

ENVIRONMENTAL ENTERPRISE GROUP
 CITY OF CLARKSVILLE WWTP - OUTFALL 001
 NPDES PERMIT NO. AR0022187
 AFIN NO. 36-00038
 BIOMONITORING REPORTING
 TEST DATE: 08/25/15

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.	11.02%

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. TP6C.	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.	7.90%

ENVIRONMENTAL ENTERPRISE GROUP
CITY OF CLARKSVILLE WWTP
OUTFALL 001

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

Ceriodaphnia dubia
Pimephales promelas

August 25, 2015

Reviewed by: _____

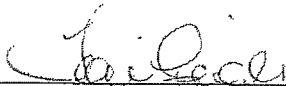

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TABLE OF CONTENTS

TOXICITY TEST REPORT 1

SUMMARY 3

CERIODAPHNIA DUBIA SURVIVAL AND REPRODUCTION SUMMARY 4

CERIODAPHNIA DUBIA STATISTICAL ANALYSES 8

PIMEPHALES PROMELAS SURVIVAL AND GROWTH SUMMARY 9

PIMEPHALES PROMELAS STATISTICAL ANALYSES 12

APPENDIX A: RAW DATA 13

APPENDIX B: REFERENCE TOXICANTS 14

APPENDIX C: CHAIN OF CUSTODY SHEETS 15

TOXICITY TEST REPORT - CHRONIC

Client Environmental Enterprise Group Sample..... Outfall 001
Facility City of Clarksville WWTP Laboratory I.D. 24436
Permit No. NPDES AR0022187 Begin Date August 25, 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 August 25, August 27, and August 29, 2015. Effluent samples were collected and composited from Outfall 001 using an automatic sampler by facility personnel. Two toxicity tests were requested: a seven-day *Ceriodaphnia dubia* survival and reproduction test (EPA Method 1002.0), and a seven-day *Pimephales promelas* larval survival and growth test (EPA Method 1000.0). Test organisms, procedures and quality assurance requirements were in accordance with the EPA manual, "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition" (EPA-821-R-02-013).

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

TEST SETUP

Ceriodaphnia dubia



The seven-day *Ceriodaphnia dubia* survival and reproduction test was initiated at 1420 hours, August 25, 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 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 1610 hours, September 1, 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: 10.6%**
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	08/24/15 08/26/15 08/28/15
LAB ID #	24436	DATE RECEIVED	08/25/15 08/27/15 08/29/15
TEST TYPE	7 Day Chronic	BEGIN DATE/TIME	08/25/15 1420
TEST ORGANISM	<i>Ceriodaphnia dubia</i>	END DATE/TIME	09/01/15 1420
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	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	
08/26/15	A	A	A	A	A	A	A	A	A	A	
08/27/15	A	A	A	A	A	A	A	A	A	A	
08/28/15	A	A	A	A	A	A	A	A	A	A	
08/29/15	A	A	A	A	A	A	A	A	A	A	
08/30/15	4	2	5	2	5	2	5	5	4	4	
08/31/15	6	9	11	8	6	9	8	11	10	11	
09/01/15	14	12	14	13	15	11	13	15	14	15	
09/01/15	24	23	30	23	26	24	25	30	26	30	
x# Young		26.1				C.V.		11.05%			
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	
08/26/15	A	A	A	A	A	A	A	A	A	A	
08/27/15	A	A	A	A	A	A	A	A	A	A	
08/28/15	A	A	A	A	A	A	A	A	A	A	
08/29/15	A	A	A	A	A	A	A	A	A	A	
08/30/15	4	2	5	4	3	5	2	3	2	5	
08/31/15	9	7	10	9	8	8	11	8	8	6	
09/01/15	13	9	15	13	11	13	13	11	10	11	
09/01/15	27	22	28	26	25	25	27	26	25	26	
x# Young		25.7				C.V.		6.37%			
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	
08/26/15	A	A	A	A	A	A	A	A	A	A	
08/27/15	A	A	A	A	A	A	A	A	A	A	
08/28/15	A	A	A	A	A	A	A	A	A	A	
08/29/15	A	A	A	A	A	A	A	A	A	A	
08/30/15	5	5	2	5	5	4	2	2	5	5	
08/31/15	8	6	6	7	11	8	11	8	8	10	
09/01/15	13	11	8	12	16	12	13	10	13	15	
09/01/15	25	23	21	27	30	24	27	23	27	29	
x# Young		25.6				C.V.		11.23%			
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	
08/26/15	A	A	A	A	A	A	A	A	A	A	
08/27/15	A	A	A	A	A	A	A	A	A	A	
08/28/15	A	A	A	A	A	A	A	A	A	A	
08/29/15	A	A	A	A	A	A	A	A	A	A	
08/30/15	4	4	3	5	4	3	4	5	2	4	
08/31/15	8	7	10	11	11	8	6	8	6	10	
09/01/15	14	15	14	12	14	14	12	15	13	13	
09/01/15	26	26	27	28	29	25	22	28	21	27	
x# Young		25.9				C.V.		10.04%			
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

