

Arkansas Analytical, Inc.

Toxicity Test Results

City of Hope
Permit Number: AR0038466
AFIN # 29-00034
Second Quarter 2015

Fathead Minnow, *Pimephales promelas*, Larval Survival and Growth Test
Test 1000.0

Ceriodaphnia dubia, Survival and Reproduction Test
Test 1002.0

Prepared for: **Kim Holston**
City of Hope
P.O. Box 667
Hope, Arkansas 71802

Prepared by: Arkansas Analytical, Inc.
11701 I-30, Bldg 1 Suite 115
Little Rock, Arkansas 72209
Lab Number K1505003

Tuesday, June 02, 2015

Introduction

This report contains test results for toxicity testing for the City of Hope WWTP. The NPDES permit number is AR0038466. The facility is located as follows: 3307 Hwy 67 West, Hope, AR 71801, West on Highway 67 to County Road 381, then 1 mile south on 381 to WWTP in Hempstead County, Arkansas.

The permit requires chronic biomonitoring testing for *Pimephales promelas* and *Ceriodaphnia dubia* once per quarter. The test results in this report represent the second quarter of 2015.

Plant Operations

To be provided by permittee.

Source of Effluent and Dilution Water

Effluent samples were collected as follows:

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	5-17-15, 0600	5-18-15, 0600
Sample #2:	5-19-15, 0600	5-20-15, 0600
Sample #3:	5-21-15, 0600	5-22-15, 6000

Samples were composites collected at the final discharge of Outfall 001, City of Hope effluent.

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

Sample Receiving Information:	Date, Time Sample(s) Received	Temperature (°C) upon receipt
Sample #1:	5-18-15, 1520	4
Sample #2:	5-20-15, 1638	10 (on ice)
Sample #3:	5-22-15, 1550	1

Chain of custody documentation is located in Appendix A.

The dilution water used in the toxicity tests was moderately hard synthetic. 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 32%, 42%, 56%, 75%, and 100%. The low-flow effluent concentration (**critical dilution**) was defined as **100% 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. There were no deviations from the reference method. The test chambers were 500 ml plastic cups, and each chamber contained ten organisms in a test solution volume of 250 mls. 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 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 mls 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 < 24 hour old Fathead Minnows, *Pimephales promelas*, which were purchased from Aquatox; a copy of the organism history is provided in Appendix E.

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 *Ceriodaphnia dubia*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	100%	X	
Average of 15 or more young per surviving female	16.9	X	
At least 60% of surviving females should have produced 3 broods	90%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	25.3%	X	

TEST ACCEPTANCE CRITERIA for *Pimephales promelas*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	98%	X	
The percent coefficient of variation between replicates must be 40% or less for survival	4.56%	X	
Minimum of 0.25 mg average dry weight of surviving controls	0.554	X	
The percent coefficient of variation between replicates must be 40% or less for growth	16.1%	X	

Reference Toxicant

The reference toxicant used was Potassium Chloride prepared in-house. The tests were performed using moderately hard synthetic 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 F.

Summary of Results
City of Hope

<i>Ceriodaphnia dubia</i>		<i>Pimephales promelas</i>	
NOEC / LOEC Survival	100% / NA	NOEC / LOEC survival	100% / NA
NOEC / LOEC Reproduction	100% / NA	NOEC / LOEC growth	100% / NA
Mean number of neonates (critical dilution)	15.8	%CV survival (critical dilution)	0.00%
%CV Reproduction (critical dilution)	21.5%	Mean dry weight (critical dilution) in milligrams	0.651
		%CV growth (critical dilution)	13.2%
PMSD Reproduction	26.9%	PMSD Growth	19.6%

Conclusion

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

The permit issued to the City of Hope, specifies that the critical dilution is 100% effluent. The effluent samples did not exhibit lethal or sublethal effects at the critical dilution, and, as such, passed both portions of the test.


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

The permit issued to the City of Hope, specifies the critical dilution is 100% effluent. The effluent samples did not exhibit lethal or sublethal effects at the critical dilution, and, as such, passed both portions of the test.

Biomonitoring Analysts:

Ryan Hudgin / Ken Pigue / Will Lindsey / Melissa Bird

Reviewed by:


Tracy Bounds, lab manager

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

PERMITTEE: City of Hope

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	5-17-15, 0600	5-18-15, 0600
Sample #2:	5-19-15, 0600	5-20-15, 0600
Sample #3:	5-21-15, 0600	5-22-15, 6000

Test initiated (date, time): 5-19-15 , 1440 Test terminated (date, time): 5-26-15, 1415

Dilution water used: Moderately Hard Synthetic

DATA TABLE FOR FATHEAD MINNOW SURVIVAL

Effluent Conc %	Percent Survival in Replicate Chambers					Mean Percent Survival				CV %
	A	B	C	D	E	24 hours	48 hours	7 days		
0%	100	90	100	100	100	100	100	98	4.56	
32%	100	100	100	100	100	100	100	100		
42%	100	100	100	100	100	100	100	100		
56%	100	100	100	100	100	100	100	100		
75%	100	100	100	100	100	100	100	100		
100%	100	100	100	100	100	100	100	100	0.00	

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%	0.449	0.485	0.574	0.588	0.674	0.554	16.1
32%	0.539	0.661	0.567	0.693	0.703	0.633	
42%	0.689	0.674	0.552	0.677	0.567	0.632	
56%	0.603	0.574	0.515	0.658	0.572	0.584	
75%	0.574	0.721	0.593	0.589	0.658	0.627	
100%	0.605	0.587	0.730	0.575	0.757	0.651	13.2

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, (100%) 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, (100%) 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)= 100 % effluent
b) NOEC growth (parameter TPP6C)= 100 % effluent
c) Coefficient of variation (parameter TQP6C)= 16.1 %

6. Enter Whole Effluent Toxicity: 100 %

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING
Ceriodaphnia dubia SURVIVAL AND REPRODUCTION

Permittee: City of Hope

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	5-17-15, 0600	5-18-15, 0600
Sample #2:	5-19-15, 0600	5-20-15, 0600
Sample #3:	5-21-15, 0600	5-22-15, 6000

Test initiated (date, time): 5-19-15, 0935 Test terminated (date, time): 5-26-15, 1040

Dilution water used: Moderately Hard Synthetic

Ceriodaphnia dubia SURVIVAL AND REPRODUCTION
NUMBER OF YOUNG PRODUCED PER FEMALE @ TEST TERMINATION

PERCENT EFFLUENT

Replicate	0%	32%	42%	56%	75%	100%
A	23	14	12	11	X0	13
B	10	15	15	12	15	19
C	12	11	15	13	11	22
D	14	X0	14	15	13	13
E	19	14	21	13	22	15
F	17	16	17	21	8	19
G	19	16	8	16	11	13
H	14	11	12	12	17	13
I	19	11	9	11	11	13
J	22	15	11	12	20	18
Mean	16.9	12.3	13.4	13.6	12.8	15.8
Mean/surviving female	16.9	13.7	13.4	13.6	14.2	15.8
CV%*	25.3					21.5

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 Hope

PERCENT SURVIVAL

PERCENT EFFLUENT	0%	32%	42%	56%	75%	100%
Time of Reading: 24 HOURS	100	100	100	100	100	100
48 HOURS	100	100	100	100	100	100
Test termination	100	90	100	100	90	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, (100%): 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, (100%): 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)= 100 % effluent

b) NOEC reproduction (parameter TPP3B)= 100 % effluent

c) Coefficient of variation (parameter TQP3B)= 25.3 %

6. Enter Whole Effluent Toxicity: 100 %

APPENDIX A

Chain of Custody Forms



8100 NATIONAL DRIVE, LITTLE ROCK, AR 72209
 501-562-8139 800-331-8139
 FAX 501-562-7025

CHAIN OF CUSTODY RECORD

TURN AROUND TIME
 RUSH 24HR. 48 HR.
 5 DAY REG
 OTHER _____

FOR LAB/OFFICE USE ONLY

LAB # 18163.0001B
 CLIENT # 15020
 P.O.# _____

STANDARD METHODS PRESERVATION PER EPA 40 CFR
 C 4= COOL TO 4.C
 S<2= SULFURIC ACID TO pH<2
 N<2= NITRIC ACID TO pH<2
 T= THIOSULFATE FOR DECHLORINATION
 W= WINKLER AZIDE MODIFICATION
 P= MEMBRANE ELECTRODE
 NaOH= pH >12

NAME OF COMPANY, CITY, OR PROJECT: City of Hope PROJECT NO.: West Plant SAMPLER(S) NAME: (PRINT) S. Ross 11091382

SAMPLE NO:	SAMPLE ID AND/OR COLLECTION LOCATION	START	END	COMP	FIELD ANALYSIS				D.O (W)	CONTAINER TYPE	ANALYSIS REQUIRED
		DATE/TIME	DATE/TIME	GRAB	pH	TEMP	FLOW	CL2	D.O(P)	PRESERVATIVE	
	W P F E	5-17-15/16:2	5-18-15/16:4	24hr C						6, 1/2 gal. p C4	K1505 - W.E.T. 003 A
METHOD OF SHIPMENT (CIRCLE)		FIELD CALIBRATION RECORD				Samples Received at Arkansas Analytical Relinquished By: Sorrells Date/Time: 5-18-15.1520 Received By: <u>Sydney James</u> Custody Seals: Yes <input checked="" type="checkbox"/> No <input type="checkbox"/> Containers Correct: <input checked="" type="checkbox"/> COC/Labels Agree: <input checked="" type="checkbox"/> Received on Ice: <input checked="" type="checkbox"/> Temperature on Receipt: _____ °C Temperature Gun ID: HHT # 2 _____					
FED EX WALK IN SRA UPS OTHER	pH 7	7.00									
	pH 4	4.01									
	pH 10	10.00									
TYPE OF SAMPLE(S): (CIRCLE)		D.O			FIELD ANALYSIS CONDUCTED BY: (CIRCLE) SRA CLIENT						
WATER SOIL W/W SLUDGE OTHER											

RELINQUISHED BY: [Signature] DATE/TIME: 5-18-15 / 9:48am RECEIVED BY: [Signature] DATE/TIME: May 18, 2015 9:48am

8100 NATIONAL DRIVE, LITTLE ROCK, AR 72209
 501-562-8139 800-331-8139
 FAX 501-562-7025

CHAIN OF CUSTODY RECORD

TURN AROUND TIME
 RUSH 24HR. 48 HR.
 5 DAY REG
 OTHER _____

FOR LAB/OFFICE USE ONLY

LAB # 18163.0003 B
 CLIENT # 15020
 P.O.# _____

STANDARD METHODS PRESERVATION PER EPA 40 CFR
 C 4= COOL TO 4.C
 S<2= SULFURIC ACID TO pH<2
 N<2= NITRIC ACID TO pH<2
 T= THIOSULFATE FOR DECHLORINATION
 W= WINKLER AZIDE MODIFICATION
 P= MEMBRANE ELECTRODE
 NaOH= pH >12

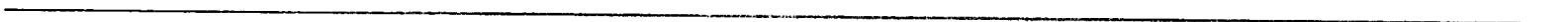
NAME OF COMPANY, CITY, OR PROJECT: City of Hope PROJECT NO: West Plant SAMPLER(S) NAME: (PRINT) ISCO AUTO SAMPLER

SAMPLE NO:	SAMPLE ID AND/OR COLLECTION LOCATION	START	END	COMP	FIELD ANALYSIS				D.O (W)	CONTAINER TYPE	ANALYSIS REQUIRED																		
		DATE/TIME	DATE/TIME	GRAB	pH	TEMP	FLOW	CL2	D.O(P)	PRESERVATIVE																			
	W P F E	5/21/15 6 AM	5/22/15 6 AM	24hr C						6, 1/2 gal. p C4	K1505 - W.E.T. 003 RE																		
METHOD OF SHIPMENT (CIRCLE)		FIELD CALIBRATION RECORD				<p>Samples Received at Arkansas Analytical Relinquished By: <u>Sorrells</u> Date/Time: <u>5-22-15, 1550</u> Received By: <u>Sydney James</u></p> <table border="1"> <tr> <td>Custody Seals:</td> <td>Yes</td> <td>No</td> </tr> <tr> <td>Containers Correct:</td> <td><input checked="" type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> <tr> <td>COC/Labels Agree:</td> <td><input checked="" type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> <tr> <td>Received on Ice:</td> <td><input checked="" type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> <tr> <td>Temperature on Receipt:</td> <td colspan="2"><u>16</u></td> </tr> <tr> <td>Temperature Gun ID:</td> <td colspan="2"><u>HHT # 2</u></td> </tr> </table>						Custody Seals:	Yes	No	Containers Correct:	<input checked="" type="checkbox"/>	<input type="checkbox"/>	COC/Labels Agree:	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Received on Ice:	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Temperature on Receipt:	<u>16</u>		Temperature Gun ID:	<u>HHT # 2</u>	
Custody Seals:	Yes	No																											
Containers Correct:	<input checked="" type="checkbox"/>	<input type="checkbox"/>																											
COC/Labels Agree:	<input checked="" type="checkbox"/>	<input type="checkbox"/>																											
Received on Ice:	<input checked="" type="checkbox"/>	<input type="checkbox"/>																											
Temperature on Receipt:	<u>16</u>																												
Temperature Gun ID:	<u>HHT # 2</u>																												
FED EX WALK IN SRA UPS OTHER	pH 7	7.00																											
	pH 4	4.01																											
	pH 10	10.00																											
	D.O																												
TYPE OF SAMPLE(S): (CIRCLE)						FIELD ANALYSIS CONDUCTED BY: (CIRCLE) SRA CLIENT																							
WATER SOIL W/W SLUDGE OTHER																													

RELINQUISHED BY: [Signature] DATE/TIME: 5-22-15 0945 RECEIVED BY: [Signature] DATE/TIME: 5-22-15 9:45
 RELINQUISHED BY: _____ DATE/TIME: _____ RECEIVED BY (LAB): [Signature] DATE/TIME: 5-22-15 9:45
TEMP 03.7°C

APPENDIX B

Effluent and Dilution Water Data



Biomonitoring Quality Control Benchsheet

Analyst	RA	RA	RA	RA	RA	RA	RA	RA
Date	5-2-15	5-13-15	5-14-15	5-15-15	5-16-17	5-17-15	5-18-15	5-19-15
pH Meter ID	AR60							
LIN pH 4 Buffer	1401167							
LIN pH 7 Buffer	1401173							
LIN pH 10 Buffer	1401168							
Slope (>90%)	95.6%	94.7%	96.1%	94.9%	95.8%	95.0%	97.1%	95.4%

Dissolved O ₂ Meter	0.0.1305							
Meter Reading	8.36	8.41	8.71	8.92	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	AR60							
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	OR 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

Analyst	RH	RH	RH	WL	mb	JP	RH	RH
Date	5-20-15	5-21-15	5-22-15	5-23-15	5-24-15	5/25/15	5/26/15	5/27/15
pH Meter ID								
LIN pH 4 Buffer	1401167							
LIN pH 7 Buffer	1401173							
LIN pH 10 Buffer	1401168							
Slope (>90%)	95.9%	94.9%	96.1%	93.9%	94.6%	94.4	96%	94.2%

Dissolved O ₂ Meter	D.O. 1305							
Meter Reading	8.51	8.21	8.46	8.65	8.56	8.81	8.64	8.38
Temp.	24	25	24	22	22	21.0	23	24
Chart Value at Temp.	8.418	8.263	8.418	8.743	8.743	8.915	8.578	8.418
Difference	0.108	0.053	0.058	0.09	0.183		0.078	0.038
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	AR60							
Meter Reading	23	24	24	22	22	20.25	23	
Thermometer Reading	22	24	23	23	22	20.5	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)			<5					
STD Result			104					
T.V. / %REC			100/104%					
Acceptance Criteria			93.5-108.5% Recovery					

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

Conductivity Meter ID	Com 02							
Blank (<1)			<1					
STD Result			1420					
T.V. / %REC			142/100%					
Acceptance Criteria			99.2-104.0% Recovery					

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

Revision 0
Effective Date 01APR15

APPENDIX C

Fathead minnow raw data and statistics



Pimephales promelas

FATHEAD MINNOW

SURVIVAL DATA FOR LARVAL SURVIVAL AND GROWTH TEST (CHRONIC)

LAB #: K1505003			TEST START		DATE	5/19/15	TIME	1440				
CLIENT: Hope			TEST END		DATE	5/26/15	TIME	1415				
ANALYST: RH			AGE AND SOURCE OF MINNOWS					< 24 hrs old, Aquatox				
DAY(NUMBER SURVIVING)												
SURVIVAL												
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONTROL	A	10	10	10	10	10	10	10	10	100%	98.0%	4.56
	B	10	10	10	10	10	9	9	9	90%		
	C	10	10	10	10	10	10	10	10	100%		
	D	10	10	10	10	10	10	10	10	100%		
	E	10	10	10	10	10	10	10	10	100%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	10	10	10	10	10	10	100%	100.0%	
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	10	10	10	10	10	10	100%		
	D	10	10	10	10	10	10	10	10	100%		
	E	10	10	10	10	10	10	10	10	100%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	10	10	10	10	10	10	100%	100.0%	
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	10	10	10	10	10	10	100%		
	D	10	10	10	10	10	10	10	10	100%		
	E	10	10	10	10	10	10	10	10	100%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	10	10	10	10	10	10	100%	100.0%	
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	10	10	10	10	10	10	100%		
	D	10	10	10	10	10	9	10	10	100%		
	E	10	10	10	10	10	10	10	10	100%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	10	10	10	10	10	10	100%	100.0%	
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	10	10	10	10	10	10	100%		
	D	10	10	10	10	10	10	10	10	100%		
	E	10	10	10	10	10	10	10	10	100%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	10	10	10	10	10	10	100%	100.0%	0.00
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	10	10	10	10	10	10	100%		
	D	10	10	10	10	10	10	10	10	100%		
	E	10	10	10	10	10	10	10	10	100%		
ANALYST:		RH	RH	KP	RH	WL	MB	KP	RH			
DATE:		5/19/15	5/20/15	5/21/15	5/22/15	5/23/15	5/24/15	5/25/15	5/26/15			
TIME:		1440	1715	1500	1145	1215	910	1400	1415			

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

REMARKS:

AA# K1505003,FATHEAD MINNOW SURV.,CHRONIC, 5-19-15
File: C:\COPYTO~1\TOXSTAT\FHSURV~1. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.021

W = 0.416

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# K1505003,FATHEAD MINNOW SURV.,CHRONIC, 5-19-15
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# K1505003, FATHEAD MINNOW SURV., CHRONIC, 5-19-15
 FILE: C:\COPYTO~1\TOXSTAT\FHSURV~1.
 TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	1.0000	1.4120
1	CONTROL	2	0.9000	1.2490
1	CONTROL	3	1.0000	1.4120
1	CONTROL	4	1.0000	1.4120
1	CONTROL	5	1.0000	1.4120
2	32 % EFFLUENT	1	1.0000	1.4120
2	32 % EFFLUENT	2	1.0000	1.4120
2	32 % EFFLUENT	3	1.0000	1.4120
2	32 % EFFLUENT	4	1.0000	1.4120
2	32 % EFFLUENT	5	1.0000	1.4120
3	42 % EFFLUENT	1	1.0000	1.4120
3	42 % EFFLUENT	2	1.0000	1.4120
3	42 % EFFLUENT	3	1.0000	1.4120
3	42 % EFFLUENT	4	1.0000	1.4120
3	42 % EFFLUENT	5	1.0000	1.4120
4	56 % EFFLUENT	1	1.0000	1.4120
4	56 % EFFLUENT	2	1.0000	1.4120
4	56 % EFFLUENT	3	1.0000	1.4120
4	56 % EFFLUENT	4	1.0000	1.4120
4	56 % EFFLUENT	5	1.0000	1.4120
5	75 % EFFLUENT	1	1.0000	1.4120
5	75 % EFFLUENT	2	1.0000	1.4120
5	75 % EFFLUENT	3	1.0000	1.4120
5	75 % EFFLUENT	4	1.0000	1.4120
5	75 % EFFLUENT	5	1.0000	1.4120
6	100 % EFFLUENT	1	1.0000	1.4120
6	100 % EFFLUENT	2	1.0000	1.4120
6	100 % EFFLUENT	3	1.0000	1.4120
6	100 % EFFLUENT	4	1.0000	1.4120
6	100 % EFFLUENT	5	1.0000	1.4120

AA# K1505003, FATHEAD MINNOW SURV., CHRONIC, 5-19-15
 File: C:\COPYTO~1\TOXSTAT\FHSURV~1. Transform: ARC SINE(SQUARE ROOT(Y))

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	1.379				
2	32 % EFFLUENT	1.412	30.00	16.00	5.00	
3	42 % EFFLUENT	1.412	30.00	16.00	5.00	
4	56 % EFFLUENT	1.412	30.00	16.00	5.00	
5	75 % EFFLUENT	1.412	30.00	16.00	5.00	
6	100 % EFFLUENT	1.412	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:		K1505003		TEST DATES (BEGIN / END):		5/19/15 - 5/26/15	
CLIENT:		Hope		WEIGHING DATE / TIME:		5/27/2015 1110	
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	0.98123	0.97674	0.00449	10	0.449	AVG DRY
	B	0.94656	0.94171	0.00485	10	0.485	WEIGHT (mg)
	C	0.95110	0.94536	0.00574	10	0.574	0.554
	D	0.98812	0.98224	0.00588	10	0.588	CV
	E	0.98066	0.97392	0.00674	10	0.674	16.1
32%	A	0.99521	0.98982	0.00539	10	0.539	AVG DRY
	B	0.96635	0.95974	0.00661	10	0.661	WEIGHT (mg)
	C	0.96542	0.95975	0.00567	10	0.567	0.633
	D	0.95936	0.95243	0.00693	10	0.693	CV
	E	0.99716	0.99013	0.00703	10	0.703	
42%	A	0.98555	0.97866	0.00689	10	0.689	AVG DRY
	B	1.02747	1.02073	0.00674	10	0.674	WEIGHT (mg)
	C	0.96884	0.96332	0.00552	10	0.552	0.632
	D	1.00714	1.00037	0.00677	10	0.677	CV
	E	0.99954	0.99387	0.00567	10	0.567	
56%	A	0.98392	0.97789	0.00603	10	0.603	AVG DRY
	B	0.97127	0.96553	0.00574	10	0.574	WEIGHT (mg)
	C	0.98939	0.98424	0.00515	10	0.515	0.584
	D	0.98640	0.97982	0.00658	10	0.658	CV
	E	0.97711	0.97139	0.00572	10	0.572	
75%	A	0.95757	0.95183	0.00574	10	0.574	AVG DRY
	B	0.97045	0.96324	0.00721	10	0.721	WEIGHT (mg)
	C	0.98371	0.97778	0.00593	10	0.593	0.627
	D	0.99258	0.98669	0.00589	10	0.589	CV
	E	0.97228	0.96570	0.00658	10	0.658	
100%	A	0.99268	0.98663	0.00605	10	0.605	AVG DRY
	B	1.00556	0.99969	0.00587	10	0.587	WEIGHT (mg)
	C	0.97996	0.97266	0.00730	10	0.730	0.651
	D	1.01298	1.00723	0.00575	10	0.575	CV
	E	0.99530	0.98773	0.00757	10	0.757	13.2

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA# K1505003, FATHEAD MINNOW GROWTH CHRONIC, 5-19-15

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

Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.127

W = 0.936

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# K1505003, FATHEAD MINNOW GROWTH CHRONIC, 5-19-15

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

Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 1.47

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# K1505003, FATHEAD MINNOW GROWTH CHRONIC, 5-19-15
 FILE: C:\COPYTO~1\TOXSTAT\FHGROWTH.
 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.4490	0.4490
1	CONTROL	2	0.4850	0.4850
1	CONTROL	3	0.5740	0.5740
1	CONTROL	4	0.5880	0.5880
1	CONTROL	5	0.6740	0.6740
2	32 % EFFLUENT	1	0.5390	0.5390
2	32 % EFFLUENT	2	0.6610	0.6610
2	32 % EFFLUENT	3	0.5670	0.5670
2	32 % EFFLUENT	4	0.6930	0.6930
2	32 % EFFLUENT	5	0.7030	0.7030
3	42 % EFFLUENT	1	0.6890	0.6890
3	42 % EFFLUENT	2	0.6740	0.6740
3	42 % EFFLUENT	3	0.5520	0.5520
3	42 % EFFLUENT	4	0.6770	0.6770
3	42 % EFFLUENT	5	0.5670	0.5670
4	56 % EFFLUENT	1	0.6030	0.6030
4	56 % EFFLUENT	2	0.5740	0.5740
4	56 % EFFLUENT	3	0.5150	0.5150
4	56 % EFFLUENT	4	0.6580	0.6580
4	56 % EFFLUENT	5	0.5720	0.5720
5	75 % EFFLUENT	1	0.5740	0.5740
5	75 % EFFLUENT	2	0.7210	0.7210
5	75 % EFFLUENT	3	0.5930	0.5930
5	75 % EFFLUENT	4	0.5890	0.5890
5	75 % EFFLUENT	5	0.6580	0.6580
6	100 % EFFLUENT	1	0.6050	0.6050
6	100 % EFFLUENT	2	0.5870	0.5870
6	100 % EFFLUENT	3	0.7300	0.7300
6	100 % EFFLUENT	4	0.5750	0.5750
6	100 % EFFLUENT	5	0.7570	0.7570

AA# K1505003, FATHEAD MINNOW GROWTH CHRONIC, 5-19-15
 File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.033	0.007	1.255
Within (Error)	24	0.127	0.005	
Total	29	0.161		

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

AA# K1505003, FATHEAD MINNOW GROWTH CHRONIC, 5-19-15

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	0.554	0.554		
2	32 % EFFLUENT	0.633	0.633	-1.706	
3	42 % EFFLUENT	0.632	0.632	-1.688	
4	56 % EFFLUENT	0.584	0.584	-0.660	
5	75 % EFFLUENT	0.627	0.627	-1.584	
6	100 % EFFLUENT	0.651	0.651	-2.101	

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

AA# K1505003, FATHEAD MINNOW GROWTH CHRONIC, 5-19-15

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	32 % EFFLUENT	5	0.109	19.6	-0.079
3	42 % EFFLUENT	5	0.109	19.6	-0.078
4	56 % EFFLUENT	5	0.109	19.6	-0.030
5	75 % EFFLUENT	5	0.109	19.6	-0.073
6	100 % EFFLUENT	5	0.109	19.6	-0.097

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

SURVIVAL AND REPRODUCTION TEST

Ceriodaphnia dubia

Discharger: Hope AFIN # 29-00034
 Location: Outfall 001
 Date Sample Collected: 5 - 18/20/22 - 15

Lab Number/s
K1505003

Analyst: RH
 Test Start - Date/Time: 5-19-15,0935
 Test Stop - Date/Time: 5-26-15, 1040

Conc 1		Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	
%	Day	A	B	C	D	E	F	G	H	I	J					
MHS	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	0	10	0.0	RH
	3	0	0	0	0	0	0	0	0	1	0	1	10	0.1	RH	
	4	1	2	0	2	1	0	0	3	0	5	14	10	1.4	RH	
	5	8	0	5	0	10	8	4	6	7	8	56	10	5.6	RH	
	6	6	3	7	8	0	4	8	2	5	0	43	10	4.3	RH	
	7	8	5	0	4	8	5	7	3	6	9	55	10	5.5	RH	
	8															
Total		23	10	12	14	19	17	19	14	19	22	169		Avg. = 16.9		
														C.V. = 25.3		

Conc 2		Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	
%	Day	A	B	C	D	E	F	G	H	I	J					
32%	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	0	10	0.0	RH
	3	0	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	4	1	0	2	0	0	0	4	3	3	1	14	10	1.4	RH	
	5	2	7	3	X	3	4	4	6	5	8	40	10	4.0	RH	
	6	10	2	4		8	0	0	2	3	6	35	10	3.5	RH	
	7	1	6	2		3	12	8	0	0	2	34	10	3.4	RH	
	8															
Total		14	15	11	0	14	16	16	11	11	15	123		Avg. = 13.7		
														C.V. = 15.5		

Conc 3		Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	
%	Day	A	B	C	D	E	F	G	H	I	J					
42%	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	
	3	1	1	0	0	0	0	0	0	0	0	2	10	0.2	RH	
	4	1	0	2	2	2	1	1	0	0	0	9	10	0.9	RH	
	5	0	4	5	3	6	9	7	2	3	3	42	10	4.2	RH	
	6	9	7	1	1	8	0	0	4	6	3	39	10	3.9	RH	
	7	1	3	7	8	5	7	0	6	0	5	42	10	4.2	RH	
	8															
Total		12	15	15	14	21	17	8	12	9	11	134		Avg. = 13.4		
														C.V. = 28.8		

Conc 4		Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	
%	Day	A	B	C	D	E	F	G	H	I	J					
56%	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	
	3	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH	
	4	4	0	0	2	3	0	1	1	2	2	15	10	1.5	RH	
	5	3	12	8	7	5	9	6	8	0	0	58	10	5.8	RH	
	6	2	0	5	1	2	6	4	3	4	8	35	10	3.5	RH	
	7	2	0	0	5	3	6	5	0	5	2	28	10	2.8	RH	
	8															
Total		11	12	13	15	13	21	16	12	11	12	136		Avg. = 13.6		
														C.V. = 22.5		

Conc 5		Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	
%	Day	A	B	C	D	E	F	G	H	I	J					
75%	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	
	3	0	0	1	0	0	0	0	0	0	0	1	10	0.1	RH	
	4	0	1	1	1	1	4	3	2	0	4	17	10	1.7	RH	
	5	0	4	5	8	9	2	2	8	6	5	49	10	4.9	RH	
	6	X	7	3	2	8	2	6	0	1	5	34	10	3.4	RH	
	7		3	1	2	4	0	0	7	4	6	27	10	2.7	RH	
	8															
Total		0	15	11	13	22	8	11	17	11	20	128		Avg. = 14.2		
														C.V. = 32.7		

Conc 6		Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	
%	Day	A	B	C	D	E	F	G	H	I	J					
100%	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	
	3	0	0	0	0	0	0	0	0	1	0	1	10	0.1	RH	
	4	0	2	0	0	3	5	0	2	2	1	15	10	1.5	RH	
	5	6	9	12	8	8	3	8	8	10	7	77	10	7.7	RH	
	6	6	7	5	1	0	2	5	0	0	2	28	10	2.8	RH	
	7	1	1	5	4	6	9	0	3	0	8	37	10	3.7	RH	
	8															
Total		13	19	22	13	15	19	13	13	13	18	158		Avg. = 15.8		
														C.V. = 21.5		

AA # K1505003, C.DUBIA CHRONIC, REPRODUCTION, 5-19-15
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 # K1505003, C.DUBIA CHRONIC, REPRODUCTION, 5-19-15
File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 6.01

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
32	9	1	10
TOTAL	19	1	20

CRITICAL FISHER'S VALUE (10,10,10) (p=0.05) IS 6. b VALUE IS 9.
 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
42	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
56	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
75	9	1	10
TOTAL	19	1	20

CRITICAL FISHER'S VALUE (10,10,10) (p=0.05) IS 6. b VALUE IS 9.
 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
100	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	32	10	1	
2	42	10	0	
3	56	10	0	
4	75	10	1	
5	100	10	0	

TITLE: AA # K1505003, C.DUBIA CHRONIC, REPRODUCCION, 5-19-15
FILE: C:\COPYTO~1\TOXSTAT\C.DUB
TRANSFORM: NO TRANSFORMATION

NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	23.0000	23.0000
1	CONTROL	2	10.0000	10.0000
1	CONTROL	3	12.0000	12.0000
1	CONTROL	4	14.0000	14.0000
1	CONTROL	5	19.0000	19.0000
1	CONTROL	6	17.0000	17.0000
1	CONTROL	7	19.0000	19.0000
1	CONTROL	8	14.0000	14.0000
1	CONTROL	9	19.0000	19.0000
1	CONTROL	10	22.0000	22.0000
2	32 % EFFLUENT	1	14.0000	14.0000
2	32 % EFFLUENT	2	15.0000	15.0000
2	32 % EFFLUENT	3	11.0000	11.0000
2	32 % EFFLUENT	4	0.0000	0.0000
2	32 % EFFLUENT	5	14.0000	14.0000
2	32 % EFFLUENT	6	16.0000	16.0000
2	32 % EFFLUENT	7	16.0000	16.0000
2	32 % EFFLUENT	8	11.0000	11.0000
2	32 % EFFLUENT	9	11.0000	11.0000
2	32 % EFFLUENT	10	15.0000	15.0000
3	42 % EFFLUENT	1	12.0000	12.0000
3	42 % EFFLUENT	2	15.0000	15.0000
3	42 % EFFLUENT	3	15.0000	15.0000
3	42 % EFFLUENT	4	14.0000	14.0000
3	42 % EFFLUENT	5	21.0000	21.0000
3	42 % EFFLUENT	6	17.0000	17.0000
3	42 % EFFLUENT	7	8.0000	8.0000
3	42 % EFFLUENT	8	12.0000	12.0000
3	42 % EFFLUENT	9	9.0000	9.0000
3	42 % EFFLUENT	10	11.0000	11.0000
4	56 % EFFLUENT	1	11.0000	11.0000
4	56 % EFFLUENT	2	12.0000	12.0000
4	56 % EFFLUENT	3	13.0000	13.0000
4	56 % EFFLUENT	4	15.0000	15.0000
4	56 % EFFLUENT	5	13.0000	13.0000
4	56 % EFFLUENT	6	21.0000	21.0000
4	56 % EFFLUENT	7	16.0000	16.0000
4	56 % EFFLUENT	8	12.0000	12.0000

4	56 % EFFLUENT	9	11.0000	11.0000
4	56 % EFFLUENT	10	12.0000	12.0000
5	75 % EFFLUENT	1	0.0000	0.0000
5	75 % EFFLUENT	2	15.0000	15.0000
5	75 % EFFLUENT	3	11.0000	11.0000
5	75 % EFFLUENT	4	13.0000	13.0000
5	75 % EFFLUENT	5	22.0000	22.0000
5	75 % EFFLUENT	6	8.0000	8.0000
5	75 % EFFLUENT	7	11.0000	11.0000
5	75 % EFFLUENT	8	17.0000	17.0000
5	75 % EFFLUENT	9	11.0000	11.0000
5	75 % EFFLUENT	10	20.0000	20.0000
6	100 % EFFLUENT	1	13.0000	13.0000
6	100 % EFFLUENT	2	19.0000	19.0000
6	100 % EFFLUENT	3	22.0000	22.0000
6	100 % EFFLUENT	4	13.0000	13.0000
6	100 % EFFLUENT	5	15.0000	15.0000
6	100 % EFFLUENT	6	19.0000	19.0000
6	100 % EFFLUENT	7	13.0000	13.0000
6	100 % EFFLUENT	8	13.0000	13.0000
6	100 % EFFLUENT	9	13.0000	13.0000
6	100 % EFFLUENT	10	18.0000	18.0000

AA # K1505003, C.DUBIA CHRONIC, REPRODUCCION, 5-19-15

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

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	163.933	32.787	1.691
Within (Error)	54	1047.000	19.389	
Total	59	1210.933		

Critical F value = 2.45 (0.05,5,40)

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All equal

AA # K1505003, C.DUBIA CHRONIC, REPRODUCCION, 5-19-15

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

Transform: NO TRANSFORMATION

DUNNETT'S TEST

TABLE 1 OF 2

H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	16.900	16.900		
2	32 % EFFLUENT	12.300	12.300	2.336	*
3	42 % EFFLUENT	13.400	13.400	1.777	
4	56 % EFFLUENT	13.600	13.600	1.676	
5	75 % EFFLUENT	12.800	12.800	2.082	
6	100 % EFFLUENT	15.800	15.800	0.559	

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

AA # K1505003, C.DUBIA CHRONIC, REPRODUCCION, 5-19-15

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

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	10			
2	32 % EFFLUENT	10	4.549	26.9	4.600
3	42 % EFFLUENT	10	4.549	26.9	3.500
4	56 % EFFLUENT	10	4.549	26.9	3.300
5	75 % EFFLUENT	10	4.549	26.9	4.100
6	100 % EFFLUENT	10	4.549	26.9	1.100

APPENDIX E

Organism History

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

TEST ORGANISM HISTORY

DATE SHIPPED 5/19¹⁹/15 CLIENT ARK ANAESTHETICAL

Purchase Order #: _____

SPECIES: Pimephales promelas

Quantity Shipped: 550⁺ CST

Age: HATCHED 5/18/15 15-1600

Brood Stock Source: Anderson Farms, AR

Culture Water: Groundwater

Hardness (Mg/l CaCO3): =160

Dissolved Oxygen (Mg/l): 8.5

Temperature (°C): 25.1 °C

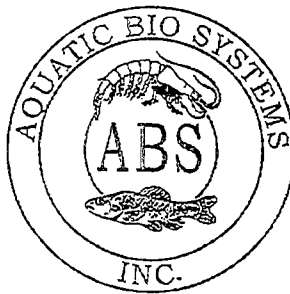
Feeding: ARTEMIA

Comments: _____

Shipped Via: Federal Express UPS Overnight Shuttle

Packaged By: _____

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



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

ORGANISM HISTORY

DATE: 11/25/2013

SPECIES: Ceriodaphnia dubia

AGE: > 3 day

LIFE STAGE: Adult

HATCH DATE: Variable


BEGAN FEEDING: Immediately

FOOD: YTC, Selenastrum sp.

Water Chemistry Record:

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

Comments:

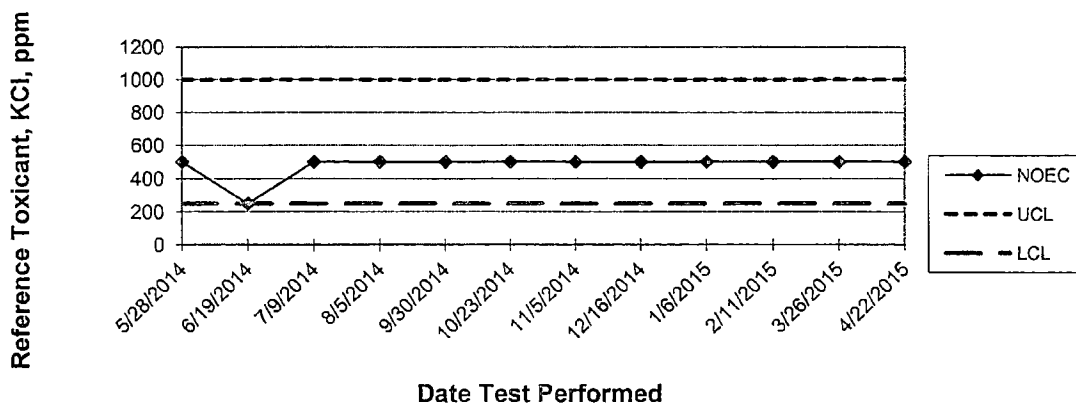


Facility Supervisor

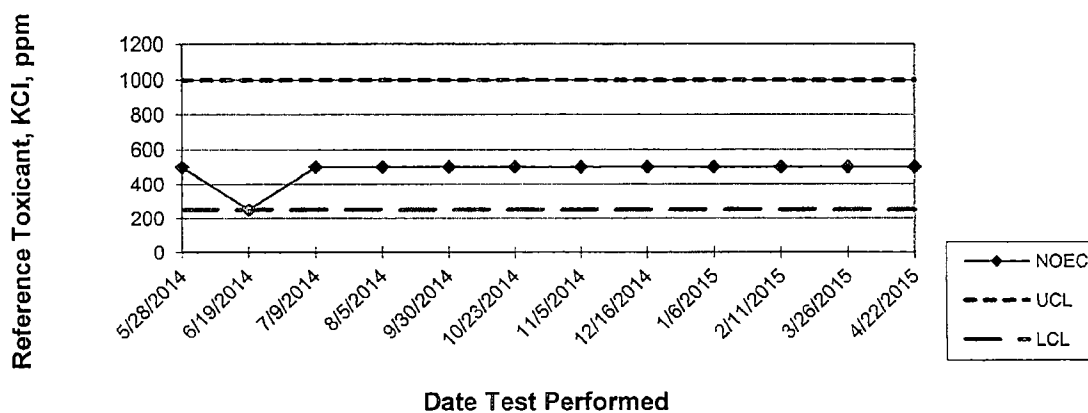
APPENDIX F

Quality Assurance Charts

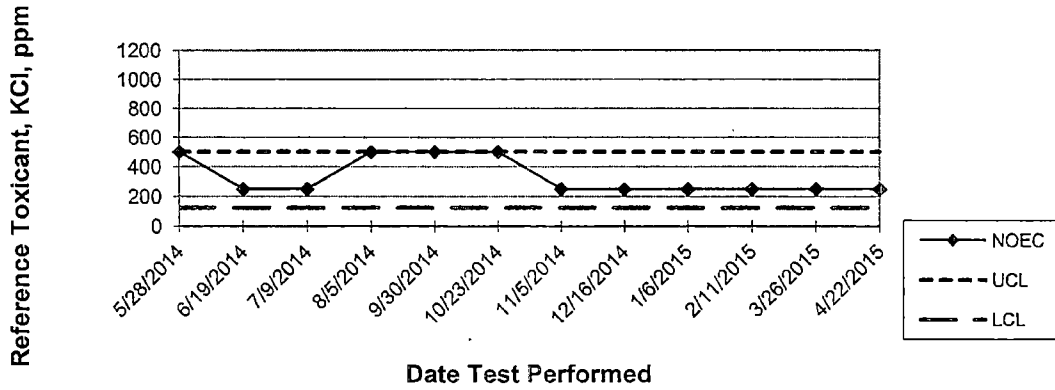
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW SURVIVAL 7 Day
QUALITY ASSURANCE



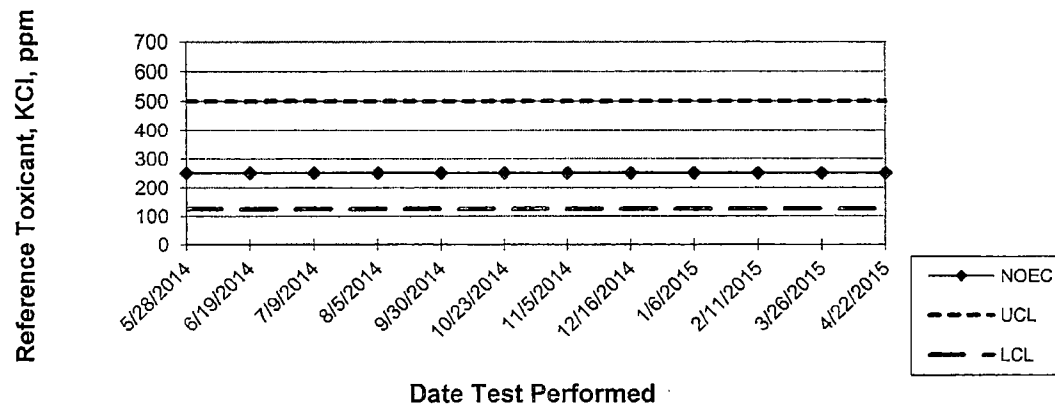
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW GROWTH 7 Day
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
CERIODAPHНИЯ DUBIA SURVIVAL
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
CERIODAPHНИЯ DUBIA REPRODUCTION
QUALITY ASSURANCE



Arkansas Analytical, Inc.

Toxicity Test Results

City of Hope
Permit Number: AR0038466
AFIN # 29-00034
Third Quarter 2015

Fathead Minnow, *Pimephales promelas*, Larval Survival and Growth Test
Test 1000.0

Ceriodaphnia dubia, Survival and Reproduction Test
Test 1002.0

Prepared for: **Kim Holston**
City of Hope
P.O. Box 667
Hope, Arkansas 71802

Prepared by: Arkansas Analytical, Inc.
11701 I-30, Bldg 1 Suite 115
Little Rock, Arkansas 72209
Lab Number K1508006

Thursday, August 27, 2015

Introduction

This report contains test results for toxicity testing for the City of Hope WWTP. The NPDES permit number is AR0038466. The facility is located as follows: 3307 Hwy 67 West, Hope, AR 71801, West on Highway 67 to County Road 381, then 1 mile south on 381 to WWTP in Hempstead County, Arkansas.

The permit requires chronic biomonitoring testing for *Pimephales promelas* and *Ceriodaphnia dubia* once per quarter. The test results in this report represent the third quarter of 2015.

Plant Operations

To be provided by permittee.

Source of Effluent and Dilution Water

Effluent samples were collected as follows:

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	8-16-15, 0700	8-17-15, 0700
Sample #2:	8-18-15, 0700	8-19-15, 0700
Sample #3:	8-20-15, 0700	8-21-15, 0700

Samples were composites collected at the final discharge of Outfall 001, City of Hope effluent.

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

Sample Receiving Information:	Date, Time Sample(s) Received	Temperature (°C) upon receipt
Sample #1:	8-17-15, 1613	4
Sample #2:	8-19-15, 1325	4
Sample #3:	8-21-15, 1123	3

Chain of custody documentation is located in Appendix A.

The dilution water used in the toxicity tests was moderately hard synthetic. 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 32%, 42%, 56%, 75%, and 100%. The low-flow effluent concentration (**critical dilution**) was defined as **100% 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. There were no deviations from the reference method. The test chambers were 500 ml plastic cups, and each chamber contained ten organisms in a test solution volume of 250 mls. 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 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 mls 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 E.

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 *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	15.7	X	
At least 60% of surviving females should have produced 3 broods	90%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	34.1%	X	

TEST ACCEPTANCE CRITERIA for *Pimephales promelas*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	98%	X	
The percent coefficient of variation between replicates must be 40% or less for survival	4.56%	X	
Minimum of 0.25 mg average dry weight of surviving controls	0.842	X	
The percent coefficient of variation between replicates must be 40% or less for growth	11.8%	X	

Reference Toxicant

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

REFERENCE TOXICANT

<i>Ceriodaphnia dubia</i> 7/22/15 – 7/29/15		<i>Pimephales promelas</i> 7/22/15 – 7/29/15	
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 F.

Summary of Results
City of Hope

<i>Ceriodaphnia dubia</i>		<i>Pimephales promelas</i>	
NOEC / LOEC Survival	100% / NA	NOEC / LOEC survival	100% / NA
NOEC / LOEC Reproduction	100% / NA	NOEC / LOEC growth	100% / NA
Mean number of neonates (critical dilution)	15.2	%CV survival (critical dilution)	4.56%
%CV Reproduction (critical dilution)	26.8%	Mean dry weight (critical dilution) in milligrams	0.779
		%CV growth (critical dilution)	9.46%
PMSD Reproduction	35.2%	PMSD Growth	18.8%

Conclusion

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

The permit issued to the City of Hope, specifies that the critical dilution is 100% effluent. The effluent samples did not exhibit lethal or sublethal effects at the critical dilution, and, as such, passed both portions of the test.


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

The permit issued to the City of Hope, specifies the critical dilution is 100% effluent. The effluent samples did not exhibit lethal or sublethal effects at the critical dilution, and, as such, passed both portions of the test.

Biomonitoring Analysts:

Ryan Hudgin / Alfred Tennison / Hallie Freyaldenhoven

Reviewed by:


Tracy Bounds, lab manager

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

PERMITTEE: City of Hope

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	8-16-15, 0700	8-17-15, 0700
Sample #2:	8-18-15, 0700	8-19-15, 0700
Sample #3:	8-20-15, 0700	8-21-15, 0700

Test initiated (date, time): 8-18-15, 1450 Test terminated (date, time): 8-25-15, 1445

Dilution water used: Moderately Hard Synthetic

DATA TABLE FOR FATHEAD MINNOW SURVIVAL

Effluent Conc %	Percent Survival in Replicate Chambers						Mean Percent Survival			CV %
	A	B	C	D	E		24 hours	48 hours	7 days	
0%	100	100	90	100	100		100	98	98	4.56
32%	90	100	100	90	100		100	96	96	
42%	90	100	100	100	90		100	98	96	
56%	70	90	100	100	100		98	96	92	
75%	100	100	90	90	100		98	98	96	
100%	90	100	100	100	100		100	98	98	4.56

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%	0.927	0.834	0.714	0.784	0.953		0.842	11.8
32%	0.606	0.714	0.866	0.689	0.998		0.775	
42%	0.928	0.905	0.812	0.833	0.930		0.882	
56%	0.792	0.862	1.077	0.977	0.904		0.922	
75%	0.832	0.984	0.850	0.704	0.963		0.867	
100%	0.785	0.891	0.720	0.705	0.792		0.779	9.46

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, (100%) 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, (100%) 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)= 100 % effluent
b) NOEC growth (parameter TPP6C)= 100 % effluent
c) Coefficient of variation (parameter TQP6C)= 11.8 %

6. Enter Whole Effluent Toxicity: 100 %

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING
Ceriodaphnia dubia SURVIVAL AND REPRODUCTION

Permittee: City of Hope

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	8-16-15, 0700	8-17-15, 0700
Sample #2:	8-18-15, 0700	8-19-15, 0700
Sample #3:	8-20-15, 0700	8-21-15, 0700

Test initiated (date, time): 8-18-15, 1140 Test terminated (date, time): 8-25-15, 0945

Dilution water used: Moderately Hard Synthetic

Ceriodaphnia dubia SURVIVAL AND REPRODUCTION
NUMBER OF YOUNG PRODUCED PER FEMALE @ TEST TERMINATION

PERCENT EFFLUENT

Replicate	0%	32%	42%	56%	75%	100%
A	10	15	15	10	10	9
B	9	13	13	8	9	17
C	19	8	20	17	14	17
D	13	18	9	9	21	19
E	24	17	10	17	11	10
F	16	8	19	12	10	21
G	17	22	8	8	8	19
H	11	11	13	21	16	13
I	X1	15	17	11	14	12
J	22	17	13	12	20	15
Mean	14.2	14.4	13.7	12.5	13.3	15.2
Mean/surviving female	15.7	14.4	13.7	12.5	13.3	15.2
CV%*	34.1					26.8

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 Hope

PERCENT SURVIVAL

PERCENT EFFLUENT	0%	32%	42%	56%	75%	100%
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, (100%): 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, (100%): 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)= 100 % effluent

b) NOEC reproduction (parameter TPP3B)= 100 % effluent

c) Coefficient of variation (parameter TQP3B)= 34.1 %

6. Enter Whole Effluent Toxicity: 100 %

APPENDIX A

Chain of Custody Forms

APPENDIX B

Effluent and Dilution Water Data

Biomonitoring Quality Control Benchsheet

Analyst	RH	RH	RH	RH	RH	TZ	TF	RH
Date	8-17-15	8-18-15	8-19-15	8-20-15	8-21-15	8/22/15	8-23-15	8-24-15
pH Meter ID	AR60							
LIN pH 4 Buffer	1500706							
LIN pH 7 Buffer	1500707							
LIN pH 10 Buffer	1500708							
Slope (>90%)	90.8%	96.8	94.7%	90.2%	98%	98.7	99.3	98.5

Dissolved O ₂ Meter	001305							
Meter Reading	8.71	8.62	8.66	8.71	8.68	8.65	8.68	8.67
Temp.	21	22	22	22	22	22	22	22
Chart Value at Temp.	8.743	8.743	8.743	8.743	8.743	8.743	8.743	8.743
Difference	<0.20	0.123	0.083	0.033	0.063	0.093	0.063	0.073
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	AR60							
Meter Reading	22	22	22	22	22	22	22	22
Thermometer Reading	22	22	21	21	22	22	22	22
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)	5mg/L		5mg/L					
STD Result	100		96					
T.V. / %REC	100/100%		100/96%					
Acceptance Criteria			93.5-108.5% Recovery					

Hardness								
Blank (<2mg/L)	2mg/L		2mg/L					
STD. Result	96		102					
T.V. / %REC	100/96%		100/102%					
Acceptance Criteria			90.0-105.5% Recovery					

Conductivity Meter ID	60102							
Blank (<1)	<1		1					
STD Result	1433		1433					
T.V. / %REC	1412/101%		1412/101%					
Acceptance Criteria			99.2-104.0% Recovery					

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

Revision 0
Effective Date 01APR15

Biomonitoring Quality Control Benchsheet

Analyst	RH	RH	RH	RH				
Date	8-25-15	8-26-15	8-27-15	8-28-15				
pH Meter ID	AR60							
LIN pH 4 Buffer	1590706							
LIN pH 7 Buffer	1590707							
LIN pH 10 Buffer	1590708							
Slope (>90%)	99.07	95.97						

Dissolved O ₂ Meter	DO 1305							
Meter Reading	8.72	8.79						
Temp.	22	21						
Chart Value at Temp.	8.743	8.915						
Difference	0.023	0.163						
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	AR60							
Meter Reading	22	21						
Thermometer Reading	22	22						
Thermometer ID	16							
Acceptance Criteria	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C

Alkalinity								
Blank (<5mg/L)	<5mg/L							
STD Result	106							
T.V. / %REC	100 / 106%							
Acceptance Criteria	93.5-108.5% Recovery							

Hardness								
Blank (<2mg/L)	<2mg/L							
STD. Result	92							
T.V. / %REC	100 / 92%							
Acceptance Criteria	90.0-105.5% Recovery							

Conductivity Meter ID	60102							
Blank (<1)	<1							
STD Result	1436							
T.V. / %REC	100 / 1436%							
Acceptance Criteria	99.2-104.0% Recovery							

Chlorine Meter ID	OR 830							
Blank (<0.05mg/L)	<0.05							
STD Result	0.21							
T.V. / % REC	0.21 / 100%							
Acceptance Criteria	100.0-120% Recovery							

Revision 0
Effective Date 01APR15

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Fathead Minnow

Lab # / Sample ID K1508006

Test Start (Date/Time) 8-18-15 1450

Client: Hope

Test End (Date/Time) 8-25-15 1445

Day of Test

		1	2	3	4	5	6	7	notes
Control	msd	8-18	8-19	8-20	8-21	8-22	8-23	8-24	
D.O. (mg/L)	INITIAL	8.6	8.6	8.9	8.8	8.7	8.6	8.8	
	FINAL	7.2	7.2	7.5	8.3	8.4	6.3	7.3	
pH (s.u.)	INITIAL	7.9	7.8	7.7	7.8	6.7	6.3	8.2	
	FINAL	6.3	7.3	7.2	7.4	8.5	8.8	8.4	
temp (C)	INITIAL	22	22	22	22	21	21	22	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		58							
HARDNESS (mg/L)		86							
CONDUCTIVITY (umhc)		422							
CHLORINE (mg/L)		0.05							
CONC:	32								
D.O. (mg/L)	INITIAL	8.7	8.4	8.6	8.6	9.0	8.7	8.8	
	FINAL	7.9	6.9	7.3	8.2	8.4	6.3	7.5	
pH (s.u.)	INITIAL	7.8	7.4	7.6	7.3	8.0	8.3	8.1	
	FINAL	6.5	7.4	7.1	7.5	8.5	8.1	8.4	
temp (C)	INITIAL	22	22	22	22	21	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	42								
D.O. (mg/L)	INITIAL	8.8	8.6	8.8	8.6	8.8	8.5	8.9	
	FINAL	7.1	7.3	7.3	8.2	8.3	6.8	7.5	
pH (mg/L)	INITIAL	7.6	7.3	7.5	7.5	8.0	8.2	8.1	
	FINAL	6.8	7.4	7.1	7.6	8.0	8.6	8.6	
temp (C)	INITIAL	22	22	22	22	22	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	56								
D.O. (mg/L)	INITIAL	8.7	8.4	8.9	8.9	8.7	8.4	9.0	
	FINAL	7.1	7.4	7.4	8.1	8.3	6.7	7.1	
pH (s.u.)	INITIAL	7.6	7.1	7.5	7.3	8.0	8.1	8.1	
	FINAL	6.7	7.5	7.0	7.6	8.5	8.5	8.2	
temp (C)	INITIAL	21	22	22	22	23	24	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	75								
D.O. (mg/L)	INITIAL	8.9	8.4	9.0	9.1	8.7	8.4	9.1	
	FINAL	7.3	7.3	7.5	8.1	8.5	6.8	7.8	
pH (s.u.)	INITIAL	7.9	7.1	7.9	7.2	7.9	8.0	8.0	
	FINAL	7.0	7.6	7.3	7.7	8.4	8.3	8.3	
temp (C)	INITIAL	21	23	22	21	23	25	23	
	FINAL	25	25	25	25	25	25	25	
CONC:	100								
D.O. (mg/L)	INITIAL	8.8	8.5	9.1	9.1	9.0	8.5	9.3	
	FINAL	7.5	7.5	7.2	8.1	8.3	7.0	8.1	
pH (s.u.)	INITIAL	7.3	7.1	7.5	7.1	7.3	8.4	7.6	
	FINAL	7.3	7.8	7.4	7.9	8.5	8.0	7.8	
temp (C)	INITIAL	21	23	22	21	25	25	23	
	FINAL	25	25	25	25	25	25	25	
CONC:	100 %	A	A	A	B	B	C	C	
ALKALINITY (mg/L)		160			172		204		
HARDNESS (mg/L)		42			42		42		
CONDUCTIVITY (umhc)		1020			1062		1060		
CHLORINE (mg/L)		0.05							

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Ceriodaphnia Dubia

Lab # / Sample ID K15 08006

Test Start (Date/Time) 8-18-15

1140

Client: Hope

Test End (Date/Time) 8-25-15

0945

Day of Test

		1	2	3	4	5	6	7	notes
Control	→ HS	8-18	8-19	8-20	8-21	8-22	8-23	8-24	
D.O. (mg/L)	INITIAL	8.6	8.6	8.9	8.8	8.7	8.6	8.9	
	FINAL	8.2	8.3	8.3	8.7	8.5	7.5	8.2	
pH (s.u.)	INITIAL	7.9	7.8	7.7	7.8	6.7	8.3	8.2	
	FINAL	7.2	7.4	7.5	7.2	7.6	7.8	7.6	
temp (C)	INITIAL	22	22	22	22	21	21	22	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		58							
HARDNESS (mg/L)		86							
CONDUCTIVITY (umhc)		422							
CHLORINE (mg/L)		20.05							
CONC:		32							
D.O. (mg/L)	INITIAL	8.7	8.4	8.6	8.6	9.0	8.7	8.8	
	FINAL	8.3	8.5	8.9	8.6	8.5	7.6	8.2	
pH (s.u.)	INITIAL	7.8	7.4	7.6	7.3	8.0	8.3	8.1	
	FINAL	7.3	7.0	7.3	7.4	7.5	7.7	7.7	
temp (C)	INITIAL	22	22	22	22	21	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:		42							
D.O. (mg/L)	INITIAL	8.8	8.6	8.8	8.6	8.8	8.5	8.9	
	FINAL	8.6	8.4	8.2	8.8	8.5	7.8	8.3	
pH (mg/L)	INITIAL	7.6	7.3	7.5	7.5	8.0	8.2	8.1	
	FINAL	7.3	7.2	7.4	7.6	7.5	7.8	7.5	
temp (C)	INITIAL	22	22	22	22	22	23	22	
	FINAL	25	25	25	25	25	25	25	
CONC:		56							
D.O. (mg/L)	INITIAL	8.7	8.4	8.9	8.9	8.7	8.4	9.0	
	FINAL	8.6	8.4	8.4	8.8	8.9	7.8	8.4	
pH (s.u.)	INITIAL	7.6	7.1	7.5	7.3	8.0	8.1	8.1	
	FINAL	7.4	7.4	7.4	7.7	7.5	7.4	7.4	
temp (C)	INITIAL	21	22	22	22	23	24	22	
	FINAL	25	25	25	25	25	25	25	
CONC:		75							
D.O. (mg/L)	INITIAL	8.4	8.4	9.0	9.1	8.7	8.4	9.1	
	FINAL	8.8	8.4	8.4	8.9	8.5	7.9	8.2	
pH (s.u.)	INITIAL	7.4	7.1	7.4	7.2	7.9	8.0	8.0	
	FINAL	7.4	7.7	7.5	7.9	7.5	7.7	7.2	
temp (C)	INITIAL	21	23	22	21	23	25	23	
	FINAL	25	25	25	25	25	25	25	
CONC:		100							
D.O. (mg/L)	INITIAL	8.8	8.5	9.1	9.1	9.0	7.5	9.3	
	FINAL	8.8	8.4	8.3	8.9	8.4	7.8	8.5	
pH (s.u.)	INITIAL	7.3	7.1	7.5	7.1	7.3	8.4	7.6	
	FINAL	7.4	8.0	7.7	8.0	7.3	8.1	7.0	
temp (C)	INITIAL	21	23	22	21	25	25	23	
	FINAL	25	25	25	25	25	25	25	
CONC:	100 %	A	A	A	B	B	C	C	
ALKALINITY (mg/L)		160			172		204		
HARDNESS (mg/L)		42			42		42		
CONDUCTIVITY (umhc)		1020			1062		1066		
CHLORINE (mg/L)		20.05							

APPENDIX C

Fathead minnow raw data and statistics

Pimephales promelas

FATHEAD MINNOW

SURVIVAL DATA FOR LARVAL SURVIVAL AND GROWTH TEST (CHRONIC)

LAB #: K1508006			TEST START		DATE	8/18/15	TIME	1450				
CLIENT: Hope			TEST END		DATE	8/25/15	TIME	1445				
ANALYST: RH/TT/HF			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	10	10	10	10	10	10	10	10	100%	98.0%	4.56
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	9	9	9	9	9	9	90%		
	D	10	10	10	10	10	10	10	10	100%		
	E	10	10	10	10	10	10	10	10	100%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	9	9	9	9	9	9	90%	96.0%	
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	10	10	10	10	10	10	100%		
	D	10	10	9	9	9	9	9	9	90%		
	E	10	10	10	10	10	10	10	10	100%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	9	9	9	9	9	9	90%	96.0%	
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	10	10	10	10	10	10	100%		
	D	10	10	10	10	10	10	10	10	100%		
	E	10	10	10	9	9	9	9	9	90%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	9	9	9	7	7	7	7	70%	92.0%	
	B	10	10	9	9	9	9	9	9	90%		
	C	10	10	10	10	10	10	10	10	100%		
	D	10	10	10	10	10	10	10	10	100%		
	E	10	10	10	10	10	10	10	10	100%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	10	10	10	10	10	10	100%	96.0%	
	B	10	10	10	10	10	10	10	10	100%		
	C	10	9	9	9	9	9	9	9	90%		
	D	10	10	10	10	9	9	9	9	90%		
	E	10	10	10	10	10	10	10	10	100%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	9	9	9	9	9	9	90%	98.0%	4.56
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	10	10	10	10	10	10	100%		
	D	10	10	10	10	10	10	10	10	100%		
	E	10	10	10	10	10	10	10	10	100%		
ANALYST:		RH	RH	RH	RH	TT	HF	RH	RH			
DATE:		8/18/15	8/19/15	8/20/15	8/21/15	8/22/15	8/23/15	8/24/15	8/25/15			
TIME:		1450	1415	1110	1030	1700	1630	1330	1445			

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

REMARKS:

AA# K1508006, FATHEAD MINNOW SURV.,CHRONIC, 8-18-15
File: C:\COPYTO~1\TOXSTAT\FHSURV~1. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.274

W = 0.838

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# K1508006, FATHEAD MINNOW SURV.,CHRONIC, 8-18-15
File: C:\COPYTO~1\TOXSTAT\FHSURV~1. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 5.52

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# K1508006, FATHEAD MINNOW SURV.,CHRONIC, 8-18-15
 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	1.4120
1	CONTROL	2	1.0000	1.4120
1	CONTROL	3	0.9000	1.2490
1	CONTROL	4	1.0000	1.4120
1	CONTROL	5	1.0000	1.4120
2	32 % EFFLUENT	1	0.9000	1.2490
2	32 % EFFLUENT	2	1.0000	1.4120
2	32 % EFFLUENT	3	1.0000	1.4120
2	32 % EFFLUENT	4	0.9000	1.2490
2	32 % EFFLUENT	5	1.0000	1.4120
3	42 % EFFLUENT	1	0.9000	1.2490
3	42 % EFFLUENT	2	1.0000	1.4120
3	42 % EFFLUENT	3	1.0000	1.4120
3	42 % EFFLUENT	4	1.0000	1.4120
3	42 % EFFLUENT	5	0.9000	1.2490
4	56 % EFFLUENT	1	0.7000	0.9912
4	56 % EFFLUENT	2	0.9000	1.2490
4	56 % EFFLUENT	3	1.0000	1.4120
4	56 % EFFLUENT	4	1.0000	1.4120
4	56 % EFFLUENT	5	1.0000	1.4120
5	75 % EFFLUENT	1	1.0000	1.4120
5	75 % EFFLUENT	2	1.0000	1.4120
5	75 % EFFLUENT	3	0.9000	1.2490
5	75 % EFFLUENT	4	0.9000	1.2490
5	75 % EFFLUENT	5	1.0000	1.4120
6	100 % EFFLUENT	1	0.9000	1.2490
6	100 % EFFLUENT	2	1.0000	1.4120
6	100 % EFFLUENT	3	1.0000	1.4120
6	100 % EFFLUENT	4	1.0000	1.4120
6	100 % EFFLUENT	5	1.0000	1.4120

AA# K1508006, FATHEAD MINNOW SURV.,CHRONIC, 8-18-15
 File: C:\COPYTO~1\TOXSTAT\FHSURV~1. Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.024	0.005	0.417
Within (Error)	24	0.274	0.011	
Total	29	0.297		

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

AA# K1508006, FATHEAD MINNOW SURV., CHRONIC, 8-18-15

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

Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST

TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	1.379	0.980		
2	32 % EFFLUENT	1.347	0.960	0.483	
3	42 % EFFLUENT	1.347	0.960	0.483	
4	56 % EFFLUENT	1.295	0.920	1.246	
5	75 % EFFLUENT	1.347	0.960	0.483	
6	100 % EFFLUENT	1.379	0.980	0.000	

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

AA# K1508006, FATHEAD MINNOW SURV., CHRONIC, 8-18-15

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

Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST

TABLE 2 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	5			
2	32 % EFFLUENT	5	0.082	8.4	0.020
3	42 % EFFLUENT	5	0.082	8.4	0.020
4	56 % EFFLUENT	5	0.082	8.4	0.060
5	75 % EFFLUENT	5	0.082	8.4	0.020
6	100 % EFFLUENT	5	0.082	8.4	0.000

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:		K1508006		TEST DATES (BEGIN / END):		8/18/15 - 8/25/15	
CLIENT:		Hope		WEIGHING DATE / TIME:		8/26/2015 1430	
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	1.01042	1.00115	0.00927	10	0.927	AVG DRY WEIGHT (mg)
	B	1.00930	1.00096	0.00834	10	0.834	
MHS	C	1.01413	1.00699	0.00714	10	0.714	0.842
	D	1.00427	0.99643	0.00784	10	0.784	CV
	E	1.01186	1.00233	0.00953	10	0.953	
CONC:	A	0.99128	0.98522	0.00606	10	0.606	AVG DRY WEIGHT (mg)
	B	1.01644	1.00930	0.00714	10	0.714	
32%	C	1.01036	1.00170	0.00866	10	0.866	0.775
	D	0.99670	0.98981	0.00689	10	0.689	CV
	E	1.02121	1.01123	0.00998	10	0.998	
CONC:	A	1.01906	1.00978	0.00928	10	0.928	AVG DRY WEIGHT (mg)
	B	1.00932	1.00027	0.00905	10	0.905	
42%	C	1.02300	1.01488	0.00812	10	0.812	0.882
	D	1.00855	1.00022	0.00833	10	0.833	CV
	E	0.99558	0.98628	0.00930	10	0.930	
CONC:	A	1.01405	1.00613	0.00792	10	0.792	AVG DRY WEIGHT (mg)
	B	1.01198	1.00336	0.00862	10	0.862	
56%	C	1.03197	1.02120	0.01077	10	1.077	0.922
	D	1.01029	1.00052	0.00977	10	0.977	CV
	E	1.02062	1.01158	0.00904	10	0.904	
CONC:	A	1.02012	1.01180	0.00832	10	0.832	AVG DRY WEIGHT (mg)
	B	1.03593	1.02609	0.00984	10	0.984	
75%	C	1.00812	0.99962	0.00850	10	0.850	0.867
	D	1.01959	1.01255	0.00704	10	0.704	CV
	E	1.02556	1.01593	0.00963	10	0.963	
CONC:	A	1.01856	1.01071	0.00785	10	0.785	AVG DRY WEIGHT (mg)
	B	1.02073	1.01182	0.00891	10	0.891	
100%	C	0.99855	0.99135	0.00720	10	0.720	0.779
	D	1.01564	1.00859	0.00705	10	0.705	CV
	E	1.01298	1.00506	0.00792	10	0.792	

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA# K1508006, FATHEAD MINNOW GROWTH CHRONIC, 8-18-15

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

Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.270

W = 0.976

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# K1508006, FATHEAD MINNOW GROWTH CHRONIC, 8-18-15

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

Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 4.37

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# K1508006, FATHEAD MINNOW GROWTH CHRONIC, 8-18-15
 FILE: C:\COPYTO~1\TOXSTAT\FHGROWTH.
 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.9270	0.9270
1	CONTROL	2	0.8340	0.8340
1	CONTROL	3	0.7140	0.7140
1	CONTROL	4	0.7840	0.7840
1	CONTROL	5	0.9530	0.9530
2	32 % EFFLUENT	1	0.6060	0.6060
2	32 % EFFLUENT	2	0.7140	0.7140
2	32 % EFFLUENT	3	0.8660	0.8660
2	32 % EFFLUENT	4	0.6890	0.6890
2	32 % EFFLUENT	5	0.9980	0.9980
3	42 % EFFLUENT	1	0.9280	0.9280
3	42 % EFFLUENT	2	0.9050	0.9050
3	42 % EFFLUENT	3	0.8120	0.8120
3	42 % EFFLUENT	4	0.8330	0.8330
3	42 % EFFLUENT	5	0.9300	0.9300
4	56 % EFFLUENT	1	0.7920	0.7920
4	56 % EFFLUENT	2	0.8620	0.8620
4	56 % EFFLUENT	3	1.0770	1.0770
4	56 % EFFLUENT	4	0.9770	0.9770
4	56 % EFFLUENT	5	0.9040	0.9040
5	75 % EFFLUENT	1	0.8320	0.8320
5	75 % EFFLUENT	2	0.9840	0.9840
5	75 % EFFLUENT	3	0.8500	0.8500
5	75 % EFFLUENT	4	0.7040	0.7040
5	75 % EFFLUENT	5	0.9630	0.9630
6	100 % EFFLUENT	1	0.7850	0.7850
6	100 % EFFLUENT	2	0.8910	0.8910
6	100 % EFFLUENT	3	0.7200	0.7200
6	100 % EFFLUENT	4	0.7050	0.7050
6	100 % EFFLUENT	5	0.7920	0.7920

AA# K1508006, FATHEAD MINNOW GROWTH CHRONIC, 8-18-15
 File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.086	0.017	1.527
Within (Error)	24	0.270	0.011	
Total	29	0.356		

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

AA# K1508006, FATHEAD MINNOW GROWTH CHRONIC, 8-18-15

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	0.842	0.842		
2	32 % EFFLUENT	0.775	0.775	1.011	
3	42 % EFFLUENT	0.882	0.882	-0.585	
4	56 % EFFLUENT	0.922	0.922	-1.193	
5	75 % EFFLUENT	0.867	0.867	-0.361	
6	100 % EFFLUENT	0.779	0.779	0.951	

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

AA# K1508006, FATHEAD MINNOW GROWTH CHRONIC, 8-18-15

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	32 % EFFLUENT	5	0.158	18.8	0.068
3	42 % EFFLUENT	5	0.158	18.8	-0.039
4	56 % EFFLUENT	5	0.158	18.8	-0.080
5	75 % EFFLUENT	5	0.158	18.8	-0.024
6	100 % EFFLUENT	5	0.158	18.8	0.064

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

SURVIVAL AND REPRODUCTION TEST

Ceriodaphnia dubia

Discharger: Hope AFIN # 29-00034

Location: Outfall 001

Date Sample Collected: 8 - 17/19/21- 15

Lab Number/s

K1508006

Analyst: RH

Test Start - Date/Time: 8-18-15, 1140

Test Stop - Date/Time: 8-25-15, 0945

Conc 1		Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	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
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	3	1	0	0	0	0	0	0	0	0	0	1	10	0.1	RH
	4	4	1	2	3	3	2	0	0	1	0	16	10	1.6	RH
	5	4	2	0	6	7	6	2	7	0	4	38	10	3.8	RH
	6	1	0	10	2	4	3	6	0	X	8	34	10	3.4	RH
	7	0	6	7	2	10	5	9	4		10	53	10	5.3	RH
	8														
Total		10	9	19	13	24	16	17	11	1	22	142		Avg. = 15.7	
										X				C.V. = 34.1	

Conc 4		Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
56%	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
	3	0	0	1	0	0	0	0	0	0	0	1	10	0.1	RH
	4	0	2	5	3	3	2	0	5	2	0	22	10	2.2	RH
	5	5	0	8	1	2	3	0	1	0	2	22	10	2.2	RH
	6	5	2	3	5	6	7	0	10	2	6	46	10	4.6	RH
	7	0	4	0	0	6	0	8	5	7	4	34	10	3.4	RH
	8												10	0.0	
Total		10	8	17	9	17	12	8	21	11	12	125		Avg. = 12.5	
														C.V. = 35.2	

Conc 2		Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
32%	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
	3	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	4	0	1	2	4	5	0	1	2	3	5	23	10	2.3	RH
	5	5	6	1	0	7	2	4	7	5	1	38	10	3.8	RH
	6	5	6	5	12	0	6	8	0	7	5	54	10	5.4	RH
	7	5	0	0	2	5	0	9	2	0	6	29	10	2.9	RH
	8														
Total		15	13	8	18	17	8	22	11	15	17	144		Avg. = 14.4	
														C.V. = 31.1	

Conc 5		Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
75%	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
	3	0	0	0	0	0	0	1	0	0	0	1	10	0.1	RH
	4	4	0	1	0	3	1	0	1	0	5	15	10	1.5	RH
	5	6	0	6	6	2	3	7	8	0	1	39	10	3.9	RH
	6	0	3	7	12	6	6	0	5	9	10	58	10	5.8	RH
	7	0	6	0	3	0	0	0	2	5	4	20	10	2.0	RH
	8												10	0.0	RH
Total		10	9	14	21	11	10	8	16	14	20	133		Avg. = 13.3	
														C.V. = 34.2	

Conc 3		Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
42%	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
	3	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	4	0	0	2	0	4	0	1	1	0	0	8	10	0.8	RH
	5	0	6	8	2	0	3	0	7	8	0	34	10	3.4	RH
	6	11	7	8	5	6	8	7	5	0	7	64	10	6.4	RH
	7	4	0	2	2	0	8	0	0	9	6	31	10	3.1	RH
	8														
Total		15	13	20	9	10	19	8	13	17	13	137		Avg. = 13.7	
														C.V. = 29.8	

Conc 6		Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
100%	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
	3	0	0	0	0	0	0	0	1	0	0	1	10	0.1	RH
	4	0	1	2	4	2	5	2	3	0	0	19	10	1.9	RH
	5	7	8	0	1	0	7	0	1	5	9	38	10	3.8	RH
	6	1	3	9	7	8	1	10	8	2	6	55	10	5.5	RH
	7	1	5	6	7	0	8	7	0	5	0	39	10	3.9	RH
	8												10	0.0	RH
Total		9	17	17	19	10	21	19	13	12	15	152		Avg. = 15.2	
														C.V. = 26.8	

AA # K1508006, C.DUBIA CHRONIC, REPRODUCCION, 8-18-15

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 # K1508006, C.DUBIA CHRONIC, REPRODUCCION, 8-18-15

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

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 3.86

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

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

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

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	1	9	10
32	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
42	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
56	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
75	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
100	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	32	10	0	
2	42	10	0	

3	56	10	0
4	75	10	0
5	100	10	0

TITLE: AA # K1508006, C.DUBIA CHRONIC, REPRODUCCION, 8-18-15
 FILE: C:\COPYTO~1\TOXSTAT\C.DUB
 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	10.0000	10.0000
1	CONTROL	2	9.0000	9.0000
1	CONTROL	3	19.0000	19.0000
1	CONTROL	4	13.0000	13.0000
1	CONTROL	5	24.0000	24.0000
1	CONTROL	6	16.0000	16.0000
1	CONTROL	7	17.0000	17.0000
1	CONTROL	8	11.0000	11.0000
1	CONTROL	9	1.0000	1.0000
1	CONTROL	10	22.0000	22.0000
2	32 % EFFLUENT	1	15.0000	15.0000
2	32 % EFFLUENT	2	13.0000	13.0000
2	32 % EFFLUENT	3	8.0000	8.0000
2	32 % EFFLUENT	4	18.0000	18.0000
2	32 % EFFLUENT	5	17.0000	17.0000
2	32 % EFFLUENT	6	8.0000	8.0000
2	32 % EFFLUENT	7	22.0000	22.0000
2	32 % EFFLUENT	8	11.0000	11.0000
2	32 % EFFLUENT	9	15.0000	15.0000
2	32 % EFFLUENT	10	17.0000	17.0000
3	42 % EFFLUENT	1	15.0000	15.0000
3	42 % EFFLUENT	2	13.0000	13.0000
3	42 % EFFLUENT	3	20.0000	20.0000
3	42 % EFFLUENT	4	9.0000	9.0000
3	42 % EFFLUENT	5	10.0000	10.0000
3	42 % EFFLUENT	6	19.0000	19.0000
3	42 % EFFLUENT	7	8.0000	8.0000
3	42 % EFFLUENT	8	13.0000	13.0000
3	42 % EFFLUENT	9	17.0000	17.0000
3	42 % EFFLUENT	10	13.0000	13.0000
4	56 % EFFLUENT	1	10.0000	10.0000
4	56 % EFFLUENT	2	8.0000	8.0000
4	56 % EFFLUENT	3	17.0000	17.0000
4	56 % EFFLUENT	4	9.0000	9.0000
4	56 % EFFLUENT	5	17.0000	17.0000
4	56 % EFFLUENT	6	12.0000	12.0000
4	56 % EFFLUENT	7	8.0000	8.0000
4	56 % EFFLUENT	8	21.0000	21.0000
4	56 % EFFLUENT	9	11.0000	11.0000
4	56 % EFFLUENT	10	12.0000	12.0000
5	75 % EFFLUENT	1	10.0000	10.0000
5	75 % EFFLUENT	2	9.0000	9.0000
5	75 % EFFLUENT	3	14.0000	14.0000

5	75 %	EFFLUENT	4	21.0000	21.0000
5	75 %	EFFLUENT	5	11.0000	11.0000
5	75 %	EFFLUENT	6	10.0000	10.0000
5	75 %	EFFLUENT	7	8.0000	8.0000
5	75 %	EFFLUENT	8	16.0000	16.0000
5	75 %	EFFLUENT	9	14.0000	14.0000
5	75 %	EFFLUENT	10	20.0000	20.0000
6	100 %	EFFLUENT	1	9.0000	9.0000
6	100 %	EFFLUENT	2	17.0000	17.0000
6	100 %	EFFLUENT	3	17.0000	17.0000
6	100 %	EFFLUENT	4	19.0000	19.0000
6	100 %	EFFLUENT	5	10.0000	10.0000
6	100 %	EFFLUENT	6	21.0000	21.0000
6	100 %	EFFLUENT	7	19.0000	19.0000
6	100 %	EFFLUENT	8	13.0000	13.0000
6	100 %	EFFLUENT	9	12.0000	12.0000
6	100 %	EFFLUENT	10	15.0000	15.0000

AA # K1508006, C.DUBIA CHRONIC, REPRODUCCION, 8-18-15
 File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	43.883	8.777	0.375
Within (Error)	54	1262.300	23.376	
Total	59	1306.183		

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

AA # K1508006, C.DUBIA CHRONIC, REPRODUCCION, 8-18-15
 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	14.200	14.200		
2	32 % EFFLUENT	14.400	14.400	-0.092	
3	42 % EFFLUENT	13.700	13.700	0.231	
4	56 % EFFLUENT	12.500	12.500	0.786	
5	75 % EFFLUENT	13.300	13.300	0.416	
6	100 % EFFLUENT	15.200	15.200	-0.462	

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

AA # K1508006, C.DUBIA CHRONIC, REPRODUCCION, 8-18-15

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	32 % EFFLUENT	10	4.995	35.2	-0.200
3	42 % EFFLUENT	10	4.995	35.2	0.500
4	56 % EFFLUENT	10	4.995	35.2	1.700
5	75 % EFFLUENT	10	4.995	35.2	0.900
6	100 % EFFLUENT	10	4.995	35.2	-1.000

APPENDIX E

Organism History

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

TEST ORGANISM HISTORY

DATE SHIPPED 8/18/15 CLIENT ARK ANALYTICAL

Purchase Order #: _____

SPECIES: Pimephales promelas

Quantity Shipped: 300⁺ CST
15-1600

Age: HATCHED 8/16/15

Brood Stock Source: Anderson Farms, AR

Culture Water: Groundwater

Hardness (Mg/l CaCO₃): 160

Dissolved Oxygen (Mg/l): 8.5

Temperature (°C): 25.1

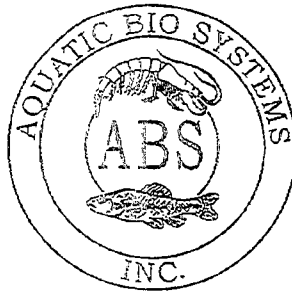
Feeding: ARTEMIA

Comments: _____

Shipped Via: Federal Express UPS Overnight Shuttle

Packaged By: _____

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



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

ORGANISM HISTORY

DATE: 11/25/2013

SPECIES: Ceriodaphnia dubia

AGE: > 3 day

LIFE STAGE: Adult


HATCH DATE: Variable

BEGAN FEEDING: Immediately

FOOD: YTC, Selenastrum sp.

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

Comments:

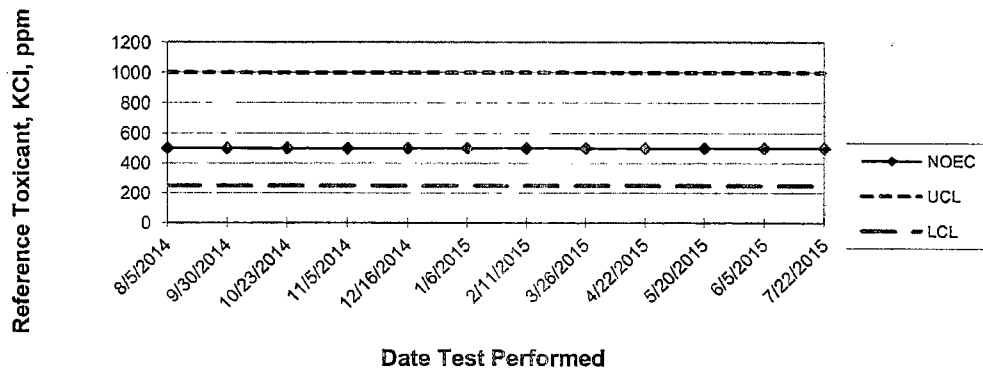


Facility Supervisor

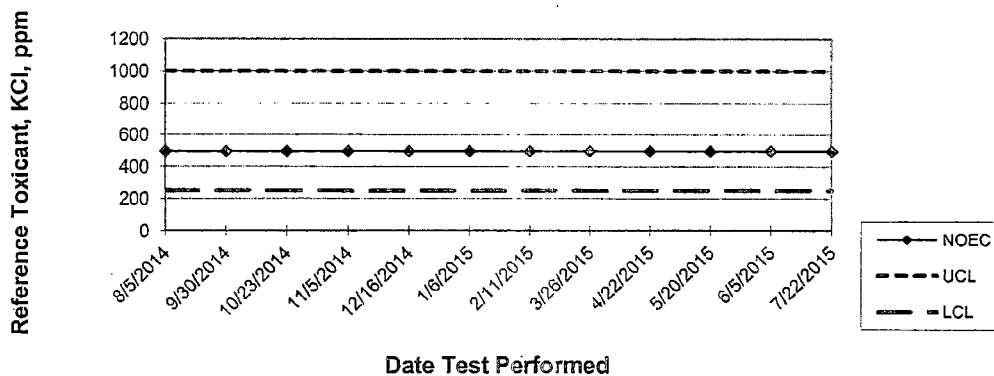
APPENDIX F

Quality Assurance Charts

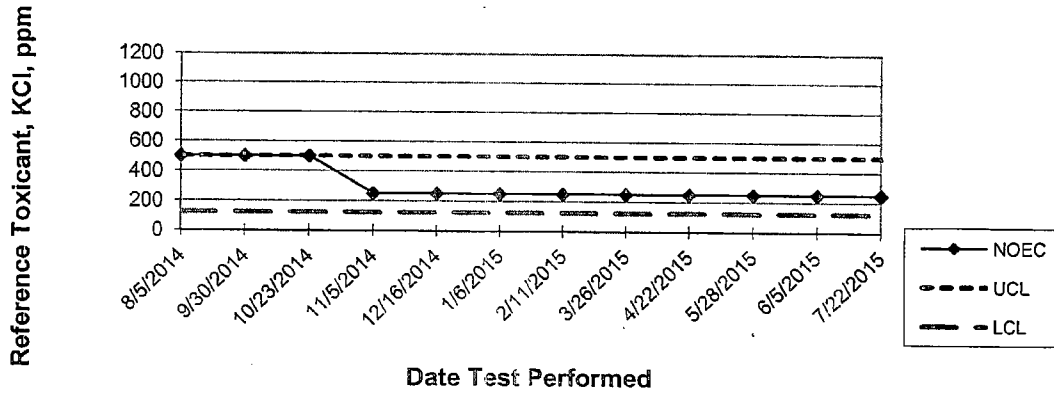
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW SURVIVAL 7 Day
QUALITY ASSURANCE



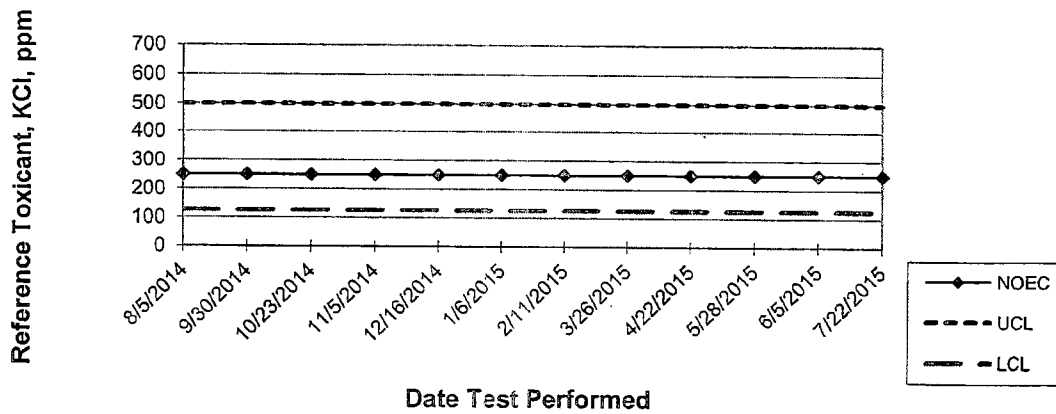
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW GROWTH 7 Day
QUALITY ASSURANCE



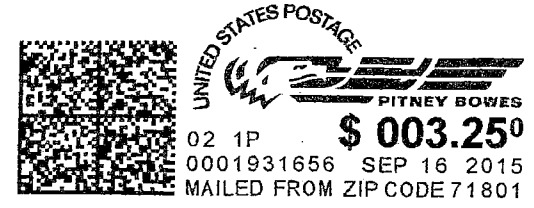
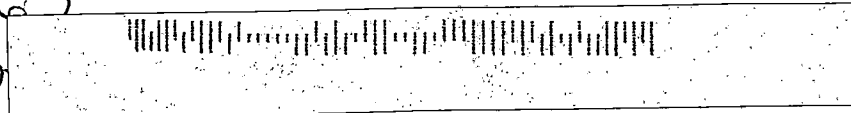
ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE



City of Hope
PO Box 617
Hope, AR 7



APED
NPPES Enforcement Section
5301 - Northshore Drive
N. Little Rock, AR
72218-5317