1	7.8	7.6	7.6	7.3	7.3		
		25 ± 1		DO (mg/L)	8.4	8.3	8.4	8.4	8.4	8.4		
IC	5/10	24 Hr	1	pH	6.9	6.9	6.9	6.8	6.8	6.7		
		25 ± 1		DO (mg/L)	7.8	7.7	7.7	7.7	7.7	7.7		
		Renew	1	pH	6.9	6.8	6.8	6.6	6.6	6.5		
				DO (mg/L)	8.2	8.3	8.3	8.5	8.5	8.6		
RR	5/11	48 Hr	1	pH	7.3	7.2	7.1	7.0	6.9	6.9		
		25 ± 1		DO (mg/L)	7.8	7.4	7.1	7.0	7.2	7.2		
		Renew	1	pH	8.0	7.8	7.5	7.4	7.2	7.2		
				DO (mg/L)	8.0	8.3	8.5	8.6	8.6	8.6		
MM	5/12	72 Hr	1	pH	7.8	7.6	7.4	7.3	7.2	7.0		
		25 ± 1		DO (mg/L)	8.2	7.8	7.7	7.5	7.5	7.5		
		Renew	2	pH	7.1	6.9	6.9	6.9	6.9	6.8		
				DO (mg/L)	8.3	8.2	8.4	8.5	8.5	8.6		
IC/JC	5/13	96 Hr	2	pH	7.3	7.2	7.2	7.0	7.0	6.9		
		25 ± 1		DO (mg/L)	7.7	7.5	7.5	7.5	7.5	7.5		
		Renew	3/2	pH	7.1	7.0	7.0	6.8	6.8	6.7		
				DO (mg/L)	8.4	8.6	8.8	9.1	9.1	9.2		
AR/IC	5/14	120 Hr	3/2	pH	6.1	6.2	6.2	6.3	6.3	6.3		
		25 ± 1		DO (mg/L)	8.5	7.9	7.9	7.5	7.5	7.4		
		Renew	3	pH	6.3	6.2	6.2	6.2	6.2	6.2		
				DO (mg/L)	8.8	9.0	9.0	9.2	9.2	9.4		
AR/IC	5/15	144 Hr	3	pH	6.7	6.6	6.6	6.6	6.6	6.4		
		25 ± 1		DO (mg/L)	8.2	7.6	7.6	7.5	7.5	7.4		
		Renew	3	pH	6.6	6.5	6.5	6.5	6.5	6.4		
				DO (mg/L)	8.7	8.9	8.9	9.0	9.0	9.2		
JR/GS	5/16	168 Hr	3	pH	7.8	7.6	7.3	7.3	7.0	7.0		
		25 ± 1		DO (mg/L)	7.8	7.6	7.4	7.4	7.4	7.4		

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.

