



MENA WATER UTILITIES

701 MENA STREET ~ MENA, ARKANSAS ~ 71953
PH (479) 394-2761 ~ FAX (479) 394-5053

October 22 2013
Shane Byrum
NPDES Permit Section, Water Division

RE: Bio- Monitoring Frequency Reduction
City Of Mena number AR0036692

Dear Mr. Byrum:

1. Attached are copies of quarterly bio monitoring results for the year 2013, from Huther and Associates, which is the City of Mena's wet testing lab for bio monitoring.
2. The second attachment is an email between Mary Barnett ADEQ, and Bruce Huther of Huther and Associates, subj: City of Mena bio test.

Since shifting labs in September 2012, the City of Mena has successfully passed 6 quarters of bio monitoring passing both lethal and sub lethal on both species as documented in attachment one.

With reference to attachment two, the last quarter of 2013 (Oct 2013) only two of the three samples made it to lab due to courier mistake, conversation is as listed.

The City is writing this letter to request bio monitoring frequency reduction, as listed in Part 2, of permit number AR0036692 to annually vice quarterly.

Sincerely,

 22 OCT 13
Mike Spencer
Mena Wastewater Supervisor

Mike Spencer

2

From: HUTHER ASSOCIATES <huther@flash.net>
Sent: Monday, October 21, 2013 4:44 PM
To: Mike Spencer
Subject: Fw: City of Mena bio test

Mike,

Save for your records. Reports went out today.

Bruce

----- Forwarded Message -----

From: "Barnett, Mary" <BARNETT@adeq.state.ar.us>
To: "HUTHER ASSOCIATES (huther@flash.net)" <huther@flash.net>
Sent: Monday, October 21, 2013 4:26 PM
Subject: RE: City of Mena bio test

Bruce,

As per EPA's Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition, October 2002, an effluent sample should not be used for renewal more than 72 hours after first use. However, the manual also states 'If shipping problems (e.g., unsuccessful Saturday delivery) are encountered with renewal samples after a test has been initiated, the permitting authority may allow the continued use of the most recently used sample for test renewal.

Due to unintended extenuating circumstances involving the sample courier, and since the facility typically passes all four WET testing endpoints, the Department has determined Mena's October 2013 test is an acceptable test.

The Department encourages the facility and laboratory to work with the couriers to ensure that all subsequent WET test samples are received within EPA's sampling handling requirements.

Mary Barnett
Ecologist Coordinator
501-682-0666

From: HUTHER ASSOCIATES [mailto:huther@flash.net]
Sent: Wednesday, October 16, 2013 4:16 PM
To: Clem, Sarah
Subject: City of Mena bio test

Sara,

Huther and Associates recently conducted chronic C. dubia and P. promelas tests for the City of Mena (AR0036692). Samples were shipped to our lab via UPS. The third sample, which was clearly labeled "Saturday Delivery", did not arrive until Tuesday (test termination date). UPS sent the sample to Kentucky by accident. There was enough sample #2 to keep the tests going with daily renewals. Both tests passed lethal and sublethal endpoints.

The chronic manual, Page 32, 8.5.4, last sentence does address potential shipping problems and states that the permitting authority may allow the continued use of the most recently used sample (isolated situations).

Please let me know if you will accept these tests (tests met all other test acceptability criteria) or should the facility retest.

Thank you for your consideration,

Bruce Huther
Huther and Associates
(940) 387-1025
huther@flash.net

**CITY OF MENA WWTF
OUTFALL 001**

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

Ceriodaphnia dubia
Pimephales promelas

January 15, 2013

Reviewed by: _____


Toni Nesbitt, QA/QC Officer
Huther & Associates, Inc.
1156 North Bonnie Brae
Denton, Texas 76201
(940) 387-1025, Fax: (940) 387-1036

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TOXICITY TEST REPORT - CHRONIC

ClientCity of Mena WWTF
Permit No.NPDES AR0036692
SampleOutfall 001

Laboratory I.D.20520
Begin DateJanuary 15, 2013

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 United Parcel Service courier to Huth & Associates on January 15, January 17, and January 19, 2013. Effluent samples were collected and composited from Outfall 001 using an automatic sampler. Two toxicity tests were requested: a seven-day *Ceriodaphnia dubia* survival and reproduction test (EPA Method 1002.0), and a seven-day *Pimephales promelas* larval survival and growth test (EPA Method 1000.0). Test organisms, procedures and quality assurance requirements were in accordance with the EPA manual, "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition" (EPA-821-R-02-013).

The effluent 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 1555 hours, January 15, 2013. 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 1555 hours, January 22, 2013. Survival and reproduction data were statistically analyzed ($p = 0.05$) according to EPA procedures to determine the Lowest Observable Effect Concentration (LOEC) and the No Observable Effect Concentration (NOEC).

SURVIVAL***Ceriodaphnia dubia***

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

LOEC: Not Applicable

NOEC: 100% Effluent

REPRODUCTION***Ceriodaphnia dubia***

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

LOEC: Not Applicable

PMSD: 13.9%

NOEC: 100% Effluent

TEST SETUP***Pimephales promelas***

The seven-day *Pimephales promelas* larval survival and growth test was initiated at 1540 hours, January 15, 2013. 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 1540 hours, January 22, 2013. 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
NOEC: 100% Effluent

PMSD: 10.8%

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 Mena WWTF	SAMPLE TYPE 24 Hour Composite
NPDES # AR0036692	DATE COLLECTED 01/14/13 01/16/13 01/18/13
LAB ID # 20520	DATE RECEIVED 01/15/13 01/17/13 01/19/13
TEST TYPE 7 Day Chronic	BEGIN DATE/TIME 01/15/13 1555
TEST ORGANISM <i>Ceriodaphnia dubia</i>	END DATE/TIME 01/22/13 1555
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. candl.
DILUTION WATER Laboratory Adjusted	TECHNICIAN T. Nesbitt

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	
01/16/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
01/17/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
01/18/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
01/19/13	A	A	A	5	A	A	4	4	A	A	
	0	0	0	5	0	0	4	4	0	0	
01/20/13	3	2	4	12	2	3	4	4	2	3	
	8	8	8	A	5	8	8	9	7	8	
01/21/13	11	8	12	12	7	9	12	13	9	9	
	12	11	12	13	10	11	13	13	12	10	
01/22/13	23	19	24	25	17	20	25	26	21	19	
x # Young 21.8 C.V. 14.10% 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	
01/16/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
01/17/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
01/18/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
01/19/13	A	5	A	8	A	5	A	A	A	4	
	0	5	0	8	0	5	0	0	0	4	
01/20/13	5	A	6	A	4	A	4	5	4	A	
	5	5	5	5	4	5	4	5	4	4	
01/21/13	11	8	11	10	8	7	8	10	8	11	
	18	13	17	16	12	12	10	15	12	15	
01/22/13	12	13	14	12	13	12	11	13	14	10	
	28	26	31	28	25	24	21	28	26	25	
x # Young 28.2 C.V. 10.48% 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	
01/16/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
01/17/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
01/18/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
01/19/13	A	A	A	A	A	A	A	4	5	3	
	0	0	0	0	0	0	0	4	5	3	
01/20/13	4	5	3	4	5	4	2	A	A	A	
	4	5	3	4	5	4	2	4	5	3	
01/21/13	8	9	8	8	7	10	8	8	10	7	
	12	14	9	12	12	14	8	12	15	10	
01/22/13	13	14	12	13	12	14	12	13	14	12	
	25	28	21	25	24	26	20	25	28	22	
x # Young 24.7 C.V. 12.38% 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	
01/16/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
01/17/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
01/18/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
01/19/13	A	5	4	2	3	A	3	4	A	4	
	0	5	4	2	3	0	3	4	0	4	
01/20/13	2	A	A	A	A	2	A	A	5	A	
	2	5	4	2	3	2	5	4	5	4	
01/21/13	8	10	10	7	9	8	8	7	8	8	
	8	16	14	9	12	8	11	11	14	12	
01/22/13	11	14	13	12	13	12	14	12	13	14	
	18	28	27	21	25	20	25	23	27	26	
x # Young 24.2 C.V. 13.75% x% Survival 100% C.V. 0.00%											

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

ex 1: A alive today
 4 total young to date

ex 2: 5 alive, 5 young today
 12 total young to date

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

City of Mena WWTF

Lab ID# 20520

Test Date: January 15, 2013

75% Effluent

Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
01/16/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
01/17/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
01/18/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
01/19/13	A	4	A	A	A	A	A	A	A	4
	0	4	0	0	0	0	0	0	0	4
01/20/13	4	A	4	5	3	4	5	3	3	A
	4	4	4	5	3	4	5	3	3	4
01/21/13	10	9	8	8	7	8	6	7	7	8
	14	13	10	11	10	12	11	10	10	12
01/22/13	14	14	12	13	12	13	11	12	13	14
	28	27	22	24	22	25	22	22	23	26
x # Young 24.1 C.V. 9.47% 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
01/16/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
01/17/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
01/18/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
01/19/13	2	3	A	A	A	A	3	4	3	5
	2	3	0	0	0	0	3	4	3	5
01/20/13	A	A	5	6	2	3	A	A	A	A
	2	3	5	6	2	3	3	4	3	5
01/21/13	8	7	8	9	7	7	7	7	6	10
	10	10	13	15	9	10	10	11	9	15
01/22/13	14	12	13	14	12	13	12	13	12	14
	24	22	26	29	21	23	22	24	21	29
x # Young 24.1 C.V. 12.44% 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

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

City of Mena WWTF

Lab ID# 20520

Test Date: January 15, 2013

WET CHEMISTRY MEASUREMENTS

Date	Time	Temp	Samp. No.	pH of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
01/15/13	Start	25.0	1	7.86	7.66	7.54	7.48	7.39	7.20	STC
01/16/13	24 Hr.	24.3	1	8.03	7.99	7.93	7.89	7.87	7.81	STC
01/16/13	Renew	24.8	1	7.76	7.67	7.47	7.39	7.30	7.22	STC
01/17/13	48 Hr.	24.2	1	8.03	7.88	7.90	7.86	7.83	7.80	CTT
01/17/13	Renew	25.0	2	7.71	7.58	7.49	7.42	7.35	7.30	CTT
01/18/13	72 Hr.	24.3	2	7.89	7.80	7.74	7.71	7.68	7.65	CTT
01/18/13	Renew	24.5	2	7.73	7.52	7.40	7.31	7.20	7.08	CTT
01/19/13	96 Hr.	24.3	2	7.93	7.73	7.71	7.53	7.44	7.37	CTT
01/19/13	Renew	25.0	3	8.21	7.65	7.51	7.42	7.33	7.26	CTT
01/20/13	120 Hr.	24.6	3	8.16	8.02	8.10	7.95	7.91	7.85	STC
01/20/13	Renew	24.6	3	7.73	7.61	7.45	7.32	7.24	7.11	STC
01/21/13	144 Hr.	24.7	3	7.55	7.49	7.47	7.72	7.73	7.71	STC
01/21/13	Renew	24.6	3	7.88	7.57	7.43	7.40	7.22	7.20	STC
01/22/13	168 Hr.	24.8	3	8.26	8.04	7.95	7.88	7.81	7.77	STC

Date	Time	Temp	Samp. No.	DO (mg/L) of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
01/15/13	Start	25.0	1	7.71	7.64	7.68	7.76	7.88	8.08	STC
01/16/13	24 Hr.	24.3	1	8.34	8.27	8.30	8.32	8.26	8.25	STC
01/16/13	Renew	24.8	1	8.89	8.70	8.85	8.86	8.89	8.89	STC
01/17/13	48 Hr.	24.2	1	7.70	7.83	7.72	7.67	7.64	7.68	CTT
01/17/13	Renew	25.0	2	8.03	7.98	7.96	7.96	8.00	8.01	CTT
01/18/13	72 Hr.	24.3	2	8.01	7.96	7.74	7.70	7.67	7.66	CTT
01/18/13	Renew	24.5	2	8.22	8.10	8.02	7.97	7.94	7.98	CTT
01/19/13	96 Hr.	24.3	2	8.55	8.57	8.63	8.66	8.74	8.62	CTT
01/19/13	Renew	25.0	3	8.76	7.98	7.97	8.03	8.15	8.22	CTT
01/20/13	120 Hr.	24.6	3	8.17	8.16	7.92	8.03	8.01	8.03	STC
01/20/13	Renew	24.6	3	7.70	7.58	7.53	7.58	7.62	7.65	STC
01/21/13	144 Hr.	24.7	3	7.86	7.74	7.65	7.69	8.05	8.23	STC
01/21/13	Renew	24.6	3	8.40	8.79	8.62	8.33	8.46	8.40	STC
01/22/13	168 Hr.	24.8	3	8.56	8.37	8.17	8.11	8.10	7.97	STC

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

City of Mena WWTF

Lab ID# 20520

Test Date: January 15, 2013

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
01/15/13	1	7.20	8.08	32	38	262	<0.01	N/A	TN
01/17/13	2	7.30	8.01	36	42	259	<0.01	N/A	TN
01/19/13	3	7.23	8.01	38	44	270	<0.01	N/A	TN
01/15/13	Con	7.86	7.71	48	38	238	-	-	TN

¹ Measurements taken in 100% solution.

CERIODAPHNIA DUBIA STATISTICAL ANALYSES
 Reproduction

Summary Statistics on Transformed Data Table 1 of 2

Grp	Identification	N	Min	Max	Mean
1	Control	10	17.000	26.000	21.900
2	32% Effluent	10	21.000	31.000	26.200
3	42% Effluent	10	20.000	29.000	24.700
4	56% Effluent	10	19.000	29.000	24.200
5	75% Effluent	10	22.000	28.000	24.100
6	100% Effluent	10	21.000	29.000	24.100

Summary Statistics on Transformed Data Table 2 of 2

Grp	Identification	Variance	Sd	Sem	C.V.%
1	Control	9.656	3.107	0.983	14.19
2	32% Effluent	7.511	2.741	0.867	10.46
3	42% Effluent	9.344	3.057	0.967	12.38
4	56% Effluent	11.067	3.327	1.052	13.75
5	75% Effluent	5.211	2.283	0.722	9.47
6	100% Effluent	8.989	2.998	0.948	12.44

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	16	19	17	4

Calculated Chi-Square goodness of fit test statistic = 1.2451
 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 = 1.40

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	95.600	19.120	2.216
Within (Error)	54	466.000	8.630	
Total	59	561.600		

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

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

Grp	Identification	Transformed Mean	Mean Calculated In Original Units	T Stat	Sig
1	Control	21.900	21.900		
2	32% Effluent	26.200	26.200	-3.273	
3	42% Effluent	24.700	24.700	-2.131	
4	56% Effluent	24.200	24.200	-1.751	
5	75% Effluent	24.100	24.100	-1.675	
6	100% Effluent	24.100	24.100	-1.675	

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

Dunnett's Test - Table 1 of 2 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	3.035	13.9	-4.300
3	42% Effluent	10	3.035	13.9	-2.800
4	56% Effluent	10	3.035	13.9	-2.300
5	75% Effluent	10	3.035	13.9	-2.200
6	100% Effluent	10	3.035	13.9	-2.200

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

CLIENT	City of Mena WWTF	SAMPLE TYPE	24 Hour Composite
NPDES #	AR0036692	DATE COLLECTED	01/14/13 01/16/13 01/18/13
LAB ID #	20520	DATE RECEIVED	01/15/13 01/17/13 01/19/13
TEST TYPE	7 Day Chronic	BEGIN DATE/TIME	01/15/13 1540
TEST ORGANISM	<i>Pimephales promelas</i>	END DATE/TIME	01/22/13 1540
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. candl.
DILUTION WATER	Laboratory Adjusted	TECHNICIAN	M. Hörner

SURVIVAL SUMMARY

Conc.	01/16/13					01/17/13					01/18/13					01/19/13					01/20/13				
	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.	01/21/13					01/22/13					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	0.4250	0.4790	0.4450	0.4830	0.4460	0.4556	5.42
32%	0.4850	0.5020	0.4160	0.5030	0.5000	0.4812	7.72
42%	0.4760	0.4900	0.4230	0.4860	0.5040	0.4758	6.55
56%	0.4960	0.5020	0.4480	0.4620	0.4750	0.4766	4.76
75%	0.4560	0.4170	0.5030	0.4920	0.5040	0.4744	7.91
100%	0.4470	0.4830	0.4920	0.4060	0.5060	0.4668	8.65

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

City of Mena WWTF

Lab ID# 20520

Test Date: January 15, 2013

WET CHEMISTRY MEASUREMENTS

Date	Time	Temp	Samp. No.	pH of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
01/15/13	Start	25.0	1	7.86	7.66	7.54	7.48	7.39	7.20	STC
01/16/13	24 Hr.	24.6	1	7.83	7.75	7.70	7.67	7.65	7.64	STC
01/16/13	Renew	24.8	1	7.76	7.67	7.47	7.39	7.30	7.22	STC
01/17/13	48 Hr.	24.5	1	7.87	7.78	7.74	7.73	7.67	7.67	CTT
01/17/13	Renew	25.0	2	7.71	7.58	7.49	7.42	7.35	7.30	CTT
01/18/13	72 Hr.	24.6	2	7.95	7.82	7.75	7.74	7.68	7.67	CTT
01/18/13	Renew	24.5	2	7.73	7.52	7.40	7.31	7.20	7.08	CTT
01/19/13	96 Hr.	24.6	2	8.02	7.84	7.76	7.74	7.72	7.70	CTT
01/19/13	Renew	25.0	3	8.21	7.65	7.51	7.42	7.33	7.26	CTT
01/20/13	120 Hr.	24.4	3	7.96	7.89	7.83	7.79	7.76	7.70	STC
01/20/13	Renew	24.6	3	7.73	7.61	7.45	7.32	7.24	7.11	STC
01/21/13	144 Hr.	24.6	3	7.91	7.84	7.80	7.76	7.74	7.73	STC
01/21/13	Renew	24.6	3	7.88	7.57	7.43	7.40	7.22	7.20	STC
01/22/13	168 Hr.	24.5	3	7.89	7.82	7.79	7.74	7.73	7.70	STC

Date	Time	Temp	Samp. No.	DO (mg/L) of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
01/15/13	Start	25.0	1	7.71	7.64	7.68	7.76	7.88	8.08	STC
01/16/13	24 Hr.	24.6	1	8.05	8.04	8.01	8.00	7.97	7.97	STC
01/16/13	Renew	24.8	1	8.89	8.70	8.85	8.86	8.89	8.89	STC
01/17/13	48 Hr.	24.5	1	8.62	8.63	8.59	8.58	8.60	8.56	CTT
01/17/13	Renew	25.0	2	8.03	7.98	7.96	7.96	8.00	8.01	CTT
01/18/13	72 Hr.	24.6	2	7.74	7.76	7.72	7.72	7.72	7.70	CTT
01/18/13	Renew	24.5	2	8.22	8.10	8.02	7.97	7.94	7.98	CTT
01/19/13	96 Hr.	24.6	2	7.88	7.84	7.90	7.88	7.93	7.92	CTT
01/19/13	Renew	25.0	3	8.76	7.98	7.97	8.03	8.15	8.22	CTT
01/20/13	120 Hr.	24.4	3	7.84	7.79	7.74	7.73	7.69	7.65	STC
01/20/13	Renew	24.6	3	7.70	7.58	7.53	7.58	7.62	7.65	STC
01/21/13	144 Hr.	24.6	3	8.20	8.22	8.21	8.22	8.17	8.16	STC
01/21/13	Renew	24.6	3	8.40	8.79	8.62	8.33	8.46	8.40	STC
01/22/13	168 Hr.	24.5	3	7.82	7.62	7.62	7.61	7.60	7.56	STC

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

City of Mena WWTF

Lab ID# 20520

Test Date: January 15, 2013

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
01/15/13	1	7.20	8.08	32	38	262	<0.01	N/A	TN
01/17/13	2	7.30	8.01	36	42	259	<0.01	N/A	TN
01/19/13	3	7.23	8.01	38	44	270	<0.01	N/A	TN
01/15/13	Con	7.86	7.71	48	38	238	-	-	TN

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	0.425	0.483	0.456
2	32% Effluent	5	0.416	0.503	0.481
3	42% Effluent	5	0.423	0.504	0.476
4	56% Effluent	5	0.448	0.502	0.477
5	75% Effluent	5	0.417	0.504	0.474
6	100% Effluent	5	0.406	0.506	0.467

Shapiro - Wilk's Test For Normality

D = 0.026

W = 0.880

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	0.025	0.011	5.42
2	32% Effluent	0.001	0.037	0.017	7.72
3	42% Effluent	0.001	0.031	0.014	6.55
4	56% Effluent	0.001	0.023	0.010	4.76
5	75% Effluent	0.001	0.038	0.017	7.91
6	100% Effluent	0.002	0.040	0.018	8.65

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	0.481	35.00	16.00	5.00	
3	42% Effluent	0.476	33.00	16.00	5.00	
4	56% Effluent	0.477	34.00	16.00	5.00	
5	75% Effluent	0.474	33.00	16.00	5.00	
6	100% Effluent	0.467	32.50	16.00	5.00	

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

No statistically significant difference

**APPENDIX A
RAW DATA**

7-DAY CERIODAPHNIA DUBIA SURVIVAL & REPRODUCTION

DAILY RAW DATA TABLE

PAGE 1 OF 2CLIENT MenaOUTFALL 001LAB ID # 20520ConSTART DATE/TIME 1-15-13 TN 1555END DATE/TIME 1-22-13 NL 1555

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
1/16	A	A	A	A	A	A	A	A	A	A	MH	1555
1/17	A	A	A	A	A	A	A	A	A	A	NL	1345
1/18	A	A	A	A	A	A	A	A	A	A	MH	1430
1/19	A	A	A	S	A	A	4	4	A	A	Jh	1215
1/20	3	2	4	7	2	3	A	A	2	3	Jh	1130
1/21	8	6	8	A	5	6	8	9	7	6	JL	1050
1/22	12	11	12	13	10	11	13	13	12	10	NL	1555
	23	19	24	25	17	20	25	26	21	19		

 $\bar{x}$ # Young w/o Dead = 21.9 CV% = 14.19 $\bar{x}$ # Young w/Dead = CV% = $\bar{x}$ % Survival = 100.0 CV% = 0.00

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
1/16	A	A	A	A	A	A	A	A	A	A	MH	1555
1/17	A	A	A	A	A	A	A	A	A	A	NL	1345
1/18	A	A	A	A	A	A	A	A	A	A	MH	1430
1/19	A	5	A	6	A	5	A	A	A	4	Jh	1215
1/20	5	A	6	A	4	A	4	5	4	A	Jh	1130
1/21	11	8	11	10	8	7	6	10	8	11	JL	1050
1/22	12	13	14	12	13	12	11	13	14	10	NL	1555
	28	26	31	28	25	24	21	28	26	25		

 $\bar{x}$ # Young w/o Dead = 26.2 CV% = 10.46 $\bar{x}$ # Young w/Dead = CV% = $\bar{x}$ % Survival = 100.0 CV% = 0.0042

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
1/16	A	A	A	A	A	A	A	A	A	A	MH	1555
1/17	A	A	A	A	A	A	A	A	A	A	NL	1345
1/18	A	A	A	A	A	A	A	A	A	A	MH	1430
1/19	A	A	A	A	A	A	4	5	3		Jh	1215
1/20	4	5	3	4	5	4	2	A	A	A	Jh	1130
1/21	8	9	6	8	7	10	6	8	10	7	JL	1050
1/22	13	14	12	13	12	14	12	13	14	12	NL	1555
	25	28	21	25	24	28	20	25	29	22		

 $\bar{x}$ # Young w/o Dead = 24.7 CV% = 12.78 $\bar{x}$ # Young w/Dead = CV% = $\bar{x}$ % Survival = 100.0 CV% = 0.0056

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
1/16	A	A	A	A	A	A	A	A	A	A	MH	1555
1/17	A	A	A	A	A	A	A	A	A	A	NL	1345
1/18	A	A	A	A	A	A	A	A	A	A	MH	1430
1/19	A	5	4	2	3	A	3	4	A	4	Jh	1215
1/20	2	A	A	A	A	2	A	A	5	A	Jh	1130
1/21	6	10	10	7	9	6	8	7	9	8	JL	1050
1/22	11	14	13	12	13	12	14	12	13	14	NL	1555
	19	29	27	21	25	20	25	23	27	26		

 $\bar{x}$ # Young w/o Dead = 24.2 CV% = 12.75 $\bar{x}$ # Young w/Dead = CV% = $\bar{x}$ % Survival = 100.0 CV% = 0.00

PAGE 2 OF 2

Mena

901

26520

1-15-13 TN 1555

1-22-13 NL 1555

75

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

100

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

$$\bar{x} \% \text{ Survival} = \quad \quad \quad CV\% =$$
$$\bar{x} \% \text{ Survival} = \quad \quad \quad CV\% =$$

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

CLIENT/FACILITY Mena
 OUTFALL # 001 PROJECT # 20520
 ORGANISM ID# ppo-13-015

DATE/TIME STARTED 1-15-13 MH 1540
 DATE/TIME ENDED 1-22-13 1540

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
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	1-16-13 GL 1540					1-17-13 GL 1000					1-18-13 MH 1010					1-19-13 NL 0920					1-20-13 NL 0915				

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	1-21-13 GL 0935					1-22-13 TN 1540						

7-DAY CHRONIC TOXICITY TEST
PIMEPHALES PROMELAS (fathead minnow) MEAN WEIGHT/REP

Client 2/1/13
Project# 20520

Date/Time Start 1/15/13 1540
Date/Time End 1/22/13 1540

% Effluent	Rep A	Rep B	Rep C	Rep D	Rep E	$\bar{x}$	C.V.%	Analyst
0	.4250	.4790	.4450	.4830	.4460	.4556	5.42	BA
32	.4850	.5020	.4160	.5030	.5000	.4812	7.72	
42	.4760	.4900	.4230	.4860	.5040	.4758	6.55	
56	.4960	.5020	.4480	.4620	.4750	.4766	4.76	
75	.4560	.4170	.5070	.4920	.5040	.4744	7.91	
100	.4470	.4830	.4920	.4060	.5060	.4668	8.65	↓

Huthur and Associates, Inc.

environmental toxicologists, biologists, and consultants

Client / Facility Mena
 Lab ID Number 20520
 Outfall Number 001
 Test Date 1-15-13

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
1/15	1	7.20	8.08	32	38	262	20.01	Na	TN
1/17	2	7.30	8.01	36	42	259	S	S	S
1/19	3	7.23	8.01	38	44	270	S	S	S
1/15	CON	7.86	7.71	48	38	238	-	-	S

INITIAL CHEMISTRY MEASUREMENTS @ RECEIVING WATER

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

Notes:

