Arkansas Analytical, Inc.

Toxicity Test Results
City of De Queen
NPDES PERMIT NUMBER: AR0021733
Second Quarter 2015
AFIN # 67-00023

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

Ceriodaphnia dubia, Survival and Reproduction Test
Test 1002.0

Prepared for: **Mr. Mike Sims**
City of DeQueen
P.O. Box 730
DeQueen, Arkansas 71832

Prepared by: Arkansas Analytical, Inc.
11701 I-30, Bldg 1, Suite 115
Little Rock, Arkansas 72209
Lab Number K1506003

Thursday, June 25, 2015

Introduction

This report contains test results for toxicity testing for the City of DeQueen. The NPDES permit number is AR0021733. The facility is located 1/8 mile south from intersection of Coulter Ave. and south of 9th Street on Philip Cox Blvd, in Section 36, Township 8 South, Range 32 West in Sevier County, Arkansas. The discharge is to receiving waters named: an unnamed ditch around pond to Bear Creek to Little River to Red River in Segment 1C of the Red River Basin.

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

Plant Operations

To be provided by permittee.

Source of Effluent and Dilution Water

Effluent samples were collected as follows:

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	6-16-15, 1100	6-17-15, 0900
Sample #2:	6-17-15, 1000	6-18-15, 0800
Sample #3:	6-21-15, 0800	6-22-15, 0800

The samples were composites collected at the final discharge from City of DeQueen Wastewater Plant outfall.

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

Sample Receiving Information:	Date, Time Sample(s) Received	Temperature Upon Receipt (°C)
Sample #1:	6-18-15, 1020	1
Sample #2:	6-19-15, 0951	1
Sample #3:	6-23-15, 1330	2

Chain of custody documentation is located in Appendix A.

The permit designates the receiving water to be used as dilution water for the toxicity tests. Due to its earlier characterization as toxic, synthetic dilution water was substituted.

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. The alternate method suggested in the method (11.3.4.5) for combating pathogen interference, was run in place of the original fathead minnow test. The test chambers were 30 ml plastic cups with 20 ml of test solution. Each chamber contained 2 organisms. The total number of fish was 40 per test solution. The fish were then combined to perform growth analysis. 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 also 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 ml 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	17.9	X	
At least 60% of surviving females should have produced 3 broods	90%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	21.8	X	

TEST ACCEPTANCE CRITERIA for *Pimephales promelas*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	95%	X	
The percent coefficient of variation between replicates must be 40% or less for survival	7.21	X	
Minimum of 0.25 mg average dry weight of surviving controls	0.636	X	
The percent coefficient of variation between replicates must be 40% or less for growth	18.9	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 5/28/15 – 6/4/15		*Pimephales promelas* 5/28/15 – 6/4/15	
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 DeQueen

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)	21.4	%CV survival (critical dilution)	0.00
%CV Reproduction (critical dilution)	14.1	Mean dry weight (critical dilution) in milligrams	0.726
		%CV growth (critical dilution)	10.7
PMSD Reproduction	22.6	PMSD Growth	19.8

Conclusion

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

The permit issued to the City of DeQueen, AR0021733, 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 DeQueen, AR0021733, specifies that the **critical dilution is 100% effluent**. The effluent samples **did not** exhibit lethal effects or sublethal effects at the critical dilution, and, as such, **passed** both portions of the test.

Biomonitoring Analysts:

Ryan Hudgin / Kenneth Pigue / Alfred Tennison

Reviewed by:

Tracy Bounds

Tracy Bounds, lab manager

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

PERMITTEE: City of DeQueen **NPDES #: AR0021733**

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	6-16-15, 1100	6-17-15, 0900
Sample #2:	6-17-15, 1000	6-18-15, 0800
Sample #3:	6-21-15, 0800	6-22-15, 0800

Test initiated (date, time): 6-18-15, 1410 Test terminated (date, time): 6-25-15, 1340

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%	87.5	100	87.5	100	100		97.5	97.5	95	7.21
32%	100	100	87.5	87.5	100		97.5	97.5	95	
42%	100	100	87.5	87.5	100		97.5	97.5	95	
56%	100	100	100	87.5	100		97.5	97.5	97.5	
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 %	A	B	C	D	E		Mean Dry Weight	CV%
0%	0.535	0.701	0.491	0.673	0.781		0.636	18.9
32%	0.636	0.734	0.670	0.564	0.704		0.662	
42%	0.621	0.746	0.580	0.705	0.830		0.696	
56%	0.652	0.644	0.766	0.589	0.754		0.681	
75%	0.676	0.718	0.630	0.762	0.683		0.694	
100%	0.752	0.759	0.661	0.635	0.825		0.726	10.7

Average Dry Weight in milligrams in replicate chambers
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)= _____18.9_____%

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING
Ceriodaphnia dubia SURVIVAL AND REPRODUCTION

PERMITTEE: City of DeQueen **NPDES #: AR0021733**

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	6-16-15, 1100	6-17-15, 0900
Sample #2:	6-17-15, 1000	6-18-15, 0800
Sample #3:	6-21-15, 0800	6-22-15, 0800

Test initiated (date, time): 6-18-15, 1425 Test terminated (date, time): 6-25-15, 1015

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	21	13	20	11	19
B	15	16	17	10	20	25
C	18	21	18	20	19	19
D	27	17	21	25	28	18
E	16	17	16	22	21	21
F	18	16	22	20	16	27
G	17	23	18	19	15	20
H	20	15	18	23	17	19
I	15	18	24	17	26	22
J	20	27	22	20	22	24
Mean	17.9	19.1	18.9	19.6	19.5	21.4
Mean/surviving female	17.9	19.1	18.9	19.6	19.5	21.4
CV%*	21.8					14.1

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 DeQueen **NPDES #: AR0021733**

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)= _____**21.8**_____%

APPENDIX A

Chain of Custody Forms

Arkansas Analytical Inc.

