

Arkansas Analytical, Inc.

Toxicity Test Results

CITY of SHERIDAN
NPDES PERMIT NUMBER: AR0034347
First Quarter 2014
AFIN # 27-00022

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 Fitzgerald**
City of Sheridan
P.O.Box 486
Sheridan, Arkansas 72150

Prepared by: Arkansas Analytical, Inc.
11701 I-30, Bldg 1, Suite 115
Little Rock, Arkansas 72209
Lab Number K1403001

Thursday, March 20, 2014

Introduction

This report contains test results for toxicity testing for the City of Sheridan, NPDES permit number AR0034347. The plant is located in the Southeast ¼ of the Northwest ¼ of Section 11, Township 5 South, Range 13 West, in Grant County, Arkansas. The discharge is to receiving waters named Big Creek to Hurricane Creek, then to the Saline River in Segment 2C of the Ouachita River Basin.

The permit requires chronic biomonitoring testing quarterly for *Ceriodaphnia dubia* and *Pimephales promelas*. The test results in this report represent the testing of the first quarter of 2014.

Plant Operations

To be provided by permittee.

Source of Effluent and Dilution Water

Effluent sample was a composite and collected as follows:

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	3-5-14, 1314	3-6-14, 1214
Sample #2	3-6-14, 1407	3-7-14, 1307
Sample #3	3-9-14, 1000	3-10-14, 0900

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:	3-6-14, 1524	2
Sample #2	3-7-14, 1450	1
Sample #3	3-10-14, 1353	2

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 because of either zero flow conditions or due to an earlier characterization of the receiving water as being toxic.

The dilution water used in the toxicity tests was synthetic moderately hard. It was prepared using Elga Maxima ultra pure water according to EPA specifications. Each batch 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 6%, 8%, 11%, 14%, and 19%. The low-flow effluent concentration (**critical dilution**) was defined as **14% 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 < 48 hour old Fathead Minnows, *Pimephales promelas*, which were purchased from Aquatox; a copy of the organism history is provided in Appendix D.

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 *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	0.445	X	
The percent coefficient of variation between replicates must be 40% or less for growth	9.76%	X	

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	16.7	X	
At least 60% of surviving females should have produced 3 broods	100%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	19.2%	X	

Reference Toxicant

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

REFERENCE TOXICANT

<i>Ceriodaphnia dubia</i> 3/12 – 19/14		<i>Pimephales promelas</i> 3/12 – 19/14	
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 E.

Summary of Results

<i>Pimephales promelas</i>	
NOEC / LOEC survival	19% / NA
NOEC / LOEC growth	19% / NA
%CV survival (critical dilution)	0.00%
Mean dry weight (critical dilution) in milligrams	0.479
%CV growth (critical dilution)	7.92%
PMSD Growth	15.5%
<i>Ceriodaphnia dubia</i>	
NOEC / LOEC survival	19% /N/A
NOEC / LOEC reproduction	19% /N/A
Mean number of neonates (critical dilution)	16.8
%CV Reproduction (critical dilution)	18.6%
PMSD Reproduction	22.8%

Conclusion

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

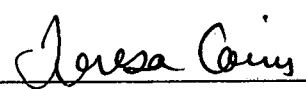
The permit issued to the City of Sheridan, AR0034347, specifies that the **critical dilution is 14% effluent**. The effluent samples **did not** exhibit lethal or sublethal effects at the critical dilution, and, as such, **passed** the test.

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

The permit issued to the City of Sheridan, AR0034347, specifies that the **critical dilution is 14% effluent**. The effluent samples **did not** exhibit lethal or sublethal effects at the critical dilution, and, as such, **passed** the test.

Biomonitoring Analysts:


Ryan Hudgin


Teresa Coins

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

PERMITTEE: City of Sheridan

NPDES #: AR0034347

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	3-5-14, 1314	3-6-14, 1214
Sample #2	3-6-14, 1407	3-7-14, 1307
Sample #3	3-9-14, 1000	3-10-14, 0900

Test initiated (date, time): 3-6-14, 1620 Test terminated (date, time): 3-13-14, 1130

Dilution water used: Moderately Hard Synthetic

DATA TABLE FOR FATHEAD MINNOW SURVIVAL

Effluent Conc %	Percent Survival in Replicate Chambers						Mean Percent Survival			
	A	B	C	D	E		24 hours	48 hours	7 days	CV %
0%	100	87.5	100	100	100		100	100	97.5	0.0%
6.0%	100	100	100	100	100		100	100	100	
8.0%	100	100	100	100	100		100	100	100	
11.0%	100	100	100	100	100		100	100	100	
14.0%	100	100	100	100	100		100	100	100	0.0%
19.0%	100	100	100	87.5	87.5		100	100	95	

DATA TABLE FOR GROWTH OF FATHEAD MINNOWS

Average Dry Weight in milligrams in replicate chambers

Effluent Conc %	A	B	C	D	E		Mean Dry Weight	CV%
0%	0.376	0.461	0.450	0.495	0.441		0.445	9.76%
6.0%	0.395	0.399	0.458	0.512	0.505		0.454	
8.0%	0.442	0.399	0.410	0.494	0.442		0.437	
11.0%	0.421	0.547	0.469	0.479	0.569		0.497	
14.0%	0.474	0.440	0.484	0.458	0.540		0.479	7.92%
19.0%	0.466	0.461	0.493	0.395	0.471		0.457	

Coefficient of Variation = standard deviation / mean * 100

SUMMARY 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, (14.0%) 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, (14.0%) 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)= 19 % effluent
b) NOEC growth (parameter TPP6C)= 19 % effluent
c) Coefficient of variation (parameter TQP6C)= 9.76 %

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING
Ceriodaphnia dubia SURVIVAL AND REPRODUCTION

Permittee: City of Sheridan

NPDES #: AR0034347

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	3-5-14, 1314	3-6-14, 1214
Sample #2	3-6-14, 1407	3-7-14, 1307
Sample #3	3-9-14, 1000	3-10-14, 0900

Test initiated (date, time): 3-6-14, 1615 Test terminated (date, time): 3-13-14, 0945

Dilution water used: Moderately Hard Synthetic

Ceriodaphnia dubia SURVIVAL AND REPRODUCTION

NUMBER OF YOUNG PRODUCED PER FEMALE @ TEST TERMINATION

PERCENT EFFLUENT

Replicate	0%	6%	8%	11%	14%	19%
A	16	20	17	12	16	18
B	15	20	18	16	18	11
C	18	17	10	17	20	17
D	11	14	18	16	22	19
E	21	14	17	19	18	23
F	16	16	9	12	14	16
G	14	21	12	21	12	21
H	22	11	17	18	13	19
I	17	14	21	20	17	7
J	17	14	12	11	18	15
Mean	16.7	16.1	15.1	16.2	16.8	16.6
Mean/surviving female	16.7	16.1	15.1	16.2	16.8	16.6
CV%*	19.2				18.6	

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: City of Sheridan

NPDES #: AR0034347

PERCENT SURVIVAL

PERCENT EFFLUENT	0%	6%	8%	11%	14%	19%
Time of Reading: 24 HOURS	100	100	100	100	100	100
48 HOURS	100	100	100	100	100	100
Test termination	100	100	100	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, (36.3%): 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, (36.3%): 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:

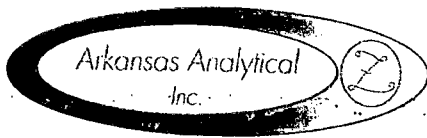
a) NOEC survival (parameter TOP3B)= 19 % effluent

b) NOEC reproduction (parameter TPP3B)= 19 % effluent

c) Coefficient of variation (parameter TQP3B)= 19.2 %

APPENDIX A

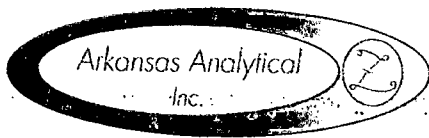
Chain of Custody Forms



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

CHAIN OF CUSTODY RECORD

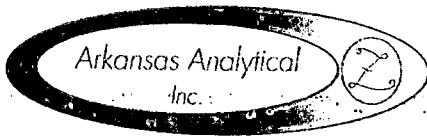
CLIENT INFORMATION		BILLING INFORMATION		Project Description		Turnaround Time		Preservation Codes:															
Sheridan Waterworks		Sheridan Waterworks		Chronic Toxicity		1 Day (100%)		1. Cool, 4 Degrees Centigrade					4. Thiosulfate for Dechlorination										
104 W High St.		P.O. Box 486				2 Day (50%)		2. Sulfuric Acid (H ₂ SO ₄), pH < 2					5. Hydrochloric Acid(HCl)										
Sheridan, AR 72150		Sheridan, AR 72150		Reporting Information		3 Day (25%)		3. Nitric Acid (HNO ₃), pH < 2					6. Sodium Hydroxide (NaOH), pH > 12										
Attn: David Fitzgerald				Telephone: 870-942-2722		Routine		TEST PARAMETERS										Bottle Type Code					
				Fax: 870-942-1937		Preservative Code: 1												G = Glass; P = Plastic					
				Email: sheridanwater@windstream.net		Bottle Type: P												V = Septum; A = Amber					
<i>Allen Parker</i> Sampler(s) Signature				<i>Allen Parker</i> Sampler(s) Printed								Chronic Toxicity										Arkansas Analytical Work Order Number:	
Field Number	SAMPLE COLLECTION		Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION																
	3/5-6/14	1214		X	24	Water	Final Discharge												K1403001A				
1. Relinquished by: (Signature)			Date/Time		2. Received by: (Signature)			SAMPLE CONDITION UPON RECEIPT IN LAB						REMARKS / SAMPLE COMMENTS									
<i>Allen Parker</i>			1524 3-6-14		<i>[Signature]</i>			1. CUSTODY SEALS: <input checked="" type="checkbox"/> Yes ___ No 2. CONTAINERS CORRECT: <input type="checkbox"/> Yes ___ No 3. COC/LABELS AGREE: <input type="checkbox"/> Yes ___ No 4. RECEIVED ON ICE: <input type="checkbox"/> Yes ___ No 5. TEMPERATURE ON RECEIPT: <i>2°C</i> 6. TEMPERATURE GUN ID: <i>HHT#2</i>															
3. Relinquished by: (Signature)			Date/Time		4. Received by lab: (Signature)			FOR COMPLETION BY LAB ONLY															
<i>[Signature]</i>					<i>Amanda Forbush</i>																		



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 Little Rock, AR 72209
 PHONE: 501-455-3233
 FAX: 501-455-6118

CHAIN OF CUSTODY RECORD

CLIENT INFORMATION		BILLING INFORMATION		Project Description		Turnaround Time		Preservation Codes:											
Sheridan Waterworks		Sheridan Waterworks		Chronic Toxicity		1 Day (100%)		1. Cool, 4 Degrees Centigrade				4. Thiosulfate for Dechlorination							
104 W High St.		P.O. Box 486				2 Day (50%)		2. Sulfuric Acid (H ₂ SO ₄), pH < 2				5. Hydrochloric Acid(HCl)							
Sheridan, AR 72150		Sheridan, AR 72150		Reporting Information		3 Day (25%)		3. Nitric Acid (HNO ₃), pH < 2				6. Sodium Hydroxide (NaOH), pH > 12							
Attn: David Fitzgerald				Telephone: 870-942-2722		Routine		TEST PARAMETERS								Bottle Type Code			
				Fax: 870-942-1937		Preservative Code: 1		1								G = Glass; P = Plastic			
				Email: sheridanwater@windstream.net		Bottle Type: P		P								V = Septum; A = Amber			
<i>Allen Parker</i> Sampler(s) Signature				Allen Parker Sampler(s) Printed				Chronic Toxicity		Arkansas Analytical Work Order Number: AP 3-7-14 K1403001B K1403001B									
Field Number		SAMPLE COLLECTION Date/s Time/s		Grab	Comp	Number of Bottles	Sample Matrix											SAMPLE IDENTIFICATION/ DESCRIPTION	
		3/6-7/14	1407-1307		X	24	Water	Final Discharge		X									
1. Relinquished by: (Signature)		Date/Time		2. Received by: (Signature)		SAMPLE CONDITION UPON RECEIPT IN LAB				REMARKS / SAMPLE COMMENTS									
<i>Allen Parker</i>		1450 3-7-14				1. CUSTODY SEALS: <input checked="" type="checkbox"/> Yes ___ No 2. CONTAINERS CORRECT: <input type="checkbox"/> Yes ___ No 3. COC/LABELS AGREE: <input type="checkbox"/> Yes ___ No 4. RECEIVED ON ICE: <input type="checkbox"/> Yes ___ No 5. TEMPERATURE ON RECEIPT: 1°C 6. TEMPERATURE GUN ID: HHT #2													
3. Relinquished by: (Signature)		Date/Time		4. Received by lab: (Signature)		FOR COMPLETION BY LAB ONLY													
				<i>Sydney James</i>															



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		BILLING INFORMATION		Project Description		Turnaround Time		Preservation Codes:									
Sheridan Waterworks		Sheridan Waterworks		Chronic Toxicity		1 Day (100%)		1. Cool, 4 Degrees Centigrade				4. Thiosulfate for Dechlorination					
104 W High St.		P.O. Box 486				2 Day (50%)		2. Sulfuric Acid (H ₂ SO ₄), pH < 2				5. Hydrochloric Acid(HCl)					
Sheridan, AR 72150		Sheridan, AR 72150		Reporting Information		3 Day (25%)		3. Nitric Acid (HNO ₃), pH < 2				6. Sodium Hydroxide (NaOH), pH > 12					
Attn: David Fitzgerald				Telephone: 870-942-2722		Routine		TEST PARAMETERS								Bottle Type Code	
				Fax: 870-942-1937		Preservative Code: 1		1								G = Glass; P = Plastic	
				Email: sheridanwater@windstream.net		Bottle Type: P		P								V = Septum; A = Amber	
Allen Parker				Allen Parker												Arkansas Analytical Work Order Number:	
Sampler(s) Signature				Sampler(s) Printed													
Field Number	SAMPLE COLLECTION		Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION		Chronic Toxicity								
	3/9-10/14	1000-0900		X	24	Water	Final Discharge		X						K1403001C		
1. Relinquished by: (Signature)			Date/Time		2. Received by: (Signature)			SAMPLE CONDITION UPON RECEIPT IN LAB				REMARKS / SAMPLE COMMENTS					
Allen Parker			1353 3-10-14		[Signature]			1. CUSTODY SEALS: <input checked="" type="checkbox"/> Yes ___ No									
3. Relinquished by: (Signature)			Date/Time		4. Received by lab: (Signature)			2. CONTAINERS CORRECT: <input type="checkbox"/> Yes ___ No									
[Signature]			[Signature]		Sidney J. James			3. COC/LABELS AGREE: <input type="checkbox"/> Yes ___ No									
								4. RECEIVED ON ICE: <input type="checkbox"/> Yes ___ No									
								5. TEMPERATURE ON RECEIPT: 2°C									
								6. TEMPERATURE GUN ID: HHT #2									
FOR COMPLETION BY LAB ONLY																	

APPENDIX B

Effluent and Dilution Water Data

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Fathead Minnow

Lab # / Sample ID K1403001

Test Start (Date/Time) 3-6-14 11:20

Client: Sheridan

Test End (Date/Time) 3-13-14 11:30

Day of Test

		1	2	3	4	5	6	7	notes/remarks
Control	MHS551	3-6	3-7	3-8	3-9	3-10	3-11	3-12	
D.O. (mg/L)	INITIAL	8.5	8.4	8.1	8.6	8.5	8.4	8.3	
	FINAL	8.1	8.2	8.4	8.0	7.8	7.6	7.8	
pH (s.u.)	INITIAL	7.6	7.5	7.6	7.8	7.7	7.8	7.7	
	FINAL	7.2	7.8	8.0	7.7	7.8	7.6	7.7	
temp (C)	INITIAL	22	23	22.2	21.3	22	23	22	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		64							
HARDNESS (mg/L)		94							
CONDUCTIVITY (umhos/cm)		325							
CHLORINE (mg/L)		<0.05							
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.4	8.7	8.7	8.6	8.7	8.8	
	FINAL	8.1	8.1	8.3	7.8	7.8	7.6	7.5	
pH (s.u.)	INITIAL	7.4	7.5	7.6	7.9	7.8	7.7	7.8	
	FINAL	7.3	7.8	8.0	8.0	7.7	7.7	7.9	
temp (C)	INITIAL	23	22	22.5	22.6	23	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.5	8.5	8.6	8.6	8.8	8.8	8.8	
	FINAL	8.1	8.0	8.4	8.1	7.7	7.5	7.8	
pH (mg/L)	INITIAL	7.4	7.5	7.6	7.9	7.8	7.8	7.8	
	FINAL	7.3	7.9	8.0	7.6	7.7	7.6	7.6	
temp (C)	INITIAL	22	23	22.7	22.8	23	23	23	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.5	8.6	8.5	8.6	8.6	8.7	8.6	
	FINAL	8.0	7.9	8.3	8.0	7.7	7.7	7.5	
pH (s.u.)	INITIAL	7.3	7.5	7.6	7.8	7.8	7.5	7.5	
	FINAL	7.2	7.9	8.0	7.5	7.7	7.7	7.4	
temp (C)	INITIAL	22	23	22.9	22.9	23	23	23	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.5	8.5	8.6	8.6	8.6	8.6	
	FINAL	8.1	7.9	8.2	7.9	7.6	7.5	7.8	
pH (s.u.)	INITIAL	7.3	7.4	7.6	7.8	7.7	7.8	7.7	
	FINAL	7.2	7.9	8.0	7.5	7.7	7.6	7.6	
temp (C)	INITIAL	23	22	23.2	23.0	22	23	22	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.5	8.4	8.5	8.6	8.8	8.7	8.6	
	FINAL	8.0	7.9	8.2	8.0	7.6	7.5	7.5	
pH (s.u.)	INITIAL	7.2	7.3	7.6	7.8	7.7	7.8	7.6	
	FINAL	7.3	7.9	8.0	7.5	7.6	7.5	7.8	
temp (C)	INITIAL	22	23	23.4	23.5	23	22	23	
	FINAL	25	25	25	25	25	25	25	
CONC: 100%		A	A	A	B	B	C	C	
ALKALINITY (mg/L)		184			94		122		
HARDNESS (mg/L)		54			68		100		
CONDUCTIVITY (umhos/cm)		445			435		438		
CHLORINE (mg/L)		<0.05							

MHS

6

8

11

14

19

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Ceriodaphnia Dubia

Lab # / Sample ID K140300

Test Start (Date/Time) 3-6-14

1615

Client: Sheridan

Test End (Date/Time) 3-13-14

0945

Day of Test

		1	2	3	4	5	6	7	notes/remarks
Control	MHS551	3-6	3-7	3-8	3-9	3-10	3-11	3-12	
D.O. (mg/L)	INITIAL	8.5	8.4	8.1	8.6	8.5	8.4	8.3	
	FINAL	8.6	8.4	8.3	8.0	7.9	8.3	8.4	
pH (s.u.)	INITIAL	7.6	7.5	7.6	7.8	7.7	7.8	7.7	
	FINAL	7.5	8.1	7.7	7.9	7.8	8.0	7.9	
temp (C)	INITIAL	22	23	22.2	21.3	22	23	22	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		64							
HARDNESS (mg/L)		94							
CONDUCTIVITY (umhos/cm)		325							
CHLORINE (mg/L)		<0.05							
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.4	8.7	8.7	8.6	8.7	8.8	
	FINAL	8.5	8.3	8.2	8.0	7.9	8.2	8.3	
pH (s.u)	INITIAL	7.4	7.5	7.6	7.9	7.8	7.7	7.8	
	FINAL	7.6	8.1	7.8	7.9	7.8	7.9	7.9	
temp (C)	INITIAL	23	22	22.5	22.6	23	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.5	8.5	8.6	8.6	8.8	8.8	8.8	
	FINAL	8.4	8.3	8.2	8.2	7.9	8.4	8.3	
pH (mg/L)	INITIAL	7.4	7.5	7.6	7.9	7.8	7.8	7.8	
	FINAL	7.5	8.0	7.7	8.1	7.8	7.9	7.9	
temp (C)	INITIAL	22	23	22.7	22.8	23	23	23	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.5	8.6	8.5	8.6	8.6	8.7	8.6	
	FINAL	8.5	8.3	8.2	8.0	8.0	8.5	8.4	
pH (s.u.)	INITIAL	7.3	7.5	7.6	7.8	7.8	7.5	7.5	
	FINAL	7.6	8.0	7.8	7.8	7.9	7.9	7.8	
temp (C)	INITIAL	22	23	22.9	22.9	23	23	23	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.4	8.5	8.5	8.6	8.6	8.6	8.6	
	FINAL	8.4	8.3	8.3	8.1	7.8	8.5	8.5	
pH (s.u.)	INITIAL	7.3	7.4	7.6	7.8	7.7	7.8	7.7	
	FINAL	7.5	8.0	8.0	7.7	7.6	7.9	7.9	
temp (C)	INITIAL	23	22	23.2	23.0	22	23	22	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.5	8.4	8.5	8.6	8.8	8.7	8.6	
	FINAL	8.5	8.3	8.2	8.0	7.9	8.4	8.3	
pH (s.u.)	INITIAL	7.2	7.3	7.6	7.8	7.7	7.8	7.6	
	FINAL	7.5	8.0	8.0	7.8	7.7	7.8	7.9	
temp (C)	INITIAL	22	23	23.4	23.5	23	22	23	
	FINAL	25	25	25	25	25	25	25	
CONC: 100%									
		A	A	A	B	B	C	C	
ALKALINITY (mg/L)		184			94		122		
HARDNESS (mg/L)		54			68		100		
CONDUCTIVITY (umhos/cm)		445			435		438		
CHLORINE (mg/L)		<0.05							

APPENDIX C

Fathead minnow raw data and statistics

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		TEST START DATE		TIME							
CLIENT		TEST END DATE		TIME							
AGE AND SOURCE OF MINNOWS											
DAY (NUMBER SURVIVING)										SURVIVAL	
REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
MHS	CONC: A	8	8	8	8	8	8	8	100%	97.5%	5.73%
	B	8	8	8	8	8	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	8	100%		
6	CONC: A	8	8	8	8	8	8	8	100%	100%	
	B	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	100%		
	D	8	8	8	8	8	8	8	100%		
	E	8	8	8	8	8	8	8	100%		
8	CONC: A	8	8	8	8	8	8	8	100%	100%	
	B	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	100%		
	D	8	8	8	8	8	8	8	100%		
	E	8	8	8	8	8	8	8	100%		
11	CONC: A	8	8	8	8	8	8	8	100%	100%	
	B	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	100%		
	D	8	8	8	8	8	8	8	100%		
	E	8	8	8	8	8	8	8	100%		
14	CONC: A	8	8	8	8	8	8	8	100%	100%	0%
	B	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	100%		
	D	8	8	8	8	8	8	8	100%		
	E	8	8	8	8	8	8	8	100%		
19	CONC: A	8	8	8	8	8	8	8	100%	95%	
	B	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	100%		
	D	8	8	8	8	8	8	7	87.5%		
	E	8	8	8	8	8	8	7	87.5%		
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 K1403001 TEST START DATE 3-6-14 TIME 1620
 CLIENT Sheridan A TEST END DATE 3-13-14 TIME 1130
 AGE AND SOURCE OF MINNOWS

		DAY (NUMBER SURVIVING)								SURVIVAL	
REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
MHS	CONC: A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1	1		
6	CONC: A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1	1		
8	CONC: A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1	1		
11	CONC: A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1	1		
14	CONC: A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1	1		
19	CONC: A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1	1		
ANALYST		RH	RH	TC	TC	RH	RH	RH	RH		
DATE:		3-6-14	3-7-14	3-8-14	3-9-14	3-10-14	3-11-14	3-12-14	3-13-14		
TIME:		1620	1100	1420	1035	1130	1130	1500	1130		

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 Sheridan B		TEST END DATE		TIME						
		AGE AND SOURCE OF MINNOWS							SURVIVAL	
		DAY (NUMBER SURVIVING)								
REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
MHS	CONC: A	2	2	2	2	2	2	1	1	
	B	1	1	1	1	1	2	2		
	C	1	1	1	1	1	2	2		
	D	1	1	1	1	1	2	2		
	E	1	1	1	1	1	2	2		
6	CONC: A	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1		
8	CONC: A	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1		
11	CONC: A	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1		
14	CONC: A	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1		
19	CONC: A	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1		
	E	1	1	1	1	1	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 Sheridan C		TEST END DATE		TIME							
AGE AND SOURCE OF MINNOWS											
DAY (NUMBER SURVIVING)										SURVIVAL	
REP #	start	1	2	3	4	5	6	7%	MEAN %	CV	
MHS	CONC: A	2	2	2	2	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓			
	E										
6	CONC: A	2	2	2	2	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓			
	E										
8	CONC: A	2	2	2	2	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓			
	E										
11	CONC: A	2	2	2	2	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓			
	E										
14	CONC: A	2	2	2	2	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓			
	E										
19	CONC: A	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 Sheridan D		TEST END DATE		TIME						
AGE AND SOURCE OF MINNOWS										
DAY (NUMBER SURVIVING)										
REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
MHS	CONC: A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C									
	D	↓	↓	↓	↓	↓	↓	↓		
	E									
6	CONC: A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C									
	D	↓	↓	↓	↓	↓	↓	↓		
	E									
8	CONC: A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C									
	D	↓	↓	↓	↓	↓	↓	↓		
	E									
11	CONC: A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C									
	D	↓	↓	↓	↓	↓	↓	↓		
	E									
14	CONC: A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C									
	D	↓	↓	↓	↓	↓	↓	↓		
	E									
19	CONC: A	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 Sheridan E		TEST END	DATE	TIME							
AGE AND SOURCE OF MINNOWS											
DAY (NUMBER SURVIVING)							SURVIVAL				
REP #	start	1	2	3	4	5	6	7%	MEAN %	CV	
MHS	CONC: A	2	2	2	2	2	3	2	2		
	B	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓			
	E										
6	CONC: A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓			
	E										
8	CONC: A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓			
	E										
11	CONC: A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓			
	E										
14	CONC: A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓			
	E										
19	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

AA# K1403001, FATHEAD MINNOW SURVIVAL, CHRONIC, 3-6-14

File: C:\COPYTO~1\TOXSTAT\FHSURV~1.

Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.067

W = 0.714

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# K1403001, FATHEAD MINNOW SURVIVAL, CHRONIC, 3-6-14

File: C:\COPYTO~1\TOXSTAT\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# K1403001, FATHEAD MINNOW SURVIVAL, CHRONIC, 3-6-14
 FILE: C:\COPYTO~1\TOXSTAT\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	0.8750	1.2094
1	CONTROL	3	1.0000	1.3931
1	CONTROL	4	1.0000	1.3931
1	CONTROL	5	1.0000	1.3931
2	6 % EFFLUENT	1	1.0000	1.3931
2	6 % EFFLUENT	2	1.0000	1.3931
2	6 % EFFLUENT	3	1.0000	1.3931
2	6 % EFFLUENT	4	1.0000	1.3931
2	6 % EFFLUENT	5	1.0000	1.3931
3	8 % EFFLUENT	1	1.0000	1.3931
3	8 % EFFLUENT	2	1.0000	1.3931
3	8 % EFFLUENT	3	1.0000	1.3931
3	8 % EFFLUENT	4	1.0000	1.3931
3	8 % EFFLUENT	5	1.0000	1.3931
4	11 % EFFLUENT	1	1.0000	1.3931
4	11 % EFFLUENT	2	1.0000	1.3931
4	11 % EFFLUENT	3	1.0000	1.3931
4	11 % EFFLUENT	4	1.0000	1.3931
4	11 % EFFLUENT	5	1.0000	1.3931
5	14 % EFFLUENT	1	1.0000	1.3931
5	14 % EFFLUENT	2	1.0000	1.3931
5	14 % EFFLUENT	3	1.0000	1.3931
5	14 % EFFLUENT	4	1.0000	1.3931
5	14 % EFFLUENT	5	1.0000	1.3931
6	19 % EFFLUENT	1	1.0000	1.3931
6	19 % EFFLUENT	2	1.0000	1.3931
6	19 % EFFLUENT	3	1.0000	1.3931
6	19 % EFFLUENT	4	0.8750	1.2094
6	19 % EFFLUENT	5	0.8750	1.2094

AA# K1403001, FATHEAD MINNOW SURVIVAL, CHRONIC, 3-6-14
 File: C:\COPYTO~1\TOXSTAT\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	1.356				
2	6 % EFFLUENT	1.393	30.00	16.00	5.00	
3	8 % EFFLUENT	1.393	30.00	16.00	5.00	
4	11 % EFFLUENT	1.393	30.00	16.00	5.00	
5	14 % EFFLUENT	1.393	30.00	16.00	5.00	
6	19 % EFFLUENT	1.320	25.00	16.00	5.00	

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

Pimephales promelas

FATHEAD MINNOW

TEST 1000.0

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:		K1403001				TEST DATES (BEGIN / END):		3/6 - 13/14	
CLIENT:		Sheridan				WEIGHING DATE / TIME:		3/14/2014 1115	
ANALYSTS:		RH				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	0.97802	0.97501	0.00301	8	0.376	AVG DRY WEIGHT (mg)	0.445	CV
	B	1.02754	1.02385	0.00369	8	0.461			
	C	0.99469	0.99109	0.00360	8	0.450			
	D	0.97019	0.96623	0.00396	8	0.495			
	E	0.97527	0.97174	0.00353	8	0.441	9.76		
6%	A	0.99330	0.99014	0.00316	8	0.395	AVG DRY WEIGHT (mg)	0.454	CV
	B	0.98817	0.98498	0.00319	8	0.399			
	C	0.94314	0.93948	0.00366	8	0.458			
	D	0.98492	0.98082	0.00410	8	0.512			
	E	0.97828	0.97424	0.00404	8	0.505			
8%	A	0.97064	0.96710	0.00354	8	0.442	AVG DRY WEIGHT (mg)	0.438	CV
	B	0.95882	0.95563	0.00319	8	0.399			
	C	0.97799	0.97471	0.00328	8	0.410			
	D	0.97756	0.97361	0.00395	8	0.494			
	E	0.97506	0.97152	0.00354	8	0.442			
11%	A	0.95191	0.94854	0.00337	8	0.421	AVG DRY WEIGHT (mg)	0.497	CV
	B	0.97698	0.97260	0.00438	8	0.547			
	C	0.97181	0.96806	0.00375	8	0.469			
	D	0.95571	0.95188	0.00383	8	0.479			
	E	0.96523	0.96068	0.00455	8	0.569			
14%	A	0.97291	0.96912	0.00379	8	0.474	AVG DRY WEIGHT (mg)	0.479	CV
	B	0.98768	0.98416	0.00352	8	0.440			
	C	0.98410	0.98023	0.00387	8	0.484			
	D	0.99380	0.99014	0.00366	8	0.458			
	E	0.97504	0.97072	0.00432	8	0.540	7.92		
19%	A	0.95542	0.95169	0.00373	8	0.466	AVG DRY WEIGHT (mg)	0.457	CV
	B	1.00157	0.99788	0.00369	8	0.461			
	C	0.97319	0.96925	0.00394	8	0.493			
	D	0.94993	0.94677	0.00316	8	0.395			
	E	0.97163	0.96786	0.00377	8	0.471			

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA# K1403001, FATHEAD MINNOW GROWTH CHRONIC, 3-6-14

File: C:\COPYTO~1\TOXSTAT\FHGROWTH.

Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.051

W = 0.952

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# K1403001, FATHEAD MINNOW GROWTH CHRONIC, 3-6-14

File: C:\COPYTO~1\TOXSTAT\FHGROWTH.

Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 1.84

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# K1403001, FATHEAD MINNOW GROWTH CHRONIC, 3-6-14
 FILE: C:\COPYTO~1\TOXSTAT\FHGWGROWTH.
 TRANSFORM: NO TRANSFORMATION

NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.3760	0.3760
1	CONTROL	2	0.4610	0.4610
1	CONTROL	3	0.4500	0.4500
1	CONTROL	4	0.4950	0.4950
1	CONTROL	5	0.4410	0.4410
2	6 % EFFLUENT	1	0.3950	0.3950
2	6 % EFFLUENT	2	0.3990	0.3990
2	6 % EFFLUENT	3	0.4580	0.4580
2	6 % EFFLUENT	4	0.5120	0.5120
2	6 % EFFLUENT	5	0.5050	0.5050
3	8 % EFFLUENT	1	0.4420	0.4420
3	8 % EFFLUENT	2	0.3990	0.3990
3	8 % EFFLUENT	3	0.4100	0.4100
3	8 % EFFLUENT	4	0.4940	0.4940
3	8 % EFFLUENT	5	0.4420	0.4420
4	11 % EFFLUENT	1	0.4210	0.4210
4	11 % EFFLUENT	2	0.5470	0.5470
4	11 % EFFLUENT	3	0.4690	0.4690
4	11 % EFFLUENT	4	0.4790	0.4790
4	11 % EFFLUENT	5	0.5690	0.5690
5	14 % EFFLUENT	1	0.4740	0.4740
5	14 % EFFLUENT	2	0.4400	0.4400
5	14 % EFFLUENT	3	0.4840	0.4840
5	14 % EFFLUENT	4	0.4580	0.4580
5	14 % EFFLUENT	5	0.5400	0.5400
6	19 % EFFLUENT	1	0.4660	0.4660
6	19 % EFFLUENT	2	0.4610	0.4610
6	19 % EFFLUENT	3	0.4930	0.4930
6	19 % EFFLUENT	4	0.3950	0.3950
6	19 % EFFLUENT	5	0.4710	0.4710

AA# K1403001, FATHEAD MINNOW GROWTH CHRONIC, 3-6-14
 File: C:\COPYTO~1\TOXSTAT\FHGWGROWTH. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.013	0.003	1.179
Within (Error)	24	0.051	0.002	
Total	29	0.064		

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

AA# K1403001, FATHEAD MINNOW GROWTH CHRONIC, 3-6-14

File: C:\COPYTO~1\TOXSTAT\FHGROWTH.

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	0.445	0.445		
2	6 % EFFLUENT	0.454	0.454	-0.315	
3	8 % EFFLUENT	0.437	0.437	0.246	
4	11 % EFFLUENT	0.497	0.497	-1.793	
5	14 % EFFLUENT	0.479	0.479	-1.184	
6	19 % EFFLUENT	0.457	0.457	-0.431	

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

AA# K1403001, FATHEAD MINNOW GROWTH CHRONIC, 3-6-14

File: C:\COPYTO~1\TOXSTAT\FHGROWTH.

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	5			
2	6 % EFFLUENT	5	0.069	15.5	-0.009
3	8 % EFFLUENT	5	0.069	15.5	0.007
4	11 % EFFLUENT	5	0.069	15.5	-0.052
5	14 % EFFLUENT	5	0.069	15.5	-0.035
6	19 % EFFLUENT	5	0.069	15.5	-0.013

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

SURVIVAL AND REPRODUCTION TEST

Ceriodaphnia dubia

Discharger: Sheridan Lab Number/s: K1403001
 Location: See Col

Analyst: RH
 Test Start - Date/Time: 3-6-14 1615
 Test Stop - Date/Time: 3-13-14 0945

Conc %	Day	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		A	B	C	D	E	F	G	H	I	J				
MHS	1	0	0	0	0	0	0	0	0	0	0	0	10	0	TC
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	0	0	0	1	1	0	0	0	2	1	5	10	0.5	
	4	5	0	4	1	2	6	4	5	0	3	30	10	3.0	
	5	4	2	1	4	5	6	1	7	8	0	38	10	3.8	
	6	7	4	8	2	8	1	7	0	4	11	52	10	5.2	
	7	0	9	5	3	5	3	2	10	3	2	42	10	4.2	
	8														
Total		16	15	18	11	21	16	14	22	17	17	167		$\bar{X}=16.7$ $CV=19.2$	

Conc %	Day	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		A	B	C	D	E	F	G	H	I	J				
11	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	0	0	0	0	0	0	0	0	0	0	0	10	0	
	4	1	1	5	6	4	0	0	3	7	2	29	10	2.9	
	5	5	3	0	6	7	3	7	8	0	0	39	10	3.9	
	6	5	8	9	4	1	5	3	2	9	0	46	10	4.6	
	7	1	4	3	0	7	4	11	5	4	8	47	10	4.7	
	8														
Total		12	16	17	16	19	12	21	18	20	11	162		$\bar{X}=16.2$ $CV=21.7$	

Conc %	Day	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		A	B	C	D	E	F	G	H	I	J				
6	1	0	0	0	0	0	0	0	0	0	0	0	10	0	TC
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	2	0	1	0	0	0	0	0	1	1	5	10	0.5	
	4	1	0	2	6	2	4	4	2	0	0	21	10	2.1	
	5	3	8	0	0	2	1	6	0	3	7	30	10	3.0	
	6	11	8	9	6	0	5	7	4	10	6	66	10	6.6	
	7	3	4	5	2	10	6	4	5	0	0	39	10	3.9	
	8														
Total		20	20	17	14	14	16	21	11	14	14	161		$\bar{X}=16.1$ $CV=20.6$	

Conc %	Day	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		A	B	C	D	E	F	G	H	I	J				
14	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	0	0	0	1	0	3	10	0.3	
	4	6	5	4	5	4	3	0	0	0	5	32	10	3.2	
	5	5	7	7	1	0	0	2	6	9	5	42	10	4.2	
	6	4	0	5	12	8	1	7	3	5	8	53	10	5.3	
	7	0	5	4	4	6	10	3	4	2	0	38	10	3.8	
	8														
Total		16	18	20	22	18	14	12	13	17	18	168		$\bar{X}=16.8$ $CV=18.6$	

Conc %	Day	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		A	B	C	D	E	F	G	H	I	J				
8	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	0	0	0	0	0	1	0	0	2	4	10	0.4	
	4	1	5	0	2	1	0	2	6	3	4	24	10	2.4	
	5	6	0	0	2	5	2	3	4	7	6	41	10	4.1	
	6	4	11	4	5	2	0	1	2	9	0	38	10	3.8	
	7	5	2	6	3	9	7	5	5	2	0	44	10	4.4	
	8														
Total		17	18	10	18	17	9	12	17	21	12	151		$\bar{X}=15.1$ $CV=26.6$	

Conc %	Day	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		A	B	C	D	E	F	G	H	I	J				
19	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	0	0	0	0	0	1	0	0	0	3	10	0.3	
	4	6	1	0	4	0	3	3	6	4	5	32	10	3.2	
	5	7	5	6	0	9	7	8	6	0	0	42	10	4.2	
	6	0	0	8	9	7	8	9	5	3	6	55	10	5.5	
	7	4	5	3	6	6	4	0	2	0	4	34	10	3.4	
	8														
Total		18	11	17	19	23	16	21	19	7	15	166		$\bar{X}=16.6$ $CV=28.4$	

X = Dead

AA # K1403001, C. DUBIA CHRONIC, REPRODUCCION, 3-6-14
File: C:\COPYTO~1\TOXSTAT\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 # K1403001, C. DUBIA CHRONIC, REPRODUCCION, 3-6-14
File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 2.34

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
6	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
8	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
11	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
14	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
19	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	6	10	0	
2	8	10	0	
3	11	10	0	
4	14	10	0	
5	19	10	0	

TITLE: AA # K1403001, C. DUBIA CHRONIC, REPRODUCCION, 3-6-14
 FILE: C:\COPYTO~1\TOXSTAT\C.DUB
 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	16.0000	16.0000
1	CONTROL	2	15.0000	15.0000
1	CONTROL	3	18.0000	18.0000
1	CONTROL	4	11.0000	11.0000
1	CONTROL	5	21.0000	21.0000
1	CONTROL	6	16.0000	16.0000
1	CONTROL	7	14.0000	14.0000
1	CONTROL	8	22.0000	22.0000
1	CONTROL	9	17.0000	17.0000
1	CONTROL	10	17.0000	17.0000
2	6 % EFFLUENT	1	20.0000	20.0000
2	6 % EFFLUENT	2	20.0000	20.0000
2	6 % EFFLUENT	3	17.0000	17.0000
2	6 % EFFLUENT	4	14.0000	14.0000
2	6 % EFFLUENT	5	14.0000	14.0000
2	6 % EFFLUENT	6	16.0000	16.0000
2	6 % EFFLUENT	7	21.0000	21.0000
2	6 % EFFLUENT	8	11.0000	11.0000
2	6 % EFFLUENT	9	14.0000	14.0000
2	6 % EFFLUENT	10	14.0000	14.0000
3	8 % EFFLUENT	1	17.0000	17.0000
3	8 % EFFLUENT	2	18.0000	18.0000
3	8 % EFFLUENT	3	10.0000	10.0000
3	8 % EFFLUENT	4	18.0000	18.0000
3	8 % EFFLUENT	5	17.0000	17.0000
3	8 % EFFLUENT	6	9.0000	9.0000
3	8 % EFFLUENT	7	12.0000	12.0000
3	8 % EFFLUENT	8	17.0000	17.0000
3	8 % EFFLUENT	9	21.0000	21.0000
3	8 % EFFLUENT	10	12.0000	12.0000
4	11 % EFFLUENT	1	12.0000	12.0000
4	11 % EFFLUENT	2	16.0000	16.0000
4	11 % EFFLUENT	3	17.0000	17.0000
4	11 % EFFLUENT	4	16.0000	16.0000
4	11 % EFFLUENT	5	19.0000	19.0000
4	11 % EFFLUENT	6	12.0000	12.0000
4	11 % EFFLUENT	7	21.0000	21.0000
4	11 % EFFLUENT	8	18.0000	18.0000

4	11 % EFFLUENT	9	20.0000	20.0000
4	11 % EFFLUENT	10	11.0000	11.0000
5	14 % EFFLUENT	1	16.0000	16.0000
5	14 % EFFLUENT	2	18.0000	18.0000
5	14 % EFFLUENT	3	20.0000	20.0000
5	14 % EFFLUENT	4	22.0000	22.0000
5	14 % EFFLUENT	5	18.0000	18.0000
5	14 % EFFLUENT	6	14.0000	14.0000
5	14 % EFFLUENT	7	12.0000	12.0000
5	14 % EFFLUENT	8	13.0000	13.0000
5	14 % EFFLUENT	9	17.0000	17.0000
5	14 % EFFLUENT	10	18.0000	18.0000
6	19 % EFFLUENT	1	18.0000	18.0000
6	19 % EFFLUENT	2	11.0000	11.0000
6	19 % EFFLUENT	3	17.0000	17.0000
6	19 % EFFLUENT	4	19.0000	19.0000
6	19 % EFFLUENT	5	23.0000	23.0000
6	19 % EFFLUENT	6	16.0000	16.0000
6	19 % EFFLUENT	7	21.0000	21.0000
6	19 % EFFLUENT	8	19.0000	19.0000
6	19 % EFFLUENT	9	7.0000	7.0000
6	19 % EFFLUENT	10	15.0000	15.0000

AA # K1403001, C. DUBIA CHRONIC, REPRODUCCION, 3-6-14
 File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	19.750	3.950	0.290
Within (Error)	54	735.500	13.620	
Total	59	755.250		

Critical F value = 2.45 (0.05,5,40)
 Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All equal

AA # K1403001, C. DUBIA CHRONIC, REPRODUCCION, 3-6-14
 File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 1 OF 2 H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	16.700	16.700		
2	6 % EFFLUENT	16.100	16.100	0.364	
3	8 % EFFLUENT	15.100	15.100	0.969	
4	11 % EFFLUENT	16.200	16.200	0.303	
5	14 % EFFLUENT	16.800	16.800	-0.061	
6	19 % EFFLUENT	16.600	16.600	0.061	

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

AA # K1403001, C. DUBIA CHRONIC, REPRODUCTION, 3-6-14

File: C:\COPYTO~1\TOXSTAT\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	6 % EFFLUENT	10	3.813	22.8	0.600
3	8 % EFFLUENT	10	3.813	22.8	1.600
4	11 % EFFLUENT	10	3.813	22.8	0.500
5	14 % EFFLUENT	10	3.813	22.8	-0.100
6	19 % EFFLUENT	10	3.813	22.8	0.100

APPENDIX E

Organism History

AQUATOX, INC.

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

TEST ORGANISM HISTORY

DATE SHIPPED 3/6/14 CLIENT AR Analytical

Purchase Order #: _____ Ryan

SPECIES: Pimephales promelas

Quantity Shipped: 240 +

Age: hatched 3/5/14 15-1600
CT

Brood Stock Source: Anderson Farms, AR

Culture Water: Groundwater 160

Hardness (Mg/l CaCO3): _____

Dissolved Oxygen (Mg/l): 8.1

Temperature (°C): 25.10C

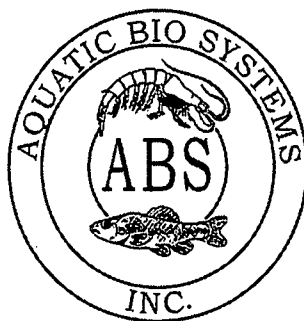
Feeding: Artemia

Comments: _____

Shipped Via: Federal Express UPS Overnight Shuttle

Packaged By: _____

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



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

ORGANISM HISTORY

DATE: 11/25/2013

SPECIES: Ceriodaphnia dubia

AGE: > 3 day

LIFE STAGE: Adult


HATCH DATE: Variable

BEGAN FEEDING: Immediately

FOOD: YTC, Selenastrum sp.

Water Chemistry Record:	Current	Range
TEMPERATURE:	<u>22°C</u>	<u>22-26°C</u>
SALINITY/CONDUCTIVITY:	<u>--</u>	<u>--</u>
TOTAL HARDNESS (as CaCO ₃):	<u>94 mg/l</u>	<u>76-130 mg/l</u>
TOTAL ALKALINITY (as CaCO ₃):	<u>65 mg/l</u>	<u>65-100 mg/l</u>
pH:	<u>7.98</u>	<u>7.50-8.20</u>

Comments:

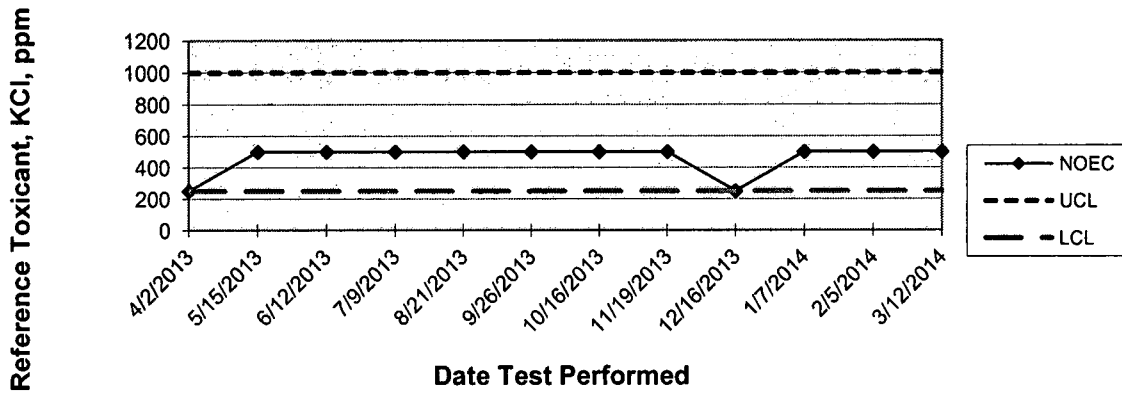


Facility Supervisor

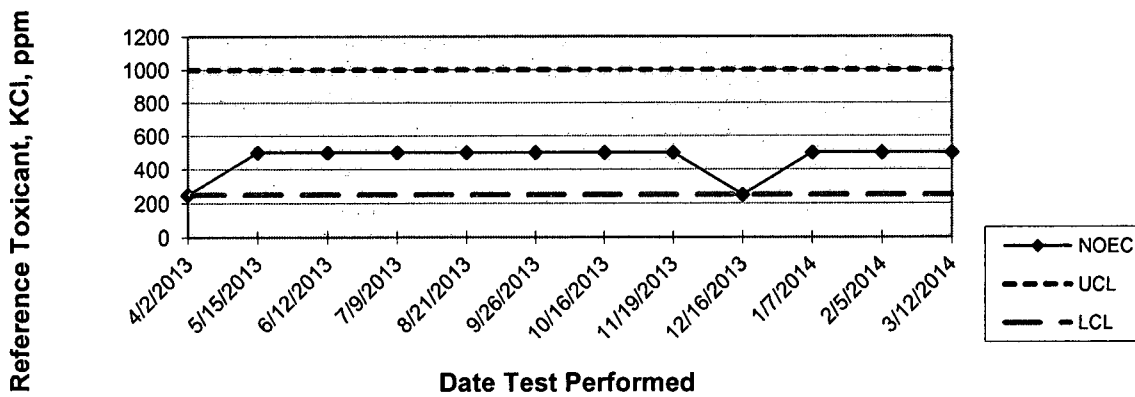
APPENDIX F

Quality Assurance Charts

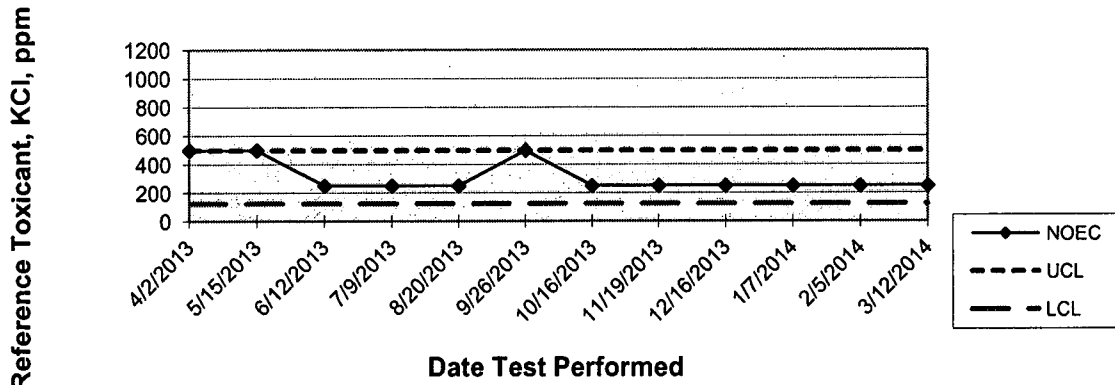
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW SURVIVAL 7 Day
QUALITY ASSURANCE



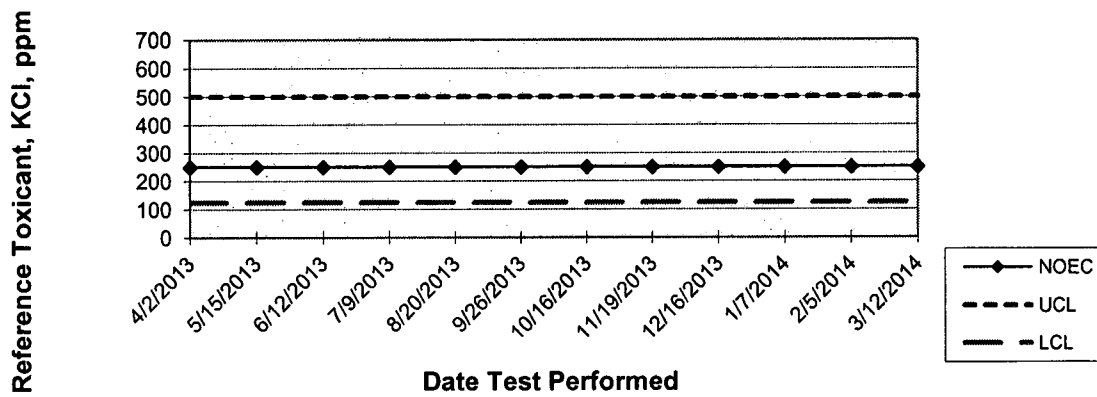
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW GROWTH 7 Day
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE



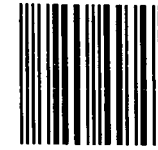
Sheridan Water Works
PO Box 486
Sheridan, AR 72150

PLACE STICKER AT TOP OF ENVELOPE TO THE RIGHT
OF THE RETURN ADDRESS, FOLD AT DOTTED LINE

CERTIFIED MAIL™



7012 2920 0000 8597 3407



U.S. POSTAGE
PAID
SHERIDAN, AR
72150
APR 17, 14
AMOUNT

\$8.66
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72118

ADEQ
NPDES Enforcement Branch
5301 Northshore Dr
No Little Rock, AR 72118-5317

RETURN RECEIPT
REQUESTED

