

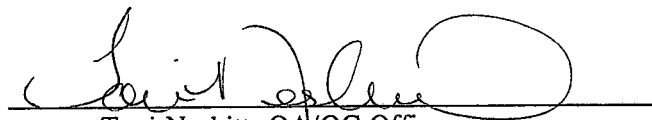
CITY OF MENA WWTF
OUTFALL 001

Chronic Biomonitoring Report
Permit Number NPDES AR0036692
AFIN Number 57-00042

Ceriodaphnia dubia
Pimephales promelas

January 14, 2014

Reviewed by:



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TOXICITY TEST REPORT - CHRONIC

ClientCity of Mena WWTF Laboratory I.D.21912
Permit No. NPDES AR0036692 Begin DateJanuary 14, 2014
SampleOutfall 001

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 City of Mena WWTF were delivered by UPS courier to Huther & Associates on January 14, January 16, and January 20, 2014. Effluent samples were collected from Outfall 001 using an automatic sampler and were manually composited 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, 22nd 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 1500 hours, January 14, 2014. Five concentrations were prepared (32%, 42%, 56%, 75%, and 100% effluent) utilizing distilled, deionized laboratory water reconstituted to match the hardness, alkalinity and pH of the receiving stream (unnamed tributary of Prairie Creek). The test was conducted in 25 mL distilled water rinsed plastic beakers containing 15 mL of solution (one organism per beaker, ten beakers per concentration). *C. dubia* neonates were less than 24 hours old and within eight hours of the same age at test initiation. Neonates were placed in beakers following a randomized block test design. Fresh solutions were prepared and renewed daily. Daily feeding consisted of 0.5 mL *Selenastrum capricornutum* and cerophyll per test chamber. The test proceeded for seven days during which survival, reproduction and water quality data were collected daily.

A control of 10 replicate beakers containing one neonate each in distilled, deionized, reconstituted water (same as diluent) was conducted concurrently with the test. There was 100% survival in the control. The test ended at 1500 hours, January 21, 2014. Survival and reproduction data were statistically analyzed ($p = 0.05$) according to EPA procedures to determine the Lowest Observable Effect Concentration (LOEC) and the No Observable Effect Concentration (NOEC).

SURVIVAL
Ceriodaphnia dubia

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

LOEC: Not Applicable
NOEC: 100% Effluent

REPRODUCTION
Ceriodaphnia dubia

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

LOEC: Not Applicable
NOEC: 100% Effluent

PMSD: 6.6%

TEST SETUP
Pimephales promelas



The seven-day *Pimephales promelas* larval survival and growth test was initiated at 1530 hours, January 14, 2014. Five concentrations were prepared (32%, 42%, 56%, 75%, and 100% effluent) utilizing distilled, deionized laboratory water reconstituted to match the hardness, alkalinity and pH of the receiving stream (unnamed tributary of Prairie 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 1530 hours, January 21, 2014. 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: 100% Effluent

GROWTH
Pimephales promelas

P. promelas growth data failed Shapiro Wilk's test for normality at the 0.01 alpha level (0.900). Bartlett's test for homogeneity is sensitive to non-normal data and should not be performed on the non-normally distributed data. Therefore, a nonparametric test was performed on the data. Steel's Many-One Rank 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: 11.5%**
NOEC: 100% Effluent

SUMMARY

There were no statistically significant differences between the control and the critical low flow concentration (100% effluent) for *C. dubia* survival and reproduction and *P. promelas* survival and growth. Based on biomonitoring requirements for Outfall 001 contained in Permit Number NPDES AR0036692 for City of Mena WWTF, Outfall 001 passed for this testing period.

Huthner and Associates

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

CLIENT: City of Menard, WTF
 NPDES #: AR0036692
 LAB ID #: 21912
 TEST TYPE: 7-Day Chronic
 TEST ORGANISM: *Ceriodaphnia dubia*
 ORGANISM AGE: 24 Hours
 ORGANISM SOURCE: In House
 RECEIVING WATER: unnamed tributary of Prairie Creek
 DILUTION WATER: Laboratory Adjusted

SAMPLE TYPE: 24-Hour Composite
 DATE COLLECTED: 01/13/14, 01/15/14, 01/17/14
 DATE RECEIVED: 01/14/14, 01/16/14, 01/20/14
 BEGIN DATE/TIME: 01/14/14, 1500
 END DATE/TIME: 01/21/14, 1500
 TEST TEMPERATURE (°C): 25 ± 1
 PHOTO PERIOD: 16-hr Light/8-hr Dark
 LIGHT INTENSITY: 50-100 ft-cnd
 TECHNICIAN: N. Lehr

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
01/15/14	A	A	A	A	A	A	A	A	A	A
01/16/14	A	A	A	A	A	A	A	A	A	A
01/17/14	A	A	A	A	A	A	A	A	A	A
01/18/14	A	A	A	A	A	A	A	A	A	A
01/19/14	2	3	2	2	4	3	2	2	2	2
01/20/14	7	7	8	6	7	8	7	7	9	7
01/21/14	13	12	12	11	12	12	13	13	12	12
x # Young 21.9 C.V. 5.47% 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
01/15/14	A	A	A	A	A	A	A	A	A	A
01/16/14	A	A	A	A	A	A	A	A	A	A
01/17/14	A	A	A	A	A	A	A	A	A	A
01/18/14	A	A	A	A	A	A	A	A	A	A
01/19/14	3	2	3	3	4	2	2	3	2	2
01/20/14	10	8	8	7	9	9	6	8	8	9
01/21/14	13	14	14	12	12	13	13	12	13	12
x # Young 23.6 C.V. 6.38% 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
01/15/14	A	A	A	A	A	A	A	A	A	A
01/16/14	A	A	A	A	A	A	A	A	A	A
01/17/14	A	A	A	A	A	A	A	A	A	A
01/18/14	A	A	A	A	A	A	A	A	A	A
01/19/14	4	3	2	2	3	2	3	4	2	3
01/20/14	7	9	9	8	8	7	9	8	8	8
01/21/14	14	13	13	12	12	15	12	13	13	13
x # Young 23.9 C.V. 4.16% x% Survival 100% C.V. 0.00%										

56% Effluent										
Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
01/15/14	A	A	A	A	A	A	A	A	A	A
01/16/14	A	A	A	A	A	A	A	A	A	A
01/17/14	A	A	A	A	A	A	A	A	A	A
01/18/14	A	A	A	A	A	A	A	A	A	A
01/19/14	3	2	4	3	3	2	4	2	3	2
01/20/14	8	8	6	6	7	7	9	9	9	10
01/21/14	13	14	12	12	12	14	14	13	14	12
x # Young 23.7 C.V. 7.72% 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

