Arkansas Analytical, Inc.

Toxicity Test Results

MAGCOBAR MINE SITE
NPDES PERMIT NUMBER: AR0049794
April, 2012
AFIN# 00-00348

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

Ceriodaphnia dubia, Survival and Reproduction Test
Test 1002.0

Prepared for: **Mr. David Friedman**
EEMA O&M Services Group
P.O. Box 232
Kulpsville, PA 19443

Prepared by: Arkansas Analytical, Inc.
11701 I-30, Bldg 1, Suite 115
Little Rock, Arkansas 72209
Lab Number K1204003

Thursday, April 26, 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 April 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:	4-11-12, 0710	4-12-12, 0710
Sample #2:	4-12-12, 0910	4-13-12, 0910
Sample #3:	4-16-12, 0805	4-17-12, 0805

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:	4-12-12, 1333	4
*Sample #2:	4-13-12, 1340	4
Sample #3:	4-17-12, 1350	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	15.0	X	
At least 60% of surviving females should have produced 3 broods	90%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	15.4%	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	0.413	X	
The percent coefficient of variation between replicates must be 40% or less for growth	6.51%	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 3/15-22/12		*Pimephales promelas* 3/15-22/12	
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	100% / NA	NOEC / LOEC survival	100% / NA
NOEC / LOEC Reproduction	100% / NA	NOEC / LOEC growth	100% / NA
Mean number of neonates (critical dilution)	16.5	%CV survival (critical dilution)	0.00%
%CV Reproduction (critical dilution)	21.2%	Mean dry weight (critical dilution) in milligrams	0.580
		%CV growth (critical dilution)	8.88%
PMSD Reproduction	29.6	PMSD Growth	14.9

Conclusion

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

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

Chronic static renewal survival and reproduction test using *Ceriodaphnia dubia,* (Method 1002.0).

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

Biomonitoring Analysts:

Ken Pigue

Allen Parker

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

PERMITTEE: Magcobar Mine Site **NPDES #: AR0049794**

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	4-11-12, 0710	4-12-12, 0710
Sample #2:	4-12-12, 0910	4-13-12, 0910
Sample #3:	4-16-12, 0805	4-17-12, 0805

Test initiated (date, time): 4-12-12, 1350 Test terminated (date, time): 4-19-12, 1100

Dilution water used: Soft Synthetic

DATA TABLE FOR FATHEAD MINNOW SURVIVAL

Percent Survival in Replicate Chambers **Mean Percent Survival**

DATA TABLE FOR GROWTH OF FATHEAD MINNOWS

Effluent Conc %	A	B	C	D	E		24 hours	48 hours	7 days	CV %
0%	100	100	87.5	87.5	100		100	100	95	7.21
32%	100	87.5	100	100	100		100	100	97.5	
42%	100	100	100	87.5	87.5		100	100	95	
56%	87.5	100	100	100	87.5		100	97.5	95	
75%	87.5	87.5	100	100	100		100	95	95	
100%	100	100	100	100	100		100	100	100	0.00

SUMMARY

Effluent Conc %	A	B	C	D	E		Mean Dry Weight	CV%
0%	0.450	0.429	0.405	0.399	0.381		0.413	6.51
32%	0.531	0.532	0.561	0.521	0.561		0.541	
42%	0.486	0.566	0.524	0.525	0.485		0.517	
56%	0.469	0.575	0.532	0.454	0.484		0.503	
75%	0.504	0.532	0.502	0.639	0.570		0.549	
100%	0.660	0.590	0.563	0.520	0.566		0.580	8.88

Coefficient of Variation = standard deviation / mean * 100

REPORTING FORMS FOR CHRONIC BIOMONITORING FATHEAD MINNOW LARVAE GROWTH AND SURVIVAL
Pimephales promelas

1. Dunnett's procedure or Steel's Many-One Rank Test as appropriate:
   Is the mean survival at 7 days significantly different (p=0.05) than the control survival for:

   a) LOW FLOW OR CRITICAL DILUTION, (100%) YES _____ NO___**X**___

2. Dunnett's Procedure
   Is the mean dry weight (growth) at 7 days significantly different (p=0.05) than the control's dry weight (growth) for:

   a) LOW FLOW OR CRITICAL DILUTION, (100%) YES_____ NO___**X**___

3. If NO was answered to 1.a) enter [0] otherwise enter [1] (parameter TLP6C): _____**0**_____

4. If NO was answered to 2.a) enter [0] otherwise enter [1] (parameter TGP6C): _____**0**_____

5. Enter percentage corresponding to each parameter below:

   a) NOEC survival (parameter TOP6C)= _____**100**____% effluent

   b) NOEC growth (parameter TPP6C)= _____**100**_____% effluent

   c) Coefficient of variation (parameter TQP6C)= _____**8.88**_____%

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:	4-11-12, 0710	4-12-12, 0710
Sample #2:	4-12-12, 0910	4-13-12, 0910
Sample #3:	4-16-12, 0805	4-17-12, 0805

Test initiated (date, time): 4-12-12, 1345 Test terminated (date, time): 4-19-12, 0910

