

CITY OF MENA WWTF
OUTFALL 001

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

Ceriodaphnia dubia
Pimephales promelas

September 22, 2015

Reviewed by: _____

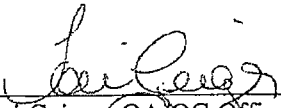

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TOXICITY TEST REPORT - CHRONIC

Client City of Mena WWTF
Permit No. NPDES AR0036692

SampleOutfall 001
Laboratory I.D. 24668
Begin Date September 22, 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 City of Mena WWTF were picked up at the facility by Huthier & Associates on September 21, September 23, and September 25, 2015. Effluent samples from Outfall 001 were collected and composited 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, 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 1300 hours, September 22, 2015. 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 1300 hours, September 29, 2015. Survival and reproduction data were statistically analyzed ($p = 0.05$) according to EPA procedures to determine the Lowest Observable Effect Concentration (LOEC) and the No Observable Effect Concentration (NOEC).

SURVIVAL***Ceriodaphnia dubia***

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

LOEC: Not Applicable

NOEC: 100% Effluent

REPRODUCTION***Ceriodaphnia dubia***

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

LOEC: Not Applicable

NOEC: 100% Effluent

PMSD: 9.7%

TEST SETUP***Pimephales promelas***

The seven-day *Pimephales promelas* larval survival and growth test was initiated at 1615 hours, September 22, 2015. 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 1615 hours, September 29, 2015. At test termination, all larvae were sacrificed, dried for 24-hours, and weighed. Survival and growth (weight) data were statistically analyzed ($p = 0.05$) according to EPA procedures to determine the Lowest Observable Effect Concentration (LOEC) and the No Observable Effect Concentration (NOEC).

SURVIVAL
Pimephales promelas

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

LOEC: Not Applicable
NOEC: 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: 10.9%**
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.

Huth and Associates

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

CLIENT City of Mena WWTF SAMPLE TYPE 24 Hour Composite
 NPDES # AR0036692 DATE COLLECTED 09/21/15 09/23/15 09/25/15
 LAB ID # 24668 DATE RECEIVED 09/21/15 09/23/15 09/25/15
 TEST TYPE 7 Day Chronic BEGIN DATE/TIME 09/22/15 1300
 TEST ORGANISM *Ceriodaphnia dubia* END DATE/TIME 09/29/15 1300
 ORGANISM AGE < 24 Hours TEST TEMPERATURE (°C) 25 ± 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. cndl.
 DILUTION WATER Laboratory TECHNICIAN Z. Geiger

SURVIVAL & REPRODUCTION SUMMARY

Control

Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
09/23/15	A	A	A	A	A	A	A	A	A	A
09/24/15	A	A	A	A	A	A	A	A	A	A
09/25/15	A	A	A	A	A	A	A	A	A	A
09/26/15	0	0	0	0	0	0	0	0	0	0
09/27/15	3	4	4	2	5	5	4	5	2	4
09/28/15	6	7	11	7	11	8	7	7	9	8
09/29/15	9	11	16	9	16	13	11	12	11	12
	14	14	13	15	11	13	11	14	11	13
	23	25	28	24	27	26	22	26	22	25
x # Young		24.8		C.V.		8.24%				
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
09/23/15	A	A	A	A	A	A	A	A	A	A
09/24/15	A	A	A	A	A	A	A	A	A	A
09/25/15	A	A	A	A	A	A	A	A	A	A
09/26/15	0	0	0	0	0	0	0	0	0	0
09/27/15	2	3	4	5	4	3	5	3	5	2
09/28/15	9	7	9	6	6	6	8	8	9	6
09/29/15	11	10	13	11	10	9	13	11	14	8
	12	12	15	15	11	14	15	11	14	15
	23	22	28	26	21	23	28	22	26	23
x # Young		24.4		C.V.		11.46%				
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
09/23/15	A	A	A	A	A	A	A	A	A	A
09/24/15	A	A	A	A	A	A	A	A	A	A
09/25/15	A	A	A	A	A	A	A	A	A	A
09/26/15	0	0	0	0	0	0	0	0	0	0
09/27/15	3	4	4	2	5	5	3	5	3	3
09/28/15	8	8	9	9	11	10	9	7	8	7
09/29/15	11	10	13	11	16	15	12	12	11	10
	12	14	15	13	13	11	12	13	13	12
	23	24	28	24	29	26	24	25	24	22
x # Young		24.9		C.V.		8.77%				
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
09/23/15	A	A	A	A	A	A	A	A	A	A
09/24/15	A	A	A	A	A	A	A	A	A	A
09/25/15	A	A	A	A	A	A	A	A	A	A
09/26/15	0	0	0	0	0	0	0	0	0	0
09/27/15	2	4	5	3	5	4	2	3	5	3
09/28/15	7	8	9	7	11	10	7	11	8	7
09/29/15	9	12	14	10	16	14	9	14	13	10
	14	11	13	13	15	12	12	11	14	14
	23	23	27	23	31	26	21	25	27	24
x # Young		25.0		C.V.		11.47%				
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

City of Mena WWTF

Lab ID# 24668

Test Date: September 22, 2015

75% Effluent										
Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
09/23/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
09/24/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
09/25/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
09/26/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
09/27/15	3	5	2	5	3	3	5	4	4	4
	3	5	2	5	3	3	5	4	4	4
09/28/15	6	8	9	6	10	9	6	11	6	6
	9	13	11	11	13	12	11	15	10	10
09/29/15	14	13	14	14	11	12	12	14	15	12
	23	26	25	25	24	24	23	29	25	22
<p>x# Young 24.6 C.V. 7.95%</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
09/23/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
09/24/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
09/25/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
09/26/15	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
09/27/15	2	3	4	3	4	2	4	3	3	4
	2	3	4	3	4	2	4	3	3	4
09/28/15	9	8	10	8	11	8	7	7	9	6
	11	11	14	11	15	10	11	10	12	10
09/29/15	13	14	14	14	11	12	14	12	13	13
	24	25	28	25	26	22	25	22	25	23
<p>x# Young 24.5 C.V. 7.51%</p> <p>x% Survival 30% C.V. 161.02%</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

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

City of Mena WWTF

Lab ID# 24668

Test Date: September 22, 2015

WET CHEMISTRY MEASUREMENTS

Date	Time	Temp	Samp. No.	pH of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
09/22/15	Start	25.0	1	7.54	7.14	7.04	6.88	6.68	6.47	GP
09/23/15	24 Hr.	24.8	1	7.72	7.53	7.28	7.16	7.06	7.02	TB
09/23/15	Renew	25.0	1	7.71	7.39	7.25	7.07	6.87	6.70	TB
09/24/15	48 Hr.	24.9	1	7.79	7.57	7.50	7.34	7.21	6.97	GP
09/24/15	Renew	25.0	2	7.69	7.48	7.40	7.21	7.01	6.83	GP
09/25/15	72 Hr.	24.9	2	7.44	7.08	7.03	6.92	6.80	6.61	GP
09/25/15	Renew	25.0	2	7.50	7.15	7.10	6.95	6.81	6.61	GP
09/26/15	96 Hr.	24.8	2	7.78	7.59	7.62	7.49	7.84	7.90	CA
09/26/15	Renew	25.0	3	7.53	7.40	7.69	7.30	7.52	7.10	CA
09/27/15	120 Hr.	24.7	3	7.22	7.02	7.79	7.45	7.53	7.01	CA
09/27/15	Renew	25.0	3	7.63	7.46	7.75	7.84	7.83	7.26	CA
09/28/15	144 Hr.	24.7	3	7.79	7.58	7.33	7.18	7.12	7.08	RK
09/28/15	Renew	24.6	3	7.16	7.14	7.20	7.15	7.06	7.00	RK
09/29/15	168 Hr.	24.8	3	7.62	7.26	7.16	7.08	6.92	6.87	GP

Date	Time	Temp	Samp. No.	DO (mg/L) of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
09/22/15	Start	25.0	1	7.98	8.46	8.44	8.46	8.58	8.62	GP
09/23/15	24 Hr.	24.8	1	8.22	8.22	8.31	8.16	7.78	7.98	TB
09/23/15	Renew	25.0	1	8.28	8.44	8.39	8.28	8.27	8.37	TB
09/24/15	48 Hr.	24.9	1	7.84	8.77	8.71	8.70	8.86	8.62	GP
09/24/15	Renew	25.0	2	7.87	7.96	8.14	8.22	8.02	8.62	GP
09/25/15	72 Hr.	24.9	2	8.39	7.33	7.81	7.95	8.05	8.30	GP
09/25/15	Renew	25.0	2	8.18	7.53	7.84	7.85	8.09	8.26	GP
09/26/15	96 Hr.	24.8	2	8.23	8.97	8.35	8.36	8.69	8.85	CA
09/26/15	Renew	25.0	3	7.90	7.80	8.66	7.75	7.83	8.11	CA
09/27/15	120 Hr.	24.7	3	7.98	7.60	9.00	8.13	8.50	8.37	CA
09/27/15	Renew	25.0	3	7.50	8.59	7.64	8.00	8.07	7.71	CA
09/28/15	144 Hr.	24.7	3	8.49	7.82	8.41	8.13	8.11	8.07	RK
09/28/15	Renew	24.6	3	7.82	8.15	8.12	8.12	8.09	8.35	RK
09/29/15	168 Hr.	24.8	3	7.55	8.38	7.18	8.40	7.12	7.05	GP

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

City of Mena WWTF

Lab ID# 24668

Test Date: September 22, 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
09/22/15	1	6.47	8.62	28	16	188	<0.01	N/A	TG
09/24/15	2	6.83	8.62	28	12	203	<0.01	N/A	TG
09/26/15	3	7.10	8.11	32	14	164	<0.01	N/A	TG
09/22/15	Con	7.54	7.98	40	32	181	-	-	TG

¹ Measurements taken in 100% solution.

Huther and Associates, Inc.
 Begin Date: September 22, 2015
 Lab I.D.# 24668

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	24.800
2	32% Effluent	10	21.000	28.000	24.400
3	42% Effluent	10	22.000	29.000	24.900
4	56% Effluent	10	21.000	31.000	25.000
5	75% Effluent	10	22.000	29.000	24.600
6	100% Effluent	10	22.000	28.000	24.500

Summary Statistics on Transformed Data Table 2 of 2

Grp	Identification	Variance	Sd	Sem	C.V.%
1	Control	4.178	2.044	0.646	8.24
2	32% Effluent	7.822	2.797	0.884	11.46
3	42% Effluent	4.767	2.183	0.690	8.77
4	56% Effluent	8.222	2.867	0.907	11.47
5	75% Effluent	3.822	1.955	0.618	7.95
6	100% Effluent	3.389	1.841	0.582	7.51

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	0	19	23	14	4

Calculated Chi-Square goodness of fit test statistic = 5.4213
 Table Chi-Square value (alpha = 0.01) = 13.277

Data Pass normality test. Continue analysis.

Bartlett's Test For Homogeneity of Variance

Calculated B1 statistic = 1.97

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	2.800	0.560	0.104
Within (Error)	54	289.800	5.367	
Total	59	292.600		

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		T Stat	Sig
			Original Units	Calculated In		
1	Control	24.800	24.800			
2	32% Effluent	24.400	24.400		0.386	
3	42% Effluent	24.900	24.900		-0.097	
4	56% Effluent	25.000	25.000		-0.193	
5	75% Effluent	24.600	24.600		0.193	
6	100% Effluent	24.500	24.500		0.290	

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	2.393	9.7	0.400
3	42% Effluent	10	2.393	9.7	-0.100
4	56% Effluent	10	2.393	9.7	-0.200
5	75% Effluent	10	2.393	9.7	0.200
6	100% Effluent	10	2.393	9.7	0.300

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

CLIENT	City of Menā WWTF	SAMPLE TYPE	24 Hour Composite
NPDES #	AR0036692	DATE COLLECTED	09/21/15 09/23/15 09/25/15
LAB ID #	24668	DATE RECEIVED	09/21/15 09/23/15 09/25/15
TEST TYPE	7 Day Chronic	BEGIN DATE/TIME	09/22/15 1615
TEST ORGANISM	<i>Pimephales promelas</i>	END DATE/TIME	09/29/15 1615
ORGANISM AGE	< 24 Hours	TEST TEMPERATURE (°C)	25 ± 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. cndl.
DILUTION WATER	Laboratory	TECHNICIAN	T. Burton

SURVIVAL SUMMARY

Conc.	09/23/15					09/24/15					09/25/15					09/26/15					09/27/15				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Con	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
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.	09/28/15					09/29/15					x % Survival	C.V. %
	A	B	C	D	E	A	B	C	D	E		
Con	8	8	8	8	8	8	8	8	8	8	100.0	0.00
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	0.0	0.00

MEAN DRY WEIGHT PER REP

% Effluent	Rep A	Rep B	Rep C	Rep D	Rep E	x	C.V. %
Con	0.4810	0.4270	0.5010	0.4160	0.4200	0.4490	8.73
32%	0.4760	0.4150	0.4820	0.4960	0.4500	0.4638	6.89
42%	0.4920	0.4560	0.4270	0.5030	0.4810	0.4718	6.47
56%	0.4450	0.4760	0.4960	0.4260	0.5040	0.4694	7.09
75%	0.4960	0.4800	0.4240	0.4910	0.4800	0.4742	6.10
100%	0.4270	0.4710	0.5040	0.4440	0.4920	0.4676	6.88

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

City of Mena WWTF

Lab ID# 24668

Test Date: September 22, 2015

WET CHEMISTRY MEASUREMENTS

Date	Time	Temp	Samp. No.	pH of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
09/22/15	Start	25.0	1	7.54	7.14	7.04	6.88	6.68	6.47	GP
09/23/15	24 Hr.	25.1	1	7.29	7.13	6.99	6.84	6.79	6.65	TB
09/23/15	Renew	25.0	1	7.71	7.39	7.25	7.07	6.87	6.70	TB
09/24/15	48 Hr.	25.1	1	7.43	7.15	7.17	7.08	6.96	6.71	GP
09/24/15	Renew	25.0	2	7.69	7.48	7.40	7.21	7.01	6.83	GP
09/25/15	72 Hr.	25.2	2	7.63	7.38	7.22	7.11	6.97	6.81	GP
09/25/15	Renew	25.0	2	7.50	7.15	7.10	6.95	6.81	6.61	GP
09/26/15	96 Hr.	24.7	2	7.31	7.39	7.98	7.94	7.96	7.00	CA
09/26/15	Renew	25.0	3	7.53	7.40	7.69	7.30	7.52	7.10	CA
09/27/15	120 Hr.	24.9	3	7.22	7.24	7.80	7.76	7.50	7.44	CA
09/27/15	Renew	25.0	3	7.63	7.46	7.75	7.84	7.83	7.26	CA
09/28/15	144 Hr.	24.8	3	7.54	7.23	7.28	7.11	7.05	7.06	RK
09/28/15	Renew	24.6	3	7.16	7.14	7.20	7.15	7.06	7.00	RK
09/29/15	168 Hr.	25.1	3	7.38	7.14	7.04	6.91	6.87	6.65	GP

Date	Time	Temp	Samp. No.	DO (mg/L) of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
09/22/15	Start	25.0	1	7.98	8.46	8.44	8.46	8.58	8.62	GP
09/23/15	24 Hr.	25.1	1	8.18	7.98	8.03	7.92	7.88	7.45	TB
09/23/15	Renew	25.0	1	8.28	8.44	8.39	8.28	8.27	8.37	TB
09/24/15	48 Hr.	25.1	1	8.06	7.09	7.29	7.47	7.72	8.00	GP
09/24/15	Renew	25.0	2	7.87	7.96	8.14	8.22	8.02	8.62	GP
09/25/15	72 Hr.	25.2	2	8.29	8.50	7.45	8.42	7.35	8.13	GP
09/25/15	Renew	25.0	2	8.18	7.53	7.84	7.85	8.09	8.26	GP
09/26/15	96 Hr.	24.7	2	7.52	8.39	8.47	7.72	8.98	7.74	CA
09/26/15	Renew	25.0	3	7.90	7.80	8.66	7.75	7.83	8.11	CA
09/27/15	120 Hr.	24.9	3	8.65	7.69	8.85	8.92	8.84	8.68	CA
09/27/15	Renew	25.0	3	7.50	8.59	7.64	8.00	8.07	7.71	CA
09/28/15	144 Hr.	24.8	3	8.94	7.63	7.84	8.21	8.20	8.64	RK
09/28/15	Renew	24.6	3	7.82	8.15	8.12	8.12	8.09	8.35	RK
09/29/15	168 Hr.	25.1	3	7.63	7.81	7.45	7.51	7.45	8.10	GP

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

City of Mena WWTF

Lab ID# 24668

Test Date: September 22, 2015

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
09/22/15	1	6.47	8.62	28	16	188	<0.01	N/A	TG
09/24/15	2	6.83	8.62	28	12	203	<0.01	N/A	TG
09/26/15	3	7.10	8.11	32	14	164	<0.01	N/A	TG
09/22/15	Con	7.54	7.98	40	32	181	-	-	TG

¹ Measurements taken in 100% solution.

PIMEPHALES PROMELAS STATISTICAL ANALYSES
 Growth

Summary Statistics on Transformed Data Table 1 of 2

Grp	Identification	N	Min	Max	Mean
1	Control	5	0.416	0.501	0.449
2	32% Effluent	5	0.415	0.496	0.464
3	42% Effluent	5	0.427	0.503	0.472
4	56% Effluent	5	0.426	0.504	0.469
5	75% Effluent	5	0.424	0.496	0.474
6	100% Effluent	5	0.427	0.504	0.468

Summary Statistics on Transformed Data Table 2 of 2

Grp	Identification	Variance	Sd	Sem	C.V.%
1	Control	0.002	0.039	0.018	8.73
2	32% Effluent	0.001	0.032	0.014	6.89
3	42% Effluent	0.001	0.031	0.014	6.47
4	56% Effluent	0.001	0.033	0.015	7.09
5	75% Effluent	0.001	0.029	0.013	6.10
6	100% Effluent	0.001	0.032	0.014	6.88

Shapiro - Wilk's Test For Normality

D = 0.026

W = 0.931

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 = 0.41

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

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

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

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.002	0.000	0.379
Within (Error)	24	0.026	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

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

Grp	Identification	Transformed Mean	Mean	T Stat	Sig
			Calculated In Original Units		
1	Control	0.449	0.449		
2	32% Effluent	0.464	0.464	-0.713	
3	42% Effluent	0.472	0.472	-1.098	
4	56% Effluent	0.469	0.469	-0.983	
5	75% Effluent	0.474	0.474	-1.214	
6	100% Effluent	0.468	0.468	-0.896	

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

No statistically significant difference

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

Grp	Identification	Num of Reps	Minimum Sig Diff	% of Control	Difference from Control
			(In Orig. Units)		
1	Control	5			
2	32% Effluent	5	0.049	10.9	-0.015
3	42% Effluent	5	0.049	10.9	-0.023
4	56% Effluent	5	0.049	10.9	-0.020
5	75% Effluent	5	0.049	10.9	-0.025
6	100% Effluent	5	0.049	10.9	-0.019

**APPENDIX A
RAW DATA**

7-DAY CERIODAPHNIA DUBIA SURVIVAL & REPRODUCTION

DAILY RAW DATA TABLE

PAGE 1 OF 2

CLIENT Mena

START DATE/TIME 9-22-15 ZG 1300

OUTFALL 001

END DATE/TIME 9-29-15 RK 1300

LAB ID # 24668

CON

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
9/23	A	A	A	A	A	A	A	A	A	A	ZG	1300
9/24	A	A	A	A	A	A	A	A	A	A	ZG	1420
9/25	A	A	A	A	A	A	A	A	A	A	MH	1030
9/26	A	A	A	A	A	A	A	A	A	A	ZG	1320
9/27	3	4	4	2	5	5	4	5	2	4	ZG	1000
9/28	6	7	11	7	11	8	7	7	9	8	MH	1330
9/29	14	14	13	15	11	13	11	14	11	13	RK	1300

026

\bar{x} # Young w/o Dead = 24.8 CV% = 8.24

\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
9/23	A	A	A	A	A	A	A	A	A	A	ZG	1300
9/24	A	A	A	A	A	A	A	A	A	A	ZG	1420
9/25	A	A	A	A	A	A	A	A	A	A	MH	1030
9/26	A	A	A	A	A	A	A	A	A	A	ZG	1320
9/27	2	3	4	5	4	3	5	3	5	2	ZG	1000
9/28	9	7	9	6	6	6	8	8	9	6	MH	1330
9/29	12	12	15	15	11	14	15	11	14	5	RK	1300

\bar{x} # Young w/o Dead = 24.4 CV% = 11.46

\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
9/23	A	A	A	A	A	A	A	A	A	A	ZG	1300
9/24	A	A	A	A	A	A	A	A	A	A	ZG	1420
9/25	A	A	A	A	A	A	A	A	A	A	MH	1030
9/26	A	A	A	A	A	A	A	A	A	A	ZG	1320
9/27	3	4	4	2	5	5	3	5	3	3	ZG	1000
9/28	8	6	9	9	11	10	9	7	8	7	MH	1330
9/29	12	14	15	13	13	11	12	13	13	12	RK	1300

\bar{x} # Young w/o Dead = 24.9 CV% = 8.77

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

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

56

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
9/23	A	A	A	A	A	A	A	A	A	A	ZG	1300
9/24	A	A	A	A	A	A	A	A	A	A	ZG	1420
9/25	A	A	A	A	A	A	A	A	A	A	MH	1030
9/26	A	A	A	A	A	A	A	A	A	A	ZG	1320
9/27	2	4	5	3	5	4	2	3	5	3	ZG	1000
9/28	7	8	9	7	11	10	7	11	8	7	MH	1330
9/29	14	11	13	13	15	12	12	11	14	14	RK	1300

\bar{x} # Young w/o Dead = 25.0 CV% = 11.47

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

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

OTG 9/30

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

CLIENT/FACILITY Mena
 OUTFALL # 001 PROJECT # 24668
 ORGANISM ID# PPO-15-264

DATE/TIME STARTED 7-22-15 TB 1615
 DATE/TIME ENDED 9-29-15 TB 1615

Conc.	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					
32	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					
56	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					
100	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8					
Initials Date/Time	9-23-15 MH 1615					9-24-15 TB 0835					9-25-15 TB 0855					9-26-15 ZG 0855					9-27-15 RF0840				

Conc.	A	B	C	D	E	A	B	C	D	E	Mean Survival	C.V. %
CON	8	8	8	8	8	8	8	8	8	8	100.0	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	9-28-15 RF 0900					9-29-15 TB 1615						

Client / Facility Mend
 Lab ID Number 24668
 Outfall Number 001
 Test Date 9-22-15

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
9/22	1	6.47	8.62	28	16	188	10.01	Na	TE
9/24	2	6.83	8.102	28	12	203	5	5	5
9/26	3	7.10	8.11	32	14	264	5	5	5
9/22	CON	7.54	7.98	40	32	181	—	—	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

Notes:

**APPENDIX B
REFERENCE TOXICANTS**

CHRONIC REFERENCE TOXICANT TEST RESULTS

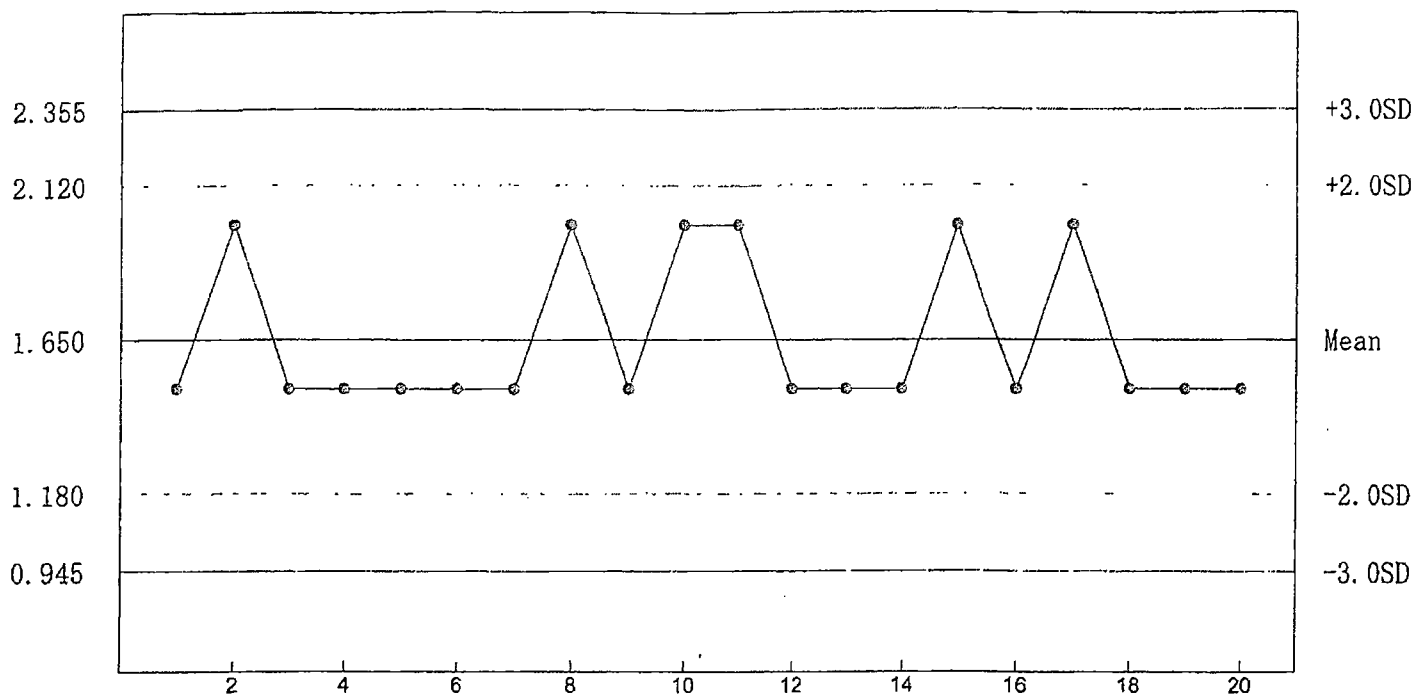
SPECIES: *Ceriodaphnia dubia*
 CHEMICAL: Sodium Chloride
 DURATION: 7-Days
 TEST NUMBER: 9
 TEST DATE: 09/02/15 - 09/09/15
 1610 Hrs - 1610 Hrs
 STATISTICAL METHOD: Dunnetts/Steels

CONCENTRATION (g/L)	NUMBER EXPOSED	NUMBER DEAD
0.5	10	0
1.0	10	0
1.5	10	0
2.0	10	6
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

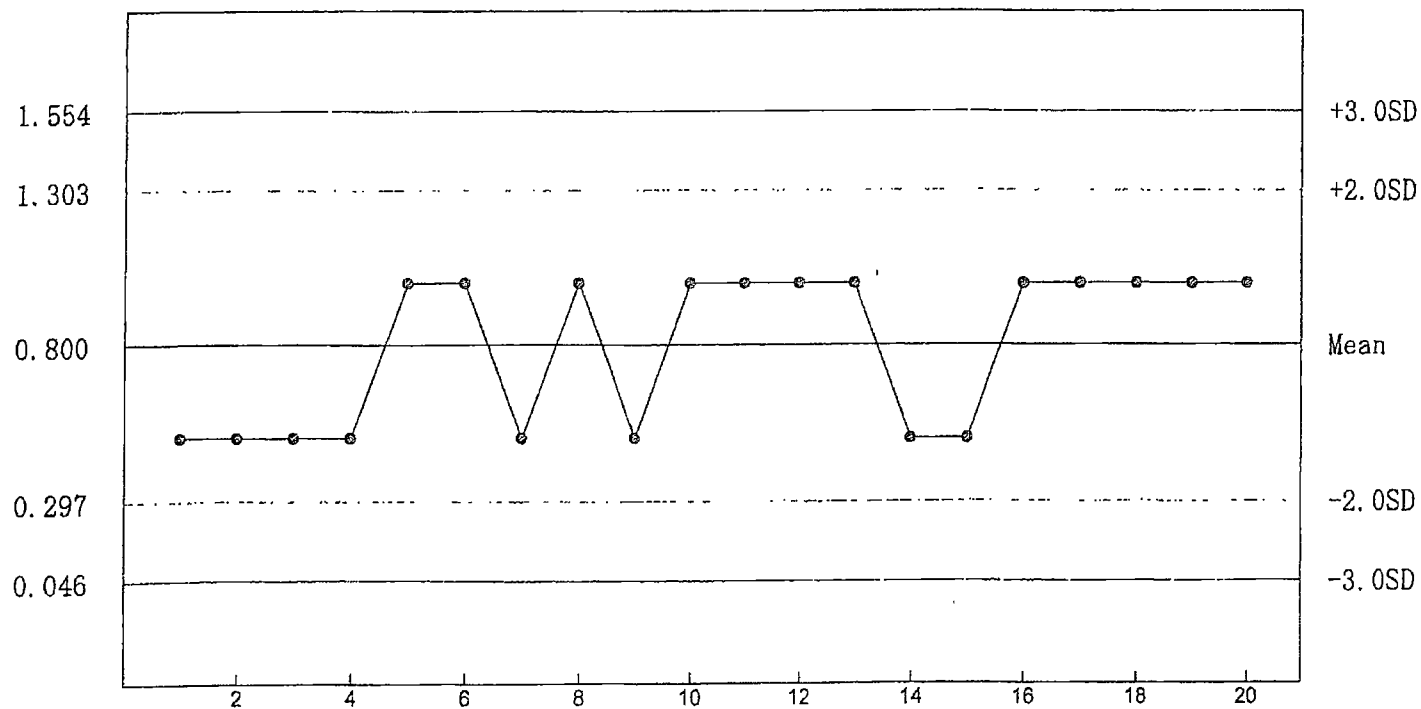
C. dubia Survival - NOEC



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= 0.800 SD= 0.251 CV= 31.41% Min= 0.500 Max= 1.000

