

# Arkansas Analytical, Inc.

## Toxicity Test Results

**CITY of SHERIDAN**  
**NPDES PERMIT NUMBER: AR0034347**  
**Second 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.  
11701 I-30, Bldg 1, Suite 115  
Little Rock, Arkansas 72209  
**Lab Number K1505002**

Friday, May 22, 2015

## **Introduction**

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

The permit requires chronic biomonitoring testing quarterly for *Ceriodaphnia dubia* and *Pimephales promelas*. The test results in this report represent the testing of the second 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:	5-11-15, 1224	5-12-15, 1124
Sample #2	5-13-15, 1434	5-14-15, 1334
Sample #3	5-14-15, 1350	5-15-15, 1250

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:	5-12-15, 1510	5
Sample #2	5-14-15,1501	2
Sample #3	5-15-15, 1555	1

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	90%	X	
The percent coefficient of variation between replicates must be 40% or less for survival	15.2%	X	
Minimum of 0.25 mg average dry weight of surviving controls	0.911	X	
The percent coefficient of variation between replicates must be 40% or less for growth	16.6%	X	

#### TEST ACCEPTANCE CRITERIA for *Ceriodaphnia dubia*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	100%	X	
Average of 15 or more young per surviving female	15.7	X	
At least 60% of surviving females should have produced 3 broods	80%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	30.0%	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> 4/22/15 – 4/29/15		<i>Pimephales promelas</i> 4/22/15 – 4/29/15	
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)	14.6	%CV survival (critical dilution)	0.00%
%CV Reproduction (critical dilution)	31.2%	Mean dry weight (critical dilution) in milligrams	0.984
		%CV growth (critical dilution)	7.06%
PMSD Reproduction	27.7%	PMSD Growth	18.5%

### Conclusion

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

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

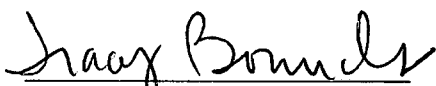
Chronic static renewal survival and reproduction test using *Ceriodaphnia dubia*, (Method 1002.0)

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

Biomonitoring Analyst:

Ryan Hudgin

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:	5-11-15, 1224	5-12-15, 1124
Sample #2	5-13-15, 1434	5-14-15, 1334
Sample #3	5-14-15, 1350	5-15-15, 1250

Test initiated (date, time): 5-13-15, 1015      Test terminated (date, time): 5-20-15, 1400

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%	75	100	100	75	100		100	95	90	15.2%
10%	100	87.5	100	100	100		100	97.5	97.5	
13%	100	100	100	100	100		100	100	100	
17%	100	100	75	100	87.5		97.5	92.5	92.5	
23%	100	100	100	100	100		100	100	100	0.00%
31%	87.5	100	100	100	100		97.5	97.5	97.5	

**DATA TABLE FOR GROWTH OF FATHEAD MINNOWS**

Effluent Conc %	Average Dry Weight in milligrams in replicate chambers						Mean Dry Weight	CV%
	A	B	C	D	E			
0%	0.704	0.944	1.112	0.841	0.954		0.911	16.6%
10%	1.093	1.105	1.258	1.021	1.013		1.098	
13%	0.939	1.131	1.129	0.854	1.000		1.011	
17%	1.156	0.925	0.781	1.073	0.955		0.978	
23%	0.930	0.913	1.076	1.034	0.967		0.984	7.06%
31%	0.935	0.980	1.111	1.029	1.011		1.013	

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)=     16.6     %



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:	5-11-15, 1224	5-12-15, 1124
Sample #2	5-13-15, 1434	5-14-15, 1334
Sample #3	5-14-15, 1350	5-15-15, 1250

Test initiated (date, time): 5-13-15, 0930    Test terminated (date, time): 5-20-15, 1105

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	12	10	18	21	16	10
B	23	15	17	20	17	17
C	15	16	18	8	15	17
D	15	12	13	14	10	13
E	19	13	12	13	24	10
F	8	16	8	12	7	17
G	20	22	9	18	14	20
H	16	8	19	12	13	18
I	10	9	10	16	13	10
J	19	14	13	17	17	16
Mean	15.7	13.5	13.7	15.1	14.6	14.8
Mean/surviving female	15.7	13.5	13.7	15.1	14.6	14.8
CV%*	30.0				31.2	

X= Dead Adult; M= Male (Not considered in statistics)

\*Coefficient of Variation = standard deviation/ mean \* 100; CV% calculation based on young per surviving female

**SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING**  
*Ceriodaphnia dubia* SURVIVAL AND REPRODUCTION

**Permittee: City of Sheridan**

**NPDES #: AR0034347**

PERCENT SURVIVAL

PERCENT EFFLUENT	0%	10%	13%	17%	23%	31%
Time of Reading: 24 HOURS	100	100	100	100	100	100
48 HOURS	100	100	100	100	100	100
Test termination	100	100	100	100	100	100

1. Fisher's Exact Test:

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

a) LOW FLOW OR CRITICAL DILUTION, (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:

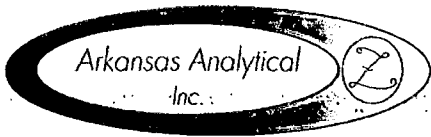
a) NOEC survival (parameter TOP3B)=  31  % effluent

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

c) Coefficient of variation (parameter TQP3B)=  31.2  %







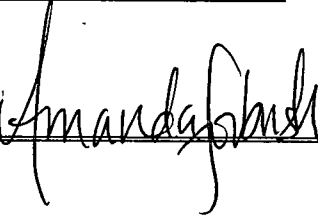
APPENDIX A

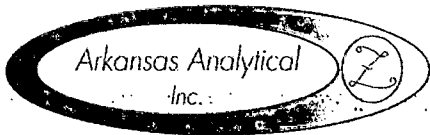
Chain of Custody Forms



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

# CHAIN OF CUSTODY RECORD

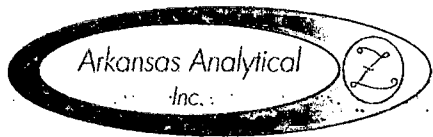
CLIENT INFORMATION		BILLING INFORMATION		Project Description		Turnaround Time		Preservation Codes:															
Sheridan Waterworks		Sheridan Waterworks		Chronic Toxicity		1 Day (100%)		1. Cool, 4 Degrees Centigrade					4. Thiosulfate for Dechlorination										
104 W High St.		P.O. Box 486				2 Day (50%)		2. Sulfuric Acid (H <sub>2</sub> SO <sub>4</sub> ), pH < 2					5. Hydrochloric Acid(HCl)										
Sheridan, AR 72150		Sheridan, AR 72150		Reporting Information		3 Day (25%)		3. Nitric Acid (HNO <sub>3</sub> ), 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					
 Sampler(s) Signature				 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														
	5/11-12/15	1224-1124		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													
		1510 5-12-15				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: 5°C 6. TEMPERATURE GUN ID: HHT#2																	
3. Relinquished by: (Signature)		Date/Time		4. Received by lab: (Signature)		FOR COMPLETION BY LAB ONLY																	
																							



