

Arkansas Analytical, Inc.

Toxicity Test Results

MAGCOBAR MINE SITE
NPDES PERMIT NUMBER: AR0049794
December, 2009
AFIN# 00-00348

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

Ceriodaphnia dubia, Survival and Reproduction Test
Test 1002.0

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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 December of 2009.

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:	12-13-09, 0912	12-14-09, 0912
Sample #2:	12-14-09, 0943	12-15-09, 0943
Sample #3:	12-16-09, 0845	12-17-09, 0845

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:	12-14-09, 1250	6
Sample #2:	12-15-09, 1233	4
Sample #3:	12-17-09, 1224	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 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	90%	X	
Average of 15 or more young per surviving female	15.2	X	
At least 60% of surviving females should have produced 3 broods	77.8%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	26.2%	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	0.521	X	
The percent coefficient of variation between replicates must be 40% or less for growth	19.3%	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> 11/18-25/09		<i>Pimephales promelas</i> 11/18-25/09	
NOEC Survival:	250 ppm KCl	NOEC Survival:	500 ppm KCl
LOEC Survival:	500 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 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)	12.3	%CV survival (critical dilution)	5.73%
%CV Reproduction (critical dilution)	30.4%	Mean dry weight (critical dilution) in milligrams	0.561
		%CV growth (critical dilution)	21.8%
PMSD Reproduction	29.0	PMSD Growth	27.0

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

Biomonitoring Analysts:



 Ken Pigue

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:	12-13-09, 0912	12-14-09, 0912
Sample #2:	12-14-09, 0943	12-15-09, 0943
Sample #3:	12-16-09, 0845	12-17-09, 0845

Test initiated (date, time): 12-15-09, 1630 Test terminated (date, time): 12-22-09, 1130

Dilution water used: Soft Synthetic

DATA TABLE FOR FATHEAD MINNOW SURVIVAL

Percent Survival in Replicate Chambers

Mean Percent Survival

DATA TABLE FOR GROWTH OF FATHEAD MINNOWS

Effluent Conc %	A	B	C	D	E		24 hours	48 hours	7 days	CV %
0%	100	100	87.5	100	100		100	100	97.5	5.73
32%	100	100	100	100	87.5		100	100	97.5	
42%	87.5	100	100	100	100		100	100	97.5	
56%	100	100	100	100	100		100	100	100	
75%	100	100	87.5	100	100		100	100	97.5	
100%	100	87.5	100	100	100		100	100	97.5	5.73

SUMMARY

Effluent Conc %	A	B	C	D	E		Mean Dry Weight	CV%
0%	0.386	0.488	0.520	0.545	0.665		0.521	19.4
32%	0.604	0.618	0.778	0.762	0.629		0.678	
42%	0.573	0.567	0.739	0.770	0.545		0.639	
56%	0.438	0.412	0.562	0.414	0.556		0.476	
75%	0.532	0.524	0.556	0.502	0.567		0.536	
100%	0.473	0.441	0.701	0.506	0.682		0.561	21.7

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)= _____ 21.7 %

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:	12-13-09, 0912	12-14-09, 0912
Sample #2:	12-14-09, 0943	12-15-09, 0943
Sample #3:	12-16-09, 0845	12-17-09, 0845

Test initiated (date, time): 12-15-09, 1350 Test terminated (date, time): 12-22-09, 0830

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	10	19	15	16	11	8
B	15	21	16	10	20	13
C	x0	16	15	15	14	15
D	16	14	20	15	21	7
E	8	16	18	14	18	13
F	14	13	17	22	14	15
G	20	18	16	15	15	14
H	18	22	14	14	12	18
I	18	21	15	12	14	7
J	18	21	17	20	12	13
Mean	13.7	18.1	16.3	15.3	15.1	12.3
Mean/surviving female	15.2	18.1	16.3	15.3	15.1	12.3
CV%*	26.2					30.4

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	100	100
48 HOURS	90	100	100	100	100	100
Test termination	90	100	100	100	100	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)= 30.4 %

