

Arkansas Analytical, Inc.

Toxicity Test Results

City of Hope
Permit Number: AR0038466
AFIN # 29-00034
Third Quarter 2014

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
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Prepared by: Arkansas Analytical, Inc.
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Little Rock, Arkansas 72209
Lab Number K1408005

Thursday, August 28, 2014

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 2014.

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:	8-17-14, 0600	8-18-14, 0600
Sample #2:	8-19-14, 0600	8-20-14, 0600
Sample #3:	8-21-14, 0700	8-22-14, 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:	8-19-14, 1130	4
Sample #2:	8-21-14, 1105	4
Sample #3:	8-22-14, 1625	4

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	17.1	X	
At least 60% of surviving females should have produced 3 broods	80%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	27.5%	X	

TEST ACCEPTANCE CRITERIA for *Pimephales promelas*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	100%	X	
The percent coefficient of variation between replicates must be 40% or less for survival	0.00%	X	
Minimum of 0.25 mg average dry weight of surviving controls	0.692	X	
The percent coefficient of variation between replicates must be 40% or less for growth	10.1%	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> 8/5/14 – 8/12/14		<i>Pimephales promelas</i> 8/5/14 – 8/12/14	
NOEC Survival:	500 ppm KCl	NOEC Survival:	500 ppm KCl
LOEC Survival:	1000 ppm KCl	LOEC Survival:	1000 ppm KCl
NOEC Reproduction:	250 ppm KCl	NOEC Growth:	500 ppm KCl
LOEC Reproduction:	500 ppm KCl	LOEC Growth:	1000 ppm KCl

Quality Assurance charts are provided in Appendix 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)	16.6	%CV survival (critical dilution)	0%
%CV Reproduction (critical dilution)	17.6%	Mean dry weight (critical dilution) in milligrams	0.878
		%CV growth (critical dilution)	13.4%
PMSD Reproduction	24.4%	PMSD Growth	19.7%

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 / Chris Turney

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:	8-17-14, 0600	8-18-14, 0600
Sample #2:	8-19-14, 0600	8-20-14, 0600
Sample #3:	8-21-14, 0700	8-22-14, 0600

Test initiated (date, time): 8-19-14, 1330 Test terminated (date, time): 8-26-14, 1500

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	100	100	100		100	100	100	0.00
32%	100	100	100	100	90		100	100	98	
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%	0.709	0.614	0.792	0.705	0.638		0.692	10.1
32%	0.688	0.713	0.754	0.617	0.560		0.666	
42%	0.572	0.656	0.754	0.666	0.730		0.676	
56%	0.777	0.668	0.912	0.739	0.924		0.804	
75%	0.704	0.755	0.904	0.777	0.899		0.808	
100%	0.954	0.812	0.782	0.795	1.049		0.878	13.4

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)= 13.4 %

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:	8-17-14, 0600	8-18-14, 0600
Sample #2:	8-19-14, 0600	8-20-14, 0600
Sample #3:	8-21-14, 0700	8-22-14, 0600

Test initiated (date, time): 8-19-14, 1430 Test terminated (date, time): 8-26-14, 1000

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	16	15	14	18	19	12
B	11	14	18	12	13	13
C	22	17	17	17	23	18
D	17	11	17	22	13	21
E	15	19	16	10	10	17
F	12	16	12	14	10	20
G	24	17	20	21	12	14
H	16	8	17	10	13	18
I	24	16	8	15	19	17
J	14	20	23	16	18	16
Mean	17.1	15.3	16.2	15.5	15.0	16.6
Mean/surviving female	17.1	15.3	16.2	15.5	15.0	16.6
CV%*	27.5					17.6

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	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)= 27.5 %

6. Enter Whole Effluent Toxicity: 100 %

APPENDIX A

Chain of Custody Forms

SORRELLS RESEARCH ASSOCIATES, INC

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 # 17407.0001 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

110913k2

NAME OF COMPANY, CITY, OR PROJECT: City of Hope PROJECT NO: _____ SAMPLER(S) NAME: (PRINT) ISO Auto

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	
	<u>WIFE</u>	<u>6AM 8-17-14</u>	<u>6AM 8-18-14</u>	<u>24HR COMP</u>		<u>CA</u>				<u>6 1/2oz Plastic</u>	<u>K1408005 - A</u> <u>W.E.T</u>

	Yes	No
Custody Seals:	<input checked="" type="checkbox"/>	<input type="checkbox"/>
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:		4°C
Temperature Gun ID:	HHT #2	

METHOD OF SHIPMENT (CIRCLE) FED EX WALK IN SRA UPS OTHER	FIELD CALIBRATION RECORD pH 7 pH 4 pH 10 D.O	NOTES/COMMENTS/OBSERVATIONS <u>Temp GRA 4.3°</u>
TYPE OF SAMPLE(S): (CIRCLE) WATER SOIL W/W SLUDGE OTHER	FIELD ANALYSIS CONDUCTED BY: (CIRCLE) SRA CLIENT	

RELINQUISHED BY: [Signature] DATE/TIME: 8/18/14 @ 12:36pm RECEIVED BY: [Signature] DATE/TIME: 8.18.14 1236

