

Arkansas Department of Environmental Quality  
5301 Northshore Drive  
North Little Rock, AR 72118

OCTOBER 22, 2012

**WATER DIVISION**  
**NPDES Enforcement Section**

**RE: City of De Queen Wastewater Plant, NPDES Permit AR0021733  
AFIN 67-00023, Sept. 2012 DMR, SSO Report & 3<sup>rd</sup> Quarter WET**

Michael Sims  
City of De Queen  
Wastewater Superintendent  
PO Box 730  
De Queen AR 71832





# Arkansas Analytical, Inc.

Toxicity Test Results  
City of DeQueen  
NPDES PERMIT NUMBER: AR0021733  
Third Quarter 2012  
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 K1209008**

Wednesday, October 10, 2012

## **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 9<sup>th</sup> 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 2012.

## **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:	9-25-12, 0800	9-26-12, 0800
Sample #2:	9-26-12, 0800	9-27-12, 0800
Sample #3:	9-30-12, 0900	10-1-12, 0900

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:	9-27-12, 0945	1
Sample #2:	9-28-12, 1000	1
Sample #3:	10-3-12, 1145	12**

\*\* As per Mary Barnett test was allowed to continue although sample #3 was received outside the temperature limits. This was due to a delay in delivery by the courier service.

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 < 24 hour old Fathead Minnows, *Pimephales promelas*, which were purchased from Aquatox; a copy of the organism history is provided in Appendix E.

The organisms used in Test 1002.0 were < 24 hour old *Ceriodaphnia dubia* neonates, (all born within the same eight hours), obtained from an in-house culture. An organism history is provided in Appendix E.

## Quality Assurance

### Test Acceptability

#### TEST ACCEPTANCE CRITERIA for *Ceriodaphnia dubia*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	100%	X	
Average of 15 or more young per surviving female	16.9	X	
At least 60% of surviving females should have produced 3 broods	100%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	21.9	X	

#### TEST ACCEPTANCE CRITERIA for *Pimephales promelas*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	92.5%	X	
The percent coefficient of variation between replicates must be 40% or less for survival	7.40	X	
Minimum of 0.25 mg average dry weight of surviving controls	0.424	X	
The percent coefficient of variation between replicates must be 40% or less for growth	13.6	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> 8/9-16/12		<i>Pimephales promelas</i> 8/9-16/12	
NOEC Survival:	500 ppm KCl	NOEC Survival:	500 ppm KCl
LOEC Survival:	1000 ppm KCl	LOEC Survival:	1000 ppm KCl
NOEC Reproduction:	125 ppm KCl	NOEC Growth:	500 ppm KCl
LOEC Reproduction:	250 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)	20.1	%CV survival (critical dilution)	5.73
%CV Reproduction (critical dilution)	23.6	Mean dry weight (critical dilution) in milligrams	0.462
		%CV growth (critical dilution)	11.1
PMSD Reproduction	24.3	PMSD Growth	22.2

### 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

  
\_\_\_\_\_  
Melissa Bird

  
\_\_\_\_\_  
Kenneth Pigue

**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:	9-25-12, 0800	9-26-12, 0800
Sample #2:	9-26-12, 0800	9-27-12, 0800
Sample #3:	9-30-12, 0900	10-1-12, 0900

Test initiated (date, time): 9-27-12, 1415      Test terminated (date, time): 10-4-12, 1150

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%	87.5	87.5	100	100	87.5		100	97.5	92.5	7.40
32%	87.5	100	100	100	100		97.5	97.5	97.5	
42%	87.5	100	100	100	100		100	100	97.5	
56%	87.5	87.5	100	87.5	100		97.5	95	92.5	
75%	87.5	100	100	100	87.5		100	97.5	95	
100%	100	100	100	87.5	100		100	100	97.5	5.73

**DATA TABLE FOR GROWTH OF FATHEAD MINNOWS**

Effluent Conc %	A	B	C	D	E		Mean Dry Weight	CV%
0%	0.433	0.340	0.400	0.490	0.459		0.424	13.6
32%	0.320	0.426	0.477	0.433	0.511		0.433	
42%	0.459	0.336	0.446	0.505	0.554		0.460	
56%	0.501	0.404	0.503	0.458	0.544		0.482	
75%	0.470	0.360	0.397	0.431	0.528		0.437	
100%	0.400	0.474	0.539	0.463	0.436		0.462	11.1

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)=     13.6     %

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING  
FATHEAD MINNOW LARVAE GROWTH AND SURVIVAL  
*Pimephales promelas*

1. Dunnett's procedure or Steel's Many-One Rank Test as appropriate:  
Is the mean survival at 7 days significantly different ( $p=0.05$ ) than the control survival for:  
a) LOW FLOW OR CRITICAL DILUTION, (100%)    YES \_\_\_\_\_    NO X
  
2. Dunnett's Procedure  
Is the mean dry weight (growth) at 7 days significantly different ( $p=0.05$ ) than the control's dry weight (growth) for:  
a) LOW FLOW OR CRITICAL DILUTION, (100%)    YES \_\_\_\_\_    NO X
  
3. If NO was answered to 1.a) enter [0] otherwise enter [1] (parameter TLP6C): \_\_\_\_\_ 0
  
4. If NO was answered to 2.a) enter [0] otherwise enter [1] (parameter TGP6C): \_\_\_\_\_ 0
  
5. Enter percentage corresponding to each parameter below:  
a) NOEC survival (parameter TOP6C)= \_\_\_\_\_ 100 \_\_\_\_\_ % effluent  
b) NOEC growth (parameter TPP6C)= \_\_\_\_\_ 100 \_\_\_\_\_ % effluent  
c) Coefficient of variation (parameter TQP6C)= \_\_\_\_\_ 13.6 \_\_\_\_\_ %

**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:

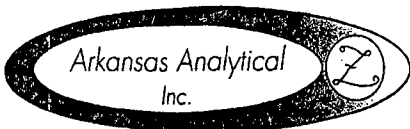
a) NOEC survival (parameter TOP3B)= 100 % effluent

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

c) Coefficient of variation (parameter TQP3B)= 23.6 %

APPENDIX A

Chain of Custody Forms



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		24 Hour		1. Cool, 4 Degrees Centigrade				4. Thiosulfate for Dechlorination							
514 South 9th		P.O. Box 730				48 Hour		2. Sulfuric Acid (H <sub>2</sub> SO <sub>4</sub> ), pH < 2				5. Hydrochloric Acid(HCl)							
DeQueen, AR 71832		DeQueen, AR 71832		Reporting Information		72 Hour		3. Nitric Acid (HNO <sub>3</sub> ), pH < 2				6. Sodium Hydroxide (NaOH), pH > 12							
Attn: Mike Sims				Telephone: 870-642-5231		Routine		TEST PARAMETERS								Bottle Type Code			
				Fax: 870-642-3117		Preservative Code: 1		1								G = Glass; P = Plastic			
				Email: msims@cityofdequeen.com		Bottle Type: P		P								V = Septum; A = Amber			
Sampler(s) Signature				Sampler(s) Printed <i>MS</i>				Chronic Biomonitoring										Arkansas Analytical Work Order Number: K1209-008A	
Field Number	SAMPLE COLLECTION		Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION												
	Date/s	Time/s																	
	9-25/26-12	8:10/8:20		X		Water	DQ Bio	X											
1. Relinquished by: (Signature)		Date/Time		2. Received by: (Signature)		SAMPLE CONDITION UPON RECEIPT IN LAB						REMARKS / SAMPLE COMMENTS							
		9-26-12		UPS		1. CUSTODY SEALS: <input checked="" type="checkbox"/> Yes ___ No						P.O. Number: 67050							
		11:45 am				2. CONTAINERS CORRECT: <input type="checkbox"/> Yes ___ No													
3. Relinquished by: (Signature)		Date/Time		4. Received by lab: (Signature)		3. COC/LABELS AGREE: <input type="checkbox"/> Yes ___ No													
UPS		9/27/12		Sydney James		4. PRESERVATION CONFIRMED: <input type="checkbox"/> Yes ___ No													
Revision 1		0945				5. RECEIVED ON ICE: <input type="checkbox"/> Yes ___ No													
12/17/10						6. TEMPERATURE ON RECEIPT: 100													
														FOR COMPLETION BY LAB ONLY					







## Norma James

---

**From:** Barnett, Mary [BARNETT@adeq.state.ar.us]  
**Sent:** Thursday, October 04, 2012 7:36 AM  
**To:** Norma James  
**Subject:** RE: city of dequeen WET temp excursion

Norma,

Due to unintended extenuating circumstances involving the sample courier, and since the facility generally has a history of passing WET tests, the Department has determined the third sample of the 3<sup>rd</sup> quarter test is an acceptable sample.

The Department encourages the facility and laboratory to work with the couriers to ensure that all subsequent WET test samples are received within EPA's sampling handing requirements.

Mary Barnett  
Ecologist  
501-682-0666

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**From:** Norma James [mailto:njames@arkansasanalytical.com]  
**Sent:** Wednesday, October 03, 2012 12:18 PM  
**To:** Barnett, Mary  
**Cc:** 'Ken Pigue'; 'Lessie Redican'; 'Sydney James'  
**Subject:** city of dequeen WET temp excursion

Mary,

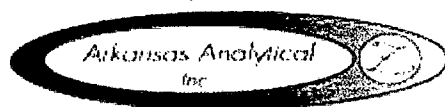
As discussed with Ken earlier yesterday, we are continuing to run the current WET test for the City of DeQueen although the temperature of the third sample received was 12degrees C. We will note such in the report. Delivery of the sample was delayed by third party courier service, causing the temp to be elevated. DeQueen usually does a good job of icing down the samples.

Let me know if you need any further information.

Thank you for your assistance.

Norma

*Norma James, President*



11701 Interstate 30  
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Business Hours - Mon-Thur - 8am-5pm, Fri - 8am-4pm

Think for yourselves and let others enjoy the privilege to do so too. Voltaire.

This e-mail and any attachments are confidential. If you receive this message in error or are not the intended recipient, you should not retain,

APPENDIX B

Effluent and Dilution Water Data

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Fathead Minnow

Lab # / Sample ID

Test Start (Date/Time) 9-27-18 1415

Client: Dr Queen

Test End (Date/Time) 10-4-12 1150

Day of Test

		1	2	3	4	5	6	7	notes/remarks
Control	MHS551	9-27	9-28	9-29	9-30	10-1	10-2	10-3	
D.O. (mg/L)	INITIAL	8.6	8.5	8.5	8.6	8.7	8.9	8.9	
	FINAL	7.915	8.0	6.6	7.7	7.4	7.4	5.9	
pH (s.u.)	INITIAL	8.0	7.8	8.0	8.2	7.9	7.9	7.8	
	FINAL	7.3	8.0	8.2	8.25	7.6	7.4	7	
temp (C)	INITIAL	22	22	21.9	21.0	21	21	21	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		62							
HARDNESS (mg/L)		84							
CONDUCTIVITY (umhos/cm)		294							
CHLORINE (mg/L)		<0.05							
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.8	8.9	8.8	8.7	8.8	8.8	
	FINAL	7.5	8.1	6.7	7.7	7.3	7.5	7.4	
pH (s.u.)	INITIAL	7.8	7.6	7.9	8.1	7.4	7.8	7.4	
	FINAL	7.4	7.9	7.7	7.5	7.6	7.4	7.4	
temp (C)	INITIAL	22	21	22.5	22.0	22	21	21	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.8	9.1	9.0	8.5	8.7	8.8	
	FINAL	7.515	8.2	6.8	7.6	7.2	7.5	7.5	
pH (mg/L)	INITIAL	7.7	7.7	8.0	7.8	7.4	7.4	7.7	
	FINAL	7.4	7.9	7.7	7.5	7.2	7.4	7.4	
temp (C)	INITIAL	22	21	23.4	24.0	22	22	21	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.6	9.3	9.0	8.7	8.7	8.8	
	FINAL	7.5	8.1	6.6	7.9	7.4	6.9	7.1	
pH (s.u.)	INITIAL	7.7	7.3	7.8	7.7	7.4	7.2	7.3	
	FINAL	7.4	8.0	7.6	7.5	7.5	7.4	7.3	
temp (C)	INITIAL	21	21	24.1	25.2	22	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.7	8.7	9.2	8.9	8.7	8.8	8.9	
	FINAL	7.9	8.1	6.6	7.7	7.4	7.4	6.9	
pH (s.u.)	INITIAL	7.7	7.5	7.7	7.5	7.3	7.4	7.5	
	FINAL	7.4	7.5	7.6	7.0	7.4	7.2	7.4	
temp (C)	INITIAL	22	21	24.8	26.0	23	21	21	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.7	8.6	8.7	8.9	8.6	8.9	8.9	
	FINAL	7.6	8.2	6.6	7.6	6.9	7.3	7.5	
pH (s.u.)	INITIAL	7.5	7.2	7.7	7.4	7.3	7.2	7.2	
	FINAL	7.4	7.5	7.5	7.1	7.3	7.3	7.3	
temp (C)	INITIAL	21	22	23.5	25.2	20	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC: 100%		A	A	A	B	B	B	C	
ALKALINITY (mg/L)		22			24			26	
HARDNESS (mg/L)		40			32			26	
CONDUCTIVITY (umhos/cm)		917			957			806	
CHLORINE (mg/L)		<0.05			<0.05			<0.05	

