

**ENVIRONMENTAL ENTERPRISE GROUP
CITY OF CLARKSVILLE WWTP
OUTFALL 001**

Chronic Biomonitoring Report
Permit Number NPDES AR0022187
AFIN 36-00038

Ceriodaphnia dubia
Pimephales promelas

March 21, 2017

Reviewed by:



Ryan Kasper, QA/QC Officer
Huther & Associates, Inc.
1156 North Bonnie Brae
Denton, Texas 76201
(940) 387-1025, Fax: (940) 387-1036

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TOXICITY TEST REPORT - CHRONIC

Client Environmental Enterprise Group Sample Outfall 001
 Facility City of Clarksville WWTP Laboratory I.D. 26728
 Permit No. NPDES AR0022187 Begin Date March 21, 2017

Results: **Pass** *Ceriodaphnia dubia* survival and reproduction and *Pimephales promelas* survival and growth at the low flow concentration (100% effluent).

SAMPLE COLLECTION

Composite effluent samples from Environmental Enterprise Group, City of Clarksville WWTP were delivered by Federal Express courier to Huther & Associates on March 21, March 23, and March 25, 2017. Effluent samples were collected and composited from Outfall 001 using an automatic sampler by facility personnel. Two toxicity tests were requested: a seven-day *Ceriodaphnia dubia* survival and reproduction test (EPA Method 1002.0), and a seven-day *Pimephales promelas* larval survival and growth test (EPA Method 1000.0). Test organisms, procedures and quality assurance requirements were in accordance with the EPA manual, "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition" (EPA-821-R-02-013).

The effluent and receiving water samples were analyzed for total residual chlorine (Standard Methods, 22nd Edition, 4500-Cl D) and contained <0.01 mg/L, <0.01 mg/L, and <0.01 mg/L, respectively. Effluent and receiving dilution water hardness, alkalinity, conductivity, pH, and dissolved oxygen data were collected and recorded.

TEST SETUP
Ceriodaphnia dubia



The seven-day *Ceriodaphnia dubia* survival and reproduction test was initiated at 1610 hours, March 21, 2017. Five concentrations were prepared (32%, 42%, 56%, 75%, and 100% effluent) utilizing receiving water (Lake Dardanelle) as dilution water. The test was conducted in 25 mL distilled water rinsed plastic beakers containing 15 mL of solution (one neonate per beaker, ten beakers per concentration). *C. dubia* neonates were less than 24-hours-old and within eight hours of the same age at test initiation. Neonates were placed in beakers following a randomized block test design. Fresh solutions were prepared and renewed daily. Daily feeding consisted of 0.5 mL *Selenastrum capricornutum* and cerophyll per test chamber. The test proceeded for seven days during which survival, reproduction and water quality data were collected daily.

A true control of ten replicate chambers containing one neonate each in receiving water was conducted concurrently with the test. There was 100% survival in the true control. In addition, a performance control of ten replicate chambers containing one neonate each in synthetic laboratory water was conducted concurrently with the test. The purpose of the performance control was to assess the health of the test organisms and to identify receiving water toxicity. The performance control data was not used in the statistical analysis of the test data. There was 100% survival in the performance control. The test ended at 1610 hours, March 28, 2017. Survival and reproduction data were statistically analyzed ($p = 0.05$) according to EPA procedures to determine the Lowest Observable Effect Concentration (LOEC) and the No Observable Effect Concentration (NOEC).

SURVIVAL***Ceriodaphnia dubia***

There was 100% survival to *C. dubia* in all of the effluent concentrations tested. Therefore, statistical analyses were not required to determine a no effect concentration.

LOEC: Not Applicable

NOEC: 100% Effluent

REPRODUCTION***Ceriodaphnia dubia***

C. dubia reproduction data were normally distributed at the 0.01 alpha level (13.277) using Chi-Square test for normality. Reproduction data were homogeneous using Bartlett's test at the 0.01 alpha level (15.09) without data transformations. Therefore, a parametric test was performed on the homogeneous data. Dunnett's test on *C. dubia* reproduction data demonstrated that there were no statistically significant differences between the control and any of the effluent concentrations.

LOEC: Not Applicable

NOEC: 100% Effluent

PMSD: 9.5%

TEST SETUP***Pimephales promelas***

The seven-day *Pimephales promelas* larval survival and growth test was initiated at 1510 hours, March 21, 2017. Five concentrations were prepared (32%, 42%, 56%, 75%, and 100% effluent) utilizing receiving water (Lake Dardanelle) as dilution water. The test was conducted in 300 mL distilled water rinsed plastic beakers containing 250 mL of solution (eight larvae per beaker, five beakers per concentration). *P. promelas* larvae were less than 24-hours-old at test initiation and originated from a minimum of three in-house spawnings. Fresh solutions were prepared and renewed daily. Larvae in each test chamber were fed <24-hour-old *Artemia* (brine shrimp) three times per day. The test proceeded for seven days during which survival and water quality data were collected daily.

Huther and Associates

7-Day/3 Brood *Ceriodaphnia dubia* Survival and Reproduction Chronic Toxicity Test

CLIENT : EEG, City of Clarksville WWTP SAMPLE TYPE : 24 Hour Composite
 NPDES # : AR0022187 DATE COLLECTED : 03/20/17 03/22/17 03/24/17
 LAB ID # : 26728 DATE RECEIVED : 03/21/17 03/23/17 03/25/17
 TEST TYPE : 7 Day Chronic BEGIN DATE/TIME : 03/21/17 1610
 TEST ORGANISM : *Ceriodaphnia dubia* END DATE/TIME : 03/28/17 1610
 ORGANISM AGE : < 24-hours TEST TEMPERATURE (°C) : 25 ± 1
 ORGANISM SOURCE : In House PHOTO PERIOD : 16-hr. Light 8-hr. Dark
 RECEIVING WATER : Lake Dardanelle LIGHT INTENSITY : 50-100 ft. cndL
 DILUTION WATER : Lake Dardanelle TECHNICIAN : T. Burton

SURVIVAL & REPRODUCTION SUMMARY

Performance Control

| Date | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 | Rep 6 | Rep 7 | Rep 8 | Rep 9 | Rep 10 |
|--|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|
| 03/22/17 | A | A | A | A | A | A | A | A | A | A |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 03/23/17 | A | A | A | A | A | A | A | A | A | A |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 03/24/17 | A | A | A | A | A | A | A | A | A | A |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 03/25/17 | 2 | 5 | 3 | 4 | 2 | 3 | 3 | 3 | 2 | 4 |
| | A | A | A | A | A | A | A | A | A | A |
| 03/26/17 | 2 | 5 | 3 | 4 | 2 | 3 | 3 | 3 | 2 | 4 |
| | 10 | 11 | 9 | 10 | 8 | 6 | 10 | 10 | 11 | 8 |
| 03/27/17 | 12 | 16 | 12 | 14 | 10 | 9 | 13 | 13 | 13 | 12 |
| | 13 | 13 | 12 | 12 | 14 | 12 | 13 | 15 | 13 | 12 |
| 03/28/17 | 25 | 29 | 24 | 26 | 24 | 21 | 26 | 28 | 26 | 24 |
| x # Young 25 3 C.V. 8.95% x% Survival 100% C.V. 0.00% | | | | | | | | | | |

True Control

| Date | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 | Rep 6 | Rep 7 | Rep 8 | Rep 9 | Rep 10 |
|--|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|
| 03/22/17 | A | A | A | A | A | A | A | A | A | A |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 03/23/17 | A | A | A | A | A | A | A | A | A | A |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 03/24/17 | A | A | A | A | A | A | A | A | A | A |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 03/25/17 | 2 | 3 | 2 | 3 | 4 | 3 | 3 | 3 | 4 | 4 |
| | A | A | A | A | A | A | A | A | A | A |
| 03/26/17 | 2 | 3 | 2 | 3 | 4 | 3 | 3 | 3 | 4 | 4 |
| | 8 | 6 | 11 | 9 | 11 | 10 | 9 | 7 | 6 | 6 |
| 03/27/17 | 10 | 9 | 13 | 12 | 15 | 13 | 12 | 10 | 10 | 10 |
| | 12 | 12 | 13 | 14 | 12 | 14 | 13 | 13 | 13 | 12 |
| 03/28/17 | 22 | 21 | 26 | 26 | 27 | 27 | 25 | 23 | 23 | 22 |
| x # Young 24 2 C.V. 9.30% x% Survival 100% C.V. 0.00% | | | | | | | | | | |

32% Effluent

| Date | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 | Rep 6 | Rep 7 | Rep 8 | Rep 9 | Rep 10 |
|--|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|
| 03/22/17 | A | A | A | A | A | A | A | A | A | A |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 03/23/17 | A | A | A | A | A | A | A | A | A | A |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 03/24/17 | A | A | A | A | A | A | A | A | A | A |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 03/25/17 | 4 | 4 | 2 | 2 | 3 | 2 | 3 | 3 | 4 | 3 |
| | A | A | A | A | A | A | A | A | A | A |
| 03/26/17 | 4 | 4 | 2 | 2 | 3 | 2 | 3 | 3 | 4 | 3 |
| | 7 | 7 | 9 | 10 | 10 | 6 | 11 | 9 | 8 | 6 |
| 03/27/17 | 11 | 11 | 11 | 12 | 13 | 8 | 14 | 12 | 12 | 9 |
| | 15 | 12 | 13 | 13 | 13 | 12 | 14 | 12 | 13 | 13 |
| 03/28/17 | 26 | 23 | 24 | 25 | 26 | 20 | 28 | 24 | 25 | 22 |
| x # Young 24 3 C.V. 9.31% x% Survival 100% C.V. 0.00% | | | | | | | | | | |

42% Effluent

| Date | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 | Rep 6 | Rep 7 | Rep 8 | Rep 9 | Rep 10 |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|
| 03/22/17 | A | A | A | A | A | A | A | A | A | A |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 03/23/17 | A | A | A | A | A | A | A | A | A | A |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 03/24/17 | A | A | A | A | A | A | A | A | A | A |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 03/25/17 | 3 | 2 | 2 | 2 | 4 | 3 | 4 | 2 | 3 | 4 |
| | A | A | A | A | A | A | A | A | A | A |
| 03/26/17 | 3 | 2 | 2 | 2 | 4 | 3 | 4 | 2 | 3 | 4 |
| | 7 | 10 | 9 | 6 | 11 | 8 | 11 | 6 | 6 | 8 |
| 03/27/17 | 10 | 12 | 11 | 8 | 15 | 11 | 15 | 8 | 9 | 12 |
| | 12 | 13 | 13 | 14 | 12 | 14 | 13 | 12 | 13 | 13 |
| 03/28/17 | 22 | 25 | 24 | 22 | 27 | 25 | 28 | 20 | 22 | 25 |
| x # Young 24 0 C.V. 10.39% x% Survival 100% C.V. 0.00% | | | | | | | | | | |

where: A = Alive
 5 = Alive, 5 young
 D = Dead
 D5 = 5 Young, Female died

ex 1:

| |
|---|
| A |
| 4 |

 alive today
 total young to date

ex 2:

| |
|----|
| 5 |
| 12 |

 alive, 5 young today
 total young to date

Huther and Associates
7-Day/3 Brood *Ceriodaphnia dubia* Survival and Reproduction Chronic Toxicity Test

EEG, Clarksville WWTP

Lab ID# 26728

Test Date: March 21, 2017

56% Effluent

| Date | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 | Rep 6 | Rep 7 | Rep 8 | Rep 9 | Rep 10 |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|
| 03/22/17 | A | A | A | A | A | A | A | A | A | A |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 03/23/17 | A | A | A | A | A | A | A | A | A | A |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 03/24/17 | A | A | A | A | A | A | A | A | A | A |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 03/25/17 | 4 | 2 | 3 | 3 | 2 | 4 | 2 | 3 | 3 | 3 |
| | 4 | 2 | 3 | 3 | 2 | 4 | 2 | 3 | 3 | 3 |
| 03/26/17 | A | A | A | A | A | A | A | A | A | A |
| | 4 | 2 | 3 | 3 | 2 | 4 | 2 | 3 | 3 | 3 |
| 03/27/17 | 7 | 8 | 6 | 10 | 9 | 7 | 10 | 10 | 6 | 10 |
| | 11 | 10 | 9 | 13 | 11 | 11 | 12 | 13 | 9 | 13 |
| 03/28/17 | 12 | 14 | 13 | 13 | 12 | 13 | 14 | 13 | 13 | 13 |
| | 23 | 24 | 22 | 26 | 23 | 24 | 26 | 26 | 22 | 26 |
| x# Young 24.2 C.V. 6.97% x% Survival 100% C.V. 0.00% | | | | | | | | | | |

75% Effluent

| Date | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 | Rep 6 | Rep 7 | Rep 8 | Rep 9 | Rep 10 |
|--|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|
| 03/22/17 | A | A | A | A | A | A | A | A | A | A |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 03/23/17 | A | A | A | A | A | A | A | A | A | A |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 03/24/17 | A | A | A | A | A | A | A | A | A | A |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 03/25/17 | 2 | 4 | 3 | 3 | 3 | 2 | 4 | 4 | 2 | 2 |
| | 2 | 4 | 3 | 3 | 3 | 2 | 4 | 4 | 2 | 2 |
| 03/26/17 | A | A | A | A | A | A | A | A | A | A |
| | 2 | 4 | 3 | 3 | 3 | 2 | 4 | 4 | 2 | 2 |
| 03/27/17 | 7 | 8 | 9 | 9 | 11 | 6 | 10 | 6 | 7 | 9 |
| | 9 | 12 | 12 | 12 | 14 | 8 | 14 | 10 | 9 | 11 |
| 03/28/17 | 14 | 14 | 12 | 13 | 14 | 12 | 13 | 13 | 12 | 13 |
| | 23 | 26 | 24 | 25 | 28 | 20 | 27 | 23 | 21 | 24 |
| x# Young 24.1 C.V. 10.43% x% Survival 100% C.V. 0.00% | | | | | | | | | | |

100% Effluent

| Date | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 | Rep 6 | Rep 7 | Rep 8 | Rep 9 | Rep 10 |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|
| 03/22/17 | A | A | A | A | A | A | A | A | A | A |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 03/23/17 | A | A | A | A | A | A | A | A | A | A |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 03/24/17 | A | A | A | A | A | A | A | A | A | A |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 03/25/17 | 3 | 3 | 2 | 4 | 2 | 3 | 4 | 2 | 4 | 3 |
| | 3 | 3 | 2 | 4 | 2 | 3 | 4 | 2 | 4 | 3 |
| 03/26/17 | A | A | A | A | A | A | A | A | A | A |
| | 3 | 3 | 2 | 4 | 2 | 3 | 4 | 2 | 4 | 3 |
| 03/27/17 | 6 | 8 | 10 | 10 | 7 | 8 | 11 | 8 | 10 | 9 |
| | 9 | 11 | 12 | 14 | 9 | 11 | 15 | 10 | 14 | 12 |
| 03/28/17 | 13 | 12 | 12 | 14 | 13 | 13 | 12 | 14 | 12 | 13 |
| | 22 | 23 | 24 | 28 | 22 | 24 | 27 | 24 | 26 | 25 |
| x# Young 24.5 C.V. 8.22% x% Survival 100% C.V. 0.00% | | | | | | | | | | |

where: A = Alive

5 = Alive, 5 young

D = Dead

D5 = 5 Young, Female died

ex 1:

| | |
|---|---------------------|
| A | alive today |
| 4 | total young to date |

ex 2:

| | |
|----|----------------------|
| 5 | alive, 5 young today |
| 12 | total young to date |

Huthner and Associates
7-Day/3 Brood *Ceriodaphnia dubia* Survival and Reproduction Chronic Toxicity Test

EEG, Clarksville WWTP

Lab ID# 26728

Test Date: March 21, 2017

WET CHEMISTRY MEASUREMENTS

| Date | Time | Temp | Samp. No. | pH of Solution | | | | | | Analyst | |
|----------|---------|------|-----------|----------------|------|------|------|------|------|---------|------|
| | | | | PCON | TCON | 32% | 42% | 56% | 75% | | 100% |
| 03/21/17 | Start | 25.0 | 1 | 8.44 | 7.57 | 7.47 | 7.41 | 7.38 | 7.35 | 7.18 | LT |
| 03/22/17 | 24 Hr. | 25.5 | 1 | 8.40 | 7.63 | 7.54 | 7.47 | 7.41 | 7.32 | 7.16 | RP |
| 03/22/17 | Renew | 25.0 | 1 | 8.37 | 7.64 | 7.60 | 7.53 | 7.45 | 7.36 | 7.14 | RP |
| 03/23/17 | 48 Hr. | 25.1 | 1 | 8.39 | 7.81 | 7.46 | 7.39 | 7.35 | 7.26 | 7.07 | LT |
| 03/23/17 | Renew | 25.2 | 2 | 8.40 | 7.70 | 7.43 | 7.35 | 7.33 | 7.27 | 7.02 | LT |
| 03/24/17 | 72 Hr. | 25.0 | 2 | 8.37 | 7.73 | 7.61 | 7.53 | 7.48 | 7.39 | 7.07 | HN |
| 03/24/17 | Renew | 24.8 | 2 | 8.27 | 7.67 | 7.59 | 7.48 | 7.44 | 7.29 | 7.07 | HN |
| 03/25/17 | 96 Hr. | 25.2 | 2 | 7.66 | 7.60 | 7.56 | 7.57 | 7.51 | 7.43 | 7.20 | LT |
| 03/25/17 | Renew | 25.2 | 3 | 7.72 | 7.23 | 7.54 | 7.48 | 7.44 | 7.37 | 7.20 | LT |
| 03/26/17 | 120 Hr. | 24.0 | 3 | 8.38 | 7.73 | 7.67 | 7.57 | 7.49 | 7.40 | 7.08 | HN |
| 03/26/17 | Renew | 23.6 | 3 | 8.39 | 7.71 | 7.67 | 7.54 | 7.47 | 7.38 | 7.07 | HN |
| 03/27/17 | 144 Hr. | 24.5 | 3 | 8.40 | 7.80 | 7.73 | 7.59 | 7.51 | 7.38 | 7.06 | HN |
| 03/27/17 | Renew | 24.1 | 3 | 8.37 | 7.73 | 7.71 | 7.58 | 7.50 | 7.38 | 7.06 | HN |
| 03/28/17 | 168 Hr. | 24.4 | 3 | 8.41 | 8.16 | 8.02 | 7.93 | 7.87 | 7.72 | 7.36 | LT |

| Date | Time | Temp | Samp. No. | DO (mg/L) of Solution | | | | | | Analyst | |
|----------|---------|------|-----------|-----------------------|------|------|------|------|------|---------|------|
| | | | | PCON | TCON | 32% | 42% | 56% | 75% | | 100% |
| 03/21/17 | Start | 25.0 | 1 | 8.40 | 8.53 | 8.53 | 8.51 | 8.41 | 8.48 | 8.79 | LT |
| 03/22/17 | 24 Hr. | 25.5 | 1 | 7.41 | 7.65 | 7.84 | 7.99 | 8.12 | 7.57 | 7.23 | RP |
| 03/22/17 | Renew | 25.0 | 1 | 7.33 | 8.26 | 8.85 | 7.47 | 8.74 | 8.26 | 8.56 | RP |
| 03/23/17 | 48 Hr. | 25.1 | 1 | 7.92 | 8.21 | 8.28 | 8.23 | 8.36 | 8.37 | 8.37 | LT |
| 03/23/17 | Renew | 25.2 | 2 | 7.94 | 8.34 | 8.49 | 8.23 | 8.58 | 8.61 | 8.52 | LT |
| 03/24/17 | 72 Hr. | 25.0 | 2 | 8.21 | 8.71 | 8.57 | 8.04 | 8.20 | 8.15 | 8.44 | HN |
| 03/24/17 | Renew | 24.8 | 2 | 8.71 | 8.66 | 8.53 | 7.55 | 8.34 | 8.24 | 8.28 | HN |
| 03/25/17 | 96 Hr. | 25.2 | 2 | 8.71 | 8.23 | 8.77 | 8.85 | 8.89 | 8.91 | 8.72 | LT |
| 03/25/17 | Renew | 25.2 | 3 | 8.01 | 8.82 | 8.73 | 8.78 | 8.94 | 8.74 | 8.88 | LT |
| 03/26/17 | 120 Hr. | 24.0 | 3 | 8.76 | 7.74 | 7.65 | 7.93 | 7.61 | 7.99 | 8.14 | HN |
| 03/26/17 | Renew | 23.6 | 3 | 8.90 | 7.87 | 7.62 | 8.35 | 7.58 | 7.62 | 8.30 | HN |
| 03/27/17 | 144 Hr. | 24.5 | 3 | 8.94 | 7.63 | 8.79 | 8.85 | 8.67 | 8.82 | 8.70 | HN |
| 03/27/17 | Renew | 24.1 | 3 | 7.52 | 8.99 | 8.87 | 8.74 | 8.65 | 8.96 | 8.95 | HN |
| 03/28/17 | 168 Hr. | 24.4 | 3 | 8.95 | 8.98 | 8.54 | 8.62 | 8.89 | 8.61 | 7.42 | LT |

Huther and Associates
7-Day/3 Brood *Ceriodaphnia dubia* Survival and Reproduction Chronic Toxicity Test

EEG, Clarksville WWTP

Lab ID# 26728

Test Date: March 21, 2017

INITIAL CHEMISTRY MEASUREMENTS @ 100% EFFLUENT

| Date | Samp. No. | pH ¹ | DO ¹ | Hardness mg/L CaCO ₃ ¹ | Alkalinity mg/L CaCO ₃ ¹ | Conduct. μS/cm ¹ | Resid. Cl ₂ mg/L ¹ | Dechlor(mL) Na ₂ S ₂ O ₃ mg/L ¹ | Analyst |
|----------|-----------|-----------------|-----------------|--|--|-----------------------------|--|---|---------|
| 03/21/17 | 1 | 7.18 | 8.79 | 48 | 26 | 430 | <0.01 | N/A | RK |
| 03/23/17 | 2 | 7.02 | 8.52 | 60 | 20 | 404 | <0.01 | N/A | RK |
| 03/25/17 | 3 | 7.20 | 8.88 | 60 | 24 | 391 | <0.01 | N/A | RK |

INITIAL CHEMISTRY MEASUREMENTS @ RECEIVING WATER

| Date | Samp. No. | pH ¹ | DO ¹ | Hardness mg/L CaCO ₃ ¹ | Alkalinity mg/L CaCO ₃ ¹ | Conduct. μS/cm ¹ | Resid. Cl ₂ mg/L ¹ | Dechlor(mL) Na ₂ S ₂ O ₃ mg/L ¹ | Analyst |
|----------|-----------|-----------------|-----------------|--|--|-----------------------------|--|---|---------|
| 03/21/17 | RS1 | 7.57 | 8.53 | 252 | 134 | 1250 | <0.01 | N/A | RK |
| 03/23/17 | RS2 | 7.70 | 8.34 | 248 | 130 | 1244 | <0.01 | N/A | RK |
| 03/25/17 | RS3 | 7.23 | 8.82 | 252 | 136 | 1282 | <0.01 | N/A | RK |

¹ Measurements taken in 100% solution.

CERIODAPHNIA DUBIA STATISTICAL ANALYSES
 Reproduction

Summary Statistics on Transformed Data Table 1 of 2

| Grp | Identification | N | Min | Max | Mean |
|-----|----------------|----|--------|--------|--------|
| 1 | Control | 10 | 21.000 | 27.000 | 24.200 |
| 2 | 32% Effluent | 10 | 20.000 | 28.000 | 24.300 |
| 3 | 42% Effluent | 10 | 20.000 | 28.000 | 24.000 |
| 4 | 56% Effluent | 10 | 22.000 | 26.000 | 24.200 |
| 5 | 75% Effluent | 10 | 20.000 | 28.000 | 24.100 |
| 6 | 100% Effluent | 10 | 22.000 | 28.000 | 24.500 |

Summary Statistics on Transformed Data Table 2 of 2

| Grp | Identification | Variance | Sd | Sem | C.V.% |
|-----|----------------|----------|-------|-------|-------|
| 1 | Control | 5.067 | 2.251 | 0.712 | 9.30 |
| 2 | 32% Effluent | 5.122 | 2.263 | 0.716 | 9.31 |
| 3 | 42% Effluent | 6.222 | 2.494 | 0.789 | 10.39 |
| 4 | 56% Effluent | 2.844 | 1.687 | 0.533 | 6.97 |
| 5 | 75% Effluent | 6.322 | 2.514 | 0.795 | 10.43 |
| 6 | 100% Effluent | 4.056 | 2.014 | 0.637 | 8.22 |

Chi-Square Test For Normality: Actual And Expected Frequencies

| Interval | < -1.5 | -1.5 to -0.5 | -0.5 to 0.5 | >0.5 to 1.5 | >1.5 |
|----------|--------|--------------|-------------|-------------|-------|
| Expected | 4.020 | 14.520 | 22.920 | 14.520 | 4.020 |
| Observed | 3 | 18 | 20 | 15 | 4 |

Calculated Chi-Square goodness of fit test statistic = 1.4808

Table Chi-Square value (alpha = 0.01) = 13.277

Data **Pass** normality test. Continue analysis.

Bartlett's Test For Homogeneity of Variance

Calculated BI statistic = 1.80

Table Chi-square value = 15.09 (alpha = 0.01, DF = 5)

Table Chi-square value = 11.07 (alpha = 0.05, DF = 5)

Data **Pass** BI homogeneity test at 0.01 level. Continue analysis.

ANOVA Table

| SOURCE | DF | SS | MS | F |
|----------------|----|---------|-------|-------|
| Between | 5 | 1.483 | 0.297 | 0.060 |
| Within (Error) | 54 | 266.700 | 4.939 | |
| Total | 59 | 268.183 | | |

Critical F value = 2.45 (0.05,5,40)

Since F < Critical F Fail to Reject Ho: All equal

Dunnett's Test - Table 1 of 2 Ho:Control<Treatment

| Grp | Identification | Transformed | Mean | T Stat | Sig |
|-----|----------------|-------------|------------------------------|--------|-----|
| | | Mean | Calculated In Original Units | | |
| 1 | Control | 24.200 | 24.200 | | |
| 2 | 32% Effluent | 24.300 | 24.300 | -0.101 | |
| 3 | 42% Effluent | 24.000 | 24.000 | 0.201 | |
| 4 | 56% Effluent | 24.200 | 24.200 | 0.000 | |
| 5 | 75% Effluent | 24.100 | 24.100 | 0.101 | |
| 6 | 100% Effluent | 24.500 | 24.500 | -0.302 | |

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

No statistically significant difference

Dunnett's Test - Table 2 of 2 Ho:Control<Treatment

| Grp | Identification | Num of Reps | Minimum | Sig | % of Control | Difference from Control |
|-----|----------------|-------------|-----------------------|------|--------------|-------------------------|
| | | | Diff (In Orig. Units) | Diff | | |
| 1 | Control | 10 | | | | |
| 2 | 32% Effluent | 10 | 2.296 | 9.5 | 9.5 | -0.100 |
| 3 | 42% Effluent | 10 | 2.296 | 9.5 | 9.5 | 0.200 |
| 4 | 56% Effluent | 10 | 2.296 | 9.5 | 9.5 | 0.000 |
| 5 | 75% Effluent | 10 | 2.296 | 9.5 | 9.5 | 0.100 |
| 6 | 100% Effluent | 10 | 2.296 | 9.5 | 9.5 | -0.300 |

Huthier and Associates
7-Day *Pimephales promelas* Survival and Growth Chronic Toxicity Test

CLIENT EEG, City of Clarksville WWTP SAMPLE TYPE 24 Hour Composite
 NPDES # AR0022187 DATE COLLECTED 03/20/17 03/22/17 03/24/17
 LAB ID # 26728 DATE RECEIVED 03/21/17 03/23/17 03/25/17
 TEST TYPE 7 Day Chronic BEGIN DATE/TIME 03/21/17 1510
 TEST ORGANISM *Pimephales promelas* END DATE/TIME 03/28/17 1510
 ORGANISM AGE <24-hours TEST TEMPERATURE (°C) 25 ± 1
 ORGANISM SOURCE In House PHOTO PERIOD 16-hr Light 8-hr Dark
 RECEIVING WATER Lake Dardanelle LIGHT INTENSITY 50-100 ft. endl.
 DILUTION WATER Lake Dardanelle TECHNICIAN B. Bacon

SURVIVAL SUMMARY

| Conc. | 03/22/17 | | | | | 03/23/17 | | | | | 03/24/17 | | | | | 03/25/17 | | | | | 03/26/17 | | | | |
|-------|----------|---|---|---|---|----------|---|---|---|---|----------|---|---|---|---|----------|---|---|---|---|----------|---|---|---|---|
| | A | B | C | D | E | A | B | C | D | E | A | B | C | D | E | A | B | C | D | E | A | B | C | D | E |
| Pcon | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | |
| Tcon | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | |
| 32% | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | |
| 42% | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | |
| 56% | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | |
| 75% | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | |
| 100% | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | |

| Conc. | 03/27/17 | | | | | 03/28/17 | | | | | x % Survival | C.V. % |
|-------|----------|---|---|---|---|----------|---|---|---|---|--------------|--------|
| | A | B | C | D | E | A | B | C | D | E | | |
| Pcon | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 100.0 | 0.00 |
| Tcon | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 100.0 | 0.00 |
| 32% | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 100.0 | 0.00 |
| 42% | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 100.0 | 0.00 |
| 56% | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 100.0 | 0.00 |
| 75% | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 100.0 | 0.00 |
| 100% | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 100.0 | 0.00 |

MEAN DRY WEIGHT PER REP

| % Effluent | Rep A | Rep B | Rep C | Rep D | Rep E | x | C.V.% |
|------------|--------|--------|--------|--------|--------|--------|-------|
| Pcon | 0.4860 | 0.4250 | 0.4950 | 0.4460 | 0.4700 | 0.4644 | 6.21 |
| Tcon | 0.4520 | 0.4650 | 0.4320 | 0.4250 | 0.4810 | 0.4510 | 5.12 |
| 32% | 0.4720 | 0.4850 | 0.4440 | 0.4620 | 0.4950 | 0.4716 | 4.22 |
| 42% | 0.4150 | 0.4570 | 0.4920 | 0.4830 | 0.4720 | 0.4638 | 6.52 |
| 56% | 0.4950 | 0.4260 | 0.4770 | 0.4530 | 0.4910 | 0.4684 | 6.16 |
| 75% | 0.4650 | 0.5010 | 0.4230 | 0.4850 | 0.4800 | 0.4708 | 6.30 |
| 100% | 0.4750 | 0.4920 | 0.4130 | 0.5020 | 0.4950 | 0.4754 | 7.63 |

Huthner and Associates
7-Day *Pimephales promelas* Survival and Growth Chronic Toxicity Test

EEG, Clarksville WWTP

Lab ID# 26728

Test Date: March 21, 2017

WET CHEMISTRY MEASUREMENTS

| Date | Time | Temp | Samp. No. | pH of Solution | | | | | | Analyst | |
|----------|---------|------|-----------|----------------|------|------|------|------|------|---------|------|
| | | | | PCON | TCON | 32% | 42% | 56% | 75% | | 100% |
| 03/21/17 | Start | 25.0 | 1 | 8.44 | 7.57 | 7.47 | 7.41 | 7.38 | 7.35 | 7.18 | LT |
| 03/22/17 | 24 Hr. | 25.6 | 1 | 8.23 | 7.90 | 7.72 | 7.65 | 7.61 | 7.42 | 7.09 | RP |
| 03/22/17 | Renew | 25.0 | 1 | 8.37 | 7.64 | 7.60 | 7.53 | 7.45 | 7.36 | 7.14 | RP |
| 03/23/17 | 48 Hr. | 25.7 | 1 | 7.88 | 7.64 | 7.62 | 7.55 | 7.50 | 7.35 | 7.10 | LT |
| 03/23/17 | Renew | 25.2 | 2 | 8.40 | 7.70 | 7.43 | 7.35 | 7.33 | 7.27 | 7.02 | LT |
| 03/24/17 | 72 Hr. | 25.3 | 2 | 8.11 | 7.85 | 7.76 | 7.67 | 7.59 | 7.40 | 7.12 | HN |
| 03/24/17 | Renew | 24.8 | 2 | 8.27 | 7.67 | 7.59 | 7.48 | 7.44 | 7.29 | 7.07 | HN |
| 03/25/17 | 96 Hr. | 25.2 | 2 | 8.15 | 7.83 | 7.73 | 7.62 | 7.52 | 7.42 | 7.24 | LT |
| 03/25/17 | Renew | 25.2 | 3 | 7.72 | 7.23 | 7.54 | 7.48 | 7.44 | 7.37 | 7.20 | LT |
| 03/26/17 | 120 Hr. | 24.0 | 3 | 8.08 | 8.15 | 7.85 | 7.81 | 7.67 | 7.49 | 7.11 | HN |
| 03/26/17 | Renew | 23.6 | 3 | 8.39 | 7.71 | 7.67 | 7.54 | 7.47 | 7.38 | 7.07 | HN |
| 03/27/17 | 144 Hr. | 24.2 | 3 | 8.16 | 7.91 | 7.68 | 7.62 | 7.55 | 7.38 | 7.02 | HN |
| 03/27/17 | Renew | 24.1 | 3 | 8.37 | 7.73 | 7.71 | 7.58 | 7.50 | 7.38 | 7.06 | HN |
| 03/28/17 | 168 Hr. | 24.0 | 3 | 7.98 | 7.85 | 7.74 | 7.50 | 7.45 | 7.31 | 7.05 | LT |

| Date | Time | Temp | Samp. No. | DO (mg/L) of Solution | | | | | | Analyst | |
|----------|---------|------|-----------|-----------------------|------|------|------|------|------|---------|------|
| | | | | PCON | TCON | 32% | 42% | 56% | 75% | | 100% |
| 03/21/17 | Start | 25.0 | 1 | 8.40 | 8.53 | 8.53 | 8.51 | 8.41 | 8.48 | 8.79 | LT |
| 03/22/17 | 24 Hr. | 25.6 | 1 | 7.70 | 8.86 | 8.53 | 8.84 | 8.90 | 8.67 | 8.82 | RP |
| 03/22/17 | Renew | 25.0 | 1 | 7.33 | 8.26 | 8.85 | 7.47 | 8.74 | 8.26 | 8.56 | RP |
| 03/23/17 | 48 Hr. | 25.7 | 1 | 8.39 | 8.24 | 8.43 | 7.78 | 8.18 | 8.27 | 8.09 | LT |
| 03/23/17 | Renew | 25.2 | 2 | 7.94 | 8.34 | 8.49 | 8.23 | 8.58 | 8.61 | 8.52 | LT |
| 03/24/17 | 72 Hr. | 25.3 | 2 | 8.56 | 8.51 | 8.37 | 8.35 | 8.62 | 7.00 | 7.01 | HN |
| 03/24/17 | Renew | 24.8 | 2 | 8.71 | 8.66 | 8.53 | 7.55 | 8.34 | 8.24 | 8.28 | HN |
| 03/25/17 | 96 Hr. | 25.2 | 2 | 7.94 | 8.42 | 8.34 | 8.52 | 8.26 | 8.38 | 8.21 | LT |
| 03/25/17 | Renew | 25.2 | 3 | 8.01 | 8.82 | 8.73 | 8.78 | 8.94 | 8.74 | 8.88 | LT |
| 03/26/17 | 120 Hr. | 24.0 | 3 | 8.35 | 8.17 | 7.34 | 8.30 | 8.51 | 8.62 | 8.00 | HN |
| 03/26/17 | Renew | 23.6 | 3 | 8.90 | 7.87 | 7.62 | 8.35 | 7.58 | 7.62 | 8.30 | HN |
| 03/27/17 | 144 Hr. | 24.2 | 3 | 7.08 | 7.00 | 7.54 | 8.20 | 8.94 | 8.17 | 6.99 | HN |
| 03/27/17 | Renew | 24.1 | 3 | 7.52 | 8.99 | 8.87 | 8.74 | 8.65 | 8.96 | 8.95 | HN |
| 03/28/17 | 168 Hr. | 24.0 | 3 | 8.65 | 8.46 | 8.42 | 8.29 | 8.36 | 7.67 | 7.13 | LT |

Huthner and Associates
7-Day *Pimephales promelas* Survival and Growth Chronic Toxicity Test

EEG, Clarksville WWTP

Lab ID# 26728

Test Date: March 21, 2017

INITIAL CHEMISTRY MEASUREMENTS @ 100% EFFLUENT

| Date | Samp. No. | pH ¹ | DO ¹ | Hardness mg/L CaCO ₃ ¹ | Alkalinity mg/L CaCO ₃ ¹ | Conduct. μS/cm ¹ | Resid. Cl ₂ mg/L ¹ | Dechlor(mL) Na ₂ S ₂ O ₃ mg/L ¹ | Analyst |
|----------|-----------|-----------------|-----------------|--|--|-----------------------------|--|---|---------|
| 03/21/17 | 1 | 7.18 | 8.79 | 48 | 26 | 430 | <0.01 | N/A | RK |
| 03/23/17 | 2 | 7.02 | 8.52 | 60 | 20 | 404 | <0.01 | N/A | RK |
| 03/25/17 | 3 | 7.20 | 8.88 | 60 | 24 | 391 | <0.01 | N/A | RK |

INITIAL CHEMISTRY MEASUREMENTS @ RECEIVING WATER

| Date | Samp. No. | pH ¹ | DO ¹ | Hardness mg/L CaCO ₃ ¹ | Alkalinity mg/L CaCO ₃ ¹ | Conduct. μS/cm ¹ | Resid. Cl ₂ mg/L ¹ | Dechlor(mL) Na ₂ S ₂ O ₃ mg/L ¹ | Analyst |
|----------|-----------|-----------------|-----------------|--|--|-----------------------------|--|---|---------|
| 03/21/17 | RS1 | 7.57 | 8.53 | 252 | 134 | 1250 | <0.01 | N/A | RK |
| 03/23/17 | RS2 | 7.70 | 8.34 | 248 | 130 | 1244 | <0.01 | N/A | RK |
| 03/25/17 | RS3 | 7.23 | 8.82 | 252 | 136 | 1282 | <0.01 | N/A | RK |

¹ Measurements taken in 100% solution.

PIMEPHALES PROMELAS STATISTICAL ANALYSES
 Growth

Summary Statistics on Transformed Data Table 1 of 2

| Grp | Identification | N | Min | Max | Mean |
|-----|----------------|---|-------|-------|-------|
| 1 | Control | 5 | 0.425 | 0.481 | 0.451 |
| 2 | 32% Effluent | 5 | 0.444 | 0.495 | 0.472 |
| 3 | 42% Effluent | 5 | 0.415 | 0.492 | 0.464 |
| 4 | 56% Effluent | 5 | 0.426 | 0.495 | 0.468 |
| 5 | 75% Effluent | 5 | 0.423 | 0.501 | 0.471 |
| 6 | 100% Effluent | 5 | 0.413 | 0.502 | 0.475 |

Summary Statistics on Transformed Data Table 2 of 2

| Grp | Identification | Variance | Sd | Sem | C.V. % |
|-----|----------------|----------|-------|-------|--------|
| 1 | Control | 0.001 | 0.023 | 0.010 | 5.12 |
| 2 | 32% Effluent | 0.000 | 0.020 | 0.009 | 4.22 |
| 3 | 42% Effluent | 0.001 | 0.030 | 0.014 | 6.52 |
| 4 | 56% Effluent | 0.001 | 0.029 | 0.013 | 6.16 |
| 5 | 75% Effluent | 0.001 | 0.030 | 0.013 | 6.30 |
| 6 | 100% Effluent | 0.001 | 0.036 | 0.016 | 7.63 |

Shapiro - Wilk's Test For Normality

D = 0.019

W = 0.901

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.

Bartlett's Test For Homogeneity of Variance

Calculated B1 statistic = 1.58

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.

ANOVA Table

| SOURCE | DF | SS | MS | F |
|----------------|----|-------|-------|-------|
| Between | 5 | 0.002 | 0.000 | 0.461 |
| Within (Error) | 24 | 0.019 | 0.001 | |
| Total | 29 | 0.021 | | |

Critical F value = 2.62 (0.05,5,24)

Since F < Critical F Fail to Reject Ho: All equal

Dunnett's Test - Table 1 of 2 Ho:Control<Treatment

| Grp | Identification | Transformed | Mean | T Stat | Sig |
|-----|----------------|-------------|------------------------------|--------|-----|
| | | Mean | Calculated In Original Units | | |
| 1 | Control | 0.451 | 0.451 | | |
| 2 | 32% Effluent | 0.472 | 0.472 | -1.143 | |
| 3 | 42% Effluent | 0.464 | 0.464 | -0.710 | |
| 4 | 56% Effluent | 0.468 | 0.468 | -0.966 | |
| 5 | 75% Effluent | 0.471 | 0.471 | -1.099 | |
| 6 | 100% Effluent | 0.475 | 0.475 | -1.354 | |

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

No statistically significant difference

Dunnett's Test - Table 2 of 2 Ho:Control<Treatment

| Grp | Identification | Num of Reps | Minimum | % of Control | Difference from Control |
|-----|----------------|-------------|---------------------------|--------------|-------------------------|
| | | | Sig Diff (In Orig. Units) | | |
| 1 | Control | 5 | | | |
| 2 | 32% Effluent | 5 | 0.043 | 9.4 | -0.021 |
| 3 | 42% Effluent | 5 | 0.043 | 9.4 | -0.013 |
| 4 | 56% Effluent | 5 | 0.043 | 9.4 | -0.017 |
| 5 | 75% Effluent | 5 | 0.043 | 9.4 | -0.020 |
| 6 | 100% Effluent | 5 | 0.043 | 9.4 | -0.024 |

**APPENDIX A
RAW DATA**

7-DAY CERIODAPHNIA DUBIA SURVIVAL & REPRODUCTION
DAILY RAW DATA TABLE
PAGE 1 OF 2

CLIENT EEG-Clarksville

START DATE/TIME 3-21-17 TB 1610

OUTFALL 001

END DATE/TIME 3-28-17 TB 1610

LAB ID # 26728

Pcon

| Date | Rep1 | Rep2 | Rep3 | Rep4 | Rep5 | Rep6 | Rep7 | Rep8 | Rep9 | Rep10 | Analyst | Time |
|------|------|------|------|------|------|------|------|------|------|-------|---------|--------------|
| 3/22 | A | A | A | A | A | A | A | A | A | A | TB | 1610 |
| 3/23 | A | A | A | A | A | A | A | A | A | A | MH | 1330 |
| 3/24 | A | A | A | A | A | A | A | A | A | A | MH | 1150 |
| 3/25 | 2 | 5 | 3 | 4 | 2 | 3 | 3 | 3 | 2 | 4 | TB | 1215 1025 |
| 3/26 | A | A | A | A | A | A | A | A | A | A | TB | 1100 |
| 3/27 | 10 | 11 | 9 | 10 | 8 | 6 | 10 | 10 | 11 | 8 | MH | 1300 |
| 3/28 | 13 | 13 | 12 | 12 | 14 | 12 | 13 | 15 | 13 | 12 | TB | 1610 |
| | 25 | 29 | 24 | 26 | 24 | 21 | 26 | 28 | 26 | 24 | | |

\bar{x} # Young w/o Dead = 25.3 CV% = 8.95

\bar{x} # Young w/Dead = CV% =

\bar{x} % Survival = 100 CV% = 0.00

Tcon

| Date | Rep1 | Rep2 | Rep3 | Rep4 | Rep5 | Rep6 | Rep7 | Rep8 | Rep9 | Rep10 | Analyst | Time |
|------|------|------|------|------|------|------|------|------|------|-------|---------|--------------|
| 3/22 | A | A | A | A | A | A | A | A | A | A | TB | 1610 |
| 3/23 | A | A | A | A | A | A | A | A | A | A | MH | 1330 |
| 3/24 | A | A | A | A | A | A | A | A | A | A | MH | 1150 |
| 3/25 | 2 | 3 | 2 | 3 | 4 | 3 | 3 | 3 | 4 | 4 | TB | 1215 1025 |
| 3/26 | A | A | A | A | A | A | A | A | A | A | TB | 1100 |
| 3/27 | 8 | 6 | 11 | 9 | 11 | 10 | 9 | 7 | 6 | 6 | MH | 1300 |
| 3/28 | 12 | 12 | 13 | 14 | 12 | 14 | 13 | 13 | 13 | 12 | TB | 1610 |
| | 22 | 21 | 24 | 26 | 27 | 27 | 25 | 23 | 23 | 22 | | |

\bar{x} # Young w/o Dead = 24.2 CV% = 9.30

\bar{x} # Young w/Dead = CV% =

\bar{x} % Survival = 100 CV% = 0.00

32

| Date | Rep1 | Rep2 | Rep3 | Rep4 | Rep5 | Rep6 | Rep7 | Rep8 | Rep9 | Rep10 | Analyst | Time |
|------|------|------|------|------|------|------|------|------|------|-------|---------|------|
| 3/22 | A | A | A | A | A | A | A | A | A | A | TB | 1610 |
| 3/23 | A | A | A | A | A | A | A | A | A | A | MH | 1330 |
| 3/24 | A | A | A | A | A | A | A | A | A | A | MH | 1150 |
| 3/25 | 4 | 4 | 2 | 2 | 3 | 2 | 3 | 3 | 4 | 3 | TB | 1215 |
| 3/26 | A | A | A | A | A | A | A | A | A | A | TB | 1100 |
| 3/27 | 7 | 7 | 9 | 10 | 10 | 6 | 11 | 9 | 8 | 6 | MH | 1300 |
| 3/28 | 15 | 12 | 13 | 13 | 13 | 12 | 14 | 12 | 13 | 13 | TB | 1610 |
| | 26 | 23 | 24 | 25 | 26 | 20 | 28 | 24 | 25 | 22 | | |

\bar{x} # Young w/o Dead = 24.3 CV% = 9.31

\bar{x} # Young w/Dead = CV% =

\bar{x} % Survival = 100 CV% = 0.00

42

| Date | Rep1 | Rep2 | Rep3 | Rep4 | Rep5 | Rep6 | Rep7 | Rep8 | Rep9 | Rep10 | Analyst | Time |
|------|------|------|------|------|------|------|------|------|------|-------|---------|------|
| 3/22 | A | A | A | A | A | A | A | A | A | A | TB | 1610 |
| 3/23 | A | A | A | A | A | A | A | A | A | A | MH | 1330 |
| 3/24 | A | A | A | A | A | A | A | A | A | A | MH | 1150 |
| 3/25 | 3 | 2 | 2 | 2 | 4 | 3 | 4 | 2 | 3 | 4 | TB | 1215 |
| 3/26 | A | A | A | A | A | A | A | A | A | A | TB | 1100 |
| 3/27 | 7 | 10 | 9 | 6 | 11 | 8 | 11 | 6 | 6 | 8 | MH | 1300 |
| 3/28 | 12 | 13 | 13 | 14 | 12 | 14 | 13 | 12 | 13 | 13 | TB | 1610 |
| | 22 | 25 | 24 | 22 | 27 | 25 | 28 | 20 | 22 | 25 | | |

\bar{x} # Young w/o Dead = 24.0 CV% = 10.39

\bar{x} # Young w/Dead = CV% =

\bar{x} % Survival = 100 CV% = 0.00

075/25/17

7-DAY CHRONIC TOXICITY TEST
FIMEPHALES PROMELAS (fathead minnow) SURVIVAL

CLIENT/FACILITY EEG-CLARKSVILLE DATE/TIME STARTED 3-21-17 BB 1510
 OUTFALL # 001 PROJECT # 26728 DATE/TIME ENDED 3-28-17 BB 1510
 ORGANISM ID# PRO-17-079

| Conc. | 3-22-17 BB 1510 | | | | | 3-23-17 RP 0830 | | | | | 3-24-17 RP 0950 | | | | | 3-25-17 BB 0835 | | | | | 3-26-17 BB 0915 | | | | | | | | | |
|-----------------------|-----------------|---|---|---|---|-----------------|---|---|---|---|-----------------|---|---|---|---|-----------------|---|---|---|---|-----------------|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | A | B | C | D | E | A | B | C | D | E | A | B | C | D | E | A | B | C | D | E | | | | | |
| Pcon | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| Tcon | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| 32 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| 42 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| 56 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| 75 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| 100 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| Initials Date/Time | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

| Conc. | 3-27-17 RP 0910 | | | | | 3-28-17 BB 1510 | | | | | Mean Survival | C.V. % |
|-----------------------|-----------------|---|---|---|---|-----------------|---|---|---|---|---------------|--------|
| | A | B | C | D | E | A | B | C | D | E | | |
| Pcon | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 100.0 | 0.00 |
| Tcon | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 100.0 | 0.00 |
| 32 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 100.0 | 0.00 |
| 42 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 100.0 | 0.00 |
| 56 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 100.0 | 0.00 |
| 75 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 100.0 | 0.00 |
| 100 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 100.0 | 0.00 |
| Initials Date/Time | 3-27-17 RP 0910 | | | | | 3-28-17 BB 1510 | | | | | | |

Client / Facility EEG-Clarksville 001

Lab ID Number 26728

Outfall Number 001

Test Date 3-21-17

INITIAL CHEMISTRY MEASUREMENTS @ 100% EFFLUENT

| Date | Samp. No. | pH | DO | Hardness mg/L CaCO ₃ ¹ | Alkalinity mg/L CaCO ₃ ¹ | Conduct. umhos/cm ¹ | Resid. Cl ₂ mg/L ¹ | Dechlor(mL) Na ₂ S ₂ O ₃ mg/L ¹ | Analyst |
|------|-----------|------|------|--|--|--------------------------------|--|---|---------|
| 3-21 | 1 | 7.18 | 8.79 | 48 | 26 | 430 | <0.01 | N/A | RK |
| 3-23 | 2 | 7.02 | 8.52 | 60 | 20 | 404 | ↓ | ↓ | ↓ |
| 3-25 | 3 | 7.20 | 8.88 | 60 | 24 | 391 | ↓ | ↓ | ↓ |

INITIAL CHEMISTRY MEASUREMENTS @ RECEIVING WATER

| Date | Samp. No. | pH | DO | Hardness mg/L CaCO ₃ ¹ | Alkalinity mg/L CaCO ₃ ¹ | Conduct. umhos/cm ¹ | Resid. Cl ₂ mg/L ¹ | Dechlor(mL) Na ₂ S ₂ O ₃ mg/L ¹ | Analyst |
|------|-----------|------|------|--|--|--------------------------------|--|---|---------|
| 3-21 | RS1 | 7.57 | 8.53 | 252 | 134 | 1250 | <0.01 | N/A | RK |
| 3-23 | RS2 | 7.70 | 8.34 | 248 | 130 | 1244 | ↓ | ↓ | ↓ |
| 3-25 | RS3 | 7.23 | 8.82 | 252 | 136 | 1282 | ↓ | ↓ | ↓ |

Notes:

**APPENDIX B
REFERENCE TOXICANTS**

CHRONIC REFERENCE TOXICANT TEST RESULTS

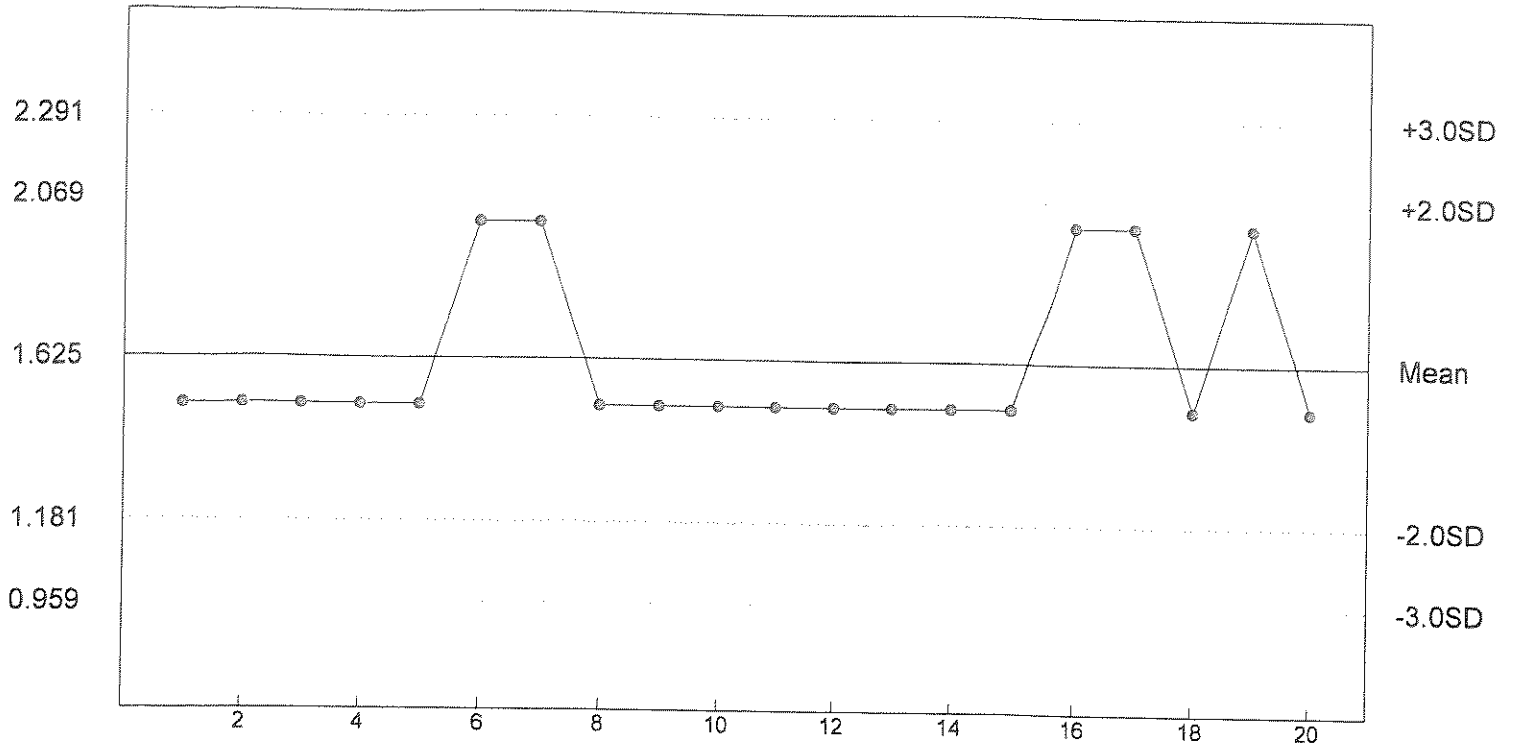
SPECIES: *Ceriodaphnia dubia*
 CHEMICAL: Sodium Chloride
 DURATION: 7-Days
 TEST NUMBER: 03
 TEST DATE: 03/02/17 - 03/09/17
 1420 Hrs - 1420 Hrs
 STATISTICAL METHOD: Dunnetts/Steels

| CONCENTRATION (g/L) | NUMBER EXPOSED | NUMBER DEAD |
|---------------------|----------------|-------------|
| 0.5 | 10 | 0 |
| 1.0 | 10 | 0 |
| 1.5 | 10 | 1 |
| 2.0 | 10 | 4 |
| 2.5 | 10 | 10 |
| 3.0 | 10 | 10 |

| LOEC FOR SURVIVAL | NOEC FOR SURVIVAL | LOEC FOR REPRODUCTION | NOEC FOR REPRODUCTION |
|-------------------|-------------------|-----------------------|-----------------------|
| 2.0 g/L | 1.5 g/L | 1.5 g/L | 1.0 g/L |

Reference Tox Sodium Chloride g/L

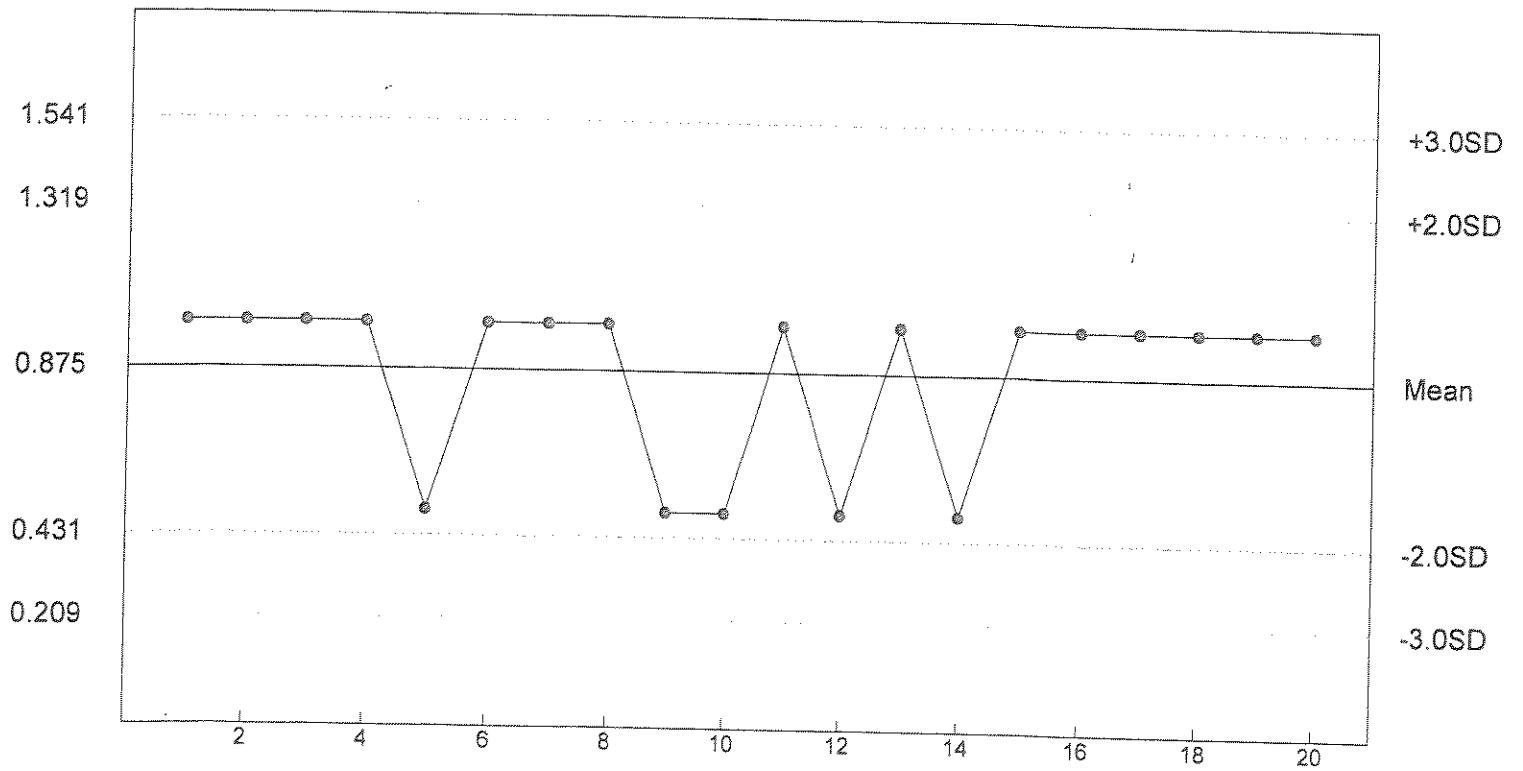
C. dubia Survival - NOEC



n= 20 Mean= 1.625 SD= 0.222 CV= 13.67% Min= 1.500 Max= 2.000

Reference Tox Sodium Chloride g/L

C. dubia Reproduction - NOEC



n= 20 Mean= 0.875 SD= 0.222 CV= 25.39% Min= 0.500 Max= 1.000

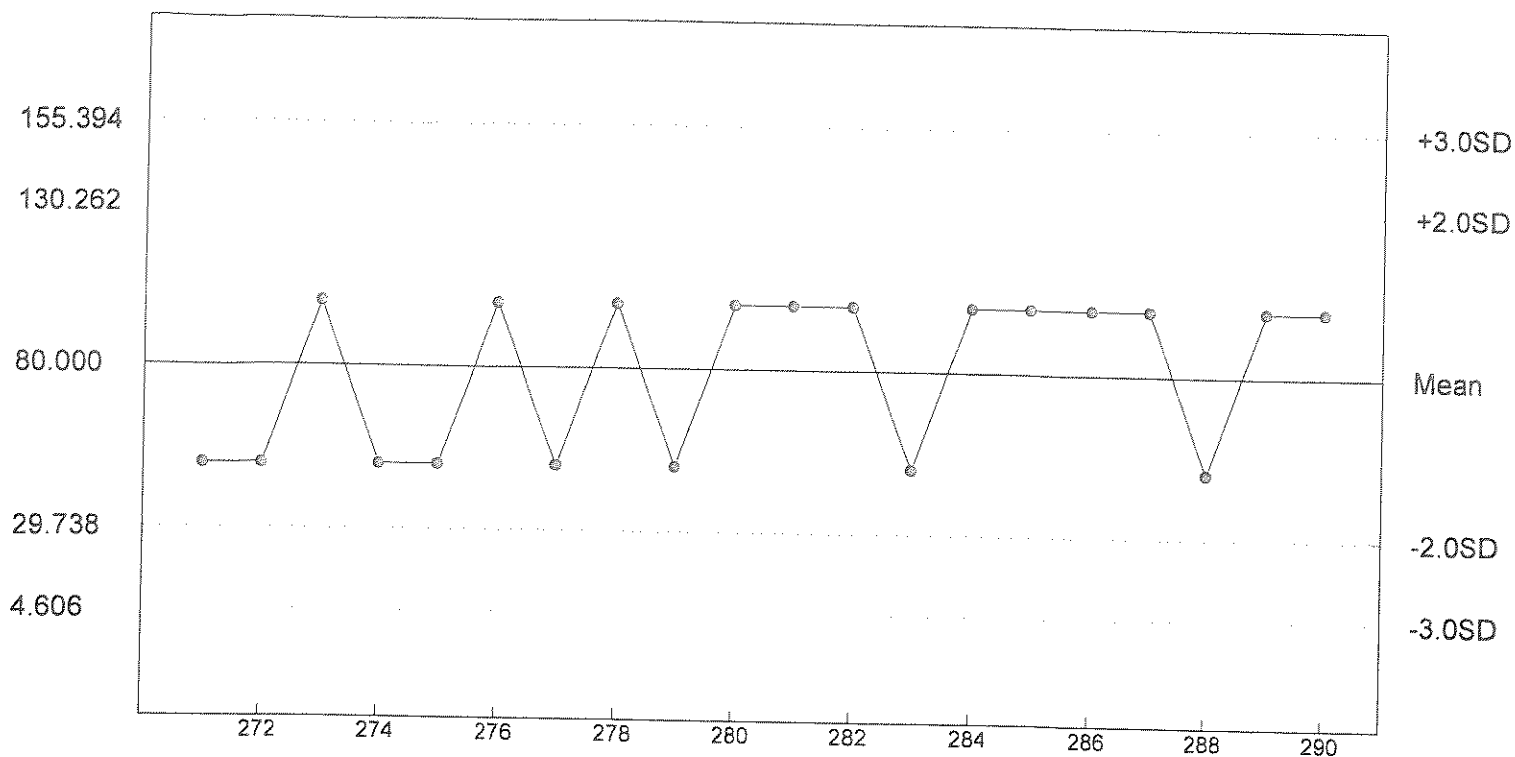
CHRONIC REFERENCE TOXICANT TEST RESULTS

SPECIES: *Pimephales promelas*
 CHEMICAL: Copper Nitrate
 DURATION: 7-Days
 TEST NUMBER: 03
 TEST DATE: 03/02/17 - 03/09/17
 1530 Hrs - 1530 Hrs
 STATISTICAL METHOD: Dunnetts/Steels

| CONCENTRATION (ug/L) | NUMBER EXPOSED | NUMBER DEAD |
|----------------------|----------------|-------------|
| 25 | 40 | 0 |
| 50 | 40 | 0 |
| 100 | 40 | 11 |
| 200 | 40 | 20 |
| 400 | 40 | 40 |
| 800 | 40 | 40 |

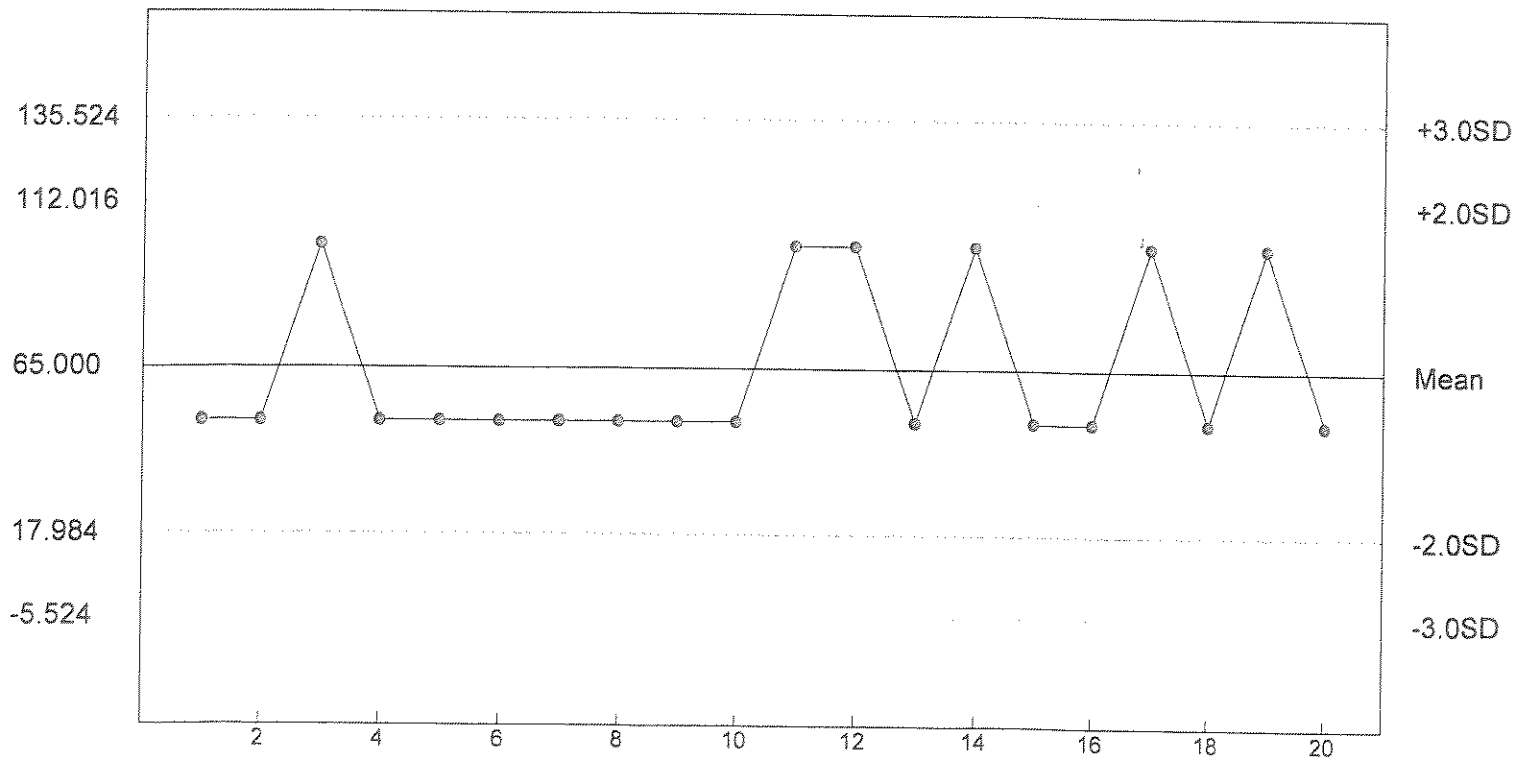
| LOEC FOR SURVIVAL | NOEC FOR SURVIVAL | LOEC FOR GROWTH | NOEC FOR GROWTH |
|-------------------|-------------------|-----------------|-----------------|
| 200 ug/L | 100 ug/L | 100 mg/L | 50 ug/L |

Reference Tox Copper Nitrate ug/L
P. promelas Chronic Survival - NOEC



n= 20 Mean= 80.000 SD= 25.131 CV= 31.41% Min= 50.000 Max= 100.000

Reference Tox Copper Nitrate ug/L
P. promelas Growth - NOEC



n= 20 Mean= 65.000 SD= 23.508 CV= 36.17% Min= 50.000 Max= 100.000

APPENDIX C
CHAIN OF CUSTODY SHEETS



Environmental Enterprise Group, Inc.
 220 North Knoxville, Suite 200
 Russellville, Arkansas 72801
 (479) 968-6767 Fax (479) 968-1956

L444-052298

| Company Name: | | Phone #: | | Requested Analysis | | | | | | | | | | Laboratory Control Number | | Remarks (Please note special detection limits below.) | | | | | | | | | | | | | | |
|-------------------------------------|------|-------------------|-------|------------------------------|-------|-------------------------|-----------------|------------------|------|---------|-----|-----------|------|---------------------------|---------------|--|-------|---------|------|-------|--|---------|--|-------|--|---------|--|-------|--|--|
| Clarksville Light and Water | | (479) 754-6241 | | 7-Day Chronic Bio-Monitoring | | | | | | | | | | 0317157 | | | | | | | | | | | | | | | | |
| Address: | | Fax #: | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| P.O. Box 1807 Clarksville, AR 72830 | | (479) 754-8181 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Project Name or Number: | | Purchase Order #: | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bio-Monitoring | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Sampling Personnel Signature(s): | | Printed: | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PamSmith | | PamSmith | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Sample I.D. | Date | Time | Comp. | Cont. Type | | | # of Containers | Method Preserved | | | | | | | Sample Matrix | | | Date | Time | | | | | | | | | | | |
| | | | | Plast. | Glass | | | H2SO4 | HNO3 | NaOH | HCL | Ice | None | Water | Soil | Air | Sudge | | | Other | | | | | | | | | | |
| Outfall 001 | 0721 | 3-23-17 | X | X | | | 1 | X | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Relinquished by: | | Date: | | Time: | | Received By: | | Time: | | Date: | | Time: | | Date: | | Time: | | Date: | | Time: | | Date: | | Time: | | Date: | | Time: | | |
| PamSmith | | 3-24-17 | | 0722 | | J. Stecyk | | 0722 | | 3-24-17 | | J. Stecyk | | 0722 | | 3-24-17 | | 3-24-17 | | 1200 | | 3-25-17 | | 1200 | | 3-25-17 | | 1200 | | |
| Received by: | | Date: | | Time: | | Relinquished By: | | Time: | | Date: | | Time: | | Date: | | Time: | | Date: | | Time: | | Date: | | Time: | | Date: | | Time: | | |
| Megan Heston | | 3-24-17 | | 0722 | | J. Stecyk | | 0722 | | 3-24-17 | | J. Stecyk | | 0722 | | 3-24-17 | | 3-24-17 | | 1600 | | 3-25-17 | | 1200 | | 3-25-17 | | 1200 | | |
| Relinquished by: | | Date: | | Time: | | Received by Laboratory: | | Time: | | Date: | | Time: | | Date: | | Time: | | Date: | | Time: | | Date: | | Time: | | Date: | | Time: | | |
| Megan Heston | | 3-24-17 | | 0800 | | T. Per | | 0800 | | 3-24-17 | | T. Per | | 0800 | | 3-24-17 | | 3-24-17 | | 1200 | | 3-25-17 | | 1200 | | 3-25-17 | | 1200 | | |
| Comments: | | | | | | Fed Ex | | | | | | | | | | | | | | | | | | | | | | | | |

TEYE
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IRI

244-05298

Environmental Enterprise Group, Inc.
220 North Knoxville, Suite 200
Russellville, Arkansas 72801
(479) 968-6767 Fax (479) 968-1956



| | | | | | | | | | | | | | | | | | | |
|-------------------------------------|------------------------|-------------------|---------|-------|------------|-------------------------|------------------|---------------|------|-------|---------|-------|-------|------|-----|--------|-------|--|
| Company Name: | | Phone #: | | | | | | | | | | | | | | | | |
| Clarksville Light and Water | | (479) 754-6241 | | | | | | | | | | | | | | | | |
| Address: | | Fax #: | | | | | | | | | | | | | | | | |
| P.O. Box 1807 Clarksville, AR 72830 | | (479) 754-8181 | | | | | | | | | | | | | | | | |
| Project Name or Number: | | Purchase Order #: | | | | | | | | | | | | | | | | |
| Bio-Monitoring | | | | | | | | | | | | | | | | | | |
| Sampling Personnel Signature(s): | | Printed: | | | | | | | | | | | | | | | | |
| <i>William Summers</i> | | William Summers | | | | | | | | | | | | | | | | |
| Sample I.D. | Date | Time | Comp. | Grab | Cont. Type | # of Containers | Method Preserved | Sample Matrix | | | | | | | | | | |
| Receiving Water | 3-24-17 | 0657 | | X | Glass | 1 | H2SO4 | HNO3 | NAOH | HCL | Ice | None | Water | Soil | Air | Sludge | Other | |
| | | | | | | | | | | | X | | | | | | | |
| | | | | | | | | | | | | | | | | | | |
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| | | | | | | | | | | | | | | | | | | |
| Relinquished by: | <i>William Summers</i> | Date: | 3-24-17 | Time: | 0722 | Received By: | <i>Stacyman</i> | | | Date: | 3/24/17 | Time: | 0800 | | | | | |
| Received by: | <i>Megan Hatcher</i> | Date: | 3-24-17 | Time: | 0722 | Relinquished By: | <i>Stacyman</i> | | | Date: | 3/24/17 | Time: | 1000 | | | | | |
| Relinquished by: | <i>Megan Hatcher</i> | Date: | 3-24-17 | Time: | 0800 | Received by Laboratory: | <i>Megan BH</i> | | | Date: | 3/25/17 | Time: | 1200 | | | | | |
| Comments: | | | | | | | | | | | | | | | | | | |

ENVIRONMENTAL ENTERPRISE GROUP
 CITY OF CLARKSVILLE WWTP – OUTFALL 001
 NPDES PERMIT NO. AR0022187
 AFIN NO. 36-00038
 BIOMONITORING REPORTING
 TEST DATE: 03/21/17

II. *Ceriodaphnia dubia*

| | Response |
|--|-----------------|
| A. If the No Observed Effect Concentration (NOEC) for survival is less than the critical dilution, enter a “1”; otherwise, enter a “0”. Parameter No. TLP3B. | 0 |
| B. If the No Observed Effect Concentration (NOEC) for reproduction is less than the critical dilution, enter a “1”; otherwise, enter a “0”. Parameter No. TGP3B. | 0 |
| C. Report the NOEC value for survival, Parameter No. TOP3B. | 100% |
| D. Report the NOEC value for reproduction, Parameter No. TPP3B. | 100% |
| E. Report the highest (critical dilution or control) Coefficient of Variation, Parameter No. TQP3B. | 9.30% |

I. *Pimephales promelas*

| | Response |
|--|-----------------|
| A. If the No Observed Effect Concentration (NOEC) for survival is less than the critical dilution, enter a “1”; otherwise, enter a “0”. Parameter No. TLP6C. | 0 |
| B. If the No Observed Effect Concentration (NOEC) for growth is less than the critical dilution, enter a “1”; otherwise, enter a “0”. Parameter No. TGP6C. | 0 |
| C. Report the NOEC value for survival, Parameter No. TOP6C. | 100% |
| D. Report the NOEC value for growth, Parameter No. TPP6C. | 100% |
| E. Report the highest (critical dilution or control) Coefficient of Variation, Parameter No. TQP6C. | 7.63% |