

Arkansas Analytical, Inc.

Toxicity Test Results

**MAGCOBAR MINE SITE
NPDES PERMIT NUMBER: AR0049794
January, 2011
AFIN# 00-00348**

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 Friedman**
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Monday, January 31, 2011

Introduction

This report contains test results for toxicity testing for the Magcobar Mine Site. The NPDES permit number is AR0049794. The facility is located one mile northeast of Magnet Cove in Sections 10, 11, 14, & 15, Township 3 South, Range 17 West in Hot Springs County, Arkansas. The facility discharges into Chamberlain Creek, thence to Cove Creek, thence to Ouachita River in Segment 2F of the Ouachita River Basin.

The permit requires chronic biomonitoring testing bi-monthly for both *Ceriodaphnia dubia* and *Pimephales promelas*. The test results in this report represent the testing for January of 2011.

Plant Operations

To be provided by permittee.

Source of Effluent and Dilution Water

Effluent samples were collected as follows:

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	1-12-11, 0905	1-13-11, 0905
Sample #2:	1-13-11, 1030	1-14-11, 1030
Sample #3:	1-17-11, 0840	1-18-11, 0840

The samples were composites collected at the final discharge from the Magcobar mine site.

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:	1-13-11, 1441	3
Sample #2:	1-14-11, 1507	4
Sample #3:	1-18-11, 1318	3

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 either because zero flow conditions existed or due to an earlier characterization of the receiving water as being toxic.

Each sample was analyzed for pH, hardness, total alkalinity, and conductivity. Results are provided in Appendix B.

Dilution Series

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

Test Methods

EPA Method 1000.0, Fathead Minnow, *Pimephales promelas*, Larval Survival and Growth Test, was used in this bioassay. Larvae are exposed in a static renewal system for seven days and the results are based on the survival and growth (increase in weight) of the larvae. The alternate method suggested in the method (11.3.4.5) for combating pathogen interference, was run in place of the original fathead minnow test. The test chambers were 30 ml plastic cups with 20 ml of test solution. Each chamber contained 2 organisms. The total number of fish was 40 per test solution. The fish were then combined to perform growth analysis. The test temperature was 25 degrees Centigrade. Raw data and statistics are provided in Appendix C.

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

Test Organisms

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

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

Quality Assurance

Test Acceptability

TEST ACCEPTANCE CRITERIA for *Ceriodaphnia dubia*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	100%	X	
Average of 15 or more young per surviving female	16.1	X	
At least 60% of surviving females should have produced 3 broods	80%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	18.6%	X	

TEST ACCEPTANCE CRITERIA for *Pimephales promelas*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	100%	X	
The percent coefficient of variation between replicates must be 40% or less for survival	0.00%	X	
Minimum of 0.25 mg average dry weight of surviving controls	0.322	X	
The percent coefficient of variation between replicates must be 40% or less for growth	8.56%	X	

Reference Toxicant

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

REFERENCE TOXICANT

<i>Ceriodaphnia dubia</i> 12/16-23/10		<i>Pimephales promelas</i> 12/16-23/10	
NOEC Survival:	250 ppm KCl	NOEC Survival:	500 ppm KCl
LOEC Survival:	500 ppm KCl	LOEC Survival:	1000 ppm KCl
NOEC Reproduction:	250 ppm KCl	NOEC 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

Magcobar Mine Site

<i>Ceriodaphnia dubia</i>		<i>Pimephales promelas</i>	
NOEC / LOEC Survival	100% / NA	NOEC / LOEC survival	100% / NA
NOEC / LOEC Reproduction	100% / NA	NOEC / LOEC growth	100% / NA
Mean number of neonates (critical dilution)	17.3	%CV survival (critical dilution)	0.00 %
%CV Reproduction (critical dilution)	25.4%	Mean dry weight (critical dilution) in milligrams	0.518
		%CV growth (critical dilution)	6.20%
PMSD Reproduction	30.3	PMSD Growth	21.5

Conclusion

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

The permit issued to the Magcobar Mine Site, AR0049794, specifies that the **critical dilution is 100% 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 Magcobar Mine Site, AR0049794, specifies that the **critical dilution is 100% effluent**. The effluent samples did not exhibit lethal or sublethal effects at the critical dilution, and, as such, **passed** both the portions of the test.

Biomonitoring Analysts:


Ken Pigue
Allen Parker

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

PERMITTEE: Magcobar Mine Site

NPDES #: AR0049794

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	1-12-11, 0905	1-13-11, 0905
Sample #2:	1-13-11, 1030	1-14-11, 1030
Sample #3:	1-17-11, 0840	1-18-11, 0840

Test initiated (date, time): 1-14-11, 1110 Test terminated (date, time): 1-21-11, 0830

Dilution water used: Soft Synthetic

DATA TABLE FOR FATHEAD MINNOW SURVIVAL

Percent Survival in Replicate Chambers	Mean Percent Survival
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DATA TABLE FOR GROWTH OF FATHEAD MINNOWS

Effluent Conc %	A	B	C	D	E		24 hours	48 hours	7 days	CV %
0%	100	100	100	100	100		100	100	100	0.00
32%	100	100	100	100	100		100	100	100	
42%	100	100	100	100	100		100	100	100	
56%	100	100	100	100	100		100	100	100	
75%	100	100	100	100	100		100	100	100	
100%	100	100	100	100	100		100	100	100	0.00

SUMMARY

Effluent Conc %	A	B	C	D	E		Mean Dry Weight	CV%
0%	0.309	0.293	0.367	0.319	0.325		0.323	8.56
32%	0.414	0.422	0.452	0.459	0.484		0.446	
42%	0.420	0.429	0.438	0.502	0.566		0.471	
56%	0.400	0.439	0.539	0.563	0.409		0.470	
75%	0.446	0.446	0.439	0.469	0.577		0.475	
100%	0.466	0.537	0.509	0.536	0.544		0.518	6.20

Coefficient of Variation = standard deviation / mean * 100

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, (100%) YES _____ NO X _____
2. Dunnett's Procedure
Is the mean dry weight (growth) at 7 days significantly different ($p=0.05$) than the control's dry weight (growth) for:
a) LOW FLOW OR CRITICAL DILUTION, (100%) YES _____ NO X _____
3. If NO was answered to 1.a) 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)= _____ 100 _____ % effluent
b) NOEC growth (parameter TPP6C)= _____ 100 _____ % effluent
c) Coefficient of variation (parameter TQP6C)= _____ 8.56 _____ %