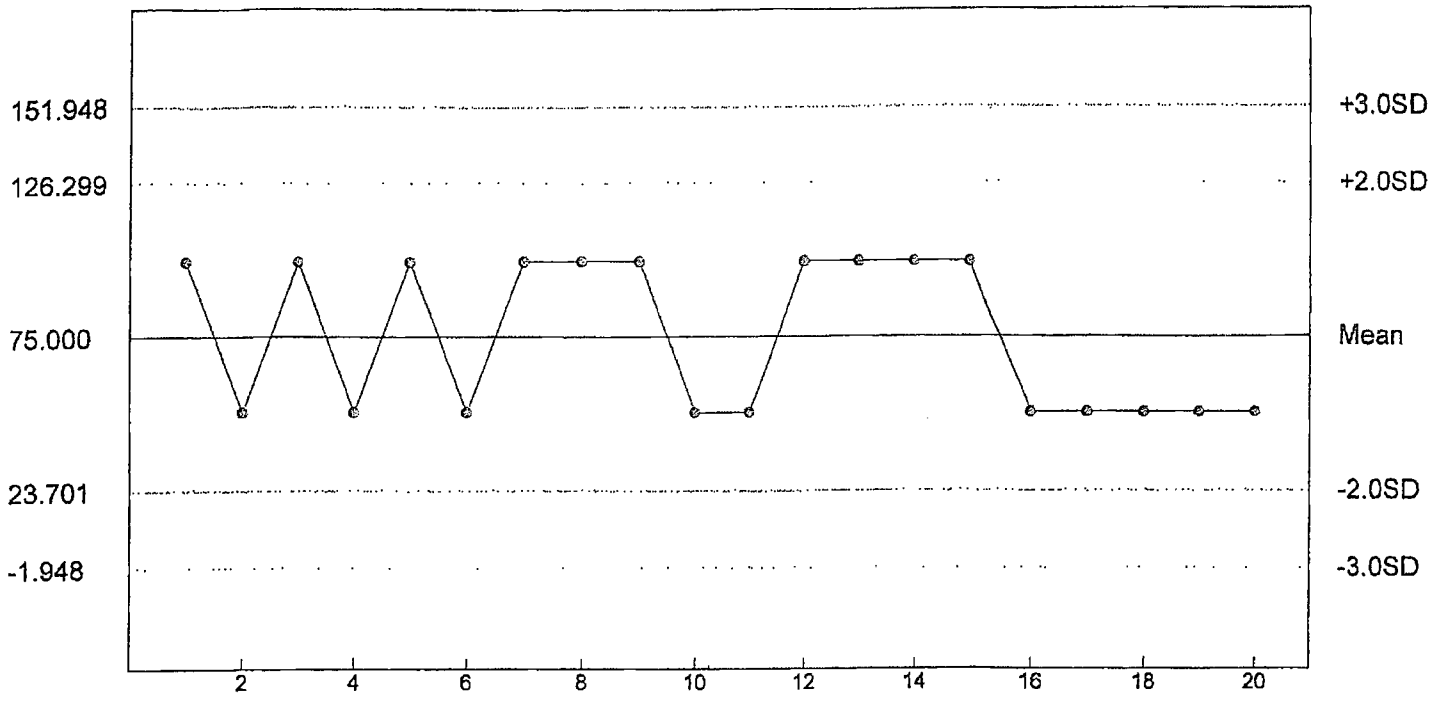
CHRONIC REFERENCE TOXICANT TEST RESULTS

SPECIES: *Pimephales promelas*
 CHEMICAL: Copper Nitrate
 DURATION: 7-Days
 TEST NUMBER: 9
 TEST DATE: 09/02/15 - 09/09/15
 1530 Hrs - 1530 Hrs
 STATISTICAL METHOD: Dunnetts/Steels

CONCENTRATION (ug/L)	NUMBER EXPOSED	NUMBER DEAD
25	40	0
50	40	0
100	40	10
200	40	28
400	40	39
800	40	40

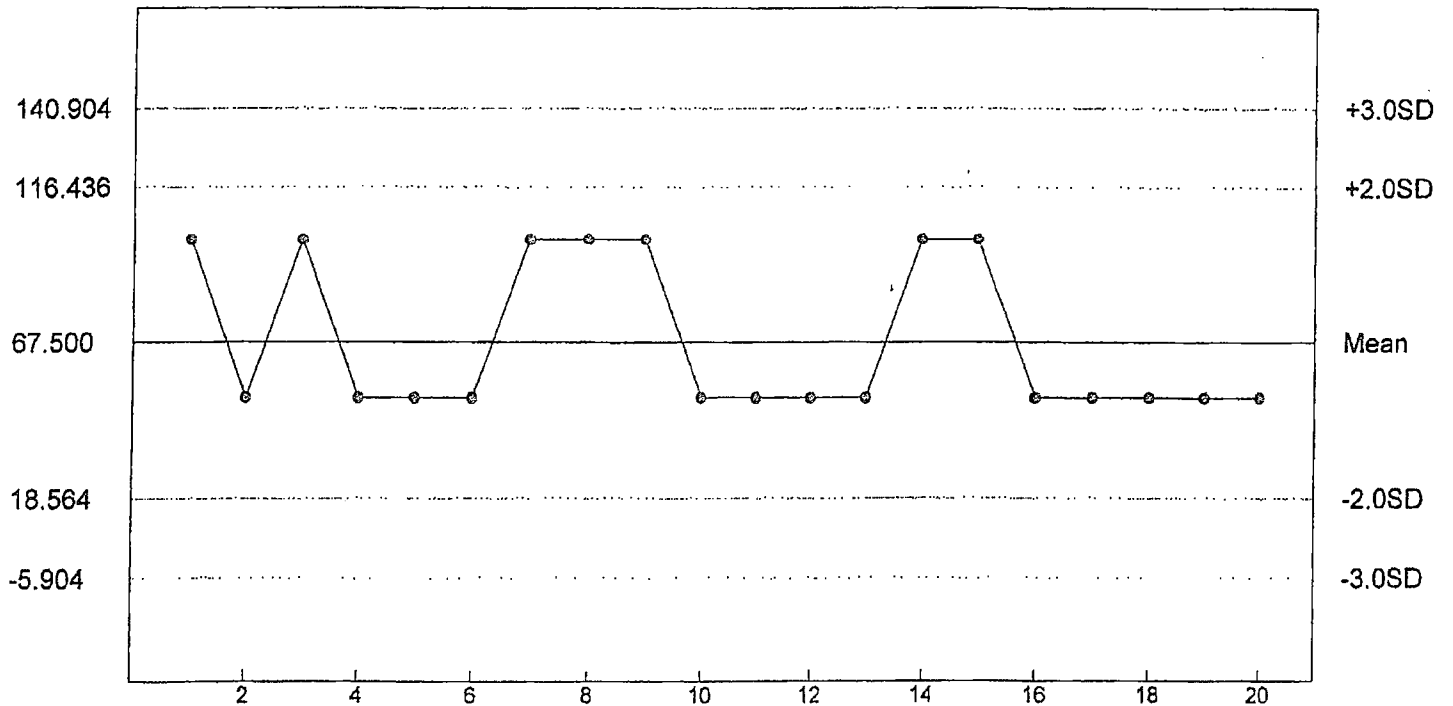
LOEC FOR SURVIVAL	NOEC FOR SURVIVAL	LOEC FOR GROWTH	NOEC FOR GROWTH
100 ug/L	50 ug/L	100 ug/L	50 ug/L

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



n= 20 Mean= 75.000 SD= 25.649 CV= 34.20% 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

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

CHAIN OF CUSTODY RECORD

PROJECT # 24608 PROJECT NAME Mena PERMIT# AR 0036092

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.	
OUT 1	Mike	0800 25 SEPT 15	0800 21 SEPT 15	24	AUTO	—	AUTO	1

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 C/F

NAME OF RECEIVING WATER unt of Prairie Creek

DILUTION WATER USED FOR THIS TEST Lab

RELINQUISHED BY: Mike DATE: 21 SEPT TIME: 1000 RECEIVED BY AT THIS DATE/TIME Rance @ Huther

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 _____

RECEIVED: Rance DATE: 9/21/15 TIME: 1900 SAMPLE TEMP. @ RECEIPT. 9°

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

CHAIN OF CUSTODY RECORD

PROJECT # 24608 PROJECT NAME Mena PERMIT# AR6036692

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.	
1	M/K	0400 22 SEP	0800 23 SEP	24	AUT	—	AUTO	1

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 7day C/F
 NAME OF RECEIVING WATER ent of prairie creek
 DILUTION WATER USED FOR THIS TEST lab

RELINQUISHED BY: Mike Sencer DATE: 22 SEP TIME: 1000 RECEIVED BY AT THIS DATE/TIME: Rance@Huther

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 _____

RECEIVED: Rance Parrott DATE: 9/23/15 TIME: 1800 SAMPLE TEMP. @ RECEIPT. 4°

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

CHAIN OF CUSTODY RECORD

PROJECT # 246068 PROJECT NAME Mena PERMIT# AR0036092

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.	
OUTFALL 1	MIKE	24 SEPT 15 6:00	25 SEPT 15 6:00	24	AUTO	—	—	1

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 C/F
 NAME OF RECEIVING WATER ent of Prairie Creek
 DILUTION WATER USED FOR THIS TEST Lab

RELINQUISHED BY: Mike Spencer DATE: 25 SEPT TIME: 8:00 RECEIVED BY AT THIS DATE/TIME HUTHER DRIVER @ Broken Bow
 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 _____
 RECEIVED: Rance Barnett DATE: 9/25/15 TIME: 1730 SAMPLE TEMP. @ RECEIPT. 4°

**CITY OF MENA WWTF
 NPDES PERMIT NO. AR0036692
 AFIN 57-00042
 BIOMONITORING REPORTING
 TEST DATE: 09/22/15**

<i>Ceriodaphnia dubia</i>	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	8.24%
Report Parameter No. 22414 (lowest NOEC value) for <i>Ceriodaphnia dubia</i> .	100%

<i>Pimephales promelas</i>	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 growth. Parameter TPP6C	100%
D. If the NOEC for growth 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	8.73%
Report Parameter No. 22414 (lowest NOEC value) for <i>Pimephales promelas</i> .	100%

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