

# Bio-Aquatic Testing, Inc.



TCEQ TNI Accredited

**City of Cumming  
WPCP  
OUTFALL 001**

## Chronic Biomonitoring Report

**62453**

*Ceriodaphnia dubia*  
*Pimephales promelas*

**June 14, 2016**

*Approved by:* Joshua Reed

*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.**

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**TOXICITY TEST REPORT - Chronic**

Client:	Cumming, City of	Sample:	001
Facility:	WPCP	Laboratory Number:	62453
Permit No.	GA0046019	Date:	June 14, 2016

*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 Cumming, WPCP, were received on June 14, 2016, June 16, 2016, and June 20, 2016. Effluent samples were collected from Outfall 001 by facility personnel. Sample three was received and used out of hold time.

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 16:49 hours on June 14, 2016. Five effluent concentrations of 6%, 12%, 24%, 48% and 95% 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 11:49 hours on June 22, 2016. 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: 95% 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: 95% Effluent****TEST PROCEDURES:***Pimephales promelas***EPA METHOD: 1000**

The seven-day Chronic *Pimephales promelas* survival and growth test was initiated at 17:30 hours on June 14, 2016. Five effluent concentrations of 6%, 12%, 24%, 48% and 95% 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 11:04 hours on June 21, 2016. 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 control and any of the effluent concentrations tested.

**LOEC: Not Calculable (Q)**

**NOEC: 95% 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. 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: 95% Effluent**

# BIO-AQUATIC TESTING, INC.

## TOXICITY TEST

**Chronic**

*Ceriodaphnia dubia*

**Lab ID:** 62453

**Client:** Cumming, City of

WPCP

**Permit Number:** NPDES GA0046019

**Test Temperature (oC):**  $25 \pm 1$

**Sample Type:** Composite

**Photo Period:** 16 hours light, 8 hours dark

**Outfall Name:** 001

**Dilution Water:** synthetic

**Receiving Water Name:**

**Begin Date:** 6/14/2016

**End Date:** 6/22/2016

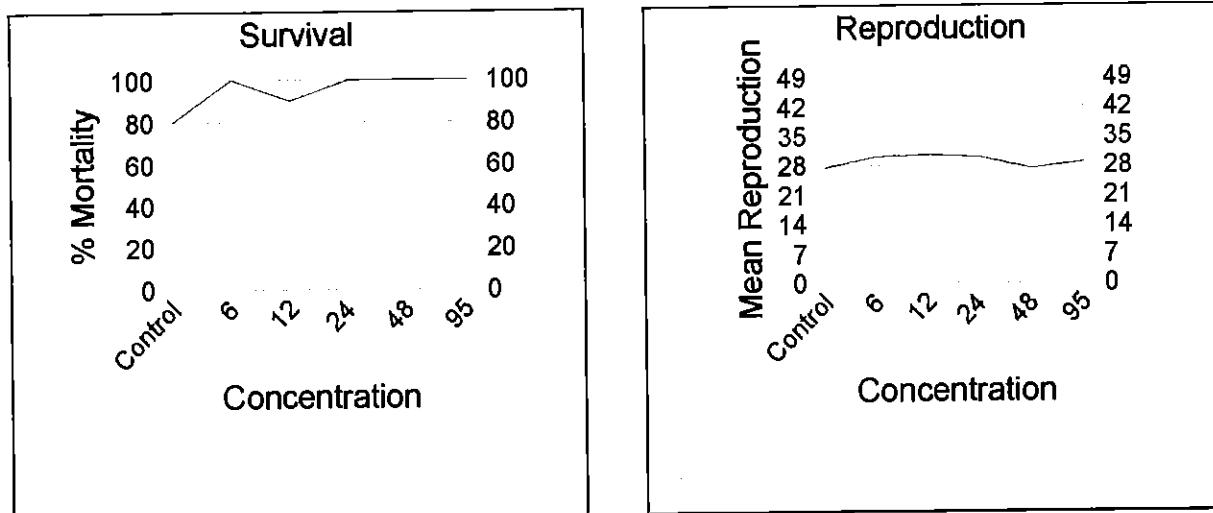
Test Start Time: 16:49

Test End Time: 11:49

### SURVIVAL AND REPRODUCTION TABLE

FEMALE #	Control	6 %	12 %	24 %	48 %	95 %
1	29	26	29	43	29	32
2	D- 14	28	28	27	27	28
3	D- 19	33	34	29	23	22
4	24	31	31	27	24	25
5	26	31	29	32	23	25
6	34	30	D- 9	24	28	27
7	28	30	30	28	37	22
8	27	28	35	31	28	30
9	26	32	31	29	28	39
10	26	32	28	30	25	38
Mean	27.5	30.1	30.5	30	27.2	28.8
C.V%	10.9	7.2	8.2	16.9	15	20.8
Var	9.142	4.766	6.277	26	16.844	36.177
Std.Dev.	3.023	2.183	2.505	5.099	4.104	6.014
Max	34	33	35	43	37	39
Min	24	26	28	24	23	22

### Concentration Response Relationships



# BIO-AQUATIC TESTING, INC.

Control

## Survival and Reproduction

6

Date	1	2	3	4	5	6	7	8	9	10
6/15	A	A	A	A	A	A	A	A	A	A
6/16	A	A	A	A	A	A	A	A	A	A
6/17	A	3	A	3	3	A	4	A	3	3
6/18	A	A	4	A	A	8	A	A	A	A
6/19	3	D11	9	8	9	11	10	11	9	10
6/20	11	D	D6	13	14	15	14	16	14	13
	14	14	19	24	26	34	28	27	26	26
6/21	A	D	D	A	A	A	A	A	A	A
	14	14	19	24	26	34	28	27	26	26
6/22	15	D	D	A	A	A	A	A	A	A
	29	14	19	24	26	34	28	27	26	26

Mean: 27.50

CV% 10.90

Var. 9.14

Max 34

Std.Dev. 3.02

Min 24

12

Date	1	2	3	4	5	6	7	8	9	10
6/15	A	A	A	A	A	A	A	A	A	A
6/16	A	A	A	A	A	A	A	A	A	A
6/17	4	3	A	5	3	A	A	A	3	3
6/18	A	A	8	A	A	9	4	7	A	A
6/19	11	10	12	11	10	D	11	13	13	10
6/20	14	15	14	15	16	D	15	15	15	15
	29	28	34	31	29	9	30	35	31	28
6/21	A	A	A	A	A	D	A	A	A	A
	29	28	34	31	29	9	30	35	31	28
6/22	A	A	A	A	A	D	A	A	A	A
	29	28	34	31	29	9	30	35	31	28

Mean: 30.50

CV% 8.20

Var. 6.28

Max 35

Std.Dev. 2.51

Min 28

48

Date	1	2	3	4	5	6	7	8	9	10
6/15	A	A	A	A	A	A	A	A	A	A
6/16	A	A	A	A	A	A	A	A	A	A
6/17	3	3	A	3	A	A	A	3	4	3
6/18	A	A	3	A	4	4	A	A	A	A
6/19	A	8	9	8	7	11	9	10	11	9
6/20	11	A	A	13	12	13	14	15	13	13
	14	11	12	24	23	28	23	28	28	25
6/21	A	A	A	A	A	A	A	A	A	A
	14	11	12	24	23	28	23	28	28	25
6/22	15	16	11	A	A	A	14	A	A	A
	29	27	23	24	23	28	37	28	28	25

Mean: 27.20

CV% 15.00

Var. 16.84

Max 37

Std.Dev. 4.10

Min 23

Date	1	2	3	4	5	6	7	8	9	10
6/15	A	A	A	A	A	A	A	A	A	A
6/16	A	A	A	A	A	A	A	A	A	A
6/17	3	3	A	4	3	A	4	A	5	5
6/18	A	A	9	A	A	6	A	3	A	A
6/19	10	11	10	12	12	10	11	10	11	12
6/20	13	14	14	15	16	14	15	15	16	15
	26	28	33	31	31	30	30	28	32	32
6/21	A	A	A	A	A	A	A	A	A	A
	26	28	33	31	31	30	30	28	32	32
6/22	A	A	A	A	A	A	A	A	A	A
	26	28	33	31	31	30	30	28	32	32

Mean: 30.10

CV% 7.20

Var. 4.77

Max 33

Std.Dev. 2.18

Min 26

24

Date	1	2	3	4	5	6	7	8	9	10
6/15	A	A	A	A	A	A	A	A	A	A
6/16	A	A	A	A	A	A	A	A	A	A
6/17	A	A	A	A	A	A	3	A	A	3
6/18	A	A	4	A	6	A	A	4	3	A
6/19	11	10	11	12	11	10	11	12	11	12
6/20	14	17	14	15	15	14	14	15	15	15
	25	27	29	27	32	24	28	31	29	30
6/21	18	A	A	A	A	A	A	A	A	A
	43	27	29	27	32	24	28	31	29	30
6/22	A	A	A	A	A	A	A	A	A	A
	43	27	29	27	32	24	28	31	29	30

