Arkansas Analytical, Inc.

Toxicity Test Results

MAGCOBAR MINE SITE
NPDES PERMIT NUMBER: AR0049794
June, 2012
AFIN# 00-00348
***C. dubia* Control Failure**

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 K1206005

Monday, July 02, 2012

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 June of 2012.

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:	6-13-12, 0845	6-14-12, 0845
Sample #2:	6-14-12, 0910	6-15-12, 0910
Sample #3:	6-18-12, 0845	6-19-12, 0845

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

The following information was collected upon immediate receipt of the samples at the laboratory:

Sample Receiving Information:	Date, Time Sample(s) Received	Temperature Upon Receipt (°C)
*Sample #1:	6-14-12, 1347	4
*Sample #2:	6-15-12, 1516	4
Sample #3:	6-19-12, 1315	4

Chain of custody documentation is located in Appendix A.

The permit designates the receiving water to be used as dilution water for the toxicity tests. Synthetic dilution water was substituted either because zero flow conditions existed or due to an earlier characterization of the receiving water as being toxic.

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

Dilution Series

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

Test Methods

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

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

Test Organisms

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

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

Quality Assurance

Test Acceptability

TEST ACCEPTANCE CRITERIA for *Ceriodaphnia dubia*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	90%	X	
Average of 15 or more young per surviving female	7.0		X
At least 60% of surviving females should have produced 3 broods	44.4%		X
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	83.6%		X

TEST ACCEPTANCE CRITERIA for *Pimephales promelas*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	97.5%	X	
The percent coefficient of variation between replicates must be 40% or less for survival	5.73%	X	
Minimum of 0.25 mg average dry weight of surviving controls	0.572	X	
The percent coefficient of variation between replicates must be 40% or less for growth	8.47%	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

Ceriodaphnia dubia 5/8-15/12		*Pimephales promelas* 5/8-15/12	
NOEC Survival:	250 ppm KCl	NOEC Survival:	500 ppm KCl
LOEC Survival:	500 ppm KCl	LOEC Survival:	1000 ppm KCl
NOEC Reproduction:	250 ppm KCl	NOEC Growth:	500 ppm KCl
LOEC Reproduction:	500 ppm KCl	LOEC Growth:	1000 ppm KCl

Quality Assurance charts are provided in Appendix F.

Summary of Results
Magcobar Mine Site

Ceriodaphnia dubia		*Pimephales promelas*	
NOEC / LOEC Survival	NA% / NA	NOEC / LOEC survival	100% / NA
NOEC / LOEC Reproduction	NA% / NA	NOEC / LOEC growth	100% / NA
Mean number of neonates (critical dilution)	6.7	%CV survival (critical dilution)	7.21%
%CV Reproduction (critical dilution)	71.9%	Mean dry weight (critical dilution) in milligrams	0.738
		%CV growth (critical dilution)	9.5%
PMSD Reproduction	77.9	PMSD Growth	26.7

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**. There was a control failure associated with C. dubia, therefore a retest must be performed.

Biomonitoring Analysts:

Ken Pigue

Allen Parker (KP)

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:	6-13-12, 0845	6-14-12, 0845
Sample #2:	6-14-12, 0910	6-15-12, 0910
Sample #3:	6-18-12, 0845	6-19-12, 0845

Test initiated (date, time): 6-14-12, 1550 Test terminated (date, time): 6-21-12, 1330

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	87.5	100		100	97.5	97.5	5.73
32%	100	100	100	100	100		100	100	100	
42%	100	100	75	100	100		100	100	95	
56%	87.5	100	100	100	87.5		95	95	95	
75%	100	100	100	87.5	100		100	100	97.5	
100%	100	87.5	87.5	100	100		100	97.5	95	7.21

SUMMARY

Effluent Conc %	A	B	C	D	E		Mean Dry Weight	CV%
0%	0.514	0.598	0.561	0.548	0.640		0.572	8.47
32%	0.615	0.601	0.630	0.605	0.627		0.616	
42%	0.598	0.906	0.476	0.698	0.505		0.637	
56%	0.501	0.585	0.551	0.681	0.524		0.568	
75%	0.626	0.657	0.800	0.580	0.660		0.665	
100%	0.855	0.724	0.667	0.716	0.726		0.738	9.46

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)= _____**9.46**_____%

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:	6-13-12, 0845	6-14-12, 0845
Sample #2:	6-14-12, 0910	6-15-12, 0910
Sample #3:	6-18-12, 0845	6-19-12, 0845

Test initiated (date, time): 6-14-12, 1600 Test terminated (date, time): 6-23-12, 1150

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	2	10	4	10	13	13
B	0	11	3	5	1	0
C	4	8	3	9	8	x0
D	11	6	11	0	9	10
E	0	17	1	11	0	8
F	17	6	8	6	x0	13
G	11	9	0	5	3	5
H	10	2	12	4	2	2
I	x0	6	13	3	4	2
J	8	2	10	7	9	7
Mean	6.3	7.7	6.5	6.0	4.9	6.0
Mean/surviving female	7.0	7.7	6.5	6.0	5.4	6.7
CV%*	83.6					71.9

X= Dead Adult; M= Male (Not considered in statistics)
*Coefficient of Variation = standard deviation/ mean * 100; CV% calculation based on young per surviving female

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING
Ceriodaphnia dubia SURVIVAL AND REPRODUCTION

Permittee: 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	90	100	100	100	90	90

1. Fisher's Exact Test:
   Is the mean survival at test termination significantly different (p=0.05) than the control survival for:

   a) LOW FLOW OR CRITICAL DILUTION, (100%): YES_____ NO_____

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_____

3. If NO was answered to 1.a) enter [0] otherwise enter [1] (parameter TLP3B):_____**NA**_____

4. If NO was answered to 2.a) enter [0] otherwise enter [1] (parameter TGP3B):_____**NA**_____

5. Enter percentage corresponding to each parameter below:

   a) NOEC survival (parameter TOP3B)= _____**NA**____ % effluent

   b) NOEC reproduction (parameter TPP3B)= _____**NA**_____% effluent

   c) Coefficient of variation (parameter TQP3B)= _____**NA**_____%

APPENDIX A

Chain of Custody Forms

Arkansas Analytical Inc.

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_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)		
Attn: Bill McAlister	Attn: Amber Rich	Fax: 501-467-8687	Preservative Code:	TEST PARAMETERS	Bottle Type Code
		Email: dave.friedman@eema-inc.com; bmcalister@eema-inc.com; bhorton@eema-inc.com	Bottle Type:		G = Glass; P = Plastic; V = Septum; A = Amber

Sampler(s) Signature: Bill McAlister

Sampler(s) Printed: Bill McAlister

Field Number	SAMPLE COLLECTION Date/s	Time/s	Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION	Chronic Biomonitoring	Arkansas Analytical Work Order Number:
FD-1 Comp.	6/14/2012	8:45 AM		X	4	W	Facility Discharge	X	K1206-005A

1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)	SAMPLE CONDITION UPON RECEIPT IN LAB	REMARKS / SAMPLE COMMENTS
Bill McAlister	6-14-12 1347		1. CUSTODY SEALS: ✓Yes ___No	
			2. CONTAINERS CORRECT: ___Yes ___No	
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)	3. COC/LABELS AGREE: ___Yes ___No	
		Sydney James	4. PRESERVATION CONFIRMED: ___Yes ___No	
			5. RECEIVED ON ICE: ___Yes ___No	
			6. TEMPERATURE ON RECEIPT: 4°C	
			FOR COMPLETION BY LAB ONLY	

Arkansas Analytical Inc.

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
EEMA O & M Services Group	EEMA O & M Services Group	Magcobar Mine Site	24 Hour
Magcobar Mine Site	P.O. Box 732	Biomonitoring Sample	48 Hour
P.O. Box 699	Kulpsville, PA 19443	**Reporting Information**	72 Hour
Malvern, AR 72104		Telephone: 501-467-8355	Routine (5 Day)
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

Preservation Codes:

1. Cool, 4 Degrees Centigrade
2. Sulfuric Acid (H_2SO_4), pH < 2
3. Nitric Acid (HNO_3), pH < 2
4. Thiosulfate for Dechlorination
5. Hydrochloric Acid(HCl)
6. Sodium Hydroxide (NaOH), pH > 12

Sampler(s) Signature: Bill McAlister

Sampler(s) Printed: Bill McAlister

Field Number	SAMPLE COLLECTION Date/s	Time/s	Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION	TEST PARAMETERS: Chronic Biomonitoring	Bottle Type Code
FD-2 Comp.	6/15/2012	9:10 AM		X	3	W	Facility Discharge	X	005 B

Bottle Type Code: G = Glass; P = Plastic; V = Septum; A = Amber

Arkansas Analytical Work Order Number: K1206-

1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)
Bill McAlister	6-15-12 1516	
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)
		Sydney James

SAMPLE CONDITION UPON RECEIPT IN LAB

1. CUSTODY SEALS:	✓ Yes	No
2. CONTAINERS CORRECT:	Yes	No
3. COC/LABELS AGREE:	Yes	No
4. PRESERVATION CONFIRMED:	Yes	No
5. RECEIVED ON ICE:	Yes	No
6. TEMPERATURE ON RECEIPT:	4°C	

FOR COMPLETION BY LAB ONLY

REMARKS / SAMPLE COMMENTS

Arkansas Analytical Inc.

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
EEMA O & M Services Group	EEMA O & M Services Group	Magcobar Mine Site	24 Hour
Magcobar Mine Site	P.O. Box 732	Biomonitoring Sample	48 Hour
P.O. Box 699	Kulpsville, PA 19443	**Reporting Information**	72 Hour
Malvern, AR 72104		Telephone: 501-467-8355	Routine (5 Day)
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

Preservation Codes:

1. Cool, 4 Degrees Centigrade
2. Sulfuric Acid (H_2SO_4), pH < 2
3. Nitric Acid (HNO_3), pH < 2
4. Thiosulfate for Dechlorination
5. Hydrochloric Acid(HCl)
6. Sodium Hydroxide (NaOH), pH > 12

Bottle Type Code: G = Glass, P = Plastic, V = Septum, A = Amber

Sampler(s) Signature: Bill McAlister

Sampler(s) Printed: Bill McAlister

Arkansas Analytical Work Order Number: K1206-005C

Field Number	SAMPLE COLLECTION Date/s	Time/s	Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION	TEST PARAMETERS: Chronic Biomonitoring
FD-1 Comp.	6/19/2012	8:45 AM		X	3	W	Facility Discharge	X

1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)
Bill McAlister	6-19-12 1315	
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)
		Sydney James

SAMPLE CONDITION UPON RECEIPT IN LAB

1. CUSTODY SEALS:	✓ Yes	No
2. CONTAINERS CORRECT:	Yes	No
3. COC/LABELS AGREE:	Yes	No
4. PRESERVATION CONFIRMED:	Yes	No
5. RECEIVED ON ICE:	Yes	No
6. TEMPERATURE ON RECEIPT:	4°C	