RELINQUISHED BY: _____ DATE/TIME: _____ RECEIVED BY (LAB): [Signature] DATE/TIME: _____

Don't know lab 112A relative

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

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

LAB # 07407.0002 B
 CLIENT # _____
 P.O.# _____

NAME OF COMPANY, CITY, OR PROJECT

PROJECT NO:

SAMPLER(S) NAME: (PRINT)

11091302

City of Hope

ISCO Automatic

MPL# ID:	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		
<u>0002 B</u>	<u>WPFE</u>	<u>6am</u> 8-19-14	<u>6am</u> 8-20-14	<u>24hr</u> Comp		<u>CA</u>					<u>6 1/2 gal. Plastic</u>	<u>K1408005-B</u> <u>W.E.T</u>

	Yes	No
Custody Seals:	<input checked="" type="checkbox"/>	<input type="checkbox"/>
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:	4°C	
Temperature Gun ID:	HHT # 2	

METHOD OF SHIPMENT (CIRCLE)

FIELD CALIBRATION RECORD

NOTES/COMMENTS/OBSERVATIONS

FED EX WALK IN SRA UPS OTHER

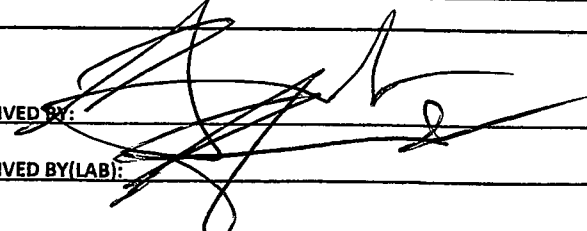
pH 7
pH 4
pH 10
D.O

Rec'd @ AA 8/21/14 Amanda Johnson @ 1105

TYPE OF SAMPLE(S): (CIRCLE)

WATER SOIL W/W SLUDGE OTHER

FIELD ANALYSIS CONDUCTED BY: (CIRCLE) SRA CLIENT

RELINQUISHED BY: Carol Smith DATE/TIME: 8/20/14 @ 12:07pm RECEIVED BY:  DATE/TIME: 8.20.14 1211

RELINQUISHED BY: _____ DATE/TIME: _____ RECEIVED BY (LAB): _____ DATE/TIME: 8.20.14 1645

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

PAGE 01

CHAIN OF CUSTODY RECORD

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

FOR LAB/OFFICE USE ONLY

LAB # 17407.0003B
 CLIENT # 15020
 P.O.# _____

STANDARD METHODS PRESERVATION PER EPA 40 CFR

C4= COOL TO 4C
 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

PROJECT NO.

SAMPLER(S) NAME: (PRINT)

LAB USE

City of Hope

K1408-005-C

ISCO Auto SAMPLER

SAMPLE ID AND/OR COLLECTION LOCATION	START DATE/TIME	END DATE/TIME	COMP GRAB	FIELD ANALYSIS				D.O (W) D.O (P)	CONTAINER TYPE PRESERVATIVE	ANALYSIS REQUIRED
				pH	TEMP	FLOW	CL2			
<u>WPFE</u>	<u>7 AM 8-21-14</u>	<u>6 AM 8-22-14</u>	<u>Comp</u>		<u>4</u>				<u>6 - 1/2 gallon plastic containers</u>	<u>W.E.T</u>

AR Analytical - 8/22/14
 Custody Seals: X Yes No
 Containers Correct: X
 COC/Labels Agree: X
 Received on Ice: X
 Temperature on Receipt: 4°C
 Temperature Gun ID: HHT # 2

METHOD OF SHIPMENT (CIRCLE)

FED EX WALK IN SRA UPS OTHER

FIELD CALIBRATION RECORD

pH 7
 pH 4
 pH 10
 D.O

NOTES/COMMENTS/OBSERVATIONS

Temp @ Lab 5°C

TYPE OF SAMPLE(S): (CIRCLE)

WATER SOIL W/W SLUDGE OTHER

rec @ AR Analytical - Sydney James, 8-22-14, 1625
 FIELD ANALYSIS CONDUCTED BY: (CIRCLE) SBA CLIENT

RELINQUISHED BY: Kim Holston

DATE/TIME: 8/22/14 @ 12:20 P

RECEIVED BY: Danny Riddle

DATE/TIME: 8-22-14 12:18

RELINQUISHED BY:

DATE/TIME:

RECEIVED BY(LAB):

DATE/TIME:

08/22/2014 16:26 5015627025



APPENDIX B

Effluent and Dilution Water Data

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Fathead Minnow

Lab # / Sample ID K1408005

Test Start (Date/Time) 8-19-14

1330

Client: Hope

Test End (Date/Time) 8-26-14

1500

Day of Test

		1	2	3	4	5	6	7	notes
Control	1415	8-19	8-20	8-21	8-22	8-23	8-24	8-25	
D.O. (mg/L)	INITIAL	8.4	8.8	8.6	8.4	8.7	8.6	8.7	
	FINAL	8.3	7.9	8.5	8.0	8.4	7.0	7.7	
pH (s.u.)	INITIAL	7.8	7.9	8.1	7.9	7.7	8.1	8.2	
	FINAL	7.7	7.8	8.2	7.5	7.9	7.8	8.0	
temp (C)	INITIAL	22	22	22	22	23.1	22.5	22	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		60							
HARDNESS (mg/L)		92							
CONDUCTIVITY (umhc)		379							
CHLORINE (mg/L)		<0.05							
CONC:	32								
D.O. (mg/L)	INITIAL	8.4	8.8	8.5	8.6	8.7	8.6	8.7	
	FINAL	8.5	8.2	8.3	8.3	8.4	7.2	7.5	
pH (s.u.)	INITIAL	7.4	7.7	7.9	8.0	7.9	7.8	8.1	
	FINAL	7.8	8.0	8.1	8.0	8.1	7.6	7.8	
temp (C)	INITIAL	22	22	22	22	23.1	23.4	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	42								
D.O. (mg/L)	INITIAL	8.5	8.8	8.3	8.7	8.8	8.7	8.8	
	FINAL	8.3	8.2	8.4	8.4	8.4	7.7	7.2	
pH (mg/L)	INITIAL	7.4	7.6	7.6	7.8	7.5	7.8	7.9	
	FINAL	7.6	8.1	8.0	8.1	8.1	7.6	7.9	
temp (C)	INITIAL	22	22	22	22	24.3	24.0	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	56								
D.O. (mg/L)	INITIAL	8.2	8.9	8.4	8.4	8.8	8.6	8.8	
	FINAL	8.4	8.1	8.2	8.4	8.3	7.2	7.4	
pH (s.u.)	INITIAL	7.4	7.4	7.5	7.7	7.9	7.7	7.7	
	FINAL	7.8	8.2	7.9	8.2	8.2	7.7	7.8	
temp (C)	INITIAL	22	22	22	22	25.1	24.4	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	75								
D.O. (mg/L)	INITIAL	8.4	9.1	8.5	8.5	8.8	8.9	8.9	
	FINAL	8.1	8.2	8.3	8.3	8.4	7.3	7.3	
pH (s.u.)	INITIAL	7.3	7.5	7.5	7.4	7.4	7.5	7.5	
	FINAL	7.5	8.3	7.5	8.2	8.2	7.8	7.6	
temp (C)	INITIAL	22	21	21	22	25.0	24.7	21	
	FINAL	25	25	25	25	25	25	25	
CONC:	100								
D.O. (mg/L)	INITIAL	8.5	9.0	9.2	8.4	8.9	8.8	9.0	
	FINAL	8.3	8.1	8.3	8.2	8.3	6.9	7.5	
pH (s.u.)	INITIAL	7.6	7.4	7.4	7.5	7.4	7.5	7.4	
	FINAL	7.6	8.3	7.5	8.2	8.2	7.8	7.4	
temp (C)	INITIAL	22	21	21	22	25.1	25.0	21	
	FINAL	25	25	25	25	25	25	25	
CONC:	100 %	A	A	A	B	B	C	C	
ALKALINITY (mg/L)		112			48		56		
HARDNESS (mg/L)		52			68		60		
CONDUCTIVITY (umhc)		912			575		768		
CHLORINE (mg/L)		<0.05			<0.05		<0.05		

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Ceriodaphnia Dubia

Lab # / Sample ID **K1408005**

Test Start (Date/Time)

8-19-14

1430

Client:

Hope

Test End (Date/Time)

8-26-14

1000

Day of Test

		1	2	3	4	5	6	7	notes
Control	MHS	8-19	8-20	8-21	8-22	8-23	8-24	8-25	
D.O. (mg/L)	INITIAL	8.4	8.8	8.6	8.4	8.6	8.6	8.7	
	FINAL	8.4	8.4	8.6	8.4	8.3	8.3	8.6	
pH (s.u.)	INITIAL	7.8	7.9	8.1	7.9	7.8	8.1	8.2	
	FINAL	7.9	8.0	7.8	7.8	8.0	8.0	8.3	
temp (C)	INITIAL	22	22	22	22	21.9	22.5	22	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		60							
HARDNESS (mg/L)		92							
CONDUCTIVITY (umhc)		379							
CHLORINE (mg/L)		0.05							
CONC:	32								
D.O. (mg/L)	INITIAL	8.4	8.8	8.5	8.6	8.7	8.6	8.7	
	FINAL	8.4	8.4	8.5	8.5	8.4	8.2	9.0	
pH (s.u.)	INITIAL	7.4	7.7	7.9	8.0	7.4	7.8	8.1	
	FINAL	7.7	8.1	8.2	8.0	7.8	8.1	8.3	
temp (C)	INITIAL	22	22	22	22	23.1	23.4	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	42								
D.O. (mg/L)	INITIAL	8.5	8.8	8.3	8.7	8.7	8.7	8.8	
	FINAL	8.4	8.5	8.7	8.5	8.3	8.4	8.4	
pH (mg/L)	INITIAL	7.4	7.6	7.6	7.8	7.5	7.8	7.9	
	FINAL	7.5	8.3	8.3	8.2	7.6	8.3	8.3	
temp (C)	INITIAL	22	22	22	22	23.8	24.0	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	56								
D.O. (mg/L)	INITIAL	8.2	8.9	8.4	8.4	8.8	8.6	8.8	
	FINAL	8.3	8.5	8.8	8.5	8.4	8.3	8.5	
pH (s.u.)	INITIAL	7.4	7.4	7.5	7.7	7.5	7.7	7.7	
	FINAL	7.6	8.3	8.4	8.2	7.8	8.3	8.2	
temp (C)	INITIAL	22	22	22	22	24.3	24.4	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	75								
D.O. (mg/L)	INITIAL	8.4	9.1	8.5	8.5	8.8	8.9	8.9	
	FINAL	8.1	8.5	8.6	8.5	8.4	8.4	8.7	
pH (s.u.)	INITIAL	7.3	7.5	7.5	7.4	7.4	7.5	7.5	
	FINAL	7.5	8.4	8.4	8.3	7.5	8.3	8.4	
temp (C)	INITIAL	22	21	21	22	25	24.7	21	
	FINAL	25	25	25	25	25	25	25	
CONC:	100								
D.O. (mg/L)	INITIAL	8.5	9.0	8.2	8.4	8.9	8.8	9.0	
	FINAL	8.1	8.5	8.6	8.5	8.4	8.4	8.6	
pH (s.u.)	INITIAL	7.6	7.4	7.4	7.5	7.4	7.5	7.4	
	FINAL	7.5	8.5	8.4	8.3	7.5	8.4	8.4	
temp (C)	INITIAL	22	21	21	22	25.1	25.0	21	
	FINAL	25	25	25	25	25	25	25	
CONC:	100 %	A	A	A	B	B	C	C	
ALKALINITY (mg/L)		112			48		56		
HARDNESS (mg/L)		52			68		60		
CONDUCTIVITY (umhc)		912			575		768		
CHLORINE (mg/L)		0.05			0.05		0.05		

APPENDIX C

Fathead minnow raw data and statistics

FATHEAD MINNOW

SURVIVAL DATA FOR LARVAL SURVIVAL AND GROWTH TEST (CHRONIC)

LAB #: K1408005		TEST START		DATE	8/19/14	TIME	1330					
CLIENT: Hope		TEST END		DATE	8/26/14	TIME	1500					
ANALYST: RH/CT		AGE AND SOURCE OF MINNOWS										
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%	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%		
MHS	A	10	10	10	10	10	10	10	10	100%	98.0%	
	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%		
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%		
32%	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%		
42%	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%		
56%	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%		
75%	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%		
100%	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	RH	RH	CT	CT	RH	RH			
DATE:		8/19/14	8/20/14	8/21/14	8/22/14	8/23/14	8/24/14	8/25/14	8/26/14			
TIME:		1330	1400	1340	1500	1000	1055	1345	1500			

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

REMARKS:

AA# K1408005, FATHEAD MINNOW SURVIVAL, CHRONIC, 8-19-14
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# K1408005, FATHEAD MINNOW SURVIVAL, CHRONIC, 8-19-14
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# K1408005, FATHEAD MINNOW SURVIVAL, CHRONIC, 8-19-14
 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	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	0.9000	1.2490
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# K1408005, FATHEAD MINNOW SURVIVAL, CHRONIC, 8-19-14
 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	1.412				
2	32 % EFFLUENT	1.379	25.00	16.00	5.00	
3	42 % EFFLUENT	1.412	27.50	16.00	5.00	
4	56 % EFFLUENT	1.412	27.50	16.00	5.00	
5	75 % EFFLUENT	1.412	27.50	16.00	5.00	
6	100 % EFFLUENT	1.412	27.50	16.00	5.00	

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

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:		K1408005			TEST DATES (BEGIN / END):		8/19 - 8/26/14	
CLIENT:		Hope			WEIGHING DATE / TIME:		8/27/2014 1500	
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	1.00369	0.99660	0.00709	10	0.709	AVG DRY	
	B	1.02140	1.01526	0.00614	10	0.614	WEIGHT (mg)	
	C	1.00998	1.00206	0.00792	10	0.792	0.692	
	D	1.01506	1.00801	0.00705	10	0.705	CV	
	E	1.00155	0.99517	0.00638	10	0.638	10.08	
32%	A	1.02226	1.01538	0.00688	10	0.688	AVG DRY	
	B	0.99381	0.98668	0.00713	10	0.713	WEIGHT (mg)	
	C	1.02570	1.01816	0.00754	10	0.754	0.666	
	D	0.99125	0.98508	0.00617	10	0.617	CV	
	E	1.01724	1.01164	0.00560	10	0.560		
42%	A	1.01308	1.00736	0.00572	10	0.572	AVG DRY	
	B	0.95304	0.94648	0.00656	10	0.656	WEIGHT (mg)	
	C	0.97417	0.96663	0.00754	10	0.754	0.676	
	D	0.98677	0.98011	0.00666	10	0.666	CV	
	E	0.97117	0.96387	0.00730	10	0.730		
56%	A	0.98128	0.97351	0.00777	10	0.777	AVG DRY	
	B	0.96523	0.95855	0.00668	10	0.668	WEIGHT (mg)	
	C	1.02168	1.01256	0.00912	10	0.912	0.804	
	D	1.05008	1.04269	0.00739	10	0.739	CV	
	E	1.01560	1.00636	0.00924	10	0.924		
75%	A	1.00372	0.99668	0.00704	10	0.704	AVG DRY	
	B	1.01797	1.01042	0.00755	10	0.755	WEIGHT (mg)	
	C	1.00554	0.99650	0.00904	10	0.904	0.808	
	D	1.02353	1.01576	0.00777	10	0.777	CV	
	E	1.00391	0.99492	0.00899	10	0.899		
100%	A	1.01978	1.01024	0.00954	10	0.954	AVG DRY	
	B	1.02228	1.01416	0.00812	10	0.812	WEIGHT (mg)	
	C	1.01146	1.00364	0.00782	10	0.782	0.878	
	D	1.02209	1.01414	0.00795	10	0.795	CV	
	E	1.02277	1.01228	0.01049	10	1.049	13.41	