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

City of Mena WWTF

Lab ID# 21912

Test Date: January 14, 2014

75% Effluent

Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
01/15/14	A	A	A	A	A	A	A	A	A	A
01/16/14	A	A	A	A	A	A	A	A	A	A
01/17/14	A	A	A	A	A	A	A	A	A	A
01/18/14	A	A	A	A	A	A	A	A	A	A
01/19/14	3	3	2	2	3	4	3	3	2	4
01/20/14	7	8	9	9	6	6	8	8	8	7
01/21/14	13	14	12	13	12	14	14	13	12	12
<p>x# Young 23.4 C.V. 5.41%</p> <p>x% Survival 100% C.V. 0.00%</p>										

100% Effluent

Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
01/15/14	A	A	A	A	A	A	A	A	A	A
01/16/14	A	A	A	A	A	A	A	A	A	A
01/17/14	A	A	A	A	A	A	A	A	A	A
01/18/14	A	A	A	A	A	A	A	A	A	A
01/19/14	4	4	2	3	2	3	4	3	2	3
01/20/14	9	6	7	8	7	10	7	9	9	8
01/21/14	14	13	13	12	14	12	13	13	12	14
<p>x# Young 24.0 C.V. 6.21%</p> <p>x% Survival 100% C.V. 0.00%</p>										

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

ex 1:

A
4

 alive today
total young to date

ex 2:

5
12

 alive, 5 young today
total young to date

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

City of Mena WWTF

Lab ID# 21912

Test Date: January 14, 2014

WET CHEMISTRY MEASUREMENTS

Date	Time	Temp	Samp No	pH of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
01/14/14	Start	25.0	1	8.81	8.16	7.72	7.54	7.38	7.15	STC
01/15/14	24 Hr	24.0	1	7.63	7.58	7.52	7.49	7.46	7.36	GZK
01/15/14	Renew	24.0	1	7.70	7.54	7.45	7.41	7.29	7.15	GZK
01/16/14	48 Hr	24.4	1	8.32	7.70	7.55	7.46	7.40	7.34	SCC
01/16/14	Renew	24.1	2	7.78	7.61	7.47	7.26	7.25	7.24	SCC
01/17/14	72 Hr	24.0	2	7.65	7.54	7.35	7.32	7.22	7.20	SCC
01/17/14	Renew	24.0	2	8.56	7.86	7.56	7.36	7.29	7.24	SCC
01/18/14	96 Hr	24.0	2	8.24	8.14	7.86	7.76	7.42	7.34	SCC
01/18/14	Renew	24.0	2	8.11	7.87	7.57	7.43	7.32	7.24	SCC
01/19/14	120 Hr	24.2	2	7.84	7.76	7.70	7.66	7.59	7.57	STC
01/19/14	Renew	24.0	2	7.58	7.45	7.33	7.23	7.12	7.02	STC
01/20/14	144 Hr	24.0	2	7.84	7.76	7.68	7.59	7.53	7.49	STC
01/20/14	Renew	25.0	3	7.70	7.52	7.44	7.31	7.15	7.05	STC
01/21/14	168 Hr	24.0	3	7.74	7.65	7.60	7.54	7.49	7.42	GZK

Date	Time	Temp	Samp No	DO (mg/L) of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
01/14/14	Start	25.0	1	7.90	8.37	7.63	8.23	8.40	8.65	STC
01/15/14	24 Hr	24.0	1	8.09	7.77	7.58	7.82	7.86	7.21	GZK
01/15/14	Renew	24.0	1	7.53	7.90	7.87	8.23	8.21	8.65	GZK
01/16/14	48 Hr	24.4	1	8.53	8.07	8.28	8.07	8.16	8.01	SCC
01/16/14	Renew	24.1	2	8.16	8.10	8.48	8.13	8.29	8.71	SCC
01/17/14	72 Hr	24.0	2	8.21	8.33	8.32	8.41	8.45	8.66	SCC
01/17/14	Renew	24.0	2	8.13	8.55	8.31	8.56	8.38	8.71	SCC
01/18/14	96 Hr	24.0	2	8.19	8.34	8.47	8.56	8.63	8.84	SCC
01/18/14	Renew	24.0	2	8.45	8.50	8.52	8.60	8.59	8.71	SCC
01/19/14	120 Hr	24.2	2	8.80	8.20	8.08	8.08	8.02	7.93	STC
01/19/14	Renew	24.0	2	8.24	8.02	8.56	8.31	8.32	8.23	STC
01/20/14	144 Hr	24.0	2	8.50	8.12	7.76	7.73	7.91	7.57	STC
01/20/14	Renew	25.0	3	8.56	7.83	8.25	7.86	7.85	8.70	STC
01/21/14	168 Hr	24.0	3	7.44	7.37	7.44	7.52	7.46	7.72	GZK

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

City of Mena WWTF

Lab ID# 21912

Test Date: January 14, 2014

INITIAL CHEMISTRY MEASUREMENTS @ 100% EFFLUENT

Date	Samp No	pH	DO	Hardness mg/L CaCO ₃	Alkalinity mg/L CaCO ₃	Conduct umhos/cm	Resid Cl ₂ mg/L	Dechlor (mL) Na ₂ S ₂ O ₃ mg/L	Analyst
01/14/14	1	7.15	8.65	20	24	142	<0.01	N/A	TN
01/16/14	2	7.24	8.71	24	26	163	<0.01	N/A	TN
01/20/14	3	7.05	8.70	20	28	167	<0.01	N/A	TN
01/14/14	Con	8.81	7.90	40	30	182	-	-	TN

¹ Measurements taken in 100% solution.

Huther and Associates, Inc.
 Begin Date: January 14, 2014
 Lab I.D.# 21912

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	23.000	21.900
2	32% Effluent	10	21.000	26.000	23.600
3	42% Effluent	10	22.000	25.000	23.900
4	56% Effluent	10	21.000	27.000	23.700
5	75% Effluent	10	21.000	25.000	23.400
6	100% Effluent	10	22.000	27.000	24.000

Summary Statistics on Transformed Data Table 2 of 2

Grp	Identification	Variance	Sd	Sem	C.V.%
1	Control	1.433	1.197	0.379	5.47
2	32% Effluent	2.267	1.506	0.476	6.38
3	42% Effluent	0.989	0.994	0.314	4.16
4	56% Effluent	3.344	1.829	0.578	7.72
5	75% Effluent	1.600	1.265	0.400	5.41
6	100% Effluent	2.222	1.491	0.471	6.21

Chi-Square Test For Normality: Actual And Expected Frequencies

Interval	< -1.5	-1.5 to -0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
Expected	4.020	14.520	22.920	14.520	4.020
Observed	4	13	26	14	3

Calculated Chi-Square goodness of fit test statistic = 0.8505
 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 = 3.82

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	29.883	5.977	3.025
Within (Error)	54	106.700	1.976	
Total	59	136.583		

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

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

Grp	Identification	Transformed Mean	Mean	T Stat	Sig
			Calculated In Original Units		
1	Control	21.900	21.900		
2	32% Effluent	23.600	23.600	-2.704	
3	42% Effluent	23.900	23.900	-3.181	
4	56% Effluent	23.700	23.700	-2.863	
5	75% Effluent	23.400	23.400	-2.386	
6	100% Effluent	24.000	24.000	-3.341	

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

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

Grp	Identification	Num of Reps	Minimum Sig Diff	% of Control	Difference
			(In Orig. Units)		from Control
1	Control	10			
2	32% Effluent	10	1.452	6.6	-1.700
3	42% Effluent	10	1.452	6.6	-2.000
4	56% Effluent	10	1.452	6.6	-1.800
5	75% Effluent	10	1.452	6.6	-1.500
6	100% Effluent	10	1.452	6.6	-2.100

Huther and Associates

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

CLIENT City of Menominee WWTF SAMPLE TYPE 24 Hour Composite
 NPDES # AR0036692 DATE COLLECTED 01/13/14, 01/15/14, 01/17/14
 LAB ID # 21912 DATE RECEIVED 01/14/14, 01/16/14, 01/20/14
 TEST TYPE 7 Day Chronic BEGIN DATE/TIME 01/14/14 1530
 TEST ORGANISM *Pimephales promelas* END DATE/TIME 01/21/14 1530
 ORGANISM AGE < 24 Hours TEST TEMPERATURE (°C) 25 ± 0.1
 ORGANISM SOURCE In House PHOTO PERIOD 16 hr Light 8 hr Dark
 RECEIVING WATER Unnamed tributary of Prairie Creek LIGHT INTENSITY 50-100 ft cnd
 DILUTION WATER Laboratory Adjusted TECHNICIAN M. Horner

SURVIVAL SUMMARY

Conc.	01/15/14					01/16/14					01/17/14					01/18/14					01/19/14				
	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
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.	01/20/14					01/21/14					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
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. %
Con	0.4190	0.4820	0.4670	0.4920	0.4210	0.4562	7.50
32%	0.4680	0.4250	0.5040	0.4490	0.5030	0.4698	7.31
42%	0.4360	0.4720	0.5060	0.4780	0.4290	0.4642	6.84
56%	0.4680	0.4690	0.4150	0.5020	0.5030	0.4714	7.60
75%	0.4960	0.4240	0.5030	0.4860	0.4250	0.4668	8.37
100%	0.4750	0.5030	0.4940	0.4160	0.4900	0.4756	7.32

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

City of Mena WWTF

Lab ID# 21912

Test Date: January 14, 2014

WET CHEMISTRY MEASUREMENTS

Date	Time	Temp	Samp No.	pH of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
01/14/14	Start	25.0	1	8.81	8.16	7.72	7.54	7.38	7.15	STC
01/15/14	24 Hr	24.0	1	7.42	7.37	7.31	7.26	7.20	7.10	GZK
01/15/14	Renew	24.0	1	7.70	7.54	7.45	7.41	7.29	7.15	GZK
01/16/14	48 Hr	24.3	1	7.46	7.41	7.38	7.34	7.29	7.22	SCC
01/16/14	Renew	24.1	2	7.78	7.61	7.47	7.26	7.25	7.24	SCC
01/17/14	72 Hr	24.1	2	7.84	7.65	7.54	7.49	7.40	7.33	SCC
01/17/14	Renew	24.0	2	8.56	7.86	7.56	7.36	7.29	7.24	SCC
01/18/14	96 Hr	24.0	2	7.78	7.61	7.54	7.44	7.41	7.32	SCC
01/18/14	Renew	24.0	2	8.11	7.87	7.57	7.43	7.32	7.24	SCC
01/19/14	120 Hr	24.2	2	7.57	7.54	7.48	7.47	7.36	7.28	STC
01/19/14	Renew	24.0	2	7.58	7.45	7.33	7.23	7.12	7.02	STC
01/20/14	144 Hr	24.0	2	7.41	7.42	7.39	7.36	7.27	7.17	STC
01/20/14	Renew	25.0	3	8.56	7.83	8.25	7.86	7.85	7.05	STC
01/21/14	168 Hr	24.0	3	7.40	7.43	7.39	7.33	7.30	7.20	STC

Date	Time	Temp	Samp No.	DO (mg/L) of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
01/14/14	Start	25.0	1	7.90	8.37	7.63	8.23	8.40	8.65	STC
01/15/14	24 Hr	24.0	1	8.49	8.49	8.44	8.46	8.36	8.23	GZK
01/15/14	Renew	24.0	1	7.53	7.90	7.87	8.23	8.21	8.65	GZK
01/16/14	48 Hr	24.3	1	8.39	8.10	8.15	8.28	8.36	8.20	SCC
01/16/14	Renew	24.1	2	8.16	8.10	8.48	8.13	8.29	8.71	SCC
01/17/14	72 Hr	24.1	2	8.62	8.27	8.10	8.16	8.10	7.90	SCC
01/17/14	Renew	24.0	2	8.13	8.55	8.31	8.56	8.38	8.71	SCC
01/18/14	96 Hr	24.0	2	8.29	8.43	8.38	8.19	8.84	8.58	SCC
01/18/14	Renew	24.0	2	8.45	8.50	8.52	8.60	8.59	8.71	SCC
01/19/14	120 Hr	24.2	2	8.24	8.28	7.87	8.01	8.31	7.52	STC
01/19/14	Renew	24.0	2	8.24	8.02	8.56	8.31	8.32	8.23	STC
01/20/14	144 Hr	24.0	2	7.71	8.36	8.29	8.12	7.84	7.56	STC
01/20/14	Renew	25.0	3	8.56	7.83	8.25	7.86	7.85	8.70	STC
01/21/14	168 Hr	24.0	3	7.77	7.79	7.78	7.71	8.16	7.80	STC

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

City of Mena WWTF

Lab ID# 21912

Test Date: January 14, 2014

INITIAL CHEMISTRY MEASUREMENTS @ 100% EFFLUENT

Date	Samp No.	pH	DO	Hardness mg/L CaCO ₃	Alkalinity mg/L CaCO ₃	Conduct umhos/cm	Resid Cl ₂ mg/L	Dechlor(mL) Na ₂ S ₂ O ₃ mg/L	Analyst
01/14/14	1	7.15	8.65	20	24	142	<0.01	N/A	TN
01/16/14	2	7.24	8.71	24	26	163	<0.01	N/A	TN
01/20/14	3	7.05	8.70	20	28	167	<0.01	N/A	TN
01/14/14	Con	8.81	7.90	40	30	182			

Measurements taken in 100% solution.