FOR COMPLETION BY LAB ONLY

REMARKS / SAMPLE COMMENTS

APPENDIX B

Effluent and Dilution Water Data

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING — Fathead Minnow

Lab # / Sample ID: K12060005 — Test Start (Date/Time): 6/14/12

Client: Westfa — Test End (Date/Time): 6/22/12

		Day of Test							
		1	2	3	4	5	6	7	notes/remarks
Control	MHS551	6/14	6/15	6/16	6/17	6/18	6/19	6/20	
D.O. (mg/L)	INITIAL	8.3	2.9 8.3	8.5	8.4	8.5	8.4	7.1	
	FINAL	8.3	8.5	8.3	7.8	6.9	7.4	7.4	
pH (s.u.)	INITIAL	7.7	7.9	7.9	7.7	7.5	6.9	7.0	
	FINAL	7.2	7.9	8.0	6.9	6.7	6.6	7.6	
temp (C)	INITIAL	21.9	22.6	22.6	22.2	22.1	22.5	23.1	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
ALKALINITY (mg/L)		30						30	
HARDNESS (mg/L)		42						38	
CONDUCTIVITY (umhos/cm)		162						158	
CHLORINE (mg/L)		<0.05						<0.05	
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.3	8.5	8.6	8.7	8.5	7.3	
	FINAL	8.3	8.4	8.4	7.5	6.7	6.6	6.8	
pH (s.u)	INITIAL	7.4	7.4	7.6	7.4	7.3	7.2	6.9	
	FINAL	7.1	7.6	7.7	7.0	6.8	6.9	7.1	
temp (C)	INITIAL	21.9	23.3	22.8	22.5	22.3	22.9	23.9	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.4	8.4	8.6	8.9	8.6	7.5	
	FINAL	8.2	8.4	8.3	7.4	6.7	5.8	6.6	
pH (mg/L)	INITIAL	7.5	7.4	7.6	7.5	7.3	7.3	7.1	
	FINAL	7.2	7.7	7.6	7.1	6.9	7.0	7.2	
temp (C)	INITIAL	22.0	24.3	23.0	22.7	22.5	23.2	24.1	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.4	8.4	8.6	9.1	8.6	7.9	
	FINAL	8.2	8.4	8.2	7.5	6.6	5.6	6.6	
pH (s.u.)	INITIAL	7.4	7.4	7.5	7.6	7.3	7.3	7.1	
	FINAL	7.2	7.6	7.5	7.1	6.9	7.0	7.1	
temp (C)	INITIAL	21.8	25.1	23.1	22.9	22.8	23.7	24.7	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.5	8.4	8.7	9.3	8.7	8.4	
	FINAL	8.2	8.4	8.2	7.5	6.5	5.2	6.5	
pH (s.u.)	INITIAL	7.3	7.4	7.4	7.5	7.4	7.3	7.1	
	FINAL	7.2	7.5	7.5	7.0	6.9	7.0	7.0	
temp (C)	INITIAL	21.6	25.9	24.4	23.1	22.8	24.0	25.3	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.7	8.3	8.8	9.7	8.9	8.7	
	FINAL	8.2	8.4	8.2	7.5	6.6	5.8	6.6	
pH (s.u.)	INITIAL	7.3	7.3	7.3	7.4	7.3	7.2	7.1	
	FINAL	7.1	7.4	7.3	7.0	6.9	6.9	7.0	
temp (C)	INITIAL	22.0	26.8	24.4	23.2	23.3	24.4	25.9	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:	**100%**	A	A	A	B	B	C	C	
ALKALINITY (mg/L)		10			14		12		
HARDNESS (mg/L)		>600			>600		>600		
CONDUCTIVITY (umhos/cm)		1983			1991		2020		
CHLORINE (mg/L)		<0.05			<0.05		<0.05		

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING									Cerodaphnia Dubia
Lab # / Sample ID		K1206005			Test Start (Date/Time)		6/14/12		
Client:		Weston			Test End (Date/Time)		6/22/12		
		Day of Test							
		1	2	3	4	5	6	7	notes/remarks
Control	MHS551	6/14	6/15	6/16	6/17	6/18	6/19	6/20	
D.O. (mg/L)	INITIAL	8.3	8.3	8.5	8.4	8.5	8.4	7.1	
	FINAL	8.4	8.4	8.0	8.0	8.1	8.0	8.1	
pH (s.u.)	INITIAL	7.7	7.9	7.9	7.7	7.5	6.9	7.0	
	FINAL	7.8	7.9	6.5	6.5 7.0	6.9	7.8	7.2	
temp (C)	INITIAL	21.9	22.6	22.6	22.7	22.1	22.5	23.1	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
ALKALINITY (mg/L)		30						30	
HARDNESS (mg/L)		42						38	
CONDUCTIVITY (umhos/cm)		162						158	
CHLORINE (mg/L)		<0.05						<0.05	
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.3	8.5	8.6	8.7	8.5	7.3	
	FINAL	8.3	8.3	8.0	8.2	8.1	7.9	8.2	
pH (s.u)	INITIAL	7.4	7.4	7.6	7.4	7.3	7.2	6.9	
	FINAL	7.4	7.5	6.5	6.8 6.9	6.9	7.4	7.4	
temp (C)	INITIAL	21.9	23.3	22.8	22.5	22.3	22.9	23.9	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.4	8.4	8.6	8.9	8.6	7.5	
	FINAL	8.3	8.3	8.0	8.5	8.1	7.9	8.2	
pH (mg/L)	INITIAL	7.5	7.4	7.6	7.5	7.3	7.3	7.1	
	FINAL	7.4	7.5	6.6	6.6 7.0	7.0	7.4	7.4	
temp (C)	INITIAL	22.0	24.3	23.0	22.7	22.5	23.2	24.1	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.4	8.4	8.6	9.1	8.6	7.9	
	FINAL	8.3	8.3	8.0	8.0 8.3	8.1	7.9	8.1	
pH (s.u.)	INITIAL	7.4	7.4	7.5	7.6	7.3	7.3	7.1	
	FINAL	7.4	7.5	6.8	6.8 7.1	7.0	7.4	7.3	
temp (C)	INITIAL	21.8	25.1	23.1	22.9	22.8	23.7	24.2	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.5	8.4	8.7	9.3	8.7	8.4	
	FINAL	8.3	8.2	7.9	7.9 8.3	8.1	7.9	8.1	
pH (s.u.)	INITIAL	7.3	7.4	7.4	7.5	7.4	7.3	7.1	
	FINAL	7.4	7.4	6.9	6.9 7.1	7.0	7.4	7.3	
temp (C)	INITIAL	21.6	25.9	24.4	23.1	22.8	24.0	25.3	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.7	8.3	88	9.7	8.9	8.7	
	FINAL	8.4	8.2	7.9	7.9	8.1	8.1	8.1	
pH (s.u.)	INITIAL	7.3	7.3	7.3	7.4	7.3	7.2	7.1	
	FINAL	7.3	7.3	7.0	7.0	7.0	7.3	7.1	
temp (C)	INITIAL	22.0	26.8	27.4	23.2	23.3	24.4	25.1	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:	100%	A	A	A	B	B	C	C	
ALKALINITY (mg/L)		10			14		12		
HARDNESS (mg/L)		>600			>600		>600		
CONDUCTIVITY (umhos/cm)		1983			1991		2020		
CHLORINE (mg/L)		<0.05			<0.05		<0.05		

Pg 2 of 2 Continued

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING									Cerodaphnia Dubia
Lab # / Sample ID		K1206005			Test Start (Date/Time)		6/14/12		
Client:		Weston			Test End (Date/Time)		6/22/12		
		Day of Test							
		0	1 2	3	4	5	6	7	notes/remarks
Control	MHS551	6/21	6/22						
D.O. (mg/L)	INITIAL	8.2	8.2						
	FINAL	8.5							
pH (s.u.)	INITIAL	6.0	7.5						
	FINAL	7.8							
temp (C)	INITIAL	22.4	22.7						
	FINAL	25.0							
ALKALINITY (mg/L)		30							
HARDNESS (mg/L)		38							
CONDUCTIVITY (umhos/cm)		158							
CHLORINE (mg/L)		<0.05							
CONC:									
D.O. (mg/L)	INITIAL	8.2	8.3						
	FINAL	8.8							
pH (s.u)	INITIAL	6.3	7.8						
	FINAL	7.9							
temp (C)	INITIAL	22.5	22.7						
	FINAL	25.0							
CONC:									
D.O. (mg/L)	INITIAL	8.2	8.3						
	FINAL	8.8							
pH (mg/L)	INITIAL	6.6	8.0						
	FINAL	7.8							
temp (C)	INITIAL	22.4	23.2						
	FINAL	25.0							
CONC:									
D.O. (mg/L)	INITIAL	8.3	8.3						
	FINAL	8.8							
pH (s.u.)	INITIAL	6.9	8.2						
	FINAL	7.8							
temp (C)	INITIAL	23.0	23.4						
	FINAL	25.0							
CONC:									
D.O. (mg/L)	INITIAL	8.5	8.3						
	FINAL	8.8							
pH (s.u.)	INITIAL	6.9	8.4						
	FINAL	7.8							
temp (C)	INITIAL	23.2	23.6						
	FINAL	25.0							
CONC:									
D.O. (mg/L)	INITIAL	8.8	8.2						
	FINAL	8.9							
pH (s.u.)	INITIAL	6.8	8.5						
	FINAL	7.5							
temp (C)	INITIAL	23.4	24.1						
	FINAL	25.0							
CONC:	**100%**	C							
ALKALINITY (mg/L)		12							
HARDNESS (mg/L)		>600							
CONDUCTIVITY (umhos/cm)		2020							
CHLORINE (mg/L)		<0.05							

APPENDIX C

Fathead minnow raw data and statistics

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID: 1C1206005 TEST START DATE: 6/14/12 TIME: 1550

CLIENT: Weston TEST END DATE: 6/21/12 TIME: 1330

AGE AND SOURCE OF MINNOWS: Summary Payne

DAY (NUMBER SURVIVING)

	REP #	start	1	2	3	4	5	6	7	SURVIVAL %	MEAN %	CV
CONC: 0	A	8	8	8	8	8	8	8	8	100	97.5	5.73
	B			8	8	8	8	8	8	100		
	C			8	8	8	8	8	8	100		
	D			7	7	7	7	7	7	87.5		
	E			8	8	8	8	8	8	100		
CONC: 32	A	8	8	8	8	8	8	8	8	100	100	
	B								8	100		
	C								8	100		
	D								8	100		
	E								8	100		
CONC: 42	A	8	8	8	8	8	8	8	8	100	95	
	B							8	8	100		
	C							6	6	75		
	D							8	8	100		
	E							8	8	100		
CONC: 56	A	8	7	7	7	7	7	7	7	87.5	95	
	B		8	8	8	8	8	8	8	100		
	C		8	8	8	8	8	8	8	100		
	D		8	8	8	8	8	8	8	100		
	E		7	7	7	7	7	7	7	87.5		
CONC: 75	A	8	8	8	8	8	8	8	8	100	97.5	
	B							8	8	100		
	C							8	8	100		
	D							7	7	87.5		
	E							8	8	100		
CONC: 100	A	8	8	8	8	8	8	8	8	100	95	7.21
	B			7	7	7	7	7	7	87.5		
	C			8	7	7	7	7	7	87.5		
	D			8	8	8	8	8	8	100		
	E			8	8	8	8	8	8	100		
ANALYST		KP										
DATE:												
TIME:												

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID K12D6005 TEST START DATE 6/14/12 TIME 1550

CLIENT Weston A TEST END DATE 6/21/12 TIME 1330

AGE AND SOURCE OF MINNOWS

D A Y (NUMBER SURVIVING) SURVIVAL

	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 0	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 3	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 6	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 12	A	2	1	1	1	1	1	1(C)1	1			
	B		2	2	2	2	2	2	2			
	C		2									
	D		2									
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 25	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 50	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E											
ANALYST		KP	KP	AP	AP	AP	AP	AP/IC	AP			
DATE:		6/14/12	6/15/12	6-16-12	6/17/12	6/18/12	6/19/12	6/20/12	6/21/12			
TIME:		1550	0930	1020				1100	1330			

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID K12060 TEST START DATE 6/14/12 TIME 1550

CLIENT Weston TEST END DATE TIME

AGE AND SOURCE OF MINNOWS B

D A Y (NUMBER SURVIVING) SURVIVAL

	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 0	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 3%	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 6%	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 9%	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 15%	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 30%	A	2	2	1	1	1	1	1	1			
	B			2	2	2	2	2	2			
	C											
	D											
	E											
ANALYST		IP		AP	AP	AP	AP	AP/IL	AP			
DATE:		6/14/12		6-16-12	6/17/12	6/18/12	6/19/12	6/20/12	6/21/12			
TIME:		1550		1020					1330			

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID K12060 TEST START DATE 6/14/12 TIME 1550

CLIENT Weston TEST END DATE TIME

AGE AND SOURCE OF MINNOWS C

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	2			
	B											
	C											
	D											
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 3.2	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 4.2	A	2	2	2	2	2	2	0	0			
	B							2	2			
	C											
	D											
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 5.4	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 7.5	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 10.0	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D			AP 6/17/12	1	1	1	1	1			
	E											
ANALYST		ICP		AP	AP	AP	AP	AP	AP			
DATE:		6/14/12	6/15/12	6/16/12	6/17/12	6/18/12	6/19/12	6/20/12	6/21/12			
TIME:		1550										

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID K12060 TEST START DATE 6/14/12 TIME 1550

CLIENT Weston - D TEST END DATE TIME

AGE AND SOURCE OF MINNOWS

		D A Y (NUMBER SURVIVING)								SURVIVAL		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 0	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											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 3.2	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											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 4.2	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											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 5.4	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											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 7.5	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											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 10	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		IP						AP	AP			
DATE:		6/14/12						6/20/12	6/21/12			
TIME:		1550										

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID K12060 TEST START DATE 6/14/12 TIME 1550

CLIENT Weston TEST END DATE TIME

AGE AND SOURCE OF MINNOWS

D A Y (NUMBER SURVIVING) SURVIVAL

	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 6	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 32	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 42	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 56	A	2	2	2	2	2	2	2	2			
	B		1	1	1	1	1	1	1			
	C			2	2	2	2	2	2			
	D			1	1	1	1	1	1			
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 75	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 100	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E											
ANALYST		TCP										
DATE:		6/14/12										
TIME:		1550										

