

Arkansas Analytical, Inc.

Toxicity Test Results

CITY of SHERIDAN
NPDES PERMIT NUMBER: AR0034347
Fourth Quarter 2013
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**
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Prepared by: Arkansas Analytical, Inc.
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Lab Number K1311008

Tuesday, December 10, 2013

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 ¼ of the Northwest ¼ 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 2013.

Plant Operations

To be provided by permittee.

Source of Effluent and Dilution Water

Effluent sample was a composite and collected as follows:

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	11-24-13, 0900	11-25-13, 0800

* There were no second or third samples collected. Discharge flow was stopped due to low creek 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:	11-25-13, 1525	1

* There were no second or third samples collected. Discharge flow was stopped due to low creek flow.

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 < 48 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	0.394	X	
The percent coefficient of variation between replicates must be 40% or less for growth	9.92%	X	

TEST ACCEPTANCE CRITERIA for *Ceriodaphnia dubia*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	80%	X	
Average of 15 or more young per surviving female	17.1	X	
At least 60% of surviving females should have produced 3 broods	100%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	15.1%	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> 11/19/13 – 11/26/13		<i>Pimephales promelas</i> 11/19/13 – 11/26/13	
NOEC Survival:	250 ppm KCl	NOEC Survival:	500 ppm KCl
LOEC Survival:	500ppm 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>Pimephales promelas</i>	
NOEC / LOEC survival	19% / NA
NOEC / LOEC growth	19% / NA
%CV survival (critical dilution)	97.5%
Mean dry weight (critical dilution) in milligrams	0.440
%CV growth (critical dilution)	10.48%
PMSD Growth	14.5%
<i>Ceriodaphnia dubia</i>	
NOEC / LOEC survival	19% /N/A
NOEC / LOEC reproduction	19% /N/A
Mean number of neonates (critical dilution)	14.0
%CV Reproduction (critical dilution)	15.6%
PMSD Reproduction	40.4%

Conclusion

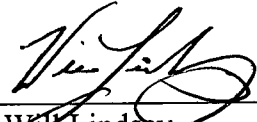
Chronic static renewal larval survival and growth test using fathead minnow, *Pimephales promelas*, (Method 1000.0)

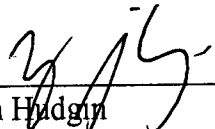
The permit issued to the City of Sheridan, AR0034347, specifies that the **critical dilution is 14% effluent**. The effluent samples **did not** exhibit lethal or sublethal effects at the critical dilution, and, as such, **passed** the test.

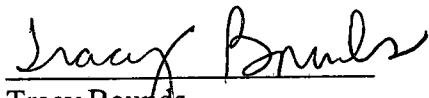
Chronic static renewal survival and reproduction test using *Ceriodaphnia dubia*, (Method 1002.0)

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:


 Will Lindsey


 Ryan Hudgin


 Tracy Bounds

**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:	11-24-13, 0900	11-25-13, 0800

* There were no second or third samples collected. Discharge flow was stopped due to low creek flow.

Test initiated (date, time): 11-26-13, 1430 Test terminated (date, time): 12-3-13, 1400

Dilution water used: Moderately Hard Synthetic

DATA TABLE FOR FATHEAD MINNOW SURVIVAL

Effluent Conc %	Percent Survival in Replicate Chambers						Mean Percent Survival			
	A	B	C	D	E		24 hours	48 hours	7 days	CV%
0%	100	100	100	87.5	100		100	100	97.5	5.73%
6.0%	87.5	100	100	100	100		100	100	97.5	
8.0%	87.5	75	100	87.5	87.5		100	100	87.5	
11.0%	100	100	75	100	100		97.5	97.5	95	
14.0%	87.5	100	100	100	100		100	100	97.5	5.73%
19.0%	100	100	100	100	100		100	100	100	

DATA TABLE FOR GROWTH OF FATHEAD MINNOWS

Average Dry Weight in milligrams in replicate chambers

Effluent Conc %	A	B	C	D	E		Mean Dry Weight	CV%
0%	0.442	0.385	0.394	0.412	0.336		0.394	9.92%
6.0%	0.390	0.410	0.454	0.440	0.409		0.421	
8.0%	0.384	0.336	0.417	0.347	0.401		0.377	
11.0%	0.437	0.451	0.416	0.366	0.493		0.433	
14.0%	0.413	0.398	0.425	0.450	0.515		0.440	10.48%
19.0%	0.416	0.361	0.456	0.403	0.406		0.408	

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) = 10.48 %

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:	11-24-13, 0900	11-25-13, 0800

* There were no second or third samples collected. Discharge flow was stopped due to low creek flow.

Test initiated (date, time): 11-26-13, 1515 Test terminated (date, time): 12-3-13, 0950

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	20	17	16	15	20	16
B	19	11	17	18	11	14
C	X0	X0	19	21	20	15
D	15	19	12	17	18	14
E	18	13	12	11	13	19
F	12	13	15	18	12	14
G	X0	14	8	17	16	10
H	17	11	11	17	13	15
I	17	17	13	X0	17	10
J	19	14	11	13	X0	15
Mean	13.7	12.9	13.4	14.7	14.0	14.2
Mean/surviving female	17.1	14.3	13.4	16.3	15.6	14.2
CV%*	15.1				22.1	

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	80	90	100	90	90	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, (14.0%): 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, (14.0%): 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)= 22.1 %

APPENDIX A

Chain of Custody Forms

APPENDIX B

Effluent and Dilution Water Data

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Fathead Minnow

Lab # / Sample ID **K1311008**

Test Start (Date/Time) **11-26-13 1430**

Client: **Sheridan**

Test End (Date/Time) **12-3-13 1400**

		Day of Test							notes/remarks
		1	2	3	4	5	6	7	
Control	MHS551	11-26	11-27	11-28	11-29	11-30	12-1	12-2	
D.O. (mg/L)	INITIAL	9.1	8.8	8.9	8.6	8.4	8.7	8.6	MHS # 709
	FINAL	7.8	8.1 8.1	8.1	8.1	8.3	7.9	8.1	MHS # 710
pH (s.u.)	INITIAL	7.7	7.5	8.2	8.0	7.9	8.2	8.1	
	FINAL	7.2	8.1	8.0	8.0	8.1	8.0	7.6	
temp (C)	INITIAL	22	22	21	22.3	22	22	22	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		64				62			
HARDNESS (mg/L)		86				80			
CONDUCTIVITY (umhos/cm)		287				261			
CHLORINE (mg/L)		<0.05							
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.6	8.9	8.9	8.8	8.7	8.5	
	FINAL	7.7	8.28 8.0	8.0	8.08 8.3	7.9	8.0	8.0	
pH (s.u.)	INITIAL	7.4	7.5	8.1	8.0	8.0	8.2	7.9	
	FINAL	7.1	8.2	7.9	7.98 8.2	8.2	8.2	7.5	
temp (C)	INITIAL	22	21	21	22.5	23	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.6	8.7	8.8	8.8	8.8	8.6	
	FINAL	7.8	8.28 8.0	8.0	8.08 8.4	7.8	8.1	8.1	
pH (mg/L)	INITIAL	8.3	7.9	8.2	8.1	7.9	8.3	8.1	
	FINAL	7.1	8.2	7.9	7.98 8.2	7.9	7.8	7.8	
temp (C)	INITIAL	21	22	22	22.7	23	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.2	8.6	8.7	8.7	8.7	8.8	8.7	
	FINAL	7.9	8.28 8.0	8.0	8.08 8.4	7.9	8.0	8.0	
pH (s.u.)	INITIAL	8.2	8.0	8.1	8.0	7.9	8.3	8.2	
	FINAL	7.2	8.2	7.9	7.98 8.2	7.8	7.8	7.3	
temp (C)	INITIAL	21	22	22	23.1	23	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.2	8.6	8.7	8.7	8.7	8.8	8.6	
	FINAL	7.7	8.28 8.0	8.0	8.08 8.4	8.0	8.1	8.1	
pH (s.u.)	INITIAL	8.2	8.1	8.0	8.0	7.8	8.2	8.1	
	FINAL	7.2	8.1	8.0	8.08 8.2	7.8	7.8	7.6	
temp (C)	INITIAL	21	22	22	23.4	23	22	23	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.3	8.9	8.7	8.7	8.7	8.9	8.8	
	FINAL	7.9	8.28 8.0	8.0	8.08 8.4	7.9	7.9	7.9	
pH (s.u.)	INITIAL	8.1	8.1	8.1	8.1	7.9	8.2	8.3	
	FINAL	7.1	8.2	8.1	8.18 8.3	8.3	8.0	8.5	
temp (C)	INITIAL	22	21	22	23.8	23	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:									
100%		A	A	A	A	A	A	A	
ALKALINITY (mg/L)		30							
HARDNESS (mg/L)		92							
CONDUCTIVITY (umhos/cm)		477							
CHLORINE (mg/L)		0.08							

MHS

6

8

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CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING										Cerodaphnia Dubia
Lab # / Sample ID K13 11008					Test Start (Date/Time) 11-26-13 1515					
Client: Sheridan					Test End (Date/Time) 12-3-13 0950					
		Day of Test								
		1	2	3	4	5	6	7	notes/remarks	
Control	MHS551	11-26	11-27	11-28	11-29	11-30	12-1	12-2		
D.O. (mg/L)	INITIAL	9.1	8.8	8.9	8.6	8.4	8.7	8.6	MHS # 709	
	FINAL	8.4	8.4	8.2	8.1	8.0	7.9	7.8	MHS # 710	
pH (s.u.)	INITIAL	7.7	7.5	7.2	8.0	7.9	8.2	8.1		
	FINAL	7.2	8.1	8.0	8.1	8.0	7.5	7.8		
temp (C)	INITIAL	22	22	21	22.3	22	22	22		
	FINAL	25	25	25	25	25	25	25		
ALKALINITY (mg/L)		64				62				
HARDNESS (mg/L)		86				80				
CONDUCTIVITY (umhos/cm)		287				261				
CHLORINE (mg/L)		40.05								
CONC:										
D.O. (mg/L)	INITIAL	8.4	8.6	8.9	8.9	8.8	8.7	8.5		
	FINAL	8.4	8.5	8.4	8.1	8.0	8.1	7.9		
pH (s.u.)	INITIAL	7.4	7.5	8.1	8.0	8.0	8.2	7.9		
	FINAL	7.0	8.2	8.1	8.1	8.0	8.1	8.1		
temp (C)	INITIAL	22	21	21	22.5	23	22	22		
	FINAL	25	25	25	25	25	25	25		
CONC:										
D.O. (mg/L)	INITIAL	8.4	8.6	8.7	8.8	8.8	8.8	8.6		
	FINAL	8.2	8.6	8.5	8.0	8.0	8.0	8.1		
pH (mg/L)	INITIAL	8.3	7.9	8.2	8.1	7.9	8.3	8.1		
	FINAL	7.2	8.3	8.1	7.9	8.0	8.0	8.0		
temp (C)	INITIAL	21	22	22	22.7	23	22	22		
	FINAL	25	25	25	25	25	25	25		
CONC:										
D.O. (mg/L)	INITIAL	8.2	8.6	8.7	8.7	8.7	8.8	8.7		
	FINAL	8.2	8.6	8.6	8.1	8.2	8.2	8.1		
pH (s.u.)	INITIAL	8.2	8.0	8.1	8.0	7.9	8.3	8.2		
	FINAL	7.2	8.3	8.1	8.0	8.2	8.3	8.2		
temp (C)	INITIAL	21	22	22	23.1	23	22	22		
	FINAL	25	25	25	25	25	25	25		
CONC:										
D.O. (mg/L)	INITIAL	8.2	8.6	8.7	8.7	8.7	8.8	8.6		
	FINAL	8.4	8.7	8.6	8.2	8.2	8.3	8.2		
pH (s.u.)	INITIAL	8.2	8.1	8.0	8.0	7.8	8.2	8.1		
	FINAL	7.3	8.3	8.1	7.9	8.1	8.2	8.1		
temp (C)	INITIAL	21	22	22	23.4	23	22	23		
	FINAL	25	25	25	25	25	25	25		
CONC:										
D.O. (mg/L)	INITIAL	8.3	8.9	8.7	8.7	8.7	8.9	8.8		
	FINAL	8.3	8.8	8.8	8.1	8.0	8.0	8.2		
pH (s.u.)	INITIAL	8.1	8.1	8.1	8.1	7.9	8.2	8.3		
	FINAL	7.2	8.3	8.1	8.2	8.1	8.2	8.1		
temp (C)	INITIAL	22	21	22	23.8	23	22	22		
	FINAL	25	25	25	25	25	25	25		
CONC: 100%										
ALKALINITY (mg/L)		30								
HARDNESS (mg/L)		92								
CONDUCTIVITY (umhos/cm)		477								
CHLORINE (mg/L)		0.08								

MHS

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APPENDIX C

Fathead minnow raw data and statistics

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID **K1311008** TEST START DATE **11-26-13** TIME **1430**
 CLIENT **Sheridan** TEST END DATE **12-3-13** TIME **1400**

AGE AND SOURCE OF MINNOWS

DAY (NUMBER SURVIVING)

SURVIVAL

CONC:	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
8	A	8	8	8	8	8	8	8	100%	97.5%	5.73%
	B	I	8	8	8	8	8	8	100%		
	C	I	8	8	8	8	8	8	100%		
	D	I	8	8	8	8	8	7	87.5%		
	E	I	8	8	8	8	8	8	100%		
6	A	8	8	8	8	8	8	7	87.5%	97.5%	
	B	I	8	8	8	8	8	8	100%		
	C	I	8	8	8	8	8	8	100%		
	D	I	8	8	8	8	8	8	100%		
	E	I	8	8	8	8	8	8	100%		
11	A	8	8	8	7	7	7	7	87.5%	87.5%	
	B	I	8	8	7	6	6	6	75%		
	C	I	8	8	8	8	8	8	100%		
	D	I	8	8	8	8	7	7	87.5%		
	E	I	8	8	8	8	8	7	87.5%		
14	A	8	8	8	8	8	8	8	100%	97.5%	5.73%
	B	I	8	8	8	8	8	8	100%		
	C	I	8	8	8	8	8	8	100%		
	D	I	8	8	8	8	8	8	100%		
	E	I	8	8	8	8	8	8	100%		
19	A	8	8	8	8	8	8	8	100%	100%	0%
	B	I	8	8	8	8	8	8	100%		
	C	I	8	8	8	8	8	8	100%		
	D	I	8	8	8	8	8	8	100%		
	E	I	8	8	8	8	8	8	100%		
ANALYST:											
DATE:											
TIME:											

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID K1311008 TEST START DATE 11-26-13 TIME 1430
 CLIENT Sheridan TEST END DATE 12-3-13 TIME 1400
 AGE AND SOURCE OF MINNOWS

		DAY (NUMBER SURVIVING)							SURVIVAL		
CONC:	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
mlfS	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1	1		
6	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	2	2		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	2	2		
	E	1	1	1	1	1	1	1	1		
8	A	2	2	2	1	1	1	1	1		
	B	1	1	1	2	2	2	2	2		
	C	1	1	1	1	2	2	2	2		
	D	1	1	1	1	2	2	2	2		
	E	1	1	1	1	1	1	1	1		
11	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1	1		
14	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	2	2	2	2		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	2	2	2	2		
	E	1	1	1	1	1	1	1	1		
19	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1	1		
ANALYST		RIF	RH	K.R.	TC	tb	tb	RH	RH		
DATE:		11-26-13	11-27-13	11-28-13	11-29-13	11-30-13	12-1-13	12-2-13	12-3-13		
TIME:		1430	1430	1400	1255	1320	1310	1315	1400		

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		TEST START		DATE		TIME					
CLIENT		TEST END		DATE		TIME					
AGE AND SOURCE OF MINNOWS											
DAY (NUMBER SURVIVING)								SURVIVAL			
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
5	A	2	2	2	2	2	2	2	2		
	B										
	C										
	D										
	E										
6	A	2	2	2	2	2	2	2	2		
	B										
	C										
	D										
	E										
8	A	2	2	2	1	1	1	1	1		
	B				2	1	1	1	1		
	C					2	2	2	2		
	D					2	2	2	2		
	E										
11	A	2	2	2	2	2	2	2	2		
	B										
	C										
	D										
	E										
14	A	2	2	2	2	2	2	2	2		
	B										
	C										
	D										
	E										
19	A	2	2	2	2	2	2	2	2		
	B										
	C										
	D										
	E										
ANALYST											
DATE:											
TIME:											

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		TEST START		DATE		TIME					
CLIENT		TEST END		DATE		TIME					
AGE AND SOURCE OF MINNOWS											
DAY (NUMBER SURVIVING)								SURVIVAL			
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
mts	A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓	↓		
6	A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓	↓		
8	A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓	↓		
11	A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	2	1	1	1		
	C	↓	↓	↓	↓	2	2	2	2		
	D	↓	↓	↓	↓	1	1	1	1		
	E	↓	↓	↓	↓	↓	↓	↓	↓		
14	A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓	↓		
19	A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓	↓		
ANALYST											
DATE:											
TIME:											

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		TEST START		DATE		TIME					
CLIENT		TEST END		DATE		TIME					
AGE AND SOURCE OF MINNOWS											
DAY (NUMBER SURVIVING)								SURVIVAL			
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
MHS	A	2	2	2	2	2	2	1	1		
	B							2	2		
	C							2	2		
	D							2	2		
	E										
6	A	2	2	2	2	2	2	2	2		
	B										
	C										
	D										
	E										
8	A	2	2	2	2	2	2	2	2		
	B							2	2		
	C							1	1		
	D							2	2		
	E										
11	A	2	2	2	2	2	2	2	2		
	B										
	C										
	D										
	E										
14	A	2	2	2	2	2	2	2	2		
	B										
	C										
	D										
	E										
19	A	2	2	2	2	2	2	2	2		
	B										
	C										
	D										
	E										
ANALYST											
DATE:											
TIME:											

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID K1311008 TEST START DATE _____ TIME _____
 TEST END DATE _____ TIME _____
 CLIENT Sheridan AGE AND SOURCE OF MINNOWS _____
 DAY (NUMBER SURVIVING)

		start	1	2	3	4	5	6	7%	MEAN %	CV
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
	A	2	2	2	2	2	2	2			
	B										
	C										
	D										
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
	A	2	0	2	2	2	2	2			
	B										
	C										
	D										
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
	A	2	2	2	2	2	2	2			
	B										
	C										
	D										
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
	A	2	2	2	2	2	2	2			
	B										
	C										
	D										
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
	A	2	2	2	2	2	2	2			
	B										
	C										
	D										
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
	A	2	2	2	2	2	2	2			
	B										
	C										
	D										
ANALYST											
DATE:											
TIME:											

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

AA# K1311008, FATHEAD MINNOW SURVIVAL, CHRONIC, 11-26-13

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

Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.237

W = 0.797

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# K1311008, FATHEAD MINNOW SURVIVAL, CHRONIC, 11-26-13

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

Transform: ARC SINE(SQUARE ROOT(Y))

Hartley's test for homogeneity of variance

Bartlett's test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.

Additional transformations are useless.

TITLE: AA# K1311008, FATHEAD MINNOW SURVIVAL, CHRONIC, 11-26-13
 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	0.8750	1.2094
2	6 % EFFLUENT	2	1.0000	1.3931
2	6 % EFFLUENT	3	1.0000	1.3931
2	6 % EFFLUENT	4	1.0000	1.3931
2	6 % EFFLUENT	5	1.0000	1.3931
3	8 % EFFLUENT	1	0.8750	1.2094
3	8 % EFFLUENT	2	0.7500	1.0472
3	8 % EFFLUENT	3	1.0000	1.3931
3	8 % EFFLUENT	4	0.8750	1.2094
3	8 % EFFLUENT	5	0.8750	1.2094
4	11 % EFFLUENT	1	1.0000	1.3931
4	11 % EFFLUENT	2	1.0000	1.3931
4	11 % EFFLUENT	3	0.7500	1.0472
4	11 % EFFLUENT	4	1.0000	1.3931
4	11 % EFFLUENT	5	1.0000	1.3931
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	1.0000	1.3931
6	19 % EFFLUENT	4	1.0000	1.3931
6	19 % EFFLUENT	5	1.0000	1.3931

AA# K1311008, FATHEAD MINNOW SURVIVAL, CHRONIC, 11-26-13
 File: C:\COPYTO~1\TOXSTAT\FHSURV~1. Transform: ARC SINE(SQUARE ROOT(Y))

STEEL'S MANY-ONE RANK TEST - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	1.356				
2	6 % EFFLUENT	1.356	27.50	16.00	5.00	
3	8 % EFFLUENT	1.214	19.50	16.00	5.00	
4	11 % EFFLUENT	1.324	27.00	16.00	5.00	
5	14 % EFFLUENT	1.356	27.50	16.00	5.00	
6	19 % EFFLUENT	1.393	30.00	16.00	5.00	

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

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:		K1311008				TEST DATES (BEGIN / END):		11/26/13 - 12/3/13	
CLIENT:		Sheridan				WEIGHING DATE / TIME:		12/4/2013 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	1.01830	1.01476	0.00354	8	0.442	AVG DRY		
	B	1.01993	1.01685	0.00308	8	0.385	WEIGHT (mg)		
	C	1.03943	1.03628	0.00315	8	0.394			0.394
	D	1.01778	1.01448	0.00330	8	0.412	CV		
	E	1.02754	1.02485	0.00269	8	0.336			9.92
CONC: 6%	A	1.02194	1.01882	0.00312	8	0.390	AVG DRY		
	B	1.03048	1.02720	0.00328	8	0.410	WEIGHT (mg)		
	C	0.99230	0.98867	0.00363	8	0.454			0.420
	D	1.03132	1.02780	0.00352	8	0.440	CV		
	E	1.01874	1.01547	0.00327	8	0.409			
CONC: 8%	A	1.04857	1.04550	0.00307	8	0.384	AVG DRY		
	B	1.01747	1.01478	0.00269	8	0.336	WEIGHT (mg)		
	C	1.02158	1.01824	0.00334	8	0.417			0.377
	D	1.00744	1.00466	0.00278	8	0.347	CV		
	E	1.01729	1.01408	0.00321	8	0.401			
CONC: 11%	A	1.02053	1.01703	0.00350	8	0.437	AVG DRY		
	B	1.04429	1.04068	0.00361	8	0.451	WEIGHT (mg)		
	C	1.02471	1.02138	0.00333	8	0.416			0.433
	D	1.02106	1.01813	0.00293	8	0.366	CV		
	E	1.01594	1.01200	0.00394	8	0.493			
CONC: 14%	A	1.03384	1.03054	0.00330	8	0.413	AVG DRY		
	B	1.01706	1.01388	0.00318	8	0.398	WEIGHT (mg)		
	C	0.99470	0.99130	0.00340	8	0.425			0.440
	D	1.00566	1.00206	0.00360	8	0.450	CV		
	E	1.00952	1.00540	0.00412	8	0.515			10.48
CONC: 19%	A	1.00188	0.99855	0.00333	8	0.416	AVG DRY		
	B	1.00085	0.99796	0.00289	8	0.361	WEIGHT (mg)		
	C	0.99683	0.99318	0.00365	8	0.456			0.409
	D	1.01355	1.01033	0.00322	8	0.403	CV		
	E	0.98003	0.97678	0.00325	8	0.406			7.7

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA# K1311008, FATHEAD MINNOW GROWTH CHRONIC, 11-26-13

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

Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.035

W = 0.987

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# K1311008, FATHEAD MINNOW GROWTH CHRONIC, 11-26-13

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

Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 1.65

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# K1311008, FATHEAD MINNOW GROWTH CHRONIC, 11-26-13
 FILE: C:\COPYTO~1\TOXSTAT\FHGROWTH.
 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.4420	0.4420
1	CONTROL	2	0.3850	0.3850
1	CONTROL	3	0.3940	0.3940
1	CONTROL	4	0.4120	0.4120
1	CONTROL	5	0.3360	0.3360
2	6 % EFFLUENT	1	0.3900	0.3900
2	6 % EFFLUENT	2	0.4100	0.4100
2	6 % EFFLUENT	3	0.4540	0.4540
2	6 % EFFLUENT	4	0.4400	0.4400
2	6 % EFFLUENT	5	0.4090	0.4090
3	8 % EFFLUENT	1	0.3840	0.3840
3	8 % EFFLUENT	2	0.3360	0.3360
3	8 % EFFLUENT	3	0.4170	0.4170
3	8 % EFFLUENT	4	0.3470	0.3470
3	8 % EFFLUENT	5	0.4010	0.4010
4	11 % EFFLUENT	1	0.4370	0.4370
4	11 % EFFLUENT	2	0.4510	0.4510
4	11 % EFFLUENT	3	0.4160	0.4160
4	11 % EFFLUENT	4	0.3660	0.3660
4	11 % EFFLUENT	5	0.4930	0.4930
5	14 % EFFLUENT	1	0.4130	0.4130
5	14 % EFFLUENT	2	0.3980	0.3980
5	14 % EFFLUENT	3	0.4250	0.4250
5	14 % EFFLUENT	4	0.4500	0.4500
5	14 % EFFLUENT	5	0.5150	0.5150
6	19 % EFFLUENT	1	0.4160	0.4160
6	19 % EFFLUENT	2	0.3610	0.3610
6	19 % EFFLUENT	3	0.4560	0.4560
6	19 % EFFLUENT	4	0.4030	0.4030
6	19 % EFFLUENT	5	0.4060	0.4060

AA# K1311008, FATHEAD MINNOW GROWTH CHRONIC, 11-26-13
 File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.014	0.003	1.945
Within (Error)	24	0.035	0.001	
Total	29	0.050		

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

AA# K1311008, FATHEAD MINNOW GROWTH CHRONIC, 11-26-13

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	0.394	0.394		
2	6 % EFFLUENT	0.421	0.421	-1.105	
3	8 % EFFLUENT	0.377	0.377	0.692	
4	11 % EFFLUENT	0.433	0.433	-1.599	
5	14 % EFFLUENT	0.440	0.440	-1.912	
6	19 % EFFLUENT	0.408	0.408	-0.602	

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

AA# K1311008, FATHEAD MINNOW GROWTH CHRONIC, 11-26-13

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	0.057	14.5	-0.027
3	8 % EFFLUENT	5	0.057	14.5	0.017
4	11 % EFFLUENT	5	0.057	14.5	-0.039
5	14 % EFFLUENT	5	0.057	14.5	-0.046
6	19 % EFFLUENT	5	0.057	14.5	-0.015

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

Ceriodaphnia dubia

SURVIVAL AND REPRODUCTION TEST

Discharger: Sweden Lab Number/s: K1311008
Location: See Ed
Date Sample Collected: See Ed

Analyst: KH
Test Start - Date/Time: 11-26-13 1515
Test Stop - Date/Time: 12-3-13 0950

Conc 1		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	0	0	0	0	0	0	X	0	0	0	0	7	0	
	4	4	5	X	3	2	1		3	2	4	24	8	3	
	5	7	3		0	4	6		1	3	5	31	8	3.9	
	6	0	3		7	5	5		6	4	5	35	8	4.4	
	7	9	8		5	7	0		7	6	5	47	8	5.9	
	8														$\bar{x} = 17.1$
	Total	20	19	X0	15	18	12	X0	17	17	19	137			$CV = 15.1$

3/5

Conc 2		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	0	0	0	0	0	0	0	0	0	0	0	10	0	
	4	4	3	X	4	2	4	2	2	4	3	29	10	2.9	
	5	1	2		5	5	6	0	5	3	4	31	9	3.4	
	6	7	2		3	6	0	6	0	5	7	36	9	4	
	7	5	4		7	0	3	6	4	5	0	34	9	3.8	
	8														$\bar{x} = 14.3$
	Total	17	17	X0	19	13	13	14	11	17	14	129			$CV = 19.4$

6

Conc 3		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	0	0	0	0	0	0	0	0	0	0	0	10	0	
	4	3	4	3	3	5	1	3	2	3	0	27	10	2.7	
	5	3	0	5	6	2	3	5	0	6	6	36	10	3.6	
	6	4	4	8	1	5	5	0	1	2	2	37	10	3.7	
	7	1	9	3	2	0	6	0	8	2	3	34	10	3.4	
	8														$\bar{x} = 13.4$
	Total	16	17	19	17	17	15	8	11	13	11	134			$CV = 24.7$

8

X = DEAD; Y = MALE

Conc 4		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	0	0	0	0	0	0	0	0	0	X	0	9	0	
	4	6	4	3	3	3	2	3	3	4		28	9	3.1	
	5	4	2	5	6	6	4	7	2		3	33	9	3.7	
	6	5	6	8	5	4	6	0	4		5	43	9	4.8	
	7	0	6	5	3	4	6	7	7		5	43	9	4.8	
	8														$\bar{x} = 16.3$
	Total	15	18	21	17	11	18	17	17	X0	13	147			$CV = 18.1$

11

Conc 5		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	0	0	0	0	0	0	0	0	0	0	0	10	0	
	4	4	4	3	3	2	0	2	2	3	X	23	9	2.6	
	5	0	5	4	6	5	4	5	3	2		34	9	3.8	
	6	9	2	7	0	6	4	2	8	7		45	9	5	
	7	7	6	6	9	0	4	7	0	5		38	9	4.2	
	8														$\bar{x} = 15.6$
	Total	20	11	26	18	13	12	16	13	17		190			$CV = 22.1$

14

Conc 6		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	0	0	0	0	0	0	0	0	0	0	0	10	0	
	4	4	4	1	2	5	1	2	3	3	4	29	10	2.9	
	5	6	3	6	5	3	0	5	4	0	4	36	10	3.6	
	6	4	3	3	0	5	5	3	6	3	2	33	10	3.3	
	7	2	3	5	7	6	8	0	2	4	5	44	10	4.4	
	8														$\bar{x} = 14.2$
	Total	16	14	15	14	19	19	10	15	10	15	142			$CV = 18.7$

19

RH

12-4

AA # K1311008, C. DUBIA CHRONIC, REPRODUCCION, 11-26-13
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 # K1311008, C. DUBIA CHRONIC, REPRODUCCION, 11-26-13
File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 11.54

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		
	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	2	8	10
6	1	9	10
TOTAL	3	17	20

CRITICAL FISHER'S VALUE (10,10,2) ($p=0.05$) IS LESS THAN 0. b VALUE IS 1.
NO SIGNIFICANT DIFFERENCE

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	2	8	10
8	0	10	10
TOTAL	2	18	20

CRITICAL FISHER'S VALUE (10,10,2) ($p=0.05$) IS LESS THAN 0. b VALUE IS 0.
NO SIGNIFICANT DIFFERENCE

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	2	8	10
11	1	9	10
TOTAL	3	17	20

CRITICAL FISHER'S VALUE (10,10,2) (p=0.05) IS LESS THAN 0. b VALUE IS 1.
 NO SIGNIFICANT DIFFERENCE

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	2	8	10
14	1	9	10
TOTAL	3	17	20

CRITICAL FISHER'S VALUE (10,10,2) (p=0.05) IS LESS THAN 0. b VALUE IS 1.
 NO SIGNIFICANT DIFFERENCE

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	2	8	10
19	0	10	10
TOTAL	2	18	20

CRITICAL FISHER'S VALUE (10,10,2) (p=0.05) IS LESS THAN 0. b VALUE IS 0.
 NO SIGNIFICANT DIFFERENCE

SUMMARY OF FISHER'S EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	10	2	
1	6	10	1	
2	8	10	0	

3	11	10	1
4	14	10	1
5	19	10	0

TITLE: AA # K1311008, C. DUBIA CHRONIC, REPRODUCCION, 11-26-13
 FILE: C:\COPYTO~1\TOXSTAT\C.DUB
 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	20.0000	20.0000
1	CONTROL	2	19.0000	19.0000
1	CONTROL	3	0.0000	0.0000
1	CONTROL	4	15.0000	15.0000
1	CONTROL	5	18.0000	18.0000
1	CONTROL	6	12.0000	12.0000
1	CONTROL	7	0.0000	0.0000
1	CONTROL	8	17.0000	17.0000
1	CONTROL	9	17.0000	17.0000
1	CONTROL	10	19.0000	19.0000
2	6 % EFFLUENT	1	17.0000	17.0000
2	6 % EFFLUENT	2	11.0000	11.0000
2	6 % EFFLUENT	3	0.0000	0.0000
2	6 % EFFLUENT	4	19.0000	19.0000
2	6 % EFFLUENT	5	13.0000	13.0000
2	6 % EFFLUENT	6	13.0000	13.0000
2	6 % EFFLUENT	7	14.0000	14.0000
2	6 % EFFLUENT	8	11.0000	11.0000
2	6 % EFFLUENT	9	17.0000	17.0000
2	6 % EFFLUENT	10	14.0000	14.0000
3	8 % EFFLUENT	1	16.0000	16.0000
3	8 % EFFLUENT	2	17.0000	17.0000
3	8 % EFFLUENT	3	19.0000	19.0000
3	8 % EFFLUENT	4	12.0000	12.0000
3	8 % EFFLUENT	5	12.0000	12.0000
3	8 % EFFLUENT	6	15.0000	15.0000
3	8 % EFFLUENT	7	8.0000	8.0000
3	8 % EFFLUENT	8	11.0000	11.0000
3	8 % EFFLUENT	9	13.0000	13.0000
3	8 % EFFLUENT	10	11.0000	11.0000
4	11 % EFFLUENT	1	15.0000	15.0000
4	11 % EFFLUENT	2	18.0000	18.0000
4	11 % EFFLUENT	3	21.0000	21.0000
4	11 % EFFLUENT	4	17.0000	17.0000
4	11 % EFFLUENT	5	11.0000	11.0000
4	11 % EFFLUENT	6	18.0000	18.0000
4	11 % EFFLUENT	7	17.0000	17.0000
4	11 % EFFLUENT	8	17.0000	17.0000
4	11 % EFFLUENT	9	0.0000	0.0000
4	11 % EFFLUENT	10	13.0000	13.0000
5	14 % EFFLUENT	1	20.0000	20.0000
5	14 % EFFLUENT	2	11.0000	11.0000
5	14 % EFFLUENT	3	20.0000	20.0000

5	14 % EFFLUENT	4	18.0000	18.0000
5	14 % EFFLUENT	5	13.0000	13.0000
5	14 % EFFLUENT	6	12.0000	12.0000
5	14 % EFFLUENT	7	16.0000	16.0000
5	14 % EFFLUENT	8	13.0000	13.0000
5	14 % EFFLUENT	9	17.0000	17.0000
5	14 % EFFLUENT	10	0.0000	0.0000
6	19 % EFFLUENT	1	16.0000	16.0000
6	19 % EFFLUENT	2	14.0000	14.0000
6	19 % EFFLUENT	3	15.0000	15.0000
6	19 % EFFLUENT	4	14.0000	14.0000
6	19 % EFFLUENT	5	19.0000	19.0000
6	19 % EFFLUENT	6	14.0000	14.0000
6	19 % EFFLUENT	7	10.0000	10.0000
6	19 % EFFLUENT	8	15.0000	15.0000
6	19 % EFFLUENT	9	10.0000	10.0000
6	19 % EFFLUENT	10	15.0000	15.0000

AA # K1311008, C. DUBIA CHRONIC, REPRODUCCION, 11-26-13
 File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	19.883	3.977	0.139
Within (Error)	54	1547.100	28.650	
Total	59	1566.983		

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

AA # K1311008, C. DUBIA CHRONIC, REPRODUCCION, 11-26-13
 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	13.700	13.700		
2	6 % EFFLUENT	12.900	12.900	0.334	
3	8 % EFFLUENT	13.400	13.400	0.125	
4	11 % EFFLUENT	14.700	14.700	-0.418	
5	14 % EFFLUENT	14.000	14.000	-0.125	
6	19 % EFFLUENT	14.200	14.200	-0.209	

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

AA # K1311008, C. DUBIA CHRONIC, REPRODUCCION, 11-26-13

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	5.530	40.4	0.800
3	8 % EFFLUENT	10	5.530	40.4	0.300
4	11 % EFFLUENT	10	5.530	40.4	-1.000
5	14 % EFFLUENT	10	5.530	40.4	-0.300
6	19 % EFFLUENT	10	5.530	40.4	-0.500

APPENDIX E

Organism History

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

TEST ORGANISM HISTORY

DATE SHIPPED 11-26-13 CLIENT Arkansas Analytical

Purchase Order #: _____

SPECIES: Pimephales promelas

Quantity Shipped: 240⁺

Age: hatched = 11/26 1500 hrs 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

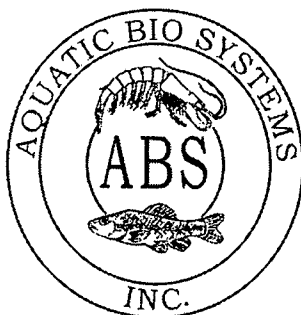
Feeding: ARTIFICIAL

Comments: _____

Shipped Via: Federal Express UPS Overnight Shuttle

Packaged By: Ull

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: 7/23/2013

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>24°C</u>	<u>22-26°C</u>
SALINITY/CONDUCTIVITY:	<u>--</u>	<u>--</u>
TOTAL HARDNESS (as CaCO ₃):	<u>118 mg/l</u>	<u>76-120 mg/l</u>
TOTAL ALKALINITY (as CaCO ₃):	<u>85 mg/l</u>	<u>65-105 mg/l</u>
pH:	<u>7.97</u>	<u>7.40-8.20</u>

Comments:

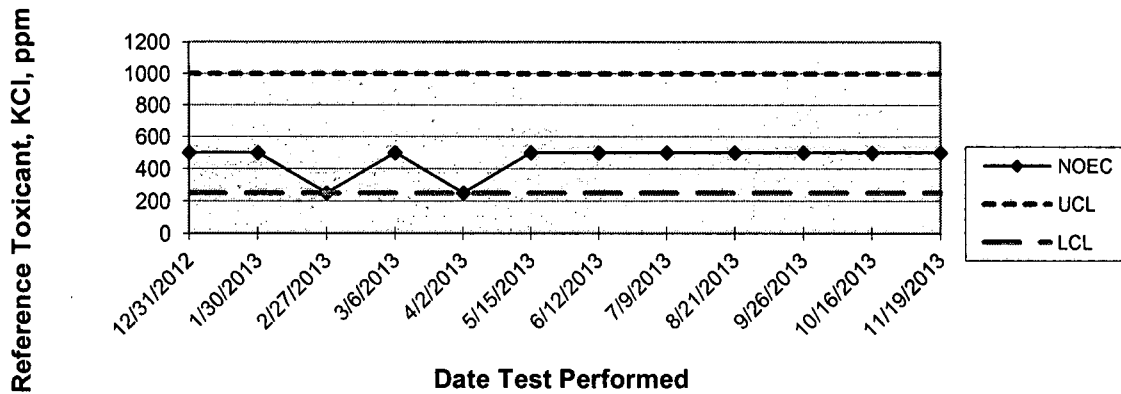


Facility Supervisor

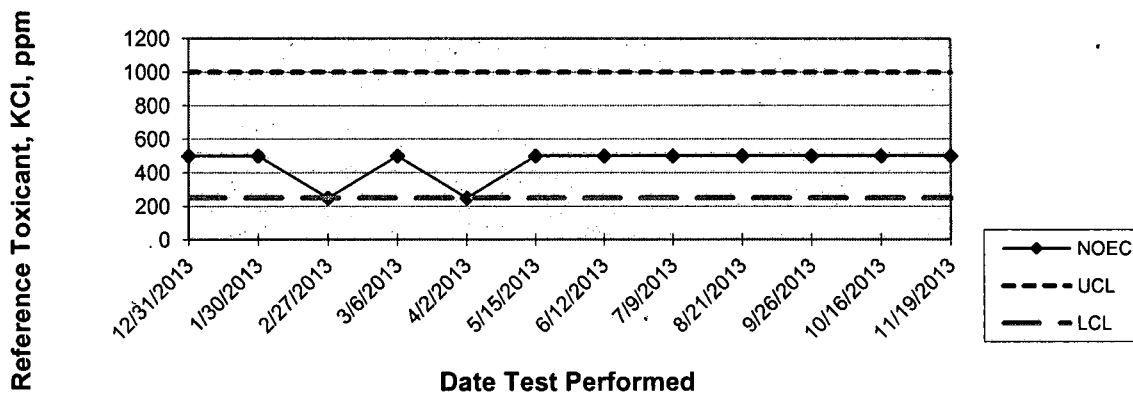
APPENDIX F

Quality Assurance Charts

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

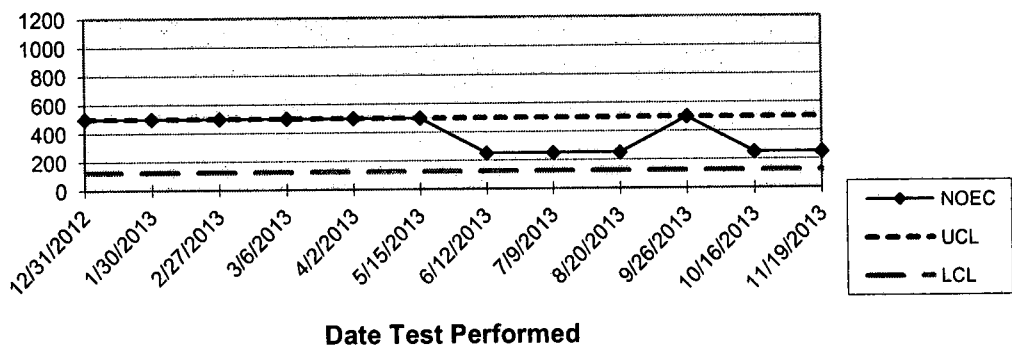


ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW GROWTH 7 Day
QUALITY ASSURANCE



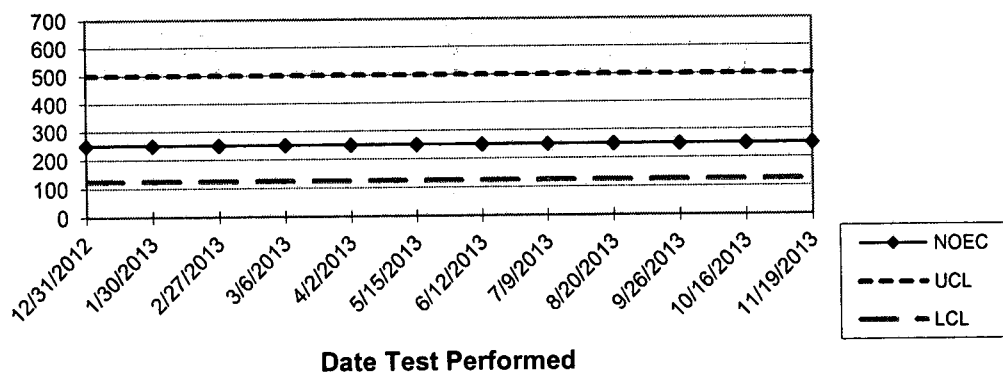
ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE

Reference Toxicant, KCl, ppm



ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE

Reference Toxicant, KCl, ppm



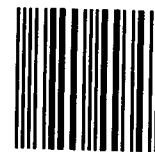
Sheridan Water Works
PO Box 486
Sheridan, AR 72150-0486

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OF THE RETURN ADDRESS, FOLD AT DOTTED LINE

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