

Arkansas Analytical, Inc.

Toxicity Test Results

City of Hope
Permit Number: AR0038466
AFIN # 29-00034
Fourth 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: **Bobby Arney**
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 K1511006

Thursday, December 3, 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 fourth 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:	11-15-15, 0600	11-16-15, 0600
Sample #2:	11-17-15, 0600	11-18-15, 0600
Sample #3:	11-19-15, 0600	11-20-15, 0600

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:	11-16-15, 1007	1
Sample #2:	11-18-15, 1602	4
Sample #3:	11-20-15, 0945	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 < 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	100%	X	
Average of 15 or more young per surviving female	15.6	X	
At least 60% of surviving females should have produced 3 broods	70%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	29.2%	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	0.588	X	
The percent coefficient of variation between replicates must be 40% or less for growth	14.1%	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> 11/10/15 – 11/17/15		<i>Pimephales promelas</i> 11/10/15 – 11/17/15	
NOEC Survival:	250 ppm KCl	NOEC Survival:	500 ppm KCl
LOEC Survival:	500 ppm KCl	LOEC Survival:	1000 ppm KCl
NOEC Reproduction:	250 ppm KCl	NOEC Growth:	500 ppm KCl
LOEC Reproduction:	500 ppm KCl	LOEC Growth:	1000 ppm KCl

Quality Assurance charts are provided in Appendix 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	0.655
		%CV growth (critical dilution)	15.9%
PMSD Reproduction	32.4%	PMSD Growth	20.3%

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.

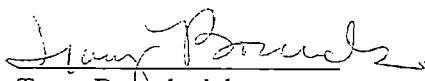
Chronic static renewal survival and reproduction test using *Ceriodaphnia dubia*, (Method 1002.0)

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 / Shannon Turney / 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:	11-15-15, 0600	11-16-15, 0600
Sample #2:	11-17-15, 0600	11-18-15, 0600
Sample #3:	11-19-15, 0600	11-20-15, 0600

Test initiated (date, time): 11-17-15, 1330 Test terminated (date, time): 11-24-15, 1610

Dilution water used: Moderately Hard Synthetic

DATA TABLE FOR FATHEAD MINNOW SURVIVAL

Effluent Conc %	Percent Survival in Replicate Chambers						Mean Percent Survival			
	A	B	C	D	E		24 hours	48 hours	7 days	CV %
0%	100	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%	90	100	90	90	100		100	98	94	
75%	100	100	90	90	100		100	100	96	
100%	100	90	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%	0.678	0.567	0.597	0.460	0.639		0.588	14.1
32%	0.664	0.656	0.752	0.602	0.647		0.664	
42%	0.657	0.713	0.541	0.650	0.602		0.633	
56%	0.502	0.812	0.653	0.651	0.672		0.658	
75%	0.712	0.659	0.634	0.613	0.686		0.661	
100%	0.620	0.515	0.778	0.624	0.736		0.655	15.9

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)= 15.9 %

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:	11-15-15, 0600	11-16-15, 0600
Sample #2:	11-17-15, 0600	11-18-15, 0600
Sample #3:	11-19-15, 0600	11-20-15, 0600

Test initiated (date, time): 11-17-15, 1100 Test terminated (date, time): 11-24-15, 1030

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	17	11	10	18	13
B	15	13	9	14	16	20
C	15	9	12	17	11	11
D	15	9	8	13	20	20
E	20	17	12	11	17	13
F	9	19	11	X0	9	21
G	11	22	15	21	20	9
H	19	X2	17	18	20	14
I	21	18	18	7	18	16
J	21	13	11	16	7	15
Mean	15.6	14.0	12.4	12.7	15.6	15.2
Mean/surviving female	15.6	14.0	12.4	14.1	15.6	15.2
CV%*	29.2					26.6

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	90	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

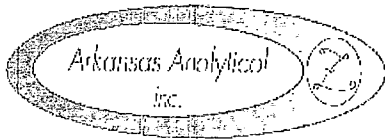
c) Coefficient of variation (parameter TQP3B)= 29.2 %

6. Enter Whole Effluent Toxicity: 100 %

APPENDIX A

Chain of Custody Forms





8100 National Dr.
 Little Rock, AR 72209
 PHONE: 501-455-3233
 FAX: 501-455-6118

CHAIN OF CUSTODY RECORD

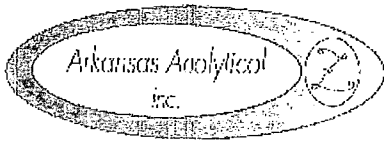
CLIENT INFORMATION		BILLING INFORMATION		Project Description		Turnaround Time		Preservation Codes:											
City of Hope		City of Hope		West Plant Toxicity Sample		1 Day (100%)		1. Cool, 4 Degrees Centigrade					4. Thio sulfate for Dechlorination						
1603 West 3rd St.		P.O. Box 667				2 Day (50%)		2. Sulfuric Acid (H ₂ SO ₄), pH < 2					5. Hydrochloric Acid(HCl)						
Hope, AR 71801		Hope, AR 71801		Reporting Information		3 Day (25%)		3. Nitric Acid (HNO ₃), pH < 2					6. Sodium Hydroxide (NaOH), pH > 12						
Attn: Bobby Arney				Telephone: 870-722-8644		5 Day (Initial)		TEST PARAMETERS										Bottle Size Code	
				Fax: 870-722-2511		Preservative Code: 1												G= Glass; P= Plastic	
				Email: wmalh@hmcnetkansas.net		Bottle Type: P												W= Sealed; A= Amber	
Sampler(s) Signature				Sampler(s) Printed												Arkansas Analytical Work Order Number:			
Field Number		SAMPLE COLLECTION Date/s Time/s		Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION						Chronic Toxicity	K1511006				
		11-15-15 / 11-16-15			X	6	Water	Final Effluent Composite - Day						X	A				
1. Relinquished by: (Signature)		Date/Time		2. Received by: (Signature)		SAMPLE CONDITION UPON RECEPTION						REMARKS / SAMPLE COMMENTS							
		11-16-15 10:07				1. CUSTODY SEALS: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No													
3. Relinquished by: (Signature)		Date/Time		4. Received by: (Signature)		2. CONTAINERS CORRECT: <input type="checkbox"/> Yes <input type="checkbox"/> No													
				Sydney James		3. COC/LABELS AGREE: <input type="checkbox"/> Yes <input type="checkbox"/> No													
						4. RECEIVED ON ICE: <input type="checkbox"/> Yes <input type="checkbox"/> No													
						5. TEMPERATURE ON RECEIPT: 1°C													
						6. TEMPERATURE GUID: HHT #2													
Revision: 2		2/14/14		FOR COMPLETION BY LAB ONLY															