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

Pimephales promelas **FATHEAD MINNOW** TEST 1000.0

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:	K1102004				TEST DATES (BEGIN / END):		2/16-23/11	
CLIENT:	EEMA- Alternate Method				WEIGHING DATE / TIME:		2/28/1, 1100	
ANALYSTS:	KP				DRYING TEMP (DEGREES C):		60	
SAMPLE ID:	SEE COC				DRYING TIME (HOURS):		24	
	REP #	FINAL DRY WEIGHT TIN+LARVAE (g)	INITIAL WEIGHT TIN (g)	TOTAL DRY WEIGHT OF LARVAE (g)	NUMBER OF LARVAE	DRY WEIGHT OF LARVAE (mg)		
CONTROL	A	1.01378	1.00967	0.00411	8	0.514	AVG DRY	
	B	1.02131	1.01653	0.00478	8	0.598	WEIGHT (mg)	
	C	1.03121	1.02672	0.00449	8	0.561		0.572
	D	1.00225	0.99787	0.00438	8	0.548	CV	
	E	1.01890	1.01378	0.00512	8	0.640		8.5
CONC:	A	1.00500	1.00008	0.00492	8	0.615	AVG DRY	
	B	1.00176	0.99695	0.00481	8	0.601	WEIGHT (mg)	
32%	C	1.01399	1.00895	0.00504	8	0.630		0.616
	D	1.00218	0.99734	0.00484	8	0.605	CV	
	E	0.99501	0.98999	0.00502	8	0.627		
CONC:	A	0.99963	0.99485	0.00478	8	0.598	AVG DRY	
	B	0.98264	0.97539	0.00725	8	0.906	WEIGHT (mg)	
42%	C	1.03450	1.03069	0.00381	8	0.476		0.637
	D	1.00869	1.00311	0.00558	8	0.698	CV	
	E	1.02088	1.01684	0.00404	8	0.505		
CONC:	A	0.99566	0.99165	0.00401	8	0.501	AVG DRY	
	B	1.01307	1.00839	0.00468	8	0.585	WEIGHT (mg)	
56%	C	1.00277	0.99836	0.00441	8	0.551		0.568
	D	1.03345	1.02800	0.00545	8	0.681	CV	
	E	0.99105	0.98686	0.00419	8	0.524		
CONC:	A	1.00415	0.99914	0.00501	8	0.626	AVG DRY	
	B	0.99769	0.99243	0.00526	8	0.657	WEIGHT (mg)	
75%	C	1.01067	1.00427	0.00640	8	0.800		0.665
	D	1.01322	1.00858	0.00464	8	0.580	CV	
	E	0.98585	0.98057	0.00528	8	0.660		
CONC:	A	0.96692	0.96008	0.00684	8	0.855	AVG DRY	
	B	0.99223	0.98644	0.00579	8	0.724	WEIGHT (mg)	
100%	C	1.01752	1.01218	0.00534	8	0.667		0.738
	D	0.97736	0.97163	0.00573	8	0.716	CV	
	E	1.02004	1.01423	0.00581	8	0.726		9.5

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA# K1206005, FATHEAD MINNOW, CHRONIC, 6-14-12
File: Z:/toxstat/monte\FHSURV. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.231

W = 0.779

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# K1206005, FATHEAD MINNOW, CHRONIC, 6-14-12
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# K1206005, FATHEAD MINNOW, CHRONIC, 6-14-12
FILE: Z:/toxstat/monte\FHSURV.
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

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

3	42	% EFFLUENT	1	1.0000	1.3931
3	42	% EFFLUENT	2	1.0000	1.3931
3	42	% EFFLUENT	3	0.7500	1.0472
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	0.8750	1.2094
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	0.8750	1.2094
5	75	% EFFLUENT	5	1.0000	1.3931
6	100	% EFFLUENT	1	1.0000	1.3931
6	100	% EFFLUENT	2	0.8750	1.2094
6	100	% EFFLUENT	3	0.8750	1.2094
6	100	% EFFLUENT	4	1.0000	1.3931
6	100	% EFFLUENT	5	1.0000	1.3931

AA# K1206005, FATHEAD MINNOW, CHRONIC, 6-14-12
File: Z:/toxstat/monte\FHSURV. Transform: ARC SINE(SQUARE ROOT(Y))

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	1.356				
2	32 % EFFLUENT	1.393	30.00	16.00	5.00	
3	42 % EFFLUENT	1.324	27.00	16.00	5.00	
4	56 % EFFLUENT	1.320	25.00	16.00	5.00	
5	75 % EFFLUENT	1.356	27.50	16.00	5.00	
6	100 % EFFLUENT	1.320	25.00	16.00	5.00	

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

AA# K1206005 FATHEAD MINNOW GROWTH CHRONIC, 6-14-12
File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.254

W = 0.885

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# K1206005 FATHEAD MINNOW GROWTH CHRONIC, 6-14-12
File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 20.42

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

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

TITLE: AA# K1206005 FATHEAD MINNOW GROWTH CHRONIC, 6-14-12
FILE: Z:\TOXSTAT\MONTE\FHGR.
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.5140	0.7994
1	CONTROL	2	0.5980	0.8840
1	CONTROL	3	0.5610	0.8466
1	CONTROL	4	0.5480	0.8335
1	CONTROL	5	0.6400	0.9273
2	32 % EFFLUENT	1	0.6150	0.9014
2	32 % EFFLUENT	2	0.6010	0.8871
2	32 % EFFLUENT	3	0.6300	0.9169
2	32 % EFFLUENT	4	0.6050	0.8912
2	32 % EFFLUENT	5	0.6270	0.9138
3	42 % EFFLUENT	1	0.5980	0.8840
3	42 % EFFLUENT	2	0.9060	1.2592
3	42 % EFFLUENT	3	0.4760	0.7614

3	42 % EFFLUENT	4	0.6980	0.9890
3	42 % EFFLUENT	5	0.5050	0.7904
4	56 % EFFLUENT	1	0.5010	0.7864
4	56 % EFFLUENT	2	0.5850	0.8708
4	56 % EFFLUENT	3	0.5510	0.8365
4	56 % EFFLUENT	4	0.6810	0.9706
4	56 % EFFLUENT	5	0.5240	0.8094
5	75 % EFFLUENT	1	0.6260	0.9128
5	75 % EFFLUENT	2	0.6570	0.9451
5	75 % EFFLUENT	3	0.8000	1.1071
5	75 % EFFLUENT	4	0.5800	0.8657
5	75 % EFFLUENT	5	0.6600	0.9483
6	100 % EFFLUENT	1	0.8550	1.1801
6	100 % EFFLUENT	2	0.7240	1.0177
6	100 % EFFLUENT	3	0.6670	0.9557
6	100 % EFFLUENT	4	0.7160	1.0088
6	100 % EFFLUENT	5	0.7260	1.0199

AA# K1206005 FATHEAD MINNOW GROWTH CHRONIC, 6-14-12
File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.117	0.023	2.211
Within (Error)	24	0.254	0.011	
Total	29	0.371		

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

AA# K1206005 FATHEAD MINNOW GROWTH CHRONIC, 6-14-12
File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	0.858	0.572		
2	32 % EFFLUENT	0.902	0.616	-0.675	
3	42 % EFFLUENT	0.937	0.637	-1.208	
4	56 % EFFLUENT	0.855	0.568	0.052	
5	75 % EFFLUENT	0.956	0.665	-1.500	
6	100 % EFFLUENT	1.036	0.738	-2.738	

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

AA# K1206005 FATHEAD MINNOW GROWTH CHRONIC, 6-14-12

File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	5			
2	32 % EFFLUENT	5	0.153	26.7	-0.043
3	42 % EFFLUENT	5	0.153	26.7	-0.064
4	56 % EFFLUENT	5	0.153	26.7	0.004
5	75 % EFFLUENT	5	0.153	26.7	-0.092
6	100 % EFFLUENT	5	0.153	26.7	-0.165

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

Control Failure

Cerodaphnia dubia — **SURVIVAL AND REPRODUCTION TEST**

Discharger: Western — Lab Number/s: K170.0005

Location:

Date Sample Collected:

Analyst: GHH/TC GKP

Test Start - Date/ Time: 6/14/17 1600

Test Stop - Date/Time: 6/23/17 1150

Conc 1 (0%)

Day	A	B	C	D	E	F	G	H	I	J	No. of Young	No. of Adult	Young/ Adult	Analyst
1	0	0	0	0	0	0	0	0	0	0	0	10	0	KP
2	0	0	0	0	0	0	0	0	0	0	0	10	0	
3	0	0	0	0	0	0	0	0	X	0	0	9	0	
4	1	0	0	4	0	3	2	1	0	0	11	9	1.2	
5	0	0	0	0	0	0	0	0	0	0	0	9	0	
6	0	0	3	3	0	6	3	4	=	0	19	9	2.1	
7	0	0	1	4	0	6	4	5	=	6	26	9	2.9	
8	1	0	0	0	0	2	2	0	=	2	7	9	0.8	
Total	2	0	4	11	0	17	11	10	X0	8	63			

Conc 2 (32%) — $\bar{x}$ = 7.0, CV = 83

Day	A	B	C	D	E	F	G	H	I	J	No. of Young	No. of Adult	Young/ Adult	Analyst
1	0	0	0	0	0	0	0	0	0	0	0	16	0	
2	0	0	0	0	0	0	0	0	0	0	0	18	0	
3	0	0	0	0	0	0	0	0	0	0	0	18	0	
4	1	1	2	1	4	0	2	0	0	0	11	10	1.1	
5	1	0	0	1	1	0	0	0	0	0	3	10	0.3	
6	0	2	0	0	3	2	0	0	6	2	15	10	1.5	
7	6	5	5	4	8	0	4	2	0	0	34	10	3.4	
8	2	3	1	0	1	4	3	0	0	0	14	10	1.4	
Total	10	11	8	6	17	6	9	2	6	2	77			

Conc 3 (42%)

Day	A	B	C	D	E	F	G	H	I	J	No. of Young	No. of Adult	Young/ Adult	Analyst
1	0	0	0	0	0	0	0	0	0	0	0	10	0	
2	0	0	0	0	0	0	0	0	0	0	0	10	0	
3	0	0	0	0	0	0	0	0	0	0	0	10	0	
4	0	0	1	3	0	1	0	3	1	2	11	10	1.1	
5	0	0	1	1	0	0	0	0	0	0	2	10	0.2	
6	0	0	0	0	0	0	0	0	0	0	0	10	0	
7	0	1	1	5	1	4	0	6	9	6	33	10	3.3	
8	0	1	0	2	0	3	0	3	3	2	14	10		
Total	4	3	3	11	1	8	0	12	13	10	65			

X= DEAD; Y= MALE

Conc 4 (56%)

Day	A	B	C	D	E	F	G	H	I	J	No. of Young	No. of Adult	Young/ Adult	Analyst
1	0	0	0	0	0	0	0	0	0	0	0	10	0	
2	0	0	0	0	0	0	0	0	0	0	0	10	0	
3	0	0	0	0	0	0	0	0	0	0	0	10	0	
4	0	0	2	0	2	0	4	0	0	3	11	10	1.1	
5	0	0	0	0	1	0	0	0	0	0	1	10	0.1	
6	0	3	2	0	0	0	0	2	3	0	10	10	1.0	
7	6	7	5	0	8	5	1	3	0	4	32	10	3.2	
8	4	1	0	0	0	1	0	0	0	0	6	10	0.6	
Total	10	5	9	0	11	6	5	4	3	7	60			

Conc 5 (75%)

Day	A	B	C	D	E	F	G	H	I	J	No. of Young	No. of Adult	Young/ Adult	Analyst
1	0	0	0	0	0	0	0	0	0	0	0	10	0	
2	0	0	0	0	0	+	0	0	0	0	0	10	0	
3	0	0	0	0	0	X	0	0	0	0	0	9	0	
4	2	0	2	1	0	0	0	0	0	1	7	9	0.8	
5	0	0	0	1	0	0	0	0	0	0	1	9	0.1	
6	1	0	0	0	0	—	0	0	0	0	1	9	0.1	
7	3	0	2	3	0	—	0	0	0	2	14	9	1.6	
8	3	1	4	4	0	—	3	2	4	5	26	9	2.9	
Total	13	1	8	9	0	X0	3	2	4	9	49			

Conc 6 (100%)

Day	A	B	C	D	E	F	G	H	I	J	No. of Young	No. of Adult	Young/ Adult	Analyst
1	0	0	0	0	0	0	0	0	0	0	0	10	0	
2	0	0	0	0	0	0	0	0	0	0	0	10	0	
3	0	0	0	0	0	0	0	0	0	0	0	9	0	
4	4	0	0	0	1	2	1	0	0	2	10	9	1.1	
5	0	0	0	1	1	4	0	0	0	0	6	9	0.7	
6	2	0	—	0	0	0	0	1	0	0	3	9	0.3	
7	3	0	—	4	3	4	0	0	0	5	19	9	2.1	
8	4	0	—	5	3	3	4	1	2	0	22	9	2.4	
Total	13	0	X0	10	8	13	5	2	2	7	60			

$\bar{x}$ = 6.75

CV = ~~61.98~~ 71.9

AA # K1206005, C. DUBIA CHRONIC, REPRODUCION, 6-14-12
File: Z:\TOXSTAT\MONTE\CD. Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

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

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

Total number of replicates = 60

AA # K1206005, C. DUBIA CHRONIC, REPRODUCION, 6-14-12
File: Z:\TOXSTAT\MONTE\CD. Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 2.82

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.

FISHER'S EXACT TEST

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

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

FISHER'S EXACT TEST

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

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

FISHER'S EXACT TEST

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

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

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF ALIVE	NUMBER OF DEAD	TOTAL ANIMALS
CONTROL	9	1	10
75%	9	1	10
TOTAL	18	2	20

CRITICAL FISHER'S VALUE (10,10,9) (p=0.05) IS 4. b VALUE IS 9.
Since b is greater than 4 there is no significant difference
between CONTROL and TREATMENT at the 0.05 level.

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF ALIVE	NUMBER OF DEAD	TOTAL ANIMALS
CONTROL	9	1	10
100%	9	1	10
TOTAL	18	2	20

CRITICAL FISHER'S VALUE (10,10,9) (p=0.05) IS 4. b VALUE IS 9.
Since b is greater than 4 there is no significant difference
between CONTROL and TREATMENT at the 0.05 level.

SUMMARY OF FISHER'S EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	10	1	

1	32%	10	0
2	42%	10	0
3	56%	10	0
4	75%	10	1
5	100%	10	1

TITLE: AA # K1206005, C. DUBIA CHRONIC, REPRODUCION, 6-14-12
FILE: Z:\TOXSTAT\MONTE\CD.
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	2.0000	2.0000
1	CONTROL	2	0.0000	0.0000
1	CONTROL	3	4.0000	4.0000
1	CONTROL	4	11.0000	11.0000
1	CONTROL	5	0.0000	0.0000
1	CONTROL	6	17.0000	17.0000
1	CONTROL	7	11.0000	11.0000
1	CONTROL	8	10.0000	10.0000
1	CONTROL	9	0.0000	0.0000
1	CONTROL	10	8.0000	8.0000
2	32 % EFFLUENT	1	10.0000	10.0000
2	32 % EFFLUENT	2	11.0000	11.0000
2	32 % EFFLUENT	3	8.0000	8.0000
2	32 % EFFLUENT	4	6.0000	6.0000
2	32 % EFFLUENT	5	17.0000	17.0000
2	32 % EFFLUENT	6	6.0000	6.0000
2	32 % EFFLUENT	7	9.0000	9.0000
2	32 % EFFLUENT	8	2.0000	2.0000
2	32 % EFFLUENT	9	6.0000	6.0000
2	32 % EFFLUENT	10	2.0000	2.0000
3	42 % EFFLUENT	1	4.0000	4.0000
3	42 % EFFLUENT	2	3.0000	3.0000
3	42 % EFFLUENT	3	3.0000	3.0000
3	42 % EFFLUENT	4	11.0000	11.0000
3	42 % EFFLUENT	5	1.0000	1.0000
3	42 % EFFLUENT	6	8.0000	8.0000
3	42 % EFFLUENT	7	0.0000	0.0000
3	42 % EFFLUENT	8	12.0000	12.0000
3	42 % EFFLUENT	9	13.0000	13.0000
3	42 % EFFLUENT	10	10.0000	10.0000
4	56 % EFFLUENT	1	10.0000	10.0000
4	56 % EFFLUENT	2	5.0000	5.0000
4	56 % EFFLUENT	3	9.0000	9.0000
4	56 % EFFLUENT	4	0.0000	0.0000
4	56 % EFFLUENT	5	11.0000	11.0000
4	56 % EFFLUENT	6	6.0000	6.0000
4	56 % EFFLUENT	7	5.0000	5.0000
4	56 % EFFLUENT	8	4.0000	4.0000
4	56 % EFFLUENT	9	3.0000	3.0000
4	56 % EFFLUENT	10	7.0000	7.0000
5	75 % EFFLUENT	1	13.0000	13.0000

5	75 % EFFLUENT	2	1.0000	1.0000
5	75 % EFFLUENT	3	8.0000	8.0000
5	75 % EFFLUENT	4	9.0000	9.0000
5	75 % EFFLUENT	5	0.0000	0.0000
5	75 % EFFLUENT	6	0.0000	0.0000
5	75 % EFFLUENT	7	3.0000	3.0000
5	75 % EFFLUENT	8	2.0000	2.0000
5	75 % EFFLUENT	9	4.0000	4.0000
5	75 % EFFLUENT	10	9.0000	9.0000
6	100 % EFFLUENT	1	13.0000	13.0000
6	100 % EFFLUENT	2	0.0000	0.0000
6	100 % EFFLUENT	3	0.0000	0.0000
6	100 % EFFLUENT	4	10.0000	10.0000
6	100 % EFFLUENT	5	8.0000	8.0000
6	100 % EFFLUENT	6	13.0000	13.0000
6	100 % EFFLUENT	7	5.0000	5.0000
6	100 % EFFLUENT	8	2.0000	2.0000
6	100 % EFFLUENT	9	2.0000	2.0000
6	100 % EFFLUENT	10	7.0000	7.0000

AA # K1206005, C. DUBIA CHRONIC, REPRODUCION, 6-14-12
File: Z:\TOXSTAT\MONTE\CD. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	41.133	8.227	0.365
Within (Error)	54	1217.600	22.548	
Total	59	1258.733		

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

AA # K1206005, C. DUBIA CHRONIC, REPRODUCION, 6-14-12
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	6.300	6.300		
2	32 % EFFLUENT	7.700	7.700	-0.659	
3	42 % EFFLUENT	6.500	6.500	-0.094	
4	56 % EFFLUENT	6.000	6.000	0.141	
5	75 % EFFLUENT	4.900	4.900	0.659	
6	100 % EFFLUENT	6.000	6.000	0.141	

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

AA # K1206005, C. DUBIA CHRONIC, REPRODUCION, 6-14-12
File: Z:\TOXSTAT\MONTE\CD. Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	10			
2	32 % EFFLUENT	10	4.905	77.9	-1.400
3	42 % EFFLUENT	10	4.905	77.9	-0.200
4	56 % EFFLUENT	10	4.905	77.9	0.300
5	75 % EFFLUENT	10	4.905	77.9	1.400
6	100 % EFFLUENT	10	4.905	77.9	0.300

AA # K1206005, C. DUBIA CHRONIC, REPRODUCION, 6-14-12
File: Z:\TOXSTAT\MONTE\CD. Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	6.300				
2	32 % EFFLUENT	7.700	112.50	75.00	10.00	
3	42 % EFFLUENT	6.500	110.00	75.00	10.00	
4	56 % EFFLUENT	6.000	105.50	75.00	10.00	
5	75 % EFFLUENT	4.900	99.50	75.00	10.00	
6	100 % EFFLUENT	6.000	105.00	75.00	10.00	

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

APPENDIX E

Organism History

AQUATOX, INC.

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

TEST ORGANISM HISTORY

DATE SHIPPED 6/13/12 **CLIENT** AR Analytical Lab

Purchase Order #: _____

SPECIES: Pimephales promelas

Quantity Shipped: ~~550~~ 604 830

Age: Hatched 6/12/12 1500 EST

Brood Stock Source: Anderson Farms, AR

Culture Water: Groundwater

Hardness (Mg/l CaCO3): 160

Dissolved Oxygen (Mg/1): 8.2

Temperature (°C): 25.1°C

Feeding: Artemia

Comments: _____

Shipped Via: Federal Express UPS Overnight Shuttle

Packaged By: _____

1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524

Toll Free: 800/331-5916
Tel:970/484-5091 Fax:970/484-2514

ORGANISM HISTORY

DATE: 6/22/09

SPECIES: *Ceriodaphnia dubia*

AGE: Variable

LIFE STAGE: Adult

HATCH DATE: Variable

BEGAN FEEDING: Immediately

FOOD: YTC, *Selenastrum* sp.

Water Chemistry Record:	Current	Range
TEMPERATURE:	25°C	20-25°C
SALINITY/CONDUCTIVITY:	--	--
TOTAL HARDNESS (as $CaCO_3$):	142 mg/l	86-124 mg/l
TOTAL ALKALINITY (as $CaCO_3$):	100 mg/l	65-130 mg/l
pH:	7.92	7.56-8.35

Comments:

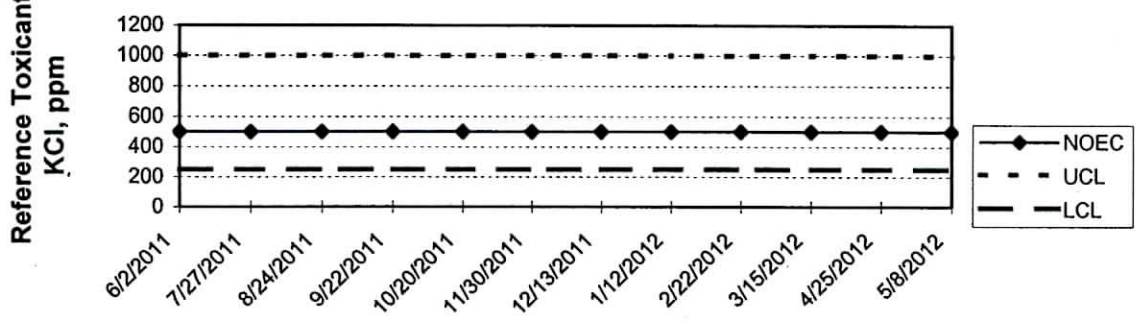
Facility Supervisor

APPENDIX F

Quality Assurance Charts

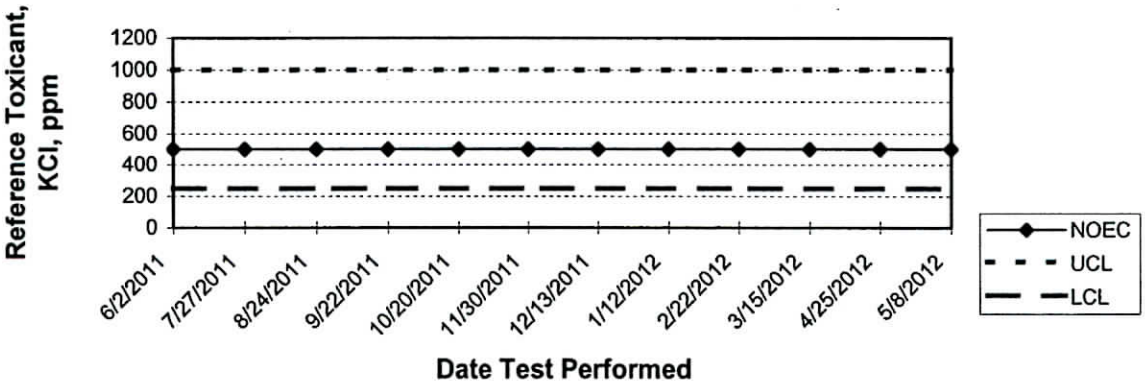
ARKANSAS ANALYTICAL, INC.

FATHEAD MINNOW SURVIVAL
QUALITY ASSURANCE



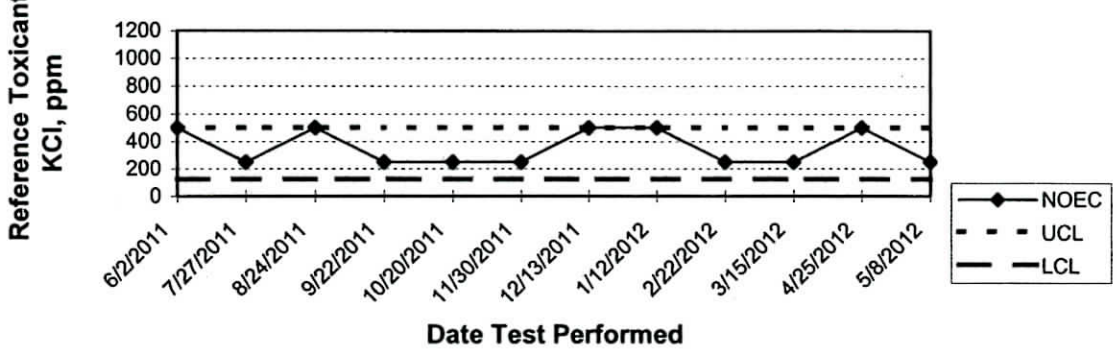
ARKANSAS ANALYTICAL, INC.

FATHEAD MINNOW GROWTH
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.

CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.

CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE