

# Arkansas Analytical, Inc.

## Toxicity Test Results

**MAGCOBAR MINE SITE**  
**NPDES PERMIT NUMBER: AR0049794**  
**December, 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**  
**EEMA O&M Services Group**  
**P.O. Box 232**  
**Kulpsville, PA 19443**

Prepared by: Arkansas Analytical, Inc.  
11701 I-30, Bldg 1, Suite 115  
Little Rock, Arkansas 72209  
**Lab Number K1112004**

Friday, December 16, 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 December 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:	12-7-11, 0745	12-8-11, 0745
Sample #2:	12-8-11, 0820	12-9-11, 0820
Sample #3:	12-12-11, 0715	12-13-11, 0715

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:	12-8-11, 1200	3
*Sample #2:	12-9-11, 1322	4
Sample #3:	12-13-11, 1350	3

\* Storage temperature exceeded specifications on 12/9/11

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	16.4	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	17.8%	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	0.319	X	
The percent coefficient of variation between replicates must be 40% or less for growth	12.9%	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> 11/30/11-12/7/11		<i>Pimephales promelas</i> 11/30/11-12/7/11	
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)	18.9	%CV survival (critical dilution)	0.00%
%CV Reproduction (critical dilution)	32.1%	Mean dry weight (critical dilution) in milligrams	0.456
		%CV growth (critical dilution)	9.07%
PMSD Reproduction	38.9	PMSD Growth	21.5

### Conclusion

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

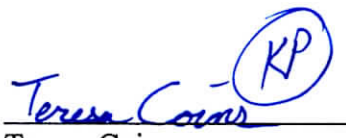
The permit issued to the Magcobar Mine Site, AR0049794, specifies that the **critical dilution is 100% effluent**. The effluent samples did not exhibit lethal effects or sublethal effects at the critical dilution, and, as such, **passed** both portions of the test.

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

  
Teresa Coins

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:	12-7-11, 0745	12-8-11, 0745
Sample #2:	12-8-11, 0820	12-9-11, 0820
Sample #3:	12-12-11, 0715	12-13-11, 0715

Test initiated (date, time): 12-8-11, 1515      Test terminated (date, time): 12-15-11, 0940

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	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%	0.372	0.318	0.271	0.287	0.344		0.318	12.9
32%	0.447	0.449	0.323	0.351	0.363		0.387	
42%	0.526	0.431	0.382	0.394	0.430		0.433	
56%	0.496	0.407	0.410	0.477	0.444		0.447	
75%	0.456	0.480	0.350	0.362	0.445		0.419	
100%	0.504	0.437	0.420	0.421	0.497		0.456	9.07

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)=     12.9     %



**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:	12-7-11, 0745	12-8-11, 0745
Sample #2:	12-8-11, 0820	12-9-11, 0820
Sample #3:	12-12-11, 0715	12-13-11, 0715

Test initiated (date, time): 12-8-11, 1400      Test terminated (date, time): 12-15-11, 0900

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	17	20	15	24	14	21
B	19	22	22	11	x0	15
C	16	16	19	23	15	26
D	14	20	13	17	x0	30
E	19	11	15	13	14	15
F	18	26	22	13	31	x7
G	17	19	7	20	x14	17
H	11	15	13	x0	18	10
I	20	10	16	17	18	19
J	13	20	15	16	18	17
Mean	16.4	17.9	15.7	15.4	14.2	17.7
Mean/surviving female	16.4	17.9	15.7	17.1	18.3	18.9
CV%*	17.8					32.1

X= Dead Adult; M= Male (Not considered in statistics)

\*Coefficient of Variation = standard deviation/ mean \* 100; CV% calculation based on young per surviving female

**SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING**  
*Ceriodaphnia dubia* SURVIVAL AND REPRODUCTION

Permittee: 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	100	100	100	90	80	100
Test termination	100	100	100	90	70	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, (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)= 32.1 %

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 RECORD

CLIENT INFORMATION				Project Description			Turnaround Time		Preservation Codes:												
EEMA O & M Services Group		EEMA O & M Services Group		Magcobar Mine Site			24 Hour		1. Cool, 4 Degrees Centigrade				4. Thiosulfate for Dechlorination								
Magcobar Mine Site		P.O. Box 732		Biomonitoring Sample			48 Hour		2. Sulfuric Acid (H <sub>2</sub> SO <sub>4</sub> ), pH < 2				5. Hydrochloric Acid (HCl)								
P.O. Box 699		Kulpsville, PA 19443		<b>Reporting Information</b>			72 Hour		3. Nitric Acid (HNO <sub>3</sub> ), pH < 2				6. Sodium Hydroxide (NaOH), pH > 12								
Malvern, AR 72104					Telephone: 501-467-8355			Routine (5 Day)		TEST PARAMETERS								Bottle Type Code			
Attn: Bill McAlister		Attn: Amber Rich		Fax: 501-467-8687			Preservative Code:		1										G = Glass; P = Plastic		
				Email: dave.friedman@eema-inc.com; bmcalister@eema-inc.com; bhorton@eema-inc.com			Bottle Type:		P										V = Septum; A = Amber		
<i>Bill McAlister</i> <b>Sampler(s) Signature</b>				<i>Bill McAlister</i> <b>Sampler(s) Printed</b>								Chronic Biomonitoring								Arkansas Analytical Work Order Number:	
														K1112004 B							
Field Number	SAMPLE COLLECTION Date/s		Time/s		Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION												
FD-1 Comp.	12/9/2011		8:20 AM			X	3	W	Facility Discharge					X							
1. Relinquished by: (Signature)		Date/Time		2. Received by: (Signature)			SAMPLE CONDITION UPON RECEIPT IN LAB					REMARKS / SAMPLE COMMENTS									
<i>Bill McAlister</i>		12-9-11 1322		<i>[Signature]</i>			1. CUSTODY SEALS: <input checked="" type="checkbox"/> Yes ___ No														
							2. CONTAINERS CORRECT: <input type="checkbox"/> Yes ___ No														
							3. COC/LABELS AGREE: <input type="checkbox"/> Yes ___ No														
3. Relinquished by: (Signature)		Date/Time		4. Received by lab: (Signature)			4. PRESERVATION CONFIRMED: <input type="checkbox"/> Yes ___ No														
<i>[Signature]</i>				<i>Sydney James</i>			5. RECEIVED ON ICE: <input checked="" type="checkbox"/> Yes ___ No														
							6. TEMPERATURE ON RECEIPT: <input checked="" type="checkbox"/> 4°C														
FOR COMPLETION BY LAB ONLY																					



