

Arkansas Analytical, Inc.

Toxicity Test Results

**CITY of SHERIDAN
NPDES PERMIT NUMBER: AR0034347
Fourth Quarter 2015
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.**
8100 National Drive
Little Rock, Arkansas 72209
Lab Number K1511010

Tuesday, December 22, 2015

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 fourth quarter of 2015.

Plant Operations

To be provided by permittee.

Source of Effluent and Dilution Water

Effluent sample was collected as follows:

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	11-18-15, 1126	11-19-15, 1026
Sample #2	11-19-15, 1105	11-20-15, 0605

*Due to a cessation of flow, only two samples were collected.

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:	11-19-15, 1216	1
Sample #2	11-20-15, 1512	0

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 10%, 13%, 17%, 23%, and 31%. The low-flow effluent concentration (**critical dilution**) was defined as **23% 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	95%	X	
The percent coefficient of variation between replicates must be 40% or less for survival	11.8%	X	
Minimum of 0.25 mg average dry weight of surviving controls	0.615	X	
The percent coefficient of variation between replicates must be 40% or less for growth	10.8%	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	80%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	28.5%	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> 11/10-17/15		<i>Pimephales promelas</i> 11/10-17/15	
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>Ceriodaphnia dubia</i>		<i>Pimephales promelas</i>	
NOEC / LOEC Survival	31% / NA	NOEC / LOEC survival	31% / NA
NOEC / LOEC Reproduction	31% / NA	NOEC / LOEC growth	31% / NA
Mean number of neonates (critical dilution)	19.6	%CV survival (critical dilution)	7.21%
%CV Reproduction (critical dilution)	13.4%	Mean dry weight (critical dilution) in milligrams	0.587
		%CV growth (critical dilution)	5.16%
PMSD Reproduction	26.3%	PMSD Growth	18.5%

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 23% effluent**. The effluent samples **did not** exhibit lethal or sublethal effects at the critical dilution, and, as such, **passed** the test.

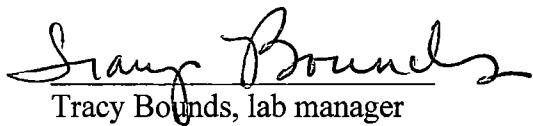
Chronic static renewal survival and reproduction test using *Ceriodaphnia dubia*, (Method 1002.0)

The permit issued to the City of Sheridan, AR0034347, specifies that the **critical dilution is 23% effluent**. The effluent samples **did not** exhibit lethal or sublethal effects at the critical dilution, and, as such, **passed** the test.

Biomonitoring Analyst:

Ryan Hudgin, Tracy Bounds, Jason Bird, Melissa Bird, Chris Turney

Reviewed by:



Tracy Bounds, lab manager

**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:	11-18-15, 1126	11-19-15, 1026
Sample #2	11-19-15, 1105	11-20-15, 0605

Test initiated (date, time): 11-19-15, 1335 Test terminated (date, time): 11-26-15, 0920

Dilution water used: Moderately Hard Synthetic

DATA TABLE FOR FATHEAD MINNOW SURVIVAL

Effluent Conc %	Percent Survival in Replicate Chambers					Mean Percent Survival				CV %
	A	B	C	D	E	24 hours	48 hours	7 days		
0%	75	100	100	100	100	95	95	95	11.8%	
10%	100	87.5	100	75	100	100	97.5	92.5		
13%	100	87.5	100	87.5	100	100	97.5	95		
17%	87.5	100	87.5	87.5	100	97.5	97.5	92.5		
23%	100	87.5	100	87.5	100	100	100	95	7.21%	
31%	100	87.5	100	87.5	87.5	100	100	92.5		

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%	0.516	0.601	0.690	0.606	0.659	0.614	10.8%
10%	0.610	0.485	0.709	0.536	0.666	0.601	
13%	0.535	0.503	0.710	0.520	0.713	0.596	
17%	0.554	0.581	0.638	0.526	0.730	0.606	
23%	0.586	0.573	0.561	0.577	0.639	0.587	5.16%
31%	0.559	0.581	0.650	0.573	0.540	0.581	

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, (23%) 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, (23%) 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)= **31** % effluent
 - b) NOEC growth (parameter TPP6C)= **31** % effluent
 - c) Coefficient of variation (parameter TQP6C)= **10.8** %

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:	11-18-15, 1126	11-19-15, 1026
Sample #2	11-19-15, 1105	11-20-15, 0605

Test initiated (date, time): 11-19-15, 1445 Test terminated (date, time): 11-25-15, 1500

Dilution water used: Moderately Hard Synthetic

***Ceriodaphnia dubia* SURVIVAL AND REPRODUCTION**

NUMBER OF YOUNG PRODUCED PER FEMALE @ TEST TERMINATION

PERCENT EFFLUENT

Replicate	0%	10%	13%	17%	23%	31%
A	18	17	11	17	23	19
B	12	11	13	12	18	23
C	11	10	13	16	16	22
D	17	14	14	26	20	18
E	10	17	15	15	23	17
F	19	17	12	19	22	24
G	17	20	11	7	21	15
H	16	3	18	13	18	16
I	25	15	13	15	16	16
J	22	18	25	20	19	19
Mean	16.7	14.2	14.5	16.0	19.6	18.9
Mean/surviving female	16.7	14.2	14.5	16.0	19.6	18.9
CV%*	28.5				13.4	

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%	10%	13%	17%	23%	31%
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, (23%): 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, (23%): 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)= **31** % effluent

b) NOEC reproduction (parameter TPP3B)= **31** % effluent

c) Coefficient of variation (parameter TQP3B)= **28.5** %

APPENDIX A

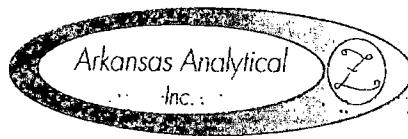
Chain of Custody Forms



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 104 W High St. Sheridan, AR 72150	Attn: David Fitzgerald	Sheridan Waterworks P.O. Box 486 Sheridan, AR 72150		Chronic Toxicity Reporting Information Telephone: 870-942-2722 Fax: 870-942-1937 Email: sheridanwater@windstream.net		1 Day (100%) 2 Day (50%) 3 Day (25%) Routine	1. Cool, 4 Degrees Centigrade 2. Sulfuric Acid (H_2SO_4), pH < 2 3. Nitric Acid (HNO_3), pH < 2			4. Thiosulfate for Dechlorination 5. Hydrochloric Acid (HCl) 6. Sodium Hydroxide (NaOH), pH > 12			
Field Number	SAMPLE COLLECTION		SAMPLE		TEST PARAMETERS						Bottle Type Code		
	Date/s	Time/s	Grab	Comp	Number of Bottles	Sample Matrix							G = Glass; P = Plastic
	11/18-19/15	1126-1026		X	24	Water							V = Septum; A = Amber
Sampler(s) Signature		Sampler(s) Printed		Chronic Toxicity						Arkansas Analytical Work Order Number:			
<i>Allen Parker</i>		<i>Allen Parker</i>								K15110107			
1. Relinquished by: (Signature)		Date/Time	2. Received by: (Signature)		SAMPLE CONDITION UPON RECEIPT IN LAB						REMARKS / SAMPLE COMMENTS		
<i>Allen Parker</i>		1216 11-19-15	<i>/</i>		1. CUSTODY SEALS: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No								
3. Relinquished by: (Signature)		Date/Time	4. Received by lab: (Signature)		2. CONTAINERS CORRECT: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No								
<i>/</i>			<i>BDG</i>		3. COC/LABELS AGREE: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No								
					4. RECEIVED ON ICE: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No								
					5. TEMPERATURE ON RECEIPT: <i>1°C</i>								
					6. TEMPERATURE GUN ID: <i>HHT772</i>								
FOR COMPLETION BY LAB ONLY													