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA# K1408005, FATHEAD MINNOW GROWTH CHRONIC, 8-19-14
File: C:\COPYTO~1\TOXSTAT\FHGGROWTH. Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.201

W = 0.948

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# K1408005, FATHEAD MINNOW GROWTH CHRONIC, 8-19-14
File: C:\COPYTO~1\TOXSTAT\FHGGROWTH. Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 1.89

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# K1408005, FATHEAD MINNOW GROWTH CHRONIC, 8-19-14
 FILE: C:\COPYTO~1\TOXSTAT\FHGROWTH.
 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.7090	0.7090
1	CONTROL	2	0.6140	0.6140
1	CONTROL	3	0.7920	0.7920
1	CONTROL	4	0.7050	0.7050
1	CONTROL	5	0.6380	0.6380
2	32 % EFFLUENT	1	0.6880	0.6880
2	32 % EFFLUENT	2	0.7130	0.7130
2	32 % EFFLUENT	3	0.7540	0.7540
2	32 % EFFLUENT	4	0.6170	0.6170
2	32 % EFFLUENT	5	0.5600	0.5600
3	42 % EFFLUENT	1	0.5720	0.5720
3	42 % EFFLUENT	2	0.6560	0.6560
3	42 % EFFLUENT	3	0.7540	0.7540
3	42 % EFFLUENT	4	0.6660	0.6660
3	42 % EFFLUENT	5	0.7300	0.7300
4	56 % EFFLUENT	1	0.7770	0.7770
4	56 % EFFLUENT	2	0.6680	0.6680
4	56 % EFFLUENT	3	0.9120	0.9120
4	56 % EFFLUENT	4	0.7390	0.7390
4	56 % EFFLUENT	5	0.9240	0.9240
5	75 % EFFLUENT	1	0.7040	0.7040
5	75 % EFFLUENT	2	0.7550	0.7550
5	75 % EFFLUENT	3	0.9040	0.9040
5	75 % EFFLUENT	4	0.7770	0.7770
5	75 % EFFLUENT	5	0.8990	0.8990
6	100 % EFFLUENT	1	0.9540	0.9540
6	100 % EFFLUENT	2	0.8120	0.8120
6	100 % EFFLUENT	3	0.7820	0.7820
6	100 % EFFLUENT	4	0.7950	0.7950
6	100 % EFFLUENT	5	1.0490	1.0490

AA# K1408005, FATHEAD MINNOW GROWTH CHRONIC, 8-19-14
 File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.193	0.039	4.609
Within (Error)	24	0.201	0.008	
Total	29	0.394		

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

AA# K1408005, FATHEAD MINNOW GROWTH CHRONIC, 8-19-14

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	0.692	0.692		
2	32 % EFFLUENT	0.666	0.666	0.435	
3	42 % EFFLUENT	0.676	0.676	0.276	
4	56 % EFFLUENT	0.804	0.804	-1.942	
5	75 % EFFLUENT	0.808	0.808	-2.008	
6	100 % EFFLUENT	0.878	0.878	-3.228	

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

AA# K1408005, FATHEAD MINNOW GROWTH CHRONIC, 8-19-14

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	0.137	19.7	0.025
3	42 % EFFLUENT	5	0.137	19.7	0.016
4	56 % EFFLUENT	5	0.137	19.7	-0.112
5	75 % EFFLUENT	5	0.137	19.7	-0.116
6	100 % EFFLUENT	5	0.137	19.7	-0.187

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- 18/20/22 - 14

Lab Number/s
K1408005

Analyst: RH
 Test Start - Date/Time: 8-19-14, 1430
 Test Stop - Date/Time: 8-26-14, 1000

Conc	1	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		%	Day	A	B	C	D	E	F	G	H				
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	2	1	0	0	0	0	1	0	3	0	7	10	0.7	RH
	4	4	0	0	8	2	5	6	0	5	8	38	10	3.8	RH
	5	7	8	5	6	2	1	0	8	4	6	47	10	4.7	RH
	6	3	0	8	2	7	6	10	0	3	0	39	10	3.9	RH
	7	0	2	9	1	4	0	7	8	9	0	40	10	4.0	RH
	8														
	Total	16	11	22	17	15	12	24	16	24	14	171		Avg. = 17.1	
														C.V. = 27.5	

Conc	4	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		%	Day	A	B	C	D	E	F	G	H				
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	1	0	2	0	0	1	0	0	0	0	4	10	0.4	RH
	4	0	0	0	1	8	7	6	4	6	5	37	10	3.7	RH
	5	5	6	7	11	2	4	2	1	0	5	43	10	4.3	RH
	6	12	6	3	4	0	2	3	5	9	3	47	10	4.7	RH
	7	0	0	5	6	0	0	10	0	0	3	24	10	2.4	RH
	8												10	0.0	
	Total	18	12	17	22	10	14	21	10	15	16	155		Avg. = 15.5	
														C.V. = 26.9	

Conc	2	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		%	Day	A	B	C	D	E	F	G	H				
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	2	2	0	1	0	0	2	7	10	0.7	RH
	4	0	5	0	0	1	8	9	3	1	7	34	10	3.4	RH
	5	7	0	3	9	6	1	2	0	6	4	38	10	3.8	RH
	6	1	4	12	0	1	6	2	0	0	3	29	10	2.9	RH
	7	7	5	2	0	9	1	3	5	9	4	45	10	4.5	RH
	8														
	Total	15	14	17	11	19	16	17	8	16	20	153		Avg. = 15.3	
														C.V. = 23.5	

Conc	5	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		%	Day	A	B	C	D	E	F	G	H				
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	2	1	1	0	0	0	1	0	0	0	5	10	0.5	RH
	4	4	4	8	3	0	0	1	2	8	7	37	10	3.7	RH
	5	6	0	5	3	3	6	0	5	0	3	31	10	3.1	RH
	6	0	3	0	5	6	3	7	3	9	2	38	10	3.8	RH
	7	7	5	9	2	1	1	3	3	2	6	39	10	3.9	RH
	8												10	0.0	RH
	Total	19	13	23	13	10	10	12	13	19	18	150		Avg. = 15.0	
														C.V. = 29.5	

Conc	3	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		%	Day	A	B	C	D	E	F	G	H				
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	3	3	0	0	1	1	0	0	0	0	8	10	0.8	RH
	4	8	2	5	0	0	2	4	0	6	8	35	10	3.5	RH
	5	0	1	5	12	7	0	0	6	2	3	36	10	3.6	RH
	6	2	6	5	0	2	4	12	6	0	3	40	10	4.0	RH
	7	1	6	2	5	6	5	4	5	0	9	43	10	4.3	RH
	8														
	Total	14	18	17	17	16	12	20	17	8	23	162		Avg. = 16.2	
														C.V. = 25.7	

Conc	6	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		%	Day	A	B	C	D	E	F	G	H				
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	2	0	1	0	0	2	1	6	10	0.6	RH
	4	1	0	0	8	6	5	2	3	4	5	34	10	3.4	RH
	5	0	4	11	1	2	10	9	8	5	3	53	10	5.3	RH
	6	0	8	2	7	1	4	3	6	4	0	35	10	3.5	RH
	7	11	1	5	3	8	0	0	1	2	7	38	10	3.8	RH
	8												10	0.0	RH
	Total	12	13	18	21	17	20	14	18	17	16	166		Avg. = 16.6	
														C.V. = 17.6	

AA # K1408005, C.DUBIA CHRONIC, REPRODUCCION, 8-19-14
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 # K1408005, C.DUBIA CHRONIC, REPRODUCCION, 8-19-14
File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 2.34

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	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
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	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	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	0	
2	42	10	0	
3	56	10	0	
4	75	10	0	
5	100	10	0	

TITLE: AA # K1408005, C.DUBIA CHRONIC, REPRODUCCION, 8-19-14
 FILE: C:\COPYTO~1\TOXSTAT\C.DUB
 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	16.0000	16.0000
1	CONTROL	2	11.0000	11.0000
1	CONTROL	3	22.0000	22.0000
1	CONTROL	4	17.0000	17.0000
1	CONTROL	5	15.0000	15.0000
1	CONTROL	6	12.0000	12.0000
1	CONTROL	7	24.0000	24.0000
1	CONTROL	8	16.0000	16.0000
1	CONTROL	9	24.0000	24.0000
1	CONTROL	10	14.0000	14.0000
2	32 % EFFLUENT	1	15.0000	15.0000
2	32 % EFFLUENT	2	14.0000	14.0000
2	32 % EFFLUENT	3	17.0000	17.0000
2	32 % EFFLUENT	4	11.0000	11.0000
2	32 % EFFLUENT	5	19.0000	19.0000
2	32 % EFFLUENT	6	16.0000	16.0000
2	32 % EFFLUENT	7	17.0000	17.0000
2	32 % EFFLUENT	8	8.0000	8.0000
2	32 % EFFLUENT	9	16.0000	16.0000
2	32 % EFFLUENT	10	20.0000	20.0000
3	42 % EFFLUENT	1	14.0000	14.0000
3	42 % EFFLUENT	2	18.0000	18.0000
3	42 % EFFLUENT	3	17.0000	17.0000
3	42 % EFFLUENT	4	17.0000	17.0000
3	42 % EFFLUENT	5	16.0000	16.0000
3	42 % EFFLUENT	6	12.0000	12.0000
3	42 % EFFLUENT	7	20.0000	20.0000
3	42 % EFFLUENT	8	17.0000	17.0000
3	42 % EFFLUENT	9	8.0000	8.0000
3	42 % EFFLUENT	10	23.0000	23.0000
4	56 % EFFLUENT	1	18.0000	18.0000
4	56 % EFFLUENT	2	12.0000	12.0000
4	56 % EFFLUENT	3	17.0000	17.0000
4	56 % EFFLUENT	4	22.0000	22.0000
4	56 % EFFLUENT	5	10.0000	10.0000
4	56 % EFFLUENT	6	14.0000	14.0000
4	56 % EFFLUENT	7	21.0000	21.0000
4	56 % EFFLUENT	8	10.0000	10.0000

4	56 % EFFLUENT	9	15.0000	15.0000
4	56 % EFFLUENT	10	16.0000	16.0000
5	75 % EFFLUENT	1	19.0000	19.0000
5	75 % EFFLUENT	2	13.0000	13.0000
5	75 % EFFLUENT	3	23.0000	23.0000
5	75 % EFFLUENT	4	13.0000	13.0000
5	75 % EFFLUENT	5	10.0000	10.0000
5	75 % EFFLUENT	6	10.0000	10.0000
5	75 % EFFLUENT	7	12.0000	12.0000
5	75 % EFFLUENT	8	13.0000	13.0000
5	75 % EFFLUENT	9	19.0000	19.0000
5	75 % EFFLUENT	10	18.0000	18.0000
6	100 % EFFLUENT	1	12.0000	12.0000
6	100 % EFFLUENT	2	13.0000	13.0000
6	100 % EFFLUENT	3	18.0000	18.0000
6	100 % EFFLUENT	4	21.0000	21.0000
6	100 % EFFLUENT	5	17.0000	17.0000
6	100 % EFFLUENT	6	20.0000	20.0000
6	100 % EFFLUENT	7	14.0000	14.0000
6	100 % EFFLUENT	8	18.0000	18.0000
6	100 % EFFLUENT	9	17.0000	17.0000
6	100 % EFFLUENT	10	16.0000	16.0000

AA # K1408005, C.DUBIA CHRONIC, REPRODUCCION, 8-19-14

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

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	33.350	6.670	0.410
Within (Error)	54	879.500	16.287	
Total	59	912.850		

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

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

AA # K1408005, C.DUBIA CHRONIC, REPRODUCCION, 8-19-14

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	17.100	17.100		
2	32 % EFFLUENT	15.300	15.300	0.997	
3	42 % EFFLUENT	16.200	16.200	0.499	
4	56 % EFFLUENT	15.500	15.500	0.887	
5	75 % EFFLUENT	15.000	15.000	1.164	
6	100 % EFFLUENT	16.600	16.600	0.277	

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

AA # K1408005, C.DUBIA CHRONIC, REPRODUCTION, 8-19-14
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	4.169	24.4	1.800
3	42 % EFFLUENT	10	4.169	24.4	0.900
4	56 % EFFLUENT	10	4.169	24.4	1.600
5	75 % EFFLUENT	10	4.169	24.4	2.100
6	100 % EFFLUENT	10	4.169	24.4	0.500

APPENDIX E

Organism History

AQUATOX, INC.

416 TWIN POINTS ROAD
HOT SPRINGS, ARKANSAS 71913

501-520-0560

Ar Analyst ceyl

TEST ORGANISM HISTORY

Ar Analyst

DATE SHIPPED 8/19/14 CLIENT ~~Aquatox~~

Purchase Order #: _____ Reyer

SPECIES: Pimephales promelas

Quantity Shipped: 550

Age: hatched 8/18/14 15-16oz

Brood Stock Source: Anderson Farms, AR CST

Culture Water: Groundwater

Hardness (Mg/l CaCO3): 160

Dissolved Oxygen (Mg/l): 8.2

Temperature (°C): 25.1°C

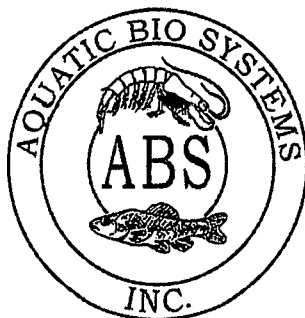
Feeding: AT

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:

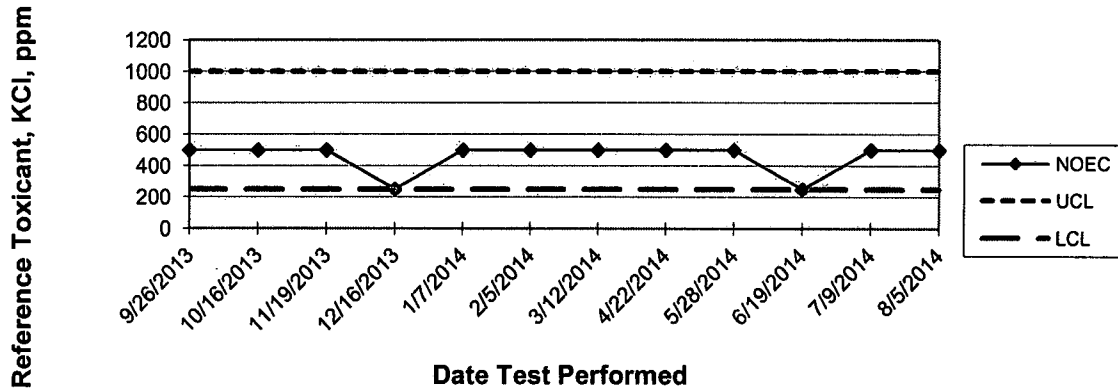


Facility Supervisor

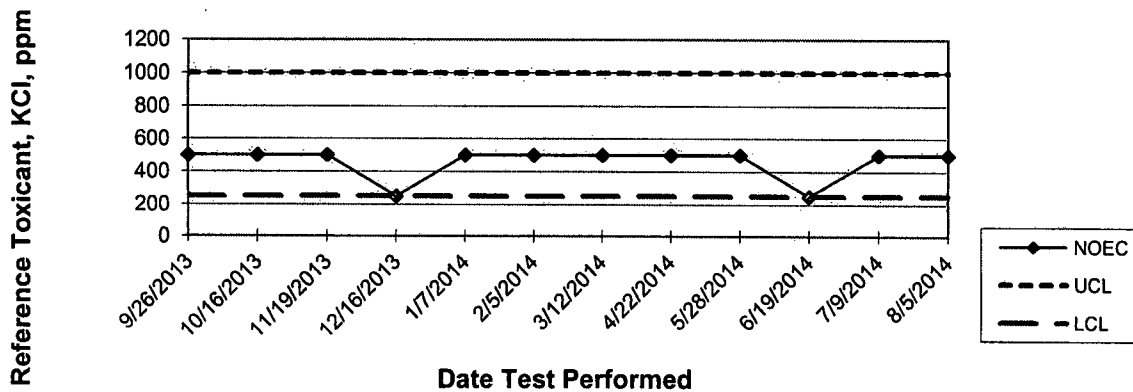
APPENDIX F

Quality Assurance Charts

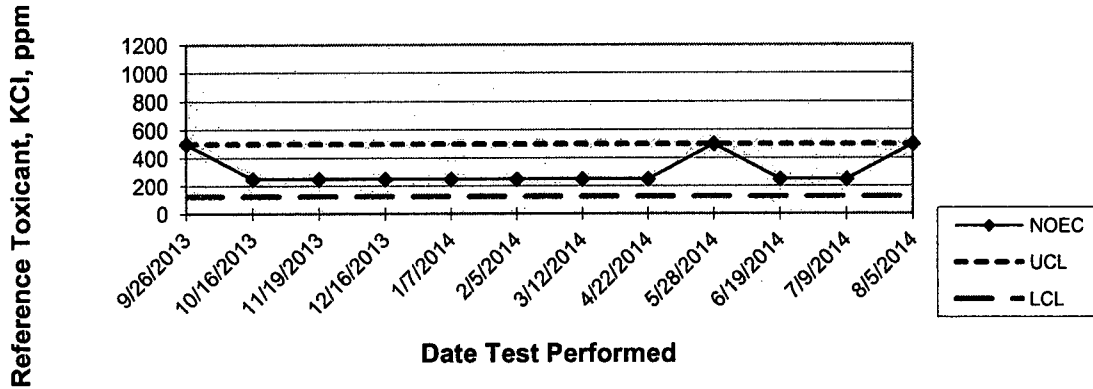
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW SURVIVAL 7 Day
QUALITY ASSURANCE



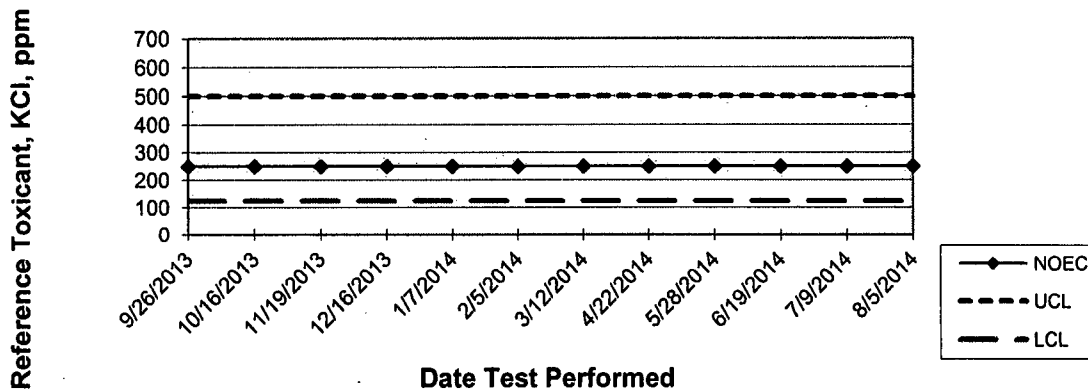
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW GROWTH 7 Day
QUALITY ASSURANCE



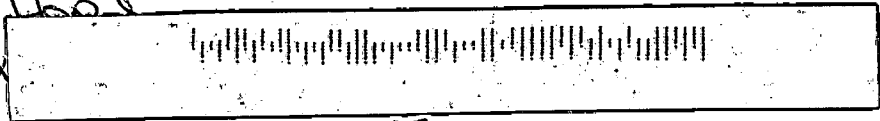
ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE



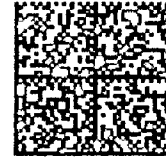
ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE



City of Hope
PO Box



Hope, AR 71802-0667



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