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	16	16	14	17	20	19
B	16	14	15	17	18	19
C	15	13	x9	16	x0	19
D	14	14	16	16	17	16
E	9	19	12	x0	9	18
F	17	12	13	14	18	20
G	15	13	x0	17	10	12
H	15	18	15	11	14	12
I	16	21	12	12	16	19
J	17	17	13	16	15	11
Mean	15.0	15.7	11.9	13.6	13.6	16.5
Mean/surviving female	15.0	15.7	13.8	15.1	15.2	16.5
CV%*	15.4					21.2

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	90	100	100	100
Test termination	100	100	80	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)= _____**21.2**_____%

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:
								1									K1204-
FD-1 Comp.	4/12/2010	7:10 AM		X	5	W	Facility Discharge	X									003A

1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)	SAMPLE CONDITION UPON RECEIPT IN LAB	REMARKS / SAMPLE COMMENTS
Bill McAlister	4-12-12 1333		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
		Email: dave.friedman@eema-inc.com; bmcalister@eema-inc.com; bhorton@eema-inc.com	Bottle Type

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	Sampler(s) Printed

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 (G = Glass, P = Plastic, V = Septum, A = Amber)	Arkansas Analytical Work Order Number: K1204-
								1		
								P		
FD-1 Comp.	4/13/2010	9:10 AM		X	4	W	Facility Discharge	X		003B

1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)
Barry Cutts	4.13.12, 1340	
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)
		Sydney Johns

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:	3°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

Sampler(s) Signature: Larry Curtis

Sampler(s) Printed: LARRY CURTIS

Field Number	SAMPLE COLLECTION Date/s	Time/s	Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION	Chronic Biomonitoring	TEST PARAMETERS	Bottle Type Code
FD-1 Comp.	4/17/2012	8:05 AM		X	4	W	Facility Discharge	X		C

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

Arkansas Analytical Work Order Number: K1204003

1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)
Larry Curtis		
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)
	1350 4/17/12	Amanda Forbis

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: K1204003 — Test Start (Date/Time): 4/12/12

Client: Wesla — Test End (Date/Time): 4/19/12

		Day of Test							
		1	2	3	4	5	6	7	notes/remarks
Control	MHS551	4/12	4/13	4/14	4/15	4/16	4/17	4/18	
D.O. (mg/L)	INITIAL	8.6	8.5	8.5	8.5	8.4	6.7	7.2	
	FINAL	8.1	8.0	8.1	7.7	8.2	8.1	8.2	
pH (s.u.)	INITIAL	7.7	7.7	7.8	7.8	7.9	7.8	7.7	
	FINAL	7.5	7.4	7.6	7.5	7.2	7.0	7.6	
temp (C)	INITIAL	21.3	21.5	22.6	23.1	22.1	23.4	23.1	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
ALKALINITY (mg/L)		32							
HARDNESS (mg/L)		42							
CONDUCTIVITY (umhos/cm)		159							
CHLORINE (mg/L)		<0.05							
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.6	8.7	8.7	8.4	7.1	7.4	
	FINAL	7.9	8.0	7.9	7.6	7.9	8.1	8.7	
pH (s.u)	INITIAL	7.4	7.4	7.5	7.4	7.4	7.4	7.4	
	FINAL	7.3	7.3	7.5-7.8	7.3	7.0	7.0	7.2	
temp (C)	INITIAL	21.0	23.6	23.1	23.6	22.2	23.8	23.5	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.7	8.9	8.9	8.4	7.4	7.8	
	FINAL	8.0	8.0	8.0	7.6	7.9	8.0	8.1	
pH (mg/L)	INITIAL	7.4	7.4	7.5	7.4	7.4	7.4	7.3	
	FINAL	7.2	7.2	7.3	7.1	7.0	7.2	7.2	
temp (C)	INITIAL	21.0	24.2	23.4	23.8	22.4	24.2	23.8	
	FINAL	25.0	25.0	24.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.8	9.0	9.0	8.4	7.7	8.1	
	FINAL	8.0	8.1	7.9	7.4	8.0	7.9	8.0	
pH (s.u.)	INITIAL	7.3	7.3	7.4	7.3	7.4	7.5	7.3	
	FINAL	7.1	7.2	7.3	7.1	7.2	7.2	7.2	
temp (C)	INITIAL	20.9	24.6	23.6	24.0	22.4	24.6	24.2	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.8	9.2	9.3	8.4	8.1	8.5	
	FINAL	8.0	8.0	7.8	7.4	7.9	7.9	8.0	
pH (s.u.)	INITIAL	7.3	7.3	7.3	7.2	7.3	7.4	7.4	
	FINAL	7.1	7.2	7.2	7.0	7.2	7.2	7.2	
temp (C)	INITIAL	20.9	25.1	23.8	24.8	24.7	25.0	24.6	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.8	9.6	10.2	8.4	8.5	8.6	
	FINAL	8.0	8.0	7.9	7.4	7.9	7.9	8.0	
pH (s.u.)	INITIAL	7.2	7.1	6.9	6.8	7.1	7.5	7.4	
	FINAL	7.1	7.0	7.0	6.9	7.1	7.1	7.1	
temp (C)	INITIAL	20.8	25.4	24.0	24.6	22.5	25.9	25.1	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:	**100%**	A	A	A, B	B	B	C	C	
ALKALINITY (mg/L)		6	—	—	6	—	12	—	
HARDNESS (mg/L)		>600	—	—	>600	—	>600	—	
CONDUCTIVITY (umhos/cm)		1927	—	—	1941	—	1984	—	
CHLORINE (mg/L)		<0.05	—	—	<0.05	—	<0.05	—	