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

EEG, City of Clarksville WWTP

Lab ID# 24436

Test Date: August 25, 2015

56% Effluent

Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
08/26/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
08/27/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
08/28/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
08/29/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
08/30/15	4	4	2	3	5	2	3	5	5	3
	4	4	2	3	5	2	3	5	5	3
08/31/15	9	8	8	9	11	10	6	7	7	10
	13	12	10	12	16	12	9	12	12	13
	15	13	14	11	15	12	12	12	14	14
09/01/15	28	25	24	23	31	24	21	24	25	27
x# Young 25.3 C.V. 11.19% 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
08/26/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
08/27/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
08/28/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
08/29/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
08/30/15	4	5	5	4	2	5	4	2	5	3
	4	5	5	4	2	5	4	2	5	3
08/31/15	8	10	8	7	6	6	6	11	7	6
	12	15	13	11	8	11	10	13	12	9
	14	15	12	13	14	14	12	12	14	15
09/01/15	26	30	25	24	22	25	22	25	26	24
x# Young 24.9 C.V. 9.17% 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
08/26/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
08/27/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
08/28/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
08/29/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
08/30/15	4	2	4	4	2	4	5	3	3	3
	4	2	4	4	2	4	5	3	3	3
08/31/15	8	9	8	7	6	11	6	11	8	10
	12	11	12	11	8	15	11	14	11	13
	13	12	12	15	12	14	15	15	12	13
09/01/15	25	23	24	26	20	29	26	29	23	26
x# Young 25.1 C.V. 11.02% x% Survival 100% C.V. 0.00%										

where: A = Alive ex 1:

A

 alive today ex 2:

5

 alive, 5 young today

 S = Alive, 5 young

4

 total young to date

12

 total young to date

 D = Dead

 D5 = 5 Young, Female died

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

EEG, City of Clarksville WWTP

Lab ID# 24436

Test Date: August 25, 2015

WET CHEMISTRY MEASUREMENTS

Date	Time	Temp	Samp. No.	pH of Solution							Analyst
				PCON	TCON	32%	42%	56%	75%	100%	
08/25/15	Start	25.0	1	7.73	7.54	7.50	7.48	7.47	7.42	7.33	TB
08/26/15	24 Hr.	24.8	1	7.93	7.84	7.66	7.54	7.51	7.36	7.44	TB
08/26/15	Renew	25.0	1	7.82	7.49	7.46	7.41	7.53	7.56	7.41	TB
08/27/15	48 Hr.	24.9	1	7.43	7.23	7.27	7.41	7.32	7.31	7.28	GP
08/27/15	Renew	25.0	2	7.73	7.26	7.21	7.22	7.20	7.19	7.13	GP
08/28/15	72 Hr.	24.9	2	7.37	7.09	7.14	7.16	7.14	7.12	7.10	GP
08/28/15	Renew	25.0	2	7.53	7.08	7.09	7.08	7.08	7.07	7.04	GP
08/29/15	96 Hr.	24.8	2	7.15	7.47	7.83	7.88	7.88	7.33	7.47	CA
08/29/15	Renew	25.0	3	7.78	7.33	7.68	7.85	7.01	7.40	7.19	CA
08/30/15	120 Hr.	24.5	3	7.69	7.68	7.71	7.20	7.00	7.50	7.55	CA
08/30/15	Renew	25.0	3	7.62	7.84	7.61	7.86	7.28	7.60	7.13	CA
08/31/15	144 Hr.	25.0	3	7.96	7.89	7.67	7.58	7.59	7.41	7.48	RK
08/31/15	Renew	24.8	3	7.78	7.20	7.15	7.14	7.12	7.16	7.12	RK
09/01/15	168 Hr.	25.0	3	7.96	7.73	7.68	7.64	7.60	7.56	7.53	GP

Date	Time	Temp	Samp. No.	DO (mg/L) of Solution							Analyst
				PCON	TCON	32%	42%	56%	75%	100%	
08/25/15	Start	25.0	1	8.61	8.36	8.32	8.31	8.24	8.23	8.21	TB
08/26/15	24 Hr.	24.8	1	8.44	8.28	8.23	8.20	8.18	8.14	8.09	TB
08/26/15	Renew	25.0	1	8.55	8.42	8.30	8.36	8.29	8.27	7.96	TB
08/27/15	48 Hr.	24.9	1	6.90	7.04	7.19	7.35	7.44	7.35	7.79	GP
08/27/15	Renew	25.0	2	8.60	7.52	8.32	8.52	8.08	8.54	7.42	GP
08/28/15	72 Hr.	24.9	2	8.51	7.70	7.93	7.97	8.63	8.29	8.25	GP
08/28/15	Renew	25.0	2	8.02	7.43	7.77	7.55	7.66	7.99	8.30	GP
08/29/15	96 Hr.	24.8	2	8.75	8.19	7.68	8.53	8.61	8.89	8.93	CA
08/29/15	Renew	25.0	3	7.63	8.29	8.66	8.55	8.43	8.38	8.14	CA
08/30/15	120 Hr.	24.5	3	8.81	7.57	8.61	8.31	8.22	8.94	8.45	CA
08/30/15	Renew	25.0	3	8.07	8.83	8.14	8.26	8.44	8.05	8.51	CA
08/31/15	144 Hr.	25.0	3	7.6	8.80	7.81	7.72	7.68	8.03	7.62	RK
08/31/15	Renew	24.8	3	8.51	8.21	7.96	7.87	7.99	8.14	8.19	RK
09/01/15	168 Hr.	25.0	3	8.75	8.18	6.81	6.59	7.96	8.45	8.37	GP

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

EEG, City of Clarksville WWTP

Lab ID# 24436

Test Date: August 25, 2015

INITIAL CHEMISTRY MEASUREMENTS @ 100% EFFLUENT

Date	Samp. No.	pH	DO	Hardness mg/L CaCO ₃ ¹	Alkalinity mg/L CaCO ₃ ¹	Conduct. umhos/cm ¹	Resid. Cl ₂ mg/L ¹	Dechlor(mL) Na ₂ S ₂ O ₃ mg/L ¹	Analyst
08/25/15	1	7.33	8.21	68	52	427	<0.01	N/A	TG
08/27/15	2	7.13	7.42	72	62	468	<0.01	N/A	TG
08/29/15	3	7.19	8.14	76	54	407	<0.01	N/A	TG

¹ Measurements taken in 100% solution.

INITIAL CHEMISTRY MEASUREMENTS @ RECEIVING WATER

Date	Samp. No.	Ph	DO	Hardness mg/L CaCO ₃ ¹	Alkalinity mg/L CaCO ₃ ¹	Conduct. umhos/cm ¹	Resid. Cl ₂ mg/L ¹	Dechlor(mL) Na ₂ S ₂ O ₃ mg/L ¹	Analyst
08/25/15	RS1	7.54	8.36	124	90	769	<0.01	N/A	TG
08/27/15	RS2	7.26	7.52	128	100	675	<0.01	N/A	TG
08/29/15	RS3	7.33	8.29	124	98	799	<0.01	N/A	TG

Huther and Associates, Inc.
 Begin Date: August 25, 2015
 Lab I.D.# 24436

CERIODAPHNIA DUBIA STATISTICAL ANALYSES
 Reproduction

Summary Statistics on Transformed Data Table 1 of 2

Grp	Identification	N	Min	Max	Mean
1	Control	10	22.000	28.000	25.700
2	32% Effluent	10	21.000	30.000	25.600
3	42% Effluent	10	21.000	29.000	25.900
4	56% Effluent	10	21.000	31.000	25.300
5	75% Effluent	10	22.000	30.000	24.900
6	100% Effluent	10	20.000	29.000	25.100

Summary Statistics on Transformed Data Table 2 of 2

Grp	Identification	Variance	Sd	Sem	C.V. %
1	Control	2.678	1.636	0.517	6.37
2	32% Effluent	8.267	2.875	0.909	11.23
3	42% Effluent	6.767	2.601	0.823	10.04
4	56% Effluent	8.011	2.830	0.895	11.19
5	75% Effluent	5.211	2.283	0.722	9.17
6	100% Effluent	7.656	2.767	0.875	11.02

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	5	9	32	11	3

Calculated Chi-Square goodness of fit test statistic = 7.0467
 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 = 3.37

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	7.283	1.457	0.226
Within (Error)	54	347.300	6.431	
Total	59	354.583		

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	T Stat	Sig
		Mean	Calculated In Original Units		
1	Control	25.700	25.700		
2	32% Effluent	25.600	25.600	0.088	
3	42% Effluent	25.900	25.900	-0.176	
4	56% Effluent	25.300	25.300	0.353	
5	75% Effluent	24.900	24.900	0.705	
6	100% Effluent	25.100	25.100	0.529	

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	32% Effluent	10	2.620	10.2	0.100
3	42% Effluent	10	2.620	10.2	-0.200
4	56% Effluent	10	2.620	10.2	0.400
5	75% Effluent	10	2.620	10.2	0.800
6	100% Effluent	10	2.620	10.2	0.600

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	08/24/15 08/26/15 08/28/15
LAB ID #	24436	DATE RECEIVED	08/25/15 08/27/15 08/29/15
TEST TYPE	7 Day Chronic	BEGIN DATE/TIME	08/25/15 1610
TEST ORGANISM	<i>Pimephales promelas</i>	END DATE/TIME	09/01/15 1610
ORGANISM AGE	< 24 Hours	TEST TEMPERATURE (°C)	25 ± 1
ORGANISM SOURCE	In House	PHOTO PERIOD	16-hr. Light 8-hr. Dark
RECEIVING WATER	Lake Dardanelle	LIGHT INTENSITY	50-100 ft. cndl.
DILUTION WATER	Lake Dardanelle	TECHNICIAN	M. Horner

SURVIVAL SUMMARY

Conc.	08/26/15					08/27/15					08/28/15					08/29/15					08/30/15				
	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.	08/31/15					09/01/15					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	0.4950	0.4270	0.5020	0.4810	0.5010	0.4812	6.53
Tcon	0.4260	0.4450	0.4710	0.4150	0.4860	0.4486	6.64
32%	0.5020	0.4500	0.4910	0.4260	0.4710	0.4680	6.57
42%	0.4860	0.4250	0.5040	0.4460	0.5010	0.4724	7.44
56%	0.4960	0.4510	0.4820	0.5040	0.4960	0.4858	4.32
75%	0.4150	0.5030	0.4760	0.4920	0.4880	0.4748	7.33
100%	0.5020	0.4200	0.4860	0.4950	0.4370	0.4680	7.90

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

EEG, City of Clarksville WWTP

Lab ID# 24436

Test Date: August 25, 2015

WET CHEMISTRY MEASUREMENTS

Date	Time	Temp	Samp. No.	pH of Solution						Analyst	
				PCON	TCON	32%	42%	56%	75%		100%
08/25/15	Start	25.0	1	7.73	7.54	7.50	7.48	7.47	7.42	7.33	TB
08/26/15	24 Hr.	25.5	1	7.81	7.69	7.64	7.57	7.51	7.49	7.45	TB
08/26/15	Renew	25.0	1	7.82	7.49	7.46	7.41	7.53	7.56	7.41	TB
08/27/15	48 Hr.	25.2	1	7.68	7.61	7.50	7.48	7.43	7.37	7.29	GP
08/27/15	Renew	25.0	2	7.73	7.26	7.21	7.22	7.20	7.19	7.13	GP
08/28/15	72 Hr.	25.4	2	7.71	7.67	7.59	7.55	7.50	7.44	7.38	GP
08/28/15	Renew	25.0	2	7.53	7.08	7.09	7.08	7.08	7.07	7.04	GP
08/29/15	96 Hr.	24.8	2	7.66	7.63	7.58	7.57	7.52	7.47	7.44	CA
08/29/15	Renew	25.0	3	7.78	7.33	7.68	7.85	7.01	7.40	7.19	CA
08/30/15	120 Hr.	25.1	3	7.87	7.76	7.9	7.89	7.76	7.64	7.08	CA
08/30/15	Renew	25.0	3	7.62	7.84	7.61	7.86	7.28	7.60	7.13	CA
08/31/15	144 Hr.	25.2	3	7.86	7.84	7.74	7.70	7.65	7.60	7.54	RK
08/31/15	Renew	24.8	3	7.78	7.20	7.15	7.14	7.12	7.16	7.12	RK
09/01/15	168 Hr.	25.3	3	7.76	7.76	7.64	7.55	7.55	7.49	7.41	GP

Date	Time	Temp	Samp. No.	DO (mg/L) of Solution						Analyst	
				PCON	TCON	32%	42%	56%	75%		100%
08/25/15	Start	25.0	1	8.61	8.36	8.32	8.31	8.24	8.23	8.21	TB
08/26/15	24 Hr.	25.5	1	8.44	8.28	8.26	8.41	8.30	8.18	8.06	TB
08/26/15	Renew	25.0	1	8.55	8.42	8.30	8.36	8.29	8.27	7.96	TB
08/27/15	48 Hr.	25.2	1	8.39	8.71	8.81	8.22	8.03	8.65	8.38	GP
08/27/15	Renew	25.0	2	8.60	7.52	8.32	8.52	8.08	8.54	7.42	GP
08/28/15	72 Hr.	25.4	2	7.89	7.53	8.61	8.35	8.69	8.74	7.66	GP
08/28/15	Renew	25.0	2	8.02	7.43	7.77	7.55	7.66	7.99	8.30	GP
08/29/15	96 Hr.	24.8	2	8.21	8.35	8.42	8.28	8.22	8.31	8.35	CA
08/29/15	Renew	25.0	3	7.63	8.29	8.66	8.55	8.43	8.38	8.14	CA
08/30/15	120 Hr.	25.1	3	8.13	8.82	8.94	8.62	8.17	8.53	8.32	CA
08/30/15	Renew	25.0	3	8.07	8.83	8.14	8.26	8.44	8.05	8.51	CA
08/31/15	144 Hr.	25.2	3	8.03	8.31	8.44	8.55	8.08	8.22	8.13	RK
08/31/15	Renew	24.8	3	8.51	8.21	7.96	7.87	7.99	8.14	8.19	RK
09/01/15	168 Hr.	25.3	3	7.41	8.44	8.24	7.85	7.97	7.87	8.46	GP

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

EEG, City of Clarksville WWTP

Lab ID# 24436

Test Date: August 25, 2015

INITIAL CHEMISTRY MEASUREMENTS @ 100% EFFLUENT

Date	Samp. No.	pH	DO	Hardness mg/L CaCO ₃ ¹	Alkalinity mg/L CaCO ₃ ¹	Conduct. umhos/cm ¹	Resid. Cl ₂ mg/L ¹	Dechlor(mL) Na ₂ S ₂ O ₃ mg/L ¹	Analyst
08/25/15	1	7.33	8.21	68	52	427	<0.01	N/A	TG
08/27/15	2	7.13	7.42	72	62	468	<0.01	N/A	TG
08/29/15	3	7.19	8.14	76	54	407	<0.01	N/A	TG

INITIAL CHEMISTRY MEASUREMENTS @ RECEIVING WATER

Date	Samp. No.	Ph	DO	Hardness mg/L CaCO ₃ ¹	Alkalinity mg/L CaCO ₃ ¹	Conduct. umhos/cm ¹	Resid. Cl ₂ mg/L ¹	Dechlor(mL) Na ₂ S ₂ O ₃ mg/L ¹	Analyst
08/25/15	RS1	7.54	8.36	124	90	769	<0.01	N/A	TG
08/27/15	RS2	7.26	7.52	128	100	675	<0.01	N/A	TG
08/29/15	RS3	7.33	8.29	124	98	799	<0.01	N/A	TG

¹ Measurements taken in 100% solution.

Huther and Associates, Inc.
 Begin Date: August 25, 2015
 Lab I.D.# 24436

PIMEPHALES PROMELAS STATISTICAL ANALYSES
 Growth

Summary Statistics on Transformed Data Table 1 of 2

Grp	Identification	N	Min	Max	Mean
1	Control	5	0.415	0.486	0.449
2	32% Effluent	5	0.426	0.502	0.468
3	42% Effluent	5	0.425	0.504	0.472
4	56% Effluent	5	0.451	0.504	0.486
5	75% Effluent	5	0.415	0.503	0.475
6	100% Effluent	5	0.420	0.502	0.468

Summary Statistics on Transformed Data Table 2 of 2

Grp	Identification	Variance	Sd	Sem	C.V.%
1	Control	0.001	0.030	0.013	6.64
2	32% Effluent	0.001	0.031	0.014	6.57
3	42% Effluent	0.001	0.035	0.016	7.44
4	56% Effluent	0.000	0.021	0.009	4.32
5	75% Effluent	0.001	0.035	0.016	7.33
6	100% Effluent	0.001	0.037	0.017	7.90

Shapiro - Wilk's Test For Normality

D = 0.024

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 = 1.33

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	0.004	0.001	0.732
Within (Error)	24	0.024	0.001	
Total	29	0.028		

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	T Stat	Sig
		Mean	Calculated In Original Units		
1	Control	0.449	0.449		
2	32% Effluent	0.468	0.468	-0.963	
3	42% Effluent	0.472	0.472	-1.181	
4	56% Effluent	0.486	0.486	-1.846	
5	75% Effluent	0.475	0.475	-1.300	
6	100% Effluent	0.468	0.468	-0.963	

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)	Difference	
				% of Control	from Control
1	Control	5			
2	32% Effluent	5	0.048	10.6	-0.019
3	42% Effluent	5	0.048	10.6	-0.024
4	56% Effluent	5	0.048	10.6	-0.037
5	75% Effluent	5	0.048	10.6	-0.026
6	100% Effluent	5	0.048	10.6	-0.019

**APPENDIX A
RAW DATA**

7-DAY CERIODAPHНИЯ DUBIA SURVIVAL & REPRODUCTION

DAILY RAW DATA TABLE

PAGE 1 OF 2

CLIENT EEG-Clarksville
 OUTFALL 001
 LAB ID # 24436

START DATE/TIME 8-25-15 ZG 1420
 END DATE/TIME 9-1-15 RK 1420

PCON

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
8/26	A	A	A	A	A	A	A	A	A	A	ZG	1420
8/27	A	A	A	A	A	A	A	A	A	A	ZG	1430
8/28	A	A	A	A	A	A	A	A	A	A	MH	1440
8/29	A	A	A	A	A	A	A	A	A	A	ZG	1510
8/30	4	2	5	2	5	2	5	5	4	4	RK	1400
8/31	6	9	11	8	6	9	8	11	10	11	MH	1415
9/1	14	12	14	13	15	13	12	14	12	15	RK	1420

\bar{x} # Young w/o Dead = 26.1 CV% = 11.05
 \bar{x} # Young w/Dead = CV% =
 \bar{x} % Survival = 100 CV% = 0.00

TCON

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
8/26	A	A	A	A	A	A	A	A	A	A	ZG	1420
8/27	A	A	A	A	A	A	A	A	A	A	ZG	1430
8/28	A	A	A	A	A	A	A	A	A	A	MH	1440
8/29	A	A	A	A	A	A	A	A	A	A	ZG	1510
8/30	4 2	5 2	5 2	5 4	5 3	5 2	5 2	5 2	5 3	5 3	RK	1400
8/31	9	7	10	9	8	8	11	8	8	6	MH	1415
9/1	14	13	13	13	14	12	14	15	15	15	RK	1420

\bar{x} # Young w/o Dead = 25.7 CV% = 6.37
 \bar{x} # Young w/Dead = CV% =
 \bar{x} % Survival = 100 CV% = 0.00

32

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
8/26	A	A	A	A	A	A	A	A	A	A	ZG	1420
8/27	A	A	A	A	A	A	A	A	A	A	ZG	1430
8/28	A	A	A	A	A	A	A	A	A	A	MH	1440
8/29	A	A	A	A	A	A	A	A	A	A	ZG	1510
8/30	5	5	2	5	5	4	2	2	5	5	RK	1400
8/31	8	6	6	7	11	8	11	8	8	10	MH	1415
9/1	12	12	13	15	14	12	14	13	14	14	RK	1420

\bar{x} # Young w/o Dead = 25.6 CV% = 11.23
 \bar{x} # Young w/Dead = CV% =
 \bar{x} % Survival = 100 CV% = 0.00

42

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
8/26	A	A	A	A	A	A	A	A	A	A	ZG	1420
8/27	A	A	A	A	A	A	A	A	A	A	ZG	1430
8/28	A	A	A	A	A	A	A	A	A	A	MH	1440
8/29	A	A	A	A	A	A	A	A	A	A	ZG	1510
8/30	4	4	3	5	4	3	4	5	2	4	RK	1400
8/31	8	7	10	11	11	8	6	8	6	10	MH	1415
9/1	14	15	14	12	14	14	12	15	13	13	RK	1420

\bar{x} # Young w/o Dead = 25.9 CV% = 10.04
 \bar{x} # Young w/Dead = CV% =
 \bar{x} % Survival = 100 CV% = 0.00

① RK 8/30 ② TG 9/2

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

CLIENT/FACILITY EEG-Clarksville
 OUTFALL # 001 PROJECT # 24436
 ORGANISM ID# PP0-15-236

DATE/TIME STARTED 8-25-15 MH 1610
 DATE/TIME ENDED 9-1-15 26 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
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
TCOW	8	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
Initials Date/Time	8-26-15 MH 1600					8-27-15 26 0950					8-28-15 MH 0825					8-29-15 26 0855					8-30-15 TB 0845				

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
TCOW	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	8-31-15 MH 0945					9-1-15 26 1610						

Client / Facility EEG Clarksville
 Lab ID Number 24436
 Outfall Number 001
 Test Date 8/25/15

INITIAL CHEMISTRY MEASUREMENTS @ 100% EFFLUENT

Date	Samp. No.	pH	DO	Hardness mg/L CaCO ₃ ¹	Alkalinity mg/L CaCO ₃ ¹	Conduct. umhos/cm ¹	Resid. Cl ₂ mg/L ¹	Dechlor(mL) Na ₂ S ₂ O ₃ mg/L ¹	Analyst
8/25	1	7.33	8.21	68	52	427	20.01	Na	TG
8/27	2	7.13	7.42	72	62	468	5	5	5
8/29	3	7.19	8.14	76	54	407	5	5	5

INITIAL CHEMISTRY MEASUREMENTS @ RECEIVING WATER

Date	Samp. No.	pH	DO	Hardness mg/L CaCO ₃ ¹	Alkalinity mg/L CaCO ₃ ¹	Conduct. umhos/cm ¹	Resid. Cl ₂ mg/L ¹	Dechlor(mL) Na ₂ S ₂ O ₃ mg/L ¹	Analyst
8/25	RS1	7.54	8.36	124	90	769	20.01	Na	TG
8/27	RS2	7.26	7.52	128	100	1675	5	5	5
8/29	RS3	7.33	8.29	124	98	799	5	5	5

Notes:

OTG 8/31

**APPENDIX B
REFERENCE TOXICANTS**

CHRONIC REFERENCE TOXICANT TEST RESULTS

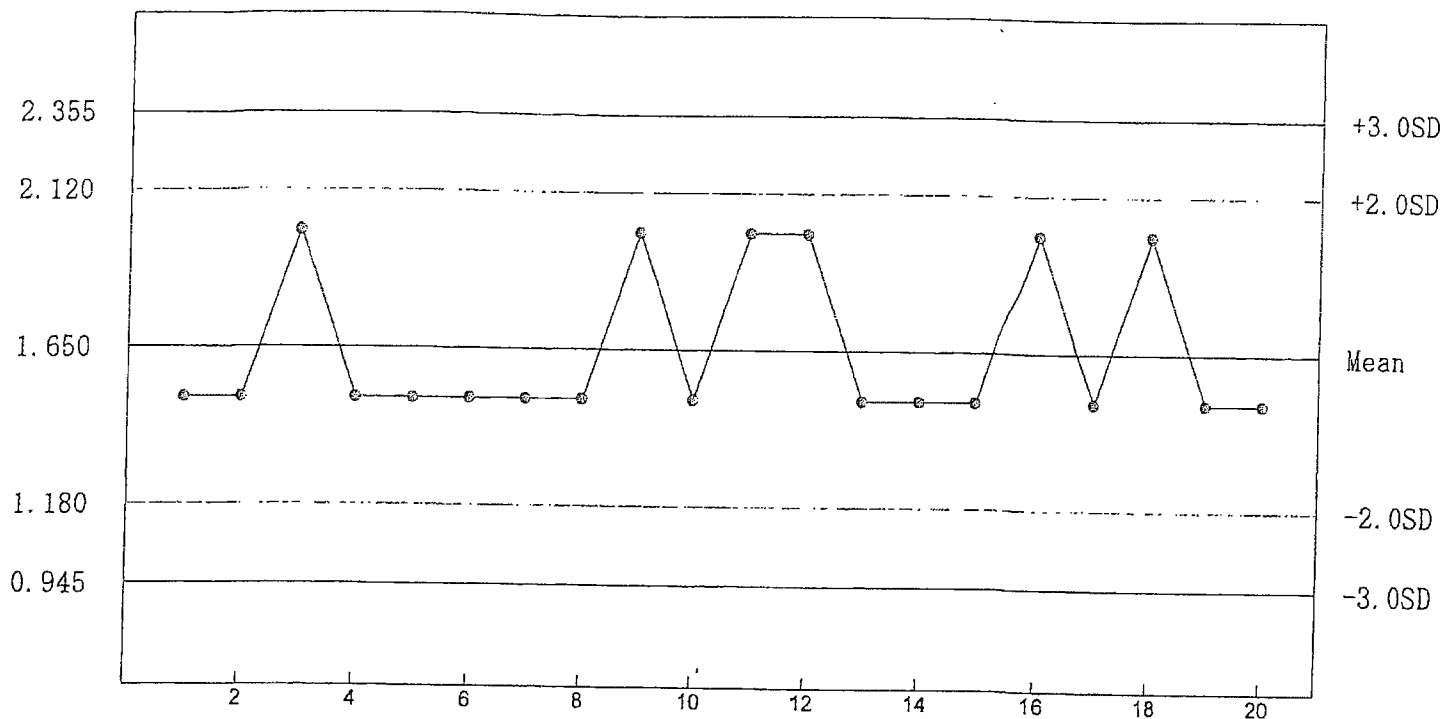
SPECIES: *Ceriodaphnia dubia*
 CHEMICAL: Sodium Chloride
 DURATION: 7-Days
 TEST NUMBER: 8
 TEST DATE: 08/05/15 - 08/12/15
 1500 Hrs - 1500 Hrs
 STATISTICAL METHOD: Dunnetts/Steels

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
2.0 g/L	1.5 g/L	1.5 g/L	1.0 g/L

Reference Tox Sodium Chloride g/L

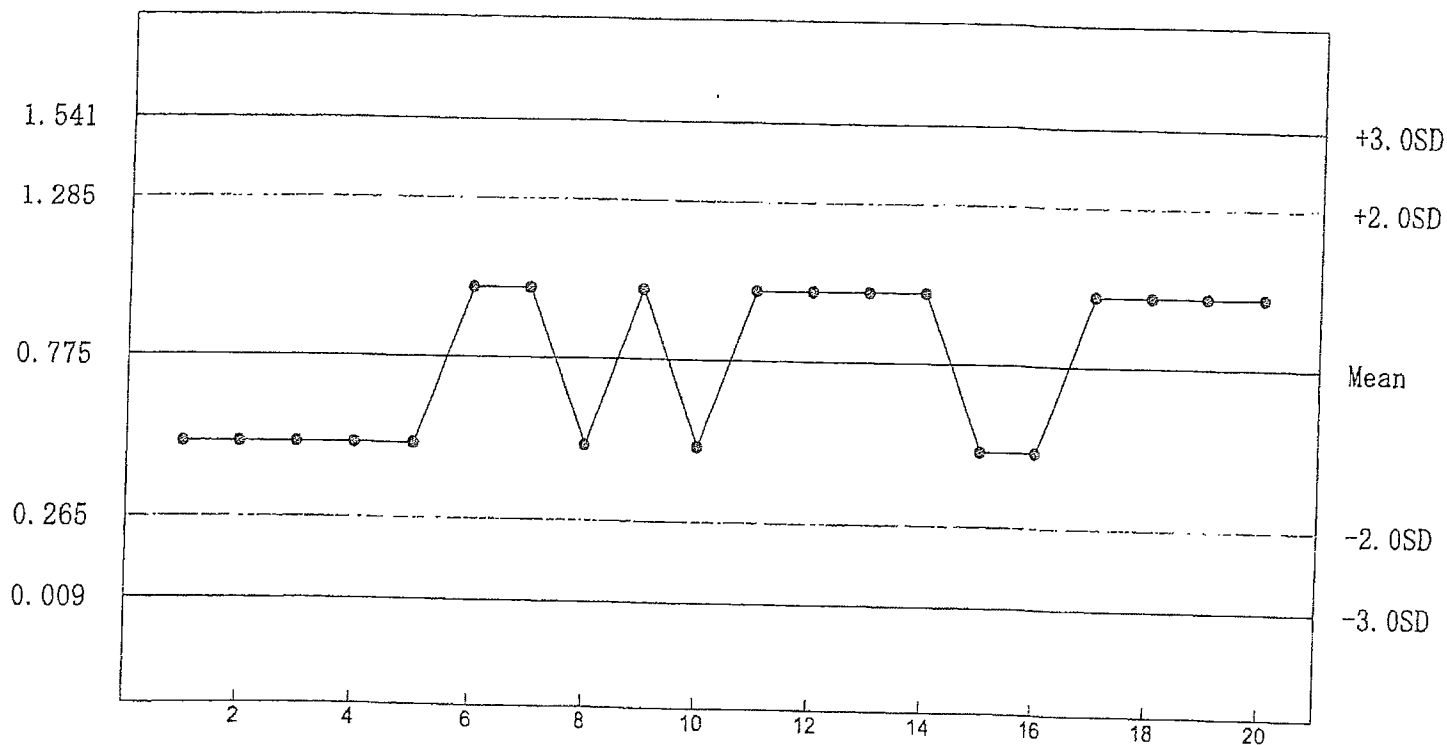
C. dubia Survival - NOEC



n= 20 Mean= 1.650 SD= 0.235 CV= 14.25% Min= 1.500 Max= 2.000

Reference Tox Sodium Chloride g/L

C. dubia Reproduction - NOEC



n= 20 Mean= 0.775 SD= 0.255 CV= 32.93% Min= 0.500 Max= 1.000

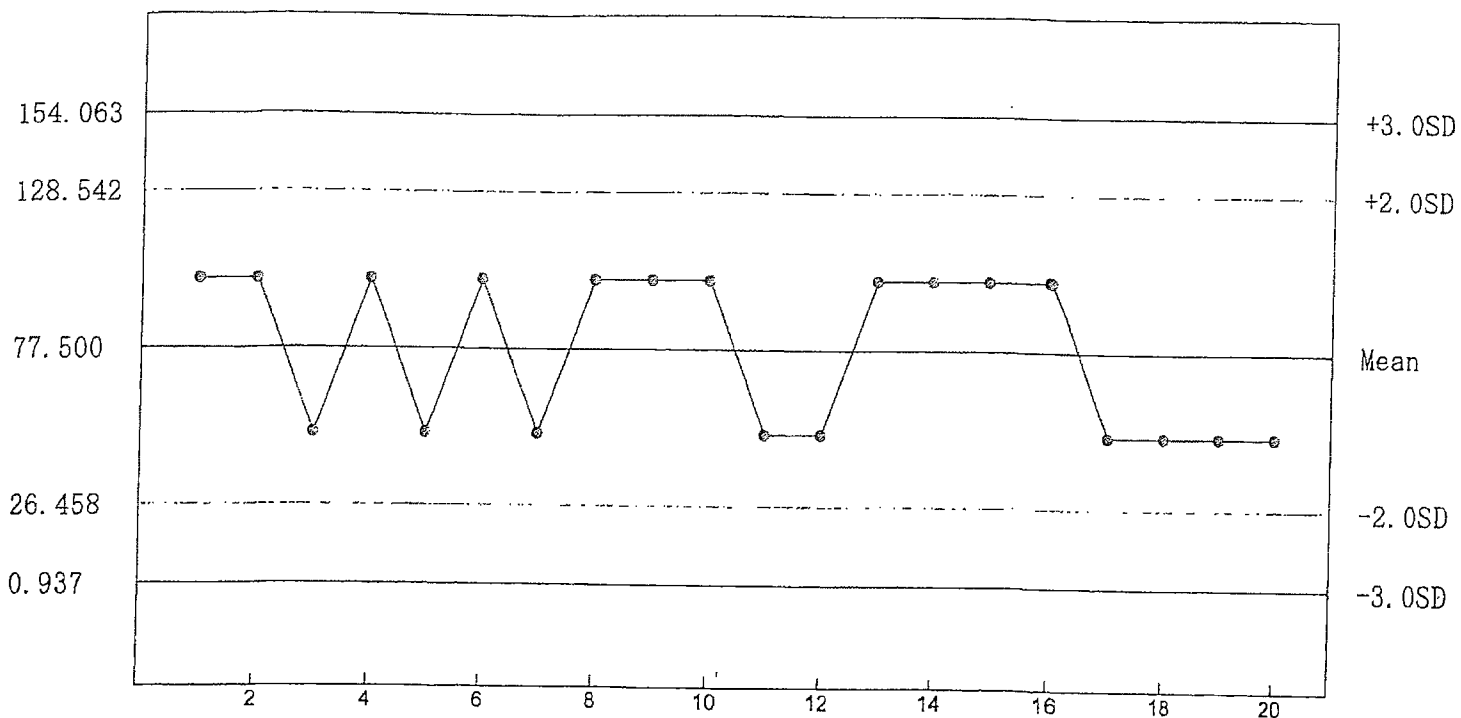
CHRONIC REFERENCE TOXICANT TEST RESULTS

SPECIES: *Pimephales promelas*
 CHEMICAL: Copper Nitrate
 DURATION: 7-Days
 TEST NUMBER: 8
 TEST DATE: 08/05/15 - 08/12/15
 1540 Hrs - 1540 Hrs
 STATISTICAL METHOD: Dunnetts/Steels

CONCENTRATION (ug/L)	NUMBER EXPOSED	NUMBER DEAD
25	40	0
50	40	3
100	40	15
200	40	25
400	40	40
800	40	40

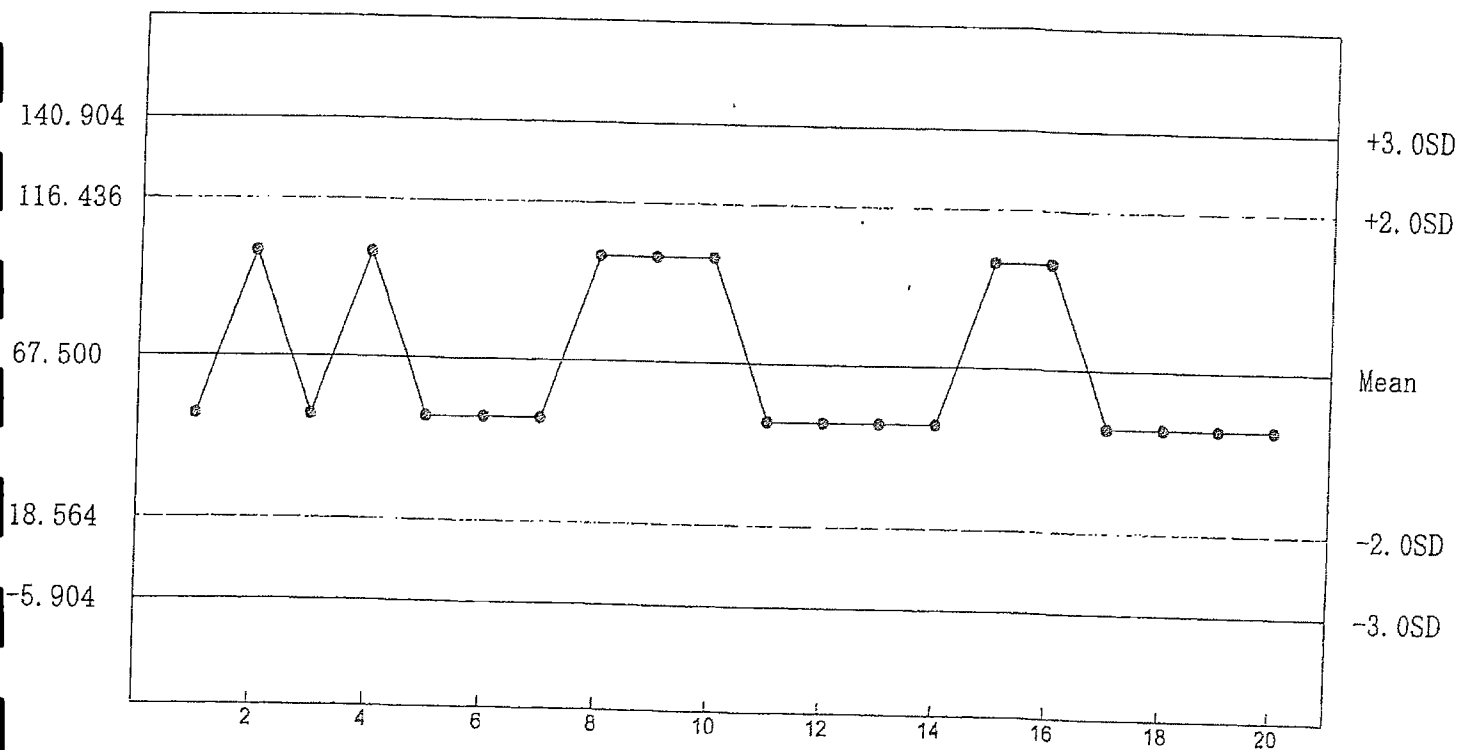
LOEC FOR SURVIVAL	NOEC FOR SURVIVAL	LOEC FOR GROWTH	NOEC FOR GROWTH
100 ug/L	50 ug/L	100 ug/L	50 ug/L

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



n= 20 Mean= 77.500 SD= 25.521 CV= 32.93% Min= 50.000 Max= 100.000

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



n= 20 Mean= 67.500 SD= 24.468 CV= 36.25% Min= 50.000 Max= 100.000

APPENDIX C
CHAIN OF CUSTODY SHEETS



L444-050111

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

Company Name:		Phone #:		Requested Analysis												7-Day Chronic Bio-Monitoring	Laboratory Control Number	Remarks (Please note special detection limits below.)					
Clarksville Light and Water		(479) 754-6241																					
Address:		Fax #:																					
P.O. Box 1807 Clarksville, AR 72830		(479) 754-8181																					
Project Name or Number:				Purchase Order #:																			
Bio-Monitoring																							
Sampling Personnel Signature(s):						Printed:																	
<i>Parsha Russell</i>						Parsha Russell																	
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		
Outfall 001	8-25-15 8-26-15	0730 0735	X		X		1						X	X					X	0815224			
Relinquished by:				Date:		Time:		Received By:				Date:		Time:									
<i>Parsha Russell</i>				8-26-15		1213		<i>Stacy</i>				8/26/15		1330									
Received by:				Date:		Time:		Relinquished By:				Date:		Time:									
<i>Lochlan</i>				8-26-15		1213		<i>Stacy</i>				8/26/15		1600									
Relinquished by:				Date:		Time:		Received by Laboratory:				Date:		Time:									
<i>Lochlan</i>				8-26-15		1330		<i>Matt Houser</i>				8-27-15		1035									
Comments: -0.8°C UPS																							

ENVIRONMENTAL ENTERPRISE GROUP
 CITY OF CLARKSVILLE WWTP – OUTFALL 001
 NPDES PERMIT NO. AR0022187
 AFIN NO. 36-00038
 BIOMONITORING REPORTING
 TEST DATE: 08/25/15

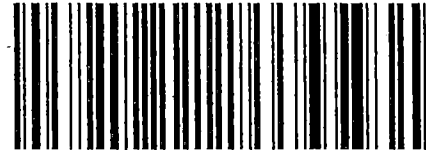
II. *Ceriodaphnia dubia*

	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.	11.02%

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. TP6C.	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.	7.90%

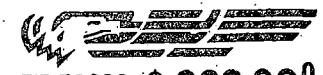
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