

# Arkansas Analytical, Inc.

## Toxicity Test Results

**MAGCOBAR MINE SITE  
NPDES PERMIT NUMBER: AR0049794  
April, 2010  
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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P.O. Box 232  
Kulpsville, PA 19443**

Prepared by: **Arkansas Analytical, Inc.  
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Little Rock, Arkansas 72209  
Lab Number K1004002**

Monday, May 03, 2010

## **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 April of 2010.

## **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:	4-14-10, 0818	4-15-10, 0818
Sample #2:	4-15-10, 0905	4-16-10, 0905
Sample #3:	4-19-10, 0835	4-20-10, 0835

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:	4-15-10, 1521	4
Sample #2:	4-16-10, 1421	4
Sample #3:	4-20-10, 1455	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	100%	X	
Average of 15 or more young per surviving female	17.7	X	
At least 60% of surviving females should have produced 3 broods	70%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	32.0%	X	

TEST ACCEPTANCE CRITERIA for *Pimephales promelas*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	95%	X	
The percent coefficient of variation between replicates must be 40% or less for survival	7.21%	X	
Minimum of 0.25 mg average dry weight of surviving controls	0.396	X	
The percent coefficient of variation between replicates must be 40% or less for growth	11.2%	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> 3/31/10-4/7/10		<i>Pimephales promelas</i> 3/31/10-4/7/10	
NOEC Survival:	250 ppm KCl	NOEC Survival:	250 ppm KCl
LOEC Survival:	500 ppm KCl	LOEC Survival:	500 ppm KCl
NOEC Reproduction:	250 ppm KCl	NOEC Growth:	250 ppm KCl
LOEC Reproduction:	500 ppm KCl	LOEC Growth:	500 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)	18.5	%CV survival (critical dilution)	7.21 %
%CV Reproduction (critical dilution)	27.7%	Mean dry weight (critical dilution) in milligrams	0.519
		%CV growth (critical dilution)	15.9%
PMSD Reproduction	34.6	PMSD Growth	30.7

### 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

**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:	4-14-10, 0818	4-15-10, 0818
Sample #2:	4-15-10, 0905	4-16-10, 0905
Sample #3:	4-19-10, 0835	4-20-10, 0835

Test initiated (date, time): 4-16-10, 1040      Test terminated (date, time): 4-23-10, 1340

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%	87.5	100	100	87.5	100		100	100	95	7.21
32%	87.5	75	75	100	100		100	100	87.5	
42%	75	87.5	87.5	100	100		100	90	90	
56%	87.5	100	100	100	100		100	97.5	97.5	
75%	100	100	100	75	100		100	100	95	
100%	87.5	87.5	100	100	100		100	100	95	7.21

**SUMMARY**

Effluent Conc %	A	B	C	D	E		Mean Dry Weight	CV%
0%	0.405	0.323	0.391	0.435	0.425		0.396	11.2
32%	0.360	0.331	0.325	0.536	0.528		0.416	
42%	0.425	0.399	0.276	0.548	0.550		0.440	
56%	0.512	0.539	0.455	0.479	0.651		0.527	
75%	0.504	0.564	0.392	0.507	0.545		0.502	
100%	0.427	0.541	0.439	0.574	0.612		0.519	15.9

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)= \_\_\_\_\_ 15.9 \_\_\_\_\_ %

**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:	4-14-10, 0818	4-15-10, 0818
Sample #2:	4-15-10, 0905	4-16-10, 0905
Sample #3:	4-19-10, 0835	4-20-10, 0835

Test initiated (date, time): 4-16-10, 1045      Test terminated (date, time): 4-23-10, 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	15	20	17	x3	x7	7
B	18	13	13	16	9	20
C	12	8	27	23	15	15
D	15	21	22	24	6	18
E	27	18	26	16	16	21
F	26	17	20	9	7	15
G	18	20	17	14	18	21
H	13	22	19	19	12	20
I	11	15	13	20	25	23
J	22	31	17	23	24	25
Mean	17.7	18.5	19.1	16.7	13.9	18.5
Mean/surviving female	17.7	18.5	19.1	18.2	14.7	18.5
CV%*	32.0					27.7

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	100	100	100
Test termination	100	100	100	90	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)=   32.0   %

## **APPENDIX A**

### **Chain of Custody Forms**

# CHAIN OF CUSTODY RECORD

CLIENT INFORMATION					Project Description	Turnaround Time	Preservation Codes:								
EEMA O & M Services Group		EEMA O & M Services Group			Magcoabar Mine Site	24 Hour	1. Cool, 4 Degrees Centigrade			4. Thiosulfate for Dechlorination					
Magcoabar Mine Site		P.O. Box 732			Biomonitoring Sample	48 Hour	2. Sulfuric Acid ( $H_2SO_4$ ), pH < 2			5. Hydrochloric Acid(HCl)					
P.O. Box 699		Kulpsville, PA 19443			Reporting Information	72 Hour	3. Nitric Acid ( $HNO_3$ ), pH < 2			6. Sodium Hydroxide (NaOH), pH > 12					
Malvern, AR 72104					Telephone: 501-467-8355	Routine (5 Day)	TEST PARAMETERS								
Attn: Bill McAlister		Attn: Amber Rich			Fax: 501-467-8687	Preservative Code	1			Bottle Type Code					
					Email: dave.friedman@eema-inc.com; bmcalister@eema-inc.com; bhorton@eema-inc.com	Bottle Type	P			G = Glass; P = Plastic V = Septum; A = Amber					
Bill McAlister			Bill McAlister											Arkansas Analytical Work Order Number: K1004002 A	
Sampler(s) Signature			Sampler(s) Printed												
Field Number	SAMPLE COLLECTION			Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION						Chronic Biomonitoring	
	Date/s	Time/s						Facility Discharge							
FD-1 Comp.	4/15/2010	8:18 AM		X	4	W		X							
1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)		SAMPLE CONDITION UPON RECEIPT IN LAB								REMARKS / SAMPLE COMMENTS			
Bill McAlister	4-15-10 1501	Sydney James		1. CUSTODY SEALS: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 2. CONTAINERS CORRECT: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 3. COC/LABELS AGREE: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 4. PRESERVATION CONFIRMED: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 5. RECEIVED ON ICE: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 6. TEMPERATURE ON RECEIPT: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No  4°C											
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)		FOR COMPLETION BY LAB ONLY											

# 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 ( $H_2SO_4$ ), pH < 2			5. Hydrochloric Acid(HCl)			
P.O. Box 699	Kulpsville, PA 19443				<b>Reporting Information</b>	72 Hour	3. Nitric Acid ( $HNO_3$ ), pH < 2			6. Sodium Hydroxide (NaOH), pH > 12			
Malvern, AR 72104					Telephone: 501-467-8355	Routine (5 Day)	TEST PARAMETERS						
Attn: Bill McAlister	Attn: Amber Rich				Fax: 501-467-8687	Preservative Code:	1					Bottle Type Code	
				Email: dave.friedman@eema-inc.com, brmcalister@eema-inc.com, bhorton@eema-inc.com		Bottle Type:	P					G = Glass; P = Plastic V = Septum; A = Amber	
<i>Bill Mc Alister</i>			<i>Bill Mc Alister</i>			Chronic Biomonitoring						Arkansas Analytical Work Order Number:	
Sampler(s) Signature			Sampler(s) Printed									K1004002	
Field Number	SAMPLE COLLECTION		Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION						
FD-2 Comp.	Date/s	Time/s		X	3	W	Facility Discharge	X				B	
1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)	SAMPLE CONDITION UPON RECEIPT IN LAB						REMARKS / SAMPLE COMMENTS				
<i>Bill Mc Alister</i>	4-16-10 1421		1. CUSTODY SEALS: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 2. CONTAINERS CORRECT: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 3. COC/LABELS AGREE: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 4. PRESERVATION CONFIRMED: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 5. RECEIVED ON ICE: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 6. TEMPERATURE ON RECEIPT: <input checked="" type="checkbox"/> 4°C										
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)	FOR COMPLETION BY LAB ONLY										
		<i>Sarah E. Ronse</i>											

# CHAIN OF CUSTODY RECORD

CLIENT INFORMATION			Project Description	Turnaround Time	Preservation Codes:								
EEMA O & M Services Group	EEMA O & M Services Group		Magcoabar Mine Site	24 Hour	1. Cool, 4 Degrees Centigrade				4. Thiosulfate for Dechlorination				
Magcoabar Mine Site	P.O. Box 732		Biomonitoring Sample	48 Hour	2. Sulfuric Acid ( $H_2SO_4$ ), pH < 2				5. Hydrochloric Acid(HCl)				
P.O. Box 699	Kulpsville, PA 19443		Reporting Information				3. Nitric Acid ( $HNO_3$ ), pH < 2				6. Sodium Hydroxide (NaOH), pH > 12		
Malvern, AR 72104			Telephone: 501-467-8355				TEST PARAMETERS				Bottle Type Code		
Attn: Bill McAlister	Attn: Amber Rich		Fax: 501-467-8687				Preservative Code:	1					
			Email: dave.friedman@eema-inc.com; bmcalister@eema-inc.com; bhorton@eema-inc.com				Bottle Type:	P				G = Glass, P = Plastic	
												V = Septum: A = Amber	

<i>Bill McAlister</i>		<i>Bill McAlister</i>										Arkansas Analytical Work Order Number:  <b>K1004002</b>					
Sampler(s) Signature		Sampler(s) Printed															
Field Number	SAMPLE COLLECTION		Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION		Chronic Biomonitoring								
	Date/s	Time/s					Facility Discharge			X							
FD-1 Comp.	4/20/2010	8:35 AM	X	4	W											C	

1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)	SAMPLE CONDITION UPON RECEIPT IN LAB						REMARKS / SAMPLE COMMENTS					
<i>Bill McAlister</i>	4-20-10 1455		1. CUSTODY SEALS: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 2. CONTAINERS CORRECT: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 3. COC/LABELS AGREE: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 4. PRESERVATION CONFIRMED: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 5. RECEIVED ON ICE: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 6. TEMPERATURE ON RECEIPT: <b>40°C</b>											
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)												
		<i>Sarah E Ronse</i>												
FOR COMPLETION BY LAB ONLY														