APPENDIX B
REFERENCE TOXICANTS

CHRONIC REFERENCE TOXICANT TEST RESULTS

SPECIES: *Ceriodaphnia dubia*

CHEMICAL: Sodium Chloride

DURATION: 7-Days

TEST NUMBER: 1

TEST DATE/TIME: 01/02/13 - 01/09/13
1540 Hrs - 1540 Hrs

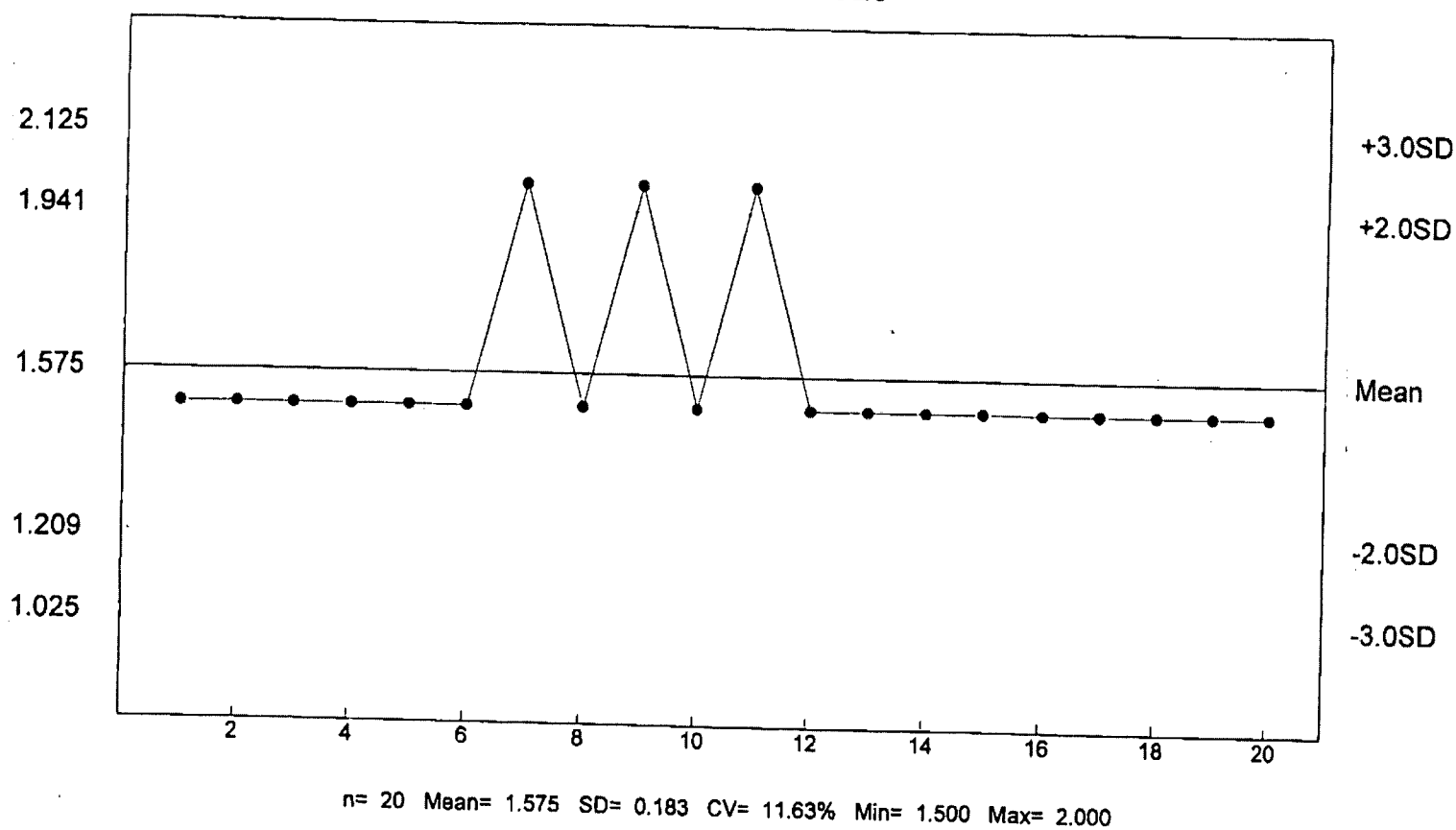
STATISTICAL METHOD: Fishers, Dunnetts/Steels

CONCENTRATION (g/L)	NUMBER EXPOSED	NUMBER DEAD
0.5	10	0
1.0	10	0
1.5	10	2
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	1.5 g/L	1.0 g/L

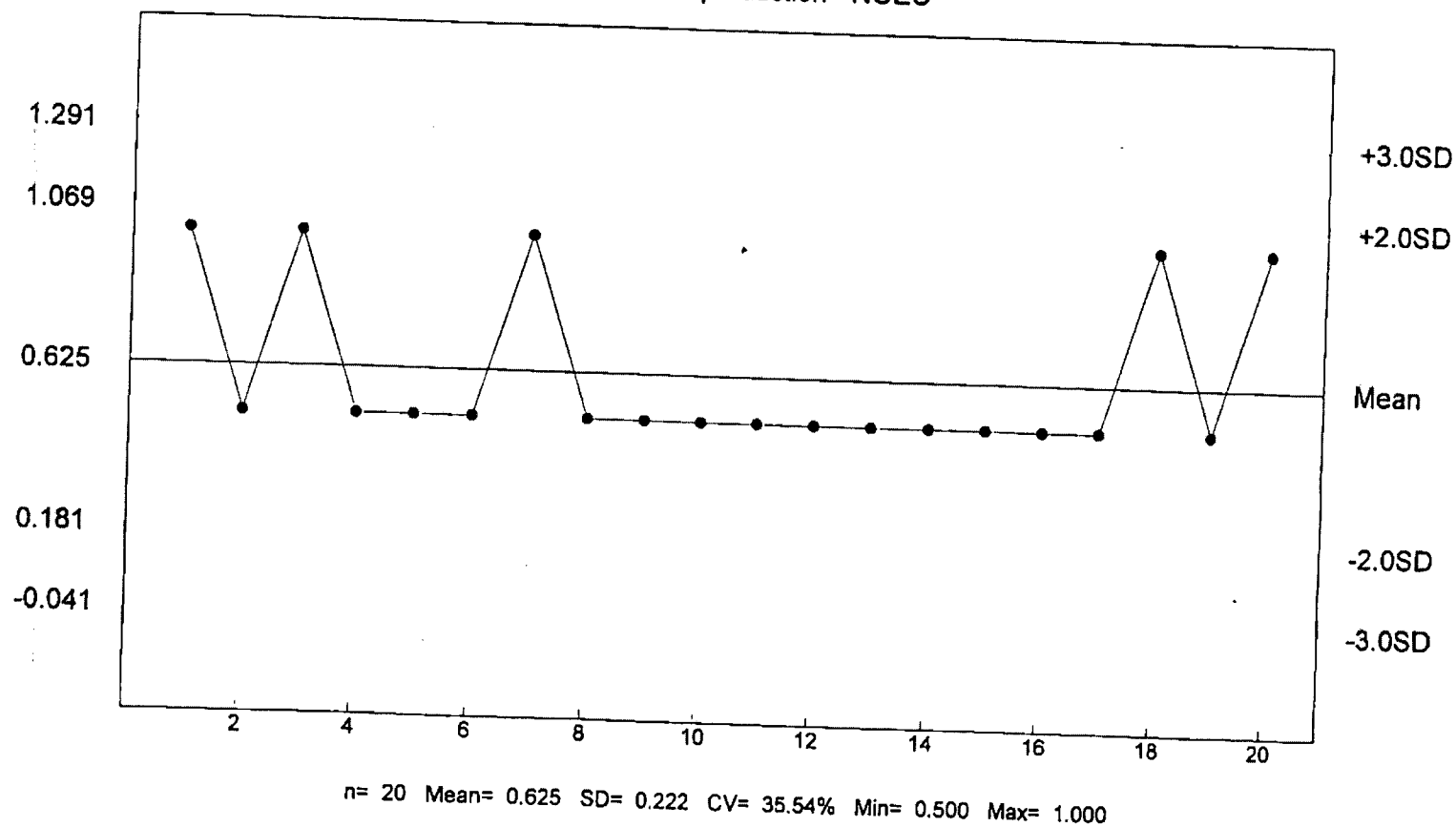
Reference Tox Sodium Chloride g/L

C. dubia Survival - NOEC



Reference Tox Sodium Chloride g/L

C. dubia Reproduction - NOEC



CHRONIC REFERENCE TOXICANT TEST RESULTS

SPECIES: *Pimephales promelas*

CHEMICAL: Copper Nitrate

DURATION: 7-Days

TEST NUMBER: 1

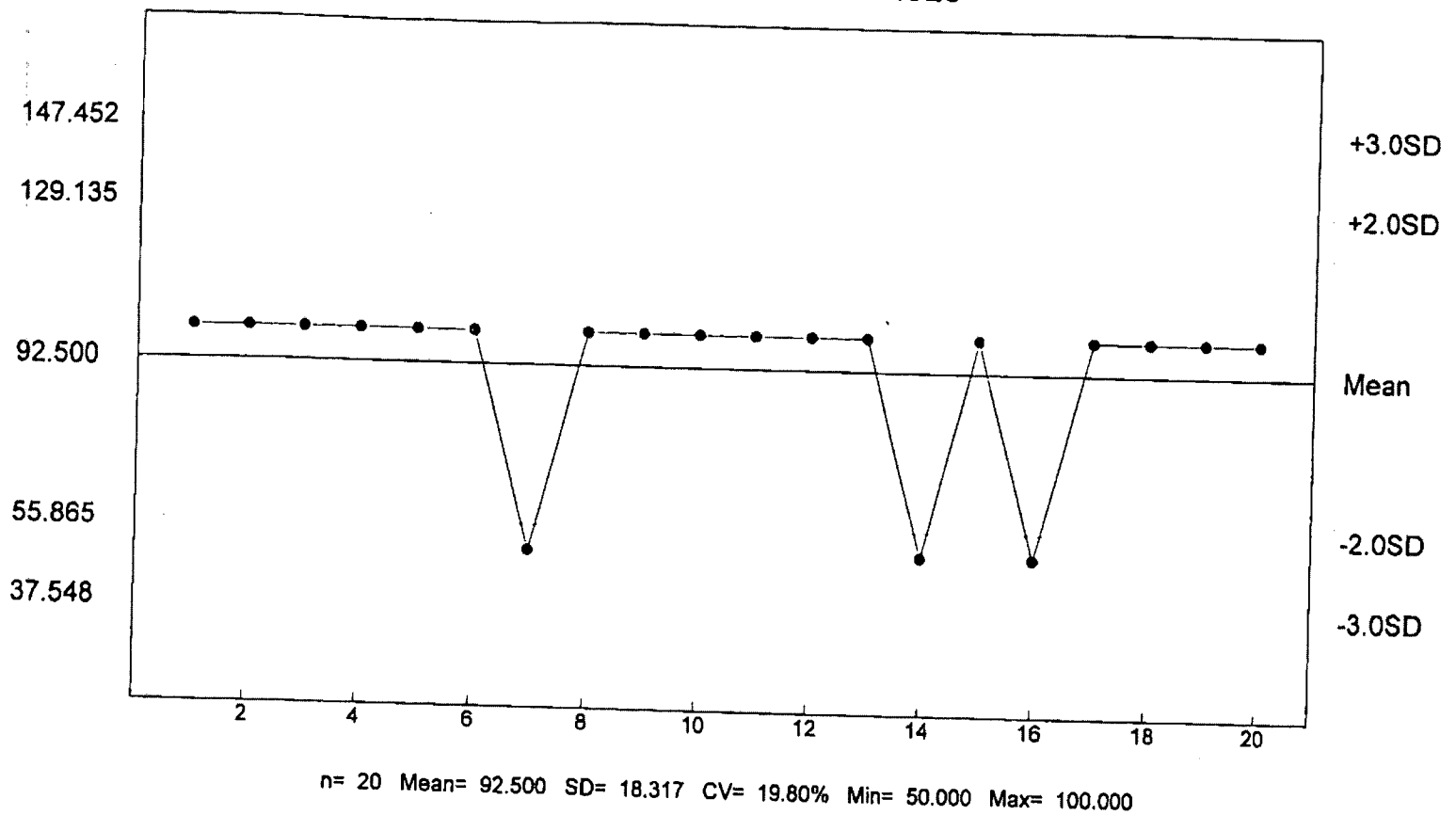
TEST DATE/TIME: 01/03/13 - 01/10/13
1025 Hrs - 1025 Hrs

STATISTICAL METHOD: Dunnetts/Steels

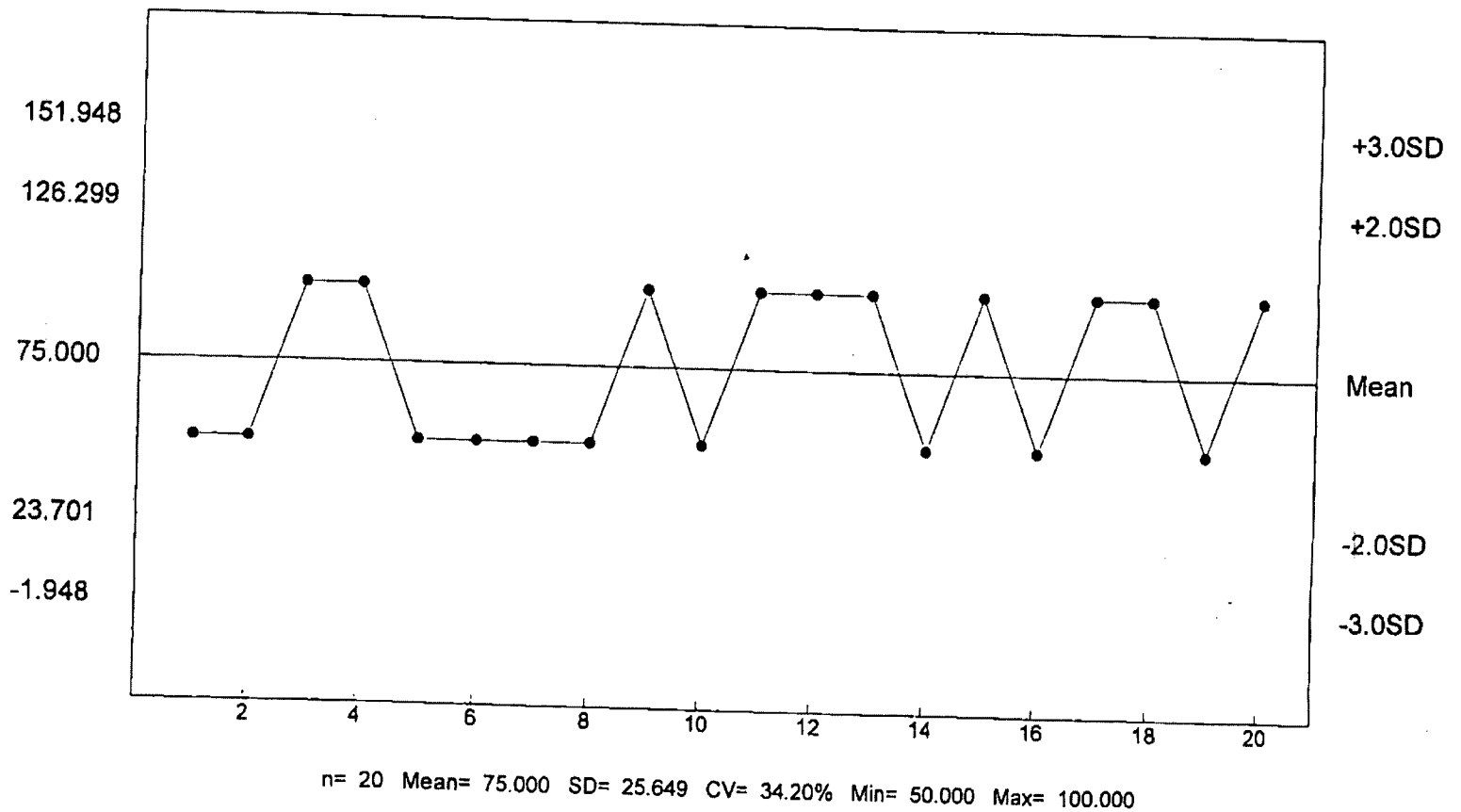
CONCENTRATION (ug/L)	NUMBER EXPOSED	NUMBER DEAD
12.5	40	0
25	40	0
50	40	0
100	40	4
200	40	31
400	40	40
800	40	40

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

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



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



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 # 20520 PROJECT NAME Mena PERMIT# NPDES AR003605

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	Mike	1200 ^{13 JAN} 2013	1200 ^{14 JAN} 2013	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 unnamed trib. to Prairie Creek

DILUTION WATER USED FOR THIS TEST Lab

RELINQUISHED BY: Mike DATE: 14 JAN 13 TIME: 1500 RECEIVED BY AT THIS DATE/TIME: Office Store 1530 14 JAN 13

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: Matt Horner DATE: 1-15-13 TIME: 1035 SAMPLE TEMP. @ RECEIPT. 0.8

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

CHAIN OF CUSTODY RECORD

PROJECT # 20520 PROJECT NAME Mena PERMIT# NPDES 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.	
001	Jeff	15 JAN 2013 1200	16 JAN 2013 1200	24	X			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 unnamed trib. to
Prairie Creek
DILUTION WATER USED FOR THIS TEST Lab

RELINQUISHED BY: Jeff DATE: 16 JAN 13 TIME: 1230 RECEIVED BY AT THIS DATE/TIME: 16 JAN 13/1230
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: Matt Jorner DATE: 1-17-13 TIME: 1010 SAMPLE TEMP. @ RECEIPT. 1.8

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

CHAIN OF CUSTODY RECORD

PROJECT # 20520 PROJECT NAME Mena PERMIT# NPDES AP0036692

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	17 JAN 2013 0800	18 JAN 2013 0800	24	X			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 Unnamed Trib. to
Prairie Creek

DILUTION WATER USED
FOR THIS TEST Lab

RELINQUISHED BY: Jeff DATE: 18 JAN 13 TIME: 0930 RECEIVED BY AT THIS DATE/TIME: LIPS 18 JAN 2013
0930

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: Intake Rg DATE: 1-19-13 TIME: 0950 SAMPLE TEMP. @ RECEIPT: 3.2°

**CITY OF MENA WWTF
NPDES PERMIT NO. AR0036692
AFIN 57-00042
BIOMONITORING REPORTING
TEST DATE: 01/15/13**

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	14.19%
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	8.65%
Report Parameter No. 22414 (lowest NOEC value) for <i>Pimephales promelas</i> .	100%

**CITY OF MENA WWTF
OUTFALL 001**

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

Ceriodaphnia dubia
Pimephales promelas

April 9, 2013

Reviewed by:

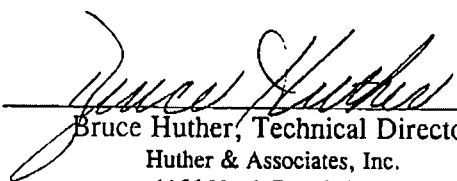

Bruce Huther, Technical Director
Huther & Associates, Inc.
1156 North Bonnie Brae
Denton, Texas 76201
(940) 387-1025, Fax: (940) 387-1036

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TOXICITY TEST REPORT - CHRONIC

ClientCity of Mena WWTF
Permit No.NPDES AR0036692
SampleOutfall 001

Laboratory I.D.20875
Begin Date April 9, 2013

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 United Parcel Service courier to Huth & Associates on April 9, April 11, and April 13, 2013. Effluent samples were collected and composited from Outfall 001 using an automatic sampler. Two toxicity tests were requested: a seven-day *Ceriodaphnia dubia* survival and reproduction test (EPA Method 1002.0), and a seven-day *Pimephales promelas* larval survival and growth test (EPA Method 1000.0). Test organisms, procedures and quality assurance requirements were in accordance with the EPA manual, "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition" (EPA-821-R-02-013).

The effluent 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 1310 hours, April 9, 2013. 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 1310 hours, April 16, 2013. 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

Utilization of the Fishers Exact test on *C. dubia* survival data detected no statistically significant differences between the control and any of the effluent concentrations tested.

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 failed Bartlett's test for homogeneity at the 0.01 alpha level (15.09). A nonparametric test was performed on the heterogeneous data. Steel's Many-One Rank test on *C. dubia* reproduction data demonstrated there were no statistically significant differences between the control and any of the effluent concentrations.

LOEC: Not Applicable
NOEC: 100% Effluent

PMSD: 15.0%

TEST SETUP
Pimephales promelas



The seven-day *Pimephales promelas* larval survival and growth test was initiated at 1530 hours, April 9, 2013. 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, April 16, 2013. 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: 12.1%

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.

Huthier 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 04/08/13 04/10/13 04/12/13
LAB ID # 20875	DATE RECEIVED 04/09/13 04/11/13 04/13/13
TEST TYPE 7 Day Chronic	BEGIN DATE/TIME 04/09/13 1310
TEST ORGANISM <i>Ceriodaphnia dubia</i>	END DATE/TIME 04/16/13 1310
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. endl.
DILUTION WATER Laboratory Adjusted	TECHNICIAN M. Horner

SURVIVAL & REPRODUCTION SUMMARY

Control											
Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10	
04/10/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
04/11/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
04/12/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
04/13/13	5	5	4	5	5	4	3	3	2	3	
	5	5	4	5	5	4	3	3	2	3	
04/14/13	A	A	7	A	A	6	8	A	6	7	
	5	5	11	5	5	10	9	3	8	10	
	8	10	A	8	7	A	A	9	A	A	
04/15/13	13	15	11	13	12	10	9	12	8	10	
	12	14	13	12	13	11	12	11	12	13	
04/16/13	25	29	24	25	25	21	21	23	20	23	
x # Young 23.6 C.V. 11.16% x% Survival 100% C.V. 0.00%											

32% Effluent											
Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10	
04/10/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
04/11/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
04/12/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
04/13/13	4	3	4	3	3	3	3	4	2	3	
	4	3	4	3	3	3	3	4	2	3	
04/14/13	8	8	A	A	A	A	A	A	A	8	
	12	9	4	3	3	3	3	4	2	9	
	A	A	9	8	7	6	9	7	8	A	
04/15/13	12	9	13	11	10	9	12	11	10	9	
	14	13	15	12	13	12	13	13	14	12	
04/16/13	26	22	28	23	23	21	25	24	24	21	
x # Young 23.7 C.V. 9.34% x% Survival 100% C.V. 0.00%											

42% Effluent											
Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10	
04/10/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
04/11/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
04/12/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
04/13/13	3	4	4	3	4	5	3	4	4	3	
	3	4	4	3	4	5	3	4	4	3	
04/14/13	8	A	7	8	A	A	8	A	A	A	
	9	4	11	9	4	5	8	4	4	3	
	A	8	A	A	9	7	A	9	10	7	
04/15/13	9	12	11	9	13	12	9	13	14	10	
	14	13	12	15	12	13	15	14	13	12	
04/16/13	23	25	23	24	25	25	24	27	27	22	
x # Young 24.5 C.V. 8.73% 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	
04/10/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
04/11/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
04/12/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
04/13/13	5	4	2	3	3	3	2	4	3	4	
	5	4	2	3	3	3	2	4	3	4	
04/14/13	A	8	A	7	8	8	8	7	6	7	
	5	12	2	10	9	9	8	11	9	11	
	9	A	6	A	A	A	A	A	A	A	
04/15/13	14	12	8	10	9	9	8	11	9	11	
	14	15	12	12	15	12	13	14	13	12	
04/16/13	28	27	20	22	24	21	21	25	22	23	
x # Young 23.3 C.V. 11.45% x% Survival 100% C.V. 0.00%											

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

ex 1:

A
4

 alive today
 total young to date

ex 2:

5
12

 alive, 5 young today
 total young to date

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

City of Mena WWTF

Lab ID# 20875

Test Date: April 9, 2013

75% Effluent										
Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
04/10/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/11/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/12/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/13/13	4	3	4	3	4	2	3	2	3	5
	4	3	4	3	4	2	3	2	3	5
04/14/13	A	6	7	9	8	A	6	A	A	A
	4	9	11	12	12	2	9	2	3	5
04/15/13	6	A	A	A	A	7	A	7	6	9
	10	9	11	12	12	9	9	9	9	14
04/16/13	12	12	14	13	15	13	14	13	12	13
	22	21	25	25	27	22	23	22	21	27
x # Young 23.5 C.V. 9.88% x% Survival 100% C.V. 0.00%										

100% Effluent										
Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
04/10/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/11/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/12/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
04/13/13	4	3	3	3	3	D-4	4	3	5	4
	4	3	3	3	3	4	4	3	5	4
04/14/13	9	A	A	7	6	D	8	A	A	A
	13	3	3	10	9	4	12	3	5	4
04/15/13	A	8	6	A	A	D	A	6	7	9
	13	11	9	10	9	4	12	9	12	13
04/16/13	13	12	12	14	14	D	12	14	15	13
	26	23	21	24	23	D-4	24	23	27	26
x # Young 24.1 C.V. 7.88% x% Survival 90% C.V. 35.14%										

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

ex 1:

A	alive today
4	total young to date

ex 2:

5	alive, 5 young today
12	total young to date

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

City of Mena WWTF

Lab ID# 20875

Test Date: April 9, 2013

WET CHEMISTRY MEASUREMENTS

Date	Time	Temp	Samp. No.	pH of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
04/09/13	Start	25.0	1	7.65	7.55	7.42	7.36	7.32	6.88	STC
04/10/13	24 Hr.	25.4	1	7.57	7.49	7.45	7.44	7.41	7.37	STC
04/10/13	Renew	25.0	1	7.88	7.67	7.51	7.42	7.34	7.17	STC
04/11/13	48 Hr.	24.5	1	8.02	7.82	7.71	7.66	7.61	7.53	CM
04/11/13	Renew	25.0	2	7.67	7.59	7.43	7.35	7.20	7.00	CM
04/12/13	72 Hr.	24.5	2	7.85	7.68	7.59	7.54	7.49	7.36	CM
04/12/13	Renew	24.1	2	7.75	7.60	7.52	7.43	7.31	7.17	CM
04/13/13	96 Hr.	24.8	2	7.67	7.60	7.47	7.37	7.25	7.07	CM
04/13/13	Renew	25.0	3	7.75	7.62	7.41	7.29	7.05	6.85	CM
04/14/13	120 Hr.	25.0	3	7.85	7.81	7.74	7.67	7.63	7.50	STC
04/14/13	Renew	25.1	3	7.70	7.55	7.36	7.22	7.10	6.98	STC
04/15/13	144 Hr.	25.8	3	7.83	7.68	7.57	7.51	7.44	7.35	STC
04/15/13	Renew	25.9	3	7.57	7.45	7.33	7.30	7.16	7.05	STC
04/16/13	168 Hr.	25.8	3	7.86	7.71	7.61	7.52	7.45	7.36	STC

Date	Time	Temp	Samp. No.	DO (mg/L) of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
04/09/13	Start	25.0	1	8.12	8.07	8.14	8.20	8.18	8.32	STC
04/10/13	24 Hr.	25.4	1	7.75	7.74	7.75	7.72	7.70	7.60	STC
04/10/13	Renew	25.0	1	8.15	8.10	8.06	7.94	8.09	8.02	STC
04/11/13	48 Hr.	24.5	1	7.67	7.66	7.67	7.67	7.68	7.68	CM
04/11/13	Renew	25.0	2	8.49	8.39	8.34	8.29	8.29	8.29	CM
04/12/13	72 Hr.	24.5	2	7.76	7.88	7.95	8.00	8.01	7.91	CM
04/12/13	Renew	24.1	2	8.05	7.97	7.93	7.90	7.92	7.96	CM
04/13/13	96 Hr.	24.8	2	8.19	8.16	8.15	8.24	8.36	8.58	CM
04/13/13	Renew	25.0	3	7.64	7.58	7.69	7.80	8.06	8.31	CM
04/14/13	120 Hr.	25.0	3	8.51	8.51	8.47	8.44	8.48	8.46	STC
04/14/13	Renew	25.1	3	8.44	8.38	8.46	8.54	8.65	8.77	STC
04/15/13	144 Hr.	25.8	3	8.20	8.05	7.97	7.89	7.86	7.81	STC
04/15/13	Renew	25.9	3	7.80	8.35	8.40	8.37	8.47	8.50	STC
04/16/13	168 Hr.	25.8	3	8.51	8.55	8.51	8.56	8.55	8.51	STC

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

City of Mena WWTF

Lab ID# 20875

Test Date: April 9, 2013

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
04/09/13	1	6.88	8.32	20	36	147	<0.01	N/A	TN
04/11/13	2	6.89	8.33	24	40	148	<0.01	N/A	TN
04/13/13	3	6.85	8.31	20	44	150	<0.01	N/A	TN
04/09/13	Con	7.65	8.12	44	40	197	-	-	TN

¹ Measurements taken in 100% solution.

CERIODAPHNIA DUBIA STATISTICAL ANALYSES
Survival

Fisher's Exact Test			
Identification	Number of Alive	Dead	Total
Control	10	0	10
32% Effluent	10	0	10
Total	20	0	20

Critical fisher's value (10,10,10) ($p=0.05$) is 6. B value is 10.
 Since B is greater than 6 there is **No Significant Difference** between control and treatment at the 0.05 level.

Fisher's Exact Test			
Identification	Number of Alive	Dead	Total
Control	10	0	10
75% Effluent	10	0	10
Total	20	0	20

Critical fisher's value (10,10,10) ($p=0.05$) is 6. B value is 10.
 Since B is greater than 6 there is **No Significant Difference** between control and treatment at the 0.05 level.

Fisher's Exact Test			
Identification	Number of Alive	Dead	Total
Control	10	0	10
42% Effluent	10	0	10
Total	20	0	20

Critical fisher's value (10,10,10) ($p=0.05$) is 6. B value is 10.
 Since B is greater than 6 there is **No Significant Difference** between control and treatment at the 0.05 level.

Fisher's Exact Test			
Identification	Number of Alive	Dead	Total
Control	10	0	10
100% Effluent	9	1	10
Total	19	1	20

Critical fisher's value (10,10,10) ($p=0.05$) is 6. B value is 9.
 Since B is greater than 6 there is **No Significant Difference** between control and treatment at the 0.05 level.

Fisher's Exact Test			
Identification	Number of Alive	Dead	Total
Control	10	0	10
56% Effluent	10	0	10
Total	20	0	20

Critical fisher's value (10,10,10) ($p=0.05$) is 6. B value is 10.
 Since B is greater than 6 there is **No Significant Difference** between control and treatment at the 0.05 level.