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING
Ceriodaphnia dubia SURVIVAL AND REPRODUCTION

Permittee: Magcobar Mine Site

NPDES #: AR0049794

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	1-12-11, 0905	1-13-11, 0905
Sample #2:	1-13-11, 1030	1-14-11, 1030
Sample #3:	1-17-11, 0840	1-18-11, 0840

Test initiated (date, time): 1-14-11, 1045 Test terminated (date, time): 1-21-11, 0815

Dilution water used: Soft Synthetic

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

Replicate	0%	32%	42%	56%	75%	100%
A	19	20	18	17	27	23
B	16	14	24	9	21	20
C	20	19	23	19	18	23
D	10	14	22	23	17	19
E	16	17	16	14	16	13
F	19	20	20	19	11	9
G	15	23	16	21	x0	16
H	16	10	10	21	22	16
I	17	14	14	13	17	15
J	13	19	20	14	17	19
Mean	16.1	17.0	18.3	17.0	16.6	17.3
Mean/surviving female	16.1	17.0	18.3	17.0	18.4	17.3
CV%*	18.6					25.4

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: Magcobar Mine Site

NPDES #: AR0049794

PERCENT SURVIVAL

PERCENT EFFLUENT	0%	32%	42%	56%	75%	100%
Time of Reading: 24 HOURS	100	100	100	100	90	100
48 HOURS	100	100	100	100	90	100
Test termination	100	100	100	100	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, (100%): YES _____ NO **X** _____

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

Is the mean number of young produced per female significantly different ($p=0.05$) than the controls number of young per female for:

a) LOW FLOW OR CRITICAL DILUTION, (100%): 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)= **100** % effluent

b) NOEC reproduction (parameter TPP3B)= **100** % effluent

c) Coefficient of variation (parameter TQP3B)= **25.4** %

APPENDIX A

Chain of Custody Forms



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

CHAIN OF CUSTODY FORM



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CHAIN OF CUSTODY FORM

APPENDIX B

Effluent and Dilution Water Data

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING								Fathead Minnow	
Lab # / Sample ID		K101004			Test Start (Date/Time)		1/14/11		
Client:		Weston			Test End (Date/Time)		1/21/11		
Day of Test									
		1	2	3	4	5	6	7	notes/remarks
Control	MHS551	1/14	1/15	1/16	1/17	1/18	1/19	1/20	
D.O. (mg/L)	INITIAL	78	8.3	8.6	8.2	87	86	85	
	FINAL	8.2	8.1	7.5	7.6	7.6	7.6	8.3	
pH (s.u.)	INITIAL	7.9	8.0	7.9	7.9	8.0	7.9	7.7	
	FINAL	7.6	7.5	7.4	7.8	7.7	7.6	7.7	
temp (C)	INITIAL	20.8	21.4	20.5	21.6	22.4	22.5	22.4	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
ALKALINITY (mg/L)		36							
HARDNESS (mg/L)		44							
CONDUCTIVITY (umhos/cm)		173							
CHLORINE (mg/L)		<0.05							
CONC:									
D.O. (mg/L)	INITIAL	7.9	8.7	8.9	8.6	8.6	8.6	8.5	
	FINAL	7.9	7.6	7.5	7.7	7.5	7.4	8.3	
pH (s.u.)	INITIAL	7.5	7.6	7.7	7.5	7.8	7.7	7.3	
	FINAL	7.3	8.0	7.4	7.5	7.5	7.4	7.7	
temp (C)	INITIAL	20.4	22.4	20.8	21.9	23.1	23.2	22.8	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.0	8.6	8.9	8.8	8.7	8.7	8.5	
	FINAL	8.0	7.7	7.5	7.6	7.5	7.7	8.3	
pH (mg/L)	INITIAL	7.5	7.7	7.7	7.5	7.6	7.7	7.4	
	FINAL	7.3	7.4	7.2	7.6	7.5	7.4	7.4	
temp (C)	INITIAL	20.2	23.2	21.1	22.1	23.2	23.3	22.8	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.0	8.6	8.9	8.8	8.6	8.7	8.6	
	FINAL	7.9	7.7	7.5	7.6	7.5	7.7	8.3	
pH (s.u.)	INITIAL	7.5	7.7	7.7	7.5	7.6	7.7	7.4	
	FINAL	7.5	7.4	7.2	7.6	7.3	7.5	7.7	
temp (C)	INITIAL	20.5	23.8	21.5	21.3	23.4	23.6	23.3	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.2	8.5	8.9	8.8	8.2	8.8	8.2	
	FINAL	8.1	7.7	7.6	7.6	7.5	7.6	8.3	
pH (s.u.)	INITIAL	7.6	7.7	7.7	7.5	7.6	7.7	7.4	
	FINAL	7.6	7.4	7.2	7.5	7.2	7.2	7.6	
temp (C)	INITIAL	20.4	24.5	21.6	22.3	23.7	23.9	23.6	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.7	8.9	8.8	8.6	8.8	8.9	
	FINAL	8.0	7.6	7.5	7.6	7.4	7.6	8.4	
pH (s.u.)	INITIAL	7.6	7.7	7.7	7.5	7.5	7.6	7.3	
	FINAL	7.6	7.3	7.1	7.5	7.2	7.6	7.5	
temp (C)	INITIAL	20.2	25.4	22.0	22.7	24.1	24.4	23.9	
	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)		190 ± 18	+1	-1	20	-1	20	-1	
HARDNESS (mg/L)		58 ± 60	+1	-1	7600	-1	7600	-1	
CONDUCTIVITY (umhos/cm)		5891 ± 2100	+1	-1	7120	-1	7120	-1	
CHLORINE (mg/L)		<0.05	+1	-1	<0.05	-1	<0.05	-1	

