

Arkansas Analytical, Inc.

Toxicity Test Results

**MAGCOBAR MINE SITE
NPDES PERMIT NUMBER: AR0049794
June, 2010
AFIN# 00-00348**

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

Ceriodaphnia dubia, Survival and Reproduction Test
Test 1002.0

Prepared for: **Mr. David Friedman**
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Lab Number K1006012

Thursday, July 08, 2010

Introduction

This report contains test results for toxicity testing for the Magcobar Mine Site. The NPDES permit number is AR0049794. The facility is located one mile northeast of Magnet Cove in Sections 10, 11, 14, & 15, Township 3 South, Range 17 West in Hot Springs County, Arkansas. The facility discharges into Chamberlain Creek, thence to Cove Creek, thence to Ouachita River in Segment 2F of the Ouachita River Basin.

The permit requires chronic biomonitoring testing bi-monthly for both *Ceriodaphnia dubia* and *Pimephales promelas*. The test results in this report represent the testing for June of 2010.

Plant Operations

To be provided by permittee.

Source of Effluent and Dilution Water

Effluent samples were collected as follows:

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	6-16-10, 0825	6-17-10, 0825
Sample #2:	6-17-10, 0910	6-18-10, 0910
Sample #3:	6-21-10, 1145	6-22-10, 1145

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

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

Sample Receiving Information:	Date, Time Sample(s) Received	Temperature Upon Receipt (°C)
Sample #1:	6-17-10, 1400	5
Sample #2:	6-18-10, 1437	4
Sample #3:	6-22-10, 1423	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	15.4	X	
At least 60% of surviving females should have produced 3 broods	80%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	32.4%	X	

TEST ACCEPTANCE CRITERIA for *Pimephales promelas*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	97.5%	X	
The percent coefficient of variation between replicates must be 40% or less for survival	5.73%	X	
Minimum of 0.25 mg average dry weight of surviving controls	0.251	X	
The percent coefficient of variation between replicates must be 40% or less for growth	20.0%	X	

Reference Toxicant

The reference toxicant used was Potassium Chloride prepared in-house. The tests were performed using moderately hard synthetic as dilution water. The results of the reference toxicant were:

REFERENCE TOXICANT

<i>Ceriodaphnia dubia</i> 5/13-20/10		<i>Pimephales promelas</i> 5/13-20/10	
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

<i>Ceriodaphnia dubia</i>		<i>Pimephales promelas</i>	
NOEC / LOEC Survival	100% / NA	NOEC / LOEC survival	100% / NA
NOEC / LOEC Reproduction	100% / NA	NOEC / LOEC growth	100% / NA
Mean number of neonates (critical dilution)	15.5	%CV survival (critical dilution)	5.73 %
%CV Reproduction (critical dilution)	33.5%	Mean dry weight (critical dilution) in milligrams	0.430
		%CV growth (critical dilution)	17.1%
PMSD Reproduction	33.1	PMSD Growth	29.6

Conclusion

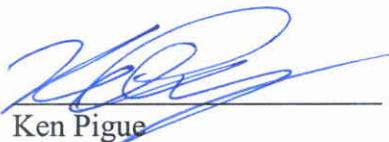
Chronic static renewal larval survival and growth test using fathead minnow, *Pimephales promelas*, (Method 1000.0).

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

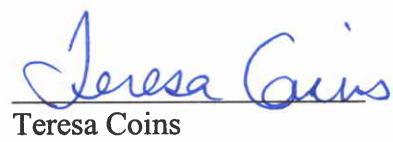
Chronic static renewal survival and reproduction test using *Ceriodaphnia dubia*, (Method 1002.0).

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

Biomonitoring Analysts:



Ken Pigue



Teresa Coins



Ruth Haldeman

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

PERMITTEE: Magcobar Mine Site

NPDES #: AR0049794

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	6-16-10, 0825	6-17-10, 0825
Sample #2:	6-17-10, 0910	6-18-10, 0910
Sample #3:	6-21-10, 1145	6-22-10, 1145

Test initiated (date, time): 6-17-10, 1620

Test terminated (date, time): 6-24-10, 1000

Dilution water used: Soft Synthetic

DATA TABLE FOR FATHEAD MINNOW SURVIVAL

Percent Survival in Replicate Chambers **Mean Percent Survival**
DATA TABLE FOR GROWTH OF FATHEAD MINNOWS

Effluent Conc %	A	B	C	D	E		24 hours	48 hours	7 days	CV %
0%	100	100	100	87.5	100		100	100	97.5	5.73
32%	100	100	100	100	100		100	100	100	
42%	100	100	100	87.5	100		100	100	97.5	
56%	100	100	100	100	100		100	100	100	
75%	100	100	100	87.5	100		100	100	97.5	
100%	100	100	100	87.5	100		100	97.5	97.5	5.73

SUMMARY

Effluent Conc %	A	B	C	D	E		Mean Dry Weight	CV%
0%	0.234	0.274	0.270	0.174	0.305		0.251	20
32%	0.305	0.333	0.406	0.325	0.427		0.359	
42%	0.380	0.385	0.330	0.314	0.357		0.353	
56%	0.336	0.437	0.509	0.340	0.400		0.404	
75%	0.382	0.435	0.424	0.352	0.511		0.421	
100%	0.400	0.489	0.419	0.329	0.514		0.430	17.1

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)= _____ 20.0 _____ %

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING
Ceriodaphnia dubia SURVIVAL AND REPRODUCTION

Permittee: Magcobar Mine Site

NPDES #: AR0049794

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	6-16-10, 0825	6-17-10, 0825
Sample #2:	6-17-10, 0910	6-18-10, 0910
Sample #3:	6-21-10, 1145	6-22-10, 1145

Test initiated (date, time): 6-17-10, 1610 Test terminated (date, time): 6-24-10, 0935

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	9	16	14	14	17	20
B	9	13	12	11	20	6
C	26	7	23	2	9	24
D	15	17	x0	13	13	15
E	13	12	17	15	14	15
F	14	15	13	15	10	17
G	20	19	20	2	13	19
H	16	10	x6	12	13	9
I	16	15	12	14	14	16
J	16	12	16	17	15	14
Mean	15.4	13.6	13.3	11.5	13.8	15.5
Mean/surviving female	15.4	13.6	15.9	11.5	13.8	15.5
CV%*	32.4					33.5

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	100	100	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 _____

2. Dunnett's Procedure or Steel's Many One Rank Test:

Is the mean number of young produced per female significantly different ($p=0.05$) than the controls number of young per female for:

a) LOW FLOW OR CRITICAL DILUTION, (100%): YES _____ NO _____

3. If NO was answered to 1.a) enter [0] otherwise enter [1] (parameter TLP3B): **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)= **33.5** %

APPENDIX A

Chain of Custody Forms

CHAIN OF CUSTODY RECORD

CLIENT INFORMATION					Project Description		Turnaround Time	Preservation Codes:								
EEMA O & M Services Group	EEMA O & M Services Group				Magcoabar Mine Site		24 Hour	1. Cool, 4 Degrees Centigrade			4. Thiosulfate for Dechlorination					
Magcoabar Mine Site	P.O. Box 732				Biomonitoring Sample		48 Hour	2. Sulfuric Acid (H_2SO_4), pH < 2			5. Hydrochloric Acid (HCl)					
P.O. Box 699	Kulpsville, PA 19443				Reporting Information		72 Hour	3. Nitric Acid (HNO_3), pH < 2			6. Sodium Hydroxide (NaOH), pH > 12					
Malvern, AR 72104					Telephone: 501-467-8355		Routine (5 Day)	TEST PARAMETERS						Bottle Type Code		
Attn: Bill McAlister	Attn: Amber Rich				Fax: 501-467-8687		Preservative Code:	1							G = Glass; P = Plastic	
					Email: dave.friedman@eema-inc.com; bmcallister@eema-inc.com; bhorton@eema-inc.com		Bottle Type:	P							V = Septum; A = Amber	
<i>Bill McAlister</i> Sampler(s) Signature			<i>Bill McAlister</i> Sampler(s) Printed					Chronic Biomonitoring							Arkansas Analytical Work Order Number:	
Field Number	SAMPLE COLLECTION		Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION		X							K1004012
FD-1 Comp.	Date/s	Time/s			4	W	Facility Discharge									A
1. Relinquished by: (Signature)		Date/Time		2. Received by: (Signature)		SAMPLE CONDITION UPON RECEIPT IN LAB				REMARKS / SAMPLE COMMENTS						
<i>Bill McAlister</i>		6-17-10 1400				1. CUSTODY SEALS: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 2. CONTAINERS CORRECT: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 3. COC/LABELS AGREE: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 4. PRESERVATION CONFIRMED: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 5. RECEIVED ON ICE: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 6. TEMPERATURE ON RECEIPT: <input checked="" type="checkbox"/> S.C.										
3. Relinquished by: (Signature)		Date/Time		4. Received by lab: (Signature)												
FOR COMPLETION BY LAB ONLY																

CHAIN OF CUSTODY RECORD

CLIENT INFORMATION				Project Description		Turnaround Time	Preservation Codes:							
EEMA O & M Services Group	EEMA O & M Services Group			Magcoabar Mine Site		24 Hour	1. Cool, 4 Degrees Centigrade			4. Thiosulfate for Dechlorination				
Magcoabar Mine Site	P.O. Box 732			Biomonitoring Sample		48 Hour	2. Sulfuric Acid (H_2SO_4), pH < 2			5. Hydrochloric Acid (HCl)				
P.O. Box 699	Kulpsville, PA 19443			Reporting Information		72 Hour	3. Nitric Acid (HNO_3), pH < 2			6. Sodium Hydroxide (NaOH), pH > 12				
Malvern, AR 72104				Telephone: 501-467-8355		Routine (5 Day)	TEST PARAMETERS						Bottle Type Code	
Attn: Bill McAlister	Attn: Amber Rich			Fax: 501-467-8687		Preservative Code:	1						G = Glass; P = Plastic	
				Email: dave.friedman@eema-inc.com; bmcallister@eema-inc.com; bhorton@eema-inc.com		Bottle Type:	P						V = Septum; A = Amber	
<i>Bill M. McAlister</i>			<i>Bill M. McAlister</i>			Chronic Biomonitoring							Arkansas Analytical Work Order Number:	
Sampler(s) Signature			Sampler(s) Printed										<i>K1006012</i>	
Field Number	SAMPLE COLLECTION		Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION							
FD-2 Comp.	Date/s	Time/s	X		3	W	Facility Discharge						X	<i>B</i>
1. Relinquished by: (Signature)	Date/Time		2. Received by: (Signature)		SAMPLE CONDITION UPON RECEIPT IN LAB						REMARKS / SAMPLE COMMENTS			
<i>Bill M. McAlister</i>	<i>6-18-10</i> <i>1437</i>				1. CUSTODY SEALS: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 2. CONTAINERS CORRECT: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 3. COC/LABELS AGREE: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 4. PRESERVATION CONFIRMED: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 5. RECEIVED ON ICE: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 6. TEMPERATURE ON RECEIPT: <i>4°C</i>									
3. Relinquished by: (Signature)	Date/Time		4. Received by lab: (Signature)											
FOR COMPLETION BY LAB ONLY														



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

CHAIN OF CUSTODY RECORD

Client Information				Project Description		Turnaround Time	Preservation Codes:										
EEMA O & M Services Group		EEMA O & M Services Group		Magcobar Mine Site			1. Cool, 4 Degrees Centigrade			4. Thiosulfate for Dechlorination							
Magcobar Mine Site		P.O. Box 732		Biomonitoring Sample			2. Sulfuric Acid (H_2SO_4), pH < 2			5. Hydrochloric Acid(HCl)							
P.O. Box 699		Kulpsville, PA 19443		Reporting Information			3. Nitric Acid (HNO_3), pH < 2			6. Sodium Hydroxide (NaOH), pH > 12							
Malvern, AR 72104				Telephone: 501-467-8355			TEST PARAMETERS										
Attn: Bill McAlister		Attn: Amber Rich		Fax: 501-467-8687 Email: dave.friedman@eema-inc.com; bmcalister@eema-inc.com; bharton@eema-inc.com			Preservative Code:	1						Bottle Type Code			
<i>Bill McAlister</i>		<i>Bill McAlister</i>								G = Glass; P = Plastic							
Sampler(s) Signature				Sampler(s) Printed										V = Septum; A = Amber			
Field Number	SAMPLE COLLECTION			Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION									
	Date/s	Time/s						Facility Discharge									
FD-1 Comp.	6/22/2010	11:45 AM		X	4	W										X	<i>K100612 C</i>
1. Relinquished by: (Signature)	Date/Time			2. Received by: (Signature)		SAMPLE CONDITION UPON RECEIPT IN LAB								REMARKS / SAMPLE COMMENTS			
<i>Bill McAlister</i>	6-22-10 1423					1. CUSTODY SEALS: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 2. CONTAINERS CORRECT: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 3. COC/LABELS AGREE: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 4. PRESERVATION CONFIRMED: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 5. RECEIVED ON ICE: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 6. TEMPERATURE ON RECEIPT: <i>4°C</i>											
3. Relinquished by: (Signature)	Date/Time			4. Received by lab: (Signature)		FOR COMPLETION BY LAB ONLY											
				<i>Sarah E. Rose</i>													

APPENDIX B

Effluent and Dilution Water Data

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING								Fathead Minnow	
Lab # / Sample ID		Test Start (Date/Time)				6/17/10			
Client		Test End (Date/Time)				6/24/10			
Day of Test									
		1	2	3	4	5	6	7	notes/remarks
Control		6/17	6/18	6/19	6/20	6/21	6/22	6/23	
D.O. (mg/L)	INITIAL	6.6	8.0	8.0	8.4	7.53	8.6	8.5	
	FINAL	7.8	8.1	9.0	7.7	7.99	7.7	7.29	
pH (s.u.)	INITIAL	7.9	7.7	7.6	7.3	7.6	7.6	7.7	
	FINAL	7.7	6.9	6.4	7.0	7.86	7.7	7.57	
temp (C)	INITIAL	21.9	21.8	22.3	21.5	22.0	21.4	22.3	
	FINAL	25.0	25.0	25.0	25.0	23.1	25.0	21.9	
ALKALINITY (mg/L)		38							
HARDNESS (mg/L)		56							
CONDUCTIVITY (umhos/cm)		157							
CHLORINE (mg/L)		0.05							
CONC:									
D.O. (mg/L)	INITIAL	7.6	7.1	8.0	8.4	8.64	8.6	8.5	
	FINAL	7.8	6.0	9.3	7.5	8.01	7.7	6.94	
pH (s.u.)	INITIAL	7.8	7.3	7.2	6.9	7.33	7.3	7.2	
	FINAL	7.2	6.7	6.5	6.8	7.44	7.2	7.31	
temp (C)	INITIAL	21.8	22.7	22.6	21.6	22.1	21.5	22.3	
	FINAL	25.0	25.0	25.0	25.0	23.1	25.0	21.7	
CONC:								8.0	
D.O. (mg/L)	INITIAL	7.9	7.8	8.2	8.4	8.68	8.6	8.5	
	FINAL	7.7	5.8	9.2	7.5	8.08	7.6	6.54	
pH (mg/L)	INITIAL	7.8	7.3	7.2	7.0	7.15	7.2	7.3	
	FINAL	7.2	10.7	6.6	7.0	7.4	7.2	7.27	
temp (C)	INITIAL	21.8	22.9	22.8	21.5	21.4	21.5	22.9	
	FINAL	25.0	25.0	25.0	25.0	22.3	25.0	21.6	
CONC:									
D.O. (mg/L)	INITIAL	8.1	7.9	8.4	8.5	8.75	8.8	8.7	
	FINAL	7.7	5.8	9.4	7.93	8.01	7.4	6.74	
pH (s.u.)	INITIAL	7.7	7.3	7.2	6.9	7.3	7.1	7.2	
	FINAL	7.2	6.6	6.6	7.0	7.44	7.1	7.19	
temp (C)	INITIAL	21.8	23.2	23.0	21.5	22.2	21.5	22.5	
	FINAL	25.0	25.0	25.0	25.0	22.7	25.0	21.8	
CONC:									
D.O. (mg/L)	INITIAL	8.5	8.2	8.6	8.6	8.83	8.8	8.8	
	FINAL	7.7	5.8	9.1	7.80	8.06	7.7	7.22	
pH (s.u.)	INITIAL	7.7	7.2	7.1	7.0	6.93	7.0	7.2	
	FINAL	7.1	6.6	6.5	7.05	7.38	7.1	7.12	
temp (C)	INITIAL	21.6	23.4	23.3	21.5	22.3	21.6	22.5	
	FINAL	25.0	25.0	25.0	25.0	23.6	25.0	22.3	
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.5	9.0	8.8	8.96	8.7	8.8	
	FINAL	7.5	6.0	9.5	7.83	7.99	7.8	7.33	
pH (s.u.)	INITIAL	7.6	7.1	7.0	6.7	6.8	6.9	7.1	
	FINAL	7.0	6.5	6.5	6.94	7.3	7.6	7.0	
temp (C)	INITIAL	21.9	23.6	23.5	21.5	21.5	24.9	22.4	
	FINAL	25.0	25.0	25.0	25.0	23.2	25.0	22.0	
CONC: 100%		A	A	A	B	C	C		
ALKALINITY (mg/L)		4		1	2	1	6		
HARDNESS (mg/L)		600+		1	600+	1	600+	1	
CONDUCTIVITY (umhos/cm)		1938		1	1973	1	1993	1	
CHLORINE (mg/L)		0.05		1	0.05	1	0.05	1	

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING								Cerodaphnia Dubia
Lab # / Sample ID K10060 M2				Test Start (Date/Time) 6/17/10				
Client Weston				Test End (Date/Time) 6/24/10				
Day of Test								
	1	2	3	4	5	6	7	notes/remarks
Control	6/17	6/18	6/19	6/20	6/21	6/22	6/23	
D.O. (mg/L)	INITIAL	6.6	7.0	8.0	8.4	8.5	8.6	85
	FINAL	8.1	8.1	7.8	7.8	7.7	7.8	
pH (s.u.)	INITIAL	7.9	7.7	7.4	7.3	7.9	7.6	77
	FINAL	7.8	7.6	7.7	7.6	7.9	7.8	
temp (C)	INITIAL	21.9	21.8	22.3	21.5	22.0	21.4	22.3
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	
ALKALINITY (mg/L)	3.8							1
HARDNESS (mg/L)	5.6							1
CONDUCTIVITY (umhos/cm)	157							1
CHLORINE (mg/L)	<0.05							1
CONC:								
D.O. (mg/L)	INITIAL	7.6	7.1	8.0	8.4	8.6	8.6	85
	FINAL	8.1	8.1	7.7	7.8	7.6	7.7	
pH (s.u.)	INITIAL	7.8	7.3	7.2	6.9	7.3	7.3	72
	FINAL	7.3	7.3	7.4	7.6	7.5	7.5	
temp (C)	INITIAL	21.8	22.7	22.6	21.6	22.1	21.5	22.3
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:								
D.O. (mg/L)	INITIAL	7.9	7.8	8.2	8.4	8.0	8.6	85
	FINAL	8.1	8.1	7.7	7.9	7.7	7.7	
pH (mg/L)	INITIAL	7.8	7.3	7.2	7.0	7.2	7.2	73
	FINAL	7.4	7.3	7.4	7.5	7.5	7.5	
temp (C)	INITIAL	21.8	22.9	22.8	21.5	21.4	21.5	22.4
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:								
D.O. (mg/L)	INITIAL	8.1	7.9	8.4	8.5	8.8	8.8	87
	FINAL	8.0	8.1	7.6	7.9	7.7	7.6	
pH (s.u.)	INITIAL	7.7	7.3	7.2	6.9	7.3	7.1	72
	FINAL	7.4	7.3	7.3	7.5	7.5	7.4	
temp (C)	INITIAL	21.8	23.2	23.0	21.5	22.2	21.5	22.5
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:								
D.O. (mg/L)	INITIAL	8.5	8.2	8.6	8.6	8.8	8.8	88
	FINAL	8.1	8.0	7.7	8.0	7.8	7.6	
pH (s.u.)	INITIAL	7.7	7.2	7.1	7.0	6.9	7.0	72
	FINAL	7.3	7.2	7.3	7.4	7.4	7.4	
temp (C)	INITIAL	21.6	23.4	23.3	21.5	22.3	21.6	22.5
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	
CONC:								
D.O. (mg/L)	INITIAL	8.6	8.5	9.0	8.8	8.9	8.7	88
	FINAL	8.2	8.0	7.6	8.0	7.8	7.5	
pH (s.u.)	INITIAL	7.6	7.1	7.0	6.7	6.8	6.9	71
	FINAL	7.2	7.2	7.2	7.4	7.3	7.3	
temp (C)	INITIAL	21.9	23.6	23.5	21.5	21.5	21.9	22.4
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	
CONC: 100%	A	A	A	B	B	C	C	
ALKALINITY (mg/L)	4		1	2	1	0	1	
HARDNESS (mg/L)	600+		1	600+	1	600+	1	
CONDUCTIVITY (umhos/cm)	1938		1	1973	1	1993	1	
CHLORINE (mg/L)	<0.05		1	<0.05	1	<0.05	1	

APPENDIX C

Fathead minnow raw data and statistics

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID	K1006012	TEST START DATE	6/17/10	TIME	1620					
CLIENT	Winston Summary	TEST END DATE	6/24/10	TIME	1000					
AGE AND SOURCE OF MINNOWS										
DAY (NUMBER SURVIVING)										
REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC:	A	8	8	8	8	8	8			
	B									
	C									
	D									
	E									
CONC:	A	8	8	8	8	8	8	8	97.5	5.73
	B									
	C									
	D									
	E									
CONC:	A	8	8	8	8	8	8	8	100	
	B									
	C									
	D									
	E									
CONC:	A	8	8	8	8	8	8	8	97.5	
	B									
	C									
	D									
	E									
CONC:	A	8	8	8	8	8	8	8	100	
	B									
	C									
	D									
	E									
CONC:	A	8	8	8	8	8	8	8	97.5	
	B									
	C									
	D									
	E									
CONC:	A	8	8	8	8	8	8	8	97.5	
	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	6/17/10	TIME	1620						
CLIENT	Weston	TEST END DATE									
AGE AND SOURCE OF MINNOWS											
DAY (NUMBER SURVIVING)											
	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC: 0	A	2	2	2	3	2	2	2			
	B										
	C										
	D										
	E										
	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC: 3L	A	2	2	2	2	2	2	2			
	B										
	C										
	D										
	E										
	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC: 12	A	2	2	2	2	2	2	2			
	B										
	C										
	D										
	E										
	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC: 56	A	2	2	2	2	2	2	2			
	B										
	C										
	D										
	E										
	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC: 75	A	2	2	2	2	2	2	2			
	B										
	C										
	D										
	E										
	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC: 66	A	2	2	2	2	2	2	2			
	B										
	C										
	D										
	E										
ANALYST		KP	KP	KP	TC	KD	KP	KP			
DATE:		6/17/10	6/18/10	6/19/10	6/20/10	6/21/10	6/22/10	6/23/10	6/24/10		
TIME:		1620	1115	1120	1245	1330	1315	1500	1000		

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 6/17/10 TIME 1620CLIENT Weston

TEST END DATE

TIME

AGE AND SOURCE OF MINNOWS

DAY (NUMBER SURVIVING)

SURVIVAL

	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC: 0	A	2	2	2	2	2	2	2			
	B										
	C										
	D										
	E										
CONC: 32	A	2	2	2	2	2	2	2			
	B										
	C										
	D										
	E										
CONC: 42	A	2	2	2	2	2	2	2			
	B										
	C										
	D										
	E										
CONC: 52	A	2	2	2	2	2	2	2			
	B										
	C										
	D										
	E										
CONC: 75	A	2	2	2	2	2	2	2			
	B										
	C										
	D										
	E										
CONC: 100	A	2	2	2	2	2	2	2			
	B										
	C										
	D										
	E										
ANALYST		KP	KP		Tc						
DATE:		6/17/10	6/18/10		6/20/10						
TIME:		1620	1115		1245						

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID

TEST START DATE 6/17/10 TIME 1620

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	7	7	2	2	7	7	7	7			
B											
C											
D											
E	—										

CONC: 3L

A	2	2	7	2	7	7	7	7			
B											
C											
D	—	—	—	—	—	—	—	—			
E											

CONC: 4L

A	7	2	7	2	7	7	7	7			
B											
C											
D	—	—	—	—	—	—	—	—			
E											

CONC: 5L

A	7	2	7	2	7	7	7	7			
B											
C											
D	—	—	—	—	—	—	—	—			
E											

CONC: 5L

A	7	2	7	2	7	7	7	7			
B											
C											
D	—	—	—	—	—	—	—	—			
E											

CONC: 10L

A	7	2	7	2	7	7	7	7			
B											
C											
D	—	—	—	—	—	—	—	—			
E											

ANALYST

JCP

DATE:

6/17/10

TIME:

1120

JCP

6/18/10

1115

TC

6/20/10

1245

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID

TEST START DATE 6/17/10 TIME 1620

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	7	7	7	2	7	2	2	2		
	B								2		
	C								2		
	D	—	—	—	—	—	—	—	1		
	E										
CONC: 32	A	2	2	2	2	2	2	2	2		
	B										
	C										
	D	—	—	—	—	—	—	—	—		
	E										
CONC: 47	A	2	2	2	2	2	2	2	2		
	B										
	C								2		
	D	—	—	—	—	—	—	—	2		
	E										
CONC: 56	A	2	2	2	2	2	2	2	2		
	B								2		
	C								2		
	D	—	—	—	—	—	—	—	2		
	E										
CONC: 75	A	2	2	2	2	2	2	2	2		
	B								2		
	C								2		
	D	—	—	—	—	—	—	—	2		
	E										
CONC: 106	A	2	2	2	2	2	2	2	2		
	B								2		
	C								2		
	D	—	—	—	—	—	—	—	2		
	E										
ANALYST	KP	KP		TC							
DATE:	6/17/10	6/18/10		6/20/10							
TIME:	1120	1115		1245							

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID

TEST START DATE 6/17/10 TIME 1620

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	7	7	2	2	2	2	2	2		
	B										
	C										
	D	1	1	1	1	1	1	1			
	E	—	—	—	—	—	—	—			
CONC: 3	A	2	7	2	2	2	2	2	2		
	B										
	C										
	D	1	1	1	1	1	1	1			
	E										
CONC: 42	A	2	2	2	2	2	2	2	2		
	B										
	C										
	D	1	1	1	1	1	1	1			
	E										
CONC: 56	A	2	2	2	2	2	2	2	2		
	B										
	C										
	D	1	1	1	1	1	1	1			
	E										
CONC: 75	A	2	2	2	2	2	2	2	2		
	B										
	C										
	D	1	1	1	1	1	1	1			
	E										
CONC: 100	A	2	2	2	2	2	2	2	2		
	B										
	C										
	D	1	1	1	1	1	1	1			
	E										
ANALYST		ICP	ICP		TC						
DATE:		6/17/10	6/18/10		6/20/10						
TIME:		1620	1115		1245						

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

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:			TEST DATES (BEGIN / END): 6/17-24/10			
CLIENT:			WEIGHING DATE / TIME: 6/25/10, 1400			
ANALYSTS:			DRYING TEMP (DEGREES C): 60			
SAMPLE ID:			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	0.99172	0.98985	0.00187	8	0.234
	B	0.99267	0.99048	0.00219	8	0.274
	C	0.98231	0.98015	0.00216	8	0.270
	D	0.98880	0.98741	0.00139	8	0.174
	E	1.00148	0.99904	0.00244	8	0.305
CONC:	A	0.98732	0.98488	0.00244	8	0.305
32%	B	0.97291	0.97025	0.00266	8	0.333
	C	0.97233	0.96908	0.00325	8	0.406
	D	0.99568	0.99308	0.00260	8	0.325
	E	0.99512	0.99170	0.00342	8	0.427
CONC:	A	0.99043	0.98739	0.00304	8	0.380
42%	B	0.99976	0.99668	0.00308	8	0.385
	C	0.98186	0.97922	0.00264	8	0.330
	D	0.96636	0.96385	0.00251	8	0.314
	E	0.97599	0.97313	0.00286	8	0.357
CONC:	A	0.99533	0.99264	0.00269	8	0.336
56%	B	1.00036	0.99686	0.00350	8	0.437
	C	1.00437	1.00030	0.00407	8	0.509
	D	1.00433	1.00161	0.00272	8	0.340
	E	1.00555	1.00235	0.00320	8	0.400
CONC:	A	0.99310	0.99004	0.00306	8	0.382
75%	B	0.97483	0.97135	0.00348	8	0.435
	C	0.98579	0.98240	0.00339	8	0.424
	D	0.99718	0.99436	0.00282	8	0.352
	E	0.99298	0.98889	0.00409	8	0.511
CONC:	A	0.99738	0.99418	0.00320	8	0.400
100%	B	0.99297	0.98906	0.00391	8	0.489
	C	0.97542	0.97207	0.00335	8	0.419
	D	0.96208	0.95945	0.00263	8	0.329
	E	0.99405	0.98994	0.00411	8	0.514

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

Pimephales promelas

FATHEAD MINNOW

TEST 1000.0

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #: <u>1006012</u>	CLIENT: <u>Weston</u>	ANALYSTS: <u>KP</u>	SAMPLE ID:	TEST DATES (BEGIN / END): <u>6/17-24/10</u>		
				WEIGHING DATE / TIME: <u>6/25/10, 1400</u>		
				DRYING TEMP (DEGREES C): <u>60</u>		
				DRYING TIME (HOURS): <u>24</u>		
	FINAL DRY WEIGHT TIN+LARVAE (g)	INITIAL WEIGHT TIN (g)	TOTAL DRY WEIGHT OF LARVAE (g)	NUMBER OF LARVAE	DRY WEIGHT OF LARVAE (mg)	
REP#						
CONTROL	A 31	0.99172	0.98985			AVG DRY WEIGHT (mg)
	B 32	0.99267	0.99048			
	C 33	0.98231	0.98015			
	D 34	0.98880	0.98741			CV
	E 35	1.00148	0.99904			
CONC: 32	A 36	0.98732	0.98488			AVG DRY WEIGHT (mg)
	B 37	0.97291	0.97025			
	C 38	0.97233	0.96908			
	D 39	0.99568	0.99308			CV
	E 40	0.99512	0.99170			
CONC: 42	A 41	0.99043	0.98739			AVG DRY WEIGHT (mg)
	B 42	0.99976	0.99668			
	C 43	0.98186	0.97922			
	D 44	0.96636	0.96385			CV
	E 45	0.97599	0.97313			
CONC: 52	A 46	0.99533	0.99264			AVG DRY WEIGHT (mg)
	B 47	1.00036	0.99686			
	C 48	1.00437	1.00030			
	D 49	1.00433	1.00161			CV
	E 50	1.00555	1.00235			
CONC: 75	A 51	0.99310	0.99004			AVG DRY WEIGHT (mg)
	B 52	0.97483	0.97135			
	C 53	0.98579	0.98240			
	D 54	0.99718	0.99436			CV
	E 55	0.99298	0.98889			
CONC: 100	A 56	0.99738	0.99418			AVG DRY WEIGHT (mg)
	B 57	0.99297	0.98906			
	C 58	0.97542	0.97207			
	D 59	0.96208	0.95945			CV
	E 60	0.99405	0.98994			

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA# K1006012, FATHEAD MINNOW SURVIVAL, CHRONIC, 6-17-10
File: Z:\TOXSTAT\MONTE\FHSURV. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.108

W = 0.596

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# K1006012, FATHEAD MINNOW SURVIVAL, CHRONIC, 6-17-10
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# K1006012, FATHEAD MINNOW SURVIVAL, CHRONIC, 6-17-10
FILE: Z:\TOXSTAT\MONTE\FHSURV.
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

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

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

AA# K1006012, FATHEAD MINNOW SURVIVAL, CHRONIC, 6-17-10
 File: Z:\TOXSTAT\MONTE\FHSURV. Transform: ARC SINE(SQUARE ROOT(Y))

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

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

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

AA# K1006012, FATHEAD MINNOW GROWTH CHRONIC, 6-17-10
File: Z:/toxstat/monte\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.090

W = 0.979

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# K1006012, FATHEAD MINNOW GROWTH CHRONIC, 6-17-10
File: Z:/toxstat/monte\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 2.70

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# K1006012, FATHEAD MINNOW GROWTH CHRONIC, 6-17-10
FILE: Z:/toxstat/monte\FHGR. NUMBER OF GROUPS: 6
TRANSFORM: ARC SINE(SQUARE ROOT(Y))

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.2340	0.5049
1	CONTROL	2	0.2740	0.5509
1	CONTROL	3	0.2700	0.5464
1	CONTROL	4	0.1740	0.4303
1	CONTROL	5	0.3050	0.5851
2	32 % EFFLUENT	1	0.3050	0.5851
2	32 % EFFLUENT	2	0.3330	0.6151
2	32 % EFFLUENT	3	0.4060	0.6908
2	32 % EFFLUENT	4	0.3250	0.6066
2	32 % EFFLUENT	5	0.4270	0.7121
3	42 % EFFLUENT	1	0.3800	0.6642
3	42 % EFFLUENT	2	0.3850	0.6694
3	42 % EFFLUENT	3	0.3300	0.6119
3	42 % EFFLUENT	4	0.3140	0.5948
3	42 % EFFLUENT	5	0.3570	0.6404
4	56 % EFFLUENT	1	0.3360	0.6183

4	56 % EFFLUENT	2	0.4370	0.7222
4	56 % EFFLUENT	3	0.5090	0.7944
4	56 % EFFLUENT	4	0.3400	0.6225
4	56 % EFFLUENT	5	0.4000	0.6847
5	75 % EFFLUENT	1	0.3820	0.6663
5	75 % EFFLUENT	2	0.4350	0.7202
5	75 % EFFLUENT	3	0.4240	0.7091
5	75 % EFFLUENT	4	0.3520	0.6351
5	75 % EFFLUENT	5	0.5110	0.7964
6	100 % EFFLUENT	1	0.4000	0.6847
6	100 % EFFLUENT	2	0.4890	0.7744
6	100 % EFFLUENT	3	0.4190	0.7040
6	100 % EFFLUENT	4	0.3290	0.6109
6	100 % EFFLUENT	5	0.5140	0.7994

AA# K1006012, FATHEAD MINNOW GROWTH CHRONIC, 6-17-10
 File: Z:/toxstat/monte\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.125	0.025	6.661
Within (Error)	24	0.090	0.004	
Total	29	0.215		

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

AA# K1006012, FATHEAD MINNOW GROWTH CHRONIC, 6-17-10
 File: Z:/toxstat/monte\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	0.524	0.251		
2	32 % EFFLUENT	0.642	0.359	-3.059	
3	42 % EFFLUENT	0.636	0.353	-2.908	
4	56 % EFFLUENT	0.688	0.404	-4.259	
5	75 % EFFLUENT	0.705	0.421	-4.697	
6	100 % EFFLUENT	0.715	0.430	-4.937	

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

AA# K1006012, FATHEAD MINNOW GROWTH CHRONIC, 6-17-10
 File: Z:/toxstat/monte\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	5			
2	32 % EFFLUENT	5	0.075	29.6	-0.108
3	42 % EFFLUENT	5	0.075	29.6	-0.102
4	56 % EFFLUENT	5	0.075	29.6	-0.153
5	75 % EFFLUENT	5	0.075	29.6	-0.169
6	100 % EFFLUENT	5	0.075	29.6	-0.179

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

Cerodaphnia dubia

Discharger: Winston

SURVIVAL AND REPRODUCTION TEST

Lab Number/s:
1b06012

Analyst:

KPTest Start - Date/ Time: 6/17/10 16:10Test Stop - Date/Time: 6/24/10 09:35

Date Sample Collected:

Conc 1		Replicate										No. of Young	No. of Adult	Young/ Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
0	1	0	0	0	0	0	0	0	0	0	0	10	0	KP	
	2	0	0	0	0	0	0	0	0	0	0	10	0	TC	
	3	0	0	1	0	0	0	4	0	0	1	0	0.6	KP	
	4	2	2	2	5	4	3	1	0	4	6	29	0	2.9	KP
	5	5	3	8	9	9	5	3	7	9	9	67	10	6.7	KP
	6	2	4	15	1	0	6	12	9	3	0	52	10	5.2	KP
	7														
	8														
Total		9	9	26	15	13	14	20	16	11	6	154	CV = 32.4		

Conc 2		Replicate										No. of Young	No. of Adult	Young/ Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
52	1	6	0	9	0	0	0	0	6	0	0	16	0		
	2	0	0	8	0	0	0	0	0	0	0	10	0	TC	
	3	0	0	0	6	0	0	1	0	1	0	10	0.2		
	4	5	5	0	4	4	3	1	4	5	2	33	10	3.3	
	5	9	7	1	6	5	5	3	2	7	3	48	10	4.8	
	6	2	1	6	7	3	7	14	4	2	7	53	10	5.3	
	7														
	8														
Total		16	13	7	17	12	15	9	10	15	12	136			

Conc 3		Replicate										No. of Young	No. of Adult	Young/ Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
52	1	0	0	0	0	0	0	0	0	0	8	10	0		
	2	0	0	0	0	0	0	0	0	0	8	9	0	TC	
	3	0	0	3	-	0	0	1	0	0	1	5	9	0.6	
	4	4	2	1	-	4	6	1	6	4	5	33	9	3.7	
	5	7	6	6	-	7	4	5	8	6	3	44	8	5.5	
	6	3	4	13	-	6	3	13	-	2	7	51	8	6.4	
	7														
	8														
Total		14	12	23	X0	17	13	20	X0	12	16	133			

X= DEAD; Y= MALE

Conc 4		Replicate										No. of Young	No. of Adult	Young/ Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
56	1	0	0	0	0	0	0	0	0	0	0	8	10	0	
	2	0	0	0	0	0	0	0	0	0	0	8	10	0	TC
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	
	4	5	3	1	4	3	3	2	3	3	2	79	10	2.9	
	5	7	2	1	4	9	8	0	1	2	9	49	10	4.9	
	6	2	6	0	5	3	4	0	8	3	6	37	10	3.7	
	7														
	8														
Total		14	11	2	13	15	15	2	12	14	17	115			

Conc 5		Replicate										No. of Young	No. of Adult	Young/ Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
75	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	TC
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	
	4	2	4	6	4	4	3	1	3	1	4	37	10	3.7	
	5	8	7	2	6	4	4	5	3	5	6	50	10	5.0	
	6	2	9	1	3	6	3	7	7	8	5	51	10	5.1	
	7														
	8														
Total		17	20	9	13	19	10	13	13	14	15	138			

Conc 6		Replicate										No. of Young	No. of Adult	Young/ Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
160	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	8	0.8	TC
	3	0	0	4	0	0	0	4	9	9	9	8	10	0.8	
	4	5	1	1	4	1	4	1	9	9	9	28	10	2.8	
	5	9	5	5	4	7	8	6	7	5	8	64	10	6.4	
	6	6	0	14	7	7	5	8	1	5	2	55	10	5.5	
	7														
	8														
Total		20	6	24	15	15	17	19	9	16	14	155			

$$\bar{x} = 15.5$$

$$CV = 33.5$$

AA # K1006012 C. DUBIA CHRONIC, REPRODUCTION, 6-17-10
File: Z:\TOXSTAT\MONTE\CD. Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

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

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

Total number of replicates = 60

AA # K1006012 C. DUBIA CHRONIC, REPRODUCTION, 6-17-10
File: Z:\TOXSTAT\MONTE\CD. Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 6.13

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%	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

NUMBER OF

IDENTIFICATION	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
75%	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

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	0	
4	75%	10	0	
5	100%	10	0	

TITLE: AA # K1006012 C. DUBIA CHRONIC, REPRODUCTION, 6-17-10

FILE: Z:\TOXSTAT\MONTE\CD.

TRANSFORM: NO TRANSFORMATION

NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	9.0000	9.0000
1	CONTROL	2	9.0000	9.0000
1	CONTROL	3	26.0000	26.0000
1	CONTROL	4	15.0000	15.0000
1	CONTROL	5	13.0000	13.0000
1	CONTROL	6	14.0000	14.0000
1	CONTROL	7	20.0000	20.0000
1	CONTROL	8	16.0000	16.0000
1	CONTROL	9	16.0000	16.0000
1	CONTROL	10	16.0000	16.0000
2	32 % EFFLUENT	1	16.0000	16.0000
2	32 % EFFLUENT	2	13.0000	13.0000
2	32 % EFFLUENT	3	7.0000	7.0000
2	32 % EFFLUENT	4	17.0000	17.0000
2	32 % EFFLUENT	5	12.0000	12.0000
2	32 % EFFLUENT	6	15.0000	15.0000
2	32 % EFFLUENT	7	19.0000	19.0000
2	32 % EFFLUENT	8	10.0000	10.0000
2	32 % EFFLUENT	9	15.0000	15.0000
2	32 % EFFLUENT	10	12.0000	12.0000
3	42 % EFFLUENT	1	14.0000	14.0000
3	42 % EFFLUENT	2	12.0000	12.0000
3	42 % EFFLUENT	3	23.0000	23.0000
3	42 % EFFLUENT	4	0.0000	0.0000
3	42 % EFFLUENT	5	17.0000	17.0000
3	42 % EFFLUENT	6	13.0000	13.0000
3	42 % EFFLUENT	7	20.0000	20.0000
3	42 % EFFLUENT	8	6.0000	6.0000
3	42 % EFFLUENT	9	12.0000	12.0000
3	42 % EFFLUENT	10	16.0000	16.0000
4	56 % EFFLUENT	1	14.0000	14.0000
4	56 % EFFLUENT	2	11.0000	11.0000
4	56 % EFFLUENT	3	2.0000	2.0000
4	56 % EFFLUENT	4	13.0000	13.0000
4	56 % EFFLUENT	5	15.0000	15.0000
4	56 % EFFLUENT	6	15.0000	15.0000
4	56 % EFFLUENT	7	2.0000	2.0000
4	56 % EFFLUENT	8	12.0000	12.0000

4	56 %	EFFLUENT	9	14.0000	14.0000
4	56 %	EFFLUENT	10	17.0000	17.0000
5	75 %	EFFLUENT	1	17.0000	17.0000
5	75 %	EFFLUENT	2	20.0000	20.0000
5	75 %	EFFLUENT	3	9.0000	9.0000
5	75 %	EFFLUENT	4	13.0000	13.0000
5	75 %	EFFLUENT	5	14.0000	14.0000
5	75 %	EFFLUENT	6	10.0000	10.0000
5	75 %	EFFLUENT	7	13.0000	13.0000
5	75 %	EFFLUENT	8	13.0000	13.0000
5	75 %	EFFLUENT	9	14.0000	14.0000
5	75 %	EFFLUENT	10	15.0000	15.0000
6	100 %	EFFLUENT	1	20.0000	20.0000
6	100 %	EFFLUENT	2	6.0000	6.0000
6	100 %	EFFLUENT	3	24.0000	24.0000
6	100 %	EFFLUENT	4	15.0000	15.0000
6	100 %	EFFLUENT	5	15.0000	15.0000
6	100 %	EFFLUENT	6	17.0000	17.0000
6	100 %	EFFLUENT	7	19.0000	19.0000
6	100 %	EFFLUENT	8	9.0000	9.0000
6	100 %	EFFLUENT	9	16.0000	16.0000
6	100 %	EFFLUENT	10	14.0000	14.0000

AA # K1006012 C. DUBIA CHRONIC, REPRODUCTION, 6-17-10
 File: Z:\TOXSTAT\MONTE\CD. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	110.150	22.030	0.906
Within (Error)	54	1313.500	24.324	
Total	59	1423.650		

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

AA # K1006012 C. DUBIA CHRONIC, REPRODUCTION, 6-17-10
 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	15.400	15.400		
2	32 % EFFLUENT	13.600	13.600	0.816	
3	42 % EFFLUENT	13.300	13.300	0.952	
4	56 % EFFLUENT	11.500	11.500	1.768	
5	75 % EFFLUENT	13.800	13.800	0.725	
6	100 % EFFLUENT	15.500	15.500	-0.045	

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

AA # K1006012 C. DUBIA CHRONIC, REPRODUCTION, 6-17-10
File: Z:\TOXSTAT\MONTE\CD. Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	10			
2	32 % EFFLUENT	10	5.095	33.1	1.800
3	42 % EFFLUENT	10	5.095	33.1	2.100
4	56 % EFFLUENT	10	5.095	33.1	3.900
5	75 % EFFLUENT	10	5.095	33.1	1.600
6	100 % EFFLUENT	10	5.095	33.1	-0.100

AA # K1006012 C. DUBIA CHRONIC, REPRODUCTION, 6-17-10
File: Z:\TOXSTAT\MONTE\CD. Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	15.400				
2	32 % EFFLUENT	13.600	95.00	75.00	10.00	
3	42 % EFFLUENT	13.300	97.00	75.00	10.00	
4	56 % EFFLUENT	11.500	85.50	75.00	10.00	
5	75 % EFFLUENT	13.800	93.50	75.00	10.00	
6	100 % EFFLUENT	15.500	108.50	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 6-17-10 CLIENT Arkansas Analytics

Purchase Order #: _____

SPECIES: Rimphichthys promelas Mysidopsis bahia Cyprinodon variegatus

Quantity Shipped: 850+ 15wet _____

Age: 24 hrs C/17 _____

Brood Stock Source: Arkansas Farms, AR _____

Culture Water: Groundwater Artificial Salts Artificial Salts

Hardness (Mg/l CaCO₃) 160 Salinity (ppt) _____

Dissolved Oxygen (Mg/l): 7.9 _____

Feeding: ATMIL _____

Comments: _____

Shipped Via: Federal Express UPS Overnight Shuttle

Packaged By: cw _____

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 CaCO₃): 142 mg/l 86-124 mg/l

TOTAL ALKALINITY (as CaCO₃): 100 mg/l 65-130 mg/l

pH: 7.92 7.56-8.35

Comments:

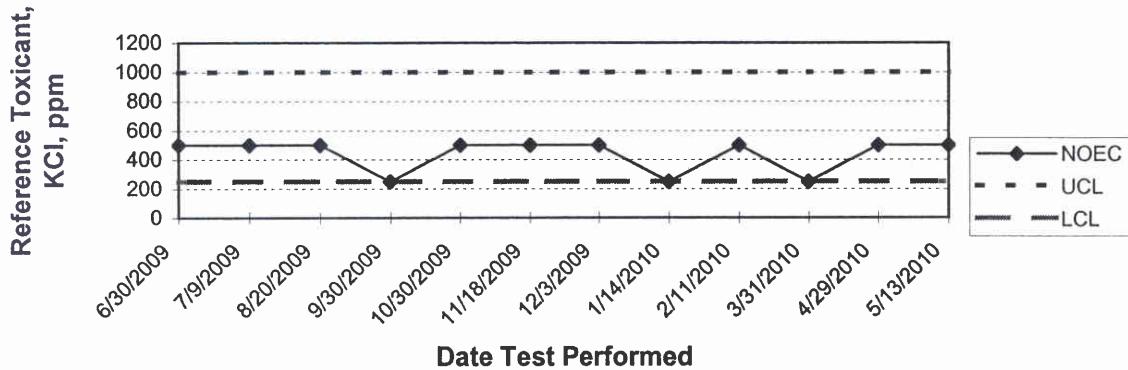


Facility Supervisor

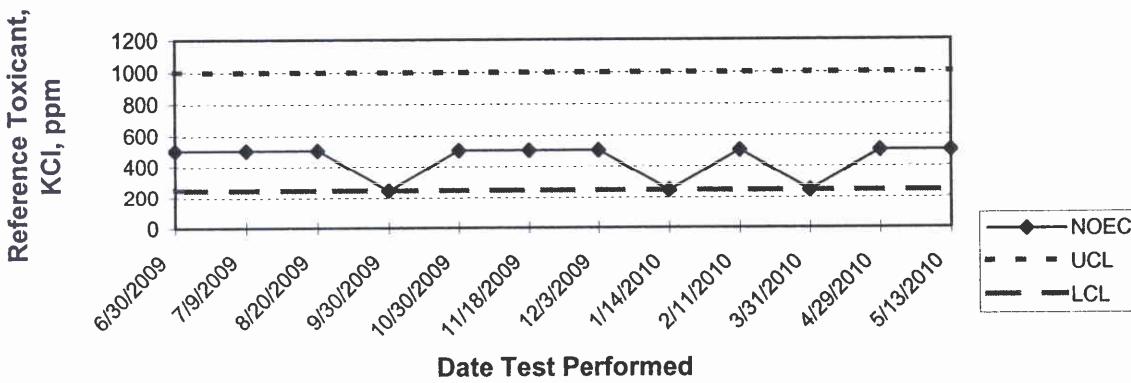
APPENDIX F

Quality Assurance Charts

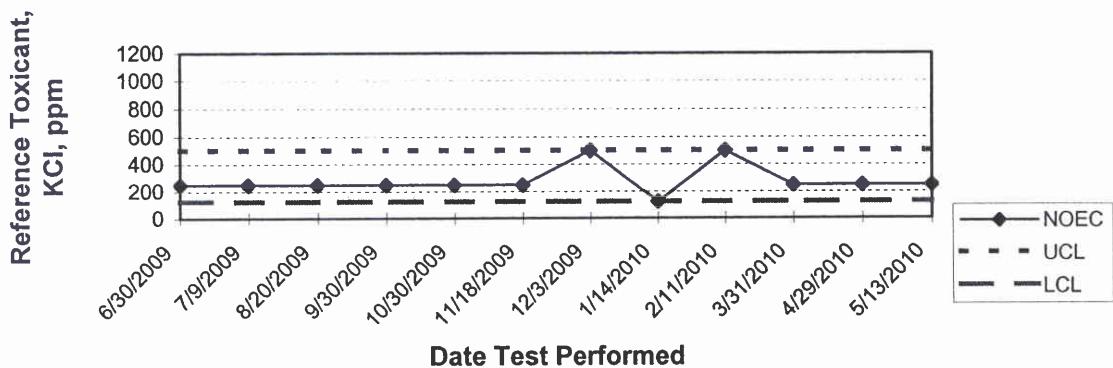
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW SURVIVAL
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW GROWTH
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE

