

Arkansas Analytical, Inc.

Toxicity Test Results

CITY of SHERIDAN
NPDES PERMIT NUMBER: AR0034347
First Quarter 2016
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 K1601005

Thursday, February 4, 2016

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 2016.

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:	1-11-16, 1403	1-12-16, 1303
Sample #2:	1-12-16, 1520	1-13-16, 1420
Sample #3:	1-13-16, 1634	1-14-16, 1534

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:	1-12-16, 1635	6
Sample #2:	1-14-16, 0827	0
Sample #3:	1-14-16, 1700	6

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 13%, 17%, 23%, 31%, and 41%. The low-flow effluent concentration (**critical dilution**) was defined as **31% 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	0.730	X	
The percent coefficient of variation between replicates must be 40% or less for growth	8.68%	X	

TEST ACCEPTANCE CRITERIA for *Ceriodaphnia dubia*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	90%	X	
Average of 15 or more young per surviving female	16.0	X	
At least 60% of surviving females should have produced 3 broods	60%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	18.2%	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> 12/1/15-12/9/15		<i>Pimephales promelas</i> 12/1/15-12/8/15	
NOEC Survival:	500 ppm KCl	NOEC Survival:	500 ppm KCl
LOEC Survival:	1000 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	41% / NA	NOEC / LOEC survival	41% / NA
NOEC / LOEC Reproduction	41% / NA	NOEC / LOEC growth	41% / NA
Mean number of neonates (critical dilution)	24.7	%CV survival (critical dilution)	0%
%CV Reproduction (critical dilution)	19.0%	Mean dry weight (critical dilution) in milligrams	0.756
		%CV growth (critical dilution)	7.48%
PMSD Reproduction	32.1%	PMSD Growth	12.2%

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 31% effluent**. The effluent samples **did not** exhibit lethal or sublethal effects at the critical dilution, and, as such, **passed** the test.

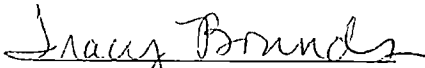
Chronic static renewal survival and reproduction test using *Ceriodaphnia dubia*, (Method 1002.0)

The permit issued to the City of Sheridan, AR0034347, specifies that the **critical dilution is 31% effluent**. The effluent samples **did not** exhibit lethal or sublethal effects at the critical dilution, and, as such, **passed** the test.

Biomonitoring Analyst:

Tracy Bounds, Melissa Bird, Chris Turney, Zabrina Ruggles, Shannon 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:	1-11-16, 1403	1-12-16, 1303
Sample #2:	1-12-16, 1520	1-13-16, 1420
Sample #3:	1-13-16, 1634	1-14-16, 1534

Test initiated (date, time): 1-13-16, 1030 Test terminated (date, time): 1-20-16, 1150

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%	87.5	100	100	100	100		97.5	97.5	97.5	5.73%
13%	100	87.5	100	87.5	100		97.5	97.5	95	
17%	100	100	100	100	87.5		100	100	97.5	
23%	100	100	100	100	100		100	100	100	
31%	100	100	100	100	100		100	100	100	0.00%
41%	100	100	100	87.5	87.5		97.5	97.5	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%	0.626	0.719	0.774	0.784	0.746		0.730	8.68%
13%	0.739	0.679	0.671	0.651	0.678		0.684	
17%	0.748	0.776	0.770	0.742	0.650		0.737	
23%	0.758	0.730	0.750	0.821	0.724		0.757	
31%	0.781	0.696	0.749	0.838	0.714		0.756	7.48%
41%	0.730	0.751	0.861	0.662	0.684		0.738	

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)= 41 % effluent
b) NOEC growth (parameter TPP6C)= 41 % effluent
c) Coefficient of variation (parameter TQP6C)= 8.68 %

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:	1-11-16, 1403	1-12-16, 1303
Sample #2:	1-12-16, 1520	1-13-16, 1420
Sample #3:	1-13-16, 1634	1-14-16, 1534

Test initiated (date, time): 1-13-16, 1000 Test terminated (date, time): 1-19-16, 1600

Dilution water used: Moderately Hard Synthetic

Ceriodaphnia dubia SURVIVAL AND REPRODUCTION

NUMBER OF YOUNG PRODUCED PER FEMALE @ TEST TERMINATION

PERCENT EFFLUENT

Replicate	0%	13%	17%	23%	31%	41%
A	13	17	30	20	18	29
B	19	22	24	28	26	32
C	16	23	25	25	18	28
D	16	20	25	27	22	26
E	14	22	26	23	23	26
F	13	19	26	25	31	25
G	15	24	26	27	26	28
H	22	27	23	24	25	27
I	X 11	18	20	23	26	30
J	16	27	23	22	32	X 0
Mean	15.5	21.9	24.8	24.4	24.7	25.1
Mean/surviving female	16.0	21.9	24.8	24.4	24.7	27.9
CV%*	18.2				19.0	

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%	13%	17%	23%	31%	41%
Time of Reading: 24 HOURS	100	100	100	100	100	100
48 HOURS	100	100	100	100	100	100
Test termination	90	100	100	100	100	90

1. Fisher's Exact Test:

Is the mean survival at test termination significantly different (p=0.05) than the control survival for:

a) LOW FLOW OR CRITICAL DILUTION, (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:

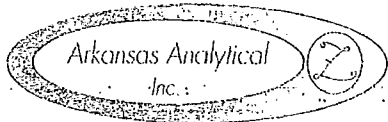
a) NOEC survival (parameter TOP3B)= 41 % effluent

b) NOEC reproduction (parameter TPP3B)= 41 % effluent

c) Coefficient of variation (parameter TQP3B)= 19.0 %

APPENDIX A

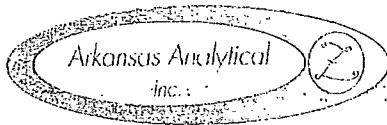
Chain of Custody Forms



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

CHAIN OF CUSTODY RECORD

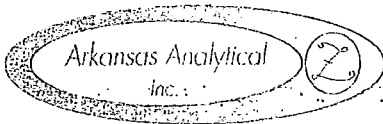
CLIENT INFORMATION		BILLING INFORMATION		Project Description		Turnaround Time		Preservation Codes:											
Sheridan Waterworks		Sheridan Waterworks		Chronic Toxicity		1 Day (100%)		1. Cool, 4 Degrees Centigrade				4. Thiosulfate for Dechlorination							
104 W High St.		P.O. Box 486				2 Day (50%)		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		Bottle Type: P		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				Allen Parker Sampler(s) Printed				Chronic Toxicity Arkansas Analytical Work Order Number: K1601-005A											
Field Number	SAMPLE COLLECTION		Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION												
	Date/s	Time/s																	
	1/12/16	1403-1303		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>		1635 1-12-16		_____		1. CUSTODY SEALS: <input checked="" type="checkbox"/> Yes ___ No 2. CONTAINERS CORRECT: <input checked="" type="checkbox"/> Yes ___ No 3. COC/LABELS AGREE: <input checked="" type="checkbox"/> Yes ___ No 4. RECEIVED ON ICE: <input checked="" type="checkbox"/> Yes ___ No 5. TEMPERATURE ON RECEIPT: 62 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							
				Telephone: 870-942-2722		Routine		TEST PARAMETERS										
Attn: David Fitzgerald				Fax: 870-942-1937		Preservative Code: 1												
				Email: sheridanwater@windstream.net		Bottle Type: P												
<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											
	1/12-13/16	1520-1420		X	24	Water	Final Discharge		X									
													K1601005.3					
1. Relinquished by: (Signature)		Date/Time		2. Received by: (Signature)		SAMPLE CONDITION UPON RECEIPT IN LAB				REMARKS / SAMPLE COMMENTS								
<i>Allen Parker</i>		0827 1-14-16		_____		1. CUSTODY SEALS: <input checked="" type="checkbox"/> Yes ___ No												
						2. CONTAINERS CORRECT: <input checked="" type="checkbox"/> Yes ___ No												
						3. COC/LABELS AGREE: <input checked="" type="checkbox"/> Yes ___ No												
3. Relinquished by: (Signature)		Date/Time		4. Received by lab: (Signature)		4. RECEIVED ON ICE: <input checked="" type="checkbox"/> Yes ___ No												
_____				<i>Jammy Riddle</i>		5. TEMPERATURE ON RECEIPT: 0°C												
						6. TEMPERATURE GUN ID: HHT#2												
FOR COMPLETION BY LAB ONLY																		