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 K912008

Test Start (Date/Time) 12/15/09

Client Weston

Test End (Date/Time) 12/22/09

Day of Test

		1	2	3	4	5	6	7	notes/remarks
Control		12/15	12/16	12/17	12/18	12/19	12/20	12/21	
D.O. (mg/L)	INITIAL	8.4	8.5	8.4	8.5	8.6	8.7	8.6	
	FINAL	7.7	7.8	7.7	7.8	8.0	7.7	7.6	
pH (s.u.)	INITIAL	7.8	7.7	7.6	7.7	7.8	7.8	7.6	
	FINAL	7.7	7.5	7.7	7.5	6.8	6.9	7.1	
temp (C)	INITIAL	26.8	26.3	21.1	21.3	22.7	21.3	22.7	
	FINAL	25.8	25.0	25.0	25.0	25	25.0	25.0	
ALKALINITY (mg/L)		3.2							
HARDNESS (mg/L)		4.8							
CONDUCTIVITY (umhos/cm)		146							
CHLORINE (mg/L)		0.05							
CONC:									
D.O. (mg/L)	INITIAL	8.5	8.6	8.5	8.4	8.5	8.4	8.5	
	FINAL	7.8	7.6	7.5	7.8	8.0	7.8	7.6	
pH (s.u.)	INITIAL	7.8	7.7	7.6	7.8	7.4	7.7	7.3	
	FINAL	7.7	7.6	7.2	7.3	6.7	6.6	6.8	
temp (C)	INITIAL	20.6	19.9	21.2	22.2	22.1	21.5	23.1	
	FINAL	25.0	25.0	25.0	25.0	25	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.5	8.5	8.4	8.5	8.7	8.5	
	FINAL	7.9	7.6	7.7	7.9	8.1	8.0	7.4	
pH (mg/L)	INITIAL	7.5	7.4	7.6	7.2	7.4	6.7	7.3	
	FINAL	7.6	7.5	7.2	7.3	6.8	6.7	6.8	
temp (C)	INITIAL	19.6	20.0	21.3	22.3	22.2	21.4	23.1	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.5	8.4	8.5	8.6	8.9	8.7	
	FINAL	7.9	7.7	7.7	7.8	8.2	8.0	7.4	
pH (s.u.)	INITIAL	7.5	7.4	7.5	7.3	7.4	6.8	7.2	
	FINAL	7.6	7.6	7.2	7.4	6.9	6.7	6.7	
temp (C)	INITIAL	19.5	20.0	21.5	22.2	22.7	21.4	23.2	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.7	8.5	8.5	8.5	8.6	8.3	8.7	
	FINAL	8.0	7.7	7.5	7.9	8.1	8.1	7.4	
pH (s.u.)	INITIAL	7.4	7.3	7.5	7.2	7.3	6.9	7.2	
	FINAL	7.6	7.6	7.5	7.3	7.0	6.7	6.7	
temp (C)	INITIAL	19.7	20.1	21.4	22.1	22.2	21.4	23.4	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.8	8.5	8.6	8.6	8.6	8.9	8.8	
	FINAL	7.9	7.7	7.5	8.0	8.1	8.1	7.5	
pH (s.u.)	INITIAL	7.3	7.3	7.4	7.2	7.3	6.7	7.1	
	FINAL	7.5	7.5	7.1	7.4	6.9	6.7	6.7	
temp (C)	INITIAL	26.6	20.3	21.4	21.9	22.6	21.3	23.8	
	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)		10			8		6		
HARDNESS (mg/L)		2600			2600		2600		
CONDUCTIVITY (umhos/cm)		1957			20300		20500		
CHLORINE (mg/L)		0.05			0.05		0.05		

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Cerodaphnia Dubia

Lab # / Sample ID K9/2008

Test Start (Date/Time) 12/15/09

Client Horroles - Weston

Test End (Date/Time) 12/22/09

		Day of Test							notes/remarks
		1	2	3	4	5	6	7	
Control		12/15	12/16	12/17	12/18	12/19	12/20	12/21	
D.O. (mg/L)	INITIAL	84	85	84	85	86	87	86	
	FINAL	76	73	79	77	77	76		
pH (s.u.)	INITIAL	7.8	7.7	7.6	7.7	7.8	7.7	7.6	
	FINAL	7.7	7.5	7.6	8.0	7.8	7.9		
temp (C)	INITIAL	20.8	20.3	21.1	21.3	22.0	21.3	22.7	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0		
ALKALINITY (mg/L)		32							
HARDNESS (mg/L)		48							
CONDUCTIVITY (umhos/cm)		146							
CHLORINE (mg/L)		0.05							
CONC:									
D.O. (mg/L)	INITIAL	85	86	85	84	85	84	85	
	FINAL	78	78	79	76	77	77		
pH (s.u.)	INITIAL	7.8	7.7	7.6	7.3	7.4	6.6	7.3	
	FINAL	7.3	7.4	7.5	7.6	7.4	7.6		
temp (C)	INITIAL	20.6	19.9	21.2	22.2	22.1	21.5	22.0	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0		
CONC:									
D.O. (mg/L)	INITIAL	86	85	85	84	83	87	85	
	FINAL	78	77	80	77	77	78		
pH (mg/L)	INITIAL	7.5	7.4	7.6	7.2	7.4	7.7	7.3	
	FINAL	7.2	7.4	7.4	7.5	7.4	7.5		
temp (C)	INITIAL	19.6	20.0	21.3	22.3	22.2	21.4	23.1	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0		
CONC:									
D.O. (mg/L)	INITIAL	86	85	84	85	86	89	87	
	FINAL	77	76	80	75	77	78		
pH (s.u.)	INITIAL	7.5	7.4	7.5	7.3	7.4	6.8	7.2	
	FINAL	7.2	7.4	7.2	7.5	7.4	7.5		
temp (C)	INITIAL	19.5	20.0	21.3	22.2	22.2	21.4	23.2	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0		
CONC:									
D.O. (mg/L)	INITIAL	87	85	85	85	86	83	87	
	FINAL	77	77	80	74	77	79		
pH (s.u.)	INITIAL	7.4	7.3	7.5	7.2	7.3	6.9	7.2	
	FINAL	7.1	7.5	7.7	7.4	7.3	7.5		
temp (C)	INITIAL	19.7	20.1	21.4	22.1	22.2	21.4	23.6	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0		
CONC:									
D.O. (mg/L)	INITIAL	88	85	86	86	87	89	88	
	FINAL	78	79	79	76	77	80		
pH (s.u.)	INITIAL	7.3	7.3	7.4	7.2	7.3	6.7	7.1	
	FINAL	7.1	7.5	7.2	7.3	7.2	7.6		
temp (C)	INITIAL	20.0	20.3	21.4	21.9	22.5	21.3	23.8	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0		
CONC:		100%	A	A	A	B	B	C	C
ALKALINITY (mg/L)		10			8	7	6	7	
HARDNESS (mg/L)		2600			2600	4	2600	7	
CONDUCTIVITY (umhos/cm)		1957			20300	4	20500	7	
CHLORINE (mg/L)		0.05			0.05	7	0.05	7	