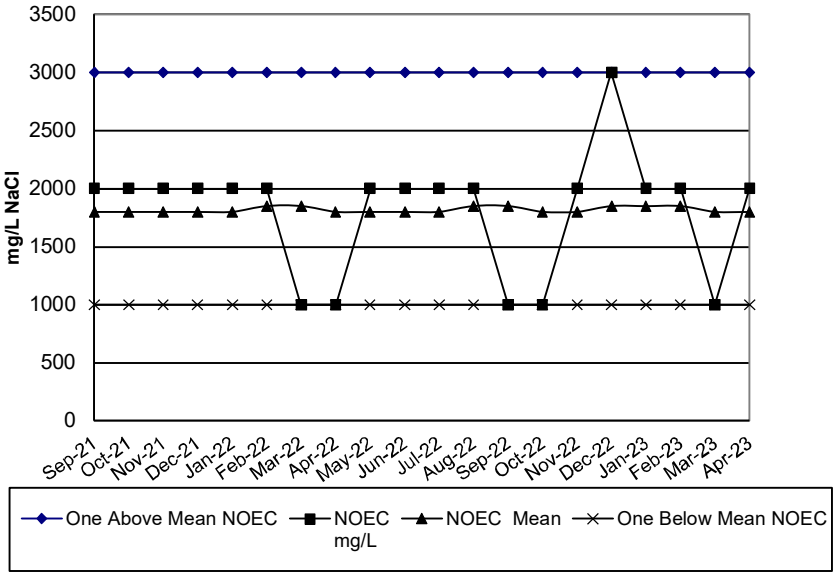
CHRONIC REFERENCE TOXICANT TEST RESULTS

DILUTION WATER:	Standard Synthetic Freshwater						
CHEMICAL:	Sodium Chloride						
DURATION:	3-Brood Chronic						
TEST NUMBER:	338						
PROJECT NUMBER:	86827						
START DATE:	4/25/2023						
START TIME:	16:15						
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	1	10	10
TEST METHODS:	As listed in EPA-821-R-02-013						
STATISTICAL METHODS:	SURVIVAL: Fisher's Exact Test REPRODUCTION: ANOVA-Dunnetts						

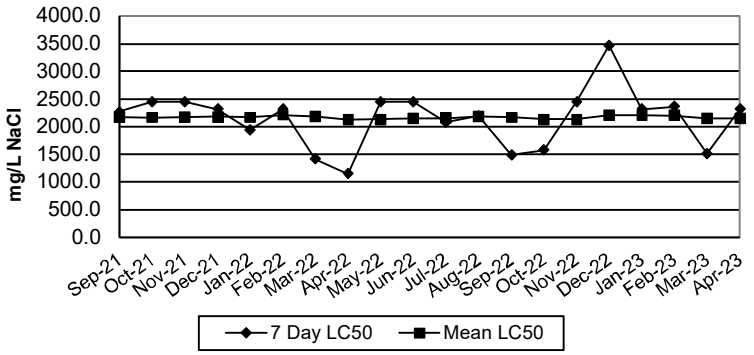
NOEC FOR SURVIVAL:	2000	mg/L
LOEC FOR SURVIVAL:	3000	mg/L
NOEC FOR REPRODUCTION:	500	mg/L
LOEC FOR REPRODUCTION:	1000	mg/L

PMSD: 20.6

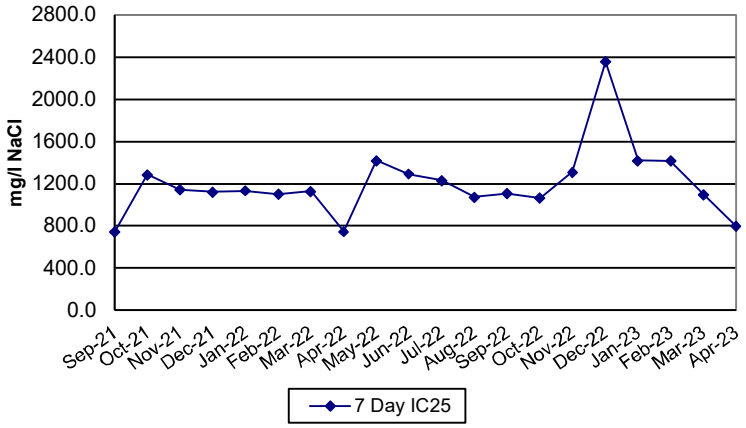
Ceriodaphnia Chronic Survival Control Chart



