Arkansas Analytical, Inc.

Toxicity Test Results

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

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

Ceriodaphnia dubia, Survival and Reproduction Test
Test 1002.0

Prepared for: **Kim Holston**
City of Hope
P.O. Box 667
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Prepared by: Arkansas Analytical, Inc.
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Little Rock, Arkansas 72209
Lab Number K1502003

Wednesday, February 25, 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 first 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:	2-15-15, 0600	2-16-15, 0600
Sample #2:	2-17-15, 0600	2-18-15, 0600
Sample #3:	2-19-15, 0600	2-20-15, 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:	2-17-15, 1415	1
Sample #2:	2-19-15, 0908	4
Sample #3:	2-20-15, 1641	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	16.5	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	19.4%	X	

TEST ACCEPTANCE CRITERIA for *Pimephales promelas*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	94%	X	
The percent coefficient of variation between replicates must be 40% or less for survival	5.83%	X	
Minimum of 0.25 mg average dry weight of surviving controls	0.671	X	
The percent coefficient of variation between replicates must be 40% or less for growth	6.03%	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

Ceriodaphnia dubia 2/11/15 – 2/18/15		*Pimephales promelas* 2/11/15 – 2/18/15	
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

Ceriodaphnia dubia		*Pimephales promelas*	
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)	16.8	%CV survival (critical dilution)	0.00%
%CV Reproduction (critical dilution)	25.8%	Mean dry weight (critical dilution) in milligrams	0.783
		%CV growth (critical dilution)	5.50%
PMSD Reproduction	25.3%	PMSD Growth	18.9%

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 / Hallie Freyaldenhoven

Reviewed by:

Tracy Bounds

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

Test initiated (date, time): 2-17-15, 1445 Test terminated (date, time): 2-24-15, 1410

Dilution water used: Moderately Hard Synthetic

DATA TABLE FOR FATHEAD MINNOW SURVIVAL

	Percent Survival in Replicate Chambers						**Mean Percent Survival**			
Effluent Conc %	A	B	C	D	E		24 hours	48 hours	7 days	CV %
0%	100	90	100	90	90		100	96	94	5.83
32%	100	100	90	90	100		98	98	96	
42%	90	100	100	90	90		98	98	94	
56%	80	100	100	100	100		100	98	96	
75%	90	80	100	100	90		98	96	92	
100%	100	100	100	100	100		100	100	100	0.00

DATA TABLE FOR GROWTH OF FATHEAD MINNOWS

Average Dry Weight in milligrams in replicate chambers

Effluent Conc %	A	B	C	D	E		Mean Dry Weight	CV%
0%	0.625	0.653	0.672	0.669	0.735		0.671	6.03
32%	0.624	0.836	0.806	0.650	0.697		0.723	
42%	0.695	0.641	0.736	0.746	0.609		0.685	
56%	0.773	0.776	0.808	0.821	0.832		0.802	
75%	0.809	0.690	0.535	0.974	0.818		0.765	
100%	0.773	0.755	0.801	0.737	0.847		0.783	5.50

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)= _____**6.03**_____%

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

Test initiated (date, time): 2-17-15, 1520 Test terminated (date, time): 2-24-15, 1315

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	13	16	13	11	14	17
B	18	19	7	10	16	10
C	12	15	16	16	19	16
D	17	18	13	19	21	21
E	15	18	15	22	7	12
F	17	6	16	18	16	19
G	21	9	11	18	19	13
H	21	19	16	15	13	24
I	18	10	21	11	15	20
J	13	12	13	20	12	16
Mean	16.5	14.2	14.1	16.0	15.2	16.8
Mean/surviving female	16.5	14.2	14.1	16.0	15.2	16.8
CV%*	19.4					25.8

X=Dead Adult; M= Male (Not considered in statistics)
*Coefficient of Variation = standard deviation/ mean * 100; CV% calculation based on young per surviving female

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING
Ceriodaphnia dubia SURVIVAL AND REPRODUCTION

Permittee: City of Hope

PERCENT SURVIVAL

PERCENT EFFLUENT	0%	32%	42%	56%	75%	100%
Time of Reading: 24 HOURS	100	100	100	100	100	100
48 HOURS	100	100	100	100	100	100
Test termination	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)= _____**25.8**_____%

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 # 17888.0001B
CLIENT # 15020
P.O.#_____

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

AME OF COMPANY, CITY, OR PROJECT: City of Hope
PROJECT NO:
SAMPLER(S) NAME: (PRINT) ISCO Automatic Sampler
110913k2

MPLE O:	SAMPLE ID AND/ OR COLLECTION LOCATION	START DATE/TIME	END DATE/TIME	COMP GRAB	FIELD ANALYSIS pH	TEMP	FLOW	CL2	D,O (W) D.O(P)	CONTAINER TYPE PRESERVATIVE	ANALYSIS REQUIRED
	WPFE	6AM 2-15-15	6AM 2-16-15	24 HC							K1502-003 BIO A

METHOD OF SHIPMENT (CIRCLE)
FED EX WALK IN SRA UPS OTHER

TYPE OF SAMPLE(S): (CIRCLE)
WATER SOIL W/W SLUDGE OTHER

FIELD CALIBRATION RECORD

pH 7			
pH 4			
pH 10			
D.O			

Samples Received at Arkansas Analytical
Relinquished By: Sorrells
Date/Time: 2-17-15, 1415
Received By: Sydney James

	Yes	No
Custody Seals:	✓	
Containers Correct:		
COC/Labels Agree:		
Received on Ice:		
Temperature on Receipt:	1°C	
Temperature Gun ID:	HHT # 2	

FIELD ANALYSIS CONDUCTED BY: (CIRCLE) SRA CLIENT

2-17-15
RELINQUISHED BY: Calvin Ware DATE/TIME: 2-18-15 11:20A RECEIVED BY: Danny Riddle DATE/TIME:
RELINQUISHED BY: DATE/TIME: RECEIVED BY(LAB): DATE/TIME: 2/17/15 11:40

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 # [redacted] 17888.0028
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: West Plant
SAMPLER(S) NAME: (PRINT) ISCO MACHINE

SAMPLE NO:	SAMPLE ID AND/ OR COLLECTION LOCATION	START DATE/TIME	END DATE/TIME	COMP GRAB	FIELD ANALYSIS pH	TEMP	FLOW	CL2	D,O (W) D.O(P)	CONTAINER TYPE PRESERVATIVE	ANALYSIS REQUIRED
											K1502003-B
	W P F E	2-17-15 6 Am	2-18-15 6 Am	24hr C						6, 1/2 gal. p C4	W.E.T.

