

ENVIRONMENTAL ENTERPRISE GROUP  
 CITY OF CLARKSVILLE WWTP – OUTFALL 002  
 NPDES PERMIT NO. AR0022187  
 AFIN NO. 36-00038  
 BIOMONITORING REPORTING  
 TEST DATE: 04/19/16

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.	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.	11.63%

I. *Pimephales promelus*

	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.	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.	7.60%

ENVIRONMENTAL ENTERPRISE GROUP  
CITY OF CLARKSVILLE WWTP  
OUTFALL 002

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

*Ceriodaphnia dubia*  
*Pimephales promelas*

April 19, 2016

Reviewed by:


  
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TOXICITY TEST REPORT - CHRONIC

Client .....	Environmental Enterprise Group	Sample .....	Outfall 002
Facility .....	City of Clarksville WWTP	Laboratory I.D. ....	24435
Permit No. ....	NPDES AR0022187	Begin Date .....	April 19, 2016

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 United Parcel Service courier to Huther & Associates on April 19, April 21, and April 23, 2016. Effluent samples were collected and composited from Outfall 002 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 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 1620 hours, April 19, 2016. 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 ten 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 1620 hours, April 26, 2016. 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**                      **PMSD: 11.3%**  
**NOEC: 25% Effluent**

**TEST SETUP**  
*Pimephales promelas*



The seven-day *Pimephales promelas* larval survival and growth test was initiated at 1545 hours, April 19, 2016. 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 1545 hours, April 26, 2016. 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: 9.9%**  
**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.

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	04/18/16 04/20/16 04/22/16
LAB ID #	24435	DATE RECEIVED	04/19/16 04/21/16 04/23/16
TEST TYPE	7 Day Chronic	BEGIN DATE/TIME	04/19/16 1620
TEST ORGANISM	<i>Ceriodaphnia dubia</i>	END DATE/TIME	04/26/16 1620
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. cpdl
DILUTION WATER	Laboratory	TECHNICIAN	M. Homer

**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
04/20/16	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/21/16	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/22/16	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/23/16	2	3	5	2	5	4	4	2	2	5
	2	3	5	2	5	4	4	2	2	5
04/24/16	A	A	A	6	A	A	A	A	A	A
	2	3	5	8	5	4	4	2	2	5
04/25/16	7	9	8	A	6	8	8	7	9	10
	9	12	13	8	11	12	12	9	11	15
04/26/16	12	13	11	11	14	11	12	13	12	12
	21	25	24	19	25	23	24	22	23	27
x # Young 23.3					C.V. 9.71%					
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
04/20/16	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/21/16	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/22/16	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/23/16	3	2	2	3	2	5	4	4	4	5
	3	2	2	3	2	5	4	4	4	5
04/24/16	7	6	5	6	7	A	9	A	8	10
	10	8	8	9	9	5	13	4	12	15
04/25/16	A	A	A	A	A	8	A	8	A	A
	10	8	8	9	9	13	13	12	12	15
04/26/16	13	14	13	12	14	11	15	13	13	14
	23	22	21	21	23	24	28	25	25	29
x # Young 24.1					C.V. 11.31%					
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
04/20/16	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/21/16	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/22/16	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/23/16	5	4	2	3	2	5	2	3	2	3
	5	4	2	3	2	5	2	3	2	3
04/24/16	A	9	6	10	7	11	A	9	6	8
	5	13	8	13	9	16	2	12	8	11
04/25/16	8	A	A	A	A	A	7	A	A	A
	13	13	8	13	9	16	9	12	8	11
04/26/16	13	15	12	13	12	14	13	13	14	11
	26	28	20	26	21	30	22	25	22	22
x # Young 24.2					C.V. 13.61%					
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
04/20/16	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/21/16	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/22/16	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/23/16	4	3	2	3	3	2	5	2	5	4
	4	3	2	3	3	2	5	2	5	4
04/24/16	9	10	7	8	8	6	A	6	A	A
	13	13	9	11	11	8	5	8	5	4
04/25/16	A	A	A	A	A	A	7	A	8	9
	13	13	9	11	11	8	12	8	13	13
04/26/16	12	11	14	15	12	15	13	13	13	12
	25	24	23	26	23	23	25	21	26	25
x # Young 24.1					C.V. 6.62%					
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# 24435

Test Date: April 19, 2016

11% Effluent										
Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
04/20/16	A	A	A	A	A	A	A	A	A	A
04/20/16	0	0	0	0	0	0	0	0	0	0
04/21/16	A	A	A	A	A	A	A	A	A	A
04/21/16	0	0	0	0	0	0	0	0	0	0
04/22/16	A	A	A	A	A	A	A	A	A	A
04/22/16	0	0	0	0	0	0	0	0	0	0
04/23/16	2	3	2	2	4	3	5	4	3	3
04/23/16	2	3	2	2	4	3	5	4	3	3
04/24/16	8	6	A	6	10	8	7	8	6	7
04/24/16	10	9	2	8	14	11	12	12	9	10
04/25/16	A	A	7	A	A	A	A	A	A	A
04/25/16	10	9	9	8	14	11	12	12	9	10
04/26/16	15	11	12	11	14	14	13	12	14	13
04/26/16	25	20	21	19	28	25	25	24	23	23
		x# Young		23.3		C.V.		11.63%		
		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
04/20/16	A	A	A	A	A	A	A	A	A	A
04/20/16	0	0	0	0	0	0	0	0	0	0
04/21/16	A	A	A	A	A	A	A	A	A	A
04/21/16	0	0	0	0	0	0	0	0	0	0
04/22/16	A	A	A	A	A	A	A	A	A	A
04/22/16	0	0	0	0	0	0	0	0	0	0
04/23/16	3	2	2	4	2	3	2	4	3	3
04/23/16	3	2	2	4	2	3	2	4	3	3
04/24/16	9	6	6	7	7	7	6	10	9	6
04/24/16	12	8	8	11	9	10	8	14	12	9
04/25/16	A	A	A	A	A	A	A	A	A	A
04/25/16	12	8	8	11	9	10	8	14	12	9
04/26/16	13	12	13	14	15	12	13	14	12	13
04/26/16	25	20	21	25	24	22	21	28	24	22
		x# Young		23.2		C.V.		10.52%		
		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# 24435

Test Date: April 19, 2016

**WET CHEMISTRY MEASUREMENTS**

Date	Time	Temp	Samp. No.	pH of Solution						Analyst
				CON	5%	6%	8%	11%	25%	
04/19/16	Start	25.0	1	7.70	7.69	7.67	7.66	7.67	7.63	LT
04/20/16	24 Hr	25.4	1	7.65	7.63	7.62	7.61	7.60	7.56	TB
04/20/16	Renew	25.4	1	7.66	7.63	7.64	7.67	7.63	7.58	TB
04/21/16	48 Hr	25.0	1	7.93	7.91	7.88	7.86	7.84	7.78	LT
04/21/16	Renew	25.0	2	7.88	7.88	7.84	7.84	7.82	7.79	LT
04/22/16	72 Hr	25.1	2	7.89	7.82	7.78	7.77	7.72	7.67	RU
04/22/16	Renew	25.0	2	7.85	7.81	7.78	7.78	7.76	7.66	RU
04/23/16	96 Hr	25.2	2	8.34	8.28	8.25	8.26	8.20	8.08	LT
04/23/16	Renew	24.9	3	8.75	8.66	8.67	8.61	8.58	8.42	LT
04/24/16	120 Hr	25.6	3	8.01	7.94	7.94	7.95	7.94	7.88	RU
04/24/16	Renew	25.3	3	8.85	8.77	8.72	8.65	8.57	8.27	RU
04/25/16	144 Hr	25.4	3	8.10	8.03	8.02	8.01	7.99	7.98	RU
04/25/16	Renew	25.4	3	8.70	8.58	8.63	8.62	8.61	8.60	RU
04/26/16	168 Hr	26.0	3	7.58	7.59	7.59	7.58	7.58	7.62	LT

Date	Time	Temp	Samp. No.	DO (mg/L) of Solution						Analyst
				CON	5%	6%	8%	11%	25%	
04/19/16	Start	25.0	1	7.98	7.30	7.35	7.76	7.20	7.09	LT
04/20/16	24 Hr	25.4	1	7.45	7.49	7.62	7.67	7.69	7.69	TB
04/20/16	Renew	25.4	1	8.47	8.23	7.83	7.77	7.81	7.79	TB
04/21/16	48 Hr	25.0	1	6.62	7.79	7.99	7.82	7.56	7.80	LT
04/21/16	Renew	25.0	2	8.06	7.12	7.60	7.51	8.23	7.59	LT
04/22/16	72 Hr	25.1	2	7.20	7.53	7.54	7.50	7.63	7.91	RU
04/22/16	Renew	25.0	2	8.54	8.47	7.92	7.85	7.69	7.55	RU
04/23/16	96 Hr	25.2	2	7.48	7.53	7.53	7.48	7.82	7.73	LT
04/23/16	Renew	24.9	3	8.02	8.31	7.74	7.72	7.77	8.19	LT
04/24/16	120 Hr	25.6	3	7.00	7.10	7.08	7.26	7.23	7.15	RU
04/24/16	Renew	25.3	3	8.52	8.41	7.82	8.03	8.16	7.63	RU
04/25/16	144 Hr	25.4	3	8.43	8.02	8.03	8.15	7.64	8.07	RU
04/25/16	Renew	25.4	3	8.21	8.25	8.64	8.10	7.95	7.45	RU
04/26/16	168 Hr	26.0	3	8.39	8.47	8.74	8.34	8.75	8.80	LT

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

EEG, City of Clarksville WWTP

Lab ID# 24435

Test Date: April 19, 2016

**INITIAL CHEMISTRY MEASUREMENTS @ 100% EFFLUENT**

Date	Samp. No.	pH <sup>1</sup>	DO <sup>1</sup>	Hardness mg/L CaCO <sub>3</sub> <sup>1</sup>	Alkalinity mg/L CaCO <sub>3</sub>	Conduct. μS/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>2</sup>	Analyst
04/19/16	1	7.38	7.84	60	38	222	<0.01	N/A	RK
04/21/16	2	7.69	8.61	60	44	209	<0.01	N/A	RK
04/23/16	3	7.82	8.24	64	40	205	<0.01	N/A	RK
04/19/16	Con	7.70	7.98	104	62	364	-	-	RK

<sup>1</sup> 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	19.000	27.000	23.300
2	5% Effluent	10	21.000	29.000	24.100
3	6% Effluent	10	20.000	30.000	24.200
4	8% Effluent	10	21.000	26.000	24.100
5	11% Effluent	10	19.000	28.000	23.300
6	25% Effluent	10	20.000	28.000	23.200

Summary Statistics on Transformed Data Table 2 of 2

Grp	Identification	Variance	Sd	Sem	C.V.%
1	Control	5.122	2.263	0.716	9.71
2	5% Effluent	7.433	2.726	0.862	11.31
3	6% Effluent	10.844	3.293	1.041	13.61
4	8% Effluent	2.544	1.595	0.504	6.62
5	11% Effluent	7.344	2.710	0.857	11.63
6	25% Effluent	5.956	2.440	0.772	10.52

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	18	16	5

Calculated Chi-Square goodness of fit test statistic = 2.5387

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 = 4.60

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	11.400	2.280	0.349
Within (Error)	54	353.200	6.541	
Total	59	364.600		

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	Transformed Mean	Mean Calculated In Original Units	T Stat	Sig
1	Control	23.300	23.300		
2	5% Effluent	24.100	24.100	-0.699	
3	6% Effluent	24.200	24.200	-0.787	
4	8% Effluent	24.100	24.100	-0.699	
5	11% Effluent	23.300	23.300	0.000	
6	25% Effluent	23.200	23.200	0.087	

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

No statistically significant difference

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

Grp	Identification	Num of Reps	Minimum Diff (In Orig. Units)	Sig % of Control	Difference from Control
1	Control	10			
2	5% Effluent	10	2.642	11.3	-0.800
3	6% Effluent	10	2.642	11.3	-0.900
4	8% Effluent	10	2.642	11.3	-0.800
5	11% Effluent	10	2.642	11.3	0.000
6	25% Effluent	10	2.642	11.3	0.100

Huthner 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	04/18/16, 04/20/16, 04/22/16
LAB ID #	24435	DATE RECEIVED	04/19/16, 04/21/16, 04/23/16
TEST TYPE	7-Day Chronic	BEGIN DATE/TIME	04/19/16, 1545
TEST ORGANISM	<i>Pimephales promelas</i>	END DATE/TIME	04/26/16, 1545
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	G. Price