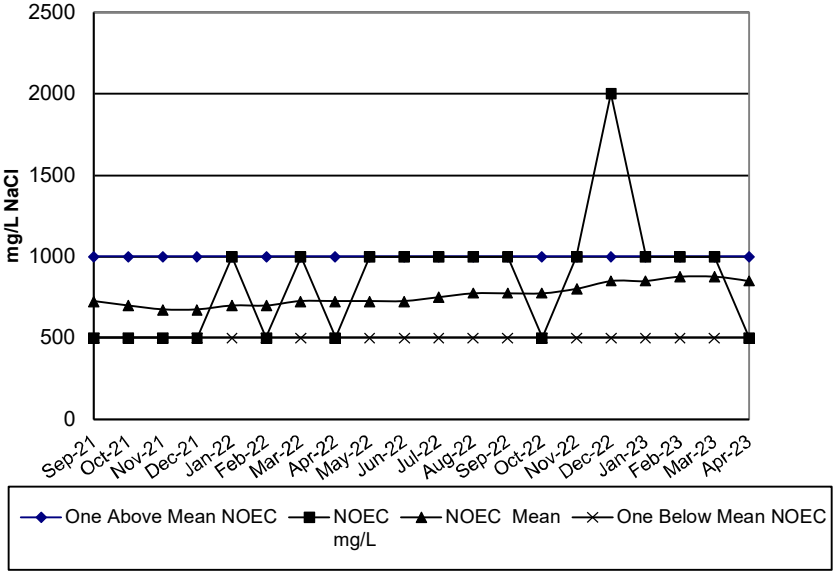
Ceriodaphnia 7-Day LC50



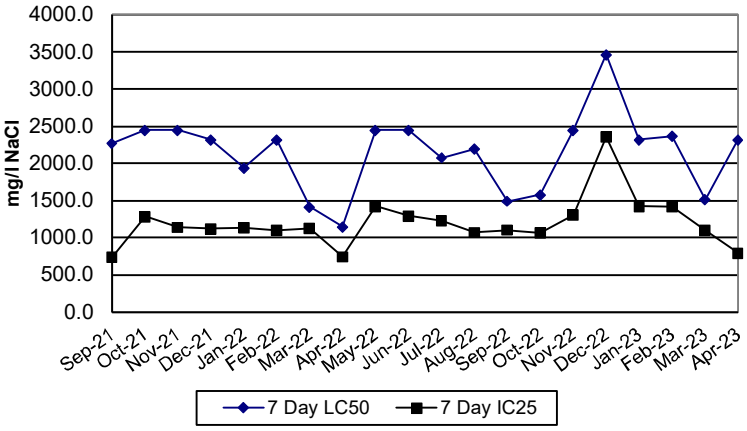
Ceriodaphnia 7-Day IC25



Ceriodaphnia Chronic Reproduction Control Chart



Ceriodaphnia 7-Day LC50 & IC25



Appendix B

Pimephales promelas

BIO-AQUATIC TESTING, INC.