11701 Interstate 30, Bldg. 1, Ste. 115
Little Rock, AR 72209
PHONE: 501-455-3233
FAX: 501-455-6118

CHAIN OF CUSTODY RECORD

CLIENT INFORMATION	BILLING	Project Description	Turnaround Time	Preservation Codes:
City of DeQueen Wastewater Plant	City of DeQueen Wastewater Plant	Chronic Toxicity	1 Day (100%)	1. Cool, 4 Degrees Centigrade; 4. Thiosulfate for Dechlorination
514 South 9th	P.O. Box 730		2 Day (50%)	2. Sulfuric Acid (H_2SO_4), pH < 2; 5. Hydrochloric Acid(HCl)
DeQueen, AR 71832	DeQueen, AR 71832	Reporting Information	3 Day (25%)	3. Nitric Acid (HNO_3), pH < 2; 6. Sodium Hydroxide (NaOH), pH > 12
		Telephone: 870-642-5231	Routine	TEST PARAMETERS
Attn: Mike Sims		Fax: 870-642-3117	Preservative Code:	1
		Email: msims@cityofdequeen.com	Bottle Type:	P

Sampler(s) Signature: MS

Sampler(s) Printed: MS

Field Number	SAMPLE COLLECTION Date/s	Time/s	Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION	Chronic Biomonitoring	Bottle Type Code (G = Glass; P = Plastic; V = Septum; A = Amber)	Arkansas Analytical Work Order Number:
	6-16-17-15	11:00-9:40		X	4	Water	DQ Toxicity	X	A	K1506003

1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)
	6-17-15 16:45	UPS
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)
UPS	6 18 15 1020	Amanda Jobush

SAMPLE CONDITION UPON RECEIPT IN LAB

1. CUSTODY SEALS:	✓ Yes	___ No
2. CONTAINERS CORRECT:	___ Yes	___ No
3. COC/LABELS AGREE:	___ Yes	___ No
4. RECEIVED ON ICE:	___ Yes	___ No
5. TEMPERATURE ON RECEIPT:	1°C	
6. TEMPERATURE GUN ID:	HHT#2	

FOR COMPLETION BY LAB ONLY

REMARKS / SAMPLE COMMENTS

PO# 71414

Revision 2
2/14/14

Arkansas Analytical inc.

11701 Interstate 30, Bldg. 1, Ste. 115
Little Rock, AR 72209
PHONE: 501-455-3233
FAX: 501-455-6118

CHAIN OF CUSTODY RECORD

CLIENT INFORMATION	BILLING	Project Description	Turnaround Time
City of DeQueen Wastewater Plant	City of DeQueen Wastewater Plant	Chronic Toxicity	1 Day (100%)
514 South 9th	P.O. Box 730		2 Day (50%)
DeQueen, AR 71832	DeQueen, AR 71832	Reporting Information	3 Day (25%)
		Telephone: 870-642-5231	Routine
Attn: Mike Sims		Fax: 870-642-3117	Preservative Code:
		Email: msims@cityofdequeen.com	Bottle Type:

Preservation Codes:
1. Cool, 4 Degrees Centigrade
2. Sulfuric Acid (H_2SO_4), pH < 2
3. Nitric Acid (HNO_3), pH < 2
4. Thiosulfate for Dechlorination
5. Hydrochloric Acid(HCl)
6. Sodium Hydroxide (NaOH), pH > 12

Sampler(s) Signature: [signature]

Sampler(s) Printed: MS

Field Number	SAMPLE COLLECTION Date/s	Time/s	Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION	TEST PARAMETERS: Chronic Biomonitoring	Bottle Type Code
	6-17-18-15	10:00 - 8:00		X		Water	DQ Toxicity	X	B

TEST PARAMETERS: Preservative Code: 1; Bottle Type: P

Bottle Type Code: G = Glass; P = Plastic; V = Septum; A = Amber

Arkansas Analytical Work Order Number: K1501003

1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)
[signature]	6-18-15 11:45am	UPS
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)
UPS	6-19-15 0957	Amanda Johnson

SAMPLE CONDITION UPON RECEIPT IN LAB

1. CUSTODY SEALS:	√ Yes	No
2. CONTAINERS CORRECT:	Yes	No
3. COC/LABELS AGREE:	Yes	No
4. RECEIVED ON ICE:	Yes	No
5. TEMPERATURE ON RECEIPT:	1°C	
6. TEMPERATURE GUN ID:	IR#2	

FOR COMPLETION BY LAB ONLY

REMARKS / SAMPLE COMMENTS: PO# 71414

Revision 2
2/14/14

Arkansas Analytical Inc.

11701 Interstate 30, Bldg. 1, Ste. 115
Little Rock, AR 72209
PHONE: 501-455-3233
FAX: 501-455-6118

CHAIN OF CUSTODY RECORD

CLIENT INFORMATION	BILLING	Project Description	Turnaround Time
City of DeQueen Wastewater Plant	City of DeQueen Wastewater Plant	Chronic Toxicity	1 Day (100%)
514 South 9th	P.O. Box 730		2 Day (50%)
DeQueen, AR 71832	DeQueen, AR 71832	**Reporting Information**	3 Day (25%)
		Telephone: 870-642-5231	Routine
Attn: Mike Sims		Fax: 870-642-3117	Preservative Code:
		Email: msims@cityofdequeen.com	Bottle Type:

Preservation Codes:

1. Cool, 4 Degrees Centigrade
2. Sulfuric Acid (H_2SO_4), pH < 2
3. Nitric Acid (HNO_3), pH < 2
4. Thiosulfate for Dechlorination
5. Hydrochloric Acid(HCl)
6. Sodium Hydroxide (NaOH), pH > 12

TEST PARAMETERS									Bottle Type Code
1									G = Glass; P = Plastic
P									V = Septum; A = Amber

Sampler(s) Signature

Sampler(s) Printed

Field Number	SAMPLE COLLECTION Date/s	Time/s	Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION	Chronic Biomonitoring								Arkansas Analytical Work Order Number:
	6-21/22-15	8:00 AM - 8:00 PM		X		Water	DQ Toxicity	X								K1506003 C