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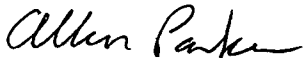






# CHAIN OF CUSTODY RECORD

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 <sub>2</sub> SO <sub>4</sub> ), pH < 2				5. Hydrochloric Acid(HCl)							
Sheridan, AR 72150		Sheridan, AR 72150		Reporting Information		3 Day (25%)		3. Nitric Acid (HNO <sub>3</sub> ), 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			
<i>Allen Parker</i> Sampler(s) Signature				Allen Parker Sampler(s) Printed				Chronic Toxicity										Arkansas Analytical Work Order Number:	
Field Number		SAMPLE COLLECTION		Number of Bottles		SAMPLE IDENTIFICATION/ DESCRIPTION													
		Date/s Time/s		Grab Comp															
		5/13-14/15 1434-1334		X		24 Water Final Discharge		X								K1505002B			
1. Relinquished by: (Signature)		Date/Time		2. Received by: (Signature)		SAMPLE CONDITION UPON RECEIPT IN LAB						REMARKS / SAMPLE COMMENTS							
<i>Allen Parker</i>		1501 5-14-15				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: 2°C 6. TEMPERATURE GUN ID: HHT# 2													
3. Relinquished by: (Signature)		Date/Time		4. Received by lab: (Signature)		FOR COMPLETION BY LAB ONLY													
				<i>Amanda Johnson</i>															



11701 Interstate 30, Bldg. 1, Ste. 115  
 Little Rock, AR 72209  
 PHONE: 501-455-3233  
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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 <sub>2</sub> SO <sub>4</sub> ), pH < 2					5. Hydrochloric Acid(HCl)						
Sheridan, AR 72150		Sheridan, AR 72150		Reporting Information		3 Day (25%)		3. Nitric Acid (HNO <sub>3</sub> ), 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												G = Glass; P = Plastic	
				Email: sheridanwater@windstream.net		Bottle Type: P												V = Septum; A = Amber	
 Sampler(s) Signature				 Sampler(s) Printed										Chronic Toxicity  Arkansas Analytical Work Order Number:					
Field Number	SAMPLE COLLECTION		Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION												
	5/14-15/15	1350-1250		X	24	Water	Final Discharge												K15050020
1. Relinquished by: (Signature)		Date/Time		2. Received by: (Signature)		SAMPLE CONDITION UPON RECEIPT IN LAB				REMARKS / SAMPLE COMMENTS									
		1555 5-15-15				1. CUSTODY SEALS: <input checked="" type="checkbox"/> Yes ___ No													
3. Relinquished by: (Signature)		Date/Time		4. Received by lab: (Signature)		2. CONTAINERS CORRECT: <input type="checkbox"/> Yes ___ No													
						3. COC/LABELS AGREE: <input type="checkbox"/> Yes ___ No													
						4. RECEIVED ON ICE: <input type="checkbox"/> Yes ___ No													
						5. TEMPERATURE ON RECEIPT: <input type="checkbox"/> Yes ___ No													
						6. TEMPERATURE GUN ID: <input type="checkbox"/> Yes ___ No													
						FOR COMPLETION BY LAB ONLY													

APPENDIX B

Effluent and Dilution Water Data

**Biomonitoring Quality Control Benchsheet**

Analyst	RA	RH	RA	RA	RA	RA	RA	RA
Date	5-12-15	5-13-15	5-14-15	5-15-15	5-16-17	5-17-15	5-18-15	5-19-15
pH Meter ID	AK60							
LIN pH 4 Buffer	1401167							
LIN pH 7 Buffer	1401173							
LIN pH 10 Buffer	1401168							
Slope (>90%)	95.6%	94.7%	96.1%	94.4%	95.8%	95.0%	97.1%	95.4%

Dissolved O <sub>2</sub> Meter	0.0.1305							
Meter Reading	8.36	8.41	8.71	8.42	8.66	8.62	8.38	8.62
Temp.	24	24	22	24	22	22	24	23
Chart Value at Temp.	8.418	8.418	8.743	8.418	8.743	8.743	8.418	8.578
Difference	0.058	0.008	0.033	0.018	0.083	0.123	0.038	0.058
Acceptance Criteria	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L

Temp. Meter ID	AK60							
Meter Reading	23	24	23	24	23	22	24	23
Thermometer Reading	23	23	22	23	22	22	23	23
Thermometer ID	PB							
Acceptance Criteria	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C

Alkalinity								
Blank (<5mg/L)								
STD Result								
T.V. / %REC								
Acceptance Criteria	93.5-108.5% Recovery							

Hardness								
Blank (<2mg/L)								
STD. Result								
T.V. / %REC								
Acceptance Criteria	90.0-105.5% Recovery							

Conductivity Meter ID	Con 02							
Blank (<1)								
STD Result								
T.V. / %REC								
Acceptance Criteria	99.2-104.0% Recovery							

Chlorine Meter ID	DR 820							
Blank (<0.05mg/L)								
STD Result								
T.V. / % REC								
Acceptance Criteria	100.0-120% Recovery							

Revision 0  
Effective Date 01APR15



**Biomonitoring Quality Control Benchsheet**

<b>Analyst</b>	RH	RH	RH						
<b>Date</b>	5-20-15	5-21-15	5-22-15						
<b>pH Meter ID</b>									
LIN pH 4 Buffer	1401167								
LIN pH 7 Buffer	1401173								
LIN pH 10 Buffer	1401168								
<b>Slope (&gt;90%)</b>	95.9%	94.9%	96.1%						

<b>Dissolved O<sub>2</sub> Meter</b>	D.O. 1305								
<b>Meter Reading</b>	8.51	8.21	8.46						
<b>Temp.</b>	24	25	24						
<b>Chart Value at Temp.</b>	8.418	8.263	8.418						
<b>Difference</b>	0.108	0.053	0.058						
<b>Acceptance Criteria</b>	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L