Carrollton, TX

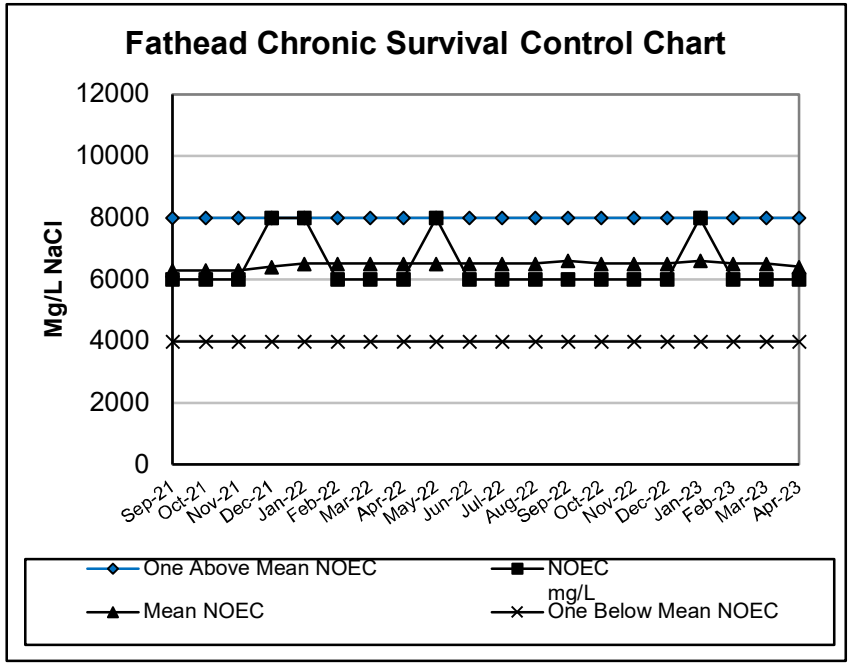
REFERENCE TOXICANTS

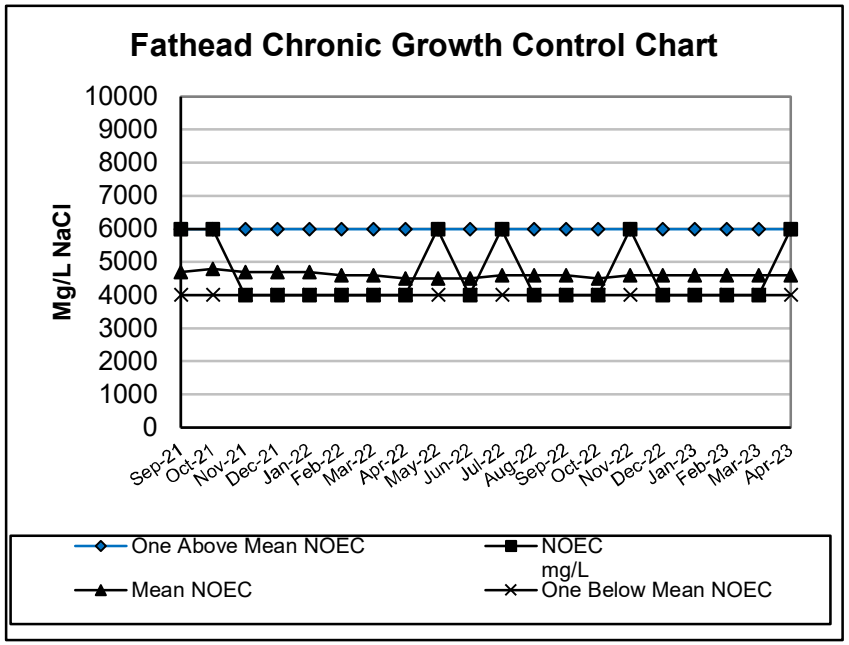
Bio-Aquatic Testing conducts reference toxicant testing monthly for organisms cultured in-house. For studies requiring purchased organisms, reference toxicant testing is performed simultaneously. Reference toxicant testing validates data and measures organism consistency. Only reagent grade chemicals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

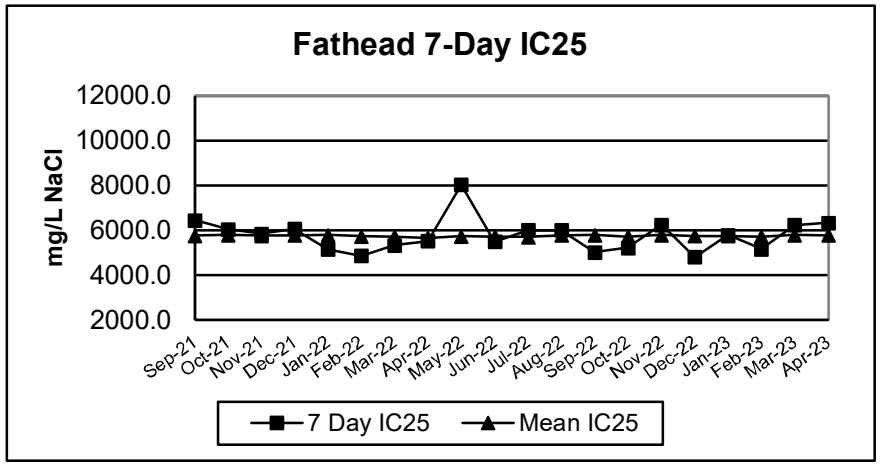
CHRONIC REFERENCE TOXICANT TEST RESULTS

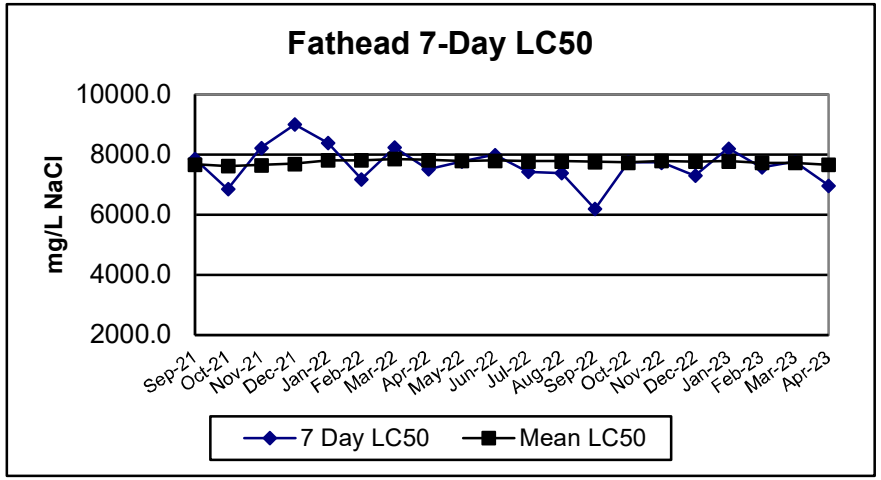
DILUTION WATER:	Standard Synthetic Freshwater						
CHEMICAL:	Sodium Chloride						
DURATION:	7 Days						
TEST NUMBER:	378						
PROJECT NUMBER:	86825						
START DATE:	4/26/2023						
START TIME:	17:25						
TOTAL NUMBER EXPOSED:	40 organisms per concentration						
CONCENTRATIONS (mg/L):	CON	2000	4000	6000	8000	10000	12000
NUMBER DEAD PER CONCENTRATION:	3	0	0	7	31	40	40
TEST METHODS:	As listed in EPA-821-R-02-013						
STATISTICAL METHODS:	SURVIVAL: Steel's Many-One Rank Test GROWTH: ANOVA-Dunnetts w/Bonf. Adj.						
NOEC FOR SURVIVAL:	6000 mg/L						
LOEC FOR SURVIVAL:	8000 mg/L						
NOEC FOR GROWTH:	6000 mg/L						
LOEC FOR GROWTH:	8000 mg/L						

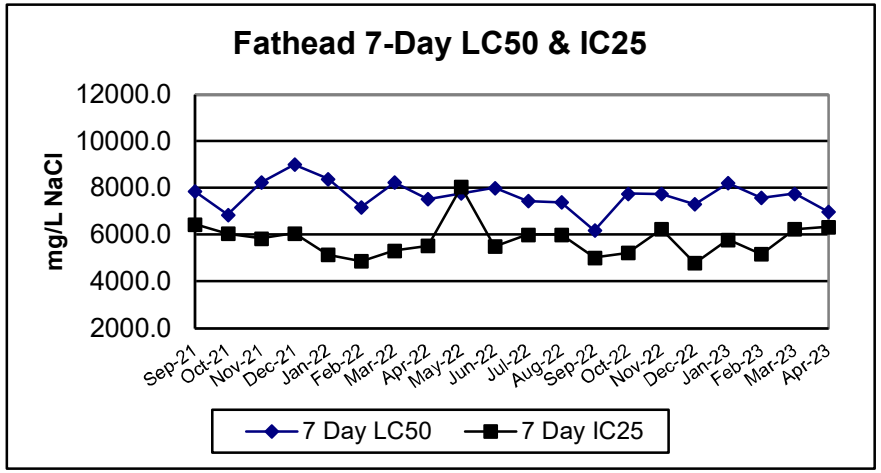
PMSD: 25.6


Fathead Chronic Survival Control Chart
Mg/L NaCl
12000
10000
8000
6000
4000
2000
0
Sep-21 Oct-21 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
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
Sep-21 Oct-21 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
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
Sep-21 Oct-21 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
7 Day IC25
Mean IC25


Fathead 7-Day LC50
mg/L NaCl
10000.0
8000.0
6000.0
4000.0
2000.0
Sep-21 Oct-21 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
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
Sep-21 Oct-21 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
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.

