

Arkansas Analytical, Inc.

Toxicity Test Results

City of Hope
Permit Number: AR0038466
AFIN # 29-00034
Second 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
Hope, Arkansas 71802

Prepared by: Arkansas Analytical, Inc.
11701 I-30, Bldg 1 Suite 115
Little Rock, Arkansas 72209
Lab Number K1405004

Wednesday, May 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 second 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:	5-18-14, 0600	5-19-14, 0600
Sample #2:	5-20-14, 0600	5-21-14, 0600
Sample #3:	5-22-14, 0600	5-23-14, 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:	5-19-14, 1423	4
Sample #2:	5-22-14, 0955	4
Sample #3:	5-23-14, 1310	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 < 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	15.9	X	
At least 60% of surviving females should have produced 3 broods	100%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	12.0%	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	0.589	X	
The percent coefficient of variation between replicates must be 40% or less for growth	12.8%	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> 4/22/14 – 4/29/14		<i>Pimephales promelas</i> 4/22/14 – 4/29/14	
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)	17.1	%CV survival (critical dilution)	0%
%CV Reproduction (critical dilution)	33.9%	Mean dry weight (critical dilution) in milligrams	0.717
		%CV growth (critical dilution)	8.54%
PMSD Reproduction	24.2%	PMSD Growth	16.1%

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)

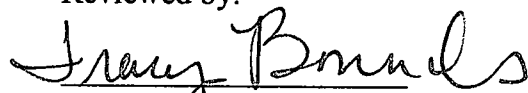
The permit issued to the City of Hope, specifies the **critical dilution is 100% effluent**. The effluent samples **did not** exhibit lethal or sublethal effects at the critical dilution, and, as such, **passed** both portions of the test.

Biomonitoring Analysts:

Ryan Hudgin

Kenneth Rood

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:	5-18-14, 0600	5-19-14, 0600
Sample #2:	5-20-14, 0600	5-21-14, 0600
Sample #3:	5-22-14, 0600	5-23-14, 0600

Test initiated (date, time): 5-20-14, 1330 Test terminated (date, time): 5-27-14, 1330

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%	90	100	100	100	100		100	98	98	
42%	100	100	100	100	100		100	100	100	
56%	100	100	90	100	100		100	100	98	
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%	0.559	0.519	0.718	0.579	0.571		0.589	12.80
32%	0.602	0.665	0.686	0.669	0.583		0.641	
42%	0.710	0.708	0.631	0.575	0.717		0.668	
56%	0.739	0.688	0.522	0.704	0.707		0.672	
75%	0.719	0.803	0.750	0.715	0.732		0.744	
100%	0.725	0.672	0.806	0.732	0.648		0.717	8.54

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)= _____ 12.8 %

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:	5-18-14, 0600	5-19-14, 0600
Sample #2:	5-20-14, 0600	5-21-14, 0600
Sample #3:	5-22-14, 0600	5-23-14, 0600

Test initiated (date, time): 5-20-14, 1100 Test terminated (date, time): 5-27-14, 1200

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	19	11	18	17	25
B	19	12	17	12	12	20
C	16	18	13	13	15	12
D	15	14	16	15	17	20
E	13	16	11	18	15	20
F	14	17	19	16	13	20
G	17	10	23	15	22	15
H	18	10	9	19	11	10
I	17	17	12	17	19	7
J	14	14	17	15	18	22
Mean	15.9	14.7	14.8	15.8	15.9	17.1
Mean/surviving female	15.9	14.7	14.8	15.8	15.9	17.1
CV%*	12.0					33.9

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)= 33.9 %

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 # 17104.0001B - K1405004A

CLIENT # ISOZO

P.O.# _____

STANDARD METHODS PRESERVATION PER EPA 40 CFR

C4= 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

PROJECT NO:

SAMPLER(S) NAME: (PRINT)

11051342

City of Hope

ISCO Auto Sampler

SAMPLE ID AND/OR COLLECTION LOCATION	START DATE/TIME	END DATE/TIME	COMP GRAB	FIELD ANALYSIS				D.O (W)	CONTAINER TYPE	ANALYSIS REQUIRED	
	DATE/TIME	DATE/TIME	GRAB	pH	TEMP	FLOW	CL2	D.O(P)	PRESERVATIVE		
<u>WPFE</u>	<u>6 AM 5/18/14</u>	<u>6 AM 5/19/14</u>	<u>24H Comp</u>		<u>CA</u>				<u>8 1/2 gal. Plastic</u>	<u>W.E.T</u>	
METHOD OF SHIPMENT (CIRCLE)	FIELD CALIBRATION RECORD			NOTES/COMMENTS/OBSERVATIONS							
FED EX WALK IN SRA UPS OTHER	pH 7			<u>Temp @ Lab 6°</u>							
	pH 4										
	pH 10										
	D.O										
TYPE OF SAMPLE(S): (CIRCLE)				RELINQUISHED BY: <u>Carol Smith</u>				RECEIVED BY: <u>Sydney James</u>			
WATER SOIL W/W SLUDGE OTHER				DATE/TIME: <u>5/19/14 @ 12:30pm</u>				DATE/TIME: <u>5-19-14 14:23</u>			

AR Analytical - 5/19/14

	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"/>
Reservation Confirmed:	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Received on Ice:	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Temperature on Receipt:	<input checked="" type="checkbox"/>	<input type="checkbox"/>

4°C - HHT #2

RELINQUISHED BY: Carol Smith DATE/TIME: 5/19/14 @ 12:30pm RECEIVED BY: Sammy Riddle DATE/TIME: 5-19-14 12:30

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 # 17164.0002B

CLIENT # _____

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

11091362

NAME OF COMPANY, CITY, OR PROJECT

PROJECT NO:

SAMPLER(S) NAME: (PRINT)

Hope City of

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	
	WPFE	5/20/14	5/21/14							4gal C4	K1405004-B WET
		6am	6am								

METHOD OF SHIPMENT (CIRCLE) FED EX WALK IN SRA UPS OTHER	FIELD CALIBRATION RECORD			NOTES/COMMENTS/OBSERVATIONS
	pH 7	7.00		All containers at C4
	pH 4	4.01		
	pH 10	10.00		
	D.O			
TYPE OF SAMPLE(S): (CIRCLE) WATER SOIL W/W SLUDGE OTHER				FIELD ANALYSIS CONDUCTED BY: (CIRCLE) SRA CLIENT

	Yes	No
Custody Seals:	X	
Containers Correct:	X	
COC/Labels Agree:	X	
Preservation Confirmed:	X	
Received on Ice:	X	
Temperature on Receipt:	4°C	HHT#2

RELINQUISHED BY: [Signature] DATE/TIME: 5-22-14 0955 RECEIVED BY: [Signature] DATE/TIME: 5-21-14 1334

RELINQUISHED BY: Amanda Johnson DATE/TIME: 5/22/14 0955 RECEIVED BY(LAB): [Signature] DATE/TIME: 5-21-14 1645

APPENDIX B

Effluent and Dilution Water Data

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Fathead Minnow

Lab # / Sample ID **K1405004** Test Start (Date/Time) **5-20-14** **1330**
 Client: **Hope** Test End (Date/Time) **5-27-14** **1330**

Day of Test

		1	2	3	4	5	6	7 Alt	notes
Control	1415	5-20	5-21	5-22	5-23	5-24	5-25	5-26	8.6
D.O. (mg/L)	INITIAL	8.4	8.6	8.5	8.7	8.5	8.5	8.6	
	FINAL	7.2	7.9	7.5	8.1	8.3	7.5	7.5	
pH (s.u.)	INITIAL	7.8	8.0	8.0	7.9	7.7	7.9	7.8	
	FINAL	7.9	8.0	7.8	7.7	7.7	8.2	7.7	
temp (C)	INITIAL	22	23	24	24	22	22	23	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		58							
HARDNESS (mg/L)		88							
CONDUCTIVITY (umhc)		339							
CHLORINE (mg/L)		<0.05							
CONC: 32	1418								
D.O. (mg/L)	INITIAL	8.5	8.8	8.7	8.5	8.5	8.5	8.6	
	FINAL	7.1	7.6	7.6	7.8	8.1	7.2	7.4	
pH (s.u.)	INITIAL	7.7	8.1	7.8	7.8	7.4	7.9	8.0	
	FINAL	7.9	8.1	7.7	7.8	8.0	7.8	8.2	
temp (C)	INITIAL	23	23	24	24	24	22	23	
	FINAL	25	25	25	25	25	25	25	
CONC: 42									
D.O. (mg/L)	INITIAL	8.4	8.9	8.7	8.2	8.2	8.4	8.5	
	FINAL	6.9	7.5	7.7	7.7	8.1	7.1	7.3	
pH (mg/L)	INITIAL	7.5	8.0	7.7	7.8	7.5	7.8	7.7	
	FINAL	7.9	8.1		7.9	8.0	8.0	8.0	
temp (C)	INITIAL	23	23	24	24	25	22	23	
	FINAL	25	25	25	25	25	25	25	
CONC: 76									
D.O. (mg/L)	INITIAL	8.2	8.9	9.1	8.1	8.2	8.3	8.6	
	FINAL	7.1	7.5	7.6	7.6	7.9	7.3	7.3	
pH (s.u.)	INITIAL	7.8	7.9	7.4	7.6	7.6	7.8	7.7	
	FINAL	8.0	8.0	7.8	7.9	8.1	7.9	7.8	
temp (C)	INITIAL	23	24	24	24	24	23	23	
	FINAL	25	25	25	25	25	25	25	
CONC: 75									
D.O. (mg/L)	INITIAL	8.3	9.0	8.9	8.3	8.1	8.1	8.5	
	FINAL	7.0	7.5	7.6	7.6	7.9	7.4	7.2	
pH (s.u.)	INITIAL	7.6	8.0	7.5	7.7	7.4	7.8	7.9	
	FINAL	7.9	8.1	7.9	8.0	8.1	8.0	7.7	
temp (C)	INITIAL	23	24	25	25	27	23	24	
	FINAL	25	25	25	25	25	25	25	
CONC: 100									
D.O. (mg/L)	INITIAL	8.4	9.0	9.0	7.8	7.7	8.0	8.5	
	FINAL	7.1	7.7	7.5	7.6	7.9	7.1	7.5	
pH (s.u.)	INITIAL	7.5	8.0	7.5	7.8	7.8	7.9	7.9	
	FINAL	7.9	8.2	7.6	7.1	8.2	7.9	7.7	
temp (C)	INITIAL	23	24	25	25	28	23	24	
	FINAL	25	25	25	25	25	25	25	
CONC: 100 %									
ALKALINITY (mg/L)		100			110		130		
HARDNESS (mg/L)		190			56		64		
CONDUCTIVITY (umhc)		648			711		739		
CHLORINE (mg/L)		<0.05							

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Ceriodaphnia Dubia

Lab # / Sample ID K1405004

Test Start (Date/Time) 5-20-14 1100

Client: Hope

Test End (Date/Time) 5-27-14 1200

Day of Test

		1	2	3	4	5	6	7	notes
Control	<u>MHS</u>	<u>5-20</u>	<u>5-21</u>	<u>5-22</u>	<u>5-23</u>	<u>5-24</u>	<u>5-25</u>	<u>5-26</u>	
D.O. (mg/L)	INITIAL	<u>8.4</u>	<u>8.6</u>	<u>8.5</u>	<u>8.7</u>	<u>8.5</u>	<u>8.5</u>	<u>8.6</u>	
	FINAL	<u>8.2</u>	<u>8.1</u>	<u>8.0</u>	<u>7.9</u>	<u>8.0</u>	<u>7.8</u>	<u>7.7</u>	
pH (s.u.)	INITIAL	<u>7.8</u>	<u>8.0</u>	<u>8.0</u>	<u>7.9</u>	<u>7.7</u>	<u>7.9</u>	<u>7.8</u>	
	FINAL	<u>7.9</u>	<u>7.5</u>	<u>8.0</u>	<u>7.7</u>	<u>7.8</u>	<u>7.8</u>	<u>8.0</u>	
temp (C)	INITIAL	<u>22</u>	<u>23</u>	<u>24</u>	<u>24</u>	<u>22</u>	<u>22</u>	<u>23</u>	
	FINAL	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	
ALKALINITY (mg/L)		<u>58</u>							
HARDNESS (mg/L)		<u>88</u>							
CONDUCTIVITY (umhd)		<u>339</u>							
CHLORINE (mg/L)		<u><0.05</u>							
CONC:	<u>32</u>								
D.O. (mg/L)	INITIAL	<u>8.5</u>	<u>8.8</u>	<u>8.7</u>	<u>8.5</u>	<u>8.5</u>	<u>8.5</u>	<u>8.6</u>	
	FINAL	<u>8.3</u>	<u>8.1</u>	<u>8.0</u>	<u>7.7</u>	<u>8.1</u>	<u>7.9</u>	<u>7.6</u>	
pH (s.u.)	INITIAL	<u>7.7</u>	<u>8.1</u>	<u>7.8</u>	<u>7.8</u>	<u>7.4</u>	<u>7.9</u>	<u>8.0</u>	
	FINAL	<u>7.8</u>	<u>8.0</u>	<u>7.9</u>	<u>7.8</u>	<u>7.6</u>	<u>8.1</u>	<u>8.0</u>	
temp (C)	INITIAL	<u>23</u>	<u>23</u>	<u>24</u>	<u>24</u>	<u>24</u>	<u>22</u>	<u>23</u>	
	FINAL	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	
CONC:	<u>42</u>								
D.O. (mg/L)	INITIAL	<u>8.4</u>	<u>8.9</u>	<u>8.7</u>	<u>8.2</u>	<u>8.2</u>	<u>8.4</u>	<u>8.5</u>	
	FINAL	<u>8.4</u>	<u>8.0</u>	<u>8.1</u>	<u>8.0</u>	<u>8.0</u>	<u>7.7</u>	<u>7.8</u>	
pH (mg/L)	INITIAL	<u>7.5</u>	<u>8.0</u>	<u>7.7</u>	<u>7.8</u>	<u>7.5</u>	<u>7.8</u>	<u>7.7</u>	
	FINAL	<u>7.8</u>	<u>7.8</u>	<u>7.9</u>	<u>8.0</u>	<u>7.5</u>	<u>8.0</u>	<u>7.8</u>	
temp (C)	INITIAL	<u>23</u>	<u>23</u>	<u>24</u>	<u>24</u>	<u>25</u>	<u>22</u>	<u>23</u>	
	FINAL	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	
CONC:	<u>56</u>								
D.O. (mg/L)	INITIAL	<u>8.2</u>	<u>8.9</u>	<u>9.1</u>	<u>8.1</u>	<u>8.2</u>	<u>8.3</u>	<u>8.6</u>	
	FINAL	<u>8.2</u>	<u>8.1</u>	<u>8.0</u>	<u>8.1</u>	<u>7.8</u>	<u>7.8</u>	<u>7.9</u>	
pH (s.u.)	INITIAL	<u>7.8</u>	<u>7.9</u>	<u>7.4</u>	<u>7.6</u>	<u>7.6</u>	<u>7.8</u>	<u>7.7</u>	
	FINAL	<u>8.0</u>	<u>8.1</u>	<u>8.1</u>	<u>7.6</u>	<u>7.7</u>	<u>7.9</u>	<u>7.9</u>	
temp (C)	INITIAL	<u>23</u>	<u>24</u>	<u>24</u>	<u>24</u>	<u>26</u>	<u>23</u>	<u>23</u>	
	FINAL	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	
CONC:	<u>75</u>								
D.O. (mg/L)	INITIAL	<u>8.3</u>	<u>9.0</u>	<u>8.9</u>	<u>8.3</u>	<u>8.1</u>	<u>8.1</u>	<u>8.5</u>	
	FINAL	<u>8.2</u>	<u>8.1</u>	<u>8.1</u>	<u>7.6</u>	<u>8.0</u>	<u>7.5</u>	<u>7.7</u>	
pH (s.u.)	INITIAL	<u>7.6</u>	<u>8.0</u>	<u>7.5</u>	<u>7.7</u>	<u>7.4</u>	<u>7.8</u>	<u>7.9</u>	
	FINAL	<u>7.8</u>	<u>8.2</u>	<u>7.9</u>	<u>7.6</u>	<u>7.6</u>	<u>7.8</u>	<u>7.8</u>	
temp (C)	INITIAL	<u>23</u>	<u>24</u>	<u>25</u>	<u>25</u>	<u>27</u>	<u>23</u>	<u>24</u>	
	FINAL	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	
CONC:	<u>106</u>								
D.O. (mg/L)	INITIAL	<u>8.4</u>	<u>9.0</u>	<u>9.0</u>	<u>7.8</u>	<u>7.7</u>	<u>8.0</u>	<u>8.5</u>	
	FINAL	<u>8.0</u>	<u>8.1</u>	<u>8.2</u>	<u>8.0</u>	<u>7.9</u>	<u>7.4</u>	<u>7.7</u>	
pH (s.u.)	INITIAL	<u>7.5</u>	<u>8.0</u>	<u>7.5</u>	<u>7.8</u>	<u>7.8</u>	<u>7.9</u>	<u>7.9</u>	
	FINAL	<u>7.6</u>	<u>8.1</u>	<u>8.2</u>	<u>7.7</u>	<u>7.8</u>	<u>8.0</u>	<u>7.7</u>	
temp (C)	INITIAL	<u>23</u>	<u>24</u>	<u>25</u>	<u>25</u>	<u>28</u>	<u>23</u>	<u>24</u>	
	FINAL	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	<u>25</u>	
CONC:	<u>100 %</u>	<u>A</u>	<u>A</u>	<u>A</u>	<u>B</u>	<u>B</u>	<u>C</u>	<u>C</u>	
ALKALINITY (mg/L)		<u>100</u>			<u>110</u>		<u>130</u>		
HARDNESS (mg/L)		<u>190</u>			<u>56</u>		<u>61</u>		
CONDUCTIVITY (umhd)		<u>648</u>			<u>711</u>		<u>739</u>		
CHLORINE (mg/L)		<u><0.05</u>							

APPENDIX C

Fathead minnow raw data and statistics

SURVIVAL DATA FOR LARVAL SURVIVAL AND GROWTH TEST (CHRONIC)

LAB #: K1405004				TEST START		DATE	5/20/14	TIME	1330			
CLIENT: Hope				TEST END		DATE	5/27/14	TIME	1330			
ANALYST: RH				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%	98.0%	4.56
	B	10	10	10	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	9	9	9	9	9	9	90%	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	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%	98.0%	
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	10	10	10	10	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	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	RH	RH	KR	KR	RH	RH			
DATE:		5/20/14	5/21/14	5/22/14	5/23/14	5/24/14	5/25/14	5/26/14	5/27/14			
TIME:		1330	1530	1030	1030	930	1130	930	1330			

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

REMARKS:

Pimephales promelas

FATHEAD MINNOW

TEST 1000.0

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:		K1405004				TEST DATES (BEGIN / END):		5/20 - 27/14	
CLIENT:		Hope				WEIGHING DATE / TIME:		5/28/2014 1330	
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	1.00414	0.99855	0.00559	10	0.559		AVG DRY	
	B	0.99708	0.99189	0.00519	10	0.519		WEIGHT (mg)	
	C	1.00638	0.99920	0.00718	10	0.718			0.589
	D	1.00485	0.99906	0.00579	10	0.579		CV	
	E	0.98633	0.98062	0.00571	10	0.571			12.83
32%	A	1.00178	0.99576	0.00602	10	0.602		AVG DRY	
	B	1.03029	1.02364	0.00665	10	0.665		WEIGHT (mg)	
	C	1.03501	1.02815	0.00686	10	0.686			0.641
	D	1.01684	1.01015	0.00669	10	0.669		CV	
	E	1.00952	1.00369	0.00583	10	0.583			
42%	A	1.01110	1.00400	0.00710	10	0.710		AVG DRY	
	B	1.01170	1.00462	0.00708	10	0.708		WEIGHT (mg)	
	C	0.99904	0.99273	0.00631	10	0.631			0.668
	D	1.01427	1.00852	0.00575	10	0.575		CV	
	E	1.01938	1.01221	0.00717	10	0.717			
56%	A	1.01871	1.01132	0.00739	10	0.739		AVG DRY	
	B	1.02208	1.01520	0.00688	10	0.688		WEIGHT (mg)	
	C	1.02644	1.02122	0.00522	10	0.522			0.672
	D	1.01126	1.00422	0.00704	10	0.704		CV	
	E	1.01537	1.00830	0.00707	10	0.707			
75%	A	1.00444	0.99725	0.00719	10	0.719		AVG DRY	
	B	1.01157	1.00354	0.00803	10	0.803		WEIGHT (mg)	
	C	0.96038	0.95288	0.00750	10	0.750			0.744
	D	0.99843	0.99128	0.00715	10	0.715		CV	
	E	0.95877	0.95145	0.00732	10	0.732			
100%	A	1.02252	1.01527	0.00725	10	0.725		AVG DRY	
	B	1.00643	0.99971	0.00672	10	0.672		WEIGHT (mg)	
	C	1.00641	0.99835	0.00806	10	0.806			0.717
	D	1.02130	1.01398	0.00732	10	0.732		CV	
	E	1.01843	1.01195	0.00648	10	0.648			8.54

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA# K1405004, FATHEAD MINNOW GROWTH CHRONIC, 5-20-14

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

Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.097

W = 0.989

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# K1405004, FATHEAD MINNOW GROWTH CHRONIC, 5-20-14

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

Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 3.43

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# K1405004, FATHEAD MINNOW GROWTH CHRONIC, 5-20-14
 FILE: C:\COPYTO~1\TOXSTAT\FHGROWTH.
 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.5590	0.5590
1	CONTROL	2	0.5190	0.5190
1	CONTROL	3	0.7180	0.7180
1	CONTROL	4	0.5790	0.5790
1	CONTROL	5	0.5710	0.5710
2	32 % EFFLUENT	1	0.6020	0.6020
2	32 % EFFLUENT	2	0.6650	0.6650
2	32 % EFFLUENT	3	0.6860	0.6860
2	32 % EFFLUENT	4	0.6690	0.6690
2	32 % EFFLUENT	5	0.5830	0.5830
3	42 % EFFLUENT	1	0.7100	0.7100
3	42 % EFFLUENT	2	0.7080	0.7080
3	42 % EFFLUENT	3	0.6310	0.6310
3	42 % EFFLUENT	4	0.5750	0.5750
3	42 % EFFLUENT	5	0.7170	0.7170
4	56 % EFFLUENT	1	0.7390	0.7390
4	56 % EFFLUENT	2	0.6880	0.6880
4	56 % EFFLUENT	3	0.5220	0.5220
4	56 % EFFLUENT	4	0.7040	0.7040
4	56 % EFFLUENT	5	0.7070	0.7070
5	75 % EFFLUENT	1	0.7190	0.7190
5	75 % EFFLUENT	2	0.8030	0.8030
5	75 % EFFLUENT	3	0.7500	0.7500
5	75 % EFFLUENT	4	0.7150	0.7150
5	75 % EFFLUENT	5	0.7320	0.7320
6	100 % EFFLUENT	1	0.7250	0.7250
6	100 % EFFLUENT	2	0.6720	0.6720
6	100 % EFFLUENT	3	0.8060	0.8060
6	100 % EFFLUENT	4	0.7320	0.7320
6	100 % EFFLUENT	5	0.6480	0.6480

AA# K1405004, FATHEAD MINNOW GROWTH CHRONIC, 5-20-14
 File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.075	0.015	3.723
Within (Error)	24	0.097	0.004	
Total	29	0.171		

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

AA# K1405004, FATHEAD MINNOW GROWTH CHRONIC, 5-20-14

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	0.589	0.589		
2	32 % EFFLUENT	0.641	0.641	-1.291	
3	42 % EFFLUENT	0.668	0.668	-1.970	
4	56 % EFFLUENT	0.672	0.672	-2.064	
5	75 % EFFLUENT	0.744	0.744	-3.854	
6	100 % EFFLUENT	0.717	0.717	-3.176	

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

AA# K1405004, FATHEAD MINNOW GROWTH CHRONIC, 5-20-14

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	0.095	16.1	-0.052
3	42 % EFFLUENT	5	0.095	16.1	-0.079
4	56 % EFFLUENT	5	0.095	16.1	-0.083
5	75 % EFFLUENT	5	0.095	16.1	-0.155
6	100 % EFFLUENT	5	0.095	16.1	-0.127

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

SURVIVAL AND REPRODUCTION TEST

Ceriodaphnia dubia

Discharger: Hope AFIN # 29-00034												Lab Number/s				Analyst: RH																
Location: Outfall 001												K1405004				Test Start - Date/Time: 5-20-14, 1100																
Date Sample Collected: 5-19/21/23 -14																Test Stop - Date/Time: 5-27-14, 1200																
Conc	1	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	Conc	4	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	
%	Day	A	B	C	D	E	F	G	H	I	J					%	Day	A	B	C	D	E	F	G	H	I	J					
MHS	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH	56%	1	0	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH		2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH	
	3	1	1	0	0	0	0	0	0	3	0	2	7	10	0.7		RH	3	0	0	0	0	2	0	0	2	0	1	5	10	0.5	RH
	4	4	3	6	5	2	0	7	1	1	2	31	10	3.1	RH		4	1	1	0	8	3	4	3	7	6	2	35	10	3.5	RH	
	5	7	2	1	1	5	6	4	0	3	6	35	10	3.5	RH		5	8	5	0	7	4	6	4	3	2	9	48	10	4.8	RH	
	6	0	5	9	4	0	5	0	9	5	0	37	10	3.7	RH		6	6	2	2	0	8	5	2	5	3	3	36	10	3.6	RH	
	7	4	8	0	5	6	3	6	5	8	4	49	10	4.9	RH		7	3	4	11	0	1	1	6	2	6	0	34	10	3.4	RH	
	8																	8											10	0.0		
Total		16	19	16	15	13	14	17	18	17	14	159		Avg. = 15.9	Total		18	12	13	15	18	16	15	19	17	15	158		Avg. = 15.8			
														C.V. = 12.0														C.V. = 14.2				
Conc	2	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	Conc	5	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	
%	Day	A	B	C	D	E	F	G	H	I	J				%	Day	A	B	C	D	E	F	G	H	I	J						
32%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH	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		2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH	
	3	3	2	0	0	0	0	0	0	0	0	0	5	10	0.5		RH	3	0	0	3	0	0	1	1	1	0	0	6	10	0.6	RH
	4	0	5	4	5	0	0	2	1	1	3	21	10	2.1	RH		4	6	5	5	7	0	0	8	0	2	3	36	10	3.6	RH	
	5	6	3	9	5	8	2	0	0	6	4	43	10	4.3	RH		5	4	7	0	0	10	3	8	6	8	3	49	10	4.9	RH	
	6	9	1	5	4	6	10	8	6	5	5	59	10	5.9	RH		6	2	0	6	2	5	0	3	4	7	2	31	10	3.1	RH	
	7	1	1	0	0	2	5	0	3	5	2	19	10	1.9	RH		7	5	0	1	8	0	9	2	0	2	10	37	10	3.7	RH	
	8																8											10	0.0	RH		
Total		19	12	18	14	16	17	10	10	17	14	147		Avg. = 14.7	Total		17	12	15	17	15	13	22	11	19	18	159		Avg. = 15.9			
														C.V. = 22.0														C.V. = 21.3				
Conc	3	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	Conc	6	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	
%	Day	A	B	C	D	E	F	G	H	I	J				%	Day	A	B	C	D	E	F	G	H	I	J						
42%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH	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		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	0	1	10	0.1		RH	3	0	0	1	1	0	0	2	0	0	1	5	10	0.5	RH
	4	5	0	0	2	0	7	6	0	1	3	24	10	2.4	RH		4	8	6	4	0	5	5	7	0	3	2	40	10	4.0	RH	
	5	2	6	5	0	3	8	7	9	5	0	45	10	4.5	RH		5	1	4	7	7	1	9	3	8	4	6	50	10	5.0	RH	
	6	0	10	3	10	8	3	8	0	6	7	55	10	5.5	RH		6	9	6	0	9	10	0	3	0	0	8	45	10	4.5	RH	
	7	3	1	5	4	0	1	2	0	0	7	23	10	2.3	RH		7	7	4	0	3	4	6	0	2	0	5	31	10	3.1	RH	
	8																8											10	0.0	RH		
Total		11	17	13	16	11	19	23	9	12	17	148		Avg. = 14.6	Total		25	20	12	20	20	20	15	10	7	22	171		Avg. = 17.1			
														C.V. = 29.3														C.V. = 33.9				

AA # K1405004, C. DUBIA CHRONIC, REPRODUCCION, 5-20-14
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 # K1405004, C. DUBIA CHRONIC, REPRODUCCION, 5-20-14
File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 13.94

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 # K1405004, C. DUBIA CHRONIC, REPRODUCCION, 5-20-14
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	19.0000	19.0000
1	CONTROL	3	16.0000	16.0000
1	CONTROL	4	15.0000	15.0000
1	CONTROL	5	13.0000	13.0000
1	CONTROL	6	14.0000	14.0000
1	CONTROL	7	17.0000	17.0000
1	CONTROL	8	18.0000	18.0000
1	CONTROL	9	17.0000	17.0000
1	CONTROL	10	14.0000	14.0000
2	32 % EFFLUENT	1	19.0000	19.0000
2	32 % EFFLUENT	2	12.0000	12.0000
2	32 % EFFLUENT	3	18.0000	18.0000
2	32 % EFFLUENT	4	14.0000	14.0000
2	32 % EFFLUENT	5	16.0000	16.0000
2	32 % EFFLUENT	6	17.0000	17.0000
2	32 % EFFLUENT	7	10.0000	10.0000
2	32 % EFFLUENT	8	10.0000	10.0000
2	32 % EFFLUENT	9	17.0000	17.0000
2	32 % EFFLUENT	10	14.0000	14.0000
3	42 % EFFLUENT	1	11.0000	11.0000
3	42 % EFFLUENT	2	17.0000	17.0000
3	42 % EFFLUENT	3	13.0000	13.0000
3	42 % EFFLUENT	4	16.0000	16.0000
3	42 % EFFLUENT	5	11.0000	11.0000
3	42 % EFFLUENT	6	19.0000	19.0000
3	42 % EFFLUENT	7	23.0000	23.0000
3	42 % EFFLUENT	8	9.0000	9.0000
3	42 % EFFLUENT	9	12.0000	12.0000
3	42 % EFFLUENT	10	17.0000	17.0000
4	56 % EFFLUENT	1	18.0000	18.0000
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	18.0000	18.0000
4	56 % EFFLUENT	6	16.0000	16.0000
4	56 % EFFLUENT	7	15.0000	15.0000
4	56 % EFFLUENT	8	19.0000	19.0000

4	56 %	EFFLUENT	9	17.0000	17.0000
4	56 %	EFFLUENT	10	15.0000	15.0000
5	75 %	EFFLUENT	1	17.0000	17.0000
5	75 %	EFFLUENT	2	12.0000	12.0000
5	75 %	EFFLUENT	3	15.0000	15.0000
5	75 %	EFFLUENT	4	17.0000	17.0000
5	75 %	EFFLUENT	5	15.0000	15.0000
5	75 %	EFFLUENT	6	13.0000	13.0000
5	75 %	EFFLUENT	7	22.0000	22.0000
5	75 %	EFFLUENT	8	11.0000	11.0000
5	75 %	EFFLUENT	9	19.0000	19.0000
5	75 %	EFFLUENT	10	18.0000	18.0000
6	100 %	EFFLUENT	1	25.0000	25.0000
6	100 %	EFFLUENT	2	20.0000	20.0000
6	100 %	EFFLUENT	3	12.0000	12.0000
6	100 %	EFFLUENT	4	20.0000	20.0000
6	100 %	EFFLUENT	5	20.0000	20.0000
6	100 %	EFFLUENT	6	20.0000	20.0000
6	100 %	EFFLUENT	7	15.0000	15.0000
6	100 %	EFFLUENT	8	10.0000	10.0000
6	100 %	EFFLUENT	9	7.0000	7.0000
6	100 %	EFFLUENT	10	22.0000	22.0000

AA # K1405004, C. DUBIA CHRONIC, REPRODUCCION, 5-20-14

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

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	38.600	7.720	0.557
Within (Error)	54	748.000	13.852	
Total	59	786.600		

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

Since F < Critical F FAIL TO REJECT Ho: All equal

AA # K1405004, C. DUBIA CHRONIC, REPRODUCCION, 5-20-14

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

Transform: NO TRANSFORMATION

DUNNETT'S TEST

TABLE 1 OF 2

Ho: Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	15.900	15.900		
2	32 % EFFLUENT	14.700	14.700	0.721	
3	42 % EFFLUENT	14.800	14.800	0.661	
4	56 % EFFLUENT	15.800	15.800	0.060	
5	75 % EFFLUENT	15.900	15.900	0.000	
6	100 % EFFLUENT	17.100	17.100	-0.721	

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

AA # K1405004, C. DUBIA CHRONIC, REPRODUCCION, 5-20-14

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

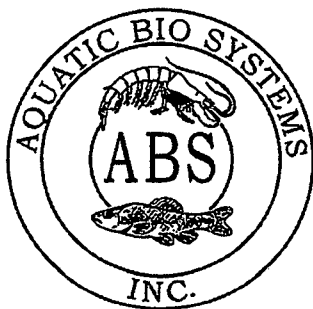
DUNNETT'S TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	10			
2	32 % EFFLUENT	10	3.845	24.2	1.200
3	42 % EFFLUENT	10	3.845	24.2	1.100
4	56 % EFFLUENT	10	3.845	24.2	0.100
5	75 % EFFLUENT	10	3.845	24.2	0.000
6	100 % EFFLUENT	10	3.845	24.2	-1.200

APPENDIX E

Organism History

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

AQUATOX, INC.

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

TEST ORGANISM HISTORY

DATE SHIPPED 5/20/14 CLIENT AR Analytical

Purchase Order #: _____ Reyn

SPECIES: Pimephales promelas

Quantity Shipped: 600

Age: Hatched 5/18/14 1500

Brood Stock Source: Anderson Farms, AR

Culture Water: Groundwater 160

Hardness (Mg/l CaCO3): _____

Dissolved Oxygen (Mg/l): 8.2

Temperature (°C): 25.1°C

Feeding: After

Comments: _____

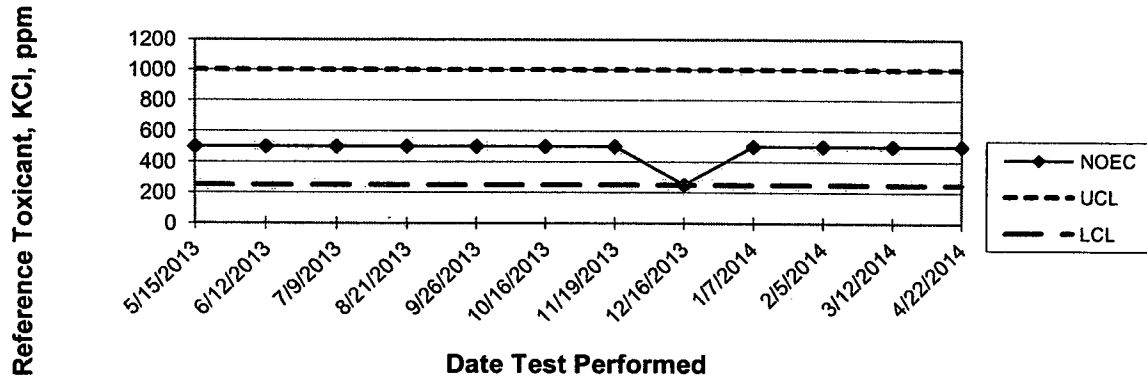
Shipped Via: Federal Express UPS Overnight Shuttle

Packaged By: _____

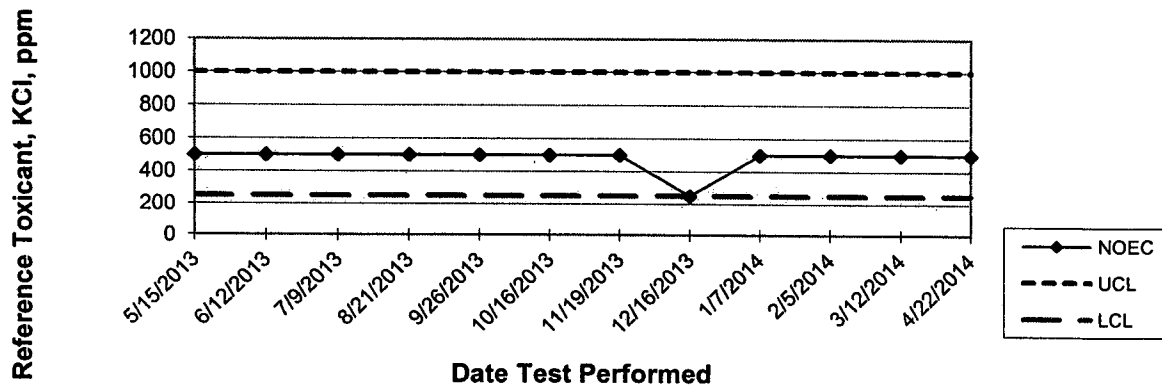
APPENDIX F

Quality Assurance Charts

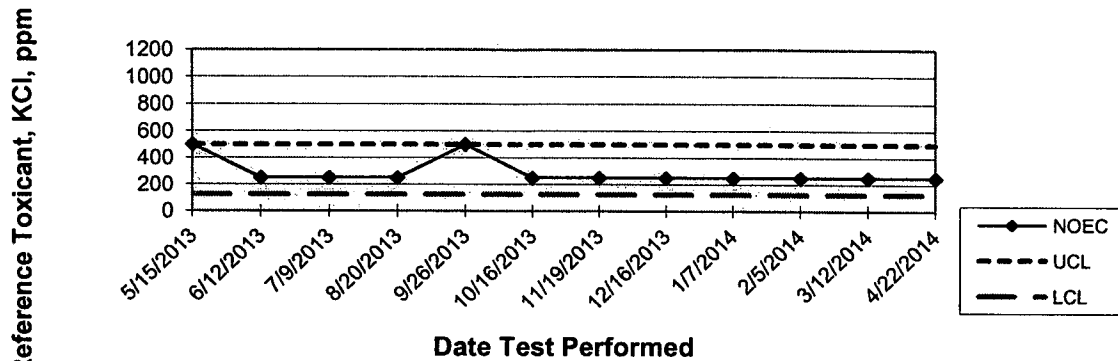
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW SURVIVAL 7 Day
QUALITY ASSURANCE



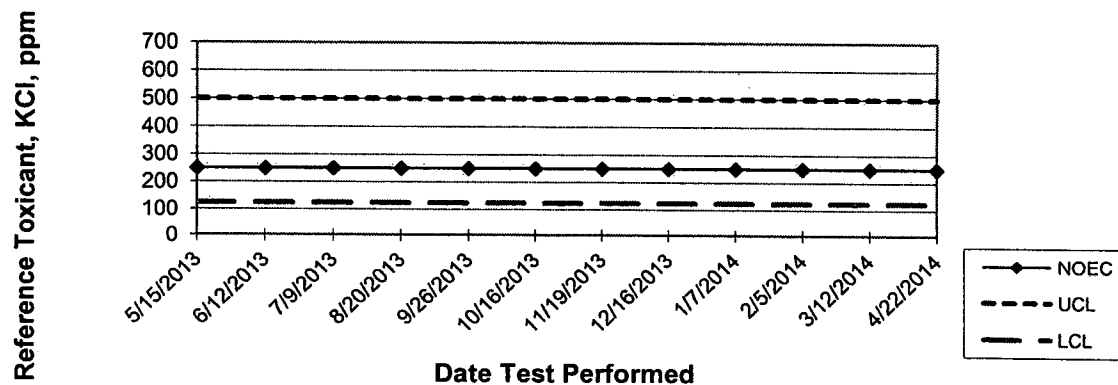
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW GROWTH 7 Day
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE



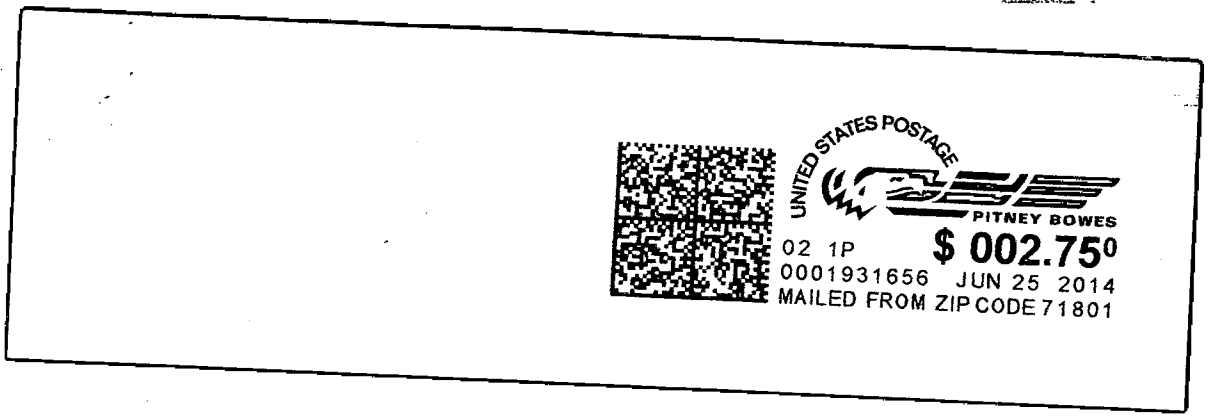
ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE



City of Hope
PO BOX 667

Hope, AR

71802-0667



ADEQ

NPDES Enforcement Section

5301 Northshore Drive

North Little Rock, AR

72218-5317