Samples Received at Arkansas Analytical
Relinquished By: Sorrells
Date/Time: 2-19-15 0905
Received By: Amanda Fowler

	Yes	No
Custody Seals:	X	
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)	FIELD CALIBRATION RECORD			
FED EX WALK IN SRA UPS OTHER	pH 7	7.00		
	pH 4	4.01		
	pH 10	10.00		
	D.O			
TYPE OF SAMPLE(S): (CIRCLE)				
WATER SOIL W/W SLUDGE OTHER				

NOTE: All c

COC rec'd w/ whiteout on ft - AJF 2/19/15

FIELD ANALYSIS CONDUCTED BY: (CIRCLE) SRA CLIENT

RELINQUISHED BY: Jim Sorrells DATE/TIME: RECEIVED BY: DATE/TIME: 2.18.15 1311
RELINQUISHED BY: Jim Sorrells DATE/TIME: RECEIVED BY(LAB): DATE/TIME: 2.18.15 1700

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 # 17888.0003B
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: West Plant
SAMPLER(S) NAME: (PRINT) ISCO MACHINE

SAMPLE NO:	SAMPLE ID AND/ OR COLLECTION LOCATION	START DATE/TIME	END DATE/TIME	COMP GRAB	FIELD ANALYSIS pH	TEMP	FLOW	CL2	D,O (W) D.O(P)	CONTAINER TYPE PRESERVATIVE	ANALYSIS REQUIRED
	WPFE	2-19-15 6 AM	2-20-15 6 AM	24hr C						6, 1/2 gal. p C4	K1502 - W.E.T. 003C

COC received w/ White cut on it - 2/20/15 - 8

Samples Received at Arkansas Analytical
Relinquished By: Sorrells
Date/Time: 2-20-15, 1641
Received By: Sydney Jaimes

	Yes	No
Custody Seals:	X	
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	7.00		
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

RELINQUISHED BY: _____ DATE/TIME: _____ RECEIVED BY: _____ DATE/TIME: _____

RELINQUISHED BY: Kim Holston DATE/TIME: 2-20-15 1330 RECEIVED BY(LAB): Danny Riddle DATE/TIME: 2/20/15 1330

APPENDIX B

Effluent and Dilution Water Data

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING — Fathead Minnow

Lab # / Sample ID: K1502003 — Test Start (Date/Time): 2-17-15 1445

Client: Hope — Test End (Date/Time): 2-24-15 1410

		Day of Test							
		1	2	3	4	5	6	7	notes
Control	mH5	2-17	2-18	2-19	2-20	2-21	2-22	2-23	
D.O. (mg/L)	INITIAL	8.6	8.8	8.9	8.9	8.7	8.9	8.7	
	FINAL	8.2	7.4	7.7	8.7	8.1	7.7	7.3	
pH (s.u.)	INITIAL	7.8	8.2	8.3	8.1	8.2	8.2	8.3	
	FINAL	7.5	7.4	7.5	7.4	7.6	8.1	7.5	
temp (C)	INITIAL	21	22	22	22	21	20	21	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		58							
HARDNESS (mg/L)		87							
CONDUCTIVITY (umhc		396							
CHLORINE (mg/L)		<0.05							
CONC:	32								
D.O. (mg/L)	INITIAL	8.9	8.9	8.8	8.8	8.8	9.0	8.7	
	FINAL	8.3	7.3	7.7	7.8	8.1	7.9	6.9	
pH (s.u)	INITIAL	7.6	7.9	8.1	7.9	8.2	8.0	8.2	
	FINAL	7.8	7.7	7.8	7.5	7.9	8.1	7.5	
temp (C)	INITIAL	23	22	23	22	21	23	23	
	FINAL	25	25	25	25	25	25	25	
CONC:	42								
D.O. (mg/L)	INITIAL	8.8	9.0	8.9	8.9	8.8	8.9	8.8	
	FINAL	8.1	7.4	7.7	7.8	7.9	8.4	7.5	
pH (mg/L)	INITIAL	7.6	7.7	7.9	8.0	8.2	8.0	8.1	
	FINAL	8.0	8.0	8.1	7.9	8.0	8.1	7.6	
temp (C)	INITIAL	24	23	25	21	20	25	25	
	FINAL	25	25	25	25	25	25	25	
CONC:	56								
D.O. (mg/L)	INITIAL	9.1	9.0	9.1	8.8	8.8	8.9	9.0	
	FINAL	8.2	7.1	7.7	7.9	7.9	8.3	7.4	
pH (s.u.)	INITIAL	7.6	7.6	7.8	7.9	8.3	7.9	8.0	
	FINAL	8.1	8.0	8.4	8.0	8.0	7.9	7.7	
temp (C)	INITIAL	25	23	25	21	20	26	26	
	FINAL	25	25	25	25	25	25	25	
CONC:	75								
D.O. (mg/L)	INITIAL	9.0	9.0	8.9	9.1	8.8	9.4	9.1	
	FINAL	8.0	7.2	7.7	8.0	7.9	8.2	7.3	
pH (s.u.)	INITIAL	7.6	7.6	7.7	7.8	8.3	7.8	7.9	
	FINAL	8.2	8.2	8.4	8.1	8.0	7.9	7.8	
temp (C)	INITIAL	25	24	26	20	20	27	28	
	FINAL	25	25	25	25	25	25	25	
CONC:	100								
D.O. (mg/L)	INITIAL	9.1	9.1	9.0	9.1	8.9	8.7	9.0	
	FINAL	8.1	7.5	7.5	8.0	7.8	8.3	7.3	
pH (s.u.)	INITIAL	7.7	7.5	7.6	7.8	8.3	7.5	7.8	
	FINAL	8.3	8.2	8.4	8.1	8.1	7.8	7.8	
temp (C)	INITIAL	27	25	27	20	20	27	28	
	FINAL	25	25	25	25	25	25	25	
CONC:	100 %	A	A	A	B	B	C	C	
ALKALINITY (mg/L)		198			82		104		
HARDNESS (mg/L)		36			46		48		
CONDUCTIVITY (umhc		1023			601		753		
CHLORINE (mg/L)		<0.05							

