

Arkansas Analytical, Inc.

Toxicity Test Results

CITY of SHERIDAN
NPDES PERMIT NUMBER: AR0034347
First 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.
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Little Rock, Arkansas 72209
Lab Number K1503001

Thursday, March 19, 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 $\frac{1}{4}$ of the Northwest $\frac{1}{4}$ 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 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:	3-1-15, 0900	3-2-15, 0800
Sample #2	3-2-15, 1152	3-3-15, 1052
Sample #3	3-3-15, 1530	3-4-15, 1430

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

Sample Receiving Information:	Date, Time Sample(s) Received	Temperature Upon Receipt (°C)
Sample #1:	3-2-15, 1253	4
Sample #2	3-3-15, 1653	3
Sample #3	3-4-15, 1605	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 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	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	0.602	X	
The percent coefficient of variation between replicates must be 40% or less for growth	14.6%	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	16.8	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	22.1%	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> 2/11/15 – 2/18/15		<i>Pimephales promelas</i> 2/11/15 – 2/18/15	
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)	15.5	%CV survival (critical dilution)	5.73%
%CV Reproduction (critical dilution)	22.0%	Mean dry weight (critical dilution) in milligrams	0.614
		%CV growth (critical dilution)	9.88%
PMSD Reproduction	27.0%	PMSD Growth	13.4%

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 Analysts:

Ryan Hudgin / Tracy Bounds / Kenneth Pigue

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:	3-1-15, 0900	3-2-15, 0800
Sample #2	3-2-15, 1152	3-3-15, 1052
Sample #3	3-3-15, 1530	3-4-15, 1430

Test initiated (date, time): 3-3-15, 1400 Test terminated (date, time): 3-10-15, 1120

Dilution water used: Moderately Hard Synthetic

DATA TABLE FOR FATHEAD MINNOW SURVIVAL

Effluent Conc %	Percent Survival in Replicate Chambers						Mean Percent Survival			
	A	B	C	D	E		24 hours	48 hours	7 days	CV %
0%	100	87.5	100	100	100		100	100	97.5	5.73%
10%	100	100	100	87.5	87.5		100	97.5	95.0	
13%	87.5	100	100	100	100		100	97.5	97.5	
17%	87.5	100	100	87.5	100		100	97.5	95.0	
23%	100	100	87.5	100	100		100	97.5	97.5	5.73%
31%	100	100	100	100	100		100	100	100	

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%	0.611	0.456	0.640	0.691	0.611		0.602	14.6%
10%	0.699	0.634	0.660	0.551	0.622		0.633	
13%	0.604	0.609	0.667	0.593	0.646		0.624	
17%	0.556	0.560	0.551	0.517	0.633		0.563	
23%	0.600	0.562	0.581	0.717	0.606		0.613	9.88%
31%	0.649	0.599	0.653	0.630	0.636		0.633	

Coefficient of Variation = standard deviation / mean * 100

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING
FATHEAD MINNOW LARVAE GROWTH AND SURVIVAL
Pimephales promelas

1. Dunnett's procedure or Steel's Many-One Rank Test as appropriate:
Is the mean survival at 7 days significantly different ($p=0.05$) than the control survival for:
a) LOW FLOW OR CRITICAL DILUTION, (14.0%) YES _____ NO X

2. Dunnett's Procedure
Is the mean dry weight (growth) at 7 days significantly different ($p=0.05$) than the control's dry weight (growth) for:
a) LOW FLOW OR CRITICAL DILUTION, (14.0%) YES _____ NO X

3. If NO was answered to 1.a) enter [0] otherwise enter [1] (parameter TLP6C): 0

4. If NO was answered to 2.a) enter [0] otherwise enter [1] (parameter TGP6C): 0

5. Enter percentage corresponding to each parameter below:
a) NOEC survival (parameter TOP6C)= 31 % effluent
b) NOEC growth (parameter TPP6C)= 31 % effluent
c) Coefficient of variation (parameter TQP6C)= 14.6 %

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING
Ceriodaphnia dubia SURVIVAL AND REPRODUCTION

Permittee: City of Sheridan

NPDES #: AR0034347

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	3-1-15, 0900	3-2-15, 0800
Sample #2	3-2-15, 1152	3-3-15, 1052
Sample #3	3-3-15, 1530	3-4-15, 1430

Test initiated (date, time): 3-3-15, 1100 Test terminated (date, time): 3-10-15, 1000

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	15	11	12	7	8	14
B	13	14	19	11	19	8
C	15	11	14	23	17	14
D	19	15	14	13	16	14
E	18	12	11	12	18	17
F	21	15	17	13	11	14
G	12	19	18	18	17	6
H	15	7	15	10	17	15
I	X3	15	14	17	15	15
J	23	16	10	13	17	18
Mean	15.4	13.5	14.4	13.7	15.5	13.5
Mean/surviving female	16.8	13.5	14.4	13.7	15.5	13.5
CV%*	22.1				22.0	

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%	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	90	100	100	100	100	100

1. Fisher's Exact Test:

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

a) LOW FLOW OR CRITICAL DILUTION, (36.3%): YES _____ NO X _____

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

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

a) LOW FLOW OR CRITICAL DILUTION, (36.3%): YES _____ NO X _____

3. If NO was answered to 1.a) enter [0] otherwise enter [1] (parameter TLP3B): 0 _____

4. If NO was answered to 2.a) enter [0] otherwise enter [1] (parameter TGP3B): 0 _____

5. Enter percentage corresponding to each parameter below:

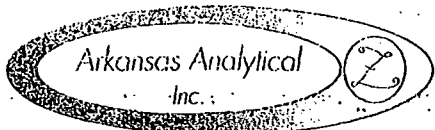
a) NOEC survival (parameter TOP3B)= 31 % effluent

