Arkansas Analytical, Inc.

Toxicity Test Results

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

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

Ceriodaphnia dubia, Survival and Reproduction Test
Test 1002.0

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 K910002

Thursday, November 12, 2009

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 October of 2009.

Plant Operations

To be provided by permittee.

Source of Effluent and Dilution Water

Effluent samples were collected as follows:

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	10-14-09, 0905	10-15-09, 0905
Sample #2:	10-15-09, 0815	10-16-09, 0815
Sample #3:	10-19-09, 0912	10-20-09, 0912

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:	10-15-09, 1327	3
Sample #2:	10-16-09, 1240	4
Sample #3:	10-20-09, 1342	3

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	16.3	X	
At least 60% of surviving females should have produced 3 broods	77.8%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	22.3%	X	

TEST ACCEPTANCE CRITERIA for *Pimephales promelas*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	100%	X	
The percent coefficient of variation between replicates must be 40% or less for survival	0.00%	X	
Minimum of 0.25 mg average dry weight of surviving controls	0.366	X	
The percent coefficient of variation between replicates must be 40% or less for growth	11.8%	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 8/20-27/09		*Pimephales promelas* 8/20-27/09	
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)	12.4	%CV survival (critical dilution)	87.5%
%CV Reproduction (critical dilution)	33.6%	Mean dry weight (critical dilution) in milligrams	0.535
		%CV growth (critical dilution)	13.1%
PMSD Reproduction	34.6	PMSD Growth	22.3

Conclusion

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

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

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

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

Biomonitoring Analysts:

Ken Pigue

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

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

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	10-14-09, 0905	10-15-09, 0905
Sample #2:	10-15-09, 0815	10-16-09, 0815
Sample #3:	10-19-09, 0912	10-20-09, 0912

Test initiated (date, time): 10-15-09, 1430 Test terminated (date, time): 10-22-09, 0955

Dilution water used: Soft Synthetic

DATA TABLE FOR FATHEAD MINNOW SURVIVAL

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

DATA TABLE FOR GROWTH OF FATHEAD MINNOWS

Effluent Conc %	A	B	C	D	E		24 hours	48 hours	7 days	CV %
0%	100	100	100	100	100		100	100	100	0.00
32%	100	100	87.5	100	100		100	100	97.5	
42%	100	87.5	100	100	100		100	100	97.5	
56%	100	87.5	87.5	100	100		100	100	95	
75%	100	100	100	87.5	75		100	100	92.5	
100%	75	87.5	100	100	75		95	95	87.5	14.29

SUMMARY

Effluent Conc %	A	B	C	D	E		Mean Dry Weight	CV%
0%	0.385	0.311	0.375	0.422	0.336		0.366	11.8
32%	0.558	0.526	0.468	0.380	0.559		0.498	
42%	0.576	0.585	0.566	0.524	0.425		0.535	
56%	0.634	0.560	0.520	0.526	0.531		0.554	
75%	0.545	0.520	0.503	0.546	0.469		0.517	
100%	0.441	0.554	0.625	0.494	0.563		0.535	13.1

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)= _____**13.1**_____%

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:	10-14-09, 0905	10-15-09, 0905
Sample #2:	10-15-09, 0815	10-16-09, 0815
Sample #3:	10-19-09, 0912	10-20-09, 0912

Test initiated (date, time): 10-15-09, 1400 Test terminated (date, time): 10-22-09, 0915

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	22	10	15	20	9	6
B	14	20	11	5	10	12
C	12	16	16	11	2	x0
D	x10	19	15	x0	22	8
E	12	22	5	12	10	11
F	19	18	11	17	16	11
G	21	17	17	14	7	16
H	15	4	8	15	14	14
I	16	11	19	3	x7	14
J	16	16	13	13	12	20
Mean	15.7	15.3	13.0	11.0	10.9	11.2
Mean/surviving female	16.3	15.3	13.0	12.2	11.3	12.4
CV%*	22.3					33.6

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

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING
Ceriodaphnia dubia SURVIVAL AND REPRODUCTION

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

PERCENT SURVIVAL

PERCENT EFFLUENT	0%	32%	42%	56%	75%	100%
Time of Reading: 24 HOURS	100	100	100	100	100	100
48 HOURS	100	100	100	90	100	100
Test termination	90	100	100	90	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___**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)= _____**33.6**_____%

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
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: K910002-

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-1 Comp.	10/15/2009	9:05 AM		X	4	W	Facility Discharge	X	A

1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)
Bill McAlister	10-15-09 1327	
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)
	10-15-09 1327	

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

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: K9100002

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-2 Comp.	10/16/2009	8:15 AM		X	3	W	Facility Discharge-2	X		B

1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)
Bill McAlister	10-16-09	
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)
	10-16-09 1240	Darlene Rowe

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
FEMA O & M Services Group	FEMA 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 McAllister	Attn: Amber Rich	Fax: 501-467-8697	Preservative Code: 1
		Email: jfriedman@eema-inc.com; bmcallister@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 McAllister

Sampler(s) Printed: Bill McAllister

Arkansas Analytical Work Order Number: K910002

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-1 Comp	10/20/2009	9:12 AM		X	3	W	Facility Discharge-1	X	C

1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)
Bill McAllister	10-20-09 1342	
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:	3°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: K9100022 — Test Start (Date/Time): 10/15/09

Client: Weston — Test End (Date/Time): 10/22/09

		Day of Test 1	2	3	4	5	6	7	notes/remarks
Control	SS	10/15	10/16	10/17	10/18	10/19	10/20	10/21	
D.O. (mg/L)	INITIAL	7.4	7.4	8.5	8.5	83	82	83	
	FINAL	6.3	8.1	8.2	74	77	80	82	
pH (s.u.)	INITIAL	7.2	7.2	7.3	7.6	79	78	77	
	FINAL	7.5	7.4	7.5	74	76	77	75	
temp (C)	INITIAL	22.3	22.0	21.3	22.0	21.7	22.2	22.0	
	FINAL	25.0	25.0	25.0	250	250	250	250	
ALKALINITY (mg/L)		32	—	—	—	—	—	↑	
HARDNESS (mg/L)		56	—	—	—	—	—	↑	
CONDUCTIVITY (umhos/cm)		174	—	—	—	—	—	↑	
CHLORINE (mg/L)		<0.05	—	—	—	—	—	↑	
CONC:	32								
D.O. (mg/L)	INITIAL	8.1	7.9	8.4	8.7	84	82	83	
	FINAL	6.4	7.7	7.9	77	77	80	82	
pH (s.u)	INITIAL	6.6	7.0	6.8	7.1	73	74	73	
	FINAL	7.0	6.9	7.4	72	73	73	72	
temp (C)	INITIAL	23.1	22.5	21.0	22.4	21.5	22.6	22.2	
	FINAL	25.0	25.0	25.0	250	250	250	250	
CONC:	42								
D.O. (mg/L)	INITIAL	8.2	8.0	8.8	8.8	85	83	84	
	FINAL	7.2	7.5	7.8	77	76	80	82	
pH (mg/L)	INITIAL	6.6	6.7	6.9	7.2	73	74	73	
	FINAL	7.1	6.9	7.2	69	72	73	71	
temp (C)	INITIAL	23.2	23.0	20.8	22.5	21.5	23.0	22.4	
	FINAL	25.0	25.0	25.0	250	250	250	250	
CONC:	56								
D.O. (mg/L)	INITIAL	8.4	8.2	8.9	9.0	87	85	85	
	FINAL	7.2	7.1	7.6	76	76	79	81	
pH (s.u.)	INITIAL	6.6	6.6	6.9	7.2	73	74	74	
	FINAL	7.1	6.9	7.1	6.9	72	73	72	
temp (C)	INITIAL	24.0	23.6	20.5	22.7	21.5	23.3	22.5	
	FINAL	25.0	25.0	25.0	250	250	250	250	
CONC:	75								
D.O. (mg/L)	INITIAL	8.6	8.4	8.8	9.2	87	85	84	
	FINAL	6.8	7.1	7.9	75	75	79	81	
pH (s.u.)	INITIAL	6.6	6.5	6.9	7.1	72	73	74	
	FINAL	7.0	6.9	7.1	6.9	70	73	72	
temp (C)	INITIAL	24.5	23.4	20.4	22.9	21.6	23.2	22.6	
	FINAL	25.0	25.0	25.0	250	250	250	250	
CONC:	100								
D.O. (mg/L)	INITIAL	9.0	8.7	9.0	9.5	89	86	84	
	FINAL	7.1	7.4	8.0	75	75	78	80	
pH (s.u.)	INITIAL	6.5	6.5	6.9	7.1	77	75	73	
	FINAL	7.0	7.0	7.1	6.9	70	72	72	
temp (C)	INITIAL	25.2	24.1	20.2	23.1	21.9	23.4	22.8	
	FINAL	25.0	25.0	25.0	250	250	250	250	
CONC:	100%	A	A	A	B	B	C	C	
ALKALINITY (mg/L)		14	—	↑	8	↑	12	↑	
HARDNESS (mg/L)		>600	—	↑	>600	↑	>600	↑	
CONDUCTIVITY (umhos/cm)		1889	—	↑	1910	↑	1977	↑	
CHLORINE (mg/L)		<0.05	—	↑	<0.05	↑	<0.05	↑	

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING — Cerodaphnia Dubia

Lab # / Sample ID: IC910002 — Test Start (Date/Time): 10-15-09 / 1400

Client: Weston — Test End (Date/Time): 10/22/09

		Day of Test 1	2	3	4	5	6	7	notes/remarks
Control	SS Ctl	10/15	10/16	10/17	10/18	10/19	10/20	10/21	
D.O. (mg/L)	INITIAL	7.4	7.4	~~8.7~~ 8.5	85	83	82	83	
	FINAL	8.0	8.3	76	75	74	73		
pH (s.u.)	INITIAL	7.2	7.2	~~7.6~~ 7.3	76	79	78	77	
	FINAL	7.4	7.5	78	77	81	73		
temp (C)	INITIAL	22.3	22.0	~~24.5~~ 21.3	220	217	222	220	
	FINAL	25.0	25.0	250	250	250	250		
ALKALINITY (mg/L)		32							
HARDNESS (mg/L)		56							
CONDUCTIVITY (umhos/cm)		174							
CHLORINE (mg/L)		<0.05							
CONC:	32								
D.O. (mg/L)	INITIAL	8.1	7.9	~~8.4~~ 8.4	89	84	82	83	
	FINAL	8.3	8.0	74	75	78	73		
pH (s.u)	INITIAL	6.6	7.0	~~7.5~~ 6.8	71	73	74	73	
	FINAL	7.1	7.2	72	73	73	74		
temp (C)	INITIAL	23.1	22.5	~~24.5~~ 21.0	224	215	226	222	
	FINAL	25.0	25.0	250	250	250	250		
CONC:	42								
D.O. (mg/L)	INITIAL	8.2	8.0	~~8.1~~ 8.8	88	85	83	84	
	FINAL	8.4	7.9	74	75	77	74		
pH (mg/L)	INITIAL	6.6	6.7	~~7.5~~ 6.9	72	73	74	73	
	FINAL	7.1	7.2	73	73	73	74		
temp (C)	INITIAL	23.2	23.0	~~24.8~~	225	215	230	224	
	FINAL	25.0	25.0	250	250	250	250		
CONC:	56								
D.O. (mg/L)	INITIAL	8.4	8.2	~~7.7~~ 8.9	90	87	85	85	
	FINAL	8.4	7.9	74	76	76	75		
pH (s.u.)	INITIAL	6.6	6.6	6.9	72	73	74	74	
	FINAL	7.0	7.2	72	72	73	72		
temp (C)	INITIAL	24.0	23.9	20.5	227	215	233	225	
	FINAL	25.0	25.0	250	250	250	250		
CONC:	75								
D.O. (mg/L)	INITIAL	8.6	8.4	8.8	92	87	85	84	
	FINAL	8.2	7.9	73	77	77	75		
pH (s.u.)	INITIAL	6.6	6.5	6.9	71	72	73	74	
	FINAL	7.0	7.1	72	72	72	72		
temp (C)	INITIAL	24.5	23.9	20.4	229	216	232	226	
	FINAL	25.0	25.0	250	250	250	250		
CONC:	100								
D.O. (mg/L)	INITIAL	9.0	8.7	9.0	95	89	86	84	
	FINAL	8.1	7.9	76	77	76	74		
pH (s.u.)	INITIAL	6.5	6.5	6.9	71	72	73	73	
	FINAL	7.0	7.1	72	72	72	70		
temp (C)	INITIAL	25.2	24.1	20.2	231	219	234	228	
	FINAL	25.0	25.0	250	250	250	250		
CONC:	100%	A	A	A	B	B	C	C	
ALKALINITY (mg/L)		44	↓	↓	8	↓	12	↓	
HARDNESS (mg/L)		>600	↓	↓	>600	↓	>600	↓	
CONDUCTIVITY (umhos/cm)		1889	↓	↓	1910	↓	1977	↓	
CHLORINE (mg/L)		<0.05	↓	↓	<0.05	↓	<005	↓	

APPENDIX C

Fathead minnow raw data and statistics

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID K9K0602 TEST START DATE 10/15/09 TIME 1'

CLIENT Weston Summary TEST END DATE 10/22/09 TIME 1

AGE AND SOURCE OF MINNOWS

			DAY (NUMBER SURVIVING)							SURVIVAL		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 0	A	8	8	8	8	8	8	8	8	100	95	11.8
	B									100		
	C									100		
	D									100		
	E		8	8	8	8	8	8	6	75 100	100	0.00
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 32	A	8	8	8	8	8	8	8	8	100	95	
	B					8	8	8	8	100		
	C					8	8	7	7	87.5		
	D				8	8	8	8	8	100	97.5	
	E				8	8	8	8	8	100 87.5		
	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	87.5		
	C		8	8	8	8	8	8	8	100		
	D		8	8	8	8	8	8	8	87.5 100	97.5	
	E		8	8	8	8	8	8	8	100		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 56	A	8	8	8	8	8	8	8	8	100	97.5	
	B				7	7	7	7	7	100 87.5		
	C						7	7	7	87.5 100		
	D						8	8	8	87.5 100	95	
	E						8	8	8	100		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 75	A	8	8	8	8	8	8	8	8	100	92.5	
	B		8	8	8	8	8	8	8	100		
	C		8	8	8	8	8	8	8	100		
	D		8	8	8	8	7	7	7	87.5		
	E		8	8	8	8	8	7	6	75		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 100	A	8	6	6	6	6	6	6	6	75	87.5	14.3
	B		8	8	8	8	7	7	7	87.5		
	C		8	8	8	8	8	8	8	100		
	D		8	8	8	8	8	8	8	100		
	E		8	8	8	8	8	8	6	75		
ANALYST												
DATE:												
TIME:												

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID	TEST START DATE 10/15/09 TIME 1430	
CLIENT Weston A	TEST END DATE 10/22/09 TIME 0955	
	AGE AND SOURCE OF MINNOWS	
	D A Y (NUMBER SURVIVING)	SURVIVAL

	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	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:	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:	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:	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:	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:	A	2	0	0	0	0	0	0	0	0		
	B		2	2	2	2	2	2	2			
	C											
	D											
	E											
ANALYST		KP	KP	KP	KP	KP	KP	KP	KP			
DATE:		10/15/09	10/16	10/17	10/18	10/19	10/20	10/21	10/22			
TIME:		1430	1330	1400	1320	1115	1310	1410	~~1500~~ 0955			

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID | TEST START DATE | TIME

CLIENT 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:	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E	1	1	1	1	1	1	1	1			
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E	1	1	1	1	1	1	1	1			
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D		1	1	1	1	1	1	1			
	E	1										
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	2	2	2	2	2	2	2	2			
	B											
	C				1	1	1	1	1			
	D		1	1	1	1	1	1	1			
	E	1										
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	2	2	2	2	2	2	2	2			
	B											
	C						1	1	1			
	D		1	1	1	1	2	2	2			
	E	1										
ANALYST												
DATE:												
TIME:												

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID			TEST START DATE		TIME							
CLIENT	Wis	on C	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:	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E	1	1	1	1	1	1	1	1			
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	2	2	2	2	2	2	2	2			
	B											
	C							2	2			
	D					1	1	2	2			
	E	1	1	1	1							
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D		1	1	1	1	1	1	1			
	E	1										
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	2	2	1	1	1	1	1	1			
	B			2	2	2	2	2	2			
	C											
	D		1	1	1	1	1	1	1			
	E	1										
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D		1	1	1	1	1	1	1			
	E	1										
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D		1	1	1	1	1	1	1			
	E	1	1									
ANALYST												
DATE:												
TIME:												

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID	TEST START DATE		TIME
CLIENT 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:	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:	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:	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:	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:	A	2	2	2	2	2	2	2	2			
	B											
	C						2	2	2			
	D						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											
	C											
	D											
	E											
ANALYST												
DATE:												
TIME:												

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID				TEST START DATE			TIME						
CLIENT Weston E				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:	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:	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D		2									
	E		1									
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	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:	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:	A	2	2	2	2	2	2	2	1			
	B								2			
	C											
	D							2	2			
	E											
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	2	2	2	2	2	2	2	0			
	B								2			
	C								2			
	D								2			
	E											
ANALYST												
DATE:												
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:	K910002			TEST DATES (BEGIN / END):		10/15-22/09	
CLIENT:	EEMA			WEIGHING DATE / TIME:		10/30/09, 1545	
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	0.99357	0.99049	0.00308	8	0.385	AVG DRY
	B	1.00009	0.99760	0.00249	8	0.311	WEIGHT (mg)
	C	0.98142	0.97842	0.00300	8	0.375	0.366
	D	0.98710	0.98372	0.00338	8	0.422	CV
	E	1.01431	1.01162	0.00269	8	0.336	11.8
CONC:	A	0.97279	0.96833	0.00446	8	0.558	AVG DRY
	B	1.00980	1.00559	0.00421	8	0.526	WEIGHT (mg)
32%	C	1.00557	1.00183	0.00374	8	0.468	0.498
	D	1.00449	1.00145	0.00304	8	0.380	CV
	E	0.99549	0.99102	0.00447	8	0.559	
CONC:	A	0.99655	0.99194	0.00461	8	0.576	AVG DRY
	B	0.99281	0.98813	0.00468	8	0.585	WEIGHT (mg)
42%	C	1.00135	0.99682	0.00453	8	0.566	0.535
	D	1.00185	0.99766	0.00419	8	0.524	CV
	E	1.01262	1.00922	0.00340	8	0.425	
CONC:	A	0.98011	0.97504	0.00507	8	0.634	AVG DRY
	B	0.98838	0.98390	0.00448	8	0.560	WEIGHT (mg)
56%	C	1.01536	1.01120	0.00416	8	0.520	0.554
	D	1.00717	1.00296	0.00421	8	0.526	CV
	E	1.00914	1.00489	0.00425	8	0.531	
CONC:	A	1.00241	0.99805	0.00436	8	0.545	AVG DRY
	B	1.01072	1.00656	0.00416	8	0.520	WEIGHT (mg)
75%	C	1.00254	0.99852	0.00402	8	0.503	0.517
	D	0.99778	0.99341	0.00437	8	0.546	CV
	E	0.99916	0.99541	0.00375	8	0.469	
CONC:	A	0.96072	0.95719	0.00353	8	0.441	AVG DRY
	B	0.99916	0.99473	0.00443	8	0.554	WEIGHT (mg)
100%	C	1.00272	0.99772	0.00500	8	0.625	0.535
	D	1.00132	0.99737	0.00395	8	0.494	CV
	E	0.99129	0.98679	0.00450	8	0.563	13.1

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

Pimephales promelas **FATHEAD MINNOW** TEST 1000.0

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s: K910062
CLIENT: Weston
ANALYSTS:
SAMPLE ID:

TEST DATES (BEGIN / END): 10/15-22/09
WEIGHING DATE / TIME: 10/30/09 1545
DRYING TEMP (DEGREES C): 60
DRYING TIME (HOURS): 24

	REP#	FINAL DRY WEIGHT TIN+LARVAE (g)	INTIAL WEIGHT TIN (g)	TOTAL DRY WEIGHT OF LARVAE (g)	NUMBER OF LARVAE	DRY WEIGHT OF LARVAE (mg)	
CONTROL	A 31	0.99357	0.99049				AVG DRY WEIGHT (mg)
	B 32	1.00009	0.99760				
	C 33	0.98142	0.97842				
	D 34	0.98710	0.98372				CV
	E 35	1.01431	1.01162				
CONC:	A 36	0.97279	.96833				AVG DRY WEIGHT (mg)
	B 37	1.00980	1.00559				
	C 38	1.00557	1.00183				
	D 39	1.00449	1.00145				CV
	E 40	0.99549	0.99102				
CONC:	A 41	0.99655	0.99184				AVG DRY WEIGHT (mg)
	B 42	0.99281	0.98803				
	C 43	1.00135	0.99682				
	D 44	1.00185	0.99766				CV
	E 45	1.01262	1.00922				
CONC:	A 46	0.98011	0.97504				AVG DRY WEIGHT (mg)
	B 47	0.98838	0.98390				
	C 48	1.01536	1.01120				
	D 49	1.00717	1.00296				CV
	E 50	1.00914	1.00489				
CONC:	A 51	1.00243	0.99805				AVG DRY WEIGHT (mg)
	B 52	1.01072	1.00656				
	C 53	1.00254	0.99852				
	D 54	0.99778	0.99341				CV
	E 55	0.99916	0.99541				
CONC:	A 56	0.96072	0.95719				AVG DRY WEIGHT (mg)
	B 57	0.99916	0.99473				
	C 58	1.00272	0.99772				
	D 59	1.00132	0.99797				CV
	E 60	0.99129	0.98679				

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

51 — 1.00241

AA# K910002, FATHEAD MINNOW SURVIVAL, CHRONIC, 10-15-09
File: H:\TOXSTAT\MONTE\FHSURV~1. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.311

W = 0.929

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# K910002, FATHEAD MINNOW SURVIVAL, CHRONIC, 10-15-09
File: H:\TOXSTAT\MONTE\FHSURV~1. Transform: ARC SINE(SQUARE ROOT(Y))

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

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

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

TITLE: AA# K910002, FATHEAD MINNOW SURVIVAL, CHRONIC, 10-15-09
FILE: H:\TOXSTAT\MONTE\FHSURV~1.
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	1.0000	1.3931
1	CONTROL	2	1.0000	1.3931
1	CONTROL	3	1.0000	1.3931
1	CONTROL	4	1.0000	1.3931
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	0.8750	1.2094
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	0.8750	1.2094
3	42 % EFFLUENT	3	1.0000	1.3931

3	42 % EFFLUENT	4	1.0000	1.3931
3	42 % EFFLUENT	5	1.0000	1.3931
4	56 % EFFLUENT	1	1.0000	1.3931
4	56 % EFFLUENT	2	0.8750	1.2094
4	56 % EFFLUENT	3	0.8750	1.2094
4	56 % EFFLUENT	4	1.0000	1.3931
4	56 % EFFLUENT	5	1.0000	1.3931
5	75 % EFFLUENT	1	1.0000	1.3931
5	75 % EFFLUENT	2	1.0000	1.3931
5	75 % EFFLUENT	3	1.0000	1.3931
5	75 % EFFLUENT	4	0.8750	1.2094
5	75 % EFFLUENT	5	0.7500	1.0472
6	100 % EFFLUENT	1	0.7500	1.0472
6	100 % EFFLUENT	2	0.8750	1.2094
6	100 % EFFLUENT	3	1.0000	1.3931
6	100 % EFFLUENT	4	1.0000	1.3931
6	100 % EFFLUENT	5	0.7500	1.0472

AA# K910002, FATHEAD MINNOW SURVIVAL, CHRONIC, 10-15-09
File: H:\TOXSTAT\MONTE\FHSURV~1. Transform: ARC SINE(SQUARE ROOT(Y))

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	1.393				
2	32 % EFFLUENT	1.356	25.00	16.00	5.00	
3	42 % EFFLUENT	1.356	25.00	16.00	5.00	
4	56 % EFFLUENT	1.320	22.50	16.00	5.00	
5	75 % EFFLUENT	1.287	22.50	16.00	5.00	
6	100 % EFFLUENT	1.218	20.00	16.00	5.00	

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

AA# K910002, FATHEAD MINNOW GROWTH CHRONIC, 10-15-09
File: w Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.082

W = 0.961

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# K910002, FATHEAD MINNOW GROWTH CHRONIC, 10-15-09
File: w Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 3.53

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# K910002, FATHEAD MINNOW GROWTH CHRONIC, 10-15-09
FILE: w
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.3850	0.6694
1	CONTROL	2	0.3110	0.5916
1	CONTROL	3	0.3750	0.6591
1	CONTROL	4	0.4220	0.7071
1	CONTROL	5	0.3360	0.6183
2	32 % EFFLUENT	1	0.5580	0.8435
2	32 % EFFLUENT	2	0.5260	0.8114
2	32 % EFFLUENT	3	0.4680	0.7534
2	32 % EFFLUENT	4	0.3800	0.6642
2	32 % EFFLUENT	5	0.5590	0.8445
3	42 % EFFLUENT	1	0.5760	0.8617
3	42 % EFFLUENT	2	0.5850	0.8708
3	42 % EFFLUENT	3	0.5660	0.8516
3	42 % EFFLUENT	4	0.5240	0.8094
3	42 % EFFLUENT	5	0.4250	0.7101
4	56 % EFFLUENT	1	0.6340	0.9211

4	56 % EFFLUENT	2	0.5600	0.8455
4	56 % EFFLUENT	3	0.5200	0.8054
4	56 % EFFLUENT	4	0.5260	0.8114
4	56 % EFFLUENT	5	0.5310	0.8164
5	75 % EFFLUENT	1	0.5450	0.8305
5	75 % EFFLUENT	2	0.5200	0.8054
5	75 % EFFLUENT	3	0.5030	0.7884
5	75 % EFFLUENT	4	0.5460	0.8315
5	75 % EFFLUENT	5	0.4690	0.7544
6	100 % EFFLUENT	1	0.4410	0.7263
6	100 % EFFLUENT	2	0.5540	0.8395
6	100 % EFFLUENT	3	0.6250	0.9117
6	100 % EFFLUENT	4	0.4940	0.7794
6	100 % EFFLUENT	5	0.5630	0.8486

AA# K910002, FATHEAD MINNOW GROWTH CHRONIC, 10-15-09
File: w Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.122	0.024	7.110
Within (Error)	24	0.082	0.003	
Total	29	0.204		

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

AA# K910002, FATHEAD MINNOW GROWTH CHRONIC, 10-15-09
File: w 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	0.649	0.366		
2	32 % EFFLUENT	0.783	0.498	-3.629	
3	42 % EFFLUENT	0.821	0.535	-4.637	
4	56 % EFFLUENT	0.840	0.554	-5.156	
5	75 % EFFLUENT	0.802	0.517	-4.131	
6	100 % EFFLUENT	0.821	0.535	-4.647	

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

AA# K910002, FATHEAD MINNOW GROWTH CHRONIC, 10-15-09
File: w 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	0.082	22.3	-0.132
3	42 % EFFLUENT	5	0.082	22.3	-0.169
4	56 % EFFLUENT	5	0.082	22.3	-0.188
5	75 % EFFLUENT	5	0.082	22.3	-0.151
6	100 % EFFLUENT	5	0.082	22.3	-0.170

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

SURVIVAL AND REPRODUCTION TEST

Cerodaphnia dubia

Discharger: Weston

Location:

Date Sample Collected:

Lab Number/s: K910003

Analyst: KP

Test Start - Date/ Time: 10-15-09 / 1400

Test Stop - Date/Time: 10/22/09, 0915

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	2	2	0	0	0	0	1	0	6	10	0.6	KP
4	4	3	3	3	0	3	3	1	2	2	26	10	2.6	KP
5	7	0	2	5	X	4	6	5	7	2	42	9	4.7	KP
6	11	10	9	5	7	8	6	8	7	9	83	9	9.2	KP
7														
8														
Total	22	14	16	20	12	19	21	15	16	16	157			

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	0	0	0	1	2	0	0	0	2	0	5	10	0.5	
4	6	4	3	3	6	5	3	1	1	2	34	10	3.4	
5	3	6	6	4	7	5	5	2	6	7	51	10	5.1	
6	1	10	7	11	7	8	9	1	2	7	63	10	6.3	
7														
8														
Total	10	20	16	19	22	18	17	4	11	16	153			

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	1	1	0	0	0	3	3	1	1	2	11	10	1.1	
4	2	2	5	4	1	2	2	3	0	0	21	10	2.1	
5	3	4	4	5	0	5	6	4	7	2	40	10	4.0	
6	4	4	7	6	4	2	6	0	11	4	53	10	5.3	
7														
8														
Total	15	11	16	15	5	11	17	8	19	13	130			

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	X	0	0	0	0	0	0	0	9	0	
3	4	5	1	—	0	0	0	2	1	1	14	9	1.5	
4	5	0	0	—	2	3	3	1	2	2	18	9	2.0	
5	5	0	0	—	3	4	3	6	6	3	28	9	3.1	
6	6	8	10	—	7	10	4	6	8	7	50	9	5.6	
7														
8														
Total	20	5	11	X0	12	17	14	15	3	13	110			

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	6	10	0	
2	0	0	0	0	0	0	0	0	0	0	0	10	0	
3	0	1	0	0	1	1	0	0	0	0	3	10	0.3	
4	2	3	0	5	3	2	0	5	1	X 1	30	9	3.3	
5	3	0	0	8	3	5	0	2	—	5	21	9	2.9	
6	4	4	2	9	3	8	7	7	—	6	50	9	5.6	
7														
8														
Total	9	10	2	22	10	16	7	14	X7	12	109			

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	1	2	0	0	1	0	2	0	3	0	9	10	0.9	
4	1	0	4	0	0	0	2	3	0	1	10	9	1.1	
5	3	2	—	4	3	3	5	2	4	6	32	9	3.6	
6	1	8	—	4	7	8	7	9	10	7	61	9	6.8	
7														
8														
Total	6	12	X0	8	11	11	16	14	14	20	112			

X̄ = 12.4

CV = 33.6

AA # K100002 C. DUBIA CHRONIC, REPRODUCION, 10-15-09
File: H:/toxstat/monte\C.DUB Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

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

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

Total number of replicates = 60

AA # K100002 C. DUBIA CHRONIC, REPRODUCION, 10-15-09
File: H:/toxstat/monte\C.DUB Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 2.63

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

Data PASS B1 homogeneity test at 0.01 level. Continue analysis.

FISHER'S EXACT TEST

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

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

FISHER'S EXACT TEST

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

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

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF ALIVE	NUMBER OF DEAD	TOTAL ANIMALS
CONTROL	9	1	10
56%	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
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%1	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	1
4	75%	10	1
5	100%1	10	1

TITLE: AA # K100002 C. DUBIA CHRONIC, REPRODUCION, 10-15-09
FILE: H:/toxstat/monte\C.DUB
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	22.0000	22.0000
1	CONTROL	2	14.0000	14.0000
1	CONTROL	3	12.0000	12.0000
1	CONTROL	4	10.0000	10.0000
1	CONTROL	5	12.0000	12.0000
1	CONTROL	6	19.0000	19.0000
1	CONTROL	7	21.0000	21.0000
1	CONTROL	8	15.0000	15.0000
1	CONTROL	9	16.0000	16.0000
1	CONTROL	10	16.0000	16.0000
2	32 % EFFLUENT	1	10.0000	10.0000
2	32 % EFFLUENT	2	20.0000	20.0000
2	32 % EFFLUENT	3	16.0000	16.0000
2	32 % EFFLUENT	4	19.0000	19.0000
2	32 % EFFLUENT	5	22.0000	22.0000
2	32 % EFFLUENT	6	18.0000	18.0000
2	32 % EFFLUENT	7	17.0000	17.0000
2	32 % EFFLUENT	8	4.0000	4.0000
2	32 % EFFLUENT	9	11.0000	11.0000
2	32 % EFFLUENT	10	16.0000	16.0000
3	42 % EFFLUENT	1	15.0000	15.0000
3	42 % EFFLUENT	2	11.0000	11.0000
3	42 % EFFLUENT	3	16.0000	16.0000
3	42 % EFFLUENT	4	15.0000	15.0000
3	42 % EFFLUENT	5	5.0000	5.0000
3	42 % EFFLUENT	6	11.0000	11.0000
3	42 % EFFLUENT	7	17.0000	17.0000
3	42 % EFFLUENT	8	8.0000	8.0000
3	42 % EFFLUENT	9	19.0000	19.0000
3	42 % EFFLUENT	10	13.0000	13.0000
4	56 % EFFLUENT	1	20.0000	20.0000
4	56 % EFFLUENT	2	5.0000	5.0000
4	56 % EFFLUENT	3	11.0000	11.0000
4	56 % EFFLUENT	4	0.0000	0.0000
4	56 % EFFLUENT	5	12.0000	12.0000
4	56 % EFFLUENT	6	17.0000	17.0000
4	56 % EFFLUENT	7	14.0000	14.0000
4	56 % EFFLUENT	8	15.0000	15.0000
4	56 % EFFLUENT	9	3.0000	3.0000
4	56 % EFFLUENT	10	13.0000	13.0000

5	75 % EFFLUENT	1	9.0000	9.0000
5	75 % EFFLUENT	2	10.0000	10.0000
5	75 % EFFLUENT	3	2.0000	2.0000
5	75 % EFFLUENT	4	22.0000	22.0000
5	75 % EFFLUENT	5	10.0000	10.0000
5	75 % EFFLUENT	6	16.0000	16.0000
5	75 % EFFLUENT	7	7.0000	7.0000
5	75 % EFFLUENT	8	14.0000	14.0000
5	75 % EFFLUENT	9	7.0000	7.0000
5	75 % EFFLUENT	10	12.0000	12.0000
6	100 % EFFLUENT	1	6.0000	6.0000
6	100 % EFFLUENT	2	12.0000	12.0000
6	100 % EFFLUENT	3	0.0000	0.0000
6	100 % EFFLUENT	4	8.0000	8.0000
6	100 % EFFLUENT	5	11.0000	11.0000
6	100 % EFFLUENT	6	11.0000	11.0000
6	100 % EFFLUENT	7	16.0000	16.0000
6	100 % EFFLUENT	8	14.0000	14.0000
6	100 % EFFLUENT	9	14.0000	14.0000
6	100 % EFFLUENT	10	20.0000	20.0000

AA # K100002 C. DUBIA CHRONIC, REPRODUCION, 10-15-09
File: H:/toxstat/monte\C.DUB Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	240.950	48.190	1.739
Within (Error)	54	1496.700	27.717	
Total	59	1737.650		

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

AA # K100002 C. DUBIA CHRONIC, REPRODUCION, 10-15-09
File: H:/toxstat/monte\C.DUB Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	15.700	15.700		
2	32 % EFFLUENT	15.300	15.300	0.170	
3	42 % EFFLUENT	13.000	13.000	1.147	
4	56 % EFFLUENT	11.000	11.000	1.996	
5	75 % EFFLUENT	10.900	10.900	2.039	
6	100 % EFFLUENT	11.200	11.200	1.911	

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

AA # K100002 C. DUBIA CHRONIC, REPRODUCION, 10-15-09
File: H:/toxstat/monte\C.DUB 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	5.439	34.6	0.400
3	42 % EFFLUENT	10	5.439	34.6	2.700
4	56 % EFFLUENT	10	5.439	34.6	4.700
5	75 % EFFLUENT	10	5.439	34.6	4.800
6	100 % EFFLUENT	10	5.439	34.6	4.500

AA # K100002 C. DUBIA CHRONIC, REPRODUCION, 10-15-09
File: H:/toxstat/monte\C.DUB Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	15.700				
2	32 % EFFLUENT	15.300	107.50	75.00	10.00	
3	42 % EFFLUENT	13.000	89.50	75.00	10.00	
4	56 % EFFLUENT	11.000	84.00	75.00	10.00	
5	75 % EFFLUENT	10.900	77.00	75.00	10.00	
6	100 % EFFLUENT	11.200	80.00	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 10-14-09 Arkansas Analytical

SPECIES Pimephales promelas

QUANTITY SHIPPED 530+ & 100+

AGE/LIFE STAGE 24 hrs 10/14 1500+ & 5 Days old 10/14

BROODSTOCK SOURCE Aquatox Inc, AR

CULTURE WATER groundwater

ALKALINITY (Mg/l as $CaCO_3$) =180

HARDNESS (Mg/l as $CaCO_3$)/Salinity (ppt) =160

FEEDING Artemia

COMMENTS

PACKAGED BY

BILL HALL PRINTERS 3171

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

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

ORGANISM HISTORY

DATE: 6/22/09

SPECIES: *Ceriodaphnia dubia*

AGE: Variable

LIFE STAGE: Adult

HATCH DATE: Variable

BEGAN FEEDING: Immediately

FOOD: YTC, *Selenastrum* sp.

Water Chemistry Record:	Current	Range
TEMPERATURE:	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:

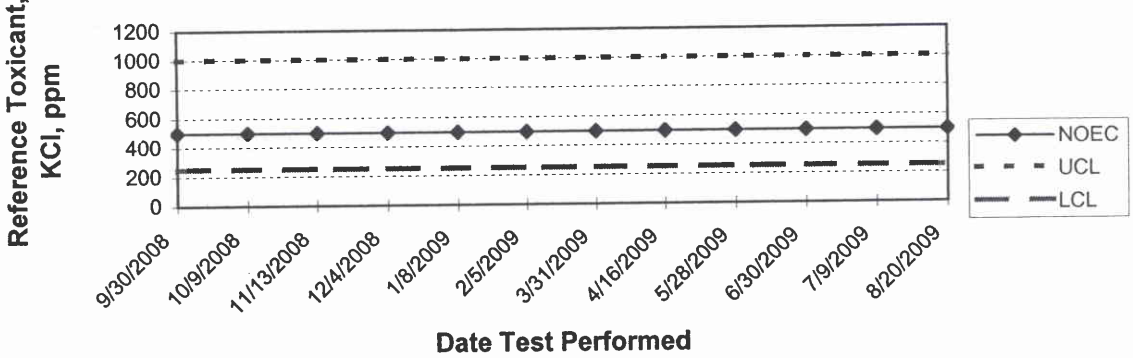
Facility Supervisor

APPENDIX F

Quality Assurance Charts

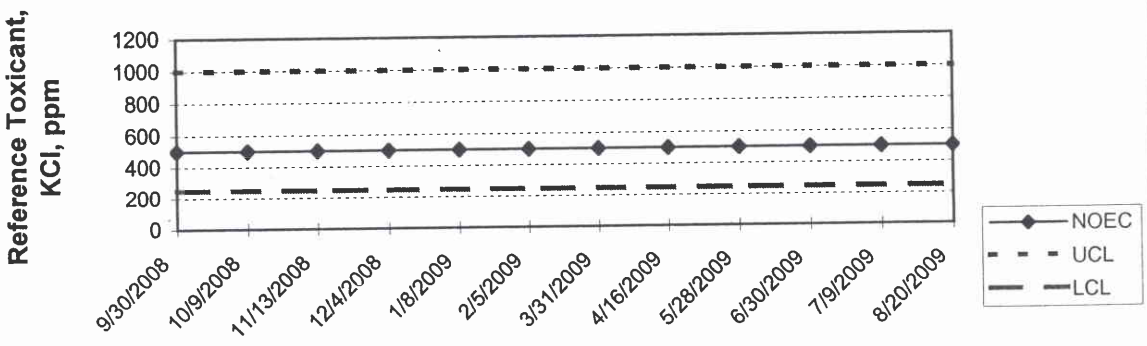
ARKANSAS ANALYTICAL, INC.

FATHEAD MINNOW SURVIVAL
QUALITY ASSURANCE



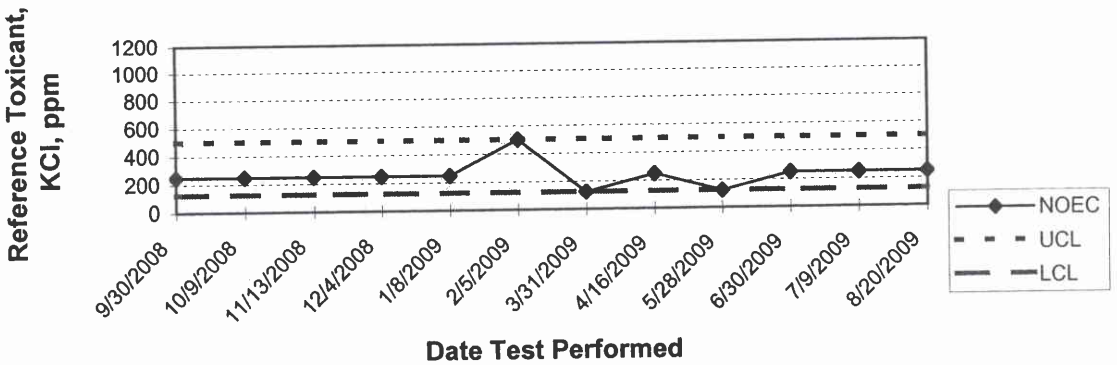
ARKANSAS ANALYTICAL, INC.

FATHEAD MINNOW GROWTH
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.

CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.

CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE