

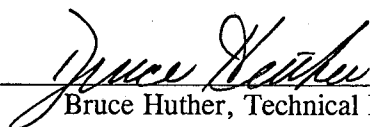
**ENVIRONMENTAL ENTERPRISE GROUP  
CITY OF CLARKSVILLE WWTP  
OUTFALL 001**

Chronic Biomonitoring Report  
Permit Number NPDES AR0022187  
AFIN Number 36-00038

*Ceriodaphnia dubia*  
*Pimephales promelas*

March 12, 2015

Reviewed by:



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TOXICITY TEST REPORT - CHRONIC

Client .....Environmental Enterprise Group
Facility .....City of Clarksville WWTP
Permit No. .... NPDES AR0022187

Sample .....Outfall 001
Laboratory I.D. ....23880
Begin Date .....March 12, 2015

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

SAMPLE COLLECTION

Composite effluent samples from Environmental Enterprise Group, City of Clarksville WWTP were delivered by United Parcel Service courier to Huthur & Associates on March 12, March 14, and March 17, 2015. Effluent samples were collected and composited from Outfall 001 using an automatic sampler. 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 1650 hours, March 12, 2015. 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 1650 hours, March 19, 2015. 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**                      **PMSD: 9.6%**  
**NOEC: 100% Effluent**

**TEST SETUP**  
*Pimephales promelas*



The seven-day *Pimephales promelas* larval survival and growth test was initiated at 1520 hours, March 12, 2015. 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.

A true control of five replicate chambers of eight larvae each in receiving water was conducted concurrently with the test. There was 100% survival in the true control. In addition, a performance control of five replicate chambers of eight larvae 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 larvae 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. At the end of the test, all larvae were sacrificed, dried, and weighed. The test ended at 1520 hours, March 19, 2015. Survival and growth (weight) 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**  
*Pimephales promelas*

There was 100% survival to *P. promelas* 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**

**GROWTH**  
*Pimephales promelas*

*P. promelas* growth data were normally distributed at the 0.01 alpha level (0.900) using Shapiro Wilk's test for normality. Growth 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 *P. promelas* growth data demonstrated that there were no statistically significant differences between the control and any of the effluent concentrations.

**LOEC: Not Applicable**                      **PMSD: 9.5%**  
**NOEC: 100% Effluent**

**SUMMARY**

There were no statistically significant differences between the control and the critical low flow concentration (100% effluent) for *C. dubia* survival and reproduction and *P. promelas* survival and growth. Based on biomonitoring requirements for Outfall 001 contained in Permit Number NPDES AR0022187 for Environmental Enterprise Group, City of Clarksville WWTP, Outfall 001 **passed** for this testing period.

Huthner 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 03/11/15 03/13/15 03/16/15  
 LAB ID # 23880 DATE RECEIVED 03/12/15 03/14/15 03/17/15  
 TEST TYPE 7-Day Chronic BEGIN DATE/TIME 03/12/15 1650  
 TEST ORGANISM *Ceriodaphnia dubia* END DATE/TIME 03/19/15 1650  
 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 Z. Geiger

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
03/13/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/14/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/15/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/16/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/17/15	3	2	2	2	4	3	2	3	2	4
	3	2	2	2	4	3	2	3	2	4
03/18/15	8	6	6	7	8	6	9	6	7	6
	11	8	8	9	12	9	11	9	9	10
03/19/15	12	12	13	14	13	11	13	12	12	14
	23	20	21	23	25	20	24	21	21	24
x# Young 22.2 C.V. 8.17% 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
03/13/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/14/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/15/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/16/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/17/15	3	2	2	4	3	2	4	3	3	2
	3	2	2	4	3	2	4	3	3	2
03/18/15	8	7	6	8	7	6	9	7	9	6
	11	9	8	12	10	8	13	10	12	8
03/19/15	12	13	11	13	13	11	13	12	13	12
	23	22	19	25	23	19	26	22	25	20
x# Young 22.4 C.V. 11.18% 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
03/13/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/14/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/15/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/16/15	2	3	A	A	A	A	A	A	A	A
	2	3	0	0	0	0	0	0	0	0
03/17/15	A	A	3	5	2	4	4	3	2	2
	2	3	3	5	2	4	4	3	2	2
03/18/15	8	7	8	10	7	9	7	9	10	9
	10	10	11	15	9	13	11	12	12	11
03/19/15	14	12	13	12	11	12	12	13	13	12
	24	22	24	27	20	25	23	25	25	23
x# Young 23.8 C.V. 8.12% 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
03/13/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/14/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/15/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/16/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/17/15	3	2	4	2	4	2	3	4	2	3
	3	2	4	2	4	2	3	4	2	3
03/18/15	8	7	9	8	9	6	8	8	7	9
	11	9	13	10	13	8	11	12	9	12
03/19/15	13	12	13	12	14	12	13	12	13	13
	24	21	26	22	27	20	24	24	22	25
x# Young 23.5 C.V. 9.46% 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, City of Clarksville WWTP

Lab ID# 23880

Test Date: March 12, 2015

56% Effluent

Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
03/13/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/14/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/15/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/16/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/17/15	2	2	3	4	2	4	2	3	5	2
	2	2	3	4	2	4	2	3	5	2
03/18/15	6	8	6	7	7	9	7	9	8	10
	8	10	9	11	9	13	9	12	13	12
03/19/15	12	13	12	12	14	13	12	14	12	13
	20	23	21	23	23	26	21	26	25	25
x# Young 23.3                      C.V. 9.28% 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
03/13/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/14/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/15/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/16/15	A	A	A	2	A	A	A	A	A	A
	0	0	0	2	0	0	0	0	0	0
03/17/15	4	3	2	A	3	2	2	3	2	4
	4	3	2	2	3	2	2	3	2	4
03/18/15	9	10	9	7	8	7	6	7	6	9
	13	13	11	9	11	9	8	10	8	13
03/19/15	14	12	12	14	13	12	14	13	15	12
	27	25	23	23	24	21	22	23	23	25
x# Young 23.6                      C.V. 7.26% 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
03/13/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/14/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/15/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/16/15	A	A	A	A	4	A	A	2	A	A
	0	0	0	0	4	0	0	2	0	0
03/17/15	2	3	5	4	2	A	3	2	A	3
	2	3	5	4	2	4	3	2	2	3
03/18/15	7	7	10	8	6	8	7	9	8	9
	9	10	15	12	8	12	10	11	10	12
03/19/15	12	13	12	14	14	12	13	12	12	13
	21	23	27	26	22	24	23	23	22	25
x# Young 23.6                      C.V. 8.04% 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

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

EEG, City of Clarksville WWTP

Lab ID# 23880

Test Date: March 12, 2015

**WET CHEMISTRY MEASUREMENTS**

Date	Time	Temp	Samp. No.	pH of Solution							Analyst
				PCON	TCON	32%	42%	56%	75%	100%	
03/12/15	Start	25.0	1	8.22	7.16	7.15	7.12	7.08	7.05	7.02	CS
03/13/15	24 Hr.	25.6	1	7.85	7.83	7.82	7.79	7.76	7.74	7.71	CS
03/13/15	Renew	25.6	1	8.25	8.22	7.25	7.23	7.19	7.14	7.08	CS
03/14/15	48 Hr.	25.5	1	7.64	7.66	7.65	7.63	7.62	7.61	7.60	CS
03/14/15	Renew	25.5	2	8.22	7.20	7.19	7.16	7.12	7.08	7.03	CS
03/15/15	72 Hr.	25.7	2	8.01	8.03	8.04	8.02	8.00	7.98	8.00	EMS
03/15/15	Renew	25.7	2	8.23	8.26	8.11	8.92	8.47	8.29	8.76	EMS
03/16/15	96 Hr.	25.8	2	7.72	7.78	7.83	7.92	7.96	7.98	8.02	EMS
03/16/15	Renew	25.8	2	8.14	8.14	8.16	8.26	8.25	8.24	8.22	EMS
03/17/15	120 Hr.	25.6	2	7.96	7.82	7.80	7.79	7.76	7.75	7.74	CS
03/17/15	Renew	25.6	3	8.52	7.22	7.20	7.16	7.12	7.08	7.06	CS
03/18/15	144 Hr.	25.1	3	7.95	7.80	7.75	7.71	7.68	7.66	7.60	CS
03/18/15	Renew	25.1	3	8.17	7.82	7.77	7.78	7.66	7.65	7.60	TG
03/19/15	168 Hr.	25.2	3	7.95	7.82	7.74	7.73	7.71	7.69	7.66	CS

Date	Time	Temp	Samp. No.	DO (mg/L) of Solution							Analyst
				PCON	TCON	32%	42%	56%	75%	100%	
03/12/15	Start	25.0	1	8.84	8.67	8.55	8.69	8.74	8.81	8.27	CS
03/13/15	24 Hr.	25.6	1	8.89	8.07	8.77	8.35	8.30	8.42	8.04	CS
03/13/15	Renew	25.6	1	8.92	8.28	8.49	8.78	8.88	8.59	8.03	CS
03/14/15	48 Hr.	25.5	1	8.87	8.71	8.61	8.14	8.33	8.95	8.26	CS
03/14/15	Renew	25.5	2	8.84	8.25	8.83	8.54	8.91	8.66	8.51	CS
03/15/15	72 Hr.	25.7	2	8.16	8.19	8.20	8.23	8.77	8.54	8.17	CS
03/15/15	Renew	25.7	2	8.24	8.63	8.93	8.92	8.18	8.11	8.93	EMS
03/16/15	96 Hr.	25.8	2	8.49	8.34	8.34	8.27	8.17	8.19	8.32	EMS
03/16/15	Renew	25.8	2	8.62	8.79	8.70	8.76	8.72	8.93	8.77	EMS
03/17/15	120 Hr.	25.6	2	8.68	8.35	8.36	8.84	8.44	8.50	8.24	CS
03/17/15	Renew	25.6	3	8.03	8.76	8.38	8.76	8.32	8.41	8.08	CS
03/18/15	144 Hr.	25.1	3	8.05	8.09	8.12	8.14	8.16	8.33	8.12	CS
03/18/15	Renew	25.1	3	8.43	8.74	8.74	8.69	8.52	8.17	7.53	TG
03/19/15	168 Hr.	25.2	3	8.23	8.69	8.18	8.31	8.39	8.51	8.50	CS



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

EEG, City of Clarksville WWTP

Lab ID# 23880

Test Date: March 12, 2015

**INITIAL CHEMISTRY MEASUREMENTS @ 100% EFFLUENT**

Date	Samp. No.	pH	DO	Hardness mg/L CaCO <sub>3</sub> <sup>1</sup>	Alkalinity mg/L CaCO <sub>3</sub> <sup>1</sup>	Conduct. umhos/cm <sup>1</sup>	Resid. Cl <sub>2</sub> mg/L <sup>1</sup>	Dechlor(mL) Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> mg/L <sup>1</sup>	Analyst
03/12/15	1	7.02	8.27	68	50	242	<0.01	N/A	TG
03/14/15	2	7.03	8.51	72	52	274	<0.01	N/A	TG
03/17/15	3	7.06	8.08	76	50	278	<0.01	N/A	TG

<sup>1</sup> Measurements taken in 100% solution.