U.S.E.P.A., 2012. Tropical Collector Urchin, *Tripneustes gratilla* (First Edition) U.S. Environmental Protection Agency, Office of Research and Development and Region 9, EPA-600-R-12-022.

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 : 86882

Revision 2 Effective Date 9/25/2017

Please Fill Out C-O-C by Completing Sections A, B, & C.

P.O. No:

Client: Mena, City of

Facility: WWTP

Permit No: AR0036692 **Outfall:** 001

Client Contact: MIKE SPENCER

Client Phone: 479-234-2592

A. SCHEDULED TEST(s):

Chronic	Ceriodaphnia dubia
Chronic	Pimephales promelas

Dilution Series: 32 45 56 80 100

Include Semi-annual 24hr Acute Test? No

To Ship the 1st Sample on: 5/8/2023

A.1 Check Sample # For Chronic and 48 Hour Acute Tests : ____ First, ____ Second, or ____ Third.

Check the type of test(s) required, if different from the Scheduled Test(s) in "A":

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

Notes: 2nd Qtr -CH

B.

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	8 May	8 May	0800	0900	Comp		1
2 Prairie Ck	RS	7 May		0800		Grab		1
3								

C.

	Relinquished By:	Date	Time	Received By:	Date	Time
1	L Hopper	8 May 23	1000	Rance Barnes	5-8-23	1000
2	Rance Parnell	5-8-23	1630	Amy Ponyne	5/9/23	1045
3						

Huther Sample Login

Date/ Time: 5/9/23 1045 Tech: AP IR Gun#: 47.0

Dilution Water: ◉ Receiving Stream ○ Synthetic Lab

Dechlor.: ○ Yes ◉ No

Effluent Parameters:

Temp: 4.0 (C)	pH: 6.6		Hd: 45 mg/l		
Cl_2: <0.1 mg/l	DO: 10.6 mg/l		Alk: 15 mg/l		
NH3: Ammonia <0.25 mg/l	Int. Salinity/ Conductivity: 106 ppt/uS		Adj. Sal: ppt		
Condition: colormetric	Other:				

Receiving Stream Parameters:

Temp: 3.9 (C)	pH: 6.7		Hd: 32 mg/l (LR)		
Cl_2: <0.1 mg/l	DO: 9.4 mg/l		Alk: 10 mg/l (OK)		
NH3: Ammonia <0.25 mg/l	Int. Salinity/ Conductivity: 39 ppt/uS		Adj. Sal: ppt		
Condition: good	Other:				

Bio-Aquatic Lab ID: 86882

35 of 42

Report Date: 06/05/2023 Revision 0

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 : 86882

Revision 2 Effective Date 9/25/2017

Please Fill Out C-O-C by Completing Sections A, B, & C. **P.O. No:**

Client: Mena, City of

Facility: WWTP

Permit No: AR0036692 **Outfall:** 001

Client Contact: MIKE SPENCER

Client Phone: 479-234-2592

A. SCHEDULED TEST(s):

Chronic	Ceriodaphnia dubia
Chronic	Pimephales promelas

To Ship the 1st Sample on: 5/8/2023

Dilution Series: 32 45 56 80 100

Include Semi-annual 24hr Acute Test? No

A.1

Check Sample # For Chronic and 48 Hour Acute Tests : _____ First, _____ Second, or _____ Third.

Check the type of test(s) required, if different from the Scheduled Test(s) in "A":

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

Notes: 2nd Qtr -CH

B.

	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	E	MAY 9 2023	MAY 10 2023	0800	0800	Comp	M Spencer	1
2	PRAIRIE CK	RS	7 MAY 2023			0800	GRAB	M Spencer	1
3									

C.