**SURVIVAL SUMMARY**

Conc.	04/20/16					04/21/16					04/22/16					04/23/16					04/24/16				
	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.	04/25/16					04/26/16					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	0.4290	0.4610	0.4350	0.4270	0.4510	0.4406	3.36
5%	0.4860	0.4950	0.5010	0.4170	0.4620	0.4722	7.25
6%	0.4450	0.4570	0.4960	0.4820	0.4900	0.4740	4.64
8%	0.5020	0.4720	0.4840	0.5040	0.4270	0.4778	6.56
11%	0.4560	0.4180	0.4960	0.5020	0.4460	0.4636	7.60
25%	0.4810	0.4970	0.5040	0.4260	0.4950	0.4806	6.58

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

EEG, City of Clarksville WWTP

Lab ID# 24435

Test Date: April 19, 2016

**WET CHEMISTRY MEASUREMENTS**

Date	Time	Temp	Samp. No.	pH of Solution						Analyst
				CON	5%	6%	8%	11%	25%	
04/19/16	Start	25.0	1	7.70	7.69	7.67	7.66	7.67	7.63	LT
04/20/16	24 Hr	25.8	1	7.29	7.30	7.37	7.38	7.17	7.31	TB
04/20/16	Renew	25.4	1	7.66	7.63	7.64	7.67	7.63	7.58	TB
04/21/16	48 Hr	25.5	1	7.34	7.40	7.47	7.54	7.55	7.28	LT
04/21/16	Renew	25.0	2	7.88	7.88	7.84	7.84	7.82	7.79	LT
04/22/16	72 Hr	25.5	2	7.92	7.88	7.87	7.87	7.87	7.86	RU
04/22/16	Renew	25.0	2	7.85	7.81	7.78	7.78	7.76	7.66	RU
04/23/16	96 Hr	25.3	2	7.47	7.57	7.53	7.47	7.53	7.46	LT
04/23/16	Renew	25.0	3	8.75	8.66	8.67	8.61	8.58	8.42	LT
04/24/16	120 Hr	25.9	3	7.95	7.84	7.85	7.83	7.75	7.73	RU
04/24/16	Renew	25.3	3	8.85	8.77	8.72	8.65	8.57	8.27	RU
04/25/16	144 Hr	25.8	3	7.75	7.82	7.76	7.80	7.69	7.64	RU
04/25/16	Renew	25.4	3	8.70	8.58	8.63	8.62	8.61	8.60	RU
04/26/16	168 Hr	25.0	3	7.98	7.90	7.98	7.99	7.95	7.96	LT

Date	Time	Temp	Samp. No.	DO (mg/L) of Solution						Analyst
				CON	5%	6%	8%	11%	25%	
04/19/16	Start	25.0	1	7.98	7.30	7.35	7.76	7.20	7.09	LT
04/20/16	24 Hr	25.8	1	7.69	7.58	7.29	7.32	7.37	6.97	TB
04/20/16	Renew	25.4	1	8.47	8.23	7.83	7.77	7.81	7.79	TB
04/21/16	48 Hr	25.5	1	8.26	7.55	7.23	7.45	7.34	7.66	LT
04/21/16	Renew	25.0	2	8.06	7.12	7.60	7.51	8.23	7.59	LT
04/22/16	72 Hr	25.5	2	8.23	8.34	8.32	8.19	8.11	7.84	RU
04/22/16	Renew	25.0	2	8.54	8.47	7.92	7.85	7.69	7.55	RU
04/23/16	96 Hr	25.3	2	7.98	7.68	8.27	7.65	7.57	8.08	LT
04/23/16	Renew	25.0	3	8.02	8.31	7.74	7.72	7.77	8.19	LT
04/24/16	120 Hr	25.9	3	7.84	8.06	8.04	7.92	7.47	7.55	RU
04/24/16	Renew	25.3	3	8.52	8.41	7.82	8.03	8.16	7.63	RU
04/25/16	144 Hr	25.8	3	8.80	8.59	8.47	7.90	7.98	7.94	RU
04/25/16	Renew	25.4	3	8.21	8.25	8.64	8.10	7.95	7.45	RU
04/26/16	168 Hr	25.0	3	8.15	8.84	8.71	8.59	8.75	8.71	LT

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

EEG, City of Clarksville WWTP

Lab ID# 24435

Test Date: April 19, 2016

**INITIAL CHEMISTRY MEASUREMENTS @ 100% EFFLUENT**

Date	Samp. No.	pH <sup>1</sup>	DO <sup>1</sup>	Hardness mg/L CaCO <sub>3</sub> <sup>1</sup>	Alkalinity mg/L CaCO <sub>3</sub> <sup>1</sup>	Conduct. μS/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>4</sub> mg/L <sup>1</sup>	Analyst
04/19/16	1	7.38	7.84	60	38	222	<0.01	N/A	RK
04/21/16	2	7.69	8.61	60	44	209	<0.01	N/A	RK
04/23/16	3	7.82	8.24	64	40	205	<0.01	N/A	RK
04/19/16	Con	7.70	7.98	104	62	364	-	-	RK

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

*PIMEPHALES PROMELAS* STATISTICAL ANALYSES  
 Growth - Transformations

Summary Statistics on Transformed Data Table 1 of 2

Grp	Identification	N	Min	Max	Mean
1	Control	5	0.427	0.461	0.441
2	5% Effluent	5	0.417	0.501	0.472
3	6% Effluent	5	0.445	0.496	0.474
4	8% Effluent	5	0.427	0.504	0.478
5	11% Effluent	5	0.418	0.502	0.464
6	25% Effluent	5	0.426	0.504	0.481

Summary Statistics on Transformed Data Table 2 of 2

Grp	Identification	Variance	Sd	Sem	C.V.%
1	Control	0.000	0.015	0.007	3.36
2	5% Effluent	0.001	0.034	0.015	7.25
3	6% Effluent	0.000	0.022	0.010	4.64
4	8% Effluent	0.001	0.031	0.014	6.56
5	11% Effluent	0.001	0.035	0.016	7.60
6	25% Effluent	0.001	0.032	0.014	6.58

Shapiro - Wilk's Test For Normality

D = 0.020

W = 0.914

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 = 3.33

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	0.005	0.001	1.269
Within (Error)	24	0.020	0.001	
Total	29	0.026		

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	Calculated In		
1	Control	0.441	0.441		
2	5% Effluent	0.472	0.472	-1.714	
3	6% Effluent	0.474	0.474	-1.811	
4	8% Effluent	0.478	0.478	-2.017	
5	11% Effluent	0.464	0.464	-1.247	
6	25% Effluent	0.481	0.481	-2.169	

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	Minimum Sig		Difference
			Reps	Diff	
1	Control	5			
2	5% Effluent	5	0.044	9.9	-0.032
3	6% Effluent	5	0.044	9.9	-0.033
4	8% Effluent	5	0.044	9.9	-0.037
5	11% Effluent	5	0.044	9.9	-0.023
6	25% Effluent	5	0.044	9.9	-0.040

APPENDIX A  
RAW DATA



7-DAY CERIODAPHNIA DUBIA SURVIVAL & REPRODUCTION

DAILY RAW DATA TABLE

PAGE 1 OF 2

CLIENT EEG - Clarksville 002

START DATE/TIME 4-19-16 MH 1620

OUTFALL 002

END DATE/TIME 4-26-16 RK 1620

LAB ID # 24435

COW

5

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
4/20	A	A	A	A	A	A	A	A	A	A	BB	1620
4/21	A	A	A	A	A	A	A	A	A	A	MH	1435
4/22	A	A	A	A	A	A	A	A	A	A	TB	1510
4/23	2	3	5	2	5	4	4	2	2	5	MH	1335
4/24	A	A	A	6	A	A	A	A	A	A	MH	1000
4/25	7	9	8	A	6	8	8	7	9	10	TB	1045
4/26	12	13	11	11	14	11	12	13	12	12	RK	1620
	21	25	24	19	25	23	24	22	23	27		

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
4/20	A	A	A	A	A	A	A	A	A	A	BB	1620
4/21	A	A	A	A	A	A	A	A	A	A	MH	1435
4/22	A	A	A	A	A	A	A	A	A	A	TB	1510
4/23	3	2	2	3	2	5	4	4	4	5	MH	1335
4/24	7	6	6	6	7	A	9	A	8	10	MH	1000
4/25	A	A	A	A	A	8	A	8	A	A	TB	1045
4/26	13	14	13	12	14	11	15	13	13	14	RK	1620
	23	22	21	21	23	24	28	25	25	29		

$\bar{x}$  # Young w/o Dead = 23.3 CV% = 9.71

$\bar{x}$  # Young w/o Dead = 24.1 CV% = 11.31

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

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

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

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

6

8

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
4/20	A	A	A	A	A	A	A	A	A	A	BB	1620
4/21	A	A	A	A	A	A	A	A	A	A	MH	1435
4/22	A	A	A	A	A	A	A	A	A	A	TB	1510
4/23	5	4	2	3	2	5	2	3	2	3	MH	1335
4/24	A	9	6	10	7	11	A	9	6	8	MH	1000
4/25	8	A	A	A	A	A	7	A	A	A	TB	1045
4/26	13	15	12	13	12	14	13	13	14	11	RK	1620
	26	28	20	26	21	30	22	25	22	22		

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
4/20	A	A	A	A	A	A	A	A	A	A	BB	1620
4/21	A	A	A	A	A	A	A	A	A	A	MH	1435
4/22	A	A	A	A	A	A	A	A	A	A	TB	1510
4/23	4	3	2	3	3	2	5	2	5	4	MH	1335
4/24	9	10	7	8	8	6	A	6	A	A	MH	1000
4/25	A	A	A	A	A	A	7	A	8	9	TB	1045
4/26	12	11	14	15	12	15	13	13	13	12	RK	1620
	25	24	23	26	23	23	25	21	26	25		

$\bar{x}$  # Young w/o Dead = 24.2 CV% = 13.61

$\bar{x}$  # Young w/o Dead = 24.1 CV% = 6.62

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

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

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

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

7-DAY CERIODAPHnia DUBIA SURVIVAL & REPRODUCTION

DAILY RAW DATA TABLE

PAGE 2 OF 2

CLIENT EEG-Clarksuille 002  
 OUTFALL 002  
 LAB ID # 24435

START DATE/TIME 4-19-16 MH 1620  
 END DATE/TIME 4-26-16 RK 1620

11

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
4/20	A	A	A	A	A	A	A	A	A	A	BB	1620
4/21	A	A	A	A	A	A	A	A	A	A	MH	1435
4/22	A	A	A	A	A	A	A	A	A	A	TB	1510
4/23	2	3	2	2	4	3	5	4	3	3	MH	1335
4/24	8	6	6	6	10	8	7	8	6	7	MH	1000
4/25	A	A	7	A	A	A	A	A	A	A	TB	1045
4/26	15	11	12	11	14	14	13	12	14	13	RK	1620
	25	20	21	19	28	25	25	24	23	23		

$\bar{x}$  # Young w/o Dead = 23.3 CV% = 11.63  
 $\bar{x}$  # Young w/Dead = CV% =  
 $\bar{x}$  % Survival = 100.0 CV% = 0.00

25

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
4/20	A	A	A	A	A	A	A	A	A	A	BB	1620
4/21	A	A	A	A	A	A	A	A	A	A	MH	1435
4/22	A	A	A	A	A	A	A	A	A	A	TB	1510
4/23	3	2	2	4	2	3	2	4	3	3	MH	1335
4/24	9	6	6	7	7	7	6	10	9	6	MH	1000
4/25	A	A	A	A	A	A	A	A	A	A	TB	1045
4/26	13	12	13	14	15	12	13	14	12	13	RK	1620
	25	20	21	25	24	22	21	28	24	22		

$\bar{x}$  # Young w/o Dead = 23.2 CV% = 10.52  
 $\bar{x}$  # Young w/Dead = CV% =  
 $\bar{x}$  % Survival = 100.0 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 # 24435  
 ORGANISM ID# pp7-16-102

DATE/TIME STARTED 4/19/16 GP 1545  
 DATE/TIME ENDED 4/26/16 GP 1545

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	4/20/16 GP 1545					4/21/16 GP 1040					4-22-16 BR 0910					4/2/16 GP 0835					4/24/16 GP 0830									

Conc.	A					B					Mean 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
Initials Date/Time	4-25-16 TB 0830					4/26/16 GP 1545						