Revision 1

11/30/10

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING								Cerodaphnia Dubia
Lab # / Sample ID			Test Start (Date/Time)					1/24/11
Client: Weston			Test End (Date/Time)					1/21/11
			Day of Test					
			1	2	3	4	5	notes/remarks
Control	MHS551		1/14	1/15	1/16	1/17	1/18	1/19
D.O. (mg/L)	INITIAL	78	8.8	8.6	8.2	8.7	8.6	8.5
	FINAL	8.3	8.4	8.3	8.2	8.9	8.9	8.9
pH (s.u.)	INITIAL	7.9	8.0	7.9	7.9	8.0	7.9	7.7
	FINAL	7.7	7.8	7.8	7.9	8.0	7.9	7.9
temp (C)	INITIAL	20.8	21.4	20.5	21.6	20.4	22.5	22.4
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0
ALKALINITY (mg/L)		36						1
HARDNESS (mg/L)		44						1
CONDUCTIVITY (umhos/cm)		173						1
CHLORINE (mg/L)		<0.05						1
CONC:								
D.O. (mg/L)	INITIAL	7.9	8.7	8.9	8.6	8.6	8.6	8.5
	FINAL	8.3	8.4	8.2	8.2	8.9	8.9	8.7
pH (s.u.)	INITIAL	7.5	7.6	7.7	7.5	7.8	7.7	7.3
	FINAL	7.6	7.7	7.6	7.2	7.4	7.5	7.5
temp (C)	INITIAL	20.4	22.4	20.8	21.9	23.1	23.2	22.8
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0
CONC:								
D.O. (mg/L)	INITIAL	8.0	8.6	8.9	8.8	8.7	8.7	8.5
	FINAL	8.3	8.3	8.3	8.1	8.9	8.0	8.8
pH (mg/L)	INITIAL	7.5	7.7	7.7	7.5	7.6	7.7	7.9
	FINAL	7.6	7.7	7.7	7.4	7.4	7.5	7.6
temp (C)	INITIAL	20.2	23.2	21.1	22.1	23.2	23.3	22.8
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0
CONC:								
D.O. (mg/L)	INITIAL	8.0	8.6	8.9	8.8	8.6	8.7	8.6
	FINAL	8.3	8.3	8.2	8.0	8.1	8.0	8.7
pH (s.u.)	INITIAL	7.5	7.7	7.7	7.5	7.6	7.7	7.4
	FINAL	7.6	7.6	7.7	7.5	7.4	7.5	7.5
temp (C)	INITIAL	20.3	23.8	21.5	21.3	23.4	23.6	23.3
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0
CONC:								
D.O. (mg/L)	INITIAL	8.2	8.5	8.9	8.8	8.2	8.3	8.8
	FINAL	8.3	8.4	8.2	8.1	8.1	8.6	8.7
pH (s.u.)	INITIAL	7.6	7.7	7.7	7.5	7.5	7.6	7.4
	FINAL	7.6	7.6	7.6	7.5	7.5	7.6	7.5
temp (C)	INITIAL	20.4	24.5	21.6	22.3	23.7	23.9	23.6
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0
CONC:								
D.O. (mg/L)	INITIAL	8.6	8.7	8.9	8.8	8.6	8.8	8.9
	FINAL	8.3	8.4	8.2	8.1	8.1	8.0	8.8
pH (s.u.)	INITIAL	7.6	7.7	7.7	7.5	7.5	7.6	7.3
	FINAL	7.5	7.5	7.6	7.5	7.5	7.6	7.4
temp (C)	INITIAL	20.2	25.4	22.0	22.7	24.1	24.4	23.9
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0
CONC: 100%								
ALKALINITY (mg/L)		18			20		20	1
HARDNESS (mg/L)		2606			2600		2600	1
CONDUCTIVITY (umhos/cm)		2100			2120		2120	1
CHLORINE (mg/L)		<0.05			<0.05		<0.05	1

APPENDIX C

Fathead minnow raw data and statistics

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID	K101004	TEST START DATE	11/11/11	TIME	1110						
CLIENT	Weston	TEST END DATE	11/11/11	TIME	0830						
AGE AND SOURCE OF MINNOWS											
CONC:	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC: 100	A	8	8	8	8	8	8	8	100		
	B	1	1	1	1	1	1	1	100		
	C	1	1	1	1	1	1	1	100		
	D	1	1	1	1	1	1	1	100		
	E	1	1	1	1	1	1	1	100		
CONC: 30	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	8	8	8	8	8	8	8	100		
	B	1	1	1	1	1	1	1	100		
	C	1	1	1	1	1	1	1	100		
	D	1	1	1	1	1	1	1	100		
	E	1	1	1	1	1	1	1	100		
CONC: 40	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	8	8	8	8	8	8	8	100		
	B	1	1	1	1	1	1	1	100		
	C	1	1	1	1	1	1	1	100		
	D	1	1	1	1	1	1	1	100		
	E	1	1	1	1	1	1	1	100		
CONC: 50	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	8	8	8	8	8	8	8	100		
	B	1	1	1	1	1	1	1	100		
	C	1	1	1	1	1	1	1	100		
	D	1	1	1	1	1	1	1	100		
	E	1	1	1	1	1	1	1	100		
CONC: 75	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	8	8	8	8	8	8	8	100		
	B	1	1	1	1	1	1	1	100		
	C	1	1	1	1	1	1	1	100		
	D	1	1	1	1	1	1	1	100		
	E	1	1	1	1	1	1	1	100		
CONC: 100	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	8	8	8	8	8	8	8	100		
	B	1	1	1	1	1	1	1	100		
	C	1	1	1	1	1	1	1	100		
	D	1	1	1	1	1	1	1	100		
	E	1	1	1	1	1	1	1	100		
ANALYST		X P									
DATE:											
TIME:											

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID	K1101064	TEST START DATE	1/14/11	TIME	11:00				
CLIENT	Weston	TEST END DATE	1/21/11	TIME	0830				
AGE AND SOURCE OF MINNOWS									
A									
			DAY	(NUMBER SURVIVING)			SURVIVAL		
CONC:	REP #	start	1	2	3	4	5	6	7 %
0	A	2	2	2	2	2	2	2	
	B	1	1	1	1	1	1	1	
	C	1	1	1	1	1	1	1	
	D	1	1	1	1	1	1	1	
	E	1	1	1	1	1	1	1	
CONC: 5%	REP #	start	1	2	3	4	5	6	7 %
5%	A	2	2	2	2	2	2	2	
	B	1	1	1	1	1	1	1	
	C	1	1	1	1	1	1	1	
	D	1	1	1	1	1	1	1	
	E	1	1	1	1	1	1	1	
CONC: 4%	REP #	start	1	2	3	4	5	6	7 %
4%	A	2	2	2	2	2	2	2	
	B	1	1	1	1	1	1	1	
	C	1	1	1	1	1	1	1	
	D	1	1	1	1	1	1	1	
	E	1	1	1	1	1	1	1	
CONC: 5%	REP #	start	1	2	3	4	5	6	7 %
5%	A	2	2	2	2	2	2	2	
	B	1	1	1	1	1	1	1	
	C	1	1	1	1	1	1	1	
	D	1	1	1	1	1	1	1	
	E	1	1	1	1	1	1	1	
CONC: 75%	REP #	start	1	2	3	4	5	6	7 %
75%	A	2	2	2	2	2	2	2	
	B	1	1	1	1	1	1	1	
	C	1	1	1	1	1	1	1	
	D	1	1	1	1	1	1	1	
	E	1	1	1	1	1	1	1	
CONC: 100%	REP #	start	1	2	3	4	5	6	7 %
100%	A	2	2	2	2	2	2	2	
	B	1	1	1	1	1	1	1	
	C	1	1	1	1	1	1	1	
	D	1	1	1	1	1	1	1	
	E	1	1	1	1	1	1	1	
ANALYST		KP	AP	AP	KP	KP	KP	KP	
DATE:		1/14/11	1/15/11	1/16/11	1/17/11	1/18/11	1/19/11	1/20/11	1/21/11
TIME:		11:00	1000	1030	1040	11:15	11:30	0926	0830