PIMEPHALES PROMELAS STATISTICAL ANALYSES
 Growth

Summary Statistics on Transformed Data Table 1 of 2

Grp	Identification	N	Min	Max	Mean
1	Control	5	0.419	0.492	0.456
2	32% Effluent	5	0.425	0.504	0.470
3	42% Effluent	5	0.429	0.506	0.464
4	56% Effluent	5	0.415	0.503	0.471
5	75% Effluent	5	0.424	0.503	0.467
6	100% Effluent	5	0.416	0.503	0.476

Shapiro - Wilk's Test For Normality

D = 0.030
 W = 0.898
 Critical W (P = 0.05) (n = 30) = 0.927
 Critical W (P = 0.01) (n = 30) = 0.900
 Data Fail normality test. Try another transformation.

Warning - The first three homogeneity tests are sensitive to non-normal data and should not be performed.

Summary Statistics on Transformed Data Table 2 of 2

Grp	Identification	Variance	Sd	Sem	C.V.%
1	Control	0.001	0.034	0.015	7.50
2	32% Effluent	0.001	0.034	0.015	7.31
3	42% Effluent	0.001	0.032	0.014	6.84
4	56% Effluent	0.001	0.036	0.016	7.60
5	75% Effluent	0.002	0.039	0.017	8.37
6	100% Effluent	0.001	0.035	0.016	7.32

Steel's Many-One Rank Test - Ho:Control < Treatment

Grp	Identification	Transformed Mean	Rank Sum	Crit. Value	Df	Sig
1	Control	0.456				
2	32% Effluent	0.470	32.00	16.00	5.00	
3	42% Effluent	0.464	30.00	16.00	5.00	
4	56% Effluent	0.471	31.00	16.00	5.00	
5	75% Effluent	0.467	33.00	16.00	5.00	
6	100% Effluent	0.476	32.00	16.00	5.00	

Critical values use k = 5, are 1 tailed, and alpha = 0.05

**APPENDIX A
RAW DATA**

7-DAY CERIODAPHNIA DUBIA SURVIVAL & REPRODUCTION

DAILY RAW DATA TABLE

CLIENT Mena

START DATE/TIME 1-14-14 NL 1500

OUTFALL 001

END DATE/TIME 1-21-14 ZG 1500

LAB ID # 21912

Con

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
1/15	A	A	A	A	A	A	A	A	A	A	ZG	1500
1/16	A	A	A	A	A	A	A	A	A	A	NL	1445
1/17	A	A	A	A	A	A	A	A	A	A	NL	1340
1/18	A	A	A	A	A	A	A	A	A	A	MH	1340
1/19	2	3	2	2	4	3	2	2	2	2	MH	0945
1/20	7	7	8	6	7	8	7	7	9	7	ZG	1030
1/21	13	12	12	11	12	12	13	13	12	12	ZG	1500
	22	22	22	19	23	23	22	22	23	21		

\bar{x} # Young w/o Dead = 21.9 CV% = 5.47
 \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
1/15	A	A	A	A	A	A	A	A	A	A	ZG	1500
1/16	A	A	A	A	A	A	A	A	A	A	NL	1445
1/17	A	A	A	A	A	A	A	A	A	A	NL	1340
1/18	A	A	A	A	A	A	A	A	A	A	MH	1340
1/19	3	2	3	3	4	2	2	3	2	2	MH	0945
1/20	10	8	8	7	9	9	6	8	8	9	ZG	1030
1/21	13	14	14	12	12	13	13	12	13	12	ZG	1500
	21	24	25	22	25	24	21	23	23	23		

\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

42

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
1/15	A	A	A	A	A	A	A	A	A	A	ZG	1500
1/16	A	A	A	A	A	A	A	A	A	A	NL	1445
1/17	A	A	A	A	A	A	A	A	A	A	NL	1340
1/18	A	A	A	A	A	A	A	A	A	A	MH	1340
1/19	4	3	2	2	3	2	3	4	2	3	MH	0945
1/20	7	9	9	8	8	7	9	8	8	8	ZG	1030
1/21	14	13	13	12	12	15	12	13	13	13	ZG	1500
	25	25	24	22	23	24	24	25	23	24		

\bar{x} # Young w/o Dead = 23.9 CV% = 4.16
 \bar{x} # Young w/Dead = CV% =
 \bar{x} % Survival = 100.0 CV% = 0.00

56

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
1/15	A	A	A	A	A	A	A	A	A	A	ZG	1500
1/16	A	A	A	A	A	A	A	A	A	A	NL	1445
1/17	A	A	A	A	A	A	A	A	A	A	NL	1340
1/18	A	A	A	A	A	A	A	A	A	A	MH	1340
1/19	3	2	4	3	3	2	4	2	3	2	MH	0945
1/20	8	8	6	6	7	7	9	9	9	10	ZG	1030
1/21	13	14	12	12	12	14	14	13	14	12	ZG	1500
	24	24	22	21	22	23	27	24	26	24		

\bar{x} # Young w/o Dead = 23.7 CV% = 7.72
 \bar{x} # Young w/Dead = CV% =
 \bar{x} % Survival = 100.0 CV% = 0.00

7-DAY CERIODAPHНИЯ DUBIA SURVIVAL & REPRODUCTION

DAILY RAW DATA TABLE

CLIENT Mena

START DATE/TIME 1-14-14 NL 1500

OUTFALL 001

END DATE/TIME 1-21-14 ZG 1500

LAB ID # 21912

75

100

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
1/15	A	A	A	A	A	A	A	A	A	A	ZG	1500
1/16	A	A	A	A	A	A	A	A	A	A	NL	1445
1/17	A	A	A	A	A	A	A	A	A	A	NL	1340
1/18	A	A	A	A	A	A	A	A	A	A	MH	1340
1/19	3	3	2	2	3	4	3	3	2	4	MH	0945
1/20	7	8	9	9	6	6	8	8	8	7	ZG	1030
1/21	13	14	12	13	12	14	14	13	12	12	ZG	1500
	23	25	23	24	21	24	25	24	22	23		