Summary of Fisher's Exact Tests				
Grp	Identification	Number Exposed	Number Dead	Sig ($P=0.5$)
	Control	10	0	
1	32% Effluent	10	0	
2	42% Effluent	10	0	
3	56% Effluent	10	0	
4	75% Effluent	10	0	
5	100% Effluent	10	1	

No statistically significant difference

CERIODAPHNIA DUBIA STATISTICAL ANALYSES
 Reproduction

Summary Statistics on Transformed Data Table 1 of 2

Grp	Identification	N	Min	Max	Mean
1	Control	10	20.000	29.000	23.600
2	32% Effluent	10	21.000	28.000	23.700
3	42% Effluent	10	22.000	27.000	24.500
4	56% Effluent	10	20.000	28.000	23.300
5	75% Effluent	10	21.000	27.000	23.500
6	100% Effluent	10	4.000	27.000	22.100

Summary Statistics on Transformed Data Table 2 of 2

Grp	Identification	Variance	Sd	Sem	C.V.%
1	Control	6.933	2.633	0.833	11.16
2	32% Effluent	4.900	2.214	0.700	9.34
3	42% Effluent	2.722	1.650	0.522	6.73
4	56% Effluent	7.122	2.669	0.844	11.45
5	75% Effluent	5.389	2.321	0.734	9.88
6	100% Effluent	43.656	6.607	2.089	29.90

Bartlett's Test For Homogeneity of Variance

Calculated B1 statistic = 24.60

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

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

Data Fail B1 homogeneity test at 0.01 level. Try another transformation.

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

Grp	Identification	Transformed Mean	Rank Sum	Crit. Value	Df	Sig
1	Control	23.600				
2	32% Effluent	23.700	106.50	75.00	10.00	
3	42% Effluent	24.500	117.50	75.00	10.00	
4	56% Effluent	23.300	100.50	75.00	10.00	
5	75% Effluent	23.500	105.00	75.00	10.00	
6	100% Effluent	22.100	107.00	75.00	10.00	

Critical values use k = 5, are 1 tailed, and alpha = 0.05
 No statistically significant difference

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	2	16	23	12	7

Calculated Chi-Square goodness of fit test statistic = 3.8126

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

Data Pass normality test. Continue analysis.

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

CLIENT	City of Mena WWTF	SAMPLE TYPE	24 Hour Composite
NPDES #	AR0036692	DATE COLLECTED	04/08/13 04/10/13 04/12/13
LAB ID #	20875	DATE RECEIVED	04/09/13 04/11/13 04/13/13
TEST TYPE	7 Day Chronic	BEGIN DATE/TIME	04/09/13 1530
TEST ORGANISM	<i>Pimephales promelas</i>	END DATE/TIME	04/16/13 1530
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. endl.
DILUTION WATER	Laboratory Adjusted	TECHNICIAN	J. Lopez

SURVIVAL SUMMARY

Conc.	04/10/13					04/11/13					04/12/13					04/13/13					04/14/13				
	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.	04/15/13					04/16/13					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	0.4690	0.4120	0.4830	0.4560	0.4720	0.4584	6.03
32%	0.4960	0.4280	0.5040	0.4570	0.4920	0.4754	6.74
42%	0.4450	0.5060	0.4240	0.4920	0.5030	0.4740	7.84
56%	0.4150	0.5040	0.4470	0.5060	0.4170	0.4578	9.81
75%	0.4920	0.5030	0.4160	0.4820	0.5030	0.4792	7.60
100%	0.4070	0.4520	0.5030	0.5020	0.4970	0.4722	8.93

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

City of Mena WWTF

Lab ID# 20875

Test Date: April 9, 2013

WET CHEMISTRY MEASUREMENTS

Date	Time	Temp	Samp. No.	pH of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
04/09/13	Start	25.0	1	7.65	7.55	7.42	7.36	7.32	6.88	STC
04/10/13	24 Hr.	25.5	1	8.02	7.89	7.76	7.71	7.68	7.57	STC
04/10/13	Renew	25.0	1	7.88	7.67	7.51	7.42	7.34	7.17	STC
04/11/13	48 Hr.	24.4	1	7.93	7.71	7.61	7.57	7.48	7.33	CM
04/11/13	Renew	25.0	2	7.67	7.59	7.43	7.35	7.20	7.00	CM
04/12/13	72 Hr.	24.4	2	8.14	7.80	7.61	7.55	7.45	7.30	CM
04/12/13	Renew	24.1	2	7.75	7.60	7.52	7.43	7.31	7.17	CM
04/13/13	96 Hr.	24.8	2	7.90	7.72	7.61	7.52	7.46	7.34	CM
04/13/13	Renew	25.0	3	7.75	7.62	7.41	7.29	7.05	6.85	CM
04/14/13	120 Hr.	25.3	3	7.82	7.72	7.66	7.63	7.55	7.40	STC
04/14/13	Renew	25.1	3	7.70	7.55	7.36	7.22	7.10	6.98	STC
04/15/13	144 Hr.	25.8	3	7.45	7.30	7.18	7.12	7.06	6.96	STC
04/15/13	Renew	25.9	3	7.57	7.45	7.33	7.30	7.16	7.05	STC
04/16/13	168 Hr.	25.8	3	7.95	7.75	7.64	7.60	7.56	7.46	STC

Date	Time	Temp	Samp. No.	DO (mg/L) of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
04/09/13	Start	25.0	1	8.12	8.07	8.14	8.20	8.18	8.32	STC
04/10/13	24 Hr.	25.5	1	8.92	8.77	8.45	8.19	8.55	8.40	STC
04/10/13	Renew	25.0	1	8.15	8.10	8.06	7.94	8.09	8.02	STC
04/11/13	48 Hr.	24.4	1	7.58	7.61	7.53	7.53	7.53	7.54	CM
04/11/13	Renew	25.0	2	8.49	8.39	8.34	8.29	8.29	8.29	CM
04/12/13	72 Hr.	24.4	2	8.55	8.49	8.41	8.43	8.37	8.37	CM
04/12/13	Renew	24.1	2	8.05	7.97	7.93	7.90	7.92	7.96	CM
04/13/13	96 Hr.	24.8	2	8.51	8.55	8.56	8.64	8.61	8.59	CM
04/13/13	Renew	25.0	3	7.64	7.58	7.69	7.80	8.06	8.31	CM
04/14/13	120 Hr.	25.3	3	8.10	8.14	8.04	8.11	8.15	8.23	STC
04/14/13	Renew	25.1	3	8.44	8.38	8.46	8.54	8.65	8.77	STC
04/15/13	144 Hr.	25.8	3	7.70	7.83	7.80	7.86	7.85	7.91	STC
04/15/13	Renew	25.9	3	7.80	8.35	8.40	8.37	8.47	8.50	STC
04/16/13	168 Hr.	25.8	3	8.34	8.40	8.40	8.42	8.44	8.38	STC

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

City of Mena WWTF

Lab ID# 20875

Test Date: April 9, 2013

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
04/09/13	1	6.88	8.32	20	36	147	<0.01	N/A	TN
04/11/13	2	6.89	8.33	24	40	148	<0.01	N/A	TN
04/13/13	3	6.85	8.31	20	44	150	<0.01	N/A	TN
04/09/13	Con	7.65	8.12	44	40	197	-	-	TN

¹Measurements taken in 100% solution.

PIMEPHALES PROMELAS STATISTICAL ANALYSES
 Growth

Summary Statistics on Transformed Data Table 1 of 2

Grp	Identification	N	Min	Max	Mean
1	Control	5	0.412	0.483	0.458
2	32% Effluent	5	0.428	0.504	0.475
3	42% Effluent	5	0.424	0.506	0.474
4	56% Effluent	5	0.415	0.506	0.458
5	75% Effluent	5	0.416	0.503	0.479
6	100% Effluent	5	0.407	0.503	0.472

Shapiro - Wilk's Test For Normality

D = 0.033

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	0.028	0.012	6.03
2	32% Effluent	0.001	0.032	0.014	6.74
3	42% Effluent	0.001	0.037	0.017	7.84
4	56% Effluent	0.002	0.045	0.020	9.81
5	75% Effluent	0.001	0.036	0.016	7.60
6	100% Effluent	0.002	0.042	0.019	8.93

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

Grp	Identification	Transformed Mean	Rank Sum	Crit. Value	Df	Sig
1	Control	0.458				
2	32% Effluent	0.475	33.00	16.00	5.00	
3	42% Effluent	0.474	32.00	16.00	5.00	
4	56% Effluent	0.458	28.00	16.00	5.00	
5	75% Effluent	0.479	35.00	16.00	5.00	
6	100% Effluent	0.472	31.00	16.00	5.00	

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

No statistically significant difference

**APPENDIX A
RAW DATA**

7-DAY CERIODAPHNIA DUBIA SURVIVAL & REPRODUCTION
DAILY RAW DATA TABLE
PAGE 1 OF 2

CLIENT Mena
OUTFALL 001
LAB ID # 20875

START DATE/TIME 4-9-13 MH 1310
END DATE/TIME 4-16-13 NL 1310

Can

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
4/10	A	A	A	A	A	A	A	A	A	A	ZG	1210
4/11	A	A	A	A	A	A	A	A	A	A	NL	1530
4/12	A	A	A	A	A	A	A	A	A	A	NL	0945
4/13	5	5	4	5	5	4	3	3	2	3	JL	1225
4/14	A	A	7	A	A	6	6	A	6	7	TJ	1105
4/15	8	10	9	8	7	A	A	9	A	A	NL	1315
4/16	12	14	13	12	13	11	12	11	12	13	NL	1310
	25	29	24	25	25	21	21	23	20	27		

$\bar{x}$ # Young w/o Dead = 23.6 CV% = 11.16

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

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

32

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
4/10	A	A	A	A	A	A	A	A	A	A	ZG	1210
4/11	A	A	A	A	A	A	A	A	A	A	NL	1530
4/12	A	A	A	A	A	A	A	A	A	A	NL	0945
4/13	4	3	4	3	3	3	3	4	2	3	JL	1225
4/14	8	6	A	A	A	A	A	A	6		TJ	1105
4/15	A	A	9	8	7	6	9	7	8	A	NL	1315
4/16	14	13	15	12	13	12	13	13	14	12	NL	1310
	26	22	28	23	23	21	25	24	24	21		

$\bar{x}$ # Young w/o Dead = 23.7 CV% = 9.34

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

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

42

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
4/10	A	A	A	A	A	A	A	A	A	A	ZG	1210
4/11	A	A	A	A	A	A	A	A	A	A	NL	1530
4/12	A	A	A	A	A	A	A	A	A	A	NL	0945
4/13	3	4	4	3	4	5	3	4	4	3	JL	1225
4/14	6	A	7	6	A	A	6	A	A	A	TJ	1105
4/15	A	8	A	A	9	7	A	9	10	7	NL	1315
4/16	14	13	15	12	13	15	14	13	12		NL	1310
	23	25	23	21	25	25	24	27	27	22		

$\bar{x}$ # Young w/o Dead = 24.5 CV% = 6.73

$\bar{x}$ # 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
4/10	A	A	A	A	A	A	A	A	A	A	ZG	1210
4/11	A	A	A	A	A	A	A	A	A	A	NL	1530
4/12	A	A	A	A	A	A	A	A	A	A	NL	0945
4/13	5	4	2	3	3	3	2	4	3	4	JL	1225
4/14	A	8	A	7	6	6	6	7	6	7	TJ	1105
4/15	9	A	6	A	A	A	A	A	A	A	NL	1315
4/16	14	15	12	12	15	12	13	14	13	12	NL	1310
	28	27	20	22	24	21	21	25	22	23		

$\bar{x}$ # Young w/o Dead = 23.3 CV% = 11.45

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

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

PAGE 2 OF 2

$$\bar{x} \% \text{ Survival} = \quad \quad \quad CV\% =$$

Chronic Toxicity Summary Form

Permitee: Mona
 Or fall: 001
 ID No.: 20875
 In Time/Date: 4-9-13 1310

Ceriodaphnia dubia
 Chemical Parameters Chart

End Date/Time: 4-16-13 1310

Date	Time	Temp.	Samp. No.	pH of Solution						Analyst
				con	32	42	56	75	100	
4/9	Start	23.0	1	7.83	7.55	7.42	7.36	7.32	6.88	SE
4/10	24 Hr.	25.4	1	7.57	7.49	7.45	7.44	7.41	7.37	SE
4/10	Renew	25.0	1	7.88	7.67	7.51	7.42	7.34	7.17	SE
4/11	48 Hr.	24.5	1	8.02	7.82	7.71	7.66	7.61	7.53	CM
4/11	Renew	25.0	2	7.67	7.59	7.43	7.35	7.20	7.00	CM
4/12	72 Hr.	24.5	2	7.85	7.68	7.59	7.54	7.49	7.36	CM
4/12	Renew	24.1	2	7.75	7.60	7.52	7.43	7.31	7.17	CM
4/13	96 Hr.	24.8	2	7.67	7.60	7.47	7.37	7.25	7.07	CM
4/13	Renew	25.0	3	7.75	7.62	7.41	7.29	7.05	6.85	CM
4/14	120 Hr.	25.0	3	7.85	7.81	7.74	7.67	7.63	7.50	SE
4/14	Renew	25.1	3	7.70	7.55	7.36	7.22	7.10	6.98	SE
4/15	144 Hr.	25.8	3	7.83	7.68	7.57	7.51	7.44	7.35	SE
4/15	Renew	25.9	3	7.57	7.43	7.33	7.30	7.16	7.05	SE
4/16	168 Hr.	25.8	3	7.86	7.71	7.61	7.52	7.45	7.36	SE

Date	Time	Temp.	Samp. No.	DO(mg/L) of Solution						Analyst
				con	32	42	56	75	100	
4/9	Start	25.0	1	8.50	8.07	8.14	8.20	8.18	8.32	SE
4/10	24 Hr.	25.4	1	7.75	7.74	7.75	7.72	7.70	7.60	SE
4/10	Renew	25.0	1	8.15	8.16	8.06	7.94	8.09	8.02	SE
4/11	48 Hr.	24.5	1	7.67	7.66	7.67	7.67	7.68	7.68	CM
4/11	Renew	25.0	2	8.49	8.39	8.34	8.29	8.29	8.29	CM
4/12	72 Hr.	24.5	2	7.76	7.88	7.95	8.00	8.01	7.91	CM
4/12	Renew	24.1	2	8.05	7.97	7.93	7.90	7.92	7.96	CM
4/13	96 Hr.	24.8	2	8.19	8.16	8.15	8.24	8.36	8.58	CM
4/13	Renew	25.0	3	7.64	7.58	7.69	7.80	8.06	8.31	CM
4/14	120 Hr.	25.0	3	8.51	8.51	8.47	8.44	8.48	8.46	SE
4/14	Renew	25.1	3	8.44	8.38	8.46	8.54	8.63	8.77	SE
4/15	144 Hr.	25.8	3	8.20	8.05	7.97	7.89	7.86	7.81	SE
4/15	Renew	25.9	3	7.80	8.35	8.40	8.37	8.47	8.50	SE
4/16	168 Hr.	25.8	3	8.51	8.55	8.51	8.56	8.55	8.51	SE

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

CLIENT/FACILITY Mena
 OUTFALL # 001 PROJECT # 20875
 ORGANISM ID# PPG-13-099

DATE/TIME STARTED 4-9-13 02 1530
 DATE/TIME ENDED 4-16-13 02 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
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	4-10-13 02 1530					4-11-13 02 100					4-12-13 26 0825					4-13-13 02 0825					4-14-13 02 0850				

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	4-15-13 26 10445					4-16-13 02 1530						

7-DAY CHRONIC TOXICITY TEST
PIMEPHALES PROMELAS (fathead minnow) MEAN WEIGHT/REP

Client Mema
Project# 20875

Date/Time Start 4/9/13 1530
Date/Time End 4/16/13 1530

% Effluent	Rep A	Rep B	Rep C	Rep D	Rep E	$\bar{x}$	C.V.%	Analyst
CN	.4690	.4120	.4830	.4560	.4220	.4584	6.03	BH
32	.4960	.4280	.5040	.4570	.4920	.4754	6.74	
72	.4450	.5060	.4240	.4920	.5030	.4740	7.84	
56	.4150	.5040	.4470	.5060	.4170	.4578	9.81	
75	.4920	.5030	.4160	.4820	.5030	.4792	7.60	
100	.4070	.4520	.5030	.5020	.4970	.4722	8.93	V

Chronic Toxicity Summary Form

Permittee:

Mena

Outfall:

001

5 ID No.:

20875

Begin Time/Date:

4/9/13 1530

End Date/Time:

4/16/13 1530

Pimephales promelas

Chemical Parameters Chart

Date	Time	Temp.	Samp. No.	pH of Solution						Analyst
				Con	32	42	56	72	100	
4/9	Start	25.0	1	7.65	7.55	7.42	7.36	7.32	6.88	Se
4/10	24 Hr.	25.5	1	8.02	7.89	7.76	7.71	7.68	7.57	Se
4/10	Renew	25.0	1	7.88	7.67	7.51	7.42	7.34	7.17	Se
4/11	48 Hr.	24.4	1	7.92	7.71	7.61	7.57	7.48	7.33	CM
4/11	Renew	25.0	2	7.67	7.59	7.43	7.35	7.20	7.00	CM
4/12	72 Hr.	24.4	2	8.14	7.80	7.61	7.55	7.45	7.30	CM
4/12	Renew	24.1	2	7.75	7.60	7.52	7.43	7.31	7.17	CM
4/13	96 Hr.	24.8	2	7.90	7.72	7.61	7.52	7.46	7.34	CM
4/13	Renew	25.0	3	7.75	7.62	7.41	7.29	7.05	6.85	CM
4/14	120 Hr.	25.3	3	7.82	7.72	7.66	7.63	7.55	7.40	Se
4/14	Renew	25.1	3	7.70	7.55	7.36	7.22	7.10	6.98	Se
4/15	144 Hr.	25.8	3	7.45	7.30	7.18	7.12	7.06	6.96	Se
4/15	Renew	25.9	3	7.57	7.45	7.33	7.30	7.16	7.05	Se
4/16	168 Hr.	25.8	3	7.95	7.75	7.64	7.60	7.56	7.46	Se

Date	Time	Temp.	Samp. No.	DO(mg/L) of Solution						Analyst
				Con	32	42	56	72	100	
4/9	Start	25.0	1	8.12	8.07	8.14	8.20	8.18	8.32	Se
4/10	24 Hr.	25.5	1	8.92	8.77	8.45	8.19	8.55	8.40	Se
4/10	Renew	25.0	1	8.15	8.10	8.06	7.94	8.09	8.02	Se
4/11	48 Hr.	24.4	1	7.58	7.61	7.53	7.53	7.53	7.54	CM
4/11	Renew	25.0	2	8.49	8.39	8.34	8.24	8.29	8.29	CM
4/12	72 Hr.	24.4	2	8.55	8.49	8.41	8.43	8.37	8.37	CM
4/12	Renew	24.1	2	8.05	7.97	7.93	7.90	7.92	7.96	CM
4/13	96 Hr.	24.8	2	8.51	8.55	8.56	8.64	8.61	8.59	CM
4/13	Renew	25.0	3	7.64	7.58	7.69	7.80	8.06	8.31	CM
4/14	120 Hr.	25.3	3	8.10	8.14	8.04	8.11	8.15	8.23	Se
4/14	Renew	25.1	3	8.44	8.38	8.46	8.54	8.65	8.77	Se
4/15	144 Hr.	25.8	3	7.70	7.83	7.80	7.86	7.85	7.91	Se
4/15	Renew	25.9	3	7.80	8.35	8.40	8.37	8.47	8.50	Se
4/16	168 Hr.	25.8	3	8.34	8.40	8.40	8.42	8.44	8.38	Se

Huth and Associates, Inc.

environmental toxicologists, biologists, and consultants

Client / Facility

Mena

Lab ID Number

20875

Outfall Number

001

Test Date

4-9-13

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
4/9	1	6.88	8.32	20	36	147	60.01	Na	TN
4/11	2	6.89	8.33	24	40	148	S	S	S
4/13	3	6.85	8.31	20	44	150	S	S	S
4/9	CON	7.65	8.12	44	40	197	—	—	C

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: 4

TEST DATE/TIME: 04/01/13 - 04/08/13
1600 Hrs - 1600 Hrs

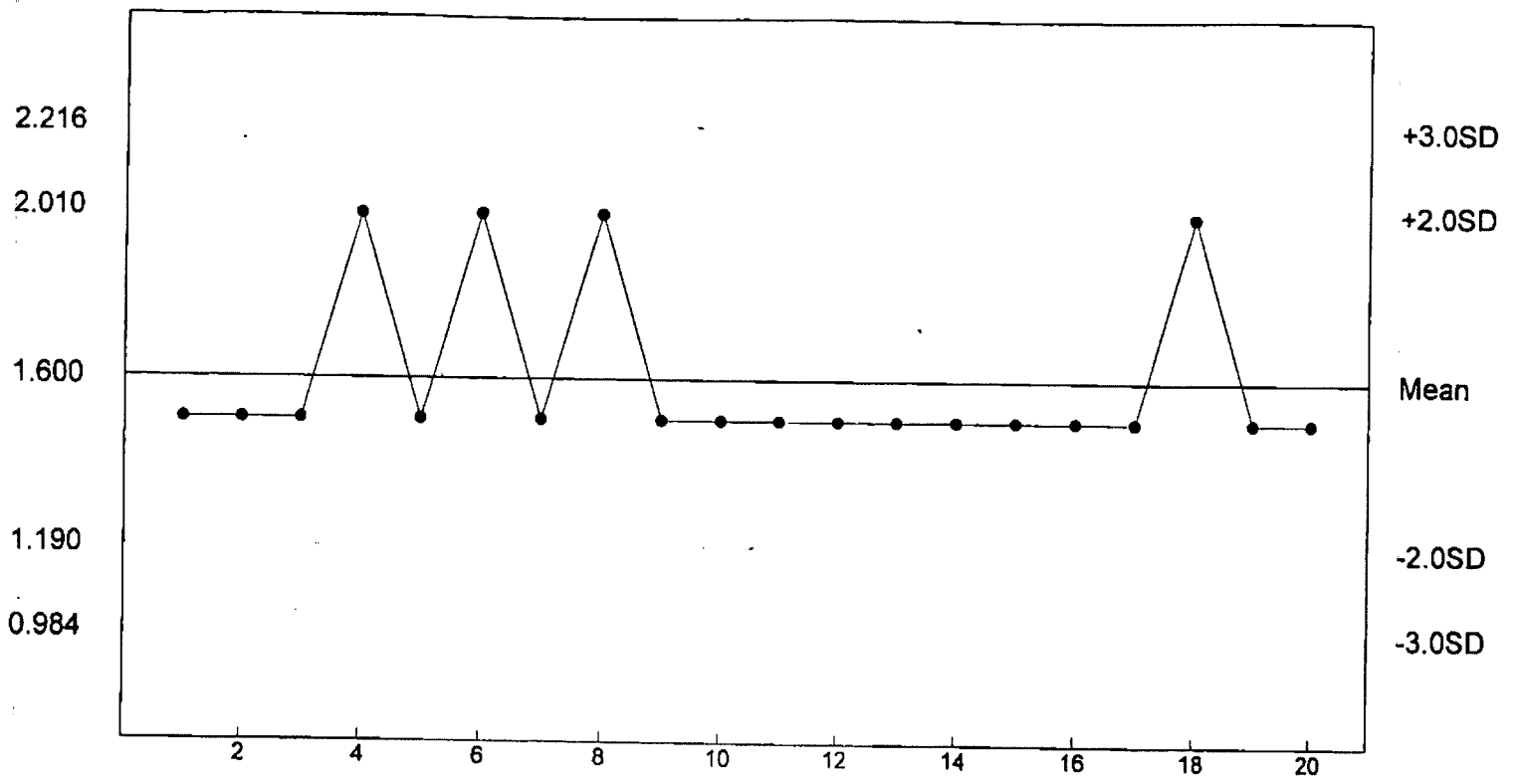
STATISTICAL METHOD: Fishers, Dunnetts/Steels

CONCENTRATION (g/L)	NUMBER EXPOSED	NUMBER DEAD
0.5	10	0
1.0	10	0
1.5	10	0
2.0	10	7
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	1.5 g/L	1.0 g/L

Reference Tox Sodium Chloride g/L

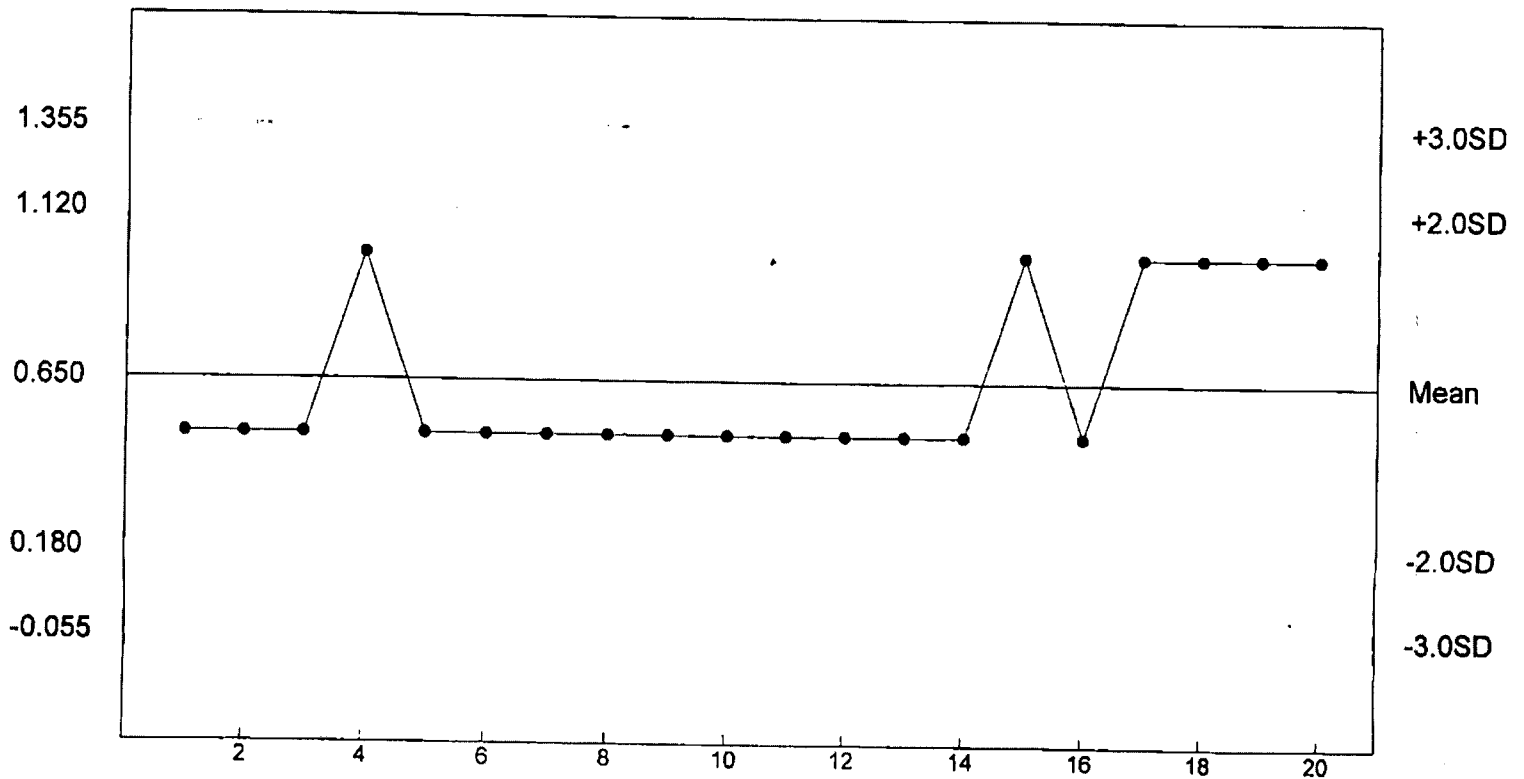
C. dubia Survival - NOEC



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

Reference Tox Sodium Chloride g/L

C. dubia Reproduction - NOEC



n= 20 Mean= 0.650 SD= 0.235 CV= 36.17% Min= 0.500 Max= 1.000

CHRONIC REFERENCE TOXICANT TEST RESULTS

SPECIES: *Pimephales promelas*

CHEMICAL: Copper Nitrate

DURATION: 7-Days

TEST NUMBER: 4

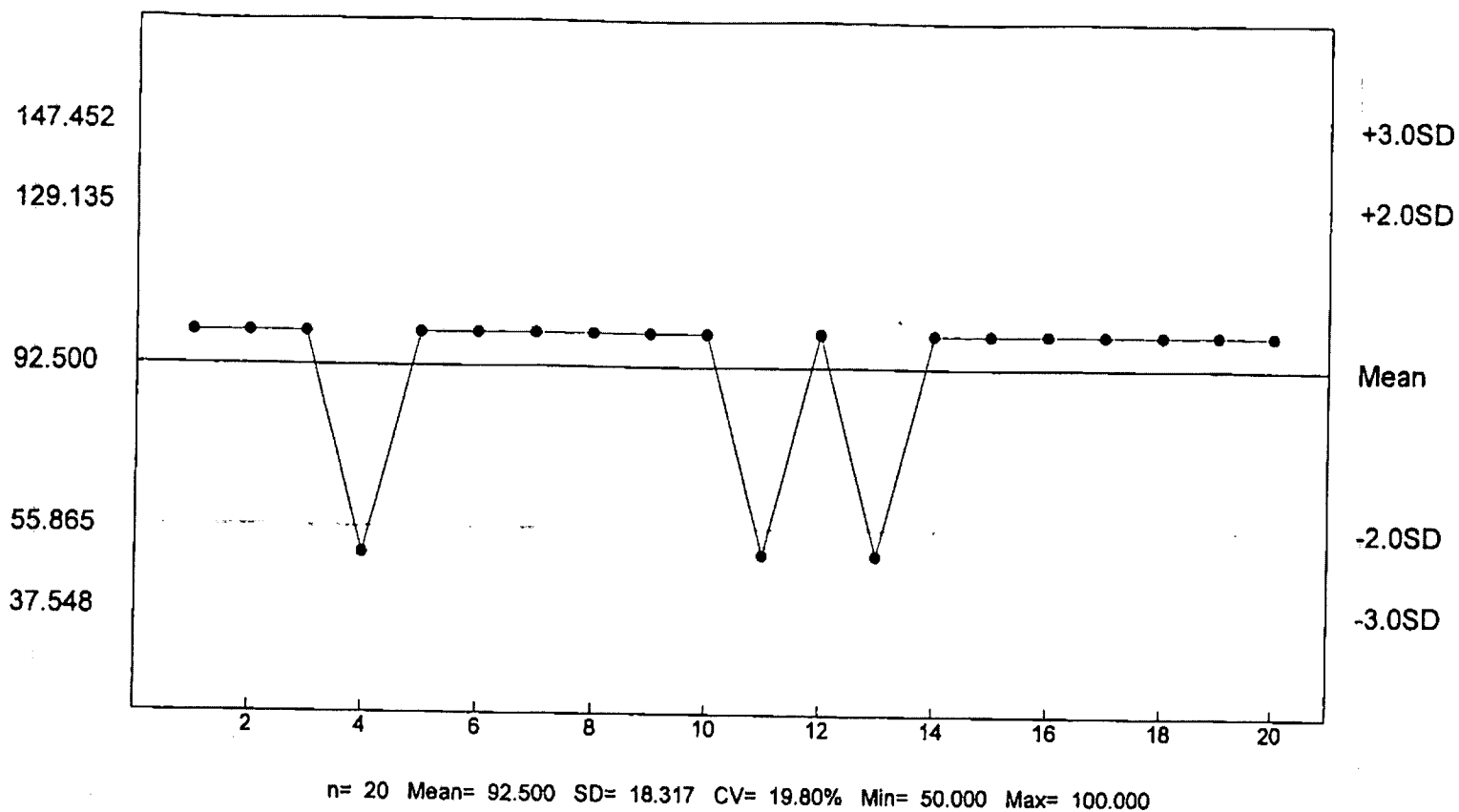
TEST DATE/TIME: 04/01/13 - 04/08/13
1600 Hrs - 1600 Hrs

STATISTICAL METHOD: Dunnetts/Steels

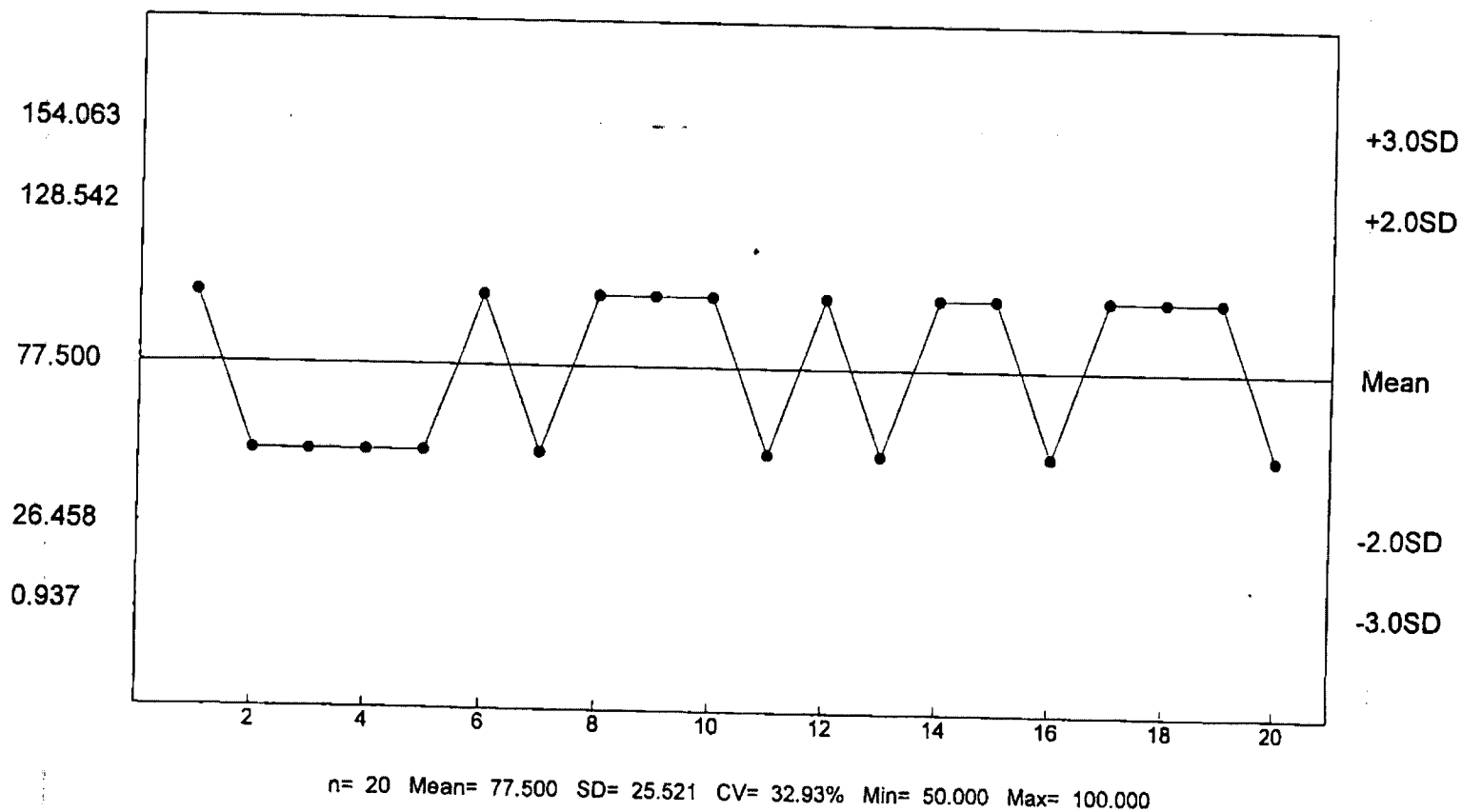
CONCENTRATION (ug/L)	NUMBER EXPOSED	NUMBER DEAD
12.5	40	0
25	40	0
50	40	1
100	40	6
200	40	22
400	40	36
800	40	40

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



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



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 # 20875 PROJECT NAME Mena PERMIT# NPDES AR0031669

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	07 APR 2013 1200	08 APR 2013 1200	24	X			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 CF
NAME OF RECEIVING WATER unnamed trib. of Prairie Creek

DILUTION WATER USED FOR THIS TEST Lab

RELINQUISHED BY: Jeff DATE: 08 APR 13 TIME: 1445 RECEIVED BY AT THIS DATE/TIME 08 APR 13/1445 MENA OFFICE STORE 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: Jeff DATE: 4-9-13 TIME: 1005 SAMPLE TEMP. @ RECEIPT. 3.4

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

CHAIN OF CUSTODY RECORD

PROJECT # 20875 PROJECT NAME Mona 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.	
<u>001</u>	<u>Jeff</u>	<u>9 APR 2013</u> <u>1200</u>	<u>10 APR 2013</u> <u>1200</u>	<u>24</u>	<u>X</u>			<u>1</u>

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 trib. of
Prairie Creek

DILUTION WATER USED FOR THIS TEST Lab

RELINQUISHED BY: Jeff DATE: 10 APR 13 TIME: 1315 RECEIVED BY AT THIS DATE/TIME 10 APR 13 / 1315
UPS - Office Store

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: Matt Horner DATE: 4-11-13 TIME: 0955 SAMPLE TEMP. @ RECEIPT. -0.4

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

CHAIN OF CUSTODY RECORD

PROJECT # 20875 PROJECT NAME Mena PERMIT# NPDES AR003669

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	Jef	11 APR 2013 0700	12 APR 2013 0700	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 trib. of
Prairie Creek

DILUTION WATER USED FOR THIS TEST Lab

RELINQUISHED BY: [Signature] DATE: 12 APR 13 TIME: 0930 RECEIVED BY AT THIS DATE/TIME: 12 APR 13 0930

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: [Signature] DATE: 4-13-13 TIME: 0900 SAMPLE TEMP. @ RECEIPT. -0.6°

**CITY OF MENA WWTF
NPDES PERMIT NO. AR0036692
AFIN 57-00042
BIOMONITORING REPORTING
TEST DATE: 04/09/13**

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**

11.16%

Report Parameter No. 22414 (lowest NOEC value) for *Ceriodaphnia dubia*.

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**

8.93%

Report Parameter No. 22414 (lowest NOEC value) for *Pimephales promelas*.

100%

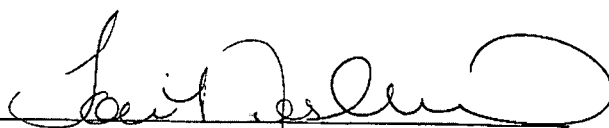
**CITY OF MENA WWTF
OUTFALL 001**

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

Ceriodaphnia dubia
Pimephales promelas

July 9, 2013

Reviewed by: _____



Toni Nesbitt, QA/QC Officer
Huther & Associates, Inc.
1156 North Bonnie Brae
Denton, Texas 76201
(940) 387-1025, Fax: (940) 387-1036

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TOXICITY TEST REPORT - CHRONIC

Client City of Mena WWTF Laboratory I.D.21229
Permit No. NPDES AR0036692 Begin Date July 9, 2013
Sample Outfall 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 United Parcel Service courier to Huthur & Associates on July 9, July 11, and July 13, 2013. Effluent samples were collected and composited from Outfall 001 using an automatic sampler. Two toxicity tests were requested: a seven-day *Ceriodaphnia dubia* survival and reproduction test (EPA Method 1002.0), and a seven-day *Pimephales promelas* larval survival and growth test (EPA Method 1000.0). Test organisms, procedures and quality assurance requirements were in accordance with the EPA manual, "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition" (EPA-821-R-02-013).

The effluent 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 1415 hours, July 9, 2013. 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 1415 hours, July 16, 2013. Survival and reproduction data were statistically analyzed ($p = 0.05$) according to EPA procedures to determine the Lowest Observable Effect Concentration (LOEC) and the No Observable Effect Concentration (NOEC).

SURVIVAL***Ceriodaphnia dubia***

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

LOEC: Not Applicable

NOEC: 100% Effluent

REPRODUCTION***Ceriodaphnia dubia***

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

LOEC: Not Applicable

PMSD: 9.7%

NOEC: 100% Effluent

TEST SETUP***Pimephales promelas***

The seven-day *Pimephales promelas* larval survival and growth test was initiated at 1500 hours, July 9, 2013. 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 1500 hours, July 16, 2013. 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: 9.2%

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 Mena WWTF	SAMPLE TYPE	24 Hour Composite
NPDES #	AR0036692	DATE COLLECTED	07/08/13 07/10/13 07/12/13
LAB ID #	21229	DATE RECEIVED	07/09/13 07/11/13 07/13/13
TEST TYPE	7 Day Chronic	BEGIN DATE/TIME	07/09/13 1415
TEST ORGANISM	<i>Ceriodaphnia dubia</i>	END DATE/TIME	07/16/13 1415
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. candl.
DILUTION WATER	Laboratory Adjusted	TECHNICIAN	M. Horner

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
07/10/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
07/11/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
07/12/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
07/13/13	2	2	2	3	2	2	3	A	A	2
	2	2	2	3	2	2	3	0	0	2
07/14/13	A	A	A	A	A	A	A	2	3	A
	2	2	2	3	2	2	3	2	3	2
07/15/13	8	7	8	7	6	8	7	7	8	8
	8	9	10	10	8	8	10	8	8	8
07/16/13	11	11	12	12	13	12	13	11	12	11
	19	20	22	22	21	20	23	20	21	19
x # Young 20.7 C.V. 6.46% 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
07/10/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
07/11/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
07/12/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
07/13/13	2	2	5	A	4	5	4	5	A	4
	2	2	5	0	4	5	4	5	0	4
07/14/13	A	8	A	2	A	A	A	A	2	A
	2	10	5	2	4	5	4	5	2	4
07/15/13	7	A	7	6	6	6	7	8	6	7
	9	10	12	8	10	11	11	13	8	11
07/16/13	11	12	13	12	13	12	13	14	11	12
	20	22	25	20	23	23	24	27	19	23
x # Young 22.6 C.V. 10.88% 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
07/10/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
07/11/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
07/12/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
07/13/13	A	2	A	4	4	5	3	4	4	2
	0	2	0	4	4	5	3	4	4	2
07/14/13	2	A	2	A	A	A	A	A	A	8
	2	2	2	4	4	5	3	4	4	8
07/15/13	8	7	8	7	6	8	7	7	8	A
	8	9	10	11	10	11	11	11	10	8
07/16/13	13	11	12	12	14	13	12	13	13	14
	21	20	22	23	24	24	23	24	23	22
x # Young 22.6 C.V. 5.97% 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
07/10/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
07/11/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
07/12/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
07/13/13	5	3	5	2	3	5	4	A	A	5
	5	3	5	2	3	5	4	0	0	5
07/14/13	7	8	A	6	A	A	7	3	2	9
	12	8	5	8	3	5	11	3	2	14
07/15/13	A	A	7	A	8	9	A	8	7	A
	12	8	12	8	11	14	11	11	9	14
07/16/13	14	12	13	15	12	12	13	14	13	14
	26	21	25	23	23	26	24	25	22	26
x # Young 24.3 C.V. 8.69% 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# 21229

Test Date: July 9, 2013

75% Effluent

Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
07/10/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
07/11/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
07/12/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
07/13/13	2	2	4	3	3	4	3	3	4	3
	2	2	4	3	3	4	3	3	4	3
07/14/13	6	7	8	A	A	A	A	A	A	A
	8	8	12	3	3	4	3	3	4	3
07/15/13	A	A	A	9	8	7	8	10	7	8
	8	9	12	12	11	11	12	13	11	11
07/16/13	13	15	14	12	15	14	14	12	14	13
	21	24	26	24	26	25	26	25	25	24
x # Young 24.6 C.V. 8.12% 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
07/10/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
07/11/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
07/12/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
07/13/13	6	4	6	4	3	4	2	3	4	4
	6	4	6	4	3	4	2	3	4	4
07/14/13	A	A	A	A	A	A	A	A	A	A
	6	4	6	4	3	4	2	3	4	4
07/15/13	8	8	6	7	8	6	7	9	6	9
	14	12	12	11	11	10	9	12	10	13
07/16/13	15	13	14	13	14	12	13	14	12	15
	29	25	26	24	25	22	22	26	22	28
x # Young 24.9 C.V. 9.92% x% Survival 100% C.V. 0.00%										

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

ex 1:

A	alive today
4	total young to date

ex 2:

5	alive, 5 young today
12	total young to date

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

City of Mena WWTF

Lab ID# 21229

Test Date: July 9, 2013

WET CHEMISTRY MEASUREMENTS

Date	Time	Temp	Samp. No.	pH of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
07/09/13	Start	25.0	1	7.88	7.79	7.67	7.50	7.27	7.18	STC
07/10/13	24 Hr.	26.0	1	7.74	7.55	7.49	7.46	7.41	7.26	GZK
07/10/13	Renew	26.0	1	7.68	7.57	7.48	7.39	7.34	7.18	GZK
07/11/13	48 Hr.	26.0	1	7.75	7.65	7.57	7.54	7.48	7.34	GZK
07/11/13	Renew	25.0	2	7.87	7.52	7.40	7.27	7.09	7.18	GZK
07/12/13	72 Hr.	26.0	2	7.54	7.41	7.36	7.31	7.23	7.14	MJK
07/12/13	Renew	25.9	2	7.38	7.32	7.19	7.12	7.03	6.79	MJK
07/13/13	96 Hr.	25.9	2	7.62	7.55	7.44	7.36	7.24	7.03	MJK
07/13/13	Renew	25.9	3	7.49	7.35	7.29	7.21	7.02	7.20	MJK
07/14/13	120 Hr.	26.0	3	8.06	7.82	7.66	7.59	7.54	7.43	STC
07/14/13	Renew	26.0	3	7.64	7.54	7.41	7.32	7.18	6.97	STC
07/15/13	144 Hr.	26.0	3	7.70	7.59	7.50	7.45	7.39	7.31	STC
07/15/13	Renew	25.9	3	7.68	7.53	7.40	7.31	7.17	6.94	STC
07/16/13	168 Hr.	26.0	3	7.67	7.57	7.52	7.48	7.40	7.31	STC

Date	Time	Temp	Samp. No.	DO (mg/L) of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
07/09/13	Start	25.0	1	8.21	8.60	8.84	8.95	8.95	8.52	STC
07/10/13	24 Hr.	26.0	1	8.54	7.90	8.24	8.49	8.07	8.49	GZK
07/10/13	Renew	26.0	1	6.87	7.39	7.01	7.18	7.16	8.52	GZK
07/11/13	48 Hr.	26.0	1	7.86	7.70	7.61	7.65	7.63	7.66	GZK
07/11/13	Renew	25.0	2	8.69	8.79	8.74	8.80	8.80	8.52	GZK
07/12/13	72 Hr.	26.0	2	6.86	6.81	6.67	6.63	6.73	6.72	MJK
07/12/13	Renew	25.9	2	7.01	7.01	6.82	6.79	6.79	6.82	MJK
07/13/13	96 Hr.	25.9	2	6.60	6.60	6.63	6.62	6.63	6.63	MJK
07/13/13	Renew	25.9	3	6.18	6.34	6.36	6.45	6.37	8.50	MJK
07/14/13	120 Hr.	26.0	3	7.58	7.60	7.97	7.99	8.06	8.06	STC
07/14/13	Renew	26.0	3	7.64	7.86	8.03	8.10	8.21	8.19	STC
07/15/13	144 Hr.	26.0	3	8.22	8.11	7.82	7.77	7.80	7.91	STC
07/15/13	Renew	25.9	3	8.41	8.25	8.28	8.43	8.43	8.36	STC
07/16/13	168 Hr.	26.0	3	8.41	8.43	8.42	8.35	8.31	8.18	STC

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

City of Mena WWTF

Lab ID# 21229

Test Date: July 9, 2013

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
07/09/13	1	7.18	8.52	24	16	141	<0.01	N/A	TN
07/11/13	2	7.19	8.53	28	16	144	<0.01	N/A	TN
07/13/13	3	7.20	8.50	28	12	141	<0.01	N/A	TN
07/09/13	Con	7.88	8.21	40	28	202	-	-	TN

¹ Measurements taken in 100% solution.

CERIODAPHNIA DUBIA STATISTICAL ANALYSES
 Reproduction

Summary Statistics on Transformed Data Table 1 of 2

Grp	Identification	N	Min	Max	Mean
1	Control	10	19.000	23.000	20.700
2	32% Effluent	10	19.000	27.000	22.600
3	42% Effluent	10	20.000	24.000	22.600
4	56% Effluent	10	21.000	28.000	24.300
5	75% Effluent	10	21.000	26.000	24.600
6	100% Effluent	10	22.000	29.000	24.900

Summary Statistics on Transformed Data Table 2 of 2

Grp	Identification	Variance	Sd	Sem	C.V.%
1	Control	1.789	1.337	0.423	6.46
2	32% Effluent	6.044	2.459	0.777	10.88
3	42% Effluent	1.822	1.350	0.427	5.97
4	56% Effluent	4.456	2.111	0.667	8.69
5	75% Effluent	2.267	1.506	0.476	6.12
6	100% Effluent	6.100	2.470	0.781	9.92

Chi-Square Test For Normality: Actual And Expected Frequencies

Interval	< -1.5	-1.5 to -0.5	-0.5 to 0.5	> 0.5 to 1.5	> 1.5
Expected	4.020	14.520	22.920	14.520	4.020
Observed	3	15	25	13	4

Calculated Chi-Square goodness of fit test statistic = 0.6227

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 = 7.10

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	129.883	25.977	6.934
Within (Error)	54	202.300	3.746	
Total	59	332.183		

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 Calculated In Original Units	T Stat	Sig
1	Control	20.700	20.700		
2	32% Effluent	22.600	22.600	-2.195	
3	42% Effluent	22.600	22.600	-2.195	
4	56% Effluent	24.300	24.300	-4.159	
5	75% Effluent	24.600	24.600	-4.506	
6	100% Effluent	24.900	24.900	-4.852	

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

No statistically significant difference

Dunnett's Test - Table 1 of 2 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	2.000	9.7	-1.900
3	42% Effluent	10	2.000	9.7	-1.900
4	56% Effluent	10	2.000	9.7	-3.600
5	75% Effluent	10	2.000	9.7	-3.900
6	100% Effluent	10	2.000	9.7	-4.200

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

CLIENT	City of Mena WWTF	SAMPLE TYPE	24 Hour Composite
NPDES #	AR0036692	DATE COLLECTED	07/08/13 07/10/13 07/12/13
LAB ID #	21229	DATE RECEIVED	07/09/13 07/11/13 07/13/13
TEST TYPE	7 Day Chronic	BEGIN DATE/TIME	07/09/13 1500
TEST ORGANISM	<i>Pimephales promelas</i>	END DATE/TIME	07/16/13 1500
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. candl.
DILUTION WATER	Laboratory Adjusted	TECHNICIAN	M. Horner

SURVIVAL SUMMARY

Conc.	07/10/13					07/11/13					07/12/13					07/13/13					07/14/13				
	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.	07/15/13					07/16/13					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	0.4200	0.4750	0.5010	0.4320	0.4260	0.4508	7.86
32%	0.4750	0.5040	0.4490	0.4670	0.4920	0.4774	4.49
42%	0.4560	0.4940	0.5030	0.4420	0.4690	0.4728	5.40
56%	0.5010	0.4470	0.4830	0.4520	0.5060	0.4778	5.71
75%	0.4590	0.5020	0.4470	0.4960	0.4720	0.4752	4.96
100%	0.4760	0.4830	0.5040	0.4200	0.4690	0.4704	6.60

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

City of Mena WWTF

Lab ID# 21229

Test Date: July 9, 2013

WET CHEMISTRY MEASUREMENTS

Date	Time	Temp	Samp. No.	pH of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
07/09/13	Start	25.0	1	7.88	7.79	7.67	7.50	7.27	7.18	STC
07/10/13	24 Hr.	26.0	1	7.85	7.70	7.59	7.51	7.40	7.22	GZK
07/10/13	Renew	26.0	1	7.68	7.57	7.48	7.39	7.34	7.18	GZK
07/11/13	48 Hr.	26.0	1	7.38	7.41	7.39	7.33	7.26	7.06	GZK
07/11/13	Renew	25.0	2	7.87	7.52	7.40	7.27	7.09	7.18	GZK
07/12/13	72 Hr.	26.0	2	7.72	7.65	7.60	7.55	7.45	7.32	MJK
07/12/13	Renew	25.9	2	7.38	7.32	7.19	7.12	7.03	6.79	MJK
07/13/13	96 Hr.	26.0	2	7.77	7.70	7.59	7.56	7.48	7.34	MJK
07/13/13	Renew	25.9	3	7.49	7.35	7.29	7.21	7.02	7.20	MJK
07/14/13	120 Hr.	26.0	3	7.73	7.64	7.55	7.50	7.46	7.34	STC
07/14/13	Renew	26.0	3	7.64	7.54	7.41	7.32	7.18	6.97	STC
07/15/13	144 Hr.	26.0	3	7.22	7.25	7.19	7.27	7.18	6.95	STC
07/15/13	Renew	25.9	3	7.68	7.53	7.40	7.31	7.17	6.94	STC
07/16/13	168 Hr.	26.0	3	7.57	7.58	7.51	7.46	7.41	7.28	STC

Date	Time	Temp	Samp. No.	DO (mg/L) of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
07/09/13	Start	25.0	1	8.21	8.60	8.84	8.95	8.95	8.52	STC
07/10/13	24 Hr.	26.0	1	8.25	7.68	7.73	7.66	7.61	7.37	GZK
07/10/13	Renew	26.0	1	6.87	7.39	7.01	7.18	7.16	8.52	GZK
07/11/13	48 Hr.	26.0	1	8.28	7.96	7.98	7.93	7.70	8.11	GZK
07/11/13	Renew	25.0	2	8.69	8.79	8.74	8.80	8.80	8.52	GZK
07/12/13	72 Hr.	26.0	2	6.72	6.71	6.68	6.87	7.07	6.96	MJK
07/12/13	Renew	25.9	2	7.01	7.01	6.82	6.79	6.79	6.82	MJK
07/13/13	96 Hr.	26.0	2	6.78	6.79	6.12	6.54	6.66	6.68	MJK
07/13/13	Renew	25.9	3	6.18	6.34	6.36	6.45	6.37	8.50	MJK
07/14/13	120 Hr.	26.0	3	8.33	8.31	7.91	7.83	7.91	7.88	STC
07/14/13	Renew	26.0	3	7.64	7.86	8.03	8.10	8.21	8.19	STC
07/15/13	144 Hr.	26.0	3	7.55	7.90	7.84	7.56	7.66	7.66	STC
07/15/13	Renew	25.9	3	8.41	8.25	8.28	8.43	8.43	8.36	STC
07/16/13	168 Hr.	26.0	3	7.62	7.63	7.84	7.98	8.12	8.30	STC

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

City of Mena WWTF

Lab ID# 21229

Test Date: July 9, 2013

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.Cl2 mg/L ¹	Dechlor(mL) Na2S2O3 mg/L ¹	Analyst
07/09/13	1	7.18	8.52	24	16	141	<0.01	N/A	TN
07/11/13	2	7.19	8.53	28	16	144	<0.01	N/A	TN
07/13/13	3	7.20	8.50	28	12	141	<0.01	N/A	TN
07/09/13	Con	7.88	8.21	40	28	202	-	-	TN

¹Measurements 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	0.420	0.501	0.451
2	32% Effluent	5	0.449	0.504	0.477
3	42% Effluent	5	0.442	0.503	0.473
4	56% Effluent	5	0.447	0.506	0.478
5	75% Effluent	5	0.447	0.502	0.475
6	100% Effluent	5	0.420	0.504	0.470

Summary Statistics on Transformed Data Table 2 of 2

Grp	Identification	Variance	Sd	Sem	C.V.%
1	Control	0.001	0.035	0.016	7.86
2	32% Effluent	0.000	0.021	0.010	4.49
3	42% Effluent	0.001	0.026	0.011	5.40
4	56% Effluent	0.001	0.027	0.012	5.71
5	75% Effluent	0.001	0.024	0.011	4.96
6	100% Effluent	0.001	0.031	0.014	6.60

Shapiro - Wilk's Test For Normality

D = 0.018

W = 0.960

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 = 1.27

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	0.003	0.001	0.669
Within (Error)	24	0.019	0.001	
Total	29	0.021		

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

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

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

Grp	Identification	Transformed Mean	Mean Calculated In Original Units	T Stat	Sig
1	Control	0.451	0.451		
2	32% Effluent	0.477	0.477	-1.514	
3	42% Effluent	0.473	0.473	-1.252	
4	56% Effluent	0.478	0.478	-1.537	
5	75% Effluent	0.475	0.475	-1.389	
6	100% Effluent	0.470	0.470	-1.115	

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

No statistically significant difference

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

Grp	Identification	Num of Reps	Minimum Sig Diff (In Orig. Units)	% of Control	Difference from Control
1	Control	5			
2	32% Effluent	5	0.041	9.2	-0.027
3	42% Effluent	5	0.041	9.2	-0.022
4	56% Effluent	5	0.041	9.2	-0.027
5	75% Effluent	5	0.041	9.2	-0.024
6	100% Effluent	5	0.041	9.2	-0.020

**APPENDIX A
RAW DATA**

7-DAY CERIODAPHNIA DUBIA SURVIVAL & REPRODUCTION
DAILY RAW DATA TABLE
PAGE 1 OF 2

CLIENT Mena
OUTFALL 001
LAB ID # 21229

START DATE/TIME 7-9-13 MH 1415
END DATE/TIME 7-16-13 NL 1415

Con

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
7/10	A	A	A	A	A	A	A	A	A	A	NL	1415
7/11	A	A	A	A	A	A	A	A	A	A	MH	1300
7/12	A	A	A	A	A	A	A	A	A	A	NL	0950
7/13	2	2	2	3	2	2	3	A	A	2	JG	1245
7/14	A	A	A	A	A	A	A	2	3	A	JG	1200
7/15	6	7	8	7	6	6	7	7	6	6	NL	1100
7/16	11	11	12	12	13	12	13	11	12	11	NL	1415

$\bar{x}$ # Young w/o Dead = 20.7 CV% = 6.46

$\bar{x}$ # 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
7/10	A	A	A	A	A	A	A	A	A	A	NL	1415
7/11	A	A	A	A	A	A	A	A	A	A	MH	1300
7/12	A	A	A	A	A	A	A	A	A	A	NL	0950
7/13	A	2	A	4	4	5	3	4	4	2	JG	1245
7/14	2	A	2	A	A	A	A	A	A	6	JG	1200
7/15	6	7	8	7	6	6	8	7	6	A	NL	1100
7/16	13	11	12	12	14	13	12	13	13	14	NL	1415

$\bar{x}$ # Young w/o Dead = 22.6 CV% = 5.97

$\bar{x}$ # 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
7/10	A	A	A	A	A	A	A	A	A	A	NL	1415
7/11	A	A	A	A	A	A	A	A	A	A	MH	1300
7/12	A	A	A	A	A	A	A	A	A	A	NL	0950
7/13	2	2	5	A	4	5	4	5	A	4	JG	1245
7/14	A	8	A	2	A	A	A	A	2	A	JG	1200
7/15	7	A	7	6	6	6	7	8	6	7	NL	1100
7/16	11	12	13	12	13	12	13	14	11	12	NL	1415

$\bar{x}$ # Young w/o Dead = 22.6 CV% = 10.88

$\bar{x}$ # 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
7/10	A	A	A	A	A	A	A	A	A	A	NL	1415
7/11	A	A	A	A	A	A	A	A	A	A	MH	1300
7/12	A	A	A	A	A	A	A	A	A	A	NL	0950
7/13	5	3	5	2	3	5	4	A	A	5	JG	1245
7/14	7	6	A	6	A	A	7	3	2	9	JG	1200
7/15	A	A	7	A	8	9	A	8	7	A	NL	1100
7/16	14	12	13	15	12	12	13	14	13	14	NL	1415

$\bar{x}$ # Young w/o Dead = 24.3 CV% = 8.69

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

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

PAGE 2 OF 2

START DATE/TIME: 7-9-13 MH 1415
END DATE/TIME: 7-16-13 NL 1415

75

$$\bar{x} \# \text{ Young w/o Dead} = 24.6 \text{ CV}\% = 6.12$$
$$\bar{x} \# \text{Young w/Dead} = \quad \quad \quad CV\% =$$
$$\bar{x} \% \text{ Survival} = 100.0 \quad CV\% = 0.00$$

100

$\bar{x}$ # Young w/o Dead = 24.9 CV% = 9.92

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

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

CLIENT/FACILITY Mena
 OUTFALL # 001 PROJECT # 21229
 ORGANISM ID# 21229 PPO-13-189
MH

DATE/TIME STARTED 7-9-13 MH 1500
 DATE/TIME ENDED 7-16-13 26 1500

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
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	7-10-13 26 1500					7-11-13 26 0830					7-12-13 26 0810					7-13-13 TN 0805					7-14-13 TN 0830				

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	7-15-13 26 0840					7-16-13 26 1500						

Huther and Associates, Inc.

environmental toxicologists, biologists, consultants

7-DAY CHRONIC TOXICITY TEST
PIMEPHALES PROMELAS (fathead minnow) MEAN WEIGHT/REP

Client Huma
Project# 21229

Date/Time Start 7/9/13 1500
Date/Time End 7/16/13 1500

% Effluent	Rep A	Rep B	Rep C	Rep D	Rep E	$\bar{x}$	C.V.%	Analyst
Con	.4200	.4750	.5010	.4320	.4260	.4508	7.86	BH
32	.4750	.5040	.4490	.4670	.4920	.4774	4.49	↓
42	.4560	.4940	.5030	.4420	.4690	.4728	5.40	
56	.5010	.4470	.4830	.4580	.5060	.4778	5.71	
75	.4590	.5020	.4470	.4960	.4720	.4752	4.96	
100	.4760	.4830	.5040	.4200	.4690	.4704	6.60	

Client / Facility Mena
 Lab ID Number 21229
 Outfall Number 001
 Test Date 7-9-13

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
7/9	1	7.18	8.52	24	16	141	20.01	16	TN
7/11	2	7.19	8.53	28	16	144	5	5	5
7/13	3	7.20	8.50	28	12	141	5	5	5
7/9	CON	7.88	8.21	40	28	202	—	—	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: 7

TEST DATE/TIME: 06/26/13 - 07/03/13
1100 Hrs - 1100 Hrs

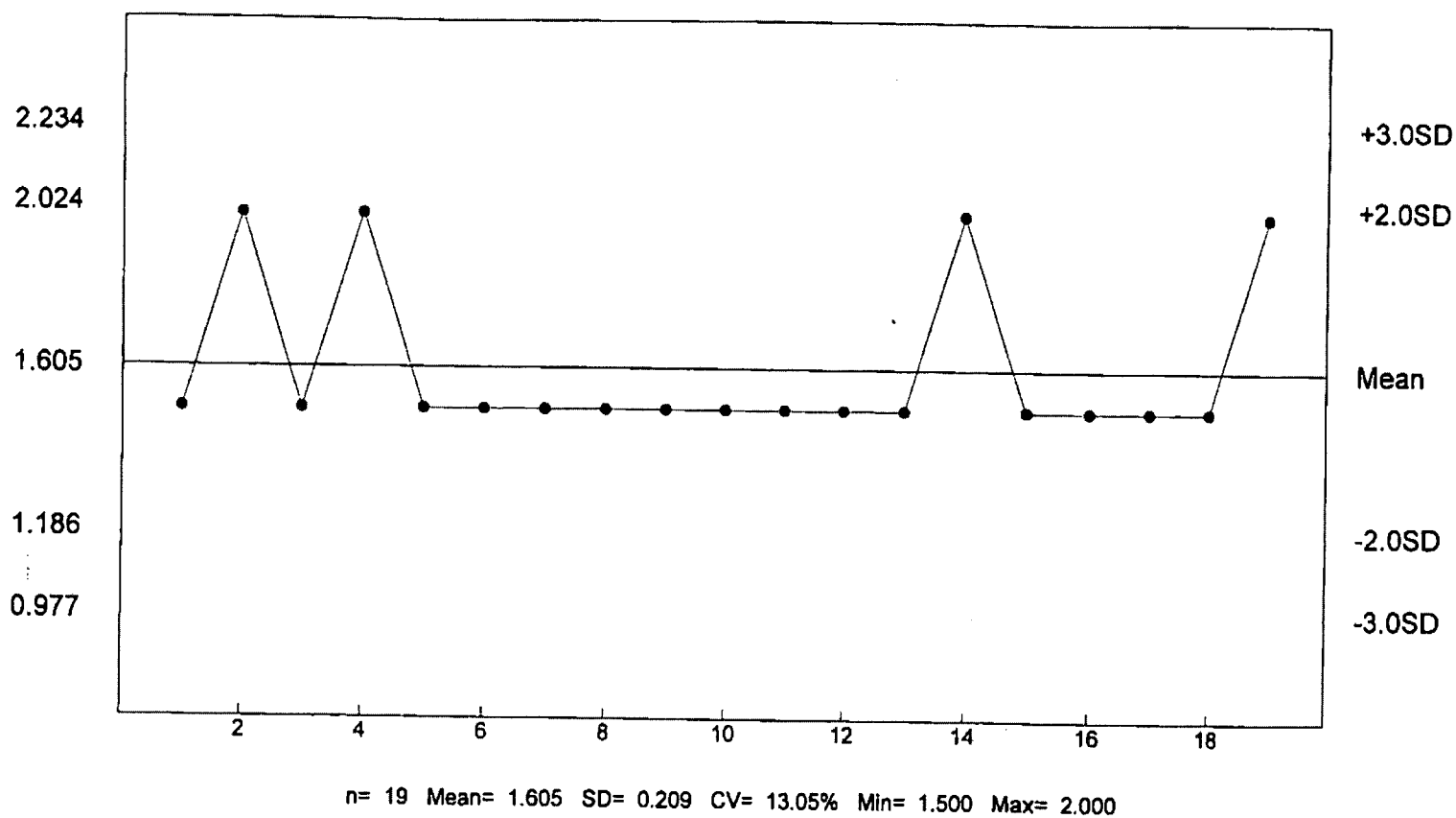
STATISTICAL METHOD: Fishers, Dunnetts/Steels

CONCENTRATION (g/L)	NUMBER EXPOSED	NUMBER DEAD
0.5	10	0
1.0	10	0
1.5	10	0
2.0	10	0
2.5	10	10
3.0	10	10
4.0	10	10

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

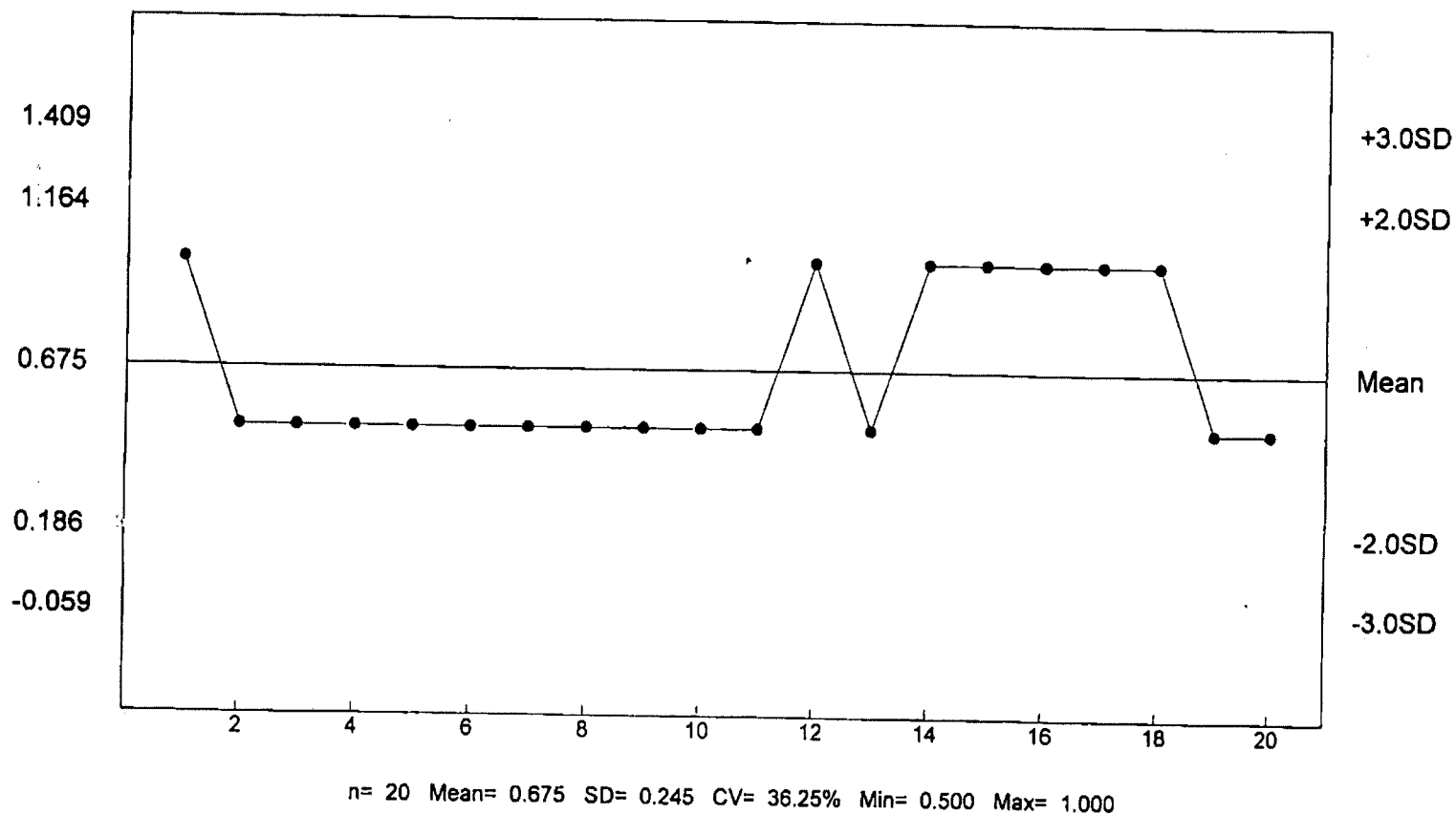
Reference Tox Sodium Chloride g/L

C. dubia Survival - NOEC



Reference Tox Sodium Chloride g/L

C. dubia Reproduction - NOEC



CHRONIC REFERENCE TOXICANT TEST RESULTS

SPECIES: *Pimephales promelas*

CHEMICAL: Copper Nitrate

DURATION: 7-Days

TEST NUMBER: 7

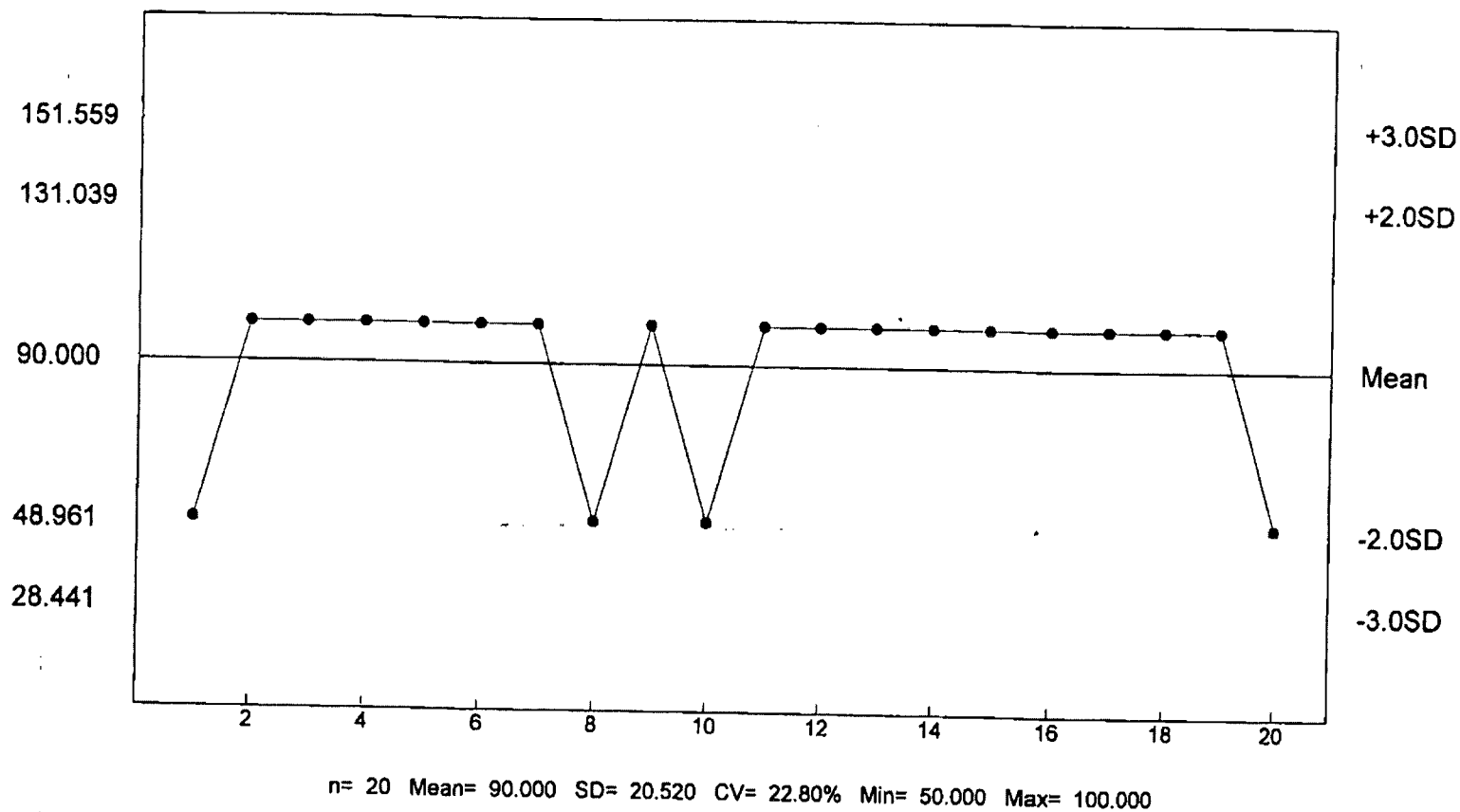
TEST DATE/TIME: 06/26/13 - 07/03/13
1500 Hrs - 1500 Hrs

STATISTICAL METHOD: Dunnetts/Steels

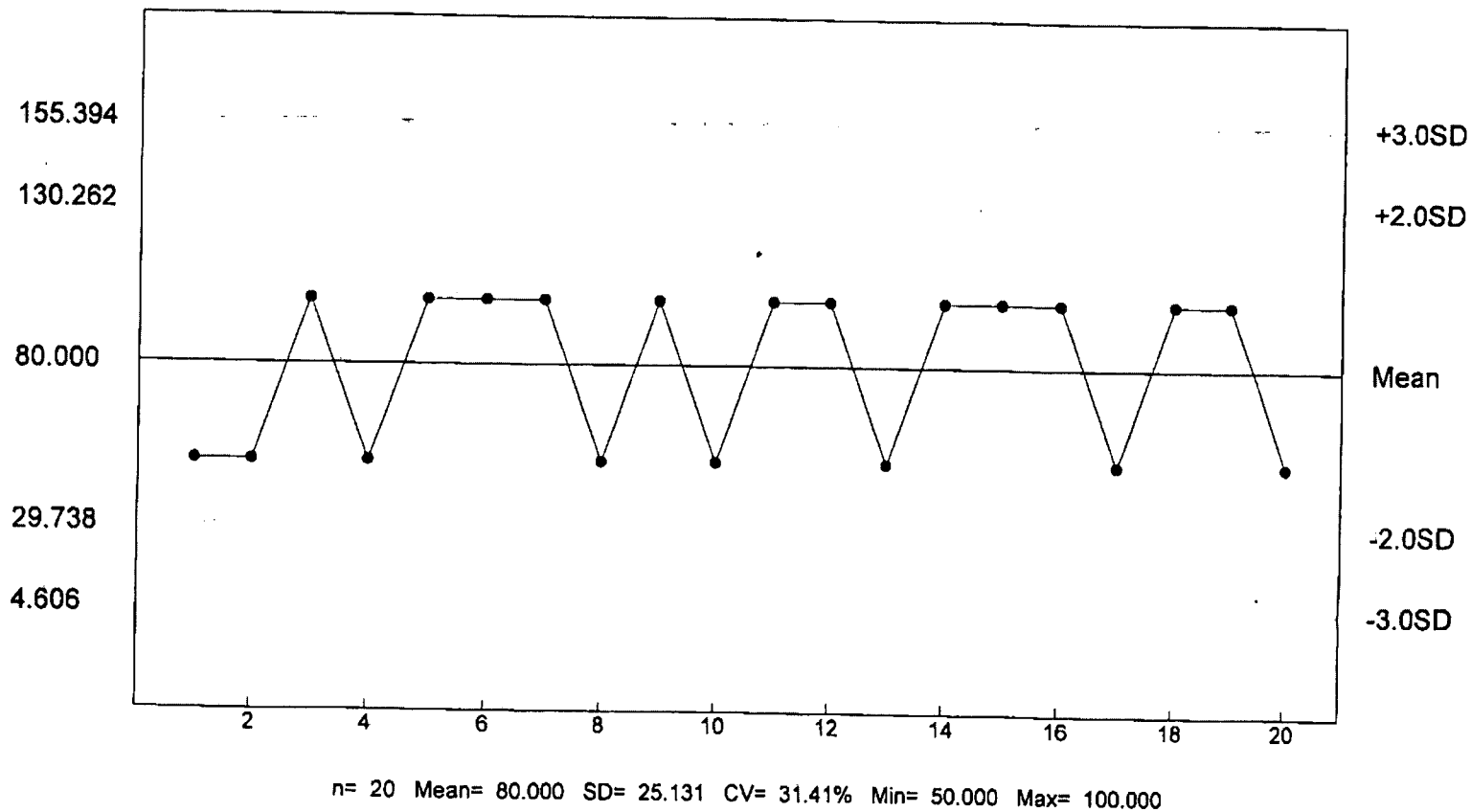
CONCENTRATION (ug/L)	NUMBER EXPOSED	NUMBER DEAD
12.5	40	0
25	40	0
50	40	3
100	40	7
200	40	27
400	40	33
800	40	40

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



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



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 # 21229 PROJECT NAME Mena PERMIT# NPDES AP0036692

OUTFALL SAMPLES

24-Hr Flow Weighted Composite ☒ Other ☐

					METHODS OF COLLECTION AND COMPOSITE			
OUTFALL NUMBER	PERSON TAKING SAMPLE	START DATE/TIME	END DATE/TIME	# OF PORTIONS COMPOSITED	AUTO COLL. AUTO COMP.	MANUAL COLL. MANUAL COMP.	AUTO COLL. MANUAL COMP.	# OF CONTAINERS TO BE SHIPPED
001	Jeff	07 JUL 2013 1200	08 JUL 2013 1200	24	X			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 CF
NAME OF RECEIVING WATER unnamed trib of Prairie Creek

DILUTION WATER USED FOR THIS TEST Lab

RELINQUISHED BY: Jeff Flay DATE: 08 JUL 13 TIME: 1500 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: Matt Gerner DATE: 7-9-13 TIME: 0920 SAMPLE TEMP. @ RECEIPT. 3.4

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

CHAIN OF CUSTODY RECORD

PROJECT # 21229 PROJECT NAME Mena PERMIT# NPDES A20036692

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	1200 09 JUL 2013	1200 10 JUL 2013	24	X			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 unnamed trib. of
Prairie Creek

DILUTION WATER USED FOR THIS TEST Lab

RELINQUISHED BY: Jeff Flynn DATE: 10 JUL 2013 TIME: 1400 RECEIVED BY AT THIS DATE/TIME UPS-Office Store Men

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: Matt Houser DATE: 7-11-13 TIME: 1130 SAMPLE TEMP. @ RECEIPT. 3.4

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

CHAIN OF CUSTODY RECORD

PROJECT # 21229 PROJECT NAME Mena PERMIT# NPDES AR00361692

OUTFALL SAMPLES

24-Hr Flow Weighted Composite ☒ Other _____

					METHODS OF COLLECTION AND COMPOSITE			# OF CONTAINERS TO BE SHIPPED
OUTFALL NUMBER	PERSON TAKING SAMPLE	START DATE/TIME	END DATE/TIME	# OF PORTIONS COMPOSITED	AUTO COLL. AUTO COMP.	MANUAL COLL. MANUAL COMP.	AUTO COLL. MANUAL COMP.	
001	Jeff	11 JUL 2013 0630	12 JUL 2013 0630	24	X			1

RECEIVING WATER SAMPLES

SAMPLE IDENTIFICATION (FOR REC'G) 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 trib of
Prairie Creek

DILUTION WATER USED FOR THIS TEST Lab

RELINQUISHED BY: Jeff DATE: 12 JUL 13 TIME: 0830 RECEIVED BY AT THIS DATE/TIME UPS Store Ft Smith 0830
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: Jeff DATE: 7-13-13 TIME: 0930

**CITY OF MENA WWTF
NPDES PERMIT NO. AR0036692
AFIN 57-00042
BIOMONITORING REPORTING
TEST DATE: 07/09/13**

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	9.92%
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	7.86%
Report Parameter No. 22414 (lowest NOEC value) for <i>Pimephales promelas</i> .	100%

**CITY OF MENA WWTF
OUTFALL 001**

**Chronic Biomonitoring Report
Permit Number NPDES AR0036692**

Ceriodaphnia dubia
Pimephales promelas

October 8, 2013

Reviewed by:

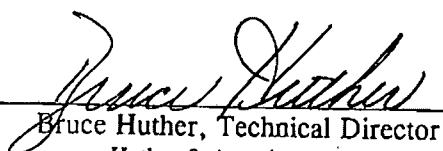

Bruce Huther, Technical Director
Huther & Associates, Inc.
1156 North Bonnie Brae
Denton, Texas 76201
(940) 387-1025, Fax: (940) 387-1036

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TOXICITY TEST REPORT - CHRONIC

Client City of Mena WWTF Laboratory I.D. 21620
Permit No. NPDES AR0036692 Begin Date October 8, 2013
Sample Outfall 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 United Parcel Service courier to Huthur & Associates on October 8 and October 10, 2013. Effluent samples were collected and composited from Outfall 001 using an automatic sampler. Two toxicity tests were requested: a seven-day *Ceriodaphnia dubia* survival and reproduction test (EPA Method 1002.0), and a seven-day *Pimephales promelas* larval survival and growth test (EPA Method 1000.0). Test organisms, procedures and quality assurance requirements were in accordance with the EPA manual, "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition" (EPA-821-R-02-013). Note: Due to shipping error, the third sample did not arrive until test termination date. There was enough sample two to conduct daily renewals.

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 1440 hours, October 8, 2013. 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 1440 hours, October 15, 2013. 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: 7.2 %

TEST SETUP***Pimephales promelas***

The seven-day *Pimephales promelas* larval survival and growth test was initiated at 1600 hours, October 8, 2013. 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 1600 hours, October 15, 2013. 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: 9.7%

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 Mena WWTF	SAMPLE TYPE	24 Hour Composite
NPDES #	AR0036692	DATE COLLECTED	10/07/13 10/09/13
LAB ID #	21620	DATE RECEIVED	10/08/13 10/10/13
TEST TYPE	7 Day Chronic	BEGIN DATE/TIME	10/08/13 1440
TEST ORGANISM	<i>Ceriodaphnia dubia</i>	END DATE/TIME	10/15/13 1440
ORGANISM AGE	< 24 Hours	TEST TEMPERATURE (°C)	25
ORGANISM SOURCE	In House	PHOTO PERIOD	16-hr. Light 8-hr. Dark
RECEIVING WATER	unnamed tributary of Prairie Creek	LIGHT INTENSITY	50-100 ft. candl.
DILUTION WATER	Laboratory Adjusted	TECHNICIAN	T. Nesbitt

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
10/09/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/10/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/11/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/12/13	3	2	2	3	2	3	4	3	2	2
	3	2	2	3	2	3	4	3	2	2
10/13/13	A	A	A	A	A	A	A	A	A	A
	3	2	2	3	2	3	4	3	2	2
10/14/13	9	7	7	8	7	10	8	7	8	8
	12	9	9	11	8	13	12	10	10	10
10/15/13	13	12	12	11	12	12	13	13	12	12
	25	21	21	22	21	26	23	22	22	22
x # Young 227 C.V. 7.50% 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
10/09/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/10/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/11/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/12/13	3	2	3	3	4	2	3	2	2	4
	3	2	3	3	4	2	3	2	2	4
10/13/13	A	A	A	A	A	A	A	A	A	A
	3	2	3	3	4	2	3	2	2	4
10/14/13	7	10	8	8	10	9	10	6	10	11
	10	12	11	11	14	11	13	8	12	15
10/15/13	13	12	12	13	12	12	12	13	12	13
	23	24	23	24	26	23	25	21	24	26
x # Young 241 C.V. 7.93% 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
10/09/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/10/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/11/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/12/13	2	3	2	4	3	2	2	3	2	4
	2	3	2	4	3	2	2	3	2	4
10/13/13	A	A	A	A	A	A	A	A	A	A
	2	3	2	4	3	2	2	3	2	4
10/14/13	8	8	8	9	8	10	10	7	7	8
	10	11	10	13	11	12	12	10	9	12
10/15/13	14	12	12	12	13	12	13	13	12	13
	24	23	22	25	24	24	26	23	21	26
x # Young 236 C.V. 5.72% x% Survival 100% C.V. 0.00%										

58% Effluent										
Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
10/09/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/10/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/11/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/12/13	2	4	3	3	2	3	2	4	3	3
	2	4	3	3	2	3	2	4	3	3
10/13/13	A	A	A	A	A	A	A	A	A	A
	2	4	3	3	2	3	2	4	3	3
10/14/13	9	9	7	9	8	10	10	8	8	9
	11	13	10	12	10	13	12	12	11	12
10/15/13	13	12	13	13	12	14	12	12	13	13
	24	26	23	26	22	27	24	24	24	25
x # Young 243 C.V. 5.50% 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# 21620

Test Date: October 8, 2013

75% Effluent

Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
10/09/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/10/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/11/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/12/13	2	3	2	4	3	4	3	3	2	3
	2	3	2	4	3	4	3	3	2	3
10/13/13	A	A	A	A	A	A	A	A	A	A
	2	3	2	4	3	4	3	3	2	3
10/14/13	8	7	8	7	7	9	9	8	9	7
	10	10	10	11	10	13	12	11	11	10
10/15/13	13	12	13	13	14	12	13	12	12	12
	23	22	23	24	24	25	25	23	23	22
x # Young 23.4 C.V. 4.59% 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
10/09/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/10/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/11/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/12/13	2	3	3	4	2	3	4	3	2	3
	2	3	3	4	2	3	4	3	2	3
10/13/13	A	A	A	A	A	A	A	A	A	A
	2	3	3	4	2	3	4	3	2	3
10/14/13	7	7	8	7	6	9	7	9	10	9
	9	10	11	11	8	12	11	12	12	12
10/15/13	12	12	13	13	12	13	13	14	13	12
	21	22	24	24	20	25	24	26	25	24
x # Young 23.5 C.V. 8.09% 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

City of Mena WWTF

Lab ID# 21620

Test Date: October 8, 2013

WET CHEMISTRY MEASUREMENTS

Date	Time	Temp	Samp. No.	pH of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
10/08/13	Start	25.0	1	7.61	7.56	7.48	7.44	7.36	6.77	STC
10/09/13	24 Hr.	24.4	1	7.61	7.50	7.44	7.40	7.32	7.27	GZK
10/09/13	Renew	24.5	1	7.57	7.51	7.46	7.40	7.30	6.96	GZK
10/10/13	48 Hr.	25.1	1	7.65	7.58	7.50	7.44	7.37	7.26	SCC
10/10/13	Renew	24.8	2	8.07	7.82	7.34	7.30	7.25	7.04	SCC
10/11/13	72 Hr.	25.1	2	7.71	7.60	7.50	7.43	7.36	7.26	SCC
10/11/13	Renew	25.1	2	7.64	7.53	7.43	7.36	7.30	6.97	SCC
10/12/13	96 Hr.	25.6	2	7.98	7.82	7.70	7.57	7.46	7.29	SCC
10/12/13	Renew	24.3	2	7.68	7.66	7.58	7.51	7.41	6.99	SCC
10/13/13	120 Hr.	25.4	2	7.71	7.62	7.53	7.47	7.39	7.26	STC
10/13/13	Renew	25.2	2	7.62	7.57	7.49	7.42	7.30	7.11	STC
10/14/13	144 Hr.	25.2	2	7.75	7.61	7.54	7.45	7.36	7.24	STC
10/14/13	Renew	25.1	2	7.58	7.53	7.47	7.39	7.26	7.00	STC
10/15/13	168 Hr.	25.0	2	7.60	7.47	7.40	7.33	7.22	7.03	GZK

Date	Time	Temp	Samp. No.	DO (mg/L) of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
10/08/13	Start	25.0	1	8.26	8.32	8.77	8.83	8.96	8.66	STC
10/09/13	24 Hr.	24.4	1	8.13	7.51	7.74	7.65	7.59	8.19	GZK
10/09/13	Renew	24.5	1	8.02	8.20	7.87	8.09	8.11	8.21	GZK
10/10/13	48 Hr.	25.1	1	8.24	8.15	8.10	8.05	7.94	8.00	SCC
10/10/13	Renew	24.8	2	8.24	8.28	8.67	8.79	8.63	8.97	SCC
10/11/13	72 Hr.	25.1	2	8.07	7.96	7.85	7.87	7.98	7.81	SCC
10/11/13	Renew	25.1	2	8.00	8.59	8.23	8.36	8.51	8.75	SCC
10/12/13	96 Hr.	25.6	2	8.21	8.00	7.96	8.19	8.19	8.20	SCC
10/12/13	Renew	24.3	2	8.08	8.04	7.95	7.91	8.17	8.75	SCC
10/13/13	120 Hr.	25.4	2	8.04	8.74	8.73	7.76	8.55	8.48	STC
10/13/13	Renew	25.2	2	8.28	8.72	8.86	8.74	8.87	8.94	STC
10/14/13	144 Hr.	25.2	2	7.64	7.72	8.77	7.92	8.07	7.77	STC
10/14/13	Renew	25.1	2	8.12	8.22	8.35	8.71	8.16	8.24	STC
10/15/13	168 Hr.	25.0	2	8.57	8.32	7.94	7.98	7.69	7.56	GZK

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

City of Mena WWTF

Lab ID# 21620

Test Date: October 8, 2013

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
10/08/13	1	6.77	8.66	24	20	203	<0.01	N/A	TN
10/10/13	2	6.97	8.75	20	24	198	<0.01	N/A	TN
10/08/13	Con	7.61	8.26	40	96	210	<0.01	N/A	TN

[†] Measurements taken in 100% solution.

CERIODAPHNIA DUBIA STATISTICAL ANALYSES
 Reproduction

Summary Statistics on Transformed Data Table 1 of 2

Grp	Identification	N	Min	Max	Mean
1	Control	10	21.000	25.000	22.700
2	32% Effluent	10	21.000	28.000	24.100
3	42% Effluent	10	21.000	25.000	23.600
4	56% Effluent	10	22.000	27.000	24.300
5	75% Effluent	10	22.000	25.000	23.400
6	100% Effluent	10	20.000	26.000	23.500

Summary Statistics on Transformed Data Table 2 of 2

Grp	Identification	Variance	Sd	Sem	C.V.%
1	Control	2.900	1.703	0.539	7.50
2	32% Effluent	3.656	1.912	0.605	7.93
3	42% Effluent	1.822	1.350	0.427	5.72
4	56% Effluent	1.789	1.337	0.423	5.50
5	75% Effluent	1.156	1.075	0.340	4.59
6	100% Effluent	3.611	1.900	0.601	8.09

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	16.000	3.200	1.286
Within (Error)	54	134.400	2.489	
Total	59	150.400		

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

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

Grp	Identification	Transformed Mean	Mean Calculated In Original Units	T Stat	Sig
1	Control	22.700	22.700		
2	32% Effluent	24.100	24.100	-1.984	
3	42% Effluent	23.600	23.600	-1.276	
4	56% Effluent	24.300	24.300	-2.268	
5	75% Effluent	23.400	23.400	-0.992	
6	100% Effluent	23.500	23.500	-1.134	

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

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	12	25	17	2

Calculated Chi-Square goodness of fit test statistic = 2.0648
 Table Chi-Square value (alpha = 0.01) = 13.277

Data Pass normality test. Continue analysis.

Bartlett's Test For Homogeneity of Variance

Calculated B1 statistic = 4.31

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.

Dunnett'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	1.630	7.2	-1.400
3	42% Effluent	10	1.630	7.2	-0.900
4	56% Effluent	10	1.630	7.2	-1.600
5	75% Effluent	10	1.630	7.2	-0.700
6	100% Effluent	10	1.630	7.2	-0.800

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

CLIENT	City of Mena WWTF	SAMPLE TYPE	24 Hour Composite
NPDES #	AR0036692	DATE COLLECTED	10/07/13 10/09/13
LAB ID #	21620	DATE RECEIVED	10/08/13 10/10/13
TEST TYPE	7 Day Chronic	BEGIN DATE/TIME	10/08/13 1600
TEST ORGANISM	<i>Pimephales promelas</i>	END DATE/TIME	10/15/13 1600
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 Adjusted	TECHNICIAN	M. Horner

SURVIVAL SUMMARY

Conc.	10/09/13					10/10/13					10/11/13					10/12/13					10/13/13				
	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.	10/14/13					10/15/13					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	0.4650	0.4290	0.4770	0.4950	0.4260	0.4584	6.58
32%	0.4890	0.5040	0.4440	0.4860	0.4920	0.4830	4.73
42%	0.4260	0.4750	0.5040	0.4460	0.4920	0.4686	6.89
56%	0.4910	0.4220	0.4650	0.4290	0.5030	0.4620	7.82
75%	0.4470	0.4860	0.5030	0.4490	0.4860	0.4742	5.25
100%	0.5030	0.4250	0.4670	0.4560	0.4910	0.4684	6.53

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

City of Mena WWTF

Lab ID# 21620

Test Date: October 8, 2013

WET CHEMISTRY MEASUREMENTS

Date	Time	Temp	Samp. No.	pH of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
10/08/13	Start	25.0	1	7.61	7.56	7.48	7.44	7.36	6.77	STC
10/09/13	24 Hr.	24.8	1	7.71	7.46	7.39	7.30	7.19	7.09	GZK
10/09/13	Renew	24.5	1	7.57	7.51	7.46	7.40	7.30	6.96	GZK
10/10/13	48 Hr.	25.0	1	7.37	7.31	7.28	7.25	7.15	7.00	SCC
10/10/13	Renew	24.8	2	8.07	7.82	7.34	7.30	7.25	7.04	SCC
10/11/13	72 Hr.	25.4	2	7.55	7.47	7.37	7.29	7.18	7.02	SCC
10/11/13	Renew	25.1	2	7.64	7.53	7.43	7.36	7.30	6.97	SCC
10/12/13	96 Hr.	25.8	2	7.53	7.45	7.36	7.33	7.26	7.13	SCC
10/12/13	Renew	24.3	2	7.68	7.66	7.58	7.51	7.41	6.99	SCC
10/13/13	120 Hr.	25.5	2	7.58	7.50	7.43	7.39	7.22	7.14	STC
10/13/13	Renew	25.2	2	7.62	7.57	7.49	7.42	7.30	7.11	STC
10/14/13	144 Hr.	25.4	2	7.33	7.35	7.32	7.29	7.20	7.10	STC
10/14/13	Renew	25.1	2	7.58	7.53	7.47	7.39	7.26	7.00	STC
10/15/13	168 Hr.	25.4	2	6.92	6.81	6.75	6.69	6.76	6.59	STC

Date	Time	Temp	Samp. No.	DO (mg/L) of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
10/08/13	Start	25.0	1	8.26	8.32	8.77	8.83	8.96	8.66	STC
10/09/13	24 Hr.	24.8	1	7.88	7.90	8.03	7.88	7.93	8.01	GZK
10/09/13	Renew	24.5	1	8.02	8.20	7.87	8.09	8.11	8.21	GZK
10/10/13	48 Hr.	25.0	1	7.97	8.01	8.29	7.89	7.35	7.75	SCC
10/10/13	Renew	24.8	2	8.24	8.28	8.67	8.79	8.63	8.97	SCC
10/11/13	72 Hr.	25.4	2	7.76	7.55	8.20	7.74	7.67	7.65	SCC
10/11/13	Renew	25.1	2	8.00	8.59	8.23	8.36	8.51	8.75	SCC
10/12/13	96 Hr.	25.8	2	7.33	8.07	8.18	7.95	8.02	7.96	SCC
10/12/13	Renew	24.3	2	8.08	8.04	7.95	7.91	8.17	8.75	SCC
10/13/13	120 Hr.	25.5	2	7.67	7.69	7.50	7.61	7.51	7.60	STC
10/13/13	Renew	25.2	2	8.28	8.72	8.86	8.74	8.87	8.94	STC
10/14/13	144 Hr.	25.4	2	8.02	7.73	8.00	7.76	7.73	7.71	STC
10/14/13	Renew	25.1	2	8.12	8.22	8.35	8.71	8.16	8.24	STC
10/15/13	168 Hr.	25.4	2	7.88	7.99	7.86	8.06	8.71	8.02	STC

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

City of Mena WWTF

Lab ID# 21620

Test Date: October 8, 2013

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
10/08/13	1	6.77	8.66	24	20	203	<0.01	N/A	TN
10/10/13	2	6.97	8.75	20	24	198	<0.01	N/A	TN
10/08/13	Con	7.61	8.26	40	96	210	<0.01	N/A	TN

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	0.426	0.495	0.458
2	32% Effluent	5	0.444	0.504	0.483
3	42% Effluent	5	0.426	0.504	0.469
4	56% Effluent	5	0.422	0.503	0.462
5	75% Effluent	5	0.447	0.503	0.474
6	100% Effluent	5	0.425	0.503	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	6.58
2	32% Effluent	0.001	0.023	0.010	4.73
3	42% Effluent	0.001	0.032	0.014	6.89
4	56% Effluent	0.001	0.036	0.016	7.82
5	75% Effluent	0.001	0.025	0.011	5.25
6	100% Effluent	0.001	0.031	0.014	6.53

Shapiro - Wilk's Test For Normality

D = 0.021

W = 0.917

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 = 1.01

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	0.433
Within (Error)	24	0.021	0.001	
Total	29	0.023		

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 Calculated In Original Units	T Stat	Sig
1	Control	0.458	0.458		
2	32% Effluent	0.483	0.483	-1.304	
3	42% Effluent	0.469	0.469	-0.541	
4	56% Effluent	0.462	0.462	-0.191	
5	75% Effluent	0.474	0.474	-0.838	
6	100% Effluent	0.468	0.468	-0.530	

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

No statistically significant difference

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

Grp	Identification	Num of Reps	Minimum Sig Diff (In Orig. Units)	% of Control	Difference from Control
1	Control	5			
2	32% Effluent	5	0.045	9.7	-0.025
3	42% Effluent	5	0.045	9.7	-0.010
4	56% Effluent	5	0.045	9.7	-0.004
5	75% Effluent	5	0.045	9.7	-0.016
6	100% Effluent	5	0.045	9.7	-0.010

**APPENDIX A
RAW DATA**

DAY CERIODAPHNIA DUBIA SURVIVAL & REPRODUCTION

DAILY RAW DATA TABLE

PAGE 1 OF 2

CLIENT Mena

START DATE/TIME 10-8-13 TN 1440

OUTFALL 001

END DATE/TIME 10-15-13 ZG 1440

LAB ID # 21620

CON

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
10/9	A	A	A	A	A	A	A	A	A	A	ZG	1440
10/10	A	A	A	A	A	A	A	A	A	A	NL	1405
10/11	A	A	A	A	A	A	A	A	A	A	ZG	1230
10/12	3	2	2	3	2	3	4	3	2	2	NL	1545
10/13	A	A	A	A	A	A	A	A	A	A	NL	1045
10/14	9	7	7	8	7	10	8	7	8	8	ZG	1445
10/15	13	12	12	11	12	12	13	13	12	12	ZG	1440

$\bar{x}$ # Young w/o Dead = 22.7 CV% = 7.50

$\bar{x}$ # 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
10/9	A	A	A	A	A	A	A	A	A	A	ZG	1440
10/10	A	A	A	A	A	A	A	A	A	A	NL	1405
10/11	A	A	A	A	A	A	A	A	A	A	ZG	1230
10/12	3	2	3	3	4	2	3	2	2	4	NL	1545
10/13	A	A	A	A	A	A	A	A	A	A	NL	1045
10/14	7	10	8	8	10	9	10	6	10	11	ZG	1445
10/15	13	12	12	13	12	12	12	13	12	13	ZG	1440

$\bar{x}$ # Young w/o Dead = 24.1 CV% = 7.93

$\bar{x}$ # 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
10/9	A	A	A	A	A	A	A	A	A	A	ZG	1440
10/10	A	A	A	A	A	A	A	A	A	A	NL	1405
10/11	A	A	A	A	A	A	A	A	A	A	ZG	1230
10/12	2	3	2	4	3	2	2	3	2	4	NL	1545
10/13	A	A	A	A	A	A	A	A	A	A	NL	1045
10/14	8	8	8	9	8	10	10	7	7	8	ZG	1445
10/15	14	12	12	12	13	12	13	13	12	13	ZG	1440

$\bar{x}$ # Young w/o Dead = 23.6 CV% = 5.72

$\bar{x}$ # 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
10/9	A	A	A	A	A	A	A	A	A	A	ZG	1440
10/10	A	A	A	A	A	A	A	A	A	A	NL	1405
10/11	A	A	A	A	A	A	A	A	A	A	ZG	1230
10/12	2	4	3	3	2	3	2	4	3	2	NL	1545
10/13	A	A	A	A	A	A	A	A	A	A	NL	1045
10/14	9	9	7	9	8	10	10	8	8	9	ZG	1445
10/15	13	12	13	13	12	14	12	12	13	13	ZG	1440

$\bar{x}$ # Young w/o Dead = 24.3 CV% = 5.50

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

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

DAY, CERIODAPHNIA DUBIA SURVIVAL & REPRODUCTION

DAILY RAW DATA TABLE

PAGE 2 OF 2

CLIENT Mena

OUTFALL DD1

LAB ID # 21620

75

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
10/9	A	A	A	A	A	A	A	A	A	A	ZG	1440
10/10	A	A	A	A	A	A	A	A	A	A	NL	1405
10/11	A	A	A	A	A	A	A	A	A	A	ZG	1230
10/12	2	3	2	4	3	4	3	3	2	3	NL	1915
10/13	A	A	A	A	A	A	A	A	A	A	NL	1045
10/14	8	7	8	7	7	9	9	8	9	7	ZG	1445
10/15	13	12	13	13	14	12	13	12	12	12	ZG	1440
	23	22	23	24	24	25	25	23	23	22		

$\bar{x}$ # Young w/o Dead = 23.4 CV% = 4.59

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

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

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

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

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

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

START DATE/TIME 10-8-13 TN 1440

END DATE/TIME 10-15-13 ZG 1440

100

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
10/9	A	A	A	A	A	A	A	A	A	A	ZG	1440
10/10	A	A	A	A	A	A	A	A	A	A	NL	1405
10/11	A	A	A	A	A	A	A	A	A	A	ZG	1230
10/12	2	3	3	4	2	3	4	3	2	3	NL	1545
10/13	A	A	A	A	A	A	A	A	A	A	NL	1045
10/14	7	7	8	7	6	9	7	9	10	9	ZG	1445
10/15	12	12	13	13	12	13	13	14	13	12	ZG	1440
	21	22	24	24	20	25	24	26	25	24		

$\bar{x}$ # Young w/o Dead = 23.5 CV% = 8.09

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

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

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

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

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

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

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

CLIENT/FACILITY Mena
 OUTFALL # 001 PROJECT # 21620
 ORGANISM ID# PPU-13-281

DATE/TIME STARTED 10-8-13 2G 1600
 DATE/TIME ENDED 10-15-13 TN 11000

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
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	10-9-13 MH 1600					10-10-13 ZK 0830					10-11-13 MH 0840					10-12-13 ZK 0910					10-13-13 ZK 0805				

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	10-14-13 ZG 0825					10-15-13 TN 11000						

7-DAY CHRONIC TOXICITY TEST
PIMEPHALES PROMELAS (fathead minnow) MEAN WEIGHT/REP

Client Mma
Project# 21620

Date/Time Start 10/8/13 1600
Date/Time End 10/15/13 1600

% Effluent	Rep A	Rep B	Rep C	Rep D	Rep E	$\bar{x}$	C.V.%	Analyst
0	.4650	.4290	.4770	.4950	.4260	.4584	6.58	BH
32	.4890	.5040	.4440	.4660	.4920	.4830	4.73	
64	.4260	.4750	.5040	.4460	.4920	.4686	6.89	
96	.4910	.4220	.4650	.4290	.5030	.4620	7.82	
75	.4470	.4860	.5030	.4490	.4860	.4742	5.25	
100	.5030	.4250	.4670	.4560	.4910	.4684	6.53	✓

Huther and Associates, Inc.

environmental toxicologists, biologists, and consultants

Client / Facility Mona
Lab ID Number 21620
Outfall Number 001
Test Date 10-8-13

INITIAL CHEMISTRY MEASUREMENTS @ 100% EFFLUENT

Date	Samp. No.	pH	DO	Hardness mg/L CaCO ₃ ¹	Alkalinity mg/L CaCO ₃ ¹	Conduct. umbos/cm ¹	Resid. Cl ₂ mg/L ¹	Dechlor(mL) Na ₂ S ₂ O ₃ mg/L ¹	Analyst
10/8	1	6.77	8.66	24	20	203	20.01	Na	TR
10/10	2	6.97	8.75	20	24	198	20.01	Na	
10/8	CON	7.61	8.26	40	96	210	—	—	

INITIAL CHEMISTRY MEASUREMENTS @ RECEIVING WATER

Date	Samp. No.	pH	DO	Hardness mg/L CaCO ₃ ¹	Alkalinity mg/L CaCO ₃ ¹	Conduct. umbos/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: 10

TEST DATE/TIME: 10/01/13 - 10/08/13
1630 Hrs - 1630 Hrs

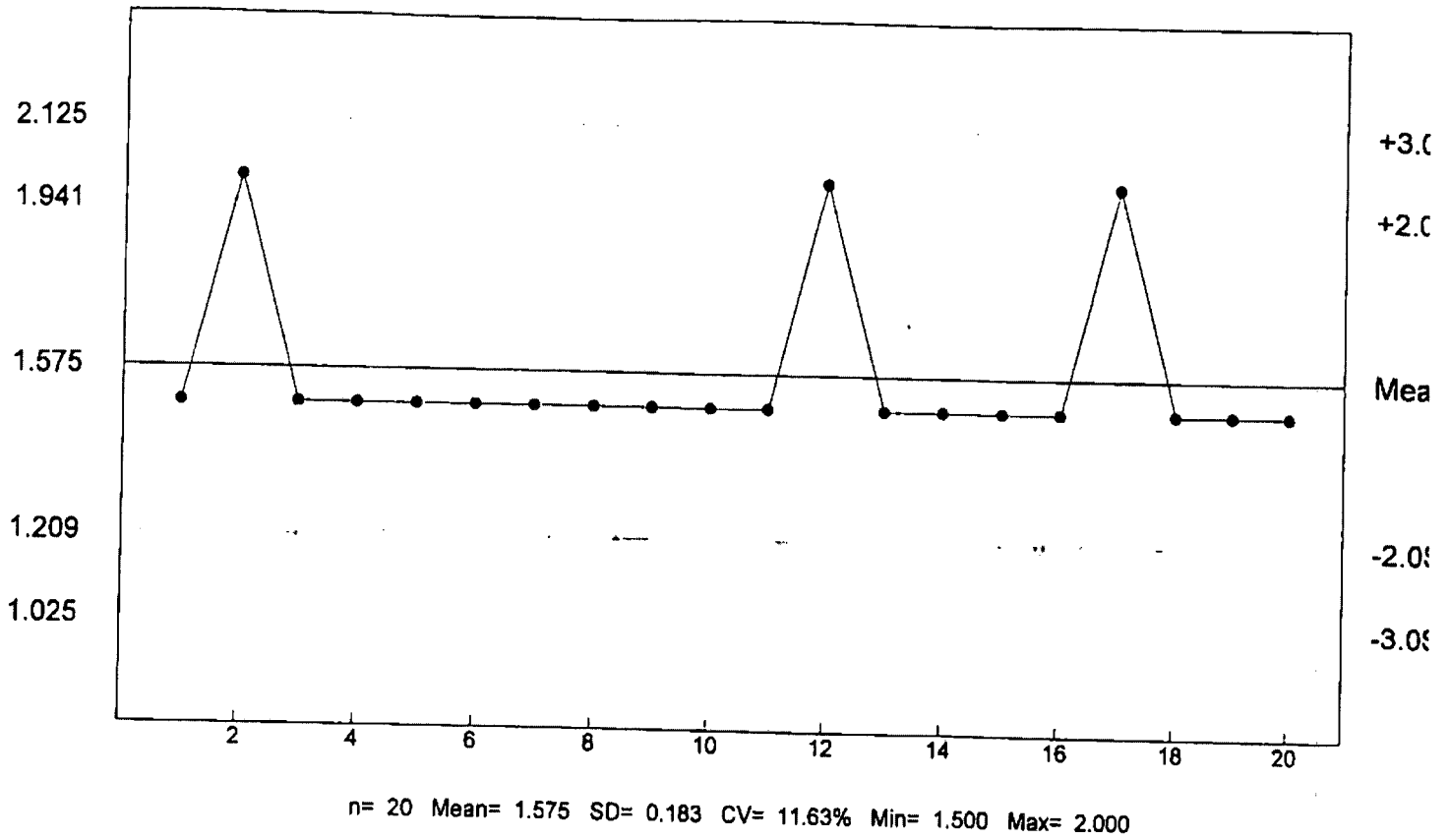
STATISTICAL METHOD: Fishers, Dunnetts/Steels

CONCENTRATION (g/L)	NUMBER EXPOSED	NUMBER DEAD
0.5	10	0
1.0	10	0
1.5	10	0
2.0	10	7
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	1.5 g/L	1.0 g/L

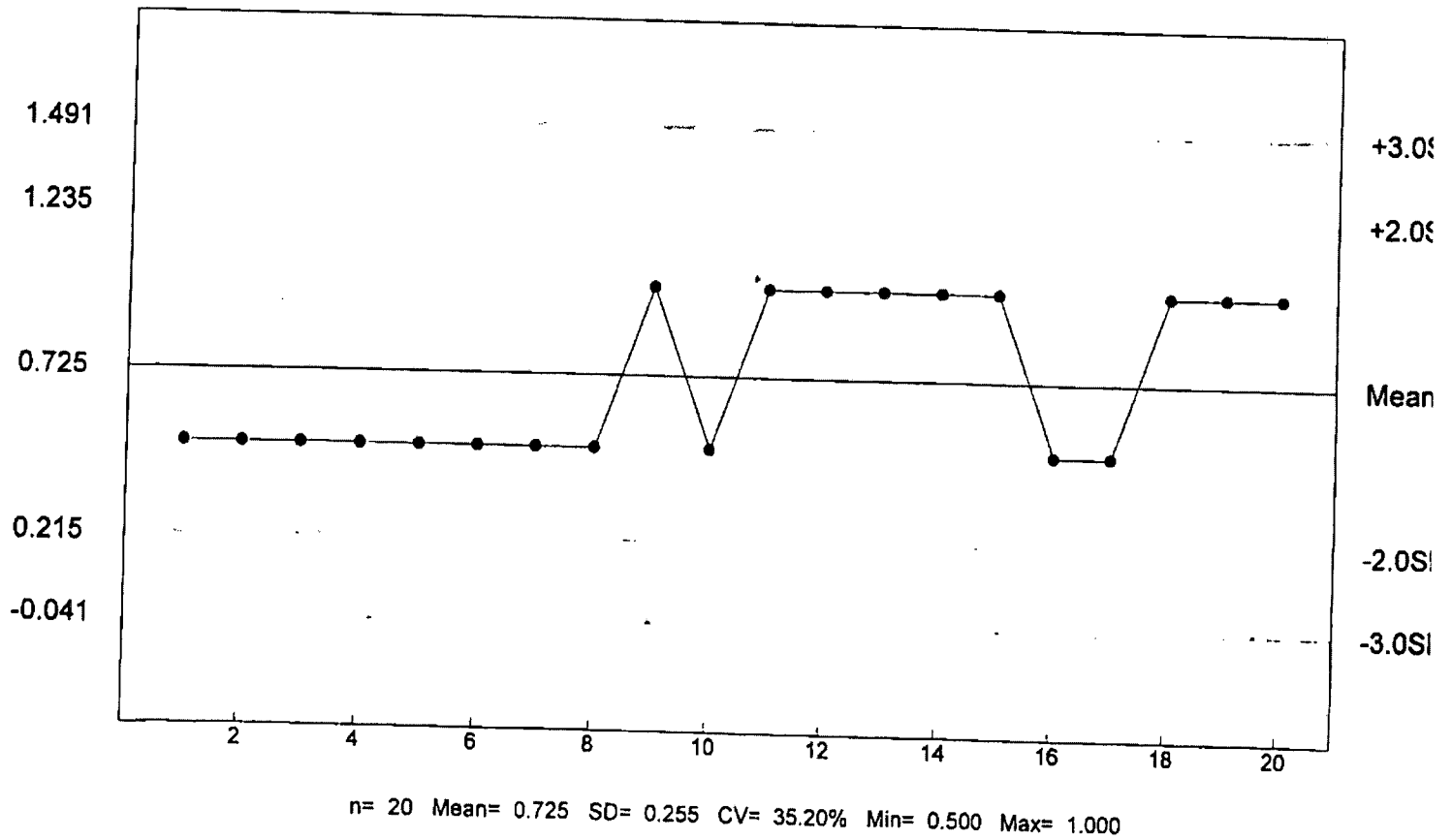
Reference Tox Sodium Chloride g/L

C. dubia Survival - NOEC



Reference Tox Sodium Chloride g/L

C. dubia Reproduction - NOEC



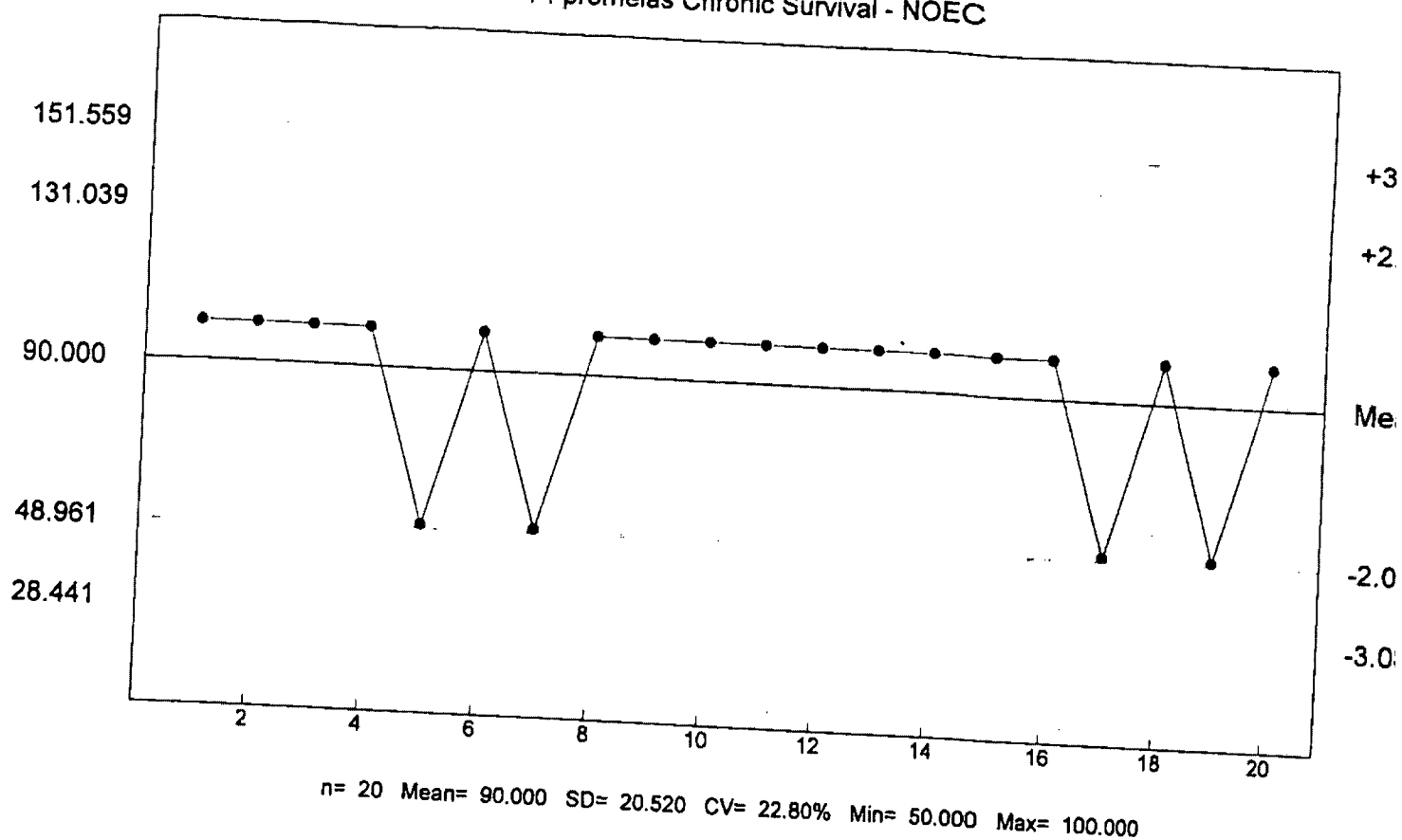
CHRONIC REFERENCE TOXICANT TEST RESULTS

SPECIES: *Pimephales promelas*
 CHEMICAL: Copper Nitrate
 DURATION: 7-Days
 TEST NUMBER: 10
 TEST DATE/TIME: 10/01/13 - 10/08/13
 1530 Hrs - 1530 Hrs
 STATISTICAL METHOD: Dunnetts/Steels

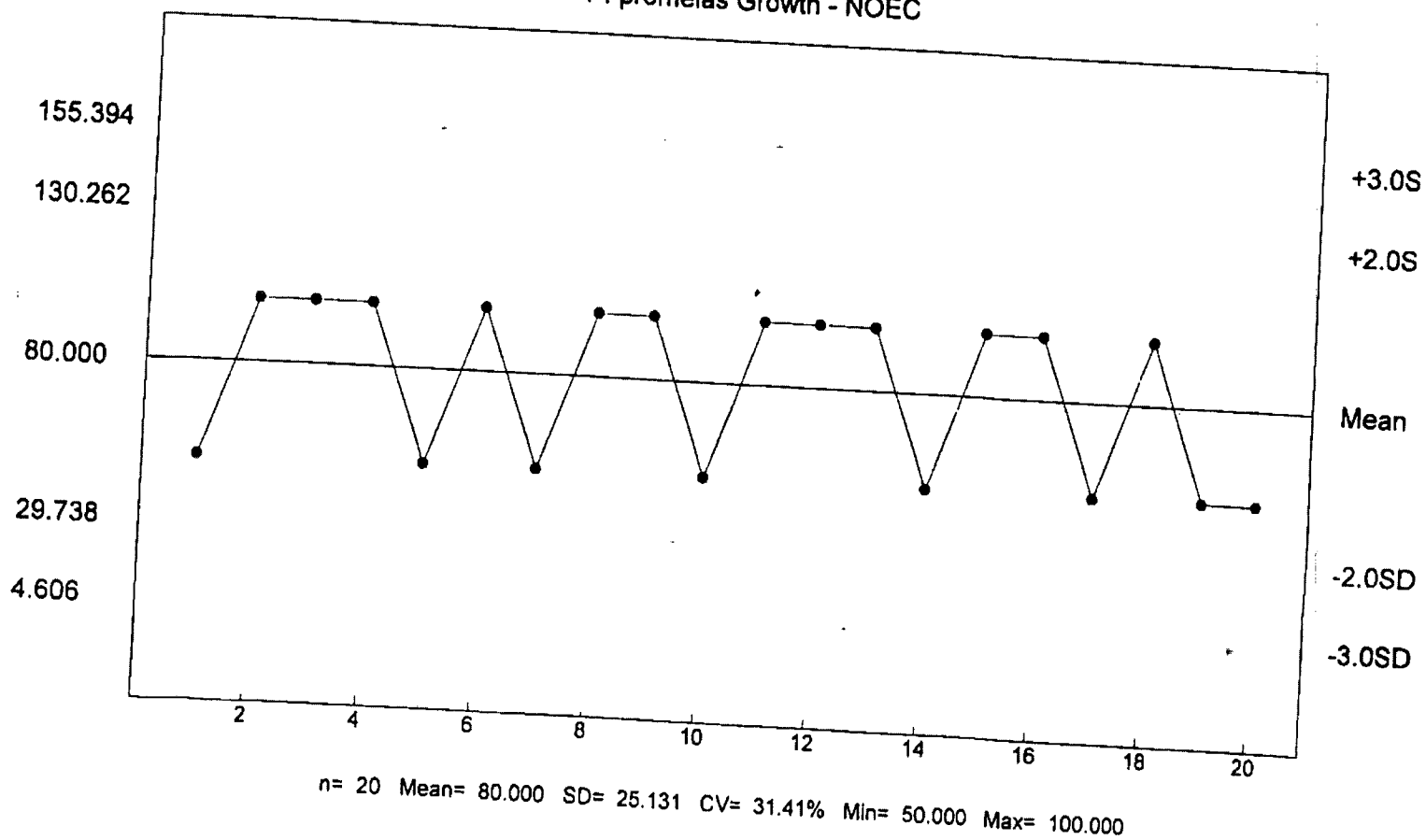
CONCENTRATION (ug/L)	NUMBER EXPOSED	NUMBER DEAD
12.5	40	0
25	40	0
50	40	0
100	40	10
200	40	23
400	40	40
800	40	40

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



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



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 # 211020 PROJECT NAME Mena PERMIT# NPDES AR003669

OUTFALL SAMPLES

24-Hr Flow Weighted Composite ☒ Other _____

					METHODS OF COLLECTION AND COMPOSITE			# OF CONTAINERS TO BE SHIPPED
OUTFALL NUMBER	PERSON TAKING SAMPLE	START DATE/TIME	END DATE/TIME	# OF PORTIONS COMPOSITED	AUTO COLL. AUTO COMP.	MANUAL COLL. MANUAL COMP.	AUTO COLL. MANUAL COMP.	
001	Jeff	06 OCT 2013 1200	07 OCT 2013 1200	24	X			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 9F
NAME OF RECEIVING WATER unnamed trib. of
Prairie Creek

DILUTION WATER USED FOR THIS TEST lab

RELINQUISHED BY: Jeff Flayman DATE: 07 OCT 2013 TIME: 1430 RECEIVED BY AT THIS DATE/TIME: Office Store - Mena
07 OCT 2013/1430

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: Matt Horner DATE: 10-8-13 TIME: 1030 SAMPLE TEMP. @ RECEIPT. 0.4

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

CHAIN OF CUSTODY RECORD

PROJECT # 21620 PROJECT NAME Mena PERMIT# NIPDES 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	08 OCT 2013 1200	09 OCT 2013 1200	24	X			1

RECEIVING WATER SAMPLES

SAMPLE IDENTIFICATION (FOR REC'G) 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/E
NAME OF RECEIVING WATER unnamed trib. of
Prairie Creek

DILUTION WATER USED FOR THIS TEST Lab

RELINQUISHED BY: Jeff Heringer DATE: 09 OCT 13 TIME: 1330 RECEIVED BY AT THIS DATE/TIME: 09 OCT 2013/1330
RELINQUISHED BY: _____ DATE: _____ TIME: _____ RECEIVED BY AT THIS DATE/TIME: OFF STORE MENA-UPS
RELINQUISHED BY: _____ DATE: _____ TIME: _____ RECEIVED BY AT THIS DATE/TIME: _____

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

RECEIVED: Matt Warner DATE: 10-10-13 TIME: 1010 SAMPLE TEMP. @ RECEIPT: 0.4

**CITY OF MENA WWTF
NPDES PERMIT NO. AR0036692
AFIN 57-00042
BIOMONITORING REPORTING
TEST DATE: 10/08/13**

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	8.09%
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	6.58%
Report Parameter No. 22414 (lowest NOEC value) for <i>Pimephales promelas</i> .	100%

**CITY OF MENA WWTF
OUTFALL 001**

Chronic Biomonitoring Report
Permit Number NPDES AR0036692

Ceriodaphnia dubia
Pimephales promelas

October 8, 2013

Reviewed by:

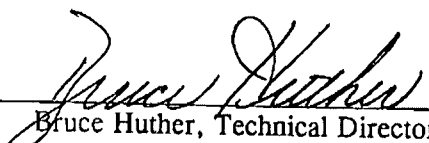

Bruce Huther, Technical Director
Huther & Associates, Inc.
1156 North Bonnie Brae
Denton, Texas 76201
(940) 387-1025, Fax: (940) 387-1036

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TOXICITY TEST REPORT - CHRONIC

Client City of Mena WWTF Laboratory I.D. 21620
Permit No. NPDES AR0036692 Begin Date October 8, 2013
Sample Outfall 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 United Parcel Service courier to Huthur & Associates on October 8 and October 10, 2013. Effluent samples were collected and composited from Outfall 001 using an automatic sampler. Two toxicity tests were requested: a seven-day *Ceriodaphnia dubia* survival and reproduction test (EPA Method 1002.0), and a seven-day *Pimephales promelas* larval survival and growth test (EPA Method 1000.0). Test organisms, procedures and quality assurance requirements were in accordance with the EPA manual, "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition" (EPA-821-R-02-013). Note: Due to shipping error, the third sample did not arrive until test termination date. There was enough sample two to conduct daily renewals.

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 1440 hours, October 8, 2013. 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 1440 hours, October 15, 2013. Survival and reproduction data were statistically analyzed ($p = 0.05$) according to EPA procedures to determine the Lowest Observable Effect Concentration (LOEC) and the No Observable Effect Concentration (NOEC).

SURVIVAL***Ceriodaphnia dubia***

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

LOEC: Not Applicable

NOEC: 100% Effluent

REPRODUCTION***Ceriodaphnia dubia***

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

LOEC: Not Applicable

PMSD: 7.2 %

NOEC: 100% Effluent

TEST SETUP***Pimephales promelas***

The seven-day *Pimephales promelas* larval survival and growth test was initiated at 1600 hours, October 8, 2013. 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 1600 hours, October 15, 2013. 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: 9.7%

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 Mena WWTF	SAMPLE TYPE	24 Hour Composite
NPDES #	AR0036692	DATE COLLECTED	10/07/13 10/09/13
LAB ID #	21620	DATE RECEIVED	10/08/13 10/10/13
TEST TYPE	7 Day Chronic	BEGIN DATE/TIME	10/08/13 1440
TEST ORGANISM	<i>Ceriodaphnia dubia</i>	END DATE/TIME	10/15/13 1440
ORGANISM AGE	< 24 Hours	TEST TEMPERATURE (°C)	25
ORGANISM SOURCE	In House	PHOTO PERIOD	16-hr. Light 8-hr. Dark
RECEIVING WATER	unnamed tributary of Prairie Creek	LIGHT INTENSITY	50-100 ft. candl.
DILUTION WATER	Laboratory Adjusted	TECHNICIAN	T. Nesbitt

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	
10/09/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
10/10/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
10/11/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
10/12/13	3	2	2	3	2	3	4	3	2	2	
	3	2	2	3	2	3	4	3	2	2	
10/13/13	A	A	A	A	A	A	A	A	A	A	
	3	2	2	3	2	3	4	3	2	2	
10/14/13	9	7	7	8	7	10	8	7	8	8	
	12	9	9	11	9	13	12	10	10	10	
10/15/13	13	12	12	11	12	12	13	13	12	12	
	25	21	21	22	21	26	26	23	22	22	
x # Young 227 C.V. 7.50% 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	
10/09/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
10/10/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
10/11/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
10/12/13	3	2	3	3	4	2	3	2	2	4	
	3	2	3	3	4	2	3	2	2	4	
10/13/13	A	A	A	A	A	A	A	A	A	A	
	3	2	3	3	4	2	3	2	2	4	
10/14/13	7	10	8	8	10	9	10	6	10	11	
	10	12	11	11	14	11	13	8	12	16	
10/15/13	13	12	12	13	12	12	12	13	12	13	
	23	24	23	24	26	23	25	21	24	28	
x # Young 241 C.V. 7.93% 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	
10/09/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
10/10/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
10/11/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
10/12/13	2	3	2	4	3	2	2	3	2	4	
	2	3	2	4	3	2	2	3	2	4	
10/13/13	A	A	A	A	A	A	A	A	A	A	
	2	3	2	4	3	2	2	3	2	4	
10/14/13	8	8	8	9	8	10	10	7	7	8	
	10	11	10	13	11	12	12	10	9	12	
10/15/13	14	12	12	12	13	12	13	13	12	13	
	24	23	22	25	24	24	26	23	21	26	
x # Young 236 C.V. 5.72% x% Survival 100% C.V. 0.00%											

58% Effluent											
Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10	
10/09/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
10/10/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
10/11/13	A	A	A	A	A	A	A	A	A	A	
	0	0	0	0	0	0	0	0	0	0	
10/12/13	2	4	3	3	2	3	2	4	3	3	
	2	4	3	3	2	3	2	4	3	3	
10/13/13	A	A	A	A	A	A	A	A	A	A	
	2	4	3	3	2	3	2	4	3	3	
10/14/13	9	9	7	9	8	10	10	8	8	9	
	11	13	10	12	10	13	12	12	11	12	
10/15/13	13	12	13	13	12	14	12	12	13	13	
	24	26	23	26	22	27	24	24	24	26	
x # Young 243 C.V. 5.50% 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

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

City of Mena WWTF

Lab ID# 21620

Test Date: October 8, 2013

75% Effluent

Date	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
10/09/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/10/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/11/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/12/13	2	3	2	4	3	4	3	3	2	3
	2	3	2	4	3	4	3	3	2	3
10/13/13	A	A	A	A	A	A	A	A	A	A
	2	3	2	4	3	4	3	3	2	3
10/14/13	8	7	8	7	7	9	9	8	9	7
	10	10	10	11	10	13	12	11	11	10
10/15/13	13	12	13	13	14	12	13	12	12	12
	23	22	23	24	24	25	25	23	23	22
x # Young 23.4 C.V. 4.59% 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
10/09/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/10/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/11/13	A	A	A	A	A	A	A	A	A	A
	0	0	0	0	0	0	0	0	0	0
10/12/13	2	3	3	4	2	3	4	3	2	3
	2	3	3	4	2	3	4	3	2	3
10/13/13	A	A	A	A	A	A	A	A	A	A
	2	3	3	4	2	3	4	3	2	3
10/14/13	7	7	8	7	6	9	7	9	10	9
	9	10	11	11	8	12	11	12	12	12
10/15/13	12	12	13	13	12	13	13	14	13	12
	21	22	24	24	20	25	24	26	25	24
x # Young 23.5 C.V. 8.09% x% Survival 100% C.V. 0.00%										

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

ex 1:

A
4

 alive today
total young to date

ex 2:

5
12

 alive, 5 young today
total young to date

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

City of Mena WWTF

Lab ID# 21620

Test Date: October 8, 2013

WET CHEMISTRY MEASUREMENTS

Date	Time	Temp	Samp. No.	pH of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
10/08/13	Start	25.0	1	7.61	7.56	7.48	7.44	7.36	6.77	STC
10/09/13	24 Hr.	24.4	1	7.61	7.50	7.44	7.40	7.32	7.27	GZK
10/09/13	Renew	24.5	1	7.57	7.51	7.46	7.40	7.30	6.96	GZK
10/10/13	48 Hr.	25.1	1	7.65	7.58	7.50	7.44	7.37	7.26	SCC
10/10/13	Renew	24.8	2	8.07	7.82	7.34	7.30	7.25	7.04	SCC
10/11/13	72 Hr.	25.1	2	7.71	7.60	7.50	7.43	7.36	7.26	SCC
10/11/13	Renew	25.1	2	7.64	7.53	7.43	7.36	7.30	6.97	SCC
10/12/13	96 Hr.	25.6	2	7.98	7.82	7.70	7.57	7.46	7.29	SCC
10/12/13	Renew	24.3	2	7.68	7.66	7.58	7.51	7.41	6.99	SCC
10/13/13	120 Hr.	25.4	2	7.71	7.62	7.53	7.47	7.39	7.26	STC
10/13/13	Renew	25.2	2	7.62	7.57	7.49	7.42	7.30	7.11	STC
10/14/13	144 Hr.	25.2	2	7.75	7.61	7.54	7.45	7.36	7.24	STC
10/14/13	Renew	25.1	2	7.58	7.53	7.47	7.39	7.26	7.00	STC
10/15/13	168 Hr.	25.0	2	7.60	7.47	7.40	7.33	7.22	7.03	GZK

Date	Time	Temp	Samp. No.	DO (mg/L) of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
10/08/13	Start	25.0	1	8.26	8.32	8.77	8.83	8.96	8.66	STC
10/09/13	24 Hr.	24.4	1	8.13	7.51	7.74	7.65	7.59	8.19	GZK
10/09/13	Renew	24.5	1	8.02	8.20	7.87	8.09	8.11	8.21	GZK
10/10/13	48 Hr.	25.1	1	8.24	8.15	8.10	8.05	7.94	8.00	SCC
10/10/13	Renew	24.8	2	8.24	8.28	8.67	8.79	8.63	8.97	SCC
10/11/13	72 Hr.	25.1	2	8.07	7.96	7.85	7.87	7.98	7.81	SCC
10/11/13	Renew	25.1	2	8.00	8.59	8.23	8.36	8.51	8.75	SCC
10/12/13	96 Hr.	25.6	2	8.21	8.00	7.96	8.19	8.19	8.20	SCC
10/12/13	Renew	24.3	2	8.08	8.04	7.95	7.91	8.17	8.75	SCC
10/13/13	120 Hr.	25.4	2	8.04	8.74	8.73	7.76	8.55	8.48	STC
10/13/13	Renew	25.2	2	8.28	8.72	8.86	8.74	8.87	8.94	STC
10/14/13	144 Hr.	25.2	2	7.64	7.72	8.77	7.92	8.07	7.77	STC
10/14/13	Renew	25.1	2	8.12	8.22	8.35	8.71	8.16	8.24	STC
10/15/13	168 Hr.	25.0	2	8.57	8.32	7.94	7.98	7.69	7.56	GZK

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

City of Mena WWTF

Lab ID# 21620

Test Date: October 8, 2013

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
10/08/13	1	6.77	8.66	24	20	203	<0.01	N/A	TN
10/10/13	2	6.97	8.75	20	24	198	<0.01	N/A	TN
10/08/13	Con	7.61	8.26	40	96	210	<0.01	N/A	TN

† Measurements taken in 100% solution.

CERIODAPHNIA DUBIA STATISTICAL ANALYSES
 Reproduction

Summary Statistics on Transformed Data Table 1 of 2

Grp	Identification	N	Min	Max	Mean
1	Control	10	21.000	25.000	22.700
2	32% Effluent	10	21.000	28.000	24.100
3	42% Effluent	10	21.000	25.000	23.600
4	56% Effluent	10	22.000	27.000	24.300
5	75% Effluent	10	22.000	25.000	23.400
6	100% Effluent	10	20.000	26.000	23.500

Summary Statistics on Transformed Data Table 2 of 2

Grp	Identification	Variance	Sd	Sem	C.V.%
1	Control	2.900	1.703	0.539	7.50
2	32% Effluent	3.656	1.912	0.605	7.93
3	42% Effluent	1.822	1.350	0.427	5.72
4	56% Effluent	1.789	1.337	0.423	5.50
5	75% Effluent	1.156	1.075	0.340	4.59
6	100% Effluent	3.611	1.900	0.601	8.09

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	12	25	17	2

Calculated Chi-Square goodness of fit test statistic = 2.0648

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

Data Pass normality test. Continue analysis.

Bartlett's Test For Homogeneity of Variance

Calculated B1 statistic = 4.31

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	16.000	3.200	1.286
Within (Error)	54	134.400	2.489	
Total	59	150.400		

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

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

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

Grp	Identification	Transformed Mean	Mean Calculated In Original Units	T Stat	Sig
1	Control	22.700	22.700		
2	32% Effluent	24.100	24.100	-1.984	
3	42% Effluent	23.600	23.600	-1.276	
4	56% Effluent	24.300	24.300	-2.268	
5	75% Effluent	23.400	23.400	-0.992	
6	100% Effluent	23.500	23.500	-1.134	

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	1.630	7.2	-1.400
3	42% Effluent	10	1.630	7.2	-0.900
4	56% Effluent	10	1.630	7.2	-1.600
5	75% Effluent	10	1.630	7.2	-0.700
6	100% Effluent	10	1.630	7.2	-0.800

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

CLIENT	City of Mena WWTF	SAMPLE TYPE	24 Hour Composite
NPDES #	AR0036692	DATE COLLECTED	10/07/13 10/09/13
LAB ID #	21620	DATE RECEIVED	10/08/13 10/10/13
TEST TYPE	7 Day Chronic	BEGIN DATE/TIME	10/08/13 1600
TEST ORGANISM	<i>Pimephales promelas</i>	END DATE/TIME	10/15/13 1600
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. candl.
DILUTION WATER	Laboratory Adjusted	TECHNICIAN	M. Horner

SURVIVAL SUMMARY

Conc.	10/09/13					10/10/13					10/11/13					10/12/13					10/13/13				
	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.	10/14/13					10/15/13					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	0.4650	0.4290	0.4770	0.4950	0.4260	0.4584	6.58
32%	0.4890	0.5040	0.4440	0.4860	0.4920	0.4830	4.73
42%	0.4260	0.4750	0.5040	0.4460	0.4920	0.4686	6.89
56%	0.4910	0.4220	0.4650	0.4290	0.5030	0.4620	7.82
75%	0.4470	0.4860	0.5030	0.4490	0.4860	0.4742	5.25
100%	0.5030	0.4250	0.4670	0.4560	0.4910	0.4684	6.53

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

City of Mena WWTF

Lab ID# 21620

Test Date: October 8, 2013

WET CHEMISTRY MEASUREMENTS

Date	Time	Temp	Samp. No.	pH of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
10/08/13	Start	25.0	1	7.61	7.56	7.48	7.44	7.36	6.77	STC
10/09/13	24 Hr.	24.8	1	7.71	7.46	7.39	7.30	7.19	7.09	GZK
10/09/13	Renew	24.5	1	7.57	7.51	7.46	7.40	7.30	6.96	GZK
10/10/13	48 Hr.	25.0	1	7.37	7.31	7.28	7.25	7.15	7.00	SCC
10/10/13	Renew	24.8	2	8.07	7.82	7.34	7.30	7.25	7.04	SCC
10/11/13	72 Hr.	25.4	2	7.55	7.47	7.37	7.29	7.18	7.02	SCC
10/11/13	Renew	25.1	2	7.64	7.53	7.43	7.36	7.30	6.97	SCC
10/12/13	96 Hr.	25.8	2	7.53	7.45	7.36	7.33	7.26	7.13	SCC
10/12/13	Renew	24.3	2	7.68	7.66	7.58	7.51	7.41	6.99	SCC
10/13/13	120 Hr.	25.5	2	7.58	7.50	7.43	7.39	7.22	7.14	STC
10/13/13	Renew	25.2	2	7.62	7.57	7.49	7.42	7.30	7.11	STC
10/14/13	144 Hr.	25.4	2	7.33	7.35	7.32	7.29	7.20	7.10	STC
10/14/13	Renew	25.1	2	7.58	7.53	7.47	7.39	7.26	7.00	STC
10/15/13	168 Hr.	25.4	2	6.92	6.81	6.75	6.69	6.76	6.59	STC

Date	Time	Temp	Samp. No.	DO (mg/L) of Solution						Analyst
				CON	32%	42%	56%	75%	100%	
10/08/13	Start	25.0	1	8.26	8.32	8.77	8.83	8.96	8.66	STC
10/09/13	24 Hr.	24.8	1	7.88	7.90	8.03	7.88	7.93	8.01	GZK
10/09/13	Renew	24.5	1	8.02	8.20	7.87	8.09	8.11	8.21	GZK
10/10/13	48 Hr.	25.0	1	7.97	8.01	8.29	7.89	7.35	7.75	SCC
10/10/13	Renew	24.8	2	8.24	8.28	8.67	8.79	8.63	8.97	SCC
10/11/13	72 Hr.	25.4	2	7.76	7.55	8.20	7.74	7.67	7.65	SCC
10/11/13	Renew	25.1	2	8.00	8.59	8.23	8.36	8.51	8.75	SCC
10/12/13	96 Hr.	25.8	2	7.33	8.07	8.18	7.95	8.02	7.96	SCC
10/12/13	Renew	24.3	2	8.08	8.04	7.95	7.91	8.17	8.75	SCC
10/13/13	120 Hr.	25.5	2	7.67	7.69	7.50	7.61	7.51	7.60	STC
10/13/13	Renew	25.2	2	8.28	8.72	8.86	8.74	8.87	8.94	STC
10/14/13	144 Hr.	25.4	2	8.02	7.73	8.00	7.76	7.73	7.71	STC
10/14/13	Renew	25.1	2	8.12	8.22	8.35	8.71	8.16	8.24	STC
10/15/13	168 Hr.	25.4	2	7.88	7.99	7.86	8.06	8.71	8.02	STC

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

City of Mena WWTF

Lab ID# 21620

Test Date: October 8, 2013

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.Cl2 mg/L ¹	Dechlor(mL) Na2S2O3 mg/L ¹	Analyst
10/08/13	1	6.77	8.66	24	20	203	<0.01	N/A	TN
10/10/13	2	6.97	8.75	20	24	198	<0.01	N/A	TN
10/08/13	Con	7.61	8.26	40	96	210	<0.01	N/A	TN

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	0.426	0.495	0.458
2	32% Effluent	5	0.444	0.504	0.483
3	42% Effluent	5	0.426	0.504	0.469
4	56% Effluent	5	0.422	0.503	0.462
5	75% Effluent	5	0.447	0.503	0.474
6	100% Effluent	5	0.425	0.503	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	6.58
2	32% Effluent	0.001	0.023	0.010	4.73
3	42% Effluent	0.001	0.032	0.014	6.89
4	56% Effluent	0.001	0.036	0.016	7.82
5	75% Effluent	0.001	0.025	0.011	5.25
6	100% Effluent	0.001	0.031	0.014	6.53

Shapiro - Wilk's Test For Normality

D = 0.021

W = 0.917

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 = 1.01

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	0.433
Within (Error)	24	0.021	0.001	
Total	29	0.023		

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 Calculated In Original Units	T Stat	Sig
1	Control	0.458	0.458		
2	32% Effluent	0.483	0.483	-1.304	
3	42% Effluent	0.469	0.469	-0.541	
4	56% Effluent	0.462	0.462	-0.191	
5	75% Effluent	0.474	0.474	-0.838	
6	100% Effluent	0.468	0.468	-0.530	

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

No statistically significant difference

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

Grp	Identification	Num of Reps	Minimum Sig Diff (In Orig. Units)	% of Control	Difference from Control
1	Control	5			
2	32% Effluent	5	0.045	9.7	-0.025
3	42% Effluent	5	0.045	9.7	-0.010
4	56% Effluent	5	0.045	9.7	-0.004
5	75% Effluent	5	0.045	9.7	-0.016
6	100% Effluent	5	0.045	9.7	-0.010

**APPENDIX A
RAW DATA**

~~DAY~~ CERIODAPHNIA DUBIA SURVIVAL & REPRODUCTION
DAILY RAW DATA TABLE
PAGE 1 OF 2

CLIENT Mena
OUTFALL 001
LAB ID # 21620

START DATE/TIME 10-8-13 TN 1440
END DATE/TIME 10-15-13 ZG 1440

CON

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
10/9	A	A	A	A	A	A	A	A	A	A	ZG	1440
10/10	A	A	A	A	A	A	A	A	A	A	NL	1405
10/11	A	A	A	A	A	A	A	A	A	A	ZG	1230
10/12	3	2	2	3	2	3	4	3	2	2	NL	1545
10/13	A	A	A	A	A	A	A	A	A	A	NL	1045
10/14	9	7	7	8	7	10	8	7	8	8	ZG	1445
10/15	13	12	12	11	12	12	13	12	12	12	ZG	1440
	25	21	21	22	21	25	25	23	22	22		

$\bar{x}$ # Young w/o Dead = 22.7 CV% = 7.50

$\bar{x}$ # 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
10/9	A	A	A	A	A	A	A	A	A	A	ZG	1440
10/10	A	A	A	A	A	A	A	A	A	A	NL	1405
10/11	A	A	A	A	A	A	A	A	A	A	ZG	1230
10/12	3	2	3	3	4	2	3	2	2	4	NL	1545
10/13	A	A	A	A	A	A	A	A	A	A	NL	1045
10/14	7	10	8	8	10	9	10	6	10	11	ZG	1445
10/15	13	12	12	13	12	12	12	13	12	13	ZG	1440
	23	24	23	24	26	23	25	21	24	28		

$\bar{x}$ # Young w/o Dead = 24.1 CV% = 7.93

$\bar{x}$ # 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
10/9	A	A	A	A	A	A	A	A	A	A	ZG	1440
10/10	A	A	A	A	A	A	A	A	A	A	NL	1405
10/11	A	A	A	A	A	A	A	A	A	A	ZG	1230
10/12	2	3	2	4	3	2	2	3	2	4	NL	1545
10/13	A	A	A	A	A	A	A	A	A	A	NL	1045
10/14	8	8	8	9	8	10	10	7	7	8	ZG	1445
10/15	14	12	12	12	13	12	13	13	12	13	ZG	1440
	24	23	22	25	24	24	25	23	21	25		

$\bar{x}$ # Young w/o Dead = 23.6 CV% = 5.72

$\bar{x}$ # 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
10/9	A	A	A	A	A	A	A	A	A	A	ZG	1440
10/10	A	A	A	A	A	A	A	A	A	A	NL	1405
10/11	A	A	A	A	A	A	A	A	A	A	ZG	1230
10/12	2	4	3	3	2	3	2	4	3	2	NL	1545
10/13	A	A	A	A	A	A	A	A	A	A	NL	1045
10/14	9	9	7	9	8	10	10	8	8	9	ZG	1445
10/15	13	12	13	13	12	14	12	12	13	13	ZG	1440
	24	25	23	25	22	27	24	24	24	25		

$\bar{x}$ # Young w/o Dead = 24.3 CV% = 5.50

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

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

DAY, CERIODAPHNIA DUBIA SURVIVAL & REPRODUCTION

DAILY RAW DATA TABLE

PAGE 2 OF 2

CLIENT Mera

OUTFALL DD1

LAB ID # 21620

75

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
10/9	A	A	A	A	A	A	A	A	A	A	ZG	1440
10/10	A	A	A	A	A	A	A	A	A	A	NL	1405
10/11	A	A	A	A	A	A	A	A	A	A	ZG	1230
10/12	2	3	2	4	3	4	3	3	2	3	NL	1915
10/13	A	A	A	A	A	A	A	A	A	A	NL	1045
10/14	8	7	8	7	7	9	9	8	9	7	ZG	1445
10/15	13	12	13	13	14	12	13	12	12	12	ZG	1440
	23	22	23	24	24	25	25	23	23	22		

$\bar{x}$ # Young w/o Dead = 23.4 CV% = 4.59

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

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

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

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

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

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

START DATE/TIME 10-8-13 TN 1440

END DATE/TIME 10-15-13 ZG 1440

100

Date	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Analyst	Time
10/9	A	A	A	A	A	A	A	A	A	A	ZG	1440
10/10	A	A	A	A	A	A	A	A	A	A	NL	1405
10/11	A	A	A	A	A	A	A	A	A	A	ZG	1230
10/12	2	3	3	4	2	3	4	3	2	3	NL	1515
10/13	A	A	A	A	A	A	A	A	A	A	NL	1045
10/14	7	7	8	7	6	9	7	9	10	9	ZG	1445
10/15	12	12	13	13	12	13	13	14	13	12	ZG	1440
	21	22	24	24	20	25	24	26	25	24		

$\bar{x}$ # Young w/o Dead = 23.5 CV% = 8.09

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

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

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

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

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

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

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

CLIENT/FACILITY Mena
 OUTFALL # 001 PROJECT # 21620
 ORGANISM ID# PPU-13-281

DATE/TIME STARTED 10-8-13 2G 1600
 DATE/TIME ENDED 10-15-13 TN 1600

Conc.	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
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	10-9-13 MH 1600					10-10-13 ZK 0830					10-11-13 MH 0840					10-12-13 ZK 0910					10-13-13 ZK 0805				

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	10-14-13 2G 0825					10-15-13 TN 1600						

7-DAY CHRONIC TOXICITY TEST
PIMEPHALES PROMELAS (fathead minnow) MEAN WEIGHT/REP

Client Mma

Date/Time Start 10/8/13 1600

Project# 21620

Date/Time End 10/15/13 1600

% Effluent	Rep A	Rep B	Rep C	Rep D	Rep E	$\bar{x}$	C.V.%	Analyst
0	.4650	.4290	.4770	.4950	.4260	.4584	6.58	BH
32	.4890	.5040	.4440	.4860	.4920	.4830	4.73	
62	.4260	.4750	.5040	.4460	.4920	.4686	6.89	
56	.4910	.4220	.4650	.4290	.5030	.4620	7.82	
75	.4470	.4860	.5030	.4490	.4860	.4742	5.25	
100	.5030	.4250	.4670	.4560	.4910	.4684	6.53	↓

Huthur and Associates, Inc.

environmental toxicologists, biologists, and consultants

Client / Facility Mena
Lab ID Number 21620
Outfall Number 00
Test Date 10-8-13

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
10/8	1	6.77	8.66	24	20	203	20.01	Na	TR
10/10	2	6.97	8.75	20	24	198	20.01	Na	
10/8	CON	7.61	8.26	40	96	210	—	—	

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: 10

TEST DATE/TIME: 10/01/13 - 10/08/13
1630 Hrs - 1630 Hrs

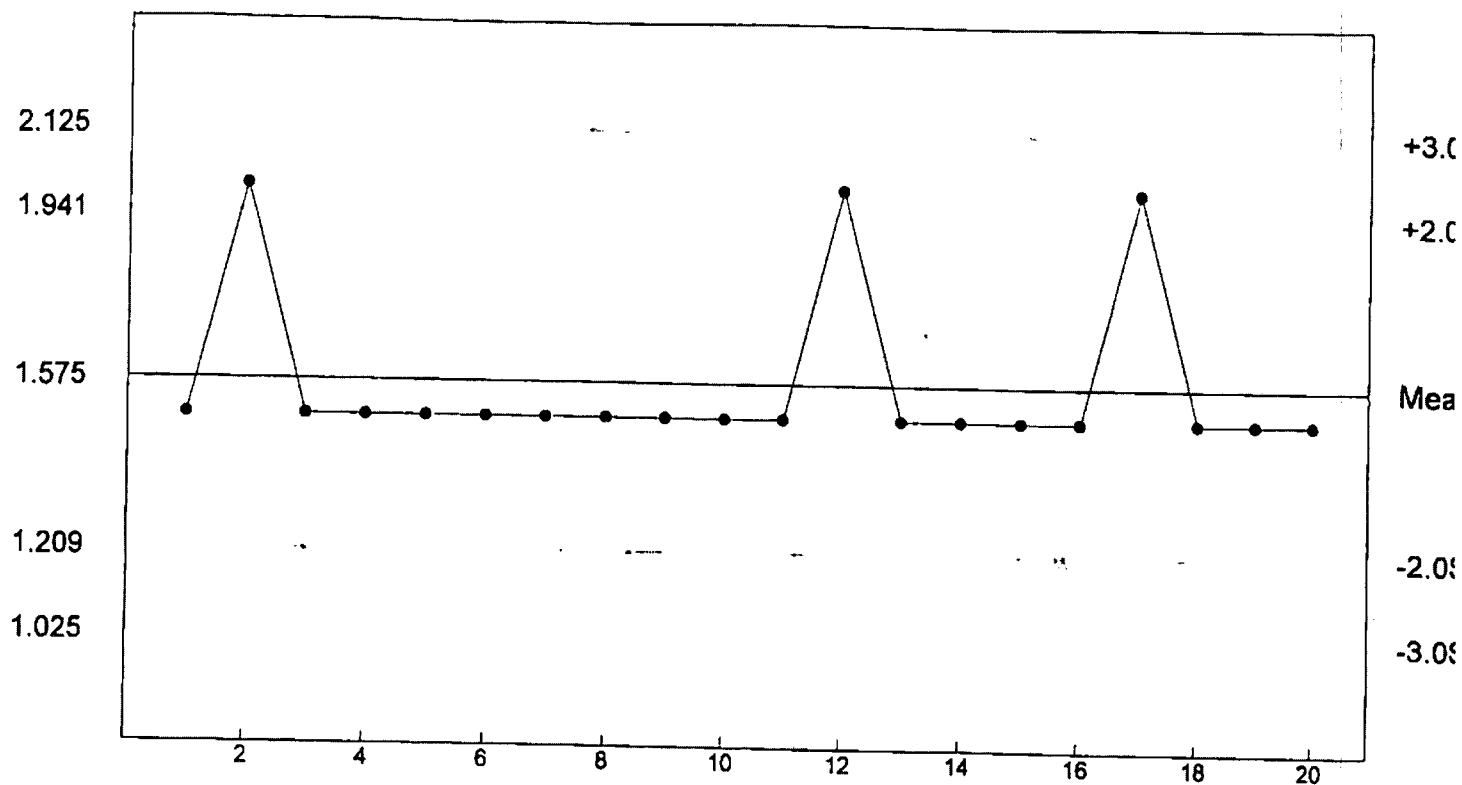
STATISTICAL METHOD: Fishers, Dunnetts/Steels

CONCENTRATION (g/L)	NUMBER EXPOSED	NUMBER DEAD
0.5	10	0
1.0	10	0
1.5	10	0
2.0	10	7
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	1.5 g/L	1.0 g/L

Reference Tox Sodium Chloride g/L

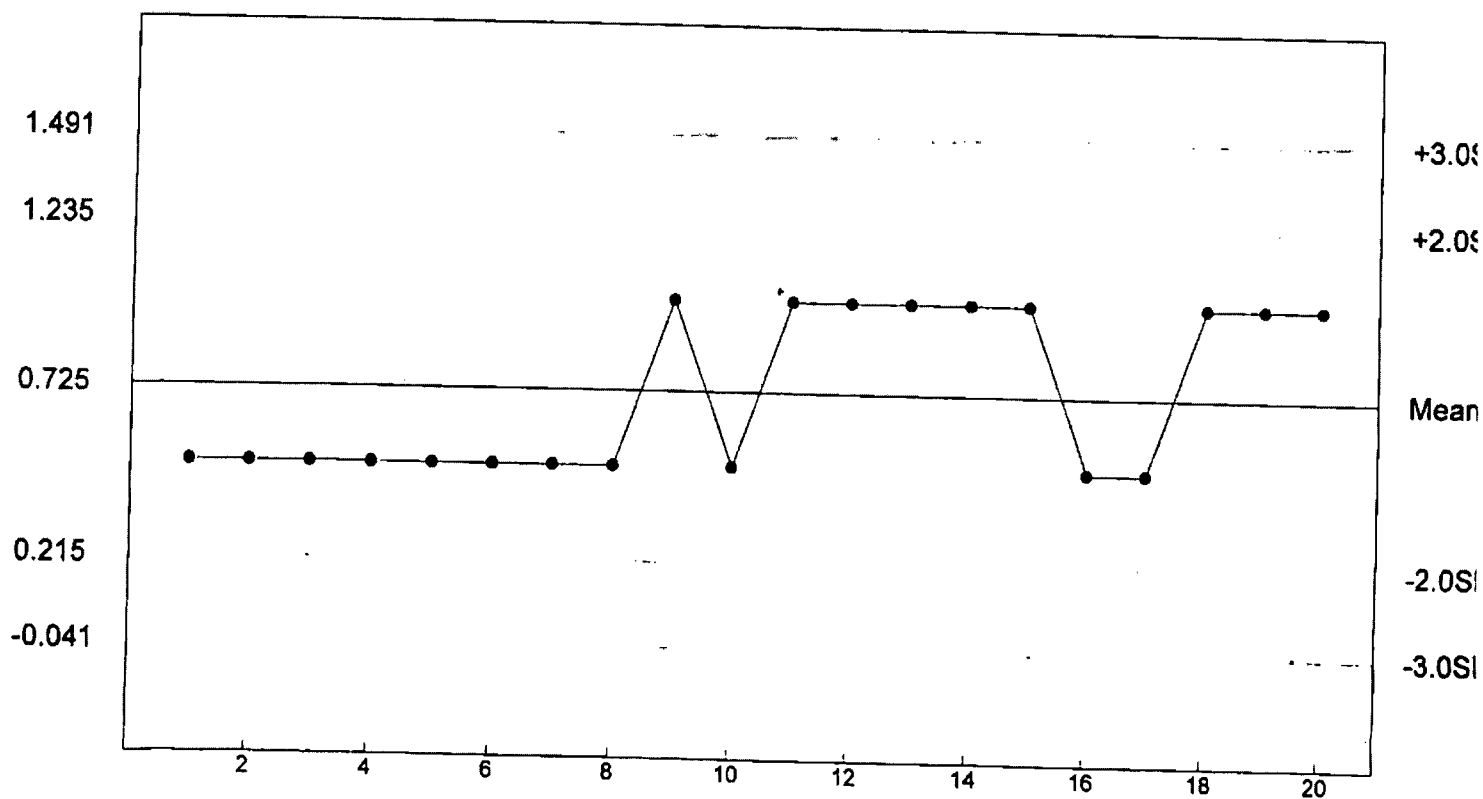
C. dubia Survival - NOEC



n= 20 Mean= 1.575 SD= 0.183 CV= 11.63% Min= 1.500 Max= 2.000

Reference Tox Sodium Chloride g/L

C. dubia Reproduction - NOEC



n= 20 Mean= 0.725 SD= 0.255 CV= 35.20% Min= 0.500 Max= 1.000

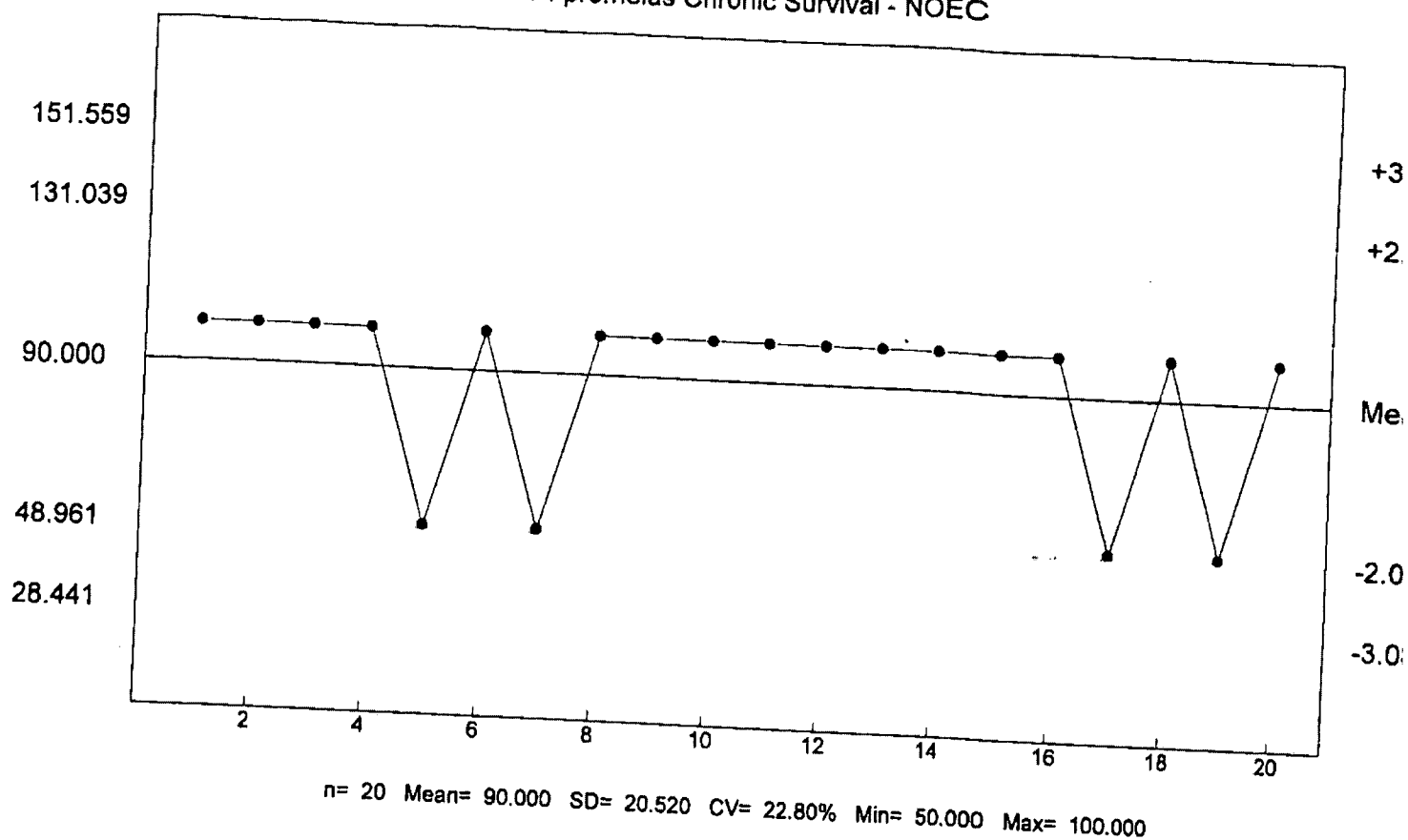
CHRONIC REFERENCE TOXICANT TEST RESULTS

SPECIES: *Pimephales promelas*
 CHEMICAL: Copper Nitrate
 DURATION: 7-Days
 TEST NUMBER: 10
 TEST DATE/TIME: 10/01/13 - 10/08/13
 1530 Hrs - 1530 Hrs
 STATISTICAL METHOD: Dunnetts/Steels

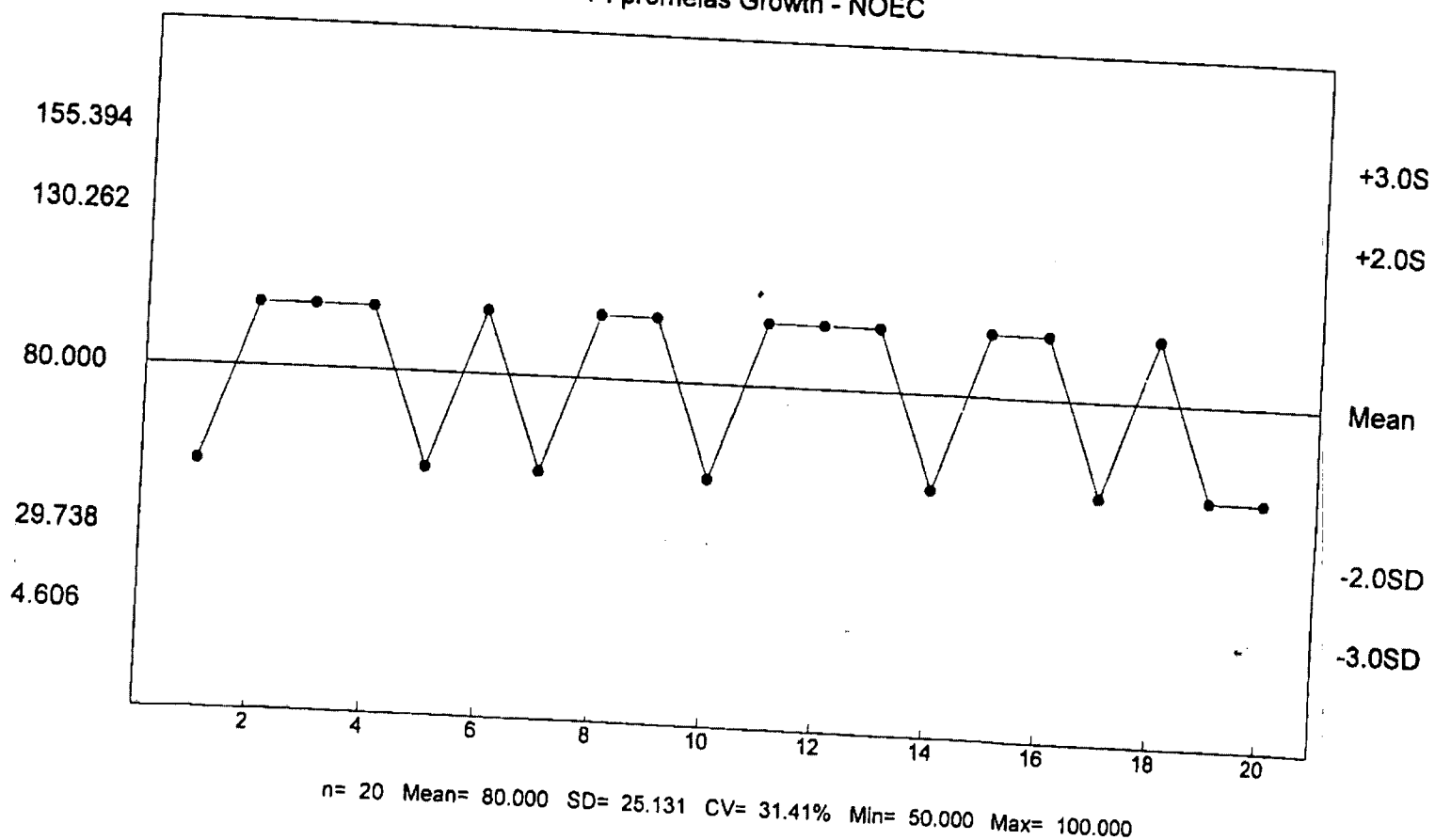
CONCENTRATION (ug/L)	NUMBER EXPOSED	NUMBER DEAD
12.5	40	0
25	40	0
50	40	0
100	40	10
200	40	23
400	40	40
800	40	40

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



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



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 # 211020 PROJECT NAME Mena PERMIT# NIPDES AR003669

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	06 OCT 2013 1200	07 OCT 2013 1200	24	X			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 9F
NAME OF RECEIVING WATER unnamed trib. of Prairie Creek

DILUTION WATER USED FOR THIS TEST lab

RELINQUISHED BY: Jeff Flay DATE: 07 OCT 2013 TIME: 1430 RECEIVED BY AT THIS DATE/TIME: Office Store - Mena 07 OCT 2013/1430

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: Matt Borne DATE: 10-8-13 TIME: 1030 SAMPLE TEMP. @ RECEIPT. 0.4

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

CHAIN OF CUSTODY RECORD

PROJECT # 21620 PROJECT NAME Mena PERMIT# NPDES AR1036692

OUTFALL SAMPLES

24-Hr Flow Weighted Composite ☒ Other _____

					METHODS OF COLLECTION AND COMPOSITE			
OUTFALL NUMBER	PERSON TAKING SAMPLE	START DATE/TIME	END DATE/TIME	# OF PORTIONS COMPOSITED	AUTO COLL. AUTO COMP.	MANUAL COLL. MANUAL COMP.	AUTO COLL. MANUAL COMP.	# OF CONTAINERS TO BE SHIPPED
001	Jeff	08 OCT 2013 1200	09 OCT 2013 1200	24	X			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/E
NAME OF RECEIVING WATER unnamed trib. of Prairie Creek

DILUTION WATER USED FOR THIS TEST Lab

RELINQUISHED BY: Jeff Heringan DATE: 09 OCT 13 TIME: 1330 RECEIVED BY AT THIS DATE/TIME 09 OCT 2013/1330 OFC STORE MENA-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: Matt Warner DATE: 10-10-13 TIME: 1010 SAMPLE TEMP. @ RECEIPT. 0.4

**CITY OF MENA WWTF
NPDES PERMIT NO. AR0036692
AFIN 57-00042
BIOMONITORING REPORTING
TEST DATE: 10/08/13**

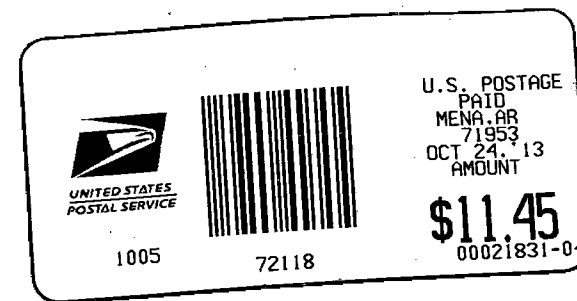
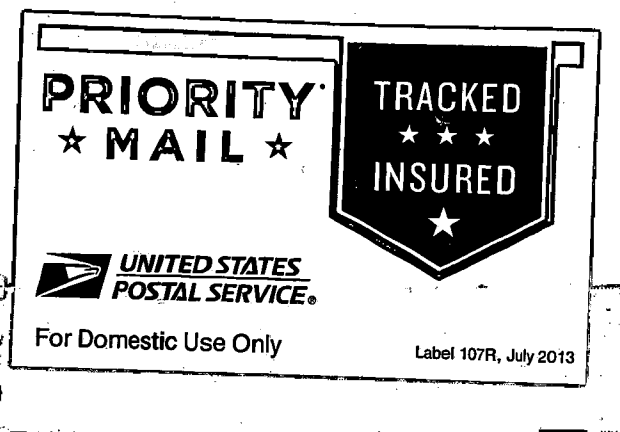
Ceriodaphnia dubia**Response**

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	8.09%
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	6.58%
Report Parameter No. 22414 (lowest NOEC value) for <i>Pimephales promelas</i> .	100%

MENA WWT P
323 Polk 53
MENA AR
71953



RETURN RECEIPT
REQUESTED

SHANE BYROM
ADEQ (NPDES)
5301 NORTH SHORE DR
NORTH LITTLE ROCK, AR
72118-5317