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

c) Coefficient of variation (parameter TQP3B)= 22.1 %

APPENDIX A

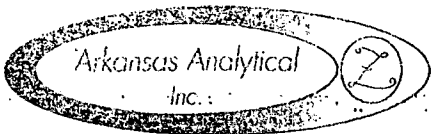
Chain of Custody Forms



11701 Interstate 30, Bldg. 1, Ste. 115
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 FAX: 501-455-6118






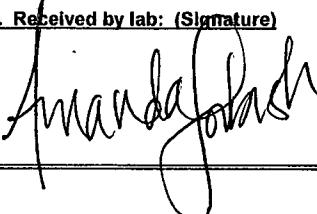
CHAIN OF CUSTODY RECORD

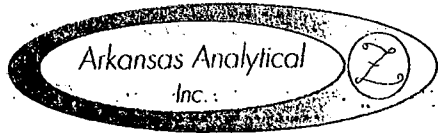
CLIENT INFORMATION		BILLING INFORMATION		Project Description		Turnaround Time		Preservation Codes:															
Sheridan Waterworks 104 W High St. Sheridan, AR 72150		Sheridan Waterworks P.O. Box 486 Sheridan, AR 72150		Chronic Toxicity		1 Day (100%) 2 Day (50%) 3 Day (25%)		1. Cool, 4 Degrees Centigrade 2. Sulfuric Acid (H ₂ SO ₄), pH < 2 3. Nitric Acid (HNO ₃), pH < 2				4. Thiosulfate for Dechlorination 5. Hydrochloric Acid (HCl) 6. Sodium Hydroxide (NaOH), pH > 12											
Attn: David Fitzgerald				Telephone: 870-942-2722		Reporting Information		Routine		TEST PARAMETERS								Bottle Type Code					
				Fax: 870-942-1937		Email: sheridanwater@windstream.net		Preservative Code: 1										G = Glass; P = Plastic V = Septum; A = Amber					
				Bottle Type: P																			
<p style="font-size: 2em; text-align: center;"><i>Allen Parker</i></p> Sampler(s) Signature				<p style="font-size: 2em; text-align: center;"><i>Allen Parker</i></p> Sampler(s) Printed								Chronic Toxicity										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																					
	3/1-2/15	0900-0800		X	24	Water	Final Discharge		X										K1502004A K1503001				
1. Relinquished by: (Signature)			Date/Time		2. Received by: (Signature)			SAMPLE CONDITION UPON RECEIPT IN LAB				REMARKS / SAMPLE COMMENTS											
<i>Allen Parker</i>			1253 3-2-15		/			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: ___ Yes ___ No 5. TEMPERATURE ON RECEIPT: 4°C 6. TEMPERATURE GUN ID: HHT #2															
3. Relinquished by: (Signature)			Date/Time		4. Received by lab: (Signature)			FOR COMPLETION BY LAB ONLY															
/			/		<i>Sydney James</i>																		



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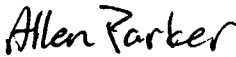




CHAIN OF CUSTODY RECORD

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								Bottle Type Code																									
Attn: David Fitzgerald				Fax: 870-942-1937		Preservative Code: 1		1								G = Glass, P = Plastic																									
				Email: sheridanwater@windstream.net		Bottle Type: P		P								V = Septum; A = Amber																									
 Sampler(s) Signature				Allen Parker Sampler(s) Printed				Chronic Toxicity		<table border="1"> <tr> <td colspan="8">Arkansas Analytical Work Order Number:</td> </tr> <tr> <td colspan="8">K1503001</td> </tr> <tr> <td colspan="8">B</td> </tr> </table>								Arkansas Analytical Work Order Number:								K1503001								B							
Arkansas Analytical Work Order Number:																																									
K1503001																																									
B																																									
Field Number	SAMPLE COLLECTION		Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION																																		
	Date/s	Time/s																																							
	3/2-3/15	1152-1052		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																															
		1653 3-3-15				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: 3°C 6. TEMPERATURE GUN ID: HHT#2																																			
3. Relinquished by: (Signature)		Date/Time		4. Received by lab: (Signature)		FOR COMPLETION BY LAB ONLY																																			
																																									



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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 = Septium; A = Amber							
 Sampler(s) Signature				 Sampler(s) Printed				Chronic Toxicity												Arkansas Analytical Work Order Number:					
Field Number		SAMPLE COLLECTION Date/s Time/s		Grab	Comp	Number of Bottles	Sample Matrix															SAMPLE IDENTIFICATION/ DESCRIPTION			
		3/3-4/15 1530-1430			X	24	Water	Final Discharge				X												K1503001C	
1. Relinquished by: (Signature)		Date/Time		2. Received by: (Signature)		SAMPLE CONDITION UPON RECEIPT IN LAB						REMARKS / SAMPLE COMMENTS													
		1605 3-4-15				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: HHT #2																			
3. Relinquished by: (Signature)		Date/Time		4. Received by lab: (Signature)		FOR COMPLETION BY LAB ONLY																			
																									

APPENDIX B

Effluent and Dilution Water Data

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Fathead Minnow

Lab #/ Sample ID K1503001

Test Start (Date/Time) 3-3-15 1400

Client: Sheridan

Test End (Date/Time) 3-10-15 1120

Day of Test

		1	2	3	4	5	6	7	notes
Control	MHS	3-3	3-4	3-5	3-6	3-7	3-8	3-9	
D.O. (mg/L)	INITIAL	8.5	8.2	8.6	8.7	8.6	8.9	8.5	
	FINAL	7.4	7.4	7.8	8.4	8.5	7.2	7.5	
pH (s.u.)	INITIAL	7.7	8.1	7.7	8.1	8.3	7.9	8.4	
	FINAL	7.6	7.7	7.7	7.5	7.9	7.6	7.7	
temp (C)	INITIAL	23	22	23	22	22.1	22.4	22	
	FINAL	25	25	25	25.0	25.0	25	25	
ALKALINITY (mg/L)		58					62		
HARDNESS (mg/L)		82					86		
CONDUCTIVITY (umhc)		396					414		
CHLORINE (mg/L)		0.05							
CONC:	10								
D.O. (mg/L)	INITIAL	8.5	7.9	8.6	8.3	8.6	8.7	8.1	
	FINAL	7.3	7.2	7.4	8.4	8.3	6.6	6.7	
pH (s.u)	INITIAL	7.8	8.0	7.8	8.0	8.2	8.0	8.2	
	FINAL	7.6	7.7	7.7	7.7	7.9	7.6	7.8	
temp (C)	INITIAL	23	22	23	22	22.2	21.6	22	
	FINAL	25	25	25	25.0	25.0	25	25	
CONC:	13								
D.O. (mg/L)	INITIAL	8.6	8.3	8.6	8.8	8.6	8.6	8.7	
	FINAL	7.6	7.1	8.0	8.4	8.5	7.2	7.6	
pH (mg/L)	INITIAL	7.9	8.0	7.8	7.9	8.2	8.0	8.1	
	FINAL	7.7	7.7	7.7	7.7	8.0	7.7	7.8	
temp (C)	INITIAL	23	22	23	22	22.4	21.8	22	
	FINAL	25	25	25	25.0	25.0	25	25	
CONC:	17								
D.O. (mg/L)	INITIAL	8.6	8.5	8.6	8.9	8.8	8.7	8.7	
	FINAL	7.7	7.1	8.0	8.4	8.7	7.9	7.7	
pH (s.u.)	INITIAL	7.9	8.0	7.9	7.9	8.2	7.9	8.1	
	FINAL	7.7	7.7	7.8	7.8	8.0	7.7	7.9	
temp (C)	INITIAL	23	22	23	22	22.3	21.8	22	
	FINAL	25	25	25	25.0	25.0	25	25	
CONC:	23								
D.O. (mg/L)	INITIAL	8.7	8.2	8.6	9.1	8.9	8.7	8.9	
	FINAL	7.5	7.7	8.0	8.3	8.2	7.1	7.4	
pH (s.u.)	INITIAL	8.0	8.0	7.9	7.9	8.2	7.9	8.0	
	FINAL	7.8	7.7	7.7	7.8	8.0	7.7	7.9	
temp (C)	INITIAL	23	23	23	22	22.7	21.6	22	
	FINAL	25	25	25	25.0	25.0	25	25	
CONC:	31								
D.O. (mg/L)	INITIAL	8.7	8.3	8.6	9.0	9.0	8.7	8.8	
	FINAL	7.5	7.2	8.1	8.1	8.0	6.9	7.5	
pH (s.u.)	INITIAL	8.0	8.0	7.9	7.9	8.1	7.9	8.0	
	FINAL	7.8	7.7	7.8	7.8	8.0	7.7	7.8	
temp (C)	INITIAL	23	23	23	22	22.8	21.8	22	
	FINAL	25	25	25	25.0	25.0	25	25	
CONC:	100 %	A	A	A	B	C	B	C	
ALKALINITY (mg/L)		68			86	70	86	70	
HARDNESS (mg/L)		66			60	60	60	60	
CONDUCTIVITY (umhc)		451			476	451	476	451	
CHLORINE (mg/L)		0.06			0.05	0.05			

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Ceriodaphnia Dubia

Lab # / Sample ID K1503001

Test Start (Date/Time) 3-3-15 1100

Client: Sheridan

Test End (Date/Time) 3-10-15 1000

		Day of Test							notes
		1	2	3	4	5	6	7	
Control	MHS	3-3	3-4	3-5	3-6	3-7	3-8	3-9	
D.O. (mg/L)	INITIAL	8.5	8.2	8.6	8.7	8.6	8.9	8.5	
	FINAL	8.3	8.4	8.5	8.5	8.0	8.3	7.9	
pH (s.u.)	INITIAL	7.7	8.1	7.7	8.1	8.3	7.9	8.4	
	FINAL	7.8	7.8	7.9	7.7	8.0	7.8	8.2	
temp (C)	INITIAL	23	22	23	22	22	22	22	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		58	---	---	---	---	62	---	
HARDNESS (mg/L)		82	---	---	---	---	86	---	
CONDUCTIVITY (umho)		396	---	---	---	---	414	---	
CHLORINE (mg/L)		0.05	---	---	---	---	---	---	
CONC:	10								
D.O. (mg/L)	INITIAL	8.5	7.9	8.6	8.3	8.6	8.7	8.1	
	FINAL	8.3	8.5	8.4	8.2	8.1	8.3	8.2	
pH (s.u.)	INITIAL	7.8	8.0	7.8	8.0	8.2	8.0	8.2	
	FINAL	7.9	7.8	7.8	7.8	8.0	8.0	8.1	
temp (C)	INITIAL	23	22	23	22	22	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	13								
D.O. (mg/L)	INITIAL	8.6	8.3	8.6	8.8	8.6	8.6	8.7	
	FINAL	8.3	8.6	8.4	8.2	8.3	8.1	8.1	
pH (mg/L)	INITIAL	7.9	8.0	7.8	7.9	8.2	8.0	8.1	
	FINAL	8.0	7.9	7.9	7.9	8.1	8.1	8.1	
temp (C)	INITIAL	23	22	23	22	22	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	17								
D.O. (mg/L)	INITIAL	8.6	8.5	8.6	8.9	8.8	8.7	8.7	
	FINAL	8.3	8.6	8.5	8.2	8.3	8.0	8.2	
pH (s.u.)	INITIAL	7.9	8.0	7.9	7.9	8.2	7.9	8.1	
	FINAL	8.0	7.9	7.9	8.0	8.1	8.0	8.2	
temp (C)	INITIAL	23	22	23	22	22	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	23								
D.O. (mg/L)	INITIAL	8.7	8.2	8.6	9.1	8.9	8.7	8.9	
	FINAL	8.3	8.6	8.4	8.2	8.2	8.0	8.2	
pH (s.u.)	INITIAL	8.0	8.0	7.9	7.9	8.2	7.9	8.0	
	FINAL	8.0	8.0	8.0	8.1	8.1	8.1	8.1	
temp (C)	INITIAL	23	23	23	22	23	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	31								
D.O. (mg/L)	INITIAL	8.7	8.3	8.6	9.0	9.0	8.7	8.8	
	FINAL	8.3	8.6	8.5	8.2	8.3	7.9	8.1	
pH (s.u.)	INITIAL	8.0	8.0	7.9	7.9	8.1	7.9	8.0	
	FINAL	8.1	8.0	8.0	8.1	8.1	8.0	8.1	
temp (C)	INITIAL	23	23	23	22	23	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	100 %	A	A	A	B	C	B	C	
ALKALINITY (mg/L)		68	---	---	86	70	86	70	
HARDNESS (mg/L)		66	---	---	60	60	60	60	
CONDUCTIVITY (umho)		451	---	---	476	451	476	451	
CHLORINE (mg/L)		0.06	---	---	0.05	---	---	---	

APPENDIX C

Fathead minnow raw data and statistics

FATHEAD MINNOW

SURVIVAL DATA FOR LARVAL SURVIVAL AND GROWTH TEST (ALTERNATE)

LAB #: K1503001			TEST START		DATE	03/03/15	TIME	1400				
CLIENT: Sheridan			TEST END		DATE	03/10/15	TIME	1120				
ANALYST: RH / TB / KP			AGE AND SOURCE OF MINNOWS		< 48 hrs old, Aquatox							
DAY(NUMBER SURVIVING)												
SURVIVAL												
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONTROL	A	8	8	8	8	8	8	8	8	100%	97.5%	5.73
	B	8	8	8	8	7	7	7	7	87.5%		
	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%		
MHS	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
	A	8	8	8	8	8	8	8	8	100%	95.0%	
	B	8	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	8	100%		
	D	8	8	8	7	7	7	7	7	87.5%		
E	8	8	7	7	7	7	7	7	87.5%			
10%	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
	A	8	8	7	7	7	7	7	7	87.5%	97.5%	
	B	8	8	8	8	8	8	8	8	100%		
	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%			
13%	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
	A	8	8	8	8	7	7	7	7	87.5%	95.0%	
	B	8	8	8	8	8	8	8	8	100%		
	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%			
17%	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
	A	8	8	8	8	8	8	8	8	100%	97.5%	5.73
	B	8	8	8	8	8	8	8	8	100%		
	C	8	8	7	7	7	7	7	7	87.5%		
	D	8	8	8	8	8	8	8	8	100%		
E	8	8	8	8	8	8	8	8	100%			
23%	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
	A	8	8	8	8	8	8	8	8	100%	100.0%	
	B	8	8	8	8	8	8	8	8	100%		
	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%			
31%	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
	A	8	8	8	8	8	8	8	8	100%	100.0%	
	B	8	8	8	8	8	8	8	8	100%		
	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%			
ANALYST:		RH	RH	TB	RH	KP	KP	RH	RH			
DATE:		3/3/15	3/4/15	3/5/15	3/6/15	3/7/15	3/8/15	3/9/15	3/10/15			
TIME:		1400	1220	1500	1300	1130	1450	1605	1120			

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

REMARKS:

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB #/SAMPLE ID	1503001	TEST START DATE	3-3-15	TIME	1400						
CLIENT	Sheridan A	TEST END DATE	3-10-15	TIME	1120						
AGE AND SOURCE OF MINNOWS											
DAY (NUMBER SURVIVING)											
	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
mHS	CONC: A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E										
10	CONC: A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E										
13	CONC: A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E										
17	CONC: A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E										
23	CONC: A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E										
31	CONC: A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E										
ANALYST		RH	RH	Hb/KR	RH	KP	KP	RH	RH		
DATE:		3-3-15	3-4-15	3-5-15	3-6-15	3-7-15	3-8-15	3-9-15	3-10-15		
TIME:		1400	1220	1500	1300	1130	1450	1605	1120		

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)											
SURVIVAL											
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
MHS	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	2	2	2	2		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	2	2	2	2		
	E										
10	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E										
13	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E										
17	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E										
23	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E										
31	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E										
ANALYST	RH										
DATE:	3-3-15										
TIME:											

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB #/SAMPLE ID		150300		TEST START DATE		3-3-15		TIME		
CLIENT		Sheridan		TEST END DATE				TIME		
AGE AND SOURCE OF MINNOWS										
DAY (NUMBER SURVIVING)										
	REP #	start	1	2	3	4	5	6	7%	MEAN % CV
mfs	CONC: A	2	2	2	2	2	2	2	2	
	B	1	1	1	1	1	1	1	1	
	C	1	1	1	1	1	1	1	1	
	D	1	1	1	1	1	1	1	1	
	E									
10	CONC: A	2	2	2	2	2	2	2	2	
	B	1	1	1	1	1	1	1	1	
	C	1	1	1	1	1	1	1	1	
	D	1	1	1	1	1	1	1	1	
	E									
13	CONC: A	2	2	2	2	2	2	2	2	
	B	1	1	1	1	1	1	1	1	
	C	1	1	1	1	1	1	1	1	
	D	1	1	1	1	1	1	1	1	
	E									
17	CONC: A	2	2	2	2	2	2	2	2	
	B	1	1	1	1	1	1	1	1	
	C	1	1	1	1	1	1	1	1	
	D	1	1	1	1	1	1	1	1	
	E									
23	CONC: A	2	2	2	2	2	2	2	2	
	B	1	1	1	1	1	1	1	1	
	C	1	1	1	1	1	1	1	1	
	D	1	1	1	1	1	1	1	1	
	E									
31	CONC: A	2	2	2	2	2	2	2	2	
	B	1	1	1	1	1	1	1	1	
	C	1	1	1	1	1	1	1	1	
	D	1	1	1	1	1	1	1	1	
	E									
ANALYST	RH									
DATE:	3-3-15									
TIME:										

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		1503001		TEST START DATE		3-3-15		TIME				
CLIENT		Sheridan D		TEST END DATE				TIME				
AGE AND SOURCE OF MINNOWS												
DAY (NUMBER SURVIVING)												
SURVIVAL												
	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV	
MHS	CONC: A	2	2	2	3	2	2	3	2			
	B	1	1	1	1	1	1	1	1			
	C	1	1	1	1	1	1	1	1			
	D	1	1	1	1	1	1	1	1			
	E	1	1	1	1	1	1	1	1			
	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV	
10	CONC: A	2	2	2	2	2	2	2	2			
	B	1	1	1	1	1	1	1	1			
	C	1	1	1	1	1	1	1	1			
	D	1	1	1	2	2	2	2	2			
	E	1	1	1	1	1	1	1	1			
	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV	
13	CONC: A	2	2	2	2	2	2	2	2			
	B	1	1	1	1	1	1	1	1			
	C	1	1	1	1	1	1	1	1			
	D	1	1	1	1	1	1	1	1			
	E	1	1	1	1	1	1	1	1			
	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV	
17	CONC: A	2	2	1	1	1	1	1	1			
	B	1	1	2	2	2	2	2	2			
	C	1	1	1	2	1	1	2	2			
	D	1	1	1	2	1	1	2	2			
	E	1	1	1	1	1	1	1	1			
	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV	
23	CONC: A	2	2	2	2	2	2	2	2			
	B	1	1	1	1	1	1	1	1			
	C	1	1	1	1	1	1	1	1			
	D	1	1	1	1	1	1	1	1			
	E	1	1	1	1	1	1	1	1			
	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV	
31	CONC: A	2	2	2	2	2	2	2	2			
	B	1	1	1	1	1	1	1	1			
	C	1	1	1	1	1	1	1	1			
	D	1	1	1	1	1	1	1	1			
	E	1	1	1	1	1	1	1	1			
ANALYST	RH											
DATE:	3-3-15											
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		TEST END DATE		TIME				
1503001		3-3-15								
CLIENT		AGE AND SOURCE OF MINNOWS								
Sheridan E										
		DAY (NUMBER SURVIVING)						SURVIVAL		
REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
CONC: 1	A	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1		
	E									
CONC: 10	A	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1		
	E									
CONC: 13	A	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1		
	E									
CONC: 17	A	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1		
	E									
CONC: 23	A	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1		
	E									
CONC: 31	A	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1		
	E									
ANALYST	RH									
DATE:	3-3-15									
TIME:										

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

AA# K1503001, FATHEAD MINNOW SURV., CHRONIC, 3-3-15

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

Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.162

W = 0.774

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# K1503001, FATHEAD MINNOW SURV., CHRONIC, 3-3-15

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

Transform: ARC SINE(SQUARE ROOT(Y))

Hartley's test for homogeneity of variance

Bartlett's test for homogeneity of variance

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

Data FAIL to meet homogeneity of variance assumption.

Additional transformations are useless.

TITLE: AA# K1503001, FATHEAD MINNOW SURV., CHRONIC, 3-3-15
 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	1.0000	1.3931
1	CONTROL	2	0.8750	1.2094
1	CONTROL	3	1.0000	1.3931
1	CONTROL	4	1.0000	1.3931
1	CONTROL	5	1.0000	1.3931
2	10 % EFFLUENT	1	1.0000	1.3931
2	10 % EFFLUENT	2	1.0000	1.3931
2	10 % EFFLUENT	3	1.0000	1.3931
2	10 % EFFLUENT	4	0.8750	1.2094
2	10 % EFFLUENT	5	0.8750	1.2094
3	13 % EFFLUENT	1	0.8750	1.2094
3	13 % EFFLUENT	2	1.0000	1.3931
3	13 % EFFLUENT	3	1.0000	1.3931
3	13 % EFFLUENT	4	1.0000	1.3931
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	1.0000	1.3931
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	1.0000	1.3931
5	23 % EFFLUENT	3	0.8750	1.2094
5	23 % EFFLUENT	4	1.0000	1.3931
5	23 % EFFLUENT	5	1.0000	1.3931
6	31 % EFFLUENT	1	1.0000	1.3931
6	31 % EFFLUENT	2	1.0000	1.3931
6	31 % EFFLUENT	3	1.0000	1.3931
6	31 % EFFLUENT	4	1.0000	1.3931
6	31 % EFFLUENT	5	1.0000	1.3931

AA# K1503001, FATHEAD MINNOW SURV., CHRONIC, 3-3-15
 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	1.356				
2	10 % EFFLUENT	1.320	25.00	16.00	5.00	
3	13 % EFFLUENT	1.356	27.50	16.00	5.00	
4	17 % EFFLUENT	1.320	25.00	16.00	5.00	
5	23 % EFFLUENT	1.356	27.50	16.00	5.00	
6	31 % EFFLUENT	1.393	30.00	16.00	5.00	

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

Pimephales promelas

FATHEAD MINNOW

TEST 1000.0

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:		K1503001				TEST DATES (BEGIN / END):		3/3/15 - 3/10/15	
CLIENT:		Sheridan				WEIGHING DATE / TIME:		3/11/2015 1300	
ANALYSTS:		RH				DRYING TEMP (DEGREES C):		60	
SAMPLE ID:						DRYING TIME (HOURS):		24	
	REP #	FINAL DRY WEIGHT TIN+LARVAE (g)	INITIAL WEIGHT TIN (g)	TOTAL DRY WEIGHT OF LARVAE (g)	NUMBER OF LARVAE	DRY WEIGHT OF LARVAE (mg)			
CONTROL	A	0.99401	0.98912	0.00489	8	0.611	AVG DRY		
	B	0.97471	0.97106	0.00365	8	0.456	WEIGHT (mg)		
	C	1.01197	1.00685	0.00512	8	0.640	0.602		
	D	1.00366	0.99813	0.00553	8	0.691	CV		
	E	0.96282	0.95793	0.00489	8	0.611	14.58		
10%	A	0.96463	0.95904	0.00559	8	0.699	AVG DRY		
	B	0.98289	0.97782	0.00507	8	0.634	WEIGHT (mg)		
	C	1.01858	1.01330	0.00528	8	0.660	0.633		
	D	0.96768	0.96327	0.00441	8	0.551	CV		
	E	0.98846	0.98348	0.00498	8	0.622			
13%	A	1.00524	1.00041	0.00483	8	0.604	AVG DRY		
	B	0.97911	0.97424	0.00487	8	0.609	WEIGHT (mg)		
	C	0.99323	0.98789	0.00534	8	0.667	0.624		
	D	0.98889	0.98415	0.00474	8	0.593	CV		
	E	0.99308	0.98791	0.00517	8	0.646			
17%	A	1.00869	1.00424	0.00445	8	0.556	AVG DRY		
	B	0.99005	0.98557	0.00448	8	0.560	WEIGHT (mg)		
	C	0.96259	0.95818	0.00441	8	0.551	0.564		
	D	0.99662	0.99248	0.00414	8	0.517	CV		
	E	0.98411	0.97905	0.00506	8	0.633			
23%	A	0.97931	0.97451	0.00480	8	0.600	AVG DRY		
	B	0.97802	0.97352	0.00450	8	0.562	WEIGHT (mg)		
	C	1.01609	1.01144	0.00465	8	0.581	0.614		
	D	1.02189	1.01615	0.00574	8	0.717	CV		
	E	1.00303	0.99818	0.00485	8	0.606	9.88		
31%	A	0.99604	0.99085	0.00519	8	0.649	AVG DRY		
	B	0.99968	0.99489	0.00479	8	0.599	WEIGHT (mg)		
	C	0.96597	0.96075	0.00522	8	0.653	0.633		
	D	0.99890	0.99386	0.00504	8	0.630	CV		
	E	0.98606	0.98097	0.00509	8	0.636			

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA# K1503001, FATHEAD MINNOW GROWTH CHRONIC, 3-3-15

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

Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.070

W = 0.955

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# K1503001, FATHEAD MINNOW GROWTH CHRONIC, 3-3-15

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

Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 8.15

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# K1503001, FATHEAD MINNOW GROWTH CHRONIC, 3-3-15
 FILE: C:\COPYTO~1\TOXSTAT\FHGROWTH.
 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.6110	0.6110
1	CONTROL	2	0.4560	0.4560
1	CONTROL	3	0.6400	0.6400
1	CONTROL	4	0.6910	0.6910
1	CONTROL	5	0.6110	0.6110
2	10 % EFFLUENT	1	0.6990	0.6990
2	10 % EFFLUENT	2	0.6340	0.6340
2	10 % EFFLUENT	3	0.6600	0.6600
2	10 % EFFLUENT	4	0.5510	0.5510
2	10 % EFFLUENT	5	0.6220	0.6220
3	13 % EFFLUENT	1	0.6040	0.6040
3	13 % EFFLUENT	2	0.6090	0.6090
3	13 % EFFLUENT	3	0.6670	0.6670
3	13 % EFFLUENT	4	0.5930	0.5930
3	13 % EFFLUENT	5	0.6460	0.6460
4	17 % EFFLUENT	1	0.5560	0.5560
4	17 % EFFLUENT	2	0.5600	0.5600
4	17 % EFFLUENT	3	0.5510	0.5510
4	17 % EFFLUENT	4	0.5170	0.5170
4	17 % EFFLUENT	5	0.6330	0.6330
5	23 % EFFLUENT	1	0.6000	0.6000
5	23 % EFFLUENT	2	0.5620	0.5620
5	23 % EFFLUENT	3	0.5810	0.5810
5	23 % EFFLUENT	4	0.7170	0.7170
5	23 % EFFLUENT	5	0.6060	0.6060
6	31 % EFFLUENT	1	0.6490	0.6490
6	31 % EFFLUENT	2	0.5990	0.5990
6	31 % EFFLUENT	3	0.6530	0.6530
6	31 % EFFLUENT	4	0.6300	0.6300
6	31 % EFFLUENT	5	0.6360	0.6360

AA# K1503001, FATHEAD MINNOW GROWTH CHRONIC, 3-3-15
 File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.018	0.004	1.197
Within (Error)	24	0.070	0.003	
Total	29	0.088		

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

AA# K1503001, FATHEAD MINNOW GROWTH CHRONIC, 3-3-15

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

Transform: NO TRANSFORMATION

DUNNETT'S TEST

TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	0.602	0.602		
2	10 % EFFLUENT	0.633	0.633	-0.917	
3	13 % EFFLUENT	0.624	0.624	-0.642	
4	17 % EFFLUENT	0.563	0.563	1.121	
5	23 % EFFLUENT	0.613	0.613	-0.333	
6	31 % EFFLUENT	0.633	0.633	-0.923	

Dunnett table value = 2.36

(1 Tailed Value, P=0.05, df=24,5)

AA# K1503001, FATHEAD MINNOW GROWTH CHRONIC, 3-3-15

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

Transform: NO TRANSFORMATION

DUNNETT'S TEST

TABLE 2 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	5			
2	10 % EFFLUENT	5	0.081	13.4	-0.031
3	13 % EFFLUENT	5	0.081	13.4	-0.022
4	17 % EFFLUENT	5	0.081	13.4	0.038
5	23 % EFFLUENT	5	0.081	13.4	-0.011
6	31 % EFFLUENT	5	0.081	13.4	-0.032

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

SURVIVAL AND REPRODUCTION TEST

Ceriodaphnia dubia

Discharger: Sheridan AFIN # 27-0022												Lab Number/s				Analyst: RH																
Location: Outfall 001												K1503001				Test Start - Date/Time: 3-3-15, 1100																
Date Sample Collected: 3 - 2/3/4 - 15																Test Stop - Date/Time: 3-10-15, 1000																
Conc	1	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	Conc	4	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	
%	Day	A	B	C	D	E	F	G	H	I	J					%	Day	A	B	C	D	E	F	G	H	I	J					
MHS	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH	17%	1	0	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH		2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH	
	3	0	0	0	0	0	1	0	0	3	0	4	10	0.4	RH		3	0	0	1	0	0	0	0	0	0	0	1	10	0.1	RH	
	4	0	0	2	3	5	1	0	3	0	3	17	10	1.7	RH		4	3	2	2	0	2	2	5	1	3	0	20	10	2.0	RH	
	5	6	4	2	7	0	8	4	4	X	8	43	10	4.3	RH		5	0	0	8	6	2	6	8	6	0	3	39	10	3.9	RH	
	6	4	9	6	0	10	5	8	1		7	50	10	5.0	RH		6	4	3	7	4	8	3	4	3	9	0	45	10	4.5	RH	
	7	5	0	5	9	3	6	0	7		5	40	10	4.0	RH		7	0	6	5	3	0	2	1	0	5	10	32	10	3.2	RH	
	8																	8												10	0.0	
	Total	15	13	15	19	18	21	12	15	3	23	154		Avg. = 16.8			Total	7	11	23	13	12	13	18	10	17	13	137		Avg. = 13.7		
									X				C.V. = 22.1														C.V. = 33.2					
Conc	2	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	Conc	5	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	
%	Day	A	B	C	D	E	F	G	H	I	J			%	Day	A	B	C	D	E	F	G	H	I	J							
10%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH	23%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH		2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH	
	3	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH		3	0	0	0	0	0	0	1	0	0	0	1	10	0.1	RH	
	4	0	3	3	0	3	3	4	4	3	0	23	10	2.3	RH		4	4	0	3	4	3	5	0	4	4	4	31	10	3.1	RH	
	5	4	0	0	4	3	0	7	0	0	8	26	10	2.6	RH		5	1	7	5	8	9	2	10	8	0	7	57	10	5.7	RH	
	6	4	5	8	0	0	7	4	3	7	0	38	10	3.8	RH		6	0	8	0	2	0	3	1	2	0	4	20	10	2.0	RH	
	7	3	6	0	11	6	5	4	0	5	8	48	10	4.8	RH		7	3	4	9	2	6	1	5	3	11	2	46	10	4.6	RH	
	8																8												10	0.0	RH	
	Total	11	14	11	15	12	15	19	7	15	16	135		Avg. = 13.5			Total	8	19	17	16	18	11	17	17	15	17	155		Avg. = 15.5		
													C.V. = 24.8														C.V. = 22.0					
Conc	3	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	Conc	6	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	
%	Day	A	B	C	D	E	F	G	H	I	J			%	Day	A	B	C	D	E	F	G	H	I	J							
13%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH	31%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH		2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH	
	3	0	0	0	0	0	0	1	1	0	0	2	10	0.2	RH		3	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH	
	4	1	2	5	0	3	3	4	0	2	1	21	10	2.1	RH		4	3	4	0	2	3	2	0	4	4	4	26	10	2.6	RH	
	5	8	2	7	3	4	7	8	5	0	0	44	10	4.4	RH		5	2	3	7	4	5	6	0	1	1	7	36	10	3.6	RH	
	6	0	8	2	5	4	0	0	6	0	4	29	10	2.9	RH		6	8	1	7	6	6	1	4	0	5	5	43	10	4.3	RH	
	7	3	7	0	6	0	7	5	3	12	5	48	10	4.8	RH		7	1	0	0	2	3	5	2	10	5	2	30	10	3.0	RH	
	8																8												10	0.0	RH	
	Total	12	19	14	14	11	17	18	15	14	10	144		Avg. = 14.4			Total	14	8	14	14	17	14	6	15	15	18	135		Avg. = 13.5		
													C.V. = 20.5														C.V. = 27.6					

AA # K1503001, C.DUBIA CHRONIC, REPRODUCCION, 3-3-15

File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

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

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

Total number of replicates = 60

AA # K1503001, C.DUBIA CHRONIC, REPRODUCCION, 3-3-15

File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 5.03

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

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

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

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	1	9	10
10	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
13	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	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	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
31	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

SUMMARY OF FISHER'S EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	10	1	
1	10	10	0	
2	13	10	0	

3	17	10	0
4	23	10	0
5	31	10	0

TITLE: AA # K1503001, C.DUBIA CHRONIC, REPRODUCCION, 3-3-15
 FILE: C:\COPYTO~1\TOXSTAT\C.DUB
 TRANSFORM: NO TRANSFORMATION

NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	15.0000	15.0000
1	CONTROL	2	13.0000	13.0000
1	CONTROL	3	15.0000	15.0000
1	CONTROL	4	19.0000	19.0000
1	CONTROL	5	18.0000	18.0000
1	CONTROL	6	21.0000	21.0000
1	CONTROL	7	12.0000	12.0000
1	CONTROL	8	15.0000	15.0000
1	CONTROL	9	3.0000	3.0000
1	CONTROL	10	23.0000	23.0000
2	10 % EFFLUENT	1	11.0000	11.0000
2	10 % EFFLUENT	2	14.0000	14.0000
2	10 % EFFLUENT	3	11.0000	11.0000
2	10 % EFFLUENT	4	15.0000	15.0000
2	10 % EFFLUENT	5	12.0000	12.0000
2	10 % EFFLUENT	6	15.0000	15.0000
2	10 % EFFLUENT	7	19.0000	19.0000
2	10 % EFFLUENT	8	7.0000	7.0000
2	10 % EFFLUENT	9	15.0000	15.0000
2	10 % EFFLUENT	10	16.0000	16.0000
3	13 % EFFLUENT	1	12.0000	12.0000
3	13 % EFFLUENT	2	19.0000	19.0000
3	13 % EFFLUENT	3	14.0000	14.0000
3	13 % EFFLUENT	4	14.0000	14.0000
3	13 % EFFLUENT	5	11.0000	11.0000
3	13 % EFFLUENT	6	17.0000	17.0000
3	13 % EFFLUENT	7	18.0000	18.0000
3	13 % EFFLUENT	8	15.0000	15.0000
3	13 % EFFLUENT	9	14.0000	14.0000
3	13 % EFFLUENT	10	10.0000	10.0000
4	17 % EFFLUENT	1	7.0000	7.0000
4	17 % EFFLUENT	2	11.0000	11.0000
4	17 % EFFLUENT	3	23.0000	23.0000
4	17 % EFFLUENT	4	13.0000	13.0000
4	17 % EFFLUENT	5	12.0000	12.0000
4	17 % EFFLUENT	6	13.0000	13.0000
4	17 % EFFLUENT	7	18.0000	18.0000
4	17 % EFFLUENT	8	10.0000	10.0000
4	17 % EFFLUENT	9	17.0000	17.0000
4	17 % EFFLUENT	10	13.0000	13.0000
5	23 % EFFLUENT	1	8.0000	8.0000
5	23 % EFFLUENT	2	19.0000	19.0000
5	23 % EFFLUENT	3	17.0000	17.0000

5	23 %	EFFLUENT	4	16.0000	16.0000
5	23 %	EFFLUENT	5	18.0000	18.0000
5	23 %	EFFLUENT	6	11.0000	11.0000
5	23 %	EFFLUENT	7	17.0000	17.0000
5	23 %	EFFLUENT	8	17.0000	17.0000
5	23 %	EFFLUENT	9	15.0000	15.0000
5	23 %	EFFLUENT	10	17.0000	17.0000
6	31 %	EFFLUENT	1	14.0000	14.0000
6	31 %	EFFLUENT	2	8.0000	8.0000
6	31 %	EFFLUENT	3	14.0000	14.0000
6	31 %	EFFLUENT	4	14.0000	14.0000
6	31 %	EFFLUENT	5	17.0000	17.0000
6	31 %	EFFLUENT	6	14.0000	14.0000
6	31 %	EFFLUENT	7	6.0000	6.0000
6	31 %	EFFLUENT	8	15.0000	15.0000
6	31 %	EFFLUENT	9	15.0000	15.0000
6	31 %	EFFLUENT	10	18.0000	18.0000

AA # K1503001, C.DUBIA CHRONIC, REPRODUCCION, 3-3-15
 File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	42.933	8.587	0.530
Within (Error)	54	874.400	16.193	
Total	59	917.333		

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

AA # K1503001, C.DUBIA CHRONIC, REPRODUCCION, 3-3-15
 File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	15.400	15.400		
2	10 % EFFLUENT	13.500	13.500	1.056	
3	13 % EFFLUENT	14.400	14.400	0.556	
4	17 % EFFLUENT	13.700	13.700	0.945	
5	23 % EFFLUENT	15.500	15.500	-0.056	
6	31 % EFFLUENT	13.500	13.500	1.056	

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

AA # K1503001, C.DUBIA CHRONIC, REPRODUCCION, 3-3-15

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	4.157	27.0	1.900
3	13 % EFFLUENT	10	4.157	27.0	1.000
4	17 % EFFLUENT	10	4.157	27.0	1.700
5	23 % EFFLUENT	10	4.157	27.0	-0.100
6	31 % EFFLUENT	10	4.157	27.0	1.900

APPENDIX E

Organism History

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

TEST ORGANISM HISTORY

DATE SHIPPED 3/3/15 CLIENT ARKANSAS ANALYTICAL

Purchase Order #: _____

SPECIES: Pimephales promelas

Quantity Shipped: 240⁺ 550⁺ 3/3/15
HATCHED

Age: HATCHED 3/1/15 15-1600^{CST}

Brood Stock Source: Anderson Farms, AR

Culture Water: Groundwater

Hardness (Mg/l CaCO₃): =160

Dissolved Oxygen (Mg/l): 8.4

Temperature (°C): 25.4

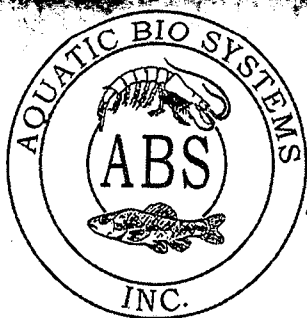
Feeding: ARTEIMA

Comments: _____

Shipped Via: Federal Express UPS Overnight Shuttle

Packaged By: _____

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



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

ORGANISM HISTORY

DATE: 11/25/2013

SPECIES: Ceriodaphnia dubia

AGE: > 3 day

LIFE STAGE: Adult

HATCH DATE: Variable


BEGAN FEEDING: Immediately

FOOD: YTC, Selenastrum sp.

Water Chemistry Record:

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

Comments:

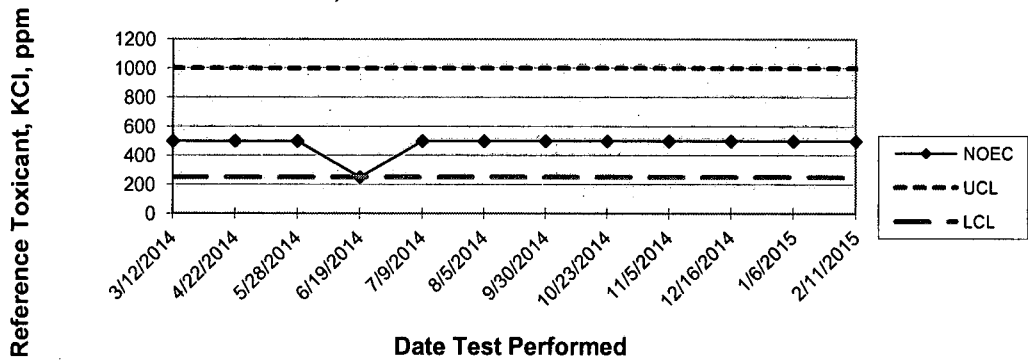


Facility Supervisor

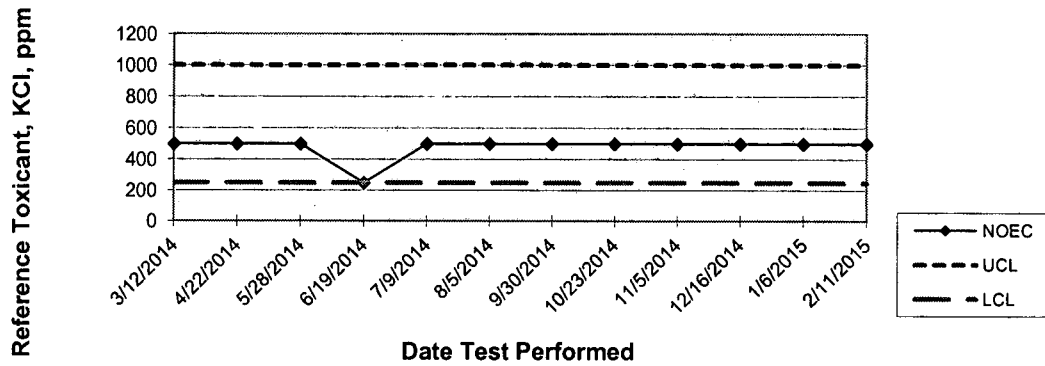
APPENDIX F

Quality Assurance Charts

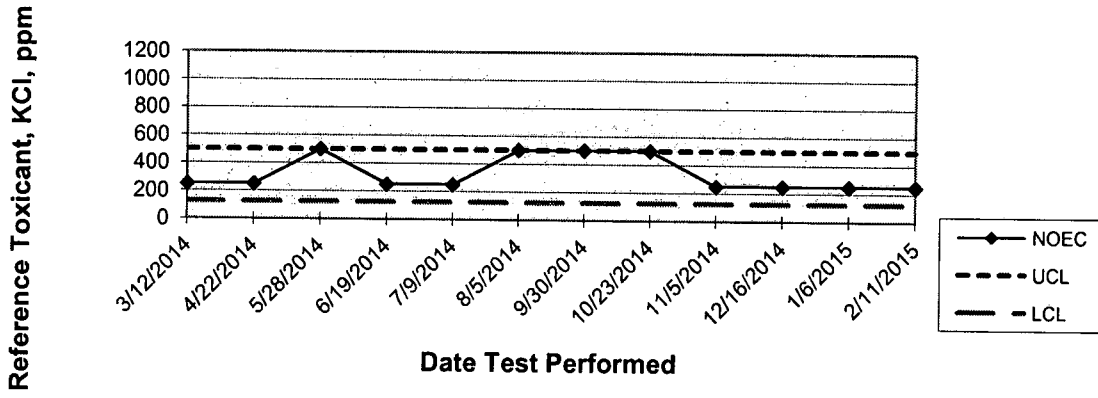
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW SURVIVAL 7 Day
QUALITY ASSURANCE



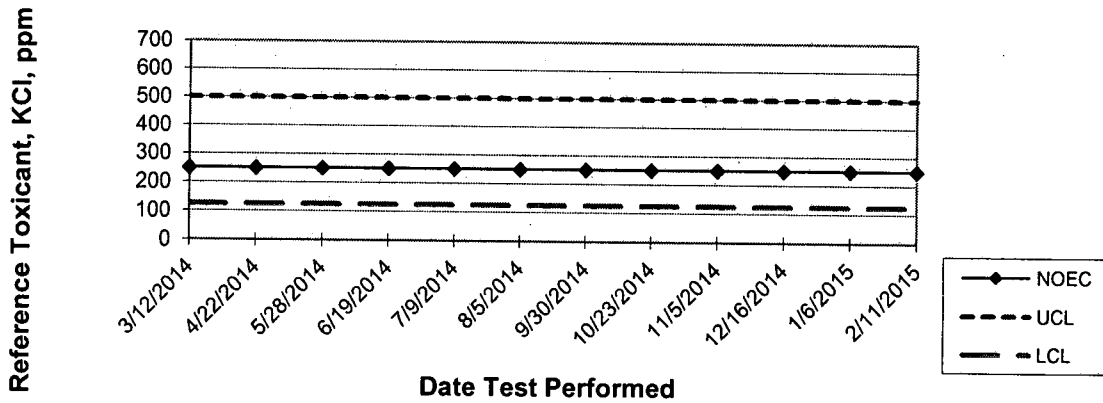
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW GROWTH 7 Day
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE




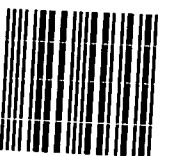
Sheridan Water Works
PO Box 486
Sheridan, AR 72150-0486

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