<b>Temp. Meter ID</b>	AR60								
<b>Meter Reading</b>	23	24	24						
<b>Thermometer Reading</b>	22	24	23						
<b>Thermometer ID</b>	PB								
<b>Acceptance Criteria</b>	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C

<b>Alkalinity</b>									
<b>Blank (&lt;5mg/L)</b>			<5						
<b>STD Result</b>			104						
<b>T.V. / %REC</b>			100/104%						
<b>Acceptance Criteria</b>			93.5-108.5% Recovery						

<b>Hardness</b>									
<b>Blank (&lt;2mg/L)</b>			<2						
<b>STD. Result</b>			94						
<b>T.V. / %REC</b>			100/94%						
<b>Acceptance Criteria</b>			90.0-105.5% Recovery						

<b>Conductivity Meter ID</b>	Com 02								
<b>Blank (&lt;1)</b>			<1						
<b>STD Result</b>			1420						
<b>T.V. / %REC</b>			1412/1007%						
<b>Acceptance Criteria</b>			99.2-104.0% Recovery						

<b>Chlorine Meter ID</b>	DR 820								
<b>Blank (&lt;0.05mg/L)</b>			<0.05						
<b>STD Result</b>			0.21						
<b>T.V. / % REC</b>			0.21/100%						
<b>Acceptance Criteria</b>			100.0-120% Recovery						

Revision 0  
Effective Date 01APR15

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Fathead Minnow

Lab # / Sample ID K1505002

Test Start (Date/Time)

5-13-15

1015

Client: Sheridan

Test End (Date/Time)

5-20-15

1400

Day of Test

		1	2	3	4	5	6	7	notes
<b>Control</b>	MHS	5-13	5-14	5-15	5-16	5-17	5-18	5-19	
D.O. (mg/L)	INITIAL	8.7	8.6	7.6	8.4	8.9	8.7	8.7	
	FINAL	7.1	7.4	7.7	8.0	7.1	7.5	7.6	
pH (s.u.)	INITIAL	7.7	8.1	7.8	8.1	8.2	8.0	8.3	
	FINAL	7.8	7.7	7.8	8.0	8.4	8.0	8.2	
temp (C)	INITIAL	23	23	23	22	21	22	22	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		58					60		
HARDNESS (mg/L)		88					96		
CONDUCTIVITY (umhd)		444					421		
CHLORINE (mg/L)		<0.05							
<b>CONC: 10</b>									
D.O. (mg/L)	INITIAL	8.4	8.6	7.3	8.5	8.8	8.7	8.5	
	FINAL	6.3	7.4	7.6	7.9	6.7	7.4	7.4	
pH (s.u.)	INITIAL	7.7	7.8	7.9	7.9	8.1	8.0	7.8	
	FINAL	7.9	7.7	7.9	8.0	8.2	8.0	8.1	
temp (C)	INITIAL	23	23	23	22	21	22	22	
	FINAL	25	25	25	25	25	25	25	
<b>CONC: 13</b>									
D.O. (mg/L)	INITIAL	8.6	8.4	7.6	8.5	9.0	8.7	8.5	
	FINAL	7.4	7.4	7.9	8.2	7.4	7.6	7.3	
pH (mg/L)	INITIAL	7.7	7.9	7.9	7.9	8.1	8.1	7.8	
	FINAL	7.9	7.8	7.9	8.0	8.0	8.1	7.9	
temp (C)	INITIAL	23	23	23	22	21	22	22	
	FINAL	25	25	25	25	25	25	25	
<b>CONC: 17</b>									
D.O. (mg/L)	INITIAL	8.6	8.7	7.8	8.5	8.9	8.7	8.6	
	FINAL	7.3	7.4	7.9	8.2	7.4	7.5	7.3	
pH (s.u.)	INITIAL	7.7	7.9	7.9	8.0	8.0	8.1	7.9	
	FINAL	7.9	7.8	7.9	8.1	7.9	8.1	7.9	
temp (C)	INITIAL	23	22	23	21	20	23	22	
	FINAL	25	25	25	25	25	25	25	
<b>CONC: 23</b>									
D.O. (mg/L)	INITIAL	8.7	8.7	7.8	8.6	9.1	8.8	8.6	
	FINAL	7.3	7.3	7.8	8.4	7.6	7.6	7.5	
pH (s.u.)	INITIAL	7.6	7.9	8.0	7.7	7.9	8.1	8.0	
	FINAL	7.9	7.8	7.9	8.1	7.9	7.8	7.6	
temp (C)	INITIAL	22	23	24	21	21	23	23	
	FINAL	25	25	25	25	25	25	25	
<b>CONC: 31</b>									
D.O. (mg/L)	INITIAL	8.7	8.7	7.9	8.6	9.1	8.8	8.7	
	FINAL	7.4	7.3	7.9	8.4	7.6	7.5	7.4	
pH (s.u.)	INITIAL	7.9	8.0	8.0	8.0	7.8	8.2	8.0	
	FINAL	7.9	7.8	7.9	8.1	7.9	7.8	7.5	
temp (C)	INITIAL	22	23	24	21	20	24	23	
	FINAL	25	25	25	25	25	25	25	
<b>CONC: 100 %</b>									
ALKALINITY (mg/L)		54			54		52		
HARDNESS (mg/L)		38			42		30		
CONDUCTIVITY (umhd)		233			255		255		
CHLORINE (mg/L)		<0.05			0.07		0.06		

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Ceriodaphnia Dubia

Lab # / Sample ID K1505002 Test Start (Date/Time) 5-13-15 0930

Client: Sheridan Test End (Date/Time) 5-20-15 1105

Day of Test

		1	2	3	4	5	6	7	notes
<b>Control</b>	<u>MHS</u>	<u>5-13</u>	<u>5-14</u>	<u>5-15</u>	<u>5-16</u>	<u>5-17</u>	<u>5-18</u>	<u>5-19</u>	
D.O. (mg/L)	INITIAL	<u>8.7</u>	<u>8.6</u>	<u>7.6</u>	<u>8.4</u>	<u>8.9</u>	<u>8.7</u>	<u>8.7</u>	
	FINAL	<u>8.5</u>	<u>8.5</u>	<u>8.0</u>	<u>8.3</u>	<u>8.4</u>	<u>8.5</u>	<u>8.0</u>	
pH (s.u.)	INITIAL	<u>7.7</u>	<u>8.1</u>	<u>7.8</u>	<u>8.1</u>	<u>8.2</u>	<u>8.0</u>	<u>8.3</u>	
	FINAL	<u>7.7</u>	<u>8.1</u>	<u>8.2</u>	<u>7.7</u>	<u>7.7</u>	<u>7.7</u>	<u>8.2</u>	
temp (C)	INITIAL	<u>23</u>	<u>23</u>	<u>23</u>	<u>22</u>	<u>21</u>	<u>22</u>	<u>22</u>	
	FINAL	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	
ALKALINITY (mg/L)		<u>58</u>	---	---	---	---	<u>60</u>	---	
HARDNESS (mg/L)		<u>88</u>	---	---	---	---	<u>96</u>	---	
CONDUCTIVITY (umho)		<u>444</u>	---	---	---	---	<u>421</u>	---	
CHLORINE (mg/L)		<u>20.05</u>	---	---	---	---	---	---	
<b>CONC:</b>	<u>10</u>								
D.O. (mg/L)	INITIAL	<u>8.4</u>	<u>8.6</u>	<u>7.3</u>	<u>8.5</u>	<u>8.8</u>	<u>8.7</u>	<u>8.5</u>	
	FINAL	<u>8.6</u>	<u>8.4</u>	<u>8.1</u>	<u>8.1</u>	<u>8.5</u>	<u>8.6</u>	<u>8.0</u>	
pH (s.u.)	INITIAL	<u>7.7</u>	<u>7.8</u>	<u>7.9</u>	<u>7.9</u>	<u>8.1</u>	<u>8.0</u>	<u>7.8</u>	
	FINAL	<u>7.5</u>	<u>8.1</u>	<u>8.1</u>	<u>7.7</u>	<u>7.7</u>	<u>7.8</u>	<u>8.2</u>	
temp (C)	INITIAL	<u>23</u>	<u>23</u>	<u>23</u>	<u>22</u>	<u>21</u>	<u>22</u>	<u>22</u>	
	FINAL	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	
<b>CONC:</b>	<u>13</u>								
D.O. (mg/L)	INITIAL	<u>8.6</u>	<u>8.4</u>	<u>7.6</u>	<u>8.5</u>	<u>9.0</u>	<u>8.7</u>	<u>8.5</u>	
	FINAL	<u>8.5</u>	<u>8.4</u>	<u>7.9</u>	<u>8.3</u>	<u>8.5</u>	<u>8.7</u>	<u>8.1</u>	
pH (mg/L)	INITIAL	<u>7.7</u>	<u>7.9</u>	<u>7.9</u>	<u>7.9</u>	<u>8.1</u>	<u>8.1</u>	<u>7.8</u>	
	FINAL	<u>7.6</u>	<u>8.0</u>	<u>7.8</u>	<u>7.8</u>	<u>7.8</u>	<u>7.5</u>	<u>8.1</u>	
temp (C)	INITIAL	<u>23</u>	<u>23</u>	<u>23</u>	<u>22</u>	<u>21</u>	<u>22</u>	<u>22</u>	
	FINAL	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	
<b>CONC:</b>	<u>17</u>								
D.O. (mg/L)	INITIAL	<u>8.6</u>	<u>8.7</u>	<u>7.8</u>	<u>8.5</u>	<u>8.9</u>	<u>8.7</u>	<u>8.6</u>	
	FINAL	<u>8.5</u>	<u>8.6</u>	<u>8.3</u>	<u>8.9</u>	<u>8.5</u>	<u>9.7</u>	<u>8.0</u>	
pH (s.u.)	INITIAL	<u>7.7</u>	<u>7.9</u>	<u>7.9</u>	<u>8.0</u>	<u>8.0</u>	<u>8.1</u>	<u>7.9</u>	
	FINAL	<u>7.8</u>	<u>7.8</u>	<u>7.9</u>	<u>7.9</u>	<u>7.8</u>	<u>7.6</u>	<u>8.1</u>	
temp (C)	INITIAL	<u>23</u>	<u>22</u>	<u>23</u>	<u>21</u>	<u>20</u>	<u>23</u>	<u>22</u>	
	FINAL	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	
<b>CONC:</b>	<u>23</u>								
D.O. (mg/L)	INITIAL	<u>8.7</u>	<u>8.7</u>	<u>7.8</u>	<u>8.6</u>	<u>9.1</u>	<u>8.8</u>	<u>8.6</u>	
	FINAL	<u>8.6</u>	<u>8.5</u>	<u>8.2</u>	<u>8.3</u>	<u>8.6</u>	<u>8.4</u>	<u>7.8</u>	
pH (s.u.)	INITIAL	<u>7.6</u>	<u>7.9</u>	<u>8.0</u>	<u>7.9</u>	<u>7.9</u>	<u>8.1</u>	<u>8.0</u>	
	FINAL	<u>7.9</u>	<u>7.7</u>	<u>8.0</u>	<u>8.0</u>	<u>7.9</u>	<u>7.7</u>	<u>8.0</u>	
temp (C)	INITIAL	<u>22</u>	<u>23</u>	<u>24</u>	<u>21</u>	<u>21</u>	<u>23</u>	<u>23</u>	
	FINAL	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	
<b>CONC:</b>	<u>31</u>								
D.O. (mg/L)	INITIAL	<u>8.7</u>	<u>8.7</u>	<u>7.9</u>	<u>8.6</u>	<u>9.1</u>	<u>8.8</u>	<u>8.7</u>	
	FINAL	<u>8.6</u>	<u>8.4</u>	<u>8.1</u>	<u>8.3</u>	<u>8.5</u>	<u>8.5</u>	<u>7.9</u>	
pH (s.u.)	INITIAL	<u>7.9</u>	<u>8.0</u>	<u>8.0</u>	<u>8.0</u>	<u>7.8</u>	<u>8.2</u>	<u>8.0</u>	
	FINAL	<u>7.9</u>	<u>7.7</u>	<u>7.7</u>	<u>8.0</u>	<u>7.9</u>	<u>7.9</u>	<u>7.8</u>	
temp (C)	INITIAL	<u>22</u>	<u>23</u>	<u>24</u>	<u>21</u>	<u>20</u>	<u>24</u>	<u>23</u>	
	FINAL	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	
<b>CONC:</b>	<u>100 %</u>								
ALKALINITY (mg/L)		<u>54</u>	---	---	<u>54</u>	---	<u>52</u>	---	
HARDNESS (mg/L)		<u>38</u>	---	---	<u>42</u>	---	<u>30</u>	---	
CONDUCTIVITY (umho)		<u>233</u>	---	---	<u>255</u>	---	<u>255</u>	---	
CHLORINE (mg/L)		<u>20.05</u>	---	---	<u>0.07</u>	---	<u>0.06</u>	---	

APPENDIX C

Fathead minnow raw data and statistics

*Pimephales promelas*

**FATHEAD MINNOW**

**SURVIVAL DATA FOR LARVAL SURVIVAL AND GROWTH TEST (ALTERNATE)**

LAB #: K1505002			TEST START		DATE	05/13/15	TIME	1015				
CLIENT: Sheridan			TEST END		DATE	05/20/15	TIME	1400				
ANALYST: RH			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	7	7	7	6	75%	90.0%	15.2
	B	8	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	8	100%		
	D	8	8	6	6	6	6	6	6	75%		
	E	8	8	8	8	8	8	8	8	100%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	8	8	8	8	8	8	8	8	100%	97.5%	
	B	8	8	7	7	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%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	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%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	8	8	8	8	8	8	8	8	100%	92.5%	
	B	8	8	8	8	8	8	8	8	100%		
	C	8	7	7	7	6	6	6	6	75%		
	D	8	8	8	8	8	8	8	8	100%		
	E	8	8	7	7	7	7	7	7	87.5%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	8	8	8	8	8	8	8	8	100%	100.0%	0.00
	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%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	8	7	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%		
ANALYST:		RH	RH	RH	RH	RH	RH	RH	RH			
DATE:		5/13/15	5/14/15	5/15/15	5/16/15	5/17/15	5/18/15	5/19/15	5/20/15			
TIME:		1015	1400	1030	1005	1250	1400	1630	1400			

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

REMARKS:

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AA# K1505002,FATHEAD MINNOW SURV.,CHRONIC, 5-13-15

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

Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

---

D = 0.295

W = 0.861

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# K1505002,FATHEAD MINNOW SURV.,CHRONIC, 5-13-15

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# K1505002, FATHEAD MINNOW SURV., CHRONIC, 5-13-15  
 FILE: C:\COPYTO~1\TOXSTAT\FHSURV~1.  
 TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.7500	1.0472
1	CONTROL	2	1.0000	1.3931
1	CONTROL	3	1.0000	1.3931
1	CONTROL	4	0.7500	1.0472
1	CONTROL	5	1.0000	1.3931
2	10 % EFFLUENT	1	1.0000	1.3931
2	10 % EFFLUENT	2	0.8750	1.2094
2	10 % EFFLUENT	3	1.0000	1.3931
2	10 % EFFLUENT	4	1.0000	1.3931
2	10 % EFFLUENT	5	1.0000	1.3931
3	13 % EFFLUENT	1	1.0000	1.3931
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	1.0000	1.3931
4	17 % EFFLUENT	2	1.0000	1.3931
4	17 % EFFLUENT	3	0.7500	1.0472
4	17 % EFFLUENT	4	1.0000	1.3931
4	17 % EFFLUENT	5	0.8750	1.2094
5	23 % EFFLUENT	1	1.0000	1.3931
5	23 % EFFLUENT	2	1.0000	1.3931
5	23 % EFFLUENT	3	1.0000	1.3931
5	23 % EFFLUENT	4	1.0000	1.3931
5	23 % EFFLUENT	5	1.0000	1.3931
6	31 % EFFLUENT	1	0.8750	1.2094
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# K1505002, FATHEAD MINNOW SURV., CHRONIC, 5-13-15  
 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	1.255				
2	10 % EFFLUENT	1.356	31.00	16.00	5.00	
3	13 % EFFLUENT	1.393	32.50	16.00	5.00	
4	17 % EFFLUENT	1.287	28.50	16.00	5.00	
5	23 % EFFLUENT	1.393	32.50	16.00	5.00	
6	31 % EFFLUENT	1.356	31.00	16.00	5.00	

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

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:		K1505002		TEST DATES (BEGIN / END):		5/13/15 - 5/20/15	
CLIENT:		Sheridan		WEIGHING DATE / TIME:		5/21/2015 1135	
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	1.03325	1.02762	0.00563	8	0.704	AVG DRY WEIGHT (mg)
	B	1.02844	1.02089	0.00755	8	0.944	
	C	1.03111	1.02221	0.00890	8	1.112	0.911
	D	1.03254	1.02581	0.00673	8	0.841	CV
	E	1.01194	1.00431	0.00763	8	0.954	
10%	A	1.00388	0.99514	0.00874	8	1.093	AVG DRY WEIGHT (mg)
	B	1.02371	1.01487	0.00884	8	1.105	
	C	1.00588	0.99582	0.01006	8	1.258	1.098
	D	1.02588	1.01771	0.00817	8	1.021	CV
	E	1.01624	1.00814	0.00810	8	1.013	
13%	A	1.06301	1.05550	0.00751	8	0.939	AVG DRY WEIGHT (mg)
	B	1.01845	1.00940	0.00905	8	1.131	
	C	1.02225	1.01322	0.00903	8	1.129	1.011
	D	1.02004	1.01321	0.00683	8	0.854	CV
	E	1.02940	1.02140	0.00800	8	1.000	
17%	A	1.05554	1.04629	0.00925	8	1.156	AVG DRY WEIGHT (mg)
	B	1.02329	1.01589	0.00740	8	0.925	
	C	1.04555	1.03930	0.00625	8	0.781	0.978
	D	1.03807	1.02949	0.00858	8	1.073	CV
	E	1.05504	1.04740	0.00764	8	0.955	
23%	A	1.02758	1.02014	0.00744	8	0.930	AVG DRY WEIGHT (mg)
	B	1.06965	1.06235	0.00730	8	0.913	
	C	1.00913	1.00052	0.00861	8	1.076	0.984
	D	1.03856	1.03029	0.00827	8	1.034	CV
	E	1.04253	1.03479	0.00774	8	0.967	
31%	A	1.03384	1.02636	0.00748	8	0.935	AVG DRY WEIGHT (mg)
	B	1.01689	1.00905	0.00784	8	0.980	
	C	1.03000	1.02111	0.00889	8	1.111	1.013
	D	1.00657	0.99834	0.00823	8	1.029	CV
	E	1.01452	1.00643	0.00809	8	1.011	