1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)	SAMPLE CONDITION UPON RECEIPT IN LAB	REMARKS / SAMPLE COMMENTS
[signature]	6-22-15 11:30 AM	~~[signature]~~ RH 6-25-15 ~~1330~~ UPS	1. CUSTODY SEALS: ✓ Yes ___ No	
			2. CONTAINERS CORRECT: ___ Yes ___ No	
			3. COC/LABELS AGREE: ___ Yes ___ No	
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)	4. RECEIVED ON ICE: ___ Yes ___ No	
UPS	6-23-15 1330	[signature]	5. TEMPERATURE ON RECEIPT: 2°C	
			6. TEMPERATURE GUN ID: IHT #2	
			FOR COMPLETION BY LAB ONLY	

Revision 2
2/14/14

Po# 71414

APPENDIX B

Effluent and Dilution Water Data

Biomonitoring Quality Control Benchsheet

Analyst	RH	RH	KP	TL	RH	RH	RH	RH
Date	6-18-15	6-19-15	6/20/15	6/21/15	6-22-15	6-23-15	6-24-15	6-25-15
pH Meter ID	AR60							
LIN pH 4 Buffer	1500706							
LIN pH 7 Buffer	1500707							
LIN pH 10 Buffer	1500708							
Slope (>90%)	90.8%	93.6%	91.2	91.2	95.2%	92.0%	90.5%	93.1%

Dissolved O_2 Meter	DO 1305							
Meter Reading	8.50	8.43	8.42	8.55	8.62	8.55	8.52	8.54
Temp.	23	23	23	23	22	23	23	23
Chart Value at Temp.	8.578	8.578	8.578	8.578	8.743	8.578	8.578	8.578
Difference	0.078	0.143	0.142	.078	0.123	0.028	0.058	0.038
Acceptance Criteria	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L

Temp. Meter ID	AR60							
Meter Reading	23	23	23	22	23	23	23	23
Thermometer Reading	22	22	22	22	22	23	22	22
Thermometer ID	PB							
Acceptance Criteria	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C

Alkalinity								
Blank (<5mg/L)	<5 mg/L						<5 mg/L	
STD Result	102						94	
T.V. / %REC	100/102%						100/94%	
Acceptance Criteria	93.5-108.5% Recovery							

Hardness								
Blank(<2mg/L)	<2mg/L						<2mg/L	
STD. Result	92						96	
T.V. /%REC	100/92%						100/96%	
Acceptance Criteria	90.0-105.5% Recovery							

Conductivity Meter ID	Con 02							
Blank (<1)	<1						<1	
STD Result	1418						1420	
T.V. / %REC	1412/100%						1412/100%	
Acceptance Criteria	99.2-104.0% Recovery							

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

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING — Fathead Minnow

Lab # / Sample ID: K1506003 — Test Start (Date/Time): 6-18-15 1410

Client: De Queen — Test End (Date/Time): 6-25-15 1340

		Day of Test							
		1	2	3	4	5	6	7	notes
Control	mHS	6-18	6-19	6-20	6-21	6-22	6-23	6-24	
D.O. (mg/L)	INITIAL	8.7	8.6	8.5	8.8	8.8	8.5	8.8	
	FINAL	6.5	6.4	8.5	7.2	7.5	7.0	6.3	
pH (s.u.)	INITIAL	8.2	7.7	7.7	7.5	7.8	7.8	7.7	
	FINAL	8.2	7.2	7.3	8.1	7.5	7.8	7.9	
temp (C)	INITIAL	23	23	23	23	22	23	23	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		60					64		
HARDNESS (mg/L)		84					90		
CONDUCTIVITY (umho		441					417		
CHLORINE (mg/L)		<0.05							
CONC: 32									
D.O. (mg/L)	INITIAL	8.7	8.4	8.1	8.9	8.7	8.3	8.4	
	FINAL	6.4	6.4	8.2	6.7	7.9	6.8	6.0	
pH (s.u)	INITIAL	8.0	7.7	7.6	7.6	7.6	7.5	7.6	
	FINAL	7.9	7.0	7.4	8.0	7.5	7.7	7.7	
temp (C)	INITIAL	23	23	24	23	22	22	23	
	FINAL	25	25	25	25	25	25	25	
CONC: 42									
D.O. (mg/L)	INITIAL	8.9	8.8	8.9	9.1	9.1	8.3	8.7	
	FINAL	6.8	7.0	8.5	7.3	7.5	7.2	6.8	
pH (mg/L)	INITIAL	8.0	7.6	7.5	7.4	7.6	7.5	7.6	
	FINAL	7.8	7.0	7.6	7.9	7.5	7.8	7.7	
temp (C)	INITIAL	22	23	25	23	22	23	22	
	FINAL	25	25	25	25	25	25	25	
CONC: 56									
D.O. (mg/L)	INITIAL	9.1	9.1	9.0	9.1	9.2	9.1	9.0	
	FINAL	6.9	7.0	8.4	7.4	7.4	7.2	6.6	
pH (s.u.)	INITIAL	7.9	7.6	7.5	7.5	7.6	7.4	7.6	
	FINAL	7.8	7.1	7.5	7.9	7.4	7.7	7.7	
temp (C)	INITIAL	23	23	24	23	22	23	22	
	FINAL	25	25	25	25	25	25	25	
CONC: 75									
D.O. (mg/L)	INITIAL	9.2	9.0	9.0	9.2	9.2	9.0	9.3	
	FINAL	7.1	7.2	8.4	7.5	7.2	7.3	6.8	
pH (s.u.)	INITIAL	7.8	7.5	7.5	7.5	7.6	7.4	7.6	
	FINAL	7.8	7.2	7.7	7.9	7.5	7.7	7.7	
temp (C)	INITIAL	22	22	24	23	22	23	22	
	FINAL	25	25	25	25	25	25	25	
CONC: 100									
D.O. (mg/L)	INITIAL	9.2	9.1	9.0	9.2	9.0	9.2	9.1	
	FINAL	7.2	7.6	8.4	7.4	7.2	7.2	6.8	
pH (s.u.)	INITIAL	7.8	7.5	7.4	7.5	7.6	7.4	7.6	
	FINAL	7.7	7.2	7.7	7.8	7.5	7.7	7.6	
temp (C)	INITIAL	22	22	27	23	22	23	21	
	FINAL	25	25	25	25	25	25	25	
CONC: 100 %		A	A	A	B	B	C	C	
ALKALINITY (mg/L)		3448RH			4850RH		50		
HARDNESS (mg/L)		50			40		54		
CONDUCTIVITY (umho		904			933		742		
CHLORINE (mg/L)		<0.05							

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING									Ceriodaphnia Dubia
Lab # / Sample ID		K150603			Test Start (Date/Time)		6-18-15		1425
Client:		De Queen			Test End (Date/Time)		6-25-15		1015
		Day of Test							
		1	2	3	4	5	6	7	notes
Control	mg H5	6-18	6-19	6-20	6-21	6-22	6-23	6-24	
D.O. (mg/L)	INITIAL	8.7	8.6	8.5	8.4	8.8	8.5	8.8	
	FINAL	8.2	8.6	8.7	8.4	8.6	8.7	8.8	
pH (s.u.)	INITIAL	8.2	7.7	7.7	7.5	7.8	7.8	7.7	
	FINAL	7.7	7.3	7.6	7.8	7.5	7.8	7.7	
temp (C)	INITIAL	23	23	23	23	22	23	23	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		60					64		
HARDNESS (mg/L)		84					90		
CONDUCTIVITY (umhc		441					417		
CHLORINE (mg/L)		<0.05							
CONC:	32								
D.O. (mg/L)	INITIAL	8.7	8.4	8.7	8.9	8.7	8.3	8.4	
	FINAL	8.2	8.6	8.5	8.5	8.5	8.5	8.3	
pH (s.u)	INITIAL	8.0	7.7	7.6	7.6	7.6	7.5	7.6	
	FINAL	7.5	7.5	7.6	7.7	7.5	7.6	7.9	
temp (C)	INITIAL	23	23	24	23	22	22	23	
	FINAL	25	25	25	25	25	25	25	
CONC:	42								
D.O. (mg/L)	INITIAL	8.9	8.8	8.9	9.1	9.1	8.3	8.7	
	FINAL	8.1	8.6	8.6	8.2	8.6	8.7	8.5	
pH (mg/L)	INITIAL	8.0	7.6	7.5	7.4	7.6	7.5	7.6	
	FINAL	7.6	7.6	7.5	7.7	7.6	7.6	7.9	
temp (C)	INITIAL	22	23	25	23	22	23	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	56								
D.O. (mg/L)	INITIAL	9.1	9.1	9.0	9.1	9.2	9.1	9.0	
	FINAL	8.2	8.5	8.5	8.2	8.6	8.7	8.7	
pH (s.u.)	INITIAL	7.9	7.6	7.5	7.5	7.6	7.4	7.6	
	FINAL	7.6	7.7	7.6	7.5	7.6	7.6	8.0	
temp (C)	INITIAL	23	23	24	23	22	23	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	75								
D.O. (mg/L)	INITIAL	9.2	9.0	9.0	9.2	9.2	9.0	9.3	
	FINAL	8.1	8.5	8.6	8.3	8.6	8.5	9.0	
pH (s.u.)	INITIAL	7.8	7.5	7.5	7.5	7.6	7.4	7.6	
	FINAL	7.6	7.7	7.7	7.4	7.6	7.6	8.0	
temp (C)	INITIAL	22	23	24	23	22	23	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	100								
D.O. (mg/L)	INITIAL	9.2	9.1	9.0	9.2	9.0	9.2	9.1	
	FINAL	8.2	8.2	8.6	8.4	8.6	8.5	8.7	
pH (s.u.)	INITIAL	7.8	7.5	7.4	7.5	7.6	7.4	7.6	
	FINAL	7.7	7.9	7.7	7.3	7.7	7.7	8.1	
temp (C)	INITIAL	22	22	24	23	22	23	21	
	FINAL	25	25	25	25	25	25	25	
CONC:	**100 %**	A	A	A	B	B	C	C	
ALKALINITY (mg/L)		34			48		50		
HARDNESS (mg/L)		50			40		54		
CONDUCTIVITY (umhc		904			933		742		
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 (ALTERNATE)

LAB #: K1506003	TEST START	DATE 6/18/15	TIME 1410
CLIENT: De Queen	TEST END	DATE 6/25/15	TIME 1340
ANALYST: RH/HF/WL	AGE AND SOURCE OF MINNOWS	< 48 hrs old, Aquatox	

		DAY(NUMBER SURVIVING)								SURVIVAL		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONTROL	A	8	7	7	7	7	7	7	7	87.5%		
	B	8	8	8	8	8	8	8	8	100%		
	C	8	8	8	7	7	7	7	7	87.5%	*95.0%*	*7.21*
MHS	D	8	8	8	8	8	8	8	8	100%		
	E	8	8	8	8	8	8	8	8	100%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	8	8	8	8	8	8	8	8	100%		
	B	8	8	8	8	8	8	8	8	100%		
	C	8	7	7	7	7	7	7	7	87.5%	*95.0%*	
32%	D	8	8	8	8	7	7	7	7	87.5%		
	E	8	8	8	8	8	8	8	8	100%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	8	8	8	8	8	8	8	8	100%		
	B	8	8	8	8	8	8	8	8	100%		
	C	8	8	8	7	7	7	7	7	87.5%	*95.0%*	
42%	D	8	7	7	7	7	7	7	7	87.5%		
	E	8	8	8	8	8	8	8	8	100%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	8	8	8	8	8	8	8	8	100%		
	B	8	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	8	100%	*97.5%*	
56%	D	8	7	7	7	7	7	7	7	87.5%		
	E	8	8	8	8	8	8	8	8	100%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	8	8	8	8	8	8	8	8	100%		
	B	8	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	8	100%	*100.0%*	
75%	D	8	8	8	8	8	8	8	8	100%		
	E	8	8	8	8	8	8	8	8	100%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	8	8	8	8	8	8	8	8	100%		
	B	8	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	8	100%	*100.0%*	*0.00*
100%	D	8	8	8	8	8	8	8	8	100%		
	E	8	8	8	8	8	8	8	8	100%		
ANALYST:		RH	RH	KP	TT	RH	RH	RH	RH			
DATE:		6/18/15	6/19/15	6/20/15	6/21/15	6/22/15	6/23/15	6/24/15	6/25/15			
TIME:		1410	1000	915	1100	1340	1520	1100	1340			

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

REMARKS:

AA# K1506003, FATHEAD MINNOW SURV.,CHRONIC, 6-18-15
File: C:\COPYTO~1\TOXSTAT\FHSURV~1. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.148

W = 0.820

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# K1506003, FATHEAD MINNOW SURV.,CHRONIC, 6-18-15
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# K1506003, FATHEAD MINNOW SURV.,CHRONIC, 6-18-15
FILE: C:\COPYTO~1\TOXSTAT\FHSURV~1.
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.8750	1.2094
1	CONTROL	2	1.0000	1.3931
1	CONTROL	3	0.8750	1.2094
1	CONTROL	4	1.0000	1.3931
1	CONTROL	5	1.0000	1.3931
2	32 % EFFLUENT	1	1.0000	1.3931
2	32 % EFFLUENT	2	1.0000	1.3931
2	32 % EFFLUENT	3	0.8750	1.2094
2	32 % EFFLUENT	4	0.8750	1.2094
2	32 % EFFLUENT	5	1.0000	1.3931
3	42 % EFFLUENT	1	1.0000	1.3931
3	42 % EFFLUENT	2	1.0000	1.3931
3	42 % EFFLUENT	3	0.8750	1.2094
3	42 % EFFLUENT	4	0.8750	1.2094
3	42 % EFFLUENT	5	1.0000	1.3931
4	56 % EFFLUENT	1	1.0000	1.3931
4	56 % EFFLUENT	2	1.0000	1.3931
4	56 % EFFLUENT	3	1.0000	1.3931
4	56 % EFFLUENT	4	0.8750	1.2094
4	56 % EFFLUENT	5	1.0000	1.3931
5	75 % EFFLUENT	1	1.0000	1.3931
5	75 % EFFLUENT	2	1.0000	1.3931
5	75 % EFFLUENT	3	1.0000	1.3931
5	75 % EFFLUENT	4	1.0000	1.3931
5	75 % EFFLUENT	5	1.0000	1.3931
6	100 % EFFLUENT	1	1.0000	1.3931
6	100 % EFFLUENT	2	1.0000	1.3931
6	100 % EFFLUENT	3	1.0000	1.3931
6	100 % EFFLUENT	4	1.0000	1.3931
6	100 % EFFLUENT	5	1.0000	1.3931

AA# K1506003, FATHEAD MINNOW SURV.,CHRONIC, 6-18-15
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	1.320				
2	32 % EFFLUENT	1.320	27.50	16.00	5.00	
3	42 % EFFLUENT	1.320	27.50	16.00	5.00	
4	56 % EFFLUENT	1.356	30.00	16.00	5.00	
5	75 % EFFLUENT	1.393	32.50	16.00	5.00	
6	100 % EFFLUENT	1.393	32.50	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:	K1506003				TEST DATES (BEGIN / END):		6/18/15 - 6/25/15	
CLIENT:	City of DeQueen				WEIGHING DATE / TIME:		6/26/2015 0900	
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	1.00328	0.99900	0.00428	8	0.535	AVG DRY	
	B	1.01007	1.00446	0.00561	8	0.701	WEIGHT (mg)	
MHS	C	1.00615	1.00222	0.00393	8	0.491		0.636
	D	1.00988	1.00450	0.00538	8	0.673	CV	
	E	1.00387	0.99762	0.00625	8	0.781		18.9
CONC:	A	1.01106	1.00597	0.00509	8	0.636	AVG DRY	
	B	1.01007	1.00420	0.00587	8	0.734	WEIGHT (mg)	
32%	C	1.01513	1.00977	0.00536	8	0.670		0.662
	D	0.99475	0.99024	0.00451	8	0.564	CV	
	E	0.99988	0.99425	0.00563	8	0.704		
CONC:	A	1.00142	0.99645	0.00497	8	0.621	AVG DRY	
	B	1.00874	1.00277	0.00597	8	0.746	WEIGHT (mg)	
42%	C	1.01293	1.00829	0.00464	8	0.580		0.697
	D	1.01885	1.01321	0.00564	8	0.705	CV	
	E	1.04210	1.03546	0.00664	8	0.830		
CONC:	A	1.00087	0.99565	0.00522	8	0.652	AVG DRY	
	B	1.01547	1.01032	0.00515	8	0.644	WEIGHT (mg)	
56%	C	1.01242	1.00629	0.00613	8	0.766		0.681
	D	0.99383	0.98912	0.00471	8	0.589	CV	
	E	1.01738	1.01135	0.00603	8	0.754		
CONC:	A	1.02967	1.02426	0.00541	8	0.676	AVG DRY	
	B	1.02183	1.01609	0.00574	8	0.718	WEIGHT (mg)	
75%	C	1.01130	1.00626	0.00504	8	0.630		0.694
	D	1.01891	1.01281	0.00610	8	0.762	CV	
	E	1.02073	1.01527	0.00546	8	0.683		
CONC:	A	1.03362	1.02760	0.00602	8	0.752	AVG DRY	
	B	1.02395	1.01788	0.00607	8	0.759	WEIGHT (mg)	
100%	C	1.02631	1.02102	0.00529	8	0.661		0.726
	D	0.99949	0.99441	0.00508	8	0.635	CV	
	E	1.01319	1.00659	0.00660	8	0.825		10.7

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA# K1506003, FATHEAD MINNOW GROWTH CHRONIC, 6-18-25
File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.172

W = 0.971

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# K1506003, FATHEAD MINNOW GROWTH CHRONIC, 6-18-25
File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 3.42

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.

TITLE: AA# K1506003, FATHEAD MINNOW GROWTH CHRONIC, 6-18-25
FILE: C:\COPYTO~1\TOXSTAT\FHGROWTH.
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.5350	0.5350
1	CONTROL	2	0.7010	0.7010
1	CONTROL	3	0.4910	0.4910
1	CONTROL	4	0.6730	0.6730
1	CONTROL	5	0.7810	0.7810
2	32 % EFFLUENT	1	0.6360	0.6360
2	32 % EFFLUENT	2	0.7340	0.7340
2	32 % EFFLUENT	3	0.6700	0.6700
2	32 % EFFLUENT	4	0.5640	0.5640
2	32 % EFFLUENT	5	0.7040	0.7040
3	42 % EFFLUENT	1	0.6210	0.6210
3	42 % EFFLUENT	2	0.7460	0.7460
3	42 % EFFLUENT	3	0.5800	0.5800
3	42 % EFFLUENT	4	0.7050	0.7050
3	42 % EFFLUENT	5	0.8300	0.8300
4	56 % EFFLUENT	1	0.6520	0.6520
4	56 % EFFLUENT	2	0.6440	0.6440
4	56 % EFFLUENT	3	0.7660	0.7660
4	56 % EFFLUENT	4	0.5890	0.5890
4	56 % EFFLUENT	5	0.7540	0.7540
5	75 % EFFLUENT	1	0.6760	0.6760
5	75 % EFFLUENT	2	0.7180	0.7180
5	75 % EFFLUENT	3	0.6300	0.6300
5	75 % EFFLUENT	4	0.7620	0.7620
5	75 % EFFLUENT	5	0.6830	0.6830
6	100 % EFFLUENT	1	0.7520	0.7520
6	100 % EFFLUENT	2	0.7590	0.7590
6	100 % EFFLUENT	3	0.6610	0.6610
6	100 % EFFLUENT	4	0.6350	0.6350
6	100 % EFFLUENT	5	0.8250	0.8250

AA# K1506003, FATHEAD MINNOW GROWTH CHRONIC, 6-18-25
File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.024	0.005	0.675
Within (Error)	24	0.172	0.007	
Total	29	0.196		

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

AA# K1506003, FATHEAD MINNOW GROWTH CHRONIC, 6-18-25
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	0.636	0.636		
2	32 % EFFLUENT	0.662	0.662	-0.475	
3	42 % EFFLUENT	0.696	0.696	-1.125	
4	56 % EFFLUENT	0.681	0.681	-0.837	
5	75 % EFFLUENT	0.694	0.694	-1.076	
6	100 % EFFLUENT	0.726	0.726	-1.686	

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

AA# K1506003, FATHEAD MINNOW GROWTH CHRONIC, 6-18-25
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	0.126	19.8	-0.025
3	42 % EFFLUENT	5	0.126	19.8	-0.060
4	56 % EFFLUENT	5	0.126	19.8	-0.045
5	75 % EFFLUENT	5	0.126	19.8	-0.058
6	100 % EFFLUENT	5	0.126	19.8	-0.090

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

SURVIVAL AND REPRODUCTION TEST

Ceriodaphnia dubia

Discharger: De Queen	**Lab Number/s**
Location: Outfall 001	***K1506003***
Date Sample Collected: 6 - 17/18/22 - 15	

Analyst: RH	
Test Start - Date/Time: 6-18-15, 1425	
Test Stop - Date/Time: 6-25-15, 1015	

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	1	0	0	0	0	0	0	1	10	0.1	RH
	4	0	5	0	0	4	6	3	2	2	2	24	10	2.4	RH
	5	10	4	3	6	0	3	6	8	5	6	51	10	5.1	RH
	6	3	6	11	10	5	0	2	6	8	4	55	10	5.5	RH
	7	0	0	4	10	7	9	6	4	0	8	48	10	4.8	RH
	8														
	Total	13	15	18	27	16	18	17	20	15	20	179		Avg. =	17.9
														C.V. =	21.8

Conc	2	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
32%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	3	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	4	1	0	4	1	0	0	4	4	0	5	19	10	1.9	RH
	5	8	6	4	8	5	8	3	0	6	10	58	10	5.8	RH
	6	12	8	9	8	6	5	9	10	12	12	91	10	9.1	RH
	7	0	2	4	0	6	3	7	1	0	0	23	10	2.3	RH
	8														
	Total	21	16	21	17	17	16	23	15	18	27	191		Avg. =	19.1
														C.V. =	20.0

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	1	0	0	0	1	10	0.1	RH
	4	1	0	1	0	3	5	4	0	2	0	16	10	1.6	RH
	5	6	11	8	3	2	3	2	8	6	12	61	10	6.1	RH
	6	6	6	9	13	8	12	10	8	13	6	91	10	9.1	RH
	7	0	0	0	5	3	2	1	2	3	4	20	10	2.0	RH
	8														
	Total	13	17	18	21	16	22	18	18	24	22	189		Avg. =	18.9
														C.V. =	17.5

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	2	0	0	0	0	0	0	0	0	0	2	10	0.2	RH
	4	0	2	6	3	4	4	1	5	0	5	30	10	3.0	RH
	5	7	0	5	6	9	9	6	8	9	5	64	10	6.4	RH
	6	10	7	9	8	8	6	8	10	6	7	79	10	7.9	RH
	7	1	1	0	8	1	1	4	0	2	3	21	10	2.1	RH
	8														
	Total	20	10	20	25	22	20	19	23	17	20	196		Avg. =	19.6
														C.V. =	20.6

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	1	0	1	4	5	5	3	2	2	6	29	10	2.9	RH
	5	1	8	6	12	7	0	0	5	8	6	53	10	5.3	RH
	6	5	11	12	8	4	11	9	5	10	8	83	10	8.3	RH
	7	4	1	0	4	5	0	3	5	6	2	30	10	3.0	RH
	8														
	Total	11	20	19	28	21	16	15	17	26	22	195		Avg. =	19.5
														C.V. =	26.2

Conc	6	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
100%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	3	0	0	0	0	0	0	1	0	0	1	2	10	0.2	RH
	4	3	5	2	6	3	6	3	1	0	0	29	10	2.9	RH
	5	9	8	5	0	7	2	6	8	11	6	62	10	6.2	RH
	6	7	12	10	7	8	13	10	9	6	14	96	10	9.6	RH
	7	0	0	2	5	3	6	0	1	5	3	25	10	2.5	RH
	8														
	Total	19	25	19	18	21	27	20	19	22	24	214		Avg. =	21.4
														C.V. =	14.1

AA # K1506003, C.DUBIA CHRONIC, REPRODUCION, 6-18-15
File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

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

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

Total number of replicates = 60

AA # K1506003, C.DUBIA CHRONIC, REPRODUCION, 6-18-15
File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 2.89

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.

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 # K1506003, C.DUBIA CHRONIC, REPRODUCION, 6-18-15
FILE: C:\COPYTO~1\TOXSTAT\C.DUB
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	13.0000	13.0000
1	CONTROL	2	15.0000	15.0000
1	CONTROL	3	18.0000	18.0000
1	CONTROL	4	27.0000	27.0000
1	CONTROL	5	16.0000	16.0000
1	CONTROL	6	18.0000	18.0000
1	CONTROL	7	17.0000	17.0000
1	CONTROL	8	20.0000	20.0000
1	CONTROL	9	15.0000	15.0000
1	CONTROL	10	20.0000	20.0000
2	32 % EFFLUENT	1	21.0000	21.0000
2	32 % EFFLUENT	2	16.0000	16.0000
2	32 % EFFLUENT	3	21.0000	21.0000
2	32 % EFFLUENT	4	17.0000	17.0000
2	32 % EFFLUENT	5	17.0000	17.0000
2	32 % EFFLUENT	6	16.0000	16.0000
2	32 % EFFLUENT	7	23.0000	23.0000
2	32 % EFFLUENT	8	15.0000	15.0000
2	32 % EFFLUENT	9	18.0000	18.0000
2	32 % EFFLUENT	10	27.0000	27.0000
3	42 % EFFLUENT	1	13.0000	13.0000
3	42 % EFFLUENT	2	17.0000	17.0000
3	42 % EFFLUENT	3	18.0000	18.0000
3	42 % EFFLUENT	4	21.0000	21.0000
3	42 % EFFLUENT	5	16.0000	16.0000
3	42 % EFFLUENT	6	22.0000	22.0000
3	42 % EFFLUENT	7	18.0000	18.0000
3	42 % EFFLUENT	8	18.0000	18.0000
3	42 % EFFLUENT	9	24.0000	24.0000
3	42 % EFFLUENT	10	22.0000	22.0000
4	56 % EFFLUENT	1	20.0000	20.0000
4	56 % EFFLUENT	2	10.0000	10.0000
4	56 % EFFLUENT	3	20.0000	20.0000
4	56 % EFFLUENT	4	25.0000	25.0000
4	56 % EFFLUENT	5	22.0000	22.0000
4	56 % EFFLUENT	6	20.0000	20.0000
4	56 % EFFLUENT	7	19.0000	19.0000
4	56 % EFFLUENT	8	23.0000	23.0000

4	56 % EFFLUENT	9	17.0000	17.0000
4	56 % EFFLUENT	10	20.0000	20.0000
5	75 % EFFLUENT	1	11.0000	11.0000
5	75 % EFFLUENT	2	20.0000	20.0000
5	75 % EFFLUENT	3	19.0000	19.0000
5	75 % EFFLUENT	4	28.0000	28.0000
5	75 % EFFLUENT	5	21.0000	21.0000
5	75 % EFFLUENT	6	16.0000	16.0000
5	75 % EFFLUENT	7	15.0000	15.0000
5	75 % EFFLUENT	8	17.0000	17.0000
5	75 % EFFLUENT	9	26.0000	26.0000
5	75 % EFFLUENT	10	22.0000	22.0000
6	100% EFFLUENT	1	19.0000	19.0000
6	100% EFFLUENT	2	25.0000	25.0000
6	100% EFFLUENT	3	19.0000	19.0000
6	100% EFFLUENT	4	18.0000	18.0000
6	100% EFFLUENT	5	21.0000	21.0000
6	100% EFFLUENT	6	27.0000	27.0000
6	100% EFFLUENT	7	20.0000	20.0000
6	100% EFFLUENT	8	19.0000	19.0000
6	100% EFFLUENT	9	22.0000	22.0000
6	100% EFFLUENT	10	24.0000	24.0000

AA # K1506003, C.DUBIA CHRONIC, REPRODUCION, 6-18-15
File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	66.400	13.280	0.864
Within (Error)	54	830.000	15.370	
Total	59	896.400		

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

AA # K1506003, C.DUBIA CHRONIC, REPRODUCION, 6-18-15
File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	17.900	17.900		
2	32 % EFFLUENT	19.100	19.100	-0.684	
3	42 % EFFLUENT	18.900	18.900	-0.570	
4	56 % EFFLUENT	19.600	19.600	-0.970	
5	75 % EFFLUENT	19.500	19.500	-0.913	
6	100% EFFLUENT	21.400	21.400	-1.996	

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

AA # K1506003, C.DUBIA CHRONIC, REPRODUCION, 6-18-15
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	4.050	22.6	-1.200
3	42 % EFFLUENT	10	4.050	22.6	-1.000
4	56 % EFFLUENT	10	4.050	22.6	-1.700
5	75 % EFFLUENT	10	4.050	22.6	-1.600
6	100% EFFLUENT	10	4.050	22.6	-3.500

APPENDIX E

Organism History

AQUATOX, INC.

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

TEST ORGANISM HISTORY

DATE SHIPPED 6/18/15 **CLIENT** ARK ANALYTICAL

Purchase Order #: _____

SPECIES: Pimephales promelas

Quantity Shipped: 240+

Age: HATCHED - 6/16/15 15-16 DS

Brood Stock Source: Anderson Farms, AR

Culture Water: Groundwater

Hardness (Mg/l CaCO3): =160

Dissolved Oxygen (Mg/1): 8.5

Temperature (°C): 25.3 °C

Feeding: ARTEMIA

Comments: _____

Shipped Via: Federal Express UPS Overnight (Shuttle)

Packaged By: _____

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

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

ORGANISM HISTORY

DATE: 11/25/2013

SPECIES: *Ceriodaphnia dubia*

AGE: > 3 day

LIFE STAGE: Adult

HATCH DATE: Variable

BEGAN FEEDING: Immediately

FOOD: YTC, *Selenastrum* sp.

Water Chemistry Record:	**Current**	**Range**
TEMPERATURE:	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

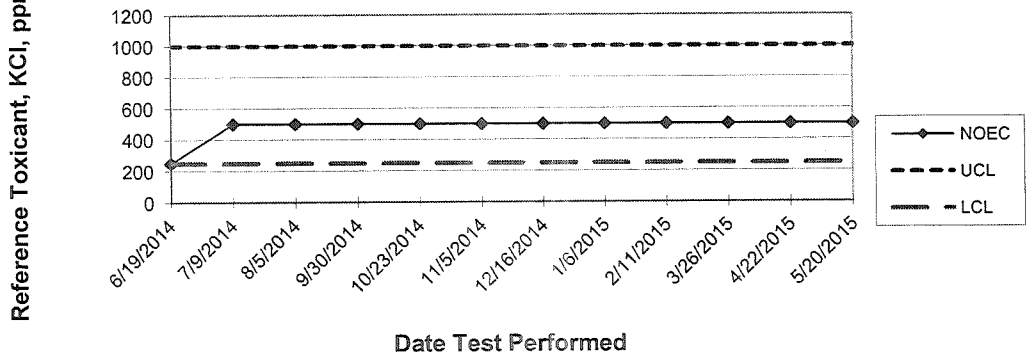
APPENDIX F

Quality Assurance Charts

ARKANSAS ANALYTICAL, INC.

FATHEAD MINNOW SURVIVAL 7 Day

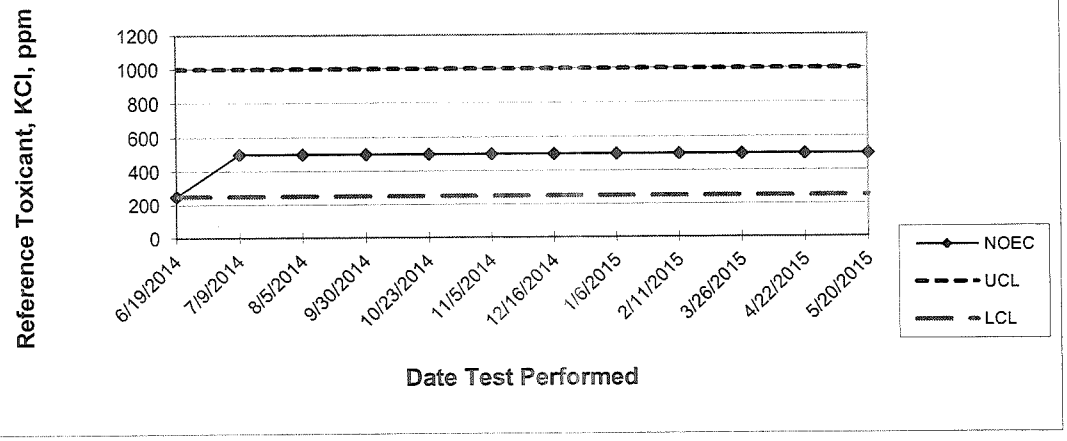
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.

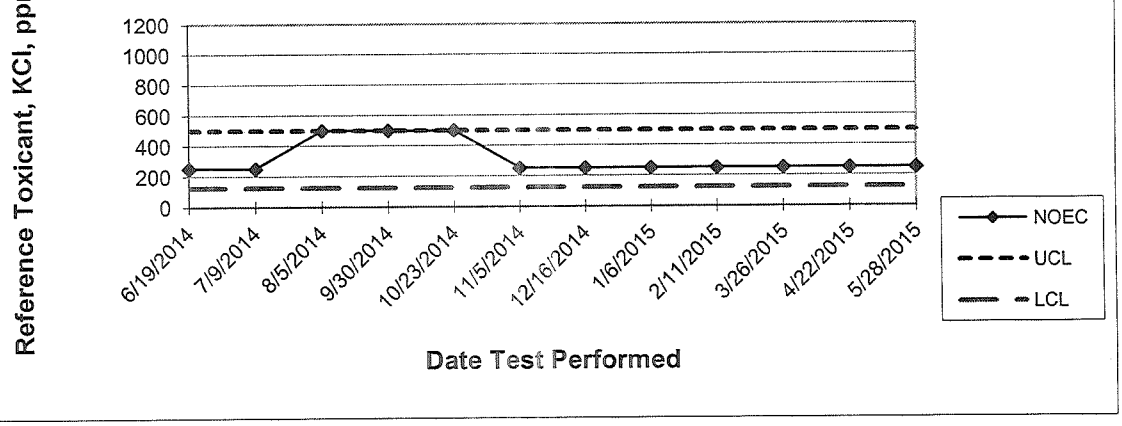
FATHEAD MINNOW GROWTH 7 Day

QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.

CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.

CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE