

Arkansas Analytical, Inc.

Toxicity Test Results
City of De Queen
NPDES PERMIT NUMBER: AR0021733
Third 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 K1508008

Friday, September 04, 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 third 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:	8-25-15, 1000	8-26-15, 1000
Sample #2:	8-26-15, 1000	8-27-15, 1000
Sample #3:	8-30-15, 0830	8-31-15, 0830

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:	8-27-15, 0958	1
Sample #2:	8-28-15, 1205	6
Sample #3:	9-1-15, 1230	1

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	16.0	X	
At least 60% of surviving females should have produced 3 broods	80%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	26.4	X	

TEST ACCEPTANCE CRITERIA for *Pimephales promelas*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	100%	X	
The percent coefficient of variation between replicates must be 40% or less for survival	0.00	X	
Minimum of 0.25 mg average dry weight of surviving controls	0.704	X	
The percent coefficient of variation between replicates must be 40% or less for growth	11.0	X	

Reference Toxicant

The reference toxicant used was Potassium Chloride prepared in-house. The tests were performed using moderately hard synthetic as dilution water. The results of the reference toxicant were:

REFERENCE TOXICANT

<i>Ceriodaphnia dubia</i> 8/5/15 – 8/12/15		<i>Pimephales promelas</i> 8/5/15 – 8/12/15	
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

<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)	19.0	%CV survival (critical dilution)	0.00
%CV Reproduction (critical dilution)	19.7	Mean dry weight (critical dilution) in milligrams	0.786
		%CV growth (critical dilution)	11.4
PMSD Reproduction	31.1	PMSD Growth	17.1

Conclusion

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

The permit issued to the City of 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 Rood

Reviewed by:


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:	8-25-15, 1000	8-26-15, 1000
Sample #2:	8-26-15, 1000	8-27-15, 1000
Sample #3:	8-30-15, 0830	8-31-15, 0830

Test initiated (date, time): 8-27-15, 1400 Test terminated (date, time): 9-3-15, 1400

Dilution water used: Moderately Hard Synthetic

DATA TABLE FOR FATHEAD MINNOW SURVIVAL

Effluent Conc %	Percent Survival in Replicate Chambers					Mean Percent Survival				CV %
	A	B	C	D	E	24 hours	48 hours	7 days		
0%	100	100	100	100	100	100	100	100	0.00	
32%	100	100	100	100	100	100	100	100		
42%	100	100	100	100	100	100	100	100		
56%	100	100	100	100	100	100	100	100		
75%	100	100	100	100	100	100	100	100		
100%	100	100	100	100	100	100	100	100	0.00	

DATA TABLE FOR GROWTH OF FATHEAD MINNOWS

Effluent Conc %	A	B	C	D	E	Mean Dry Weight	CV%
0%	0.659	0.713	0.699	0.624	0.827	0.704	11.0
32%	0.605	0.725	0.737	0.749	0.859	0.735	
42%	0.832	0.683	0.741	0.844	0.909	0.802	
56%	0.716	0.739	0.784	0.757	0.842	0.768	
75%	0.762	0.728	0.764	0.790	0.939	0.797	
100%	0.774	0.861	0.768	0.651	0.874	0.786	11.4

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)= 11.4 %

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:	8-25-15, 1000	8-26-15, 1000
Sample #2:	8-26-15, 1000	8-27-15, 1000
Sample #3:	8-30-15, 0830	8-31-15, 0830

Test initiated (date, time): 8-27-15, 1400 Test terminated (date, time): 9-3-15, 1000

Dilution water used: Moderately Hard Synthetic

Ceriodaphnia dubia SURVIVAL AND REPRODUCTION
 NUMBER OF YOUNG PRODUCED PER FEMALE @ TEST TERMINATION
 PERCENT EFFLUENT

Replicate	0%	32%	42%	56%	75%	100%
A	15	13	13	13	15	18
B	18	15	14	7	19	24
C	15	11	14	20	19	22
D	18	6	18	17	10	22
E	8	21	21	20	23	18
F	10	17	19	17	18	19
G	20	17	10	20	9	13
H	21	10	8	24	8	19
I	16	20	X4	16	20	13
J	19	9	16	12	23	22
Mean	16.0	13.9	13.7	16.6	16.4	19.0
Mean/surviving female	16.0	13.9	14.8	16.6	16.4	19.0
CV%*	26.4					19.7

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	90	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:

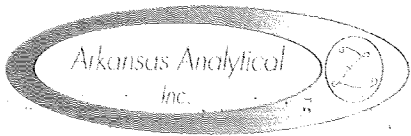
a) NOEC survival (parameter TOP3B)= 100 % effluent

b) NOEC reproduction (parameter TPP3B)= 100 % effluent

c) Coefficient of variation (parameter TQP3B)= 26.4 %



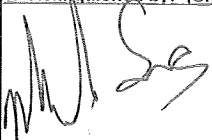
APPENDIX A

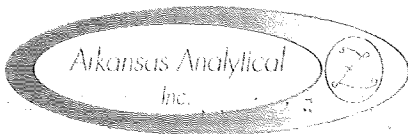
Chain of Custody Forms



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

CHAIN OF CUSTODY RECORD

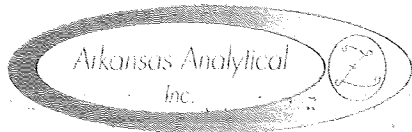
CLIENT INFORMATION		BILLING		Project Description		Turnaround Time		Preservation Codes:											
City of DeQueen Wastewater Plant		City of DeQueen Wastewater Plant		Chronic Toxicity		1 Day (100%)		1. Cool, 4 Degrees Centigrade				4. Thiosulfate for Dechlorination							
514 South 9th		P.O. Box 730				2 Day (50%)		2. Sulfuric Acid (H ₂ SO 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		Preservative Code: 1		TEST PARAMETERS								Bottle Type Code			
Attn: Mike Sims				Fax: 870-642-3117		Bottle Type: P										G = Glass; P = Plastic			
				Email: msims@cityofdequeen.com				V = Septum; A = Amber											
 Sampler(s) Signature				 Sampler(s) Printed				Chronic Biomonitoring		Arkansas Analytical Work Order Number: K1508-008A									
Field Number	SAMPLE COLLECTION		Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION												
	8-25-26-15	10:00-10:00		X		Water	DQ Toxicity		X										
1. Relinquished by: (Signature)		Date/Time		2. Received by: (Signature)		SAMPLE CONDITION UPON RECEIPT BY LAB				REMARKS / SAMPLE COMMENTS									
		8-26-15 11:36 am		UPS		1. CUSTODY SEALS: <input checked="" type="checkbox"/> Yes ___ No				P# 71769									
3. Relinquished by: (Signature)		Date/Time		4. Received by lab: (Signature)		2. CONTAINERS CORRECT: <input checked="" type="checkbox"/> Yes ___ No													
UPS		8/27/15 0958		Sydney James		3. COC/LABELS AGREE: <input checked="" type="checkbox"/> Yes ___ No													
						4. RECEIVED ON ICE: <input checked="" type="checkbox"/> Yes ___ No													
						5. TEMPERATURE ON RECEIPT: 1°C													
						6. TEMPERATURE GUN ID: HHT#2													
						FOR LABORATORY USE ONLY													



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 ₂ SO 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							
Attn: Mike Sims				Telephone: 870-642-5231		Preservative Code: 1		TEST PARAMETERS								Bottle Type Code			
				Fax: 870-642-3117		Bottle Type: P										G = Glass; P = Plastic V = Septum; A = Amber			
Email: msims@cityofdequeen.com																			
Sampler(s) Signature				Sampler(s) Printed MS				Chronic Biomonitoring										Arkansas Analytical Work Order Number: K1508008	
Field Number	SAMPLE COLLECTION		Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION												
	8-26-27-15	10:40-10:50		X		Water	DQ Toxicity												
1. Relinquished by: (Signature)		Date/Time 8-27-15 11:45am		2. Received by: (Signature) UPS		SAMPLE CONDITION UPON RECEIPT BY LAB		REMARKS / SAMPLE COMMENTS											
						1. CUSTODY SEALS: <input checked="" type="checkbox"/> Yes ___ No		PO# 71769											
						2. CONTAINERS CORRECT: <input checked="" type="checkbox"/> Yes ___ No													
						3. COC/LABELS AGREE: <input checked="" type="checkbox"/> Yes ___ No													
3. Relinquished by: (Signature) UPS		Date/Time 8/28/15 1205		4. Received by lab: (Signature) Amanda Jabush		4. RECEIVED ON ICE: ___ Yes ___ No													
						5. TEMPERATURE ON RECEIPT: 6°C													
						6. TEMPERATURE GUN ID: HHT#2													
						FOR CUSTODY USE ONLY													



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 ₂ SO 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						
Attn: Mike Sims				Telephone: 870-642-5231		Preservative Code: 1		TEST PARAMETERS								Bottle Type Code		
				Fax: 870-642-3117		Bottle Type: P										G = Glass; P = Plastic V = Septum; A = Amber		
				Email: msims@cityofdequeen.com														
Sampler(s) Signature				Sampler(s) Printed MS												Arkansas Analytical Work Order Number:		
Field Number	SAMPLE COLLECTION Date/s Time/s		Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION		Chronic Biomonitoring								K1508-008C	
	8-30-31-15 8:30-8:30			X		Water	DQ Toxicity		X									
1. Relinquished by: (Signature)			Date/Time 8-31-15 11:45am		2. Received by: (Signature) UPS			CUSTODY CHECKLIST (FOR RECEIVING LAB)				REMARKS / SAMPLE COMMENTS						
3. Relinquished by: (Signature) UPS			Date/Time 9-1-15 1230		4. Received by lab: (Signature) Sydney James			1. CUSTODY SEALS: <input checked="" type="checkbox"/> Yes ___ No 2. CONTAINERS CORRECT: <input type="checkbox"/> Yes ___ No 3. COC/LABELS AGREE: <input type="checkbox"/> Yes ___ No 4. RECEIVED ON ICE: <input type="checkbox"/> Yes ___ No 5. TEMPERATURE ON RECEIPT: 1°C 6. TEMPERATURE GUN ID: HTF #2				PS# 71769						
FOR COMPLETION BY LAB ONLY																		

APPENDIX B

Effluent and Dilution Water Data

Biomonitoring Quality Control Benchsheet

Analyst	RH	RH	RH	RH	KL	KE	RL	RH
Date	8-25-15	8-26-15	8-27-15	8-28-15	8-29-15	8-30-15	8-31-15	9-1-15
pH Meter ID	AR60	_____						
LIN pH 4 Buffer	1500706	_____						
LIN pH 7 Buffer	1500707	_____						
LIN pH 10 Buffer	1500708	_____						
Slope (>90%)	99.0%	95.7%	90.8%	90.8%	90.7%	91.3%	93.5%	92.7%