8100 National Dr.
 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:									
City of Hope		City of Hope		West Plant Toxicity Sample		1 Day (100%)		1. Cool, 4 Degrees Centigrade				4. Thio sulfate for Dechlorination					
1603 West 3rd St.		P.O. Box 667				2 Day (50%)		2. Sulfuric Acid (H ₂ SO ₄), pH < 2				5. Hydrochloric Acid (HCl)					
Hope, AR 71801		Hope, AR 71801		Reporting Information		3 Day (25%)		3. Nitric Acid (HNO ₃), pH < 2				6. Sodium Hydroxide (NaOH), pH > 12					
Attn: Bobby Arney				Telephone: 870-722-8644		5 Day (Inactivated)		TEST PARAMETERS								Bottle Urea Code	
				Fax: 870-722-2511		Preservative Code: 1										G= Glass; P= Plastic	
				Email: wslab@honnearkansas.net		Bottle Type: P		V= Serum; A= Amber		Arkansas Analytical Work Order Number: K1511006 B							
Sampler(s) Signature		Sampler(s) Printed															
Field Number	SAMPLE COLLECTION		Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/DESCRIPTION		Chronic Toxicity								
	11-17-15	6 AM			6	Water	Final Effluent Composite - Day 2		X								
	11-18-15	6 AM		X													
1. Relinquished by: (Signature)		Date/Time		2. Received by: (Signature)		SAMPLE CONDITION UPON RECEIPT IN LAB				REMARKS / SAMPLE COMMENTS							
		11-18-15 12:50				1. CUSTODY SEALS: Yes No											
						2. CONTAINERS CORRECT: Yes No											
						3. COC/LABELS AGREE: Yes No											
3. Relinquished by: (Signature)		Date/Time		4. Received by lab: (Signature)		4. RECEIVED ON ICE: Yes No											
		11-18-15 1602				5. TEMPERATURE ON RECEIPT: 4°C											
						6. TEMPERATURE GUID: HHT#2											
Revision 2 2/14/14						FOR COMPLETION BY LAB ONLY											



8100 National Dr.
 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:										
City of Hope		City of Hope		West Plant Toxicity Sample		1 Day (100%)	1. Cool, 4 Degrees Centigrade			4. Thiosulfate for Dechlorination							
1603 West 3rd St.		P.O. Box 667				2 Day (50%)	2. Sulfuric Acid (H ₂ SO ₄), pH < 2			5. Hydrochloric Acid(HCl)							
Hope, AR 71801		Hope, AR 71801		Reporting Information		3 Day (25%)	3. Nitric Acid (HNO ₃), pH < 2			6. Sodium Hydroxide (NaOH), pH > 12							
Attn: Bobby Arney				Telephone: 870-722-8644		5 Day (Routine)	TEST PARAMETERS										Bottle Type Code
				Fax: 870-722-2511		Preservative Code: 1											G= Glass; P= Plastic
				Email: wwwlab@hannarkansas.net		Bottle Type: P											Me: Septom; A= Amber
Sampler(s) Signature		Sampler(s) Printed												Arkansas Analytical Work Order Number: K1511006 C			
Field Number	SAMPLE COLLECTION Date/s Time/s		Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION DESCRIPTION					Chronic Toxicity					
	11-19-15 11-20-15 6A-6A			X	6	Water	Final Effluent Composite - Day 3					X					
1. Relinquished by: (Signature)		Date/Time	2. Received by: (Signature)		SAMPLE CONDITION FROM RECEIPT IN LAB					REMARKS / SAMPLE COMMENTS							
		11-20-15 9:45			1. CUSTODY SEALS: Yes No												
					2. CONTAINERS CORRECT: Yes No												
					3. COC/LABELS AGREE: Yes No												
3. Relinquished by: (Signature)		Date/Time	4. Received by Lab: (Signature)		4. RECEIVED ON ICE: Yes No												
			Sydney James		5. TEMPERATURE ON RECEIPT: 10												
					6. TEMPERATURE GUN ID: 177 #2												
					EQB COMPLETION BY LAB/AILY												

APPENDIX B

Effluent and Dilution Water Data



CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Fathead Minnow

Lab # / Sample ID *K15 11006*

Test Start (Date/Time)

11-17-15 1330

Client: *Hope*

Test End (Date/Time)

11-24-15 1610

Day of Test

		1	2	3	4	5	6	7	notes
Control	<i>MHS</i>	<i>11-17</i>	<i>11-18</i>	<i>11-19</i>	<i>11-20</i>	<i>11-21</i>	<i>11-22</i>	<i>11-23</i>	<i>MHS 792</i>
D.O. (mg/L)	INITIAL	8.9	8.6	8.7	8.6	8.7	8.8	8.0	
	FINAL	7.5	7.9	7.5	7.3	8.1	7.4	7.0	
pH (s.u.)	INITIAL	7.9	7.8	7.9	7.5	7.7	7.1	7.5	
	FINAL	7.5	7.1	7.3	7.4	7.4	7.4	6.9	
temp (C)	INITIAL	22	24	22	21.4	20.8	20.4	23.8	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		112							
HARDNESS (mg/L)		52							
CONDUCTIVITY (umhd)		480							
CHLORINE (mg/L)		40.05							
CONC:	<i>32</i>								
D.O. (mg/L)	INITIAL	8.9	8.8	8.9	8.6	9.1	9.0	8.7	
	FINAL	7.9	8.0	7.6	7.8	9.0	8.0	7.2	
pH (s.u.)	INITIAL	7.3	8.0	7.5	7.4	7.6	7.2	7.5	
	FINAL	7.7	7.3	7.4	7.4	7.5	7.3	6.9	
temp (C)	INITIAL	22	24	22	22.2	22.1	22.9	23.8	
	FINAL	25	25	25	25	25	25	25	
CONC:	<i>42</i>								
D.O. (mg/L)	INITIAL	8.8	8.9	8.8	8.6	9.1	9.1	8.6	
	FINAL	7.6	7.8	7.9	7.2	7.7	7.7	7.5	
pH (mg/L)	INITIAL	7.3	8.1	7.3	7.4	7.5	7.2	7.6	
	FINAL	7.7	7.6	7.6	7.5	7.6	7.3	7.0	
temp (C)	INITIAL	22	23	22	22.5	23.0	23.3	23.7	
	FINAL	25	25	25	25	25	25	25	
CONC:	<i>56</i>								
D.O. (mg/L)	INITIAL	9.0	9.1	8.8	8.6	9.0	9.1	8.3	
	FINAL	7.8	7.1	7.8	7.2	7.7	7.9	7.5	
pH (s.u.)	INITIAL	7.4	8.0	7.3	7.5	7.6	7.5	7.5	
	FINAL	7.9	7.6	7.7	7.6	7.7	7.4	7.1	
temp (C)	INITIAL	22	23	22	23	23.0	23.7	23.9	
	FINAL	25	25	25	25	25	25	25	
CONC:	<i>75</i>								
D.O. (mg/L)	INITIAL	9.2	9.2	8.9	8.6	9.0	9.1	9.3	
	FINAL	7.9	7.1	7.4	7.2	7.8	7.8	6.8	
pH (s.u.)	INITIAL	7.5	8.1	7.2	7.6	7.6	7.5	7.5	
	FINAL	7.9	7.8	7.8	7.6	7.9	7.4	7.0	
temp (C)	INITIAL	23	21	22	22.8	23.0	23.42	23.8	23.8
	FINAL	25	25	25	25	25	25	25	
CONC:	<i>100</i>								
D.O. (mg/L)	INITIAL	9.1	9.1	9.2	8.7	9.2	9.7	9.4	
	FINAL	9.1	7.5	7.5	7.3	7.8	7.8	6.8	
pH (s.u.)	INITIAL	7.5	8.0	7.1	7.6	7.6	7.5	7.5	
	FINAL	8.0	8.0	8.0	7.7	7.9	7.6	7.1	
temp (C)	INITIAL	23	21	22	22.6	25.0	25.4	24.1	
	FINAL	25	25	25	25	25	25	25	
CONC:	<i>100 %</i>	<i>A</i>	<i>A</i>	<i>A</i>	<i>B</i>	<i>B</i>	<i>C</i>	<i>C</i>	
ALKALINITY (mg/L)		112			34		64		
HARDNESS (mg/L)		52			20		48		
CONDUCTIVITY (umhd)		703			148		496		
CHLORINE (mg/L)		40.05			40.05		40.05		