Revision 1
11/30/10

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING — Cerodaphnia Dubia

Lab # / Sample ID: K12D4003 — Test Start (Date/Time): 4/12/12

Client: Weston — Test End (Date/Time): 4/19/12

		Day of Test							
		1	2	3	4	5	6	7	notes/remarks
Control	MHS551	4/12	4/13	4/14	4/15	4/16	4/17	4/18	
D.O. (mg/L)	INITIAL	8.6	8.5	8.5	8.5	8.4	6.7	7.2	
	FINAL	8.1	8.2	8.2	8.4	8.3	8.4	8.3	
pH (s.u.)	INITIAL	7.7	7.7	7.8	7.8	7.9	7.8	7.7	
	FINAL	7.7	7.7	7.6	7.9	7.5	7.8	7.3	
temp (C)	INITIAL	21.3	21.5	22.6	23.1	22.1	23.4	23.1	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
ALKALINITY (mg/L)		32							
HARDNESS (mg/L)		42							
CONDUCTIVITY (umhos/cm)		159							
CHLORINE (mg/L)		<0.05							
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.6	8.7	8.7	8.4	7.1	7.4	
	FINAL	8.0	8.1	8.1	8.2	8.3	8.4	8.2	
pH (s.u)	INITIAL	7.4	7.4	7.5	7.4	7.4	7.4	7.4	
	FINAL	7.2	7.4	7.4	7.4	7.1	7.6	7.0	
temp (C)	INITIAL	21.0	23.6	23.1	23.6	22.2	23.8	23.5	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.7	8.9	8.9	8.4	7.4	7.8	
	FINAL	8.0	8.1	8.1	8.2	8.3	8.4	8.2	
pH (mg/L)	INITIAL	7.4	7.4	7.5	7.4	7.4	7.4	7.3	
	FINAL	7.3	7.4	7.4	7.4	7.1	7.5	7.0	
temp (C)	INITIAL	21.0	24.2	23.4	23.8	22.4	24.2	23.8	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.8	9.0	9.0	8.4	7.7	8.1	
	FINAL	8.0	8.3	8.1	8.1	8.4	8.4	8.2	
pH (s.u.)	INITIAL	7.3	7.3	7.4	7.3	7.4	7.5	7.3	
	FINAL	7.2	7.3	7.4	7.4	7.2	7.4	6.9	
temp (C)	INITIAL	20.9	24.6	23.6	24.0	22.4	24.6	24.2	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.8	9.2	9.3	8.4	8.1	8.5	
	FINAL	7.9	8.1	8.1	8.1	8.3	8.4	8.2	
pH (s.u.)	INITIAL	7.3	7.3	7.3	7.2	7.3	7.4	7.4	
	FINAL	7.2	7.3	7.4	7.4	7.1	7.4	6.9	
temp (C)	INITIAL	20.9	25.1	23.8	24.8	22.7	25.0	24.6	
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.8	9.6	10.2	8.4	8.5	8.6	
	FINAL	7.9	8.1	8.0	8.3	8.4	8.4	8.2	
pH (s.u.)	INITIAL	7.2	7.1	6.9	6.8	7.1	7.5	7.4	
	FINAL	7.1	7.2	7.3	7.3	7.1	7.4	6.9	
temp (C)	INITIAL	20.8	25.4	24.0	24.6	22.5	25.9	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)		6			6		12		
HARDNESS (mg/L)		>600			>600		>600		
CONDUCTIVITY (umhos/cm)		1927			1941		1984		
CHLORINE (mg/L)		<0.05			<0.05		<0.05		

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID K1204003 TEST START DATE 4/17/12 TIME 1350

CLIENT Weston TEST END DATE 4/19/12 TIME 1100

Summary Page 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	8	8	8	8	8	8	8	8	100	95	7.21
	B	8	8	8	8	8	8	8	8	100		
	C	8	8	8	7	7	7	7	7	87.5		
	D	8	8	8	8	8	8	7	7	87.5		
	E	8	8	8	8	8	8	8	8	100		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 32	A	8	8	8	8	8	8	8	8	100	97.5	
	B	8	8	8	8	8	8	7	7	87.5		
	C	8	8	8	8	8	8	8	8	100		
	D	8	8	8	8	8	8	8	8	100		
	E	8	8	8	8	8	8	8	8	100		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 42	A	8	8	8	8	8	8	8	8	100	95	
	B	8	8	8	8	8	8	8	8	100		
	C	8	8	8	8	8	8	8	8	100		
	D	8	8	8	8	8	8	7	7	87.5		
	E	8	8	8	8	8	8	7	7	87.5		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 56	A	8	8	7	7	7	7	7	7	87.5	95	
	B	8	8	8	8	8	8	8	8	100		
	C	8	8	8	8	8	8	8	8	100		
	D	8	8	8	8	8	8	8	8	100		
	E	8	8	8	8	8	8	7	7	87.5		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 75	A	8	8	7	7	7	7	7	7	87.5	95	
	B	8	8	7	7	7	7	7	7	87.5		
	C	8	8	8	8	8	8	8	8	100		
	D	8	8	8	8	8	8	8	8	100		
	E	8	8	8	8	8	8	8	8	100		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 100	A	8	8	8	8	8	8	8	8	100	100	0.00
	B	8	8	8	8	8	8	8	8	100		
	C	8	8	8	8	8	8	8	8	100		
	D	8	8	8	8	8	8	8	8	100		
	E	8	8	8	8	8	8	8	8	100		
ANALYST												
DATE:												
TIME:												

