

Arkansas Analytical, Inc.

Toxicity Test Results

CITY of SHERIDAN
NPDES PERMIT NUMBER: AR0034347
Third 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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Thursday, October 04, 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 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 both *Ceriodaphnia dubia* and *Pimephales promelas*. The test results in this report represent the testing for the third 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:	9-17-12, 1133	9-18-12, 1033

Due to flow stoppage only one sample was collected.

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:	9-18-12, 1331	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	100%	X	
Average of 15 or more young per surviving female	17.2	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	15.9%	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	0.458	X	
The percent coefficient of variation between replicates must be 40% or less for growth	13.1%	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> 8/9-16/12		<i>Pimephales promelas</i> 8/9-16/12	
NOEC Survival:	500 ppm KCl	NOEC Survival:	500 ppm KCl
LOEC Survival:	1000 ppm KCl	LOEC Survival:	1000 ppm KCl
NOEC Reproduction:	125 ppm KCl	NOEC Growth:	500 ppm KCl
LOEC Reproduction:	250 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	8% / 11%
%CV survival (critical dilution)	15.2%
Mean dry weight (critical dilution) in milligrams	0.314
%CV growth (critical dilution)	13.0%
PMSD Growth	20.3%
<i>Ceriodaphnia dubia</i>	
NOEC / LOEC survival	19% / NA
NOEC / LOEC reproduction	19% / NA
Mean number of neonates (critical dilution)	23.0
%CV Reproduction (critical dilution)	14.9%
PMSD Reproduction	34.8

Conclusion


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

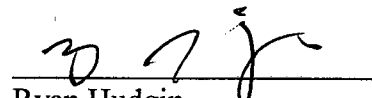
The permit issued to the City of Sheridan, AR0034347, specifies that the **critical dilution is 14% effluent**. The effluent samples **did not** exhibit lethal effects at the critical dilution, and, as such, **passed** the survival portion of the test. However, the effluent samples did exhibit sublethal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

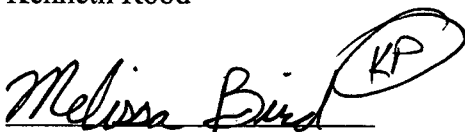
Chronic static renewal survival and reproduction test using *Ceriodaphnia dubia*, (Method 1002.0)

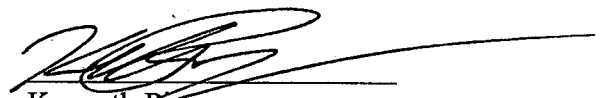
The permit issued to the City of Sheridan, AR0034347, specifies that the **critical dilution is 14% effluent**. The effluent samples **did not** exhibit lethal effects or sublethal effects at the critical dilution, and, as such, **passed** both portions of the test.

Biomonitoring Analysts:


Kenneth Rood


Ryan Hudgin


Melissa Bird


Kenneth Pigue

**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:	9-17-12, 1133	9-18-12, 1033

Test initiated (date, time): 9-19-12, 1300 Test terminated (date, time): 9-26-12, 1155

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	87.5	100	100		100	100	97.5	5.73
6.0%	100	75	100	100	87.5		95	95	92.5	
8.0%	100	100	100	100	87.5		100	100	97.5	
11.0%	87.5	100	100	100	100		100	100	97.5	
14.0%	100	75	100	75	100		100	97.5	90	15.2
19.0%	100	87.5	62.5	87.5	87.5		100	95	85	

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%	0.380	0.406	0.493	0.508	0.502		0.458	13.10
6.0%	0.464	0.324	0.443	0.491	0.366		0.418	
8.0%	0.456	0.383	0.462	0.299	0.486		0.417	
11.0%	0.265	0.296	0.323	0.433	0.431		0.350	
14.0%	0.341	0.316	0.365	0.266	0.281		0.314	13.00
19.0%	0.280	0.317	0.268	0.280	0.290		0.287	

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)= 8 % effluent
 - c) Coefficient of variation (parameter TQP6C)= 13.0 %

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:	9-17-12, 1133	9-18-12, 1033

Test initiated (date, time): 9-19-12, 1130 Test terminated (date, time): 9-26-12, 1500

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	9	17	16	17	x0
B	12	16	20	16	23	24
C	17	14	25	14	21	18
D	19	13	21	27	23	21
E	14	16	24	18	23	19
F	18	19	20	22	x0	15
G	19	23	21	18	27	31
H	15	18	21	24	28	20
I	21	23	20	17	20	32
J	18	17	21	28	25	26
Mean	17.2	16.8	21.0	20.0	20.7	20.6
Mean/surviving female	17.2	16.8	21.0	20.0	23.0	22.9
CV%*	15.9				14.9	

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	90	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)= 15.9 %

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

Test Start (Date/Time)

9-19-12 1300

Client: Sheridan

Test End (Date/Time)

9-26-12 1300-1155

Day of Test

RH 9-2

MHS

6

8

11

14

19

		1	2	3	4	5	6	7	notes/remarks
Control	MHS551	9/19	9/20	9/21	9/22	9-23	9-24	9-25	
D.O. (mg/L)	INITIAL	9.0	8.6	8.6	8.5	8.6	8.8	8.5	
	FINAL	7.9	7.8 8.5	8.4	7.7	8.3	8.1	7.8	
pH (s.u.)	INITIAL	8.4	7.7	7.7	7.8	8.1	7.9	8.2	
	FINAL	7.6	7.8	7.7	7.7	7.8	7.9	7.9	
temp (C)	INITIAL	21	21	22	22.4	22	21	22	
	FINAL	25	25	25.0	25	25	25	25	
ALKALINITY (mg/L)		58							
HARDNESS (mg/L)		82							
CONDUCTIVITY (umhos/cm)		264							
CHLORINE (mg/L)		<0.05							
CONC:									
D.O. (mg/L)	INITIAL	8.8	8.6	8.5	8.4	8.5	8.7	8.5	
	FINAL	7.6	7.8 7.8	8.0	7.6	8.0	7.9	7.8	
pH (s.u.)	INITIAL	7.9	7.8	8.0	7.5	7.9	7.9	7.9	
	FINAL	7.6	7.8	7.8	7.6	7.8	7.8	7.9	
temp (C)	INITIAL	22	22	22	22.1	23	21	22	
	FINAL	25	25	25.0	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	9.2	8.7	8.5	8.5	8.5	8.8	8.7	
	FINAL	7.6	7.8 7.8	8.1	7.6	7.8	7.8	7.8	
pH (mg/L)	INITIAL	7.8	7.9	7.9	7.6	7.8	7.8	7.9	
	FINAL	7.6	7.8	7.7	7.7	7.7	7.8	7.8	
temp (C)	INITIAL	22	22	22	22.1	23	21	22	
	FINAL	25	25	25.0	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.8	8.7	8.5	8.6	8.4	8.8	8.8	
	FINAL	7.7	7.8 7.7	7.8	7.5	7.8	7.7	7.8	
pH (s.u.)	INITIAL	7.9	8.0	7.9	7.6	7.9	7.8	7.9	
	FINAL	7.7	7.8	7.8	7.7	7.8	7.8	7.7	
temp (C)	INITIAL	22	22	22	22.3	23	21	21	
	FINAL	25	25	25.0	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.8	8.7	8.5	8.6	8.4	8.8	8.8	
	FINAL	7.7	7.8 7.7	7.8	7.5	7.8	7.7	7.7	
pH (s.u.)	INITIAL	7.8	7.9	7.9	7.6	7.9	7.8	7.8	
	FINAL	7.7	7.8	7.8	7.6	7.7	7.8	7.7	
temp (C)	INITIAL	22	22	22	22.4	24	21	21	
	FINAL	25	25	25.0	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.8	8.7	8.5	8.5	8.4	8.8	8.8	
	FINAL	7.8	7.9 7.8	7.8	7.5	7.8	7.8	7.8	
pH (s.u.)	INITIAL	7.0	7.8	7.9	7.7	7.9	7.8	7.9	
	FINAL	7.7	7.8	7.8	7.8	7.7	7.8	7.8	
temp (C)	INITIAL	22	22	22	22.4	24	21	21	
	FINAL	25	25	25.0	25	25	25	25	
CONC: 100%		A	A	A	B	B	C	C	
ALKALINITY (mg/L)		20-2 94	64						
HARDNESS (mg/L)			62						
CONDUCTIVITY (umhos/cm)			433						
CHLORINE (mg/L)			<0.05						

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Cerodaphnia Dubia

Lab # / Sample ID

Test Start (Date/Time)

9-19-12

1130

Client: Sheridan

Test End (Date/Time)

9-26-12

1500

Day of Test

		1	2	3	4	5	6	7	notes/remarks
Control	MHS551	9-19	9-20	9-21	9-22	9-23	9-24	9-25	
D.O. (mg/L)	INITIAL	9.0	8.6	8.4	8.5	8.6	8.8	8.5	
	FINAL	9.0	8.1	8.5	8.5	8.2	8.1	7.7	
pH (s.u.)	INITIAL	8.4	7.7	7.7	7.8	8.1	7.9	8.2	
	FINAL	7.8	7.7	7.9	7.9	7.9	7.9	7.7	
temp (C)	INITIAL	21	21	22	22.4	22	21	22	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		68							
HARDNESS (mg/L)		82							
CONDUCTIVITY (umhos/cm)		264							
CHLORINE (mg/L)		<0.05							
CONC:									
D.O. (mg/L)	INITIAL	8.8	8.6	8.5	8.4	8.5	8.7	8.5	
	FINAL	7.9	8.0	8.3	8.5	8.1	7.8	7.9	
pH (s.u.)	INITIAL	7.9	7.8	8.0	7.5	7.9	7.9	7.9	
	FINAL	7.8	7.8	7.9	7.9	8.0	7.9	7.6	
temp (C)	INITIAL	22	22	22	22.1	23	21	22	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	9.2	8.7	8.5	8.5	8.6	8.8	8.7	
	FINAL	7.9	8.0	8.3	8.4	8.1	7.8	7.6	
pH (mg/L)	INITIAL	7.8	7.9	7.9	7.6	7.8	7.8	7.9	
	FINAL	7.8	7.8	7.9	7.9	7.9	7.8	7.7	
temp (C)	INITIAL	22	22	22	22.1	23	21	22	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.8	8.7	8.5	8.6	8.6	8.8	8.7	
	FINAL	7.9	8.0	8.3	8.4	8.1	7.8	8.1	
pH (s.u.)	INITIAL	7.9	8.0	7.9	7.6	7.9	7.8	7.9	
	FINAL	7.9	7.8	7.9	8.0	7.9	8.0	8.2	
temp (C)	INITIAL	22	22	22	22.3	23	21	21	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.8	8.7	8.5	8.6	8.4	8.8	8.8	
	FINAL	7.8	8.0	8.3	8.4	8.1	7.8	7.9	
pH (s.u.)	INITIAL	7.8	7.9	7.9	7.6	7.9	7.8	7.8	
	FINAL	7.7	7.8	7.9	8.0	7.9	7.8	7.7	
temp (C)	INITIAL	22	22	22	22.4	24	21	21	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.8	8.7	8.5	8.5	8.4	8.8	8.8	
	FINAL	7.9	7.9	8.3	8.4	8.2	7.9	7.7	
pH (s.u.)	INITIAL	7.0	7.8	7.9	7.7	7.9	7.8	7.9	
	FINAL	7.9	7.9	7.9	8.0	8.0	7.9	7.8	
temp (C)	INITIAL	22	22	22	22.4	24	21	21	
	FINAL	25	25	25	25	25	25	25	
CONC: 100%									
ALKALINITY (mg/L)		64							
HARDNESS (mg/L)		62							
CONDUCTIVITY (umhos/cm)		933							
CHLORINE (mg/L)		<0.05							