Dissolved O ₂ Meter	DO 1305	_____						
Meter Reading	8.72	8.79	8.71	8.70	8.61	8.70	8.67	8.47
Temp.	22	21	22	22	22	22	22	23
Chart Value at Temp.	8.743	8.915	8.743	8.743	8.743	8.743	8.743	8.578
Difference	0.023	0.165	0.033	0.043	0.13	0.043	0.073	0.108
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	22	21	22	22	21	21	22	23
Thermometer Reading	22	22	22	21	21	21	22	22
Thermometer ID	FB	_____						
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							
STD Result	106							
T.V. / %REC	100/106%							
Acceptance Criteria	93.5-108.5% Recovery							

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

Conductivity Meter ID	Con 02	_____						
Blank (<1)	<1							
STD Result	1436							
T.V. / %REC	1412/107%							
Acceptance Criteria	99.2-104.0% Recovery							

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

Revision 0
Effective Date 01APR15

Biomonitoring Quality Control Benchsheet

Analyst	RH	BH	RH						
Date	9-2-15	9-3-15	9-4-15						
pH Meter ID	AR100								
LIN pH 4 Buffer	1500106								
LIN pH 7 Buffer	1500107								
LIN pH 10 Buffer	1500108								
Slope (>90%)	92.1%	92.3%							

Dissolved O ₂ Meter	101305								
Meter Reading	8.15	8.72							
Temp.	22	22							
Chart Value at Temp.	8.743	8.743							
Difference	0.093	0.023							
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	<0.2mg/L

Temp. Meter ID	AR100								
Meter Reading	22	22							
Thermometer Reading	21	22							
Thermometer ID	08								
Acceptance Criteria	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C

Alkalinity									
Blank (<5mg/L)									
STD Result									
T.V. / %REC									
Acceptance Criteria									

Hardness									
Blank (<2mg/L)									
STD. Result									
T.V. / %REC									
Acceptance Criteria									

Conductivity Meter ID	(=100)								
Blank (<1)									
STD Result									
T.V. / %REC									
Acceptance Criteria									

Chlorine Meter ID	DR 920								
Blank (<0.05mg/L)									
STD Result									
T.V. / % REC									
Acceptance Criteria									

Revision 0
Effective Date 01APR15

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Fathead Minnow

Lab # / Sample ID K1508008

Test Start (Date/Time)

8-27-15

1400

Client: D. Queen

Test End (Date/Time)

9-3-15

1400

Day of Test

		1	2	3	4	5	6	7	notes
Control	mt >	8-27	8-28	8-29	8-30	8-31	9-1	9-2	
D.O. (mg/L)	INITIAL	7.2	8.6	7.7	8.2	8.7	8.6	8.5	
	FINAL	7.0	8.3	8.2	6.9	7.3	7.9	7.7	
pH (s.u.)	INITIAL	7.8	8.0	7.9	7.9	7.7	7.8	7.9	
	FINAL	8.0	7.7	7.9	8.0	7.5	7.9	7.6	
temp (C)	INITIAL	22	23	22	22	22	22	22	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		64							
HARDNESS (mg/L)		86							
CONDUCTIVITY (umhc)		968							
CHLORINE (mg/L)		20.05							
CONC:	32								
D.O. (mg/L)	INITIAL	8.5	8.6	9.4	9.1	8.8	8.3	8.5	
	FINAL	7.0	8.1	8.1	6.1	7.5	8.2	7.9	PH
pH (s.u.)	INITIAL	7.6	8.0	7.7	7.9	7.6	7.8	7.8	
	FINAL	7.8	7.5	7.7	7.9	7.6	7.8	7.6	
temp (C)	INITIAL	22	23	24	22	21	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	42								
D.O. (mg/L)	INITIAL	8.8	8.7	8.3	8.1	8.9	8.6	8.7	
	FINAL	7.1	8.2	8.0	7.8	7.2	8.0	7.7	
pH (mg/L)	INITIAL	7.6	8.0	7.7	7.9	7.6	7.9	7.9	
	FINAL	7.7	7.8	7.9	7.9	7.6	7.7	7.6	
temp (C)	INITIAL	22	22	25	22	21	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	56								
D.O. (mg/L)	INITIAL	8.8	8.8	8.2	8.1	9.1	8.8	8.8	
	FINAL	7.2	8.0	8.0	7.6	7.3	7.9	7.8	
pH (s.u.)	INITIAL	7.5	7.8	7.7	7.9	7.5	7.8	7.8	
	FINAL	7.7	7.8	7.9	7.9	7.5	7.7	7.6	
temp (C)	INITIAL	22	22	25	23	21	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	75								
D.O. (mg/L)	INITIAL	9.3	9.0	8.1	8.0	9.2	9.1	9.0	
	FINAL	7.4	8.0	8.0	7.6	7.2	8.0	7.6	
pH (s.u.)	INITIAL	7.5	7.8	7.6	7.9	7.5	7.8	7.7	
	FINAL	7.7	7.7	7.8	7.9	7.6	7.7	7.6	
temp (C)	INITIAL	21	22	24	23	20	23	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	100								
D.O. (mg/L)	INITIAL	9.3	9.1	7.6	7.8	9.1	9.3	9.1	
	FINAL	7.5	7.9	8.0	7.6	7.3	8.0	7.7	
pH (s.u.)	INITIAL	7.4	7.7	7.7	7.9	7.7	7.8	7.6	
	FINAL	7.6	7.7	7.8	7.6	7.6	7.7	7.6	
temp (C)	INITIAL	21	22	27	23	20	23	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	100 %	A	A	A	B	B	C	C	
ALKALINITY (mg/L)		64			60		40		
HARDNESS (mg/L)		72			50		58		
CONDUCTIVITY (umhc)		962			1030		1053		
CHLORINE (mg/L)		20.05							

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Chironomidia Dubia

Lab # / Sample ID K1508008

Test Start (Date/Time)

8-27-15

1430

Client: Dr Queen

Test End (Date/Time)

9-3-15

1500

Day of Test

		1	2	3	4	5	6	7	notes
Control	ms H5	8-27	8-28	8-29	8-30	8-31	9-1	9-2	
D.O. (mg/L)	INITIAL	7.2	8.1	7.7	8.2	8.7	8.6	8.5	
	FINAL	8.5	8.4	8.2	8.5	8.5	7.7	8.3	
pH (s.u.)	INITIAL	7.8	8.0	7.9	7.9	7.7	7.9	7.9	
	FINAL	8.1	8.7	8.0	7.5	7.7	7.6	8.0	
temp (C)	INITIAL	22	23	22	22	22	22	22	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		64							
HARDNESS (mg/L)		86							
CONDUCTIVITY (umhd)		968							
CHLORINE (mg/L)		0.05							
CONC:	32								
D.O. (mg/L)	INITIAL	8.5	8.6	8.4	8.1	8.8	8.3	8.5	
	FINAL	8.4	8.1	8.1	8.3	8.5	7.7	8.2	
pH (s.u.)	INITIAL	7.6	8.0	7.7	7.9	7.6	7.8	7.8	
	FINAL	8.0	8.3	8.0	7.6	7.4	7.6	7.9	
temp (C)	INITIAL	22	23	24	22	21	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	42								
D.O. (mg/L)	INITIAL	8.8	8.7	8.3	8.1	8.9	8.6	8.7	
	FINAL	8.6	8.2	8.3	8.5	8.1	7.9	8.5	
pH (mg/L)	INITIAL	7.6	8.0	7.7	7.9	7.6	7.9	7.9	
	FINAL	8.0	7.8	8.0	7.4	7.3	7.6	8.0	
temp (C)	INITIAL	22	22	25	22	21	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	5L								
D.O. (mg/L)	INITIAL	8.8	8.8	8.2	8.1	9.1	8.8	8.8	
	FINAL	8.6	8.2	8.4	8.4	8.5	7.9	8.6	
pH (s.u.)	INITIAL	7.5	7.8	7.7	7.9	7.5	7.8	7.8	
	FINAL	7.9	7.9	7.9	7.3	7.4	7.6	8.0	
temp (C)	INITIAL	22	22	25	23	21	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	75								
D.O. (mg/L)	INITIAL	9.3	9.0	8.1	8.0	9.2	9.1	9.0	
	FINAL	8.6	8.2	8.4	7.9	8.8	8.5	8.7	
pH (s.u.)	INITIAL	7.5	7.8	7.4	7.9	7.5	7.8	7.7	
	FINAL	7.9	7.9	7.7	7.2	7.2	7.7	7.9	
temp (C)	INITIAL	21	22	26	23	20	23	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	100								
D.O. (mg/L)	INITIAL	7.3	9.1	7.6	7.9	9.1	9.3	9.1	
	FINAL	8.6	8.2	8.3	8.0	8.6	8.4	8.8	
pH (s.u.)	INITIAL	7.4	7.7	7.7	7.9	7.7	7.8	7.6	
	FINAL	7.8	7.8	7.7	7.5	7.5	7.7	7.9	
temp (C)	INITIAL	21	22	27	23	20	23	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	100 %	A	A	B	B	B	C	C	
ALKALINITY (mg/L)		54			60		90		
HARDNESS (mg/L)		72			50		58		
CONDUCTIVITY (umhd)		962			1030		1053		
CHLORINE (mg/L)		0.05							

APPENDIX C

Fathead minnow raw data and statistics

SURVIVAL DATA FOR LARVAL SURVIVAL AND GROWTH TEST (ALTERNATE)

LAB #: K1508008				TEST START		DATE	8/27/15	TIME	1400			
CLIENT: De Queen				TEST END		DATE	9/3/15	TIME	1400			
ANALYST: RH/KR				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	8	8	8	8	8	8	8	100%	100.0%	0.00
	B	8	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	8	100%		
	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%	100.0%	
	B	8	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	8	100%		
	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%	100.0%	
	B	8	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	8	100%		
	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%	100.0%	
	B	8	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	8	100%		
	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%	100.0%	
	B	8	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	8	100%		
	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%	100.0%	0.00
	B	8	8	8	8	8	8	8	8	100%		
	C	8	8	8	8	8	8	8	8	100%		
	D	8	8	8	8	8	8	8	8	100%		
	E	8	8	8	8	8	8	8	8	100%		
ANALYST:		RH	RH	KR	KR	RH	RH	RH	RH			
DATE:		8/27/15	8/28/15	8/29/15	8/30/15	8/31/15	9/1/15	9/2/15	9/3/15			
TIME:		1400	1435	1530	1430	1325	1550	1435	1400			

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

REMARKS:

AA# K1508008, FATHEAD MINNOW SURV.,CHRONIC, 8-27-15

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

Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.000

W = 0.000

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# K1508008, FATHEAD MINNOW SURV.,CHRONIC, 8-27-15

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# K1508008, FATHEAD MINNOW SURV.,CHRONIC, 8-27-15
 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	1.3931
1	CONTROL	2	1.0000	1.3931
1	CONTROL	3	1.0000	1.3931
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	1.0000	1.3931
2	32 % EFFLUENT	4	1.0000	1.3931
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	1.0000	1.3931
3	42 % EFFLUENT	4	1.0000	1.3931
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	1.0000	1.3931
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# K1508008, FATHEAD MINNOW SURV.,CHRONIC, 8-27-15
 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	1.393				
2	32 % EFFLUENT	1.393	27.50	16.00	5.00	
3	42 % EFFLUENT	1.393	27.50	16.00	5.00	
4	56 % EFFLUENT	1.393	27.50	16.00	5.00	
5	75 % EFFLUENT	1.393	27.50	16.00	5.00	
6	100 % EFFLUENT	1.393	27.50	16.00	5.00	

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

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:		K1508008		TEST DATES (BEGIN / END):		8/25/15 - 9/3/15	
CLIENT:		City of DeQueen		WEIGHING DATE / TIME:		9/4/2015 1005	
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	0.99181	0.98654	0.00527	8	0.659	AVG DRY
MHS	B	0.98767	0.98197	0.00570	8	0.713	WEIGHT (mg)
	C	0.98389	0.97830	0.00559	8	0.699	0.704
	D	1.00660	1.00161	0.00499	8	0.624	CV
	E	0.98213	0.97551	0.00662	8	0.827	11.0
CONC:	A	0.98992	0.98508	0.00484	8	0.605	AVG DRY
32%	B	1.03134	1.02554	0.00580	8	0.725	WEIGHT (mg)
	C	1.00839	1.00249	0.00590	8	0.737	0.735
	D	0.98921	0.98322	0.00599	8	0.749	CV
	E	0.99028	0.98341	0.00687	8	0.859	
CONC:	A	1.00104	0.99438	0.00666	8	0.832	AVG DRY
42%	B	1.03558	1.03012	0.00546	8	0.683	WEIGHT (mg)
	C	1.00919	1.00326	0.00593	8	0.741	0.802
	D	1.01261	1.00586	0.00675	8	0.844	CV
	E	1.01564	1.00837	0.00727	8	0.909	
CONC:	A	1.00493	0.99920	0.00573	8	0.716	AVG DRY
56%	B	1.00032	0.99441	0.00591	8	0.739	WEIGHT (mg)
	C	1.01044	1.00417	0.00627	8	0.784	0.768
	D	1.00521	0.99915	0.00606	8	0.757	CV
	E	1.00352	0.99678	0.00674	8	0.842	
CONC:	A	1.00962	1.00352	0.00610	8	0.762	AVG DRY
75%	B	0.98645	0.98063	0.00582	8	0.728	WEIGHT (mg)
	C	0.98842	0.98231	0.00611	8	0.764	0.796
	D	0.99487	0.98855	0.00632	8	0.790	CV
	E	1.01378	1.00627	0.00751	8	0.939	
CONC:	A	1.01624	1.01005	0.00619	8	0.774	AVG DRY
100%	B	1.00133	0.99444	0.00689	8	0.861	WEIGHT (mg)
	C	1.01608	1.00994	0.00614	8	0.768	0.786
	D	0.98513	0.97992	0.00521	8	0.651	CV
	E	0.99885	0.99186	0.00699	8	0.874	11.4

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA# K1508008, FATHEAD MINNOW GROWTH CHRONIC, 8-27-15
File: C:\COPYTO~1\TOXSTAT\FHGWGROWTH. Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.157

W = 0.964

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# K1508008, FATHEAD MINNOW GROWTH CHRONIC, 8-27-15
File: C:\COPYTO~1\TOXSTAT\FHGWGROWTH. Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 1.67

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# K1508008, FATHEAD MINNOW GROWTH CHRONIC, 8-27-15
 FILE: C:\COPYTO~1\TOXSTAT\FHGWGROWTH.
 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.6590	0.6590
1	CONTROL	2	0.7120	0.7120
1	CONTROL	3	0.6990	0.6990
1	CONTROL	4	0.6240	0.6240
1	CONTROL	5	0.8270	0.8270
2	32 % EFFLUENT	1	0.6050	0.6050
2	32 % EFFLUENT	2	0.7250	0.7250
2	32 % EFFLUENT	3	0.7370	0.7370
2	32 % EFFLUENT	4	0.7490	0.7490
2	32 % EFFLUENT	5	0.8590	0.8590
3	42 % EFFLUENT	1	0.8320	0.8320
3	42 % EFFLUENT	2	0.6830	0.6830
3	42 % EFFLUENT	3	0.7410	0.7410
3	42 % EFFLUENT	4	0.8440	0.8440
3	42 % EFFLUENT	5	0.9090	0.9090
4	56 % EFFLUENT	1	0.7160	0.7160
4	56 % EFFLUENT	2	0.7390	0.7390
4	56 % EFFLUENT	3	0.7840	0.7840
4	56 % EFFLUENT	4	0.7570	0.7570
4	56 % EFFLUENT	5	0.8420	0.8420
5	75 % EFFLUENT	1	0.7620	0.7620
5	75 % EFFLUENT	2	0.7280	0.7280
5	75 % EFFLUENT	3	0.7640	0.7640
5	75 % EFFLUENT	4	0.7900	0.7900
5	75 % EFFLUENT	5	0.9390	0.9390
6	100 % EFFLUENT	1	0.7740	0.7740
6	100 % EFFLUENT	2	0.8610	0.8610
6	100 % EFFLUENT	3	0.7680	0.7680
6	100 % EFFLUENT	4	0.6510	0.6510
6	100 % EFFLUENT	5	0.8740	0.8740

AA# K1508008, FATHEAD MINNOW GROWTH CHRONIC, 8-27-15
 File: C:\COPYTO~1\TOXSTAT\FHGWGROWTH. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.037	0.007	1.129
Within (Error)	24	0.157	0.007	
Total	29	0.194		

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

AA# K1508008, FATHEAD MINNOW GROWTH CHRONIC, 8-27-15

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	0.704	0.704		
2	32 % EFFLUENT	0.735	0.735	-0.602	
3	42 % EFFLUENT	0.802	0.802	-1.908	
4	56 % EFFLUENT	0.768	0.768	-1.240	
5	75 % EFFLUENT	0.797	0.797	-1.807	
6	100 % EFFLUENT	0.786	0.786	-1.591	

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

AA# K1508008, FATHEAD MINNOW GROWTH CHRONIC, 8-27-15

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	0.121	17.1	-0.031
3	42 % EFFLUENT	5	0.121	17.1	-0.098
4	56 % EFFLUENT	5	0.121	17.1	-0.063
5	75 % EFFLUENT	5	0.121	17.1	-0.092
6	100 % EFFLUENT	5	0.121	17.1	-0.081

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

SURVIVAL AND REPRODUCTION TEST

Ceriodaphnia dubia

Discharger: De Queen
 Location: Outfall 001
 Date Sample Collected: 8 - 26/27/31/ - 15

Lab Number/s
 K1508008

Analyst: RH
 Test Start - Date/Time: 8-27-15, 1400
 Test Stop - Date/Time: 9-3-15,1000

Conc	1	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		%	Day	A	B	C	D	E	F	G	H				
MHS	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	3	0	0	0	0	0	0	0	1	1	0	2	10	0.2	RH
	4	5	1	1	5	4	0	2	0	0	3	21	10	2.1	RH
	5	8	7	4	0	4	5	2	7	8	5	50	10	5.0	RH
	6	2	10	5	6	0	0	10	7	3	0	43	10	4.3	RH
	7	0	0	5	7	0	5	6	6	4	11	44	10	4.4	RH
	8														
Total		15	18	15	18	8	10	20	21	16	19	160		Avg. =	16.0
														C.V. =	26.4

Conc	4	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		%	Day	A	B	C	D	E	F	G	H				
56%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	3	0	0	0	0	0	2	0	0	0	0	2	10	0.2	RH
	4	0	0	6	3	1	0	5	4	3	2	24	10	2.4	RH
	5	5	6	0	0	8	10	8	7	2	4	50	10	5.0	RH
	6	8	0	7	12	9	6	7	8	10	6	73	10	7.3	RH
	7	0	1	7	2	0	1	0	5	1	0	17	10	1.7	RH
	8														
Total		13	7	20	17	20	17	20	24	16	12	166		Avg. =	16.6
														C.V. =	29.5

Conc	2	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		%	Day	A	B	C	D	E	F	G	H				
32%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	3	0	0	0	0	1	0	0	0	0	0	1	10	0.1	RH
	4	2	0	1	0	0	1	0	2	3	0	9	10	0.9	RH
	5	0	5	4	1	7	6	7	0	1	4	35	10	3.5	RH
	6	10	5	6	0	7	8	9	6	8	0	59	10	5.9	RH
	7	1	5	0	5	6	2	1	2	8	5	35	10	3.5	RH
	8														
Total		13	15	11	6	21	17	17	10	20	9	139		Avg. =	13.9
														C.V. =	35.5

Conc	5	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		%	Day	A	B	C	D	E	F	G	H				
75%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	3	1	0	0	0	0	0	0	0	0	0	1	10	0.1	RH
	4	4	4	0	1	0	4	5	0	2	2	22	10	2.2	RH
	5	8	6	11	2	10	5	1	6	3	7	59	10	5.9	RH
	6	1	7	8	5	9	0	3	2	8	8	51	10	5.1	RH
	7	1	2	0	2	4	9	0	0	7	6	31	10	3.1	RH
	8														
Total		15	19	19	10	23	18	9	8	20	23	164		Avg. =	16.4
														C.V. =	34.3

Conc	3	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		%	Day	A	B	C	D	E	F	G	H				
42%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	3	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	4	4	6	0	0	1	0	0	2	3	6	22	10	2.2	RH
	5	9	8	4	8	6	7	2	1	1	1	47	10	4.7	RH
	6	0	0	5	8	12	7	8	0	X	7	47	10	4.7	RH
	7	0	0	5	2	2	5	0	5		2	21	10	2.1	RH
	8														
Total		13	14	14	18	21	19	10	8	4	16	137		Avg. =	14.8
										X				C.V. =	28.5

Conc	6	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
		%	Day	A	B	C	D	E	F	G	H				
100%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	3	0	0	0	1	0	0	0	0	0	0	1	10	0.1	RH
	4	5	5	6	1	0	5	0	2	3	1	28	10	2.8	RH
	5	5	2	0	9	6	4	13	9	6	8	62	10	6.2	RH
	6	0	10	8	1	1	7	0	8	1	7	43	10	4.3	RH
	7	8	7	8	10	11	3	0	0	3	6	56	10	5.6	RH
	8														
Total		18	24	22	22	18	19	13	19	13	22	190		Avg. =	19.0
														C.V. =	19.7

AA # K1508008, C.DUBIA CHRONIC, REPRODUCTION, 8-27-15

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 # K1508008, C.DUBIA CHRONIC, REPRODUCTION, 8-27-15

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

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 1.81

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	9	1	10
TOTAL	19	1	20

CRITICAL FISHER'S VALUE (10,10,10) (p=0.05) IS 6. b VALUE IS 9.
 Since b is greater than 6 there is no significant difference
 between CONTROL and TREATMENT at the 0.05 level.

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
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	1	
3	56	10	0	
4	75	10	0	
5	100	10	0	

TITLE: AA # K1508008, C.DUBIA CHRONIC, REPRODUCCION, 8-27-15
FILE: C:\COPYTO~1\TOXSTAT\C.DUB
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	15.0000	15.0000
1	CONTROL	2	18.0000	18.0000
1	CONTROL	3	15.0000	15.0000
1	CONTROL	4	18.0000	18.0000
1	CONTROL	5	8.0000	8.0000
1	CONTROL	6	10.0000	10.0000
1	CONTROL	7	20.0000	20.0000
1	CONTROL	8	21.0000	21.0000
1	CONTROL	9	16.0000	16.0000
1	CONTROL	10	19.0000	19.0000
2	32 % EFFLUENT	1	13.0000	13.0000
2	32 % EFFLUENT	2	15.0000	15.0000
2	32 % EFFLUENT	3	11.0000	11.0000
2	32 % EFFLUENT	4	6.0000	6.0000
2	32 % EFFLUENT	5	21.0000	21.0000
2	32 % EFFLUENT	6	17.0000	17.0000
2	32 % EFFLUENT	7	17.0000	17.0000
2	32 % EFFLUENT	8	10.0000	10.0000
2	32 % EFFLUENT	9	20.0000	20.0000
2	32 % EFFLUENT	10	9.0000	9.0000
3	42 % EFFLUENT	1	13.0000	13.0000
3	42 % EFFLUENT	2	14.0000	14.0000
3	42 % EFFLUENT	3	14.0000	14.0000
3	42 % EFFLUENT	4	18.0000	18.0000
3	42 % EFFLUENT	5	21.0000	21.0000
3	42 % EFFLUENT	6	19.0000	19.0000
3	42 % EFFLUENT	7	10.0000	10.0000
3	42 % EFFLUENT	8	8.0000	8.0000
3	42 % EFFLUENT	9	4.0000	4.0000
3	42 % EFFLUENT	10	16.0000	16.0000
4	56 % EFFLUENT	1	13.0000	13.0000
4	56 % EFFLUENT	2	7.0000	7.0000
4	56 % EFFLUENT	3	20.0000	20.0000
4	56 % EFFLUENT	4	17.0000	17.0000
4	56 % EFFLUENT	5	20.0000	20.0000
4	56 % EFFLUENT	6	17.0000	17.0000
4	56 % EFFLUENT	7	20.0000	20.0000
4	56 % EFFLUENT	8	24.0000	24.0000

4	56 %	EFFLUENT	9	16.0000	16.0000
4	56 %	EFFLUENT	10	12.0000	12.0000
5	75 %	EFFLUENT	1	15.0000	15.0000
5	75 %	EFFLUENT	2	19.0000	19.0000
5	75 %	EFFLUENT	3	19.0000	19.0000
5	75 %	EFFLUENT	4	10.0000	10.0000
5	75 %	EFFLUENT	5	23.0000	23.0000
5	75 %	EFFLUENT	6	18.0000	18.0000
5	75 %	EFFLUENT	7	9.0000	9.0000
5	75 %	EFFLUENT	8	8.0000	8.0000
5	75 %	EFFLUENT	9	20.0000	20.0000
5	75 %	EFFLUENT	10	23.0000	23.0000
6	100 %	EFFLUENT	1	18.0000	18.0000
6	100 %	EFFLUENT	2	24.0000	24.0000
6	100 %	EFFLUENT	3	22.0000	22.0000
6	100 %	EFFLUENT	4	22.0000	22.0000
6	100 %	EFFLUENT	5	18.0000	18.0000
6	100 %	EFFLUENT	6	19.0000	19.0000
6	100 %	EFFLUENT	7	13.0000	13.0000
6	100 %	EFFLUENT	8	19.0000	19.0000
6	100 %	EFFLUENT	9	13.0000	13.0000
6	100 %	EFFLUENT	10	22.0000	22.0000

AA # K1508008, C.DUBIA CHRONIC, REPRODUCCION, 8-27-15
 File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	191.933	38.387	1.656
Within (Error)	54	1251.800	23.181	
Total	59	1443.733		

Critical F value = 2.45 (0.05,5,40)
 Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All equal

AA # K1508008, C.DUBIA CHRONIC, REPRODUCCION, 8-27-15
 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	16.000	16.000		
2	32 % EFFLUENT	13.900	13.900	0.975	
3	42 % EFFLUENT	13.700	13.700	1.068	
4	56 % EFFLUENT	16.600	16.600	-0.279	
5	75 % EFFLUENT	16.400	16.400	-0.186	
6	100 % EFFLUENT	19.000	19.000	-1.393	

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

AA # K1508008, C.DUBIA CHRONIC, REPRODUCCION, 8-27-15

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	4.974	31.1	2.100
3	42 % EFFLUENT	10	4.974	31.1	2.300
4	56 % EFFLUENT	10	4.974	31.1	-0.600
5	75 % EFFLUENT	10	4.974	31.1	-0.400
6	100 % EFFLUENT	10	4.974	31.1	-3.000

APPENDIX E

Organism History

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

TEST ORGANISM HISTORY

DATE SHIPPED 8/27/15 CLIENT ARK ANALYTICAL

Purchase Order #: _____

SPECIES: Pimephales promelas

Quantity Shipped: 240+ 1.5-1600
CST

Age: HATCHED 8/25/15

Brood Stock Source: Anderson Farms, AR

Culture Water: Groundwater

Hardness (Mg/l CaCO3): 160

Dissolved Oxygen (Mg/l): 8.5

Temperature (°C): 25.1

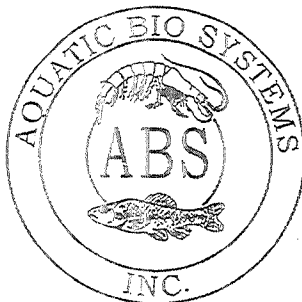
Feeding: ARTEMIA

Comments: _____

Shipped Via: Federal Express UPS Overnight Shuttle

Packaged By: _____

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



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

ORGANISM HISTORY

DATE: 11/25/2013

SPECIES: Ceriodaphnia dubia

AGE: > 3 day

LIFE STAGE: Adult

HATCH DATE: Variable

BEGAN FEEDING: Immediately

FOOD: YTC, Selenastrum sp.

Water Chemistry Record:

	Current	Range
TEMPERATURE:	<u>22°C</u>	<u>22-26°C</u>
SALINITY/CONDUCTIVITY:	<u>--</u>	<u>--</u>
TOTAL HARDNESS (as CaCO ₃):	<u>94 mg/l</u>	<u>76-130 mg/l</u>
TOTAL ALKALINITY (as CaCO ₃):	<u>65 mg/l</u>	<u>65-100 mg/l</u>
pH:	<u>7.98</u>	<u>7.50-8.20</u>

Comments:

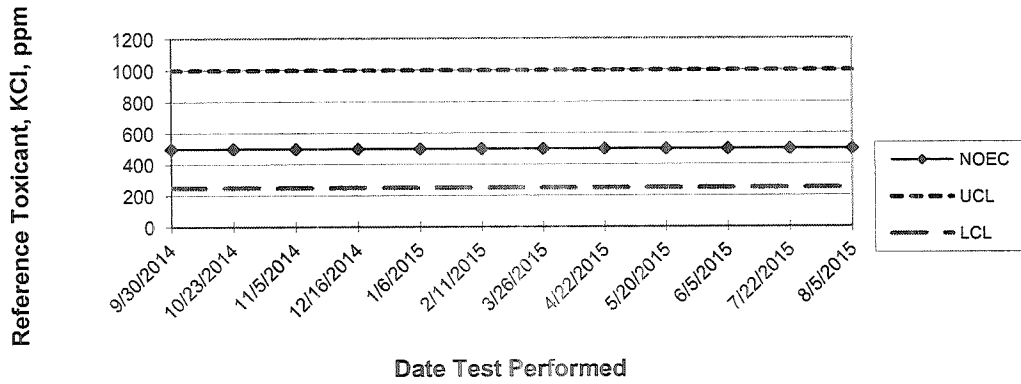


Facility Supervisor

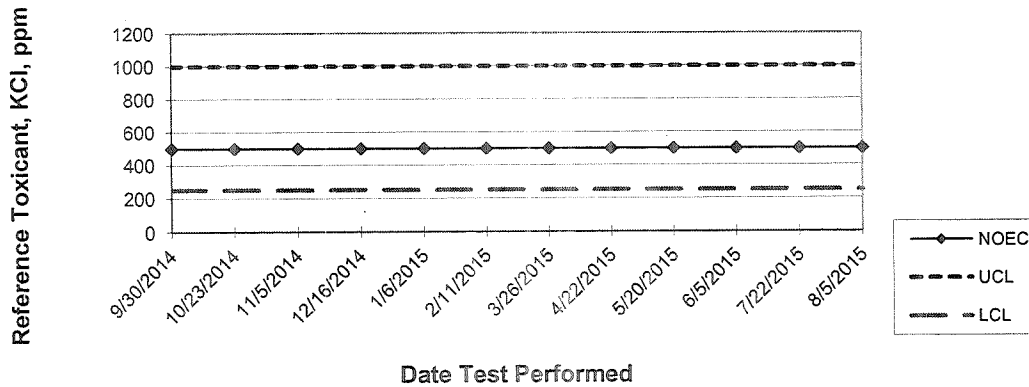
APPENDIX F

Quality Assurance Charts

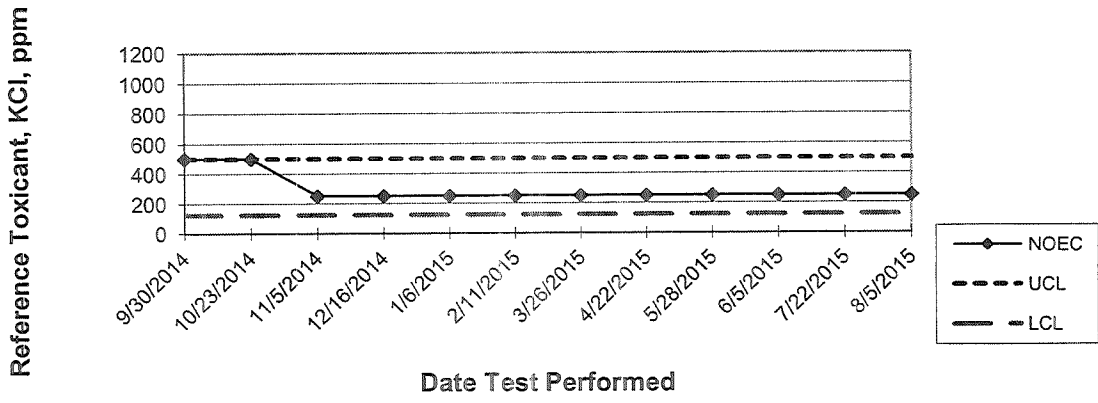
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW SURVIVAL 7 Day
QUALITY ASSURANCE



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

