

Arkansas Analytical, Inc.

Toxicity Test Results

CITY of SHERIDAN
NPDES PERMIT NUMBER: AR0034347
Second Quarter 2012
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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Lab Number K1205003

Wednesday, May 30, 2012

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 both *Ceriodaphnia dubia* and *Pimephales promelas*. The test results in this report represent the testing for the second quarter of 2012.

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:	5-21-12, 1110	5-22-12, 1010
Sample #2	5-22-12, 1017	5-23-12, 0917
Sample #3	5-23-12, 1028	5-24-12, 0928

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

Sample Receiving Information:	Date, Time Sample(s) Received	Storage Temperature (°C)
Sample #1:	5-22-12, 1137	4
Sample #2	5-23-12, 1153	4
Sample #3	5-24-12, 1105	4

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 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	80%	X	
Average of 15 or more young per surviving female	16.3	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	25.0%	X	

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	0.431	X	
The percent coefficient of variation between replicates must be 40% or less for growth	9.47%	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> 4/25/12-5/2/12		<i>Pimephales promelas</i> 4/25/12-5/2/12	
NOEC Survival:	500 ppm 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 F.

Summary of Results

<i>Pimephales promelas</i>	
NOEC / LOEC survival	19% / NA
NOEC / LOEC growth	19% / NA
%CV survival (critical dilution)	5.73%
Mean dry weight (critical dilution) in milligrams	0.381
%CV growth (critical dilution)	10.9%
PMSD Growth	15.5%
<i>Ceriodaphnia dubia</i>	
NOEC / LOEC survival	19% / NA
NOEC / LOEC reproduction	19% / NA
Mean number of neonates (critical dilution)	14.6
%CV Reproduction (critical dilution)	17.1%
PMSD Reproduction	43.7

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 effects or sublethal effects at the critical dilution, and, as such, **passed** both portions of 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 effects or sublethal effects at the critical dilution, and, as such, **passed** both portions of the test.

Biomonitoring Analysts:


Kenneth Pigue


Chris Turney

**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:	5-21-12, 1110	5-22-12, 1010
Sample #2	5-22-12, 1017	5-23-12, 0917
Sample #3	5-23-12, 1028	5-24-12, 0928

Test initiated (date, time): 5-22-12, 1415 Test terminated (date, time): 5-29-12, 1345

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	100	87.5		100	100	97.5	5.73
6.0%	100	87.5	100	100	100		100	100	97.5	
8.0%	100	100	100	100	87.5		100	100	97.5	
11.0%	100	87.5	100	87.5	100		100	100	95	
14.0%	100	87.5	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

Effluent Conc %	Average Dry Weight in milligrams in replicate chambers						Mean Dry Weight	CV%
	A	B	C	D	E			
0%	0.473	0.439	0.452	0.424	0.365		0.431	9.47
6.0%	0.446	0.289	0.411	0.353	0.453		0.390	
8.0%	0.434	0.436	0.379	0.364	0.372		0.397	
11.0%	0.424	0.349	0.348	0.367	0.435		0.385	
14.0%	0.354	0.351	0.411	0.350	0.439		0.381	10.90
19.0%	0.374	0.394	0.341	0.405	0.357		0.374	

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, (36.3%) 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, (36.3%) 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)= _____ 10.9 _____ %

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	90
Test termination	80	90	100	90	80	80

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)= 25.0 %

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 *K1205603*

Test Start (Date/Time) *5/22/12*

Client: *Skudon*

Test End (Date/Time) *5/29/12*

		Day of Test							notes/remarks
		1	2	3	4	5	6	7	
Control	MHS551	5/22	5/23	5/24	5/25/12	5/26	5/27	5/28	
D.O. (mg/L)	INITIAL	8.5	8.5	8.3	8.4	8.5	8.4	8.2	
	FINAL	7.7	7.8	8.1	8.0	7.1	7.8	8.1	
pH (s.u.)	INITIAL	7.8	7.9	8.2	8.3	7.9	8.2	8.0	
	FINAL	7.6	7.8	7.9	7.9	7.8	7.9	7.9	
temp (C)	INITIAL	22.7	22.8	22.8	23.1	23.4	22.6	22.4	
	FINAL	25.0	25.0	25.0	25	23.25	25.0	25.0	
ALKALINITY (mg/L)		56							
HARDNESS (mg/L)		84							
CONDUCTIVITY (umhos/cm)		303							
CHLORINE (mg/L)		<0.05							
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.5	8.3	8.4	8.6	8.5	8.3	
	FINAL	7.7	7.9	8.1	8.1	7.2	7.7	8.0	
pH (s.u.)	INITIAL	8.0	8.1	8.3	8.4	8.4	8.5	8.0	
	FINAL	7.6	7.7	8.0	8.0	7.8	7.8	7.9	
temp (C)	INITIAL	22.4	23.4	22.8	22.6	23.4	23.3	22.5	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.5	8.3	8.4	8.6	8.5	8.5	
	FINAL	7.5	7.9	8.0	8.1	7.2	7.7	8.0	
pH (mg/L)	INITIAL	8.0	8.3	8.4	8.4	8.5	8.6	8.0	
	FINAL	7.7	7.7	8.0	8.0	7.8	7.6	7.9	
temp (C)	INITIAL	22.3	23.3	22.8	22.9	23.4	23.8	22.5	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.5	8.3	8.4	8.6	8.5	8.5	
	FINAL	7.5	7.9	8.1	8.0	7.2	7.6	7.9	
pH (s.u.)	INITIAL	8.1	8.6	8.4	8.5	8.7	8.7	8.1	
	FINAL	7.5	7.7	7.9	8.1	7.8	7.6	7.9	
temp (C)	INITIAL	22.4	23.7	23.0	23.3	23.5	23.6	22.6	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.5	8.4	8.5	8.6	8.5	8.6	
	FINAL	7.5	8.0	8.0	8.0	7.2	7.4	7.9	
pH (s.u.)	INITIAL	8.1	8.6	8.3	8.5	8.7	8.9	8.2	
	FINAL	7.6	7.7	7.9	8.1	7.9	7.4	7.9	
temp (C)	INITIAL	22.4	23.8	22.9	23.1	23.4	23.7	22.9	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.3	8.6	8.4	8.5	8.6	8.6	8.6	
	FINAL	7.5	8.0	8.0	8.0	6.9	7.4	7.9	
pH (s.u.)	INITIAL	8.2	8.8	8.1	8.4	8.8	8.9	8.2	
	FINAL	7.2	7.7	7.8	8.1	7.8	7.4	8.0	
temp (C)	INITIAL	22.4	23.9	22.9	23.2	23.4	23.2	22.3	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:	100%	A	A	A	B	B	C	C	
ALKALINITY (mg/L)		48			52		42		
HARDNESS (mg/L)		48			50		46		
CONDUCTIVITY (umhos/cm)		289			292		292		
CHLORINE (mg/L)		<0.05			<0.05		<0.05		

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING		Cerodaphnia Dubia							
Lab # / Sample ID		K1205003			Test Start (Date/Time) 5/22/12				
Client:		Sheridan			Test End (Date/Time) 5/29/12				
		Day of Test							
		1	2	3	4	5	6	7	notes/remarks
Control	MHS551	5/22	5/23	5/24	5/25	5/26	5/27	5/28	
D.O. (mg/L)	INITIAL	8.5	8.5	8.3	8.4	8.5	8.4	8.2	
	FINAL	8.1	8.0	8.5	8.3	8.2	8.2	8.0	
pH (s.u.)	INITIAL	7.8	7.9	8.2	8.3	7.9	8.2	8.0	
	FINAL	7.8	7.9	8.1	8.2	7.9	8.0	7.9	
temp (C)	INITIAL	22.7	22.8	22.8	23.1	23.4	22.6	22.4	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
ALKALINITY (mg/L)		56							
HARDNESS (mg/L)		84							
CONDUCTIVITY (umhos/cm)		303							
CHLORINE (mg/L)		0.05							
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.5	8.3	8.4	8.6	8.5	8.3	
	FINAL	8.1	8.0	8.4	8.3	8.2	8.2	7.7	
pH (s.u.)	INITIAL	8.0	8.1	8.3	8.4	8.4	8.5	8.0	
	FINAL	7.9	7.8	8.1	8.0	8.0	8.1	7.9	
temp (C)	INITIAL	22.4	23.4	22.8	22.6	23.4	23.3	22.5	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.5	8.3	8.4	8.6	8.5	8.5	
	FINAL	8.1	8.0	8.2	8.3	8.2	8.2	7.6	
pH (mg/L)	INITIAL	8.0	8.3	8.4	8.4	8.5	8.6	8.0	
	FINAL	7.9	7.9	8.1	8.1	8.1	8.1	7.8	
temp (C)	INITIAL	22.3	23.3	22.8	22.9	23.4	23.5	22.5	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.5	8.3	8.4	8.6	8.5	8.5	
	FINAL	8.0	8.0	8.2	8.2	8.2	8.2	7.6	
pH (s.u.)	INITIAL	8.1	8.6	8.4	8.5	8.7	8.7	8.1	
	FINAL	7.9	7.9	8.1	8.1	8.1	8.1	7.8	
temp (C)	INITIAL	22.4	23.7	23.0	23.3	23.5	23.6	22.6	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.5	8.4	8.5	8.6	8.5	8.6	
	FINAL	8.0	8.0	8.2	8.1	8.2	8.2	7.6	
pH (s.u.)	INITIAL	8.1	8.6	8.3	8.5	8.7	8.9	8.2	
	FINAL	7.9	7.7	8.1	8.1	8.1	8.1	7.8	
temp (C)	INITIAL	22.4	23.8	22.9	23.1	23.4	23.9	22.9	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.3	8.6	8.4	8.5	8.6	8.6	8.6	
	FINAL	8.1	8.1	8.3	8.1	8.2	8.2	7.5	
pH (s.u.)	INITIAL	8.2	8.8	8.1	8.4	8.8	8.9	8.2	
	FINAL	7.9	7.7	8.1	8.1	8.1	8.1	7.8	
temp (C)	INITIAL	22.4	23.9	22.9	23.2	23.4	23.2	22.3	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:		100%							
		A	A	A	B	B	C	C	
ALKALINITY (mg/L)		48			52		42		
HARDNESS (mg/L)		48			50		46		
CONDUCTIVITY (umhos/cm)		289			292		292		
CHLORINE (mg/L)		0.05			0.05		0.05		

APPENDIX C

Fathead minnow raw data and statistics

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		TEST START DATE		TIME							
11205003		5/22/12		1415							
CLIENT		TEST END DATE		TIME							
Sheridan		5/29/12		1345							
AGE AND SOURCE OF MINNOWS											
Summary Page											
		DAY (NUMBER SURVIVING)						SURVIVAL			
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
0	A	8	8	8	8	8	8	8	100	97.5	5.73
	B	8	8	8	8	8	8	8	100		
	C	8	8	8	8	8	8	8	100		
	D	8	8	8	7	7	7	7	87.5		
	E	8	8	8	7	7	7	7	87.5		
6	A	8	8	8	8	8	8	8	100	97.5	
	B	8	8	8	7	7	7	7	87.5		
	C	8	8	8	8	8	8	8	100		
	D	8	8	8	8	8	8	8	100		
	E	8	8	8	8	8	8	8	100		
8	A	8	8	8	8	8	8	8	100	97.5	
	B	8	8	8	8	8	8	8	100		
	C	8	8	8	8	8	8	8	100		
	D	8	8	8	8	8	8	8	100		
	E	8	8	8	8	8	8	8	87.5		
11	A	8	8	8	8	8	8	8	100	95	
	B	8	8	8	8	8	8	8	87.5		
	C	8	8	8	8	8	8	8	100		
	D	8	8	8	8	8	8	8	87.5		
	E	8	8	8	8	8	8	8	100		
14	A	8	8	8	8	8	8	8	100	97.5	5.73
	B	8	8	8	8	8	8	8	87.5		
	C	8	8	8	8	8	8	8	100		
	D	8	8	8	8	8	8	8	100		
	E	8	8	8	8	8	8	8	100		
19	A	8	8	8	8	8	8	8	100	100	0.00
	B	8	8	8	8	8	8	8	100		
	C	8	8	8	8	8	8	8	100		
	D	8	8	8	8	8	8	8	100		
	E	8	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		K1205003		TEST START DATE		5/22/12		TIME		1415			
CLIENT		Sheridan		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		
0	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	↓	↓	↓	↓	↓	↓	↓	↓				
	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		KP	KP	KP	KP	SA	J	KP	KP				
DATE:		5/23/12	5/23/12	5/24/12	5/25/12	5/26/12	5/27	5/28	5/29				
TIME:		1415	1526	1030	1115	1050		1145	1345				

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB #/SAMPLE ID		K1205003		TEST START DATE		5/22/12		TIME		1415		
CLIENT		Sheridan B		TEST END DATE				TIME				
AGE AND SOURCE OF MINNOWS												
DAY (NUMBER SURVIVING)												
		REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
CONC: 0	A		2	2	2	2	2	2	2			
	B		2	2	2	2	2	2	2			
	C		2	2	2	2	2	2	2			
	D		2	2	2	2	2	2	2			
	E		2	2	2	2	2	2	2			
CONC: 6	A		2	2	2	2	2	2	2			
	B		2	2	2	2	2	2	2			
	C		2	2	2	2	2	2	2			
	D		2	2	2	2	2	2	2			
	E		2	2	2	2	2	2	2			
CONC: 8	A		2	2	2	2	2	2	2			
	B		2	2	2	2	2	2	2			
	C		2	2	2	2	2	2	2			
	D		2	2	2	2	2	2	2			
	E		2	2	2	2	2	2	2			
CONC: 11	A		2	2	2	2	2	2	2			
	B		2	2	2	2	2	2	2			
	C		2	2	2	2	2	2	2			
	D		2	2	2	2	2	2	2			
	E		2	2	2	2	2	2	2			
CONC: 14	A		2	2	2	2	2	2	2			
	B		2	2	2	2	2	2	2			
	C		2	2	2	2	2	2	2			
	D		2	2	2	2	2	2	2			
	E		2	2	2	2	2	2	2			
CONC: 19	A		2	2	2	2	2	2	2			
	B		2	2	2	2	2	2	2			
	C		2	2	2	2	2	2	2			
	D		2	2	2	2	2	2	2			
	E		2	2	2	2	2	2	2			
ANALYST		KP				SD	CS					
DATE:		5/22/12										
TIME:		1415										

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID K205003 TEST START DATE 5/22/12 TIME 1415
 CLIENT Sheridan TEST END DATE _____ TIME _____
 AGE AND SOURCE OF MINNOWS _____

		DAY (NUMBER SURVIVING)							SURVIVAL	
REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
CONC: 0	A	2	2	2	2	2	2	2		
	B	2	2	2	2	2	2	2		
	C	2	2	2	2	2	2	2		
	D	2	2	2	2	2	2	2		
	E	2	2	2	2	2	2	2		
CONC: 6	A	2	2	2	2	2	2	2		
	B	2	2	2	2	2	2	2		
	C	2	2	2	2	2	2	2		
	D	2	2	2	2	2	2	2		
	E	2	2	2	2	2	2	2		
CONC: 8	A	2	2	2	2	2	2	2		
	B	2	2	2	2	2	2	2		
	C	2	2	2	2	2	2	2		
	D	2	2	2	2	2	2	2		
	E	2	2	2	2	2	2	2		
CONC: 11	A	2	2	2	2	2	2	2		
	B	2	2	2	2	2	2	2		
	C	2	2	2	2	2	2	2		
	D	2	2	2	2	2	2	2		
	E	2	2	2	2	2	2	2		
CONC: 14	A	2	2	2	2	2	2	2		
	B	2	2	2	2	2	2	2		
	C	2	2	2	2	2	2	2		
	D	2	2	2	2	2	2	2		
	E	2	2	2	2	2	2	2		
CONC: 19	A	2	2	2	2	2	2	2		
	B	2	2	2	2	2	2	2		
	C	2	2	2	2	2	2	2		
	D	2	2	2	2	2	2	2		
	E	2	2	2	2	2	2	2		
ANALYST	VP									
DATE:	5/22/12									
TIME:	1415									

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		K1205003		TEST START DATE		5/22/12		TIME		1415	
CLIENT		Sheridan		TEST END DATE				TIME			
AGE AND SOURCE OF MINNOWS											
DAY (NUMBER SURVIVING)											
		start	1	2	3	4	5	6	7%	MEAN %	CV
CONC: 0	REP #	A	2	2	2	2	2	2	2		
	B	2	2	2	2	2	2	2	2		
	C	2	2	2	2	2	2	2	2		
	D	2	2	2	2	2	2	2	2		
	E	2	2	2	2	2	2	2	2		
CONC: 1	REP #	A	2	2	2	2	2	2	2		
	B	2	2	2	2	2	2	2	2		
	C	2	2	2	2	2	2	2	2		
	D	2	2	2	2	2	2	2	2		
	E	2	2	2	2	2	2	2	2		
CONC: 8	REP #	A	2	2	2	2	2	2	2		
	B	2	2	2	2	2	2	2	2		
	C	2	2	2	2	2	2	2	2		
	D	2	2	2	2	2	2	2	2		
	E	2	2	2	2	2	2	2	2		
CONC: 11	REP #	A	2	2	2	2	2	2	2		
	B	2	2	2	2	2	2	2	2		
	C	2	2	2	2	2	2	2	2		
	D	2	2	2	2	2	2	2	2		
	E	2	2	2	2	2	2	2	2		
CONC: 14	REP #	A	2	2	2	2	2	2	2		
	B	2	2	2	2	2	2	2	2		
	C	2	2	2	2	2	2	2	2		
	D	2	2	2	2	2	2	2	2		
	E	2	2	2	2	2	2	2	2		
CONC: 19	REP #	A	2	2	2	2	2	2	2		
	B	2	2	2	2	2	2	2	2		
	C	2	2	2	2	2	2	2	2		
	D	2	2	2	2	2	2	2	2		
	E	2	2	2	2	2	2	2	2		
ANALYST	VP				A		A				
DATE:	5/22/12										
TIME:	1415										

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID K1205003 TEST START DATE 5/22/12 TIME 1415
 CLIENT Sheridan 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
0	A	2	2	2	1	1	1	1	1		
	B	2	2	2	2	2	2	2	2		
	C	2	2	2	2	2	2	2	2		
	D	2	2	2	2	2	2	2	2		
	E	2	2	2	2	2	2	2	2		
6	A	2	2	2	2	2	2	2	2		
	B	2	2	2	2	2	2	2	2		
	C	2	2	2	2	2	2	2	2		
	D	2	2	2	2	2	2	2	2		
	E	2	2	2	2	2	2	2	2		
8	A	2	2	2	2	2	2	2	2		
	B	2	2	2	2	2	2	2	2		
	C	2	2	2	2	2	2	2	2		
	D	2	2	2	2	2	2	2	2		
	E	2	2	2	2	2	2	2	2		
11	A	2	2	2	2	2	2	2	2		
	B	2	2	2	2	2	2	2	2		
	C	2	2	2	2	2	2	2	2		
	D	2	2	2	2	2	2	2	2		
	E	2	2	2	2	2	2	2	2		
14	A	2	2	2	2	2	2	2	2		
	B	2	2	2	2	2	2	2	2		
	C	2	2	2	2	2	2	2	2		
	D	2	2	2	2	2	2	2	2		
	E	2	2	2	2	2	2	2	2		
19	A	2	2	2	2	2	2	2	2		
	B	2	2	2	2	2	2	2	2		
	C	2	2	2	2	2	2	2	2		
	D	2	2	2	2	2	2	2	2		
	E	2	2	2	2	2	2	2	2		
ANALYST		VP				CA	CA				
DATE:		5/22/12					5/22				
TIME:		1415					130				

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

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:		K1205003				TEST DATES (BEGIN / END):		5/22-29/12	
CLIENT:		Sheridan				WEIGHING DATE / TIME:		5-30-12, 1100	
ANALYSTS:		KP				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	0.98936	0.98558	0.00378	8	0.473	AVG DRY		
	B	0.96157	0.95806	0.00351	8	0.439	WEIGHT (mg)		
	C	0.98159	0.97797	0.00362	8	0.452			0.431
	D	1.00872	1.00533	0.00339	8	0.424	CV		
	E	0.97074	0.96782	0.00292	8	0.365			9.47
CONC:	A	0.98130	0.97773	0.00357	8	0.446	AVG DRY		
	B	0.98934	0.98703	0.00231	8	0.289	WEIGHT (mg)		
	C	0.99149	0.98820	0.00329	8	0.411			0.390
	D	1.01738	1.01456	0.00282	8	0.353	CV		
	E	0.99165	0.98803	0.00362	8	0.453			
CONC:	A	0.98270	0.97923	0.00347	8	0.434	AVG DRY		
	B	0.98747	0.98398	0.00349	8	0.436	WEIGHT (mg)		
	C	0.97117	0.96814	0.00303	8	0.379			0.397
	D	0.99881	0.99590	0.00291	8	0.364	CV		
	E	0.99409	0.99111	0.00298	8	0.372			
CONC:	A	0.99231	0.98892	0.00339	8	0.424	AVG DRY		
	B	0.98428	0.98149	0.00279	8	0.349	WEIGHT (mg)		
	C	0.98175	0.97897	0.00278	8	0.348			0.385
	D	0.99413	0.99119	0.00294	8	0.367	CV		
	E	0.96865	0.96517	0.00348	8	0.435			
CONC:	A	0.97784	0.97501	0.00283	8	0.354	AVG DRY		
	B	0.99850	0.99569	0.00281	8	0.351	WEIGHT (mg)		
	C	0.99771	0.99442	0.00329	8	0.411			0.381
	D	1.01377	1.01097	0.00280	8	0.350	CV		
	E	0.98918	0.98567	0.00351	8	0.439			10.85
CONC:	A	0.97332	0.97033	0.00299	8	0.374	AVG DRY		
	B	1.00271	0.99956	0.00315	8	0.394	WEIGHT (mg)		
	C	0.99371	0.99098	0.00273	8	0.341			0.374
	D	0.99835	0.99511	0.00324	8	0.405	CV		
	E	0.96016	0.95730	0.00286	8	0.357			6.8

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA# K1205003, FATHEAD MINNOW, CHRONIC, 5-22-12

File: Z:\TOXSTAT\MONTE\FHSURV.

Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.148

W = 0.705

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# K1205003, FATHEAD MINNOW, CHRONIC, 5-22-12

File: Z:\TOXSTAT\MONTE\FHSURV.

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# K1205003, FATHEAD MINNOW, CHRONIC, 5-22-12

FILE: Z:\TOXSTAT\MONTE\FHSURV.

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	1.0000	1.3931
1	CONTROL	5	0.8750	1.2094
2	6 % EFFLUENT	1	1.0000	1.3931
2	6 % EFFLUENT	2	0.8750	1.2094
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	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	1.0000	1.3931
5	14 %	EFFLUENT	1	1.0000	1.3931
5	14 %	EFFLUENT	2	0.8750	1.2094
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# K1205003, FATHEAD MINNOW, CHRONIC, 5-22-12

File: Z:\TOXSTAT\MONTE\FHSURV.

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	1.356	27.50	16.00	5.00	
4	11 % EFFLUENT	1.320	25.00	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 $\alpha = 0.05$

AA# K1205003 FATHEAD MINNOW GROWTH CHRONIC, 5-22-12
File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.050

W = 0.944

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# K1205003 FATHEAD MINNOW GROWTH CHRONIC, 5-22-12
File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 4.02

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# K1205003 FATHEAD MINNOW GROWTH CHRONIC, 5-22-12
FILE: Z:\TOXSTAT\MONTE\FHGR.
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.4730	0.7584
1	CONTROL	2	0.4390	0.7242
1	CONTROL	3	0.4520	0.7373
1	CONTROL	4	0.4240	0.7091
1	CONTROL	5	0.3650	0.6487
2	6 % EFFLUENT	1	0.4460	0.7313
2	6 % EFFLUENT	2	0.2890	0.5676
2	6 % EFFLUENT	3	0.4110	0.6959
2	6 % EFFLUENT	4	0.3530	0.6362
2	6 % EFFLUENT	5	0.4530	0.7383
3	8 % EFFLUENT	1	0.4340	0.7192
3	8 % EFFLUENT	2	0.4360	0.7212
3	8 % EFFLUENT	3	0.3790	0.6632
3	8 % EFFLUENT	4	0.3640	0.6477
3	8 % EFFLUENT	5	0.3720	0.6560
4	11 % EFFLUENT	1	0.4240	0.7091

4	11	% EFFLUENT	2	0.3490	0.6320
4	11	% EFFLUENT	3	0.3480	0.6310
4	11	% EFFLUENT	4	0.3670	0.6508
4	11	% EFFLUENT	5	0.4350	0.7202
5	14	% EFFLUENT	1	0.3540	0.6372
5	14	% EFFLUENT	2	0.3510	0.6341
5	14	% EFFLUENT	3	0.4110	0.6959
5	14	% EFFLUENT	4	0.3500	0.6331
5	14	% EFFLUENT	5	0.4390	0.7242
6	19	% EFFLUENT	1	0.3740	0.6580
6	19	% EFFLUENT	2	0.3940	0.6786
6	19	% EFFLUENT	3	0.3410	0.6236
6	19	% EFFLUENT	4	0.4050	0.6898
6	19	% EFFLUENT	5	0.3570	0.6404

AA# K1205003 FATHEAD MINNOW GROWTH CHRONIC, 5-22-12

File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.010	0.002	0.999
Within (Error)	24	0.050	0.002	
Total	29	0.061		

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

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All equal

AA# K1205003 FATHEAD MINNOW GROWTH CHRONIC, 5-22-12

File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST - TABLE 1 OF 2 H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	0.716	0.431		
2	6 % EFFLUENT	0.674	0.390	1.441	
3	8 % EFFLUENT	0.681	0.397	1.179	
4	11 % EFFLUENT	0.669	0.385	1.623	
5	14 % EFFLUENT	0.665	0.381	1.751	
6	19 % EFFLUENT	0.658	0.374	1.987	

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

AA# K1205003 FATHEAD MINNOW GROWTH CHRONIC, 5-22-12

File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST - TABLE 2 OF 2 H_0 : 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	0.067	15.5	0.040
3	8 % EFFLUENT	5	0.067	15.5	0.034
4	11 % EFFLUENT	5	0.067	15.5	0.046
5	14 % EFFLUENT	5	0.067	15.5	0.050
6	19 % EFFLUENT	5	0.067	15.5	0.056

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

Cerodaphnia dubia

SURVIVAL AND REPRODUCTION TEST

Discharger: Shawlan
 Location: Sec 10E
 Date Sample Collected: 5/22/12
 Lab Number/s: K1705003

Analyst: KP
 Test Start - Date/Time: 5/22/12, 1415
 Test Stop - Date/Time: 5/29/12, 1515

Conc 1	%	Day	Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
			A	B	C	D	E	F	G	H	I	J				
1		1	6	0	6	0	6	0	6	0	0	0	0	10	0	KP
2		2	0	0	0	0	0	0	0	0	0	0	0	10	0	KP
3		3	0	0	0	0	0	0	0	0	0	0	1	10	0.1	KP
4		4	1	0	0	1	0	0	1	0	1	3	9	10	0.9	KP
5		5	0	0	2	0	3	4	1	0	6	5	7	10	2.8	KP
6		6	7	3	4	0	4	6	5	3	5	3	53	47	5.9	KP
7		7	5	1	7	2	9	4	6	10	5	8	55	8	6.9	KP
8		8														
Total			13	4	13	x1	16	14	13	22	16	23	135			

Conc 2	%	Day	Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
			A	B	C	D	E	F	G	H	I	J				
1		1	0	0	0	0	0	0	0	0	0	0	0	10	0	
2		2	0	0	0	0	0	0	0	0	0	0	0	10	0	
3		3	0	0	0	0	0	0	0	0	0	0	0	10	0	
4		4	3	0	2	3	2	0	2	0	2	1	15	10	1.5	
5		5	0	4	2	4	3	5	1	0	2	4	25	10	2.5	
6		6	5	2	4	2	5	1	4	2	5	3	33	9	3.7	
7		7	6	4	8	4	11	6	7	7	8	10	67	9	7.4	
8		8														
Total			14	x6	12	17	21	12	14	9	17	18	140			

Conc 3	%	Day	Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
			A	B	C	D	E	F	G	H	I	J				
1		1	0	0	0	0	0	0	0	0	0	0	0	10	0	
2		2	0	0	0	0	0	0	0	0	0	0	0	10	0	
3		3	0	0	0	0	0	0	0	0	0	0	0	10	0	
4		4	2	0	1	0	3	0	7	2	3	7	13	10	1.3	
5		5	2	1	3	5	6	2	1	4	4	3	31	10	3.1	
6		6	5	7	3	7	4	2	3	7	3	2	43	10	4.3	
7		7	0	8	7	8	6	0	7	9	10	11	60	10	6.0	
8		8														
Total			9	16	14	20	19	4	17	22	20	17	153			

X= DEAD; Y= MALE

0

6

8

Conc 4	%	Day	Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
			A	B	C	D	E	F	G	H	I	J				
1		1	0	0	0	0	0	0	0	0	0	0	0	10	0	
2		2	0	0	0	0	0	0	0	0	0	0	0	10	0	
3		3	1	0	0	0	0	1	0	0	0	0	2	10	0.2	
4		4	0	1	0	1	3	2	1	0	2	0	10	10	1.0	
5		5	1	6	1	0	2	3	1	4	0	3	15	10	1.5	
6		6	6	2	3	2	0	5	4	7	5	4	32	10	3.2	
7		7	4	8	8	9	x0	10	9	8	7	9	72	9	8.0	
8		8														
Total			17	11	12	17	x5	21	15	19	x1	16	135			

Conc 5	%	Day	Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
			A	B	C	D	E	F	G	H	I	J				
1		1	0	0	0	0	0	0	0	0	0	0	0	10	0	
2		2	0	0	0	0	0	0	0	0	0	0	0	10	0	
3		3	0	0	0	0	0	0	0	0	0	0	0	10	0	
4		4	3	1	1	2	3	2	1	2	0	0	15	10	1.5	
5		5	2	0	1	1	3	4	0	3	1	2	17	10	1.7	
6		6	6	5	2	7	4	0	2	0	5	8	39	9	4.3	
7		7	6	7	9	3	8	x0	8	-	9	7	57	8	7.1	
8		8														
Total			17	13	13	13	18	x6	11	x5	15	17	128			

Conc 6	%	Day	Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
			A	B	C	D	E	F	G	H	I	J				
1		1	0	0	0	0	0	0	0	0	0	0	0	10	0	
2		2	0	0	0	0	0	0	0	x0	0	0	0	9	0	
3		3	0	0	0	0	0	0	0	-	0	0	2	9	0.2	
4		4	3	1	2	0	2	2	2	-	4	3	19	9	2.1	
5		5	3	0	4	2	5	4	1	-	3	3	25	9	2.8	
6		6	0	9	5	4	7	6	4	-	7	6	48	9	5.3	
7		7	6	7	8	x0	7	11	8	-	5	9	61	8	7.6	
8		8														
Total			12	17	19	x6	21	23	15	x0	19	23	155			

AA # K1205003, C. DUBIA CHRONIC, REPRODUCCION, 5-22-12
File: Z:/toxstat/monte\CD. 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 # K1205003, C. DUBIA CHRONIC, REPRODUCCION, 5-22-12
File: Z:/toxstat/monte\CD. Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 4.87

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%	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
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		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	8	2	10
19%	8	2	10
TOTAL	16	4	20

CRITICAL FISHER'S VALUE (10,10,8) (p=0.05) IS 3. b VALUE IS 8.
Since b is greater than 3 there is no significant difference
between CONTROL and TREATMENT at the 0.05 level.

SUMMARY OF FISHER'S EXACT TESTS

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

2	8%	10	0
3	11%	10	0
4	14%	10	1
5	19%	10	2

TITLE: AA # K1205003, C. DUBIA CHRONIC, REPRODUCCION, 5-22-12
 FILE: Z:/toxstat/monte\CD.
 TRANSFORM: NO TRANSFORMATION

NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	13.0000	13.0000
1	CONTROL	2	4.0000	4.0000
1	CONTROL	3	13.0000	13.0000
1	CONTROL	4	1.0000	1.0000
1	CONTROL	5	16.0000	16.0000
1	CONTROL	6	14.0000	14.0000
1	CONTROL	7	13.0000	13.0000
1	CONTROL	8	22.0000	22.0000
1	CONTROL	9	16.0000	16.0000
1	CONTROL	10	23.0000	23.0000
2	6 % EFFLUENT	1	14.0000	14.0000
2	6 % EFFLUENT	2	6.0000	6.0000
2	6 % EFFLUENT	3	12.0000	12.0000
2	6 % EFFLUENT	4	17.0000	17.0000
2	6 % EFFLUENT	5	21.0000	21.0000
2	6 % EFFLUENT	6	12.0000	12.0000
2	6 % EFFLUENT	7	14.0000	14.0000
2	6 % EFFLUENT	8	9.0000	9.0000
2	6 % EFFLUENT	9	17.0000	17.0000
2	6 % EFFLUENT	10	18.0000	18.0000
3	8 % EFFLUENT	1	9.0000	9.0000
3	8 % EFFLUENT	2	16.0000	16.0000
3	8 % EFFLUENT	3	14.0000	14.0000
3	8 % EFFLUENT	4	20.0000	20.0000
3	8 % EFFLUENT	5	19.0000	19.0000
3	8 % EFFLUENT	6	4.0000	4.0000
3	8 % EFFLUENT	7	12.0000	12.0000
3	8 % EFFLUENT	8	22.0000	22.0000
3	8 % EFFLUENT	9	20.0000	20.0000
3	8 % EFFLUENT	10	17.0000	17.0000
4	11 % EFFLUENT	1	12.0000	12.0000
4	11 % EFFLUENT	2	11.0000	11.0000
4	11 % EFFLUENT	3	12.0000	12.0000
4	11 % EFFLUENT	4	17.0000	17.0000
4	11 % EFFLUENT	5	5.0000	5.0000
4	11 % EFFLUENT	6	21.0000	21.0000
4	11 % EFFLUENT	7	15.0000	15.0000
4	11 % EFFLUENT	8	19.0000	19.0000
4	11 % EFFLUENT	9	14.0000	14.0000
4	11 % EFFLUENT	10	16.0000	16.0000
5	14 % EFFLUENT	1	17.0000	17.0000
5	14 % EFFLUENT	2	13.0000	13.0000

5	14 %	EFFLUENT	3	13.0000	13.0000
5	14 %	EFFLUENT	4	13.0000	13.0000
5	14 %	EFFLUENT	5	18.0000	18.0000
5	14 %	EFFLUENT	6	6.0000	6.0000
5	14 %	EFFLUENT	7	11.0000	11.0000
5	14 %	EFFLUENT	8	5.0000	5.0000
5	14 %	EFFLUENT	9	15.0000	15.0000
5	14 %	EFFLUENT	10	17.0000	17.0000
6	19 %	EFFLUENT	1	12.0000	12.0000
6	19 %	EFFLUENT	2	17.0000	17.0000
6	19 %	EFFLUENT	3	19.0000	19.0000
6	19 %	EFFLUENT	4	6.0000	6.0000
6	19 %	EFFLUENT	5	21.0000	21.0000
6	19 %	EFFLUENT	6	23.0000	23.0000
6	19 %	EFFLUENT	7	15.0000	15.0000
6	19 %	EFFLUENT	8	0.0000	0.0000
6	19 %	EFFLUENT	9	19.0000	19.0000
6	19 %	EFFLUENT	10	23.0000	23.0000

AA # K1205003, C. DUBIA CHRONIC, REPRODUCCION, 5-22-12
 File: Z:/toxstat/monte\CD. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	53.883	10.777	0.330
Within (Error)	54	1764.300	32.672	
Total	59	1818.183		

Critical F value = 2.45 (0.05,5,40)
 Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All equal

AA # K1205003, C. DUBIA CHRONIC, REPRODUCCION, 5-22-12
 File: Z:/toxstat/monte\CD. Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 1 OF 2 H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	13.500	13.500		
2	6 % EFFLUENT	14.000	14.000	-0.196	
3	8 % EFFLUENT	15.300	15.300	-0.704	
4	11 % EFFLUENT	14.200	14.200	-0.274	
5	14 % EFFLUENT	12.800	12.800	0.274	
6	19 % EFFLUENT	15.500	15.500	-0.782	

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

AA # K1205003, C. DUBIA CHRONIC, REPRODUCCION, 5-22-12

File: Z:/toxstat/monte\CD.

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	5.905	43.7	-0.500
3	8 % EFFLUENT	10	5.905	43.7	-1.800
4	11 % EFFLUENT	10	5.905	43.7	-0.700
5	14 % EFFLUENT	10	5.905	43.7	0.700
6	19 % EFFLUENT	10	5.905	43.7	-2.000

AA # K1205003, C. DUBIA CHRONIC, REPRODUCCION, 5-22-12

File: Z:/toxstat/monte\CD.

Transform: NO TRANSFORMATION

STEEL'S MANY-ONE RANK TEST

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	13.500				
2	6 % EFFLUENT	14.000	106.00	75.00	10.00	
3	8 % EFFLUENT	15.300	113.50	75.00	10.00	
4	11 % EFFLUENT	14.200	105.50	75.00	10.00	
5	14 % EFFLUENT	12.800	101.50	75.00	10.00	
6	19 % EFFLUENT	15.500	116.00	75.00	10.00	

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

APPENDIX E

Organism History

AQUATOX, INC.

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

10/2

TEST ORGANISM HISTORY

DATE SHIPPED 5/20/12 CLIENT AR Analytical
Ken

Purchase Order #: _____

SPECIES: Pimephales promelas

Quantity Shipped: 280

Age: Hatched 5/21/12 1500
CSJ

Brood Stock Source: Anderson Farms, AR

Culture Water: Groundwater 160

Hardness (Mg/l CaCO3): _____

Dissolved Oxygen (Mg/l): 8.2

Temperature (°C): 25-100

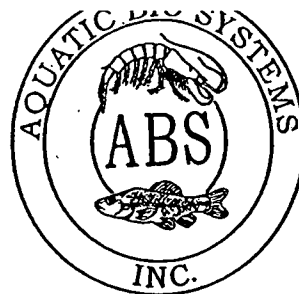
Feeding: Artemia

Comments: _____

Shipped Via: 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: 6/22/09

SPECIES: Ceriodaphnia dubia

AGE: Variable

LIFE STAGE: Adult

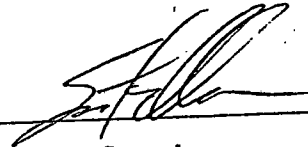
HATCH DATE: Variable

BEGAN FEEDING: Immediately

FOOD: YTC, Selenastrum sp.

Water Chemistry Record:	Current	Range
TEMPERATURE:	<u>25°C</u>	<u>20-25°C</u>
SALINITY/CONDUCTIVITY:	<u>--</u>	<u>--</u>
TOTAL HARDNESS (as CaCO ₃):	<u>142 mg/l</u>	<u>86-124 mg/l</u>
TOTAL ALKALINITY (as CaCO ₃):	<u>100 mg/l</u>	<u>65-130 mg/l</u>
pH:	<u>7.92</u>	<u>7.56-8.35</u>

Comments:

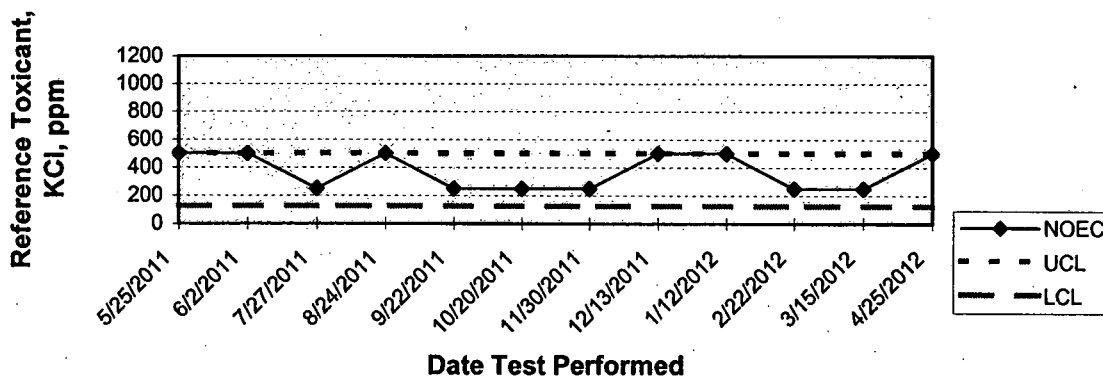


Facility Supervisor

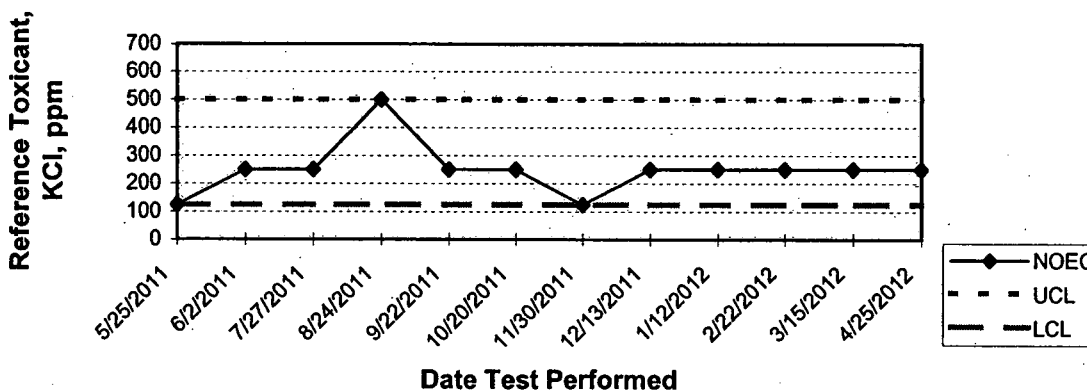
APPENDIX F

Quality Assurance Charts

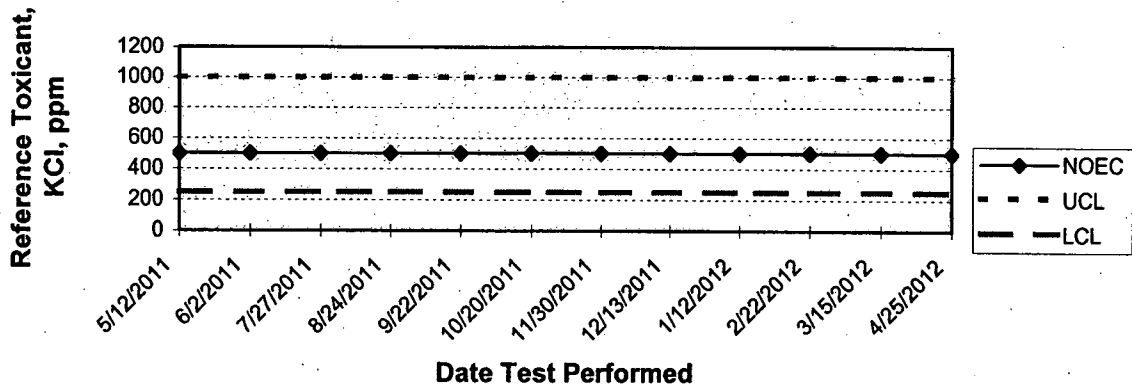
ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE



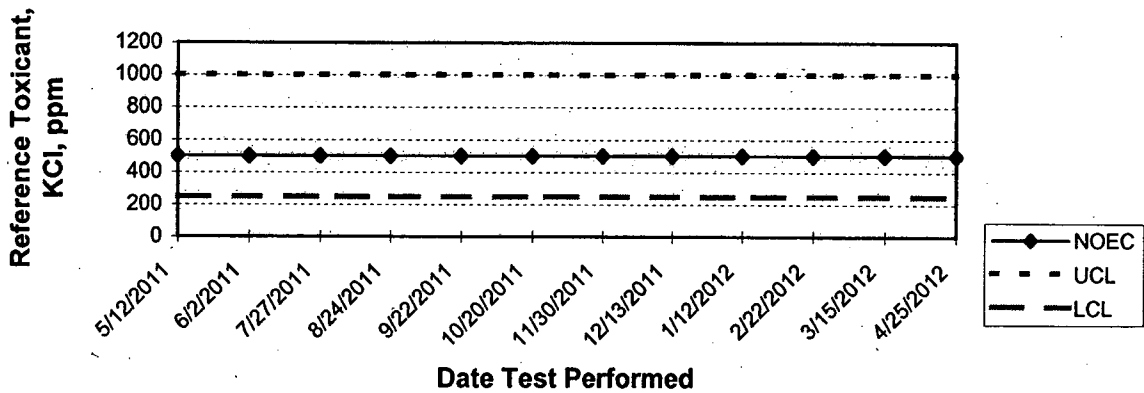
ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE



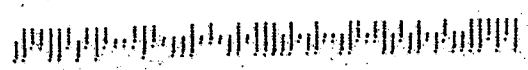
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW SURVIVAL
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW GROWTH
QUALITY ASSURANCE



SHERIDAN
PO BOX 486
SHERIDAN, AR 72150



66 RndRkAcS
CENTRAL AR PSD 721
FRI 06 JUL 2012



ADEQ
NPDES Enforcement Branch
5301 Northshore Dr
No Little Rock, AR 72118-5317