m 115

69%

8%

11%

14%

19%

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							
CLIENT		TEST END DATE		TIME							
AGE AND SOURCE OF MINNOWS											
DAY (NUMBER SURVIVING)											
CONC:	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
0	A	8	8	8	8	8	8	8	100	97.5	
	B	↓	↓	↓	↓	↓	↓	↓	100		
	C	↓	↓	↓	↓	↓	↓	↓	87.5		
	D	↓	↓	↓	↓	↓	↓	↓	100		
	E	↓	↓	↓	↓	↓	↓	↓	100		
6	A	8	8	8	8	8	8	8	100	92.5	
	B	↓	7	7	7	7	6	6	75		
	C	↓	8	8	8	8	8	8	100		
	D	↓	8	8	8	8	8	8	100		
	E	↓	7	7	7	7	7	7	87.5		
8	A	8	8	8	8	8	8	8	100	97.5	
	B	↓	↓	↓	↓	↓	↓	↓	100		
	C	↓	↓	↓	↓	↓	↓	↓	100		
	D	↓	↓	↓	↓	↓	↓	↓	100		
	E	↓	↓	↓	7	7	7	7	87.5		
11	A	8	8	8	8	8	8	7	87.5	97.5	
	B	↓	↓	↓	↓	↓	↓	↓	100		
	C	↓	↓	↓	↓	↓	↓	↓	100		
	D	↓	↓	↓	↓	↓	↓	↓	100		
	E	↓	↓	↓	↓	↓	↓	↓	100		
14	A	8	8	8	8	8	8	8	100	90	15.2
	B	↓	↓	7	7	7	7	6	75		
	C	↓	↓	8	8	8	8	8	100		
	D	↓	↓	8	8	8	7	6	75		
	E	↓	↓	8	8	8	8	8	100		
19	A	8	8	8	8	8	8	8	100	85	
	B	↓	↓	8	8	8	7	7	87.5		
	C	↓	↓	6	6	6	6	5	62.5		
	D	↓	↓	8	8	8	8	7	87.5		
	E	↓	↓	8	8	8	8	7	87.5		
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		K1209004		TEST START DATE		9-19-12		TIME		1300			
CLIENT		Sheridan A		TEST END DATE		9-26-12		TIME		1300 1155		9-26	
AGE AND SOURCE OF MINNOWS												KH	
D A Y (NUMBER SURVIVING)												SURVIVAL	
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV	
MHS	CONC: A	2	2	2	2	2	2	2	2				
	B	↓	↓	↓	↓	↓	↓	↓	↓				
	C	↓	↓	↓	↓	↓	↓	↓	↓				
	D	↓	↓	↓	↓	↓	↓	↓	↓				
	E												
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV	
6	CONC: A	2	2	2	2	2	2	2	2				
	B	↓	↓	↓	↓	↓	↓	↓	↓				
	C	↓	↓	↓	↓	↓	↓	↓	↓				
	D	↓	↓	↓	↓	↓	↓	↓	↓				
	E												
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV	
8	CONC: A	2	2	2	2	2	2	2	2				
	B	↓	↓	↓	↓	↓	↓	↓	↓				
	C	↓	↓	↓	↓	↓	↓	↓	↓				
	D	↓	↓	↓	↓	↓	↓	↓	↓				
	E												
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV	
11	CONC: A	2	2	2	2	2	2	2	2				
	B	↓	↓	↓	↓	↓	↓	↓	2				
	C	↓	↓	↓	↓	↓	↓	↓	*2				
	D	↓	↓	↓	↓	↓	↓	↓	1				
	E												
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV	
14	CONC: A	2	2	2	2	2	2	2	2				
	B	↓	↓	↓	↓	↓	↓	↓	↓				
	C	↓	↓	↓	↓	↓	↓	↓	↓				
	D	↓	↓	↓	↓	↓	↓	↓	↓				
	E												
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV	
19	CONC: A	2	2	2	2	2	2	2	2				
	B	↓	↓	↓	↓	↓	↓	↓	↓				
	C	↓	↓	↓	↓	↓	↓	↓	↓				
	D	↓	↓	↓	↓	↓	↓	↓	↓				
	E												
ANALYST		RH	K.R.	mb	mb	KH	KR	K.R.	K.R.				
DATE:		9-19-12	9-20-12	9-21	9-22	9-23	9-24	9-25	9-26				
TIME:		1300	0845	0900	0900	1230	1300	1400	1105				

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		9-19-12		TIME		1300			
CLIENT		Sheridan B		TEST END DATE		9-26-12		TIME 1155 RH 9-26			
AGE AND SOURCE OF MINNOWS											
D A Y (NUMBER SURVIVING)											
SURVIVAL											
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
mfs	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	1	1	1	2	1	1		
	B	↓	↓	2	2	2	2	2	2		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E										
19	A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	2	2	2		
	E										
ANALYST		RH	K.R.	mb	mb	RH	K.R.	K.R.	K.R.		
DATE:		9-19-12	9-20-12	9-21-12	9-22	9-23	9-24	9-25	9-26		
TIME:		1300	0930	0900	0900	1200	1045	1400	1115		

0845 K.R. 1410

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 9-19-12 TIME 1300									
CLIENT Sheridan C		TEST END DATE 9-26-12 TIME 1400 1155 9-26 RH									
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	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	↓	↓	2	2	2	↓	↓	↓		
	C	↓	↓	0	0	0	0	0	0		
	D	↓	↓	2	2	2	2	2	2		
	E										
ANALYST		RH	K.R.	mb	mb	RH	K.R.	K.R.	K.R.		
DATE:		9-19-12	9-20-12	9-21-12	9-22	9-23	9-24	9-25	9-26		
TIME:		1300	0915	0900	0900	1215	1230	1415	1125		

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 9-19-12 TIME 1300									
CLIENT Sheridan D		TEST END DATE 9-26-12 TIME 1430 1155 Rlt 9-26									
AGE AND SOURCE OF MINNOWS											
D A Y (NUMBER SURVIVING)											
SURVIVAL											
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
mHS	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	↓	↓	↓	↓	↓	2	1	1		
	E	↓	↓	↓	↓	↓	↓	↓	↓		
19	A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	2	2		
	E	↓	↓	↓	↓	↓	↓	↓	↓		
ANALYST		RH	K.R.	mb	mb	RH	K.R.	K.R.	K.L.		
DATE:		9-19-12	9-20-12	9-21	9-22	9-23	9-24	9-25	9-26		
TIME:		1300	0915	0900	0900	1130	1245	1420	1430		

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 9-19-12 TIME 1300									
CLIENT <i>Shelden E</i>		TEST END DATE 9-26-12 TIME 1300 1155 RH 9-26									
AGE AND SOURCE OF MINNOWS											
DAY (NUMBER SURVIVING)										SURVIVAL	
	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
MFS	CONC: A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E										
6	CONC: A	2	1	1	1	1	1	1	1		
	B	↓	2	2	2	2	2	2	2		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E										
8	CONC: A	2	2	2	2	1	1	1	1		
	B	↓	↓	↓	↓	2	2	2	2		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	2	2	↓	↓	↓	↓		
	E										
11	CONC: A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E										
14	CONC: A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E										
19	CONC: A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	2	2	↓	↓	↓	↓		
	E										
ANALYST		RH	K.R.	mb	mb	RH	K.R.	K.R.	K.R.		
DATE:		9-19-12	9-20-12	9-21	9-22	9-23	9-24	9-25	9-26		
TIME:		1300	0945	0900	0900	1200	1300 1130	1130	1155		

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

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:		K1209004		TEST DATES (BEGIN / END):		9/19-9/26	
CLIENT:		Sheridan		WEIGHING DATE / TIME:		9/28/12 1130	
ANALYSTS:		RH		DRYING TEMP (DEGREES C):		60	
SAMPLE ID:				DRYING TIME (HOURS):		24	
	REP #	FINAL DRY WEIGHT TIN+LARVAE (g)	INITIAL WEIGHT TIN (g)	TOTAL DRY WEIGHT OF LARVAE (g)	NUMBER OF LARVAE	DRY WEIGHT OF LARVAE (mg)	
CONTROL	A	1.00916	1.00612	0.00304	8	0.380	AVG DRY
	B	1.01557	1.01232	0.00325	8	0.406	WEIGHT (mg)
	C	1.00360	0.99966	0.00394	8	0.493	0.458
	D	1.01198	1.00792	0.00406	8	0.508	CV
	E	0.99352	0.98950	0.00402	8	0.502	13.10
CONC: 6%	A	0.99956	0.99585	0.00371	8	0.464	AVG DRY
	B	1.01683	1.01424	0.00259	8	0.324	WEIGHT (mg)
	C	1.02255	1.01901	0.00354	8	0.443	0.418
	D	1.01298	1.00905	0.00393	8	0.491	CV
	E	1.02048	1.01755	0.00293	8	0.366	
CONC: 8%	A	1.01484	1.01119	0.00365	8	0.456	AVG DRY
	B	1.03916	1.03610	0.00306	8	0.383	WEIGHT (mg)
	C	0.98513	0.98143	0.00370	8	0.462	0.417
	D	1.00260	1.00021	0.00239	8	0.299	CV
	E	0.98476	0.98087	0.00389	8	0.486	
CONC: 11%	A	1.01200	1.00988	0.00212	8	0.265	AVG DRY
	B	1.00943	1.00706	0.00237	8	0.296	WEIGHT (mg)
	C	1.01045	1.00787	0.00258	8	0.323	0.349
	D	1.01238	1.00892	0.00346	8	0.433	CV
	E	1.01674	1.01329	0.00345	8	0.431	
CONC: 14%	A	0.98892	0.98619	0.00273	8	0.341	AVG DRY
	B	0.99168	0.98915	0.00253	8	0.316	WEIGHT (mg)
	C	1.01107	1.00815	0.00292	8	0.365	0.314
	D	1.01977	1.01764	0.00213	8	0.266	CV
	E	0.99480	0.99255	0.00225	8	0.281	13.04
CONC: 19%	A	1.02215	1.01991	0.00224	8	0.280	AVG DRY
	B	1.01150	1.00896	0.00254	8	0.317	WEIGHT (mg)
	C	1.02040	1.01826	0.00214	8	0.268	0.287
	D	1.00382	1.00158	0.00224	8	0.280	CV
	E	1.01426	1.01194	0.00232	8	0.290	6.0

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA# K1209004, FATHEAD MINNOW, CHRONIC, 9-19-12
File: Z:\TOXSTAT\MONTE\FHSURV. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.442

W = 0.866

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# K1209004, FATHEAD MINNOW, CHRONIC, 9-19-12
File: Z:\TOXSTAT\MONTE\FHSURV. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 5.74

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# K1209004, FATHEAD MINNOW, CHRONIC, 9-19-12
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	0.8750	1.2094
1	CONTROL	4	1.0000	1.3931
1	CONTROL	5	1.0000	1.3931
2	6 % EFFLUENT	1	1.0000	1.3931
2	6 % EFFLUENT	2	0.7500	1.0472
2	6 % EFFLUENT	3	1.0000	1.3931
2	6 % EFFLUENT	4	1.0000	1.3931
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	0.8750	1.2094
4	11 %	EFFLUENT	2	1.0000	1.3931
4	11 %	EFFLUENT	3	1.0000	1.3931
4	11 %	EFFLUENT	4	1.0000	1.3931
4	11 %	EFFLUENT	5	1.0000	1.3931
5	14 %	EFFLUENT	1	1.0000	1.3931
5	14 %	EFFLUENT	2	0.7500	1.0472
5	14 %	EFFLUENT	3	1.0000	1.3931
5	14 %	EFFLUENT	4	0.7500	1.0472
5	14 %	EFFLUENT	5	1.0000	1.3931
6	19 %	EFFLUENT	1	1.0000	1.3931
6	19 %	EFFLUENT	2	0.8750	1.2094
6	19 %	EFFLUENT	3	0.6250	0.9117
6	19 %	EFFLUENT	4	0.8750	1.2094
6	19 %	EFFLUENT	5	0.8750	1.2094

AA# K1209004, FATHEAD MINNOW, CHRONIC, 9-19-12
 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	1.287	24.50	16.00	5.00	
3	8 % EFFLUENT	1.356	27.50	16.00	5.00	
4	11 % EFFLUENT	1.356	27.50	16.00	5.00	
5	14 % EFFLUENT	1.255	24.00	16.00	5.00	
6	19 % EFFLUENT	1.187	19.50	16.00	5.00	

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

AA# K1209004, FATHEAD MINNOW GROWTH CHRONIC, 9-19-12
File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.096

W = 0.954

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# K1209004, FATHEAD MINNOW GROWTH CHRONIC, 9-19-12
File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 6.88

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# K1209004, FATHEAD MINNOW GROWTH CHRONIC, 9-19-12
FILE: Z:\TOXSTAT\MONTE\FHGR.
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.3800	0.6642
1	CONTROL	2	0.4060	0.6908
1	CONTROL	3	0.4930	0.7784
1	CONTROL	4	0.5080	0.7934
1	CONTROL	5	0.5020	0.7874
2	6 % EFFLUENT	1	0.4640	0.7494
2	6 % EFFLUENT	2	0.3240	0.6055
2	6 % EFFLUENT	3	0.4430	0.7283
2	6 % EFFLUENT	4	0.4910	0.7764
2	6 % EFFLUENT	5	0.3660	0.6497
3	8 % EFFLUENT	1	0.4560	0.7413
3	8 % EFFLUENT	2	0.3830	0.6673
3	8 % EFFLUENT	3	0.4620	0.7474
3	8 % EFFLUENT	4	0.2990	0.5785
3	8 % EFFLUENT	5	0.4860	0.7714
4	11 % EFFLUENT	1	0.2650	0.5408

4	11 %	EFFLUENT	2	0.2960	0.5753
4	11 %	EFFLUENT	3	0.3230	0.6045
4	11 %	EFFLUENT	4	0.4330	0.7182
4	11 %	EFFLUENT	5	0.4310	0.7162
5	14 %	EFFLUENT	1	0.3410	0.6236
5	14 %	EFFLUENT	2	0.3160	0.5970
5	14 %	EFFLUENT	3	0.3650	0.6487
5	14 %	EFFLUENT	4	0.2660	0.5419
5	14 %	EFFLUENT	5	0.2810	0.5587
6	19 %	EFFLUENT	1	0.2800	0.5576
6	19 %	EFFLUENT	2	0.3170	0.5980
6	19 %	EFFLUENT	3	0.2680	0.5441
6	19 %	EFFLUENT	4	0.2800	0.5576
6	19 %	EFFLUENT	5	0.2900	0.5687

AA# K1209004, FATHEAD MINNOW GROWTH CHRONIC, 9-19-12
 File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.122	0.024	6.078
Within (Error)	24	0.096	0.004	
Total	29	0.218		

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

AA# K1209004, FATHEAD MINNOW GROWTH CHRONIC, 9-19-12
 File: Z:\TOXSTAT\MONTE\FHGR. 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	0.743	0.458		
2	6 % EFFLUENT	0.702	0.418	1.023	
3	8 % EFFLUENT	0.701	0.417	1.040	
4	11 % EFFLUENT	0.631	0.350	2.792	*
5	14 % EFFLUENT	0.594	0.314	3.715	*
6	19 % EFFLUENT	0.565	0.287	4.433	*

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

AA# K1209004, FATHEAD MINNOW GROWTH CHRONIC, 9-19-12
 File: Z:\TOXSTAT\MONTE\FHGR. 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	0.093	20.3	0.040
3	8 % EFFLUENT	5	0.093	20.3	0.041
4	11 % EFFLUENT	5	0.093	20.3	0.108
5	14 % EFFLUENT	5	0.093	20.3	0.144
6	19 % EFFLUENT	5	0.093	20.3	0.171

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

Cerodaphnia dubia

SURVIVAL AND REPRODUCTION TEST

KH / KR

Discharger: Sheridan Lab Number/s: K1201004
 Location: _____
 Date Sample Collected: _____

Analyst: _____
 Test Start - Date/Time: 7-19-12 1130
 Test Stop - Date/Time: 7-26-12 1500

Conc 1		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
MHS	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	2	3	2	3	4	3	1	0	2	2	22	10	2.2	
	4	4	3	0	3	0	2	4	5	4	0	25	10	2.5	
	5	7	0	5	4	3	6	6	10	8	5	54	10	5.4	
	6	6	6	10	9	7	7	8	0	7	11	71	10	7.1	
	7														
	8														
	Total	19	12	17	19	14	18	19	15	21	18	172		$\bar{X}=17.2$ $CV=159$	

Conc 4		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
11	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	3	0	4	3	2	1	4	0	0	17	10	1.7	
	4	4	5	3	2	0	6	6	2	4	0	44	10	4.4	
	5	7	6	4	8	6	4	11	2	8	11	67	10	6.7	
	6	5	2	7	13	9	10	0	10	5	11	72	10	7.2	
	7														
	8														
	Total	16	16	14	27	18	22	18	24	17	28	200			

Conc 2		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
6	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	1	3	3	3	4	3	1	0	18	10	1.8	
	4	4	3	4	3	0	2	4	6	5	4	35	10	3.5	
	5	5	4	5	4	3	10	8	0	8	6	53	10	5.3	
	6	0	9	4	3	10	4	7	9	9	7	62	10	6.2	
	7														
	8														
	Total	9	16	14	13	16	19	23	18	23	17	168			

Conc 5		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
14	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	3	0	4	1	2	X	2	3	0	1	16	9	1.8	
	4	0	5	3	7	4	-	6	0	9	3	42	9	4.7	
	5	5	10	3	5	8	-	11	11	8	3	64	9	7.1	
	6	9	8	11	10	9	-	8	14	3	13	85	9	9.4	
	7														
	8														
	Total	17	23	21	23	23	X	27	28	20	25	267		$\bar{X}=23.0$ $CV=14.9$	

Conc 3		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	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	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	2	1	1	3	1	0	1	2	2	0	13	10	1.3	
	4	7	6	3	0	9	5	4	7	4	4	46	10	4.6	
	5	7	4	10	7	2	7	9	4	5	8	63	10	6.3	
	6	4	9	11	11	12	8	7	8	7	9	88	10	8.8	
	7														
	8														
	Total	17	26	25	21	24	20	21	21	20	21	210			

Conc 6		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
19	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	X	0	2	1	0	2	0	2	3	2	13	9	1.4	
	4	-	5	8	4	8	5	5	0	5	10	50	9	5.6	
	5	-	7	7	9	9	4	10	5	11	0	62	9	6.9	
	6	-	10	2	8	0	6	14	12	14	15	81	9	9.0	
	7														
	8														
	Total	X	24	18	21	19	15	31	26	32	26	260			

X= DEAD; Y= MALE

AA # K1209004, C. DUBIA CHRONIC REPRODUCCION, 9-19-12
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 # K1209004, C. DUBIA CHRONIC REPRODUCCION, 9-19-12
File: Z:\TOXSTAT\MONTE\CD. Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 24.15

Table Chi-square value = 15.09 (alpha = 0.01, df = 5)
Table Chi-square value = 11.07 (alpha = 0.05, df = 5)

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

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%	9	1	10
TOTAL	19	1	20

CRITICAL FISHER'S VALUE (10,10,10) (p=0.05) IS 6. b VALUE IS 9.
 Since b is greater than 6 there is no significant difference
 between CONTROL and TREATMENT at the 0.05 level.

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
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	1	
5	19%	10	1	

TITLE: AA # K1209004, C. DUBIA CHRONIC REPRODUCTION, 9-19-12
FILE: Z:\TOXSTAT\MONTE\CD.
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	19.0000	19.0000
1	CONTROL	2	12.0000	12.0000
1	CONTROL	3	17.0000	17.0000
1	CONTROL	4	19.0000	19.0000
1	CONTROL	5	14.0000	14.0000
1	CONTROL	6	18.0000	18.0000
1	CONTROL	7	19.0000	19.0000
1	CONTROL	8	15.0000	15.0000
1	CONTROL	9	21.0000	21.0000
1	CONTROL	10	18.0000	18.0000
2	6 % EFFLUENT	1	9.0000	9.0000
2	6 % EFFLUENT	2	16.0000	16.0000
2	6 % EFFLUENT	3	14.0000	14.0000
2	6 % EFFLUENT	4	13.0000	13.0000
2	6 % EFFLUENT	5	16.0000	16.0000
2	6 % EFFLUENT	6	19.0000	19.0000
2	6 % EFFLUENT	7	23.0000	23.0000
2	6 % EFFLUENT	8	18.0000	18.0000
2	6 % EFFLUENT	9	23.0000	23.0000
2	6 % EFFLUENT	10	17.0000	17.0000
3	8 % EFFLUENT	1	17.0000	17.0000
3	8 % EFFLUENT	2	20.0000	20.0000
3	8 % EFFLUENT	3	25.0000	25.0000
3	8 % EFFLUENT	4	21.0000	21.0000
3	8 % EFFLUENT	5	24.0000	24.0000
3	8 % EFFLUENT	6	20.0000	20.0000
3	8 % EFFLUENT	7	21.0000	21.0000
3	8 % EFFLUENT	8	21.0000	21.0000
3	8 % EFFLUENT	9	20.0000	20.0000
3	8 % EFFLUENT	10	21.0000	21.0000
4	11 % EFFLUENT	1	16.0000	16.0000
4	11 % EFFLUENT	2	16.0000	16.0000
4	11 % EFFLUENT	3	14.0000	14.0000
4	11 % EFFLUENT	4	27.0000	27.0000
4	11 % EFFLUENT	5	18.0000	18.0000
4	11 % EFFLUENT	6	22.0000	22.0000
4	11 % EFFLUENT	7	18.0000	18.0000
4	11 % EFFLUENT	8	24.0000	24.0000

4	11	% EFFLUENT	9	17.0000	17.0000
4	11	% EFFLUENT	10	28.0000	28.0000
5	14	% EFFLUENT	1	17.0000	17.0000
5	14	% EFFLUENT	2	23.0000	23.0000
5	14	% EFFLUENT	3	21.0000	21.0000
5	14	% EFFLUENT	4	23.0000	23.0000
5	14	% EFFLUENT	5	23.0000	23.0000
5	14	% EFFLUENT	6	0.0000	0.0000
5	14	% EFFLUENT	7	27.0000	27.0000
5	14	% EFFLUENT	8	28.0000	28.0000
5	14	% EFFLUENT	9	20.0000	20.0000
5	14	% EFFLUENT	10	25.0000	25.0000
6	19	% EFFLUENT	1	0.0000	0.0000
6	19	% EFFLUENT	2	24.0000	24.0000
6	19	% EFFLUENT	3	18.0000	18.0000
6	19	% EFFLUENT	4	21.0000	21.0000
6	19	% EFFLUENT	5	19.0000	19.0000
6	19	% EFFLUENT	6	15.0000	15.0000
6	19	% EFFLUENT	7	31.0000	31.0000
6	19	% EFFLUENT	8	20.0000	20.0000
6	19	% EFFLUENT	9	32.0000	32.0000
6	19	% EFFLUENT	10	26.0000	26.0000

AA # K1209004, C. DUBIA CHRONIC REPRODUCCION, 9-19-12
 File: Z:\TOXSTAT\MONTE\CD. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	176.483	35.297	1.052
Within (Error)	54	1811.700	33.550	
Total	59	1988.183		

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

AA # K1209004, C. DUBIA CHRONIC REPRODUCCION, 9-19-12
 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	17.200	17.200		
2	6 % EFFLUENT	16.800	16.800	0.154	
3	8 % EFFLUENT	21.000	21.000	-1.467	
4	11 % EFFLUENT	20.000	20.000	-1.081	
5	14 % EFFLUENT	20.700	20.700	-1.351	
6	19 % EFFLUENT	20.600	20.600	-1.313	

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

AA # K1209004, C. DUBIA CHRONIC REPRODUCCION, 9-19-12
 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	5.984	34.8	0.400
3	8 % EFFLUENT	10	5.984	34.8	-3.800
4	11 % EFFLUENT	10	5.984	34.8	-2.800
5	14 % EFFLUENT	10	5.984	34.8	-3.500
6	19 % EFFLUENT	10	5.984	34.8	-3.400

AA # K1209004, C. DUBIA CHRONIC REPRODUCCION, 9-19-12
 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	17.200				
2	6 % EFFLUENT	16.800	99.50	75.00	10.00	
3	8 % EFFLUENT	21.000	143.50	75.00	10.00	
4	11 % EFFLUENT	20.000	116.00	75.00	10.00	
5	14 % EFFLUENT	20.700	137.00	75.00	10.00	
6	19 % EFFLUENT	20.600	128.50	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

TEST ORGANISM HISTORY

DATE SHIPPED 9/18/12 CLIENT AR Analytical

Purchase Order #: _____ Low

SPECIES: Pimephales promelas

Quantity Shipped: 1140

Age: hatched 9/17/12 15000g

Brood Stock Source: Anderson Farms, AR

Culture Water: Groundwater

Hardness (Mg/l CaCO3): 160

Dissolved Oxygen (Mg/l): 8.1

Temperature (°C): 25.1°C

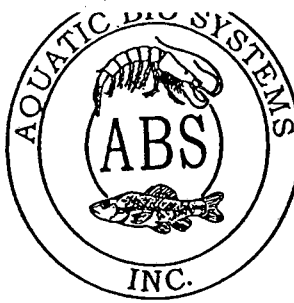
Feeding: Artemia

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:

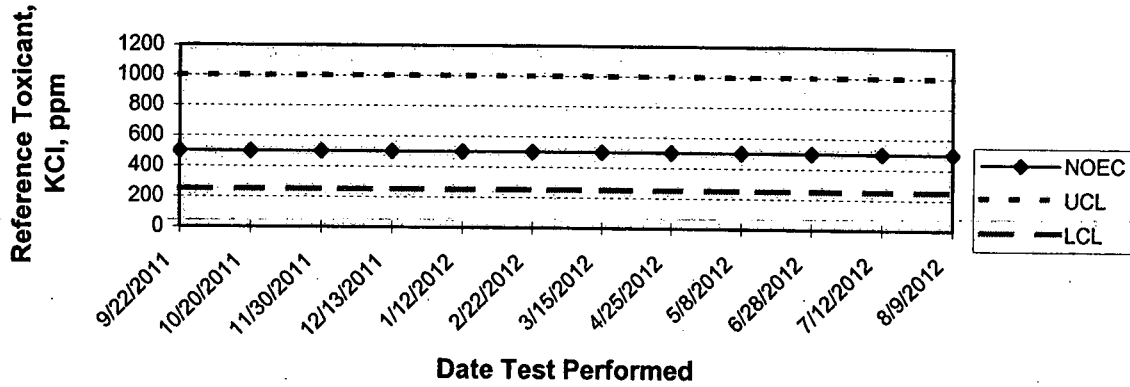


Facility Supervisor

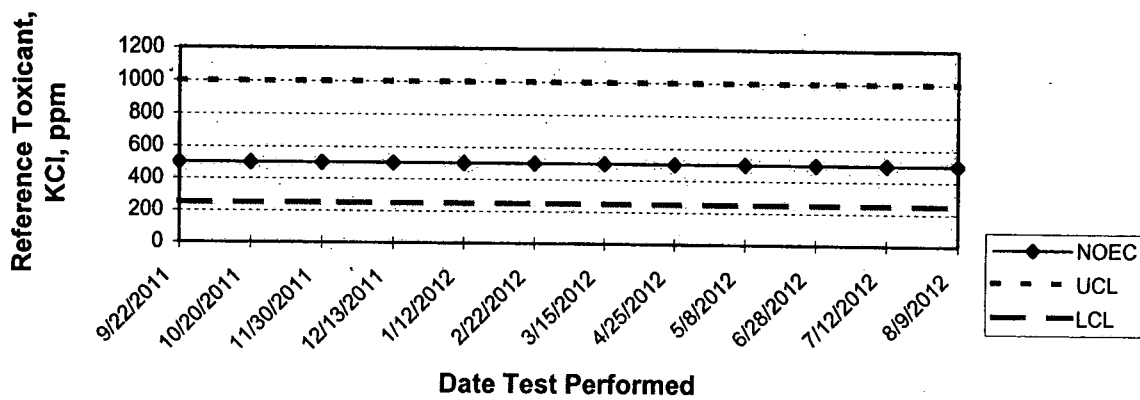
APPENDIX F

Quality Assurance Charts

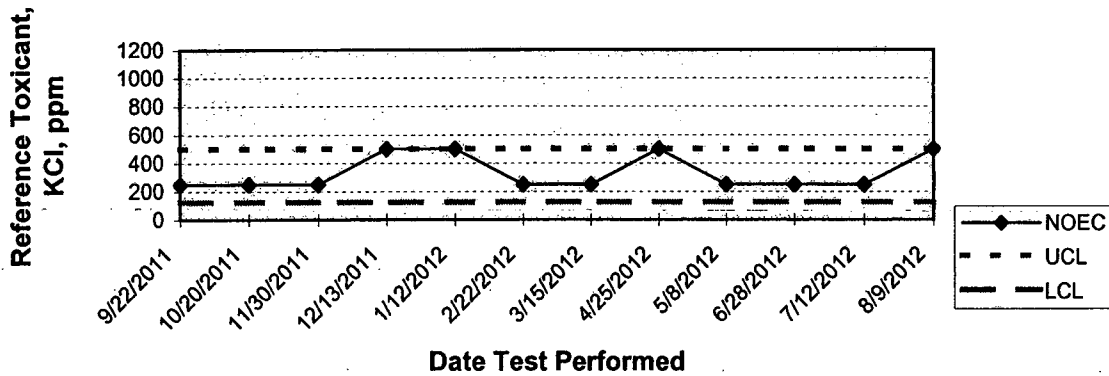
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW SURVIVAL
QUALITY ASSURANCE



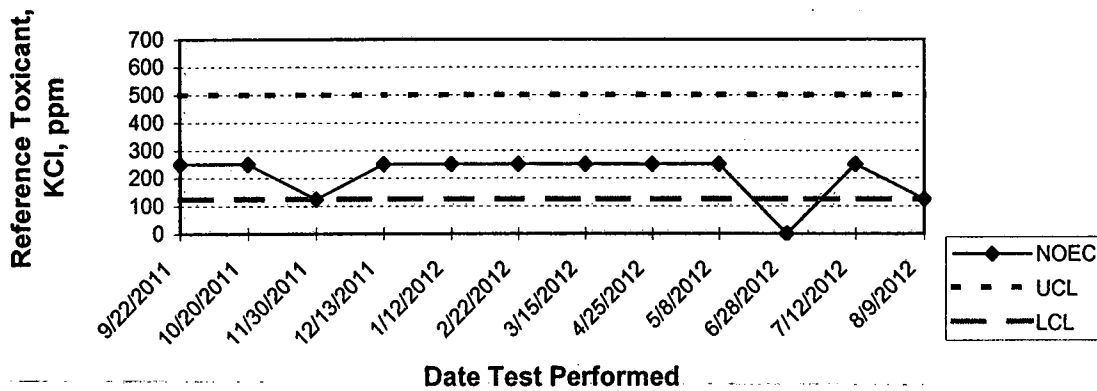
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW GROWTH
QUALITY ASSURANCE



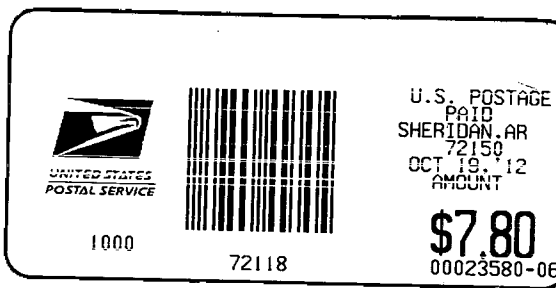
ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE



SHERIDAN WATER WORKS
PO BOX 486
SHERIDAN, AR 72150



RETURN RECEIPT
REQUESTED

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

