

Arkansas Analytical, Inc.

Toxicity Test Results

City of Wynne
NPDES PERMIT NUMBER: AR0021903
Second Quarter 2015

Fathead Minnow, *Pimephales promelas*, Larval Survival and Growth Test
Test 1000.0

Ceriodaphnia dubia, Survival and Reproduction Test
Test 1002.0

Prepared for: **Harrel Williams**
Wynne Water Utilities
121 East Merriman
Wynne, AR 72396

Prepared by: Arkansas Analytical, Inc
11701 I-30, Bldg 1 Suite 115
Little Rock, Arkansas 72209
Lab Number K1505001

Friday, May 22, 2015

Introduction

This report contains test results for the toxicity testing of Wynne facility. The NPDES permit number is AR0021903. The plant located in Wynne, Arkansas, is authorized to discharge treated municipal wastewater from the facility located as follows: approximately 1.5 miles West of Wynne on Hwy 284 to Bowden Road, thence south on Bowden Road approximately 0.25 mile in Cross County, Arkansas. The applicant's mailing address is: 121 East Merriman Avenue, Wynne, AR 72396.

Facility Coordinates: Latitude: 35° 13' 8.81"; Longitude: 90° 49' 41.25"

The permitted outfall is located at the following coordinates:

Outfall 001: Latitude: 35° 13' *OT*"; Longitude: 90° 49' 52"

The permit requires chronic biomonitoring testing once per quarter for both *Ceriodaphnia dubia* and *Pimephales promelas*. The test results in this report represent the testing for the second quarter of 2015.

Plant Operations

To be provided by permittee.

Source of Effluent and Dilution Water

Effluent samples were collected as follows:

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	5-10-15, 0700	5-11-15, 0700
Sample #2:	5-12-15, 0700	5-13-15, 0700
Sample #3:	5-14-15, 0700	5-15-15, 0700

Samples were composites collected at Outfall 001.

The following information was collected upon immediate receipt of the samples at the laboratory:

Sample Receiving Information:	Date, Time Sample(s) Received	Temperature Upon Receipt (°C)
Sample #1:	5-11-15, 1549	6
Sample #2:	5-13-15, 1638	9 (on ice)
Sample #3:	5-15-15, 1545	10 (on ice)

Chain of custody documentation is located in Appendix A.

The permit designates the receiving water to be used as dilution water for the toxicity tests. However, due to its earlier characterization as toxic, synthetic water was substituted.

The dilution water used in the toxicity tests was synthetic moderately hard. It was prepared using Elga Maxima ultra pure water according to EPA specifications. Each batch was analyzed for pH, hardness, total alkalinity, and conductivity. Results are provided in Appendix B.

Dilution Series

Five dilutions in addition to a control (0% effluent) were used in the toxicity tests. The dilutions, which were made with synthetic water, were 32%, 42%, 56%, 75%, and 100%. The low-flow effluent concentration (**critical dilution**) was defined as **100% effluent**.

Test Methods

EPA Method 1000.0, Fathead Minnow, *Pimephales promelas*, Larval Survival and Growth Test, was used in this bioassay. Larvae are exposed in a static renewal system for seven days and the results are based on the survival and growth (increase in weight) of the larvae. There were no deviations from the reference method. The test chambers were 500 ml plastic cups, and each chamber contained ten organisms in a test solution volume of 250 mls. The test temperature was 25 degrees Centigrade. Raw data and statistics are provided in Appendix C.

EPA Method 1002.0, Cladoceran, *Ceriodaphnia dubia*, Survival and Reproduction Test, was also used. Neonates are exposed in a static renewal system until at least 60% of the control organisms have produced a third brood. Results are based on the survival and reproduction of the organisms. One neonate was placed in each of ten replicate chambers using a randomizing template. Test chambers were 30 ml plastic cups filled with 15 mls of test solution. The test temperature was 25 degrees Centigrade. Raw data and statistics are provided in Appendix D.

Test Organisms

The organisms used in Test 1000.0 were < 24 hour old Fathead Minnows, *Pimephales promelas*, which were purchased from Aquatox; a copy of the organism history is provided in Appendix E.

The organisms used in Test 1002.0 were < 24 hour old *Ceriodaphnia dubia* neonates, (all born within the same eight hours), obtained from an in-house culture. An organism history is provided in Appendix E.

Quality Assurance

Test Acceptability

TEST ACCEPTANCE CRITERIA for *Ceriodaphnia dubia*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	100%	X	
Average of 15 or more young per surviving female	16.8	X	
At least 60% of surviving females should have produced 3 broods	70%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	25.8%	X	

TEST ACCEPTANCE CRITERIA for *Pimephales promelas*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	98%	X	
The percent coefficient of variation between replicates must be 40% or less for survival	4.56%	X	
Minimum of 0.25 mg average dry weight of surviving controls	0.746	X	
The percent coefficient of variation between replicates must be 40% or less for growth	7.14%	X	

Reference Toxicant

The reference toxicant used was Potassium Chloride prepared in-house. The tests were performed using moderately hard water as dilution water. The results of the reference toxicant were:

REFERENCE TOXICANT

<i>Ceriodaphnia dubia</i> 4/22/15 – 4/29/15		<i>Pimephales promelas</i> 4/22/15 – 4/29/15	
NOEC Survival:	250 ppm KCl	NOEC Survival:	500 ppm KCl
LOEC Survival:	500 ppm KCl	LOEC Survival:	1000 ppm KCl
NOEC Reproduction:	250 ppm KCl	NOEC Reproduction:	500 ppm KCl
LOEC Reproduction:	500 ppm KCl	LOEC Reproduction:	1000 ppm KCl

Quality Assurance charts are provided in Appendix F.

**Summary of Results
City of Wynne**

<i>Ceriodaphnia dubia</i>		<i>Pimephales promelas</i>	
NOEC / LOEC Survival	100% / NA	NOEC / LOEC survival	100% / NA
NOEC / LOEC Reproduction	100% / NA	NOEC / LOEC growth	100% / NA
Mean number of neonates (critical dilution)	15.5	%CV survival (critical dilution)	4.56%
%CV Reproduction (critical dilution)	28.7%	Mean dry weight (critical dilution)	0.924
PMSD Reproduction	28.4%	%CV growth (critical dilution)	2.08%
		PMSD Growth	19.8 %

Conclusion

Chronic static renewal larval survival and growth test using fathead minnow, *Pimephales promelas*, (Method 1000.0)

The permit issued to Wynne, specifies that the **critical dilution is 100% effluent**. The effluent samples did not exhibit either lethal or sublethal effects at the critical dilution, and, as such, the effluent samples **passed** both portions of the test.

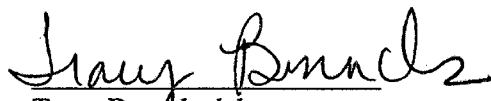
Chronic static renewal survival and reproduction test using *Ceriodaphnia dubia*, (Method 1002.0)

The permit issued to Wynne, specifies the **critical dilution is 100% effluent**. The effluent samples did not exhibit either lethal or sublethal effects at the critical dilution, and, as such, the effluent samples **passed** both portions of the test.

Biomonitoring Analyst:

Ryan Hudgin

Reviewed by:


Tracy Bounds, lab manager

**SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING
FATHEAD MINNOW LARVAE GROWTH AND SURVIVAL
*PIMEPHALES PROMELAS***

PERMITTEE: City of Wynne

NPDES #: AR0021903

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	5-10-15, 0700	5-11-15, 0700
Sample #2:	5-12-15, 0700	5-13-15, 0700
Sample #3:	5-14-15, 0700	5-15-15, 0700

Test initiated (date, time): 5-12-15, 1450 Test terminated (date, time): 5-19-15, 1330
Dilution water used: Moderately Hard Synthetic

DATA TABLE FOR FATHEAD MINNOW SURVIVAL

Effluent Conc %	Percent Survival in Replicate Chambers						Mean Percent Survival			CV %
	A	B	C	D	E		24 hours	48 hours	7 days	
0%	100	100	90	100	100		100	100	98	4.56
32%	100	100	100	100	80		100	98	96	
42%	90	100	100	100	90		100	98	96	
56%	100	90	100	100	90		98	98	96	
75%	90	100	100	100	100		100	100	98	
100%	100	100	100	90	100		98	98	98	4.56

DATA TABLE FOR GROWTH OF FATHEAD MINNOWS

Effluent Conc %	Average Dry Weight in milligrams in replicate chambers						Mean Dry Weight	CV%
	A	B	C	D	E			
0%	0.792	0.786	0.693	0.775	0.683		0.746	7.14
32%	0.850	0.747	0.828	0.865	0.788		0.816	
42%	0.707	0.697	0.753	0.841	0.624		0.724	
56%	0.852	0.734	1.115	0.751	0.822		0.855	
75%	0.691	0.937	0.999	1.088	0.849		0.913	
100%	0.920	0.924	0.896	0.949	0.931		0.924	2.08

Coefficient of Variation = standard deviation / mean * 100

SUMMARY REPORTING FOR CHRONIC BIOMONITORING
FATHEAD MINNOW LARVAE GROWTH AND SURVIVAL
PIMEPHALES PROMELAS

1. Dunnett's procedure or Steel's Many-One Rank Test as appropriate:

Is the mean survival at 7 days significantly different ($p=0.05$) than the control survival for:

a) LOW FLOW OR CRITICAL DILUTION, (100%) YES _____ NO X _____

2. Dunnett's Procedure

Is the mean dry weight (growth) at 7 days significantly different ($p=0.05$) than the control's dry weight (growth) for:

a) LOW FLOW OR CRITICAL DILUTION, (100%) YES _____ NO X _____

3. If NO was answered to 1.a) and 2.a) enter [0] otherwise enter [1]: _____ 0 _____

4. Enter the response to item 3 on DMR Form, parameter # TEP6C.

5. Enter percent effluent corresponding to each NOEC below and circle the lowest number:

a) NOEC survival = _____ 100 _____ % effluent

b) NOEC growth = _____ 100 _____ % effluent

c) Coefficient of variation (parameter TQP6C)= _____ 7.14 _____ %

6. Enter Whole Effluent Toxicity: _____ 100 _____ %

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING
Ceriodaphnia dubia SURVIVAL AND REPRODUCTION

Permittee: City of Wynne

NPDES #:AR0021903

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	5-10-15, 0700	5-11-15, 0700
Sample #2:	5-12-15, 0700	5-13-15, 0700
Sample #3:	5-14-15, 0700	5-15-15, 0700

Test initiated (date, time): 5-12-15, 1030 Test terminated (date, time): 5-19-15, 1055

Dilution water used: Moderately Hard Synthetic

Ceriodaphnia dubia SURVIVAL AND REPRODUCTION
 NUMBER OF YOUNG PRODUCED PER FEMALE @ TEST TERMINATION

PERCENT EFFLUENT

Replicate	0%	32%	42%	56%	75%	100%
A	17	11	18	18	19	14
B	12	12	13	11	18	17
C	16	11	13	14	11	10
D	20	18	11	16	20	9
E	18	14	9	19	20	19
F	17	20	14	19	14	12
G	21	16	11	6	7	13
H	7	X0	15	17	14	20
I	20	11	22	6	15	21
J	20	17	16	18	17	20
Mean	16.8	13.0	14.2	14.4	15.5	15.5
Mean/surviving female	16.8	14.4	14.2	14.4	15.5	15.5
CV%*	25.8					28.7

X= Dead Adult; M= Male (Not considered in statistics)

*Coefficient of Variation = standard deviation/ mean * 100; CV% calculation based on young per surviving female

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING
Ceriodaphnia dubia SURVIVAL AND REPRODUCTION

Permittee: City of Wynne

NPDES #: AR0021903

PERCENT SURVIVAL

PERCENT EFFLUENT	0%	32%	42%	56%	75%	100%
Time of Reading: 24 HOURS	100	90	100	100	100	100
48 HOURS	100	100	100	100	100	100
Test termination	100	90	100	100	100	100

1. Fisher's Exact Test:

Is the mean survival at test termination significantly different (p=0.05) than the control survival for:

a) LOW FLOW OR CRITICAL DILUTION, (100%) YES _____ NO X _____

2. Dunnett's Procedure or Steel's Many One Rank Test:

Is the mean number of young produced per female significantly different (p=0.05) than the control's number of young per female for:

a) LOW FLOW OR CRITICAL DILUTION, (100%): YES _____ NO X _____

3. If NO was answered to 1.a) and 2.a) enter [0] otherwise enter [1]: 0

4. Enter response to item 3 on DMR Form, parameter # TEP3B.

5. Enter percent effluent corresponding to each NOEC below and circle the lowest number:

a) NOEC survival = 100 % effluent

b) NOEC reproduction = 100 % effluent

c) Coefficient of variation (parameter TQP6C)= 28.7 %

6. Enter Whole Effluent Toxicity: 100 %

APPENDIX A

Chain of Custody Forms

TURNAROUND TIME
 RUSH 24HR. 48HR.
 5 DAY REG.
 OTHER:

FOR LAB/OFFICE USE ONLY

LAB # 18139-0002
 CLIENT # _____
 P. O. # _____

STANDARD METHODS PRESERVATION PER EPA 40 CFR

C 4 = COOL TO 4.0 C
 S<2 = SULFURIC ACID TO PH < 2
 N<2 = NITRIC ACID TO PH > 2
 T = THIOSULFATE
 W = AZIDE MODIFICATION (4500-0 C)
 P = MEMBRANE ELECTRODE (4500-0 G)
 NaOH = Ph > 12

NAME OF COMPANY, CITY, OR PROJECT:

PROJECT NO:

SAMPLER(S) SIGNATURE/PRINT

WYNNE WATER UTILITIES

Harrell Williams
 (HARRELL WILLIAMS)

SAMPLE NO.	SAMPLE COLLECTION LOCATION	START DATE/TIME	END DATE/TIME	COMP/GRAB	FIELD ANALYSIS				D.O. (W)	CONTAINER TYPE	ANALYSIS
					PH	TEMP	FLOW	CL2			
1	POST AERATION BASIN OUTFALL	5/12/15 7:00:00 AM	5/13/14 7:00 AM	COMP/24			1.103			6 - 1/2 GAL	BIO-MONITORING
											K1505-
											001B

Samples Received at Arkansas Analytical
 Relinquished By: Sorrells

Date/Time: 5-13-15, 1638
 Received By: *Suzanne James*

Custody Seals:	Yes	No
Containers Correct:	<input checked="" type="checkbox"/>	<input type="checkbox"/>
COC/Labels Agree:	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Received on Ice:	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Temperature on Receipt:	9°C	
Temperature Gun ID:	HHT #2	

METHOD OF SHIPMENT (CIRCLE) FIELD CALIBRATION RECORD

FED-EX WALK-IN SRA UPS OTHER PH 7

PH 4

TYPE OF SAMPLE(S): (CIRCLE) PH 10

WATER SOIL WW SLUDGE OTHER D. O.

FIELD ANALYSIS CONDUCTED BY: SRA CLIENT

RELINQUISHED BY: *Harrell Williams* DATE/TIME: _____ RECEIVED BY: _____ DATE/TIME: 5-13-15

RELINQUISHED BY: _____ DATE/TIME: 5-13-15 RECEIVED BY: _____ DATE/TIME: 5-13-15

APPENDIX B

Effluent and Dilution Water Data



Biomonitoring Quality Control Benchsheet

Analyst	RA	RH	RH	RF	RF	RF	RF	RF	RF
Date	5-12-15	5-13-15	5-14-15	5-15-15	5-16-17	5-17-15	5-18-15	5-19-15	
pH Meter ID	AK60								
LIN pH 4 Buffer	1401167								
LIN pH 7 Buffer	1401173								
LIN pH 10 Buffer	1401168								
Slope (>90%)	98.6%	94.7%	96.1%	94.9%	95.8%	95.0%	97.1%	95.4%	

Dissolved O ₂ Meter	O.O. 1305								
Meter Reading	8.36	8.41	8.71	8.42	8.66	8.62	8.38	8.62	
Temp.	24	24	22	24	22	22	24	23	
Chart Value at Temp.	8.418	8.418	8.743	8.418	8.743	8.743	8.418	8.578	
Difference	0.058	0.008	0.033	0.018	0.083	0.123	0.038	0.058	
Acceptance Criteria	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L

Temp. Meter ID	AR 60								
Meter Reading	23	24	23	24	23	22	24	23	
Thermometer Reading	23	23	22	23	22	22	23	23	
Thermometer ID	PB								
Acceptance Criteria	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C

Alkalinity									
Blank (<5mg/L)									
STD Result									
T.V. / %REC									
Acceptance Criteria	93.5-108.5% Recovery								

Hardness									
Blank (<2mg/L)									
STD. Result									
T.V. / %REC									
Acceptance Criteria	90.0-105.5% Recovery								

Conductivity Meter ID	Con 02								
Blank (<1)									
STD Result									
T.V. / %REC									
Acceptance Criteria	99.2-104.0% Recovery								

Chlorine Meter ID	OR 820								
Blank (<0.05mg/L)									
STD Result									
T.V. / % REC									
Acceptance Criteria	100.0-120% Recovery								

Revision 0
Effective Date 01APR15

Biomonitoring Quality Control Benchsheet

Analyst	RH	RH	RH					
Date	5-20-15	5-21-15	5-22-15					
pH Meter ID								
LIN pH 4 Buffer	1401167							
LIN pH 7 Buffer	1401173							
LIN pH 10 Buffer	1401168							
Slope (>90%)	95.9%	94.9%	96.5%					

Dissolved O ₂ Meter	D.O. 1305							
Meter Reading	8.51	8.21	8.46					
Temp.	24	25	24					
Chart Value at Temp.	8.418	8.263	8.418					
Difference	0.108	0.053	0.058					
Acceptance Criteria	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L

Temp. Meter ID	AR60							
Meter Reading	23	24	24					
Thermometer Reading	22	24	23					
Thermometer ID	PB							
Acceptance Criteria	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C

Alkalinity								
Blank (<5mg/L)			<5					
STD Result			104					
T.V. / %REC			100 / 104%					
Acceptance Criteria	93.5-108.5% Recovery							

Hardness								
Blank (<2mg/L)			<2					
STD. Result			94					
T.V. / %REC			100 / 94%					
Acceptance Criteria	90.0-105.5% Recovery							

Conductivity Meter ID	Con 02							
Blank (<1)			<1					
STD Result			1420					
T.V. / %REC			142 / 100%					
Acceptance Criteria	99.2-104.0% Recovery							

Chlorine Meter ID	DR 820							
Blank (<0.05mg/L)			<0.05					
STD Result			0.21					
T.V. / % REC			0.21 / 100%					
Acceptance Criteria	100.0-120% Recovery							

Revision 0
Effective Date 01APR15

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Fathead Minnow

Lab # / Sample ID K1505001

Test Start (Date/Time) 5-12-15 1450

Client: Wynne

Test End (Date/Time) 5-19-15 1330

		Day of Test							
		1	2	3	4	5	6	7	notes
Control	44#5	5-12	5-13	5-14	5-15	5-16	5-17	5-18	
	D.O. (mg/L)	INITIAL 8.2	8.7	8.6	7.6	8.4	8.9	8.7	
	FINAL	8.5	7.3	7.5	7.6	8.1	7.3	7.1	
pH (s.u.)	INITIAL	7.8	7.7	8.1	7.8	8.1	8.2	8.0	
	FINAL	7.6	7.9	7.8	7.7	7.9	7.8	8.3	
temp (C)	INITIAL	24	23	23	23	22	21	22	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		58						25	
HARDNESS (mg/L)		88						60	
CONDUCTIVITY (umhc)		444						96	
CHLORINE (mg/L)		20.05						421	
CONC:	32								
D.O. (mg/L)	INITIAL	8.5	8.7	8.5	7.6	8.6	9.1	8.8	
	FINAL	8.5	7.1	7.4	8.1	8.2	7.3	7.4	
pH (s.u.)	INITIAL	7.6	7.7	8.0	7.8	8.0	8.0	7.9	
	FINAL	7.7	7.9	7.5	7.7	7.8	7.8	8.3	
temp (C)	INITIAL	24	22	22	24	22	21	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	42								
D.O. (mg/L)	INITIAL	8.6	8.9	8.9	8.2	8.6	9.0	8.8	
	FINAL	8.5	7.3	7.5	7.4	7.7	7.3	7.4	
pH (mg/L)	INITIAL	7.6	7.6	7.9	7.8	8.0	8.0	7.8	
	FINAL	7.8	7.9	7.6	7.8	8.0	7.8	8.2	
temp (C)	INITIAL	23	23	23	24	22	21	23	
	FINAL	25	25	25	25	25	25	25	
CONC:	56								
D.O. (mg/L)	INITIAL	8.5	9.0	8.8	8.3	8.5	8.8	8.9	
	FINAL	8.5	7.3	7.6	7.5	7.7	7.4	7.3	
pH (s.u.)	INITIAL	7.6	7.4	7.9	7.7	7.9	8.0	7.7	
	FINAL	7.7	7.9	7.6	7.7	7.9	7.8	8.1	
temp (C)	INITIAL	22	22	22	24	22	21	24	
	FINAL	25	25	25	25	25	25	25	
CONC:	75								
D.O. (mg/L)	INITIAL	9.0	9.2	8.9	8.6	8.8	8.9	8.9	
	FINAL	8.6	7.4	7.6	7.1	7.7	7.3	7.3	
pH (s.u.)	INITIAL	7.6	7.5	7.8	7.7	7.9	8.1	7.6	
	FINAL	7.7	7.9	7.7	7.7	7.9	7.8	8.0	
temp (C)	INITIAL	22	21	22	25	22	22	25	
	FINAL	25	25	25	25	25	25	25	
CONC:	100								
D.O. (mg/L)	INITIAL	8.9	9.2	9.1	8.6	8.8	8.8	9.0	
	FINAL	8.6	7.5	7.6	7.6	7.8	7.4	7.5	
pH (s.u.)	INITIAL	7.5	7.3	7.7	7.6	7.8	8.1	7.4	
	FINAL	7.6	7.8	7.7	7.8	7.9	7.6	7.8	
temp (C)	INITIAL	22	21	22	25	21	22	25	
	FINAL	25	25	25	25	25	25	25	
CONC:	100 %								
ALKALINITY (mg/L)		A	A	A	B	B	C	C	
HARDNESS (mg/L)		50			52		48		
CONDUCTIVITY (umhc)		72			82		98		
CHLORINE (mg/L)		256			299		435		
		20.05							

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Ceriodaphnia Dubia

Lab # / Sample ID X1505001

Test Start (Date/Time) 5-12-15 1030

Client: Wyan

Test End (Date/Time) 5-19-15 1050

Day of Test

		1	2	3	4	5	6	7	notes
Control	<u>MHS</u>	<u>5-12</u>	<u>5-13</u>	<u>5-14</u>	<u>5-15</u>	<u>5-16</u>	<u>5-17</u>	<u>5-18</u>	
D.O. (mg/L)	INITIAL	<u>8.2</u>	<u>8.7</u>	<u>8.6</u>	<u>7.6</u>	<u>8.4</u>	<u>8.9</u>	<u>8.7</u>	
	FINAL	<u>8.9</u>	<u>8.2</u>	<u>8.3</u>	<u>8.1</u>	<u>8.3</u>	<u>8.0</u>	<u>8.1</u>	
pH (s.u.)	INITIAL	<u>7.8</u>	<u>7.7</u>	<u>8.1</u>	<u>7.8</u>	<u>8.1</u>	<u>8.2</u>	<u>8.0</u>	
	FINAL	<u>8.0</u>	<u>8.1</u>	<u>7.8</u>	<u>7.8</u>	<u>7.6</u>	<u>7.6</u>	<u>7.9</u>	
temp (C)	INITIAL	<u>24</u>	<u>23</u>	<u>23</u>	<u>23</u>	<u>22</u>	<u>21</u>	<u>22</u>	
	FINAL	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	
ALKALINITY (mg/L)		<u>58</u>						<u>60</u>	
HARDNESS (mg/L)		<u>88</u>						<u>96</u>	
CONDUCTIVITY (umhc)		<u>444</u>						<u>421</u>	
CHLORINE (mg/L)		<u>0.05</u>							
CONC:	<u>32</u>								
D.O. (mg/L)	INITIAL	<u>8.5</u>	<u>8.7</u>	<u>8.5</u>	<u>7.6</u>	<u>8.6</u>	<u>9.1</u>	<u>8.8</u>	
	FINAL	<u>8.5</u>	<u>8.4</u>	<u>8.1</u>	<u>8.3</u>	<u>8.1</u>	<u>8.0</u>	<u>8.2</u>	
pH (s.u.)	INITIAL	<u>7.6</u>	<u>7.7</u>	<u>8.0</u>	<u>7.8</u>	<u>8.0</u>	<u>8.0</u>	<u>7.9</u>	
	FINAL	<u>7.8</u>	<u>7.7</u>	<u>7.9</u>	<u>7.9</u>	<u>7.7</u>	<u>7.7</u>	<u>7.9</u>	
temp (C)	INITIAL	<u>24</u>	<u>22</u>	<u>22</u>	<u>24</u>	<u>22</u>	<u>21</u>	<u>22</u>	
	FINAL	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	
CONC:	<u>42</u>								
D.O. (mg/L)	INITIAL	<u>8.6</u>	<u>8.9</u>	<u>8.9</u>	<u>8.2</u>	<u>8.6</u>	<u>9.0</u>	<u>8.8</u>	
	FINAL	<u>8.5</u>	<u>8.3</u>	<u>8.3</u>	<u>8.3</u>	<u>8.4</u>	<u>9.1</u>	<u>8.2</u>	
pH (mg/L)	INITIAL	<u>7.6</u>	<u>7.6</u>	<u>7.9</u>	<u>7.8</u>	<u>8.0</u>	<u>8.0</u>	<u>7.8</u>	
	FINAL	<u>7.9</u>	<u>7.8</u>	<u>8.0</u>	<u>7.9</u>	<u>8.0</u>	<u>7.8</u>	<u>7.7</u>	
temp (C)	INITIAL	<u>23</u>	<u>23</u>	<u>23</u>	<u>21</u>	<u>22</u>	<u>21</u>	<u>23</u>	
	FINAL	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	
CONC:	<u>56</u>								
D.O. (mg/L)	INITIAL	<u>8.5</u>	<u>9.0</u>	<u>8.8</u>	<u>8.3</u>	<u>8.5</u>	<u>8.8</u>	<u>8.9</u>	
	FINAL	<u>8.6</u>	<u>8.3</u>	<u>8.4</u>	<u>8.3</u>	<u>8.4</u>	<u>8.2</u>	<u>8.3</u>	
pH (s.u.)	INITIAL	<u>7.6</u>	<u>7.4</u>	<u>7.9</u>	<u>7.7</u>	<u>7.9</u>	<u>8.0</u>	<u>7.7</u>	
	FINAL	<u>7.8</u>	<u>7.9</u>	<u>8.0</u>	<u>7.8</u>	<u>8.0</u>	<u>7.8</u>	<u>7.8</u>	
temp (C)	INITIAL	<u>22</u>	<u>22</u>	<u>22</u>	<u>21</u>	<u>22</u>	<u>21</u>	<u>24</u>	
	FINAL	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	
CONC:	<u>75</u>								
D.O. (mg/L)	INITIAL	<u>9.0</u>	<u>9.2</u>	<u>8.9</u>	<u>8.1</u>	<u>8.8</u>	<u>8.9</u>	<u>8.9</u>	
	FINAL	<u>8.7</u>	<u>8.2</u>	<u>8.4</u>	<u>8.3</u>	<u>8.3</u>	<u>8.1</u>	<u>8.2</u>	
pH (s.u.)	INITIAL	<u>7.6</u>	<u>7.3</u>	<u>7.8</u>	<u>7.7</u>	<u>7.9</u>	<u>8.1</u>	<u>7.6</u>	
	FINAL	<u>7.9</u>	<u>7.9</u>	<u>7.9</u>	<u>8.0</u>	<u>8.0</u>	<u>8.0</u>	<u>7.7</u>	
temp (C)	INITIAL	<u>22</u>	<u>21</u>	<u>22</u>	<u>25</u>	<u>22</u>	<u>22</u>	<u>25</u>	
	FINAL	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	
CONC:	<u>100</u>								
D.O. (mg/L)	INITIAL	<u>8.9</u>	<u>9.2</u>	<u>9.1</u>	<u>8.6</u>	<u>8.8</u>	<u>8.8</u>	<u>9.0</u>	
	FINAL	<u>8.7</u>	<u>8.2</u>	<u>8.6</u>	<u>8.2</u>	<u>8.3</u>	<u>8.2</u>	<u>8.4</u>	
pH (s.u.)	INITIAL	<u>7.5</u>	<u>7.3</u>	<u>7.7</u>	<u>7.6</u>	<u>7.8</u>	<u>8.1</u>	<u>7.4</u>	
	FINAL	<u>7.5</u>	<u>8.0</u>	<u>7.6</u>	<u>7.9</u>	<u>8.0</u>	<u>7.8</u>	<u>7.5</u>	
temp (C)	INITIAL	<u>22</u>	<u>21</u>	<u>22</u>	<u>25</u>	<u>21</u>	<u>22</u>	<u>25</u>	
	FINAL	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	
CONC:	<u>100 %</u>	<u>A</u>	<u>A</u>	<u>A</u>	<u>B</u>	<u>B</u>	<u>C</u>	<u>C</u>	
ALKALINITY (mg/L)		<u>50</u>			<u>52</u>		<u>48</u>		
HARDNESS (mg/L)		<u>72</u>			<u>82</u>		<u>98</u>		
CONDUCTIVITY (umhc)		<u>256</u>			<u>299</u>		<u>435</u>		
CHLORINE (mg/L)		<u>0.05</u>							

TITLE: AA# K1505001 ,FATHEAD MINNOW SURV.,CHRONIC, 5-12-15
 FILE: C:\COPYTO~1\TOXSTAT\FHSURV~1.
 TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	1.0000	1.4120
1	CONTROL	2	1.0000	1.4120
1	CONTROL	3	0.9000	1.2490
1	CONTROL	4	1.0000	1.4120
1	CONTROL	5	1.0000	1.4120
2	32 % EFFLUENT	1	1.0000	1.4120
2	32 % EFFLUENT	2	1.0000	1.4120
2	32 % EFFLUENT	3	1.0000	1.4120
2	32 % EFFLUENT	4	1.0000	1.4120
2	32 % EFFLUENT	5	0.8000	1.1071
3	42 % EFFLUENT	1	0.9000	1.2490
3	42 % EFFLUENT	2	1.0000	1.4120
3	42 % EFFLUENT	3	1.0000	1.4120
3	42 % EFFLUENT	4	1.0000	1.4120
3	42 % EFFLUENT	5	0.9000	1.2490
4	56 % EFFLUENT	1	1.0000	1.4120
4	56 % EFFLUENT	2	0.9000	1.2490
4	56 % EFFLUENT	3	1.0000	1.4120
4	56 % EFFLUENT	4	1.0000	1.4120
4	56 % EFFLUENT	5	0.9000	1.2490
5	75 % EFFLUENT	1	0.9000	1.2490
5	75 % EFFLUENT	2	1.0000	1.4120
5	75 % EFFLUENT	3	1.0000	1.4120
5	75 % EFFLUENT	4	1.0000	1.4120
5	75 % EFFLUENT	5	1.0000	1.4120
6	100 % EFFLUENT	1	1.0000	1.4120
6	100 % EFFLUENT	2	1.0000	1.4120
6	100 % EFFLUENT	3	1.0000	1.4120
6	100 % EFFLUENT	4	0.9000	1.2490
6	100 % EFFLUENT	5	1.0000	1.4120

AA# K1505001 ,FATHEAD MINNOW SURV.,CHRONIC, 5-12-15
 File: C:\COPYTO~1\TOXSTAT\FHSURV~1. Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.007	0.001	0.175
Within (Error)	24	0.202	0.008	
Total	29	0.209		

Critical F value = 2.62 (0.05,5,24)
 Since F < Critical F FAIL TO REJECT Ho: All equal

AA# K1505001 , FATHEAD MINNOW SURV., CHRONIC, 5-12-15

File: C:\COPYTO~1\TOXSTAT\FHSURV~1.

Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	1.379	0.980		
2	32 % EFFLUENT	1.351	0.960	0.489	
3	42 % EFFLUENT	1.347	0.960	0.562	
4	56 % EFFLUENT	1.347	0.960	0.562	
5	75 % EFFLUENT	1.379	0.980	0.000	
6	100 % EFFLUENT	1.379	0.980	0.000	

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

AA# K1505001 , FATHEAD MINNOW SURV., CHRONIC, 5-12-15

File: C:\COPYTO~1\TOXSTAT\FHSURV~1.

Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST - TABLE 2 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	5			
2	32 % EFFLUENT	5	0.068	6.9	0.020
3	42 % EFFLUENT	5	0.068	6.9	0.020
4	56 % EFFLUENT	5	0.068	6.9	0.020
5	75 % EFFLUENT	5	0.068	6.9	0.000
6	100 % EFFLUENT	5	0.068	6.9	0.000

Pimephales promelas

FATHEAD MINNOW

TEST 1000.0

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:		K1505001		TEST DATES (BEGIN / END):		5/12/15 - 5/19/15	
CLIENT:		Wynne		WEIGHING DATE / TIME:		5/20/2015 1130	
ANALYSTS:		RH		DRYING TEMP (DEGREES C):		60	
SAMPLE ID:				DRYING TIME (HOURS):		24	
	REP #	FINAL DRY WEIGHT TIN+LARVAE (g)	INITIAL WEIGHT TIN (g)	TOTAL DRY WEIGHT OF LARVAE (g)	NUMBER OF LARVAE	DRY WEIGHT OF LARVAE (mg)	
CONTROL	A	0.97372	0.96580	0.00792	10	0.792	AVG DRY
	B	1.03008	1.02222	0.00786	10	0.786	WEIGHT (mg)
	C	1.00401	0.99708	0.00693	10	0.693	0.746
	D	0.97807	0.97032	0.00775	10	0.775	CV
	E	1.00948	1.00265	0.00683	10	0.683	7.14
32%	A	1.01047	1.00197	0.00850	10	0.850	AVG DRY
	B	0.97852	0.97105	0.00747	10	0.747	WEIGHT (mg)
	C	1.00302	0.99474	0.00828	10	0.828	0.816
	D	0.96317	0.95452	0.00865	10	0.865	CV
	E	0.96786	0.95998	0.00788	10	0.788	
42%	A	1.01757	1.01050	0.00707	10	0.707	AVG DRY
	B	1.02778	1.02081	0.00697	10	0.697	WEIGHT (mg)
	C	1.00125	0.99372	0.00753	10	0.753	0.724
	D	1.02728	1.01887	0.00841	10	0.841	CV
	E	1.00804	1.00180	0.00624	10	0.624	
56%	A	1.01475	1.00623	0.00852	10	0.852	AVG DRY
	B	1.03646	1.02912	0.00734	10	0.734	WEIGHT (mg)
	C	0.98121	0.97006	0.01115	10	1.115	0.855
	D	0.97565	0.96814	0.00751	10	0.751	CV
	E	0.97997	0.97175	0.00822	10	0.822	
75%	A	1.00871	1.00180	0.00691	10	0.691	AVG DRY
	B	1.00793	0.99856	0.00937	10	0.937	WEIGHT (mg)
	C	1.02265	1.01266	0.00999	10	0.999	0.913
	D	1.01310	1.00222	0.01088	10	1.088	CV
	E	0.99827	0.98978	0.00849	10	0.849	
100%	A	0.97519	0.96599	0.00920	10	0.920	AVG DRY
	B	0.97618	0.96694	0.00924	10	0.924	WEIGHT (mg)
	C	1.02775	1.01879	0.00896	10	0.896	0.924
	D	0.97550	0.96601	0.00949	10	0.949	CV
	E	0.96867	0.95936	0.00931	10	0.931	2.08

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA# K1505001, FATHEAD MINNOW GROWTH CHRONIC, 5-12-15
File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.234

W = 0.951

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.

AA# K1505001, FATHEAD MINNOW GROWTH CHRONIC, 5-12-15
File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 16.87

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.

TITLE: AA# K1505001, FATHEAD MINNOW GROWTH CHRONIC, 5-12-15
 FILE: C:\COPYTO~1\TOXSTAT\FHGROWTH.
 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.7920	0.7920
1	CONTROL	2	0.7860	0.7860
1	CONTROL	3	0.6930	0.6930
1	CONTROL	4	0.7750	0.7750
1	CONTROL	5	0.6830	0.6830
2	32 % EFFLUENT	1	0.8500	0.8500
2	32 % EFFLUENT	2	0.7470	0.7470
2	32 % EFFLUENT	3	0.8280	0.8280
2	32 % EFFLUENT	4	0.8650	0.8650
2	32 % EFFLUENT	5	0.7880	0.7880
3	42 % EFFLUENT	1	0.7070	0.7070
3	42 % EFFLUENT	2	0.6970	0.6970
3	42 % EFFLUENT	3	0.7530	0.7530
3	42 % EFFLUENT	4	0.8410	0.8410
3	42 % EFFLUENT	5	0.6240	0.6240
4	56 % EFFLUENT	1	0.8520	0.8520
4	56 % EFFLUENT	2	0.7340	0.7340
4	56 % EFFLUENT	3	1.1150	1.1150
4	56 % EFFLUENT	4	0.7510	0.7510
4	56 % EFFLUENT	5	0.8220	0.8220
5	75 % EFFLUENT	1	0.6910	0.6910
5	75 % EFFLUENT	2	0.9370	0.9370
5	75 % EFFLUENT	3	0.9990	0.9990
5	75 % EFFLUENT	4	1.0880	1.0880
5	75 % EFFLUENT	5	0.8490	0.8490
6	100 % EFFLUENT	1	0.9200	0.9200
6	100 % EFFLUENT	2	0.9240	0.9240
6	100 % EFFLUENT	3	0.8960	0.8960
6	100 % EFFLUENT	4	0.9490	0.9490
6	100 % EFFLUENT	5	0.9310	0.9310

AA# K1505001, FATHEAD MINNOW GROWTH CHRONIC, 5-12-15
 File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.174	0.035	3.569
Within (Error)	24	0.234	0.010	
Total	29	0.408		

Critical F value = 2.62 (0.05,5,24)
 Since F > Critical F REJECT Ho: All equal

AA# K1505001, FATHEAD MINNOW GROWTH CHRONIC, 5-12-15

File: C:\COPYTO~1\TOXSTAT\FHGROWTH.

Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	0.746	0.746		
2	32 % EFFLUENT	0.816	0.816	-1.118	
3	42 % EFFLUENT	0.724	0.724	0.343	
4	56 % EFFLUENT	0.855	0.855	-1.746	
5	75 % EFFLUENT	0.913	0.913	-2.676	
6	100 % EFFLUENT	0.924	0.924	-2.855	

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

AA# K1505001, FATHEAD MINNOW GROWTH CHRONIC, 5-12-15

File: C:\COPYTO~1\TOXSTAT\FHGROWTH.

Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 2 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	5			
2	32 % EFFLUENT	5	0.147	19.8	-0.070
3	42 % EFFLUENT	5	0.147	19.8	0.021
4	56 % EFFLUENT	5	0.147	19.8	-0.109
5	75 % EFFLUENT	5	0.147	19.8	-0.167
6	100 % EFFLUENT	5	0.147	19.8	-0.178

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics



SURVIVAL AND REPRODUCTION TEST

Ceriodaphnia dubia

Discharger: Wynne												Lab Number/s			Analyst: RH			
Location: Outfall 001												K1505001			Test Start - Date/Time: 5-12-15, 1030			
Date Sample Collected: 5 - 11/13/15 - 15															Test Stop - Date/Time: 5-19-15, 1055			
Conc	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst				
%	Day	A	B	C	D	E	F	G	H	I	J							
MHS	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH			
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH			
	3	1	0	0	0	0	1	0	0	0	0	0	2	10	0.2	RH		
	4	1	0	0	3	4	0	5	5	5	0	23	10	2.3	RH			
	5	7	6	8	10	7	1	0	2	3	9	53	10	5.3	RH			
	6	3	6	8	1	3	10	8	0	0	2	41	10	4.1	RH			
	7	5	0	0	6	4	5	8	0	12	9	49	10	4.9	RH			
	8																	
Total		17	12	16	20	18	17	21	7	20	20	168		Avg. = 16.8				
														C.V. = 26.8				
32%	1	0	0	0	0	0	0	0	X	0	0	0	10	0.0	RH			
	2	0	0	0	0	0	0	0		0	0	0	10	0.0	RH			
	3	0	0	0	0	0	0	0		0	1	1	10	0.1	RH			
	4	2	2	0	0	5	0	3		0	6	18	10	1.8	RH			
	5	9	0	6	1	7	8	3		2	5	41	10	4.1	RH			
	6	0	10	4	12	1	7	8		0	5	47	10	4.7	RH			
	7	0	0	1	5	1	5	2		9	0	23	10	2.3	RH			
	8																	
Total		11	12	11	18	14	20	16	0	11	17	130		Avg. = 14.4				
									X					C.V. = 23.8				
42%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH			
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH			
	3	0	0	2	0	0	0	1	0	0	0	3	10	0.3	RH			
	4	1	0	4	5	2	1	0	0	5	6	24	10	2.4	RH			
	5	8	6	3	5	7	5	0	10	11	6	61	10	6.1	RH			
	6	0	5	2	1	0	0	8	2	1	0	19	10	1.9	RH			
	7	9	2	2	0	0	8	2	3	5	4	35	10	3.5	RH			
	8																	
Total		18	13	13	11	9	14	11	15	22	16	142		Avg. = 14.2				
														C.V. = 26.7				
56%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH			
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH			
	3	1	0	0	0	0	0	0	0	0	0	1	10	0.1	RH			
	4	4	1	1	5	4	0	0	3	2	6	26	10	2.6	RH			
	5	8	5	11	6	6	8	0	1	2	4	51	10	5.1	RH			
	6	5	0	2	1	5	9	6	5	0	6	39	10	3.9	RH			
	7	0	5	0	4	4	2	0	8	2	2	27	10	2.7	RH			
	8																	
Total		18	11	14	16	19	19	6	17	6	18	144		Avg. = 14.4				
														C.V. = 35.1				
75%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH			
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH			
	3	0	0	1	1	0	0	1	0	0	0	3	10	0.3	RH			
	4	3	4	0	4	1	1	1	0	0	5	25	10	2.5	RH			
	6	4	8	0	7	12	0	5	6	8	7	57	10	5.7	RH			
	6	10	5	3	1	6	8	0	1	0	3	37	10	3.7	RH			
	7	2	1	7	7	1	5	0	7	1	2	33	10	3.3	RH			
	8																	
Total		19	18	11	20	20	14	7	14	15	17	165		Avg. = 15.5				
														C.V. = 27.1				
100%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH			
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH			
	3	2	0	0	0	1	0	0	0	0	0	3	10	0.3	RH			
	4	5	0	1	1	3	3	2	4	5	6	30	10	3.0	RH			
	5	7	12	3	0	8	7	8	3	2	4	52	10	5.2	RH			
	6	0	4	6	8	7	1	3	5	11	6	51	10	5.1	RH			
	7	0	1	0	0	2	1	0	8	3	4	18	10	1.8	RH			
	8																	
Total		14	17	10	9	19	12	13	20	21	20	155		Avg. = 15.5				
														C.V. = 28.7				

AA # K1505001, C.DUBIA CHRONIC, REPRODUCTION, 5-12-15
File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

***** Shapiro - Wilk's Test is aborted *****

This test can not be performed because total number of replicates
is greater than 50.

Total number of replicates = 60

AA # K1505001, C.DUBIA CHRONIC, REPRODUCTION, 5-12-15
File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 1.70

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.

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
32	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 ANIMALS
CONTROL	10	0	10
42	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 ANIMALS
CONTROL	10	0	10
56	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 ANIMALS
CONTROL	10	0	10
75	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 ANIMALS
CONTROL	10	0	10
100	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

NUMBER	NUMBER	SIG
--------	--------	-----

GROUP	IDENTIFICATION	EXPOSED	DEAD	(P=.05)
	CONTROL	10	0	
1	32	10	1	
2	42	10	0	
3	56	10	0	
4	75	10	0	
5	100	10	0	

TITLE: AA # K1505001, C.DUBIA CHRONIC, REPRODUCCION, 5-12-15
FILE: C:\COPYTO~1\TOXSTAT\C.DUB
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	17.0000	17.0000
1	CONTROL	2	12.0000	12.0000
1	CONTROL	3	16.0000	16.0000
1	CONTROL	4	20.0000	20.0000
1	CONTROL	5	18.0000	18.0000
1	CONTROL	6	17.0000	17.0000
1	CONTROL	7	21.0000	21.0000
1	CONTROL	8	7.0000	7.0000
1	CONTROL	9	20.0000	20.0000
1	CONTROL	10	20.0000	20.0000
2	32 % EFFLUENT	1	11.0000	11.0000
2	32 % EFFLUENT	2	12.0000	12.0000
2	32 % EFFLUENT	3	11.0000	11.0000
2	32 % EFFLUENT	4	18.0000	18.0000
2	32 % EFFLUENT	5	14.0000	14.0000
2	32 % EFFLUENT	6	20.0000	20.0000
2	32 % EFFLUENT	7	16.0000	16.0000
2	32 % EFFLUENT	8	0.0000	0.0000
2	32 % EFFLUENT	9	11.0000	11.0000
2	32 % EFFLUENT	10	17.0000	17.0000
3	42 % EFFLUENT	1	18.0000	18.0000
3	42 % EFFLUENT	2	13.0000	13.0000
3	42 % EFFLUENT	3	13.0000	13.0000
3	42 % EFFLUENT	4	11.0000	11.0000
3	42 % EFFLUENT	5	9.0000	9.0000
3	42 % EFFLUENT	6	14.0000	14.0000
3	42 % EFFLUENT	7	11.0000	11.0000
3	42 % EFFLUENT	8	15.0000	15.0000
3	42 % EFFLUENT	9	22.0000	22.0000
3	42 % EFFLUENT	10	16.0000	16.0000
4	56 % EFFLUENT	1	18.0000	18.0000
4	56 % EFFLUENT	2	11.0000	11.0000
4	56 % EFFLUENT	3	14.0000	14.0000
4	56 % EFFLUENT	4	16.0000	16.0000
4	56 % EFFLUENT	5	19.0000	19.0000
4	56 % EFFLUENT	6	19.0000	19.0000
4	56 % EFFLUENT	7	6.0000	6.0000
4	56 % EFFLUENT	8	17.0000	17.0000

4	56 % EFFLUENT	9	6.0000	6.0000
4	56 % EFFLUENT	10	18.0000	18.0000
5	75 % EFFLUENT	1	19.0000	19.0000
5	75 % EFFLUENT	2	18.0000	18.0000
5	75 % EFFLUENT	3	11.0000	11.0000
5	75 % EFFLUENT	4	20.0000	20.0000
5	75 % EFFLUENT	5	20.0000	20.0000
5	75 % EFFLUENT	6	14.0000	14.0000
5	75 % EFFLUENT	7	7.0000	7.0000
5	75 % EFFLUENT	8	14.0000	14.0000
5	75 % EFFLUENT	9	15.0000	15.0000
5	75 % EFFLUENT	10	17.0000	17.0000
6	100 % EFFLUENT	1	14.0000	14.0000
6	100 % EFFLUENT	2	17.0000	17.0000
6	100 % EFFLUENT	3	10.0000	10.0000
6	100 % EFFLUENT	4	9.0000	9.0000
6	100 % EFFLUENT	5	19.0000	19.0000
6	100 % EFFLUENT	6	12.0000	12.0000
6	100 % EFFLUENT	7	13.0000	13.0000
6	100 % EFFLUENT	8	20.0000	20.0000
6	100 % EFFLUENT	9	21.0000	21.0000
6	100 % EFFLUENT	10	20.0000	20.0000

AA # K1505001, C.DUBIA CHRONIC, REPRODUCCION, 5-12-15
 File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	86.800	17.360	0.816
Within (Error)	54	1148.600	21.270	
Total	59	1235.400		

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

AA # K1505001, C.DUBIA CHRONIC, REPRODUCCION, 5-12-15
 File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 1 OF 2 Ho: Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	16.800	16.800		
2	32 % EFFLUENT	13.000	13.000	1.842	
3	42 % EFFLUENT	14.200	14.200	1.261	
4	56 % EFFLUENT	14.400	14.400	1.164	
5	75 % EFFLUENT	15.500	15.500	0.630	
6	100 % EFFLUENT	15.500	15.500	0.630	

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

AA # K1505001, C.DUBIA CHRONIC, REPRODUCTION, 5-12-15

File: C:\COPYTO~1\TOXSTAT\C.DUB

Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 2 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	10			
2	32 % EFFLUENT	10	4.764	28.4	3.800
3	42 % EFFLUENT	10	4.764	28.4	2.600
4	56 % EFFLUENT	10	4.764	28.4	2.400
5	75 % EFFLUENT	10	4.764	28.4	1.300
6	100 % EFFLUENT	10	4.764	28.4	1.300

APPENDIX E

Organism History

AQUATOX, INC.
416 TWIN POINTS ROAD
HOT SPRINGS, ARKANSAS 71913
501-520-0560

TEST ORGANISM HISTORY

DATE SHIPPED 5/13/15 CLIENT Ac Analytical
Reyn

Purchase Order #: _____

SPECIES: Pimephales promelas

Quantity Shipped: 900

Age: hatched 5/11/15 @ 15-1600
EST

Brood Stock Source: Anderson Farms, AR

Culture Water: Groundwater 160

Hardness (Mg/l CaCO3): _____

Dissolved Oxygen (Mg/l): 8.5

Temperature (°C): 25.1°C

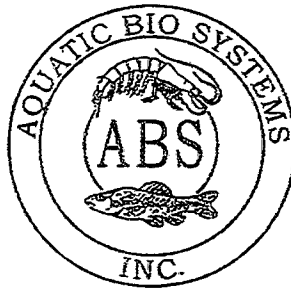
Feeding: AFM

Comments: _____

Shipped Via: KA Federal Express UPS Overnight Shuttle

Packaged By: _____

1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524



Toll Free: 800/331-5916
Tel: 970/484-5091 Fax: 970/484-2514

ORGANISM HISTORY

DATE: 11/25/2013

SPECIES: Ceriodaphnia dubia

AGE: > 3 day

LIFE STAGE: Adult

HATCH DATE: Variable


BEGAN FEEDING: Immediately

FOOD: YTC, Selenastrum sp.

Water Chemistry Record:

	Current	Range
TEMPERATURE:	<u>22°C</u>	<u>22-26°C</u>
SALINITY/CONDUCTIVITY:	<u>--</u>	<u>--</u>
TOTAL HARDNESS (as CaCO ₃):	<u>94 mg/l</u>	<u>76-130 mg/l</u>
TOTAL ALKALINITY (as CaCO ₃):	<u>65 mg/l</u>	<u>65-100 mg/l</u>
pH:	<u>7.98</u>	<u>7.50-8.20</u>

Comments:



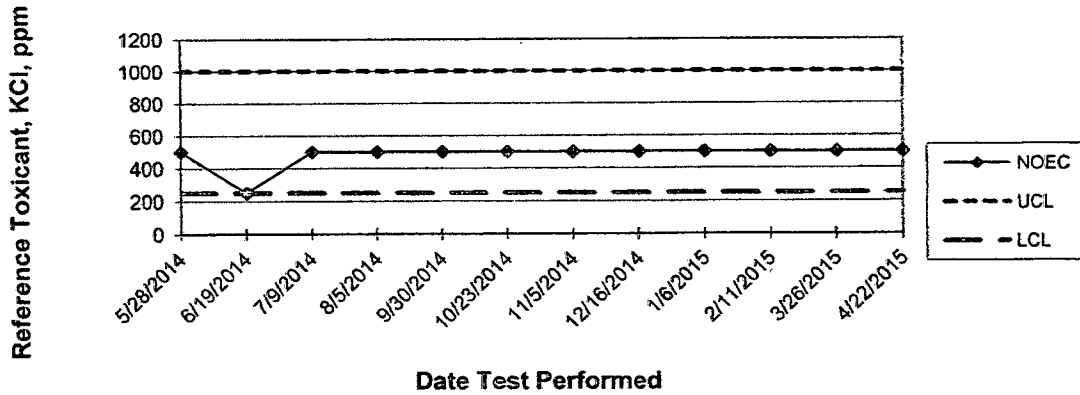
Facility Supervisor

APPENDIX F

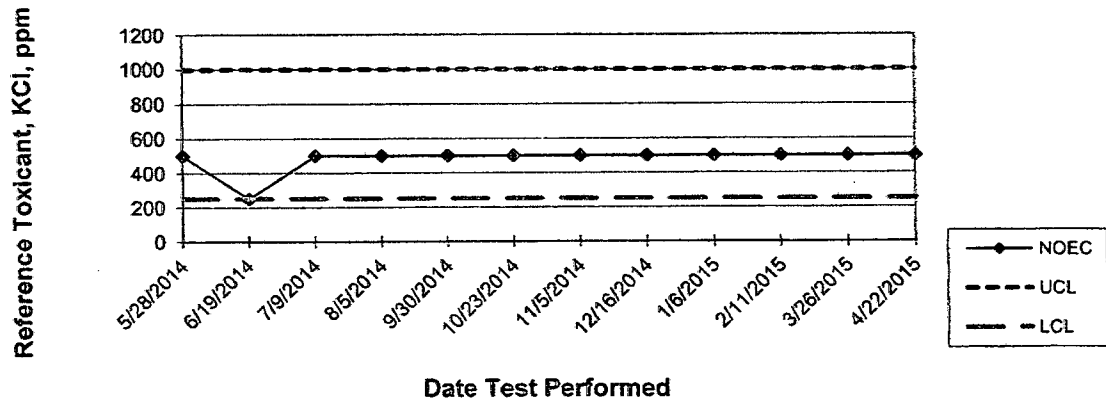
Quality Assurance Charts



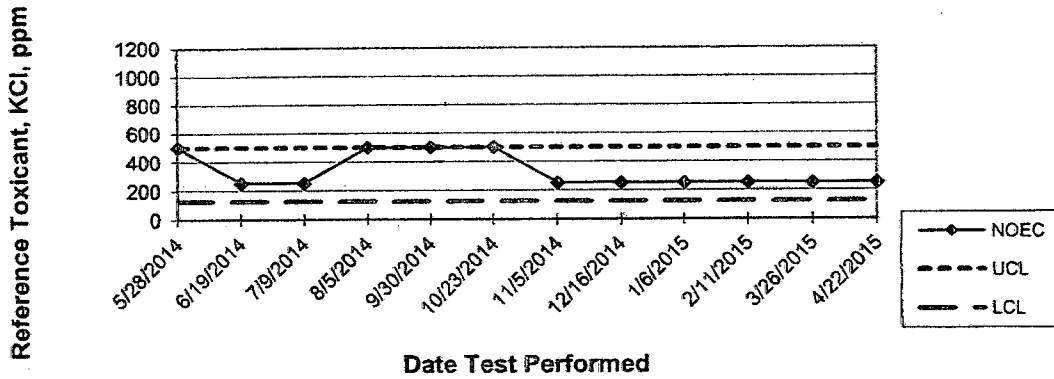
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW SURVIVAL 7 Day
QUALITY ASSURANCE



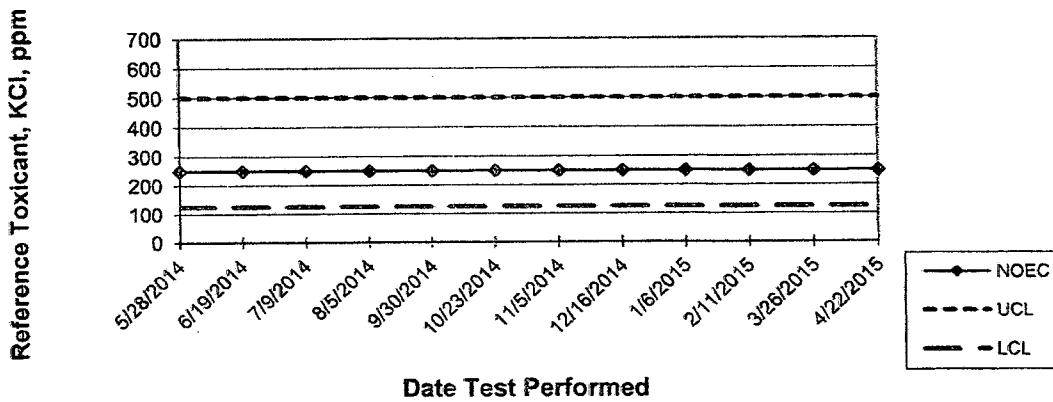
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW GROWTH 7 Day
QUALITY ASSURANCE

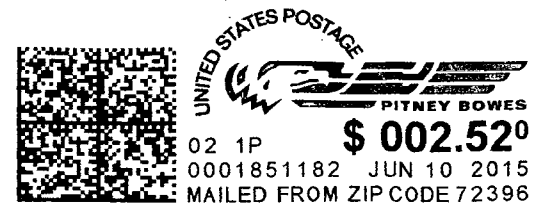
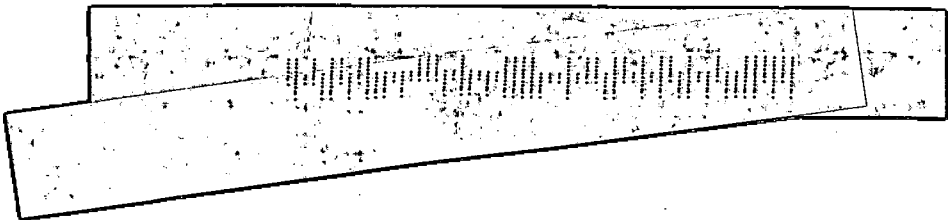


ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE





WYNNE WATER UTILITIES

121 E. MERRIMAN
WYNNE, AR 72396

(870) 238-2751
"Water is Life"



←
TO:

**Arkansas Department of Environmental Quality
NPDES Enforcement Section
5301 Northshore Dr.
North Little Rock, Arkansas 72118-5317**