**INITIAL CHEMISTRY MEASUREMENTS @ RECEIVING WATER**

Date	Samp. No.	pH	DO	Hardness mg/L CaCO <sub>3</sub> <sup>1</sup>	Alkalinity mg/L CaCO <sub>3</sub> <sup>1</sup>	Conduct. umhos/cm <sup>1</sup>	Resid. Cl <sub>2</sub> mg/L <sup>1</sup>	Dechlor(mL) Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> mg/L <sup>1</sup>	Analyst
03/12/15	RS1	7.16	8.67	68	46	298	<0.01	N/A	TG
03/14/15	RS2	7.20	8.25	64	44	214	<0.01	N/A	TG
03/17/15	RS3	7.22	8.76	68	42	232	<0.01	N/A	TG

**CERIODAPHNIA DUBIA STATISTICAL ANALYSES**  
 Reproduction

Summary Statistics on Transformed Data Table 1 of 2

Grp	Identification	N	Min	Max	Mean
1	Control	10	19.000	26.000	22.400
2	32% Effluent	10	20.000	27.000	23.800
3	42% Effluent	10	20.000	27.000	23.500
4	56% Effluent	10	20.000	26.000	23.300
5	75% Effluent	10	21.000	27.000	23.600
6	100% Effluent	10	21.000	27.000	23.600

Summary Statistics on Transformed Data Table 2 of 2

Grp	Identification	Variance	Sd	Sem	C.V. %
1	Control	6.267	2.503	0.792	11.18
2	32% Effluent	3.733	1.932	0.611	8.12
3	42% Effluent	4.944	2.224	0.703	9.46
4	56% Effluent	4.678	2.163	0.684	9.28
5	75% Effluent	2.933	1.713	0.542	7.26
6	100% Effluent	3.600	1.897	0.600	8.04

Chi-Square Test For Normality: Actual And Expected Frequencies

Interval	< -1.5	-1.5 to -0.5	-0.5 to 0.5	> 0.5 to 1.5	> 1.5
Expected	4.020	14.520	22.920	14.520	4.020
Observed	4	13	23	16	4

Calculated Chi-Square goodness of fit test statistic = 0.3105

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

Data Pass normality test. 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	12.533	2.507	0.575
Within (Error)	54	235.400	4.359	
Total	59	247.933		

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

Since  $F < \text{Critical F}$  Fail to Reject  $H_0$ : All equal

Dunnett's Test - Table 1 of 2  $H_0$ : Control < Treatment

Grp	Identification	Mean		T Stat	Sig
		Transformed Mean	Calculated In Original Units		
1	Control	22.400	22.400		
2	32% Effluent	23.800	23.800	-1.499	
3	42% Effluent	23.500	23.500	-1.178	
4	56% Effluent	23.300	23.300	-0.964	
5	75% Effluent	23.600	23.600	-1.285	
6	100% Effluent	23.600	23.600	-1.285	

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

No statistically significant difference

Dunnett's Test - Table 1 of 2  $H_0$ : Control < Treatment

Grp	Identification	Num of Reps	Minimum Sig Diff (In Orig. Units)	% of Control	Difference
					from Control
1	Control	10			
2	32% Effluent	10	2.157	9.6	-1.400
3	42% Effluent	10	2.157	9.6	-1.100
4	56% Effluent	10	2.157	9.6	-0.900
5	75% Effluent	10	2.157	9.6	-1.200
6	100% Effluent	10	2.157	9.6	-1.200

Huther 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	03/11/15 03/13/15 03/16/15
LAB ID #	23880	DATE RECEIVED	03/12/15 03/14/15 03/17/15
TEST TYPE	7 Day Chronic	BEGIN DATE/TIME	03/12/15 1520
TEST ORGANISM	<i>Pimephales promelas</i>	END DATE/TIME	03/19/15 1520
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. candl.
DILUTION WATER	Lake Dardanelle	TECHNICIAN	T. Burton

**SURVIVAL SUMMARY**

Conc.	03/13/15					03/14/15					03/15/15					03/16/15					03/17/15				
	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
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
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
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
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
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
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

Conc.	03/18/15					03/19/15					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	0.4150	0.4920	0.4810	0.4650	0.4678	6.66
Tcon	0.4420	0.4810	0.4160	0.4390	0.4910	0.4538	6.89
32%	0.4860	0.4920	0.4620	0.4910	0.4560	0.4774	3.58
42%	0.4470	0.5020	0.4750	0.4560	0.5010	0.4762	5.29
56%	0.4860	0.4450	0.4750	0.5020	0.4560	0.4728	4.83
75%	0.4920	0.4160	0.5060	0.4950	0.4480	0.4714	8.07
100%	0.4710	0.4620	0.4200	0.5040	0.4960	0.4706	7.04

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

EEG, City of Clarksville WWTP

Lab ID# 23880

Test Date: March 12, 2015

**WET CHEMISTRY MEASUREMENTS**

Date	Time	Temp	Samp. No.	pH of Solution						Analyst	
				PCON	TCON	32%	42%	56%	75%		100%
03/12/15	Start	25.0	1	8.22	7.16	7.15	7.12	7.08	7.05	7.02	CS
03/13/15	24 Hr.	25.6	1	8.41	8.29	7.74	7.69	7.59	7.57	7.41	TB
03/13/15	Renew	25.6	1	8.25	8.22	7.25	7.23	7.19	7.14	7.08	CS
03/14/15	48 Hr.	25.5	1	8.37	8.31	7.77	7.76	7.74	7.70	7.68	CS
03/14/15	Renew	25.5	2	8.22	7.20	7.19	7.16	7.12	7.08	7.03	CS
03/15/15	72 Hr.	25.4	2	8.47	8.46	8.42	8.41	8.39	8.30	8.38	EMS
03/15/15	Renew	25.4	2	8.23	8.26	8.11	8.92	8.47	8.29	8.76	EMS
03/16/15	96 Hr.	25.3	2	8.32	8.56	8.62	8.71	8.64	8.66	8.65	EMS
03/16/15	Renew	25.3	2	8.14	8.14	8.16	8.26	8.25	8.24	8.22	EMS
03/17/15	120 Hr.	25.5	2	8.25	8.24	8.23	8.22	8.21	8.19	8.18	CS
03/17/15	Renew	25.5	3	8.52	7.22	7.20	7.16	7.12	7.08	7.06	CS
03/18/15	144 Hr.	25.3	3	8.45	8.08	7.86	7.72	7.35	7.29	7.16	TG
03/18/15	Renew	25.3	3	8.17	7.82	7.77	7.78	7.66	7.65	7.60	TG
03/19/15	168 Hr.	25.5	3	8.31	8.29	7.26	7.25	7.24	7.22	7.20	CS

Date	Time	Temp	Samp. No.	DO (mg/L) of Solution						Analyst	
				PCON	TCON	32%	42%	56%	75%		100%
03/12/15	Start	25.0	1	8.84	8.67	8.55	8.69	8.74	8.81	8.27	CS
03/13/15	24 Hr.	25.6	1	8.64	8.46	7.71	8.58	7.79	8.54	8.55	TB
03/13/15	Renew	25.6	1	8.92	8.28	8.49	8.78	8.88	8.59	8.03	CS
03/14/15	48 Hr.	25.5	1	8.21	8.00	8.29	8.73	8.57	8.85	8.76	CS
03/14/15	Renew	25.5	2	8.84	8.25	8.83	8.54	8.91	8.66	8.51	CS
03/15/15	72 Hr.	25.4	2	8.11	8.10	8.09	8.54	8.77	8.76	8.81	EMS
03/15/15	Renew	25.4	2	8.24	8.63	8.93	8.92	8.18	8.11	8.93	EMS
03/16/15	96 Hr.	25.3	2	8.49	8.29	8.24	8.49	8.37	8.24	8.49	EMS
03/16/15	Renew	25.3	2	8.62	8.79	8.70	8.76	8.72	8.93	8.77	EMS
03/17/15	120 Hr.	25.5	2	8.27	8.32	8.77	8.38	8.79	8.01	8.13	CS
03/17/15	Renew	25.5	3	8.03	8.76	8.38	8.76	8.32	8.41	8.08	CS
03/18/15	144 Hr.	25.3	3	7.58	7.14	7.80	8.17	8.91	7.20	7.09	TG
03/18/15	Renew	25.3	3	8.43	8.74	8.74	8.69	8.52	8.17	7.53	TG
03/19/15	168 Hr.	25.5	3	8.11	8.74	8.57	7.81	7.53	8.70	8.41	CS

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

EEG, City of Clarksville WWTP

Lab ID# 23880

Test Date: March 12, 2015

**INITIAL CHEMISTRY MEASUREMENTS @ 100% EFFLUENT**

Date	Samp. No.	pH	DO	Hardness mg/L CaCO <sub>3</sub> <sup>1</sup>	Alkalinity mg/L CaCO <sub>3</sub> <sup>1</sup>	Conduct. umhos/cm <sup>1</sup>	Resid. Cl <sub>2</sub> mg/L <sup>1</sup>	Dechlor(mL) Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> mg/L <sup>1</sup>	Analyst
03/12/15	1	7.02	8.27	68	50	242	<0.01	N/A	TG
03/14/15	2	7.03	8.51	72	52	274	<0.01	N/A	TG
03/17/15	3	7.06	8.08	76	50	278	<0.01	N/A	TG

**INITIAL CHEMISTRY MEASUREMENTS @ RECEIVING WATER**

Date	Samp. No.	Ph	DO	Hardness mg/L CaCO <sub>3</sub> <sup>1</sup>	Alkalinity mg/L CaCO <sub>3</sub> <sup>1</sup>	Conduct. umhos/cm <sup>1</sup>	Resid. Cl <sub>2</sub> mg/L <sup>1</sup>	Dechlor(mL) Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> mg/L <sup>1</sup>	Analyst
03/12/15	RS1	7.16	8.67	68	46	298	<0.01	N/A	TG
03/14/15	RS2	7.20	8.25	64	44	214	<0.01	N/A	TG
03/17/15	RS3	7.22	8.76	68	42	232	<0.01	N/A	TG

<sup>1</sup> Measurements taken in 100% solution.

Huther and Associates, Inc.  
 Begin Date: March 12, 2014  
 Lab I.D.# 23880

**PIMEPHALES PROMELAS STATISTICAL ANALYSES**  
 Growth

Summary Statistics on Transformed Data Table 1 of 2

Grp	Identification	N	Min	Max	Mean
1	Control	5	0.416	0.491	0.454
2	32% Effluent	5	0.456	0.492	0.477
3	42% Effluent	5	0.447	0.502	0.476
4	56% Effluent	5	0.445	0.502	0.473
5	75% Effluent	5	0.416	0.506	0.471
6	100% Effluent	5	0.420	0.504	0.471

Summary Statistics on Transformed Data Table 2 of 2

Grp	Identification	Variance	Sd	Sem	C.V.%
1	Control	0.001	0.031	0.014	6.89
2	32% Effluent	0.000	0.017	0.008	3.58
3	42% Effluent	0.001	0.025	0.011	5.29
4	56% Effluent	0.001	0.023	0.010	4.83
5	75% Effluent	0.001	0.038	0.017	8.07
6	100% Effluent	0.001	0.033	0.015	7.04

Shapiro - Wilk's Test For Normality

D = 0.020

W = 0.942

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 = 2.80

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

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

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

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.002	0.000	0.440
Within (Error)	24	0.020	0.001	
Total	29	0.022		

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

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

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

Grp	Identification	Transformed Mean	Mean	T Stat	Sig
			Calculated In Original Units		
1	Control	0.454	0.454		
2	32% Effluent	0.477	0.477	-1.296	
3	42% Effluent	0.476	0.476	-1.230	
4	56% Effluent	0.473	0.473	-1.043	
5	75% Effluent	0.471	0.471	-0.966	
6	100% Effluent	0.471	0.471	-0.923	

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

No statistically significant difference

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

Grp	Identification	Num of Reps	Minimum Sig Diff (In Orig. Units)	% of Control	Difference
					from Control
1	Control	5			
2	32% Effluent	5	0.043	9.5	-0.024
3	42% Effluent	5	0.043	9.5	-0.022
4	56% Effluent	5	0.043	9.5	-0.019
5	75% Effluent	5	0.043	9.5	-0.018
6	100% Effluent	5	0.043	9.5	-0.017

**APPENDIX A  
RAW DATA**

7-DAY CERIODAPHNIA DUBIA SURVIVAL & REPRODUCTION

DAILY RAW DATA TABLE

CLIENT EEG-Clarksville  
 OUTFALL 001  
 LAB ID # 23880

START DATE/TIME 3-12-15 2G 1650  
 END DATE/TIME 3-19-15 NL 1650

PCON

TCON

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
3/13	A	A	A	A	A	A	A	A	A	A	MH	1650
3/14	A	A	A	A	A	A	A	A	A	A	TG	1530
3/15	A	A	A	A	A	A	A	A	A	A	ZG	1150
3/16	A	A	A	A	A	A	A	A	A	A	MH	1140
3/17	3	2	2	2	4	3	2	3	2	4	NL	1320
3/18	8	6	6	7	8	6	9	6	7	6	NL	1200
3/19	12	12	13	14	13	11	13	12	12	14	NL	1650
	23	20	21	23	25	20	24	21	21	24		