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

# CHAIN OF CUSTODY RECORD

CLIENT INFORMATION		Project Description		Turnaround Time	Preservation Codes:								
EEMA O & M Services Group	EEMA O & M Services Group	Magcobar Mine Site		24 Hour	1. Cool, 4 Degrees Centigrade			4. Thiosulfate for Dechlorination					
Magcobar Mine Site	P.O. Box 732	Biomonitoring Sample		48 Hour	2. Sulfuric Acid (H <sub>2</sub> SO <sub>4</sub> ), pH < 2			5. Hydrochloric Acid(HCl)					
P.O. Box 699	Kulpsville, PA 19443	Reporting Information		72 Hour	3. Nitric Acid (HNO <sub>3</sub> ), pH < 2			6. Sodium Hydroxide (NaOH), pH > 12					
Malvern, AR 72104		Telephone: 501-467-8355		Routine (5 Day)	TEST PARAMETERS						Bottle Type Code		
Attn: Bill McAlister	Attn: Amber Rich	Fax: 501-467-8687		Preservative Code:	1								G = Glass; P = Plastic V = Septum; A = Amber
		Email: dave.friedman@eema-inc.com; bmcAlister@eema-inc.com; bhorton@eema-inc.com		Bottle Type:	P								

Bill McAlister

**Sampler(s) Signature**
**Sampler(s) Printed**
Arkansas Analytical Work Order Number:

Field Number	SAMPLE COLLECTION		Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION	Chronic Biomonitoring	TEST PARAMETERS						Bottle Type Code		
	Date/s	Time/s															
FD-2 Comp.	12/8/2011	7:45 AM		X	4	W	Facility Discharge	X									K1112-004A

1. Relinquished by: (Signature)		Date/Time	2. Received by: (Signature)		SAMPLE CONDITION UPON RECEIPT IN LAB		REMARKS / SAMPLE COMMENTS
		12-8-11 1200			1. CUSTODY SEALS: <input checked="" type="checkbox"/> Yes ___ No		
3. Relinquished by: (Signature)		Date/Time			4. Received by lab: (Signature)		
					3. COC/LABELS AGREE: <input type="checkbox"/> Yes ___ No		
					4. PRESERVATION CONFIRMED: <input type="checkbox"/> Yes ___ No		
					5. RECEIVED ON ICE: <input type="checkbox"/> Yes ___ No		
					6. TEMPERATURE ON RECEIPT: 30C		
					FOR COMPLETION BY LAB ONLY		

APPENDIX B

Effluent and Dilution Water Data

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Fathead Minnow

Lab # / Sample ID K1112004

Test Start (Date/Time) 12/8/11

Client: Weston

Test End (Date/Time) 12/15/11

		Day of Test							notes/remarks
		1	2	3	4	5	6	7	
<b>Control</b>	MHS551	12/8/11	12/9	12/10	12/11	12/12	12/13	12/14	
D.O. (mg/L)	INITIAL	7.2	7.4	8.4	9.0	8.4	8.2	8.2	
	FINAL	8.2	8.6	8.6	8.3	8.2	8.1	8.6	
pH (s.u.)	INITIAL	7.5	7.7	8.0	7.8	7.7	7.8	7.9	
	FINAL	7.4	7.8	7.9	7.8	7.5	7.7	7.7	
temp (C)	INITIAL	22.8	22.6	21.2	20.1	21.2	22.9	22.5	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
ALKALINITY (mg/L)		32							
HARDNESS (mg/L)		42							
CONDUCTIVITY (umhos/cm)		151							
CHLORINE (mg/L)		<0.05							
<b>CONC:</b>									
D.O. (mg/L)	INITIAL	7.3	7.8	8.5	8.9	8.4	8.3	8.6	
	FINAL	8.1	8.4	8.3	8.3	8.2	7.9	8.0	
pH (s.u.)	INITIAL	7.3	7.3	7.5	7.6	7.3	7.3	7.5	
	FINAL	6.9	7.3	7.6	7.3	7.2	7.3	7.5	
temp (C)	INITIAL	22.1	22.8	22.1	20.9	21.9	23.2	22.7	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
<b>CONC:</b>									
D.O. (mg/L)	INITIAL	7.8	8.0	8.8	9.0	8.4	8.4	8.6	
	FINAL	8.1	8.2	8.2	8.2	8.2	7.9	8.0	
pH (mg/L)	INITIAL	7.1	7.3	7.4	7.5	7.2	7.2	7.4	
	FINAL	6.9	7.4	7.5	7.3	7.0	7.3	7.4	
temp (C)	INITIAL	22.7	22.8	22.2	20.7	21.6	23.2	23.0	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
<b>CONC:</b>									
D.O. (mg/L)	INITIAL	8.2	8.4	8.8	9.1	8.4	8.6	8.8	
	FINAL	8.1	8.3	8.4	8.2	8.1	7.9	7.9	
pH (s.u.)	INITIAL	7.1	7.2	7.4	7.5	7.3	7.2	7.4	
	FINAL	6.9	7.4	7.5	7.3	7.1	7.2	7.3	
temp (C)	INITIAL	22.8	23.1	22.2	21.1	22.1	23.4	23.2	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
<b>CONC:</b>									
D.O. (mg/L)	INITIAL	8.4	8.6	9.3	9.1	8.7	8.7	8.9	
	FINAL	8.0	8.5	8.3	8.1	8.1	7.8	7.9	
pH (s.u.)	INITIAL	7.1	7.2	7.3	7.4	7.2	7.1	7.3	
	FINAL	6.70	7.4	7.5	7.2	7.0	7.2	7.3	
temp (C)	INITIAL	22.9	23.1	22.8	21.3	22.0	23.6	23.4	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
<b>CONC:</b>									
D.O. (mg/L)	INITIAL	8.6	8.7	10.2	9.3	8.9	8.8	9.1	
	FINAL	8.0	8.4	8.3	8.1	8.0	7.8	7.9	
pH (s.u.)	INITIAL	7.0	7.1	7.1	7.3	7.7	7.1	7.3	
	FINAL	7.0	7.3	7.4	7.3	7.6	7.2	7.2	
temp (C)	INITIAL	23.1	23.2	22.2	21.3	22.2	24.3	23.7	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
<b>CONC:</b>		100%	A	A	B	B	C	C	
ALKALINITY (mg/L)		4			6		6		
HARDNESS (mg/L)		2600			2600		2600		
CONDUCTIVITY (umhos/cm)		1928			1964		1968		
CHLORINE (mg/L)		<0.05			<0.05		<0.05		



CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Cerodaphnia Dubia

Lab # / Sample ID K112004

Test Start (Date/Time) 12/8/11

Client: Weston

Test End (Date/Time) 12/15/11

Day of Test

		1	2	3	4	5	6	7	notes/remarks
<b>Control</b>	MHS551	12/8/11	12/9	12/10	12/11	12/12	12/13	12/14	
D.O. (mg/L)	INITIAL	7.2	7.4	8.4	9.0	8.4	8.2	8.2	
	FINAL	8.2	8.4	8.4	8.3	8.4	8.4	8.1	
pH (s.u.)	INITIAL	7.5	7.7	8.0	7.8	7.7	7.8	7.9	
	FINAL	7.5	7.8	7.8	8.0	7.8	7.7	7.7	
temp (C)	INITIAL	22.8	22.6	21.2	20.1	21.2	22.9	22.5	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
ALKALINITY (mg/L)		32							
HARDNESS (mg/L)		42							
CONDUCTIVITY (umhos/cm)		151							
CHLORINE (mg/L)		0.05							
<b>CONC:</b>									
D.O. (mg/L)	INITIAL	7.3	7.8	8.5	8.9	8.7	8.3	8.6	
	FINAL	8.2	8.3	8.4	8.3	8.4	8.3	8.1	
pH (s.u.)	INITIAL	7.3	7.3	7.5	7.6	7.3	7.3	7.5	
	FINAL	7.2	7.5	7.6	7.7	7.8	7.3	7.2	
temp (C)	INITIAL	22.1	22.8	22.1	20.9	21.9	23.2	22.7	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
<b>CONC:</b>									
D.O. (mg/L)	INITIAL	7.8	8.0	8.8	9.0	8.4	8.4	8.6	
	FINAL	8.2	8.3	8.4	8.2	8.4	8.3	8.0	
pH (mg/L)	INITIAL	7.1	7.3	7.4	7.5	7.2	7.2	7.4	
	FINAL	7.1	7.5	7.6	7.6	7.7	7.3	7.3	
temp (C)	INITIAL	22.7	22.8	22.2	20.7	21.6	23.2	23.0	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
<b>CONC:</b>									
D.O. (mg/L)	INITIAL	8.2	8.4	8.8	9.1	8.4	8.6	8.8	
	FINAL	8.3	8.3	8.4	8.2	8.3	8.3	7.9	
pH (s.u.)	INITIAL	7.8	7.2	7.4	7.5	7.3	7.2	7.4	
	FINAL	7.1	7.5	7.5	7.6	7.7	7.3	7.2	
temp (C)	INITIAL	22.8	23.1	22.2	21.1	22.1	23.4	23.2	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
<b>CONC:</b>									
D.O. (mg/L)	INITIAL	8.4	8.6	9.3	9.1	8.7	8.7	8.9	
	FINAL	8.3	8.3	8.4	8.3	8.4	8.3	7.9	
pH (s.u.)	INITIAL	7.1	7.2	7.3	7.4	7.2	7.1	7.3	
	FINAL	7.1	7.4	7.5	7.5	7.7	7.3	7.3	
temp (C)	INITIAL	22.9	23.1	22.8	21.3	22.0	23.6	23.4	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
<b>CONC:</b>									
D.O. (mg/L)	INITIAL	8.6	8.7	10.2	9.3	8.9	8.8	9.1	
	FINAL	8.3	8.3	8.5	8.4	8.4	8.2	7.9	
pH (s.u.)	INITIAL	7.0	7.1	7.1	7.3	7.2	7.1	7.3	
	FINAL	7.0	7.3	7.4	7.4	7.4	7.2	7.2	
temp (C)	INITIAL	23.1	23.2	22.2	21.3	22.2	24.3	23.7	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
<b>CONC: 100%</b>									
ALKALINITY (mg/L)		4			6		6		
HARDNESS (mg/L)		2600			2600		2600		
CONDUCTIVITY (umhos/cm)		1928			1964		1968		
CHLORINE (mg/L)		0.05			0.05		0.05		