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

CHAIN OF CUSTODY RECORD

CLIENT INFORMATION		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				Allen Parker Sampler(s) Printed														Arkansas Analytical Work Order Number:	
Field Number	SAMPLE COLLECTION		Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION		Chronic Toxicity										
	Date/s	Time/s																	
	1/13-14/16	1634-1534		X	24	Water	Final Discharge	X											K1601005C
1. Relinquished by: (Signature)			Date/Time		2. Received by: (Signature)			SAMPLE CONDITION UPON RECEIPT IN LAB						REMARKS / SAMPLE COMMENTS					
<i>Allen Parker</i>			1700 1-14-16					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: 6°C 6. TEMPERATURE GUN ID: HFI #2											
3. Relinquished by: (Signature)			Date/Time		4. Received by lab: (Signature)			FOR COMPLETION BY LAB ONLY											
					<i>Sydney James</i>														

APPENDIX B

Effluent and Dilution Water Data

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Fathead Minnow

Lab # / Sample ID K1601005

Test Start (Date/Time) 1-13-16 / 1030

Client: Sheridan

Test End (Date/Time) 1-20-16 / 1150

Day of Test

	1	2	3	4	5	6	7	notes
Control	1/13	1/14	1/15	1/16	1/17	1/18	1/19	
D.O. (mg/L) INITIAL	8.7	8.3	8.6	8.4	8.5	8.9	9.4	1/13 804
FINAL	7.9	7.5	7.6	7.6	7.1	6.0	6.8	1/18 805
pH (s.u.) INITIAL	8.0	8.2	8.7	7.9	7.4	7.8	8.0	
FINAL	8.0	7.7	7.9	7.3	7.0	7.5	7.5	
temp (C) INITIAL	22	22	23	22	21	21	19	
FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)	78					62		
HARDNESS (mg/L)	78					89		
CONDUCTIVITY (umhc)	322					289		
CHLORINE (mg/L)	<0.05					<0.05		
CONC:	13%							
D.O. (mg/L) INITIAL	8.7	8.4	8.4	8.3	8.2	8.9	9.7	
FINAL	8.0	7.3	7.5	7.4	6.8	6.2	6.9	
pH (s.u.) INITIAL	7.9	8.0	7.9	7.9	7.7	8.0	8.0	
FINAL	7.9	7.7	7.9	7.6	7.6	7.5	7.6	
temp (C) INITIAL	22	23	23	22	22	20	18	
FINAL	25	25	25	25	25	25	25	
CONC:	17%							
D.O. (mg/L) INITIAL	8.7	8.5	8.2	8.3	9.1	9.0	9.6	
FINAL	8.1	7.2	7.5	7.4	6.8	6.2	6.7	
pH (mg/L) INITIAL	7.9	8.0	8.0	7.8	7.8	8.0	8.0	
FINAL	8.0	7.7	7.8	7.7	7.6	7.4	7.5	
temp (C) INITIAL	22	23	23	22	21	20	18	
FINAL	25	25	25	25	25	25	25	
CONC:	23%							
D.O. (mg/L) INITIAL	8.6	8.3	8.4	8.2	8.7	8.9	9.6	
FINAL	8.1	7.3	7.7	7.4	6.9	6.1	6.8	
pH (s.u.) INITIAL	7.9	8.0	8.0	7.9	8.0	7.9	8.0	
FINAL	8.0	7.8	7.9	7.8	7.6	7.5	7.5	
temp (C) INITIAL	22	24	23	22	23	21	18	
FINAL	25	25	25	25	25	25	25	
CONC:	31%							
D.O. (mg/L) INITIAL	8.6	8.4	8.4	8.1	8.6	8.8	9.5	
FINAL	8.1	7.3	7.5	7.4	7.0	6.1	6.8	
pH (s.u.) INITIAL	7.9	7.9	7.9	7.9	8.0	7.9	8.0	
FINAL	8.0	7.7	7.8	7.8	7.6	7.4	7.5	
temp (C) INITIAL	22	24	24	22	23	21	18	
FINAL	25	25	25	25	25	25	25	
CONC:	41%							
D.O. (mg/L) INITIAL	8.5	8.3	8.4	7.9	8.5	8.9	9.9	
FINAL	8.2	7.2	7.6	7.6	7.0	6.3	6.8	
pH (s.u.) INITIAL	7.9	7.9	7.9	7.8	7.9	7.8	7.9	
FINAL	8.1	7.7	7.8	7.9	7.6	7.5	7.5	
temp (C) INITIAL	22	24	24	23	24	21	18	
FINAL	25	25	25	25	25	25	25	
CONC: 100%	A	A	B	B	C	C	C	
ALKALINITY (mg/L)	32		30		40			
HARDNESS (mg/L)	36		46		49			
CONDUCTIVITY (umhc)	291		291		289			
CHLORINE (mg/L)	0.06		0.05		<0.05			