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING									Ceriodaphnia Dubia
Lab # / Sample ID		K1502003			Test Start (Date/Time)		2-17-15	1520	
Client:		Hope			Test End (Date/Time)		2-24-15	1315	
		Day of Test							
		1	2	3	4	5	6	7	notes
Control	MHS	2-17	2-18	2-19	2-20	2-21	2-22	2-23	
D.O. (mg/L)	INITIAL	8.6	8.8	8.9	8.9	8.7	8.9	8.7	
	FINAL	8.4	8.4	8.3	9.2	9.1	8.2	8.3	
pH (s.u.)	INITIAL	7.8	8.2	8.3	8.1	8.2	8.2	8.3	
	FINAL	7.6	7.6	7.7	8.0	8.4	7.7	8.2	
temp (C)	INITIAL	21	22	22	22	21	20	21	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		58							
HARDNESS (mg/L)		82							
CONDUCTIVITY (umho		396							
CHLORINE (mg/L)		<0.05							
CONC:	32								
D.O. (mg/L)	INITIAL	8.9	8.9	8.8	8.8	8.8	9.0	8.7	
	FINAL	8.4	8.6	8.4	9.1	9.2	8.5	8.2	
pH (s.u)	INITIAL	7.6	7.9	8.1	7.9	8.2	8.0	8.2	
	FINAL	7.9	7.8	7.8	8.3	8.4	7.7	8.2	
temp (C)	INITIAL	23	22	23	22	21	23	23	
	FINAL	25	25	25	25	25	25	25	
CONC:	42								
D.O. (mg/L)	INITIAL	8.8	9.0	8.9	8.9	8.8	8.9	8.8	
	FINAL	8.4	8.4	8.3	8.8	9.0	8.3	8.0	
pH (mg/L)	INITIAL	7.6	7.7	7.9	8.0	8.2	8.0	8.1	
	FINAL	8.1	8.1	8.2	8.4	8.4	8.0	8.1	
temp (C)	INITIAL	24	23	25	21	20	25	25	
	FINAL	25	25	25	25	25	25	25	
CONC:	56								
D.O. (mg/L)	INITIAL	9.1	9.0	9.1	8.8	8.8	8.9	9.0	
	FINAL	8.5	8.4	8.3	9.0	9.2	8.3	8.1	
pH (s.u.)	INITIAL	7.6	7.6	7.8	7.9	8.3	7.9	8.0	
	FINAL	8.2	8.3	8.3	8.4	8.4	8.1	8.1	
temp (C)	INITIAL	25	23	25	21	20	26	26	
	FINAL	25	25	25	25	25	25	25	
CONC:	75								
D.O. (mg/L)	INITIAL	9.0	9.0	8.9	9.1	8.8	8.9	9.1	
	FINAL	8.4	8.3	8.3	9.2	9.1	8.3	8.0	
pH (s.u.)	INITIAL	7.6	7.6	7.7	7.8	8.3	7.8	7.9	
	FINAL	8.3	8.4	8.5	8.5	8.9	8.2	8.1	
temp (C)	INITIAL	25	24	26	20	20	27	28	
	FINAL	25	25	25	25	25	25	25	
CONC:	100								
D.O. (mg/L)	INITIAL	9.1	9.1	9.0	9.1	8.9	8.7	9.0	
	FINAL	8.5	8.4	8.3	9.0	9.2	8.3	8.1	
pH (s.u.)	INITIAL	7.7	7.5	7.6	7.8	8.3	7.5	7.8	
	FINAL	8.4	8.4	8.5	8.5	8.5	8.3	8.1	
temp (C)	INITIAL	27	25	27	20	20	27	28	
	FINAL	25	25	25	25	25	25	25	
CONC:	**100 %**	A	A	A	B	B	C	C	
ALKALINITY (mg/L)		198			82		104		
HARDNESS (mg/L)		36			46		48		
CONDUCTIVITY (umho		1023			601		753		
CHLORINE (mg/L)		<0.05							

APPENDIX C

Fathead minnow raw data and statistics

Pimephales promelas **FATHEAD MINNOW**

SURVIVAL DATA FOR LARVAL SURVIVAL AND GROWTH TEST (CHRONIC)

LAB #: K1502003			TEST START		DATE	2/17/15	TIME	1445				
CLIENT: Hope			TEST END		DATE	2/24/15	TIME	1410				
ANALYST: RH/HF			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%		
	B	10	10	10	10	10	10	9	9	90%		
	C	10	10	10	10	10	10	10	10	100%	*94.0%*	*5.83*
MHS	D	10	10	9	9	9	9	9	9	90%		
	E	10	10	9	9	9	9	9	9	90%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	10	10	10	10	10	10	100%		
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	10	9	9	9	9	9	90%	*96.0%*	
32%	D	10	9	9	9	9	9	9	9	90%		
	E	10	10	10	10	10	10	10	10	100%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	9	9	9	9	9	9	9	90%		
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	10	10	10	10	10	10	100%	*94.0%*	
42%	D	10	10	10	10	10	10	9	9	90%		
	E	10	10	10	10	10	10	9	9	90%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	9	9	9	9	8	8	80%		
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	10	10	10	10	10	10	100%	*96.0%*	
56%	D	10	10	10	10	10	9	10	10	100%		
	E	10	10	10	10	10	10	10	10	100%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	9	9	9	9	9	9	90%		
	B	10	10	10	10	10	10	8	8	80%		
	C	10	10	10	10	10	10	10	10	100%	*92.0%*	
75%	D	10	10	10	10	10	10	10	10	100%		
	E	10	9	9	9	9	9	9	9	90%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	10	10	10	10	10	10	100%		
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	10	10	10	10	10	10	100%	*100.0%*	*0.00*
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	HF	HF	RH	RH			
DATE:		2/17/15	2/18/15	2/19/15	2/20/15	2/21/15	2/22/15	2/23/15	2/24/14			
TIME:		1445	1515	1335	1000	1600	1400	1115	1410			

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

REMARKS:

AA# K1502003,FATHEAD MINNOW SURV.,CHRONIC, 2-17-15
File: C:\COPYTO~1\TOXSTAT\FHSURV~1. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.236

W = 0.926

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# K1502003,FATHEAD MINNOW SURV.,CHRONIC, 2-17-15
File: C:\COPYTO~1\TOXSTAT\FHSURV~1. Transform: ARC SINE(SQUARE ROOT(Y))

Hartley's test for homogeneity of variance
Bartlett's test for homogeneity of variance

These two tests can not be performed because at least one group has
zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

TITLE: AA# K1502003,FATHEAD MINNOW SURV.,CHRONIC, 2-17-15
FILE: C:\COPYTO~1\TOXSTAT\FHSURV~1.
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	1.0000	1.4120
1	CONTROL	2	0.9000	1.2490
1	CONTROL	3	1.0000	1.4120
1	CONTROL	4	0.9000	1.2490
1	CONTROL	5	0.9000	1.2490
2	32 % EFFLUENT	1	1.0000	1.4120
2	32 % EFFLUENT	2	1.0000	1.4120
2	32 % EFFLUENT	3	0.9000	1.2490
2	32 % EFFLUENT	4	0.9000	1.2490
2	32 % EFFLUENT	5	1.0000	1.4120
3	42 % EFFLUENT	1	0.9000	1.2490
3	42 % EFFLUENT	2	1.0000	1.4120
3	42 % EFFLUENT	3	1.0000	1.4120
3	42 % EFFLUENT	4	0.9000	1.2490
3	42 % EFFLUENT	5	0.9000	1.2490
4	56 % EFFLUENT	1	0.8000	1.1071
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	0.9000	1.2490
5	75 % EFFLUENT	2	0.8000	1.1071
5	75 % EFFLUENT	3	1.0000	1.4120
5	75 % EFFLUENT	4	1.0000	1.4120
5	75 % EFFLUENT	5	0.9000	1.2490
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# K1502003,FATHEAD MINNOW SURV.,CHRONIC, 2-17-15
File: C:\COPYTO~1\TOXSTAT\FHSURV~1. Transform: ARC SINE(SQUARE ROOT(Y))

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	1.314				
2	32 % EFFLUENT	1.347	30.00	16.00	5.00	
3	42 % EFFLUENT	1.314	27.50	16.00	5.00	
4	56 % EFFLUENT	1.351	31.00	16.00	5.00	
5	75 % EFFLUENT	1.286	26.00	16.00	5.00	
6	100 % EFFLUENT	1.412	35.00	16.00	5.00	

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

Pimephales promelas **FATHEAD MINNOW** TEST 1000.0

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:	K1502003					TEST DATES (BEGIN / END):	2/17/15 - 2/24/15	
CLIENT:	Hope					WEIGHING DATE / TIME:	2/25/2015 1030	
ANALYSTS:	RH					DRYING TEMP (DEGREES C):	60	
SAMPLE ID:						DRYING TIME (HOURS):	24	
	REP #	FINAL DRY WEIGHT TIN+LARVAE (g)	INITIAL WEIGHT TIN (g)	TOTAL DRY WEIGHT OF LARVAE (g)	NUMBER OF LARVAE	DRY WEIGHT OF LARVAE (mg)		
CONTROL	A	0.99787	0.99162	0.00625	10	0.625	AVG DRY	
	B	1.00028	0.99375	0.00653	10	0.653	WEIGHT (mg)	
MHS	C	1.00692	1.00020	0.00672	10	0.672		0.671
	D	0.99995	0.99326	0.00669	10	0.669	CV	
	E	0.96665	0.95930	0.00735	10	0.735		6.03
CONC:	A	1.00621	0.99997	0.00624	10	0.624	AVG DRY	
	B	1.01051	1.00215	0.00836	10	0.836	WEIGHT (mg)	
32%	C	1.00442	0.99636	0.00806	10	0.806		0.723
	D	0.97286	0.96636	0.00650	10	0.650	CV	
	E	1.01363	1.00666	0.00697	10	0.697		
CONC:	A	1.01557	1.00862	0.00695	10	0.695	AVG DRY	
	B	1.00023	0.99382	0.00641	10	0.641	WEIGHT (mg)	
42%	C	0.99107	0.98371	0.00736	10	0.736		0.685
	D	1.00708	0.99962	0.00746	10	0.746	CV	
	E	0.99251	0.98642	0.00609	10	0.609		
CONC:	A	1.00532	0.99759	0.00773	10	0.773	AVG DRY	
	B	1.01455	1.00679	0.00776	10	0.776	WEIGHT (mg)	
56%	C	1.02379	1.01571	0.00808	10	0.808		0.802
	D	1.00845	1.00024	0.00821	10	0.821	CV	
	E	0.96877	0.96045	0.00832	10	0.832		
CONC:	A	0.96107	0.95298	0.00809	10	0.809	AVG DRY	
	B	0.98890	0.98200	0.00690	10	0.690	WEIGHT (mg)	
75%	C	1.01185	1.00650	0.00535	10	0.535		0.765
	D	1.02179	1.01205	0.00974	10	0.974	CV	
	E	1.00892	1.00074	0.00818	10	0.818		
CONC:	A	1.02578	1.01805	0.00773	10	0.773	AVG DRY	
	B	1.01659	1.00904	0.00755	10	0.755	WEIGHT (mg)	
100%	C	0.98466	0.97665	0.00801	10	0.801		0.783
	D	1.00374	0.99637	0.00737	10	0.737	CV	
	E	1.04626	1.03779	0.00847	10	0.847		5.50

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA# K1502003, FATHEAD MINNOW GROWTH CHRONIC, 2-17-15
File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.173

W = 0.955

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# K1502003, FATHEAD MINNOW GROWTH CHRONIC, 2-17-15
File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 15.95

Table Chi-square value = 15.09 (alpha = 0.01, df = 5)
Table Chi-square value = 11.07 (alpha = 0.05, df = 5)

Data FAIL B1 homogeneity test at 0.01 level. Try another transformation.

TITLE: AA# K1502003, FATHEAD MINNOW GROWTH CHRONIC, 2-17-15
FILE: C:\COPYTO~1\TOXSTAT\FHGROWTH.
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.6250	0.6250
1	CONTROL	2	0.6530	0.6530
1	CONTROL	3	0.6720	0.6720
1	CONTROL	4	0.6690	0.6690
1	CONTROL	5	0.7350	0.7350
2	32 % EFFLUENT	1	0.6240	0.6240
2	32 % EFFLUENT	2	0.8360	0.8360
2	32 % EFFLUENT	3	0.8060	0.8060
2	32 % EFFLUENT	4	0.6500	0.6500
2	32 % EFFLUENT	5	0.6970	0.6970
3	42 % EFFLUENT	1	0.6950	0.6950
3	42 % EFFLUENT	2	0.6410	0.6410
3	42 % EFFLUENT	3	0.7360	0.7360
3	42 % EFFLUENT	4	0.7460	0.7460

3	42 % EFFLUENT	5	0.6090	0.6090
4	56 % EFFLUENT	1	0.7730	0.7730
4	56 % EFFLUENT	2	0.7760	0.7760
4	56 % EFFLUENT	3	0.8080	0.8080
4	56 % EFFLUENT	4	0.8210	0.8210
4	56 % EFFLUENT	5	0.8320	0.8320
5	75 % EFFLUENT	1	0.8090	0.8090
5	75 % EFFLUENT	2	0.6900	0.6900
5	75 % EFFLUENT	3	0.5350	0.5350
5	75 % EFFLUENT	4	0.9740	0.9740
5	75 % EFFLUENT	5	0.8180	0.8180
6	100 % EFFLUENT	1	0.7730	0.7730
6	100 % EFFLUENT	2	0.7550	0.7550
6	100 % EFFLUENT	3	0.8010	0.8010
6	100 % EFFLUENT	4	0.7370	0.7370
6	100 % EFFLUENT	5	0.8470	0.8470

AA# K1502003, FATHEAD MINNOW GROWTH CHRONIC, 2-17-15
File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.072	0.014	1.986
Within (Error)	24	0.173	0.007	
Total	29	0.245		

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

AA# K1502003, FATHEAD MINNOW GROWTH CHRONIC, 2-17-15
File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	0.671	0.671		
2	32 % EFFLUENT	0.723	0.723	-0.964	
3	42 % EFFLUENT	0.685	0.685	-0.272	
4	56 % EFFLUENT	0.802	0.802	-2.441	
5	75 % EFFLUENT	0.765	0.765	-1.756	
6	100 % EFFLUENT	0.783	0.783	-2.080	

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

AA# K1502003, FATHEAD MINNOW GROWTH CHRONIC, 2-17-15
File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	5			
2	32 % EFFLUENT	5	0.127	18.9	-0.052
3	42 % EFFLUENT	5	0.127	18.9	-0.015
4	56 % EFFLUENT	5	0.127	18.9	-0.131
5	75 % EFFLUENT	5	0.127	18.9	-0.094
6	100 % EFFLUENT	5	0.127	18.9	-0.112

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

SURVIVAL AND REPRODUCTION TEST

Ceriodaphnia dubia

Discharger: Hope AFIN # 29-00034	Lab Number/s
Location: Outfall 001	*K1502003*
Date Sample Collected: 2 - 16/18/20 - 15	

Analyst: RH	
Test Start - Date/Time: 2-17-15, 1520	
Test Stop - Date/Time: 2-24-15, 1315	

Conc	1	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
MHS	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	3	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	4	3	1	0	3	0	5	4	2	4	1	23	10	2.3	RH
	5	0	6	5	0	7	0	1	7	4	2	32	10	3.2	RH
	6	10	6	0	12	8	7	8	0	5	2	58	10	5.8	RH
	7	0	5	7	2	0	5	8	12	5	8	52	10	5.2	RH
	8														
	Total	13	18	12	17	15	17	21	21	18	13	165		Avg. =	16.5
														C.V. =	19.4

Conc	2	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
32%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	3	0	0	0	0	0	1	1	0	0	0	2	10	0.2	RH
	4	4	0	3	0	0	0	2	6	2	0	17	10	1.7	RH
	5	1	1	1	6	2	5	4	8	2	3	33	10	3.3	RH
	6	3	12	8	7	6	0	2	0	6	8	52	10	5.2	RH
	7	8	6	3	5	10	0	0	5	0	1	38	10	3.8	RH
	8														
	Total	16	19	15	18	18	6	9	19	10	12	142		Avg. =	14.2
														C.V. =	32.8

Conc	3	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
42%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	3	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	4	5	2	4	1	0	6	2	3	0	1	24	10	2.4	RH
	5	3	0	0	2	1	2	6	3	8	2	27	10	2.7	RH
	6	0	0	4	0	9	0	3	1	6	4	27	10	2.7	RH
	7	5	5	8	10	5	8	0	9	7	6	63	10	6.3	RH
	8														
	Total	13	7	16	13	15	16	11	16	21	13	141		Avg. =	14.1
														C.V. =	26.2

Conc	4	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
56%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	3	0	0	0	0	0	0	0	0	1	0	1	10	0.1	RH
	4	1	0	0	1	6	5	4	4	3	2	26	10	2.6	RH
	5	7	0	0	1	5	3	6	3	2	5	32	10	3.2	RH
	6	0	7	8	12	6	0	1	6	0	8	48	10	4.8	RH
	7	3	3	8	5	5	10	7	2	5	5	53	10	5.3	RH
	8												10	0.0	
	Total	11	10	16	19	22	18	18	15	11	20	160		Avg. =	16.0
														C.V. =	26.0

Conc	5	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
75%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	3	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	4	3	3	3	6	4	0	2	0	1	6	28	10	2.8	RH
	5	2	6	2	4	2	8	8	0	2	3	37	10	3.7	RH
	6	1	7	10	8	0	5	8	6	7	0	52	10	5.2	RH
	7	8	0	4	3	1	3	1	7	5	3	35	10	3.5	RH
	8												10	0.0	RH
	Total	14	16	19	21	7	16	19	13	15	12	152		Avg. =	15.2
														C.V. =	26.6

Conc	6	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
100%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	3	0	1	0	0	0	0	0	0	0	0	1	10	0.1	RH
	4	2	0	5	0	1	5	3	4	2	4	26	10	2.6	RH
	5	3	4	3	8	0	0	5	6	7	3	39	10	3.9	RH
	6	7	0	5	0	8	7	5	12	4	0	48	10	4.8	RH
	7	5	5	3	13	3	7	0	2	7	9	54	10	5.4	RH
	8												10	0.0	RH
	Total	17	10	16	21	12	19	13	24	20	16	168		Avg. =	16.8
														C.V. =	25.8

AA # K1502003, C.DUBIA CHRONIC, REPRODUCION, 2-17-15
File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

******** Shapiro - Wilk's Test is aborted ********

This test can not be performed because total number of replicates
is greater than 50.

Total number of replicates = 60

AA # K1502003, C.DUBIA CHRONIC, REPRODUCION, 2-17-15
File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 1.42

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

	NUMBER OF		
IDENTIFICATION	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

	NUMBER OF		
IDENTIFICATION	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 # K1502003, C.DUBIA CHRONIC, REPRODUCION, 2-17-15
FILE: C:\COPYTO~1\TOXSTAT\C.DUB
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	13.0000	13.0000
1	CONTROL	2	18.0000	18.0000
1	CONTROL	3	12.0000	12.0000
1	CONTROL	4	17.0000	17.0000
1	CONTROL	5	15.0000	15.0000
1	CONTROL	6	17.0000	17.0000
1	CONTROL	7	21.0000	21.0000
1	CONTROL	8	21.0000	21.0000
1	CONTROL	9	18.0000	18.0000
1	CONTROL	10	13.0000	13.0000
2	32 % EFFLUENT	1	16.0000	16.0000
2	32 % EFFLUENT	2	19.0000	19.0000
2	32 % EFFLUENT	3	15.0000	15.0000
2	32 % EFFLUENT	4	18.0000	18.0000
2	32 % EFFLUENT	5	18.0000	18.0000
2	32 % EFFLUENT	6	6.0000	6.0000
2	32 % EFFLUENT	7	9.0000	9.0000
2	32 % EFFLUENT	8	19.0000	19.0000
2	32 % EFFLUENT	9	10.0000	10.0000
2	32 % EFFLUENT	10	12.0000	12.0000
3	15 % EFFLUENT	1	13.0000	13.0000
3	15 % EFFLUENT	2	7.0000	7.0000
3	15 % EFFLUENT	3	16.0000	16.0000
3	15 % EFFLUENT	4	13.0000	13.0000
3	15 % EFFLUENT	5	15.0000	15.0000
3	15 % EFFLUENT	6	16.0000	16.0000
3	15 % EFFLUENT	7	11.0000	11.0000
3	15 % EFFLUENT	8	16.0000	16.0000
3	15 % EFFLUENT	9	21.0000	21.0000
3	15 % EFFLUENT	10	13.0000	13.0000
4	56 % EFFLUENT	1	11.0000	11.0000
4	56 % EFFLUENT	2	10.0000	10.0000
4	56 % EFFLUENT	3	16.0000	16.0000
4	56 % EFFLUENT	4	19.0000	19.0000
4	56 % EFFLUENT	5	22.0000	22.0000
4	56 % EFFLUENT	6	18.0000	18.0000
4	56 % EFFLUENT	7	18.0000	18.0000
4	56 % EFFLUENT	8	15.0000	15.0000

4	56 % EFFLUENT	9	11.0000	11.0000
4	56 % EFFLUENT	10	20.0000	20.0000
5	75 % EFFLUENT	1	14.0000	14.0000
5	75 % EFFLUENT	2	16.0000	16.0000
5	75 % EFFLUENT	3	19.0000	19.0000
5	75 % EFFLUENT	4	21.0000	21.0000
5	75 % EFFLUENT	5	7.0000	7.0000
5	75 % EFFLUENT	6	16.0000	16.0000
5	75 % EFFLUENT	7	19.0000	19.0000
5	75 % EFFLUENT	8	13.0000	13.0000
5	75 % EFFLUENT	9	15.0000	15.0000
5	75 % EFFLUENT	10	12.0000	12.0000
6	100 % EFFLUENT	1	17.0000	17.0000
6	100 % EFFLUENT	2	10.0000	10.0000
6	100 % EFFLUENT	3	16.0000	16.0000
6	100 % EFFLUENT	4	21.0000	21.0000
6	100 % EFFLUENT	5	12.0000	12.0000
6	100 % EFFLUENT	6	19.0000	19.0000
6	100 % EFFLUENT	7	13.0000	13.0000
6	100 % EFFLUENT	8	24.0000	24.0000
6	100 % EFFLUENT	9	20.0000	20.0000
6	100 % EFFLUENT	10	16.0000	16.0000

AA # K1502003, C.DUBIA CHRONIC, REPRODUCION, 2-17-15
File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	66.733	13.347	0.815
Within (Error)	54	884.200	16.374	
Total	59	950.933		

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

AA # K1502003, C.DUBIA CHRONIC, REPRODUCION, 2-17-15
File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	16.500	16.500		
2	32 % EFFLUENT	14.200	14.200	1.271	
3	15 % EFFLUENT	14.100	14.100	1.326	
4	56 % EFFLUENT	16.000	16.000	0.276	
5	75 % EFFLUENT	15.200	15.200	0.718	
6	100 % EFFLUENT	16.800	16.800	-0.166	

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

AA # K1502003, C.DUBIA CHRONIC, REPRODUCION, 2-17-15
File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	10			
2	32 % EFFLUENT	10	4.180	25.3	2.300
3	15 % EFFLUENT	10	4.180	25.3	2.400
4	56 % EFFLUENT	10	4.180	25.3	0.500
5	75 % EFFLUENT	10	4.180	25.3	1.300
6	100 % EFFLUENT	10	4.180	25.3	-0.300

APPENDIX E

Organism History

AQUATOX, INC.

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

TEST ORGANISM HISTORY

DATE SHIPPED 2-17-15 **CLIENT** Arkansas Anesthesia

Purchase Order #: Ryan

SPECIES: **Pimephales promelas**

Quantity Shipped: 300+

Age: Initial 2-15-15 15-1600 CST

Brood Stock Source: **Anderson Farms, AR**

Culture Water: (**Groundwater**)

Hardness (Mg/l CaCO3): ≈160

Dissolved Oxygen (Mg/1): 8.4

Temperature (°C): 25.5

Feeding: Artemia

Comments:

Shipped Via: **Federal Express** **UPS Overnight** (**Shuttle**)

Packaged By:

Bill Hall Printers 3171

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:	22°C	22-26°C
SALINITY/CONDUCTIVITY:	--	--
TOTAL HARDNESS (as $CaCO_3$):	94 mg/l	76-130 mg/l
TOTAL ALKALINITY (as $CaCO_3$):	65 mg/l	65-100 mg/l
pH:	7.98	7.50-8.20

Comments:

Facility Supervisor

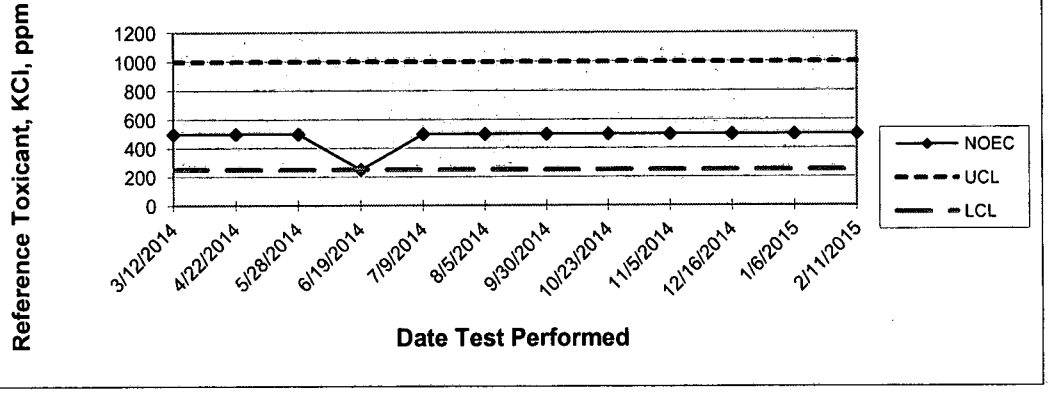
APPENDIX F

Quality Assurance Charts

ARKANSAS ANALYTICAL, INC.

FATHEAD MINNOW SURVIVAL 7 Day

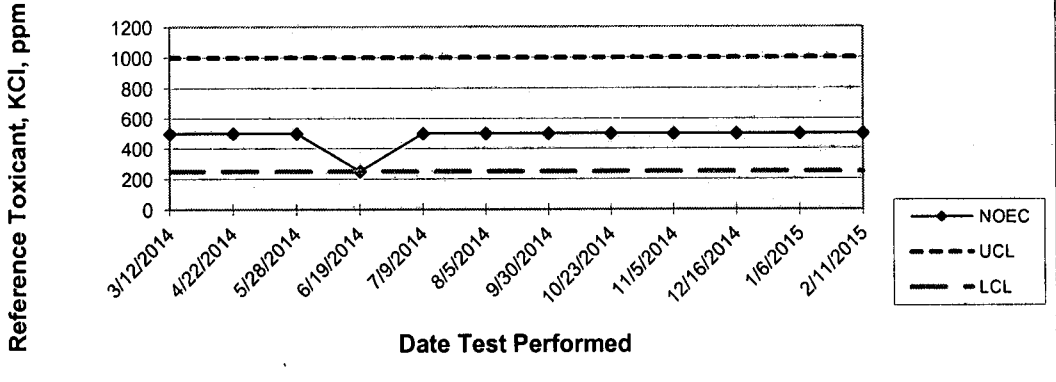
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.

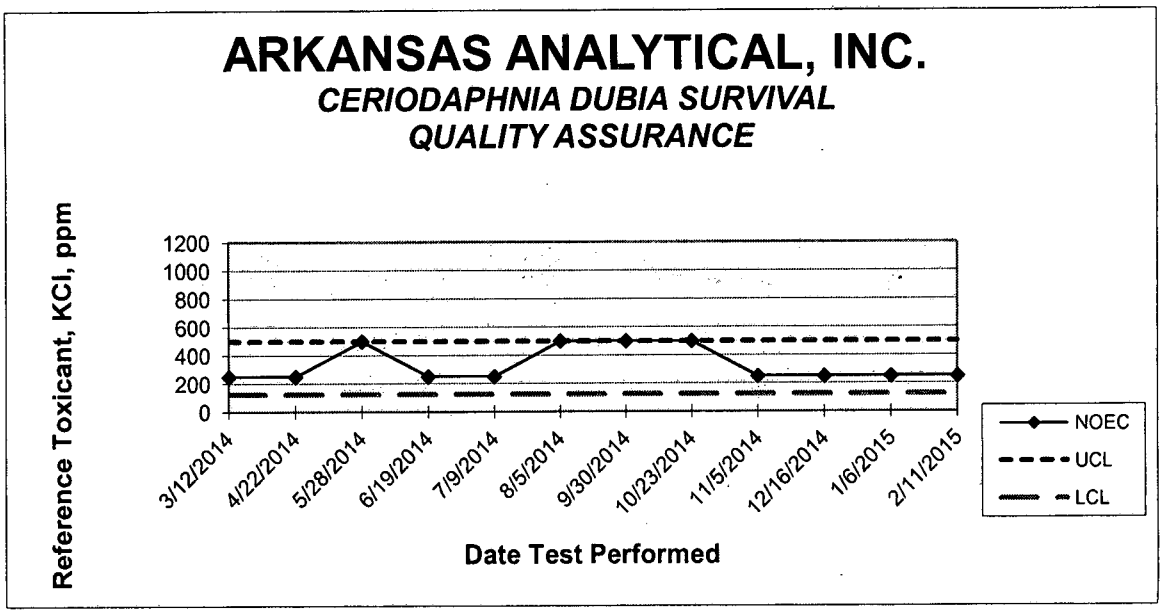
FATHEAD MINNOW GROWTH 7 Day

QUALITY ASSURANCE



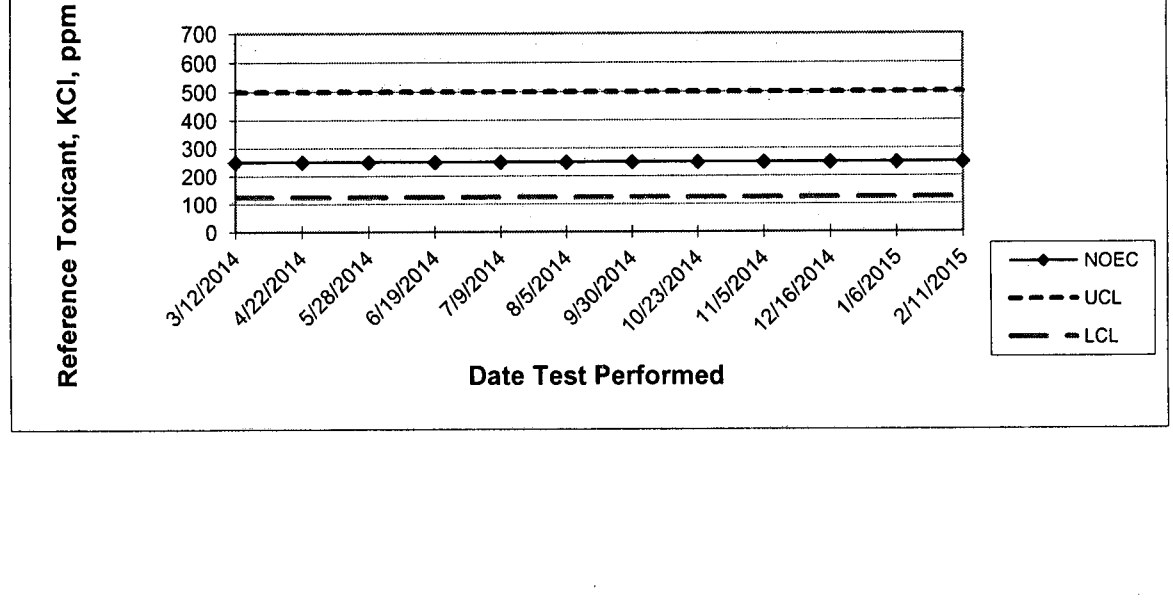
ARKANSAS ANALYTICAL, INC.

CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE

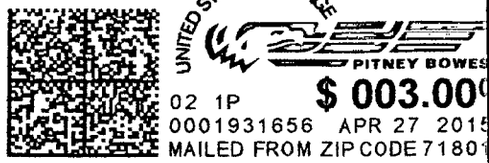


ARKANSAS ANALYTICAL, INC.

CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE



City of Hope
PO Box 667
Hope, AR
71802-0667



ADEQ
NPDES WATER SECTION
5301 Northshore Drive
North Little Rock, AR
72218-5317