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											
	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC:	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C										
	D	1	1	1	1	1	1	1	1		
	E										
CONC: 32	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C										
	D	1	1	1	1	1	1	1	1		
	E										
CONC: 42	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C										
	D	1	1	1	1	1	1	1	1		
	E										
CONC: 51	A	2	2	3	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C										
	D	1	1	1	1	1	1	1	1		
	E										
CONC: 75	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C										
	D	1	1	1	1	1	1	1	1		
	E										
CONC: 100	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C										
	D	1	1	1	1	1	1	1	1		
	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	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		
CONC: 20	A	2	2	3	2	2	1	1	1	1	1		
	B	1	1	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1	1	1	1		
CONC: 30	A	2	2	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1	1	1	1		
CONC: 40	A	2	2	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1	1	1	1		
CONC: 50	A	2	2	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1	1	1	1		
CONC: 75	A	2	2	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1	1	1	1		
CONC: 100	A	2	2	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1	1	1	1		
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	TEST END DATE		TIME	AGE AND SOURCE OF MINNOWS						
							DAY (NUMBER SURVIVING)						
CONC:	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV		
CONC: D	A	7	7	7	2	7	7	7	7				
	B	1	1	1	1	1	1	1					
	C	1	1	1	1	1	1	1					
	D	1	1	1	1	1	1	1					
	E	1	1	1	1	1	1	1					
CONC: 32	A	7	7	7	2	2	7	7	7				
	B	1	1	1	1	1	1	1					
	C	1	1	1	1	1	1	1					
	D	1	1	1	1	1	1	1					
	E	1	1	1	1	1	1	1					
CONC: 40	A	2	7	7	2	2	2	2	2				
	B	1	1	1	1	1	1	1					
	C	1	1	1	1	1	1	1					
	D	1	1	1	1	1	1	1					
	E	1	1	1	1	1	1	1					
CONC: 56	A	2	7	2	2	2	2	2	2				
	B	1	1	1	1	1	1	1					
	C	1	1	1	1	1	1	1					
	D	1	1	1	1	1	1	1					
	E	1	1	1	1	1	1	1					
CONC: 75	A	2	2	2	7	2	2	2	2				
	B	1	1	1	1	1	1	1					
	C	1	1	1	1	1	1	1					
	D	1	1	1	1	1	1	1					
	E	1	1	1	1	1	1	1					
CONC: 100	A	2	2	2	2	2	2	2	2				
	B	1	1	1	1	1	1	1					
	C	1	1	1	1	1	1	1					
	D	1	1	1	1	1	1	1					
	E	1	1	1	1	1	1	1					
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	TEST END DATE		TIME	AGE AND SOURCE OF MINNOWS							
CLIENT														
									SURVIVAL					
REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV			
CONC: E	A	2	2	2	2	2	2	2						
	B	1	1	1	1	1	1	1						
	C	1	1	1	1	1	1	1						
	D	1	1	1	1	1	1	1						
	E	1	1	1	1	1	1	1						
CONC: 3L	A	2	2	2	2	2	2	2						
	B	1	1	1	1	1	1	1						
	C	1	1	1	1	1	1	1						
	D	1	1	1	1	1	1	1						
	E	1	1	1	1	1	1	1						
CONC: 4L	A	2	2	2	2	2	2	2						
	B	1	1	1	1	1	1	1						
	C	1	1	1	1	1	1	1						
	D	1	1	1	1	1	1	1						
	E	1	1	1	1	1	1	1						
CONC: 5L	A	2	2	2	2	2	2	2						
	B	1	1	1	1	1	1	1						
	C	1	1	1	1	1	1	1						
	D	1	1	1	1	1	1	1						
	E	1	1	1	1	1	1	1						
CONC: 75	A	2	2	2	2	2	2	2						
	B	1	1	1	1	1	1	1						
	C	1	1	1	1	1	1	1						
	D	1	1	1	1	1	1	1						
	E	1	1	1	1	1	1	1						
CONC: 100	A	4	2	2	2	2	2	2						
	B	1	1	1	1	1	1	1						
	C	1	1	1	1	1	1	1						
	D	1	1	1	1	1	1	1						
	E	1	1	1	1	1	1	1						
ANALYST														
DATE:														
TIME:														

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

Pimephales promelas

FATHEAD MINNOW

TEST 1000.0

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:		TEST DATES (BEGIN / END): 6/17-24/10									
CLIENT:		WEIGHING DATE / TIME: 6/25/10, 1400									
ANALYSTS:		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	1.02880	1.02633	0.00247	8	0.309					
	B	1.03898	1.03664	0.00234	8	0.293					
	C	1.00948	1.00654	0.00294	8	0.367					
	D	1.01715	1.01460	0.00255	8	0.319					
	E	1.02657	1.02397	0.00260	8	0.325					
CONC:	A	1.01820	1.01489	0.00331	8	0.414					
32%	B	0.99886	0.99548	0.00338	8	0.422					
	C	1.02298	1.01936	0.00362	8	0.452					
	D	1.00772	1.00405	0.00367	8	0.459					
	E	1.00944	1.00557	0.00387	8	0.484					
						CV					
CONC:	A	1.02615	1.02279	0.00336	8	0.420					
42%	B	1.02908	1.02565	0.00343	8	0.429					
	C	1.02138	1.01788	0.00350	8	0.438					
	D	1.04937	1.04535	0.00402	8	0.502					
	E	1.00452	0.99999	0.00453	8	0.566					
						CV					
CONC:	A	1.01740	1.01420	0.00320	8	0.400					
56%	B	1.01022	1.00671	0.00351	8	0.439					
	C	1.02974	1.02543	0.00431	8	0.539					
	D	1.01086	1.00636	0.00450	8	0.563					
	E	1.00359	1.00032	0.00327	8	0.409					
						CV					
CONC:	A	1.01229	1.00872	0.00357	8	0.446					
75%	B	1.00879	1.00522	0.00357	8	0.446					
	C	0.99848	0.99497	0.00351	8	0.439					
	D	1.00067	0.99692	0.00375	8	0.469					
	E	1.03514	1.03052	0.00462	8	0.577					
						CV					
CONC:	A	0.99165	0.98792	0.00373	8	0.466					
100%	B	1.02293	1.01863	0.00430	8	0.537					
	C	1.02981	1.02574	0.00407	8	0.509					
	D	0.99385	0.98956	0.00429	8	0.536					
	E	0.99813	0.99378	0.00435	8	0.544					
						CV					
CV = (STANDARD DEVIATION/MEAN)*100											
REMARKS:											

Pimephales promelas

FATHEAD MINNOW

TEST 1000.0

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:	K101004			TEST DATES (BEGIN / END):
CLIENT:	Weston			WEIGHING DATE / TIME:
ANALYSTS:	KP			DRYING TEMP (DEGREES C):
SAMPLE ID:				DRYING TIME (HOURS):
REP#	FINAL DRY WEIGHT TIN+LARVAE (g)	INTIAL WEIGHT TIN (g)	TOTAL DRY WEIGHT OF LARVAE (g)	NUMBER OF LARVAE (mg)
CONTROL	A 1 1.02880	1.02633		DRY WEIGHT OF LARVAE (mg)
	B 2 1.03898	1.03164		AVG DRY WEIGHT (mg)
	C 3 1.00948	1.00654		
	D 4 1.01715	1.01460		CV
	E 5 1.02657	1.02397		
CONC:	A 6 1.01820	1.01489		AVG DRY WEIGHT (mg)
	B 7 0.99886	0.99548		
	C 8 1.02298	1.01936		
	D 9 1.00772	1.00405		CV
	E 10 1.00944	1.00557		
CONC:	A 11 1.02615	1.02279		AVG DRY WEIGHT (mg)
	B 12 1.02908	1.02565		
	C 13 1.02138	1.01788		
	D 14 1.04937	1.04535		CV
	E 15 1.00452	0.99999		
CONC:	A 16 1.01740	1.01420		AVG DRY WEIGHT (mg)
	B 17 1.01022	1.00671		
	C 18 1.02974	1.02543		
	D 19 1.01086	1.00636		CV
	E 20 1.00359	1.00032		
CONC:	A 21 1.01229	1.00872		AVG DRY WEIGHT (mg)
	B 22 1.00879	1.00522		
	C 23 0.99848	0.99497		
	D 24 1.00067	0.99692		CV
	E 25 1.03514	1.03052		
CONC:	A 26 0.99165	0.98792		AVG DRY WEIGHT (mg)
	B 27 1.02293	1.01863		
	C 28 1.01981	1.02574		
	D 29 0.99385	0.98956		CV
	E 30 0.99813	0.99378		

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA# K1101004, FATHEAD MINNOW SURVIVAL, CHRONIC, 1-14-11
File: Z:\TOXSTAT\MONTE\FHSURV. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.000

W = 0.000

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# K1101004, FATHEAD MINNOW SURVIVAL, CHRONIC, 1-14-11
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# K1101004, FATHEAD MINNOW SURVIVAL, CHRONIC, 1-14-11
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	1.0000	1.3931
2	32 % EFFLUENT	1	1.0000	1.3931
2	32 % EFFLUENT	2	1.0000	1.3931
2	32 % EFFLUENT	3	1.0000	1.3931
2	32 % EFFLUENT	4	1.0000	1.3931
2	32 % EFFLUENT	5	1.0000	1.3931

3	42 %	EFFLUENT	1	1.0000	1.3931
3	42 %	EFFLUENT	2	1.0000	1.3931
3	42 %	EFFLUENT	3	1.0000	1.3931
3	42 %	EFFLUENT	4	1.0000	1.3931
3	42 %	EFFLUENT	5	1.0000	1.3931
4	56 %	EFFLUENT	1	1.0000	1.3931
4	56 %	EFFLUENT	2	1.0000	1.3931
4	56 %	EFFLUENT	3	1.0000	1.3931
4	56 %	EFFLUENT	4	1.0000	1.3931
4	56 %	EFFLUENT	5	1.0000	1.3931
5	75 %	EFFLUENT	1	1.0000	1.3931
5	75 %	EFFLUENT	2	1.0000	1.3931
5	75 %	EFFLUENT	3	1.0000	1.3931
5	75 %	EFFLUENT	4	1.0000	1.3931
5	75 %	EFFLUENT	5	1.0000	1.3931
6	100 %	EFFLUENT	1	1.0000	1.3931
6	100 %	EFFLUENT	2	1.0000	1.3931
6	100 %	EFFLUENT	3	1.0000	1.3931
6	100 %	EFFLUENT	4	1.0000	1.3931
6	100 %	EFFLUENT	5	1.0000	1.3931

AA# K1101004, FATHEAD MINNOW SURVIVAL, CHRONIC, 1-14-11
 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	1.393				
2	32 % EFFLUENT	1.393	27.50	16.00	5.00	
3	42 % EFFLUENT	1.393	27.50	16.00	5.00	
4	56 % EFFLUENT	1.393	27.50	16.00	5.00	
5	75 % EFFLUENT	1.393	27.50	16.00	5.00	
6	100 % EFFLUENT	1.393	27.50	16.00	5.00	

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

AA# K1101004, FATHEAD MINNOW GROWTH CHRONIC, 1-14-11
File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.063

W = 0.929

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# K1101004, FATHEAD MINNOW GROWTH CHRONIC, 1-14-11
File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 6.48

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# K1101004, FATHEAD MINNOW GROWTH CHRONIC, 1-14-11
FILE: Z:\TOXSTAT\MONTE\FHGR.
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.3090	0.5894
1	CONTROL	2	0.2930	0.5720
1	CONTROL	3	0.3670	0.6508
1	CONTROL	4	0.3190	0.6002
1	CONTROL	5	0.3250	0.6066
2	32 % EFFLUENT	1	0.4140	0.6990
2	32 % EFFLUENT	2	0.4220	0.7071
2	32 % EFFLUENT	3	0.4520	0.7373
2	32 % EFFLUENT	4	0.4590	0.7444
2	32 % EFFLUENT	5	0.4840	0.7694
3	42 % EFFLUENT	1	0.4200	0.7051
3	42 % EFFLUENT	2	0.4290	0.7142
3	42 % EFFLUENT	3	0.4380	0.7232
3	42 % EFFLUENT	4	0.5020	0.7874
3	42 % EFFLUENT	5	0.5660	0.8516
4	56 % EFFLUENT	1	0.4000	0.6847

4	56 %	EFFLUENT	2	0.4390	0.7242
4	56 %	EFFLUENT	3	0.5390	0.8244
4	56 %	EFFLUENT	4	0.5630	0.8486
4	56 %	EFFLUENT	5	0.4090	0.6939
5	75 %	EFFLUENT	1	0.4460	0.7313
5	75 %	EFFLUENT	2	0.4460	0.7313
5	75 %	EFFLUENT	3	0.4390	0.7242
5	75 %	EFFLUENT	4	0.4690	0.7544
5	75 %	EFFLUENT	5	0.5770	0.8627
6	100 %	EFFLUENT	1	0.4660	0.7514
6	100 %	EFFLUENT	2	0.5370	0.8224
6	100 %	EFFLUENT	3	0.5090	0.7944
6	100 %	EFFLUENT	4	0.5360	0.8214
6	100 %	EFFLUENT	5	0.5440	0.8295

AA# K1101004, FATHEAD MINNOW GROWTH CHRONIC, 1-14-11
 File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.117	0.023	8.929
Within (Error)	24	0.063	0.003	
Total	29	0.181		

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

AA# K1101004, FATHEAD MINNOW GROWTH CHRONIC, 1-14-11
 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 CALCULATED IN	T STAT	SIG
		MEAN	ORIGINAL UNITS		
1	CONTROL	0.604	0.323		
2	32 % EFFLUENT	0.731	0.446	-3.935	
3	42 % EFFLUENT	0.756	0.471	-4.701	
4	56 % EFFLUENT	0.755	0.470	-4.667	
5	75 % EFFLUENT	0.761	0.475	-4.840	
6	100 % EFFLUENT	0.804	0.518	-6.166	

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

AA# K1101004, FATHEAD MINNOW GROWTH CHRONIC, 1-14-11
 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	32 % EFFLUENT	5	0.069	21.5	-0.124
3	42 % EFFLUENT	5	0.069	21.5	-0.148
4	56 % EFFLUENT	5	0.069	21.5	-0.147
5	75 % EFFLUENT	5	0.069	21.5	-0.153
6	100 % EFFLUENT	5	0.069	21.5	-0.196

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

Cerodaphnia dubia

Discharger: Weston
Location: K1101004

SURVIVAL AND REPRODUCTION TEST

Analyst: KPTest Start - Date/ Time: 1/14/11 1045Test Stop - Date/ Time: 1/21/11 0815

Date Sample Collected:

Conc 1		Replicate										No. of Young	No. of Adult	Young/ Adult	Analyst	Conc 4		Replicate									
%	Day	A	B	C	D	E	F	G	H	I	J					A	B	C	D	E	F	G	H	I	J		
0	1	0	0	0	0	0	0	0	0	0	0	10	8	KP	56	1	0	0	0	0	0	0	0	0			
	2	0	0	0	0	0	0	0	0	0	0	10	8	KP		2	0	0	0	0	0	0	0	0			
	3	0	0	0	0	0	2	2	0	0	4	10	0.4	KP		3	2	0	2	3	3	1	2	0			
	4	3	3	4	7	5	0	1	2	3	325	10	2.5	KP		4	1	1	3	0	0	2	2	5			
	5	3	1	0	0	7	3	3	0	0	11	10	1.1	KP		5	0	0	3	2	3	4	3	3			
	6	8	5	7	3	1	7	4	7	5	0	46	10	4.6	KP	6	6	6	5	7	4	7	6	3			
	7	6	7	9	5	4	8	5	7	9	16	75	10	7.5	KP	7	8	7	6	11	5	0	8	6			
	8														8												
Total		19	16	20	10	16	19	15	16	17	13	161	X= 16.1		Total	7	9	19	23	14	19	21	21	11			
Conc 2		Replicate										No. of Young	No. of Adult	Young/ Adult	Analyst	Conc 5		Replicate									
%	Day	A	B	C	D	E	F	G	H	I	J					%	Day	A	B	C	D	E	F	G	H	I	J
3L	1	6	0	0	0	0	0	0	0	0	0	10	0		75	1	0	0	0	0	0	0	0	0	0		
	2	0	6	6	0	0	0	0	0	0	0	10	0			2	0	3	0	0	0	0	0	0	0		
	3	0	3	3	3	0	0	0	0	0	0	10	1.3			3	1	0	2	0	3	0	0	0	0		
	4	3	2	1	2	2	0	6	5	0	0	10	2.2			4	2	5	2	2	0	4	-3	3	2		
	5	3	3	3	2	1	7	5	0	4	0	21	10	2.1		5	4	3	3	4	1	1	-4	4	0		
	6	7	7	5	2	6	7	4	3	5	6	51	10	5.1		6	2	5	4	4	3	-	8	9	0		
	7	7	6	8	7	6	9	7	5	8	63	10	7.3		7	7	6	7	8	3	-	7	0	1			
	8														8												
Total		20	14	19	14	17	20	23	10	14	19	170			Total	27	21	18	17	16	11	x0	22	11			
Conc 3		Replicate										No. of Young	No. of Adult	Young/ Adult	Analyst	Conc 6		Replicate									
%	Day	A	B	C	D	E	F	G	H	I	J					%	Day	A	B	C	D	E	F	G	H	I	J
4L	1	0	2	8	8	0	0	0	0	0	0	10	0		100	1	0	0	0	0	0	0	0	0	0		
	2	0	0	0	0	0	0	0	0	0	0	10	0			2	0	8	6	8	0	0	0	0	0		
	3	1	0	2	0	2	0	3	0	0	0	10	0.8			3	0	0	0	0	2	0	0	0	0		
	4	6	5	2	5	3	7	0	2	0	0	10	3.6			4	2	5	5	3	1	0	0	0	0		
	5	2	5	3	6	2	6	3	1	2	5	31	10	3.1		5	6	5	0	5	0	4	0	3			
	6	2	5	3	6	2	6	5	0	2	4	33	10	4.3		6	7	4	7	6	5	8	5	4			
	7	9	7	7	6	8	6	5	7	5	5	105	10	6.5		7	8	6	11	5	4	6	6	5			
	8														8												
Total		18	24	23	22	16	20	16	10	14	20	183			Total	23	20	23	19	13	9	16	16	15			

A = DEAD; Y = MALE

Revision 1
11/30/10

AA # K1101004 C. DUBIA CHRONIC, REPRODUCTION, 1-14-11
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 # K1101004 C. DUBIA CHRONIC, REPRODUCTION, 1-14-11
File: Z:/toxstat/monte\CD. Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 7.64

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

Data PASS B1 homogeneity test at 0.01 level. Continue analysis.

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
32%	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
42%	10	0	10
TOTAL	20	0	20

CRITICAL FISHER'S VALUE (10,10,10) (p=0.05) IS 6. b VALUE IS 10.

Since b is greater than 6 there is no significant difference between CONTROL and TREATMENT at the 0.05 level.

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
56%	10	0	10

TOTAL	20	0	20
-------	----	---	----

CRITICAL FISHER'S VALUE (10,10,10) (p=0.05) IS 6. b VALUE IS 10.

Since b is greater than 6 there is no significant difference between CONTROL and TREATMENT at the 0.05 level.

FISHER'S EXACT TEST

NUMBER OF

IDENTIFICATION	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
75%	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

NUMBER OF

IDENTIFICATION	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
100%	10	0	10
TOTAL	20	0	20

CRITICAL FISHER'S VALUE (10,10,10) (p=0.05) IS 6. b VALUE IS 10.

Since b is greater than 6 there is no significant difference between CONTROL and TREATMENT at the 0.05 level.

SUMMARY OF FISHER'S EXACT TESTS

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

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

TITLE: AA # K1101004 C. DUBIA CHRONIC, REPRODUCTION, 1-14-11
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	16.0000	16.0000
1	CONTROL	3	20.0000	20.0000
1	CONTROL	4	10.0000	10.0000
1	CONTROL	5	16.0000	16.0000
1	CONTROL	6	19.0000	19.0000
1	CONTROL	7	15.0000	15.0000
1	CONTROL	8	16.0000	16.0000
1	CONTROL	9	17.0000	17.0000
1	CONTROL	10	13.0000	13.0000
2	32 % EFFLUENT	1	20.0000	20.0000
2	32 % EFFLUENT	2	14.0000	14.0000
2	32 % EFFLUENT	3	19.0000	19.0000
2	32 % EFFLUENT	4	14.0000	14.0000
2	32 % EFFLUENT	5	17.0000	17.0000
2	32 % EFFLUENT	6	20.0000	20.0000
2	32 % EFFLUENT	7	23.0000	23.0000
2	32 % EFFLUENT	8	10.0000	10.0000
2	32 % EFFLUENT	9	14.0000	14.0000
2	32 % EFFLUENT	10	19.0000	19.0000
3	42 % EFFLUENT	1	18.0000	18.0000
3	42 % EFFLUENT	2	24.0000	24.0000
3	42 % EFFLUENT	3	23.0000	23.0000
3	42 % EFFLUENT	4	22.0000	22.0000
3	42 % EFFLUENT	5	16.0000	16.0000
3	42 % EFFLUENT	6	20.0000	20.0000
3	42 % EFFLUENT	7	16.0000	16.0000
3	42 % EFFLUENT	8	10.0000	10.0000
3	42 % EFFLUENT	9	14.0000	14.0000
3	42 % EFFLUENT	10	20.0000	20.0000
4	56 % EFFLUENT	1	17.0000	17.0000
4	56 % EFFLUENT	2	9.0000	9.0000
4	56 % EFFLUENT	3	19.0000	19.0000
4	56 % EFFLUENT	4	23.0000	23.0000
4	56 % EFFLUENT	5	14.0000	14.0000
4	56 % EFFLUENT	6	19.0000	19.0000
4	56 % EFFLUENT	7	21.0000	21.0000
4	56 % EFFLUENT	8	21.0000	21.0000

4	56 % EFFLUENT	9	13.0000	13.0000
4	56 % EFFLUENT	10	14.0000	14.0000
5	75 % EFFLUENT	1	27.0000	27.0000
5	75 % EFFLUENT	2	21.0000	21.0000
5	75 % EFFLUENT	3	18.0000	18.0000
5	75 % EFFLUENT	4	17.0000	17.0000
5	75 % EFFLUENT	5	16.0000	16.0000
5	75 % EFFLUENT	6	11.0000	11.0000
5	75 % EFFLUENT	7	0.0000	0.0000
5	75 % EFFLUENT	8	22.0000	22.0000
5	75 % EFFLUENT	9	17.0000	17.0000
5	75 % EFFLUENT	10	17.0000	17.0000
6	100 % EFFLUENT	1	23.0000	23.0000
6	100 % EFFLUENT	2	20.0000	20.0000
6	100 % EFFLUENT	3	23.0000	23.0000
6	100 % EFFLUENT	4	19.0000	19.0000
6	100 % EFFLUENT	5	13.0000	13.0000
6	100 % EFFLUENT	6	9.0000	9.0000
6	100 % EFFLUENT	7	16.0000	16.0000
6	100 % EFFLUENT	8	16.0000	16.0000
6	100 % EFFLUENT	9	15.0000	15.0000
6	100 % EFFLUENT	10	19.0000	19.0000

AA # K1101004 C. DUBIA CHRONIC, REPRODUCTION, 1-14-11
 File: Z:/toxstat/monte\CD. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	27.350	5.470	0.245
Within (Error)	54	1205.500	22.324	
Total	59	1232.850		

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

AA # K1101004 C. DUBIA CHRONIC, REPRODUCTION, 1-14-11
 File: Z:/toxstat/monte\CD. Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED	MEAN CALCULATED IN	T STAT	SIG
		MEAN	ORIGINAL UNITS		
1	CONTROL	16.100	16.100		
2	32 % EFFLUENT	17.000	17.000	-0.426	
3	42 % EFFLUENT	18.300	18.300	-1.041	
4	56 % EFFLUENT	17.000	17.000	-0.426	
5	75 % EFFLUENT	16.600	16.600	-0.237	
6	100 % EFFLUENT	17.300	17.300	-0.568	