## APPENDIX C

Fathead minnow raw data and statistics

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		K1112004		TEST START DATE		12/8/11		TIME		1515	
CLIENT		Western		TEST END DATE		12/15/11		TIME		0940	
AGE AND SOURCE OF MINNOWS											
Summary Page											
DAY (NUMBER SURVIVING)											
SURVIVAL											
CONC: 0	REP #	start	1	2	3	4	5	6	7	%	MEAN % CV
	A	8	8	8	8	8	8	8	8	100	100 0.00
	B	8	8	8	8	8	8	8	8	100	
	C	8	8	8	8	8	8	8	8	100	
	D	8	8	8	8	8	8	8	8	100	
	E	8	8	8	8	8	8	8	8	100	
CONC: 32	REP #	start	1	2	3	4	5	6	7	%	MEAN % CV
	A	8	8	8	8	8	8	8	8	100	100
	B	8	8	8	8	8	8	8	8	100	
	C	8	8	8	8	8	8	8	8	100	
	D	8	8	8	8	8	8	8	8	100	
	E	8	8	8	8	8	8	8	8	100	
CONC: 42	REP #	start	1	2	3	4	5	6	7	%	MEAN % CV
	A	8	8	8	8	8	8	8	8	100	100
	B	8	8	8	8	8	8	8	8	100	
	C	8	8	8	8	8	8	8	8	100	
	D	8	8	8	8	8	8	8	8	100	
	E	8	8	8	8	8	8	8	8	100	
CONC: 56	REP #	start	1	2	3	4	5	6	7	%	MEAN % CV
	A	8	8	8	8	8	8	8	8	100	100
	B	8	8	8	8	8	8	8	8	100	
	C	8	8	8	8	8	8	8	8	100	
	D	8	8	8	8	8	8	8	8	100	
	E	8	8	8	8	8	8	8	8	100	
CONC: 75	REP #	start	1	2	3	4	5	6	7	%	MEAN % CV
	A	8	8	8	8	8	8	8	8	100	100
	B	8	8	8	8	8	8	8	8	100	
	C	8	8	8	8	8	8	8	8	100	
	D	8	8	8	8	8	8	8	8	100	
	E	8	8	8	8	8	8	8	8	100	
CONC: 100	REP #	start	1	2	3	4	5	6	7	%	MEAN % CV
	A	8	8	8	8	8	8	8	8	100	100 0.00
	B	8	8	8	8	8	8	8	8	100	
	C	8	8	8	8	8	8	8	8	100	
	D	8	8	8	8	8	8	8	8	100	
	E	8	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 K1112004 TEST START DATE 12/8/11 TIME 1515  
 CLIENT Weston TEST END DATE 12/15/11 TIME 0940  
 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	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓		
CONC: 31	A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓		
CONC: 41	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: 76	A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓		
CONC: 150	A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓		
ANALYST	KP	TC	TC	KP	KP	KP	KP			
DATE:	12/8/11	12/9/11	12/10/11	12/11/11	12/12/11	12/13/11	12/14/11	12/15/11		
TIME:	1515	1330 1300	1350 1330	1245	1540	1500	1516	0940		

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		12/8/11		TIME 1515				
CLIENT <i>Weston</i>		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	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓		
	E									
CONC: 31	A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓		
	E									
CONC: 41	A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓		
	E									
CONC: 46	A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓		
	E									
CONC: 76	A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓		
	E									
CONC: 100	A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓		
	E									
ANALYST		ICP	FERP	TC	TC					
DATE:		12/8/11	12/9/11	12/10/11	12/11/11					
TIME:		1515	1230	1245	1245					

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		12/8/11		TIME 1515				
CLIENT <i>Weston</i>		TEST END DATE				TIME				
AGE AND SOURCE OF MINNOWS										
DAY (NUMBER SURVIVING)										
REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
CONC: 0	A	2	2	2	2	2	2			
	B									
	C									
	D									
	E									
CONC: 3L	A	2	2	2	2	2	2			
	B									
	C									
	D									
	E									
CONC: 4L	A	2	2	2	2	2	2			
	B									
	C									
	D									
	E									
CONC: 5L	A	2	2	2	2	2	2			
	B									
	C									
	D									
	E									
CONC: 7L	A	2	2	2	2	2	2			
	B									
	C									
	D									
	E									
CONC: 10L	A	2	2	2	2	2	2			
	B									
	C									
	D									
	E									
ANALYST	JP	TC	TC	TC						
DATE:	12/8/11	12/9/11	12/10/11	12/11/11						
TIME:	1515	1300	1245	1245						

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		12/8/11		TIME 1515					
CLIENT <u>Weston</u>		TEST END DATE				TIME					
		AGE AND SOURCE OF MINNOWS									
		DAY (NUMBER SURVIVING)								SURVIVAL	
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
0	A	2	2	2	2	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓			
	E										
3L	A	2	2	2	2	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓			
	E										
1L	A	2	2	2	2	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓			
	E										
1L	A	2	2	2	2	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓			
	E										
7L	A	2	2	2	2	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓			
	E										
10L	A	2	2	2	2	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓			
	E										
ANALYST		KP	TC	TC	TC						
DATE:		12/8/11	12/9/11	12/10/11	12/11/11						
TIME:		1515	1230	1245	1245						

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		12/8/11		TIME 1515						
CLIENT <i>Weston</i>		TEST END DATE				TIME						
AGE AND SOURCE OF MINNOWS												
DAY (NUMBER SURVIVING)												
		REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
CONC: 0	A		2	2	2	2	2	2	2			
	B		↓	↓	↓	↓	↓	↓	↓			
	C		↓	↓	↓	↓	↓	↓	↓			
	D		↓	↓	↓	↓	↓	↓	↓			
	E		↓	↓	↓	↓	↓	↓	↓			
CONC: 3L	A		2	2	2	2	2	2	2			
	B		↓	↓	↓	↓	↓	↓	↓			
	C		↓	↓	↓	↓	↓	↓	↓			
	D		↓	↓	↓	↓	↓	↓	↓			
	E		↓	↓	↓	↓	↓	↓	↓			
CONC: 1L	A		2	2	2	2	2	2	2			
	B		↓	↓	↓	↓	↓	↓	↓			
	C		↓	↓	↓	↓	↓	↓	↓			
	D		↓	↓	↓	↓	↓	↓	↓			
	E		↓	↓	↓	↓	↓	↓	↓			
CONC: 1L	A		2	2	2	2	2	2	2			
	B		↓	↓	↓	↓	↓	↓	↓			
	C		↓	↓	↓	↓	↓	↓	↓			
	D		↓	↓	↓	↓	↓	↓	↓			
	E		↓	↓	↓	↓	↓	↓	↓			
CONC: 7L	A		2	2	2	2	2	2	2			
	B		↓	↓	↓	↓	↓	↓	↓			
	C		↓	↓	↓	↓	↓	↓	↓			
	D		↓	↓	↓	↓	↓	↓	↓			
	E		↓	↓	↓	↓	↓	↓	↓			
CONC: 15L	A		2	2	2	2	2	2	2			
	B		↓	↓	↓	↓	↓	↓	↓			
	C		↓	↓	↓	↓	↓	↓	↓			
	D		↓	↓	↓	↓	↓	↓	↓			
	E		↓	↓	↓	↓	↓	↓	↓			
ANALYST		KP	TC	TC	TC							
DATE:		12/8/11	12/9/11	12/10/11	12/11/11							
TIME:		1515	1300	1230	1245							

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



**WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST**

LAB # / #s:		K1112003			TEST DATES (BEGIN / END):		12/8-15/11	
CLIENT:		EEMA			WEIGHING DATE / TIME:		12/16/11, 1355	
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	0.96536	0.96238	0.00298	8	0.372	AVG DRY	
	B	0.96874	0.96620	0.00254	8	0.318	WEIGHT (mg)	
	C	0.98938	0.98721	0.00217	8	0.271	0.319	
	D	0.99889	0.99659	0.00230	8	0.287	CV	
	E	1.01143	1.00868	0.00275	8	0.344	12.9	
CONC:	A	0.98713	0.98355	0.00358	8	0.447	AVG DRY	
	B	1.00733	1.00374	0.00359	8	0.449	WEIGHT (mg)	
	C	0.99958	0.99700	0.00258	8	0.323	0.386	
	D	1.00288	1.00007	0.00281	8	0.351	CV	
	E	0.99966	0.99676	0.00290	8	0.363		
CONC:	A	0.98741	0.98320	0.00421	8	0.526	AVG DRY	
	B	0.97306	0.96961	0.00345	8	0.431	WEIGHT (mg)	
	C	0.98663	0.98357	0.00306	8	0.382	0.433	
	D	1.01364	1.01049	0.00315	8	0.394	CV	
	E	0.96513	0.96169	0.00344	8	0.430		
CONC:	A	0.95358	0.94961	0.00397	8	0.496	AVG DRY	
	B	1.00863	1.00537	0.00326	8	0.407	WEIGHT (mg)	
	C	0.96980	0.96652	0.00328	8	0.410	0.447	
	D	0.96132	0.95750	0.00382	8	0.477	CV	
	E	0.96881	0.96526	0.00355	8	0.444		
CONC:	A	0.95652	0.95287	0.00365	8	0.456	AVG DRY	
	B	1.01035	1.00651	0.00384	8	0.480	WEIGHT (mg)	
	C	1.00364	1.00084	0.00280	8	0.350	0.419	
	D	1.00090	0.99800	0.00290	8	0.362	CV	
	E	1.00509	1.00153	0.00356	8	0.445		
CONC:	A	0.99500	0.99097	0.00403	8	0.504	AVG DRY	
	B	0.96985	0.96635	0.00350	8	0.437	WEIGHT (mg)	
	C	0.98394	0.98058	0.00336	8	0.420	0.456	
	D	1.01429	1.01092	0.00337	8	0.421	CV	
	E	1.00487	1.00089	0.00398	8	0.497	9.07	

CV = (STANDARD DEVIATION/MEAN)\*100

**REMARKS:**

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AA# K1112004, FATHEAD MINNOW, CHRONIC, 12-8-11  
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# K1112004, FATHEAD MINNOW, CHRONIC, 12-8-11  
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# K1112004, FATHEAD MINNOW, CHRONIC, 12-8-11  
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	1.4120
1	CONTROL	2	1.0000	1.4120
1	CONTROL	3	1.0000	1.4120
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	1.0000	1.4120

3	42 %	EFFLUENT	1	1.0000	1.4120
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	1.0000	1.4120
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	1.0000	1.4120
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# K1112004, FATHEAD MINNOW, CHRONIC, 12-8-11

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	1.412				
2	32 % EFFLUENT	1.412	27.50	16.00	5.00	
3	42 % EFFLUENT	1.412	27.50	16.00	5.00	
4	56 % EFFLUENT	1.412	27.50	16.00	5.00	
5	75 % EFFLUENT	1.412	27.50	16.00	5.00	
6	100 % EFFLUENT	1.412	27.50	16.00	5.00	

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

AA# K1112004, FATHEAD MINNOW GROWTH CHRONIC, 12-8-11  
File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.062

W = 0.932

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# K1112004, FATHEAD MINNOW GROWTH CHRONIC, 12-8-11  
File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 1.27

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# K1112004, FATHEAD MINNOW GROWTH CHRONIC, 12-8-11  
FILE: Z:\TOXSTAT\MONTE\FHGR.  
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.3720	0.6560
1	CONTROL	2	0.3180	0.5991
1	CONTROL	3	0.2710	0.5475
1	CONTROL	4	0.2870	0.5654
1	CONTROL	5	0.3440	0.6267
2	32 % EFFLUENT	1	0.4470	0.7323
2	32 % EFFLUENT	2	0.4490	0.7343
2	32 % EFFLUENT	3	0.3230	0.6045
2	32 % EFFLUENT	4	0.3510	0.6341
2	32 % EFFLUENT	5	0.3630	0.6466
3	42 % EFFLUENT	1	0.5260	0.8114
3	42 % EFFLUENT	2	0.4310	0.7162
3	42 % EFFLUENT	3	0.3820	0.6663
3	42 % EFFLUENT	4	0.3940	0.6786
3	42 % EFFLUENT	5	0.4300	0.7152
4	56 % EFFLUENT	1	0.4960	0.7814

4	56 %	EFFLUENT	2	0.4070	0.6919
4	56 %	EFFLUENT	3	0.4100	0.6949
4	56 %	EFFLUENT	4	0.4770	0.7624
4	56 %	EFFLUENT	5	0.4440	0.7293
5	75 %	EFFLUENT	1	0.4560	0.7413
5	75 %	EFFLUENT	2	0.4800	0.7654
5	75 %	EFFLUENT	3	0.3500	0.6331
5	75 %	EFFLUENT	4	0.3620	0.6456
5	75 %	EFFLUENT	5	0.4450	0.7303
6	100 %	EFFLUENT	1	0.5040	0.7894
6	100 %	EFFLUENT	2	0.4370	0.7222
6	100 %	EFFLUENT	3	0.4200	0.7051
6	100 %	EFFLUENT	4	0.4210	0.7061
6	100 %	EFFLUENT	5	0.4970	0.7824

AA# K1112004, FATHEAD MINNOW GROWTH CHRONIC, 12-8-11  
 File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.069	0.014	5.333
Within (Error)	24	0.062	0.003	
Total	29	0.132		

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

AA# K1112004, FATHEAD MINNOW GROWTH CHRONIC, 12-8-11  
 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	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	0.599	0.318		
2	32 % EFFLUENT	0.670	0.387	-2.213	
3	42 % EFFLUENT	0.718	0.433	-3.675	
4	56 % EFFLUENT	0.732	0.447	-4.122	
5	75 % EFFLUENT	0.703	0.419	-3.229	
6	100 % EFFLUENT	0.741	0.456	-4.403	

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

AA# K1112004, FATHEAD MINNOW GROWTH CHRONIC, 12-8-11  
 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	-0.068
3	42 % EFFLUENT	5	0.069	21.5	-0.114
4	56 % EFFLUENT	5	0.069	21.5	-0.128
5	75 % EFFLUENT	5	0.069	21.5	-0.100
6	100 % EFFLUENT	5	0.069	21.5	-0.137

APPENDIX D

*Ceriodaphnia dubia* Raw Data and Statistics

Cerodaphnia dubia

SURVIVAL AND REPRODUCTION TEST

Discharger: W. Weston Lab Number/s: K1112004

Analyst: KP  
Test Start - Date/Time: 12/8/11 1400  
Test Stop - Date/Time: 17/15/11 0900

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
0	2	0	0	0	0	0	0	0	0	0	0	0	10	0	Te
0	3	0	2	1	1	3	0	1	1	0	3	12	10	1.2	KP
0	4	2	2	1	3	4	3	2	6	1	0	18	10	1.8	KP
0	5	4	3	3	2	1	3	2	5	4	6	33	10	3.3	KP
0	6	8	6	9	5	7	3	4	0	8	4	56	10	5.6	KP
0	7	3	6	2	3	4	7	8	5	7	0	45	10	4.5	KP
0	8														
0	Total	17	19	16	14	19	18	17	11	70	13	164		$\bar{x}=16.4$	

Conc 4	% Day	Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
		A	B	C	D	E	F	G	H	I	J				
50	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
50	2	0	0	0	0	0	0	0	0	0	0	0	9	0	
50	3	2	0	1	0	1	1	9	0	2	2	13	9	1.4	
50	4	2	1	5	2	2	0	1	0	2	3	17	9	1.9	
50	5	8	1	4	7	2	5	4	0	9	6	46	9	5.1	
50	6	2	6	6	4	7	0	6	0	3	3	39	9	4.3	
50	7	5	3	7	4	2	7	5	0	4	2	39	9	4.3	
50	8														
50	Total	24	11	23	17	13	13	20	10	17	16	154			

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	
32	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
32	3	0	0	1	3	2	3	1	0	0	4	14	10	1.4	
32	4	3	3	4	1	0	1	0	4	3	0	19	10	1.9	
32	5	10	9	3	3	7	8	6	9	7	4	66	10	6.6	
32	6	5	3	5	7	2	9	5	0	6	9	45	10	4.5	
32	7	2	7	3	6	0	5	7	2	0	3	35	10	3.5	
32	8														
32	Total	20	21	16	20	11	26	19	15	10	20	179			

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	
75	2	0	0	0	0	0	0	0	0	0	0	0	8	0	
75	3	1	0	0	0	2	5	2	3	1	3	17	8	2.1	
75	4	2	0	1	0	0	4	2	1	3	1	14	8	1.8	
75	5	4	0	3	0	0	4	2	10	9	0	74	8	5.5	
75	6	6	0	4	0	0	5	9	0	0	6	73	7	5.3	
75	7	7	0	7	0	0	3	6	0	5	8	30	7	4.3	
75	8														
75	Total	14	0	15	0	19	31	14	18	18	18	142			

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	
42	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
42	3	1	1	3	3	4	4	1	0	2	3	22	10	2.2	
42	4	6	3	1	1	0	2	0	2	0	0	9	10	0.9	
42	5	4	8	5	2	4	7	6	6	8	10	60	10	6.0	
42	6	3	7	8	7	4	7	0	5	3	0	47	10	4.7	
42	7	3	4	2	0	3	2	0	0	2	2	19	10		
42	8														
42	Total	15	22	19	13	15	22	7	13	16	15	157			

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	
100	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
100	3	6	1	4	6	2	3	1	2	2	0	27	10	2.7	
100	4	1	2	1	0	3	0	4	2	1	3	17	10	1.7	
100	5	6	7	9	9	1	4	9	3	7	6	56	9	6.2	
100	6	3	8	4	9	2	0	3	5	8	8	42	9	4.7	
100	7	5	2	8	6	7	0	3	0	4	0	35	9	3.9	
100	8														
100	Total	21	15	26	30	15	17	10	19	17	17	177			

X= DEAD; Y= MALE

$\bar{x}=18.9$   
 $CV=321$



AA # K1112004, C. DUBIA CHRONIC, REPRODUCCION, 12-8-11  
File: Z:/toxstat/monte\CD. Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

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\*\*\*\*\* 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 # K1112004, C. DUBIA CHRONIC, REPRODUCCION, 12-8-11  
File: Z:/toxstat/monte\CD. Transform: NO TRANSFORMATION

---

Bartlett's test for homogeneity of variance  
Calculated B1 statistic = 12.05

---

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%	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
75%	7	3	10
TOTAL	17	3	20

CRITICAL FISHER'S VALUE (10,10,10) (p=0.05) IS 6. b VALUE IS 7.  
 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
100%	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	32%	10	0	
2	42%	10	0	
3	56%	10	1	
4	75%	10	3	
5	100%	10	1	

TITLE: AA # K1112004, C. DUBIA CHRONIC, REPRODUCTION, 12-8-11  
FILE: Z:/toxstat/monte\CD.  
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	17.0000	17.0000
1	CONTROL	2	19.0000	19.0000
1	CONTROL	3	16.0000	16.0000
1	CONTROL	4	14.0000	14.0000
1	CONTROL	5	19.0000	19.0000
1	CONTROL	6	18.0000	18.0000
1	CONTROL	7	17.0000	17.0000
1	CONTROL	8	11.0000	11.0000
1	CONTROL	9	20.0000	20.0000
1	CONTROL	10	13.0000	13.0000
2	32 % EFFLUENT	1	20.0000	20.0000
2	32 % EFFLUENT	2	22.0000	22.0000
2	32 % EFFLUENT	3	16.0000	16.0000
2	32 % EFFLUENT	4	20.0000	20.0000
2	32 % EFFLUENT	5	11.0000	11.0000
2	32 % EFFLUENT	6	26.0000	26.0000
2	32 % EFFLUENT	7	19.0000	19.0000
2	32 % EFFLUENT	8	15.0000	15.0000
2	32 % EFFLUENT	9	10.0000	10.0000
2	32 % EFFLUENT	10	20.0000	20.0000
3	42 % EFFLUENT	1	15.0000	15.0000
3	42 % EFFLUENT	2	22.0000	22.0000
3	42 % EFFLUENT	3	19.0000	19.0000
3	42 % EFFLUENT	4	13.0000	13.0000
3	42 % EFFLUENT	5	15.0000	15.0000
3	42 % EFFLUENT	6	22.0000	22.0000
3	42 % EFFLUENT	7	7.0000	7.0000
3	42 % EFFLUENT	8	13.0000	13.0000
3	42 % EFFLUENT	9	16.0000	16.0000
3	42 % EFFLUENT	10	15.0000	15.0000
4	56 % EFFLUENT	1	24.0000	24.0000
4	56 % EFFLUENT	2	11.0000	11.0000
4	56 % EFFLUENT	3	23.0000	23.0000
4	56 % EFFLUENT	4	17.0000	17.0000
4	56 % EFFLUENT	5	13.0000	13.0000
4	56 % EFFLUENT	6	13.0000	13.0000
4	56 % EFFLUENT	7	20.0000	20.0000
4	56 % EFFLUENT	8	0.0000	0.0000

4	56	%	EFFLUENT	9	17.0000	17.0000
4	56	%	EFFLUENT	10	16.0000	16.0000
5	75	%	EFFLUENT	1	14.0000	14.0000
5	75	%	EFFLUENT	2	0.0000	0.0000
5	75	%	EFFLUENT	3	15.0000	15.0000
5	75	%	EFFLUENT	4	0.0000	0.0000
5	75	%	EFFLUENT	5	14.0000	14.0000
5	75	%	EFFLUENT	6	31.0000	31.0000
5	75	%	EFFLUENT	7	14.0000	14.0000
5	75	%	EFFLUENT	8	18.0000	18.0000
5	75	%	EFFLUENT	9	18.0000	18.0000
5	75	%	EFFLUENT	10	18.0000	18.0000
6	100	%	EFFLUENT	1	21.0000	21.0000
6	100	%	EFFLUENT	2	15.0000	15.0000
6	100	%	EFFLUENT	3	26.0000	26.0000
6	100	%	EFFLUENT	4	30.0000	30.0000
6	100	%	EFFLUENT	5	15.0000	15.0000
6	100	%	EFFLUENT	6	7.0000	7.0000
6	100	%	EFFLUENT	7	17.0000	17.0000
6	100	%	EFFLUENT	8	10.0000	10.0000
6	100	%	EFFLUENT	9	19.0000	19.0000
6	100	%	EFFLUENT	10	17.0000	17.0000

AA # K1112004, C. DUBIA CHRONIC, REPRODUCCION, 12-8-11  
 File: Z:/toxstat/monte\CD. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	100.683	20.137	0.529
Within (Error)	54	2055.500	38.065	
Total	59	2156.183		

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

AA # K1112004, C. DUBIA CHRONIC, REPRODUCCION, 12-8-11  
 File: Z:/toxstat/monte\CD. 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	16.400	16.400		
2	32 % EFFLUENT	17.900	17.900	-0.544	
3	42 % EFFLUENT	15.700	15.700	0.254	
4	56 % EFFLUENT	15.400	15.400	0.362	
5	75 % EFFLUENT	14.200	14.200	0.797	
6	100 % EFFLUENT	17.700	17.700	-0.471	

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

AA # K1112004, C. DUBIA CHRONIC, REPRODUCCION, 12-8-11  
 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	6.374	38.9	-1.500
3	42 % EFFLUENT	10	6.374	38.9	0.700
4	56 % EFFLUENT	10	6.374	38.9	1.000
5	75 % EFFLUENT	10	6.374	38.9	2.200
6	100 % EFFLUENT	10	6.374	38.9	-1.300

AA # K1112004, C. DUBIA CHRONIC, REPRODUCCION, 12-8-11  
 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	16.400				
2	32 % EFFLUENT	17.900	118.50	75.00	10.00	
3	42 % EFFLUENT	15.700	98.50	75.00	10.00	
4	56 % EFFLUENT	15.400	101.50	75.00	10.00	
5	75 % EFFLUENT	14.200	95.00	75.00	10.00	
6	100 % EFFLUENT	17.700	109.00	75.00	10.00	

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

## APPENDIX E

### Organism History

**TEST ORGANISM HISTORY**

DATE SHIPPED 12-7-11 CLIENT Arkansas Analytical  
Purchase Order #: \_\_\_\_\_  
SPECIES: Pimephales promelas Mysidopsis bahia Cyprinodon variegates  
Quantity Shipped: 300<sup>+</sup> + 100<sup>+</sup>  
Age: hatched 12/7 Am - 7 days old 12/7  
Brood Stock Source: Anderson Farms, Inc  
Culture Water: Groundwater Artificial Salts Artificial Salts  
Hardness (Mg/l CaCO<sub>3</sub>) 160 Salinity (ppt) \_\_\_\_\_  
Dissolved Oxygen (Mg/l): 8.2  
Feeding: ARTIFICIAL  
Comments: 25.3 °C  
\_\_\_\_\_  
\_\_\_\_\_  
Shipped Via: Federal Express UPS Overnight Shuttle  
Packaged By: [Signature]



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, Selastrum 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 <sub>3</sub> ):	<u>142 mg/l</u>	<u>86-124 mg/l</u>
TOTAL ALKALINITY (as CaCO <sub>3</sub> ):	<u>100 mg/l</u>	<u>65-130 mg/l</u>
pH:	<u>7.92</u>	<u>7.56-8.35</u>

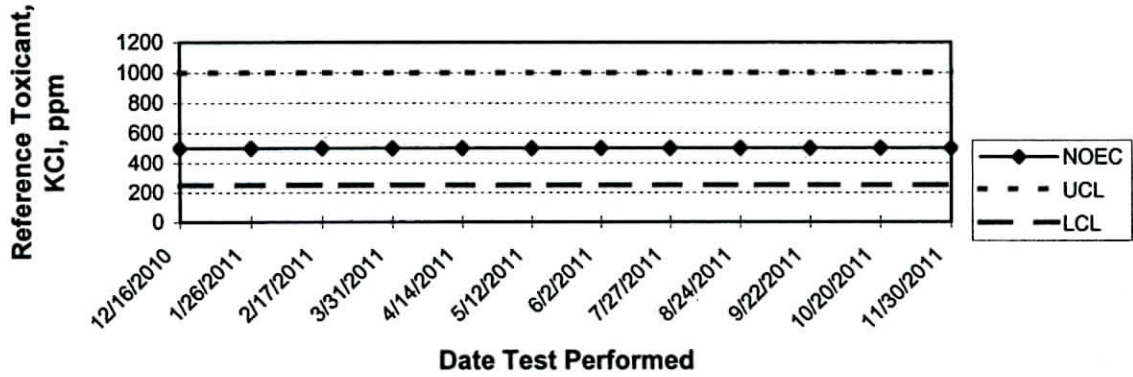
Comments:

  
\_\_\_\_\_  
Facility Supervisor

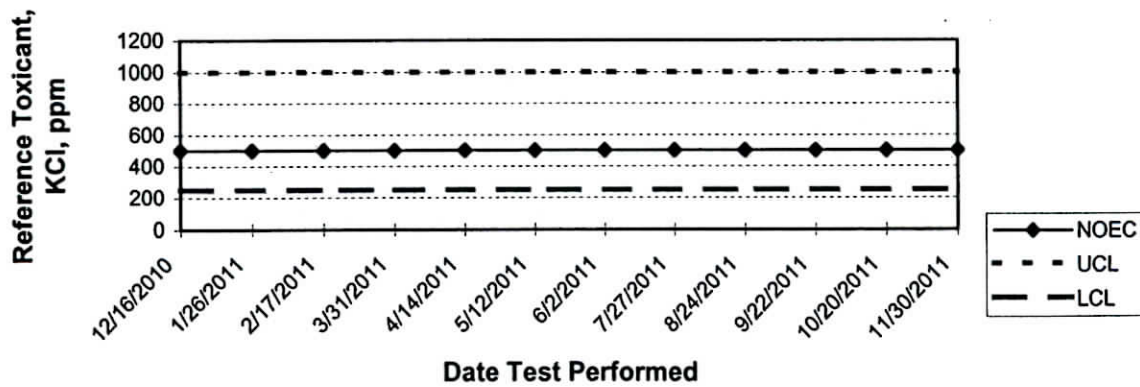
## APPENDIX F

### Quality Assurance Charts

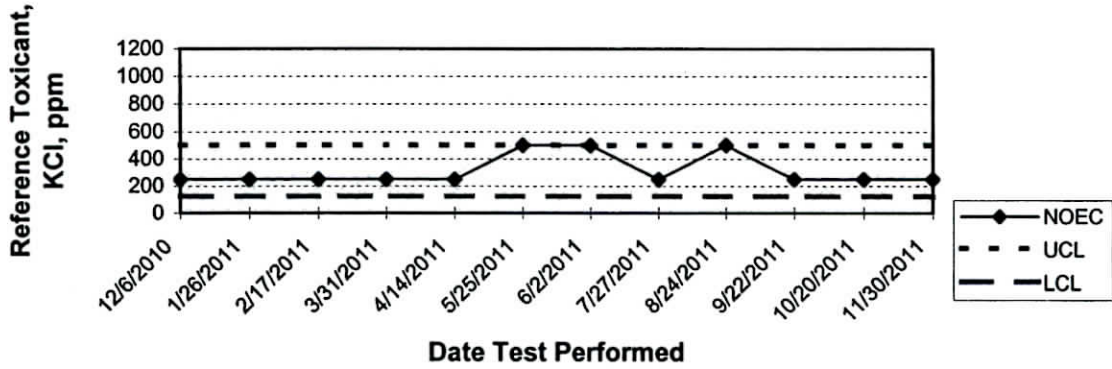
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**FATHEAD MINNOW SURVIVAL**  
**QUALITY ASSURANCE**



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