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Sheridan Waterworks 104 W High St. Sheridan, AR 72150	Attn: David Fitzgerald	Sheridan Waterworks P.O. Box 486 Sheridan, AR 72150		Chronic Toxicity Reporting Information Telephone: 870-942-2722 Fax: 870-942-1937 Email: sheridanwater@windstream.net			1 Day (100%) 2 Day (50%) 3 Day (25%)	1. Cool, 4 Degrees Centigrade 2. Sulfuric Acid (H_2SO_4), pH < 2 3. Nitric Acid (HNO_3), pH < 2	4. Thiosulfate for Dechlorination 5. Hydrochloric Acid (HCl) 6. Sodium Hydroxide (NaOH), pH > 12						
						Routine	TEST PARAMETERS						Bottle Type Code		
						Preservative Code:	1							G = Glass; P = Plastic	
						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: <i>K15110103</i>	
Field Number	SAMPLE COLLECTION		SAMPLE		IDENTIFICATION/ DESCRIPTION										
Date/s	Time/s	Grab	Comp	Number of Bottles	Sample Matrix										
11/19-20/15	1105-1005	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>	11-20-15	<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: <input type="checkbox"/> Yes _____ No _____ 6. TEMPERATURE GUN ID: <i>0°C</i> <i>HHT#2</i>						<i>Stopped discharging water @ 0700 per David Fitzgerald. Hours 0705-1005 were not collected.</i> AP 11-20-15					
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)		FOR COMPLETION BY LAB ONLY											
<i>[Signature]</i>	<i>[Signature]</i>	<i>Amanda Johnson</i>													

APPENDIX B

Effluent and Dilution Water Data

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING							Fathead Minnow	
Lab # / Sample ID K1511010			Test Start (Date/Time)		11-19-15 1335			
Client: Sheridan			Test End (Date/Time)		11-26-15 0920			
Day of Test								
	1	2	3	4	5	6	7	notes
Control	MHS	11-19	11-20	11-21	11-22	11-23	11-24	11-25 MHS
D.O. (mg/L)	INITIAL	8.7	8.6	8.7	8.9	8.6	8.7	8.2 972
	FINAL	7.5	7.3	7.1	7.4	7.7	7.1	7.4
pH (s.u.)	INITIAL	7.9	7.5	7.7	7.1	7.5	7.4	7.2
	FINAL	7.0	7.2	7.4	7.5	7.3	7.2	7.4
temp (C)	INITIAL	22	21.4	20.8	20.4	23.8	23.7	22
	FINAL	25	25	25	25	25	25	25
ALKALINITY (mg/L)	62							→
HARDNESS (mg/L)	92							→
CONDUCTIVITY (umho)	480							↓
CHLORINE (mg/L)	10.05							→
CONC:	10							
D.O. (mg/L)	INITIAL	8.9	8.5	8.6	9.1	8.6	8.8	8.6
	FINAL	7.7	5.2	7.9	7.6	7.8	7.0	7.9
pH (s.u.)	INITIAL	7.4	7.5	7.4	7.4	7.6	7.4	7.1
	FINAL	6.9	7.0	7.1	7.5	7.3	7.2	7.3
temp (C)	INITIAL	22	21.7	21.3	21.4	22	23.7	22
	FINAL	25	25	25	25	25	25	25
CONC:	13							
D.O. (mg/L)	INITIAL	9.1	8.5	9.5	9.0	8.8	8.7	8.6
	FINAL	7.8	5.3	7.9	7.7	7.9	7.0	7.8
pH (mg/L)	INITIAL	7.3	7.5	7.4	7.4	7.6	7.4	7.2
	FINAL	6.9	7.1	7.84	7.5	7.3	7.2	7.5
temp (C)	INITIAL	22	21.7	21.5	21.7	22	23.6	2222
	FINAL	25	25	25	25	25	25	25
CONC:	17							
D.O. (mg/L)	INITIAL	8.7	8.4	8.5	8.9	8.8	8.7	8.6
	FINAL	7.8	5.3	7.8	7.8	7.8	7.0	7.7
pH (s.u.)	INITIAL	7.4	7.5	7.4	7.5	7.5	7.4	7.3
	FINAL	7.0	7.1	7.4	7.5	7.2	7.1	7.4
temp (C)	INITIAL	22	22	21.8	22.1	22	23.5	22
	FINAL	25	25	25	25	25	25	25
CONC:	23							
D.O. (mg/L)	INITIAL	8.8	8.3	8.4	8.9	8.9	8.6	8.7
	FINAL	7.8	5.4	7.8	7.8	7.8	6.9	7.7
pH (s.u.)	INITIAL	7.5	7.5	7.4	7.5	7.6	7.4	7.2
	FINAL	7.1	7.1	7.5	7.4	7.2	7.1	7.5
temp (C)	INITIAL	22	22	22.1	22.3	22	23.3	22
	FINAL	25	25	25	25	25	25	25
CONC:	31							
D.O. (mg/L)	INITIAL	8.8	8.4	8.4	8.8	8.9	8.5	8.4
	FINAL	7.9	5.6	9.0	7.8	7.7	7.0	7.4
pH (s.u.)	INITIAL	7.4	7.5	7.7	7.5	7.6	7.5	7.2
	FINAL	7.2	7.1	7.5	7.5	7.3	7.2	7.4
temp (C)	INITIAL	22	21.6	22.5	23.0	22	23.2	23
	FINAL	25	25	26	25	25	25	25
CONC: 100 %	A	A	A	B	B	B	A	
ALKALINITY (mg/L)	70			64				70
HARDNESS (mg/L)	58			82				58
CONDUCTIVITY (umho)	460			461				460
CHLORINE (mg/L)	0.09			0.09				0.09

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING								Ceriodaphnia Dubia
Lab # / Sample ID K1511010				Test Start (Date/Time)		11-19-15		1445
Client: Sheridan				Test End (Date/Time)		11-25-15		1500
Day of Test								
	1	2	3	4	5	6	7	notes
Control	11-19	11-20	11-21	11-22	11-23	11-24	11-25	MHS
D.O. (mg/L) INITIAL	8.7	8.6	8.7	8.8	8.6	8.2	8.7	8.2
	FINAL	8.3	8.3	8.1	8.1	8.4	7.8	-
pH (s.u.) INITIAL	7.9	7.5	7.7	7.1	7.5	7.2	7.4	7.2
	FINAL	7.2	7.7	7.6	7.7	7.2	7.4	-
temp (C) INITIAL	22	21.4	20.8	20.4	23.8	22.24	22	
	FINAL	25	25	25	25	25	25	-
ALKALINITY (mg/L)	62							→
HARDNESS (mg/L)	92							→
CONDUCTIVITY (umho)	480							→
CHLORINE (mg/L)	0.05							→
CONC: 10								
D.O. (mg/L) INITIAL	8.9	8.5	8.6	9.1	8.6	8.6	8.8	8.6
	FINAL	8.4	8.4	8.1	8.1	8.4	7.7	-
pH (s.u.) INITIAL	7.4	7.5	7.6	7.4	7.6	7.4	7.4	7.1
	FINAL	7.3	7.7	7.7	7.6	7.3	7.5	-
temp (C) INITIAL	22	21.7	21.3	21.4	22	22.24	22	
	FINAL	25	25	25	25	25	25	-
CONC: 13								
D.O. (mg/L) INITIAL	9.1	8.5	8.5	9.0	8.8	8.6	8.6	
	FINAL	8.4	8.3	8.0	8.3	8.4	7.6	-
pH (mg/L) INITIAL	7.3	7.5	7.6	7.4	7.6	7.2	7.4	7.2
	FINAL	7.4	7.7	7.8	7.5	7.5	7.5	-
temp (C) INITIAL	22	21.7	21.5	21.7	22	22.24	22	
	FINAL	25	25	25	25	25	25	-
CONC: 17								
D.O. (mg/L) INITIAL	8.7	8.4	8.5	8.9	8.8	8.6	8.7	8.6
	FINAL	8.2	8.3	7.6	8.1	8.6	7.7	-
pH (s.u.) INITIAL	7.4	7.5	7.6	7.5	7.5	7.3	7.4	7.3
	FINAL	7.5	7.7	7.7	7.7	7.5	7.1	-
temp (C) INITIAL	22	22	21.8	22.1	22	22.24	22	
	FINAL	25	25	25	25	25	25	-
CONC: 23								
D.O. (mg/L) INITIAL	8.8	8.3	8.4	8.9	8.9	8.7	8.6	8.7
	FINAL	8.3	8.3	8.2	8.5	8.7	7.8	-
pH (s.u.) INITIAL	7.5	7.5	7.6	7.5	7.6	7.2	7.4	7.2
	FINAL	7.5	7.8	7.5	7.6	7.6	7.1	-
temp (C) INITIAL	22	22	22.1	22.3	22	22.23	22	
	FINAL	25	25	25	25	25	25	-
CONC: 31								
D.O. (mg/L) INITIAL	8.8	8.4	8.4	8.8	8.9	8.6	8.5	8.6
	FINAL	8.5	8.3	8.2	8.4	8.6	7.7	-
pH (s.u.) INITIAL	7.4	7.5	7.7	7.5	7.6	7.2	7.5	7.2
	FINAL	7.5	7.7	7.6	7.5	7.6	7.1	-
temp (C) INITIAL	22	21.6	22.5	23.0	22	23	23	
	FINAL	25	25	25	25	25	25	-
CONC: 100 %	A	A	A	B	B	A/B	A	
ALKALINITY (mg/L)	70			64			70	
HARDNESS (mg/L)	58			82			58	
CONDUCTIVITY (umho)	460			460			460	
CHLORINE (mg/L)	0.09			0.09			0.09	

APPENDIX C

Fathead minnow raw data and statistics

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID	TEST START DATE	11-19-15	TIME	1335							
CLIENT	TEST END DATE	11-26-15	TIME	0920							
	AGE AND SOURCE OF MINNOWS	248 hrs, Aquatox									
	Summary	DAY (NUMBER SURVIVING)									
REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	8	6	6	6	6	6	6	75		
O	B	8	8	8	8	8	8	8	100	95	11.8
C	8	8	8	8	8	8	8	8	100		
D	8	8	8	8	8	8	8	8	100		
E	8	8	8	8	8	8	8	8	100		
CONC:	A	8	8	8	8	8	8	8	100		
10	B	8	8	8	8	8	8	8	87.5	92.5	
C	8	8	8	8	8	8	8	8	100		
D	8	7	6	6	6	6	6	6	75		
E	8	8	8	8	8	8	8	8	100		
CONC:	A	8	8	8	8	8	8	8	100		
13	B	8	8	8	8	8	8	8	87.5	95	
C	8	8	8	8	8	8	8	8	100		
D	8	7	7	7	7	7	7	7	87.5		
E	8	8	8	8	8	8	8	8	100		
CONC:	A	8	8	8	8	7	7	7	87.5		
17	B	8	8	8	8	8	8	8	100	92.5	
C	7	7	7	7	7	7	7	7	87.5		
D	8	8	7	7	7	7	7	7	87.5		
E	8	8	8	8	8	8	8	8	100		
CONC:	A	8	8	8	8	8	8	8	100		
23	B	8	8	8	8	7	7	7	87.5	95	7.21
C	8	8	8	8	8	8	8	8	100		
D	8	8	7	7	7	7	7	7	87.5		
E	8	8	8	8	8	8	8	8	100		
CONC:	A	8	8	8	8	8	8	8	100		
31	B	8	8	8	8	8	7	7	87.5	92.5	
C	8	8	8	8	8	8	8	8	100		
D	8	8	7	7	7	7	7	7	87.5		
E	8	8	8	8	8	8	7	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		TEST START DATE	11-19-15	TIME	1335						
CLIENT	Sheldan	TEST END DATE	11-21-15	TIME	0920						
AGE AND SOURCE OF MINNOWS											
DAY (NUMBER SURVIVING)											
CONC:	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
MHS	A	2	X								
	B	1	2	2	2	=	2	2	2		
	C	1	1	1	1	1	1	1	2		
	D	1	1	1	1	+	1	1	2		
	E										
CONC:	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
10	A	2	2	2	?	?	2	2	2		
	B	1	1	1	1	1	1	1	2		
	C	1	1	1	1	1	1	1	2		
	D	1	1	1	1	+	1	1	2		
	E										
CONC:	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
13	A	2	2	2	2	?	2	2	2		
	B	1	1	1	1	1	1	1	2		
	C	1	1	1	1	1	1	1	2		
	D	+	1	1	+	+	1	1	2		
	E										
CONC:	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
17	A	2	2	2	2	=	2	2	2		
	B	1	1	1	1	=	2	2	2		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	+	2	2	2	2		
	E										
CONC:	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
23	A	2	2	2	2	2	2	3	2		
	B	1	1	1	1	1	1	1	2		
	C	1	1	1	1	1	1	1	2		
	D	1	1	1	1	1	1	1	2		
	E										
CONC:	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
31	A	2	2	2	2	3	2	2	2		
	B	1	1	1	1	1	1	1	2		
	C	1	1	1	1	1	1	1	2		
	D	1	1	1	1	1	1	1	2		
	E										
ANALYST	KF	MF	HF	SD	RH	+b	c	JB			
DATE:	11-19-15	11-20-15	11-21-15	11-22-15	11-23-15	11-24-15	11-25-15	11-26-15	11-27-15		
TIME:	1335 (100)	1600	1100	1730	1530	1115	0920				

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 11-19-15 TIME

CLIENT Sheridan

TEST END DATE

AGE AND SOURCE OF MINNOWS

DAY (NUMBER SURVIVING)

SURVIVAL

	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC: MHS	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	2		
	C	1	1	1	1	1	1	1	2		
	D	1	1	1	1	1	1	1	2		
	E										
CONC: 10	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	2	1		
	B	1	1	1	1	1	1	1	2		
	C	1	1	1	1	1	1	1	2		
	D	1	1	1	1	1	1	1	2		
CONC: 13	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	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	2		
	D	1	1	1	1	1	1	1	2		
CONC: 17	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	2		
	C	1	1	1	1	1	1	1	2		
	D	1	1	1	1	1	1	1	2		
CONC: 23	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	2	2	2		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	2	2	2		
CONC: 31	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	2	2	2		
	D	1	1	1	1	1	2	2	2		
ANALYST	KIT	HF	HF						JK	JB	
DATE:	11-19-15	11-20-15	11-21-15						11-20-15	11-21-15	
TIME:									11:55		

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 11-19-15 TIME

CLIENT Sheridan

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: 4115S	A	2	2	2	2	2	2	2			
	B	1	1	1	1	1	1	1	2		
	C	1	1	1	1	1	1	1	2		
	D	1	1	1	1	1	1	1	2		
	E										
CONC: 10	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	2		
	C	1	1	1	1	1	1	1	2		
	D	1	1	1	1	1	1	1	2		
CONC: 13	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	2		
	C	1	1	1	1	1	1	1	2		
	D	1	1	1	1	1	1	1	2		
CONC: 17	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	2		
	C	1	1	1	1	1	1	1	2		
	D	1	1	1	1	1	1	1	1		
CONC: 23	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	2		
	C	1	1	1	1	1	1	1	2		
	D	1	1	1	1	1	1	2	2		
CONC: 31	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	2		
	C	1	1	1	1	1	1	1	2		
	D	1	1	1	1	1	1	1	2		
ANALYST	KIT	MF	HF					CT	JP		
DATE:	11-19-15	11-20-15	11-21-15	W206	W23-15	W24-15	W25-15	11-26			
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 11-19-15 TIME

CLIENT Sheridan

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: WITS	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	2	2	2		
	C	1	1	1	1	1	2	2	2		
	D	1	1	1	1	1	1	2	2		
	E										
CONC: 10	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	2	2	2	2	2	2	2		
	D	1	2	1	1	1	1	1	1		
CONC: 13	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	2	2	2		
	C	1	1	1	1	1	2	2	2		
	D	1	2	1	1	1	1	1	1		
CONC: 17	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	2	2	2	2	2	2		
	D	1	2	1	1	1	2	2	2		
CONC: 23	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	1	1	1	1	1	1		
	B	1	1	2	2	2	2	2	2		
	C	1	1	1	1	1	2	2	2		
	D	1	1	1	1	1	2	2	2		
CONC: 31	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	2	2	2		
	C	1	1	1	1	1	2	2	2		
	D	1	1	1	1	1	1	1	1		
ANALYST	KIT	HF	HF								
DATE:	11-19-15	11-20-15	11-21-15	11-20-15	11-22-15	11-24-15	11-25-15	11-26-15	11-27-15		
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 11-19-15 TIME

CLIENT Sheridan

TEST END DATE

AGE AND SOURCE OF MINNOWS

DAY (NUMBER SURVIVING)

SURVIVAL

	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC: 4/15S	A	2	2	2	2	2	2	2	2		
	B	1		1				1	2		
	C	1	1		1				2		
	D	1	1	1	1	1	1	1	2		
	E										
CONC: 10	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	3	2		
	B	1		1	1			1	2		
	C	1	1		1				2		
	D	1	1	1	1	1	1	1	2		
CONC: 13	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	2	2		
	B	1		1	1			1	2		
	C	1	1		1				2		
	D	1	1	1	1	1	1	1	2		
CONC: 17	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	3	2		
	B	1		1	1			1	2		
	C	1	1		1			1	2		
	D	1	1	1	1	1	1	1	2		
CONC: 23	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	2	2		
	B	1		1	1	1	1	1	2		
	C	1	1		1	1	1	1	2		
	D	1	1	1	1	1	1	1	2		
CONC: 31	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
	A	2	2	2	2	2	2	2	2		
	B	1		1	1	1	1	1	2		
	C	1	1		1	1	1	1	1		
	D	1	1	1	1	1	1	1	2		
ANALYST	Rif	HF	HF					CJ	JB		
DATE:	11-19-15	11-20-15	11-21-15	11-22-15	11-23-15	11-24-15	11-25-15				
TIME:											

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

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s: K1511008			TEST DATES (BEGIN / END): 11/17/15 - 11/24/15					
CLIENT: Sheridan			WEIGHING DATE / TIME: 11/25/2015					
ANALYSTS: MB			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	1.02001	1.01588	0.00413	8	0.516	AVG DRY WEIGHT (mg) 0.615	
MHS	B	0.99757	0.99276	0.00481	8	0.601		
	C	1.02880	1.02328	0.00552	8	0.690		
	D	0.99191	0.98706	0.00485	8	0.606		
	E	0.99686	0.99159	0.00527	8	0.659		
CONC:		A	1.01448	1.00960	0.00488	8	0.610	AVG DRY WEIGHT (mg) 0.601
10%		B	1.01288	1.00900	0.00388	8	0.485	
		C	1.02105	1.01538	0.00567	8	0.709	
		D	1.03055	1.02626	0.00429	8	0.536	
		E	1.02101	1.01568	0.00533	8	0.666	
CONC:		A	1.03317	1.02889	0.00428	8	0.535	AVG DRY WEIGHT (mg) 0.596
13%		B	1.01329	1.00927	0.00402	8	0.503	
		C	1.00988	1.00420	0.00568	8	0.710	
		D	0.99450	0.99034	0.00416	8	0.520	
		E	0.99183	0.98613	0.00570	8	0.713	
CONC:		A	1.01703	1.01260	0.00443	8	0.554	AVG DRY WEIGHT (mg) 0.606
17%		B	1.02235	1.01770	0.00465	8	0.581	
		C	1.03620	1.03110	0.00510	8	0.638	
		D	0.98786	0.98365	0.00421	8	0.526	
		E	1.02355	1.01771	0.00584	8	0.730	
CONC:		A	0.99140	0.98671	0.00469	8	0.586	AVG DRY WEIGHT (mg) 0.587
23%		B	0.99558	0.99100	0.00458	8	0.573	
		C	1.00016	0.99567	0.00449	8	0.561	
		D	0.98586	0.98124	0.00462	8	0.577	
		E	1.04733	1.04222	0.00511	8	0.639	
CONC:		A	1.03600	1.03153	0.00447	8	0.559	AVG DRY WEIGHT (mg) 0.580
31%		B	0.99220	0.98755	0.00465	8	0.581	
		C	1.04144	1.03624	0.00520	8	0.650	
		D	1.00887	1.00429	0.00458	8	0.573	
		E	0.99108	0.98676	0.00432	8	0.540	
		CV = (STANDARD DEVIATION/MEAN)*100					7.21	

REMARKS:

AA# K1511008, FATHEAD MINNOW SURV., CHRONIC, 11-19-15
File: C:\COPYTO^1\TOXSTAT\FHSURV^1. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.355

W = 0.825

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# K1511008, FATHEAD MINNOW SURV., CHRONIC, 11-19-15
File: C:\COPYTO^1\TOXSTAT\FHSURV^1. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 1.96

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# K1511008, FATHEAD MINNOW SURV., CHRONIC, 11-19-15
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	0.7500	1.0472
1	CONTROL	2	1.0000	1.3931
1	CONTROL	3	1.0000	1.3931
1	CONTROL	4	1.0000	1.3931
1	CONTROL	5	1.0000	1.3931
2	10 % EFFLUENT	1	1.0000	1.3931
2	10 % EFFLUENT	2	0.8750	1.2094
2	10 % EFFLUENT	3	1.0000	1.3931
2	10 % EFFLUENT	4	0.7500	1.0472
2	10 % EFFLUENT	5	1.0000	1.3931
3	13 % EFFLUENT	1	1.0000	1.3931
3	13 % EFFLUENT	2	0.8750	1.2094
3	13 % EFFLUENT	3	1.0000	1.3931

3	13	% EFFLUENT	4	0.8750	1.2094
3	13	% EFFLUENT	5	1.0000	1.3931
4	17	% EFFLUENT	1	0.8750	1.2094
4	17	% EFFLUENT	2	1.0000	1.3931
4	17	% EFFLUENT	3	0.8750	1.2094
4	17	% EFFLUENT	4	0.8750	1.2094
4	17	% EFFLUENT	5	1.0000	1.3931
5	23	% EFFLUENT	1	1.0000	1.3931
5	23	% EFFLUENT	2	0.8750	1.2094
5	23	% EFFLUENT	3	1.0000	1.3931
5	23	% EFFLUENT	4	0.8750	1.2094
5	23	% EFFLUENT	5	1.0000	1.3931
6	31	% EFFLUENT	1	1.0000	1.3931
6	31	% EFFLUENT	2	0.8750	1.2094
6	31	% EFFLUENT	3	1.0000	1.3931
6	31	% EFFLUENT	4	0.8750	1.2094
6	31	% EFFLUENT	5	0.8750	1.2094

AA# K1511008, FATHEAD MINNOW SURV., CHRONIC, 11-19-15

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	1.324				
2	10 % EFFLUENT	1.287	25.50	16.00	5.00	
3	13 % EFFLUENT	1.320	26.00	16.00	5.00	
4	17 % EFFLUENT	1.283	24.00	16.00	5.00	
5	23 % EFFLUENT	1.320	26.00	16.00	5.00	
6	31 % EFFLUENT	1.283	24.00	16.00	5.00	

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

AA# K1511008, FATHEAD MINNOW GROWTH CHRONIC, 11-17-15
File: C:\COPYTO\TOXSTAT\FHGROWTH. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.142

W = 0.954

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# K1511008, FATHEAD MINNOW GROWTH CHRONIC, 11-17-15
File: C:\COPYTO\TOXSTAT\FHGROWTH. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 7.02

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# K1511008, FATHEAD MINNOW GROWTH CHRONIC, 11-17-15
FILE: C:\COPYTO\TOXSTAT\FHGROWTH.
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.5160	0.8014
1	CONTROL	2	0.6010	0.8871
1	CONTROL	3	0.6900	0.9803
1	CONTROL	4	0.6060	0.8922
1	CONTROL	5	0.6590	0.9472
2	10 % EFFLUENT	1	0.6100	0.8963
2	10 % EFFLUENT	2	0.4850	0.7704
2	10 % EFFLUENT	3	0.7090	1.0010
2	10 % EFFLUENT	4	0.5360	0.8214
2	10 % EFFLUENT	5	0.6660	0.9546
3	13 % EFFLUENT	1	0.5350	0.8204
3	13 % EFFLUENT	2	0.5030	0.7884
3	13 % EFFLUENT	3	0.7100	1.0021
3	13 % EFFLUENT	4	0.5200	0.8054
3	13 % EFFLUENT	5	0.7130	1.0054
4	17 % EFFLUENT	1	0.5540	0.8395

4	17	% EFFLUENT	2	0.5810	0.8668
4	17	% EFFLUENT	3	0.6380	0.9252
4	17	% EFFLUENT	4	0.5260	0.8114
4	17	% EFFLUENT	5	0.7300	1.0244
5	23	% EFFLUENT	1	0.5860	0.8718
5	23	% EFFLUENT	2	0.5730	0.8587
5	23	% EFFLUENT	3	0.5610	0.8466
5	23	% EFFLUENT	4	0.5770	0.8627
5	23	% EFFLUENT	5	0.6390	0.9263
6	31	% EFFLUENT	1	0.5590	0.8445
6	31	% EFFLUENT	2	0.5810	0.8668
6	31	% EFFLUENT	3	0.6500	0.9377
6	31	% EFFLUENT	4	0.5730	0.8587
6	31	% EFFLUENT	5	0.5400	0.8254

AA# K1511008, FATHEAD MINNOW GROWTH CHRONIC, 11-17-15

File: C:\COPYTO\TOXSTAT\FHGROWTH.

Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.004	0.001	0.142
Within (Error)	24	0.142	0.006	
Total	29	0.146		

Critical F value = 2.62 (0.05, 5, 24)

Since F < Critical F FAIL TO REJECT Ho: All equal

AA# K1511008, FATHEAD MINNOW GROWTH CHRONIC, 11-17-15

File: C:\COPYTO\TOXSTAT\FHGROWTH.

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	0.902	0.614		
2	10 % EFFLUENT	0.889	0.601	0.265	
3	13 % EFFLUENT	0.884	0.596	0.355	
4	17 % EFFLUENT	0.893	0.606	0.168	
5	23 % EFFLUENT	0.873	0.587	0.585	
6	31 % EFFLUENT	0.867	0.581	0.720	

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

AA# K1511008, FATHEAD MINNOW GROWTH CHRONIC, 11-17-15

File: C:\COPYTO\TOXSTAT\FHGROWTH.

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	10 % EFFLUENT	5	0.114	18.5	0.013
3	13 % EFFLUENT	5	0.114	18.5	0.018
4	17 % EFFLUENT	5	0.114	18.5	0.009
5	23 % EFFLUENT	5	0.114	18.5	0.027
6	31 % EFFLUENT	5	0.114	18.5	0.034

AA# K1511008, FATHEAD MINNOW GROWTH CHRONIC, 11-17-15

File: C:\COPYTO\TOXSTAT\FHGROWTH.

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	0.902				
2	10 % EFFLUENT	0.889	28.00	16.00	5.00	
3	13 % EFFLUENT	0.884	27.00	16.00	5.00	
4	17 % EFFLUENT	0.893	26.00	16.00	5.00	
5	23 % EFFLUENT	0.873	22.00	16.00	5.00	
6	31 % EFFLUENT	0.867	22.00	16.00	5.00	

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

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

SURVIVAL AND REPRODUCTION TEST

Ceriodaphnia dubia

Discharger: *glaucidium*

Location: Outfall

Date Sample Collected: See COC

Lab Number/s

K1511010

Conc %	Day	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		A	B	C	D	E	F	G	H	I	J				
15	1	0										0	10	0	RH
	2	0										0	10	0	RH
	3	8	0	0	0	2	6	0	0	2	0	4	10	0.4	RH
	4	5	0	3	6	6	5	4	6	6	0	43	10	4.3	RH
	5	3	5	6	4	0	10	6	4	7	5	50	10	5.0	RH
	6	12	2	5	16	4	8	6	8	10	11	70	10	7.0	mb
	7														
	8														
Total		18	12	11	17	10	19	17	16	25	22	167		$\bar{x} = 16.7$	
														$CV = 28.5$	

Conc %	Day	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		A	B	C	D	E	F	G	H	I	J				
10	1	0										0	10	0	RH
	2	0										0	10	0	RH
	3	7	0	0	0	2	0	2	0	1	0	4	10	0.4	RH
	4	1	4	5	7	5	0	2	3	2	6	35	10	3.5	RH
	5	7	3	0	6	8	7	6	0	4	3	44	10	4.4	RH
	6	6	4	5	1	4	10	12	0	8	9	59	10	5.9	mb
	7														
	8														
Total		17	11	10	14	17	17	20	3	15	18	142			

Conc %	Day	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		A	B	C	D	E	F	G	H	I	J				
13	1	0										0	10	0	RH
	2	0										0	10	0	RH
	3	2	0	2	0	0	0	0	0	0	0	4	10	0.4	RH
	4	2	5	6	5	3	0	0	7	5	8	41	10	4.1	RH
	5	0	4	5	0	2	4	5	0	6	26	10	2.6	RH	
	6	7	4	0	9	10	8	6	11	8	11	74	10	7.4	mb
	7														
	8														
Total		11	13	13	14	15	12	11	18	13	25	145			

X = Dead

Conc %	Day	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		A	B	C	D	E	F	G	H	I	J				
17	1	0										0	10	0	RH
	2	0										0	10	0	RH
	3	1	0	0	0	0	0	0	0	2	0	0	10	0.3	RH
	4	6	9	5	7	1	1	3	2	1	0	20	10	3.0	RH
	5	4	7	6	1	1	6	4	9	8	10	50	10	5.0	RH
	6	10	1	11	18	13	12	0	0	6	10	77	10	7.7	mb
	7														
	8														
Total		17	12	16	26	15	19	7	13	15	20	160			
23	1	0										1	0	0	RH
	2	0										0	10	0	RH
	3	4	5	0	0	2	0	2	0	0	0	3	10	0.3	RH
	4	4	2	6	3	5	0	8	1	5	2	40	10	4.0	RH
	5	10	9	2	5	0	11	6	8	5	8	64	10	6.4	RH
	6	18	7	8	12	14	9	7	9	10	9	89	10	8.9	mb
	7														
	8														
Total		23	18	16	20	23	22	21	18	16	19	196			
														$CV = 13.4\%$	
31	1	0										1	0	0	RH
	2	0										0	10	0	RH
	3	0	0	0	1	1	0	0	6	0	0	3	10	0.3	RH
	4	6	3	5	2	0	5	4	6	7	2	39	10	3.9	RH
	5	4	2	6	3	5	7	0	5	2	3	37	10	3.7	RH
	6	9	18	11	12	11	12	11	10	7	9	110	10	11.0	mb
	7														
	8														
Total		19	23	22	18	17	24	15	16	16	19	189			

$\bar{x} = 18.9$

$CV = 16.6$

7/14/2014 10:12 AM

AA #K1511010, CERIODAPHNIA DUBIA CHRONIC, REPRODUCTION
File: C:\COPYTO\TOXSTAT\MONTECD. 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 #K1511010, CERIODAPHNIA DUBIA CHRONIC, REPRODUCTION
File: C:\COPYTO\TOXSTAT\MONTECD. Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 5.65

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

NUMBER OF

IDENTIFICATION	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
10%	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
13%	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
17%	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
23%	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
31%	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	10%	10	0	
2	13%	10	0	
3	17%	10	0	
4	23%	10	0	
5	31%	10	0	

TITLE: AA #K1511010, CERIODAPHNIA DUBIA CHRONIC, REPRODUCTION
FILE: C:\COPYTO\TOXSTAT\MONTECD.
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	18.0000	18.0000
1	CONTROL	2	12.0000	12.0000
1	CONTROL	3	11.0000	11.0000
1	CONTROL	4	17.0000	17.0000
1	CONTROL	5	10.0000	10.0000
1	CONTROL	6	19.0000	19.0000
1	CONTROL	7	17.0000	17.0000
1	CONTROL	8	16.0000	16.0000
1	CONTROL	9	25.0000	25.0000
1	CONTROL	10	22.0000	22.0000
2	10 % EFFLUENT	1	17.0000	17.0000
2	10 % EFFLUENT	2	11.0000	11.0000
2	10 % EFFLUENT	3	10.0000	10.0000
2	10 % EFFLUENT	4	14.0000	14.0000
2	10 % EFFLUENT	5	17.0000	17.0000
2	10 % EFFLUENT	6	17.0000	17.0000
2	10 % EFFLUENT	7	20.0000	20.0000
2	10 % EFFLUENT	8	3.0000	3.0000
2	10 % EFFLUENT	9	15.0000	15.0000
2	10 % EFFLUENT	10	18.0000	18.0000
3	13 % EFFLUENT	1	11.0000	11.0000
3	13 % EFFLUENT	2	13.0000	13.0000
3	13 % EFFLUENT	3	13.0000	13.0000
3	13 % EFFLUENT	4	14.0000	14.0000
3	13 % EFFLUENT	5	15.0000	15.0000
3	13 % EFFLUENT	6	12.0000	12.0000
3	13 % EFFLUENT	7	11.0000	11.0000
3	13 % EFFLUENT	8	18.0000	18.0000
3	13 % EFFLUENT	9	13.0000	13.0000
3	13 % EFFLUENT	10	25.0000	25.0000
4	17 % EFFLUENT	1	17.0000	17.0000
4	17 % EFFLUENT	2	12.0000	12.0000
4	17 % EFFLUENT	3	16.0000	16.0000
4	17 % EFFLUENT	4	26.0000	26.0000
4	17 % EFFLUENT	5	15.0000	15.0000
4	17 % EFFLUENT	6	19.0000	19.0000
4	17 % EFFLUENT	7	7.0000	7.0000
4	17 % EFFLUENT	8	13.0000	13.0000

4	17	% EFFLUENT	9	15.0000	15.0000
4	17	% EFFLUENT	10	20.0000	20.0000
5	23	% EFFLUENT	1	23.0000	23.0000
5	23	% EFFLUENT	2	18.0000	18.0000
5	23	% EFFLUENT	3	16.0000	16.0000
5	23	% EFFLUENT	4	20.0000	20.0000
5	23	% EFFLUENT	5	23.0000	23.0000
5	23	% EFFLUENT	6	22.0000	22.0000
5	23	% EFFLUENT	7	21.0000	21.0000
5	23	% EFFLUENT	8	18.0000	18.0000
5	23	% EFFLUENT	9	16.0000	16.0000
5	23	% EFFLUENT	10	19.0000	19.0000
6	31	% EFFLUENT	1	19.0000	19.0000
6	31	% EFFLUENT	2	23.0000	23.0000
6	31	% EFFLUENT	3	22.0000	22.0000
6	31	% EFFLUENT	4	18.0000	18.0000
6	31	% EFFLUENT	5	17.0000	17.0000
6	31	% EFFLUENT	6	24.0000	24.0000
6	31	% EFFLUENT	7	15.0000	15.0000
6	31	% EFFLUENT	8	16.0000	16.0000
6	31	% EFFLUENT	9	16.0000	16.0000
6	31	% EFFLUENT	10	19.0000	19.0000

AA #K1511010, CERIODAPHNIA DUBIA CHRONIC, REPRODUCTION
 File: C:\COPYTO~1\TOXSTAT\MONTECD. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	248.150	49.630	2.747
Within (Error)	54	975.500	18.065	
Total	59	1223.650		

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

AA #K1511010, CERIODAPHNIA DUBIA CHRONIC, REPRODUCTION
 File: C:\COPYTO~1\TOXSTAT\MONTECD. Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED	MEAN CALCULATED IN		T STAT	SIG
		MEAN	ORIGINAL UNITS			
1	CONTROL	16.700	16.700			
2	10 % EFFLUENT	14.200	14.200	1.315		
3	13 % EFFLUENT	14.500	14.500	1.157		
4	17 % EFFLUENT	16.000	16.000	0.368		
5	23 % EFFLUENT	19.600	19.600	-1.526		
6	31 % EFFLUENT	18.900	18.900	-1.157		

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

AA #K1511010, CERIODAPHNIA DUBIA CHRONIC, REPRODUCTION
File: C:\COPYTO\TOXSTAT\MONTECD. 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	10 % EFFLUENT	10	4.391	26.3	2.500
3	13 % EFFLUENT	10	4.391	26.3	2.200
4	17 % EFFLUENT	10	4.391	26.3	0.700
5	23 % EFFLUENT	10	4.391	26.3	-2.900
6	31 % EFFLUENT	10	4.391	26.3	-2.200

AA #K1511010, CERIODAPHNIA DUBIA CHRONIC, REPRODUCTION
File: C:\COPYTO\TOXSTAT\MONTECD. Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	16.700				
2	10 % EFFLUENT	14.200	92.50	75.00	10.00	
3	13 % EFFLUENT	14.500	91.50	75.00	10.00	
4	17 % EFFLUENT	16.000	100.50	75.00	10.00	
5	23 % EFFLUENT	19.600	125.00	75.00	10.00	
6	31 % EFFLUENT	18.900	118.00	75.00	10.00	

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

APPENDIX E

Organism History

AQUATOX, INC.

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

TEST ORGANISM HISTORY

DATE SHIPPED 11/19/15 CLIENT ARK ANALYTICAL

Purchase Order #: _____

SPECIES: Pimephales promelas

Quantity Shipped: 440t 15-1600
CST

Age: HATCHED 11/17/15

Brood Stock Source: Anderson Farms, AR

Culture Water: Groundwater

Hardness (Mg/l CaCO₃): 2160

Dissolved Oxygen (Mg/l): 8.5

Temperature (°C): 25.1

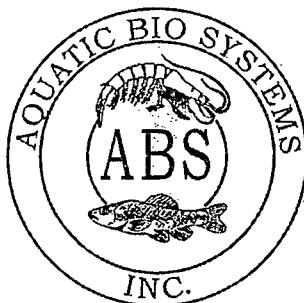
Feeding: ARTEMIA

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: 22°C 22-26°C

SALINITY/CONDUCTIVITY: -- --

TOTAL HARDNESS (as CaCO₃): 94 mg/l 76-130 mg/l

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

pH: 7.98 7.50-8.20

Comments:



Facility Supervisor

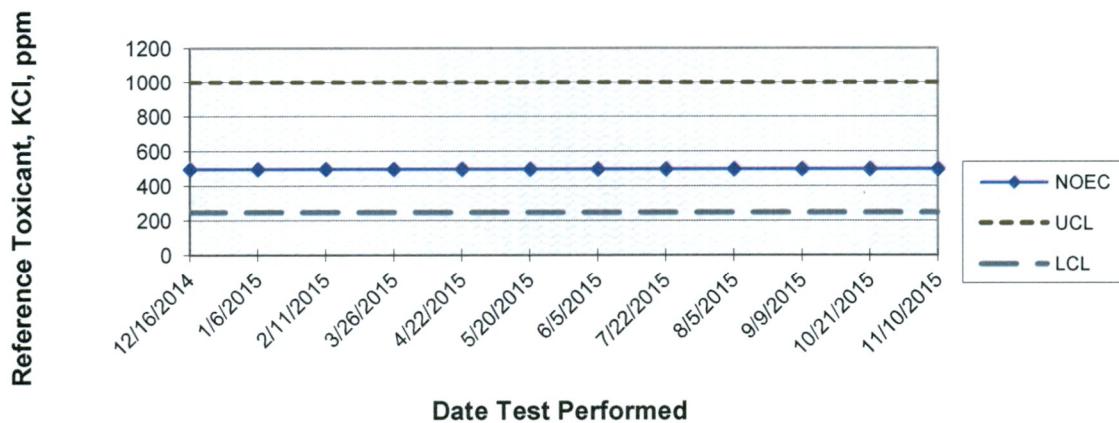
APPENDIX F

Quality Assurance Charts

ARKANSAS ANALYTICAL, INC.

FATHEAD MINNOW SURVIVAL 7 Day

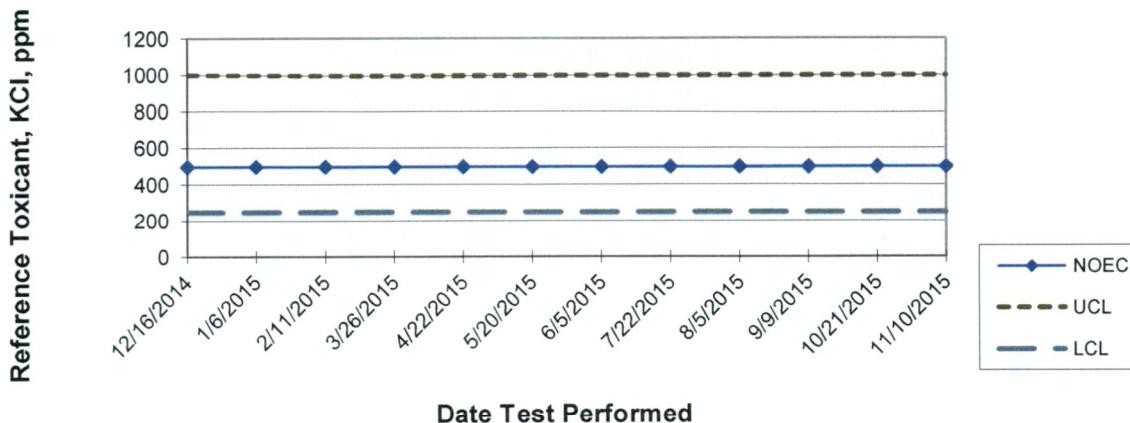
QUALITY ASSURANCE



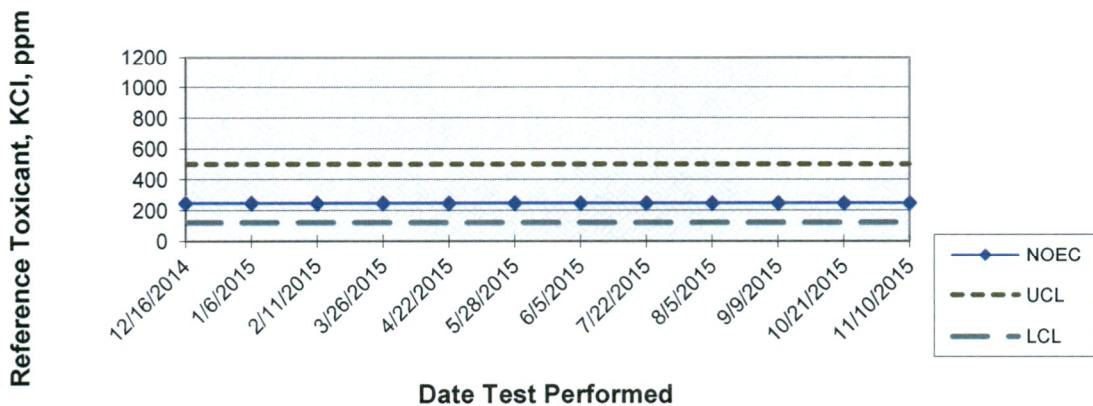
ARKANSAS ANALYTICAL, INC.

FATHEAD MINNOW GROWTH 7 Day

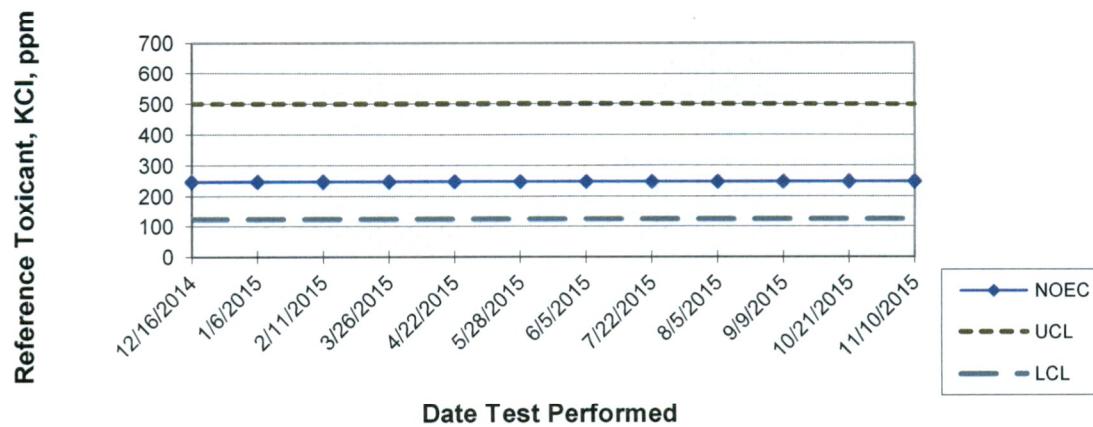
QUALITY ASSURANCE



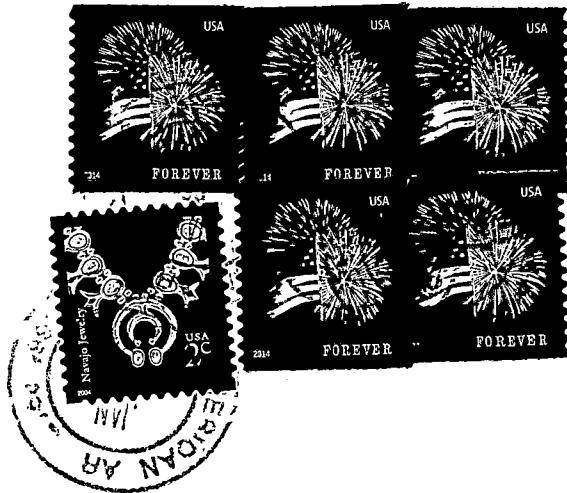
ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE



Sheridan Water Works
PO Box 486
Sheridan, AR 72150-0486



ADEQ
NPDES Enforcement Branch
5301 Northshore Drive
North Little Rock, AR 72118-5317