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

AA # K1101004 C. DUBIA CHRONIC, REPRODUCTION, 1-14-11
 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	32 % EFFLUENT	10	4.881	30.3	-0.900
3	42 % EFFLUENT	10	4.881	30.3	-2.200
4	56 % EFFLUENT	10	4.881	30.3	-0.900
5	75 % EFFLUENT	10	4.881	30.3	-0.500
6	100 % EFFLUENT	10	4.881	30.3	-1.200

AA # K1101004 C. DUBIA CHRONIC, REPRODUCTION, 1-14-11
 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	16.100				
2	32 % EFFLUENT	17.000	113.00	75.00	10.00	
3	42 % EFFLUENT	18.300	122.50	75.00	10.00	
4	56 % EFFLUENT	17.000	113.00	75.00	10.00	
5	75 % EFFLUENT	16.600	117.00	75.00	10.00	
6	100 % EFFLUENT	17.300	113.50	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 1/13/11 CLIENT Arkansas Analytical

Purchase Order #: _____

SPECIES: Pimephales promelas Mysidopsis bahia Cyprinodon variegates

Quantity Shipped: 300+ _____

Age: 24 hrs 1/13 150ct _____

Brood Stock Source: Anderson Dam, AR _____

Culture Water: Groundwater Artificial Salts Artificial Salts

Hardness (Mg/l CaCO₃) 160 Salinity (ppt) _____

Dissolved Oxygen (Mg/l): 8.1 _____

Feeding: ATTEMPT _____

Comments: _____

Shipped Via: Federal Express UPS Overnight Shuttle

Packaged By: me

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: 25°C 20-25°C

SALINITY/CONDUCTIVITY: -- --

TOTAL HARDNESS (as CaCO₃): 142 mg/l 86-124 mg/l

TOTAL ALKALINITY (as CaCO₃): 100 mg/l 65-130 mg/l

pH: 7.92 7.56-8.35

Comments:

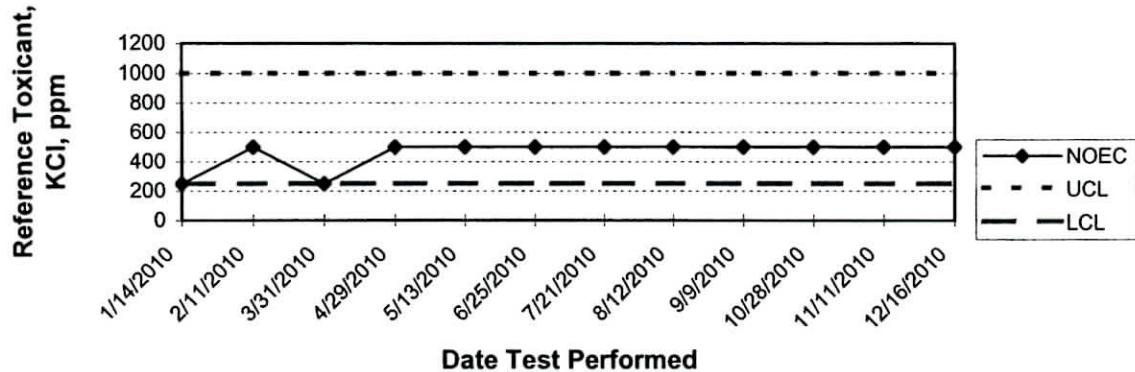


Facility Supervisor

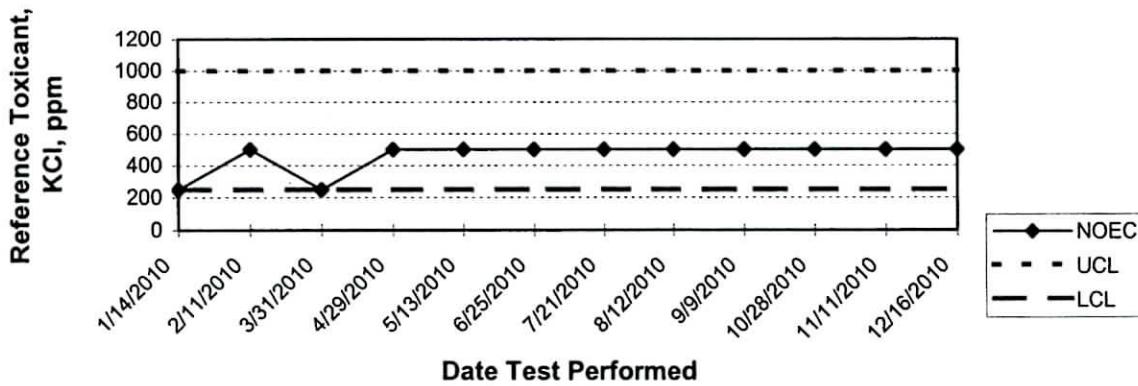
APPENDIX F

Quality Assurance Charts

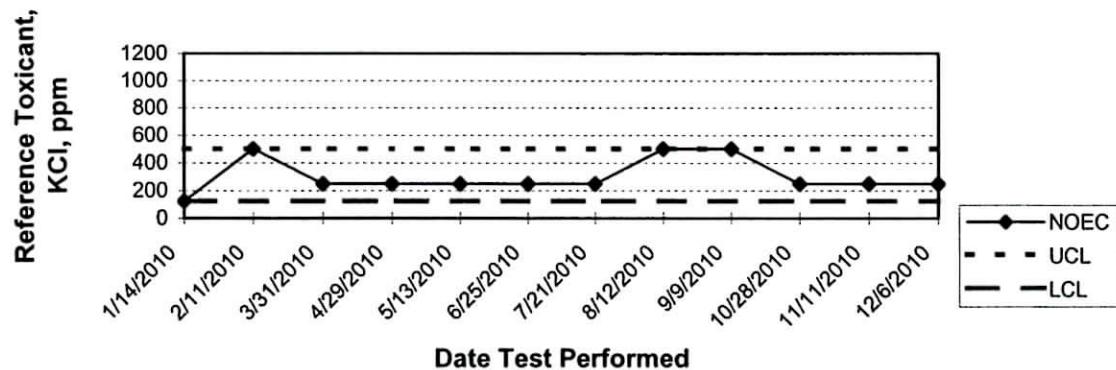
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW SURVIVAL
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW GROWTH
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE