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
1/15	A	A	A	A	A	A	A	A	A	A	ZG	1500
1/16	A	A	A	A	A	A	A	A	A	A	NL	1445
1/17	A	A	A	A	A	A	A	A	A	A	NL	1340
1/18	A	A	A	A	A	A	A	A	A	A	MH	1340
1/19	4	4	2	3	2	3	4	3	2	3	MH	0945
1/20	9	6	7	8	7	10	7	9	9	8	ZG	1030
1/21	14	13	13	12	14	12	13	13	12	14	ZG	1500
	27	23	22	23	23	25	24	25	23	25		

\bar{x} # Young w/o Dead = 23.4 CV% = 5.41

\bar{x} # Young w/o Dead = 24.0 CV% = 6.21

\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

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

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

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

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

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

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

\bar{x} % Survival = CV% =

\bar{x} % Survival = CV% =

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

CLIENT/FACILITY Mena
 OUTFALL # 001 PROJECT # 21912
 ORGANISM ID# PP0-14-013

DATE/TIME STARTED 1-14-14 ZG 1530
 DATE/TIME ENDED 01-21-14 JK 1530

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
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	1-15-14 MH 1530					01-15-14 JK 0820					1-17-14 NL 0905					1-18-14 MH 0950					01-19-14 JK 0845				

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
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	1-20-14 ZG 0815					01-21-14 JK 1530						

Client / Facility Mena
 Lab ID Number 21912
 Outfall Number 001
 Test Date 1-14-14

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
1/14	1	7.15	8.65	20	24	142	20.01	Na	TN
1/16	2	7.24	8.71	24	26	163	~	~	~
1/20	3	7.05	8.70	20	28	167	~	~	~
1/14	CON	8.81	7.90	40	30	182	—	—	~

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

Notes:

APPENDIX B
REFERENCE TOXICANTS

CHRONIC REFERENCE TOXICANT TEST RESULTS

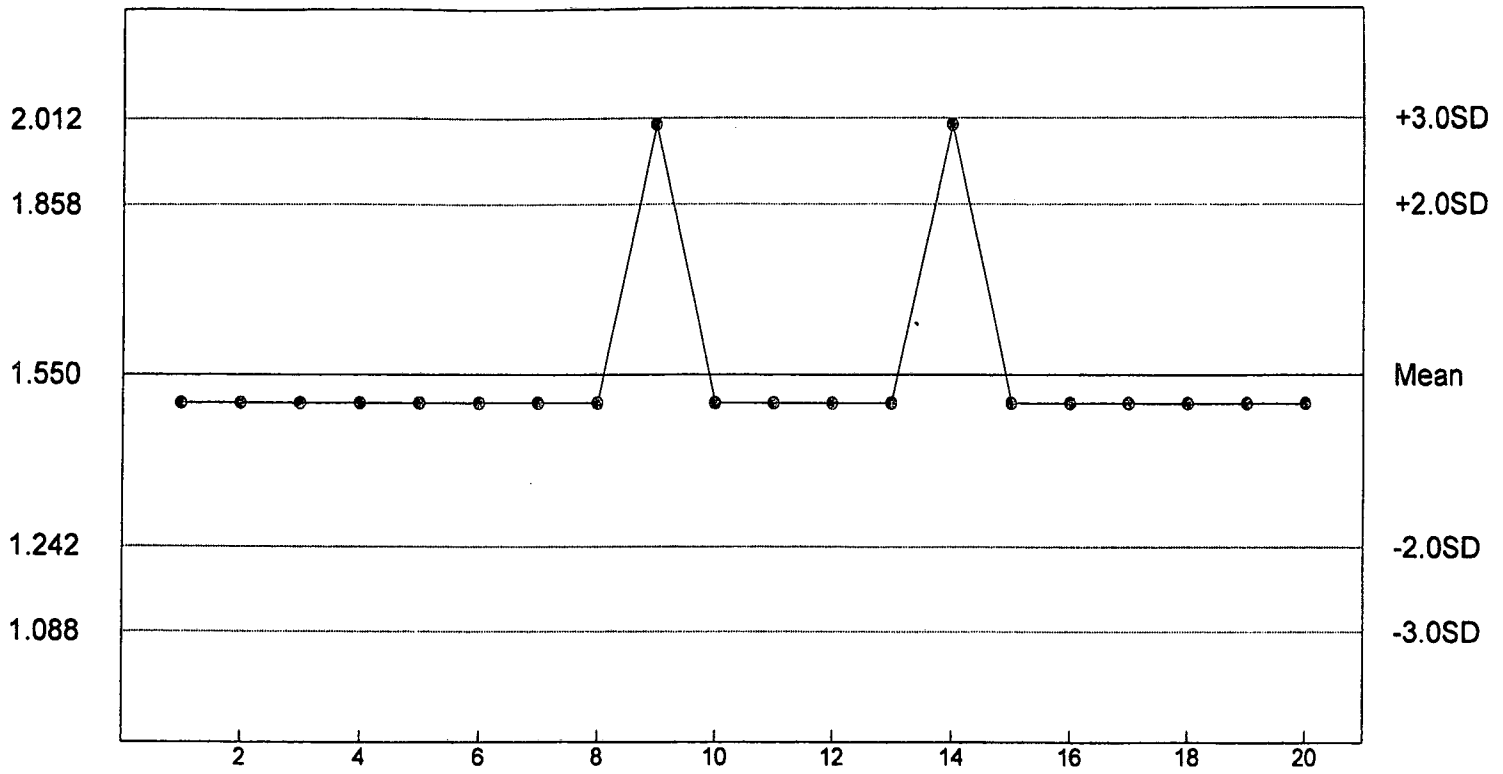
SPECIES: *Ceriodaphnia dubia*
 CHEMICAL: Sodium Chloride
 DURATION: 7-Days
 TEST NUMBER: 01
 TEST DATE/TIME: 01/03/14 - 01/10/14
 1530 Hrs - 1530 Hrs
 STATISTICAL METHOD: Fishers, Dunnetts/Steels