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Ceriodaphnia Dubia

Lab # / Sample ID K1601005

Test Start (Date/Time) 1-13-16/1000

Client: Sheridan

Test End (Date/Time) 1-19-16/1600

		Day of Test							
		1	2	3	4	5	6	7	notes
Control		1/13	1/14	1/15	1/16	1/17	1/18		1/3 804
D.O. (mg/L)	INITIAL	8.7	8.3	8.6	8.4	8.8	8.9		1/8 805
	FINAL	8.2	8.2	8.6	8.9	9.1	8.0		
pH (s.u.)	INITIAL	8.0	8.2	8.7	7.9	7.4	7.8		
	FINAL	7.9	8.1	8.3	7.6	25 8.1	7.7	10 1-A-10	
temp (C)	INITIAL	22	22	23	22	21	21	2	
	FINAL	25	25	25	25	25	25		
ALKALINITY (mg/L)		78					62		
HARDNESS (mg/L)		78					84		
CONDUCTIVITY (umhc)		322					284		
CHLORINE (mg/L)		<0.05					<0.05		
CONC:	13%								
D.O. (mg/L)	INITIAL	8.7	8.4	8.4	8.3	8.8	8.9		
	FINAL	8.0	8.4	8.7	8.9	8.9	7.9		
pH (s.u.)	INITIAL	7.9	8.0	7.9	7.9	7.7	8.0		
	FINAL	7.9	8.0	8.2	7.9	8.0	7.7		
temp (C)	INITIAL	22	23	23	22	22	20		
	FINAL	25	25	25	25	25	25		
CONC:	17%								
D.O. (mg/L)	INITIAL	8.7	8.5	8.2	8.3	9.1	9.0		
	FINAL	8.1	8.1	8.6	8.9	9.0	7.9		
pH (mg/L)	INITIAL	7.9	8.0	8.0	7.8	7.9	8.0		
	FINAL	7.9	8.0	8.1	7.9	8.0	7.7		
temp (C)	INITIAL	22	23	23	22	21	20		
	FINAL	25	25	25	25	25	25		
CONC:	23%								
D.O. (mg/L)	INITIAL	8.6	8.3	8.4	8.2	8.7	8.9		
	FINAL	7.9	8.2	8.6	8.8	9.0	7.6		
pH (s.u.)	INITIAL	7.9	8.0	8.0	7.9	8.0	7.9		
	FINAL	7.9	8.0	8.1	7.9	8.0	7.6		
temp (C)	INITIAL	22	24	22 23	22	23	21		
	FINAL	25	25	25	25	25	25		
CONC:	31%								
D.O. (mg/L)	INITIAL	8.6	8.4	8.4	8.1	8.6	8.8		
	FINAL	8.0	8.2	8.7	8.9	9.0	7.6		
pH (s.u.)	INITIAL	7.9	7.9	7.9	7.9	8.0	7.9		
	FINAL	7.9	8.0	8.1	7.9	7.9	7.6		
temp (C)	INITIAL	22	24	24	22	23	21		
	FINAL	25	25	25	25	25	25		
CONC:	41%								
D.O. (mg/L)	INITIAL	8.5	8.3	8.4	7.9	8.5	8.9		
	FINAL	8.0	8.1	8.7	8.8	8.9	7.2		
pH (s.u.)	INITIAL	7.9	7.9	7.9	7.8	7.9	7.8		
	FINAL	7.9	8.0	8.1	8.0	7.9	7.6		
temp (C)	INITIAL	22	24	24	23	24	21		
	FINAL	25	25	25	25	25	25		
CONC:	100%	A	A	B	B	C	C		
ALKALINITY (mg/L)		32		30		40			
HARDNESS (mg/L)		36		46		44			
CONDUCTIVITY (umhc)		291		291		289			
CHLORINE (mg/L)		0.06		0.05		<0.05			

APPENDIX C

Fathead minnow raw data and statistics



SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID K1601005 TEST START DATE 1-13-16 TIME 1030
 CLIENT Sheridan TEST END DATE 1-20-16 TIME 1150

AGE AND SOURCE OF MINNOWS 48 hrs Aquatox

SUMMARY		DAY (NUMBER SURVIVING)									SURVIVAL	
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV	
CONTROL	A	8	7	7	7	7	7	7	7	87.5	97.5%	5.73
	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		
13%	A	8	8	8	8	8	8	8	8	100	95%	
	B	↓	7	7	7	7	7	7	7	87.5		
	C	↓	8	8	8	8	8	8	8	100		
	D	↓	8	8	7	7	7	7	7	87.5		
	E	↓	8	8	8	8	8	8	8	100		
17%	A	8	8	8	8	8	8	8	8	100	97.5%	
	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	7	7	7	7	87.5		
23%	A	8	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		
31%	A	8	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		
41%	A	8	8	8	8	8	8	8	8	100	95%	7.21
	B	↓	8	8	8	8	8	8	8	100		
	C	↓	8	8	8	8	8	8	8	100		
	D	↓	8	8	8	8	7	7	7	87.5		
	E	↓	7	7	7	7	7	7	7	87.5		
ANALYST		tb	tb	tb	ct	tb	tb	tb	zr			
DATE:		1-13-16	1-14-16	1-15-16	1-16-16	1-17-16	1-18-16	1-19-16	1-20-16			
TIME:		1030	1330	1345	0744	1030	1600	1515	1150			

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		K1601005		TEST START DATE		TIME					
CLIENT		Sheridan		TEST END DATE		TIME					
Replicate		A		AGE AND SOURCE OF MINNOWS				SURVIVAL			
		DAY (NUMBER SURVIVING)									
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
CONT	A	2	1	1	1	1	1	1			
	B	1	2	2	2	2	2	2			
	C	1	2	2	2	2	2	2			
	D	1	2	2	2	2	2	2			
	E										
131	A	2	2	2	2	2	2	2			
	B	1	2	2	2	2	2	2			
	C	1	2	2	2	2	2	2			
	D	1	2	2	2	2	2	2			
	E										
171	A	2	2	2	2	2	2	2			
	B	1	2	2	2	2	2	2			
	C	1	2	2	2	2	2	2			
	D	1	2	2	2	2	2	2			
	E										
231	A	2	2	2	2	2	2	2			
	B	1	2	2	2	2	2	2			
	C	1	2	2	2	2	2	2			
	D	1	2	2	2	2	2	2			
	E										
311	A	2	2	2	2	2	2	2			
	B	1	2	2	2	2	2	2			
	C	1	2	2	2	2	2	2			
	D	1	2	2	2	2	2	2			
	E										
411	A	2	2	2	2	2	2	2			
	B	1	2	2	2	2	2	2			
	C	1	2	2	2	2	2	2			
	D	1	2	2	2	2	2	2			
	E										
ANALYST			tb	tb	ct	tb	tb	tb	tb		
DATE:			1-14-16	1-15-16	1-16-16	1-17-16	1-18-16	1-19-16	1-20-16		
TIME:			1330	1345	0744	1030	1600	1515	1150		

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		TEST START		DATE		TIME					
K1601005											
CLIENT		TEST END		DATE		TIME					
Sheridan											
AGE AND SOURCE OF MINNOWS											
Replicate B		DAY (NUMBER SURVIVING)						SURVIVAL			
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
control	A	2	2	2	2	2	2	2	2		
	B	1	2	2	2	2	2	2	2		
	C	1	2	2	2	2	2	2	2		
	D	1	2	2	2	2	2	2	2		
	E										
13%	A	2	2	2	2	2	2	2	2		
	B	1	2	2	2	2	2	2	2		
	C	1	2	2	2	2	2	2	2		
	D	1	1	1	1	1	1	1	1		
	E										
17%	A	2	2	2	2	2	2	2	2		
	B	1	2	2	2	2	2	2	2		
	C	1	2	2	2	2	2	2	2		
	D	1	2	2	2	2	2	2	2		
	E										
23%	A	2	2	2	2	2	2	2	2		
	B	1	2	2	2	2	2	2	2		
	C	1	2	2	2	2	2	2	2		
	D	1	2	2	2	2	2	2	2		
	E										
31%	A	2	2	2	2	2	2	2	2		
	B	1	2	2	2	2	2	2	2		
	C	1	2	2	2	2	2	2	2		
	D	1	2	2	2	2	2	2	2		
	E										
41%	A	2	2	2	2	2	2	2	2		
	B	1	2	2	2	2	2	2	2		
	C	1	2	2	2	2	2	2	2		
	D	1	2	2	2	2	2	2	2		
	E										
ANALYST			tb	tb	ct	tb	tb	tb	ER		
DATE:					1-16-16	1-17-16	1-18-16	1-19-16	1/20/16		
TIME:									1150		

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		TEST START DATE		TIME							
CLIENT		TEST END DATE		TIME							
AGE AND SOURCE OF MINNOWS											
Replicate		DAY (NUMBER SURVIVING)				SURVIVAL					
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
Control	A	Z	2	2	2	2	2	2	2		
	B	I	2	2	2	2	2	2	2		
	C	I	2	2	2	2	2	2	2		
	D	L	2	2	2	2	2	2	2		
	E										
13%	A	Z	2	2	2	2	2	2	2		
	B	Z	2	2	2	2	2	2	2		
	C	I	2	2	2	2	2	2	2		
	D	L	2	2	2	2	2	2	2		
	E										
17%	A	Z	2	2	2	2	2	2	2		
	B	I	2	2	2	2	2	2	2		
	C	I	2	2	2	2	2	2	2		
	D	L	2	2	2	2	2	2	2		
	E										
23%	A	Z	2	2	2	2	2	2	2		
	B	I	2	2	2	2	2	2	2		
	C	I	2	2	2	2	2	2	2		
	D	L	2	2	2	2	2	2	2		
	E										
31%	A	Z	2	2	2	2	2	2	2		
	B	I	2	2	2	2	2	2	2		
	C	I	2	2	2	2	2	2	2		
	D	L	2	2	2	2	2	2	2		
	E										
41%	A	Z	2	2	2	2	2	2	2		
	B	I	2	2	2	2	2	2	2		
	C	I	2	2	2	2	2	2	2		
	D	L	2	2	2	2	2	2	2		
	E										
ANALYST			tb	tb	cb	tb	tb	tb	BL		
DATE:			1-14-16	1-15-16	1-16-16	1-17-16	1-18-16	1-19-16	1/20/16		
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		TEST END		DATE		TIME					
AGE AND SOURCE OF MINNOWS											
DAY (NUMBER SURVIVING)											
		start	1	2	3	4	5	6	7%	MEAN %	CV
Control	CONC:	A	2	2	2	2	2	2	2		
		B	1	2	2	2	2	2	2		
		C	1	2	2	2	2	2	2		
		D	1	2	2	2	2	2	2		
		E									
13%	CONC:	A	2	2	2	2	2	2	2		
		B	1	2	2	2	2	2	2		
		C	1	2	2	2	2	2	2		
		D	1	2	2	1	1	1	1		
		E									
17%	CONC:	A	2	2	2	2	2	2	2		
		B	1	2	2	2	2	2	2		
		C	1	2	2	2	2	2	2		
		D	1	2	2	2	2	2	2		
		E									
23%	CONC:	A	2	2	2	2	2	2	2		
		B	1	2	2	2	2	2	2		
		C	1	2	2	2	2	2	2		
		D	1	2	2	2	2	2	2		
		E									
31%	CONC:	A	2	2	2	2	2	2	2		
		B	1	2	2	2	2	2	2		
		C	1	2	2	2	2	2	2		
		D	1	2	2	2	2	2	2		
		E									
41%	CONC:	A	2	2	2	2	2	2	2		
		B	1	2	2	2	2	2	2		
		C	1	2	2	2	1	1	1		
		D	1	2	2	2	2	2	2		
		E									
ANALYST			Hb	Hb	Ch	Hb	Hb	Hb	EL		
DATE:			1-14-16	1-15-16	1-16-16	1-17-16	1-18-16	1-19-16	1/20/16		
TIME:											

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB #/SAMPLE ID		K1601005		TEST START DATE		TIME					
CLIENT		Shen dan		TEST END DATE		TIME					
Replicate		E		AGE AND SOURCE OF MINNOWS							
		DAY (NUMBER SURVIVING)				SURVIVAL					
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
Control	A	2	2	2	2	2	2	2	2		
	B	1	2	2	2	2	2	2	2		
	C	1	2	2	2	2	2	2	2		
	D	1	2	2	2	2	2	2	2		
	E										
13%	A	2	2	2	2	2	2	2	2		
	B	1	2	2	2	2	2	2	2		
	C	1	2	2	2	2	2	2	2		
	D	1	2	2	2	2	2	2	2		
	E										
17%	A	2	2	2	2	2	2	2	2		
	B	1	2	2	2	2	1	1	1		
	C	1	2	2	2	2	2	2	2		
	D	1	2	2	2	2	2	2	2		
	E										
23%	A	2	2	2	2	2	2	2	2		
	B	1	2	2	2	2	2	2	2		
	C	1	2	2	2	2	2	2	2		
	D	1	2	2	2	2	2	2	2		
	E										
31%	A	2	2	2	2	2	2	2	2		
	B	1	2	2	2	2	2	2	2		
	C	1	2	2	2	2	2	2	2		
	D	1	2	2	2	2	2	2	2		
	E										
41%	A	2	2	2	2	2	2	2	2		
	B	1	2	2	2	2	2	2	2		
	C	1	1	1	1	1	1	1	1		
	D	1	2	2	2	2	2	2	2		
	E										
ANALYST			tb	tb	ct	tb	tb	tb	tb		
DATE:			1-14-16	1-15-16	1-16-16	1-17-16	1-18-16	1-19-16	1/20/16		
TIME:											

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

Pimephales promelas

FATHEAD MINNOW

TEST 1000.0

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB #/ #s: K1601005	TEST DATES (BEGIN / END): 1-13-16 / 1-20-16
CLIENT: Sheridan	WEIGHING DATE / TIME: 1-21-16 / 1200
ANALYSTS: Hb ZR	DRYING TEMP (DEGREES C): 60
SAMPLE ID: K1601005 → Outfall	DRYING TIME (HOURS): 24

	REP#	FINAL DRY WEIGHT TIN+LARVAE	INITIAL WEIGHT TIN (g)	TOTAL DRY WEIGHT OF LARVAE (g)	NUMBER OF LARVAE	DRY WEIGHT OF LARVAE (mg)	
CONTROL	A 1	1.03519	1.03048	0.00501	8	0.626	AVG DRY WEIGHT (mg)
.04654	B 2	1.03174	1.04079	0.00575	8	0.719	0.7298
.03128	C 3	1.03289	1.07509	0.00619	8	0.774	CV
.02335	D 4	1.01140	1.01708	0.00627	8	0.784	8.68%
	E 5	1.02366	1.01769	0.00597	8	0.746	
CONC:	A 6	1.03591	1.03000	0.00591	8	0.739	AVG DRY WEIGHT (mg)
13%	B 7	1.02645	1.02102	0.00543	8	0.679	0.6836
	C 8	1.03336	1.02799	0.00537	8	0.671	CV
	D 9	1.03157	1.02636	0.00521	8	0.651	
	E 10	1.02540	1.01998	0.00542	8	0.678	
CONC:	A 11	1.03785	1.03187	0.00598	8	0.748	AVG DRY WEIGHT (mg)
17%	B 12	0.99716	0.99095	0.00621	8	0.776	0.7372
	C 13	1.00380	0.99764	0.00616	8	0.777	CV
	D 14	1.02263	1.01669	0.00594	8	0.742	
	E 15	1.00677	1.00157	0.0052	8	0.65	
CONC:	A 16	1.02506	1.01900	0.00606	8	0.758	AVG DRY WEIGHT (mg)
23%	B 17	1.02160	1.01576	0.00584	8	0.73	0.7566
	C 18	1.02428	1.01828	0.006	8	0.75	CV
	D 19	1.03219	1.02562	0.00657	8	0.821	
	E 20	1.03215	1.02636	0.00579	8	0.724	
CONC:	A 21	1.03881	1.03256	0.00625	8	0.781	AVG DRY WEIGHT (mg)
31%	B 22	1.04848	1.03791	0.00557	8	0.696	0.7556
	C 23	1.03233	1.02634	0.00599	8	0.749	CV
	D 24	1.04349	1.03679	0.0067	8	0.838	7.48%
1.02832	E 25	1.01790	1.02261	0.00571	8	0.714	
CONC:	A 26	1.01556	1.00942	0.00584	8	0.73	AVG DRY WEIGHT (mg)
45%	B 27	1.04470	1.03869	0.00601	8	0.751	0.7376
10%	C 28	1.04792	1.04103	0.00689	8	0.861	CV
	D 29	1.03047	1.02517	0.0053	8	0.662	
04713	E 30	1.01	1.04166	0.00547	8	0.684	

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

PRINT

AA # K1601005, P. PROMELAS 7 DAY CHRONIC, 1-13-16
File: sherifh Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - wilk's test for normality

D = 0.135

W = 0.814

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 # K1601005, P. PROMELAS 7 DAY CHRONIC, 1-13-16
File: sherifh 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.

PRINT1

TITLE: AA # K1601005, P. PROMELAS 7 DAY CHRONIC, 1-13-16
 FILE: sheriff
 TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.8750	1.2094
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	13 % EFFLUENT	1	1.0000	1.3931
2	13 % EFFLUENT	2	0.8750	1.2094
2	13 % EFFLUENT	3	1.0000	1.3931
2	13 % EFFLUENT	4	0.8750	1.2094
2	13 % EFFLUENT	5	1.0000	1.3931
3	17 % EFFLUENT	1	1.0000	1.3931
3	17 % EFFLUENT	2	1.0000	1.3931
3	17 % EFFLUENT	3	1.0000	1.3931
3	17 % EFFLUENT	4	1.0000	1.3931
3	17 % EFFLUENT	5	0.8750	1.2094
4	23 % EFFLUENT	1	1.0000	1.3931
4	23 % EFFLUENT	2	1.0000	1.3931
4	23 % EFFLUENT	3	1.0000	1.3931
4	23 % EFFLUENT	4	1.0000	1.3931
4	23 % EFFLUENT	5	1.0000	1.3931
5	31 % EFFLUENT	1	1.0000	1.3931
5	31 % EFFLUENT	2	1.0000	1.3931
5	31 % EFFLUENT	3	1.0000	1.3931
5	31 % EFFLUENT	4	1.0000	1.3931
5	31 % EFFLUENT	5	1.0000	1.3931
6	41 % EFFLUENT	1	1.0000	1.3931
6	41 % EFFLUENT	2	1.0000	1.3931
6	41 % EFFLUENT	3	1.0000	1.3931
6	41 % EFFLUENT	4	0.8750	1.2094
6	41 % EFFLUENT	5	0.8750	1.2094

AA # K1601005, P. PROMELAS 7 DAY CHRONIC, 1-13-16
 File: sheriff 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	13 % EFFLUENT	1.320	25.00	16.00	5.00	
3	17 % EFFLUENT	1.356	27.50	16.00	5.00	
4	23 % EFFLUENT	1.393	30.00	16.00	5.00	
5	31 % EFFLUENT	1.393	30.00	16.00	5.00	
6	41 % EFFLUENT	1.320	25.00	16.00	5.00	

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

PRINT2

AA # K1601005, P. PROMELAS 7 DAY CHRONIC, 1-13-16
File: sherifg Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - wilk's test for normality

D = 0.099

W = 0.982

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 # K1601005, P. PROMELAS 7 DAY CHRONIC, 1-13-16
File: sherifg Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 3.86

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.

PRINT3

TITLE: AA # K1601005, P. PROMELAS 7 DAY CHRONIC, 1-13-16
 FILE: sherifg
 TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.6260	0.9128
1	CONTROL	2	0.7190	1.0121
1	CONTROL	3	0.7740	1.0754
1	CONTROL	4	0.7840	1.0874
1	CONTROL	5	0.7460	1.0426
2	13 % EFFLUENT	1	0.7390	1.0346
2	13 % EFFLUENT	2	0.6790	0.9685
2	13 % EFFLUENT	3	0.6710	0.9599
2	13 % EFFLUENT	4	0.6510	0.9388
2	13 % EFFLUENT	5	0.6780	0.9674
3	17 % EFFLUENT	1	0.7480	1.0449
3	17 % EFFLUENT	2	0.7760	1.0778
3	17 % EFFLUENT	3	0.7700	1.0706
3	17 % EFFLUENT	4	0.7420	1.0380
3	17 % EFFLUENT	5	0.6500	0.9377
4	23 % EFFLUENT	1	0.7580	1.0565
4	23 % EFFLUENT	2	0.7300	1.0244
4	23 % EFFLUENT	3	0.7500	1.0472
4	23 % EFFLUENT	4	0.8210	1.1340
4	23 % EFFLUENT	5	0.7240	1.0177
5	31 % EFFLUENT	1	0.7810	1.0838
5	31 % EFFLUENT	2	0.6960	0.9868
5	31 % EFFLUENT	3	0.7490	1.0460
5	31 % EFFLUENT	4	0.8380	1.1566
5	31 % EFFLUENT	5	0.7140	1.0065
6	41 % EFFLUENT	1	0.7300	1.0244
6	41 % EFFLUENT	2	0.7510	1.0484
6	41 % EFFLUENT	3	0.8610	1.1887
6	41 % EFFLUENT	4	0.6620	0.9504
6	41 % EFFLUENT	5	0.6840	0.9738

AA # K1601005, P. PROMELAS 7 DAY CHRONIC, 1-13-16
 File: sherifg Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.023	0.005	1.111
within (Error)	24	0.099	0.004	
Total	29	0.122		

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

PRINT3

File: sherifg 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	1.026	0.730		
2	13 % EFFLUENT	0.974	0.684	1.286	
3	17 % EFFLUENT	1.034	0.737	-0.191	
4	23 % EFFLUENT	1.056	0.757	-0.736	
5	31 % EFFLUENT	1.056	0.756	-0.736	
6	41 % EFFLUENT	1.037	0.738	-0.273	

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

AA # K1601005, P. PROMELAS 7 DAY CHRONIC, 1-13-16
 File: sherifg 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	13 % EFFLUENT	5	0.089	12.2	0.046
3	17 % EFFLUENT	5	0.089	12.2	-0.007
4	23 % EFFLUENT	5	0.089	12.2	-0.027
5	31 % EFFLUENT	5	0.089	12.2	-0.026
6	41 % EFFLUENT	5	0.089	12.2	-0.008

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

SURVIVAL AND REPRODUCTION TEST

Coriodesphnia dubia

Discharger: Sheridan Lab Number/s: K1601005 Analyst: tb, zr
 Location: Outfall Test Start - Date/Time: 1-13-16 / 1000
 Date Sample Collected: See LOC Test Stop - Date/Time: 1-19-16 / 1600

Conc %	1	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		A	B	C	D	E	F	G	H	I	J				
C 20	Day 1	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
	3	3	3	3	2	0	0	4	4	0	0	19	10	1.9	tb
	4	0	0	0	0	2	4	0	0	2	1	9	10	0.9	tb
	5	6	5	4	6	4	6	6	8	7	7	59	10	5.9	tb
	6	4	11	9	8	8	3	5	10	X2	8	68	9	6.8	tb
	7														
	8														
Total		13	19	16	16	14	13	15	22	X11	16	155		$\bar{X} = 16.0$	$CV = 18.2$

Conc %	2	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		A	B	C	D	E	F	G	H	I	J				
13	Day 1	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
	3	3	3	0	3	0	0	4	4	2	0	19	10	1.9	tb
	4	0	0	4	1	0	2	0	0	1	3	11	10	1.1	tb
	5	9	10	8	6	10	9	10	9	10	9	90	10	9.0	tb
	6	5	9	11	10	12	8	10	14	5	15	99	10	9.9	tb
	7														
	8														
Total		17	22	23	20	22	19	24	27	18	27	219			

Conc %	3	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		A	B	C	D	E	F	G	H	I	J				
17	Day 1	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
	3	4	3	4	3	3	0	3	4	3	3	30	10	3.0	tb
	4	0	0	0	0	2	3	0	0	0	1	6	10	0.6	tb
	5	11	10	9	9	8	10	9	8	11	9	93	10	9.3	tb
	6	15	11	12	13	13	13	14	11	9	8	119	10	11.9	tb
	7														
	8														
Total		30	24	25	25	26	26	26	23	20	23	248			

Conc %	4	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		A	B	C	D	E	F	G	H	I	J				
23	Day 1	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
	3	3	2	4	4	0	0	4	0	3	2	22	10	2.2	tb
	4	0	0	0	0	3	0	1	4	0	0	8	10	0.8	tb
	5	8	11	9	9	9	12	8	8	6	9	89	10	8.9	tb
	6	9	15	12	14	11	13	14	12	14	11	125	10	12.5	tb
	7														
	8														
Total		20	28	25	27	23	25	27	24	23	22	244			

Conc %	5	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		A	B	C	D	E	F	G	H	I	J				
31	Day 1	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
	3	4	4	4	3	3	4	4	3	2	4	35	10	3.5	tb
	4	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
	5	7	6	10	10	8	12	9	11	9	12	94	10	9.4	tb
	6	7	16	4	9	12	15	13	11	15	16	118	10	11.8	tb
	7														
	8														
Total		18	26	18	22	23	31	26	25	26	32	247		$\bar{X} = 24.7$	$CV = 19.0$

Conc %	6	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		A	B	C	D	E	F	G	H	I	J				
41	Day 1	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
	3	4	3	4	3	3	4	3	3	3	0	30	10	3.0	tb
	4	0	1	0	0	0	0	0	1	0	X	2	98	0.2	tb
	5	10	11	10	10	11	9	10	9	12	-	92	98	0.92	tb
	6	15	17	14	13	12	12	15	14	15	-	127	97	1.27	tb
	7														
	8														
Total		29	32	28	26	26	25	28	27	30	X0	251		$\bar{X} = 27.9$	$CV = 7.94$

X = Dead

PRINT

AA # K1601005, CERIODAPHNIA DUBIA REPRODUCTION, 1-13-16
File: sheridc 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 # K1601005, CERIODAPHNIA DUBIA REPRODUCTION, 1-13-16
File: sheridc Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 24.27

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

Data FAIL B1 homogeneity test at 0.01 level. Try another transformation.

PRINT2

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	1	9	10
13% effluent	0	10	10
TOTAL	1	19	20

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

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	1	9	10
17% effluent	0	10	10
TOTAL	1	19	20

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

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	1	9	10
23% effluent	0	10	10
TOTAL	1	19	20

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

PRINT2

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	1	9	10
31% effluent	0	10	10
TOTAL	1	19	20

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

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	9	1	10
41% effluent	9	1	10
TOTAL	18	2	20

CRITICAL FISHER'S VALUE (10,10,9) (p=0.05) IS 4. b VALUE IS 9.
Since b is greater than 4 there is no significant difference
between CONTROL and TREATMENT at the 0.05 level.

SUMMARY OF FISHER'S EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	10	1	
1	13% effluent	10	0	
2	17% effluent	10	0	
3	23% effluent	10	0	
4	31% effluent	10	0	
5	41% effluent	10	1	

PRINT2

PRINT1

TITLE: AA # K1601005, CERIODAPHNIA DUBIA REPRODUCTION, 1-13-16
 FILE: sheridc
 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	13.0000	13.0000
1	CONTROL	2	19.0000	19.0000
1	CONTROL	3	16.0000	16.0000
1	CONTROL	4	16.0000	16.0000
1	CONTROL	5	14.0000	14.0000
1	CONTROL	6	13.0000	13.0000
1	CONTROL	7	15.0000	15.0000
1	CONTROL	8	22.0000	22.0000
1	CONTROL	9	11.0000	11.0000
1	CONTROL	10	16.0000	16.0000
2	13 % EFFLUENT	1	17.0000	17.0000
2	13 % EFFLUENT	2	22.0000	22.0000
2	13 % EFFLUENT	3	23.0000	23.0000
2	13 % EFFLUENT	4	20.0000	20.0000
2	13 % EFFLUENT	5	22.0000	22.0000
2	13 % EFFLUENT	6	19.0000	19.0000
2	13 % EFFLUENT	7	24.0000	24.0000
2	13 % EFFLUENT	8	27.0000	27.0000
2	13 % EFFLUENT	9	18.0000	18.0000
2	13 % EFFLUENT	10	27.0000	27.0000
3	17 % EFFLUENT	1	30.0000	30.0000
3	17 % EFFLUENT	2	24.0000	24.0000
3	17 % EFFLUENT	3	25.0000	25.0000
3	17 % EFFLUENT	4	25.0000	25.0000
3	17 % EFFLUENT	5	26.0000	26.0000
3	17 % EFFLUENT	6	26.0000	26.0000
3	17 % EFFLUENT	7	26.0000	26.0000
3	17 % EFFLUENT	8	23.0000	23.0000
3	17 % EFFLUENT	9	20.0000	20.0000
3	17 % EFFLUENT	10	23.0000	23.0000
4	23 % EFFLUENT	1	20.0000	20.0000
4	23 % EFFLUENT	2	28.0000	28.0000
4	23 % EFFLUENT	3	25.0000	25.0000
4	23 % EFFLUENT	4	27.0000	27.0000
4	23 % EFFLUENT	5	23.0000	23.0000
4	23 % EFFLUENT	6	25.0000	25.0000
4	23 % EFFLUENT	7	27.0000	27.0000
4	23 % EFFLUENT	8	24.0000	24.0000
4	23 % EFFLUENT	9	23.0000	23.0000
4	23 % EFFLUENT	10	22.0000	22.0000
5	31 % EFFLUENT	1	18.0000	18.0000
5	31 % EFFLUENT	2	26.0000	26.0000
5	31 % EFFLUENT	3	18.0000	18.0000
5	31 % EFFLUENT	4	22.0000	22.0000
5	31 % EFFLUENT	5	23.0000	23.0000
5	31 % EFFLUENT	6	31.0000	31.0000
5	31 % EFFLUENT	7	26.0000	26.0000
5	31 % EFFLUENT	8	25.0000	25.0000
5	31 % EFFLUENT	9	26.0000	26.0000
5	31 % EFFLUENT	10	32.0000	32.0000
6	41 % EFFLUENT	1	29.0000	29.0000
6	41 % EFFLUENT	2	32.0000	32.0000
6	41 % EFFLUENT	3	28.0000	28.0000
6	41 % EFFLUENT	4	26.0000	26.0000

			PRINT1	
6	41 % EFFLUENT	5	26.0000	26.0000
6	41 % EFFLUENT	6	25.0000	25.0000
6	41 % EFFLUENT	7	28.0000	28.0000
6	41 % EFFLUENT	8	27.0000	27.0000
6	41 % EFFLUENT	9	30.0000	30.0000
6	41 % EFFLUENT	10	0.0000	0.0000

AA # K1601005, CERIODAPHNIA DUBIA REPRODUCTION, 1-13-16
 File: sherid Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	695.333	139.067	5.987
Within (Error)	54	1254.400	23.230	
Total	59	1949.733		

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

AA # K1601005, CERIODAPHNIA DUBIA REPRODUCTION, 1-13-16
 File: sherid Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 1 OF 2

Ho: Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	15.500	15.500		
2	13 % EFFLUENT	21.900	21.900	-2.969	
3	17 % EFFLUENT	24.800	24.800	-4.315	
4	23 % EFFLUENT	24.400	24.400	-4.129	
5	31 % EFFLUENT	24.700	24.700	-4.268	
6	41 % EFFLUENT	25.100	25.100	-4.454	

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

AA # K1601005, CERIODAPHNIA DUBIA REPRODUCTION, 1-13-16
 File: sherid 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	13 % EFFLUENT	10	4.979	32.1	-6.400
3	17 % EFFLUENT	10	4.979	32.1	-9.300
4	23 % EFFLUENT	10	4.979	32.1	-8.900
5	31 % EFFLUENT	10	4.979	32.1	-9.200
6	41 % EFFLUENT	10	4.979	32.1	-9.600

PRINT1

AA # K1601005, CERIODAPHNIA DUBIA REPRODUCTION, 1-13-16
 File: shericd Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	15.500				
2	13 % EFFLUENT	21.900	147.50	75.00	10.00	
3	17 % EFFLUENT	24.800	154.00	75.00	10.00	
4	23 % EFFLUENT	24.400	153.50	75.00	10.00	
5	31 % EFFLUENT	24.700	150.50	75.00	10.00	
6	41 % EFFLUENT	25.100	145.00	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 1/12/16 CLIENT ARU ANALYTICAL

Purchase Order #: _____

SPECIES: Pimephales promelas

Quantity Shipped: 1000⁺ 15-1600
CST

Age: HATCHED 1/11/16

Brood Stock Source: Anderson Farms, AR

Culture Water: Groundwater

Hardness (Mg/l CaCO₃): ≈ 160

Dissolved Oxygen (Mg/l): 8.5

Temperature (°C): 25.1

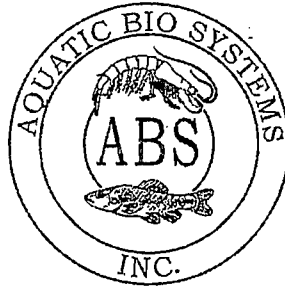
Feeding: ARTEMIA

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

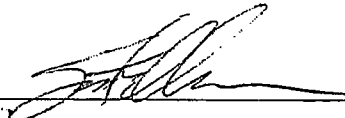
BEGAN FEEDING: Immediately

FOOD: YTC, Selenastrum sp.

Water Chemistry Record:

	Current	Range
TEMPERATURE:	<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

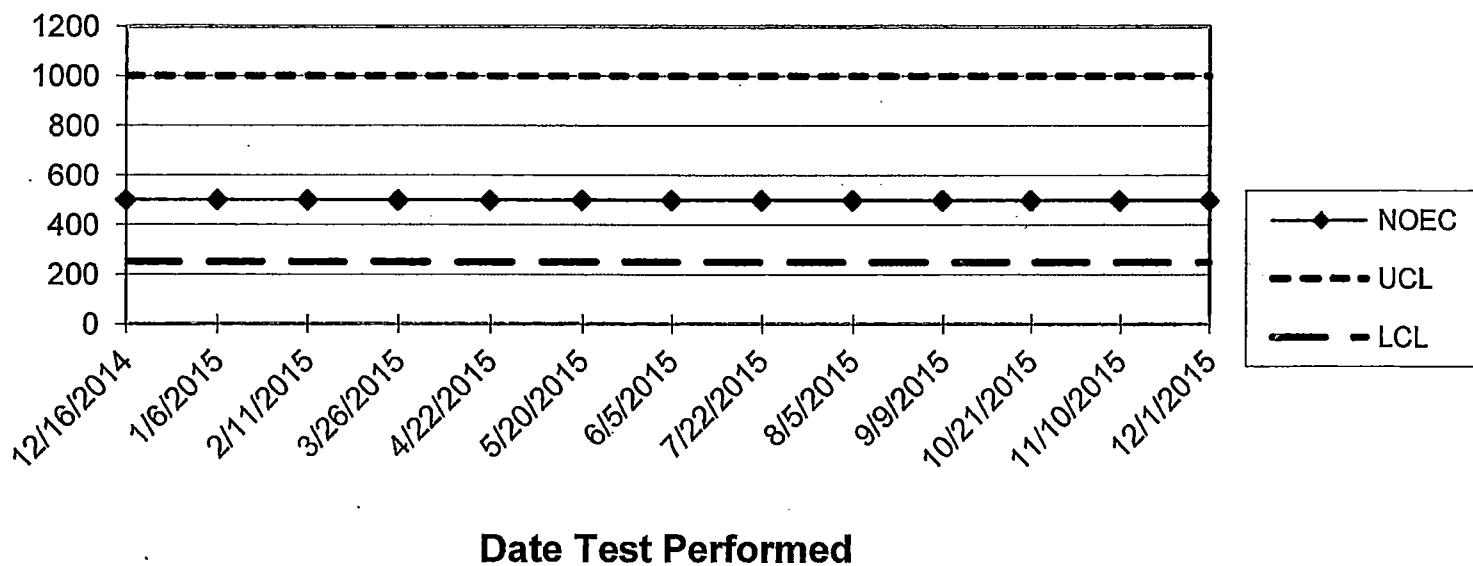
APPENDIX F

Quality Assurance Charts

ARKANSAS ANALYTICAL, INC.

FATHEAD MINNOW SURVIVAL 7 Day QUALITY ASSURANCE

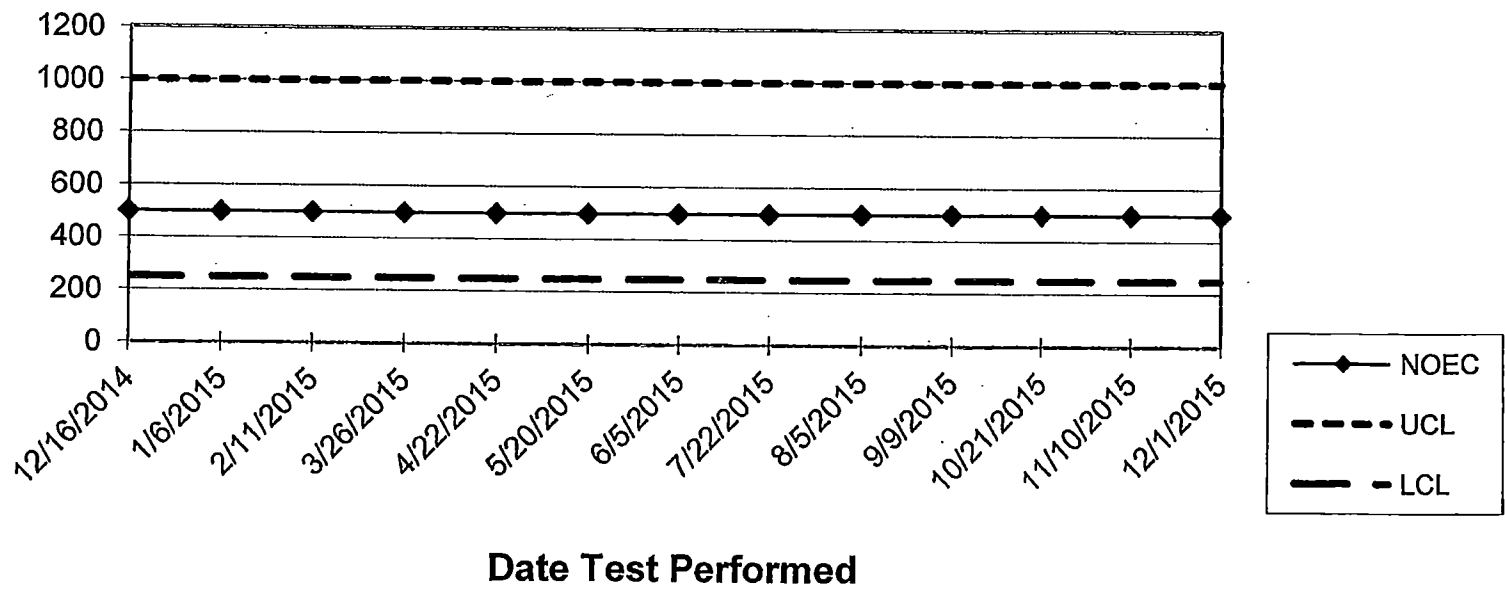
Reference Toxicant, KCl, ppm



ARKANSAS ANALYTICAL, INC.

FATHEAD MINNOW GROWTH 7 Day QUALITY ASSURANCE

Reference Toxicant, KCl, ppm

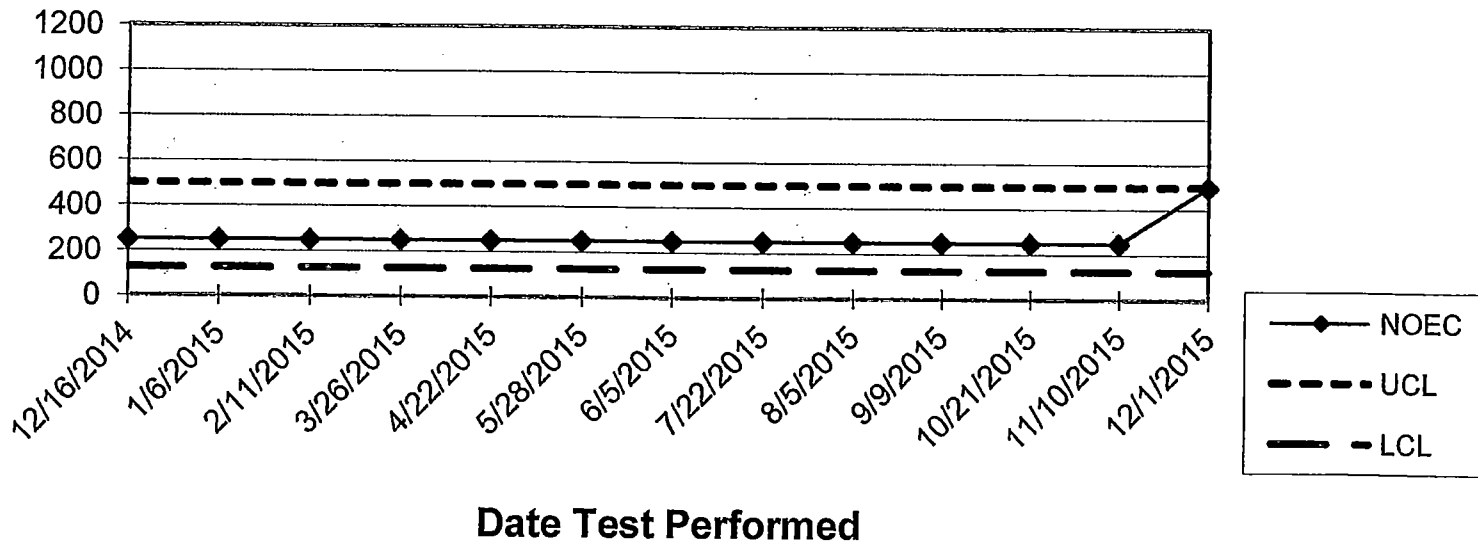


ARKANSAS ANALYTICAL, INC.

CERIODAPHNIA DUBIA SURVIVAL

QUALITY ASSURANCE

Reference Toxicant, KCl, ppm

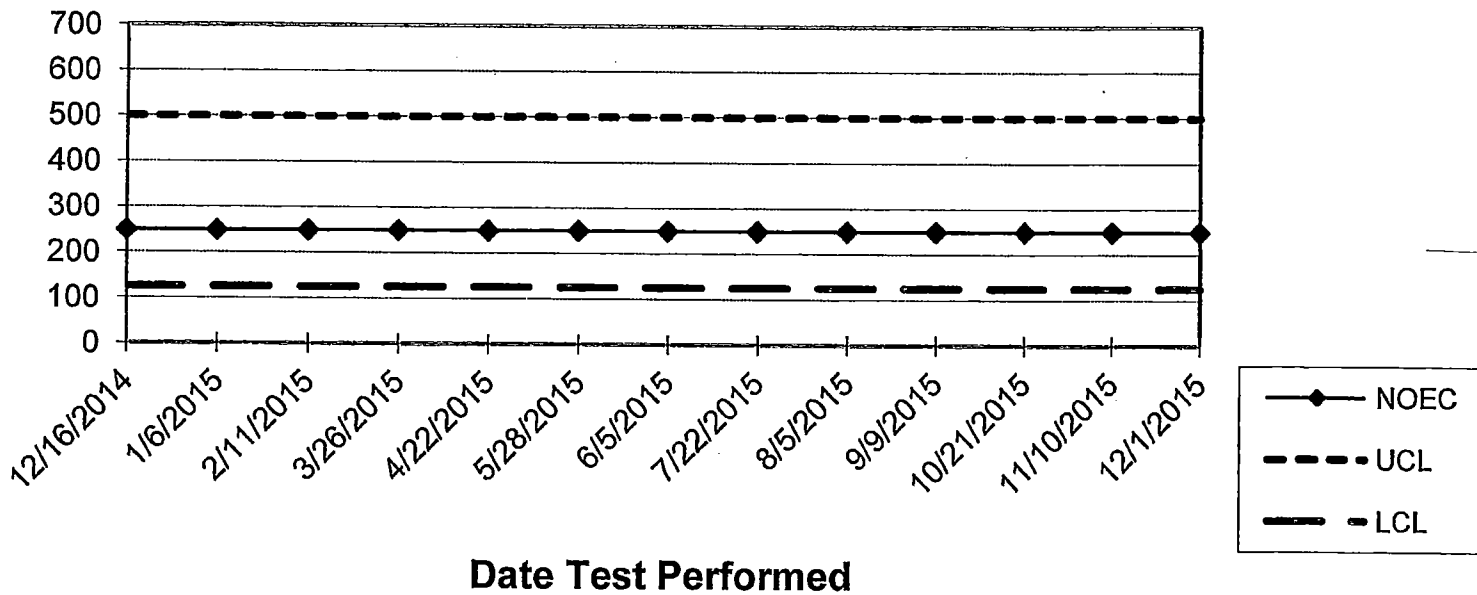


ARKANSAS ANALYTICAL, INC.

CERIODAPHНИЯ DUBIA REPRODUCTION

QUALITY ASSURANCE

Reference Toxicant, KCl, ppm



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

CERTIFIED MAIL

Sh
PC

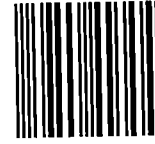
Sheridan, AR 72150



7015 3430 0001 1320 9368



1000



72118

U.S. POSTAGE
PAID
SHERIDAN, AR
72150
APR 27, 16
AMOUNT

\$8.62

R2305E125647-02

RETURN RECEIPT
REQUESTED

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