CV = (STANDARD DEVIATION/MEAN)\*100

REMARKS:

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AA# K1505002, FATHEAD MINNOW GROWTH CHRONIC, 5-13-15

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

Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

---

D = 0.307

W = 0.972

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# K1505002, FATHEAD MINNOW GROWTH CHRONIC, 5-13-15

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

Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 4.27

---

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# K1505002, FATHEAD MINNOW GROWTH CHRONIC, 5-13-15  
 FILE: C:\COPYTO~1\TOXSTAT\FHGROWTH.  
 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.7040	0.7040
1	CONTROL	2	0.9440	0.9440
1	CONTROL	3	1.1120	1.1120
1	CONTROL	4	0.8410	0.8410
1	CONTROL	5	0.9540	0.9540
2	10 % EFFLUENT	1	1.0930	1.0930
2	10 % EFFLUENT	2	1.1050	1.1050
2	10 % EFFLUENT	3	1.2580	1.2580
2	10 % EFFLUENT	4	1.0210	1.0210
2	10 % EFFLUENT	5	1.0130	1.0130
3	13 % EFFLUENT	1	0.9390	0.9390
3	13 % EFFLUENT	2	1.1310	1.1310
3	13 % EFFLUENT	3	1.1290	1.1290
3	13 % EFFLUENT	4	0.8540	0.8540
3	13 % EFFLUENT	5	1.0000	1.0000
4	17 % EFFLUENT	1	1.1560	1.1560
4	17 % EFFLUENT	2	0.9250	0.9250
4	17 % EFFLUENT	3	0.7810	0.7810
4	17 % EFFLUENT	4	1.0730	1.0730
4	17 % EFFLUENT	5	0.9550	0.9550
5	23 % EFFLUENT	1	0.9300	0.9300
5	23 % EFFLUENT	2	0.9130	0.9130
5	23 % EFFLUENT	3	1.0760	1.0760
5	23 % EFFLUENT	4	1.0340	1.0340
5	23 % EFFLUENT	5	0.9670	0.9670
6	31 % EFFLUENT	1	0.9350	0.9350
6	31 % EFFLUENT	2	0.9800	0.9800
6	31 % EFFLUENT	3	1.1110	1.1110
6	31 % EFFLUENT	4	1.0290	1.0290
6	31 % EFFLUENT	5	1.0110	1.0110

AA# K1505002, FATHEAD MINNOW GROWTH CHRONIC, 5-13-15  
 File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.093	0.019	1.449
Within (Error)	24	0.307	0.013	
Total	29	0.400		

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

AA# K1505002, FATHEAD MINNOW GROWTH CHRONIC, 5-13-15

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

Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	0.911	0.911		
2	10 % EFFLUENT	1.098	1.098	-2.613	
3	13 % EFFLUENT	1.011	1.011	-1.392	
4	17 % EFFLUENT	0.978	0.978	-0.936	
5	23 % EFFLUENT	0.984	0.984	-1.020	
6	31 % EFFLUENT	1.013	1.013	-1.428	

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

AA# K1505002, FATHEAD MINNOW GROWTH CHRONIC, 5-13-15

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

Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 2 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	5			
2	10 % EFFLUENT	5	0.169	18.5	-0.187
3	13 % EFFLUENT	5	0.169	18.5	-0.100
4	17 % EFFLUENT	5	0.169	18.5	-0.067
5	23 % EFFLUENT	5	0.169	18.5	-0.073
6	31 % EFFLUENT	5	0.169	18.5	-0.102

APPENDIX D

*Ceriodaphnia dubia* Raw Data and Statistics



AA # K1505002, C.DUBIA CHRONIC, REPRODUCCION, 5-13-15

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

Shapiro - Wilk's test for normality

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\*\*\*\*\* 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

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AA # K1505002, C.DUBIA CHRONIC, REPRODUCCION, 5-13-15

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

---

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 2.00

---

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

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

---

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

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
10	10	0	10
TOTAL	20	0	20

CRITICAL FISHER'S VALUE (10,10,10) (p=0.05) IS 6. b VALUE IS 10.  
 Since b is greater than 6 there is no significant difference  
 between CONTROL and TREATMENT at the 0.05 level.

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
13	10	0	10
TOTAL	20	0	20

CRITICAL FISHER'S VALUE (10,10,10) (p=0.05) IS 6. b VALUE IS 10.  
 Since b is greater than 6 there is no significant difference  
 between CONTROL and TREATMENT at the 0.05 level.

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
17	10	0	10

TOTAL 20 0 20

CRITICAL FISHER'S VALUE (10,10,10) (p=0.05) IS 6. b VALUE IS 10.  
 Since b is greater than 6 there is no significant difference  
 between CONTROL and TREATMENT at the 0.05 level.

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
23	10	0	10
TOTAL	20	0	20

CRITICAL FISHER'S VALUE (10,10,10) (p=0.05) IS 6. b VALUE IS 10.  
 Since b is greater than 6 there is no significant difference  
 between CONTROL and TREATMENT at the 0.05 level.

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
31	10	0	10
TOTAL	20	0	20

CRITICAL FISHER'S VALUE (10,10,10) (p=0.05) IS 6. b VALUE IS 10.  
 Since b is greater than 6 there is no significant difference  
 between CONTROL and TREATMENT at the 0.05 level.

SUMMARY OF FISHER'S EXACT TESTS

NUMBER NUMBER SIG



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

TITLE: AA # K1505002, C.DUBIA CHRONIC, REPRODUCCION, 5-13-15  
FILE: C:\COPYTO~1\TOXSTAT\C.DUB  
TRANSFORM: NO TRANSFORM NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	12.0000	12.0000
1	CONTROL	2	23.0000	23.0000
1	CONTROL	3	15.0000	15.0000
1	CONTROL	4	15.0000	15.0000
1	CONTROL	5	19.0000	19.0000
1	CONTROL	6	8.0000	8.0000
1	CONTROL	7	20.0000	20.0000
1	CONTROL	8	16.0000	16.0000
1	CONTROL	9	10.0000	10.0000
1	CONTROL	10	19.0000	19.0000
2	10 % EFFLUENT	1	10.0000	10.0000
2	10 % EFFLUENT	2	15.0000	15.0000
2	10 % EFFLUENT	3	16.0000	16.0000
2	10 % EFFLUENT	4	12.0000	12.0000
2	10 % EFFLUENT	5	13.0000	13.0000
2	10 % EFFLUENT	6	16.0000	16.0000
2	10 % EFFLUENT	7	22.0000	22.0000
2	10 % EFFLUENT	8	8.0000	8.0000
2	10 % EFFLUENT	9	9.0000	9.0000
2	10 % EFFLUENT	10	14.0000	14.0000
3	13 % EFFLUENT	1	18.0000	18.0000
3	13 % EFFLUENT	2	17.0000	17.0000
3	13 % EFFLUENT	3	18.0000	18.0000
3	13 % EFFLUENT	4	13.0000	13.0000
3	13 % EFFLUENT	5	12.0000	12.0000
3	13 % EFFLUENT	6	8.0000	8.0000
3	13 % EFFLUENT	7	9.0000	9.0000
3	13 % EFFLUENT	8	19.0000	19.0000
3	13 % EFFLUENT	9	10.0000	10.0000
3	13 % EFFLUENT	10	13.0000	13.0000
4	17 % EFFLUENT	1	21.0000	21.0000
4	17 % EFFLUENT	2	20.0000	20.0000
4	17 % EFFLUENT	3	8.0000	8.0000
4	17 % EFFLUENT	4	14.0000	14.0000
4	17 % EFFLUENT	5	13.0000	13.0000
4	17 % EFFLUENT	6	12.0000	12.0000
4	17 % EFFLUENT	7	18.0000	18.0000
4	17 % EFFLUENT	8	12.0000	12.0000

4	17 %	EFFLUENT	9	16.0000	16.0000
4	17 %	EFFLUENT	10	17.0000	17.0000
5	23 %	EFFLUENT	1	16.0000	16.0000
5	23 %	EFFLUENT	2	17.0000	17.0000
5	23 %	EFFLUENT	3	15.0000	15.0000
5	23 %	EFFLUENT	4	10.0000	10.0000
5	23 %	EFFLUENT	5	24.0000	24.0000
5	23 %	EFFLUENT	6	7.0000	7.0000
5	23 %	EFFLUENT	7	14.0000	14.0000
5	23 %	EFFLUENT	8	13.0000	13.0000
5	23 %	EFFLUENT	9	13.0000	13.0000
5	23 %	EFFLUENT	10	17.0000	17.0000
6	31 %	EFFLUENT	1	10.0000	10.0000
6	31 %	EFFLUENT	2	17.0000	17.0000
6	31 %	EFFLUENT	3	17.0000	17.0000
6	31 %	EFFLUENT	4	13.0000	13.0000
6	31 %	EFFLUENT	5	10.0000	10.0000
6	31 %	EFFLUENT	6	17.0000	17.0000
6	31 %	EFFLUENT	7	20.0000	20.0000
6	31 %	EFFLUENT	8	18.0000	18.0000
6	31 %	EFFLUENT	9	10.0000	10.0000
6	31 %	EFFLUENT	10	16.0000	16.0000

AA # K1505002, C.DUBIA CHRONIC, REPRODUCCION, 5-13-15  
 File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	35.133	7.027	0.395
Within (Error)	54	959.600	17.770	
Total	59	994.733		

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

AA # K1505002, C.DUBIA CHRONIC, REPRODUCCION, 5-13-15  
 File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORM

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	15.700	15.700		
2	10 % EFFLUENT	13.500	13.500	1.167	
3	13 % EFFLUENT	13.700	13.700	1.061	
4	17 % EFFLUENT	15.100	15.100	0.318	
5	23 % EFFLUENT	14.600	14.600	0.583	
6	31 % EFFLUENT	14.800	14.800	0.477	

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

AA # K1505002, C.DUBIA CHRONIC, REPRODUCTION, 5-13-15

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

Transform: NO TRANSFORM

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	4.355	27.7	2.200
3	13 % EFFLUENT	10	4.355	27.7	2.000
4	17 % EFFLUENT	10	4.355	27.7	0.600
5	23 % EFFLUENT	10	4.355	27.7	1.100
6	31 % EFFLUENT	10	4.355	27.7	0.900

APPENDIX E

Organism History

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

**TEST ORGANISM HISTORY**

DATE SHIPPED 5/12/15 CLIENT Ar Analytical  
Reyn

Purchase Order #: \_\_\_\_\_

SPECIES: Pimephales promelas

Quantity Shipped: 900

Age: hatched 5/11/15 @ 15-1600  
EST

Brood Stock Source: Anderson Farms, AR

Culture Water: Groundwater 160

Hardness (Mg/l CaCO<sub>3</sub>): \_\_\_\_\_

Dissolved Oxygen (Mg/l): 8.5

Temperature (°C): 25.1°C

Feeding: Artn

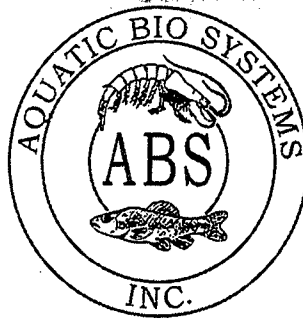
Comments: \_\_\_\_\_

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

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 <sub>3</sub> ):	<u>94 mg/l</u>	<u>76-130 mg/l</u>
TOTAL ALKALINITY (as CaCO <sub>3</sub> ):	<u>65 mg/l</u>	<u>65-100 mg/l</u>
pH:	<u>7.98</u>	<u>7.50-8.20</u>

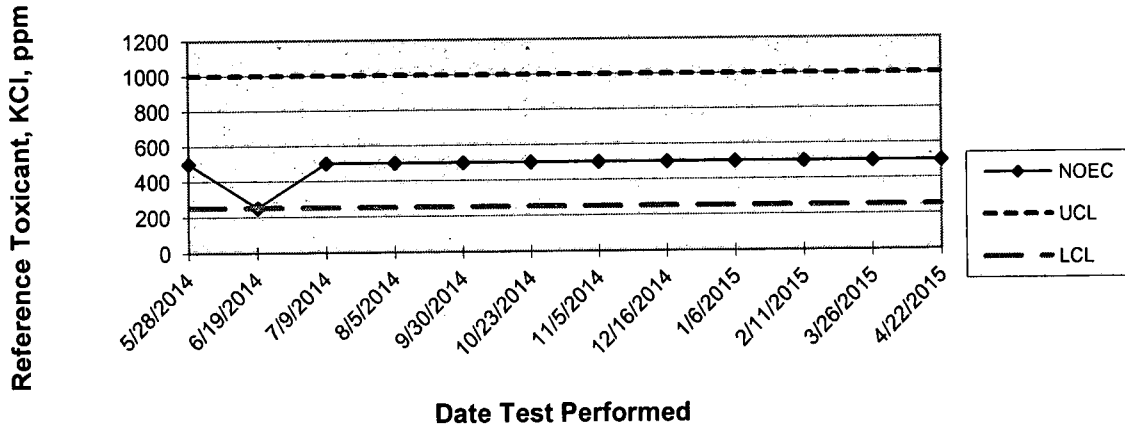
Comments:

  
\_\_\_\_\_  
Facility Supervisor

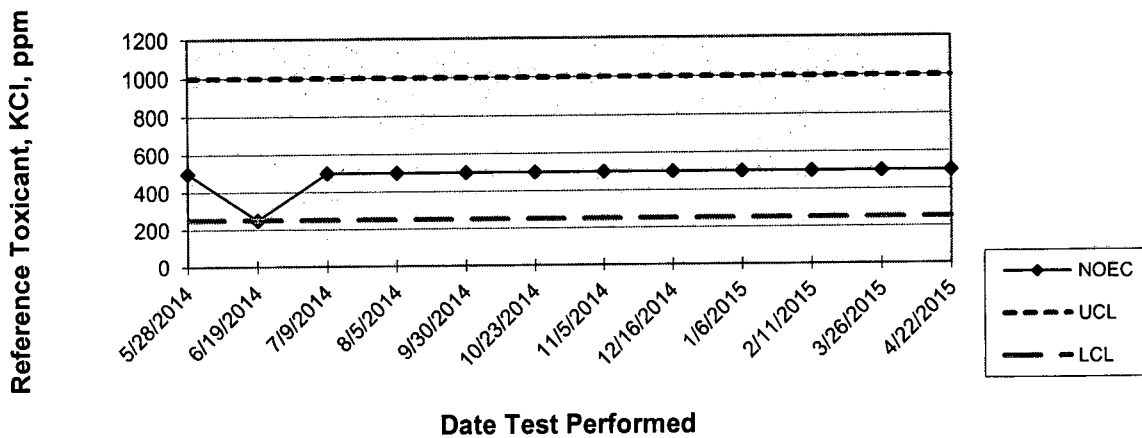
APPENDIX F

Quality Assurance Charts

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

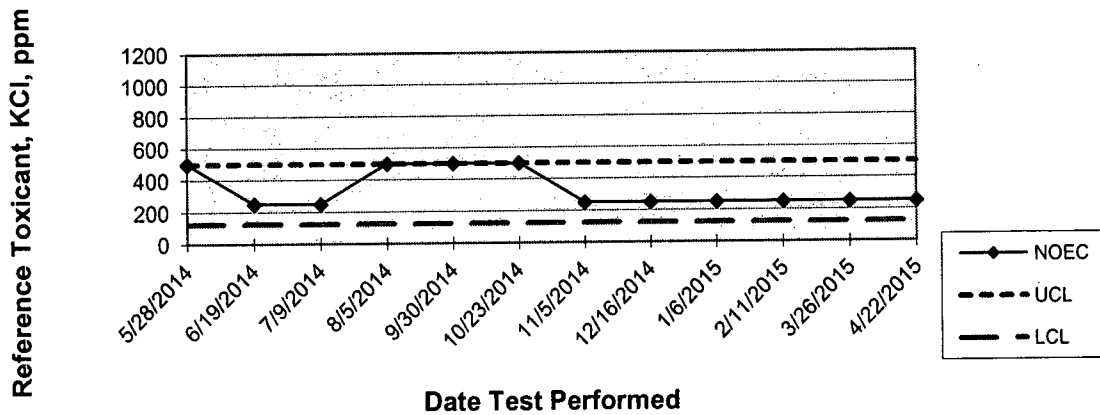


**ARKANSAS ANALYTICAL, INC.**  
**FATHEAD MINNOW GROWTH 7 Day**  
**QUALITY ASSURANCE**

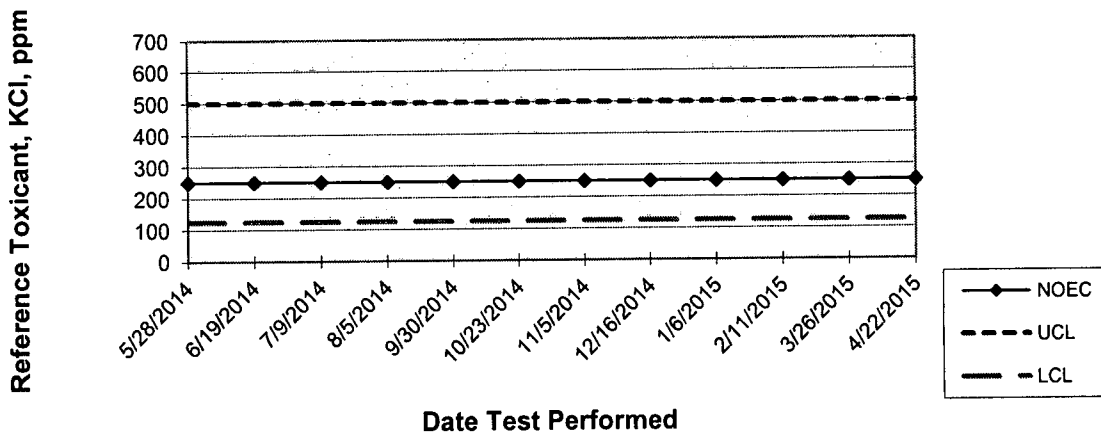




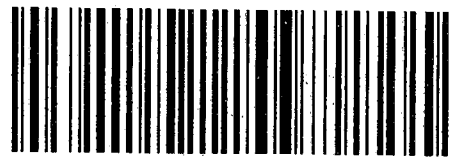
**ARKANSAS ANALYTICAL, INC.**  
**CERIODAPHNIA DUBIA SURVIVAL**  
**QUALITY ASSURANCE**



**ARKANSAS ANALYTICAL, INC.**  
**CERIODAPHNIA DUBIA REPRODUCTION**  
**QUALITY ASSURANCE**



OF THE RETURN ADDRESS, FOLD AT DOTTED LINE  
ENVELOPE TO THE RIGHT  
**CERTIFIED MAIL™**

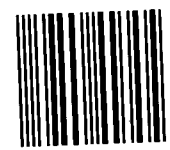


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PO Box 486  
Sheridan, AR 72150-0486



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