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
3/13	A	A	A	A	A	A	A	A	A	A	MH	1650
3/14	A	A	A	A	A	A	A	A	A	A	TG	1530
3/15	A	A	A	A	A	A	A	A	A	A	ZG	1150
3/16	A	A	A	A	A	A	A	A	A	A	MH	1140
3/17	3	2	2	4	3	2	4	3	3	2	NL	1320
3/18	8	7	6	8	7	6	9	7	9	6	NL	1200
3/19	12	13	11	13	13	11	13	12	13	12	NL	1650
	23	22	19	25	23	19	26	22	25	20		

$\bar{x}$  # Young w/o Dead = 22.2 CV% = 8.17

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

$\bar{x}$  % Survival = 100.0 CV% = 0.00

$\bar{x}$  # Young w/o Dead = 22.4 CV% = 11.18

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

$\bar{x}$  % Survival = 100.0 CV% = 0.00

32

42

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
3/13	A	A	A	A	A	A	A	A	A	A	MH	1650
3/14	A	A	A	A	A	A	A	A	A	A	TG	1530
3/15	A	A	A	A	A	A	A	A	A	A	ZG	1150
3/16	2	3	A	A	A	A	A	A	A	A	MH	1140
3/17	A	A	3	5	2	4	4	3	2	2	NL	1320
3/18	8	7	8	10	7	9	7	9	10	9	NL	1200
3/19	14	12	13	12	11	12	13	13	12		NL	1650
	24	22	24	27	20	25	23	25	23			

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
3/13	A	A	A	A	A	A	A	A	A	A	MH	1650
3/14	A	A	A	A	A	A	A	A	A	A	TG	1530
3/15	A	A	A	A	A	A	A	A	A	A	ZG	1150
3/16	A	A	A	A	A	A	A	A	A	A	MH	1140
3/17	3	2	4	2	4	2	3	4	2	3	NL	1320
3/18	8	7	9	8	9	6	8	8	7	9	NL	1200
3/19	13	12	13	12	14	12	13	12	13	13	NL	1650
	24	21	26	22	27	20	24	24	22	25		

$\bar{x}$  # Young w/o Dead = 23.8 CV% = 8.12

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

$\bar{x}$  % Survival = 100.0 CV% = 0.00

$\bar{x}$  # Young w/o Dead = 23.5 CV% = 9.46

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

$\bar{x}$  % Survival = 100.0 CV% = 0.00





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

CLIENT/FACILITY EEG-Clarksville 001  
 OUTFALL # 001 PROJECT # 23880  
 ORGANISM ID# PPO-15-070

DATE/TIME STARTED 3-12-15 TB 1520  
 DATE/TIME ENDED 3-19-15 TB 1520

Conc.	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	A	B	C	D	E					
P CON	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
T CON	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	3-13-15 TB 1520					3-14-15 TG 0925					3-15-15 TG 0915					3-16-15 TB 0850					3-17-15 TB 0900									

Conc.	A					B					Mean Survival	C.V. %
	A	B	C	D	E	A	B	C	D	E		
P CON	8	8	8	8	8	8	8	8	8	8	100.0	0.00
T CON	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	3-18-15 TB 0850					3-19-15 TB 1520						



Client / Facility EEG Clarksville Cool  
 Lab ID Number 23880  
 Outfall Number 001  
 Test Date 3-12-15

INITIAL CHEMISTRY MEASUREMENTS @ 100% EFFLUENT

Date	Samp. No.	pH	DO	Hardness mg/L CaCO <sub>3</sub> <sup>1</sup>	Alkalinity mg/L CaCO <sub>3</sub> <sup>1</sup>	Conduct. umhos/cm <sup>1</sup>	Resid. Cl <sub>2</sub> mg/L <sup>1</sup>	Dechlor(mL) Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> mg/L <sup>1</sup>	Analyst
3/12	1	7.02	8.27	68	50	242	60.01	Na	TG
3/14	2	7.03	8.51	72	52	274	§	§	§
3/17	3	7.06	8.08	76	50	278	§	§	§

INITIAL CHEMISTRY MEASUREMENTS @ RECEIVING WATER

Date	Samp. No.	pH	DO	Hardness mg/L CaCO <sub>3</sub> <sup>1</sup>	Alkalinity mg/L CaCO <sub>3</sub> <sup>1</sup>	Conduct. umhos/cm <sup>1</sup>	Resid. Cl <sub>2</sub> mg/L <sup>1</sup>	Dechlor(mL) Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> mg/L <sup>1</sup>	Analyst
3/12	RS1	7.16	8.67	68	46	298	60.01	Na	TG
3/14	RS2	7.20	8.25	64	44	214	§	§	§
3/17	RS3	7.22	8.76	68	42	232	§	§	§

Notes:

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**APPENDIX B**  
**REFERENCE TOXICANTS**

CHRONIC REFERENCE TOXICANT TEST RESULTS

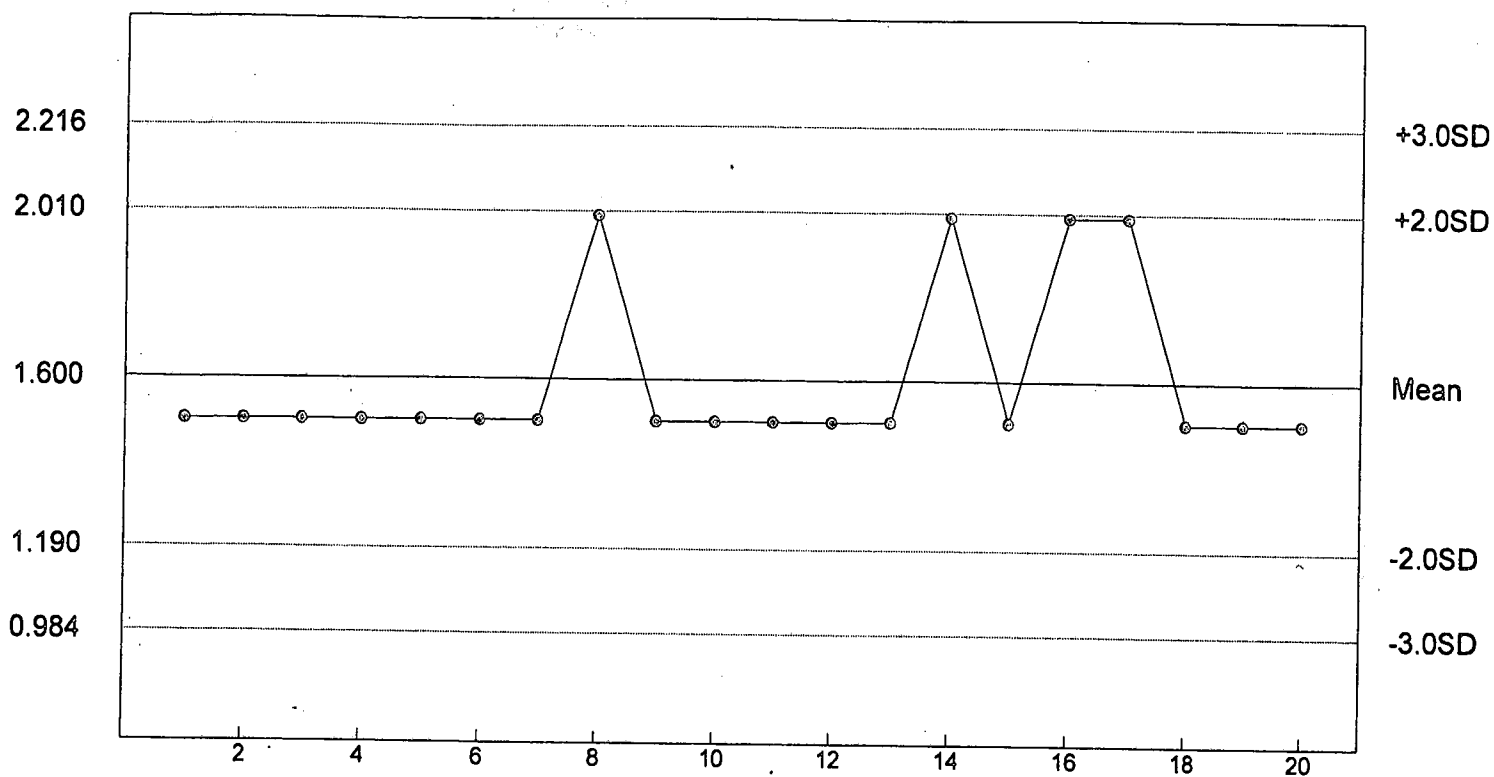
SPECIES: *Ceriodaphnia dubia*  
 CHEMICAL: Copper Nitrate  
 DURATION: 7-Days  
 TEST NUMBER: 3  
 TEST DATE: 03/04/15 - 03/11/15  
 1000 Hrs - 1000 Hrs  
 STATISTICAL METHOD: Dunnetts/Steels

CONCENTRATION (ug/L)	NUMBER EXPOSED	NUMBER DEAD
0.5	10	0
1.0	10	0
1.5	10	0
2.0	10	5
2.5	10	10
3.0	10	10

LOEC FOR SURVIVAL	NOEC FOR SURVIVAL	LOEC FOR GROWTH	NOEC FOR GROWTH
2.0 ug/L	1.5 ug/L	1.0 ug/L	0.5 ug/L

Reference Tox Sodium Chloride g/L

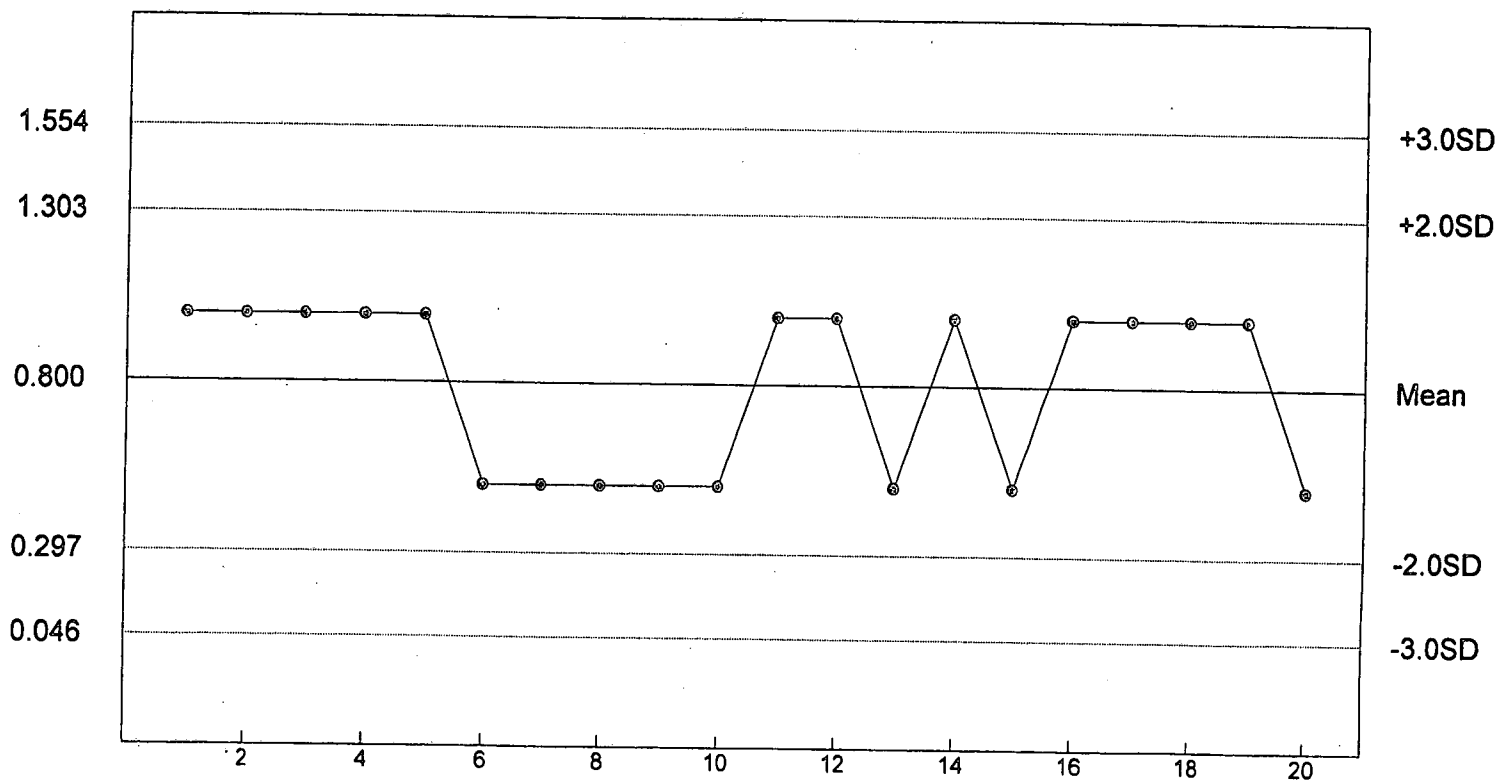
C. dubia Survival - NOEC



n= 20 Mean= 1.600 SD= 0.205 CV= 12.82% Min= 1.500 Max= 2.000

Reference Tox Sodium Chloride g/L

C. dubia Reproduction - NOEC



n= 20 Mean= 0.800 SD= 0.251 CV= 31.41% Min= 0.500 Max= 1.000

**CHRONIC REFERENCE TOXICANT TEST RESULTS**

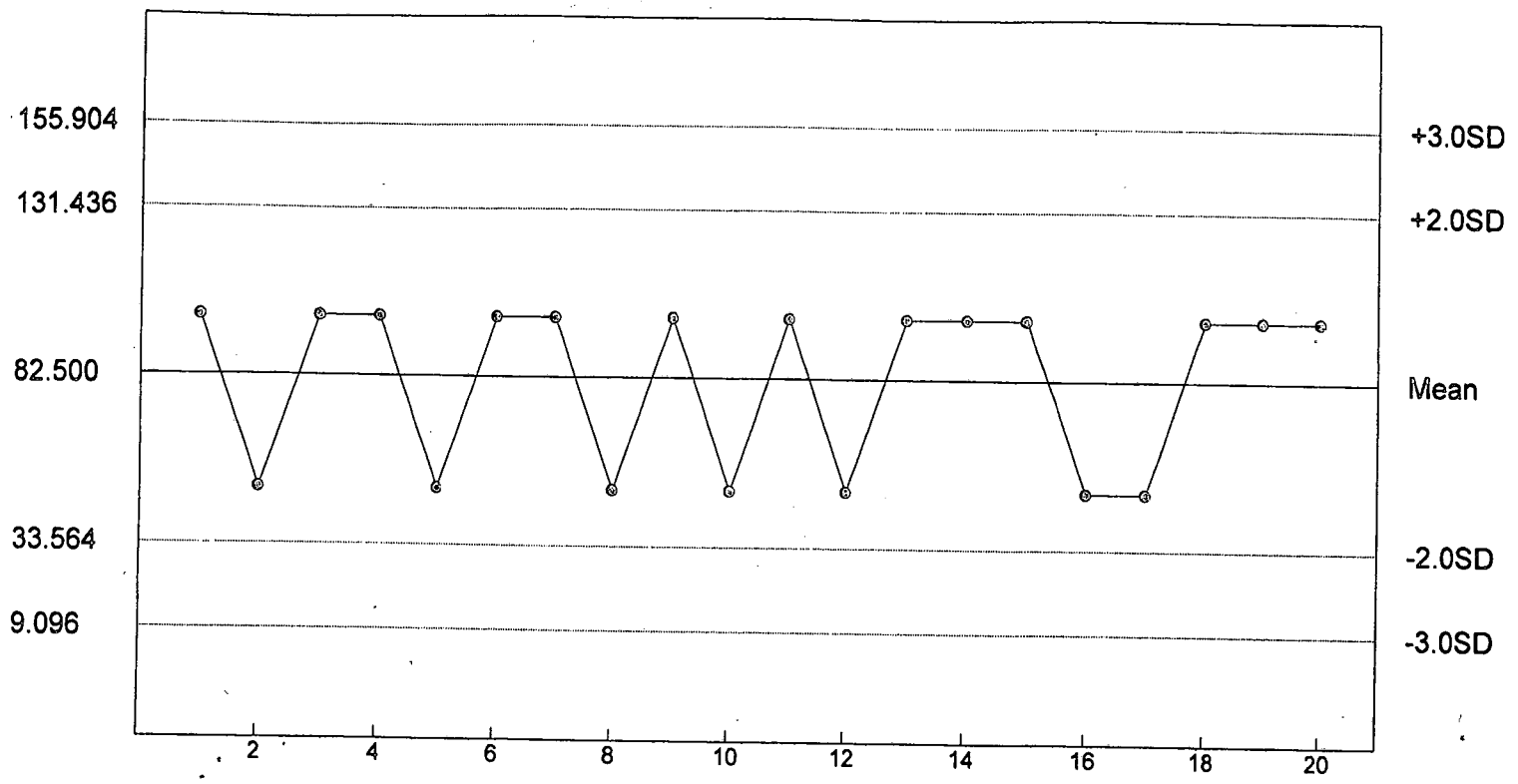
SPECIES: *Pimephales promelas*  
 CHEMICAL: Copper Nitrate  
 DURATION: 7-Days  
 TEST NUMBER: 3  
 TEST DATE: 03/04/15 - 03/11/15  
 1540 Hrs - 1540 Hrs  
 STATISTICAL METHOD: Dunnetts/Steels

CONCENTRATION (ug/L)	NUMBER EXPOSED	NUMBER DEAD
25	40	0
50	40	0
100	40	0
200	40	6
400	40	15
800	40	40

LOEC FOR SURVIVAL	NOEC FOR SURVIVAL	LOEC FOR GROWTH	NOEC FOR GROWTH
200 ug/L	100 ug/L	200 ug/L	100 ug/L

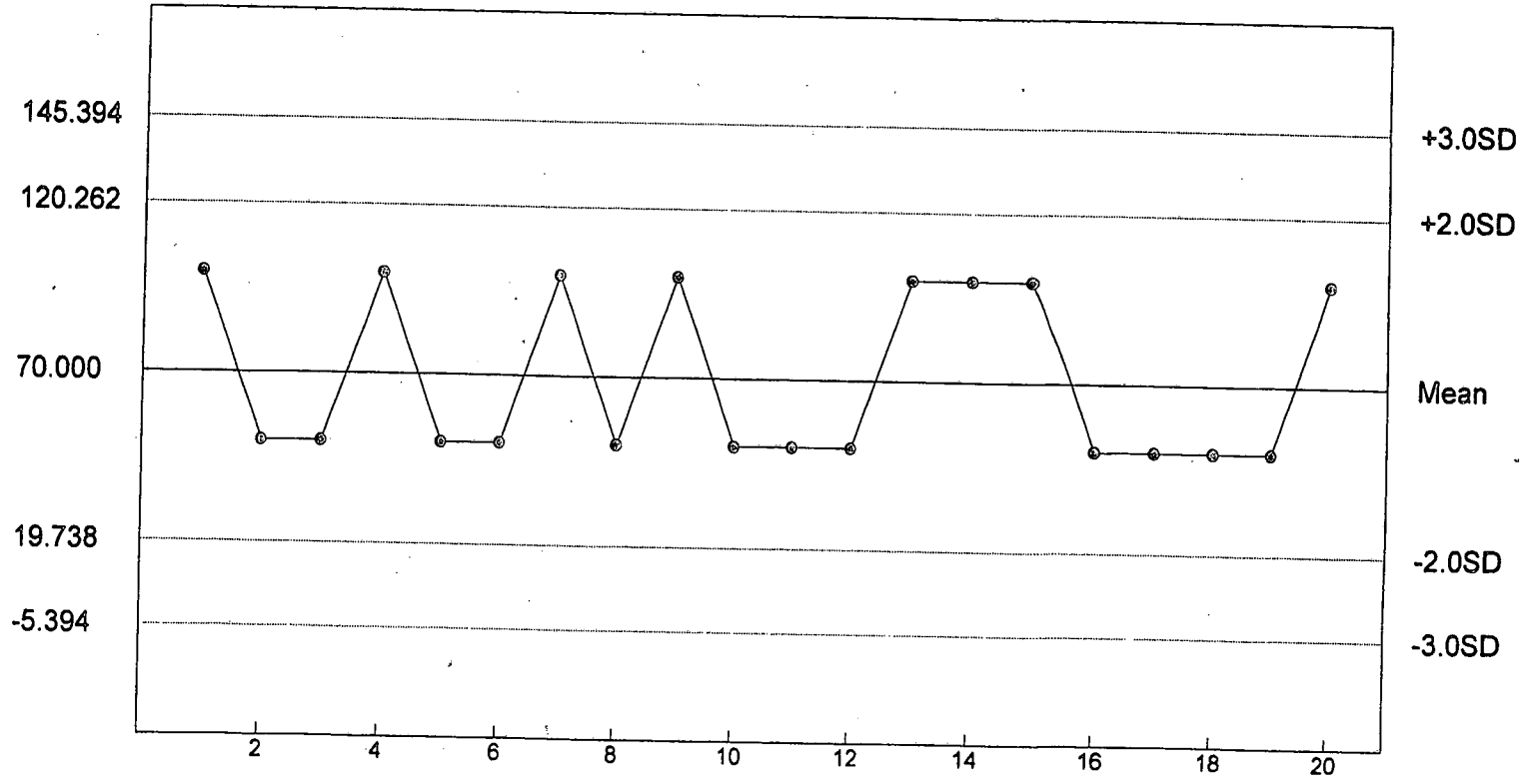


Reference Tox Copper Nitrate ug/L  
P. promelas Chronic Survival - NOEC



n= 20 Mean= 82.500 SD= 24.468 CV= 29.66% Min= 50.000 Max= 100.000

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



n= 20 Mean= 70.000 SD= 25.131 CV= 35.90% Min= 50.000 Max= 100.000

**APPENDIX C**  
**CHAIN OF CUSTODY SHEETS**















**ENVIRONMENTAL ENTERPRISE GROUP**  
**CITY OF CLARKSVILLE WWTP - OUTFALL 001**  
**NPDES PERMIT NO. AR0022187**  
**AFIN NO. 36-00038**  
**BIOMONITORING REPORTING**  
**TEST DATE: 03/12/15**

**II. *Ceriodaphnia dubia***

	<b>Response</b>
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.	11.18%

**I. *Pimephales promelas***

	<b>Response</b>
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.	7.04%

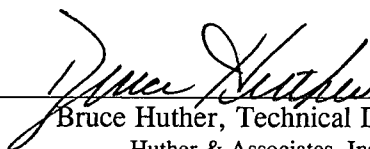
**ENVIRONMENTAL ENTERPRISE GROUP  
CITY OF CLARKSVILLE WWTP  
OUTFALL 002**

Chronic Biomonitoring Report  
Permit Number NPDES AR0022187  
AFIN Number 36-00038

*Ceriodaphnia dubia*  
*Pimephales promelas*

March 10, 2015

Reviewed by:



Bruce Huther, Technical Director

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Denton, Texas 76201  
(940) 387-1025, Fax: (940) 387-1036

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TOXICITY TEST REPORT - CHRONIC

Client .....Environmental Enterprise Group      Sample .....Outfall 002  
Facility .....City of Clarksville WWTP      Laboratory I.D. ....23879  
Permit No. .... NPDES AR0022187      Begin Date .....March 10, 2015

Results: Pass *Ceriodaphnia dubia* survival and reproduction and *Pimephales promelas* survival and growth at the critical low flow concentration (11% effluent).

SAMPLE COLLECTION

Composite effluent samples from Environmental Enterprise Group, City of Clarksville WWTP were delivered by United Parcel Service courier to Huthur & Associates on March 10, March 12, and March 14, 2015. Effluent samples were collected and composited from Outfall 002 using an automatic sampler. 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 samples were analyzed for total residual chlorine (Standard Methods, 22<sup>nd</sup> Edition, 4500-Cl D) and contained <0.01 mg/L, <0.01 mg/L, and <0.01 mg/L, respectively. Effluent and laboratory 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 1640 hours, March 10, 2015. Five concentrations were prepared (5%, 6%, 8%, 11%, and 25% effluent) utilizing distilled, deionized laboratory water reconstituted to match the hardness, alkalinity and pH of the receiving stream (Spadra Creek). The test was conducted in 25 mL distilled water rinsed plastic beakers containing 15 mL of solution (one organism 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 control of 10 replicate beakers containing one neonate each in distilled, deionized, reconstituted water (same as diluent) was conducted concurrently with the test. There was 100% survival in the control. The test ended at 1640 hours, March 17, 2015. 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: 25% 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: 25% Effluent**

**PMSD: 6.8%**

**TEST SETUP***Pimephales promelas*

The seven-day *Pimephales promelas* larval survival and growth test was initiated at 1610 hours, March 10, 2015. Five concentrations were prepared (5%, 6%, 8%, 11%, and 25% effluent) utilizing distilled, deionized laboratory water reconstituted to match the hardness, alkalinity and pH of the receiving stream (Spadra Creek). 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.

A control of five replicate chambers containing eight larvae each in distilled, deionized, reconstituted water (same as diluent) was conducted concurrently with the test. There was 100% survival in the control. The test ended at 1610 hours, February 17, 2015. At test termination, all larvae were sacrificed, dried for 24-hours, and weighed. Survival and growth (weight) 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**  
*Pimephales promelas*

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

**LOEC: Not Applicable**  
**NOEC: 25% Effluent**

**GROWTH**  
*Pimephales promelas*

*P. promelas* growth data were normally distributed at the 0.01 alpha level (0.900) using Shapiro Wilk's test for normality. Growth 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 *P. promelas* growth data demonstrated that there were no statistically significant differences between the control and any of the effluent concentrations.

**LOEC: Not Applicable**                      **PMSD: 9.8%**  
**NOEC: 25% Effluent**

**SUMMARY**

There were no statistically significant differences between the control and the critical low flow concentration (11% effluent) for *C. dubia* survival and reproduction and *P. promelas* survival and growth. Based on biomonitoring requirements for Outfall 002 contained in Permit Number NPDES AR0022187 for Environmental Enterprise Group, City of Clarksville WWTP, Outfall 002 **passed** for this testing period.

Huthner 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	03/09/15 03/11/15 03/13/15
LAB ID #	23879	DATE RECEIVED	03/10/15 03/12/15 03/14/15
TEST TYPE	7 Day Chronic	BEGIN DATE/TIME	03/10/15 1640
TEST ORGANISM	<i>Ceriodaphnia dubia</i>	END DATE/TIME	03/17/15 1640
ORGANISM AGE	< 24 Hours	TEST TEMPERATURE (°C)	25 ± 1
ORGANISM SOURCE	In House	PHOTO PERIOD	16-hr. Light 8-hr. Dark
RECEIVING WATER	Spadra Creek	LIGHT INTENSITY	50-100 ft. candl.
DILUTION WATER	Laboratory	TECHNICIAN	Z. Geiger

**SURVIVAL & REPRODUCTION SUMMARY**

Control										
Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
03/11/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/12/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/13/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/14/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/15/15	3	4	2	3	2	3	2	2	2	3
	3	4	2	3	2	3	2	2	2	3
03/16/15	8	6	7	9	7	8	7	8	10	8
	11	10	9	12	9	11	9	10	12	11
03/17/15	13	14	12	12	13	13	12	13	14	12
	24	24	21	24	22	24	21	23	26	23
x # Young 23.2                      C.V. 6.68% x% Survival 100%                      C.V. 0.00%										

5% Effluent										
Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
03/11/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/12/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/13/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/14/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/15/15	3	2	2	4	2	3	3	4	2	2
	3	2	2	4	2	3	3	4	2	2
03/16/15	8	6	9	7	6	10	8	6	7	7
	11	8	11	11	8	13	11	10	9	9
03/17/15	13	14	12	12	13	12	13	14	12	12
	24	22	23	23	21	25	24	24	21	21
x # Young 22.8                      C.V. 6.47% x% Survival 100%                      C.V. 0.00%										

6% Effluent										
Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
03/11/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/12/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/13/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/14/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/15/15	3	2	2	3	2	2	3	2	4	3
	3	2	2	3	2	2	3	2	4	3
03/16/15	8	6	7	9	7	9	7	8	7	6
	11	8	9	12	9	11	10	11	9	9
03/17/15	13	14	12	12	13	12	13	13	14	14
	24	22	21	24	22	23	23	23	25	23
x # Young 23.0                      C.V. 5.02% x% Survival 100%                      C.V. 0.00%										

8% Effluent										
Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
03/11/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/12/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/13/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/14/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/15/15	3	2	4	3	4	3	2	3	4	3
	3	2	4	3	4	3	2	3	4	3
03/16/15	8	7	9	9	7	10	8	9	7	7
	11	9	13	12	11	13	10	12	11	10
03/17/15	13	12	13	14	12	12	13	13	12	13
	24	21	26	26	23	25	23	25	23	23
x # Young 23.9                      C.V. 6.67% 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

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

EEG, City of Clarksville WWTP

Lab ID# 23879

Test Date: March 10, 2015

11% Effluent

Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
03/11/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/12/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/13/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/14/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
	3	4	2	3	4	3	3	2	2	4
03/15/15	3	4	2	3	4	3	3	2	2	4
	8	9	6	8	6	7	7	9	8	7
03/16/15	11	13	8	11	10	10	11	10	11	
	13	12	14	13	14	15	12	13	13	14
03/17/15	24	25	22	24	24	25	22	24	23	25
x# Young 23.8                      C.V. 4.77% x% Survival 100%                      C.V. 0.00%										

25% Effluent

Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
03/11/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/12/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/13/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
03/14/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
	3	2	3	2	3	2	3	2	4	3
03/15/15	3	2	3	2	3	2	3	2	4	3
	9	7	10	9	7	6	8	7	10	10
03/16/15	12	9	13	11	10	8	11	9	14	13
	13	12	14	12	13	13	12	13	12	12
03/17/15	25	21	27	23	23	21	23	22	26	25
x# Young 23.6                      C.V. 8.75% 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



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

EEG, City of Clarksville WWTP

Lab ID# 23879

Test Date: March 10, 2015

**WET CHEMISTRY MEASUREMENTS**

Date	Time	Temp	Samp. No.	pH of Solution <sup>1</sup>						Analyst
				CON	5%	6%	8%	11%	25%	
03/10/15	Start	25.0	1	8.51	8.49	8.46	8.42	8.37	8.34	CS
03/11/15	24 Hr.	25.4	1	8.03	8.00	7.96	7.92	7.87	7.86	CS
03/11/15	Renew	25.4	1	8.50	8.46	8.43	8.42	8.39	8.38	CS
03/12/15	48 Hr.	25.5	1	7.97	7.96	7.95	7.91	7.88	7.85	CS
03/12/15	Renew	25.5	2	8.22	8.19	8.20	8.21	8.22	8.23	CS
03/13/15	72 Hr.	25.6	2	8.06	8.05	8.04	8.03	8.02	8.01	TB
03/13/15	Renew	25.6	2	8.48	8.46	8.43	8.40	8.38	8.37	CS
03/14/15	96 Hr.	25.5	2	8.01	7.99	7.96	7.95	7.94	7.93	CS
03/14/15	Renew	25.5	3	8.45	8.42	8.39	8.35	8.31	8.28	CS
03/15/15	120 Hr.	25.7	3	7.71	7.96	7.84	7.62	7.60	7.58	EMS
03/15/15	Renew	25.7	3	8.48	8.37	8.11	7.98	7.97	7.89	EMS
03/16/15	144 Hr.	25.8	3	7.99	8.02	8.01	7.97	7.96	7.92	EMS
03/16/15	Renew	25.8	3	8.46	8.38	8.21	8.19	8.17	8.10	EMS
03/17/15	168 Hr.	25.6	3	8.03	8.02	7.96	7.94	7.87	7.85	CS

Date	Time	Temp	Samp. No.	DO (mg/L) of Solution <sup>1</sup>						Analyst
				CON	5%	6%	8%	11%	25%	
03/10/15	Start	25.0	1	8.60	8.26	8.52	8.40	8.77	8.80	CS
03/11/15	24 Hr.	25.4	1	8.79	8.73	8.22	8.31	8.05	8.78	CS
03/11/15	Renew	25.4	1	8.42	8.28	8.78	8.41	8.34	8.06	CS
03/12/15	48 Hr.	25.5	1	8.77	8.86	8.71	8.96	8.78	8.74	CS
03/12/15	Renew	25.5	2	8.67	8.32	8.47	8.19	8.21	8.75	CS
03/13/15	72 Hr.	25.6	2	8.12	8.98	8.66	8.48	8.08	8.21	TB
03/13/15	Renew	25.6	2	8.92	8.56	8.75	8.95	8.64	8.18	CS
03/14/15	96 Hr.	25.5	2	8.07	8.01	8.86	8.09	8.14	8.30	CS
03/14/15	Renew	25.5	3	8.25	8.42	8.73	8.79	8.46	8.52	CS
03/15/15	120 Hr.	25.7	3	8.63	8.14	8.13	8.10	8.67	8.89	EMS
03/15/15	Renew	25.7	3	8.56	8.49	8.38	8.32	8.29	8.27	EMS
03/16/15	144 Hr.	25.8	3	8.10	7.98	8.16	8.13	8.01	7.76	EMS
03/16/15	Renew	25.8	3	8.09	8.01	7.98	7.62	7.58	7.51	EMS
03/17/15	168 Hr.	25.6	3	8.08	8.14	8.01	7.45	7.51	8.24	CS

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

EEG, City of Clarksville WWTP

Lab ID# 23879

Test Date: March 10, 2015

INITIAL CHEMISTRY MEASUREMENTS @ 100% EFFLUENT

Date	Samp No.	pH	DO	Hardness mg/L CaCO <sub>3</sub> <sup>1</sup>	Alkalinity mg/L CaCO <sub>3</sub> <sup>1</sup>	Conduct. umhos/cm <sup>1</sup>	Resid. Cl <sub>2</sub> mg/L <sup>1</sup>	Dechlor(mL) Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> mg/L <sup>1</sup>	Analyst
03/10/15	1	8.34	8.13	56	52	202	<0.01	N/A	TG
03/12/15	2	8.29	8.88	52	50	280	<0.01	N/A	TG
03/14/15	3	8.15	8.76	56	52	266	<0.01	N/A	TG
03/10/15	Con	8.51	8.60	100	80	412	-	-	TG

<sup>1</sup> Measurements taken in 100% solution.

Huther and Associates, Inc.  
 Begin Date: March 10, 2015  
 Lab I.D.# 23879

**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	26.000	23.200
2	5% Effluent	10	21.000	25.000	22.800
3	6% Effluent	10	21.000	25.000	23.000
4	8% Effluent	10	21.000	26.000	23.900
5	11% Effluent	10	22.000	25.000	23.800
6	25% Effluent	10	21.000	27.000	23.600

Summary Statistics on Transformed Data Table 2 of 2

Grp	Identification	Variance	Sd	Sem	C.V.%
1	Control	2.400	1.549	0.490	6.68
2	5% Effluent	2.178	1.476	0.467	6.47
3	6% Effluent	1.333	1.155	0.365	5.02
4	8% Effluent	2.544	1.595	0.504	6.67
5	11% Effluent	1.289	1.135	0.359	4.77
6	25% Effluent	4.267	2.066	0.653	8.75

Chi-Square Test For Normality: Actual And Expected Frequencies

Interval	< -1.5	-1.5 to -0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
Expected	4.020	14.520	22.920	14.520	4.020
Observed	4	17	16	20	3

Calculated Chi-Square goodness of fit test statistic = 4.8400  
 Table Chi-Square value (alpha = 0.01) = 13.277

Data Pass normality test. Continue analysis.

Bartlett's Test For Homogeneity of Variance

Calculated B1 statistic = 4.39

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	10.083	2.017	0.864
Within (Error)	54	126.100	2.335	
Total	59	136.183		

Critical F value = 2.45 (0.05,5,40)  
 Since F < Critical F Fail to Reject Ho: All equal

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

Grp	Identification	Mean		T Stat	Sig
		Transformed Mean	Calculated In Original Units		
1	Control	23.200	23.200		
2	5% Effluent	22.800	22.800	0.585	
3	6% Effluent	23.000	23.000	0.293	
4	8% Effluent	23.900	23.900	-1.024	
5	11% Effluent	23.800	23.800	-0.878	
6	25% Effluent	23.600	23.600	-0.585	

Dunnnett table value = 2.31 (1 Tailed Value, P=0.05, DF=40,5)  
 No statistically significant difference

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

Grp	Identification	Num of Reps	Minimum Sig Diff (In Orig. Units)	% of Control	Difference from
					Control
1	Control	10			
2	5% Effluent	10	1.579	6.8	0.400
3	6% Effluent	10	1.579	6.8	0.200
4	8% Effluent	10	1.579	6.8	-0.700
5	11% Effluent	10	1.579	6.8	-0.600
6	25% Effluent	10	1.579	6.8	-0.400

Huther 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 03/09/15 03/11/15 03/13/15  
 LAB ID # 23879 DATE RECEIVED 03/10/15 03/12/15 03/14/15  
 TEST TYPE 7 Day Chronic BEGIN DATE/TIME 03/10/15 1610  
 TEST ORGANISM *Pimephales promelas* END DATE/TIME 03/17/15 1610  
 ORGANISM AGE < 24 Hours TEST TEMPERATURE (°C) 25 ± 1  
 ORGANISM SOURCE In House PHOTO PERIOD 16-hr. Light 8-hr. Dark  
 RECEIVING WATER Spadra Creek LIGHT INTENSITY 50-100 ft. candl.  
 DILUTION WATER Laboratory TECHNICIAN Z. Geiger

SURVIVAL SUMMARY

Conc.	03/11/15					03/12/15					03/13/15					03/14/15					03/15/15				
	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
Con	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
5%	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
6%	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	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
11%	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
25%	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

Conc.	03/16/15					03/17/15					x % Survival	C.V. %
	A	B	C	D	E	A	B	C	D	E		
Con	8	8	8	8	8	8	8	8	8	8	100.0	0.00
5%	8	8	8	8	8	8	8	8	8	8	100.0	0.00
6%	8	8	8	8	8	8	8	8	8	8	100.0	0.00
8%	8	8	8	8	8	8	8	8	8	8	100.0	0.00
11%	8	8	8	8	8	8	8	8	8	8	100.0	0.00
25%	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. %
Con	0.4120	0.4450	0.4620	0.4250	0.4750	0.4438	5.82
5%	0.4810	0.4440	0.4920	0.4350	0.4810	0.4666	5.43
6%	0.4960	0.4500	0.4260	0.4710	0.4950	0.4676	6.42
8%	0.5020	0.4760	0.4920	0.4160	0.5060	0.4784	7.68
11%	0.4520	0.4810	0.4950	0.4270	0.5020	0.4714	6.65
25%	0.5010	0.4450	0.4690	0.4950	0.4810	0.4782	4.67

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

EEG, City of Clarksville WWTP

Lab ID# 23879

Test Date: March 10, 2015

**WET CHEMISTRY MEASUREMENTS**

Date	Time	Temp.	Samp. No.	pH of Solution						Analyst
				CON	5%	6%	8%	11%	25%	
03/10/15	Start	25.0	1	8.51	8.49	8.46	8.42	8.37	8.34	CS
03/11/15	24 Hr.	25.5	1	8.10	8.08	8.08	8.04	8.03	8.02	CS
03/11/15	Renew	25.5	1	8.50	8.46	8.43	8.42	8.39	8.38	CS
03/12/15	48 Hr.	25.5	1	8.06	8.05	8.04	8.01	7.97	7.98	CS
03/12/15	Renew	25.5	2	8.22	8.19	8.20	8.21	8.22	8.23	CS
03/13/15	72 Hr.	25.1	2	7.96	7.95	7.85	7.88	7.91	7.92	TB
03/13/15	Renew	25.1	2	8.48	8.46	8.43	8.40	8.38	8.37	CS
03/14/15	96 Hr.	25.5	2	8.12	8.11	8.08	8.06	8.03	8.02	CS
03/14/15	Renew	25.5	3	8.45	8.42	8.39	8.35	8.31	8.28	CS
03/15/15	120 Hr.	25.4	3	8.55	8.43	8.16	7.91	7.82	7.76	EMS
03/15/15	Renew	25.4	3	8.48	8.37	8.11	7.98	7.97	7.89	EMS
03/16/15	144 Hr.	25.3	3	8.14	8.06	8.05	8.00	7.96	7.91	EMS
03/16/15	Renew	25.3	3	8.46	8.38	8.21	8.19	8.17	8.10	EMS
03/17/15	168 Hr.	25.5	3	8.47	8.48	8.49	8.47	8.46	8.45	CS

Date	Time	Temp.	Samp. No.	DO (mg/L) of Solution						Analyst
				CON	5%	6%	8%	11%	25%	
03/10/15	Start	25.0	1	8.60	8.26	8.52	8.40	8.77	8.80	CS
03/11/15	24 Hr.	25.5	1	8.62	8.71	8.65	8.83	8.71	8.21	CS
03/11/15	Renew	25.5	1	8.42	8.28	8.78	8.41	8.34	8.06	CS
03/12/15	48 Hr.	25.5	1	8.53	8.09	8.07	8.44	8.75	8.76	CS
03/12/15	Renew	25.5	2	8.67	8.32	8.47	8.19	8.21	8.75	CS
03/13/15	72 Hr.	25.1	2	8.78	8.77	8.75	8.74	8.74	8.22	TB
03/13/15	Renew	25.1	2	8.92	8.56	8.75	8.95	8.64	8.18	CS
03/14/15	96 Hr.	25.5	2	8.67	8.41	8.31	8.77	8.37	8.22	CS
03/14/15	Renew	25.5	3	8.25	8.42	8.73	8.79	8.46	8.52	CS
03/15/15	120 Hr.	25.4	3	8.81	7.57	7.66	8.06	7.79	8.22	EMS
03/15/15	Renew	25.4	3	8.56	8.49	8.38	8.32	8.29	8.27	EMS
03/16/15	144 Hr.	25.3	3	8.49	8.56	8.62	8.02	8.16	7.96	EMS
03/16/15	Renew	25.3	3	8.09	8.01	7.98	7.62	7.58	7.51	EMS
03/17/15	168 Hr.	25.5	3	8.22	7.65	8.02	7.76	8.00	7.86	CS

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

EEG, City of Clarksville WWTP

Lab ID# 23879

Test Date: March 10, 2015

INITIAL CHEMISTRY MEASUREMENTS @ 100% EFFLUENT

Date	Samp. No.	pH	DO	Hardness mg/L CaCO <sub>3</sub> <sup>1</sup>	Alkalinity mg/L CaCO <sub>3</sub> <sup>1</sup>	Conduct umhos/cm <sup>1</sup>	Resid. Cl <sub>2</sub> mg/L <sup>1</sup>	Dechlor(mL) Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> mg/L <sup>1</sup>	Analyst
03/10/15	1	8.34	8.13	56	52	202	<0.01	N/A	TG
03/12/15	2	8.29	8.88	52	50	280	<0.01	N/A	TG
03/14/15	3	8.15	8.76	56	52	266	<0.01	N/A	TG
03/10/15	Con	8.51	8.60	100	80	412	-	-	TG

<sup>1</sup>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	0.412	0.475	0.444
2	5% Effluent	5	0.435	0.492	0.467
3	6% Effluent	5	0.426	0.496	0.468
4	8% Effluent	5	0.416	0.506	0.478
5	11% Effluent	5	0.427	0.502	0.471
6	25% Effluent	5	0.445	0.501	0.478

Summary Statistics on Transformed Data Table 2 of 2

Grp	Identification	Variance	Sd	Sem	C.V.%
1	Control	0.001	0.026	0.012	5.82
2	5% Effluent	0.001	0.025	0.011	5.43
3	6% Effluent	0.001	0.030	0.013	6.42
4	8% Effluent	0.001	0.037	0.016	7.68
5	11% Effluent	0.001	0.031	0.014	6.65
6	25% Effluent	0.000	0.022	0.010	4.67

Shapiro - Wilk's Test For Normality

D = 0.020

W = 0.909

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 = 1.17

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	0.004	0.001	0.964
Within (Error)	24	0.020	0.001	
Total	29	0.024		

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	Mean		T Stat	Sig
		Transformed Mean	Calculated In Original Units		
1	Control	0.444	0.444		
2	5% Effluent	0.467	0.467	-1.243	
3	6% Effluent	0.468	0.468	-1.298	
4	8% Effluent	0.478	0.478	-1.887	
5	11% Effluent	0.471	0.471	-1.505	
6	25% Effluent	0.478	0.478	-1.876	

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

No statistically significant difference

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

Grp	Identification	Num of Reps	Minimum Sig Diff (In Orig. Units)	Difference	
				% of Control	from Control
1	Control	5			
2	5% Effluent	5	0.043	9.8	-0.023
3	6% Effluent	5	0.043	9.8	-0.024
4	8% Effluent	5	0.043	9.8	-0.035
5	11% Effluent	5	0.043	9.8	-0.028
6	25% Effluent	5	0.043	9.8	-0.034

**APPENDIX A  
RAW DATA**



7-DAY CERIODAPHNIA DUBIA SURVIVAL & REPRODUCTION

DAILY RAW DATA TABLE

PAGE 1 OF 2

CLIENT EEG-Clarksville

OUTFALL 002

LAB ID # 23879

START DATE/TIME 3-10-15 26 1640

END DATE/TIME 3-17-15 26 1640

Con

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
3/11	A	A	A	A	A	A	A	A	A	A	ZG	1640
3/12	A	A	A	A	A	A	A	A	A	A	ZG	1445
3/13	A	A	A	A	A	A	A	A	A	A	NL	1100
3/14	A	A	A	A	A	A	A	A	A	A	ZG	1210
3/15	3	4	2	3	2	3	2	2	2	3	ZG	1220
3/16	8	6	7	9	7	8	7	8	10	8	NL	1200
3/17	13	14	12	12	13	13	12	13	14	12	ZG	1640
	24	24	21	24	22	24	21	23	26	23		

$\bar{x}$  # Young w/o Dead = 23.2 CV% = 6.68

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

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

5

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
3/11	A	A	A	A	A	A	A	A	A	A	ZG	1640
3/12	A	A	A	A	A	A	A	A	A	A	ZG	1445
3/13	A	A	A	A	A	A	A	A	A	A	NL	1100
3/14	A	A	A	A	A	A	A	A	A	A	ZG	1210
3/15	3	2	2	4	2	3	3	4	2	2	ZG	1220
3/16	8	6	9	7	6	10	8	6	7	7	NL	1200
3/17	13	14	12	12	13	12	13	14	12	12	ZG	1640
	24	22	23	23	21	25	24	24	21	21		

$\bar{x}$  # Young w/o Dead = 22.8 CV% = 6.47

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

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

6

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
3/11	A	A	A	A	A	A	A	A	A	A	ZG	1640
3/12	A	A	A	A	A	A	A	A	A	A	ZG	1445
3/13	A	A	A	A	A	A	A	A	A	A	NL	1100
3/14	A	A	A	A	A	A	A	A	A	A	ZG	1210
3/15	3	2	2	3	2	2	3	2	4	3	ZG	1220
3/16	8	6	7	9	7	9	7	8	7	6	NL	1200
3/17	13	14	12	12	13	12	13	13	14	14	ZG	1640
	24	22	21	24	22	23	23	23	25	23		

$\bar{x}$  # Young w/o Dead = 23.0 CV% = 5.02

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

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

8

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
3/11	A	A	A	A	A	A	A	A	A	A	ZG	1640
3/12	A	A	A	A	A	A	A	A	A	A	ZG	1445
3/13	A	A	A	A	A	A	A	A	A	A	NL	1100
3/14	A	A	A	A	A	A	A	A	A	A	ZG	1210
3/15	3	2	4	3	4	3	2	3	4	3	ZG	1220
3/16	8	7	9	9	7	10	8	9	7	7	NL	1200
3/17	13	12	13	14	12	12	13	13	12	13	ZG	1640
	24	21	26	26	23	25	23	25	23	23		

$\bar{x}$  # Young w/o Dead = 23.9 CV% = 6.67

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

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

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

CLIENT EEG-Clarksville  
OUTFALL 002  
LAB ID # 23879

START DATE/TIME 3-10-15 26 1640  
END DATE/TIME 3-17-15 26 1640

11

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
3/11	A	A	A	A	A	A	A	A	A	A	ZG	1640
3/12	A	A	A	A	A	A	A	A	A	A	ZG	1445
3/13	A	A	A	A	A	A	A	A	A	A	NL	1100
3/14	A	A	A	A	A	A	A	A	A	A	ZG	1210
3/15	3	4	2	3	4	3	3	2	2	4	ZG	1220
3/16	8	9	6	8	6	7	7	9	8	7	NL	1200
3/17	13	12	14	12	14	15	12	13	13	14	ZG	1640
	24	25	22	24	24	25	22	24	23	25		

$\bar{x}$  # Young w/o Dead = 23.8 CV% = 4.77  
 $\bar{x}$  # Young w/Dead =          CV% =           
 $\bar{x}$  % Survival = 100 CV% = 0.00

25

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
3/11	A	A	A	A	A	A	A	A	A	A	ZG	1640
3/12	A	A	A	A	A	A	A	A	A	A	ZG	1445
3/13	A	A	A	A	A	A	A	A	A	A	NL	1100
3/14	A	A	A	A	A	A	A	A	A	A	ZG	1270
3/15	3	2	3	2	3	2	3	2	4	3	ZG	1220
3/16	9	7	10	9	7	6	8	7	10	10	NL	1200
3/17	13	12	14	12	13	13	12	13	12	12	ZG	1640
	25	21	27	23	23	21	23	22	21	25		

$\bar{x}$  # Young w/o Dead = 23.6 CV% = 8.75  
 $\bar{x}$  # Young w/Dead =          CV% =           
 $\bar{x}$  % Survival = 100 CV% = 0.00

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time

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

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time

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

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

CLIENT/FACILITY EEG-Clarksville 002  
 OUTFALL # 002 PROJECT # 23879  
 ORGANISM ID# PP0-15-068

DATE/TIME STARTED 3-10-15 TG 1610  
 DATE/TIME ENDED 3-17-15 NL 1610

Conc.	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	A	B	C	D	E					
CON	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
5	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
6	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
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	8
11	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
25	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	3-11-15 TG 1610					3-12-15 MH 0825					3-13-15 TB 0955					3-14-15 TG 0810					3-15-15 TG 0900									

Conc.	A	B	C	D	E	A	B	C	D	E	Mean Survival	C.V. %
CON	8	8	8	8	8	8	8	8	8	8	100	0.00
5	8	8	8	8	8	8	8	8	8	8	100	0.00
6	8	8	8	8	8	8	8	8	8	8	100	0.00
8	8	8	8	8	8	8	8	8	8	8	100	0.00
11	8	8	8	8	8	8	8	8	8	8	100	0.00
25	8	8	8	8	8	8	8	8	8	8	100	0.00
Initials Date/Time	3-16-15 TB 0905					3-17-15 NL 1610						



Client / Facility EEG Clarksville 002  
 Lab ID Number 23879  
 Outfall Number 002  
 Test Date 3-10-15

INITIAL CHEMISTRY MEASUREMENTS @ 100% EFFLUENT

Date	Samp. No.	pH	DO	Hardness mg/L CaCO <sub>3</sub> <sup>1</sup>	Alkalinity mg/L CaCO <sub>3</sub> <sup>1</sup>	Conduct. umhos/cm <sup>1</sup>	Resid. Cl <sub>2</sub> mg/L <sup>1</sup>	Dechlor(mL) Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> mg/L <sup>1</sup>	Analyst
3/10	1	8.34	8.13	56	52	202	40.01	Na	TG
3/12	2	8.29	8.88	52	50	280	5	5	5
3/14	3	8.15	8.76	56	52	246	5	5	5
3/10	CON	8.51	8.60	100	80	412	-	-	5

INITIAL CHEMISTRY MEASUREMENTS @ RECEIVING WATER

Date	Samp. No.	pH	DO	Hardness mg/L CaCO <sub>3</sub> <sup>1</sup>	Alkalinity mg/L CaCO <sub>3</sub> <sup>1</sup>	Conduct. umhos/cm <sup>1</sup>	Resid. Cl <sub>2</sub> mg/L <sup>1</sup>	Dechlor(mL) Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> mg/L <sup>1</sup>	Analyst

Notes:

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**APPENDIX B**  
**REFERENCE TOXICANTS**

**CHRONIC REFERENCE TOXICANT TEST RESULTS**

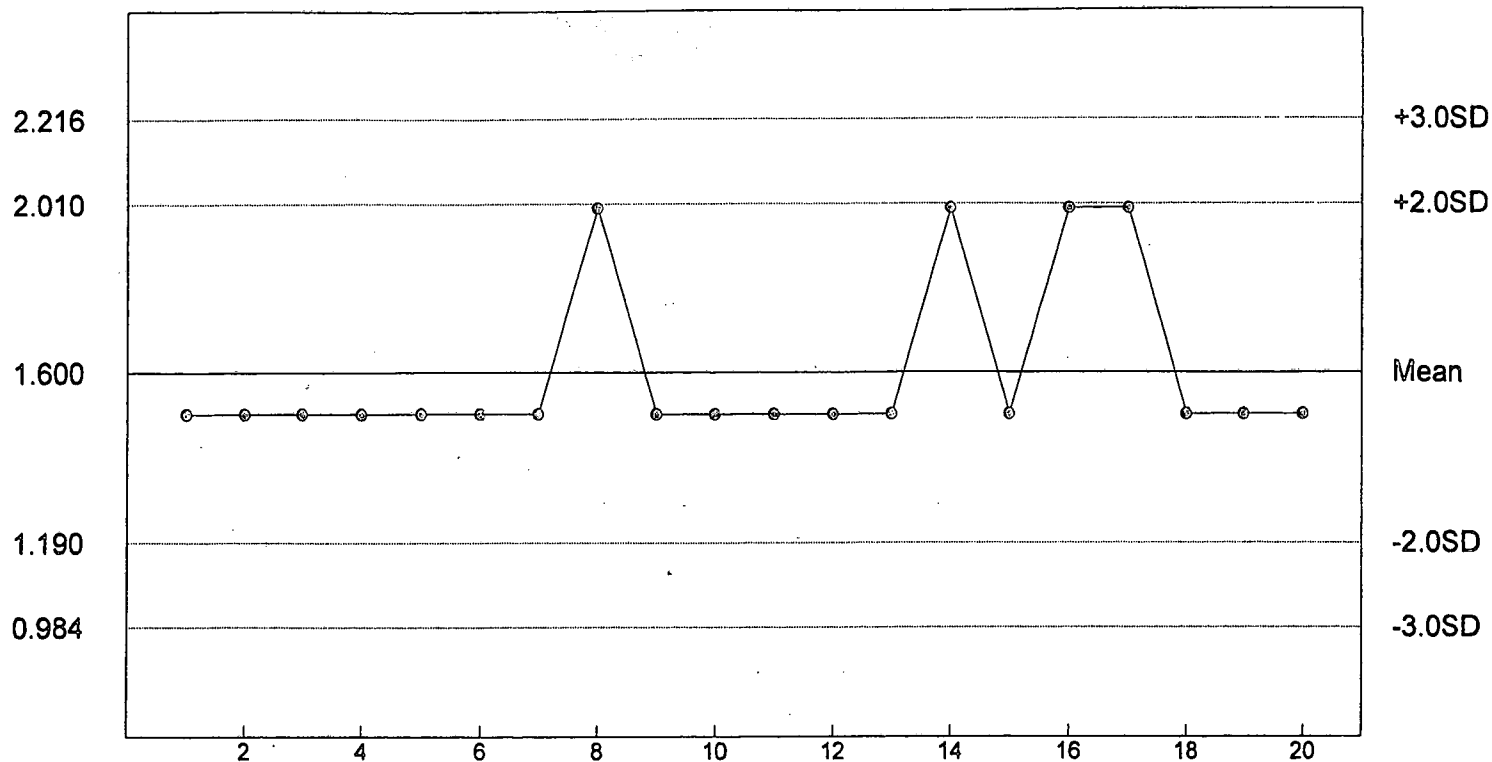
SPECIES: *Ceriodaphnia dubia*  
 CHEMICAL: Copper Nitrate  
 DURATION: 7-Days  
 TEST NUMBER: 3  
 TEST DATE: 03/04/15 - 03/11/15  
 1000 Hrs - 1000 Hrs  
 STATISTICAL METHOD: Dunnetts/Steels

CONCENTRATION (ug/L)	NUMBER EXPOSED	NUMBER DEAD
0.5	10	0
1.0	10	0
1.5	10	0
2.0	10	5
2.5	10	10
3.0	10	10

LOEC FOR SURVIVAL	NOEC FOR SURVIVAL	LOEC FOR GROWTH	NOEC FOR GROWTH
2.0 ug/L	1.5 ug/L	1.0 ug/L	0.5 ug/L

Reference Tox Sodium Chloride g/L

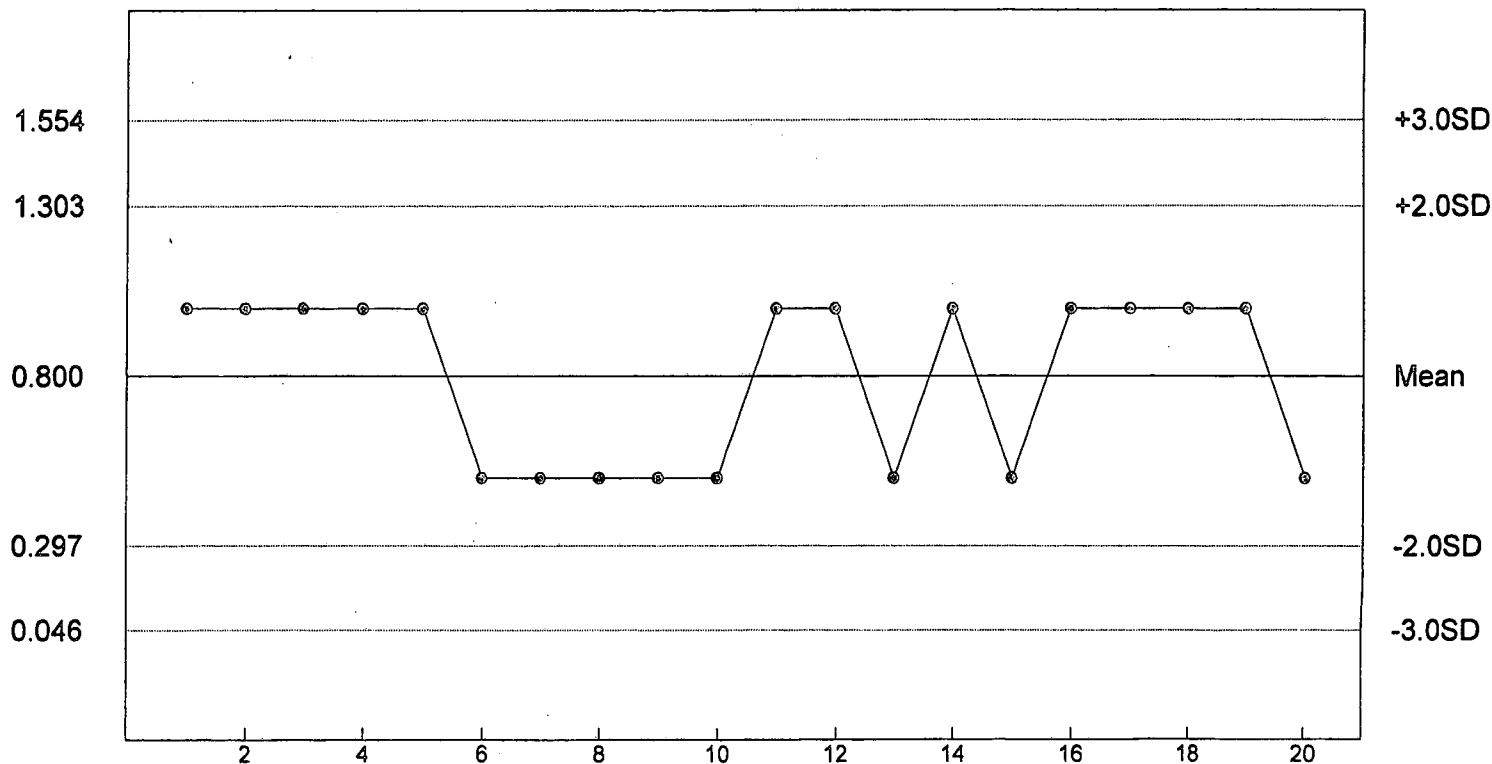
C. dubia Survival - NOEC



n= 20 Mean= 1.600 SD= 0.205 CV= 12.82% Min= 1.500 Max= 2.000

Reference Tox Sodium Chloride g/L

C. dubia Reproduction - NOEC



n= 20 Mean= 0.800 SD= 0.251 CV= 31.41% Min= 0.500 Max= 1.000



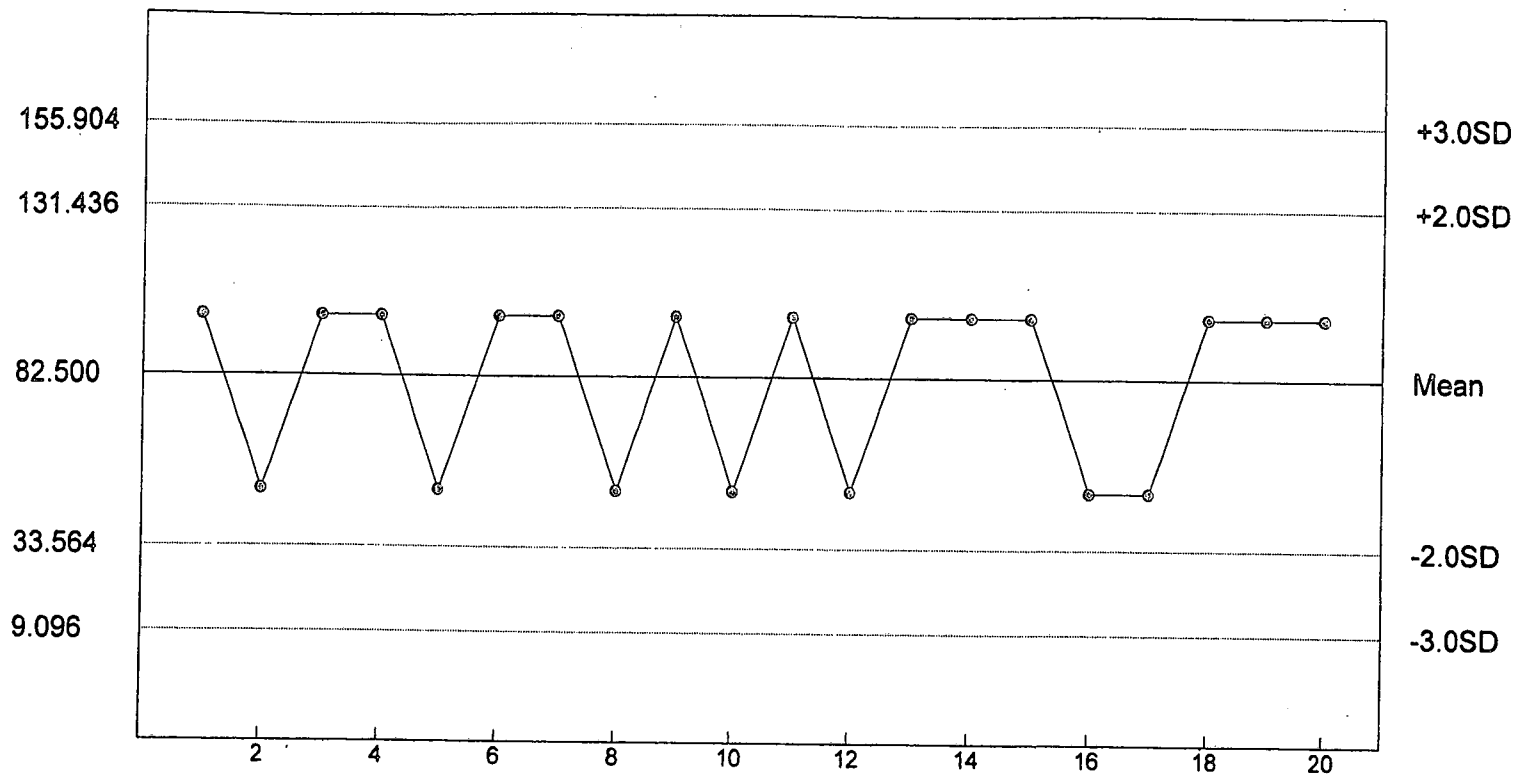
**CHRONIC REFERENCE TOXICANT TEST RESULTS**

SPECIES: *Pimephales promelas*  
 CHEMICAL: Copper Nitrate  
 DURATION: 7-Days  
 TEST NUMBER: 3  
 TEST DATE: 03/04/15 - 03/11/15  
 1540 Hrs - 1540 Hrs  
 STATISTICAL METHOD: Dunnetts/Steels

CONCENTRATION (ug/L)	NUMBER EXPOSED	NUMBER DEAD
25	40	0
50	40	0
100	40	0
200	40	6
400	40	15
800	40	40

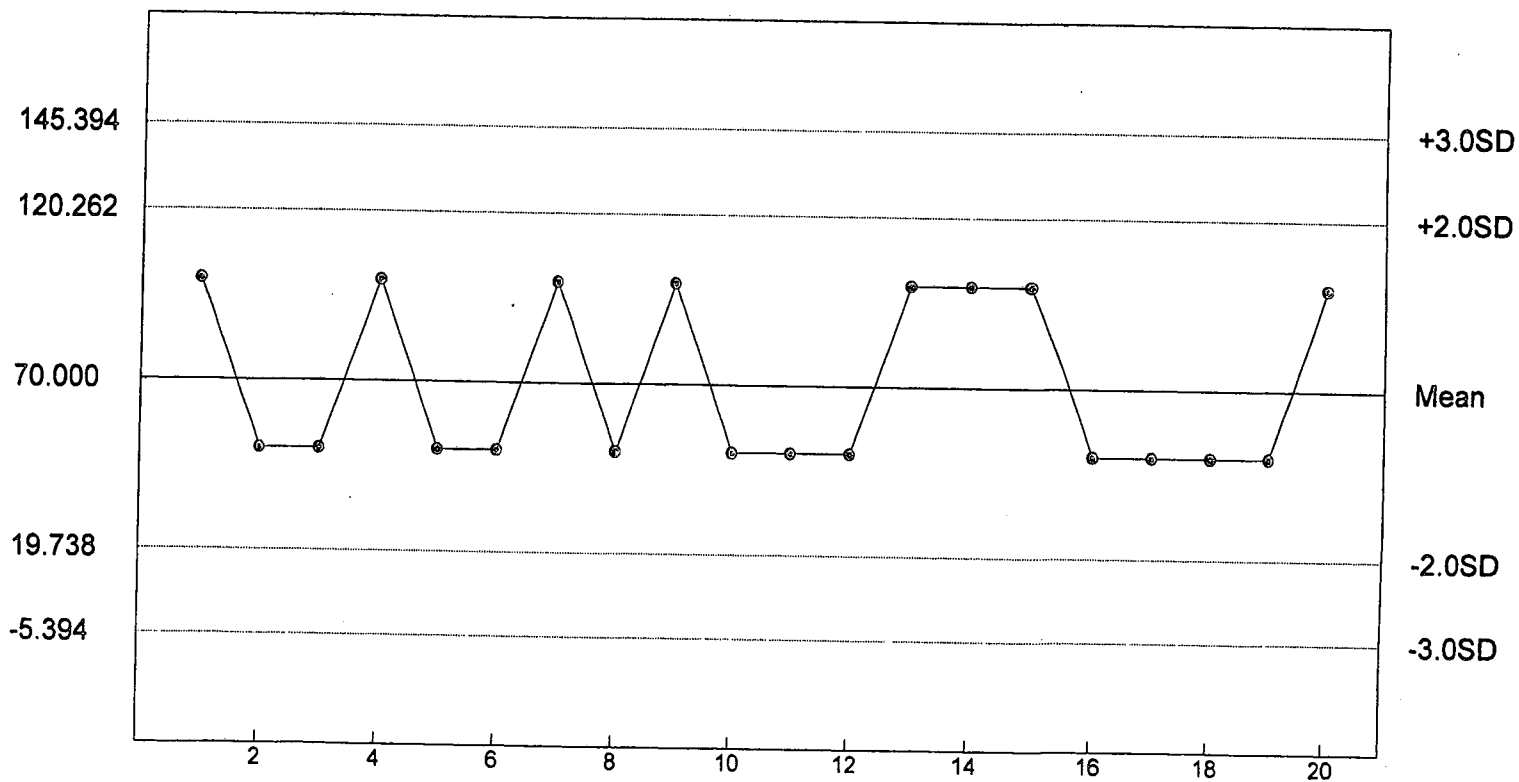
LOEC FOR SURVIVAL	NOEC FOR SURVIVAL	LOEC FOR GROWTH	NOEC FOR GROWTH
200 ug/L	100 ug/L	200 ug/L	100 ug/L

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



n= 20 Mean= 82.500 SD= 24.468 CV= 29.66% Min= 50.000 Max= 100.000

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



n= 20 Mean= 70.000 SD= 25.131 CV= 35.90% Min= 50.000 Max= 100.000

**APPENDIX C**  
**CHAIN OF CUSTODY SHEETS**

Environmental Enterprise Group, Inc.  
 220 N. Knoxville  
 Russellville, AR 72801  
 (479)968-6767 Fax (479)968-1956



Environmental Enterprise Group, Inc.  
 PROVIDING CUSTOMIZED SERVICES NATIONWIDE

L444-049488

Company Name: Clarksville Light and Water							Phone #: (479) 754-7929							Requested Analysis						Laboratory Control Number	Remarks (Please note special detection limits below.)	
Address: P.O. Box 1807, Clarksville, AR 72830							Fax #: (479) 754-8181															7-Day Chronic Bio-Monitoring Minnows
Project Name or Number:							Purchase Order #:							Bio-Monitoring	Laboratory Control Number	Remarks (Please note special detection limits below.)						
Sampling Personnel Signature(s): <i>[Signature]</i>							Printed: Andrew Zander															
Sample I.D.	Date	Time	Comp.	Grab	Cont. Type		# of Containers	Method Preserved						Sample Matrix						7-Day Chronic Bio-Monitoring Minnows	Laboratory Control Number	Remarks (Please note special detection limits below.)
					Plast.	Glass		H2SO4	HNO3	NAOH	HCL	Ice	None	Water	Soil	Air	Sludge	Other				
Outfall 002	3-8-15 3-9-15	0800 1115	X		X		1						X	X						X	0315028	
Relinquished by: <i>[Signature]</i>							Date: 3-9-15		Time: 1311		Relinquished by: <i>[Signature]</i>							Date: 3/9/15		Time: 1600		
Received by: <i>[Signature]</i>							Date: 3/9/15		Time: 1311		Received by Laboratory: <i>[Signature]</i>							Date: 3-10-15		Time: 1225		
Comments: ups							4.3°C															





**ENVIRONMENTAL ENTERPRISE GROUP**  
**CITY OF CLARKSVILLE WWTP – OUTFALL 002**  
**NPDES PERMIT NO. AR0022187**  
**AFIN NO. 36-00038**  
**BIOMONITORING REPORTING**  
**TEST DATE: 03/10/15**

**II. *Ceriodaphnia dubia***

	<b>Response</b>
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.	25%
D. Report the NOEC value for reproduction, Parameter No. TPP3B.	25%
E. Report the highest (critical dilution or control) Coefficient of Variation, Parameter No. TQP3B.	6.68%

**I. *Pimephales promelas***

	<b>Response</b>
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.	25%
D. Report the NOEC value for growth, Parameter No. TPP6C.	25%
E. Report the highest (critical dilution or control) Coefficient of Variation, Parameter No. TQP6C.	6.65%

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CLARKSVILLE, AR 72830  
PHONE (479) 754-3148

**To**

ADEQ  
5301 Northshore Drive  
North Little Rock, AR 72118