

Arkansas Analytical, Inc.

Toxicity Test Results

CITY of SHERIDAN
NPDES PERMIT NUMBER: AR0034347
Fourth Quarter 2014
AFIN # 27-00022

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

Ceriodaphnia dubia, Survival and Reproduction Test
Test 1002.0

Prepared for: **Mr. David Fitzgerald**
City of Sheridan
P.O.Box 486
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Prepared by: Arkansas Analytical, Inc.
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Little Rock, Arkansas 72209
Lab Number K1412004

Thursday, December 18, 2014

Introduction

This report contains test results for toxicity testing for the City of Sheridan, NPDES permit number AR0034347. The plant is located in the Southeast $\frac{1}{4}$ of the Northwest $\frac{1}{4}$ of Section 11, Township 5 South, Range 13 West, in Grant County, Arkansas. The discharge is to receiving waters named Big Creek to Hurricane Creek, then to the Saline River in Segment 2C of the Ouachita River Basin.

The permit requires chronic biomonitoring testing quarterly for *Ceriodaphnia dubia* and *Pimephales promelas*. The test results in this report represent the testing of the fourth quarter of 2014.

Plant Operations

To be provided by permittee.

Source of Effluent and Dilution Water

Effluent sample was collected as follows:

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	12-8-14, 1235	12-8-14, 1235

*Only one grab was collected due to the cessation of flow.

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:	12-8-14, 1400	1

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. Synthetic dilution water was substituted because of either zero flow conditions or due to an earlier characterization of the receiving water as being toxic.

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 6%, 8%, 11%, 14%, and 19%. The low-flow effluent concentration (**critical dilution**) was defined as **14% 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. The alternate method suggested in the method (11.3.4.5) for combating pathogen interference was run in place of the original fathead minnow test. The test chambers were 30 ml plastic cups with 20 ml of test solution. Each chamber contained 2 organisms. The total number of fish was 40 per test solution. The fish were then combined to perform growth analysis. 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 ml 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 D.

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 *Pimephales promelas*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	97.5%	X	
The percent coefficient of variation between replicates must be 40% or less for survival	5.73%	X	
Minimum of 0.25 mg average dry weight of surviving controls	0.634	X	
The percent coefficient of variation between replicates must be 40% or less for growth	13.1%	X	

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	16.3	X	
At least 60% of surviving females should have produced 3 broods	90%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	22.8%	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> 11/5/14 – 11/12/14		<i>Pimephales promelas</i> 11/5/14 – 11/12/14	
NOEC Survival:	500ppm KCl	NOEC Survival:	500 ppm KCl
LOEC Survival:	1000 ppm KCl	LOEC Survival:	1000 ppm KCl
NOEC Reproduction:	250 ppm KCl	NOEC Growth:	500 ppm KCl
LOEC Reproduction:	500 ppm KCl	LOEC Growth:	1000 ppm KCl

Quality Assurance charts are provided in Appendix E.

Summary of Results

<i>Ceriodaphnia dubia</i>		<i>Pimephales promelas</i>	
NOEC / LOEC Survival	19% / NA	NOEC / LOEC survival	19% / NA
NOEC / LOEC Reproduction	19% / NA	NOEC / LOEC growth	19% / NA
Mean number of neonates (critical dilution)	17.1	%CV survival (critical dilution)	5.73%
%CV Reproduction (critical dilution)	35.1%	Mean dry weight (critical dilution) in milligrams	0.662
		%CV growth (critical dilution)	11.0%
PMSD Reproduction	33.7%	PMSD Growth	17.4%

Conclusion

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

The permit issued to the City of Sheridan, AR0034347, specifies that the **critical dilution is 14% effluent**. The effluent samples **did not** exhibit lethal or sublethal effects at the critical dilution, and, as such, **passed** the test.

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

The permit issued to the City of Sheridan, AR0034347, specifies that the **critical dilution is 14% effluent**. The effluent samples **did not** exhibit lethal or sublethal effects at the critical dilution, and, as such, **passed** the test.

Biomonitoring Analysts:

Ryan Hudgin / Christopher Turney / Hallie Freyaldenhoven

Reviewed by:

Tracy Bounds (nr)
Tracy Bounds, lab manager

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

PERMITTEE: City of Sheridan

NPDES #: AR0034347

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	12-8-14, 1235	12-8-14, 1235

Test initiated (date, time): 12-9-14, 1340 Test terminated (date, time): 12-16-14, 1310

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	100	87.5	100		100	100	97.5	5.73%
6%	100	100	87.5	87.5	87.5		97.5	97.5	92.5	
8%	100	100	100	100	87.5		100	100	97.5	
11%	100	87.5	100	87.5	87.5		100	100	92.5	
14%	87.5	100	100	100	100		100	100	97.5	5.73%
19%	100	100	87.5	100	100		100	100	97.5	

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%	0.632	0.656	0.709	0.494	0.679		0.634	13.1%
6%	0.714	0.720	0.631	0.669	0.620		0.671	
8%	0.681	0.641	0.731	0.730	0.663		0.689	
11%	0.696	0.539	0.744	0.451	0.520		0.590	
14%	0.546	0.665	0.744	0.661	0.692		0.662	11.0%
19%	0.569	0.626	0.641	0.614	0.662		0.622	

Coefficient of Variation = standard deviation / mean * 100

SUMMARY REPORTING FORMS 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, (14.0%) 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, (14.0%) YES _____ NO X

3. If NO was answered to 1.a) enter [0] otherwise enter [1] (parameter TLP6C): 0

4. If NO was answered to 2.a) enter [0] otherwise enter [1] (parameter TGP6C): 0

5. Enter percentage corresponding to each parameter below:
a) NOEC survival (parameter TOP6C)= 19 % effluent
b) NOEC growth (parameter TPP6C)= 19 % effluent
c) Coefficient of variation (parameter TQP6C)= 13.1 %

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING
Ceriodaphnia dubia SURVIVAL AND REPRODUCTION

Permittee: City of Sheridan

NPDES #: AR0034347

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	12-8-14, 1235	12-8-14, 1235

Test initiated (date, time): 12-9-14, 1005 Test terminated (date, time): 12-16-14, 1130

Dilution water used: Moderately Hard Synthetic

Ceriodaphnia dubia SURVIVAL AND REPRODUCTION

NUMBER OF YOUNG PRODUCED PER FEMALE @ TEST TERMINATION

PERCENT EFFLUENT

Replicate	0%	6%	8%	11%	14%	19%
A	19	19	13	13	13	20
B	11	15	12	21	11	22
C	16	8	11	14	24	15
D	15	15	25	21	22	18
E	20	10	25	22	12	X0
F	15	21	11	10	24	18
G	14	13	20	8	23	20
H	23	12	10	14	20	13
I	12	11	14	23	13	19
J	18	13	15	13	9	10
Mean	16.3	13.7	15.6	15.9	17.1	15.5
Mean/surviving female	16.3	13.7	15.6	15.9	17.1	17.2
CV%*	22.8				35.1	

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 Sheridan

NPDES #: AR0034347

PERCENT SURVIVAL

PERCENT EFFLUENT	0%	6%	8%	11%	14%	19%
Time of Reading: 24 HOURS	100	100	100	100	100	100
48 HOURS	100	100	100	100	100	100
Test termination	100	100	100	100	100	90

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, (36.3%): 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 controls number of young per female for:

a) LOW FLOW OR CRITICAL DILUTION, (36.3%): YES _____ NO X

3. If NO was answered to 1.a) enter [0] otherwise enter [1] (parameter TLP3B): 0

4. If NO was answered to 2.a) enter [0] otherwise enter [1] (parameter TGP3B): 0

5. Enter percentage corresponding to each parameter below:

a) NOEC survival (parameter TOP3B)= 19 % effluent

b) NOEC reproduction (parameter TPP3B)= 19 % effluent

c) Coefficient of variation (parameter TQP3B)= 35.1 %

APPENDIX A

Chain of Custody Forms



11701 Interstate 30, Bldg. 1, Ste. 115
 Little Rock, AR 72209
 PHONE: 501-455-3233
 FAX: 501-455-6118

CHAIN OF CUSTODY RECORD

CLIENT INFORMATION		BILLING INFORMATION		Project Description		Turnaround Time		Preservation Codes:									
Sheridan Waterworks		Sheridan Waterworks		Chronic Toxicity		1 Day (100%)		1. Cool, 4 Degrees Centigrade				4. Thiosulfate for Dechlorination					
104 W High St.		P.O. Box 486				2 Day (50%)		2. Sulfuric Acid (H ₂ SO ₄), pH < 2				5. Hydrochloric Acid(HCl)					
Sheridan, AR 72150		Sheridan, AR 72150		Reporting Information		3 Day (25%)		3. Nitric Acid (HNO ₃), pH < 2				6. Sodium Hydroxide (NaOH), pH > 12					
Attn: David Fitzgerald				Telephone: 870-942-2722		Routine		TEST PARAMETERS								Bottle Type Code	
				Fax: 870-942-1937		Preservative Code: 1										G = Glass; P = Plastic	
				Email: sheridanwater@windstream.net		Bottle Type: P										V = Septum; A = Amber	
Allen Parker				Allen Parker				Chronic Toxicity								Arkansas Analytical Work Order Number:	
Field Number		SAMPLE COLLECTION Date/s Time/s		Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION				Chronic Toxicity					
		12/8/14 1235		X		5	Water	Final Discharge				X		K1412004A			
1. Relinquished by: (Signature)		Date/Time		2. Received by: (Signature)				SAMPLE CONDITION UPON RECEIPT IN LAB				REMARKS / SAMPLE COMMENTS					
Allen Parker		1400 12-8-14		/				1. CUSTODY SEALS: <input checked="" type="checkbox"/> Yes ___ No									
3. Relinquished by: (Signature)		Date/Time		4. Received by lab: (Signature)				2. CONTAINERS CORRECT: <input checked="" type="checkbox"/> Yes ___ No									
/		/		Sydney James				3. COC/LABELS AGREE: <input checked="" type="checkbox"/> Yes ___ No									
								4. RECEIVED ON ICE: <input checked="" type="checkbox"/> Yes ___ No									
								5. TEMPERATURE ON RECEIPT: 1°C									
								6. TEMPERATURE GUN ID: HHT #2									
FOR COMPLETION BY LAB ONLY																	

APPENDIX B

Effluent and Dilution Water Data

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Ceriodaphnia Dubia

Lab # / Sample ID K1412004

Test Start (Date/Time)

12-9-14

1005

Client: *Sheridan*

Test End (Date/Time)

12-16-14

1130

Day of Test

		1	2	3	4	5	6	7	notes
Control	<i>ml 5</i>	12-9	12-10-14	12-11	12-12	12-13	12-14	12-15	
D.O. (mg/L)	INITIAL	8.6	8.4	8.6	8.4	8.5	8.4	8.8	
	FINAL	8.4	8.1	8.3	8.8	8.9	8.4	8.5	
pH (s.u.)	INITIAL	7.7	7.7	7.6	7.7	7.9	7.8	7.9	
	FINAL	7.8	7.7	7.4	8.0	7.9	8.0	8.1	
temp (C)	INITIAL	24	22	22	23	23	23	22	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		69	→	64	→	→	→	→	
HARDNESS (mg/L)		98	→	90	→	→	→	→	
CONDUCTIVITY (umhc)		457	→	404	→	→	→	→	
CHLORINE (mg/L)		<0.05	→	→	→	→	→	→	
CONC:	<i>6</i>								
D.O. (mg/L)	INITIAL	8.5	8.3	8.3	8.4	8.4	8.0	8.6	
	FINAL	8.4	8.3	8.3	8.8	8.7	8.4	8.5	
pH (s.u.)	INITIAL	7.8	7.8	7.7	7.6	8.0	7.6	7.8	
	FINAL	7.7	7.7	7.5	7.7	7.3	8.0	8.0	
temp (C)	INITIAL	23	22	23	23	23	23	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	<i>8</i>								
D.O. (mg/L)	INITIAL	8.3	8.3	8.4	8.5	8.6	8.4	8.6	
	FINAL	8.4	8.4	8.4	8.6	9.0	8.4		
pH (mg/L)	INITIAL	7.7	7.7	7.7	7.6	8.0	7.6	7.8	
	FINAL	7.8	7.8	7.5	7.7	7.4	8.0	8.0	
temp (C)	INITIAL	23	22	23	23	23	23	21	
	FINAL	25	25	25	25	25	25	25	
CONC:	<i>11</i>								
D.O. (mg/L)	INITIAL	8.8	8.0	8.5	8.8	8.6	8.5	8.7	
	FINAL	8.5	8.4	8.5	8.7	9.0	8.4	8.7	
pH (s.u.)	INITIAL	7.7	7.8	7.8	7.7	7.9	7.7	7.9	
	FINAL	7.8	7.8	7.5	7.8	7.4	8.0	7.9	
temp (C)	INITIAL	23	22	23	23	23	23	21	
	FINAL	25	25	25	25	25	25	25	
CONC:	<i>14</i>								
D.O. (mg/L)	INITIAL	8.8	8.1	8.6	8.8	8.7	8.5	8.7	
	FINAL	8.4	8.4	8.5	8.3	9.0	8.5	8.8	
pH (s.u.)	INITIAL	7.8	7.7	7.8	7.7	7.9	7.7	7.9	
	FINAL	7.7	7.8	7.7	7.8	7.5	8.0	8.0	
temp (C)	INITIAL	24	22	23	23	23	23	21	
	FINAL	25	25	25	25	25	25	25	
CONC:	<i>19</i>								
D.O. (mg/L)	INITIAL	8.8	7.8	8.6	9.0	8.7	8.5	8.7	
	FINAL	8.5	8.6	8.5	8.4	9.0	8.5	8.8	
pH (s.u.)	INITIAL	7.7	7.8	7.8	7.7	7.9	7.8	7.8	
	FINAL	7.7	7.8	7.8	7.7	7.5	8.0	8.0	
temp (C)	INITIAL	24	23	23	23	23	23	21	
	FINAL	25	25	25	25	25	25	25	
CONC:	<i>100 %</i>								
ALKALINITY (mg/L)		80	→	→	→	→	→	→	
HARDNESS (mg/L)		78	→	→	→	→	→	→	
CONDUCTIVITY (umhc)		459	→	→	→	→	→	→	
CHLORINE (mg/L)		<0.05	→	→	→	→	→	→	

APPENDIX C

Fathead minnow raw data and statistics

Pimephales promelas

FATHEAD MINNOW

SURVIVAL DATA FOR LARVAL SURVIVAL AND GROWTH TEST (ALTERNATE)

LAB #: K1412004		TEST START								DATE	12/09/14	TIME	1340
CLIENT: Sheridan		TEST END								DATE	12/16/14	TIME	1310
ANALYST: RH / CT / HF		AGE AND SOURCE OF MINNOWS								< 24 hrs old, Aquatox			
DAY(NUMBER SURVIVING)													
		REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONTROL	A	8	8	8	8	8	8	8	8	8	100%	97.5%	5.73
	B	8	8	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	8	8	100%		
	D	8	8	8	8	8	8	8	8	7	87.5%		
	E	8	8	8	8	8	8	8	8	8	100%		
MHS	A	8	8	8	8	8	8	8	8	8	100%	92.5%	5.73
	B	8	8	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	8	8	100%		
	D	8	8	8	8	8	8	8	8	7	87.5%		
	E	8	8	8	8	8	8	8	8	8	100%		
CONC:	A	8	8	8	8	8	8	8	8	8	100%	92.5%	5.73
	B	8	8	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	8	8	100%		
	D	8	8	8	8	8	8	8	8	7	87.5%		
	E	8	8	8	8	8	8	8	8	7	87.5%		
6%	A	8	8	8	8	8	8	8	8	8	100%	97.5%	5.73
	B	8	8	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	8	8	100%		
	D	8	8	8	8	8	8	8	8	8	100%		
	E	8	8	8	8	8	8	8	8	7	87.5%		
8%	A	8	8	8	8	8	8	8	8	8	100%	92.5%	5.73
	B	8	8	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	8	8	100%		
	D	8	8	8	8	8	8	8	8	8	100%		
	E	8	8	8	8	8	8	8	8	7	87.5%		
11%	A	8	8	8	8	8	8	8	8	8	100%	97.5%	5.73
	B	8	8	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	8	7	87.5%		
	D	8	8	8	8	8	8	8	8	8	100%		
	E	8	8	8	8	8	8	8	8	7	87.5%		
14%	A	8	8	8	8	8	8	8	8	8	100%	97.5%	5.73
	B	8	8	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	8	8	100%		
	D	8	8	8	8	8	8	8	8	8	100%		
	E	8	8	8	8	8	8	8	8	8	100%		
19%	A	8	8	8	8	8	8	8	8	8	100%	97.5%	5.73
	B	8	8	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	8	8	100%		
	D	8	8	8	8	8	8	8	8	7	87.5%		
	E	8	8	8	8	8	8	8	8	8	100%		
ANALYST:		RH	RH	RH	RH	CT	HF	RH	RH				
DATE:		12/9/14	12/10/14	12/11/14	12/12/14	12/13/14	12/14/14	12/15/14	12/16/14				
TIME:		1340	1505	1500	1115	1115	1100	1340	1310				

CV= PERCENT COEFFICIENT OF VARIATION: STANDARD DEVIATION/MEAN * 100

REMARKS:

AA# K1412004, FATHEAD MINNOW SURV., CHRONIC, 12-9-14

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

Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.189

W = 0.817

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.

AA# K1412004, FATHEAD MINNOW SURV., CHRONIC, 12-9-14

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

Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 0.42

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.

TITLE: AA# K1412004, FATHEAD MINNOW SURV., CHRONIC, 12-9-14
 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	1.3931
1	CONTROL	2	1.0000	1.3931
1	CONTROL	3	1.0000	1.3931
1	CONTROL	4	0.8750	1.2094
1	CONTROL	5	1.0000	1.3931
2	6 % EFFLUENT	1	1.0000	1.3931
2	6 % EFFLUENT	2	1.0000	1.3931
2	6 % EFFLUENT	3	0.8750	1.2094
2	6 % EFFLUENT	4	0.8750	1.2094
2	6 % EFFLUENT	5	0.8750	1.2094
3	8 % EFFLUENT	1	1.0000	1.3931
3	8 % EFFLUENT	2	1.0000	1.3931
3	8 % EFFLUENT	3	1.0000	1.3931
3	8 % EFFLUENT	4	1.0000	1.3931
3	8 % EFFLUENT	5	0.8750	1.2094
4	11 % EFFLUENT	1	1.0000	1.3931
4	11 % EFFLUENT	2	0.8750	1.2094
4	11 % EFFLUENT	3	1.0000	1.3931
4	11 % EFFLUENT	4	0.8750	1.2094
4	11 % EFFLUENT	5	0.8750	1.2094
5	14 % EFFLUENT	1	0.8750	1.2094
5	14 % EFFLUENT	2	1.0000	1.3931
5	14 % EFFLUENT	3	1.0000	1.3931
5	14 % EFFLUENT	4	1.0000	1.3931
5	14 % EFFLUENT	5	1.0000	1.3931
6	19 % EFFLUENT	1	1.0000	1.3931
6	19 % EFFLUENT	2	1.0000	1.3931
6	19 % EFFLUENT	3	0.8750	1.2094
6	19 % EFFLUENT	4	1.0000	1.3931
6	19 % EFFLUENT	5	1.0000	1.3931

AA# K1412004, FATHEAD MINNOW SURV., CHRONIC, 12-9-14
 File: C:\COPYTO~1\TOXSTAT\FHSURV~1. Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.036	0.007	0.914
Within (Error)	24	0.189	0.008	
Total	29	0.225		

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

AA# K1412004, FATHEAD MINNOW SURV., CHRONIC, 12-9-14

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	1.356	0.975		
2	6 % EFFLUENT	1.283	0.925	1.309	
3	8 % EFFLUENT	1.356	0.975	0.000	
4	11 % EFFLUENT	1.283	0.925	1.309	
5	14 % EFFLUENT	1.356	0.975	0.000	
6	19 % EFFLUENT	1.356	0.975	0.000	

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

AA# K1412004, FATHEAD MINNOW SURV., CHRONIC, 12-9-14

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	6 % EFFLUENT	5	0.070	7.2	0.050
3	8 % EFFLUENT	5	0.070	7.2	0.000
4	11 % EFFLUENT	5	0.070	7.2	0.050
5	14 % EFFLUENT	5	0.070	7.2	0.000
6	19 % EFFLUENT	5	0.070	7.2	0.000

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:		K1412004		TEST DATES (BEGIN / END):		12/9/14 - 12/16/14	
CLIENT:		Sheridan		WEIGHING DATE / TIME:		12/17/2014 1530	
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	0.99767	0.99261	0.00506	8	0.632	AVG DRY
MHS	B	1.01879	1.01354	0.00525	8	0.656	WEIGHT (mg)
	C	1.02164	1.01597	0.00567	8	0.709	0.634
	D	1.00601	1.00206	0.00395	8	0.494	CV
	E	1.04105	1.03562	0.00543	8	0.679	13.14
CONC:	A	0.99517	0.98946	0.00571	8	0.714	AVG DRY
6%	B	1.02059	1.01483	0.00576	8	0.720	WEIGHT (mg)
	C	1.00452	0.99947	0.00505	8	0.631	0.671
	D	1.01191	1.00656	0.00535	8	0.669	CV
	E	1.02729	1.02233	0.00496	8	0.620	
CONC:	A	0.99856	0.99311	0.00545	8	0.681	AVG DRY
8%	B	0.99903	0.99390	0.00513	8	0.641	WEIGHT (mg)
	C	1.01628	1.01043	0.00585	8	0.731	0.689
	D	1.03272	1.02688	0.00584	8	0.730	CV
	E	1.01396	1.00866	0.00530	8	0.663	
CONC:	A	1.00129	0.99572	0.00557	8	0.696	AVG DRY
11%	B	1.00617	1.00186	0.00431	8	0.539	WEIGHT (mg)
	C	1.02102	1.01507	0.00595	8	0.744	0.590
	D	1.00232	0.99871	0.00361	8	0.451	CV
	E	1.00049	0.99633	0.00416	8	0.520	
CONC:	A	1.00402	0.99965	0.00437	8	0.546	AVG DRY
14%	B	1.00494	0.99962	0.00532	8	0.665	WEIGHT (mg)
	C	1.00513	0.99918	0.00595	8	0.744	0.662
	D	0.99887	0.99358	0.00529	8	0.661	CV
	E	1.00224	0.99670	0.00554	8	0.692	10.95
CONC:	A	1.02030	1.01575	0.00455	8	0.569	AVG DRY
19%	B	1.02451	1.01950	0.00501	8	0.626	WEIGHT (mg)
	C	1.00813	1.00300	0.00513	8	0.641	0.623
	D	1.02163	1.01672	0.00491	8	0.614	CV
	E	0.99585	0.99055	0.00530	8	0.662	

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA# K1412004, FATHEAD MINNOW GROWTH CHRONIC, 12-9-14

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

Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.130

W = 0.972

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# K1412004, FATHEAD MINNOW GROWTH CHRONIC, 12-9-14

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

Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 8.73

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.

TITLE: AA# K1412004, FATHEAD MINNOW GROWTH CHRONIC, 12-9-14
 FILE: C:\COPYTO~1\TOXSTAT\FHGROWTH.
 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.6320	0.6320
1	CONTROL	2	0.6560	0.6560
1	CONTROL	3	0.7090	0.7090
1	CONTROL	4	0.4940	0.4940
1	CONTROL	5	0.6790	0.6790
2	6 % EFFLUENT	1	0.7140	0.7140
2	6 % EFFLUENT	2	0.7200	0.7200
2	6 % EFFLUENT	3	0.6310	0.6310
2	6 % EFFLUENT	4	0.6690	0.6690
2	6 % EFFLUENT	5	0.6200	0.6200
3	8 % EFFLUENT	1	0.6810	0.6810
3	8 % EFFLUENT	2	0.6410	0.6410
3	8 % EFFLUENT	3	0.7310	0.7310
3	8 % EFFLUENT	4	0.7300	0.7300
3	8 % EFFLUENT	5	0.6630	0.6630
4	11 % EFFLUENT	1	0.6960	0.6960
4	11 % EFFLUENT	2	0.5390	0.5390
4	11 % EFFLUENT	3	0.7440	0.7440
4	11 % EFFLUENT	4	0.4510	0.4510
4	11 % EFFLUENT	5	0.5200	0.5200
5	14 % EFFLUENT	1	0.5460	0.5460
5	14 % EFFLUENT	2	0.6650	0.6650
5	14 % EFFLUENT	3	0.7440	0.7440
5	14 % EFFLUENT	4	0.6610	0.6610
5	14 % EFFLUENT	5	0.6920	0.6920
6	19 % EFFLUENT	1	0.5690	0.5690
6	19 % EFFLUENT	2	0.6260	0.6260
6	19 % EFFLUENT	3	0.6410	0.6410
6	19 % EFFLUENT	4	0.6140	0.6140
6	19 % EFFLUENT	5	0.6620	0.6620

AA# K1412004, FATHEAD MINNOW GROWTH CHRONIC, 12-9-14
 File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.033	0.007	1.206
Within (Error)	24	0.130	0.005	
Total	29	0.163		

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

AA# K1412004, FATHEAD MINNOW GROWTH CHRONIC, 12-9-14

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	0.634	0.634		
2	6 % EFFLUENT	0.671	0.671	-0.789	
3	8 % EFFLUENT	0.689	0.689	-1.184	
4	11 % EFFLUENT	0.590	0.590	0.944	
5	14 % EFFLUENT	0.662	0.662	-0.592	
6	19 % EFFLUENT	0.622	0.622	0.249	

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

AA# K1412004, FATHEAD MINNOW GROWTH CHRONIC, 12-9-14

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	6 % EFFLUENT	5	0.110	17.4	-0.037
3	8 % EFFLUENT	5	0.110	17.4	-0.055
4	11 % EFFLUENT	5	0.110	17.4	0.044
5	14 % EFFLUENT	5	0.110	17.4	-0.028
6	19 % EFFLUENT	5	0.110	17.4	0.012

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

SURVIVAL AND REPRODUCTION TEST

Ceriodaphnia dubia

Discharger: Sheridan AFIN # 27-00022												Lab Number/s				Analyst: RH																														
Location: Outfall 001												K1412004				Test Start - Date/Time: 12-9-14, 1005																														
Date Sample Collected: 12-8-14, 1235																Test Stop - Date/Time: 12-16-14, 1130																														
Conc	1	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	Conc	4	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst															
%	Day	A	B	C	D	E	F	G	H	I	J					%	Day	A	B	C	D	E	F	G	H	I	J																			
MHS	1	0	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH	11%	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	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	0	10	0.0	RH		2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH		
	3	0	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH		3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH		
	4	3	6	4	0	5	1	3	0	2	3	27	10	2.7	RH	4		4	2	6	5	2	4	1	4	4	4	36	10	3.6	RH															
	5	1	2	7	6	4	7	1	3	5	7	43	10	4.3	RH	5		9	6	7	9	7	6	0	5	10	6	65	10	6.5	RH															
	6	9	2	5	9	8	7	5	12	0	2	59	10	5.9	RH	6		0	8	0	6	8	0	4	5	8	3	42	10	4.2	RH															
	7	6	1	0	0	3	0	5	8	5	6	34	10	3.4	RH	7		0	5	1	1	5	0	3	0	1	0	16	10	1.6	RH															
	8																											10	0.0																	
	Total	19	11	16	15	20	15	14	23	12	18	163		Avg. = 16.3	Total	13	21	14	21	22	10	8	14	23	13	159		Avg. = 15.9																		
														C.V. = 22.8															C.V. = 33.9																	
Conc	2	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	Conc	5	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst															
%	Day	A	B	C	D	E	F	G	H	I	J				%	Day	A	B	C	D	E	F	G	H	I	J																				
6%	1	0	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH	14%	1	0	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	0	10	0.0	RH		2	0	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH													
	3	0	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH		3	0	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH													
	4	3	4	0	4	4	3	0	4	4	3	29	10	2.9	RH	4		3	2	3	5	0	5	3	3	2	4	30	10	3.0	RH															
	5	5	6	8	6	6	8	5	0	7	4	55	10	5.5	RH	5		1	9	8	8	2	6	7	9	8	0	58	10	5.8	RH															
	6	1	5	0	3	0	1	6	3	0	0	19	10	1.9	RH	6		6	0	7	5	0	6	8	0	3	0	35	10	3.5	RH															
	7	10	0	0	2	0	9	2	5	0	6	34	10	3.4	RH	7		3	0	6	4	10	7	5	8	0	5	48	10	4.8	RH															
	8																											10	0.0	RH																
	Total	19	15	8	15	10	21	13	12	11	13	137		Avg. = 13.7	Total	13	11	24	22	12	24	23	20	13	9	171		Avg. = 17.1																		
														C.V. = 29.0															C.V. = 35.1																	
Conc	3	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	Conc	6	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst															
%	Day	A	B	C	D	E	F	G	H	I	J				%	Day	A	B	C	D	E	F	G	H	I	J																				
8%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH	19%	1	0	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		2	0	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH														
	3	0	0	0	0	0	1	0	0	0	0	1	10	0.1	RH		3	0	0	0	1	0	0	0	0	0	0	1	10	0.1	RH															
	4	3	1	4	3	3	4	2	3	7	3	33	10	3.3	RH		4	3	3	3	6	X	3	4	3	3	3	31	10	3.1	RH															
	5	1	5	1	10	7	2	9	1	1	8	45	10	4.5	RH		5	8	7	7	1		7	8	6	3	1	48	10	4.8	RH															
	6	0	6	5	10	8	2	6	1	5	3	46	10	4.6	RH		6	6	10	5	8		6	4	3	8	6	56	10	5.6	RH															
	7	9	0	1	2	7	2	3	5	1	1	31	10	3.1	RH		7	3	2	0	2		2	4	1	5	0	19	10	1.9	RH															
	8																											10	0.0	RH																
	Total	13	12	11	25	25	11	20	10	14	15	156		Avg. = 15.6	Total	20	22	15	18	0	18	20	13	19	10	155		Avg. = 17.2																		
														C.V. = 36.5							X								C.V. = 22.3																	

AA # K1412004, C.DUBIA CHRONIC, REPRODUCCION, 12-9-14
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 # K1412004, C.DUBIA CHRONIC, REPRODUCCION, 12-9-14
File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 4.21

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
6	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
8	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
11	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
14	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
19	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.

SUMMARY OF FISHER'S EXACT TESTS

NUMBER NUMBER SIG

GROUP	IDENTIFICATION	EXPOSED	DEAD	(P=.05)
	CONTROL	10	0	
1	6	10	0	
2	8	10	0	
3	11	10	0	
4	14	10	0	
5	19	10	1	

TITLE: AA # K1412004, C.DUBIA CHRONIC, REPRODUCCION, 12-9-14
FILE: C:\COPYTO~1\TOXSTAT\C.DUB
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	19.0000	19.0000
1	CONTROL	2	11.0000	11.0000
1	CONTROL	3	16.0000	16.0000
1	CONTROL	4	15.0000	15.0000
1	CONTROL	5	20.0000	20.0000
1	CONTROL	6	15.0000	15.0000
1	CONTROL	7	14.0000	14.0000
1	CONTROL	8	23.0000	23.0000
1	CONTROL	9	12.0000	12.0000
1	CONTROL	10	18.0000	18.0000
2	6 % EFFLUENT	1	19.0000	19.0000
2	6 % EFFLUENT	2	15.0000	15.0000
2	6 % EFFLUENT	3	8.0000	8.0000
2	6 % EFFLUENT	4	15.0000	15.0000
2	6 % EFFLUENT	5	10.0000	10.0000
2	6 % EFFLUENT	6	21.0000	21.0000
2	6 % EFFLUENT	7	13.0000	13.0000
2	6 % EFFLUENT	8	12.0000	12.0000
2	6 % EFFLUENT	9	11.0000	11.0000
2	6 % EFFLUENT	10	13.0000	13.0000
3	8 % EFFLUENT	1	13.0000	13.0000
3	8 % EFFLUENT	2	12.0000	12.0000
3	8 % EFFLUENT	3	11.0000	11.0000
3	8 % EFFLUENT	4	25.0000	25.0000
3	8 % EFFLUENT	5	25.0000	25.0000
3	8 % EFFLUENT	6	11.0000	11.0000
3	8 % EFFLUENT	7	20.0000	20.0000
3	8 % EFFLUENT	8	10.0000	10.0000
3	8 % EFFLUENT	9	14.0000	14.0000
3	8 % EFFLUENT	10	15.0000	15.0000
4	11 % EFFLUENT	1	13.0000	13.0000
4	11 % EFFLUENT	2	21.0000	21.0000
4	11 % EFFLUENT	3	14.0000	14.0000
4	11 % EFFLUENT	4	21.0000	21.0000
4	11 % EFFLUENT	5	22.0000	22.0000
4	11 % EFFLUENT	6	10.0000	10.0000
4	11 % EFFLUENT	7	8.0000	8.0000
4	11 % EFFLUENT	8	14.0000	14.0000

4	11 % EFFLUENT	9	23.0000	23.0000
4	11 % EFFLUENT	10	13.0000	13.0000
5	14 % EFFLUENT	1	13.0000	13.0000
5	14 % EFFLUENT	2	11.0000	11.0000
5	14 % EFFLUENT	3	24.0000	24.0000
5	14 % EFFLUENT	4	22.0000	22.0000
5	14 % EFFLUENT	5	12.0000	12.0000
5	14 % EFFLUENT	6	24.0000	24.0000
5	14 % EFFLUENT	7	23.0000	23.0000
5	14 % EFFLUENT	8	20.0000	20.0000
5	14 % EFFLUENT	9	13.0000	13.0000
5	14 % EFFLUENT	10	9.0000	9.0000
6	19 % EFFLUENT	1	20.0000	20.0000
6	19 % EFFLUENT	2	22.0000	22.0000
6	19 % EFFLUENT	3	15.0000	15.0000
6	19 % EFFLUENT	4	18.0000	18.0000
6	19 % EFFLUENT	5	0.0000	0.0000
6	19 % EFFLUENT	6	18.0000	18.0000
6	19 % EFFLUENT	7	20.0000	20.0000
6	19 % EFFLUENT	8	13.0000	13.0000
6	19 % EFFLUENT	9	19.0000	19.0000
6	19 % EFFLUENT	10	10.0000	10.0000

AA # K1412004, C.DUBIA CHRONIC, REPRODUCCION, 12-9-14
 File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	64.083	12.817	0.453
Within (Error)	54	1528.900	28.313	
Total	59	1592.983		

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

AA # K1412004, C.DUBIA CHRONIC, REPRODUCCION, 12-9-14
 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	16.300	16.300		
2	6 % EFFLUENT	13.700	13.700	1.093	
3	8 % EFFLUENT	15.600	15.600	0.294	
4	11 % EFFLUENT	15.900	15.900	0.168	
5	14 % EFFLUENT	17.100	17.100	-0.336	
6	19 % EFFLUENT	15.500	15.500	0.336	

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

AA # K1412004, C.DUBIA CHRONIC, REPRODUCTION, 12-9-14

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	6 % EFFLUENT	10	5.497	33.7	2.600
3	8 % EFFLUENT	10	5.497	33.7	0.700
4	11 % EFFLUENT	10	5.497	33.7	0.400
5	14 % EFFLUENT	10	5.497	33.7	-0.800
6	19 % EFFLUENT	10	5.497	33.7	0.800

APPENDIX E

Organism History

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

TEST ORGANISM HISTORY

DATE SHIPPED 12-9-14 CLIENT Arkansas Analytical

Purchase Order #: _____

SPECIES: Pimephales promelas

Quantity Shipped: 840⁺

Age: hatched 12/8 15-1600 CST

Brood Stock Source: Anderson Farms, AR

Culture Water: Groundwater

Hardness (Mg/l CaCO₃): ~160

Dissolved Oxygen (Mg/l): 8.4

Temperature (°C): 25.4

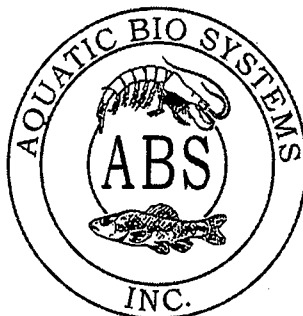
Feeding: ARTIFICIAL

Comments: _____

Shipped Via: Federal Express UPS Overnight Shuttle

Packaged By: Cell

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

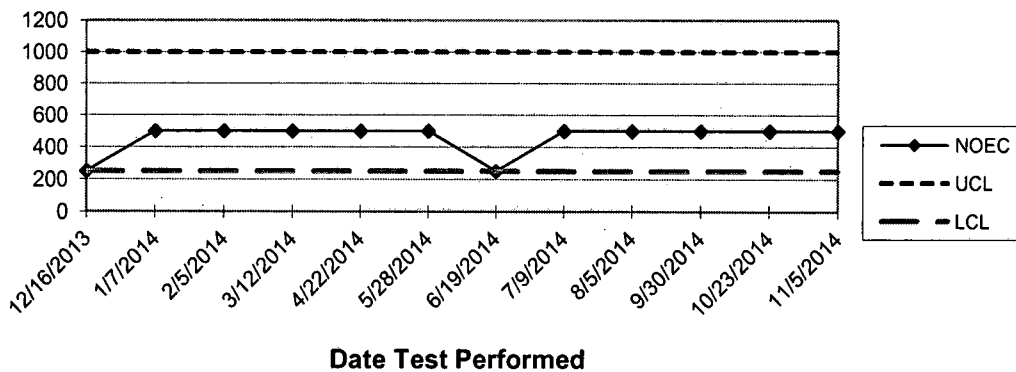
APPENDIX F

Quality Assurance Charts

ARKANSAS ANALYTICAL, INC.

FATHEAD MINNOW SURVIVAL 7 Day QUALITY ASSURANCE

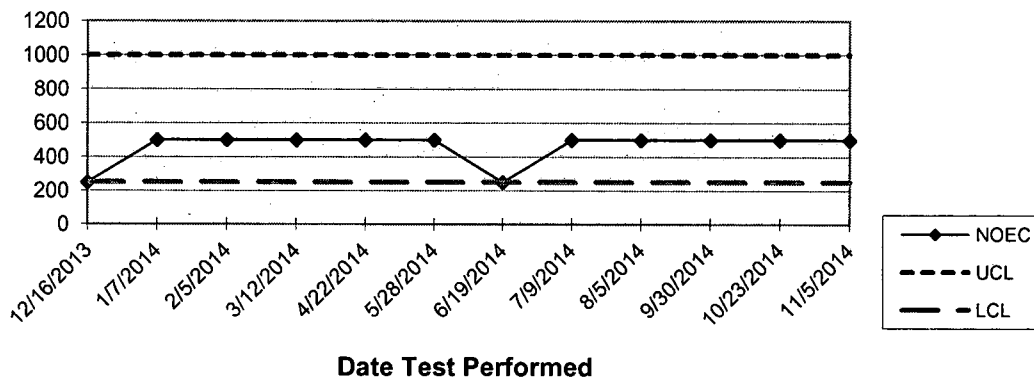
Reference Toxicant, KCl, ppm



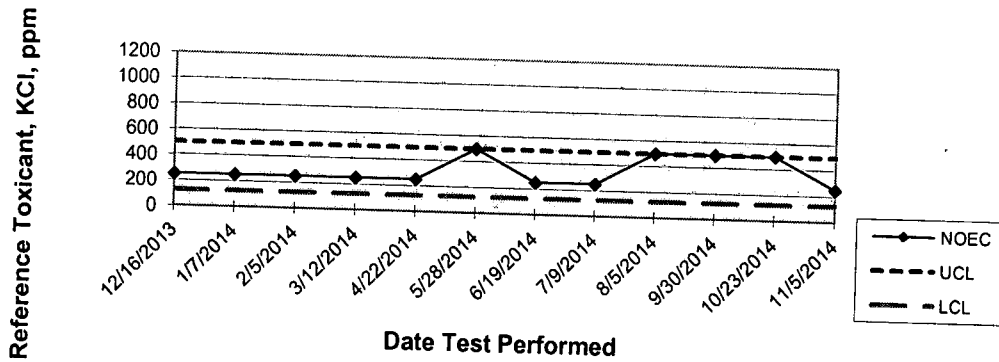
ARKANSAS ANALYTICAL, INC.

FATHEAD MINNOW GROWTH 7 Day QUALITY ASSURANCE

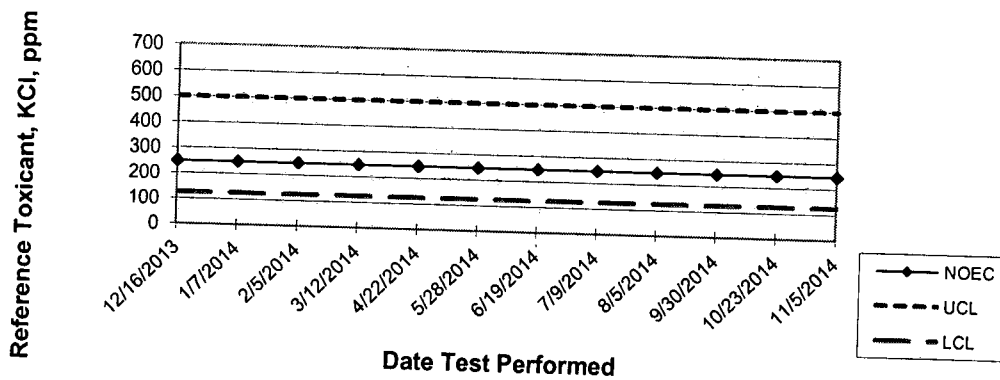
Reference Toxicant, KCl, ppm



ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE




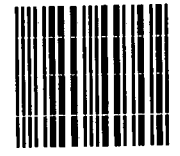
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No Little Rock, AR 72118-5317