Mean: 30.00

CV% 16.90

Var. 26.00

Max 43

Std.Dev. 5.10

Min 24

95

Date	1	2	3	4	5	6	7	8	9	10
6/15	A	A	A	A	A	A	A	A	A	A
6/16	A	A	A	A	A	A	A	A	A	A
6/17	3	A	A	3	A	4	A	A	A	A
6/18	A	A	4	A	A	5	A	A	A	A
6/19	11	6	7	9	10	9	7	A	9	10
6/20	A	A	11	13	12	13	11	13	11	14
	14	6	22	22	25	27	22	13	20	24
6/21	18	A	A	A	A	A	A	A	A	A
	32	6	22	22	25	27	22	13	20	24
6/22	A	22	A	3	A	A	17	19	14	
	32	28	22	25	25	27	22	30	39	38

Mean: 28.80

CV% 20.80

Var. 36.18

Max 39

Std.Dev. 6.01

Min 22

## BIO-AQUATIC TESTING, INC.

Chronic

CERIODAPHNIA DUBIA

SURVIVAL AND REPRODUCTION

Client: Cumming, City of - WPCP Lab ID: 62453 Culture No.: B1046016A

## TEST INSTRUCTIONS:

ORGANISMS ADDED: Date: 6-14-16 Time: 1644 Technician: 44

Photo Period 16hr Light/8hr dark

Dilution: Control

## RANDOMIZATION:

SC-10 11

DAY/TIME/ TECHNICIAN	1	2	3	4	5	6	7	8	9	10
24Hr 6-15-16 BH1305	A	-								A
48Hr 6-16-16 BH1305	A	-								A
72Hr 6-17-16 BH1310	A	3	A	3	3	A	4	A	3	3
96Hr 6-18-16 BH1111	A	A	4	A	8	A	-	A		
5 days 6-21-16 BH1105	3	9	9	8	9	11	10	11	9	10
6 days 6-20-16 BH1705	11	1	D	6	B	14	15	14	B	1413
7 days 6-21-16 BH1814	A	/	/	A				/		A
8 days 6-22-16 BH1149	5	1	/	A				/	-	A

Dilution: 6 %

	1	2	3	4	5	6	7	8	9	10	
24Hr	A	-								A	
48Hr	A	-								A	
72Hr	3	3	A	4	3	A	4	A	5	5	
96Hr	A	A	9	A	A	6	A	3	A	A	
5 days	10	11	10	12	Y	11	10	11	10	11	12
6 days	13	14	14	15	16	14	15	15	16	15	
7 days	A	-							-	A	
8 days	A	-							-	A	

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

## BIO-AQUATIC TESTING, INC.

Chronic

CERIODAPHNIA DUBIA

SURVIVAL AND REPRODUCTION

Client: Cumming, City of - WPCP Lab ID: 62453 Culture No.: \_\_\_\_\_

TEST INSTRUCTIONS: \_\_\_\_\_

Dilution:	12	%								
	1	2	3	4	5	6	7	8	9	10
24Hr	A									A
48Hr	A	-								A
72Hr	4	3	A	5	3	A	A	A	3	3
96Hr	A	A	8	A	A	9	4	7	A	A
5 days	11	10	12	4	10	0	11	13	Y <sub>12</sub>	10
6 days	14	15	14	15	16	1	15	15	15	15
7 days	A	-	-	A	A	A	-	-	A	
8 days	A	-	-	A	A	A	-	-	A	

Dilution:	24	%								
	1	2	3	4	5	6	7	8	9	10
24Hr	A	-								A
48Hr	A	-								A
72Hr	A				A	3	A	A	3	
96Hr	A	1	4	A	6	A	A	4	3	A
5 days	11	10	11	2	11	10	11	12	11	10
6 days	14	15	14	15	15	14	14	15	15	15
7 days	18	(A)								A
8 days	A	2								A

Code: Cells in numbered column 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. "L" 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: Cumming, City of - WPCP

Lab ID: 62453

Culture No.: \_\_\_\_\_

TEST INSTRUCTIONS: \_\_\_\_\_

	Dilution: 48 %									
	1	2	3	4	5	6	7	8	9	10
24Hr	A	-	-	-	-	-	-	-	-	A
48Hr	A	-	-	-	-	-	-	-	-	A
72Hr	3	3	A	3	A	A	A	3	4	3
96Hr	A	A	3	A	4	4	A	-	-	A
5 days	4	8	9	8	7	16	9	10	11	9
6 days	7	A	A	13	12	13	14	15	13	13
7 days	3	A	9	-	-	-	-	-	-	A
8 days	12	16	16	A	-	A	14	A	-	A

	Dilution: 95 %									
	1	2	3	4	5	6	7	8	9	10
24Hr	A	-	-	-	-	-	-	-	-	A
48Hr	A	-	-	-	-	-	-	-	-	A
72Hr	3	A	A	A	3	A	0	A	-	A
96Hr	A	A	0	A	A	S	A	-	-	A
5 days	11	6	7	9	10	9	7	A	9	0
6 days	A	A	11	13	12	13	11	13	11	14
7 days	18	A	-	-	-	-	-	-	-	A
8 days	A	3	0	A	3	A	-	A	17	19

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; "0" 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. "T" 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: Cumming, City of WPCP

Lab ID: 62453 Culture No.:

TEST INSTRUCTIONS: [Redacted]

## Test Temperatures

	0Hr new	24Hr old / new	48Hr old / new	72Hr old / new	96Hr old / new	5 days old / new	6 days old / new	7 days old
Control	25.1	25.2	25.3	25.4	25.5	25.6	25.7	25.8
6	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-
48	25.1	-	-	-	-	-	-	-
95	-	-	-	-	-	-	-	-
TIME/DATE TECH	6-14-16 04-1649	6-15-16 BH 310	6-16-16 BH 120	6-17-16 1310 npd	6-18-16 BH 1117	6-19-16 2 1115	6-20-16 Q nus	6-21-16 BH 1215
IR GUN ID #	011	605	611	611	605	629	605	011
SAMPLE # @ RENEWAL	1	1	1	2	2	2	3	

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

**BIO-AQUATIC TESTING, INC.**

**TOXICITY TEST**

**Chronic *Pimephales promelas***

**Client:** Cumming City of WPCP

**Lab ID:** 62453

**Permit Number:** NPDES GA0046019

**Test Temperature (oC):** 25 ± 1

**Outfall Name:** 001

**Sample Type:** Composite

**Photo Period:** 16 Hours Light  
8 Hours Dark

**Receiving Water Name:**

Test Start Time: 17:30

Test End Time: 11:04

**Begin Date:** 6/14/2016

**End Date:** 6/21/2016

**SURVIVAL**

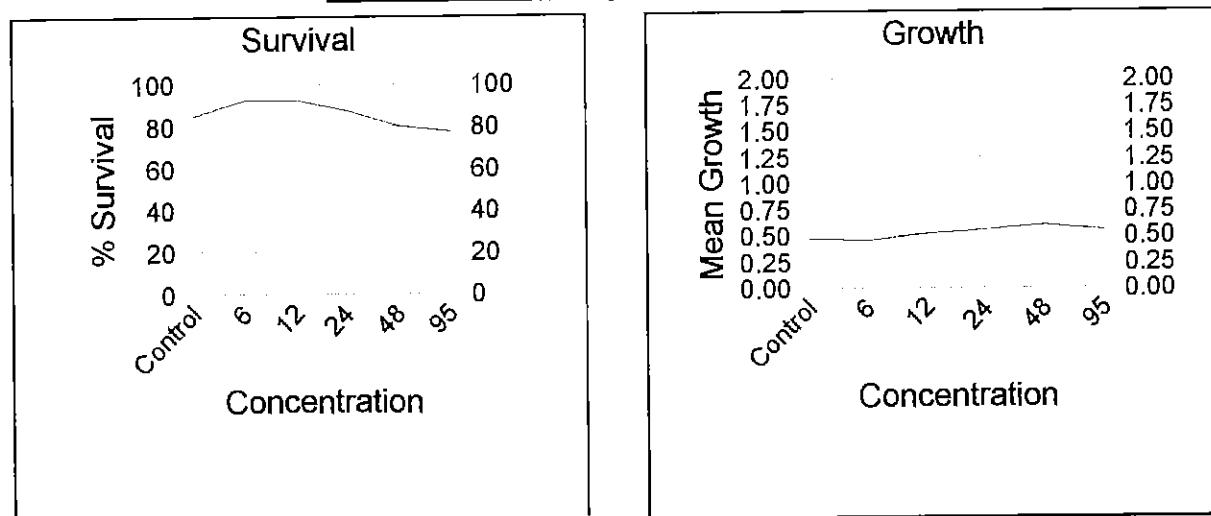
Effluent Concentration	Number Of Alive								Avg% Surv.
	6/14	6/15	6/16	6/17	6/18	6/19	6/20	6/21	
Control	A	8	8	8	8	8	7	7	85.0%
	B	8	8	8	8	8	7	7	
	C	8	8	8	8	8	8	8	
	D	8	8	8	8	8	5	5	
	E	8	8	7	7	7	7	7	
6	A	8	8	8	8	8	8	8	92.5%
	B	8	8	8	8	8	8	8	
	C	8	8	8	8	8	7	7	
	D	8	8	8	8	8	7	7	
	E	8	8	8	8	8	8	7	
12	A	8	8	8	8	8	8	8	92.5%
	B	8	8	8	8	8	6	6	
	C	8	8	8	8	8	8	8	
	D	8	8	8	8	8	8	8	
	E	8	8	8	8	8	7	7	
24	A	8	8	7	7	7	7	6	87.5%
	B	8	8	8	8	8	8	8	
	C	8	8	8	8	8	8	8	
	D	8	8	8	8	8	6	6	
	E	8	8	8	8	8	7	7	

# BIO-AQUATIC TESTING, INC.

# TOXICITY TEST

Effluent Concentration	Number Of Alive								Avg% Surv.
	6/14	6/15	6/16	6/17	6/18	6/19	6/20	6/21	
48	A	8	8	8	8	8	7	7	7
	B	8	8	7	7	7	7	7	80.0%
	C	8	8	8	8	8	6	6	
	D	8	8	8	8	8	7	6	
	E	8	8	8	8	8	7	7	
95	A	8	8	8	8	8	8	8	77.5%
	B	8	8	8	8	8	6	6	
	C	8	8	8	7	7	6	6	
	D	8	8	8	8	8	5	5	
	E	8	8	8	8	8	6	6	
	A								
	B								
	C								
	D								
	E								

## Concentration Response Relationships



## BIO-AQUATIC TESTING, INC.

Chronic

Pimephales promelas SURVIVAL

Lab ID: 62453

Client: Cumming, City of

Facility WPCP

Outfall:001

Sample Type Composite

## TEST INSTRUCTIONS:

Culture No.: P1-16-165A

Photo Period: 16hr light, 8hr dark

RANDOMIZATION: SC-5

0

Dilution:	Control	6	12	24											
DATE/TIME/ TECHNICIAN	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
6-14-16 DP 1730	8					8					8				
6-15-16 SC 1120	8					8					8				
6-16-16 SC 950	8				7	8					8			7,8	
6-17-16 MM 1049	8				7	8					8			7,8	
6-18-16 DP 0920	8				7	8					8			7,8	
6-19-16 DP 1305	7,7,8	5	7	8	7,7,8	8	8	7,7,8	8	6	8	7,7	7,8	8	6,7
6-20-16 DP 1425	7,7,8	5	7	8	7,7,8	8	8	7,7,8	8	6	8	8,7	7,8	8	6,7
6-21-16 DP 1104	7,7,8	5	7	8	7,7,8	9,8	7	7,7,8	9,6	8	9,6	7	6,8	8,6	7
Dilution:	48	95													
DATE/TIME/ TECHNICIAN	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
0Hr	8					8									
24Hr	8					8									
48Hr	8	7,8				8									
72Hr	8	7,8	-			8	8	7,8							
96Hr	8	7,8	-			8	8	7,8							
5 days	7,7,6,7,7					8	6	4,5,6							
6 days	7,7,6,6,7					8	6	6,5	6						
7 days	7,7,6,6,6	9				8	6	6,5	6						

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

## BIO-AQUATIC TESTING, INC.

Chronic

Pimephales promelas SURVIVAL

Lab ID: 62453

Client: Cumming, City of

Facility WPCP

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
Control	21/3	20/2 21/3	20/2 21/3	20/2 21/3	20/2 21/3	20/2 21/3	20/2 21/3	20/2
6	1							
12								
24								
48								
95								
TIME/DATE TECH	6-14-16 1730AP	6-15-16 82 1120	6-16-16 82 93	6-17-16 mm 1655	6-18-16 110720	6-19-16 (6-13-15)	6-20-16 103500	6-21-16 93 1101
IR GUN ID #	013	013	013	013	013	013	013	013
SAMPLE # @RENEWAL	1	1	1	2	2	2	3	3

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: Cumming, City of WPCP

Lab ID: 62453

Permit Number: GA0046019

Sample Type: Composite Outfall Name: 001

Receiving Water Name:

**Synthetic**

	ON	SN	Wt.	Avg.	Avg.
A	8	7	3.244	0.406	0.463
B	8	7	4.153	0.519	0.593
C	8	8	4.106	0.513	0.513
D	8	5	3.150	0.394	0.630
E	8	7	4.154	0.519	0.593

**6**

	ON	Wt.	Avg.
A	8	4.113	0.514
B	8	3.915	0.489
C	8	3.345	0.418
D	8	3.473	0.434
E	8	3.400	0.425

**12**

	ON	Wt.	Avg.
A	8	4.751	0.594
B	8	2.959	0.370
C	8	4.608	0.576
D	8	4.076	0.510
E	8	4.399	0.550

**24**

	ON	Wt.	Avg.
A	8	3.627	0.453
B	8	4.866	0.608
C	8	4.685	0.586
D	8	3.990	0.499
E	8	5.070	0.634

**Mean C.V. %**

0.470	13.7
-------	------

**SN Mean SN C.V. %**

0.559	12.2
-------	------

**48**

	ON	Wt.	Avg.
A	8	4.675	0.584
B	8	4.334	0.542
C	8	4.390	0.549
D	8	4.553	0.569
E	8	6.120	0.765

**Mean C.V. %**

0.602	15.4
-------	------

**95**

	ON	Wt.	Avg.
A	8	6.131	0.766
B	8	3.481	0.435
C	8	3.651	0.456
D	8	4.465	0.558
E	8	4.293	0.537

**Mean C.V. %**

0.551	23.9
-------	------

	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.

Chronic

*Pimephales promelas*

TOXICITY TEST

Client: Cumming, City of - WPCP

Lab ID: 62453

Begin Date: 6/14/2016

End Date: 6/21/2016

Organism: *Pimephales promelas*

Balance: Sartorius MP3

Analyst: MM

Weigh Date: 6-18-16

Date/Time placed in Oven: 6-21-16 1535

Date/Time removed from Oven: 1535

Control

Qty.

Wt.

A	7	3.2441
B	7	4.153
C	8	4.106
D	5	3.150
E	7	4.134

6 %  
Qty. Wt.

A	8	3.2441
B	8	3.915
C	7	3.345
D	1	3.473
E		3.400

12 %

Qty.

Wt.

A	8	4.751
B	6	2.957
C	8	4.608
D	7	4.070
E	7	4.399

24 %

Qty.

Wt.

A	6	3.622
B	8	4.866
C	8	4.685
D	6	3.990
E	7	5.070

48 %

Qty.

Wt.

A	7	4.673
B	7	4.334
C	6	4.340
D	1	4.553
E	1	4.120

95 %

Qty.

Wt.

A	8	4.131
B	6	4.131
C	6	3.631
D	5	4.405
E	6	4.223

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 Steel's 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 Shapiro Wilks Test and Bartlett's Test then Steel's 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 Steel's 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 Steel's 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 Shapiro Wilks Test or Bartlett's Test then Steel's 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 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 Steel's 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 Steel's 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 Steel's Many One Test is used. Point estimation may or TST methodology may also be used.

62453

chronic cerio repro  
File: 62453.cdr      Transform: NO TRANSFORMATION

---

chi-square test for normality: actual and expected frequencies

---

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.020	14.520	22.920	14.520	4.020
OBSERVED	3	14	31	7	5

---

Calculated Chi-Square goodness of fit test statistic = 7.2594  
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

chronic cerio repro  
File: 62453.cdr      Transform: NO TRANSFORMATION

---

Bartlett's test for homogeneity of variance  
Calculated B1 statistic = 11.47

---

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.

chronic cerio repro  
File: 62453.cdr      Transform: NO TRANSFORMATION

---

ANOVA TABLE

---

SOURCE	DF	SS	MS	F
Between	5	166.000	33.200	1.201
Within (Error)	54	1492.600	27.641	
Total	59	1658.600		

---

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

chronic cerio repro  
File: 62453.cdr      Transform: NO TRANSFORMATION

---

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

---

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	con	25.300	25.300		

---

			62453	
2	6	30.100	30.100	-2.042
3	12	28.400	28.400	-1.318
4	24	30.000	30.000	-1.999
5	48	27.200	27.200	-0.808
6	95	28.800	28.800	-1.489

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

chronic cerio repro  
File: 62453.cdr

Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	con	10			
2	6	10	5.431	21.5	-4.800
3	12	10	5.431	21.5	-3.100
4	24	10	5.431	21.5	-4.700
5	48	10	5.431	21.5	-1.900
6	95	10	5.431	21.5	-3.500

chronic fathead survival  
File: 62453.pps Transform: NO TRANSFORMATION

Shapiro - wilk's test for normality

D = 19.200

W = 0.971

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.

chronic fathead survival  
File: 62453.pps Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance  
Calculated B1 statistic = 3.38

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.

chronic fathead survival  
File: 62453.pps Transform: NO TRANSFORMATION

62453  
ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	6.267	1.253	1.567
within (Error)	24	19.200	0.800	
Total	29	25.467		

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

chronic fathead survival  
 File: 62453.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	con	6.800	6.800		
2	6	7.400	7.400	-1.061	
3	12	7.400	7.400	-1.061	
4	24	7.000	7.000	-0.354	
5	48	6.400	6.400	0.707	
6	95	6.200	6.200	1.061	

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

chronic fathead survival  
 File: 62453.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	con	5			
2	6	5	1.335	19.6	-0.600
3	12	5	1.335	19.6	-0.600
4	24	5	1.335	19.6	-0.200
5	48	5	1.335	19.6	0.400
6	95	5	1.335	19.6	0.600

chronic fathead growth  
 File: 62453.ppg      Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.183

W = 0.965

62453  
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.

chronic fathead growth  
File: 62453.ppg      Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance  
Calculated B1 statistic = 4.75

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.

chronic fathead growth  
File: 62453.ppg      Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.076	0.015	2.007
Within (Error)	24	0.183	0.008	
Total	29	0.259		

Critical F value = 2.62 (0.05, 5, 24)  
Since  $F < \text{Critical F}$  FAIL TO REJECT  $H_0$ : All equal

chronic fathead growth  
File: 62453.ppg      Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 1 OF 2       $H_0: \text{Control} < \text{Treatment}$

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	con	0.470	0.470		
2	6	0.456	0.456	0.257	
3	12	0.520	0.520	-0.902	
4	24	0.556	0.556	-1.554	
5	48	0.602	0.602	-2.384	
6	95	0.550	0.550	-1.453	

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

chronic fathead growth  
File: 62453.ppg      Transform: NO TRANSFORMATION

62453

## DUNNETT'S TEST - TABLE 2 OF 2

Ho:Control &lt; Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	con	5			
2	6	5	0.130	27.7	0.014
3	12	5	0.130	27.7	-0.050
4	24	5	0.130	27.7	-0.086
5	48	5	0.130	27.7	-0.132
6	95	5	0.130	27.7	-0.080

# Bio-Aquatic Testing, Inc.

## FRESH WATER TEST SETUP FORM

Client: Cumming, City of \_\_\_\_\_

Permit GA0046019

Facility: WPCP \_\_\_\_\_

Lab Number 62453Outfall Name: 001Number of samples 3Dilution Water: Synthetic Lab

Sx #	Rcvd Date	Rcvd Time	Sampling Dates		Sampling Times	
			Begin Date	End Date	Start	End
1	06/14/16	11:20	06/12/16	06/13/16	09:30	09:30
2	06/16/16	16:30	06/14/16	06/15/16	09:30	09:30
3	06/20/16	10:30	06/16/16	06/17/16	09:30	09:30

**Type of Test(s)**

<u>Ceriodaphnia dubia</u>	<u>Chronic</u>
<u>Pimephales promelas</u>	<u>Chronic</u>

Controls: Synthetic

Start Sx #	<u>1</u>	Date:	<u>6/14/2016</u>
Renew Sx #	<u>1</u>	Date:	<u>6/15/2016</u>
Renew Sx #	<u>1</u>	Date:	<u>6/16/2016</u>
Renew Sx #	<u>2</u>	Date:	<u>6/17/2016</u>
Renew Sx #	<u>2</u>	Date:	<u>6/18/2016</u>
Renew Sx #	<u>2</u>	Date:	<u>6/19/2016</u>
Renew Sx #	<u>3</u>	Date:	<u>6/20/2016</u>

pH Match: \_\_\_\_\_

Test Start Date: \_\_\_\_\_

Test End Date: \_\_\_\_\_

Hardness Match: \_\_\_\_\_

6/14/20166/21/2016Ceriodaphnia dubia Test Set Up: 10 Reps & 1 Organisms per RepPimephales Test Set Up: 5 Reps & 8 Organism per RepConcentrations: 6 12 24 48 95 % LF % 95Test Chemistry on these dilutions: 6 12 24 48 95**Samples received by:**

- |  |   |  |                             |
|--|---|--|-----------------------------|
| <input type="radio"/> Greyhound                  | <input type="radio"/> UPS Next Day      | <input type="radio"/> Delta Dash         | <input type="radio"/> Delta |
| <input type="radio"/> Pony Express               | <input type="radio"/> Client Delivered  | <input type="radio"/> Southwest Airlines | <input type="radio"/> DHL   |
| <input checked="" type="radio"/> Federal Express | <input type="radio"/> American Airlines | <input type="radio"/> Bio Pick Up        |                             |

Other: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

# BIO-AQUATIC TESTING, INC.

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

**Client:** Cumming, City of

**Lab ID:** 62453

**Facility:** WPCP

**Outfall:** 001

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

**Test Date:** June 14, 2016

\*\* 100 %

Effluent Sample #	Received		DeChlor (ml/L)	** Ammonia mg/L	Analyst Initials	Initial Salinity	Adjusted Salinity	Temp. Received
	Date	Time	** Residual Cl <sub>2</sub>					
1	6/14/16	11:20	<0.10	N/A	<0.25	JR	N/A	3.9
2	6/16/16	16:30	<0.10	N/A	<0.25	LZ	N/A	4.1
3	6/20/16	10:30	<0.10	N/A	<0.25	DF	N/A	2.9

**Chlorine Analysis Method:**

Hanna Ion Specific Meter #711

**Dechlorination Reagent:** 0.025 N Sodium Thiosulfate

Received			Hardness (EDTA) As mg/L CaCO <sub>3</sub>		ALKALINITY TO END POINT pH 4.50 =/- 0.05 as mg/L CaCO <sub>3</sub>		Analyst Initials
Sample #	Date	Time	CON	100	CON	100	
1	6/14/16	11:20	144.0	116.0	61.0	89.0	JAS
2	6/16/16	16:30	144.0	112.0	61.0	92.0	JAS
3	6/20/16	10:30	138.0	112.0	56.0	91.0	JAS

Date	Sample #	Values are at Highest Dilution		Values are at 100% Dilution		Analyst	Other
		Specific Conductivity as umhos/cm	Salinity (ppt)	Residual Chlorine as mg Cl <sub>2</sub> /L	1 ml 0.02N Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> /L		
6/14	Lab H <sub>2</sub> O	474	0.3	<0.10	N/A	GC	
6/15	Lab H <sub>2</sub> O	464	0.3	<0.10	N/A	GC	
6/16	Lab H <sub>2</sub> O	461	0.3	<0.10	N/A	GC	
6/17	Lab H <sub>2</sub> O	469	0.3	<0.10	N/A	GC	
6/18	Lab H <sub>2</sub> O	439	0.3	<0.10	N/A	GC	
6/19	Lab H <sub>2</sub> O	429	0.2	<0.10	N/A	MM	
6/20	Lab H <sub>2</sub> O	430	0.2	<0.10	N/A	GC	
6/14	OUTFALL*	1	536	0.3	<0.10	N/A	GC
6/15	OUTFALL*	1	553	0.3	<0.10	N/A	GC
6/16	OUTFALL*	1	552	0.3	<0.10	N/A	GC
6/17	OUTFALL*	2	559	0.3	<0.10	N/A	GC
6/18	OUTFALL*	2	549	0.3	<0.10	N/A	GC
6/19	OUTFALL*	2	546	0.3	<0.10	N/A	MM
6/20	OUTFALL*	3	588	0.3	<0.10	N/A	GC

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

# BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen

Chronic

Ceriodaphnia dubia

Client: Cumming, City of

Lab ID: 62453

Facility: WPCP

Dilution Water(s): Synthetic Lab

Outfall: 001

Test Begin Date: June 14, 2016

NR indicates that the test is non-renewal.

ANALYST	DATE	TIME	SX#	UNIT	Concentration							
					Control	6	12	24	48	95		
GC	6/14	Start	1	pH	7.9	7.9	7.7	7.7	7.7	7.6		
		25 ± 1		DO (mg/L)	8.4	7.8	7.8	7.8	7.8	7.8		
GC	6/15	24 Hr	1	pH	8.1	8.0	8.0	8.0	8.0	8.0		
		25 ± 1		DO (mg/L)	8.2	8.1	8.0	8.0	8.0	8.0		
GC	6/16	Renew	1	pH	7.8	7.8	7.7	7.7	7.6	7.6		
		48 Hr		DO (mg/L)	7.9	7.9	8.0	8.0	8.2	8.2		
GC	6/17	25 ± 1	1	pH	8.3	7.7	7.8	7.8	7.8	7.8		
		Renew		DO (mg/L)	8.1	8.1	8.1	8.1	8.1	8.1		
GC	6/17	72 Hr	1	pH	8.3	7.6	7.6	7.6	7.7	7.7		
		25 ± 1		DO (mg/L)	8.2	8.0	8.0	8.0	7.9	7.9		
GC	6/18	Renew	2	pH	7.7	7.8	7.8	7.8	7.8	7.8		
		96 Hr		DO (mg/L)	7.7	7.8	7.9	7.9	8.0	8.0		
GC	6/18	25 ± 1	2	pH	7.9	7.8	7.8	7.8	7.8	7.8		
		Renew		DO (mg/L)	7.9	7.9	7.9	7.9	7.9	7.9		
MM	6/19	120 Hr	2	pH	7.7	7.6	7.6	7.6	7.7	7.7		
		25 ± 1		DO (mg/L)	7.8	7.8	7.8	7.8	7.9	7.9		
MM	6/20	Renew	2	pH	7.9	7.9	7.8	7.8	7.7	7.7		
		144 Hr		DO (mg/L)	8.1	8.0	8.2	8.3	8.4	8.5		
MM	6/20	25 ± 1	2	pH	7.7	7.6	7.6	7.6	7.6	7.6		
		Renew		DO (mg/L)	8.0	8.0	7.9	7.9	7.9	7.9		
GC	6/21	168 Hr	3	pH	7.8	7.8	7.8	7.8	7.8	7.8		
		25 ± 1		DO (mg/L)	8.2	8.2	8.1	8.1	8.3	8.5		
GC	6/21	Renew	3	pH	7.9	7.8	7.7	7.7	7.8	7.8		
		168 Hr		DO (mg/L)	7.7	7.7	7.7	7.7	7.6	7.6		

# BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen

Chronic

*Pimephales promelas*

Client: Cumming, City of

Lab Number: 62453

Facility: WPCP

Dilution Water(s): Synthetic Lab

Outfall: 001

Test Begin Date: June 14, 2016

NR indicates that the test is non-renewal.

ANALYST	DATE	TIME	SX#	UNIT	Concentration							
					Control	6	12	24	48	95		
GC	6/14	Start	1	pH	7.9	7.9	7.7	7.7	7.7	7.6		
		25 ± 1		DO (mg/L)	8.4	7.8	7.8	7.8	7.8	7.8		
GC	6/15	24 Hr	1	pH	7.5	7.6	7.6	7.6	7.6	7.6		
		25 ± 1		DO (mg/L)	7.9	7.9	7.9	7.9	7.8	7.8		
GC	6/16	Renew	1	pH	7.8	7.8	7.7	7.7	7.6	7.6		
				DO (mg/L)	7.9	7.9	8.0	8.0	8.2	8.2		
GC	6/17	48 Hr	1	pH	7.5	7.5	7.5	7.5	7.5	7.5		
		25 ± 1		DO (mg/L)	8.2	8.0	7.9	7.9	7.7	7.7		
GC	6/18	Renew	1	pH	7.8	7.8	7.7	7.7	7.6	7.6		
				DO (mg/L)	8.0	8.0	8.2	8.2	8.4	8.4		
GC	6/19	72 Hr	1	pH	7.5	7.5	7.5	7.5	7.5	7.5		
		25 ± 1		DO (mg/L)	8.0	7.8	7.7	7.7	7.7	7.7		
GC	6/20	Renew	2	pH	7.7	7.8	7.8	7.8	7.8	7.8		
				DO (mg/L)	7.7	7.8	7.9	7.9	8.0	8.0		
MM	6/21	96 Hr	2	pH	7.6	7.6	7.5	7.5	7.5	7.5		
		25 ± 1		DO (mg/L)	7.9	7.8	7.7	7.7	7.7	7.5		
MM	6/20	Renew	2	pH	7.8	7.8	7.8	7.8	7.8	7.8		
				DO (mg/L)	7.8	7.9	8.1	8.1	8.3	8.3		
GC	6/21	120 Hr	2	pH	7.5	7.4	7.4	7.4	7.4	7.4		
		25 ± 1		DO (mg/L)	8.1	7.7	7.6	7.5	7.4	7.3		
MM	6/20	Renew	2	pH	7.9	7.9	7.8	7.8	7.7	7.7		
				DO (mg/L)	8.1	8.0	8.2	8.3	8.4	8.5		
MM	6/20	144 Hr	2	pH	7.5	7.5	7.5	7.5	7.5	7.5		
		25 ± 1		DO (mg/L)	8.0	7.8	7.8	7.8	7.7	7.6		
GC	6/21	Renew	3	pH	7.8	7.8	7.8	7.8	7.8	7.8		
				DO (mg/L)	8.2	8.2	8.1	8.1	8.3	8.5		
GC	6/21	168 Hr	3	pH	7.4	7.3	7.3	7.3	7.3	7.3		
		25 ± 1		DO (mg/L)	7.1	7.1	7.2	7.2	7.1	7.1		

## **Appendix B**

*Ceriodaphnia dubia*

### **BIO-AQUATIC TESTING, INC.**

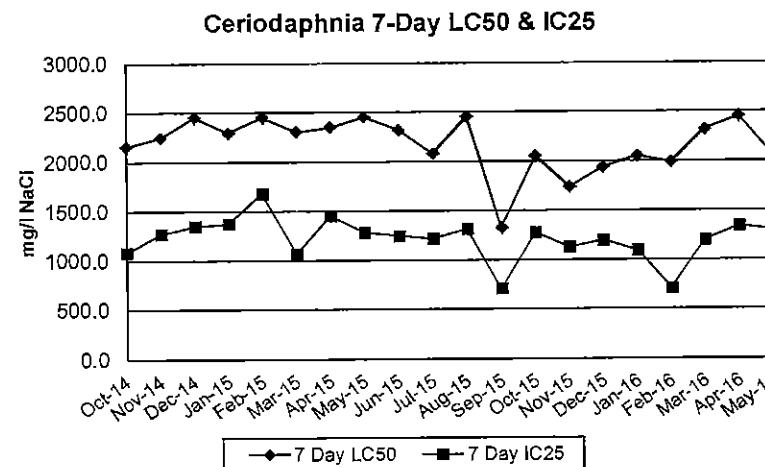
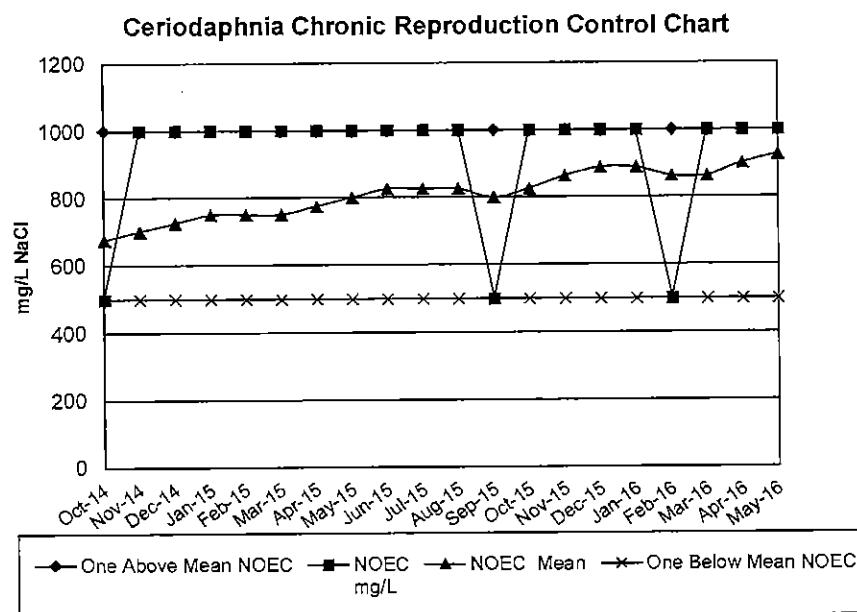
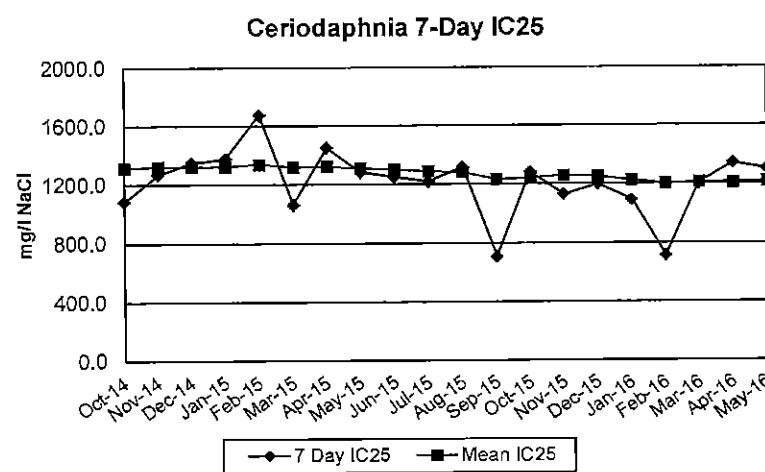
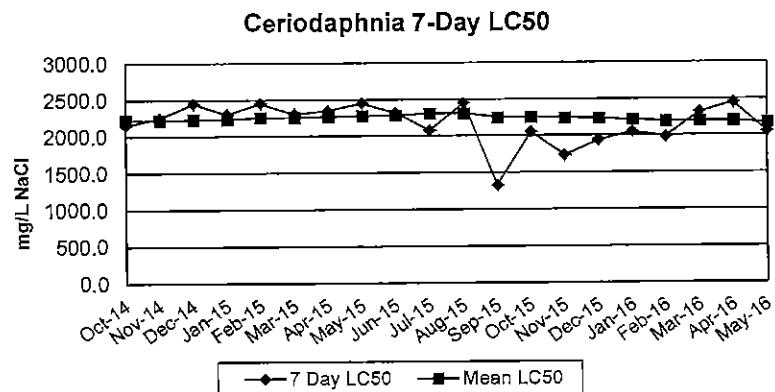
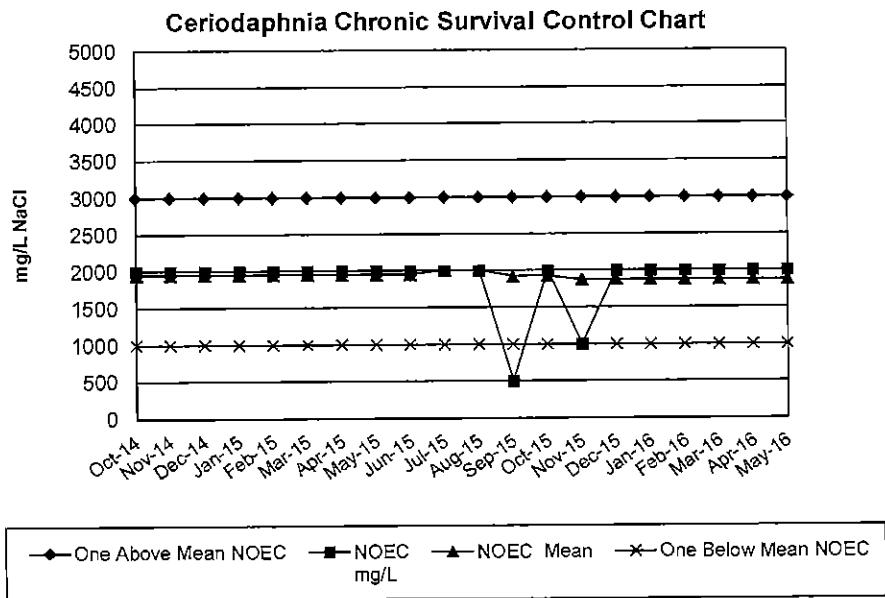
Carrollton, TX

#### **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:	255						
PROJECT NUMBER:	61884						
START DATE:	5/31/2016						
START TIME:	14:00						
TOTAL NUMBER EXPOSED:	10 organisms per concentration						
CONCENTRATIONS (mg/L):	CON	250	500	1000	2000	3000	4000
NUMBER DEAD PER CONCENTRATION:	0	0	0	1	2	10	10
TEST METHODS:	As listed in EPA-821-R-02-013						
STATISTICAL METHODS:	SURVIVAL: Fisher's Exact Test						
REPRODUCTION: ANOVA - Dunnett's							
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:	11.7						



## **Appendix B**

*Pimephales promelas*

### **BIO-AQUATIC TESTING, INC.**

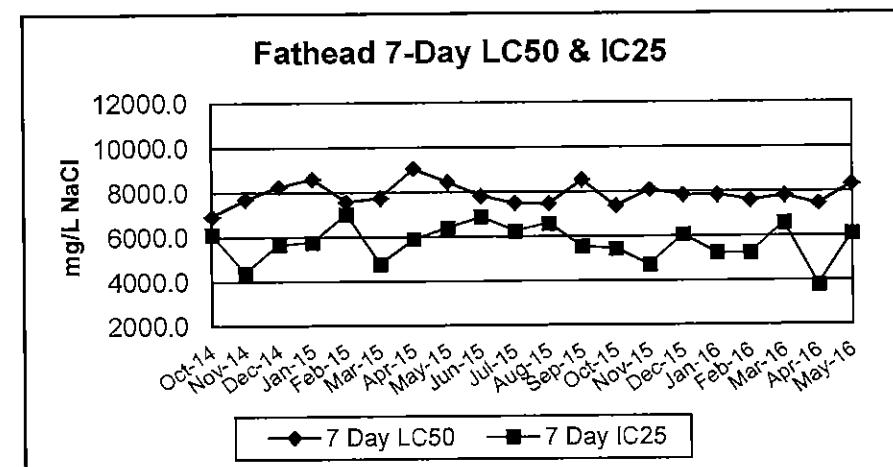
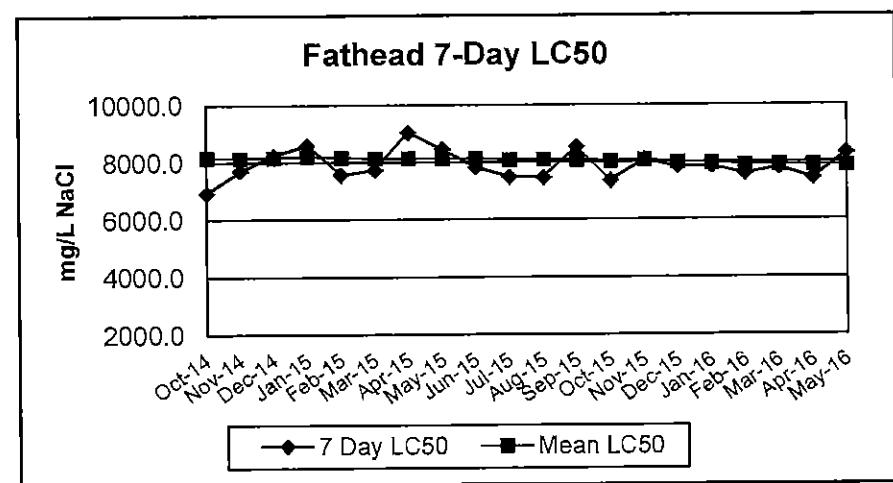
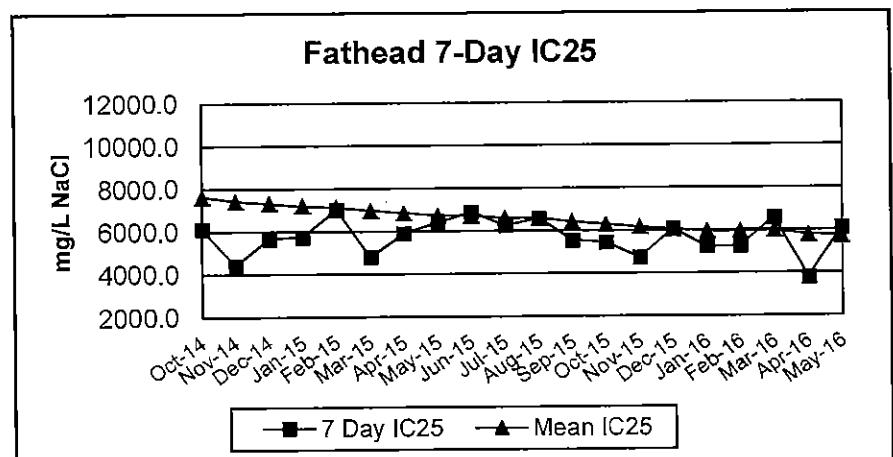
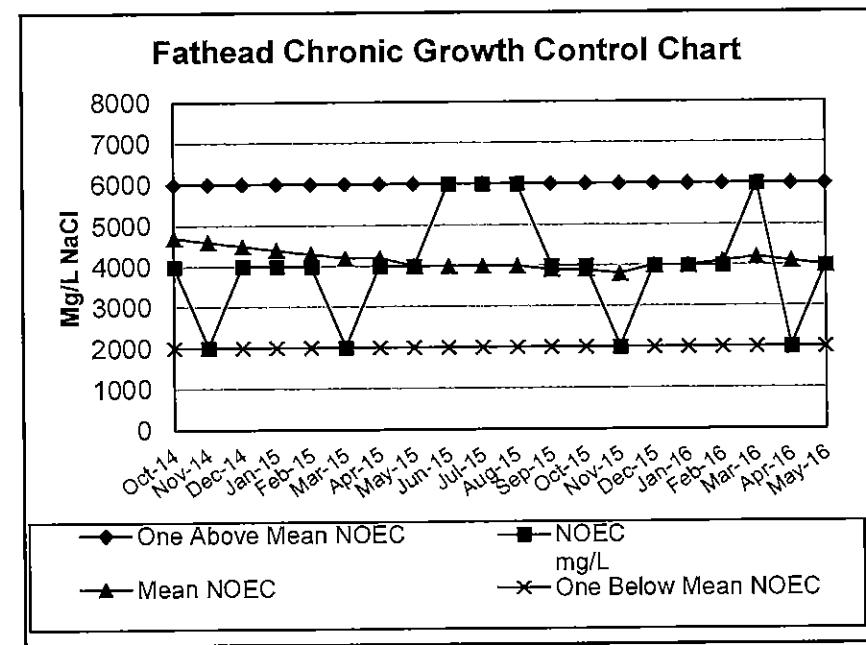
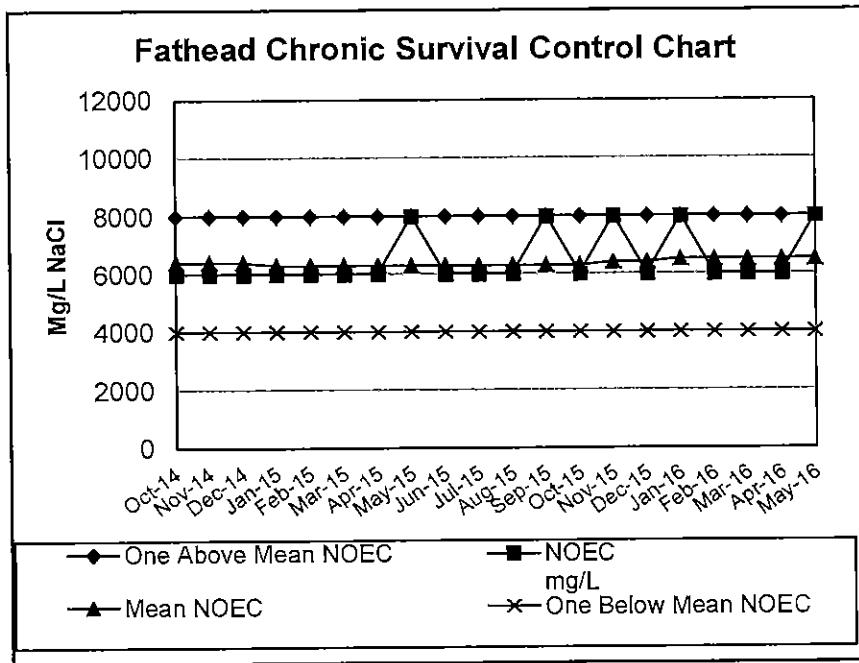
Carrollton, TX

#### **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:	295						
PROJECT NUMBER:	61896						
START DATE:	5/31/2016						
START TIME:	13:20						
TOTAL NUMBER EXPOSED:	40 organisms per concentration						
CONCENTRATIONS (mg/L):	CON	2000	4000	6000	8000	10000	12000
NUMBER DEAD PER CONCENTRATION:	4	4	3	5	14	38	40
TEST METHODS:	As listed in EPA-821-R-02-013						
STATISTICAL METHODS:	SURVIVAL: Steel's Many-One Rank Test GROWTH: ANOVA and Dunnett's Test						
NOEC FOR SURVIVAL:	8000	mg/L					
LOEC FOR SURVIVAL:	10000	mg/L					
NOEC FOR GROWTH:	4000	mg/L					
LOEC FOR GROWTH:	6000	mg/L					
PMSD:	18.4						



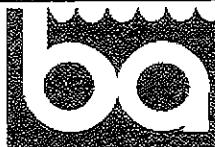
## **APPENDIX C**

### **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



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

Report Date: 07/13/2016 Revision 0

Client: Cumming, City of

Facility: WPCP

Permit No: GA0046019

Outfall: 001

Client Contact: JIMMY ANDREWS / Linda Lea

Client Phone: 770-781-2007 7-781-2018

## A. REVIEW SCHEDULED TEST(S):

Chronic	Ceriodaphnia dubia
Chronic	Pimephales promelas

Concentration: 6 12 24 48 95

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

C.	Sample ID or Location: (Outfall No. or Name)	Sample Date		Sample Time (military)		Grab or Composite	Sampled By: (Sign and Print Name)	Number Of Containers Shipped
		From	To	From	To			
1	Eff. Dischz. to Big Creek	E	6/12/16	6/13/16	9:30A	9:30A	C Linda Lea	Linda Lea
2								
3								

D.	Relinquished By:	Date	Time	Received By:	Date	Time
		6/13/15	4P			
1	Linda Lea					
2						
3						

## Bio-Aquatic Sample Login

BAT sample personnel:  Yes  NoCheck for Ammonia:  Yes  NoDechlorinate Sample:  Yes  NoDilution Water:  Receiving Stream C Synthetic Lab

Date: 6-14-16	Time: 1120	By: SC
Temperature: 39 (C) IR#: see	Int. Salinity: ppt	Adj. Salinity: ppt
Chlorine: 0 mg/l	Ammonia: 0.02 mg/l	Other: 0 mg/l
pH: 7.6	DO: 9.1 mg/l	Condition: 6 mmol/l

Bio Only.  No Sample Left. Lab Id : 62453

Sample No: 62453 -

## CHAIN OF CUSTODY

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

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

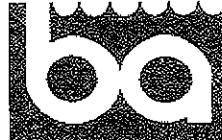
P.O. No:

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)	Selenastium (green algae)	M. beryllina (minnow)
<input type="checkbox"/> Chronic	<input type="checkbox"/> Chronic	<input type="checkbox"/> Chronic	<input type="checkbox"/> Chronic	<input type="checkbox"/> 96 Hour	<input type="checkbox"/> Chronic
<input type="checkbox"/> 96 Hour	<input type="checkbox"/> 48 Hour	<input type="checkbox"/> 96 Hour			
<input type="checkbox"/> 48 Hour	<input type="checkbox"/> 24 Hour	<input type="checkbox"/> 48 Hour			
<input type="checkbox"/> 24 Hour		<input type="checkbox"/> 24 Hour			

Notes: Annual Chronic Cerio/Fathead (BG)

Bio-Aquatic Lab ID: 62453



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

Client: Cumming, City of \_\_\_\_\_  
Facility: WPCP  
Permit No: GA0046019  
Outfall: 001  
Client Contact: JIMMY ANDREWS / Linda Lea  
Client Phone: 770-781-2007 770-781-2018

**A REVIEW SCHEDULED TEST(S):**

Chronic	Ceriodaphnia dubia
Chronic	Pimephales promelas

Concentration: 6 12 24 48 95

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

To Ship the  
1st Sample on:  
6/13/2016

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

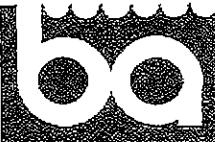
Freshwater Species					Saltwater Species	
C. dubia (water flea)	D. pulex (water flea)	D. magna (water flea)	P. promelas (minnow)	Selenastrum (green algae)	M. beryllina (minnow)	Mysidopsis (shrimp)
<input type="checkbox"/> Chronic	<input type="checkbox"/> Chronic	<input type="checkbox"/> Chronic	<input type="checkbox"/> Chronic	<input type="checkbox"/> 96 Hour	<input type="checkbox"/> Chronic	<input type="checkbox"/> Chronic
<input type="checkbox"/> 96 Hour	<input type="checkbox"/> 48 Hour	<input type="checkbox"/> 96 Hour	<input type="checkbox"/> 96 Hour			
<input type="checkbox"/> 48 Hour	<input type="checkbox"/> 24 Hour	<input type="checkbox"/> 48 Hour	<input type="checkbox"/> 48 Hour			
<input type="checkbox"/> 24 Hour		<input type="checkbox"/> 24 Hour	<input type="checkbox"/> 24 Hour			

Notes: Annual Chronic Cerio/Fathead (BG)

C	Sample ID or Location: (Outfall No. or Name)	Sample Type: E = Effluent RS = Rec. Stream S = Sediment	Sample Date		Sample Time (military)		Grab or Composite	Sampled By: (Sign and Print Name)	Number Of Containers Shipped
			From	To	From	To			
1	Eff. Dischq. to Big Creek	E	6/14/16	6/15/16	9:30A	9:30A	C	Linda Lea	1
2									
3									

D	Relinquished By:	Date	Time	Received By:		Date	Time
				Initials	Date		
1	Linda Lea	6/15/16	3P		6/16/16	6/16/16	16:30
2							
3							

Bio-Aquatic Sample Login		BAT sample personnel: <input type="radio"/> Yes <input checked="" type="radio"/> No		Date: 6/16/16	Time: 16:30	By: LZ	
<i>1st use</i> <i>6/17/16</i> <i>JL</i>		Check for Ammonia: <input type="radio"/> Yes <input type="radio"/> No		Temperature: 61.0	IR# 002	Int. Salinity: ppt	Adj. Salinity: ppt
		Dechlorinate Sample: <input type="radio"/> Yes <input checked="" type="radio"/> No		Chlorine: 20.1 mg/l	Ammonia: 10.25	Other:	
		Dilution Water: <input type="radio"/> Receiving Stream <input checked="" type="radio"/> Synthetic Lab		pH: 7.5	DO: 6.6 mg/l	Condition: Good	



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

Report Date: 07/13/2016 Revision 0

Client: Cumming, City of

Facility: WPCP

Permit No: GA0046019

Outfall: 001

Client Contact: JIMMY ANDREWS / Linda Lea

Client Phone: 770-781-2007 7-181-2018

**A. REVIEW SCHEDULED TEST(s):**

Chronic	Ceriodaphnia dubia
Chronic	Pimephales promelas

Concentration: 6 12 24 48 96

(For TX) Setup separate 24hr Acute Test?

No

To Ship the  
1st Sample on:

6/13/2016

Notes: Annual Chronic Cerio/Fathead (BG)

<b>CHAIN OF CUSTODY</b>		<input type="checkbox"/> Bio Only: No Sample Left	Lab Id: <b>62453</b>
Please Review & Complete Sections A, B, C, & D.		Sample No: <b>62453</b> -	
Check Sample No.: <input type="checkbox"/> First, <input type="checkbox"/> Second, or <input checked="" type="checkbox"/> Third.		P.O. 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)
<input type="checkbox"/> Chronic	<input type="checkbox"/> Chronic	<input type="checkbox"/> Chronic	<input type="checkbox"/> Chronic	<input type="checkbox"/> 96 Hour	<input type="checkbox"/> Chronic
<input type="checkbox"/> 96 Hour	<input type="checkbox"/> 48 Hour	<input type="checkbox"/> 96 Hour			
<input type="checkbox"/> 48 Hour	<input type="checkbox"/> 24 Hour	<input type="checkbox"/> 48 Hour			
<input type="checkbox"/> 24 Hour					

<b>C.</b> Sample ID or Location: (Outfall No. or Name)	Sample Type: E = Effluent RS = Rec. Stream S = Sediment	Sample Date	Sample Time (military)	Grab or Composite	Sampled By: (Sign and Print Name)	Number Of Containers Shipped
1 Eff. Dischgs. to Big Creek	E	6/16/16	6/17/16 9:30A	C	Linda Lea	1
2						
3						

<b>D.</b> Relinquished By:	Date	Time	Received By:	Date	Time
1 Linda Lea	6/17/16	3P	CD	6/20/16	10:30
2					
3					

Bio-Aquatic Sample Login		BAT sample personnel: <input type="radio"/> Yes <input checked="" type="radio"/> No	Date: 6/20	Time: 10:56	By: C
Rec. S US 8d O H T DCL		Check for Ammonia: <input type="radio"/> Yes <input type="radio"/> No	Temperature: 24 (C)	IR#: 002	Int. Salinity: ppt
		Dechlorinate Sample: <input type="radio"/> Yes <input checked="" type="radio"/> No	Chlorine: 0.1 mg/l	Ammonia: 0.21 mg/l	Other:
		<input type="radio"/> Receiving Stream C	pH: 7.5	DO: 9.9 mg/l	Adj. Salinity: ppt
		<input checked="" type="radio"/> Synthetic Lab			Condition: Good

# **REGULATORY AGENCY TABLES**

Appendix E

Table 1 (Sheet 1 of 4 )

## BIOMONITORING REPORT

*Ceriodaphnia dubia* SURVIVAL AND REPRODUCTION TESTPermittee: Cumming, City of - WPCPPermit No.: GA0046019Outfall No.: 001

	Date/Time	Date/Time
Dates and times	FROM: <u>6/12/2016 @09:30</u>	TO: <u>6/13/2016@ 09:30</u>
Composites were collected:	FROM: <u>6/14/2016 @09:30</u>	TO: <u>6/15/2016@ 09:30</u>
	FROM: <u>6/16/2016 @09:30</u>	TO: <u>6/17/2016@ 09:30</u>

Sample three was received and used out of hold time.

Test Initiation: Time: 16:49 Date: 6/14/2016Dilution Water Used:  Receiving Water  Synthetic Dilution WaterNUMBER OF YOUNG PRODUCED PER ADULT AT TEST TERMINATION

REPLICATE	EFFLUENT CONCENTRATION (%)					
	0%	6 %	12 %	24 %	48 %	95 %
A	29	26	29	43	29	32
B	D- 14	28	28	27	27	28
C	D- 19	33	34	29	23	22
D	24	31	31	27	24	25
E	26	31	29	32	23	25
F	34	30	D- 9	24	28	27
G	28	30	30	28	37	22
H	27	28	35	31	28	30
I	26	32	31	29	28	39
J	26	32	28	30	25	38
MEAN	27.5	30.1	30.5	30	27.2	28.8
CV % <sup>1</sup>	10.9	7.2	8.2	16.9	15	20.8
PMSD	Acceptable Range 47 or Less					21.5 %

<sup>1</sup> 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: Cumming, City of -WPCP  
 Permit No.: GA0046019  
 Outfall No.: 001

PERCENT SURVIVAL

Time of Reading	EFFLUENT CONCENTRATION (%)					
	0%	6 %	12 %	24 %	48 %	95 %
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	80.0	100.0	90.0	100.0	100.0	100.0

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 ( 95 %) : \_\_\_\_\_ YES  X  NO

If you report NO, enter a '0' on the DMR form for Parameter **TWP3B**, otherwise 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 ( 95 %) : \_\_\_\_\_ YES  X  NO

If you report NO, enter a '0' on the DMR form for Parameter **TLP3B**, otherwise 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 = 95 % Effluent (Parameter TOP3B)

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

c. NOEC Reproduction = 95 % 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: Cumming, City of - WPCP  
 Permit No.: GA0046019  
 Outfall No.: 001

	Date/Time	Date/Time
Dates and times	FROM: 6/12/2016 @ 09:30	TO: 6/13/2016 @ 09:30
Composites were collected:	FROM: 6/14/2016 @ 09:30	TO: 6/15/2016 @ 09:30
	FROM: 6/16/2016 @ 09:30	TO: 6/17/2016 @ 09:30

Sample three was received and used out of hold time.

Test Initiation: Time: 17:30 Date: 6/14/2016

Dilution Water Used:  Receiving Water  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 % <sup>1</sup>
	A	B	C	D	E		
0%	0.406	0.519	0.513	0.394	0.519	0.470	13.74
6 %	0.514	0.489	0.418	0.434	0.425	0.456	9.41
12 %	0.594	0.370	0.576	0.510	0.550	0.520	17.24
24 %	0.453	0.608	0.586	0.499	0.634	0.556	13.78
48 %	0.584	0.542	0.549	0.569	0.765	0.602	15.42
95 %	0.766	0.435	0.456	0.558	0.537	0.551	23.86
PMSD	Acceptable Range 30 or Less					27.7 %	

<sup>1</sup> 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 % <sup>1</sup>
	A	B	C	D	E	24 Hours	48 Hours	7-Day	
0%	87.5	87.5	100	62.5	87.5	100	97.5	85	16.11
6 %	100	100	87.5	87.5	87.5	100	100	92.5	7.40
12 %	100	75	100	100	87.5	100	100	92.5	12.09
24 %	75	100	100	75	87.5	100	97.5	87.5	14.29
48 %	87.5	87.5	75	75	75	100	97.5	80	8.56
95 %	100	75	75	62.5	75	100	100	77.5	17.67

Table 1 (Sheet 4 of 4)  
BIOMONITORING REPORT

*Pimephales promelas* SURVIVAL AND GROWTH TEST

Permittee: Cumming, City of WPCP  
Permit No.: GA0046019  
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 ( 95 % ) : \_\_\_\_\_ YES  NO

If you report NO, enter a '0' on the DMR form for Parameter **TWP6C**, otherwise 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 ( 95 % ) : \_\_\_\_\_ YES  NO

If you report NO, enter a '0' on the DMR form for Parameter **TLP6C**, otherwise 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:

- |                    |                      | <u>For DMR Form:</u> |
|--------------------|----------------------|----------------------|
| a. NOEC Survival = | <u>95</u> % Effluent | (Parameter TOP6C)    |
| b. LOEC Survival = | <u>Q*</u> % Effluent | (Parameter TXP6C)    |
| c. NOEC Growth =   | <u>95</u> % Effluent | (Parameter TPP6C)    |
| d. LOEC Growth =   | <u>Q*</u> % Effluent | (Parameter TYP6C)    |

Q\* refers to a value that is not calculable