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Ceriodaphnia Dubia

Lab # / Sample ID: K1511006

Test Start (Date/Time)

11-17-15

1100

Client: Hope

Test End (Date/Time)

11-24-15

1030

Day of Test

		1	2	3	4	5	6	7	notes
Control	WHS	11-17	11-18	11-19	11-20	11-21	11-22	11-23	WHS 792
D.O. (mg/L)	INITIAL	8.4	8.6	8.7	8.6	8.7	8.3	8.6	
	FINAL	8.4	8.2	8.1	8.1	8.5	8.0	7.8	
pH (s.u.)	INITIAL	7.9	7.8	7.9	7.5	7.7	7.1	7.5	
	FINAL	7.9	7.7	7.4	7.3	7.8	7.7	7.9	
temp (C)	INITIAL	22	24	22	22	20.4	20.4	23.8	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		62							
HARDNESS (mg/L)		42							
CONDUCTIVITY (umhc)		485							
CHLORINE (mg/L)		0.05							
CONC: 32									
D.O. (mg/L)	INITIAL	8.9	8.8	8.9	8.6	8.9	9.3	9.0	
	FINAL	8.4	8.5	9.2	9.0	8.5	8.3	8.1	
pH (s.u.)	INITIAL	7.3	8.0	7.5	7.4	7.6	7.1	7.3	
	FINAL	7.6	7.9	7.6	7.5	7.5	7.7	7.9	
temp (C)	INITIAL	22	24	22	22.2	22.1	22.3	23.8	
	FINAL	25	25	25	25	25	25	25	
CONC: 42									
D.O. (mg/L)	INITIAL	8.8	8.9	8.8	8.6	9.2	9.4	8.5	
	FINAL	8.3	8.5	9.2	9.0	8.5	8.3	8.5	
pH (mg/L)	INITIAL	7.3	8.1	7.3	7.4	7.5	7.0	7.2	
	FINAL	7.5	7.8	7.9	7.6	7.7	7.5	7.8	
temp (C)	INITIAL	22	23	22	22.5	23.0	23	23	
	FINAL	25	25	25	25	25	25	25	
CONC: 56									
D.O. (mg/L)	INITIAL	9.0	9.1	8.8	8.6	9.2	9.5	9.2	
	FINAL	8.5	8.4	9.2	9.0	8.7	8.4	8.4	
pH (s.u.)	INITIAL	7.4	8.0	7.3	7.5	7.6	7.0	7.2	
	FINAL	7.7	7.7	8.0	7.7	7.7	7.6	7.9	
temp (C)	INITIAL	22	23	22	23	22.7	24	23	
	FINAL	25	25	25	25	25	25	25	
CONC: 75									
D.O. (mg/L)	INITIAL	9.2	9.2	8.9	8.6	9.0	9.5	8.9	
	FINAL	8.3	8.3	9.2	9.0	8.3	8.4	8.3	
pH (s.u.)	INITIAL	7.5	8.1	7.2	7.6	7.6	7.0	7.2	
	FINAL	7.6	7.4	8.1	7.9	7.6	7.0	7.6	
temp (C)	INITIAL	23	21	22	22.9	23.2	24	22	
	FINAL	25	25	25	25	25	25	25	
CONC: 100									
D.O. (mg/L)	INITIAL	9.1	9.1	9.2	8.7	9.2	9.3	8.9	
	FINAL	8.5	8.3	9.2	9.1	8.2	8.3	8.1	
pH (s.u.)	INITIAL	7.5	8.0	7.1	7.6	7.6	7.0	7.3	
	FINAL	7.5	7.5	8.3	7.8	7.9	7.0	7.7	
temp (C)	INITIAL	23	21	22	22.6	23.0	25	22	
	FINAL	25	25	25	25	25	25	25	
CONC: 100 %		A	A	A	B	B	C	C	
ALKALINITY (mg/L)		112			84		64		
HARDNESS (mg/L)		52			52		48		
CONDUCTIVITY (umhc)		405			1248		4610		
CHLORINE (mg/L)		0.05			0.05		0.05		

APPENDIX C

Fathead minnow raw data and statistics

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		K1511006		TEST START DATE		11-17-15		TIME		1330			
CLIENT		Hope		TEST END DATE		11-24-15		TIME		1610			
				AGE AND SOURCE OF MINNOWS				248hrs Aquatex					
DAY (NUMBER SURVIVING)												SURVIVAL	
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV		
45	A	10	10	10	10	10	10	10	100	95%	45%		
	B	↓	↓	↓	↓	10	10	9	90				
	C	↓	↓	↓	↓	10	10	10	100				
	D	↓	↓	↓	↓	10	10	↓	100				
	E	↓	↓	↓	↓	10	10	↓	100				
32	A	10	10	10	10	10	10	10	100	100%			
	B	↓	↓	↓	↓	10	10	↓	100				
	C	↓	↓	↓	↓	10	10	↓	100				
	D	↓	↓	↓	↓	10	10	↓	100				
	E	↓	↓	↓	↓	10	10	↓	100				
42	A	10	10	10	10	10	10	10	100	100%			
	B	↓	↓	↓	↓	10	10	↓	100				
	C	↓	↓	↓	↓	10	10	↓	100				
	D	↓	↓	↓	↓	10	10	↓	100				
	E	↓	↓	↓	↓	10	10	↓	100				
56	A	10	10	10	10	10	10	9	90	94%			
	B	↓	↓	10	↓	10	10	10	100				
	C	↓	↓	9	↓	9	9	9	90				
	D	↓	↓	10	↓	10	10	9	90				
	E	↓	↓	10	↓	10	10	10	100				
75	A	10	10	10	10	10	10	10	100	96%			
	B	↓	↓	↓	↓	10	10	10	100				
	C	↓	↓	↓	↓	10	9	9	90				
	D	↓	↓	↓	↓	10	10	9	90				
	E	↓	↓	↓	↓	10	10	10	100				
100	A	10	10	10	10	10	10	10	100	95%	45%		
	B	↓	↓	9	9	9	9	9	90				
	C	↓	↓	10	10	10	10	10	100				
	D	↓	↓	10	↓	10	10	10	100				
	E	↓	↓	10	↓	10	10	10	100				
ANALYST		RH	RH	RH	RH	SD	SD	RH	RH				
DATE:		11-17-15	11-18-15	11-19-15	11-20-15	11-21-15	11-22-15	11-23-15	11-24-15				
TIME:		1330	1515	1530	1030	0950	1040	1600	1610				

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

Pimephales promelas

FATHEAD MINNOW

TEST 1000.0

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:		K1511006		TEST DATES (BEGIN / END):		11/17/15 - 11/24/15	
CLIENT:		Hope		WEIGHING DATE / TIME:		11/25/2015	
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	1.00790	1.00112	0.00678	10	0.678	AVG DRY
	B	1.03234	1.02667	0.00567	10	0.567	WEIGHT (mg)
	C	1.04357	1.03760	0.00597	10	0.597	0.588
	D	1.01951	1.01491	0.00460	10	0.460	CV
	E	1.01665	1.01026	0.00639	10	0.639	14.1
32%	A	1.02936	1.02272	0.00664	10	0.664	AVG DRY
	B	1.01865	1.01209	0.00656	10	0.656	WEIGHT (mg)
	C	1.00772	1.00020	0.00752	10	0.752	0.664
	D	1.02948	1.02346	0.00602	10	0.602	CV
	E	0.98846	0.98199	0.00647	10	0.647	
42%	A	1.01737	1.01080	0.00657	10	0.657	AVG DRY
	B	1.01933	1.01220	0.00713	10	0.713	WEIGHT (mg)
	C	1.02585	1.02044	0.00541	10	0.541	0.633
	D	0.99134	0.98484	0.00650	10	0.650	CV
	E	1.02119	1.01517	0.00602	10	0.602	
56%	A	0.99005	0.98503	0.00502	10	0.502	AVG DRY
	B	1.03131	1.02319	0.00812	10	0.812	WEIGHT (mg)
	C	1.03478	1.02825	0.00653	10	0.653	0.658
	D	1.02178	1.01527	0.00651	10	0.651	CV
	E	1.02181	1.01509	0.00672	10	0.672	
75%	A	1.02233	1.01521	0.00712	10	0.712	AVG DRY
	B	1.03582	1.02923	0.00659	10	0.659	WEIGHT (mg)
	C	1.01368	1.00734	0.00634	10	0.634	0.661
	D	1.01148	1.00535	0.00613	10	0.613	CV
	E	0.99536	0.98850	0.00686	10	0.686	
100%	A	1.01118	1.00498	0.00620	10	0.620	AVG DRY
	B	1.01821	1.01306	0.00515	10	0.515	WEIGHT (mg)
	C	1.02119	1.01341	0.00778	10	0.778	0.655
	D	1.02268	1.01644	0.00624	10	0.624	CV
	E	1.03513	1.02777	0.00736	10	0.736	15.9

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA#K1511006, FATHEAD MINNOW SURVIVAL, CHRONIC, 11-17-15
File: hopefhsur Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.106

W = 0.901

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#K1511006, FATHEAD MINNOW SURVIVAL, CHRONIC, 11-17-15
File: hopefhsur 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#K1511006, FATHEAD MINNOW SURVIVAL, CHRONIC, 11-17-15
 FILE: hopefhsur
 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	0.9000	1.2490
4	56 % EFFLUENT	2	1.0000	1.4120
4	56 % EFFLUENT	3	0.9000	1.2490
4	56 % EFFLUENT	4	0.9000	1.2490
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	1.0000	1.4120
6	100 % EFFLUENT	2	0.9000	1.2490
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#K1511006, FATHEAD MINNOW SURVIVAL, CHRONIC, 11-17-15
 File: hopefhsur 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	1.314	22.50	16.00	5.00	
5	75 % EFFLUENT	1.347	25.00	16.00	5.00	
6	100 % EFFLUENT	1.379	27.50	16.00	5.00	

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

AA# K1511006, FATHEAD MINNOW GROWTH, CHRONIC, 11-17-15
File: hopefhg Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.154

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# K1511006, FATHEAD MINNOW GROWTH, CHRONIC, 11-17-15
File: hopefhg Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 5.05

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# K1511006, FATHEAD MINNOW GROWTH, CHRONIC, 11-17-15
FILE: hopefhg
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.6780	0.6780
1	CONTROL	2	0.5670	0.5670
1	CONTROL	3	0.5970	0.5970
1	CONTROL	4	0.4600	0.4600
1	CONTROL	5	0.6390	0.6390
2	32 % EFFLUENT	1	0.6640	0.6640
2	32 % EFFLUENT	2	0.6560	0.6560
2	32 % EFFLUENT	3	0.7520	0.7520
2	32 % EFFLUENT	4	0.6020	0.6020
2	32 % EFFLUENT	5	0.6470	0.6470
3	42 % EFFLUENT	1	0.6570	0.6570
3	42 % EFFLUENT	2	0.7130	0.7130
3	42 % EFFLUENT	3	0.5410	0.5410
3	42 % EFFLUENT	4	0.6500	0.6500

3	42 %	EFFLUENT	5	0.6020	0.6020
4	56 %	EFFLUENT	1	0.5020	0.5020
4	56 %	EFFLUENT	2	0.8120	0.8120
4	56 %	EFFLUENT	3	0.6530	0.6530
4	56 %	EFFLUENT	4	0.6510	0.6510
4	56 %	EFFLUENT	5	0.6720	0.6720
5	75 %	EFFLUENT	1	0.7120	0.7120
5	75 %	EFFLUENT	2	0.6590	0.6590
5	75 %	EFFLUENT	3	0.6340	0.6340
5	75 %	EFFLUENT	4	0.6130	0.6130
5	75 %	EFFLUENT	5	0.6860	0.6860
6	100 %	EFFLUENT	1	0.6200	0.6200
6	100 %	EFFLUENT	2	0.5150	0.5150
6	100 %	EFFLUENT	3	0.7780	0.7780
6	100 %	EFFLUENT	4	0.6240	0.6240
6	100 %	EFFLUENT	5	0.7360	0.7360

AA# K1511006, FATHEAD MINNOW GROWTH, CHRONIC, 11-17-15
 File: hopefhg Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.021	0.004	0.659
Within (Error)	24	0.154	0.006	
Total	29	0.175		

Critical F value = 2.62 (0.05,5,24)
 Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All equal

AA# K1511006, FATHEAD MINNOW GROWTH, CHRONIC, 11-17-15
 File: hopefhg 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	0.588	0.588		
2	32 % EFFLUENT	0.664	0.664	-1.499	
3	42 % EFFLUENT	0.633	0.633	-0.876	
4	56 % EFFLUENT	0.658	0.658	-1.376	
5	75 % EFFLUENT	0.661	0.661	-1.432	
6	100 % EFFLUENT	0.655	0.655	-1.309	

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

AA# K1511006, FATHEAD MINNOW GROWTH, CHRONIC, 11-17-15
 File: hopefhg 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	0.120	20.3	-0.076
3	42 % EFFLUENT	5	0.120	20.3	-0.044
4	56 % EFFLUENT	5	0.120	20.3	-0.070
5	75 % EFFLUENT	5	0.120	20.3	-0.073
6	100 % EFFLUENT	5	0.120	20.3	-0.066

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

SURVIVAL AND REPRODUCTION TEST

Ceriodaphnia dubia													Lab Number/s			Analyst: RH, ST			
Discharger: Hape													R/5/1006			Test Start - Date/Time: 11-17-15 1100			
Location:																Test Stop - Date/Time: 11-24-15 1030			
Date Sample Collected: See CDC																			
Conc %	1	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst				
	Day	A	B	C	D	E	F	G	H	I	J								
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	RH				
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	RH				
	3	4	0	0	3	0	0	1	0	2	0	10	10	1.0	RH				
	4	0	3	3	0	6	0	0	2	6	4	24	10	2.4	RH				
	5	4	5	5	3	4	5	3	2	6	8	45	10	4.5	RH				
	6	0	7	2	9	7	4	0	9	0	1	28	10	3.9	RH				
	7	2	0	5	0	3	0	7	6	7	8	38	10	3.8	RH				
	8																		
	Total	10	15	13	15	20	9	11	19	21	21	150		1.2					
Conc %	2	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst				
	Day	A	B	C	D	E	F	G	H	I	J								
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	RH				
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	RH				
	3	0	0	0	0	0	4	2	0	0	0	6	10	0.6	RH				
	4	4	5	2	4	5	0	4	2	5	8	35	10	3.5	RH				
	5	8	7	0	5	5	6	8	0	7	8	54	10	5.4	RH				
	6	0	0	1	0	7	4	7	X	6	0	29	10	2.9	RH				
	7	5	1	5	0	1	1	X	0	3	0	16	10	1.6	RH				
	8																		
	Total	17	13	9	9	17	19	22	X2	18	13	140							
Conc %	3	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst				
	Day	A	B	C	D	E	F	G	H	I	J								
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	RH				
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	RH				
	3	1	0	1	0	0	0	2	2	0	0	6	10	0.6	RH				
	4	6	2	2	2	4	4	2	0	4	6	32	10	3.2	RH				
	5	1	7	3	2	2	2	9	10	6	5	57	10	5.7	RH				
	6	3	0	0	1	6	5	0	0	4	0	19	10	1.9	RH				
	7	0	0	1	3	0	0	2	5	4	0	15	10	1.5	RH				
	8																		
	Total	11	9	12	8	12	11	15	17	18	11	124							
Conc %	4	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst				
	Day	A	B	C	D	E	F	G	H	I	J								
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	RH				
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	RH				
	3	0	0	2	0	0	0	0	0	0	0	2	10	0.2	RH				
	4	3	3	1	4	3	0	4	3	0	4	25	10	2.5	RH				
	5	4	6	9	8	7	X	10	5	0	4	52	10	5.2	RH				
	6	3	0	3	8	7	-	2	0	0	0	9	10	0.9	RH				
	7	0	5	3	1	0	-	5	10	7	8	39	10	3.9	RH				
	8																		
	Total	10	14	17	13	11	X0	21	18	7	16	127							
Conc %	5	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst				
	Day	A	B	C	D	E	F	G	H	I	J								
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	RH				
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	RH				
	3	0	0	0	0	2	4	0	2	0	0	8	10	0.8	RH				
	4	4	4	3	4	10	4	6	0	4	4	39	10	3.9	RH				
	5	7	7	8	7	3	0	1	8	5	2	48	10	4.8	RH				
	6	1	0	0	8	2	5	8	8	0	0	32	10	3.2	RH				
	7	6	5	0	1	0	0	5	2	9	1	29	10	2.9	RH				
	8																		
	Total	18	16	11	20	17	9	20	20	18	7	150							
Conc %	6	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst				
	Day	A	B	C	D	E	F	G	H	I	J								
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	RH				
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	RH				
	3	2	3	2	0	0	3	0	0	0	0	10	10	1.0	RH				
	4	1	1	4	0	3	6	4	4	0	3	26	10	2.6	RH				
	5	3	0	5	6	4	5	1	7	2	5	40	10	4.0	RH				
	6	7	5	0	7	6	7	0	0	8	7	47	10	4.7	RH				
	7	0	3	0	7	0	0	4	3	6	0	23	10	2.3	RH				
	8																		
	Total	13	14	11	20	13	21	9	14	16	15	152		15.2					

X = Dead

12 3 15
125

0.15

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
32% effluent	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% effluent	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% effluent	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
75% effluent	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
100% effluent	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

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	10	0	
1	32% effluent	10	1	
2	42% effluent	10	0	
3	56% effluent	10	1	
4	75% effluent	10	0	
5	100% effluent	10	0	

TITLE: AA #K1511006, CERIODAPHNIA DUBIA CHRONIC, REPRODUCTION
 FILE: hopecd
 TRANSFORM: NO TRANSFORMATION

NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	10.0000	10.0000
1	CONTROL	2	15.0000	15.0000
1	CONTROL	3	15.0000	15.0000
1	CONTROL	4	15.0000	15.0000
1	CONTROL	5	20.0000	20.0000
1	CONTROL	6	9.0000	9.0000
1	CONTROL	7	11.0000	11.0000
1	CONTROL	8	19.0000	19.0000
1	CONTROL	9	21.0000	21.0000
1	CONTROL	10	21.0000	21.0000
2	32 % EFFLUENT	1	17.0000	17.0000
2	32 % EFFLUENT	2	13.0000	13.0000
2	32 % EFFLUENT	3	9.0000	9.0000
2	32 % EFFLUENT	4	9.0000	9.0000
2	32 % EFFLUENT	5	17.0000	17.0000
2	32 % EFFLUENT	6	19.0000	19.0000
2	32 % EFFLUENT	7	22.0000	22.0000
2	32 % EFFLUENT	8	2.0000	2.0000
2	32 % EFFLUENT	9	18.0000	18.0000
2	32 % EFFLUENT	10	13.0000	13.0000
3	42 % EFFLUENT	1	11.0000	11.0000
3	42 % EFFLUENT	2	9.0000	9.0000
3	42 % EFFLUENT	3	12.0000	12.0000
3	42 % EFFLUENT	4	8.0000	8.0000
3	42 % EFFLUENT	5	12.0000	12.0000
3	42 % EFFLUENT	6	11.0000	11.0000
3	42 % EFFLUENT	7	15.0000	15.0000
3	42 % EFFLUENT	8	17.0000	17.0000
3	42 % EFFLUENT	9	18.0000	18.0000
3	42 % EFFLUENT	10	11.0000	11.0000
4	56 % EFFLUENT	1	10.0000	10.0000
4	56 % EFFLUENT	2	14.0000	14.0000
4	56 % EFFLUENT	3	17.0000	17.0000
4	56 % EFFLUENT	4	13.0000	13.0000
4	56 % EFFLUENT	5	11.0000	11.0000

4	56 %	EFFLUENT	6	0.0000	0.0000
4	56 %	EFFLUENT	7	21.0000	21.0000
4	56 %	EFFLUENT	8	18.0000	18.0000
4	56 %	EFFLUENT	9	7.0000	7.0000
4	56 %	EFFLUENT	10	16.0000	16.0000
5	75 %	EFFLUENT	1	18.0000	18.0000
5	75 %	EFFLUENT	2	16.0000	16.0000
5	75 %	EFFLUENT	3	11.0000	11.0000
5	75 %	EFFLUENT	4	20.0000	20.0000
5	75 %	EFFLUENT	5	17.0000	17.0000
5	75 %	EFFLUENT	6	9.0000	9.0000
5	75 %	EFFLUENT	7	20.0000	20.0000
5	75 %	EFFLUENT	8	20.0000	20.0000
5	75 %	EFFLUENT	9	18.0000	18.0000
5	75 %	EFFLUENT	10	7.0000	7.0000
6	100 %	EFFLUENT	1	13.0000	13.0000
6	100 %	EFFLUENT	2	20.0000	20.0000
6	100 %	EFFLUENT	3	11.0000	11.0000
6	100 %	EFFLUENT	4	20.0000	20.0000
6	100 %	EFFLUENT	5	13.0000	13.0000
6	100 %	EFFLUENT	6	21.0000	21.0000
6	100 %	EFFLUENT	7	9.0000	9.0000
6	100 %	EFFLUENT	8	14.0000	14.0000
6	100 %	EFFLUENT	9	16.0000	16.0000
6	100 %	EFFLUENT	10	15.0000	15.0000

AA #K1511006, CERIODAPHNIA DUBIA CHRONIC, REPRODUCTION
File: hopecd 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 #K1511006, CERIODAPHNIA DUBIA CHRONIC, REPRODUCTION
File: hopecd Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 4.50

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 #K1511006, CERIODAPHNIA DUBIA CHRONIC, REPRODUCTION
FILE: hopecd
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	10.0000	10.0000
1	CONTROL	2	15.0000	15.0000
1	CONTROL	3	15.0000	15.0000
1	CONTROL	4	15.0000	15.0000
1	CONTROL	5	20.0000	20.0000
1	CONTROL	6	9.0000	9.0000
1	CONTROL	7	11.0000	11.0000
1	CONTROL	8	19.0000	19.0000
1	CONTROL	9	21.0000	21.0000
1	CONTROL	10	21.0000	21.0000
2	32 % EFFLUENT	1	17.0000	17.0000
2	32 % EFFLUENT	2	13.0000	13.0000
2	32 % EFFLUENT	3	9.0000	9.0000
2	32 % EFFLUENT	4	9.0000	9.0000
2	32 % EFFLUENT	5	17.0000	17.0000
2	32 % EFFLUENT	6	19.0000	19.0000

2	32	%	EFFLUENT	7	22.0000	22.0000
2	32	%	EFFLUENT	8	2.0000	2.0000
2	32	%	EFFLUENT	9	18.0000	18.0000
2	32	%	EFFLUENT	10	13.0000	13.0000
3	42	%	EFFLUENT	1	11.0000	11.0000
3	42	%	EFFLUENT	2	9.0000	9.0000
3	42	%	EFFLUENT	3	12.0000	12.0000
3	42	%	EFFLUENT	4	8.0000	8.0000
3	42	%	EFFLUENT	5	12.0000	12.0000
3	42	%	EFFLUENT	6	11.0000	11.0000
3	42	%	EFFLUENT	7	15.0000	15.0000
3	42	%	EFFLUENT	8	17.0000	17.0000
3	42	%	EFFLUENT	9	18.0000	18.0000
3	42	%	EFFLUENT	10	11.0000	11.0000
4	56	%	EFFLUENT	1	10.0000	10.0000
4	56	%	EFFLUENT	2	14.0000	14.0000
4	56	%	EFFLUENT	3	17.0000	17.0000
4	56	%	EFFLUENT	4	13.0000	13.0000
4	56	%	EFFLUENT	5	11.0000	11.0000
4	56	%	EFFLUENT	6	0.0000	0.0000
4	56	%	EFFLUENT	7	21.0000	21.0000
4	56	%	EFFLUENT	8	18.0000	18.0000
4	56	%	EFFLUENT	9	7.0000	7.0000
4	56	%	EFFLUENT	10	16.0000	16.0000
5	75	%	EFFLUENT	1	18.0000	18.0000
5	75	%	EFFLUENT	2	16.0000	16.0000
5	75	%	EFFLUENT	3	11.0000	11.0000
5	75	%	EFFLUENT	4	20.0000	20.0000
5	75	%	EFFLUENT	5	17.0000	17.0000
5	75	%	EFFLUENT	6	9.0000	9.0000
5	75	%	EFFLUENT	7	20.0000	20.0000
5	75	%	EFFLUENT	8	20.0000	20.0000
5	75	%	EFFLUENT	9	18.0000	18.0000
5	75	%	EFFLUENT	10	7.0000	7.0000
6	100	%	EFFLUENT	1	13.0000	13.0000
6	100	%	EFFLUENT	2	20.0000	20.0000
6	100	%	EFFLUENT	3	11.0000	11.0000
6	100	%	EFFLUENT	4	20.0000	20.0000
6	100	%	EFFLUENT	5	13.0000	13.0000
6	100	%	EFFLUENT	6	21.0000	21.0000
6	100	%	EFFLUENT	7	9.0000	9.0000
6	100	%	EFFLUENT	8	14.0000	14.0000
6	100	%	EFFLUENT	9	16.0000	16.0000
6	100	%	EFFLUENT	10	15.0000	15.0000

AA #K1511006, CERIODAPHNIA DUBIA CHRONIC, REPRODUCTION
 File: hopecd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	104.933	20.987	0.877
Within (Error)	54	1291.800	23.922	
Total	59	1396.733		

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

AA #K1511006, CERIODAPHNIA DUBIA CHRONIC, REPRODUCTION
 File: hopecd Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	15.600	15.600		
2	32 % EFFLUENT	13.900	13.900	0.777	
3	42 % EFFLUENT	12.400	12.400	1.463	
4	56 % EFFLUENT	12.700	12.700	1.326	
5	75 % EFFLUENT	15.600	15.600	0.000	
6	100 % EFFLUENT	15.200	15.200	0.183	

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

AA #K1511006, CERIODAPHNIA DUBIA CHRONIC, REPRODUCTION
 File: hopecd 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	5.053	32.4	1.700
3	42 % EFFLUENT	10	5.053	32.4	3.200
4	56 % EFFLUENT	10	5.053	32.4	2.900
5	75 % EFFLUENT	10	5.053	32.4	0.000
6	100 % EFFLUENT	10	5.053	32.4	0.400

AA #K1511006, CERIODAPHNIA DUBIA CHRONIC, REPRODUCTION
 File: hopecd Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	15.600				
2	32 % EFFLUENT	13.900	96.50	75.00	10.00	
3	42 % EFFLUENT	12.400	85.50	75.00	10.00	
4	56 % EFFLUENT	12.700	92.00	75.00	10.00	
5	75 % EFFLUENT	15.600	104.50	75.00	10.00	
6	100 % EFFLUENT	15.200	101.50	75.00	10.00	

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

APPENDIX E

Organism History



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

TEST ORGANISM HISTORY

DATE SHIPPED 11/17/15 CLIENT ARK ANALYTICAL

Purchase Order #: _____

SPECIES: Pimephales promelas

Quantity Shipped: 850⁺ 15-160
251

Age: HATCHED 11/15/15

Brood Stock Source: Anderson Farms, AR

Culture Water: Groundwater

Hardness (Mg/l CaCO3): 160

Dissolved Oxygen (Mg/l): 8.5

Temperature (°C): 25.1

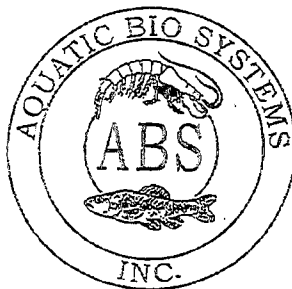
Feeding: ARTEMIA

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

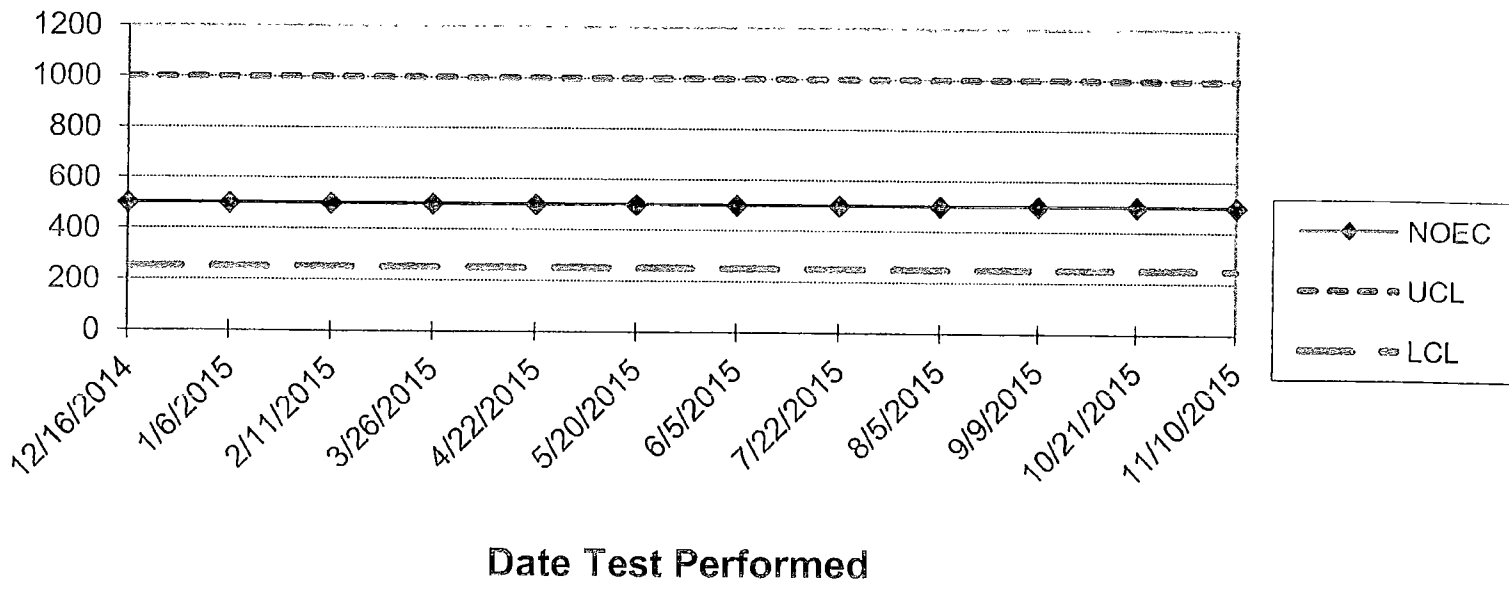
APPENDIX F

Quality Assurance Charts

ARKANSAS ANALYTICAL, INC.

FATHEAD MINNOW SURVIVAL 7 Day QUALITY ASSURANCE

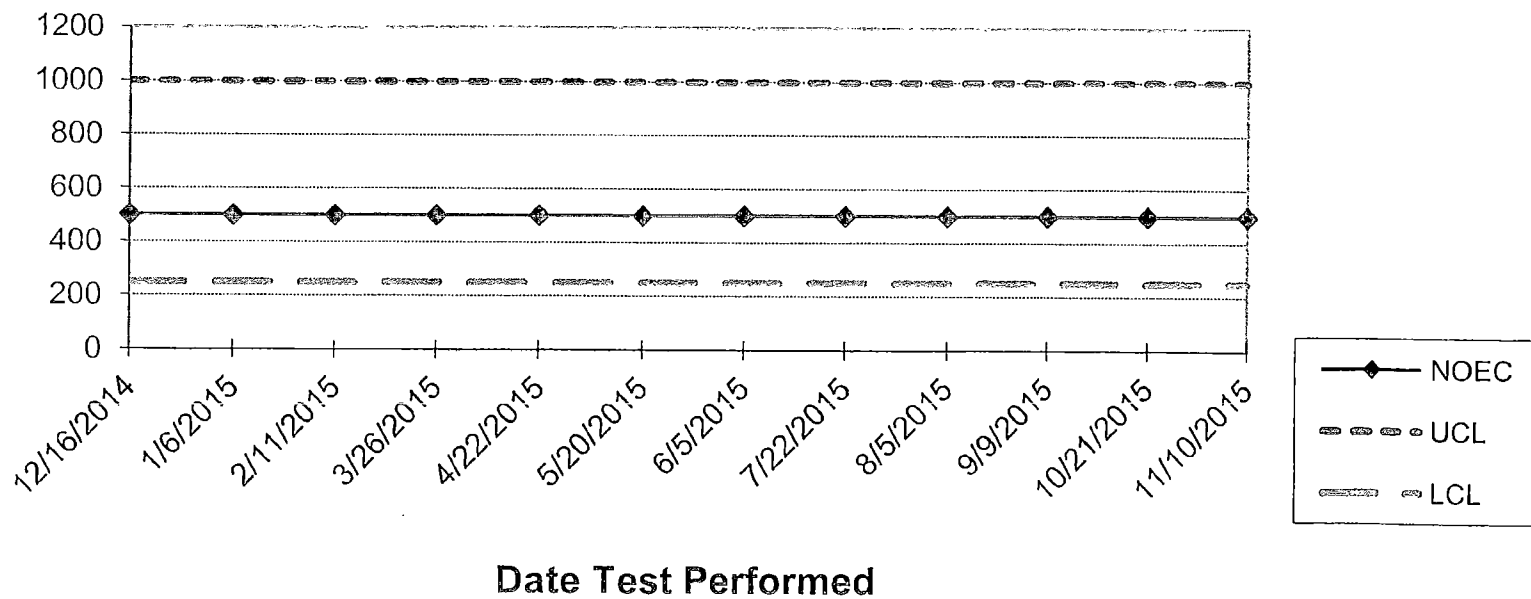
Reference Toxicant, KCl, ppm



ARKANSAS ANALYTICAL, INC.

FATHEAD MINNOW GROWTH 7 Day QUALITY ASSURANCE

Reference Toxicant, KCl, ppm

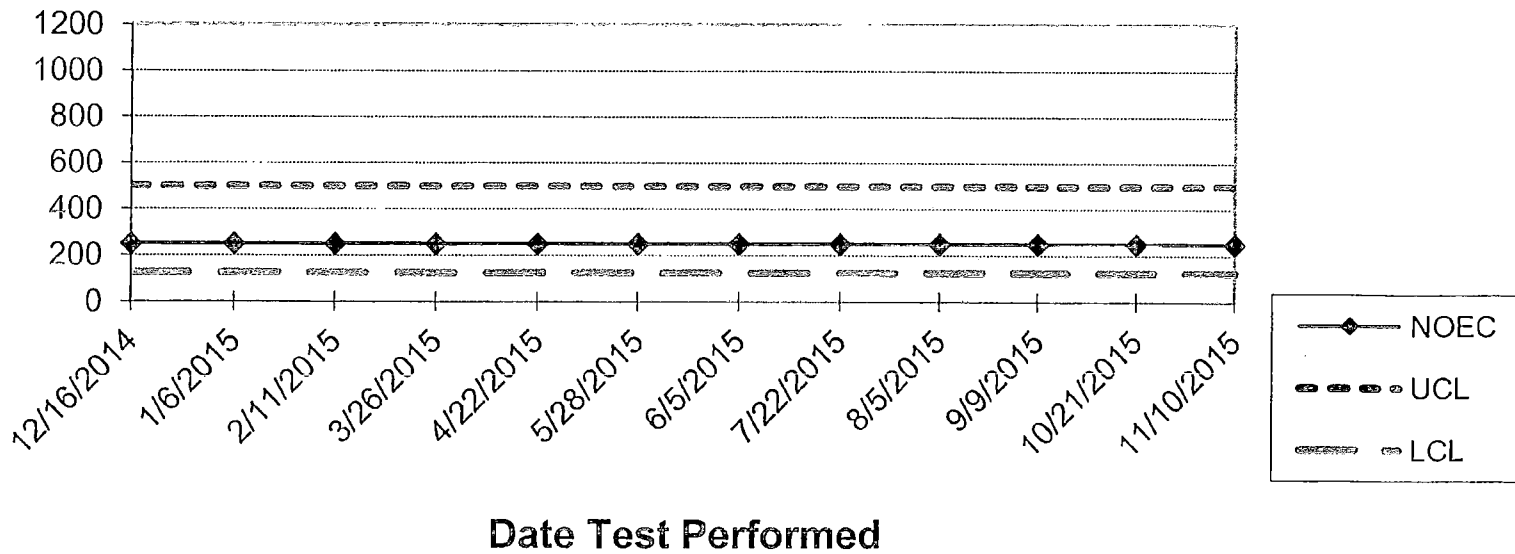


ARKANSAS ANALYTICAL, INC.

CERIODAPHNIA DUBIA SURVIVAL

QUALITY ASSURANCE

Reference Toxicant, KCl, ppm

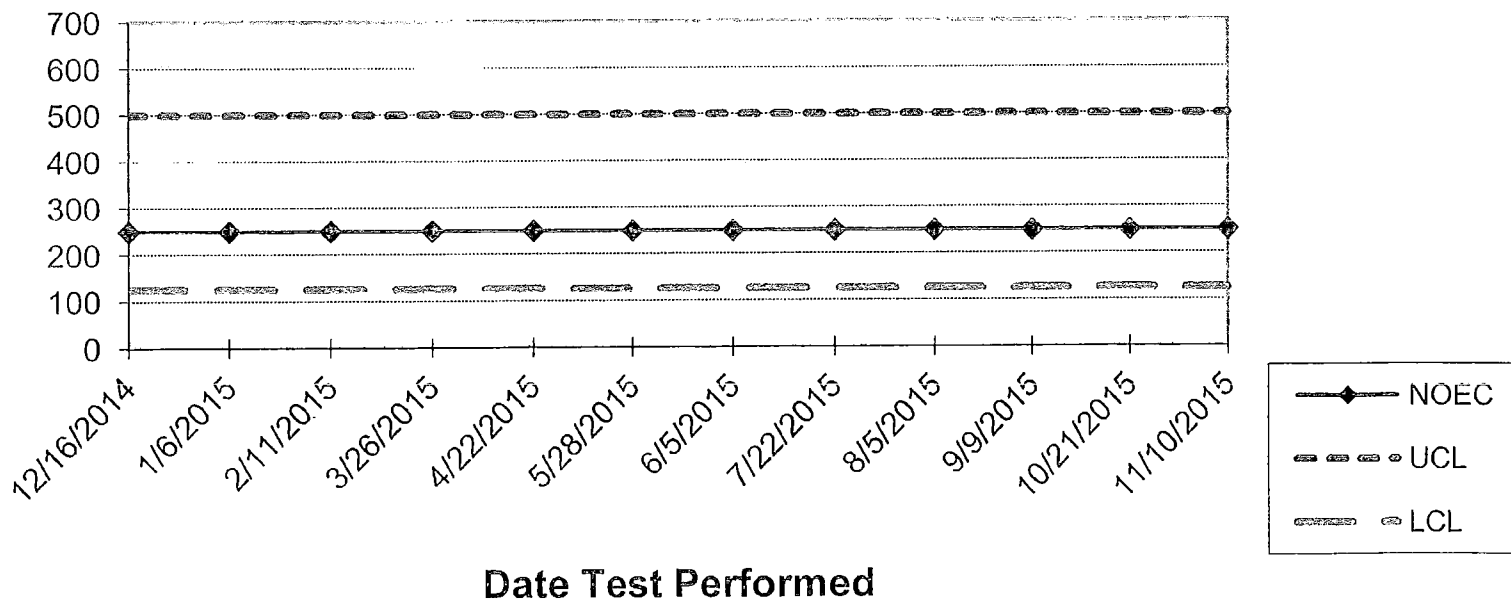


ARKANSAS ANALYTICAL, INC.

CERIODAPHNIA DUBIA REPRODUCTION

QUALITY ASSURANCE

Reference Toxicant, KCl, ppm



EC 23 2015

DEC 23



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