	Relinquished By:	Date	Time	Received By:	Date	Time
1	L Hopper	10MAY23	1000	Rance Blow	5/10/23	1000
2	[illegible]ott	5-10-23	1800	Troy Payne	5/11/23	1405
3						

Huther Sample Login

Date/Time: 5/11/23 1405 **Tech:** JP **IR Gun#:** 002

Dilution Water: ◉ Receiving Stream ○ Synthetic Lab

Dechlor.: ○ Yes ◉ No

Effluent Parameters:

Temp: 3.8 (C)	pH: 7.4	Hd: 40 mg/l
Cl_2^-: <0.1 mg/l	DO: 10.4 mg/l	Alk: 11 mg/l
NH3 (Ammonia): <0.25 mg/l	Int. Salinity/Conductivity: 129 ppt/uS	Adj. Sal: ppt
Condition: good	Other:	

Receiving Stream Parameters:

Temp: 3.9 (C)	pH: 7.2	Hd: 30 mg/l (LR)
Cl_2^-: <0.1 mg/l	DO: 10.0 mg/l	Alk: 18 mg/l (OK)
NH3 (Ammonia): <0.25 mg/l	Int. Salinity/Conductivity: 45 ppt/uS	Adj. Sal: ppt
Condition: good	Other:	

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

CHAIN OF COUSTODY

☐ Huther Only: No Sample Left

Lab Id : 86882

Revision 2 Effective Date 9/25/2017

Please Fill Out C-O-C by Completing Sections A, B, & C.

P.O. No:

Client: Mena, City of

Facility: WWTP

Permit No: AR0036692 **Outfall:** 001

Client Contact: MIKE SPENCER

Client Phone: 479-234-2592

A. SCHEDULED TEST(s):

Chronic	Ceriodaphnia dubia
Chronic	Pimephales promelas

Dilution Series: 32 45 56 80 100

To Ship the 1st Sample on: 5/8/2023

Include Semi-annual 24hr Acute Test? No

A.1 Check Sample # For Chronic and 48 Hour Acute Tests : ____ First, ____ Second, or ____ Third.

Check the type of test(s) required, if different from the Scheduled Test(s) in "A":

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

Notes: 2nd Qtr -CH

B.

	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	11MAY23	12MAY23	0800	0800	Comp	[signature]	1
2	PRAIRIE CK	RS	07MAY23		0800		GRAB	[signature]	1
3									

C.

	Relinquished By:	Date	Time	Received By:	Date	Time
1	[signature]	12MAY	1000	RANCE @ BTB	12MAY	1000
2	Rance Darrott	5-12-23	1700	[signature]	5/13/23	1055
3						

Huther Sample Login

Date/ 5/13/23 Time: 1055 Tech: JP IR Gun#: 002

Dilution Water: ◉ Receiving Stream ○ Synthetic Lab

Dechlor. ○ Yes ◉ No

Effluent Parameters:

Temp: 8.8 (C)	pH: 6.3	Hd: 49 mg/l
Cl_2^-: <0.1 mg/l	DO: 10.4 mg/l	Alk: 8 mg/l
NH3 (Ammonia): <0.25 mg/l	Int. Salinity/Conductivity: 102 ppt/uS	Adj. Sal: ppt
Condition: good	Other:	

Receiving Stream Parameters:

Temp: 8.9 (C)	pH: 6.5	Hd: 33 mg/l (LR)
Cl_2^-: <0.1 mg/l	DO: 10.3 mg/l	Alk: 14 mg/l (OK)
NH3 (Ammonia): <0.25 mg/l	Int. Salinity/Conductivity: 39 ppt/uS	Adj. Sal: ppt
Condition: good	Other:	

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:	5/7/2023 @ 08:00	TO:	5/8/2023 @ 08:00
	FROM:	5/9/2023 @ 08:00	TO:	5/10/2023 @ 08:00
	FROM:	5/11/2023 @ 08:00	TO:	5/12/2023 @ 08:00

Pimephales promelas **passed** survival and growth testing requirements when the Performance Control (PCON) was used for statistical analysis. The True Control (TCON) was invalid because it did not meet the minimum test-acceptability criteria. Synthetic water will be used as dilution water for the remainder of the permit cycle.

Test Initiation: Time: 11:55 Date: 5/9/2023

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

NUMBER OF YOUNG PRODUCED PER ADULT AT TEST TERMINATION

	EFFLUENT CONCENTRATION (%)						
REPLICATE	TCON	PCON	32 %	45 %	56 %	80 %	100 %
A	17	22	29	24	24	21	24
B	21	25	19	27	23	17	29
C	19	24	24	29	17	19	26
D	33	15	29	23	23	27	20
E	21	28	21	33	25	28	30
F	24	24	22	22	19	30	25
G	24	E	23	29	22	24	20
H	19	27	26	27	24	25	24
I	26	26	24	20	21	27	39
J	26	18	28	18	31	34	38
Surv. MEAN	23.0	23.2	24.5	25.2	22.9	25.2	27.5
Total MEAN	23.0	23.2	24.5	25.2	22.9	25.2	27.5
CV % [1]	20.2	18.3	14	18.3	16.3	20.4	24.1
PMSD	Acceptable Range 47 or Less					21.7 %	

[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	TCON	PCON	32 %	45 %	56 %	80 %	100 %
24 HOURS	100	100	100	100	100	100	100
48 HOURS	100	100	100	100	100	100	100
7-DAY	100	100	100	100	100.0	100	100

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 (= 24.1).

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 (= 0).

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:	5/7/2023 @08:00	TO:	5/8/2023@ 08:00
	FROM:	5/9/2023 @08:00	TO:	5/10/2023@ 08:00
	FROM:	5/11/2023 @08:00	TO:	5/12/2023@ 08:00

Pimephales promelas **passed** survival and growth testing requirements when the Performance Control (PCON) was used for statistical analysis. The True Control (TCON) was invalid because it did not meet the minimum test-acceptability criteria. Synthetic water will be used as dilution water for the remainder of the permit cycle.

Test Initiation: Time: 14:12 Date: 5/9/2023

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

DATA TABLE FOR GROWTH OF *Pimephales promelas*
Average Dry Weight in milligrams (mg) per replicate

Effluent Concentration	A	B	C	D	E	Mean Dry Weight (mg)	CV % [1]
TCON	0.402	0.376	0.306	0.000	0.290	0.275	58.4
PCON	0.402	0.460	0.449	0.371	0.475	0.431	10.1
32 %	0.314	0.327	0.275	0.159	0.000	0.215	63.8
45 %	0.318	0.353	0.104	0.095	0.000	0.174	88.2
56 %	0.015	0.425	0.384	0.379	0.101	0.261	72.2
80 %	0.213	0.375	0.388	0.355	0.220	0.310	27.9
100 %	0.393	0.395	0.425	0.401	0.274	0.377	15.7
PMSD	Acceptable Range 30 or Less					20.3 %	

DATA TABLE FOR SURVIVAL OF *Pimephales promelas*
Percent Survival per replicate

Effluent Concentration	A	B	C	D	E	Average % Survival 24 Hours	48 Hours	7-Day	CV % [1]
TCON	62.5	75	62.5	0	50	100	90	50	58.6
PCON	87.5	100	87.5	87.5	100	100	100	92.5	7.4
32 %	87.5	87.5	50	25	0	100	100	50	77.1
45 %	50	62.5	25	12.5	0	100	100	30	86.4
56 %	12.5	62.5	75	75	12.5	100	100	47.5	68.1
80 %	50	75	75	87.5	37.5	100	97.5	65	31.6
100 %	87.5	100	100	87.5	50	100	100	85	24.2

[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. NOEL Growth = 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 (= 15.7).

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 (= 15).