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							
19/2668		12/15/09		16:35							
CLIENT		TEST END DATE		TIME							
Weston		12/20/09		11:30							
AGE AND SOURCE OF MINNOWS											
Summary											
DAY (NUMBER SURVIVING)										SURVIVAL	
REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV	
CONC: 0	A	8	8	8	8	8	8	8	100	97.5	5.7%
	B	8	8	8	8	8	8	8	100		
	C	8	8	8	8	8	8	8	87.5		
	D	8	8	8	8	8	8	8	100		
	E	8	8	8	8	8	8	8	100		
CONC: 31	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		
CONC: 100	A	8	8	8	8	8	8	8	87.5	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	100		
CONC: 50	A	8	8	8	8	8	8	8	100	100	
	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		
CONC: 75	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	87.5		
	D	8	8	8	8	8	8	8	100		
	E	8	8	8	8	8	8	8	100		
CONC: 100	A	8	8	8	8	8	8	8	100	97.5	5.7%
	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		
ANALYST											
DATE:											
TIME:											

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		TEST START DATE		TIME						
CLIENT		TEST END DATE		TIME						
AGE AND SOURCE OF MINNOWS										
DAY (NUMBER SURVIVING)							SURVIVAL			
REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC: 6	A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓		
	E									
CONC: 32	A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓		
	E									
CONC: 42	A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓		
	E									
CONC: 56	A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓		
	E									
CONC: 75	A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓		
	E									
CONC: 160	A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓		
	E									
ANALYST	VP	VP	VP	VP	VP	VP	VP	VP		
DATE:	12/15/09	12/16	12/17	12/18/09	12/19/09	12/20	12/21	12/22/09		
TIME:	1630	1550	1415	1050	1110		1115	1130		

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										
D A Y (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: 32	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: 100	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: 50	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: 75	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: 100	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										
DATE:										
TIME:										

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		TEST START	DATE	TIME						
CLIENT		TEST END	DATE	TIME						
		AGE AND SOURCE OF MINNOWS								
		DAY (NUMBER SURVIVING)					SURVIVAL			
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: 3	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: 10	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: 50	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: 15	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: 100	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						CB				
DATE:						12-20				
TIME:										

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		TEST START	DATE	TIME						
CLIENT		TEST END	DATE	TIME						
D		AGE AND SOURCE OF MINNOWS					SURVIVAL			
		DAY (NUMBER SURVIVING)								
REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC: 0	A	2	2	2	2	2	2			
	B	2	2	2	2	2	2			
	C	2	2	2	2	2	2			
	D	2	2	2	2	2	2			
	E	2	2	2	2	2	2			
CONC: 5	A	2	2	2	2	2	2			
	B	2	2	2	2	2	2			
	C	2	2	2	2	2	2			
	D	2	2	2	2	2	2			
	E	2	2	2	2	2	2			
CONC: 42	A	2	2	2	2	2	2			
	B	2	2	2	2	2	2			
	C	2	2	2	2	2	2			
	D	2	2	2	2	2	2			
	E	2	2	2	2	2	2			
CONC: 56	A	2	2	2	2	2	2			
	B	2	2	2	2	2	2			
	C	2	2	2	2	2	2			
	D	2	2	2	2	2	2			
	E	2	2	2	2	2	2			
CONC: 75	A	2	2	2	2	2	2			
	B	2	2	2	2	2	2			
	C	2	2	2	2	2	2			
	D	2	2	2	2	2	2			
	E	2	2	2	2	2	2			
CONC: 100	A	2	2	2	2	2	2			
	B	2	2	2	2	2	2			
	C	2	2	2	2	2	2			
	D	2	2	2	2	2	2			
	E	2	2	2	2	2	2			
ANALYST						OK				
DATE:						12-20				
TIME:										

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		TEST START	DATE	TIME						
CLIENT <u>Weston</u>		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		
	C					2	2	2		
	D					2	2	2		
	E									
CONC: 32	A	2	2	2	2	2	2	2		
	B					1	1	1		
	C					2	2	2		
	D					2	2	2		
	E									
CONC: 47	A	2	2	2	2	2	2	2		
	B					2	2	2		
	C					2	2	2		
	D					2	2	2		
	E									
CONC: 51	A	2	2	2	2	2	2	2		
	B					2	2	2		
	C					2	2	2		
	D					2	2	2		
	E									
CONC: 75	A	2	2	2	2	2	2	2		
	B					2	2	2		
	C					2	2	2		
	D					2	2	2		
	E									
CONC: 100	A	2	2	2	2	2	2	2		
	B					2	2	2		
	C					2	2	2		
	D					2	2	2		
	E									
ANALYST										
DATE:						12-20				
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:		K912008		TEST DATES (BEGIN / END):		12/15-22/09	
CLIENT:		EEMA		WEIGHING DATE / TIME:		12/29/09, 1410	
ANALYSTS:		KP		DRYING TEMP (DEGREES C):		60	
SAMPLE ID:		SEE COC		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	1.02119	1.01810	0.00309	8	0.386	AVG DRY
	B	1.02721	1.02331	0.00390	8	0.488	WEIGHT (mg)
	C	1.00237	0.99821	0.00416	8	0.520	0.521
	D	1.04228	1.03792	0.00436	8	0.545	CV
	E	1.01800	1.01268	0.00532	8	0.665	19.3
CONC: 32%	A	1.00368	0.99885	0.00483	8	0.604	AVG DRY
	B	1.01001	1.00507	0.00494	8	0.618	WEIGHT (mg)
	C	1.04377	1.03755	0.00622	8	0.778	0.678
	D	1.01590	1.00980	0.00610	8	0.762	CV
	E	1.02458	1.01955	0.00503	8	0.629	
CONC: 42%	A	1.03402	1.02944	0.00458	8	0.573	AVG DRY
	B	1.00687	1.00233	0.00454	8	0.567	WEIGHT (mg)
	C	1.03128	1.02537	0.00591	8	0.739	0.639
	D	1.03509	1.02893	0.00616	8	0.770	CV
	E	1.03105	1.02669	0.00436	8	0.545	
CONC: 56%	A	1.03644	1.03294	0.00350	8	0.438	AVG DRY
	B	1.02754	1.02424	0.00330	8	0.412	WEIGHT (mg)
	C	1.03276	1.02826	0.00450	8	0.562	0.476
	D	1.04854	1.04523	0.00331	8	0.414	CV
	E	1.02176	1.01731	0.00445	8	0.556	
CONC: 75%	A	1.03764	1.03338	0.00426	8	0.532	AVG DRY
	B	1.03351	1.02932	0.00419	8	0.524	WEIGHT (mg)
	C	1.02208	1.01763	0.00445	8	0.556	0.536
	D	1.04724	1.04322	0.00402	8	0.502	CV
	E	1.01700	1.01246	0.00454	8	0.567	
CONC: 100%	A	1.01794	1.01416	0.00378	8	0.473	AVG DRY
	B	1.02448	1.02095	0.00353	8	0.441	WEIGHT (mg)
	C	1.00795	1.00234	0.00561	8	0.701	0.561
	D	1.01633	1.01228	0.00405	8	0.506	CV
	E	1.03964	1.03418	0.00546	8	0.682	21.8

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

Pimephales promelas

FATHEAD MINNOW

TEST 1000.0

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s: <u>K912008</u>	TEST DATES (BEGIN / END): <u>12/15-27/09</u>
CLIENT: <u>Weston</u>	WEIGHING DATE / TIME: <u>12/29/09 1410</u>
ANALYSTS: <u>KP</u>	DRYING TEMP (DEGREES C): <u>60</u>
SAMPLE ID:	DRYING TIME (HOURS): <u>24</u>

	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 31	1.02119	1.01810				AVG DRY WEIGHT (mg)
	B 32	1.02721	1.02331				
	C 33	1.00237	0.99821				
	D 34	1.04728	1.03792				
	E 35	1.01800	1.01268				
							CV
CONC: 32	A 36	1.00368	0.99885				AVG DRY WEIGHT (mg)
	B 37	1.01001	1.00507				
	C 38	1.04377	1.03755				
	D 39	1.01590	1.00980				
	E 40	1.02452	1.01955				
							CV
CONC: 42	A 41	1.03402	1.02944				AVG DRY WEIGHT (mg)
	B 42	1.00687	1.00233				
	C 43	1.03728	1.02537				
	D 44	1.03509	1.02893				
	E 45	1.03005	1.02669				
							CV
CONC: 56	A 46	1.03644	1.03294				AVG DRY WEIGHT (mg)
	B 47	1.02754	1.02424				
	C 48	1.03276	1.02826				
	D 49	1.04854	1.04523				
	E 50	1.02176	1.01731				
							CV
CONC: 75	A 51	1.03764	1.03338				AVG DRY WEIGHT (mg)
	B 52	1.03361	1.02932				
	C 53	1.02268	1.01763				
	D 54	1.04724	1.04322				
	E 55	1.01700	1.01246				
							CV
CONC: 100	A 56	1.01794	1.01416				AVG DRY WEIGHT (mg)
	B 57	1.02458	1.02095				
	C 58	1.00795	1.00239				
	D 59	1.01733	1.01228				
	E 60	1.03964	1.03418				
							CV

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA# K912008, FATHEAD MINNOW SURVIVAL, CHRONIC, 12-15-09
File: H:\TOXSTAT\MONTE\FHSURV~1. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.164

W = 0.558

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# K912008, FATHEAD MINNOW SURVIVAL, CHRONIC, 12-15-09
File: H:\TOXSTAT\MONTE\FHSURV~1. Transform: ARC SINE(SQUARE ROOT(Y))

Hartley's test for homogeneity of variance
Bartlett's test for homogeneity of variance

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

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

TITLE: AA# K912008, FATHEAD MINNOW SURVIVAL, CHRONIC, 12-15-09
FILE: H:\TOXSTAT\MONTE\FHSURV~1.
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	1.0000	1.4120
1	CONTROL	2	1.0000	1.4120
1	CONTROL	3	0.8750	1.2094
1	CONTROL	4	1.0000	1.4120
1	CONTROL	5	1.0000	1.4120
2	32 % EFFLUENT	1	1.0000	1.4120
2	32 % EFFLUENT	2	1.0000	1.4120
2	32 % EFFLUENT	3	1.0000	1.4120
2	32 % EFFLUENT	4	1.0000	1.4120
2	32 % EFFLUENT	5	0.8750	1.2094

3	42 %	EFFLUENT	1	0.8750	1.2094
3	42 %	EFFLUENT	2	1.0000	1.4120
3	42 %	EFFLUENT	3	1.0000	1.4120
3	42 %	EFFLUENT	4	1.0000	1.4120
3	42 %	EFFLUENT	5	1.0000	1.4120
4	56 %	EFFLUENT	1	1.0000	1.4120
4	56 %	EFFLUENT	2	1.0000	1.4120
4	56 %	EFFLUENT	3	1.0000	1.4120
4	56 %	EFFLUENT	4	1.0000	1.4120
4	56 %	EFFLUENT	5	1.0000	1.4120
5	75 %	EFFLUENT	1	1.0000	1.4120
5	75 %	EFFLUENT	2	1.0000	1.4120
5	75 %	EFFLUENT	3	0.8750	1.2094
5	75 %	EFFLUENT	4	1.0000	1.4120
5	75 %	EFFLUENT	5	1.0000	1.4120
6	100 %	EFFLUENT	1	1.0000	1.4120
6	100 %	EFFLUENT	2	0.8750	1.2094
6	100 %	EFFLUENT	3	1.0000	1.4120
6	100 %	EFFLUENT	4	1.0000	1.4120
6	100 %	EFFLUENT	5	1.0000	1.4120

AA# K912008, FATHEAD MINNOW SURVIVAL, CHRONIC, 12-15-09
 File: H:\TOXSTAT\MONTE\FHSURV~1. Transform: ARC SINE(SQUARE ROOT(Y))

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	1.372				
2	32 % EFFLUENT	1.372	27.50	16.00	5.00	
3	42 % EFFLUENT	1.372	27.50	16.00	5.00	
4	56 % EFFLUENT	1.412	30.00	16.00	5.00	
5	75 % EFFLUENT	1.372	27.50	16.00	5.00	
6	100 % EFFLUENT	1.372	27.50	16.00	5.00	

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

AA# K912008, FATHEAD MINNOW GROWTH CHRONIC, 12-15-09
 File: H:/toxstat/monte\FHGROWTH. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.216

W = 0.921

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# K912008, FATHEAD MINNOW GROWTH CHRONIC, 12-15-09
 File: H:/toxstat/monte\FHGROWTH. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 7.35

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# K912008, FATHEAD MINNOW GROWTH CHRONIC, 12-15-09
 FILE: H:/toxstat/monte\FHGROWTH.
 TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.3860	0.6704
1	CONTROL	2	0.4880	0.7734
1	CONTROL	3	0.5200	0.8054
1	CONTROL	4	0.5450	0.8305
1	CONTROL	5	0.6650	0.9535
2	32 % EFFLUENT	1	0.6040	0.8902
2	32 % EFFLUENT	2	0.6180	0.9045
2	32 % EFFLUENT	3	0.7780	1.0802
2	32 % EFFLUENT	4	0.7620	1.0612
2	32 % EFFLUENT	5	0.6290	0.9159
3	42 % EFFLUENT	1	0.5730	0.8587
3	42 % EFFLUENT	2	0.5670	0.8526
3	42 % EFFLUENT	3	0.7390	1.0346
3	42 % EFFLUENT	4	0.7700	1.0706
3	42 % EFFLUENT	5	0.5450	0.8305
4	56 % EFFLUENT	1	0.4380	0.7232

4	56 %	EFFLUENT	2	0.4120	0.6969
4	56 %	EFFLUENT	3	0.5620	0.8476
4	56 %	EFFLUENT	4	0.4140	0.6990
4	56 %	EFFLUENT	5	0.5560	0.8415
5	75 %	EFFLUENT	1	0.5320	0.8174
5	75 %	EFFLUENT	2	0.5240	0.8094
5	75 %	EFFLUENT	3	0.5560	0.8415
5	75 %	EFFLUENT	4	0.5020	0.7874
5	75 %	EFFLUENT	5	0.5670	0.8526
6	100 %	EFFLUENT	1	0.4730	0.7584
6	100 %	EFFLUENT	2	0.4410	0.7263
6	100 %	EFFLUENT	3	0.7010	0.9922
6	100 %	EFFLUENT	4	0.5060	0.7914
6	100 %	EFFLUENT	5	0.6820	0.9717

AA# K912008, FATHEAD MINNOW GROWTH CHRONIC, 12-15-09

File: H:/toxstat/monte\FHGGROWTH.

Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.155	0.031	3.454
Within (Error)	24	0.216	0.009	
Total	29	0.371		

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

Since F > Critical F REJECT Ho: All equal

AA# K912008, FATHEAD MINNOW GROWTH CHRONIC, 12-15-09

File: H:/toxstat/monte\FHGGROWTH.

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	0.807	0.521		
2	32 % EFFLUENT	0.970	0.678	-2.731	
3	42 % EFFLUENT	0.929	0.639	-2.047	
4	56 % EFFLUENT	0.762	0.476	0.750	
5	75 % EFFLUENT	0.822	0.536	-0.251	
6	100 % EFFLUENT	0.848	0.561	-0.690	

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

AA# K912008, FATHEAD MINNOW GROWTH CHRONIC, 12-15-09

File: H:/toxstat/monte\FHGGROWTH.

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	0.140	27.0	-0.157
3	42 % EFFLUENT	5	0.140	27.0	-0.118
4	56 % EFFLUENT	5	0.140	27.0	0.044
5	75 % EFFLUENT	5	0.140	27.0	-0.015
6	100 % EFFLUENT	5	0.140	27.0	-0.040

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

Cerodaphnia dubia
 Discharger: Weston
 Location: 1912008
 Date Sample Collected:

SURVIVAL AND REPRODUCTION TEST

Analyst: KP
 Test Start - Date/Time: 12/15/09, 1350
 Test Stop - Date/Time: 12/22/09, 0830

Conc 1	% Day	Replicate										No. of Young	No. of Adult	Young/Adult	Analyst		
		A	B	C	D	E	F	G	H	I	J						
0	1	0	0	0	0	0	0	0	0	0	0	0	10	0	KP		
	2	0	0	0	0	0	0	0	0	0	0	0	9	0	KP		
	3	0	2	0	0	0	1	3	0	0	2	8	9	0.9	KP		
	4	0	8	0	0	0	1	3	0	0	2	34	9	3.8	KP		
	5	0	5	0	0	0	2	4	5	6	3	34	9	3.8	KP		
	6	0	3	5	0	0	2	7	8	10	11	10	2	61	9	6.8	KP
	7																
	8																
Total		10	15	0	16	8	14	20	18	18	18	137		5=15.2	CV=76.2		

Conc 2	% Day	Replicate										No. of Young	No. of Adult	Young/Adult	Analyst	
		A	B	C	D	E	F	G	H	I	J					
32	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	3	0	0	0	0	4	2	0	17	10	1.7		
	4	0	8	0	3	5	4	5	7	3	4	7	46	10	4.6	
	5	0	6	12	5	1	2	2	2	0	4	6	40	10	4.0	
	6	0	2	6	8	8	10	6	9	15	11	8	83	10	8.3	
	7															
	8															
Total		19	21	16	14	16	13	18	22	21	21	181				

Conc 3	% Day	Replicate										No. of Young	No. of Adult	Young/Adult	Analyst	
		A	B	C	D	E	F	G	H	I	J					
42	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	2	0	0	3	0	0	1	0	2	3	11	10	1.1	
	4	0	5	6	3	0	4	0	2	1	3	6	30	10	3.0	
	5	0	5	7	4	7	1	6	5	1	9	4	43	10	4.3	
	6	0	3	9	8	10	13	9	8	12	1	4	79	10	7.9	
	7															
	8															
Total		15	16	15	20	18	17	16	14	15	17	163				

Conc 4	% Day	Replicate										No. of Young	No. of Adult	Young/Adult	Analyst	
		A	B	C	D	E	F	G	H	I	J					
56	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	0	0	3	3	0	1	4	17	10	1.7		
	4	0	5	0	7	6	4	7	1	6	0	6	42	10	4.2	
	5	0	2	5	0	3	2	4	6	1	2	0	25	10	2.5	
	6	0	9	5	7	6	8	8	5	7	9	10	74	10	7.4	
	7															
	8															
Total		16	10	15	15	14	22	15	14	12	20	153				

Conc 5	% Day	Replicate										No. of Young	No. of Adult	Young/Adult	Analyst	
		A	B	C	D	E	F	G	H	I	J					
75	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	1	0	4	3	2	1	0	1	0	12	10	1.2		
	4	0	4	9	2	0	7	2	2	4	6	3	39	10	3.9	
	5	0	4	1	3	5	0	6	3	1	0	3	26	10	2.6	
	6	0	3	5	9	12	8	4	9	5	7	6	74	10	7.4	
	7															
	8															
Total		11	20	14	21	18	14	15	12	14	12	151				

Conc 6	% Day	Replicate										No. of Young	No. of Adult	Young/Adult	Analyst	
		A	B	C	D	E	F	G	H	I	J					
100	1	0	0	0	0	0	0	0	0	0	0	0	10	0		
	2	0	0	0	0	0	0	0	0	0	0	0	10	0		
	3	0	0	0	0	0	1	3	0	0	1	5	10	0.5		
	4	0	2	6	7	3	1	6	4	1	1	0	32	10	3.2	
	5	0	1	0	7	7	6	3	7	2	1	0	23	10	2.3	
	6	0	5	6	6	3	2	5	0	5	5	6	43	10	4.3	
	7															
	8															
Total		0	13	15	7	13	15	14	18	7	13	128				

X= DEAD; Y= MALE

$\bar{x} = 17.3$
 CV = 36.4

AA # K9120086 C. DUBIA CHRONIC, REPRODUCCION, 12-15-09
File: H:/toxstat/monte\C.DUB Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

***** Shapiro - Wilk's Test is aborted *****

This test can not be performed because total number of replicates
is greater than 50.

Total number of replicates = 60

AA # K9120086 C. DUBIA CHRONIC, REPRODUCCION, 12-15-09
File: H:/toxstat/monte\C.DUB Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 12.63

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

NUMBER OF

IDENTIFICATION	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	1	9	10
32%	0	10	10
TOTAL	1	19	20

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

FISHER'S EXACT TEST

NUMBER OF

IDENTIFICATION	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	1	9	10
42%	0	10	10
TOTAL	1	19	20

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

FISHER'S EXACT TEST

NUMBER OF

IDENTIFICATION	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	1	9	10
56%	0	10	10
TOTAL	1	19	20

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

FISHER'S EXACT TEST

NUMBER OF

IDENTIFICATION	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	1	9	10
75%	0	10	10
TOTAL	1	19	20

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

FISHER'S EXACT TEST

NUMBER OF

IDENTIFICATION	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	1	9	10
100%	0	10	10
TOTAL	1	19	20

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

SUMMARY OF FISHER'S EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
1	CONTROL	10	1	
	32%	10	0	
2	42%	10	0	

3	56%	10	0
4	75%	10	0
5	100%	10	0

TITLE: AA # K9120086 C. DUBIA CHRONIC, REPRODUCCION, 12-15-09
 FILE: H:/toxstat/monte\C.DUB
 TRANSFORM: NO TRANSFORMATION

NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	10.0000	10.0000
1	CONTROL	2	15.0000	15.0000
1	CONTROL	3	0.0000	0.0000
1	CONTROL	4	16.0000	16.0000
1	CONTROL	5	8.0000	8.0000
1	CONTROL	6	14.0000	14.0000
1	CONTROL	7	20.0000	20.0000
1	CONTROL	8	18.0000	18.0000
1	CONTROL	9	18.0000	18.0000
1	CONTROL	10	18.0000	18.0000
2	32 % EFFLUENT	1	19.0000	19.0000
2	32 % EFFLUENT	2	21.0000	21.0000
2	32 % EFFLUENT	3	16.0000	16.0000
2	32 % EFFLUENT	4	14.0000	14.0000
2	32 % EFFLUENT	5	16.0000	16.0000
2	32 % EFFLUENT	6	13.0000	13.0000
2	32 % EFFLUENT	7	18.0000	18.0000
2	32 % EFFLUENT	8	22.0000	22.0000
2	32 % EFFLUENT	9	21.0000	21.0000
2	32 % EFFLUENT	10	21.0000	21.0000
3	42 % EFFLUENT	1	15.0000	15.0000
3	42 % EFFLUENT	2	16.0000	16.0000
3	42 % EFFLUENT	3	15.0000	15.0000
3	42 % EFFLUENT	4	20.0000	20.0000
3	42 % EFFLUENT	5	18.0000	18.0000
3	42 % EFFLUENT	6	17.0000	17.0000
3	42 % EFFLUENT	7	16.0000	16.0000
3	42 % EFFLUENT	8	14.0000	14.0000
3	42 % EFFLUENT	9	15.0000	15.0000
3	42 % EFFLUENT	10	17.0000	17.0000
4	56 % EFFLUENT	1	16.0000	16.0000
4	56 % EFFLUENT	2	10.0000	10.0000
4	56 % EFFLUENT	3	15.0000	15.0000
4	56 % EFFLUENT	4	15.0000	15.0000
4	56 % EFFLUENT	5	14.0000	14.0000
4	56 % EFFLUENT	6	22.0000	22.0000
4	56 % EFFLUENT	7	15.0000	15.0000
4	56 % EFFLUENT	8	14.0000	14.0000
4	56 % EFFLUENT	9	12.0000	12.0000
4	56 % EFFLUENT	10	20.0000	20.0000
5	75 % EFFLUENT	1	11.0000	11.0000
5	75 % EFFLUENT	2	20.0000	20.0000
5	75 % EFFLUENT	3	14.0000	14.0000

5	75 %	EFFLUENT	4	21.0000	21.0000
5	75 %	EFFLUENT	5	18.0000	18.0000
5	75 %	EFFLUENT	6	14.0000	14.0000
5	75 %	EFFLUENT	7	15.0000	15.0000
5	75 %	EFFLUENT	8	12.0000	12.0000
5	75 %	EFFLUENT	9	14.0000	14.0000
5	75 %	EFFLUENT	10	12.0000	12.0000
6	100 %	EFFLUENT	1	8.0000	8.0000
6	100 %	EFFLUENT	2	13.0000	13.0000
6	100 %	EFFLUENT	3	15.0000	15.0000
6	100 %	EFFLUENT	4	7.0000	7.0000
6	100 %	EFFLUENT	5	13.0000	13.0000
6	100 %	EFFLUENT	6	15.0000	15.0000
6	100 %	EFFLUENT	7	14.0000	14.0000
6	100 %	EFFLUENT	8	18.0000	18.0000
6	100 %	EFFLUENT	9	7.0000	7.0000
6	100 %	EFFLUENT	10	13.0000	13.0000

AA # K9120086 C. DUBIA CHRONIC, REPRODUCCION, 12-15-09
 File: H:/toxstat/monte\C.DUB Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	202.733	40.547	2.736
Within (Error)	54	800.200	14.819	
Total	59	1002.933		

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

AA # K9120086 C. DUBIA CHRONIC, REPRODUCCION, 12-15-09
 File: H:/toxstat/monte\C.DUB Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	13.700	13.700		
2	32 % EFFLUENT	18.100	18.100	-2.556	
3	42 % EFFLUENT	16.300	16.300	-1.510	
4	56 % EFFLUENT	15.300	15.300	-0.929	
5	75 % EFFLUENT	15.100	15.100	-0.813	
6	100 % EFFLUENT	12.300	12.300	0.813	

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

AA # K9120086 C. DUBIA CHRONIC, REPRODUCCION, 12-15-09

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	3.977	29.0	-4.400
3	42 % EFFLUENT	10	3.977	29.0	-2.600
4	56 % EFFLUENT	10	3.977	29.0	-1.600
5	75 % EFFLUENT	10	3.977	29.0	-1.400
6	100 % EFFLUENT	10	3.977	29.0	1.400

AA # K9120086 C. DUBIA CHRONIC, REPRODUCCION, 12-15-09

STEEL'S MANY-ONE RANK TEST

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	13.700				
2	32 % EFFLUENT	18.100	129.00	75.00	10.00	
3	42 % EFFLUENT	16.300	112.00	75.00	10.00	
4	56 % EFFLUENT	15.300	106.00	75.00	10.00	
5	75 % EFFLUENT	15.100	106.00	75.00	10.00	
6	100 % EFFLUENT	12.300	87.50	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 12-15-09 Arkansas Analytical

SPECIES Pimpla promelas

QUANTITY SHIPPED 830+

AGE/LIFE STAGE 424 hrs 12/15 1500ct

BROODSTOCK SOURCE Anderson Farms, Ar

CULTURE WATER groundwater

ALKALINITY (Mg/l as CaCO₃) = 180

HARDNESS (Mg/l as CaCO₃)/Salinity (ppt) = 160

FEEDING ATF MILK

COMMENTS _____

PACKAGED BY Ull

BILL HALL PRINTERS 3171

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:

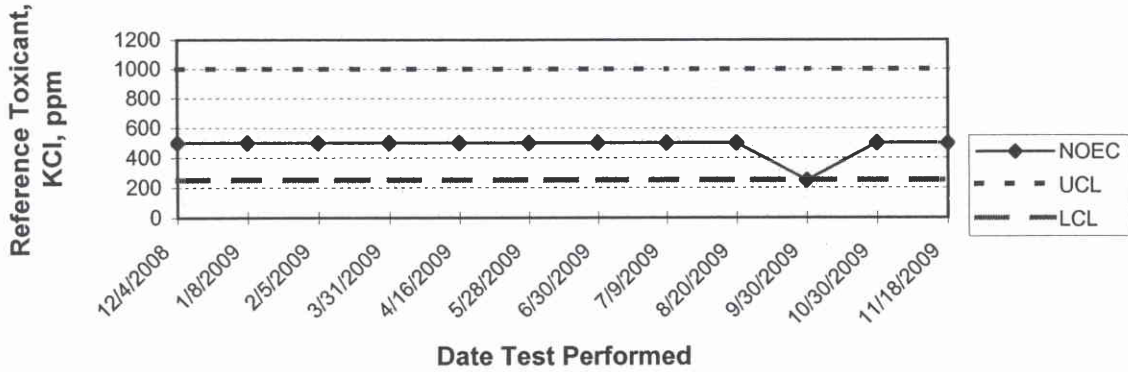


Facility Supervisor

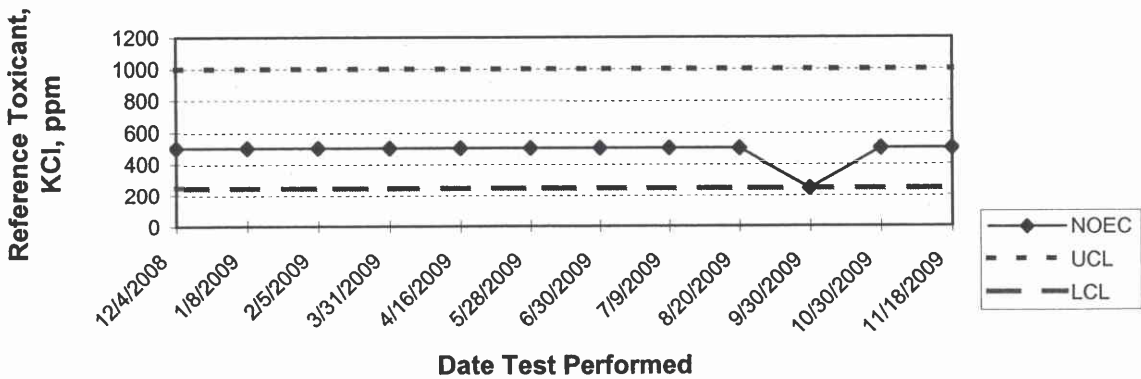
APPENDIX F

Quality Assurance Charts

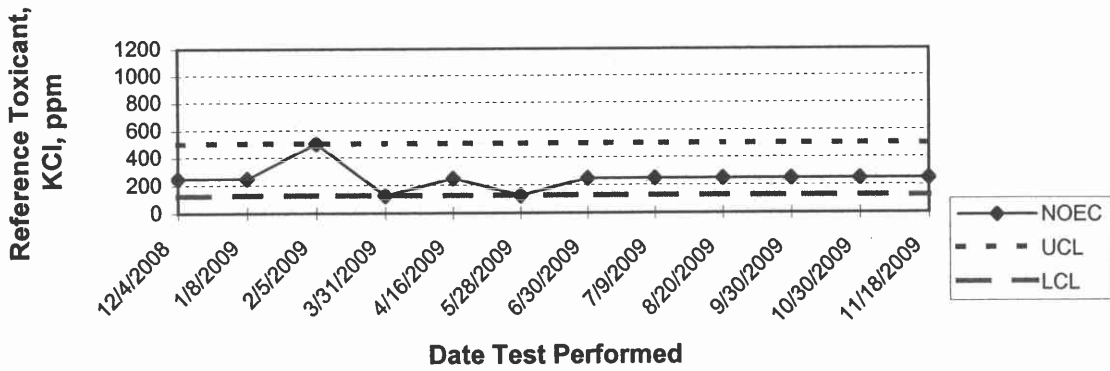
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW SURVIVAL
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW GROWTH
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE