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
4.0	10	10

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

Reference Tox Sodium Chloride g/L

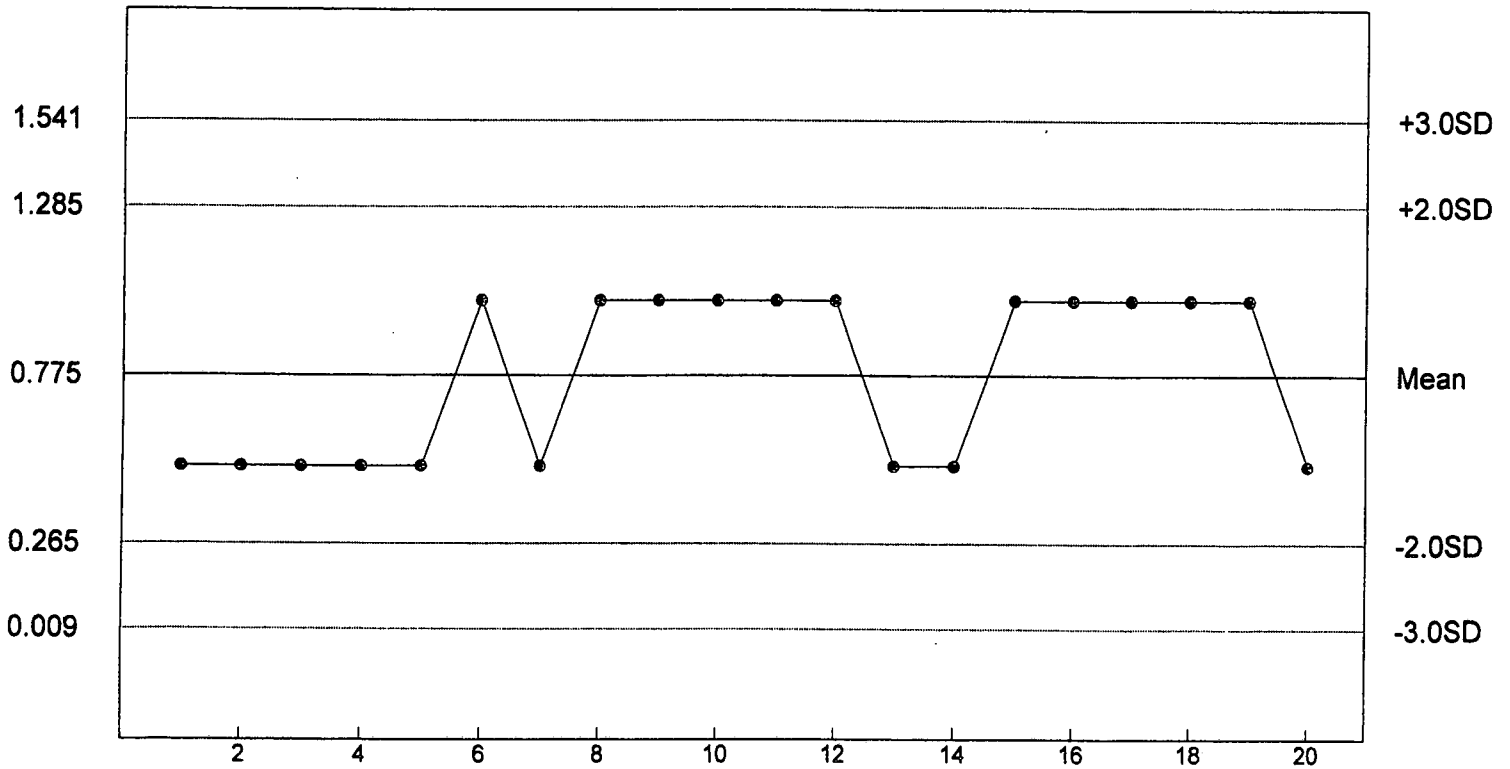
C. dubia Survival - NOEC



n= 20 Mean= 1.550 SD= 0.154 CV= 9.93% 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

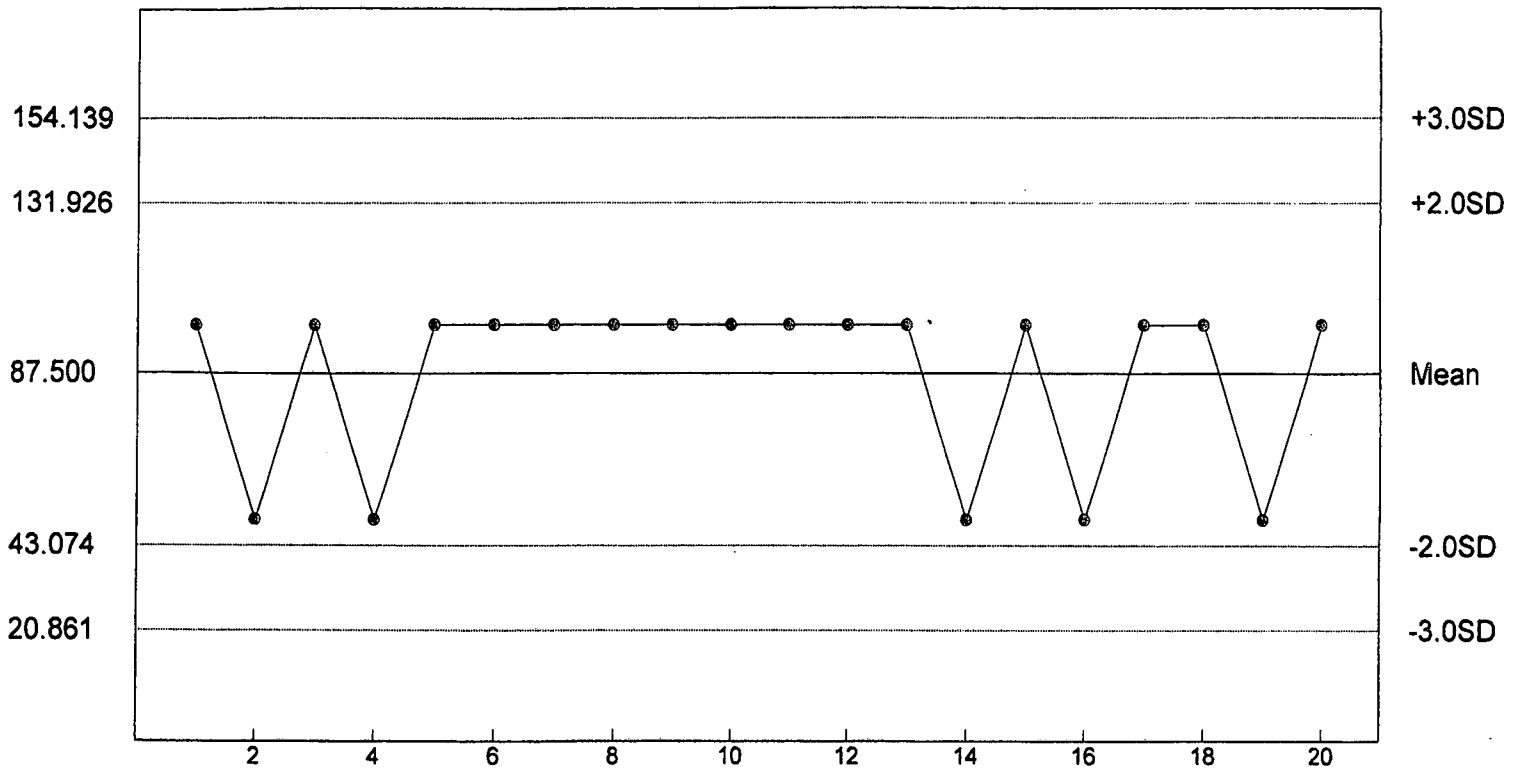
CHRONIC REFERENCE TOXICANT TEST RESULTS

SPECIES: *Pimephales promelas*
 CHEMICAL: Copper Nitrate
 DURATION: 7-Days
 TEST NUMBER: 01
 TEST DATE/TIME: 01/06/13 - 01/13/14
 1005 Hrs - 1005 Hrs
 STATISTICAL METHOD: Dunnetts/Steels

CONCENTRATION (ug/L)	NUMBER EXPOSED	NUMBER DEAD
12.5	40	0
25	40	0
50	40	0
100	40	7
200	40	26
400	40	40
800	40	40

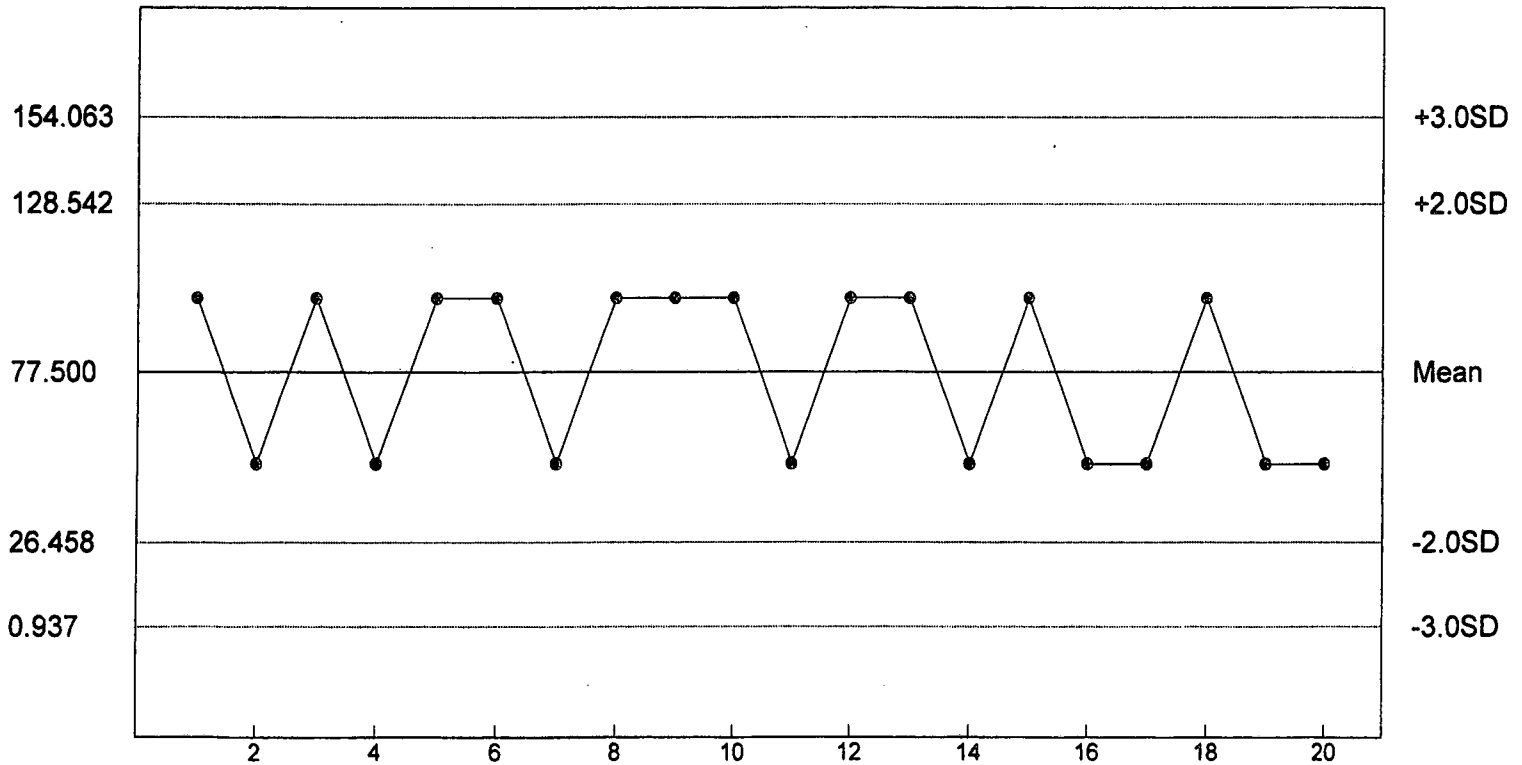
LOEC FOR SURVIVAL	NOEC FOR SURVIVAL	LOEC FOR GROWTH	NOEC FOR GROWTH
200 ug/L	100 ug/L	100 ug/L	50 ug/L

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



n= 20 Mean= 87.500 SD= 22.213 CV= 25.39% Min= 50.000 Max= 100.000

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



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

APPENDIX C
CHAIN OF CUSTODY SHEETS

HUTHER & ASSOCIATES
 1156 NORTH BONNIE BRAE STREET
 DENTON, TX 76201
 (940) 387-1025 • FAX (940) 387-1036

CHAIN OF CUSTODY RECORD

PROJECT # 21912 PROJECT NAME Mena PERMIT# NPDES AR0036692

OUTFALL SAMPLES

24-Hr Flow Weighted Composite Other _____

OUTFALL NUMBER	PERSON TAKING SAMPLE	START DATE/TIME	END DATE/TIME	# OF PORTIONS COMPOSITED	METHODS OF COLLECTION AND COMPOSITE			# OF CONTAINERS TO BE SHIPPED
					AUTO COLL. AUTO COMP.	MANUAL COLL. MANUAL COMP.	AUTO COLL. MANUAL COMP.	
001	Jeff	12 JAN 2014 1200	13 JAN 14 1200	24	X			1

RECEIVING WATER SAMPLES

SAMPLE IDENTIFICATION (FOR REC'NG) H/O GRABS: GIVE NAME OF STREAM AND LOCATION	PERSON TAKING SAMPLE	DATE	TIME	# OF CONTAINERS TO BE SHIPPED

TYPE OF TEST 7 day CF

NAME OF RECEIVING WATER unnamed tributary

DILUTION WATER USED FOR THIS TEST Lab

RELINQUISHED BY: Jeff DATE: 13 JAN 14 TIME: 1400 RECEIVED BY AT THIS DATE/TIME: 13 JAN 2014 UPS 1400 Office Store-Me

RELINQUISHED BY: _____ DATE: _____ TIME: _____ RECEIVED BY AT THIS DATE/TIME: _____

RELINQUISHED BY: _____ DATE: _____ TIME: _____ RECEIVED BY AT THIS DATE/TIME: _____

METHOD OF SHIPMENT: Greyhound _____ Pick Up _____ Client Delivered _____ Other UPS

RECEIVED: Guy JRC DATE: 01-14-14 TIME: 1037 SAMPLE TEMP. @ RECEIPT: 17

HUTHER & ASSOCIATES
 1156 NORTH BONNIE BRAE STREET
 DENTON, TX 76201
 (940) 387-1025 • FAX (940) 387-1036

CHAIN OF CUSTODY RECORD

PROJECT # 21912 PROJECT NAME Mena PERMIT# NPDES AR0036692

OUTFALL SAMPLES

24-Hr Flow Weighted Composite Other _____

OUTFALL NUMBER	PERSON TAKING SAMPLE	START DATE/TIME	END DATE/TIME	# OF PORTIONS COMPOSITED	METHODS OF COLLECTION AND COMPOSITE			# OF CONTAINERS TO BE SHIPPED
					AUTO COLL. AUTO COMP.	MANUAL COLL. MANUAL COMP.	AUTO COLL. MANUAL COMP.	
001	Jeff	15 JAN 2014 1200	15 JAN 2014 1200	24	X			

RECEIVING WATER SAMPLES

SAMPLE IDENTIFICATION (FOR REC'NG) H ₂ O GRABS, GIVE NAME OF STREAM AND LOCATION	PERSON TAKING SAMPLE	DATE	TIME	# OF CONTAINERS TO BE SHIPPED

TYPE OF TEST 7 day CF
 NAME OF RECEIVING WATER unnamed tributary
 DILUTION WATER USED FOR THIS TEST lab

RELINQUISHED BY: Jeff Hanger DATE: 15 JAN 14 TIME: 1345 RECEIVED BY AT THIS DATE/TIME: UPS (office store Mena)

RELINQUISHED BY: _____ DATE: _____ TIME: _____ RECEIVED BY AT THIS DATE/TIME: _____

RELINQUISHED BY: _____ DATE: _____ TIME: _____ RECEIVED BY AT THIS DATE/TIME: _____

METHOD OF SHIPMENT: Greyhound _____ Pick Up _____ Client Delivered _____ Other UPS

RECEIVED: Guy JAK DATE: 01-16-14 TIME: 0930 SAMPLE TEMP. @ RECEIPT: 0.9°c

HUTHER & ASSOCIATES
 1156 NORTH BONNIE BRAE STREET
 DENTON, TX 76201
 (940) 387-1025 • FAX (940) 387-1036

CHAIN OF CUSTODY RECORD

PROJECT # 21912 PROJECT NAME Mena PERMIT# NPDES AR0036692

OUTFALL SAMPLES

24-Hr Flow Weighted Composite Other _____

OUTFALL NUMBER	PERSON TAKING SAMPLE	START DATE/TIME	END DATE/TIME	# OF PORTIONS COMPOSED	METHODS OF COLLECTION AND COMPOSITE			# OF CONTAINERS TO BE SHIPPED
					AUTO COLL AUTO COMP	MANUAL COLL MANUAL COMP	AUTO COLL MANUAL COMP	
001	Jeff	16 JAN 2014 1200	17 JAN 2014 1200	24	X			1

RECEIVING WATER SAMPLES

SAMPLE IDENTIFICATION (FOR REC'NG) H2O GRABS: GIVE NAME OF STREAM AND LOCATION	PERSON TAKING SAMPLE	DATE	TIME	# OF CONTAINERS TO BE SHIPPED

TYPE OF TEST 7day CF

NAME OF RECEIVING WATER unnamed tributary

DILUTION WATER USED FOR THIS TEST Lab

RELINQUISHED BY: Jeff Flanger DATE: 17 JAN 14 TIME: 1445 RECEIVED BY AT THIS DATE/TIME 17 JAN 14 / 1445 Office Store-Mel ^{UPS}

RELINQUISHED BY: _____ DATE: _____ TIME: _____ RECEIVED BY AT THIS DATE/TIME _____

RELINQUISHED BY: _____ DATE: _____ TIME: _____ RECEIVED BY AT THIS DATE/TIME _____

METHOD OF SHIPMENT: Greyhound _____ Pick Up _____ Client Delivered _____ Other UPS

RECEIVED: Paul Singer DATE: 1-20-14 TIME: 0920 SAMPLE TEMP. @ RECEIPT. -1.2°

**CITY OF MENA WWTF
 NPDES PERMIT NO. AR0036692
 AFIN 57-00042
 BIOMONITORING REPORTING
 TEST DATE: 01/14/2014**

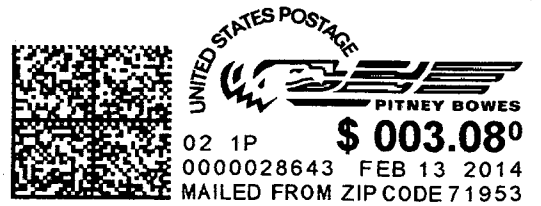
Ceriodaphnia dubia

	Response
A. If the NOEC for survival is less than the critical dilution, enter a "1"; otherwise, enter a "0". Parameter TLP3B	0
B. Report the NOEC value for survival. Parameter TOP3B	100%
C. Report the NOEC value for reproduction. Parameter TPP3B	100%
D. If the NOEC for reproduction is less than the critical dilution, enter a "1"; otherwise, enter a "0". Parameter TGP3B	0
E. Report the higher (critical dilution or control) Coefficient of Variation (CV%), Parameter TQP3B	6.21%
Report Parameter No. 22414 (lowest NOEC value) for <i>Ceriodaphnia dubia</i> .	100%

Pimephales promelas

	Response
A. If the NOEC for survival is less than the critical dilution, enter a "1"; otherwise, enter a "0". Parameter TLP6C	0
B. Report the NOEC value for survival. Parameter TOP6C	100%
C. Report the NOEC value for reproduction. Parameter TPP6C	100%
D. If the NOEC for reproduction is less than the critical dilution, enter a "1"; otherwise, enter a "0". Parameter TGP6C	0
E. Report the higher (critical dilution or control) Coefficient of Variation (CV%), Parameter TQP6C	7.50%
Report Parameter No. 22414 (lowest NOEC value) for <i>Pimephales promelas</i> .	100%

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