MHS

32

42

56

75

100

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Cerodaphnia Dubia

Lab # / Sample ID

Test Start (Date/Time) 9-27-12 1100

Client: De Queen

Test End (Date/Time) 10-4-12 1030

Day of Test

		1	2	3	4	5	6	7	notes/remarks
Control	MHS551	9-27	9-28	9-29	9-30	10-1	10-2	10-3	
D.O. (mg/L)	INITIAL	8.6	8.5	8.5	8.6	8.7	8.9	8.9	
	FINAL	8.1	<del>8.7</del> 8.27	8.2	8.1	8.1	8.1	7.8	
pH (s.u.)	INITIAL	8.0	7.8	8.0	8.2	7.9	7.9	7.8	
	FINAL	7.7	7.8	7.9	7.8	7.7	7.7	7.5	
temp (C)	INITIAL	22	22	21.3	21	21	21	21	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		62							
HARDNESS (mg/L)		84							
CONDUCTIVITY (umhos/cm)		294							
CHLORINE (mg/L)		<0.05							
<b>CONC:</b>									
D.O. (mg/L)	INITIAL	8.6	8.8	8.9	8.8	8.7	8.8	8.8	
	FINAL	8.0	8.2	8.2	8.0	8.2	8.2	7.5	
pH (s.u.)	INITIAL	7.8	7.6	7.9	8.1	7.4	7.8	7.9	
	FINAL	7.7	7.9	8.2	7.7	7.6	7.7	7.3	
temp (C)	INITIAL	22	22.2	22.5	22	22	21	21	
	FINAL	25	25	25	25	25	25	25	
<b>CONC:</b>									
D.O. (mg/L)	INITIAL	8.6	8.8	9.1	9.0	8.5	8.7	8.8	
	FINAL	8.0	8.1	8.2	8.1	8.1	8.5	7.6	
pH (mg/L)	INITIAL	7.7	7.7	8.0	7.9	7.4	7.4	7.7	
	FINAL	7.7	8.0	8.0	7.8	7.5	7.7	7.1	
temp (C)	INITIAL	22	22.2	23.4	24	22	22	21	
	FINAL	25	25	25	25	25	25	25	
<b>CONC:</b>									
D.O. (mg/L)	INITIAL	8.6	8.6	9.3	9.0	8.7	8.7	8.8	
	FINAL	8.3	8.1	8.4	8.2	8.1	8.2	7.6	
pH (s.u.)	INITIAL	7.7	7.3	7.8	7.7	7.4	7.2	7.3	
	FINAL	7.8	8.0	7.8	7.7	7.4	7.6	7.3	
temp (C)	INITIAL	21	21	24.1	25.2	22	22	22	
	FINAL	25	25	25	25	25	25	25	
<b>CONC:</b>									
D.O. (mg/L)	INITIAL	8.7	8.7	9.2	8.9	8.7	8.8	8.9	
	FINAL	8.0	8.1	8.2	8.1	8.3	8.2	7.7	
pH (s.u.)	INITIAL	7.7	7.5	7.7	7.5	7.3	7.4	7.5	
	FINAL	8.1	8.0	7.9	7.6	7.5	7.7	7.2	
temp (C)	INITIAL	22	21	24.8	26	23	21	21	
	FINAL	25	25	25	25	25	25	25	
<b>CONC:</b>									
D.O. (mg/L)	INITIAL	8.7	8.6	8.7	8.9	8.6	8.9	8.9	
	FINAL	8.1	8.1	8.2	8.0	8.0	8.2	7.6	
pH (s.u.)	INITIAL	7.5	7.2	7.7	7.4	7.3	7.2	7.2	
	FINAL	7.5	7.9	7.7	7.6	7.6	7.7	7.4	
temp (C)	INITIAL	21	22	23.5	25.2	20	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	100%	A	A	A	B	B	B	C	
ALKALINITY (mg/L)		22			24			26	
HARDNESS (mg/L)		40			32			26	
CONDUCTIVITY (umhos/cm)		917			957			806	
CHLORINE (mg/L)		<0.05			<0.05		<0.05	<0.05	

mHS

32

42

RH 9-27

42

56

75

100

## APPENDIX C

Fathead minnow raw data and statistics

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID K1709008 TEST START DATE 9/27/12 TIME 1415  
 CLIENT DeQueer TEST END DATE 10/4/12 TIME 1150

AGE AND SOURCE OF MINNOWS

		D A Y (NUMBER SURVIVING)							SURVIVAL		MEAN %	CV
CONC:	REP #	start	1	2	3	4	5	6	7 %			
0	A	8	8	8	8	8	8	7	7	87.5	92.5	7.40
	B	8	8	7	7	7	7	7	7	87.5		
	C	8	8	8	8	8	8	8	8	100		
	D	8	8	8	8	8	8	8	8	100		
	E	8	8	7	7	7	7	7	7	87.5		
32	A	8	7	7	7	7	7	7	7	87.5	97.5	
	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		
42	A	8	8	8	8	8	8	7	7	87.5	97.5	
	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		
56	A	8	8	7	7	7	7	7	7	87.5	92.5	1.92
	B	8	7	7	7	7	7	7	7	87.5		
	C	8	8	8	8	8	8	8	8	100		
	D	8	8	8	8	8	8	8	8	87.5		
	E	8	8	8	8	8	8	8	8	87.5		
75	A	8	8	7	7	7	7	7	7	87.5	95	
	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	7	7	7	7	7	7	87.5		
100	A	8	8	8	8	8	8	8	8	100	97.5	5.73
	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	87.5		
	E	8	8	8	8	8	8	8	8	100		
ANALYST												
DATE:												
TIME:												

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID TEST START DATE 9-27-12 TIME 1415  
 CLIENT Dr. Queen TEST END DATE 10-4-12 TIME 1150

AGE AND SOURCE OF MINNOWS

		DAY (NUMBER SURVIVING)								SURVIVAL	
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
1415	A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	1	1		
	C	↓	↓	↓	↓	↓	↓	2	2		
	D	↓	↓	↓	↓	↓	↓	2	2		
	E										
32	A	2	2	2	2	2	2	2	2		
	B	↓	1	1	1	1	1	1	1		
	C	↓	2	2	2	2	2	2	2		
	D	↓	2	2	2	2	2	2	2		
	E										
42	A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	1	1		
	C	↓	↓	↓	↓	↓	↓	2	2		
	D	↓	↓	↓	↓	↓	↓	2	2		
	E										
56	A	2	2	2	2	2	2	2	2		
	B	↓	↓	2	2	2	2	2	2		
	C	↓	↓	1	1	1	1	1	1		
	D	↓	↓	2	2	2	2	2	2		
	E										
75	A	2	2	2	2	2	2	2	2		
	B	↓	2	2	↓	2	↓	2	2		
	C	↓	1	2	↓	2	↓	2	2		
	D	↓	2	1	1	1	1	1	1		
	E										
100	A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E										
ANALYST		RH	RH	CH	CH	RH	RH	RH	RH		
DATE:		9-27-12	9-29-12	9-29	10-1	10-1	10-2	10-3	10-4		
TIME:		1415	1100	1200	1230	1330	1530	1500	1100		

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



SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		TEST START DATE		TIME						
CLIENT <i>Dr. Guren B</i>		TEST END DATE		TIME						
AGE AND SOURCE OF MINNOWS										
DAY (NUMBER SURVIVING)										
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN % CV
<i>mHS</i>	A	2	2	1	1	1	1	1	1	
	B	1	1	2	2	2	2	2	2	
	C	1	1	1	1	1	1	1	1	
	D	1	1	1	1	1	1	1	1	
	E									
<i>32</i>	A	2	2	2	2	2	2	2	2	
	B	1	1	1	1	1	1	1	1	
	C	1	1	1	1	1	1	1	1	
	D	1	1	1	1	1	1	1	1	
	E									
<i>42</i>	A	2	2	2	2	2	2	2	2	
	B	1	1	1	1	1	1	1	1	
	C	1	1	1	1	1	1	1	1	
	D	1	1	1	1	1	1	1	1	
	E									
<i>56</i>	A	2	2	2	2	2	2	2	2	
	B	1	2	2	2	2	2	2	2	
	C	1	1	1	1	1	1	1	1	
	D	1	2	2	2	2	2	2	2	
	E									
<i>75</i>	A	2	2	2	2	2	2	2	2	
	B	1	1	1	1	1	1	1	1	
	C	1	1	1	1	1	1	1	1	
	D	1	1	1	1	1	1	1	1	
	E									
<i>100</i>	A	2	2	2	2	2	2	2	2	
	B	1	1	1	1	1	1	1	1	
	C	1	1	1	1	1	1	1	1	
	D	1	1	1	1	1	1	1	1	
	E									
ANALYST		KH	KH	ct	ct	RH	RH	RH	RH	
DATE:		9-27-12	9-28-12	9-29-12	9-30	10-1	10-2	10-3	10-4	
TIME:		1415	1100	1200	1230	1350	1540	1510	1110	

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID TEST START DATE 9-27-12 TIME 1415  
 CLIENT De Queen C TEST END DATE 10-4-12 TIME 1150

AGE AND SOURCE OF MINNOWS

		DAY (NUMBER SURVIVING)								SURVIVAL		
CONC:	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
m HS	A	2	2	2	2	2	2	2	2			
	B	1	1	1	1	1	1	1	1			
	C	1	1	1	1	1	1	1	1			
	D	1	1	1	1	1	1	1	1			
	E											
32	A	2	2	2	2	2	2	2	2			
	B	1	1	1	1	1	1	1	1			
	C	1	1	1	1	1	1	1	1			
	D	1	1	1	1	1	1	1	1			
	E											
42	A	2	2	2	2	2	2	2	2			
	B	1	1	1	1	1	1	1	1			
	C	1	1	1	1	1	1	1	1			
	D	1	1	1	1	1	1	1	1			
	E											
56	A	2	2	2	2	2	2	2	2			
	B	1	1	1	1	1	1	1	1			
	C	1	1	1	1	1	1	1	1			
	D	1	1	1	1	1	1	1	1			
	E											
75	A	2	2	2	2	2	2	2	2			
	B	1	1	1	1	1	1	1	1			
	C	1	1	1	1	1	1	1	1			
	D	1	1	1	1	1	1	1	1			
	E											
100	A	2	2	2	2	2	2	2	2			
	B	1	1	1	1	1	1	1	1			
	C	1	1	1	1	1	1	1	1			
	D	1	1	1	1	1	1	1	1			
	E											
ANALYST		RH	RH	CF	CF	RH	RH	RH	RH			
DATE:		9-27	9-28	9-29	9-30	10-1	10-2	10-3	10-4			
TIME:		1415	1100	1200	1230	1345	1450	1520	1120			

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		TEST START DATE		TIME							
CLIENT		TEST END DATE		TIME							
AGE AND SOURCE OF MINNOWS											
DAY (NUMBER SURVIVING)										SURVIVAL	
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
mHS	A	2	2	2	2	2	2	2	3		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓	↓		
32	A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓	↓		
42	A	2	2	2	2	2	2	2	3		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓	↓		
56	A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓	↓		
75	A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓	↓		
100	A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓	↓		
ANALYST		RH	RH	U	U	RH	RH	RH	RH		
DATE:		9-27	9-28-12	9-29-12	9-30-12	10-1	10-2	10-3	10-4		
TIME:		1415	1100	1200	1200	1400	1600	1530	1130		

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID *De Queen E* TEST START DATE *9-27-12* TIME *1415*  
 CLIENT TEST END DATE *10-4-12* TIME *1150*  
 AGE AND SOURCE OF MINNOWS

		DAY (NUMBER SURVIVING)								SURVIVAL		
CONC:	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
	A	2	2	2	2	2	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓	↓			
	E	↓	↓	↓	↓	↓	↓	↓	↓			
	A	2	3	2	2	2	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓	↓			
	E	↓	↓	↓	↓	↓	↓	↓	↓			
	A	2	2	3	2	3	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓	↓			
	E	↓	↓	↓	↓	↓	↓	↓	↓			
	A	2	2	2	2	2	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓	↓			
	E	↓	↓	↓	↓	↓	↓	↓	↓			
	A	2	2	2	2	2	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓	↓			
	E	↓	↓	↓	↓	↓	↓	↓	↓			
	A	3	2	↓	2	2	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓	↓			
	E	↓	↓	↓	↓	↓	↓	↓	↓			
ANALYST		KH	KH	U	J	KH	KH	RH	KH			
DATE:		9-27	9-28-12	9-28	9-30	10-1	10-1	10-3	10-4			
TIME:		1415	1100	1200	1230	1400	1610	1540	1150			

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

**WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST**

LAB # / #s:		K1206003				TEST DATES (BEGIN / END):		9/24/12 - 10/4/12	
CLIENT:		City of DeQueen				WEIGHING DATE / TIME:		10/5/2012 1115	
ANALYSTS:		KP				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	1.00580	1.00234	0.00346	8	0.433	AVG DRY		
	B	1.03262	1.02990	0.00272	8	0.340	WEIGHT (mg)		
	C	0.98721	0.98401	0.00320	8	0.400	0.424		
	D	1.01960	1.01568	0.00392	8	0.490	CV		
	E	1.02724	1.02357	0.00367	8	0.459	13.58		
CONC: 32%	A	1.01555	1.01299	0.00256	8	0.320	AVG DRY		
	B	1.02827	1.02486	0.00341	8	0.426	WEIGHT (mg)		
	C	0.97371	0.96989	0.00382	8	0.477	0.433		
	D	1.00540	1.00194	0.00346	8	0.433	CV		
	E	0.99202	0.98793	0.00409	8	0.511			
CONC: 42%	A	0.97725	0.97358	0.00367	8	0.459	AVG DRY		
	B	0.99517	0.99248	0.00269	8	0.336	WEIGHT (mg)		
	C	0.97587	0.97230	0.00357	8	0.446	0.460		
	D	0.99737	0.99333	0.00404	8	0.505	CV		
	E	1.00386	0.99943	0.00443	8	0.554			
CONC: 56%	A	0.95360	0.94959	0.00401	8	0.501	AVG DRY		
	B	0.99032	0.98709	0.00323	8	0.404	WEIGHT (mg)		
	C	0.97688	0.97286	0.00402	8	0.503	0.482		
	D	0.99259	0.98893	0.00366	8	0.458	CV		
	E	0.99552	0.99117	0.00435	8	0.544			
CONC: 75%	A	0.99861	0.99485	0.00376	8	0.470	AVG DRY		
	B	1.01283	1.00995	0.00288	8	0.360	WEIGHT (mg)		
	C	1.00485	1.00167	0.00318	8	0.397	0.437		
	D	0.99846	0.99501	0.00345	8	0.431	CV		
	E	0.98980	0.98558	0.00422	8	0.528			
CONC: 100%	A	0.98223	0.97903	0.00320	8	0.400	AVG DRY		
	B	0.99722	0.99343	0.00379	8	0.474	WEIGHT (mg)		
	C	0.99329	0.98898	0.00431	8	0.539	0.462		
	D	0.98640	0.98270	0.00370	8	0.463	CV		
	E	0.99126	0.98777	0.00349	8	0.436	11.10		

CV = (STANDARD DEVIATION/MEAN)\*100

**REMARKS:**

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AA# K1209008, FATHEAD MINNOW, CHRONIC, 9-27-12  
File: Z:\TOXSTAT\MONTE\FHSURV. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.202

W = 0.861

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# K1209008, FATHEAD MINNOW, CHRONIC, 9-27-12  
File: Z:\TOXSTAT\MONTE\FHSURV. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 0.45

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# K1209008, FATHEAD MINNOW, CHRONIC, 9-27-12  
FILE: Z:\TOXSTAT\MONTE\FHSURV.  
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	0.8750	1.2094
1	CONTROL	3	1.0000	1.3931
1	CONTROL	4	1.0000	1.3931
1	CONTROL	5	0.8750	1.2094
2	32 % EFFLUENT	1	0.8750	1.2094
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	0.8750	1.2094
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	0.8750	1.2094
4	56 %	EFFLUENT	2	0.8750	1.2094
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	0.8750	1.2094
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	0.8750	1.2094
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	0.8750	1.2094
6	100 %	EFFLUENT	5	1.0000	1.3931

AA# K1209008, FATHEAD MINNOW, CHRONIC, 9-27-12

File: Z:\TOXSTAT\MONTE\FHSURV.

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	1.283				
2	32 % EFFLUENT	1.356	32.50	16.00	5.00	
3	42 % EFFLUENT	1.356	32.50	16.00	5.00	
4	56 % EFFLUENT	1.283	27.50	16.00	5.00	
5	75 % EFFLUENT	1.320	30.00	16.00	5.00	
6	100 % EFFLUENT	1.356	32.50	16.00	5.00	

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

AA# K1209008, FATHEAD MINNOW GROWTH CHRONIC, 9-27-12  
File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.102

W = 0.963

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# K1209008, FATHEAD MINNOW GROWTH CHRONIC, 9-27-12  
File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 1.27

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# K1209008, FATHEAD MINNOW GROWTH CHRONIC, 9-27-12  
FILE: Z:\TOXSTAT\MONTE\FHGR.  
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.4330	0.7182
1	CONTROL	2	0.3400	0.6225
1	CONTROL	3	0.4000	0.6847
1	CONTROL	4	0.4900	0.7754
1	CONTROL	5	0.4590	0.7444
2	32 % EFFLUENT	1	0.3200	0.6013
2	32 % EFFLUENT	2	0.4260	0.7111
2	32 % EFFLUENT	3	0.4770	0.7624
2	32 % EFFLUENT	4	0.4330	0.7182
2	32 % EFFLUENT	5	0.5110	0.7964
3	42 % EFFLUENT	1	0.4590	0.7444
3	42 % EFFLUENT	2	0.3360	0.6183
3	42 % EFFLUENT	3	0.4460	0.7313
3	42 % EFFLUENT	4	0.5050	0.7904
3	42 % EFFLUENT	5	0.5540	0.8395
4	56 % EFFLUENT	1	0.5010	0.7864



4	56 %	EFFLUENT	2	0.4040	0.6888
4	56 %	EFFLUENT	3	0.5030	0.7884
4	56 %	EFFLUENT	4	0.4580	0.7433
4	56 %	EFFLUENT	5	0.5440	0.8295
5	75 %	EFFLUENT	1	0.4700	0.7554
5	75 %	EFFLUENT	2	0.3600	0.6435
5	75 %	EFFLUENT	3	0.3970	0.6817
5	75 %	EFFLUENT	4	0.4310	0.7162
5	75 %	EFFLUENT	5	0.5280	0.8134
6	100 %	EFFLUENT	1	0.4000	0.6847
6	100 %	EFFLUENT	2	0.4740	0.7594
6	100 %	EFFLUENT	3	0.5390	0.8244
6	100 %	EFFLUENT	4	0.4630	0.7484
6	100 %	EFFLUENT	5	0.4360	0.7212

AA# K1209008, FATHEAD MINNOW GROWTH CHRONIC, 9-27-12  
 File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.012	0.002	0.571
Within (Error)	24	0.102	0.004	
Total	29	0.114		

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

AA# K1209008, FATHEAD MINNOW GROWTH CHRONIC, 9-27-12  
 File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	0.709	0.424		
2	32 % EFFLUENT	0.718	0.433	-0.214	
3	42 % EFFLUENT	0.745	0.460	-0.866	
4	56 % EFFLUENT	0.767	0.482	-1.411	
5	75 % EFFLUENT	0.722	0.437	-0.315	
6	100 % EFFLUENT	0.748	0.462	-0.935	

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

AA# K1209008, FATHEAD MINNOW GROWTH CHRONIC, 9-27-12  
 File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

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	0.094	22.2	-0.009
3	42 % EFFLUENT	5	0.094	22.2	-0.036
4	56 % EFFLUENT	5	0.094	22.2	-0.058
5	75 % EFFLUENT	5	0.094	22.2	-0.013
6	100 % EFFLUENT	5	0.094	22.2	-0.038

APPENDIX D

*Ceriodaphnia dubia* Raw Data and Statistics

Cerodaphnia dubia

SURVIVAL AND REPRODUCTION TEST

RH

Discharger: Dr. Ruzen Lab Number/s: K1209008  
 Location: K1209008  
 Date Sample Collected:

Analyst: RH  
 Test Start - Date/ Time: 9-27-12 1100  
 Test Stop - Date/Time: 10-4-12 1030

MHS

Conc 1		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	1	2	0	0	0	3	2	0	4	3	15	16	1.5	
	4	5	2	4	3	8	7	0	4	2	7	36	10	3.6	
	5	7	5	6	6	3	7	9	8	4	5	60	10	6.0	
	6	5	7	0	1	8	7	1	1	4	0	24	10	2.4	
	7	4	0	3	2	2	0	0	3	6	4	24	10	2.4	
	8														
	Total	24	16	13	12	22	18	12	16	20	19	169		CV=21.9	

32

Conc 2		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	2	4	0	1	0	1	3	2	0	0	13	10	1.3	
	4	6	0	4	5	6	4	3	0	6	2	36	10	3.6	
	5	9	8	5	5	11	0	8	5	7	0	58	10	5.8	
	6	1	8	10	2	0	10	5	7	5	9	57	10	5.7	
	7	3	6	1	3	3	0	4	0	4	2	20	10	2.0	
	8														
	Total	21	20	26	16	20	15	23	14	22	13	184			

42

Conc 3		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	3	3	4	5	0	4	4	2	0	0	25	10	2.5	
	4	3	5	0	0	3	0	0	2	3	4	20	10	2.0	
	5	10	10	9	12	4	8	7	7	5	3	75	10	7.5	
	6	0	1	2	3	4	5	8	8	10	4	45	10	4.5	
	7	1	1	3	1	2	X	1	1	0	0	10	9	1.1	
	8														
	Total	17	20	18	21	13	17	20	20	18	11	175			

X= DEAD; Y= MALE

Conc 4		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	4	5	4	2	2	2	1	0	3	0	23	10	2.3	
	4	7	1	0	3	0	7	5	5	4	4	27	10	2.7	
	5	5	12	6	8	6	8	8	9	0	9	71	10	7.1	
	6	10	2	3	2	9	7	3	0	11	0	47	10	4.7	
	7	0	1	2	0	4	0	5	5	6	10	33	10	3.3	
	8														
	Total	23	21	15	15	21	18	22	19	24	23	201			

Conc 5		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	2	4	0	0	0	1	4	2	0	0	13	10	1.3	
	4	3	0	3	4	4	8	4	3	2	5	35	10	3.5	
	5	6	9	7	6	0	0	12	8	2	11	61	10	6.1	
	6	15	11	8	9	5	11	3	7	8	1	78	10	7.8	
	7	0	4	0	2	3	5	1	4	4	8	31	10	3.1	
	8														
	Total	25	28	18	21	12	25	24	24	16	25	218			

Conc 6		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	2	0	1	3	0	0	2	0	5	2	15	10	1.5	
	4	2	6	3	0	5	6	7	5	3	2	39	10	3.9	
	5	8	12	9	5	4	8	11	9	8	8	82	10	8.2	
	6	2	5	2	2	0	5	3	3	2	0	24	10	2.4	
	7	5	0	6	4	5	2	7	6	1	5	41	10	4.1	
	8														
	Total	19	23	21	14	14	21	30	23	19	17	201			

$\bar{X} = 20.1$   
 $CV = 23.6$

AA # K1209008, C. DUBIA CHRONIC REPRODUCCION, 9-27-12  
File: Z:\TOXSTAT\MONTE\CD. Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

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\*\*\*\*\* 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 # K1209008, C. DUBIA CHRONIC REPRODUCCION, 9-27-12  
File: Z:\TOXSTAT\MONTE\CD. Transform: NO TRANSFORMATION

---

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 3.18

---

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 # K1209008, C. DUBIA CHRONIC REPRODUCCION, 9-27-12  
 FILE: Z:\TOXSTAT\MONTE\CD.  
 TRANSFORM: NO TRANSFORMATION

NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	22.0000	22.0000
1	CONTROL	2	16.0000	16.0000
1	CONTROL	3	13.0000	13.0000
1	CONTROL	4	12.0000	12.0000
1	CONTROL	5	21.0000	21.0000
1	CONTROL	6	18.0000	18.0000
1	CONTROL	7	12.0000	12.0000
1	CONTROL	8	16.0000	16.0000
1	CONTROL	9	20.0000	20.0000
1	CONTROL	10	19.0000	19.0000
2	32 % EFFLUENT	1	21.0000	21.0000
2	32 % EFFLUENT	2	20.0000	20.0000
2	32 % EFFLUENT	3	20.0000	20.0000
2	32 % EFFLUENT	4	16.0000	16.0000
2	32 % EFFLUENT	5	20.0000	20.0000
2	32 % EFFLUENT	6	15.0000	15.0000
2	32 % EFFLUENT	7	23.0000	23.0000
2	32 % EFFLUENT	8	14.0000	14.0000
2	32 % EFFLUENT	9	22.0000	22.0000
2	32 % EFFLUENT	10	13.0000	13.0000
3	42 % EFFLUENT	1	17.0000	17.0000
3	42 % EFFLUENT	2	20.0000	20.0000
3	42 % EFFLUENT	3	18.0000	18.0000
3	42 % EFFLUENT	4	21.0000	21.0000
3	42 % EFFLUENT	5	13.0000	13.0000
3	42 % EFFLUENT	6	17.0000	17.0000
3	42 % EFFLUENT	7	20.0000	20.0000
3	42 % EFFLUENT	8	20.0000	20.0000
3	42 % EFFLUENT	9	18.0000	18.0000
3	42 % EFFLUENT	10	11.0000	11.0000
4	56 % EFFLUENT	1	23.0000	23.0000
4	56 % EFFLUENT	2	21.0000	21.0000
4	56 % EFFLUENT	3	15.0000	15.0000
4	56 % EFFLUENT	4	15.0000	15.0000
4	56 % EFFLUENT	5	21.0000	21.0000
4	56 % EFFLUENT	6	18.0000	18.0000
4	56 % EFFLUENT	7	22.0000	22.0000
4	56 % EFFLUENT	8	19.0000	19.0000



4	56 %	EFFLUENT	9	24.0000	24.0000
4	56 %	EFFLUENT	10	23.0000	23.0000
5	75 %	EFFLUENT	1	25.0000	25.0000
5	75 %	EFFLUENT	2	28.0000	28.0000
5	75 %	EFFLUENT	3	18.0000	18.0000
5	75 %	EFFLUENT	4	21.0000	21.0000
5	75 %	EFFLUENT	5	12.0000	12.0000
5	75 %	EFFLUENT	6	25.0000	25.0000
5	75 %	EFFLUENT	7	24.0000	24.0000
5	75 %	EFFLUENT	8	24.0000	24.0000
5	75 %	EFFLUENT	9	16.0000	16.0000
5	75 %	EFFLUENT	10	25.0000	25.0000
6	100 %	EFFLUENT	1	19.0000	19.0000
6	100 %	EFFLUENT	2	23.0000	23.0000
6	100 %	EFFLUENT	3	21.0000	21.0000
6	100 %	EFFLUENT	4	14.0000	14.0000
6	100 %	EFFLUENT	5	14.0000	14.0000
6	100 %	EFFLUENT	6	21.0000	21.0000
6	100 %	EFFLUENT	7	30.0000	30.0000
6	100 %	EFFLUENT	8	23.0000	23.0000
6	100 %	EFFLUENT	9	19.0000	19.0000
6	100 %	EFFLUENT	10	17.0000	17.0000

AA # K1209008, C. DUBIA CHRONIC REPRODUCCION, 9-27-12  
 File: Z:\TOXSTAT\MONTE\CD. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	171.733	34.347	2.174
Within (Error)	54	853.200	15.800	
Total	59	1024.933		

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

AA # K1209008, C. DUBIA CHRONIC REPRODUCCION, 9-27-12  
 File: Z:\TOXSTAT\MONTE\CD. 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	16.900	16.900		
2	32 % EFFLUENT	18.400	18.400	-0.844	
3	42 % EFFLUENT	17.500	17.500	-0.338	
4	56 % EFFLUENT	20.100	20.100	-1.800	
5	75 % EFFLUENT	21.800	21.800	-2.756	
6	100 % EFFLUENT	20.100	20.100	-1.800	

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

AA # K1209008, C. DUBIA CHRONIC REPRODUCTION, 9-27-12  
File: Z:\TOXSTAT\MONTE\CD. 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	4.106	24.3	-1.500
3	42 % EFFLUENT	10	4.106	24.3	-0.600
4	56 % EFFLUENT	10	4.106	24.3	-3.200
5	75 % EFFLUENT	10	4.106	24.3	-4.900
6	100 % EFFLUENT	10	4.106	24.3	-3.200

AA # K1209008, C. DUBIA CHRONIC REPRODUCTION, 9-27-12  
File: Z:\TOXSTAT\MONTE\CD. Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	16.900				
2	32 % EFFLUENT	18.400	118.00	75.00	10.00	
3	42 % EFFLUENT	17.500	109.50	75.00	10.00	
4	56 % EFFLUENT	20.100	129.50	75.00	10.00	
5	75 % EFFLUENT	21.800	134.00	75.00	10.00	
6	100 % EFFLUENT	20.100	126.00	75.00	10.00	

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

APPENDIX E

Organism History

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

**TEST ORGANISM HISTORY**

DATE SHIPPED 9-27-12 CLIENT Arkansas Analytical

Purchase Order #: \_\_\_\_\_

SPECIES: Pimephales promelas

Quantity Shipped: 420<sup>+</sup> = 180<sup>+</sup>

Age: hatched 9/26/12 - 7 Days old 9/27

Brood Stock Source: Anderson Farms, AR

Culture Water: Groundwater

Hardness (Mg/l CaCO3): = 160

Dissolved Oxygen (Mg/l): 8.3

Temperature (°C): 25.1°C

Feeding: AFRAMIL

Comments: \_\_\_\_\_

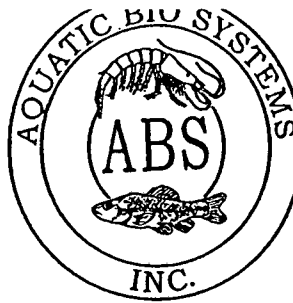
\_\_\_\_\_

\_\_\_\_\_

Shipped Via:  Federal Express  UPS Overnight  Shuttle

Packaged By: CM

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: 6/22/09

SPECIES: Ceriodaphnia dubia

AGE: Variable

LIFE STAGE: Adult


HATCH DATE: Variable

BEGAN FEEDING: Immediately

FOOD: YTC, Selenastrum sp.

Water Chemistry Record:	Current	Range
TEMPERATURE:	<u>25°C</u>	<u>20-25°C</u>
SALINITY/CONDUCTIVITY:	<u>--</u>	<u>--</u>
TOTAL HARDNESS (as CaCO <sub>3</sub> ):	<u>142 mg/l</u>	<u>86-124 mg/l</u>
TOTAL ALKALINITY (as CaCO <sub>3</sub> ):	<u>100 mg/l</u>	<u>65-130 mg/l</u>
pH:	<u>7.92</u>	<u>7.56-8.35</u>

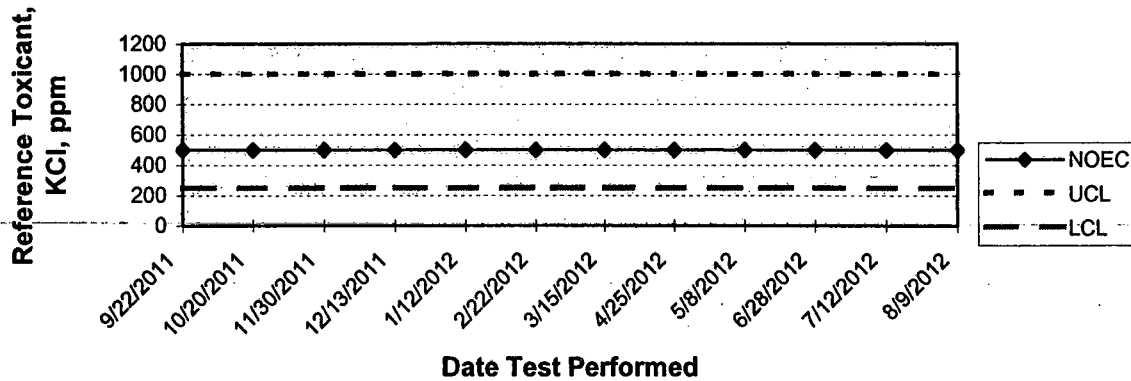
Comments:

  
\_\_\_\_\_  
Facility Supervisor

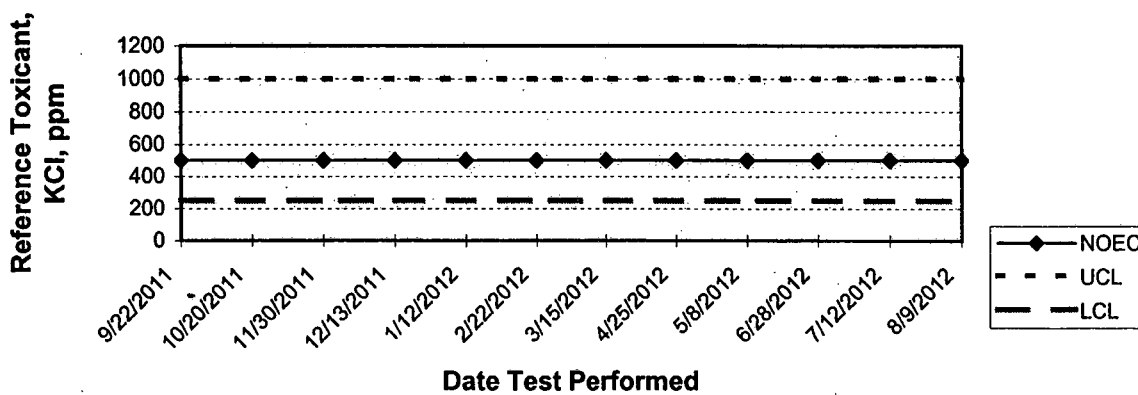
APPENDIX F

Quality Assurance Charts

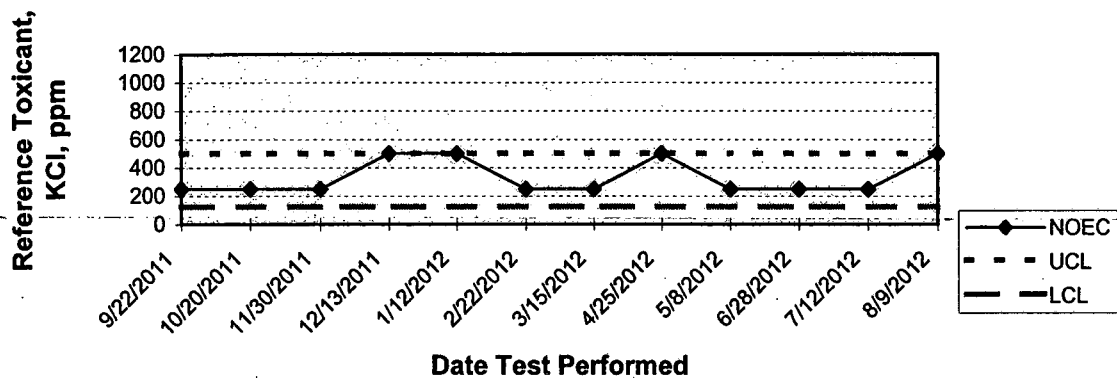
**ARKANSAS ANALYTICAL, INC.**  
**FATHEAD MINNOW SURVIVAL**  
**QUALITY ASSURANCE**



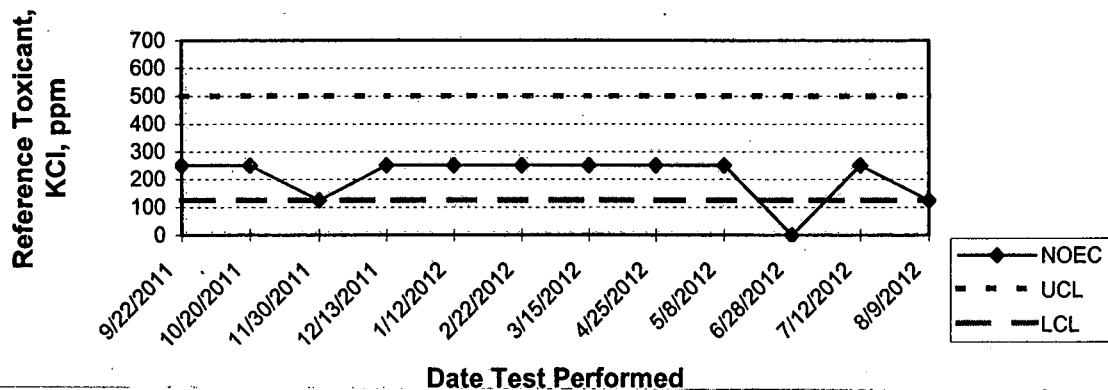
**ARKANSAS ANALYTICAL, INC.**  
**FATHEAD MINNOW GROWTH**  
**QUALITY ASSURANCE**



**ARKANSAS ANALYTICAL, INC.**  
**CERIODAPHНИЯ DUBIA SURVIVAL**  
**QUALITY ASSURANCE**



**ARKANSAS ANALYTICAL, INC.**  
**CERIODAPHНИЯ DUBIA REPRODUCTION**  
**QUALITY ASSURANCE**





# Arkansas Analytical, Inc.

Toxicity Test Results  
City of DeQueen  
NPDES PERMIT NUMBER: AR0021733  
Third Quarter 2012  
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 K1209008**

Wednesday, October 10, 2012

## **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 9<sup>th</sup> 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 2012.

## **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:	9-25-12, 0800	9-26-12, 0800
Sample #2:	9-26-12, 0800	9-27-12, 0800
Sample #3:	9-30-12, 0900	10-1-12, 0900

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:	9-27-12, 0945	1
Sample #2:	9-28-12, 1000	1
Sample #3:	10-3-12, 1145	12**

\*\* As per Mary Barnett test was allowed to continue although sample #3 was received outside the temperature limits. This was due to a delay in delivery by the courier service.

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 < 24 hour old Fathead Minnows, *Pimephales promelas*, which were purchased from Aquatox; a copy of the organism history is provided in Appendix E.

The organisms used in Test 1002.0 were < 24 hour old *Ceriodaphnia dubia* neonates, (all born within the same eight hours), obtained from an in-house culture. An organism history is provided in Appendix E.

## Quality Assurance

### Test Acceptability

#### TEST ACCEPTANCE CRITERIA for *Ceriodaphnia dubia*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	100%	X	
Average of 15 or more young per surviving female	16.9	X	
At least 60% of surviving females should have produced 3 broods	100%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	21.9	X	

#### TEST ACCEPTANCE CRITERIA for *Pimephales promelas*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	92.5%	X	
The percent coefficient of variation between replicates must be 40% or less for survival	7.40	X	
Minimum of 0.25 mg average dry weight of surviving controls	0.424	X	
The percent coefficient of variation between replicates must be 40% or less for growth	13.6	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> 8/9-16/12		<i>Pimephales promelas</i> 8/9-16/12	
NOEC Survival:	500 ppm KCl	NOEC Survival:	500 ppm KCl
LOEC Survival:	1000 ppm KCl	LOEC Survival:	1000 ppm KCl
NOEC Reproduction:	125 ppm KCl	NOEC Growth:	500 ppm KCl
LOEC Reproduction:	250 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)	20.1	%CV survival (critical dilution)	5.73
%CV Reproduction (critical dilution)	23.6	Mean dry weight (critical dilution) in milligrams	0.462
		%CV growth (critical dilution)	11.1
PMSD Reproduction	24.3	PMSD Growth	22.2

### Conclusion


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

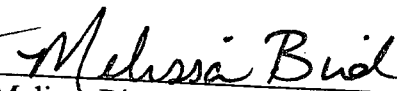
The permit issued to the City of DeQueen, AR0021733, specifies that the **critical dilution is 100% effluent**. The effluent samples **did not** exhibit lethal or sublethal effects at the critical dilution, and, as such, **passed** both portions of the test.


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

  
Melissa Bird

  
Kenneth Pigue

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:	9-25-12, 0800	9-26-12, 0800
Sample #2:	9-26-12, 0800	9-27-12, 0800
Sample #3:	9-30-12, 0900	10-1-12, 0900

Test initiated (date, time): 9-27-12, 1415      Test terminated (date, time): 10-4-12, 1150

Dilution water used:      Moderately Hard Synthetic

**DATA TABLE FOR FATHEAD MINNOW SURVIVAL**

Effluent Conc %	Percent Survival in Replicate Chambers					Mean Percent Survival			
	A	B	C	D	E	24 hours	48 hours	7 days	CV %
0%	87.5	87.5	100	100	87.5	100	97.5	92.5	7.40
32%	87.5	100	100	100	100	97.5	97.5	97.5	
42%	87.5	100	100	100	100	100	100	97.5	
56%	87.5	87.5	100	87.5	100	97.5	95	92.5	
75%	87.5	100	100	100	87.5	100	97.5	95	
100%	100	100	100	87.5	100	100	100	97.5	5.73

**DATA TABLE FOR GROWTH OF FATHEAD MINNOWS**

Effluent Conc %	A	B	C	D	E	Mean Dry Weight	CV%
0%	0.433	0.340	0.400	0.490	0.459	0.424	13.6
32%	0.320	0.426	0.477	0.433	0.511	0.433	
42%	0.459	0.336	0.446	0.505	0.554	0.460	
56%	0.501	0.404	0.503	0.458	0.544	0.482	
75%	0.470	0.360	0.397	0.431	0.528	0.437	
100%	0.400	0.474	0.539	0.463	0.436	0.462	11.1

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)= \_\_\_\_\_   13.6   %



SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING  
FATHEAD MINNOW LARVAE GROWTH AND SURVIVAL  
*Pimephales promelas*

1. Dunnett's procedure or Steel's Many-One Rank Test as appropriate:  
Is the mean survival at 7 days significantly different ( $p=0.05$ ) than the control survival for:
  - a) LOW FLOW OR CRITICAL DILUTION, (100%) YES \_\_\_\_\_ NO X
  
2. Dunnett's Procedure  
Is the mean dry weight (growth) at 7 days significantly different ( $p=0.05$ ) than the control's dry weight (growth) for:
  - a) LOW FLOW OR CRITICAL DILUTION, (100%) YES \_\_\_\_\_ NO X
  
3. If NO was answered to 1.a) enter [0] otherwise enter [1] (parameter TLP6C): 0
  
4. If NO was answered to 2.a) enter [0] otherwise enter [1] (parameter TGP6C): 0
  
5. Enter percentage corresponding to each parameter below:
  - a) NOEC survival (parameter TOP6C)= 100 % effluent
  - b) NOEC growth (parameter TPP6C)= 100 % effluent
  - c) Coefficient of variation (parameter TQP6C)= 13.6 %

**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

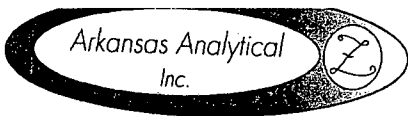
c) Coefficient of variation (parameter TQP3B)=   23.6   %

APPENDIX A

Chain of Custody Forms







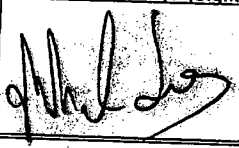
11701 Interstate 30, Bldg. 1, Ste. 115  
 Little Rock, AR 72209  
 PHONE: 501-455-3233  
 FAX: 501-455-6118

# CHAIN OF CUSTODY RECORD

<b>CLIENT INFORMATION</b>		<b>BILLING</b>		<b>Project Description</b>		<b>Turnaround Time</b>		<b>Preservation Codes:</b>						
City of DeQueen Wastewater Plant		City of DeQueen Wastewater Plant		Chronic Toxicity		24 Hour		1. Cool, 4 Degrees Centigrade			4. Thiosulfate for Dechlorination			
514 South 9th		P.O. Box 730				48 Hour		2. Sulfuric Acid (H <sub>2</sub> SO <sub>4</sub> ), pH < 2			5. Hydrochloric Acid(HCl)			
DeQueen, AR 71832		DeQueen, AR 71832		<b>Reporting Information</b>		72 Hour		3. Nitric Acid (HNO <sub>3</sub> ), pH < 2			6. Sodium Hydroxide (NaOH), pH > 12			
Attn: Mike Sims				Telephone: 870-642-5231		Routine		<b>TEST PARAMETERS</b>						
				Fax: 870-642-3117		Preservative Code:		1						Bottle Type Code
				Email: msims@cityofdequeen.com		Bottle Type:		P						G = Glass; P = Plastic V = Septum; A = Amber

Sampler(s) Signature:  MS  
 Sampler(s) Printed: MS

Field Number	SAMPLE COLLECTION		Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION	Chronic Biomonitoring	TEST PARAMETERS										Arkansas Analytical Work Order Number:			
	Date/s	Time/s																				
	7-30/11-12	9:00/9:20		X		Water	DQ Bio	X														K1209088
																						C

1. Relinquished by: (Signature) 		Date/Time 10-1-12 11:50 am		2. Received by: (Signature) UPS		SAMPLE CONDITION UPON RECEIPT IN LAB				REMARKS / SAMPLE COMMENTS			
3. Relinquished by: (Signature) UPS		Date/Time 10-3-12 11:45		4. Received by lab: (Signature) Amanda Fabish		1. CUSTODY SEALS: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 2. CONTAINERS CORRECT: <input type="checkbox"/> Yes <input type="checkbox"/> No 3. COC/LABELS AGREE: <input type="checkbox"/> Yes <input type="checkbox"/> No 4. PRESERVATION CONFIRMED: <input type="checkbox"/> Yes <input type="checkbox"/> No 5. RECEIVED ON ICE: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No 6. TEMPERATURE ON RECEIPT: 12°C				P.O. Number: 67050			

FOR COMPLETION BY LAB ONLY

## Norma James

---

**From:** Barnett, Mary [BARNETT@adeq.state.ar.us]  
**Sent:** Thursday, October 04, 2012 7:36 AM  
**To:** Norma James  
**Subject:** RE: city of dequeen WET temp excursion

Norma,

Due to unintended extenuating circumstances involving the sample courier, and since the facility generally has a history of passing WET tests, the Department has determined the third sample of the 3<sup>rd</sup> quarter test is an acceptable sample.

The Department encourages the facility and laboratory to work with the couriers to ensure that all subsequent WET test samples are received within EPA's sampling handing requirements.

Mary Barnett  
Ecologist  
501-682-0666

---

**From:** Norma James [<mailto:njames@arkansasanalytical.com>]  
**Sent:** Wednesday, October 03, 2012 12:18 PM  
**To:** Barnett, Mary  
**Cc:** 'Ken Pigue'; 'Lessie Redican'; 'Sydney James'  
**Subject:** city of dequeen WET temp excursion

Mary,

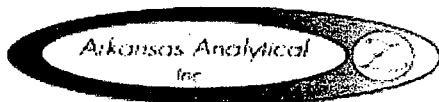
As discussed with Ken earlier yesterday, we are continuing to run the current WET test for the City of DeQueen although the temperature of the third sample received was 12degrees C. We will note such in the report. Delivery of the sample was delayed by third party courier service, causing the temp to be elevated. DeQueen usually does a good job of icing down the samples.

Let me know if you need any further information.

Thank you for your assistance.

Norma

*Norma James, President*



11701 Interstate 30  
Bldg. 1, Ste. 115  
Little Rock, AR 72209  
501-455-3233  
501-455-6118 (fax)  
501-993-1852 (cell)

Business Hours - Mon-Thur - 8am-5pm, Fri - 8am-4pm

Think for yourselves and let others enjoy the privilege to do so too. Voltaire.

This e-mail and any attachments are confidential. If you receive this message in error or are not the intended recipient, you should not retain,

APPENDIX B

Effluent and Dilution Water Data



CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Fathead Minnow

Lab # / Sample ID

Test Start (Date/Time) 9-27-18 1415

Client: Dr Queen

Test End (Date/Time) 10-4-12 1150

Day of Test

MHS

32

42

56

75

100

		1	2	3	4	5	6	7	notes/remarks
Control	MHS551	9-27	9-28	9-29	9-30	10-1	10-2	10-3	
D.O. (mg/L)	INITIAL	8.6	8.5	8.5	8.6	8.7	8.9	8.9	
	FINAL	2915	8.0	6.6	7.7	7.4	7.4	5.9	
pH (s.u.)	INITIAL	8.0	7.8	8.0	8.2	7.9	7.9	7.8	
	FINAL	7.3	8.0	8.2	8.25	7.6	7.4	7	
temp (C)	INITIAL	22	22	21.3	21.0	21	21	21	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		62							
HARDNESS (mg/L)		89							
CONDUCTIVITY (umhos/cm)		294							
CHLORINE (mg/L)		<0.05							
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.8	8.9	8.8	8.7	8.8	8.8	
	FINAL	7.5	8.1	6.7	7.7	7.3	7.5	7.4	
pH (s.u.)	INITIAL	7.8	7.6	7.9	8.1	7.4	7.8	7.4	
	FINAL	7.4	7.9	7.7	7.5	7.6	7.4	7.4	
temp (C)	INITIAL	22	21	22.5	22.0	22	21	21	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.8	9.1	9.0	8.5	8.7	8.8	
	FINAL	7.515	8.2	6.8	7.6	7.2	7.5	7.5	
pH (mg/L)	INITIAL	7.7	7.7	8.0	7.8	7.4	7.4	7.7	
	FINAL	7.4	7.9	7.7	7.5	7.2	7.4	7.4	
temp (C)	INITIAL	22	21	23.4	24.0	22	22	21	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.6	9.3	9.0	8.7	8.7	8.8	
	FINAL	7.5	8.1	6.6	7.9	7.4	6.9	7.1	
pH (s.u.)	INITIAL	7.7	7.3	7.8	7.7	7.4	7.2	7.3	
	FINAL	7.4	8.0	7.6	7.5	7.5	7.4	7.3	
temp (C)	INITIAL	21	21	24.1	25.2	22	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.7	8.7	9.2	8.9	8.7	8.8	8.9	
	FINAL	7.9	8.1	6.6	7.7	7.4	7.4	6.9	
pH (s.u.)	INITIAL	7.7	7.5	7.7	7.5	7.3	7.4	7.5	
	FINAL	7.4	7.5	7.6	7.0	7.4	7.2	7.4	
temp (C)	INITIAL	22	21	24.8	26.0	23	21	21	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.7	8.6	8.7	8.9	8.6	8.9	8.9	
	FINAL	7.6	8.2	6.6	7.6	6.9	7.3	7.5	
pH (s.u.)	INITIAL	7.5	7.2	7.7	7.4	7.3	7.2	7.2	
	FINAL	7.4	7.5	7.5	7.1	7.3	7.3	7.3	
temp (C)	INITIAL	21	22	23.5	25.2	20	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	100%	A	A	A	B	B	B	C	
ALKALINITY (mg/L)		22			24			26	
HARDNESS (mg/L)		40			32			26	
CONDUCTIVITY (umhos/cm)		917			957			806	
CHLORINE (mg/L)		<0.05			<0.05			<0.05	

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Cerodaphnia Dubia

Lab # / Sample ID

Test Start (Date/Time)

9-27-12 1100

Client: *Dr Queen*

Test End (Date/Time)

10-4-12 1030

Day of Test

		1	2	3	4	5	6	7	notes/remarks
Control	MHS551	9-27	9-28	9-29	9-30	10-1	10-2	10-3	
D.O. (mg/L)	INITIAL	8.6	8.5	8.5	8.6	8.7	8.9	8.9	
	FINAL	8.1	<del>7.8</del> 8.2	8.2	8.1	8.1	8.1	7.8	
pH (s.u.)	INITIAL	8.0	7.8	8.0	8.2	7.9	7.9	7.8	
	FINAL	7.7	7.8	7.9	7.8	7.7	7.7	7.5	
temp (C)	INITIAL	22	22	21.3	21	21	21	21	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		62							
HARDNESS (mg/L)		84							
CONDUCTIVITY (umhos/cm)		294							
CHLORINE (mg/L)		<0.05							
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.8	8.9	8.8	8.7	8.8	8.8	
	FINAL	8.0	8.2	8.2	8.0	8.2	8.2	7.5	
pH (s.u.)	INITIAL	7.8	7.6	7.9	8.1	7.4	7.8	7.9	
	FINAL	7.7	7.9	8.2	7.7	7.6	7.7	7.3	
temp (C)	INITIAL	22	<del>22</del> 22	22.5	22	22	21	21	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.8	9.1	9.0	8.5	8.7	8.8	
	FINAL	8.0	8.1	8.2	8.1	8.1	8.5	7.6	
pH (mg/L)	INITIAL	7.7	7.7	8.0	7.8	7.4	7.4	7.7	
	FINAL	7.7	8.0	8.0	7.8	7.5	7.7	7.1	
temp (C)	INITIAL	22	<del>22</del> 22	23.4	24	22	22	21	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.6	8.6	9.3	9.0	8.7	8.7	8.8	
	FINAL	8.3	8.1	8.4	8.2	8.1	8.2	7.6	
pH (s.u.)	INITIAL	7.7	7.3	7.8	7.7	7.4	7.2	7.3	
	FINAL	7.8	8.0	7.8	7.7	7.4	7.6	7.3	
temp (C)	INITIAL	21	21	24.1	25.2	22	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.7	8.7	9.2	8.9	8.7	8.8	8.9	
	FINAL	8.0	8.1	8.2	8.1	8.3	8.2	8.9	
pH (s.u.)	INITIAL	7.7	7.5	7.7	7.5	7.3	8.2	7.7	
	FINAL	8.1	8.0	7.9	7.6	7.5	7.4	7.5	
temp (C)	INITIAL	22	21	24.8	26	23	21	21	
	FINAL	25	25	25	25	25	25	25	
CONC:									
D.O. (mg/L)	INITIAL	8.7	8.6	8.7	8.9	8.6	8.9	8.9	
	FINAL	8.1	8.1	8.2	8.0	8.0	8.2	7.6	
pH (s.u.)	INITIAL	7.5	7.2	7.7	7.4	7.3	7.2	7.2	
	FINAL	7.5	7.9	7.7	7.6	7.6	7.7	7.4	
temp (C)	INITIAL	21	22	23.5	25.2	20	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC:	100%	A	A	A	B	B	B	C	
ALKALINITY (mg/L)		22			24			26	
HARDNESS (mg/L)		40			32			26	
CONDUCTIVITY (umhos/cm)		917			957			806	
CHLORINE (mg/L)		<0.05			<0.05		<0.05	<0.05	

MHS

32

42

PH 9-27  
42  
56

75

100

APPENDIX C

Fathead minnow raw data and statistics

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		TEST START DATE		TIME								
K1709008		9/27/12		1415								
CLIENT		TEST END DATE		TIME								
DeQueer		10/4/12		1150								
AGE AND SOURCE OF MINNOWS												
DAY (NUMBER SURVIVING)												
CONC:	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
0	A	8	8	8	8	8	8	7	7	87.5	92.5	7.40
	B	8	8	7	7	8	7	7	7	87.5		
	C	8	8	8	8	8	8	8	8	100		
	D	8	8	8	8	8	8	8	8	100		
	E	8	8	7	7	7	7	7	7	87.5		
32	A	8	7	7	7	7	7	7	7	87.5	97.5	
	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		
42	A	8	8	8	8	8	8	7	7	87.5	97.5	
	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		
56	A	8	8	7	7	7	7	7	7	87.5	92.5	10.14%
	B	8	7	7	7	7	7	7	7	87.5		
	C	8	8	8	8	8	8	8	8	100		
	D	8	8	8	8	8	8	8	8	87.5		
	E	8	8	8	8	8	8	8	8	87.5		
75	A	8	8	7	7	7	7	7	7	87.5	95	
	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	7	7	7	7	87.5		
100	A	8	8	8	8	8	8	8	8	100	97.5	5.73
	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	87.5		
	E	8	8	8	8	8	8	8	8	100		
ANALYST												
DATE:												
TIME:												

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID: \_\_\_\_\_ TEST START DATE: 9-27-12 TIME: 1415  
 CLIENT: Dr. Queen / TEST END DATE: 10-4-12 TIME: 1150  
 AGE AND SOURCE OF MINNOWS: \_\_\_\_\_

		DAY (NUMBER SURVIVING)						SURVIVAL			
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
41HS	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1	1		
32	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	2	2	2	2	2	2	2		
	D	1	2	2	2	2	2	2	2		
	E	1	2	2	2	2	2	2	2		
42	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1	1		
56	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1	1		
75	A	2	2	2	2	2	2	2	2		
	B	1	2	2	1	2	1	2	2		
	C	1	1	2	1	2	1	2	2		
	D	1	2	1	1	1	1	1	1		
	E	1	2	1	1	1	1	1	1		
100	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E	1	1	1	1	1	1	1	1		
ANALYST		RH	RH	CH	CH	RH	RH	RH	RH		
DATE:		9-27-12	9-29-12	9-29	10-1	10-1	10-2	10-3	10-4		
TIME:		1415	1100	1200	1230	1330	1530	1500	1100		

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID TEST START DATE 9-27-12 TIME 1415  
 CLIENT De Buren B TEST END DATE 10-4-12 TIME 1150  
 AGE AND SOURCE OF MINNOWS

		DAY (NUMBER SURVIVING)						SURVIVAL			
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
mHS	A	2	2	1	1	1	1	1	1		
	B	1	1	2	2	2	2	2	2		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E										
32	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E										
42	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E										
56	A	2	2	2	2	2	2	2	2		
	B	1	2	2	2	2	2	2	2		
	C	1	1	1	1	1	1	1	1		
	D	1	2	2	2	2	2	2	2		
	E										
75	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E										
100	A	2	2	2	2	2	2	2	2		
	B	1	1	1	1	1	1	1	1		
	C	1	1	1	1	1	1	1	1		
	D	1	1	1	1	1	1	1	1		
	E										
ANALYST		KH	KH	CH	CH	RH	RH	RH	RH		
DATE:		9-27-12	9-28-12	9-29-12	9-30	10-1	10-2	10-3	10-4		
TIME:		1415	1100	1200	1230	1350	1540	1510	1110		

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID TEST START DATE 9-27-12 TIME 1415  
 CLIENT DeQueen C TEST END DATE 10-4-12 TIME 1150  
 AGE AND SOURCE OF MINNOWS

		DAY (NUMBER SURVIVING)								SURVIVAL		
CONC:	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
mHS	A	2	2	2	2	2	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓	↓			
	E											
32	A	2	2	2	2	2	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓	↓			
	E											
42	A	2	2	2	2	2	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓	↓			
	E											
56	A	2	2	2	2	2	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓	↓			
	E											
75	A	2	2	2	2	2	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓	↓			
	E											
100	A	2	2	2	2	2	2	2	2			
	B	↓	↓	↓	↓	↓	↓	↓	↓			
	C	↓	↓	↓	↓	↓	↓	↓	↓			
	D	↓	↓	↓	↓	↓	↓	↓	↓			
	E											
ANALYST		RH	RH	ct	ct	RH	RH	RH	RH			
DATE:		9-27	9-28	9-29	9-30	10-1	10-2	10-3	10-4			
TIME:		1415	1100	1200	1230	1345	1450	1520	1120			

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		TEST START DATE		TIME							
CLIENT		TEST END DATE		TIME							
AGE AND SOURCE OF MINNOWS											
DAY (NUMBER SURVIVING)										SURVIVAL	
CONC:	REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
mHS	A	2	2	2	2	2	2	2	3		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓	↓		
32	A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓	↓		
42	A	2	2	2	2	2	2	2	3		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓	↓		
56	A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓	↓		
75	A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓	↓		
100	A	2	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓	↓		
ANALYST		RH	RH	U	U	RH	RH	RH	RH		
DATE:		9-27	9-28-12	9-29-12	9-30-12	10-1	10-2	10-3	10-4		
TIME:		1415	1100	1200	1230	1400	1600	1530	1130		

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



SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		TEST START		DATE		TIME				
De Queen E				9-27-12		1415				
CLIENT		TEST END		DATE		TIME				
				10-4-12		1150				
AGE AND SOURCE OF MINNOWS										
DAY (NUMBER SURVIVING)										
REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
CONC: A	2	2	2	2	2	2	2	2		
B	↓	↓	↓	↓	↓	↓	↓	↓		
C	↓	↓	↓	↓	↓	↓	↓	↓		
D	↓	↓	↓	↓	↓	↓	↓	↓		
E										
REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
CONC: A	2	3	2	2	2	2	2	2		
B	↓	↓	↓	↓	↓	↓	↓	↓		
C	↓	↓	↓	↓	↓	↓	↓	↓		
D	↓	↓	↓	↓	↓	↓	↓	↓		
E										
REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
CONC: A	2	2	3	2	3	2	2	2		
B	↓	↓	↓	↓	↓	↓	↓	↓		
C	↓	↓	↓	↓	↓	↓	↓	↓		
D	↓	↓	↓	↓	↓	↓	↓	↓		
E										
REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
CONC: A	2	2	2	2	2	2	2	2		
B	↓	↓	↓	↓	↓	↓	↓	↓		
C	↓	↓	↓	↓	↓	↓	↓	↓		
D	↓	↓	↓	↓	↓	↓	↓	↓		
E										
REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
CONC: A	2	2	2	2	2	2	2	2		
B	↓	↓	↓	↓	↓	↓	↓	↓		
C	↓	↓	↓	↓	↓	↓	↓	↓		
D	↓	↓	↓	↓	↓	↓	↓	↓		
E										
REP #	start	1	2	3	4	5	6	7%	MEAN %	CV
CONC: A	3	2	↓	2	2	2	2	2		
B	↓	↓	↓	↓	↓	↓	↓	↓		
C	↓	↓	↓	↓	↓	↓	↓	↓		
D	↓	↓	↓	↓	↓	↓	↓	↓		
E										
ANALYST		RH	RH	UH	UH	RH	RH	RH	RH	
DATE:		9-27	9-28-12	9-29	9-30	10-1	10-1	10-3	10-4	
TIME:		1415	1100	1200	1230	1400	1610	1540	1150	

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

**WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST**

LAB # / #s:		K1206003		TEST DATES (BEGIN / END):		9/24/12 - 10/4/12	
CLIENT:		City of DeQueen		WEIGHING DATE / TIME:		10/5/2012 1115	
ANALYSTS:		KP		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	1.00580	1.00234	0.00346	8	0.433	AVG DRY WEIGHT (mg)
	B	1.03262	1.02990	0.00272	8	0.340	
	C	0.98721	0.98401	0.00320	8	0.400	0.424
	D	1.01960	1.01568	0.00392	8	0.490	CV
	E	1.02724	1.02357	0.00367	8	0.459	
CONC: 32%	A	1.01555	1.01299	0.00256	8	0.320	AVG DRY WEIGHT (mg)
	B	1.02827	1.02486	0.00341	8	0.426	
	C	0.97371	0.96989	0.00382	8	0.477	0.433
	D	1.00540	1.00194	0.00346	8	0.433	CV
	E	0.99202	0.98793	0.00409	8	0.511	
CONC: 42%	A	0.97725	0.97358	0.00367	8	0.459	AVG DRY WEIGHT (mg)
	B	0.99517	0.99248	0.00269	8	0.336	
	C	0.97587	0.97230	0.00357	8	0.446	0.460
	D	0.99737	0.99333	0.00404	8	0.505	CV
	E	1.00386	0.99943	0.00443	8	0.554	
CONC: 56%	A	0.95360	0.94959	0.00401	8	0.501	AVG DRY WEIGHT (mg)
	B	0.99032	0.98709	0.00323	8	0.404	
	C	0.97688	0.97286	0.00402	8	0.503	0.482
	D	0.99259	0.98893	0.00366	8	0.458	CV
	E	0.99552	0.99117	0.00435	8	0.544	
CONC: 75%	A	0.99861	0.99485	0.00376	8	0.470	AVG DRY WEIGHT (mg)
	B	1.01283	1.00995	0.00288	8	0.360	
	C	1.00485	1.00167	0.00318	8	0.397	0.437
	D	0.99846	0.99501	0.00345	8	0.431	CV
	E	0.98980	0.98558	0.00422	8	0.528	
CONC: 100%	A	0.98223	0.97903	0.00320	8	0.400	AVG DRY WEIGHT (mg)
	B	0.99722	0.99343	0.00379	8	0.474	
	C	0.99329	0.98898	0.00431	8	0.539	0.462
	D	0.98640	0.98270	0.00370	8	0.463	CV
	E	0.99126	0.98777	0.00349	8	0.436	

CV = (STANDARD DEVIATION/MEAN)\*100

**REMARKS:**

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AA# K1209008, FATHEAD MINNOW, CHRONIC, 9-27-12  
File: Z:\TOXSTAT\MONTE\FHSURV. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.202

W = 0.861

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# K1209008, FATHEAD MINNOW, CHRONIC, 9-27-12  
File: Z:\TOXSTAT\MONTE\FHSURV. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 0.45

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# K1209008, FATHEAD MINNOW, CHRONIC, 9-27-12

FILE: Z:\TOXSTAT\MONTE\FHSURV.

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	0.8750	1.2094
1	CONTROL	3	1.0000	1.3931
1	CONTROL	4	1.0000	1.3931
1	CONTROL	5	0.8750	1.2094
2	32 % EFFLUENT	1	0.8750	1.2094
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	0.8750	1.2094
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	0.8750	1.2094
4	56 %	EFFLUENT	2	0.8750	1.2094
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	0.8750	1.2094
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	0.8750	1.2094
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	0.8750	1.2094
6	100 %	EFFLUENT	5	1.0000	1.3931

AA# K1209008, FATHEAD MINNOW, CHRONIC, 9-27-12

File: Z:\TOXSTAT\MONTE\FHSURV.

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	1.283				
2	32 % EFFLUENT	1.356	32.50	16.00	5.00	
3	42 % EFFLUENT	1.356	32.50	16.00	5.00	
4	56 % EFFLUENT	1.283	27.50	16.00	5.00	
5	75 % EFFLUENT	1.320	30.00	16.00	5.00	
6	100 % EFFLUENT	1.356	32.50	16.00	5.00	

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

AA# K1209008, FATHEAD MINNOW GROWTH CHRONIC, 9-27-12  
 File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.102

W = 0.963

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# K1209008, FATHEAD MINNOW GROWTH CHRONIC, 9-27-12  
 File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 1.27

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# K1209008, FATHEAD MINNOW GROWTH CHRONIC, 9-27-12  
 FILE: Z:\TOXSTAT\MONTE\FHGR.  
 TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.4330	0.7182
1	CONTROL	2	0.3400	0.6225
1	CONTROL	3	0.4000	0.6847
1	CONTROL	4	0.4900	0.7754
1	CONTROL	5	0.4590	0.7444
2	32 % EFFLUENT	1	0.3200	0.6013
2	32 % EFFLUENT	2	0.4260	0.7111
2	32 % EFFLUENT	3	0.4770	0.7624
2	32 % EFFLUENT	4	0.4330	0.7182
2	32 % EFFLUENT	5	0.5110	0.7964
3	42 % EFFLUENT	1	0.4590	0.7444
3	42 % EFFLUENT	2	0.3360	0.6183
3	42 % EFFLUENT	3	0.4460	0.7313
3	42 % EFFLUENT	4	0.5050	0.7904
3	42 % EFFLUENT	5	0.5540	0.8395
4	56 % EFFLUENT	1	0.5010	0.7864

4	56 %	EFFLUENT	2	0.4040	0.6888
4	56 %	EFFLUENT	3	0.5030	0.7884
4	56 %	EFFLUENT	4	0.4580	0.7433
4	56 %	EFFLUENT	5	0.5440	0.8295
5	75 %	EFFLUENT	1	0.4700	0.7554
5	75 %	EFFLUENT	2	0.3600	0.6435
5	75 %	EFFLUENT	3	0.3970	0.6817
5	75 %	EFFLUENT	4	0.4310	0.7162
5	75 %	EFFLUENT	5	0.5280	0.8134
6	100 %	EFFLUENT	1	0.4000	0.6847
6	100 %	EFFLUENT	2	0.4740	0.7594
6	100 %	EFFLUENT	3	0.5390	0.8244
6	100 %	EFFLUENT	4	0.4630	0.7484
6	100 %	EFFLUENT	5	0.4360	0.7212

AA# K1209008, FATHEAD MINNOW GROWTH CHRONIC, 9-27-12  
 File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.012	0.002	0.571
Within (Error)	24	0.102	0.004	
Total	29	0.114		

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

AA# K1209008, FATHEAD MINNOW GROWTH CHRONIC, 9-27-12  
 File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	0.709	0.424		
2	32 % EFFLUENT	0.718	0.433	-0.214	
3	42 % EFFLUENT	0.745	0.460	-0.866	
4	56 % EFFLUENT	0.767	0.482	-1.411	
5	75 % EFFLUENT	0.722	0.437	-0.315	
6	100 % EFFLUENT	0.748	0.462	-0.935	

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

AA# K1209008, FATHEAD MINNOW GROWTH CHRONIC, 9-27-12  
 File: Z:\TOXSTAT\MONTE\FHGR. Transform: ARC SINE(SQUARE ROOT(Y))

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	0.094	22.2	-0.009
3	42 % EFFLUENT	5	0.094	22.2	-0.036
4	56 % EFFLUENT	5	0.094	22.2	-0.058
5	75 % EFFLUENT	5	0.094	22.2	-0.013
6	100 % EFFLUENT	5	0.094	22.2	-0.038

APPENDIX D

*Ceriodaphnia dubia* Raw Data and Statistics



Cerodaphnia dubia

SURVIVAL AND REPRODUCTION TEST

RH

Discharger: Dr. Queen  
 Location: K1209008  
 Date Sample Collected:

Analyst: RH  
 Test Start - Date/Time: 9-27-12 1100  
 Test Stop - Date/Time: 10-4-12 1030

MHS

Conc 1		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	1	2	0	0	0	3	2	0	4	3	15	16	1.5	
	4	5	3	4	3	8	7	0	4	2	7	36	10	3.6	
	5	7	5	6	6	3	7	9	8	4	5	60	10	6.0	
	6	5	7	0	1	8	7	1	1	4	0	34	10	3.4	
	7	4	0	3	2	2	0	0	3	6	4	24	10	2.4	
	8														
	Total	22	16	13	12	21	18	12	16	20	19	169		CV=21.9	

Conc 4		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	4	5	4	2	2	2	1	0	3	0	23	10	2.3	
	4	4	1	0	3	0	1	5	5	4	4	27	10	2.7	
	5	5	12	6	8	6	8	8	9	0	9	71	10	7.1	
	6	10	2	3	2	9	7	3	0	11	0	47	10	4.7	
	7	0	1	2	0	4	0	5	5	6	10	33	10	3.3	
	8														
	Total	23	21	15	15	21	18	22	19	24	23	261			

32

Conc 2		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	2	4	0	1	0	1	3	2	0	0	13	10	1.3	
	4	6	0	4	5	6	4	3	0	6	2	36	10	3.6	
	5	9	8	5	5	11	0	8	5	7	0	58	10	5.8	
	6	1	8	10	2	0	10	5	7	5	9	57	10	5.7	
	7	3	6	1	3	3	0	4	0	4	2	20	10	2.0	
	8														
	Total	21	20	20	16	20	15	23	14	22	13	184			

Conc 5		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	2	4	0	0	0	1	4	2	0	0	13	10	1.3	
	4	2	0	3	4	4	8	4	3	2	5	35	10	3.5	
	5	6	9	7	6	0	0	12	8	2	11	61	10	6.1	
	6	13	11	8	9	5	11	3	7	8	1	78	10	7.8	
	7	0	4	0	2	3	5	1	4	4	8	31	10	3.1	
	8														
	Total	25	28	18	21	12	25	24	24	16	25	218			

42

Conc 3		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	3	3	4	5	0	4	4	2	0	0	25	10	2.5	
	4	3	5	0	0	3	0	0	2	3	4	20	10	2.0	
	5	10	10	9	12	4	8	7	7	5	3	75	10	7.5	
	6	0	1	2	3	4	5	8	8	10	4	45	10	4.5	
	7	1	1	3	1	2	X	1	1	0	0	10	9	1.1	
	8														
	Total	17	20	18	21	13	X	17	20	20	18	175			

Conc 6		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	2	0	1	3	0	0	2	0	5	2	15	10	1.5	
	4	2	6	3	0	5	6	7	5	3	2	39	10	3.9	
	5	8	12	9	5	4	8	11	7	8	8	82	10	8.2	
	6	2	5	2	2	0	5	3	3	2	0	24	10	2.4	
	7	5	0	6	4	5	2	7	6	1	5	41	10	4.1	
	8														
	Total	19	23	21	14	14	21	30	23	19	17	201			

X= DEAD; Y= MALE

$\bar{X} = 20.1$   
 $CV = 23.6$

AA # K1209008, C. DUBIA CHRONIC REPRODUCTION, 9-27-12  
File: Z:\TOXSTAT\MONTE\CD. Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

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\*\*\*\*\* 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

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AA # K1209008, C. DUBIA CHRONIC REPRODUCTION, 9-27-12  
File: Z:\TOXSTAT\MONTE\CD. Transform: NO TRANSFORMATION

---

Bartlett's test for homogeneity of variance  
Calculated B1 statistic = 3.18

---

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
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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 # K1209008, C. DUBIA CHRONIC REPRODUCTION, 9-27-12  
 FILE: Z:\TOXSTAT\MONTE\CD.  
 TRANSFORM: NO TRANSFORMATION

NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	22.0000	22.0000
1	CONTROL	2	16.0000	16.0000
1	CONTROL	3	13.0000	13.0000
1	CONTROL	4	12.0000	12.0000
1	CONTROL	5	21.0000	21.0000
1	CONTROL	6	18.0000	18.0000
1	CONTROL	7	12.0000	12.0000
1	CONTROL	8	16.0000	16.0000
1	CONTROL	9	20.0000	20.0000
1	CONTROL	10	19.0000	19.0000
2	32 % EFFLUENT	1	21.0000	21.0000
2	32 % EFFLUENT	2	20.0000	20.0000
2	32 % EFFLUENT	3	20.0000	20.0000
2	32 % EFFLUENT	4	16.0000	16.0000
2	32 % EFFLUENT	5	20.0000	20.0000
2	32 % EFFLUENT	6	15.0000	15.0000
2	32 % EFFLUENT	7	23.0000	23.0000
2	32 % EFFLUENT	8	14.0000	14.0000
2	32 % EFFLUENT	9	22.0000	22.0000
2	32 % EFFLUENT	10	13.0000	13.0000
3	42 % EFFLUENT	1	17.0000	17.0000
3	42 % EFFLUENT	2	20.0000	20.0000
3	42 % EFFLUENT	3	18.0000	18.0000
3	42 % EFFLUENT	4	21.0000	21.0000
3	42 % EFFLUENT	5	13.0000	13.0000
3	42 % EFFLUENT	6	17.0000	17.0000
3	42 % EFFLUENT	7	20.0000	20.0000
3	42 % EFFLUENT	8	20.0000	20.0000
3	42 % EFFLUENT	9	18.0000	18.0000
3	42 % EFFLUENT	10	11.0000	11.0000
4	56 % EFFLUENT	1	23.0000	23.0000
4	56 % EFFLUENT	2	21.0000	21.0000
4	56 % EFFLUENT	3	15.0000	15.0000
4	56 % EFFLUENT	4	15.0000	15.0000
4	56 % EFFLUENT	5	21.0000	21.0000
4	56 % EFFLUENT	6	18.0000	18.0000
4	56 % EFFLUENT	7	22.0000	22.0000
4	56 % EFFLUENT	8	19.0000	19.0000

4	56 % EFFLUENT	9	24.0000	24.0000
4	56 % EFFLUENT	10	23.0000	23.0000
5	75 % EFFLUENT	1	25.0000	25.0000
5	75 % EFFLUENT	2	28.0000	28.0000
5	75 % EFFLUENT	3	18.0000	18.0000
5	75 % EFFLUENT	4	21.0000	21.0000
5	75 % EFFLUENT	5	12.0000	12.0000
5	75 % EFFLUENT	6	25.0000	25.0000
5	75 % EFFLUENT	7	24.0000	24.0000
5	75 % EFFLUENT	8	24.0000	24.0000
5	75 % EFFLUENT	9	16.0000	16.0000
5	75 % EFFLUENT	10	25.0000	25.0000
6	100 % EFFLUENT	1	19.0000	19.0000
6	100 % EFFLUENT	2	23.0000	23.0000
6	100 % EFFLUENT	3	21.0000	21.0000
6	100 % EFFLUENT	4	14.0000	14.0000
6	100 % EFFLUENT	5	14.0000	14.0000
6	100 % EFFLUENT	6	21.0000	21.0000
6	100 % EFFLUENT	7	30.0000	30.0000
6	100 % EFFLUENT	8	23.0000	23.0000
6	100 % EFFLUENT	9	19.0000	19.0000
6	100 % EFFLUENT	10	17.0000	17.0000

AA # K1209008, C. DUBIA CHRONIC REPRODUCTION, 9-27-12  
 File: Z:\TOXSTAT\MONTE\CD. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	171.733	34.347	2.174
Within (Error)	54	853.200	15.800	
Total	59	1024.933		

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

AA # K1209008, C. DUBIA CHRONIC REPRODUCTION, 9-27-12  
 File: Z:\TOXSTAT\MONTE\CD. 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	16.900	16.900		
2	32 % EFFLUENT	18.400	18.400	-0.844	
3	42 % EFFLUENT	17.500	17.500	-0.338	
4	56 % EFFLUENT	20.100	20.100	-1.800	
5	75 % EFFLUENT	21.800	21.800	-2.756	
6	100 % EFFLUENT	20.100	20.100	-1.800	

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

AA # K1209008, C. DUBIA CHRONIC REPRODUCTION, 9-27-12  
 File: Z:\TOXSTAT\MONTE\CD. 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	4.106	24.3	-1.500
3	42 % EFFLUENT	10	4.106	24.3	-0.600
4	56 % EFFLUENT	10	4.106	24.3	-3.200
5	75 % EFFLUENT	10	4.106	24.3	-4.900
6	100 % EFFLUENT	10	4.106	24.3	-3.200

AA # K1209008, C. DUBIA CHRONIC REPRODUCTION, 9-27-12  
 File: Z:\TOXSTAT\MONTE\CD. Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	16.900				
2	32 % EFFLUENT	18.400	118.00	75.00	10.00	
3	42 % EFFLUENT	17.500	109.50	75.00	10.00	
4	56 % EFFLUENT	20.100	129.50	75.00	10.00	
5	75 % EFFLUENT	21.800	134.00	75.00	10.00	
6	100 % EFFLUENT	20.100	126.00	75.00	10.00	

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

APPENDIX E

Organism History



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

**TEST ORGANISM HISTORY**

DATE SHIPPED 9-27-12 CLIENT Arkansas Analytical

Purchase Order #: \_\_\_\_\_

SPECIES: Pimephales promelas

Quantity Shipped: 420<sup>+</sup> + 180<sup>+</sup>

Age: hatched 9/26 150x55 - 7 Days old 9/27

Brood Stock Source: Anderson Farms, AR

Culture Water: Groundwater

Hardness (Mg/l CaCO<sub>3</sub>): = 160

Dissolved Oxygen (Mg/l): 8.3

Temperature (°C): 25.1°C

Feeding: Artemia

Comments: \_\_\_\_\_

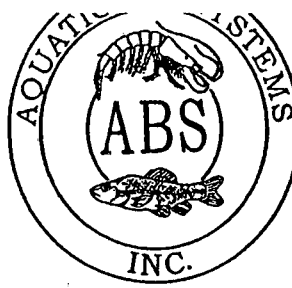
\_\_\_\_\_

\_\_\_\_\_

Shipped Via:  Federal Express  UPS Overnight  Shuttle

Packaged By: CM

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: 6/22/09

SPECIES: Ceriodaphnia dubia

AGE: Variable

LIFE STAGE: Adult

HATCH DATE: Variable

BEGAN FEEDING: Immediately

FOOD: YTC, Selenastrum sp.

### Water Chemistry Record:

	Current	Range
TEMPERATURE:	<u>25°C</u>	<u>20-25°C</u>
SALINITY/CONDUCTIVITY:	<u>--</u>	<u>--</u>
TOTAL HARDNESS (as CaCO <sub>3</sub> ):	<u>142 mg/l</u>	<u>86-124 mg/l</u>
TOTAL ALKALINITY (as CaCO <sub>3</sub> ):	<u>100 mg/l</u>	<u>65-130 mg/l</u>
pH:	<u>7.92</u>	<u>7.56-8.35</u>

Comments:

Facility Supervisor

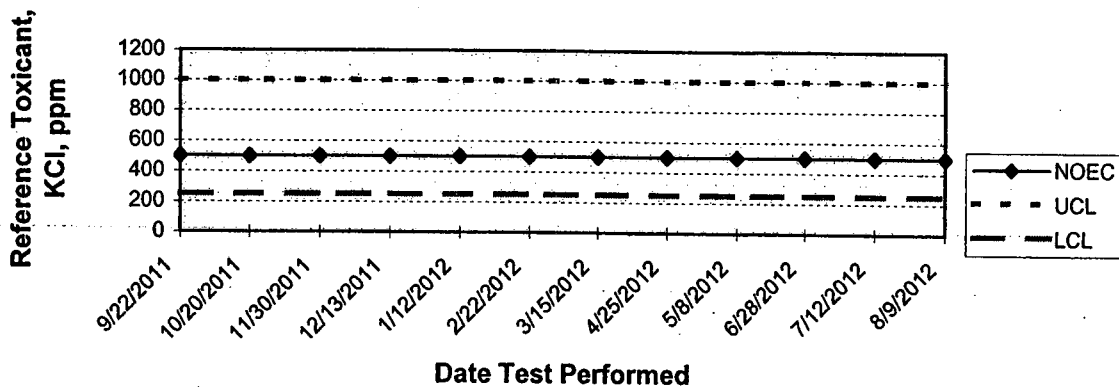
APPENDIX F

Quality Assurance Charts

# ARKANSAS ANALYTICAL, INC.

## FATHEAD MINNOW SURVIVAL

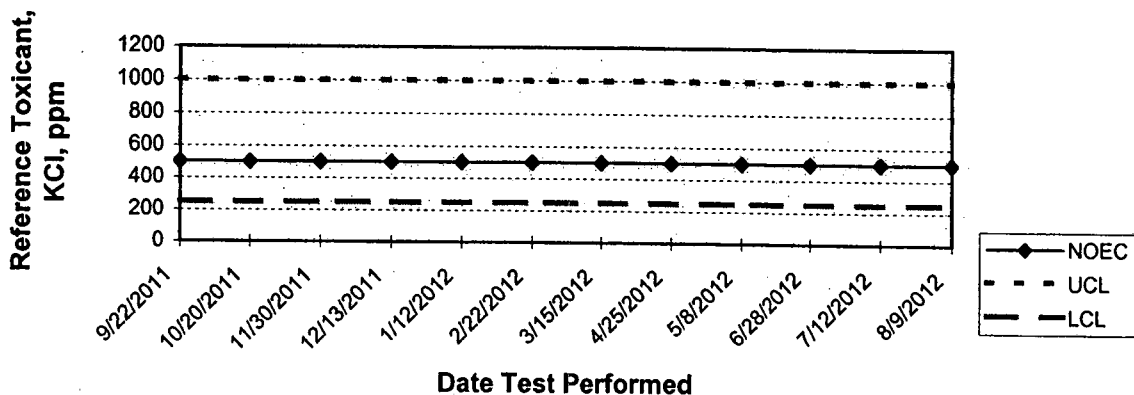
### QUALITY ASSURANCE



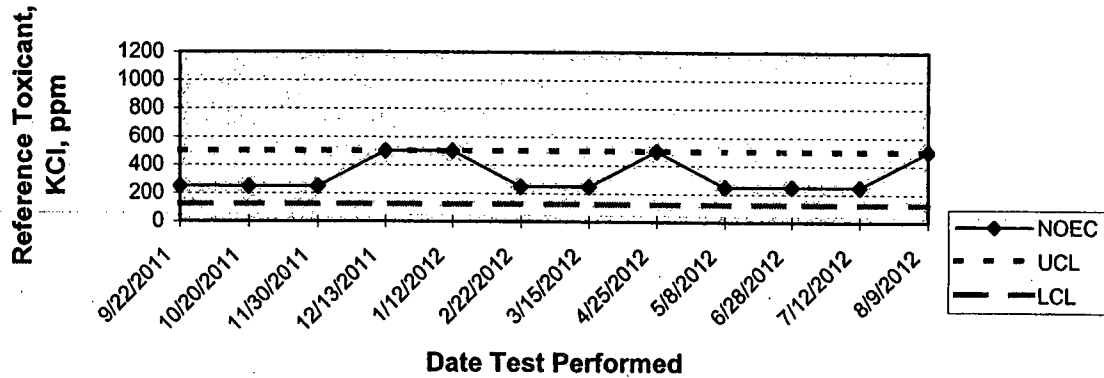
# ARKANSAS ANALYTICAL, INC.

## FATHEAD MINNOW GROWTH

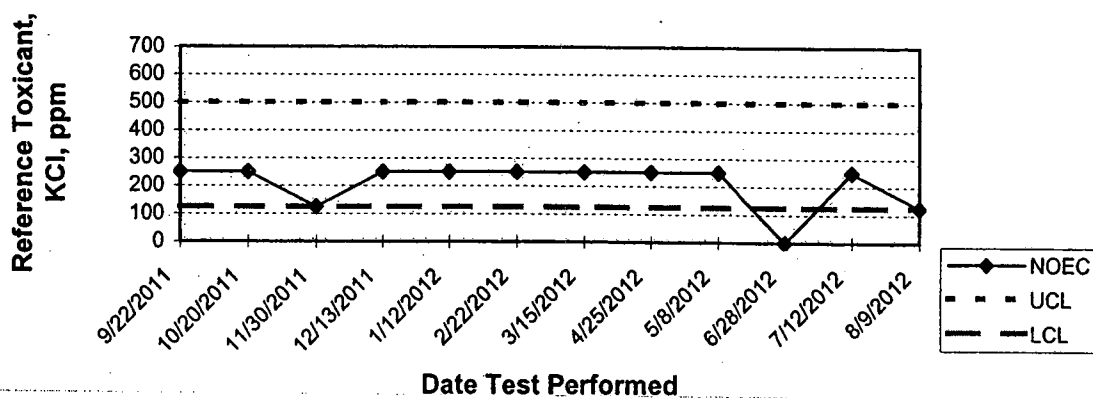
### QUALITY ASSURANCE



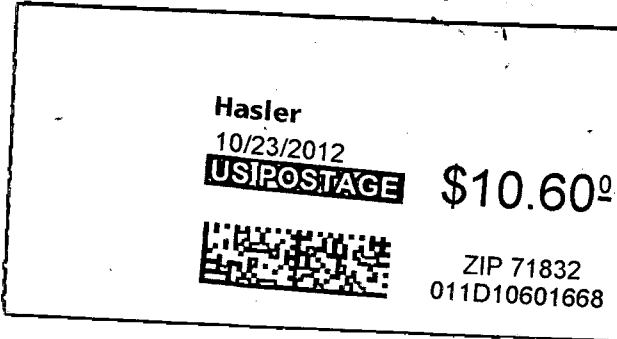
**ARKANSAS ANALYTICAL, INC.**  
**CERIODAPHНИЯ DUBIA SURVIVAL**  
**QUALITY ASSURANCE**



**ARKANSAS ANALYTICAL, INC.**  
**CERIODAPHНИЯ DUBIA REPRODUCTION**  
**QUALITY ASSURANCE**



City of DeQueen  
P.O. Box 730  
DeQueen Ar 71832



ADEQ  
NPDES Enforcement Section  
5301 Northshore Dr.  
North Little Rock Ar. 72118-5317