## APPENDIX B

### Effluent and Dilution Water Data

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING								Fathead Minnow	
Lab # / Sample ID		Test Start (Date/Time) 4/16/10							
Client Weston		Test End (Date/Time) 4/23/10							
Day of Test									
		1	2	3	4	5	6	7	notes/remarks
Control	0	4/16/10	4/17	4/18	4/19	4/20	4/21	4/22	
D.O. (mg/L)	INITIAL	84	8.48	8.40	85	84	86	85	
	FINAL	7.53	7.74	7.7	7.6	81	82	77	
pH (s.u.)	INITIAL	78	6.9	7.2	7.2	7.3	7.3	74	
	FINAL	6.94	6.99	74	70	78	76	77	
temp (C)	INITIAL	20.8	20.9	20.3	21.0	21.8	21.9	22.3	
	FINAL	25.0	25.0	250	250	250	250	250	
ALKALINITY (mg/L)		22							↑
HARDNESS (mg/L)		38.4							↑
CONDUCTIVITY (umhos/cm)		153							↓
CHLORINE (mg/L)		0.05							↑
CONC:	32								
D.O. (mg/L)	INITIAL	85	8.54	8.77	87	85	85	86	
	FINAL	7.58	7.71	7.6	77	78	82	76	
pH (s.u.)	INITIAL	77	6.9	6.89	69	67	67	70	
	FINAL	6.61	6.90	72	70	74	77	77	
temp (C)	INITIAL	20.8	21.4	21.9	21.1	21.4	22.4	22.5	
	FINAL	25.0	25.0	250	250	250	250	250	
CONC:	42								
D.O. (mg/L)	INITIAL	86	9.12	8.78	87	87	86	86	
	FINAL	7.64	7.65	76	77	78	80	77	
pH (mg/L)	INITIAL	77	6.8	7.01	76	67	68	70	
	FINAL	6.45	6.82	72	70	73	77	76	
temp (C)	INITIAL	20.7	21.8	22.5	21.2	21.4	22.4	22.6	
	FINAL	25.0	25.0	250	250	250	250	250	
CONC:	56								
D.O. (mg/L)	INITIAL	88	9.27	8.82	81	88	87	87	
	FINAL	7.75	7.65	77	78	78	81	78	
pH (s.u.)	INITIAL	76	6.6	6.99	70	66	69	70	
	FINAL	6.72	6.91	72	70	73	74	75	
temp (C)	INITIAL	20.9	22.2	23.1	21.2	21.3	22.6	22.5	
	FINAL	25.0	25.0	250	250	250	250	250	
CONC:	75								
D.O. (mg/L)	INITIAL	89	9.43	8.91	86	89	87	87	
	FINAL	7.77	7.70	77	78	77	81	78	
pH (s.u.)	INITIAL	75	6.7	7.11	70	66	69	69	
	FINAL	6.63	6.85	72	69	72	74	75	
temp (C)	INITIAL	21.0	22.7	23.8	21.2	21.4	22.8	22.6	
	FINAL	25.0	25.0	250	250	250	250	250	
CONC:	100								
D.O. (mg/L)	INITIAL	89	9.6	9.12	87	89	87	88	
	FINAL	7.76	8.42	81	76	78	81	78	
pH (s.u.)	INITIAL	73	6.5	7.05	68	66	68	69	
	FINAL	6.42	6.44	7.0	69	71	72	74	
temp (C)	INITIAL	21.3	23.0	23.8	21.4	21.6	22.1	22.7	
	FINAL	25.0	25.0	250	250	250	250	250	
CONC:	100%	A	A	A	B	B	C	C	
ALKALINITY (mg/L)		4		1	6	1	4	1	
HARDNESS (mg/L)		>600		1	>600	1	>600	1	
CONDUCTIVITY (umhos/cm)		20300		1	20500	1	20500	1	
CHLORINE (mg/L)		0.05		1	0.05	1	0.05	1	

## CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Cerodaphnia Dubia

Lab # / Sample ID K1004002

Test Start (Date/Time) 4/16/10

Client Weston

Test End (Date/Time) 4/23/10

		Day of Test							notes/remarks
		1	2	3	4	5	6	7	
Control	0	4/16/10	4/17	4/18	4/19	4/20	4/21	4/22	
D.O. (mg/L)	INITIAL	84	8.48	8.40	85	84	86	85	
	FINAL	8.21	7.85	79	76	75	78		
pH (s.u.)	INITIAL	7.8	6.9	7.2	7.7	7.3	7.3	7.4	
	FINAL	6.7	7.12	74	6.9	7.7	7.3		
temp (C)	INITIAL	20.8	20.9	20.3	21.0	21.8	21.9	22.3	
	FINAL	25.0	25.0	250	250	250	250		
ALKALINITY (mg/L)		22							
HARDNESS (mg/L)		258							
CONDUCTIVITY (umhos/cm)		153							
CHLORINE (mg/L)		0.05							
CONC:	32								
D.O. (mg/L)	INITIAL	85	8.54	8.77	87	85	85	86	
	FINAL	8.20	7.97	879	75	77	78		
pH (s.u.)	INITIAL	77	6.9	6.89	69	67	67	70	
	FINAL	6.6	6.82	70	6.6	6.8	6.8		
temp (C)	INITIAL	20.8	21.4	21.8	211	214	224	225	
	FINAL	25.0	25.0	250	250	250	250		
CONC:	42								
D.O. (mg/L)	INITIAL	86	9.12	8.78	87	87	86	86	
	FINAL	8.19	6.8	78	75	76	77		
pH (s.u.)	INITIAL	77	6.8	7.01	70	6.7	6.8	70	
	FINAL	6.7	6.79	6.8	6.6	76	6.9		
temp (C)	INITIAL	20.7	21.8	22.5	212	214	224	226	
	FINAL	25.0	25.0	250	250	250	250		
CONC:	56								
D.O. (mg/L)	INITIAL	88	9.27	8.82	86	88	87	87	
	FINAL	8.22	7.98	78	76	78	77		
pH (s.u.)	INITIAL	76	6.6	6.99	70	6.6	6.9	70	
	FINAL	6.5	6.85	6.8	6.6	78	6.9		
temp (C)	INITIAL	20.9	22.2	23.1	212	213	226	226	
	FINAL	25.0	25.0	250	250	250	250		
CONC:	75								
D.O. (mg/L)	INITIAL	89	9.43	8.91	86	89	87	87	
	FINAL	8.24	6.70	77	75	78	73		
pH (s.u.)	INITIAL	75	6.7	7.11	70	6.6	6.9	6.9	
	FINAL	6.6	6.79	6.7	6.6	78	70		
temp (C)	INITIAL	21.0	22.7	23.8	212	214	228	226	
	FINAL	25.0	25.0	250	250	250	250		
CONC:	100								
D.O. (mg/L)	INITIAL	89	9.60	9.12	87	89	87	88	
	FINAL	8.26	8.11	79	75	77	74		
pH (s.u.)	INITIAL	73	6.5	6.95	68	6.6	6.8	6.9	
	FINAL	6.42	7.08	6.9	6.6	74	72		
temp (C)	INITIAL	21.3	23.0	23.8	214	216	227	227	
	FINAL	25.0	25.0	250	250	250	250		
CONC:	100%	A	A	A	B	B	C	C	
ALKALINITY (mg/L)		4		1	6	7	4	7	
HARDNESS (mg/L)		>600		1	>600	1	>600	1	
CONDUCTIVITY (umhos/cm)		20800		1	20500	1	20500	1	
CHLORINE (mg/L)		0.05		1	0.05	1	0.05	1	

## APPENDIX C

Fathead minnow raw data and statistics

## SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID 1004002

TEST START DATE 4/16/10 TIME 1040

CLIENT Weston Summary

TEST END DATE 4/23/10 TIME 1340

## AGE AND SOURCE OF MINNOWS

## DAY (NUMBER SURVIVING)

## SURVIVAL

CONC:	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC:	A	8	8	8	7	7	7	7	7	95	7.21
	B	8	8	8	8	8	8	8	8		
	C	8	8	8	8	8	8	8	8		
	D	8	7	7	7	7	7	7	7		
	E	8	8	8	8	8	8	8	8		
CONC:	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC:	A	8	8	8	7	7	7	7	7	87.5	
	B	8	6	6	6	6	6	6	6		
	C	6	6	6	6	6	6	6	6		
	D	8	8	8	8	8	8	8	8		
	E	8	8	8	8	8	8	8	8		
CONC:	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC:	A	8	8	6	6	6	6	6	6	90	
	B	7	7	7	7	7	7	7	7		
	C	7	7	7	7	7	7	7	7		
	D	8	8	8	8	8	8	8	8		
	E	8	8	8	8	8	8	8	8		
CONC:	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC:	A	8	8	7	7	7	7	7	7	97.5	
	B	8	8	8	8	8	8	8	8		
	C	8	8	8	8	8	8	8	8		
	D	8	8	6	6	6	6	6	6		
	E	8	8	8	8	8	8	8	8		
CONC:	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC:	A	8	8	8	8	8	8	8	8	95	
	B	8	8	8	8	8	8	8	8		
	C	8	8	8	8	8	8	8	8		
	D	6	6	6	6	6	6	6	6		
	E	8	8	8	8	8	8	8	8		
CONC:	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC:	A	8	8	8	7	7	7	7	7	95	7.21
	B	8	8	8	7	7	7	7	7		
	C	8	8	8	8	8	8	8	8		
	D	8	8	8	8	8	8	8	8		
	E	8	8	8	8	8	8	8	8		
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 K1004002

TEST START DATE 4/16/10 TIME 1040

CLIENT Weston

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	1	1	2	2	2	2	2			
	C	1	1	1	1	1	1	1			
	D	1	1	2	2	2	2	2			
	E										
CONC: 2	A	2	2	2	2	2	2	2			
	B	1	1	1	1	1	1	1			
	C	1	2	2	2	2	2	2			
	D	1	1	2	2	2	2	2			
	E										
CONC: 4	A	2	2	2	2	2	2	2			
	B	1	1	1	1	1	1	1			
	C	1	2	2	2	2	2	2			
	D	1	1	1	1	1	1	1			
	E										
CONC: 50	A	2	2	1	1	1	1	1			
	B	1	2	2	2	2	2	2			
	C	1	2	2	2	2	2	2			
	D	1	2	2	2	2	2	2			
	E										
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										
CONC: 100	A	2	2	2	2	2	2	2			
	B	1	2	2	2	2	2	2			
	C	1	2	2	2	2	2	2			
	D	1	1	1	1	1	1	1			
	E										
ANALYST		KP	KP	KP	KP	KP	KP	KP	KP		
DATE:		4/16/10	4/17	4/18	4/19	4/20	4/21	4/22	4/23		
TIME:		1040	1115	1210	1115	1320	1510	1620	1340		

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	4/16/10	TIME	AGE AND SOURCE OF MINNOWS						
CLIENT		TEST END DATE		TIME							
		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	7%		
	B	1	2	2	1	2	1	1			
	C	1	2	2	1	2	1	1			
	D	1	2	2	1	2	1	1			
	E										
CONC: 3L	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	0	0	0	0			
	B	1	2	2	2	2	2	2			
	C	1	2	2	2	2	2	2			
	D	1	2	2	2	2	2	2			
CONC: 4L	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	1	1	1	1	1			
	B	1	1	2	2	2	2	2			
	C	1	1	2	2	2	2	2			
	D	1	1	2	2	2	2	2			
CONC: 5L	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	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			
CONC: 75	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	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			
CONC: 100	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	2			
	B	1	2	2	2	2	2	2			
	C	1	2	2	2	2	2	2			
	D	1	2	2	2	1	1	1			
ANALYST		KP									
DATE:		4/16/10									
TIME:											

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

## SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID K100402TEST START DATE 4/16/10 TIME

TEST END DATE

TIME

CLIENT Wester

## AGE AND SOURCE OF MINNOWS

## DAY (NUMBER SURVIVING)

## SURVIVAL

CONC:	REP #	start	DAY (NUMBER SURVIVING)							MEAN %	CV
			1	2	3	4	5	6	7 %		
CONC: 0	A	2	2	2	2	2	2	2	2	MEAN %	CV
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E										
CONC: 32	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	1	1	1	1	1		
	B	1	1	2	2	2	2	2	2		
	C	1	1	2	2	2	2	2	2		
	D	1	1	2	2	2	2	2	2		
CONC: 42	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	2	2		
	B	1	2	2	2	2	2	2	2		
	C	1	2	1	1	1	1	1	1		
	D	1	2	2	2	2	2	2	2		
CONC: 56	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
CONC: 75	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
CONC: 100	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
ANALYST		KP									
DATE:		4/16/10									
TIME:											

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

## SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID K1004002

TEST START DATE 4/16/10 TIME

TEST END DATE

TIME

CLIENT Weston

## 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	1	1	1	1	2	2	2			
	C	1	1	2	2	2	2	2			
	D	1	1	2	2	2	2	2			
	E										
CONC: 2	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	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			
CONC: 4	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	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			
CONC: 56	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	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			
CONC: 75	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	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			
CONC: 100	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	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			
ANALYST		KP									
	DATE:	4/16/10									
	TIME:										

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

## SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID K1004002TEST START DATE 4/16/10 TIMECLIENT WestonTEST END DATE  TIME

## AGE AND SOURCE OF MINNOWS

## DAY (NUMBER SURVIVING)

## SURVIVAL

CONC:	REP #	start	DAY (NUMBER SURVIVING)							MEAN %	CV
			1	2	3	4	5	6	7 %		
<u>0</u>	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E										
<u>32</u>	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E										
<u>42</u>	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E										
<u>56</u>	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E										
<u>75</u>	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E										
<u>100</u>	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E										
ANALYST		KP									
DATE:		4/16/10									
TIME:											

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

## WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #'s:		K1004002					TEST DATES (BEGIN / END):	4/16-23/10
CLIENT:		EEMA					WEIGHING DATE / TIME:	4/27/10, 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	0.99205	0.98881	0.00324	8	0.405	AVG DRY WEIGHT (mg)	
	B	1.01537	1.01279	0.00258	8	0.323		
	C	1.01670	1.01357	0.00313	8	0.391	0.396	
	D	1.01149	1.00801	0.00348	8	0.435	CV	
	E	0.99817	0.99477	0.00340	8	0.425	11.2	
CONC:	A	0.99168	0.98880	0.00288	8	0.360	AVG DRY WEIGHT (mg)	
	B	0.99791	0.99526	0.00265	8	0.331		
	C	1.03698	1.03438	0.00260	8	0.325	0.416	
	D	0.99209	0.98780	0.00429	8	0.536	CV	
	E	1.01469	1.01047	0.00422	8	0.528		
CONC:	A	0.98464	0.98124	0.00340	8	0.425	AVG DRY WEIGHT (mg)	
	B	1.02026	1.01707	0.00319	8	0.399		
	C	1.01301	1.01080	0.00221	8	0.276	0.440	
	D	0.99370	0.98932	0.00438	8	0.548	CV	
	E	0.99683	0.99243	0.00440	8	0.550		
CONC:	A	0.97411	0.97001	0.00410	8	0.512	AVG DRY WEIGHT (mg)	
	B	1.02641	1.02210	0.00431	8	0.539		
	C	0.97255	0.96891	0.00364	8	0.455	0.527	
	D	1.01088	1.00705	0.00383	8	0.479	CV	
	E	1.01721	1.01200	0.00521	8	0.651		
CONC:	A	1.00958	1.00555	0.00403	8	0.504	AVG DRY WEIGHT (mg)	
	B	1.01564	1.01113	0.00451	8	0.564		
	C	1.00901	1.00587	0.00314	8	0.392	0.502	
	D	1.00457	1.00051	0.00406	8	0.507	CV	
	E	1.00436	1.00000	0.00436	8	0.545		
CONC:	A	0.99815	0.99473	0.00342	8	0.427	AVG DRY WEIGHT (mg)	
	B	1.00291	0.99858	0.00433	8	0.541		
	C	1.00515	1.00164	0.00351	8	0.439	0.519	
	D	1.01294	1.00835	0.00459	8	0.574	CV	
	E	1.01355	1.00865	0.00490	8	0.612	15.9	

CV = (STANDARD DEVIATION/MEAN)\*100

REMARKS:

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Pimephales promelas

## FATHEAD MINNOW

TEST 1000.0

## WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #: <u>K1604002</u>	CLIENT: <u>Weston</u>	ANALYSTS: <u>KP</u>	SAMPLE ID:	TEST DATES (BEGIN / END): <u>4/16-23/10</u>	
				WEIGHING DATE / TIME: <u>4/27/10, 14:10</u>	
				DRYING TEMP (DEGREES C): <u>60</u>	
				DRYING TIME (HOURS): <u>24</u>	
	FINAL DRY WEIGHT TIN+LARVAE REP#	INITIAL WEIGHT TIN (g)	TOTAL DRY WEIGHT OF LARVAE (g)	NUMBER OF LARVAE (mg)	DRY WEIGHT OF LARVAE (mg)
CONTROL	A 1 <u>0.98881</u> B 2 <u>1.01537</u> C 3 <u>1.01670</u> D 4 <u>1.01649</u> E 5 <u>0.99817</u>	0.98881 1.01279 1.61357 1.00801 0.99477			Avg Dry Weight (mg)
CONC: 32	A 6 <u>0.991468</u> B 7 <u>0.99791</u> C 8 <u>1.03698</u> D 9 <u>0.99209</u> E 10 <u>1.01469</u>	0.98880 0.99526 1.03438 0.987180 1.01647			Avg Dry Weight (mg)
CONC: 42	A 11 <u>0.98464</u> B 12 <u>1.02026</u> C 13 <u>1.01361</u> D 14 <u>0.99370</u> E 15 <u>0.99683</u>	0.98124 1.01767 1.01080 0.98932 0.99243			Avg Dry Weight (mg)
CONC: 56	A 16 <u>0.97411</u> B 17 <u>1.02641</u> C 18 <u>0.972545</u> D 19 <u>1.01089</u> E 20 <u>1.01721</u>	0.97001 1.02210 0.96891 1.007045 1.01206			Avg Dry Weight (mg)
CONC: 75	A 21 <u>1.00958</u> B 22 <u>1.01561</u> C 23 <u>1.00901</u> D 24 <u>1.00457</u> E 25 <u>1.06436</u>	1.005545 1.01113 1.005087 1.00051 1.06600			Avg Dry Weight (mg)
CONC: 100	A 26 <u>0.99815</u> B 27 <u>1.00291</u> C 28 <u>1.00515</u> D 29 <u>1.01294</u> E 30 <u>1.01365</u>	0.99473 0.99858 1.00164 1.00235 1.00865			Avg Dry Weight (mg)
					CV

CV = (STANDARD DEVIATION/MEAN)\*100

REMARKS:

AA# K1004002, FATHEAD MINNOW SURVIVAL, CHRONIC, 4-16-10  
File: Z:\TOXSTAT\MONTE\FHSURV. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.409

W = 0.914

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# K1004002, FATHEAD MINNOW SURVIVAL, CHRONIC, 4-16-10  
File: Z:\TOXSTAT\MONTE\FHSURV. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance  
Calculated B1 statistic = 3.05

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.

TITLE: AA# K1004002, FATHEAD MINNOW SURVIVAL, CHRONIC, 4-16-10  
FILE: Z:\TOXSTAT\MONTE\FHSURV.  
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.8750	1.2094
1	CONTROL	2	1.0000	1.3931
1	CONTROL	3	1.0000	1.3931
1	CONTROL	4	0.8750	1.2094
1	CONTROL	5	1.0000	1.3931
2	32 % EFFLUENT	1	0.8750	1.2094
2	32 % EFFLUENT	2	0.7500	1.0472
2	32 % EFFLUENT	3	0.7500	1.0472
2	32 % EFFLUENT	4	1.0000	1.3931
2	32 % EFFLUENT	5	1.0000	1.3931
3	42 % EFFLUENT	1	0.7500	1.0472
3	42 % EFFLUENT	2	0.8750	1.2094
3	42 % EFFLUENT	3	0.8750	1.2094
3	42 % EFFLUENT	4	1.0000	1.3931
3	42 % EFFLUENT	5	1.0000	1.3931
4	56 % EFFLUENT	1	0.8750	1.2094

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	0.7500	1.0472
5	75 % EFFLUENT	5	1.0000	1.3931
6	100 % EFFLUENT	1	0.8750	1.2094
6	100 % EFFLUENT	2	0.8750	1.2094
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# K1004002, FATHEAD MINNOW SURVIVAL, CHRONIC, 4-16-10  
 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	1.320				
2	32 % EFFLUENT	1.218	23.00	16.00	5.00	
3	42 % EFFLUENT	1.250	24.00	16.00	5.00	
4	56 % EFFLUENT	1.356	30.00	16.00	5.00	
5	75 % EFFLUENT	1.324	29.00	16.00	5.00	
6	100 % EFFLUENT	1.320	27.50	16.00	5.00	

---

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

AA# K1004002, FATHEAD MINNOW GROWTH CHRONIC, 4-16-10  
File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.180

W = 0.962

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# K1004002, FATHEAD MINNOW GROWTH CHRONIC, 4-16-10  
File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance  
Calculated B1 statistic = 3.82

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# K1004002, FATHEAD MINNOW GROWTH CHRONIC, 4-16-10  
FILE: Z:\TOXSTAT\MONTE\FHGR.  
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.4050	0.6898
1	CONTROL	2	0.3230	0.6045
1	CONTROL	3	0.3910	0.6755
1	CONTROL	4	0.4350	0.7202
1	CONTROL	5	0.4250	0.7101
2	32 % EFFLUENT	1	0.3600	0.6435
2	32 % EFFLUENT	2	0.3310	0.6130
2	32 % EFFLUENT	3	0.3250	0.6066
2	32 % EFFLUENT	4	0.5360	0.8214
2	32 % EFFLUENT	5	0.5280	0.8134
3	42 % EFFLUENT	1	0.4250	0.7101
3	42 % EFFLUENT	2	0.3990	0.6837
3	42 % EFFLUENT	3	0.2760	0.5531
3	42 % EFFLUENT	4	0.5480	0.8335
3	42 % EFFLUENT	5	0.5500	0.8355
4	56 % EFFLUENT	1	0.5120	0.7974

4	56 % EFFLUENT	2	0.5390	0.8244
4	56 % EFFLUENT	3	0.4550	0.7403
4	56 % EFFLUENT	4	0.4790	0.7644
4	56 % EFFLUENT	5	0.6510	0.9388
5	75 % EFFLUENT	1	0.5040	0.7894
5	75 % EFFLUENT	2	0.5640	0.8496
5	75 % EFFLUENT	3	0.3920	0.6765
5	75 % EFFLUENT	4	0.5070	0.7924
5	75 % EFFLUENT	5	0.5450	0.8305
6	100 % EFFLUENT	1	0.4270	0.7121
6	100 % EFFLUENT	2	0.5410	0.8264
6	100 % EFFLUENT	3	0.4390	0.7242
6	100 % EFFLUENT	4	0.5740	0.8597
6	100 % EFFLUENT	5	0.6120	0.8984

AA# K1004002, FATHEAD MINNOW GROWTH CHRONIC, 4-16-10  
 File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

#### ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.082	0.016	2.197
Within (Error)	24	0.180	0.008	
Total	29	0.262		

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

AA# K1004002, FATHEAD MINNOW GROWTH CHRONIC, 4-16-10  
 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	0.680	0.396		
2	32 % EFFLUENT	0.700	0.416	-0.357	
3	42 % EFFLUENT	0.723	0.440	-0.788	
4	56 % EFFLUENT	0.813	0.527	-2.429	
5	75 % EFFLUENT	0.788	0.502	-1.965	
6	100 % EFFLUENT	0.804	0.519	-2.266	

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

AA# K1004002, FATHEAD MINNOW GROWTH CHRONIC, 4-16-10  
 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	0.122	30.7	-0.020
3	42 % EFFLUENT	5	0.122	30.7	-0.044
4	56 % EFFLUENT	5	0.122	30.7	-0.131
5	75 % EFFLUENT	5	0.122	30.7	-0.107
6	100 % EFFLUENT	5	0.122	30.7	-0.123

AA# K1004002, FATHEAD MINNOW GROWTH CHRONIC, 4-16-10

File: Z:\TOXSTAT\MONTE\FHGR. 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	0.680				
2	32 % EFFLUENT	0.700	28.00	16.00	5.00	
3	42 % EFFLUENT	0.723	30.50	16.00	5.00	
4	56 % EFFLUENT	0.813	40.00	16.00	5.00	
5	75 % EFFLUENT	0.788	37.00	16.00	5.00	
6	100 % EFFLUENT	0.804	39.00	16.00	5.00	

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

## APPENDIX D

### *Ceriodaphnia dubia* Raw Data and Statistics



AA # K1004002 C. DUBIA CHRONIC, REPRODUCTION, 4-16-10  
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 # K1004002 C. DUBIA CHRONIC, REPRODUCTION, 4-16-10  
File: Z:/toxstat/monte\CD. Transform: NO TRANSFORMATION

---

Bartlett's test for homogeneity of variance  
Calculated B1 statistic = 1.81

---

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

---

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	1	
4	75%	10	1	
5	100%	10	0	

TITLE: AA # K1004002 C. DUBIA CHRONIC, REPRODUCCION, 4-16-10

FILE: Z:/toxstat/monte\CD.

TRANSFORM: NO TRANSFORMATION

NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	15.0000	15.0000
1	CONTROL	2	18.0000	18.0000
1	CONTROL	3	12.0000	12.0000
1	CONTROL	4	15.0000	15.0000
1	CONTROL	5	27.0000	27.0000
1	CONTROL	6	26.0000	26.0000
1	CONTROL	7	18.0000	18.0000
1	CONTROL	8	13.0000	13.0000
1	CONTROL	9	11.0000	11.0000
1	CONTROL	10	22.0000	22.0000
2	32 % EFFLUENT	1	20.0000	20.0000
2	32 % EFFLUENT	2	13.0000	13.0000
2	32 % EFFLUENT	3	8.0000	8.0000
2	32 % EFFLUENT	4	21.0000	21.0000
2	32 % EFFLUENT	5	18.0000	18.0000
2	32 % EFFLUENT	6	17.0000	17.0000
2	32 % EFFLUENT	7	20.0000	20.0000
2	32 % EFFLUENT	8	22.0000	22.0000
2	32 % EFFLUENT	9	15.0000	15.0000
2	32 % EFFLUENT	10	31.0000	31.0000
3	42 % EFFLUENT	1	17.0000	17.0000
3	42 % EFFLUENT	2	13.0000	13.0000
3	42 % EFFLUENT	3	27.0000	27.0000
3	42 % EFFLUENT	4	22.0000	22.0000
3	42 % EFFLUENT	5	26.0000	26.0000
3	42 % EFFLUENT	6	20.0000	20.0000
3	42 % EFFLUENT	7	17.0000	17.0000
3	42 % EFFLUENT	8	19.0000	19.0000
3	42 % EFFLUENT	9	13.0000	13.0000
3	42 % EFFLUENT	10	17.0000	17.0000
4	56 % EFFLUENT	1	3.0000	3.0000
4	56 % EFFLUENT	2	16.0000	16.0000
4	56 % EFFLUENT	3	23.0000	23.0000
4	56 % EFFLUENT	4	24.0000	24.0000
4	56 % EFFLUENT	5	16.0000	16.0000
4	56 % EFFLUENT	6	9.0000	9.0000
4	56 % EFFLUENT	7	14.0000	14.0000
4	56 % EFFLUENT	8	19.0000	19.0000

4	56 %	EFFLUENT	9	20.0000	20.0000
4	56 %	EFFLUENT	10	23.0000	23.0000
5	75 %	EFFLUENT	1	7.0000	7.0000
5	75 %	EFFLUENT	2	9.0000	9.0000
5	75 %	EFFLUENT	3	15.0000	15.0000
5	75 %	EFFLUENT	4	6.0000	6.0000
5	75 %	EFFLUENT	5	16.0000	16.0000
5	75 %	EFFLUENT	6	7.0000	7.0000
5	75 %	EFFLUENT	7	18.0000	18.0000
5	75 %	EFFLUENT	8	12.0000	12.0000
5	75 %	EFFLUENT	9	25.0000	25.0000
5	75 %	EFFLUENT	10	24.0000	24.0000
6	100 %	EFFLUENT	1	7.0000	7.0000
6	100 %	EFFLUENT	2	20.0000	20.0000
6	100 %	EFFLUENT	3	15.0000	15.0000
6	100 %	EFFLUENT	4	18.0000	18.0000
6	100 %	EFFLUENT	5	21.0000	21.0000
6	100 %	EFFLUENT	6	15.0000	15.0000
6	100 %	EFFLUENT	7	21.0000	21.0000
6	100 %	EFFLUENT	8	20.0000	20.0000
6	100 %	EFFLUENT	9	23.0000	23.0000
6	100 %	EFFLUENT	10	25.0000	25.0000

AA # K1004002 C. DUBIA CHRONIC, REPRODUCTION, 4-16-10  
 File: Z:/toxstat/monte\CD. Transform: NO TRANSFORMATION

#### ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	181.400	36.280	1.029
Within (Error)	54	1903.000	35.241	
Total	59	2084.400		

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

AA # K1004002 C. DUBIA CHRONIC, REPRODUCTION, 4-16-10  
 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	17.700	17.700		
2	32 % EFFLUENT	18.500	18.500	-0.301	
3	42 % EFFLUENT	19.100	19.100	-0.527	
4	56 % EFFLUENT	16.700	16.700	0.377	
5	75 % EFFLUENT	13.900	13.900	1.431	
6	100 % EFFLUENT	18.500	18.500	-0.301	

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

AA # K1004002 C. DUBIA CHRONIC, REPRODUCTION, 4-16-10  
 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	6.133	34.6	-0.800
3	42 % EFFLUENT	10	6.133	34.6	-1.400
4	56 % EFFLUENT	10	6.133	34.6	1.000
5	75 % EFFLUENT	10	6.133	34.6	3.800
6	100 % EFFLUENT	10	6.133	34.6	-0.800

AA # K1004002 C. DUBIA CHRONIC, REPRODUCTION, 4-16-10  
 File: Z:/toxstat/monte\CD. Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	17.700				
2	32 % EFFLUENT	18.500	111.00	75.00	10.00	
3	42 % EFFLUENT	19.100	114.50	75.00	10.00	
4	56 % EFFLUENT	16.700	106.00	75.00	10.00	
5	75 % EFFLUENT	13.900	87.50	75.00	10.00	
6	100 % EFFLUENT	18.500	113.00	75.00	10.00	

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

## APPENDIX E

### Organism History

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

## TEST ORGANISM HISTORY

DATE SHIPPED 4/15/10 CLIENT Arkansas Analysis

Purchase Order #: 1001/KM

SPECIES: Pimephales promelas Mysidopsis bahia Cyprinodon variegates

Quantity Shipped: 480+ - 180+ 150

Age: 24 hrs 4/15 & Mysid 4/15

Brood Stock Source: Anderson Farms AR

Culture Water: Groundwater Artificial Salts Artificial Salts

Hardness (Mg/l CaCO<sub>3</sub>) 160 Salinity (ppt) \_\_\_\_\_

Dissolved Oxygen (Mg/l): 7.1 \_\_\_\_\_

Feeding: Artemia \_\_\_\_\_

Comments: \_\_\_\_\_

Shipped Via: Federal Express UPS Overnight Shuttle

Packaged By: LLC

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 CaCO<sub>3</sub>): 142 mg/l 86-124 mg/l

TOTAL ALKALINITY (as CaCO<sub>3</sub>): 100 mg/l 65-130 mg/l

pH: 7.92 7.56-8.35

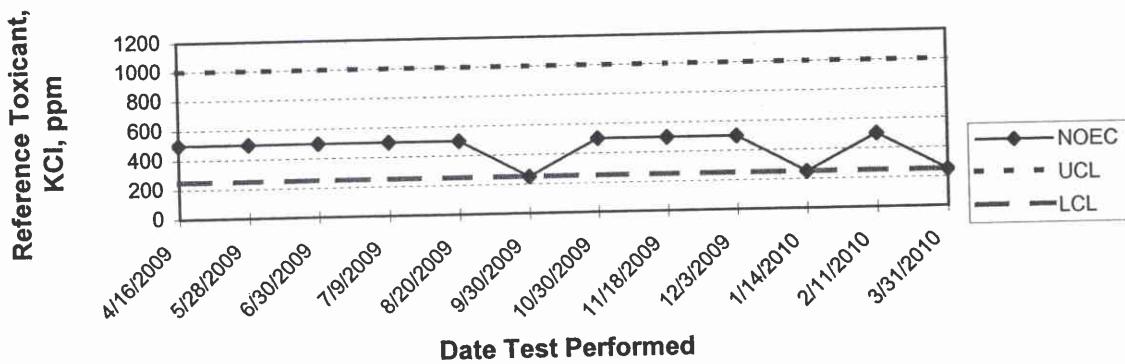
### Comments:

  
Facility Supervisor

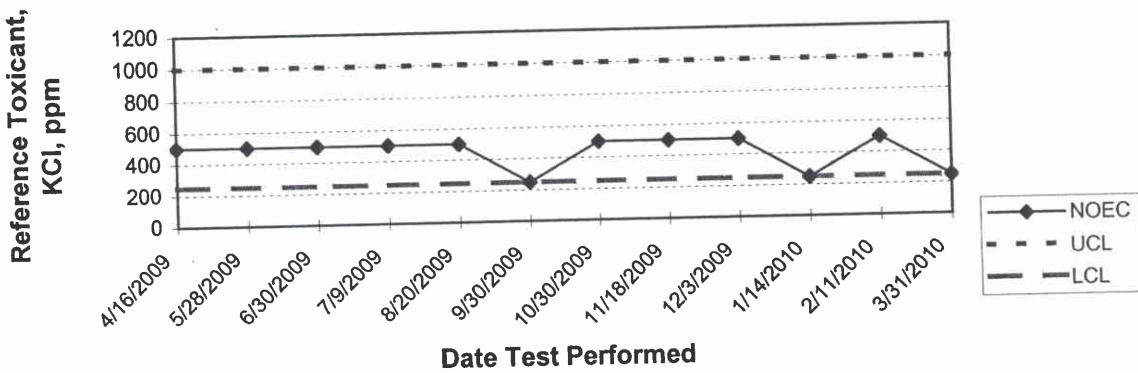
## **APPENDIX F**

### **Quality Assurance Charts**

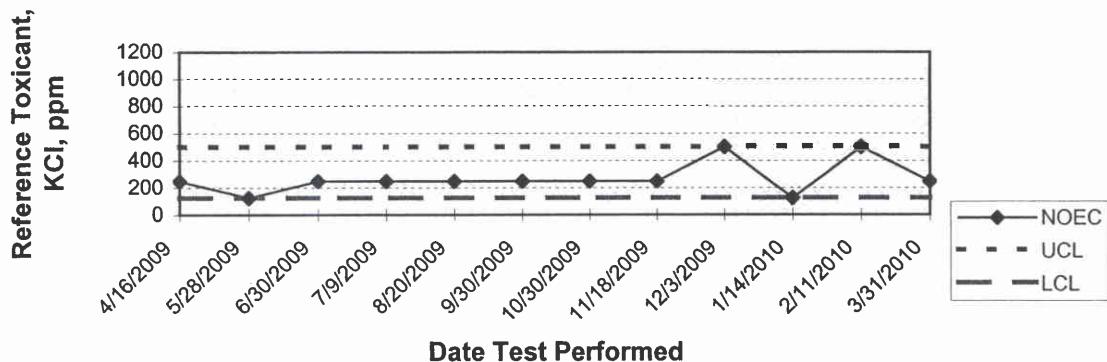
**ARKANSAS ANALYTICAL, INC.**  
**FATHEAD MINNOW SURVIVAL**  
**QUALITY ASSURANCE**



**ARKANSAS ANALYTICAL, INC.**  
**FATHEAD MINNOW GROWTH**  
**QUALITY ASSURANCE**



**ARKANSAS ANALYTICAL, INC.**  
**CERIODAPHNIA DUBIA SURVIVAL**  
**QUALITY ASSURANCE**



**ARKANSAS ANALYTICAL, INC.**  
**CERIODAPHNIA DUBIA REPRODUCTION**  
**QUALITY ASSURANCE**