Client / Facility EEG - Clarksville  
 Lab ID Number 24435  
 Outfall Number 002  
 Test Date 4-19-16

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
4-19	1	7.38	7.84	60	38	222	ca. 01	N/A	RK
4-21	2	7.69	8.61	60	44	209	↓	↓	↓
4-23	3	7.82	8.24	64	40	205	↓	↓	↓
4-19	con	7.70	7.98	104	62	364	—	—	↓

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:

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

APPENDIX B  
REFERENCE TOXICANTS

**CHRONIC REFERENCE TOXICANT TEST RESULTS**

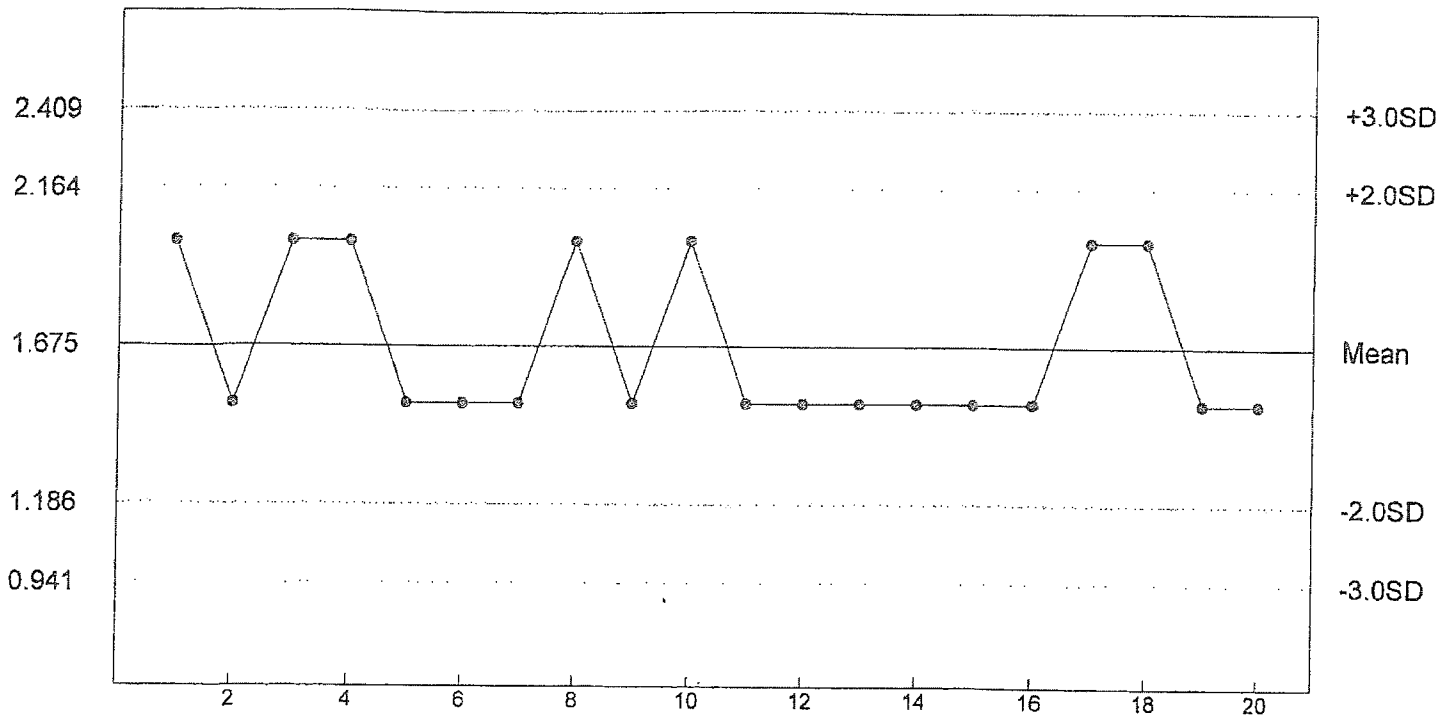
SPECIES: *Ceriodaphnia dubia*  
 CHEMICAL: Sodium Chloride  
 DURATION: 7-Days  
 TEST NUMBER: 4  
 TEST DATE: 04/06/16 - 04/13/16  
 1630 Hrs - 1630 Hrs  
 STATISTICAL METHOD: Dunnetts/Steels

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

LOEC FOR SURVIVAL	NOEC FOR SURVIVAL	LOEC FOR REPRODUCTION	NOEC FOR REPRODUCTION
1.5 g/L	1.0 g/L	1.0 g/L	0.5 g/L

Reference Tox Sodium Chloride g/L

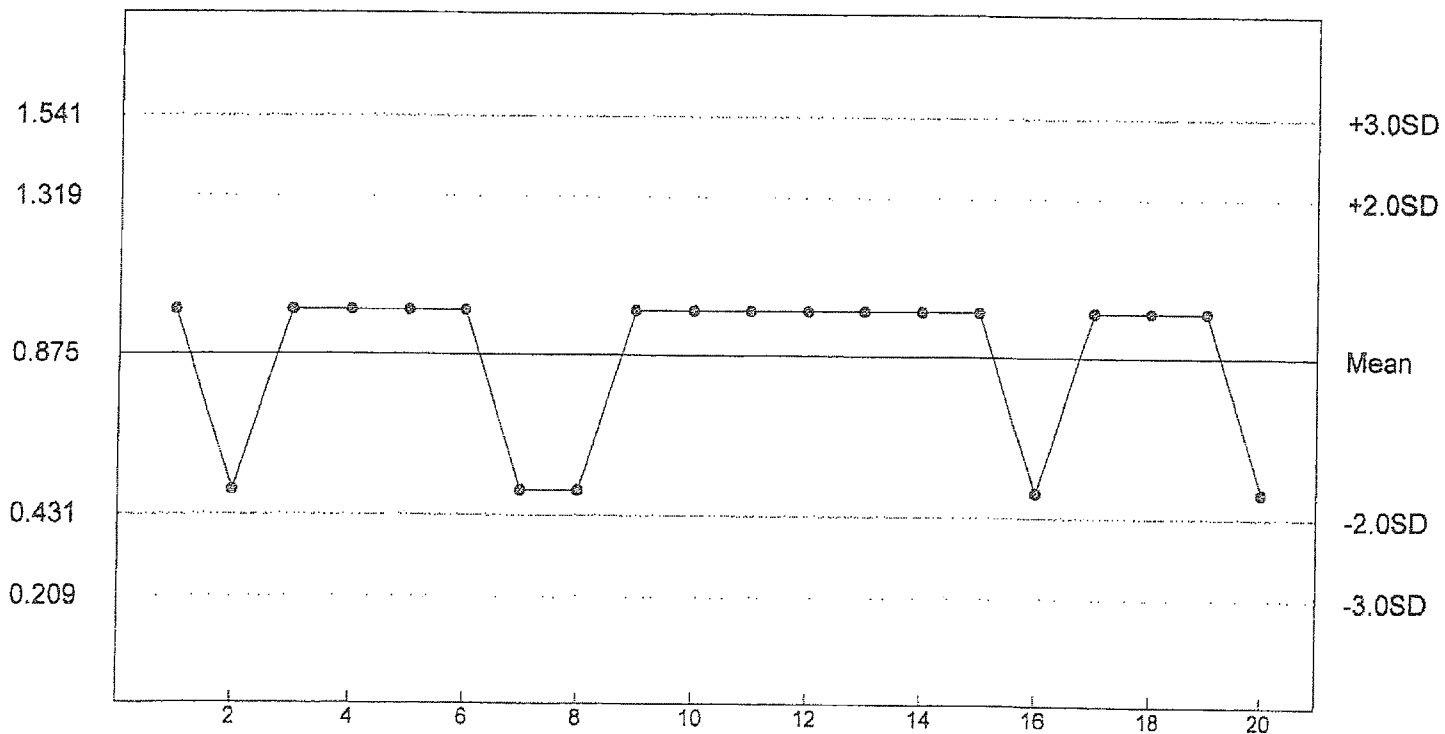
C. dubia Survival - NOEC



n= 20 Mean= 1.675 SD= 0.245 CV= 14.61% 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



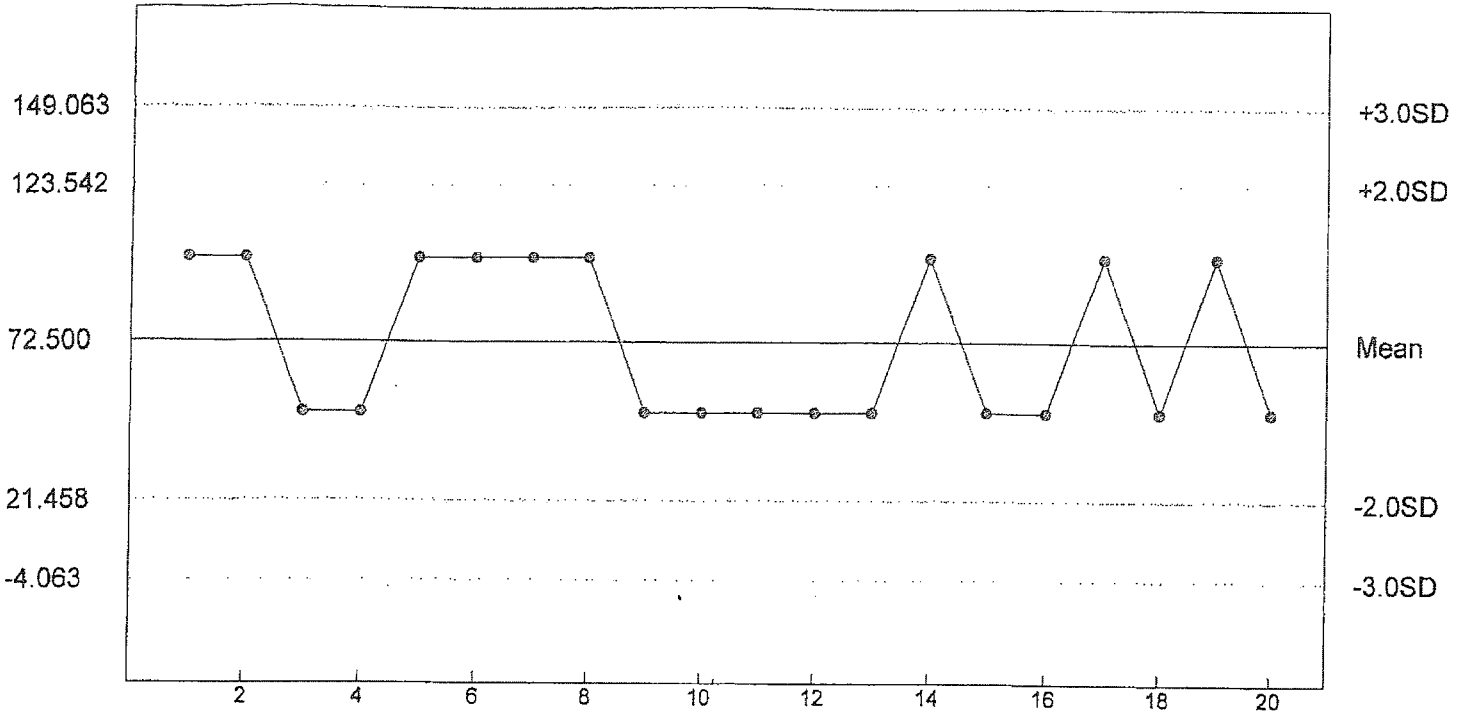
CHRONIC REFERENCE TOXICANT TEST RESULTS

SPECIES: *Pimephales promelas*  
 CHEMICAL: Copper Nitrate  
 DURATION: 7-Days  
 TEST NUMBER: 4  
 TEST DATE: 04/06/16 - 04/13/16  
 1500 Hrs - 1500 Hrs  
 STATISTICAL METHOD: Dunnetts/Steels

CONCENTRATION (ug/L)	NUMBER EXPOSED	NUMBER DEAD
25	40	0
50	40	1
100	40	12
200	40	35
400	40	40
800	40	40

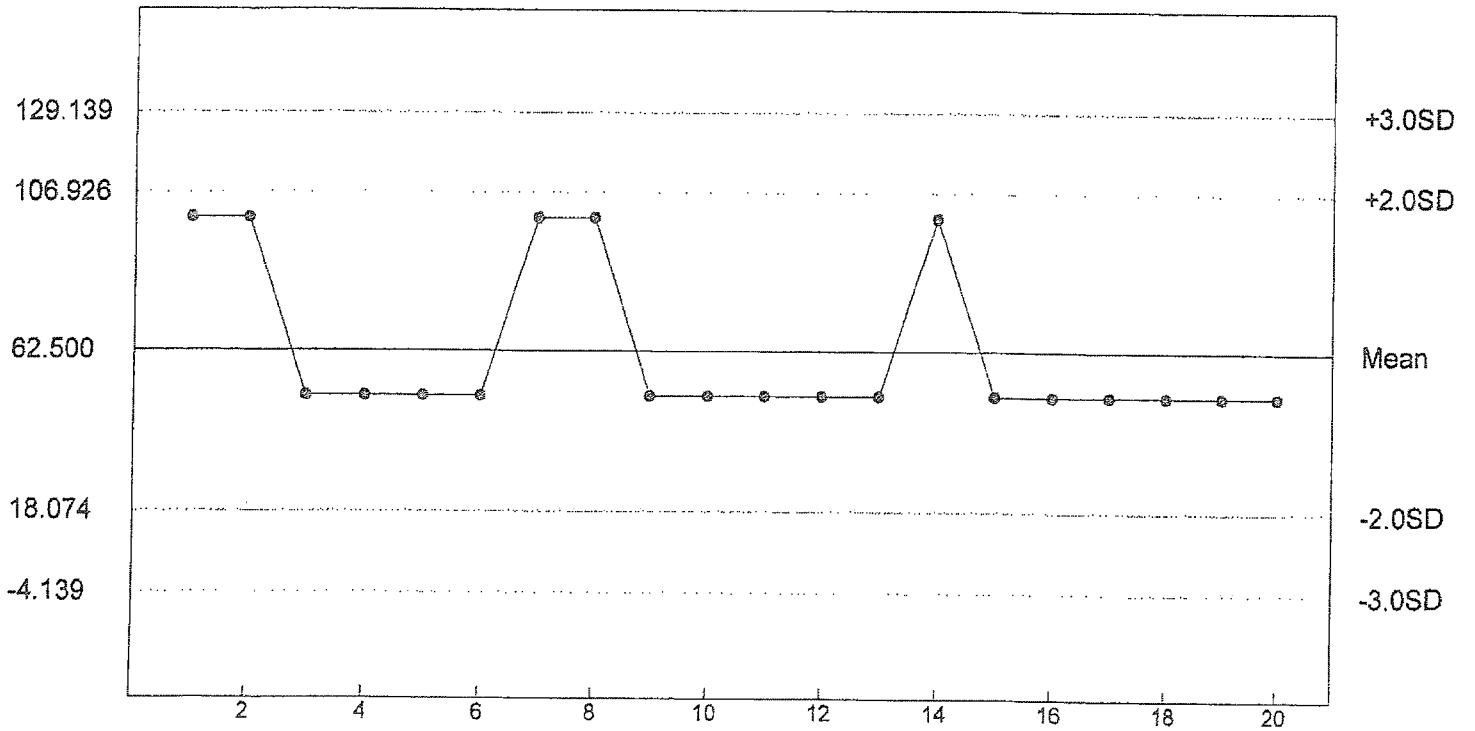
LOEC FOR SURVIVAL	NOEC FOR SURVIVAL	LOEC FOR GROWTH	NOEC FOR GROWTH
100 ug/L	50 ug/L	100 ug/L	50 ug/L

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



n= 20 Mean= 72.500 SD= 25.521 CV= 35.20% Min= 50.000 Max= 100.000

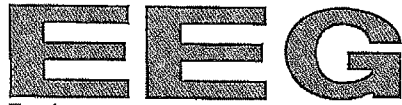
Reference Tox Copper Nitrate ug/L  
*P. promelas* Growth - NOEC



n= 20 Mean= 62.500 SD= 22.213 CV= 35.54% 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

L444-051028

Company Name: Clarksville Light and Water				Phone #: (479) 754-6241											Requested Analysis						Laboratory Control Number	Remarks (Please note special detection limits below.)																
Address: P.O. Box 1807, Clarksville, AR 72830				Fax #: (479) 754-8181																																		
Project Name or Number: Bio-Monitoring				Purchase Order #:																																		
Sampling Personnel Signature(s): <i>Megan Hatcher</i>				Printed: <i>Megan Hatcher</i>																																		
Sample I.D.	Date	Time	Comp.	Grab	Cont. Type		# of Containers	Method Preserved						Sample Matrix					7-Day Chronic Bio-Monitoring Minnows																			
					Plast.	Glass		H2SO4	HNO3	NaOH	HCL	Ice	None	Water	Soil	Air	Sludge	Other																				
Outfall 002	4-17-16 4-18-16	0730 0730	X		X		1							X	X									X													0416130	
Relinquished by: <i>Megan R Hatcher</i>							Date: 4-18-16	Time: 1000	Relinquished by: <i>Staunton</i>							Date: 4-18-16	Time: 1600																					
Received by: <i>Staunton</i>							Date: 4-18-16	Time: 1000	Received by Laboratory: <i>Matt Boman</i>							Date: 4-19-16	Time: 1100																					
Comments: <i>S 3,3°C</i>																												<i>UPS</i>										

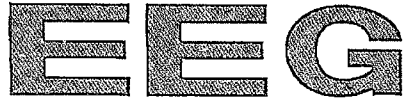
Environmental Enterprise Group, Inc.  
 220 N. Knoxville  
 Russellville, AR 72801  
 (479)968-6767 Fax (479)968-1956



LL44-051028

Company Name: Clarksville Light and Water			Phone #: (479) 754-6241						Requested Analysis									Laboratory Control Number  0416130	Remarks (Please note special detection limits below.)																										
Address: P.O. Box 1807, Clarksville, AR 72830			Fax #: (479) 754-8181																																										
Project Name or Number: Bio-Monitoring			Purchase Order #:																																										
Sampling Personnel Signature(s): Megan Hatcher			Printed: Megan Hatcher																																										
Sample I.D.	Date	Time	Comp.	Grab	Cont. Type		# of Containers	Method Preserved							Sample Matrix					7-Day Chronic Bio-Monitoring Minnows																									
					Plast.	Glass		H2SO4	HNO3	NAOH	HCL	Ice	None	Water	Soil	Air	Sudge	Other																											
Outfall 002	4-19-16 4-20-16	0730 0730	X		X		1							X	X								X																						
Relinquished by: Megan Hatcher								Date: 4-20-16 0800	Time: 0800	Relinquished by: Jhe								Date: 4/20/16	Time: 1100																										
Received by: Jhe								Date: 4/20/16	Time: 0800	Received by Laboratory:								Date: 4/21/16	Time: 1145																										
Comments: UPS Temp - 1.6																																													

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

L 444-051028

Company Name: Clarksville Light and Water												Phone #: (479) 754-6241						Requested Analysis							Laboratory Control Number		Remarks <i>(Please note special detection limits below.)</i>		
Address: P.O. Box 1807, Clarksville, AR 72830												Fax #: (479) 754-8181																	
Project Name or Number: Bio-Monitoring												Purchase Order #:																	
Sampling Personnel Signature(s): <i>Megan Hatcher</i>												Printed: <i>Megan Hatcher</i>						7-Day Chronic Bio-Monitoring Minnows											
Sample I.D.	Date	Time	Comp.	Grab	Cont. Type		# of Containers	Method Preserved					Sample Matrix																
					Plast.	Glass		H2SO4	HNO3	NAOH	HCL	Ice	None	Water	Soil	Air	Sludge											Other	
Outfall 002	4-21-16 4-22-16	0730 0730	X		X		1					X	X															X	
Relinquished by: <i>Megan Hatcher</i>								Date: 4-22-16		Time: 0920		Relinquished by: <i>Stacyner</i>							Date: 4/22/16		Time: 1100								
Received by: <i>Stacyner</i>								Date: 4/22/16		Time: 0920		Received by Laboratory: <i>Matt Stainer</i>							Date: 4-23-16		Time: 1030								
Comments: -0.7°C																			UPS										

ENVIRONMENTAL ENTERPRISE GROUP  
 CITY OF CLARKSVILLE WWTP – OUTFALL 002  
 NPDES PERMIT NO. AR0022187  
 AFIN NO. 36-00038  
 BIOMONITORING REPORTING  
 TEST DATE: 04/19/16

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.	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.	11.63%

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.	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.	7.60%

ENVIRONMENTAL ENTERPRISE GROUP  
 CITY OF CLARKSVILLE WWTP – OUTFALL 001  
 NPDES PERMIT NO. AR0022187  
 AFIN NO. 36-00038  
 BIOMONITORING REPORTING  
 TEST DATE: 04/19/16

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.	11.25%

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.	7.50%



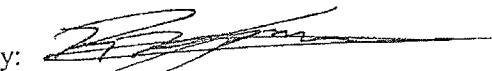
ENVIRONMENTAL ENTERPRISE GROUP  
CITY OF CLARKSVILLE WWTP  
OUTFALL 001

Chronic Biomonitoring Report  
Permit Number NPDES AR022187  
AFIN Number 36-00038

*Ceriodaphnia dubia*  
*Pimephales promelas*

April 19, 2016

Reviewed by:



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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. .... 25424  
 Permit No. .... NPDES AR022187      Begin Date ..... April 19, 2016

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 United Parcel Service courier to Huther & Associates on April 19, April 21, and April 23. 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, 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 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 1600 hours, April 19, 2016. 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 1600 hours, April 26, 2016. 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: 10.1%**  
**NOEC: 100% Effluent**

**TEST SETUP**  
*Pimephales promelas*



The seven-day *Pimephales promelas* larval survival and growth test was initiated at 1600 hours, April 19, 2016. 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 1600 hours, April 26, 2016. 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**

**NOEC: 100% Effluent**

**PMSD: 10.1%**

**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 AR022187 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 : 04/18/16 04/20/16 04/22/16  
 LAB ID # : 25424 DATE RECEIVED : 04/19/16 04/21/16 04/23/16  
 TEST TYPE : 7 Day Chronic BEGIN DATE/TIME : 04/19/16 1600  
 TEST ORGANISM : *Ceriodaphnia dubia* END DATE/TIME : 04/26/16 1600  
 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. candle  
 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
04/20/16	A	A	A	A	A	A	A	A	A	A
04/21/16	0	0	0	0	0	0	0	0	0	0
04/22/16	A	A	A	A	A	A	A	A	A	A
04/23/16	2	3	2	2	2	3	3	2	2	4
04/24/16	2	3	2	2	2	3	3	2	2	4
04/25/16	9	7	10	8	7	8	8	6	9	8
04/26/16	23	24	25	22	23	22	23	21	25	24
x# Young 23.2 C.V. 5.67% 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
04/20/16	A	A	A	A	A	A	A	A	A	A
04/21/16	0	0	0	0	0	0	0	0	0	0
04/22/16	A	A	A	A	A	A	A	A	A	A
04/23/16	2	3	3	3	4	2	3	2	3	4
04/24/16	2	3	3	3	4	2	3	2	3	4
04/25/16	8	8	7	8	9	6	10	10	7	8
04/26/16	22	24	22	23	26	19	27	27	21	23
x# Young 23.4 C.V. 11.25% 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
04/20/16	A	A	A	A	A	A	A	A	A	A
04/21/16	0	0	0	0	0	0	0	0	0	0
04/22/16	A	A	A	A	A	A	A	A	A	A
04/23/16	2	2	2	3	3	3	5	3	4	3
04/24/16	2	2	2	3	3	3	5	3	4	3
04/25/16	9	8	8	10	7	7	6	9	8	9
04/26/16	14	12	13	13	12	14	11	14	15	11
x# Young 24.0 C.V. 7.66% 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
04/20/16	A	A	A	A	A	A	A	A	A	A
04/21/16	0	0	0	0	0	0	0	0	0	0
04/22/16	A	A	A	A	A	A	A	A	A	A
04/23/16	3	3	3	2	2	5	4	4	2	3
04/24/16	3	3	3	2	2	5	4	4	2	3
04/25/16	6	10	7	8	6	8	9	9	7	8
04/26/16	22	28	22	23	19	28	27	27	22	23
x# Young 24.1 C.V. 13.04% x% Survival 100% C.V. 0.00%										

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

ex 1  

A	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, City of Clarksville WWTP

Lab ID# 25424

Test Date: April 19, 2016

56% Effluent

Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
04/20/16	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/21/16	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/22/16	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/23/16	4	3	3	2	3	3	3	2	4	3
	4	3	3	2	3	3	3	2	4	3
04/24/16	A	A	A	A	A	A	A	A	A	A
	4	3	3	2	3	3	3	2	4	3
04/25/16	7	9	8	7	6	10	7	9	7	8
	11	12	11	9	9	13	10	11	11	11
04/26/16	14	15	14	12	13	12	13	15	13	13
	25	27	25	21	22	25	23	26	24	24
x# Young 24.2                      C.V. 7.49% 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
04/20/16	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/21/16	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/22/16	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/23/16	3	2	3	2	2	2	3	4	3	4
	3	2	3	2	2	2	3	4	3	4
04/24/16	A	A	A	A	A	A	A	A	A	A
	3	2	3	2	2	2	3	4	3	4
04/25/16	8	8	8	6	10	7	9	7	8	9
	11	10	11	8	12	9	12	11	11	13
04/26/16	12	12	12	14	13	15	11	12	13	14
	23	22	23	22	25	24	23	23	24	27
x# Young 23.6                      C.V. 6.38% 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
04/20/16	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/21/16	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/22/16	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/23/16	5	3	2	4	2	2	2	2	3	5
	5	3	2	4	2	2	2	2	3	5
04/24/16	A	A	A	A	A	A	A	A	A	A
	5	3	2	4	2	2	2	2	3	5
04/25/16	10	8	9	7	7	8	6	9	9	8
	15	11	11	11	9	10	8	11	12	13
04/26/16	15	13	15	12	15	14	14	13	13	14
	30	24	26	23	24	24	22	24	25	27
x# Young 24.9                      C.V. 9.17% 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# 25424

Test Date: April 19, 2016

**WET CHEMISTRY MEASUREMENTS**

Date	Time	Temp	Samp. No.	pH of Solution						Analyst	
				PCON	TCON	32%	42%	56%	75%		100%
04/19/16	Start	25.0	1	7.96	7.43	7.28	7.24	7.20	7.12	7.06	LT
04/20/16	24 Hr.	25.4	1	7.93	7.71	7.61	7.55	7.51	7.41	7.37	TB
04/20/16	Renew	25.4	1	8.03	7.60	7.35	7.41	7.34	7.29	7.15	TB
04/21/16	48 Hr.	25.0	1	8.01	7.80	7.74	7.71	7.66	7.56	7.57	LT
04/21/16	Renew	25.0	2	8.16	7.61	7.45	7.41	7.39	7.38	7.45	LT
04/22/16	72 Hr.	25.1	2	7.91	7.51	7.31	7.33	7.29	7.24	7.22	RU
04/22/16	Renew	25.0	2	7.84	7.49	7.25	7.26	7.23	7.20	7.20	RU
04/23/16	96 Hr.	25.2	2	8.19	8.02	7.96	7.93	7.91	7.90	7.95	LT
04/23/16	Renew	25.0	3	8.24	7.80	7.68	7.61	7.60	7.62	7.63	LT
04/24/16	120 Hr.	25.6	3	8.28	8.11	8.05	7.98	7.93	7.85	7.78	RU
04/24/16	Renew	25.3	3	8.41	7.44	7.33	7.28	7.27	7.23	7.20	RU
04/25/16	144 Hr.	25.4	3	8.24	8.17	8.06	8.03	7.99	7.88	7.86	RK
04/25/16	Renew	25.4	3	8.32	7.47	7.38	7.30	7.35	7.29	7.24	RK
04/26/16	168 Hr.	26.0	3	8.14	8.03	8.01	7.97	7.94	7.88	7.82	LT

Date	Time	Temp	Samp. No.	DO (mg/L) of Solution						Analyst	
				PCON	TCON	32%	42%	56%	75%		100%
04/19/16	Start	25.0	1	7.19	7.29	7.46	7.69	7.41	7.80	7.64	LT
04/20/16	24 Hr.	25.4	1	7.74	7.39	7.38	7.42	7.38	7.53	7.52	TB
04/20/16	Renew	25.4	1	8.61	8.24	8.20	8.37	8.21	8.32	8.19	TB
04/21/16	48 Hr.	25.0	1	8.18	7.73	7.52	7.36	6.87	7.16	7.79	LT
04/21/16	Renew	25.0	2	8.88	8.08	8.13	8.32	8.25	8.53	8.52	LT
04/22/16	72 Hr.	25.1	2	8.30	8.72	8.51	8.59	8.72	8.41	8.40	RU
04/22/16	Renew	25.0	2	8.83	8.35	7.97	7.72	7.86	7.77	7.85	RU
04/23/16	96 Hr.	24.9	2	8.60	8.60	8.05	8.33	7.91	8.17	7.76	LT
04/23/16	Renew	25.0	3	7.50	7.52	7.65	7.85	8.36	8.22	7.78	LT
04/24/16	120 Hr.	25.6	3	7.19	7.43	7.72	7.30	7.08	7.06	7.21	RU
04/24/16	Renew	25.3	3	8.36	8.37	8.23	7.49	7.51	7.59	7.87	RU
04/25/16	144 Hr.	25.4	3	8.00	7.58	8.85	8.47	8.38	8.22	8.20	RK
04/25/16	Renew	25.4	3	7.74	7.70	8.61	7.73	8.47	8.44	8.04	RK
04/26/16	168 Hr.	26.0	3	8.15	8.15	8.85	8.63	8.75	8.62	8.77	LT



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

EEG, City of Clarksville WWTP

Lab ID# 25424

Test Date: April 19, 2016

**INITIAL CHEMISTRY MEASUREMENTS @ 100% EFFLUENT**

Date	Samp. No.	pH <sup>1</sup>	DO <sup>1</sup>	Hardness mg/L CaCO <sub>3</sub> <sup>1</sup>	Alkalinity mg/L CaCO <sub>3</sub> <sup>1</sup>	Conduct. μS/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
04/19/16	1	7.06	7.64	64	34	442	<0.01	N/A	RK
04/21/16	2	7.45	8.52	64	36	459	<0.01	N/A	RK
04/23/16	3	7.63	7.78	76	34	471	<0.01	N/A	RK

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

**INITIAL CHEMISTRY MEASUREMENTS @ RECEIVING WATER**

Date	Samp. No.	pH <sup>1</sup>	DO <sup>1</sup>	Hardness mg/L CaCO <sub>3</sub> <sup>1</sup>	Alkalinity mg/L CaCO <sub>3</sub> <sup>1</sup>	Conduct. μS/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
04/19/16	RS1	7.43	7.29	176	90	818	<0.01	N/A	RK
04/21/16	RS2	7.61	8.08	160	82	585	<0.01	N/A	RK
04/23/16	RS3	7.80	7.52	168	78	975	<0.01	N/A	RK

<sup>1</sup> 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	19.000	27.000	23.400
2	32% Effluent	10	22.000	27.000	24.000
3	42% Effluent	10	19.000	28.000	24.100
4	56% Effluent	10	21.000	27.000	24.200
5	75% Effluent	10	22.000	27.000	23.600
6	100% Effluent	10	22.000	30.000	24.900

Summary Statistics on Transformed Data Table 2 of 2

Grp	Identification	Variance	Sd	Sem	C.V.%
1	Control	6.933	2.633	0.833	11.25
2	32% Effluent	3.556	1.886	0.596	7.86
3	42% Effluent	9.878	3.143	0.994	13.04
4	56% Effluent	3.289	1.814	0.573	7.49
5	75% Effluent	2.267	1.506	0.476	6.38
6	100% Effluent	5.211	2.283	0.722	9.17

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	17	23	13	4

Calculated Chi-Square goodness of fit test statistic = 0.8419

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 = 6.25

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	13.733	2.747	0.529
Within (Error)	54	280.200	5.189	
Total	59	293.933		

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	Mean		T Stat	Sig
		Transformed Mean	Calculated In Original Units		
1	Control	23.400	23.400		
2	32% Effluent	24.000	24.000	-0.589	
3	42% Effluent	24.100	24.100	-0.687	
4	56% Effluent	24.200	24.200	-0.785	
5	75% Effluent	23.600	23.600	-0.196	
6	100% Effluent	24.900	24.900	-1.472	

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
			Diff (In Orig. Units)		from Control
1	Control	10			
2	32% Effluent	10	2.353	10.1	-0.600
3	42% Effluent	10	2.353	10.1	-0.700
4	56% Effluent	10	2.353	10.1	-0.800
5	75% Effluent	10	2.353	10.1	-0.200
6	100% Effluent	10	2.353	10.1	-1.500

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	04/18/16 04/20/16 04/22/16
LAB ID #	25424	DATE RECEIVED	04/19/16 04/21/16 04/23/16
TEST TYPE	7 Day Chronic	BEGIN DATE/TIME	04/19/16 1600
TEST ORGANISM	<i>Pimephales promelas</i>	END DATE/TIME	04/26/16 1600
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	B. Bacon

**SURVIVAL SUMMARY**

Conc.	04/20/16					04/21/16					04/22/16					04/23/16					04/24/16				
	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.	04/25/16					04/26/16					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	0.4460	0.4510	0.4920	0.4810	0.4450	0.4630	4.73
Tcon	0.4260	0.4450	0.5010	0.4360	0.4290	0.4474	6.89
32%	0.4820	0.4570	0.4510	0.4970	0.4860	0.4746	4.15
42%	0.4360	0.4690	0.5020	0.4450	0.4960	0.4696	6.28
56%	0.4200	0.4750	0.4260	0.5020	0.4980	0.4642	8.41
75%	0.5010	0.4460	0.4720	0.4930	0.5020	0.4828	4.94
100%	0.4860	0.4710	0.5060	0.4170	0.4450	0.4650	7.50

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

EEG, City of Clarksville WWTP

Lab ID# 25424

Test Date: April 19, 2016

**WET CHEMISTRY MEASUREMENTS**

Date	Time	Temp	Samp. No.	pH of Solution						Analyst	
				PCON	TCON	32%	42%	56%	75%		100%
04/19/16	Start	25.0	1	7.96	7.43	7.28	7.24	7.20	7.12	7.06	LT
04/20/16	24 Hr.	25.8	1	7.44	7.34	7.31	7.30	7.08	7.02	7.07	TB
04/20/16	Renew	25.4	1	8.03	7.60	7.35	7.41	7.34	7.29	7.15	TB
04/21/16	48 Hr.	25.5	1	7.62	7.55	7.60	7.50	7.42	7.36	7.33	LT
04/21/16	Renew	25.0	2	8.16	7.61	7.45	7.41	7.39	7.38	7.45	LT
04/22/16	72 Hr.	25.5	2	7.97	7.91	7.76	7.62	7.58	7.52	7.48	RU
04/22/16	Renew	25.0	2	7.84	7.49	7.25	7.26	7.23	7.20	7.20	RU
04/23/16	96 Hr.	25.3	2	7.86	7.76	7.63	7.54	7.53	7.48	7.40	LT
04/23/16	Renew	25.0	3	8.24	7.80	7.68	7.61	7.60	7.62	7.63	LT
04/24/16	120 Hr.	25.9	3	7.85	7.75	7.55	7.46	7.45	7.45	7.42	RU
04/24/16	Renew	25.3	3	8.41	7.44	7.33	7.28	7.27	7.23	7.20	RU
04/25/16	144 Hr.	25.8	3	7.67	7.44	7.36	7.30	7.30	7.31	7.34	RK
04/25/16	Renew	25.4	3	8.32	7.47	7.38	7.30	7.35	7.29	7.24	RK
04/26/16	168 Hr.	25.0	3	8.08	7.93	7.80	7.66	7.63	7.67	7.54	LT

Date	Time	Temp	Samp. No.	DO (mg/L) of Solution						Analyst	
				PCON	TCON	32%	42%	56%	75%		100%
04/19/16	Start	25.0	1	7.19	7.29	7.46	7.69	7.41	7.80	7.64	LT
04/20/16	24 Hr.	25.8	1	8.19	7.29	6.93	7.41	7.39	7.59	7.32	TB
04/20/16	Renew	25.4	1	8.61	8.24	8.20	8.37	8.21	8.32	8.19	TB
04/21/16	48 Hr.	25.5	1	8.42	8.52	7.39	7.16	7.00	6.88	7.71	LT
04/21/16	Renew	25.0	2	8.88	8.08	8.13	8.32	8.25	8.53	8.52	LT
04/22/16	72 Hr.	25.5	2	8.16	8.20	7.92	7.55	7.44	7.43	7.51	RU
04/22/16	Renew	25.0	2	8.83	8.35	7.97	7.72	7.86	7.77	7.85	RU
04/23/16	96 Hr.	25.3	2	7.82	8.15	7.81	7.00	7.67	6.73	7.46	LT
04/23/16	Renew	25.0	3	7.50	7.52	7.65	7.85	8.36	8.22	7.78	LT
04/24/16	120 Hr.	25.9	3	7.41	7.59	7.53	7.78	7.76	7.09	7.21	RU
04/24/16	Renew	25.3	3	8.36	8.37	8.23	7.49	7.51	7.59	7.87	RU
04/25/16	144 Hr.	25.8	3	8.16	7.67	7.45	7.35	7.90	7.73	7.35	RK
04/25/16	Renew	25.4	3	7.74	7.70	8.61	7.73	8.47	8.44	8.04	RK
04/26/16	168 Hr.	25.0	3	8.34	8.80	8.45	8.59	8.17	8.17	8.85	LT

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

EEG, City of Clarksville WWTP

Lab ID# 25424

Test Date: April 19, 2016

**INITIAL CHEMISTRY MEASUREMENTS @ 100% EFFLUENT**

Date	Samp. No.	pH <sup>1</sup>	DO <sup>1</sup>	Hardness mg/L CaCO <sub>3</sub> <sup>1</sup>	Alkalinity mg/L CaCO <sub>3</sub> <sup>1</sup>	Conduct $\mu$ S/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
04/19/16	1	7.06	7.64	64	34	442	<0.01	N/A	RK
04/21/16	2	7.45	8.52	64	36	459	<0.01	N/A	RK
04/23/16	3	7.63	7.78	76	34	471	<0.01	N/A	RK

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

**INITIAL CHEMISTRY MEASUREMENTS @ RECEIVING WATER**

Date	Samp. No.	pH <sup>1</sup>	DO <sup>1</sup>	Hardness mg/L CaCO <sub>3</sub> <sup>1</sup>	Alkalinity mg/L CaCO <sub>3</sub> <sup>1</sup>	Conduct $\mu$ S/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
04/19/16	RS1	7.43	7.29	176	90	818	<0.01	N/A	RK
04/21/16	RS2	7.61	8.08	160	82	585	<0.01	N/A	RK
04/23/16	RS3	7.80	7.52	168	78	975	<0.01	N/A	RK

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

Huther and Associates, Inc.  
 Begin Date: April 19, 2016  
 Lab I.D.# 25424

*PIMEPHALES PROMELAS* STATISTICAL ANALYSES  
 Growth - Transformations

Summary Statistics on Transformed Data Table 1 of 2

Grp	Identification	N	Min	Max	Mean
1	Control	5	0.426	0.501	0.447
2	32% Effluent	5	0.451	0.497	0.475
3	42% Effluent	5	0.436	0.502	0.470
4	56% Effluent	5	0.420	0.502	0.464
5	75% Effluent	5	0.446	0.502	0.483
6	100% Effluent	5	0.417	0.506	0.465

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	0.020	0.009	4.15
3	42% Effluent	0.001	0.030	0.013	6.28
4	56% Effluent	0.002	0.039	0.017	8.41
5	75% Effluent	0.001	0.024	0.011	4.94
6	100% Effluent	0.001	0.035	0.016	7.50

Shapiro - Wilk's Test For Normality

D = 0.022

W = 0.968

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 = 2.12

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	0.772
Within (Error)	24	0.022	0.001	
Total	29	0.026		

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	Mean		T Stat	Sig
		Transformed Mean	Calculated In Original Units		
1	Control	0.447	0.447		
2	32% Effluent	0.475	0.475	-1.418	
3	42% Effluent	0.470	0.470	-1.157	
4	56% Effluent	0.464	0.464	-0.876	
5	75% Effluent	0.483	0.483	-1.845	
6	100% Effluent	0.465	0.465	-0.918	

Dunnnett table value = 2.36 (1 Tailed Value, P=0.05, DF=24,5)  
 No statistically significant difference

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

Grp	Identification	Num of Reps	Minimum Sig	% of Control	Difference
			Diff (In Orig. Units)		from Control
1	Control	5			
2	32% Effluent	5	0.045	10.1	-0.027
3	42% Effluent	5	0.045	10.1	-0.022
4	56% Effluent	5	0.045	10.1	-0.017
5	75% Effluent	5	0.045	10.1	-0.035
6	100% Effluent	5	0.045	10.1	-0.018

APPENDIX A  
RAW DATA

7-DAY CERIODAPHNIA DUBIA SURVIVAL & REPRODUCTION

DAILY RAW DATA TABLE

PAGE 1 OF 2

CLIENT EEG-Clarksville 001  
 OUTFALL 001  
 LAB ID # 25424

START DATE/TIME 4-19-16 TB 1600  
 END DATE/TIME 4-26-16 RK 1600

TC02

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
4/20	A	A	A	A	A	A	A	A	A	A	MH	1600
4/21	A	A	A	A	A	A	A	A	A	A	MH	1425
4/22	A	A	A	A	A	A	A	A	A	A	TB	1615
4/23	2	3	2	2	2	3	3	2	2	4	MH	1305
4/24	A	A	A	A	A	A	A	A	A	7	MH	1030
4/25	9	7	10	8	7	8	8	6	9	A	TB	1115
4/26	12	14	13	12	14	11	12	13	14	13	RK	1600
	23	24	25	22	23	22	23	21	25	24		

$\bar{x}$  # Young w/o Dead = 23.2 CV% = 5.67

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

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

TC02

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
4/20	A	A	A	A	A	A	A	A	A	A	MH	1600
4/21	A	A	A	A	A	A	A	A	A	A	MH	1425
4/22	A	A	A	A	A	A	A	A	A	A	TB	1615
4/23	2	3	3	3	4	2	3	2	3	4	MH	1305
4/24	A	A	A	A	A	A	A	A	A	A	MH	1030
4/25	8	8	7	8	9	6	10	10	7	8	TB	1115
4/26	12	13	12	12	13	11	14	15	11	11	RK	1600
	22	24	22	23	26	27	27	21	23			

$\bar{x}$  # Young w/o Dead = 23.4 CV% = 11.25

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

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

32

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
4/20	A	A	A	A	A	A	A	A	A	A	MH	1600
4/21	A	A	A	A	A	A	A	A	A	A	MH	1425
4/22	A	A	A	A	A	A	A	A	A	A	TB	1615
4/23	2	2	2	3	3	3	5	3	4	3	MH	1305
4/24	A	A	A	A	A	A	A	A	A	A	MH	1030
4/25	9	8	8	10	7	7	6	9	8	9	TB	1115
4/26	14	12	13	13	12	14	11	14	15	11	RK	1600
	25	22	23	26	22	24	22	26	27	23		

$\bar{x}$  # Young w/o Dead = 24.0 CV% = 7.86

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

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

42

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
4/20	A	A	A	A	A	A	A	A	A	A	MH	1600
4/21	A	A	A	A	A	A	A	A	A	A	MH	1425
4/22	A	A	A	A	A	A	A	A	A	A	TB	1615
4/23	3	3	3	2	2	5	4	4	2	3	MH	1305
4/24	A	A	A	A	A	A	A	A	A	A	MH	1030
4/25	6	10	7	8	6	8	9	9	7	8	TB	1115
4/26	13	15	12	13	11	15	14	14	13	12	RK	1600
	22	28	22	23	19	28	27	27	22	23		

$\bar{x}$  # Young w/o Dead = 24.1 CV% = 13.04

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

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



7-DAY CERIODAPHNIA DUBIA SURVIVAL & REPRODUCTION

DAILY RAW DATA TABLE

PAGE 2 OF 2

CLIENT EEG-Clarksville 001  
 OUTFALL 001  
 LAB ID # 25424

START DATE/TIME 4-19-16 TB 1600  
 END DATE/TIME 4-26-16 RK 1600

56

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
4/20	A	A	A	A	A	A	A	A	A	A	MH	1600
4/21	A	A	A	A	A	A	A	A	A	A	MH	1425
4/22	A	A	A	A	A	A	A	A	A	A	TB	1615
4/23	4	3	3	2	3	3	3	2	4	3	MH	1305
4/24	A	A	A	A	A	A	A	A	A	A	MH	1030
4/25	7	9	8	7	6	10	7	9	7	8	TB	1115
4/26	14	15	14	12	13	12	13	15	13	13	RK	1600
	25	27	25	21	22	25	23	26	24	24		

$\bar{x}$  # Young w/o Dead = 24.2 CV% = 7.49  
 $\bar{x}$  # Young w/Dead = CV% =  
 $\bar{x}$  % Survival = 100.0 CV% = 0.00

75

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
4/20	A	A	A	A	A	A	A	A	A	A	MH	1600
4/21	A	A	A	A	A	A	A	A	A	A	MH	1425
4/22	A	A	A	A	A	A	A	A	A	A	TB	1615
4/23	3	2	3	2	2	2	3	4	3	4	MH	1305
4/24	A	A	A	A	A	A	A	A	A	A	MH	1030
4/25	8	8	8	6	10	7	9	7	8	9	TB	1115
4/26	12	12	12	14	13	15	11	12	13	14	RK	1600
	23	22	23	22	25	24	23	23	24	27		

$\bar{x}$  # Young w/o Dead = 23.6 CV% = 6.38  
 $\bar{x}$  # Young w/Dead = CV% =  
 $\bar{x}$  % Survival = 100.0 CV% = 0.00

100

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
4/20	A	A	A	A	A	A	A	A	A	A	MH	1600
4/21	A	A	A	A	A	A	A	A	A	A	MH	1425
4/22	A	A	A	A	A	A	A	A	A	A	TB	1615
4/23	5	3	2	4	2	2	2	2	3	5	MH	1305
4/24	A	A	A	A	A	A	A	A	A	A	MH	1030
4/25	10	8	9	7	7	8	6	9	9	8	TB	1115
4/26	15	13	15	12	15	14	14	13	13	14	RK	1600
	30	24	26	23	24	24	22	24	25	27		

$\bar{x}$  # Young w/o Dead = 24.9 CV% = 9.17  
 $\bar{x}$  # Young w/Dead = CV% =  
 $\bar{x}$  % Survival = 100.0 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% =

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

CLIENT/FACILITY EEG- Clarksville 001  
 OUTFALL # 001 PROJECT # 25424  
 ORGANISM ID# PPO-16-110

DATE/TIME STARTED 4-19-16 BJB 1100  
 DATE/TIME ENDED 4-26-16 RIK 1600

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					
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	4/20/16 GP 1600					4/21/16 GP 1110					4-22-16 BJB 0840					4/23/16 GP 0905					4/24/16 GP 0900									

Conc.	A					B					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	4-25-16 TB 0900					4-26-16 RIK 1600						



Client / Facility EEG-Clarksville  
 Lab ID Number 25424  
 Outfall Number 001  
 Test Date 4-19-16

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
4-19	1	7.06	7.64	64	34	442	20.01	N/A	RK
4-21	2	7.45	8.52	64	36	459	↓	↓	↓
4-23	3	7.63	7.78	76	34	471	↓	↓	↓

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
4-19	RS1	7.43	7.29	176	90	818	20.01	N/A	RK
4-21	RS2	7.61	8.08	160	82	585	↓	↓	↓
4-23	RS3	7.80	7.52	168	78	975	↓	↓	↓

Notes:

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APPENDIX B  
REFERENCE TOXICANTS

**CHRONIC REFERENCE TOXICANT TEST RESULTS**

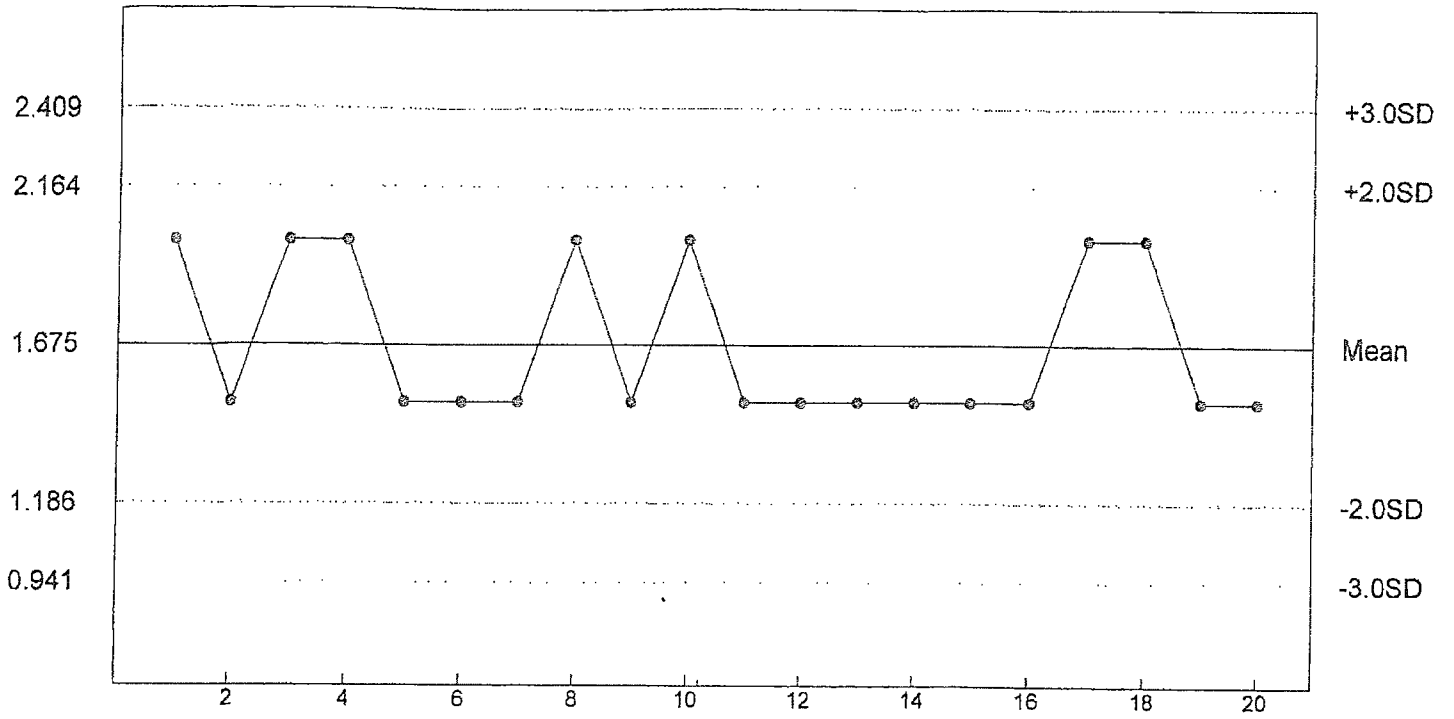
SPECIES: *Ceriodaphnia dubia*  
 CHEMICAL: Sodium Chloride  
 DURATION: 7-Days  
 TEST NUMBER: 4  
 TEST DATE: 04/06/16 - 04/13/16  
 1630 Hrs - 1630 Hrs  
 STATISTICAL METHOD: Dunnetts/Steels

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

LOEC FOR SURVIVAL	NOEC FOR SURVIVAL	LOEC FOR REPRODUCTION	NOEC FOR REPRODUCTION
1.5 g/L	1.0 g/L	1.0 g/L	0.5 g/L

Reference Tox Sodium Chloride g/L

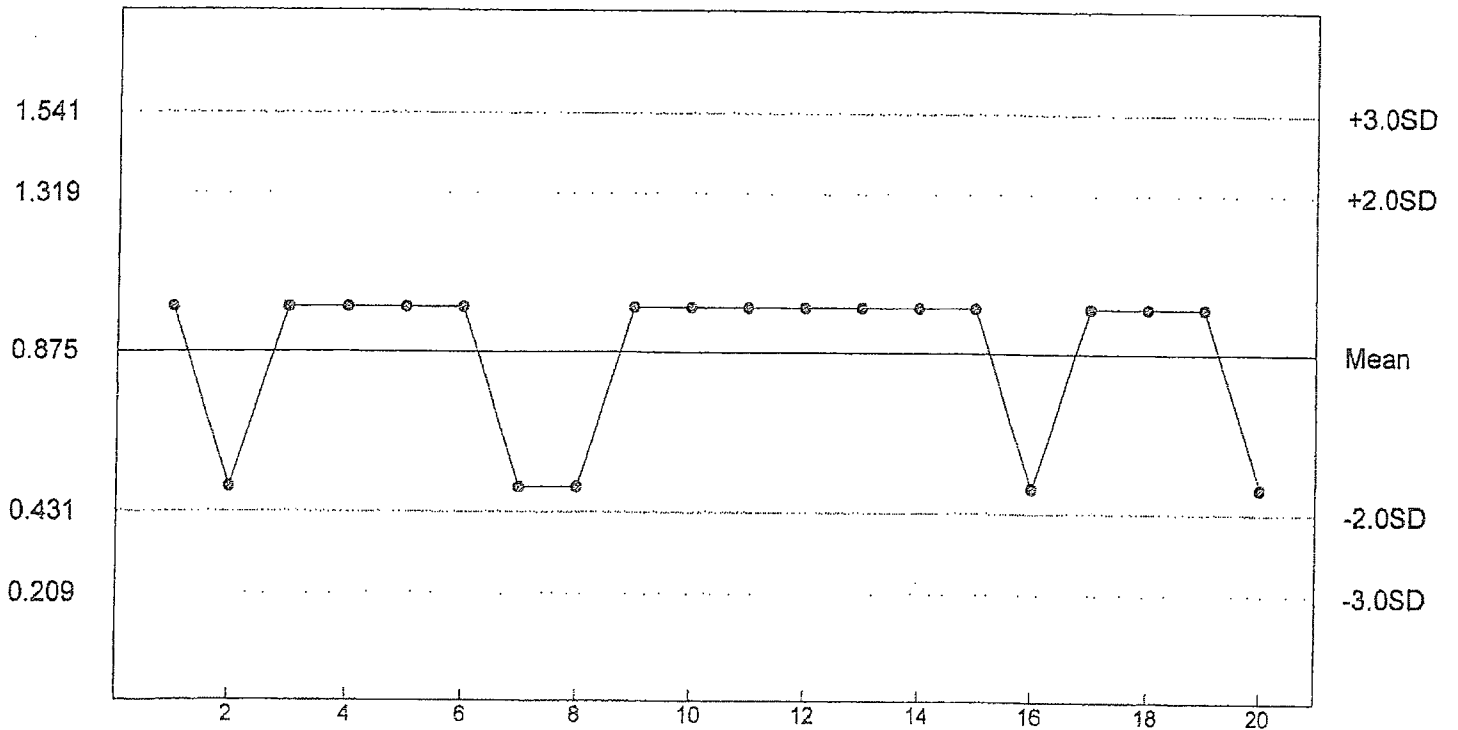
C. dubia Survival - NOEC



n= 20 Mean= 1.675 SD= 0.245 CV= 14.61% 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

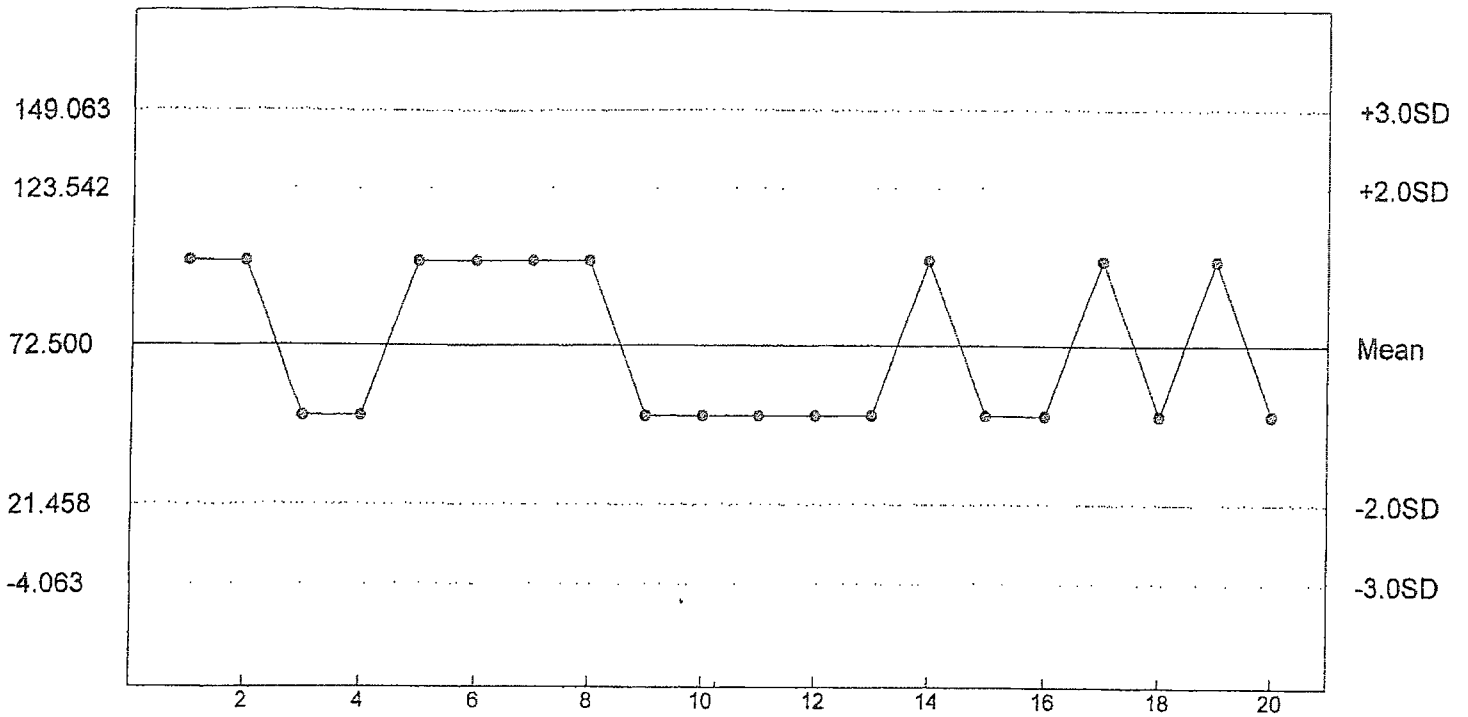
SPECIES: *Pimephales promelas*  
 CHEMICAL: Copper Nitrate  
 DURATION: 7-Days  
 TEST NUMBER: 4  
 TEST DATE: 04/06/16 - 04/13/16  
 1500 Hrs - 1500 Hrs  
 STATISTICAL METHOD: Dunnetts/Steels

CONCENTRATION (ug/L)	NUMBER EXPOSED	NUMBER DEAD
25	40	0
50	40	1
100	40	12
200	40	35
400	40	40
800	40	40

LOEC FOR SURVIVAL	NOEC FOR SURVIVAL	LOEC FOR GROWTH	NOEC FOR GROWTH
100 ug/L	50 ug/L	100 ug/L	50 ug/L

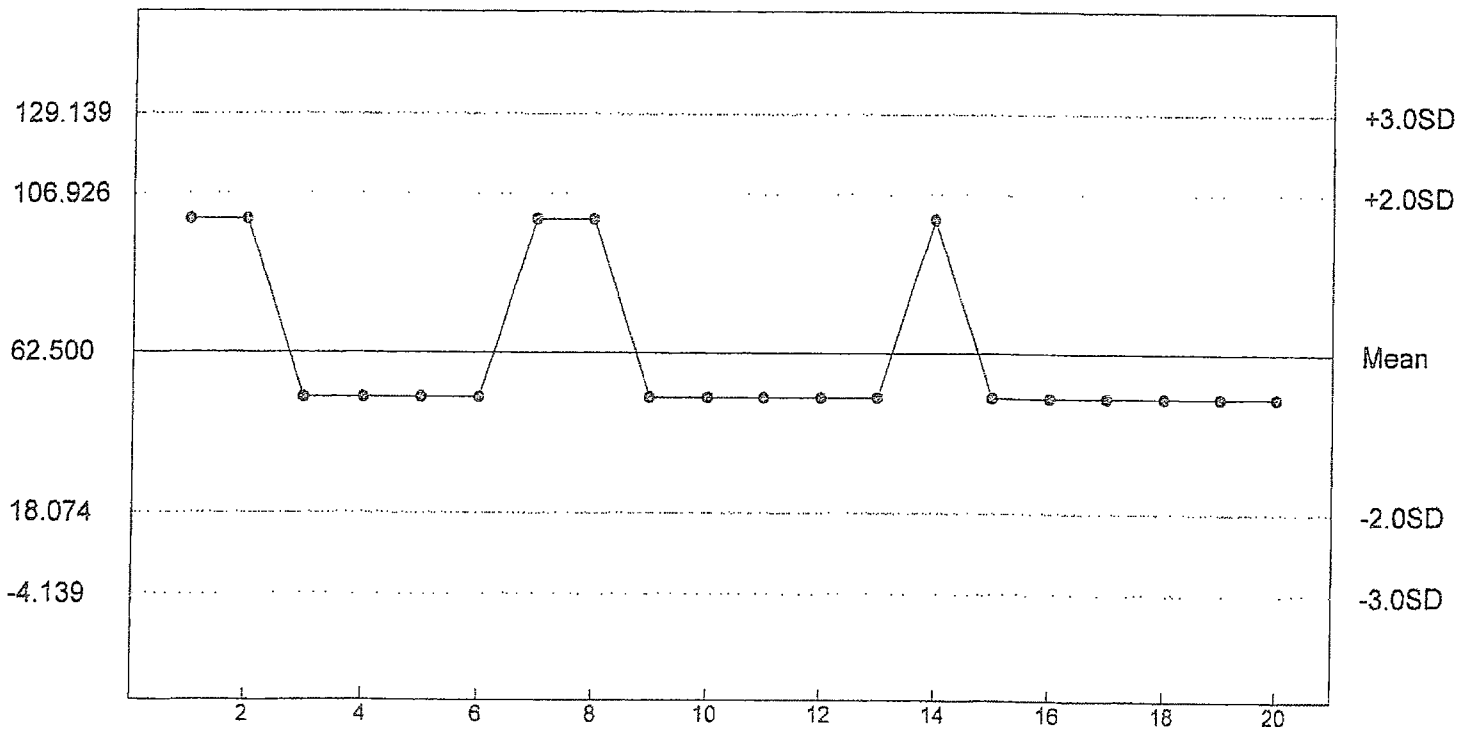


Reference Tox Copper Nitrate ug/L  
*P. promelas* Chronic Survival - NOEC



n= 20 Mean= 72.500 SD= 25.521 CV= 35.20% Min= 50.000 Max= 100.000

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



n= 20 Mean= 62.500 SD= 22.213 CV= 35.54% Min= 50.000 Max= 100.000

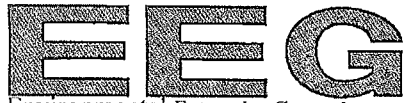
APPENDIX C  
CHAIN OF CUSTODY SHEETS



444-051029

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

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													
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>Henry Umbro</i>							Cory Yarbrough																				
Sample I.D.	Date	Time	Comp.	Grab	Cont. Type		# of Containers	Method Preserved					Sample Matrix					7-Day Chronic Bio-Monitoring									
					Plast.	Glass		H2SO4	HNO3	NAOH	HCL	Ice	None	Water	Soil	Air	Sludge				Other						
Outfall 001	4-17-16 4-18-16	0730 0744	X			X										X											
Relinquished by:							Date:		Time:		Received By:							Date:		Time:							
<i>Henry Umbro</i>							4-18-16		0852		<i>Stacy</i>							4/18/16		1000							
Received by:							Date:		Time:		Relinquished By:							Date:		Time:							
<i>Megan Hatchew</i>							4-18-16		0852		<i>Stacy</i>							4/18/16		1600							
Relinquished by:							Date:		Time:		Received by Laboratory:							Date:		Time:							
<i>Megan Hatchew</i>							4-18-16		1000		<i>Matt Horner</i>							4-19-16		1100							
Comments:																											
2.8°C																											
UPS																											



Environmental Enterprise Group, Inc.  
PROVIDING CUSTOMIZED SERVICES NATIONWIDE

L444-051029

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

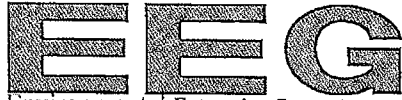
Company Name:		Phone #:		Requested Analysis												Laboratory Control Number	Remarks (Please note special detection limits below.)													
Clarksville Light and Water		(479) 754-6241		7-Day Chronic Bio-Monitoring																										
Address:		Fax #:																												
P.O. Box 1807 Clarksville, AR 72830		(479) 754-8181																												
Project Name or Number:		Purchase Order #:		<table border="1"> <tr> <th colspan="2">Method Preserved</th> <th colspan="2">Sample Matrix</th> </tr> <tr> <td>H2SO4</td> <td>HNO3</td> <td>NAOH</td> <td>HCL</td> <td>Ice</td> <td>None</td> <td>Water</td> <td>Soil</td> <td>Air</td> <td>Sludge</td> <td>Other</td> </tr> </table>												Method Preserved		Sample Matrix		H2SO4	HNO3	NAOH	HCL	Ice	None	Water	Soil	Air	Sludge	Other
Method Preserved		Sample Matrix																												
H2SO4	HNO3	NAOH	HCL													Ice	None	Water	Soil	Air	Sludge	Other								
Bio-Monitoring																														
Sampling Personnel Signature(s):		Printed:																												
<i>Henry Umbrough</i>		Gary Varbrough																												
Sample I.D.	Date	Time	Comp.	Grab	Cont. Type		# of Containers	Method Preserved		Sample Matrix		7-Day Chronic Bio-Monitoring	Laboratory Control Number	Remarks																
					Plast.	Glass		H2SO4	HNO3	NAOH	HCL				Ice	None	Water	Soil	Air	Sludge	Other									
Receiving Water	4-18-16	0740		X	X		1					X	X							046132										
Relinquished by:		Date:		Time:		Received By:		Date:		Time:		Date:		Time:																
<i>Henry Umbrough</i>		4-18-16		0852		<i>Stacyne</i>		4/18/16		1000		4/18/16		1000																
Received by:		Date:		Time:		Relinquished By:		Date:		Time:		Date:		Time:																
<i>Megan Hatcher</i>		4-18-16		0852		<i>Stacyne</i>		4/18/16		1000		4-19-16		1100																
Relinquished by:		Date:		Time:		Received by Laboratory:		Date:		Time:		Date:		Time:																
<i>Megan Hatcher</i>		4-18-16		1000		<i>[Signature]</i>		4-19-16		1100		4-19-16		1100																
Comments:																														
<i>7.8°C</i>																														



L444-051029

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

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																					
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 :																
Pam Smith					Pam Smith																					
Sample I.D.	Date	Time	Comp.	Grab	Cont. Type		# of Containers	Method Preserved						Sample Matrix					7-Day Chronic Bio-Monitoring							
					Plast.	Glass		H2SO4	HNO3	NAOH	HCL	Ice	None	Water	Soil	Air	Sludge	Other								
Outfall 001	4-19-16 4-20-16	0730 0630	X		X		1					X	X						X							
Relinquished by:								Date:	Time:	Received By:						Date:	Time:									
Pam Smith								4-20-16	6:53 AM	Shen						4/20/16	0800									
Received by:								Date:	Time:	Relinquished By:						Date:	Time:									
Megan Hatcher								4-20-16	0653	Shen						4/20/16	1100									
Relinquished by:								Date:	Time:	Received by Laboratory:						Date:	Time:									
Megan Hatcher								4-20-16	0800	[Signature]						4/21/16	1145									
Comments:																										
UPS Temp - 6.7'																										



Environmental Enterprise Group, Inc.  
PROVIDING CUSTOMIZED SERVICES NATIONWIDE

L444-051029

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

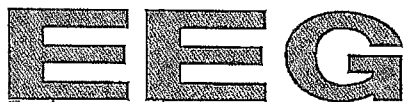
Company Name: Clarksville Light and Water							Phone #: (479) 754-6241							Requested Analysis										Laboratory Control Number	Remarks (Please note special detection limits below.)
Address: P.O. Box 1807 Clarksville, AR 72830							Fax #: (479) 754-8181							7-Day Chronic Bio-Monitoring											
Project Name or Number: Bio-Monitoring							Purchase Order #:																		
Sampling Personnel Signature(s): <i>Henry Underbrugh</i>							Printed: Gary Underbrugh																		
Sample I.D.	Date	Time	Comp.	Grab	Cont. Type		# of Containers	Method Preserved						Sample Matrix											
					Plast.	Glass		H2SO4	HNO3	NaOH	HCL	Ice	None	Water	Soil	Air	Sludge	Other							
Receiving Water	4-20-16	0609		X	X		1						X	X									X	0416132	
Relinquished by: <i>Henry Underbrugh</i>							Date: 4-20-16		Time: 0653 AM		Received By: <i>Sher</i>				Date: 4/20/16		Time: 0800								
Received by: <i>Megan Hatcher</i>							Date: 4-20-16		Time: 0653		Relinquished By: <i>Sher</i>				Date: 4/20/16		Time: 1100								
Relinquished by: <i>Megan Hatcher</i>							Date: 4-20-16		Time: 0800		Received by Laboratory:				Date: 4/21/16		Time: 1145								
Comments: UPS Temp = 0.7"																									



L444-051029

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

Company Name: Clarksville Light and Water										Phone #: (479) 754-6241										Requested Analysis						Laboratory Control Number		Remarks (Please note special detection limits below.)	
Address: P.O. Box 1807 Clarksville, AR 72830										Fax #: (479) 754-8181																			
Project Name or Number: Bio-Monitoring										Purchase Order #:										7-Day Chronic Bio-Monitoring									
Sampling Personnel Signature(s): <i>Porsha Russell</i>					Printed: <i>Porsha Russell</i>																								
Sample I.D.	Date	Time	Comp.	Grab	Cont. Type		# of Containers	Method Preserved						Sample Matrix					7-Day Chronic Bio-Monitoring										
					Plast.	Glass		H2SO4	HNO3	NaOH	HCL	Ice	None	Water	Soil	Air	Sludge	Other											
Outfall 001	4-21-16 4-22-16	0720 0720	X		X		1						X	X							X					0416131			
Relinquished by: <i>Porsha Russell</i>								Date: 4-22-16		Time: 0830		Received By: <i>Stacygnen</i>					Date: 4/22/16		Time: 0920										
Received by: <i>Megan Hatcher</i>								Date: 4-22-16		Time: 0830		Relinquished By: <i>Stacygnen</i>					Date: 4/22/16		Time: 1100										
Relinquished by: <i>Megan Hatcher</i>								Date: 4-22-16		Time: 0920		Received by Laboratory: <i>Matt Bowner</i>					Date: 4-23-16		Time: 1030										
Comments: 2.3°C																		UPS											



Environmental Enterprise Group, Inc.  
PROVIDING CUSTOMIZED SERVICES NATIONWIDE

L444-051029

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

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															
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>Gary Umbrough</i>										Gary Umbrough											
Sample I.D.	Date	Time	Comp.	Grab	Cont. Type		# of Containers	Method Preserved					Sample Matrix								
					Plast.	Glass		H2SO4	HNO3	NAOH	HCL	Ice	None	Water	Soil	Air	Sludge			Other	
Receiving Water	4-22-16	0715		X	X		1				X		X					X		041113Z	
Relinquished by:			Date:	Time:	Received By:			Date:	Time:	Relinquished By:			Date:	Time:	Received by Laboratory:			Date:	Time:		
<i>Gary Umbrough</i>			4-22-16	0830	<i>Stacy Men</i>			4/22/16	0920	<i>Stacy Men</i>			4/22/16	1100	<i>[Signature]</i>			4-23-16	1030		
Received by:			Date:	Time:	Relinquished By:			Date:	Time:	Received by Laboratory:			Date:	Time:	Comments:						
<i>Megan Hatcher</i>			4-22-16	0830	<i>Stacy Men</i>			4-22-16	0920	<i>[Signature]</i>			2.3 °C								
Relinquished by:			Date:	Time:	Received by Laboratory:			Date:	Time:	Comments:			UPS								
<i>Megan Hatcher</i>			4-22-16	0920	<i>[Signature]</i>																



ENVIRONMENTAL ENTERPRISE GROUP  
 CITY OF CLARKSVILLE WWTP – OUTFALL 001  
 NPDES PERMIT NO. AR0022187  
 AFIN NO. 36-00038  
 BIOMONITORING REPORTING  
 TEST DATE: 04/19/16

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.	11.25%

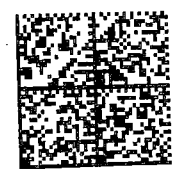
I. *Pimephales promelas*

	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.	7.50%

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400 WEST MAIN • P.O. BOX 1807  
CLARKSVILLE, AR 72830  
PHONE (479) 754-3148

**To**

Arkansas Department of Environmental Quality  
5301 Northshore Drive  
North Little Rock, AR 72118