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

APPENDIX C

Fathead minnow raw data and statistics

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID | TEST START DATE 4/12/12 TIME 1350

CLIENT Weston A | TEST END DATE 4/19/12 TIME 1100

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							2	2			
	C							2	2			
	D	1	1	1	1	1	1	2	2			
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 37	A	2	2	2	2	2	2	2	2			
	B							2	2			
	C							2	2			
	D	1	1	1	1	1	1	2	2			
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 42	A	2	2	2	2	2	2	2	2			
	B							2	2			
	C							2	2			
	D	1	1	1	1	1	1	2	2			
	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			
	C			2	2	2	2	2	2			
	D	1	1	2	2	2	2	2	2			
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 75	A	2	2	2	2	2	2	2	2			
	B			2	2	2	2	2	2			
	C			1	1	1	1	1	1			
	D	1	1	2	2	2	2	2	2			
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 100	A	2	2	2	2	2	2	2	2			
	B							2	2			
	C							2	2			
	D	1	1	1	1	1	1	2	2			
	E											
ANALYST		KP	KP	AP	AP	KP	KP	KP	KP			
DATE:		4/12/12	4/13/12	4-14-12	4-15-12	4/16/12	4/17	4/18	4/19/12			
TIME:		1350	1110	0800	1150	1430	1510	1415	1100			

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 4/12/12 TIME 1350

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

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID | TEST START DATE 4/12/12 TIME 0/350

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	2			
	B				2	2	2	2	2			
	C				2	2	2	2	2			
	D	1	1	1	1	1	1	1	1			
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 32	A	2	2	2	2	2	2	2	2			
	B						2	2	2			
	C						2	2	2			
	D	1	1	1	1	1	2	2	2			
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 42	A	2	2	2	2	2	2	2	2			
	B						2	2	2			
	C						2	2	2			
	D	1	1	1	1	1	2	2	2			
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 56	A	2	2	2	2	2	2	2	2			
	B						2	2	2			
	C						2	2	2			
	D	1	1	1	1	1	2	2	2			
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 75	A	2	2	2	2	2	2	2	2			
	B						2	2	2			
	C						2	2	2			
	D	1	1	1	1	1	2	2	2			
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 100	A	2	2	2	2	2	2	2	2			
	B						2	2	2			
	C						2	2	2			
	D	1	1	1	1	1	2	2	2			
	E											
ANALYST		KP		AP	AP							
DATE:		4/12/12		4-14-12	4-15-12							
TIME:				0800	1150							

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 4/12/12 TIME 0/350

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

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID — TEST START DATE 4/12/17 TIME 1350

CLIENT Weston E — TEST END DATE — TIME

AGE AND SOURCE OF MINNOWS

			DAY (NUMBER SURVIVING)									
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	2	2	2	2	2	2	2	2			
	B	1	1	1	1	1	2	2	2			
	C	1	1	1	1	1	2	2	2			
	D	1	1	1	1	1	2	2	2			
	E						2					
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	2	2	2	2	2	2	2	2			
	B	1	1	1	1	1	2	2	2			
	C	1	1	1	1	1	2	2	2			
	D	1	1	1	1	1	2	2	2			
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	2	2	2	2	2	2	2	2			
	B	1	1	1	1	1	2	1	1			
	C	1	1	1	1	1	2	2	2			
	D	1	1	1	1	1	2	2	2			
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	2	2	2	2	2	2	2	2			
	B	1	1	1	1	1	2	2	2			
	C	1	1	1	1	1	2	2	2			
	D	1	1	1	1	1	2	1	1			
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	2	2	2	2	2	2	2	2			
	B	1	1	1	1	1	2	2	2			
	C	1	1	1	1	1	2	2	2			
	D	1	1	1	1	1	2	2	2			
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	2	2	2	2	2	2	2	2			
	B	1	1	1	1	1	2	2	2			
	C	1	1	1	1	1	2	2	2			
	D	1	1	1	1	1	2	2	2			
	E											
ANALYST		KP										
DATE:		4/12/17										
TIME:												

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

Pimephales promelas **FATHEAD MINNOW** TEST 1000.0

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:	K1204003				TEST DATES (BEGIN / END):	4/12-19/12		
CLIENT:	EEMA				WEIGHING DATE / TIME:	4/26/12, 1540		
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	0.98899	0.98539	0.00360	8	0.450	AVG DRY	
	B	0.97963	0.97620	0.00343	8	0.429	WEIGHT (mg)	
	C	0.97770	0.97446	0.00324	8	0.405		0.413
	D	0.96544	0.96225	0.00319	8	0.399	CV	
	E	1.01496	1.01191	0.00305	8	0.381		6.51
CONC:	A	0.98245	0.97820	0.00425	8	0.531	AVG DRY	
	B	1.01033	1.00607	0.00426	8	0.532	WEIGHT (mg)	
32%	C	1.01716	1.01267	0.00449	8	0.561		0.542
	D	0.99072	0.98655	0.00417	8	0.521	CV	
	E	1.00618	1.00169	0.00449	8	0.561		
CONC:	A	0.98456	0.98067	0.00389	8	0.486	AVG DRY	
	B	0.99793	0.99340	0.00453	8	0.566	WEIGHT (mg)	
42%	C	0.98917	0.98498	0.00419	8	0.524		0.517
	D	1.01452	1.01032	0.00420	8	0.525	CV	
	E	1.02279	1.01891	0.00388	8	0.485		
CONC:	A	0.99749	0.99374	0.00375	8	0.469	AVG DRY	
	B	0.97637	0.97177	0.00460	8	0.575	WEIGHT (mg)	
56%	C	1.00441	1.00015	0.00426	8	0.532		0.503
	D	0.97370	0.97007	0.00363	8	0.454	CV	
	E	0.96975	0.96588	0.00387	8	0.484		
CONC:	A	0.96807	0.96404	0.00403	8	0.504	AVG DRY	
	B	1.00125	0.99699	0.00426	8	0.532	WEIGHT (mg)	
75%	C	0.99779	0.99377	0.00402	8	0.502		0.549
	D	0.95756	0.95245	0.00511	8	0.639	CV	
	E	0.98840	0.98384	0.00456	8	0.570		
CONC:	A	0.97519	0.96991	0.00528	8	0.660	AVG DRY	
	B	1.01535	1.01063	0.00472	8	0.590	WEIGHT (mg)	
100%	C	1.00346	0.99896	0.00450	8	0.563		0.580
	D	0.97033	0.96617	0.00416	8	0.520	CV	
	E	1.00563	1.00110	0.00453	8	0.566		8.88

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA# K1204003, FATHEAD MINNOW, CHRONIC, 4-12-12
File: Z:\TOXSTAT\MONTE\FHSURV. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.189

W = 0.774

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# K1204003, FATHEAD MINNOW, CHRONIC, 4-12-12
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# K1204003, FATHEAD MINNOW, CHRONIC, 4-12-12
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	0.8750	1.2094
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	0.8750	1.2094
2	32 % EFFLUENT	3	1.0000	1.3931
2	32 % EFFLUENT	4	1.0000	1.3931
2	32 % EFFLUENT	5	1.0000	1.3931

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

AA# K1204003, FATHEAD MINNOW, CHRONIC, 4-12-12
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	1.320				
2	32 % EFFLUENT	1.356	30.00	16.00	5.00	
3	42 % EFFLUENT	1.320	27.50	16.00	5.00	
4	56 % EFFLUENT	1.320	27.50	16.00	5.00	
5	75 % EFFLUENT	1.320	27.50	16.00	5.00	
6	100 % EFFLUENT	1.393	32.50	16.00	5.00	

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

AA# K1204003, FATHEAD MINNOW GROWTH CHRONIC, 4-12-12
File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.043

W = 0.941

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# K1204003, FATHEAD MINNOW GROWTH CHRONIC, 4-12-12
File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 5.98

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# K1204003, FATHEAD MINNOW GROWTH CHRONIC, 4-12-12
FILE: Z:\TOXSTAT\MONTE\FHGR.
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.4500	0.7353
1	CONTROL	2	0.4290	0.7142
1	CONTROL	3	0.4050	0.6898
1	CONTROL	4	0.3990	0.6837
1	CONTROL	5	0.3810	0.6652
2	32 % EFFLUENT	1	0.5310	0.8164
2	32 % EFFLUENT	2	0.5320	0.8174
2	32 % EFFLUENT	3	0.5610	0.8466
2	32 % EFFLUENT	4	0.5210	0.8064
2	32 % EFFLUENT	5	0.5610	0.8466
3	42 % EFFLUENT	1	0.4880	0.7734
3	42 % EFFLUENT	2	0.5660	0.8516
3	42 % EFFLUENT	3	0.5240	0.8094
3	42 % EFFLUENT	4	0.5250	0.8104
3	42 % EFFLUENT	5	0.4850	0.7704
4	56 % EFFLUENT	1	0.4690	0.7544

4	56 % EFFLUENT	2	0.5750	0.8607
4	56 % EFFLUENT	3	0.5320	0.8174
4	56 % EFFLUENT	4	0.4540	0.7393
4	56 % EFFLUENT	5	0.4840	0.7694
5	75 % EFFLUENT	1	0.5040	0.7894
5	75 % EFFLUENT	2	0.5320	0.8174
5	75 % EFFLUENT	3	0.5020	0.7874
5	75 % EFFLUENT	4	0.6390	0.9263
5	75 % EFFLUENT	5	0.5700	0.8556
6	100 % EFFLUENT	1	0.6600	0.9483
6	100 % EFFLUENT	2	0.5900	0.8759
6	100 % EFFLUENT	3	0.5630	0.8486
6	100 % EFFLUENT	4	0.5200	0.8054
6	100 % EFFLUENT	5	0.5660	0.8516

AA# K1204003, FATHEAD MINNOW GROWTH CHRONIC, 4-12-12
File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.084	0.017	9.369
Within (Error)	24	0.043	0.002	
Total	29	0.128		

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

AA# K1204003, FATHEAD MINNOW GROWTH CHRONIC, 4-12-12
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	0.698	0.413		
2	32 % EFFLUENT	0.827	0.541	-4.806	
3	42 % EFFLUENT	0.803	0.518	-3.926	
4	56 % EFFLUENT	0.788	0.503	-3.375	
5	75 % EFFLUENT	0.835	0.549	-5.125	
6	100 % EFFLUENT	0.866	0.580	-6.270	

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

AA# K1204003, FATHEAD MINNOW GROWTH CHRONIC, 4-12-12
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	0.062	14.9	-0.128
3	42 % EFFLUENT	5	0.062	14.9	-0.105
4	56 % EFFLUENT	5	0.062	14.9	-0.090
5	75 % EFFLUENT	5	0.062	14.9	-0.137
6	100 % EFFLUENT	5	0.062	14.9	-0.167

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

Ceriodaphnia dubia

SURVIVAL AND REPRODUCTION TEST

Discharger: Weston | Lab Number/s:

Location:

Date Sample Collected:

Analyst: KP

Test Start - Date/ Time: 4/12/12 1345

Test Stop - Date/Time: 4/19/12 09:10

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	KP
3	0	1	0	1	0	0	0	0	1	0	3	10	0.3	KP
4	1	0	0	1	0	1	2	1	1	2	9	10	0.9	KP
5	2	2	1	3	2	3	0	1	3	1	18	10	1.8	KP
6	3	5	2	4	0	6	4	3	2	2	43	10	4.3	KP
7	10	8	7	5	7	7	9	8	9	7	77	10	7.7	
8														
Total	16	16	15	14	9	17	15	13	16	17	150		X=	15.0
													CV=	15.4

Conc 2 — %: 32

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	2	0	0	0	1	1	0	1	3	1	8	10	0.8	
4	1	1	0	0	2	2	1	2	3	1	14	10	1.4	
5	0	2	1	2	3	0	2	0	0	3	13	10	1.3	
6	4	4	2	5	4	3	0	6	2	5	40	10	4.0	
7	8	7	10	7	9	7	10	9	8	7	82	10	8.2	
8														
Total	16	14	13	14	19	12	13	18	21	17	157			

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	X	0	0	2	0	9	0	
3	2	0	1	1	0	0	-	2	0	1	5	9	0.6	
4	2	2	1	0	1	2	-	3	1	1	13	9	1.4	
5	0	1	3	3	1	0	-	0	1	0	10	9	1.1	
6	4	5	4	4	3	3	-	3	6	5	37	8	4.6	
7	8	7	8	8	6	8	-	7	4	6	54	8	6.8	
8			4											
Total	14	15	X9	16	12	13	X0	15	12	13	119			

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	1	0		1	10	0.1	
4	3	1	2	3	0	2	0	2	1	1	15	10	1.5	
5	2	3	2	2	0	2	1	1	2	2	17	10	1.7	
6	5	5	8	2	0	6	7	5	4	5	47	10	5.9	
7	7	8	4	9	X0	9	9	3	4	8	56	9	6.2	
8														
Total	17	17	16	16	X0	14	17	11	12	16	136			

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	1	0	0	0	0	0	0	0	0	0	10	0	
3	1	2	0	0	0	0	0	0	0		3	10	0.3	
4	3	2	0	1	0	0	0	1	1	1	11	10	1.1	
5	6	3	0	1	2	1	3	1	0	3	20	10	2.0	
6	3	4	0	2	1	0	4	3	8	3	39	10	3.9	
7	7	7	X0	8	6	9	3	9	9	8	64	9	7.1	
8														
Total	20	18	X0	17	9	18	10	14	16	15	137			

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	2	0	0	0	0	0	0	0	0	0	2	10	0	
3	0	0	0	0	0	0	0	0	0	0	0	10	0	
4	0	3	1	0	3	1	1	2	1	3	15	10	1.5	
5	3	3	3	3	2	3	1	0	2	2	22	10	2.2	
6	6	4	7	5	6	6	4	3	5	4	50	10	5.0	
7	10	9	8	8	7	10	6	7	11	2	78	10	7.8	
8														
Total	19	19	19	16	18	20	12	12	19	11	165			

$\bar{X}$=16.5

CV=21.2

Revision 1
11/30/10

AA # K1204003, C. DUBIA CHRONIC, REPRODUCION, 4-12-12
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 # K1204003, C. DUBIA CHRONIC, REPRODUCION, 4-12-12
File: Z:/toxstat/monte\CD. Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 10.19

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%	8	2	10
TOTAL	18	2	20

CRITICAL FISHER'S VALUE (10,10,10) (p=0.05) IS 6. b VALUE IS 8.
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	2	
3	56%	10	1	
4	75%	10	1	
5	100%	10	0	

TITLE: AA # K1204003, C. DUBIA CHRONIC, REPRODUCION, 4-12-12
FILE: Z:/toxstat/monte\CD.
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	16.0000	16.0000
1	CONTROL	2	16.0000	16.0000
1	CONTROL	3	15.0000	15.0000
1	CONTROL	4	14.0000	14.0000
1	CONTROL	5	9.0000	9.0000
1	CONTROL	6	17.0000	17.0000
1	CONTROL	7	15.0000	15.0000
1	CONTROL	8	15.0000	15.0000
1	CONTROL	9	16.0000	16.0000
1	CONTROL	10	17.0000	17.0000
2	32 % EFFLUENT	1	16.0000	16.0000
2	32 % EFFLUENT	2	14.0000	14.0000
2	32 % EFFLUENT	3	13.0000	13.0000
2	32 % EFFLUENT	4	14.0000	14.0000
2	32 % EFFLUENT	5	19.0000	19.0000
2	32 % EFFLUENT	6	12.0000	12.0000
2	32 % EFFLUENT	7	13.0000	13.0000
2	32 % EFFLUENT	8	18.0000	18.0000
2	32 % EFFLUENT	9	21.0000	21.0000
2	32 % EFFLUENT	10	17.0000	17.0000
3	42 % EFFLUENT	1	14.0000	14.0000
3	42 % EFFLUENT	2	15.0000	15.0000
3	42 % EFFLUENT	3	9.0000	9.0000
3	42 % EFFLUENT	4	16.0000	16.0000
3	42 % EFFLUENT	5	12.0000	12.0000
3	42 % EFFLUENT	6	13.0000	13.0000
3	42 % EFFLUENT	7	0.0000	0.0000
3	42 % EFFLUENT	8	15.0000	15.0000
3	42 % EFFLUENT	9	12.0000	12.0000
3	42 % EFFLUENT	10	13.0000	13.0000
4	56 % EFFLUENT	1	17.0000	17.0000
4	56 % EFFLUENT	2	17.0000	17.0000
4	56 % EFFLUENT	3	16.0000	16.0000
4	56 % EFFLUENT	4	16.0000	16.0000
4	56 % EFFLUENT	5	0.0000	0.0000
4	56 % EFFLUENT	6	14.0000	14.0000
4	56 % EFFLUENT	7	17.0000	17.0000
4	56 % EFFLUENT	8	11.0000	11.0000

4	56 % EFFLUENT	9	12.0000	12.0000
4	56 % EFFLUENT	10	16.0000	16.0000
5	75 % EFFLUENT	1	20.0000	20.0000
5	75 % EFFLUENT	2	18.0000	18.0000
5	75 % EFFLUENT	3	0.0000	0.0000
5	75 % EFFLUENT	4	17.0000	17.0000
5	75 % EFFLUENT	5	9.0000	9.0000
5	75 % EFFLUENT	6	18.0000	18.0000
5	75 % EFFLUENT	7	10.0000	10.0000
5	75 % EFFLUENT	8	14.0000	14.0000
5	75 % EFFLUENT	9	16.0000	16.0000
5	75 % EFFLUENT	10	15.0000	15.0000
6	100 % EFFLUENT	1	19.0000	19.0000
6	100 % EFFLUENT	2	19.0000	19.0000
6	100 % EFFLUENT	3	19.0000	19.0000
6	100 % EFFLUENT	4	16.0000	16.0000
6	100 % EFFLUENT	5	18.0000	18.0000
6	100 % EFFLUENT	6	20.0000	20.0000
6	100 % EFFLUENT	7	12.0000	12.0000
6	100 % EFFLUENT	8	12.0000	12.0000
6	100 % EFFLUENT	9	19.0000	19.0000
6	100 % EFFLUENT	10	11.0000	11.0000

AA # K1204003, C. DUBIA CHRONIC, REPRODUCION, 4-12-12
File: Z:/toxstat/monte\CD. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	138.400	27.680	1.501
Within (Error)	54	996.000	18.444	
Total	59	1134.400		

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

AA # K1204003, C. DUBIA CHRONIC, REPRODUCION, 4-12-12
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	15.000	15.000		
2	32 % EFFLUENT	15.700	15.700	-0.364	
3	42 % EFFLUENT	11.900	11.900	1.614	
4	56 % EFFLUENT	13.600	13.600	0.729	
5	75 % EFFLUENT	13.700	13.700	0.677	
6	100 % EFFLUENT	16.500	16.500	-0.781	

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

AA # K1204003, C. DUBIA CHRONIC, REPRODUCION, 4-12-12
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	4.437	29.6	-0.700
3	42 % EFFLUENT	10	4.437	29.6	3.100
4	56 % EFFLUENT	10	4.437	29.6	1.400
5	75 % EFFLUENT	10	4.437	29.6	1.300
6	100 % EFFLUENT	10	4.437	29.6	-1.500

AA # K1204003, C. DUBIA CHRONIC, REPRODUCION, 4-12-12
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	15.000				
2	32 % EFFLUENT	15.700	106.50	75.00	10.00	
3	42 % EFFLUENT	11.900	74.50	75.00	10.00	*
4	56 % EFFLUENT	13.600	105.00	75.00	10.00	
5	75 % EFFLUENT	13.700	107.00	75.00	10.00	
6	100 % EFFLUENT	16.500	124.50	75.00	10.00	

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

APPENDIX E

Organism History

AQUATOX, INC.

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

TEST ORGANISM HISTORY

DATE SHIPPED 4/12/12 **CLIENT** AR Analytical Ken

Purchase Order #: _____

SPECIES: Pimephales promelas

Quantity Shipped: 280

Age: hatched 4/11/12 1500 CST

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:

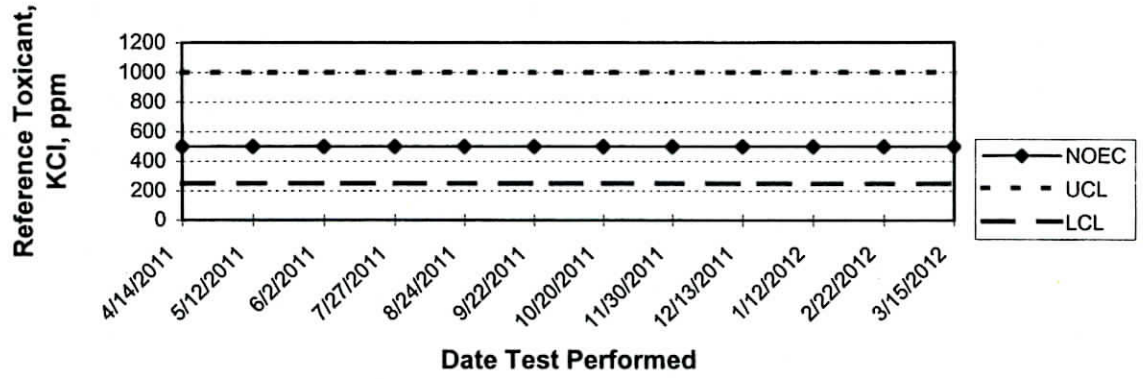
Facility Supervisor

APPENDIX F

Quality Assurance Charts

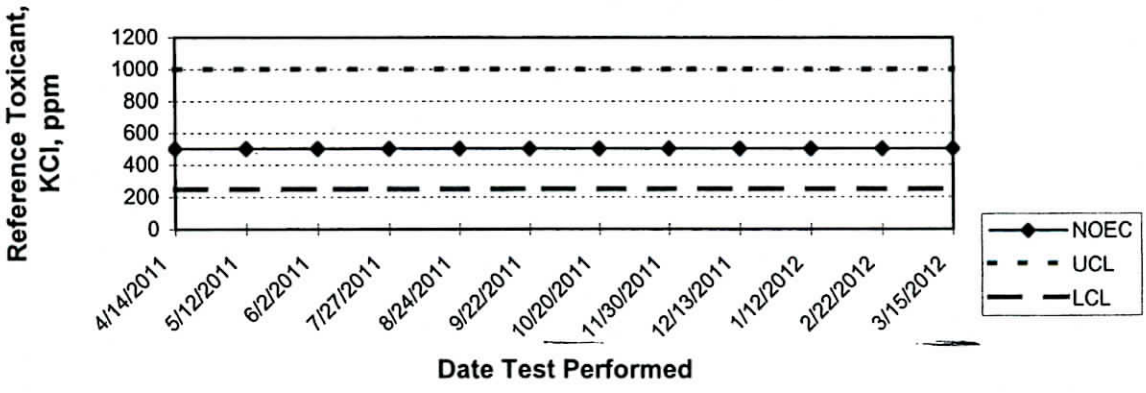
ARKANSAS ANALYTICAL, INC.

FATHEAD MINNOW SURVIVAL
QUALITY ASSURANCE



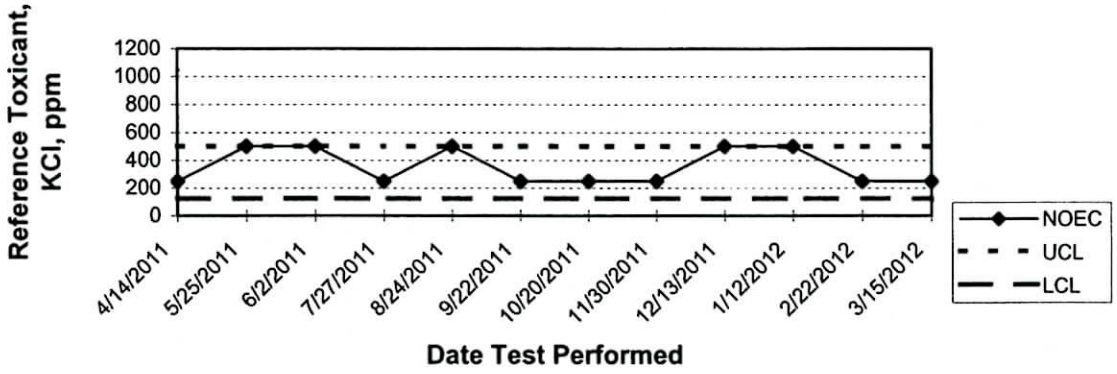
ARKANSAS ANALYTICAL, INC.

FATHEAD MINNOW GROWTH
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.

CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.

CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE