Arkansas Analytical, Inc.

Toxicity Test Results
City of De Queen
NPDES PERMIT NUMBER: AR0021733
First Quarter 2016
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.
8100 National Drive
Little Rock, Arkansas 72209
Lab Number K1603001

Tuesday, March 15, 2016

Introduction

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

The permit requires chronic biomonitoring testing quarterly for both *Ceriodaphnia dubia* and *Pimephales promelas*. The test results in this report represent the testing for the first quarter of 2016.

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:	3-1-16, 0930	3-2-16, 0730
Sample #2:	3-2-16, 1000	3-3-16, 0800
Sample #3:	3-6-16, 0800	3-7-16, 0800

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

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

Sample Receiving Information:	Date, Time Sample(s) Received	Temperature Upon Receipt (°C)
Sample #1:	3-4-16, 1003	6
Sample #2:	3-4-16, 1003	2
Sample #3:	3-8-16, 1045	1

Chain of custody documentation is located in Appendix A.

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

The dilution water used in the toxicity tests was moderately hard synthetic. It was prepared using Elga Maxima ultra pure water according to EPA specifications. Each batch was analyzed for pH, hardness, total alkalinity, and conductivity. Results are provided in Appendix B.

Dilution Series

Five dilutions in addition to a control (0% effluent) were used in the toxicity tests. The dilutions, which were made with synthetic water, were 32%, 42%, 56%, 75%, and 100%. The low-flow effluent concentration (**critical dilution**) was defined as **100% effluent**.

Test Methods

EPA Method 1000.0, Fathead Minnow, *Pimephales promelas,* Larval Survival and Growth Test, was used in this bioassay. Larvae are exposed in a static renewal system for seven days and the results are based on the survival and growth (increase in weight) of the larvae. The alternate method suggested in the method (11.3.4.5) for combating pathogen interference, was run in place of the original fathead minnow test. The test chambers were 30 ml plastic cups with 20 ml of test solution. Each chamber contained 2 organisms. The total number of fish was 40 per test solution. The fish were then combined to perform growth analysis. The test temperature was 25 degrees Centigrade. Raw data and statistics are provided in Appendix C.

EPA Method 1002.0, Cladoceran, *Ceriodaphnia dubia,* Survival and Reproduction Test, was also used. Neonates are exposed in a static renewal system until at least 60% of the control organisms have produced a third brood. Results are based on the survival and reproduction of the organisms. One neonate was placed in each of ten replicate chambers using a randomizing template. Test chambers were 30 ml plastic cups filled with 15 ml of test solution. The test temperature was 25 degrees Centigrade. Raw data and statistics are provided in Appendix D.

Test Organisms

The organisms used in Test 1000.0 were < 48 hour old Fathead Minnows, *Pimephales promelas*, which were purchased from Aquatox; a copy of the organism history is provided in Appendix E.

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

Quality Assurance

Test Acceptability

TEST ACCEPTANCE CRITERIA for *Ceriodaphnia dubia*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	90 %	X	
Average of 15 or more young per surviving female	24.8	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	39.9%	X	

TEST ACCEPTANCE CRITERIA for *Pimephales promelas*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	100%	X	
The percent coefficient of variation between replicates must be 40% or less for survival	0%	X	
Minimum of 0.25 mg average dry weight of surviving controls	0.994	X	
The percent coefficient of variation between replicates must be 40% or less for growth	8.50%	X	

Reference Toxicant

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

REFERENCE TOXICANT

Ceriodaphnia dubia 2/25/16-3/2/16		*Pimephales promelas* 2/3/16-2/10/16	
NOEC Survival:	250 ppm KCl	NOEC Survival:	500 ppm KCl
LOEC Survival:	500 ppm KCl	LOEC Survival:	1000 ppm KCl
NOEC Reproduction:	250 ppm KCl	NOEC Growth:	500 ppm KCl
LOEC Reproduction:	500 ppm KCl	LOEC Growth:	1000 ppm KCl

Quality Assurance charts are provided in Appendix F.

Summary of Results
City of DeQueen

Ceriodaphnia dubia		*Pimephales promelas*	
NOEC / LOEC Survival	100% / NA	NOEC / LOEC survival	100% / NA
NOEC / LOEC Reproduction	100% / NA	NOEC / LOEC growth	100%/ NA
Mean number of neonates (critical dilution)	28.8	%CV survival (critical dilution)	0%
%CV Reproduction (critical dilution)	25.0%	Mean dry weight (critical dilution) in milligrams	1.093
		%CV growth (critical dilution)	3.56%
PMSD Reproduction	45.0%	PMSD Growth	11.3%

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:

Tracy Bounds, Ken Rood, Zabrina Ruggles, Melissa Bird

Reviewed by:

Tracy Bounds, lab manager

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

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

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	3-1-16, 0930	3-2-16, 0730
Sample #2:	3-2-16, 1000	3-3-16, 0800
Sample #3:	3-6-16, 0800	3-7-16, 0800

Test initiated (date, time): 3-4-16, 1345 Test terminated (date, time): 3-11-16, 1145

Dilution water used: Moderately Hard Synthetic

DATA TABLE FOR FATHEAD MINNOW SURVIVAL

	Percent Survival in Replicate Chambers						**Mean Percent Survival**			
Effluent Conc %	A	B	C	D	E		24 hours	48 hours	7 days	CV %
0%	100	100	100	100	100		100	100	100	0.0
32%	100	100	100	100	100		100	100	100	
42%	100	100	100	100	100		100	100	100	
56%	100	100	100	100	100		100	100	100	
75%	100	100	100	100	100		100	100	100	
100%	100	100	100	100	100		100	100	100	0.0

DATA TABLE FOR GROWTH OF FATHEAD MINNOWS

Effluent Conc %	A	B	C	D	E		Mean Dry Weight	CV%
0%	0.982	0.859	1.086	1.013	1.030		0.994	8.50
32%	0.927	1.157	0.894	0.940	1.056		0.995	
42%	0.974	0.986	1.074	0.988	1.148		1.034	
56%	0.999	0.930	1.055	1.009	1.046		1.008	
75%	0.943	1.149	1.050	1.041	1.065		1.050	
100%	1.040	1.071	1.140	1.116	1.096		1.093	3.56

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)= _____**8.50**_____%

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING
Ceriodaphnia dubia SURVIVAL AND REPRODUCTION

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

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	3-1-16, 0930	3-2-16, 0730
Sample #2:	3-2-16, 1000	3-3-16, 0800
Sample #3:	3-6-16, 0800	3-7-16, 0800

Test initiated (date, time): 3-4-16, 0920 Test terminated (date, time): 3-11-16, 1000

Dilution water used: Moderately Hard Synthetic

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

Replicate	0%	32%	42%	56%	75%	100%
A	15	27	25	33	25	27
B	19	X9	19	20	21	27
C	23	22	21	19	22	26
D	25	23	23	23	21	24
E	X5	21	21	16	23	17
F	40	32	44	36	28	37
G	14	27	38	37	48	26
H	41	43	43	37	25	42
I	27	27	46	26	38	27
J	19	39	22	X12	47	35
Mean	22.8	27.0	30.2	25.9	29.8	28.8
Mean/surviving female	24.8	29.0	30.2	27.4	29.8	28.8
CV%*	39.9					25.0

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

APPENDIX A

Chain of Custody Forms

Arkansas Analytical Inc.

8100 National Dr.
Little Rock, AR 72209
PHONE: 501-455-3233
FAX: 501-455-6118

CHAIN OF CUSTODY RECORD

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

Preservation Codes:

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

Sampler(s) Signature: [signature]

Sampler(s) Printed: MS

Field Number	SAMPLE COLLECTION Date/s	Time/s	Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION	TEST PARAMETERS: Chronic Biomonitoring	Bottle Type Code (G = Glass; P = Plastic; V = Septum; A = Amber)	Arkansas Analytical Work Order Number: K1603001
	3-1to2-16	9:30am-7:30a		X	3	Water	Final Discharge Outfall	X	A	

1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)
[signature]	3-2-16 10:45am	FedEx
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)
FedEx	3-4-16 10:03	Danny Riddle

SAMPLE CONDITION UPON RECEIPT IN LAB

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

FOR COMPLETION BY LAB ONLY

REMARKS / SAMPLE COMMENTS

PO# 12482

Revision 3
1/4/16

Arkansas Analytical Inc.

8100 National Dr.
Little Rock, AR 72209
PHONE: 501-455-3233
FAX: 501-455-6118

CHAIN OF CUSTODY RECORD

CLIENT INFORMATION	BILLING
City of DeQueen Wastewater Plant	City of DeQueen Wastewater Plant
514 South 9th	P.O. Box 730
DeQueen, AR 71832	DeQueen, AR 71832
Attn: Mike Sims	

Project Description: Chronic Toxicity

Reporting Information
Telephone: 870-642-5231
Fax: 870-642-3117
Email: msims@cityofdequeen.com

Turnaround Time: 1 Day (100%) / 2 Day (50%) / 3 Day (25%) / Routine

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

Sampler(s) Signature:

Sampler(s) Printed:

Field Number	SAMPLE COLLECTION Date/s	Time/s	Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION	Chronic Biomonitoring	Bottle Type Code
	3-2+3-16	10:00 to 8:00		X	3	Water	Final Discharge Outfall	X	~~A~~ B

TEST PARAMETERS

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

Preservative Code: 1
Bottle Type: P

Arkansas Analytical Work Order Number: K1603001

1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)
	3-3-16 10:45 am	~~FedEx~~ FedEx
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)
FedEx	3-4-16 10:03	Tammy Riddle

SAMPLE CONDITION UPON RECEIPT IN LAB

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

FOR COMPLETION BY LAB ONLY

REMARKS / SAMPLE COMMENTS

PO# 72482

Revision 3
1/4/16

Arkansas Analytical Inc.

8100 National Dr.
Little Rock, AR 72209
PHONE: 501-455-3233
FAX: 501-455-6118

CHAIN OF CUSTODY RECORD

CLIENT INFORMATION	BILLING
City of DeQueen Wastewater Plant	City of DeQueen Wastewater Plant
514 South 9th	P.O. Box 730
DeQueen, AR 71832	DeQueen, AR 71832
Attn: Mike Sims	

Project Description: Chronic Toxicity

Reporting Information
Telephone: 870-642-5231
Fax: 870-642-3117
Email: msims@cityofdequeen.com

Turnaround Time: 1 Day (100%) / 2 Day (50%) / 3 Day (25%) / Routine

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

Sampler(s) Signature:

Sampler(s) Printed:

Field Number	SAMPLE COLLECTION Date/s	Time/s	Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION	Chronic Biomonitoring	Preservative Code: 1	Bottle Type: P	Bottle Type Code (G = Glass; P = Plastic; V = Septum; A = Amber)	Arkansas Analytical Work Order Number:
	3-6+07-16	8:40-8:40		X	3	Water	Final Discharge Outfall	X			C	KL603001

TEST PARAMETERS

1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)
	3-7-16 11:30	FedEx
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)
FedEx	3-8-16 1045	Jammy Riddle

SAMPLE CONDITION UPON RECEIPT IN LAB

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

FOR COMPLETION BY LAB ONLY

REMARKS / SAMPLE COMMENTS

PO # 72482

APPENDIX B

Effluent and Dilution Water Data

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING — Fathead Minnow

Lab # / Sample ID K1603001 — Test Start (Date/Time) 3-4-2016 / 1345

Client: DeQueen — Test End (Date/Time) 3-11-2016 / 1145

		Day of Test							
		1	2	3	4	5	6	7	notes
Control	MHS 810	3/4	3/5	3/6	3/7	3/8 *	3/9	3/10	* MHS 811
D.O. (mg/L)	INITIAL	8.7	8.3	~~8.7~~ 8.6	8.6	8.8	8.7	8.3	
	FINAL	7.7	~~8.7~~ 7.2	~~8.7~~ 7.0	6.4	6.3	5.7	7.2	
pH (s.u.)	INITIAL	7.7	7.9	7.9	7.9	7.8	8.1	8.0	
	FINAL	7.7	~~7.8~~ 7.6	7.5	7.4	7.6	7.4	7.8	
temp (C)	INITIAL	22	22	21	21	21	21	22	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		~~68~~ 54				60			
HARDNESS (mg/L)		44				72			
CONDUCTIVITY (umhc		277				284			
CHLORINE (mg/L)		<0.05				<0.05			
CONC: 32%									
D.O. (mg/L)	INITIAL	8.9	8.9	8.8	9.0	8.3	9.1	9.1	
	FINAL	7.3	6.7	6.3	5.9	6.0	5.6	7.0	
pH (s.u)	INITIAL	7.7	7.9	7.9	7.9	7.7	8.0	7.9	
	FINAL	7.8	7.6	7.5	7.4	7.6	7.5	7.8	
temp (C)	INITIAL	23	22	22	22	22	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC: ~~32~~ 42%									
D.O. (mg/L)	INITIAL	8.9	8.9	8.8	9.2	8.8	9.1	9.5	
	FINAL	7.3	6.7	6.4	5.9	6.0	5.6	7.0	
pH (mg/L)	INITIAL	7.6	7.8	7.8	7.8	7.7	7.9	7.9	
	FINAL	7.8	7.6	7.5	7.6	7.6	7.5	7.8	
temp (C)	INITIAL	24	22	23	23	22	23	22	
	FINAL	25	25	25	25	25	25	25	
CONC: 56%									
D.O. (mg/L)	INITIAL	8.9	9.0	8.8	9.3	9.0	9.1	9.6	
	FINAL	7.3	6.9	6.2	5.7	6.0	6.1	7.2	
pH (s.u.)	INITIAL	7.6	7.7	7.7	7.7	7.7	7.8	7.8	
	FINAL	7.8	7.7	7.5	7.6	7.7	7.6	7.8	
temp (C)	INITIAL	24	22	24	23	23	24	22	
	FINAL	25	25	25	25	25	25	25	
CONC: 75%									
D.O. (mg/L)	INITIAL	9.0	9.2	8.9	9.4	9.2	9.1	9.9	
	FINAL	7.3	6.7	6.2	5.8	6.0	5.8	7.11	
pH (s.u.)	INITIAL	7.5	7.6	7.7	7.6	7.6	7.7	7.7	
	FINAL	7.8	7.7	7.5	7.6	7.6	7.6	7.8	
temp (C)	INITIAL	25	22	24	24	24	25	22	
	FINAL	25	25	25	25	25	25	25	
CONC: 100%									
D.O. (mg/L)	INITIAL	9.2	9.4	9.3	9.5	9.6	9.4	10	
	FINAL	7.2	6.6	6.3	5.9	6.0	6.2	7.1	
pH (s.u.)	INITIAL	7.4	7.5	7.5	7.5	7.5	7.6	7.6	
	FINAL	7.8	7.7	7.6	7.6	7.7	7.6	7.8	
temp (C)	INITIAL	26	23	24	25	24	26	25	
	FINAL	25	25	25	25	25	25	25	
CONC: 100 %		B	A	A	B	C	C	C	
ALKALINITY (mg/L)		60	58		60	66			
HARDNESS (mg/L)		42	50		42	48			
CONDUCTIVITY (umhc		868	805		868	700			
CHLORINE (mg/L)		<0.05	<0.05		<0.05	<0.05			

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING — Ceriodaphnia Dubia

Lab # / Sample ID: K1603001 — Test Start (Date/Time): 3-4-2016 / 0920

Client: DeQueen — Test End (Date/Time): 3-11-2016 / 1000

		Day of Test 1	2	3	4	5	6	7	notes
Control	MH680	3/4	3/5	3/6	3/7	3/8 *	3/9	3/10	*MHS 811
D.O. (mg/L)	INITIAL	8.7	8.3	8.7	8.6	8.8	8.7	8.3	
	FINAL	8.2	8.6	8.7	8.1	8.0	8.0	8.1	
pH (s.u.)	INITIAL	7.7	7.9	7.9	7.9	7.8	8.1	8.0	
	FINAL	7.7	8.0	8.1	8.2	8.0	7.9	8.0	
temp (C)	INITIAL	22	22	21	21	21	21	22	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		54				60			
HARDNESS (mg/L)		64				72			
CONDUCTIVITY (umhc		277				284			
CHLORINE (mg/L)		<0.05				<0.05			
CONC: 32%									
D.O. (mg/L)	INITIAL	8.9	8.9	8.8	9.0	8.3	9.1	9.1	
	FINAL	8.0	8.5	8.4	8.2	7.7	7.8	7.8	
pH (s.u)	INITIAL	7.7	7.9	7.9	7.9	7.7	8.0	7.9	
	FINAL	7.9	7.6	8.1	8.1	8.0	8.1	7.9	
temp (C)	INITIAL	23	22	22	22	22	22	22	
	FINAL	25	25	25	25	25	25	25	
CONC: 42%									
D.O. (mg/L)	INITIAL	8.9	8.9	8.8	9.2	8.8	9.1	9.5	
	FINAL	8.0	8.3	8.2	8.6	7.5	7.8	7.7	
pH (mg/L)	INITIAL	7.6	7.8	7.8	7.8	7.7	7.9	7.9	
	FINAL	7.9	8.1	8.1	8.1	8.0	8.1	7.9	
temp (C)	INITIAL	24	22	23	23	22	23	22	
	FINAL	25	25	25	25	25	25	25	
CONC: 56%									
D.O. (mg/L)	INITIAL	8.9	9.0	8.8	9.3	9.0	9.1	9.6	
	FINAL	7.9	8.5	8.2	7.9	7.5	7.8	7.6	
pH (s.u.)	INITIAL	7.6	7.7	7.7	7.7	7.7	7.8	7.8	
	FINAL	7.9	8.1	~~8.2~~ 8.1	8.1	8.0	8.1	7.9	
temp (C)	INITIAL	24	22	24	23	23	24	22	
	FINAL	25	25	25	25	25	25	25	
CONC: 75%									
D.O. (mg/L)	INITIAL	9.0	9.2	8.9	9.4	9.2	9.1	9.9	
	FINAL	7.9	8.5	8.3	7.6	7.4	7.8	7.6	
pH (s.u.)	INITIAL	7.5	7.6	7.7	7.6	7.6	7.7	7.7	
	FINAL	8.0	8.1	8.1	8.1	8.0	8.1	~~7.6~~ 7.9	
temp (C)	INITIAL	25	22	24	24	24	25	22	
	FINAL	25	25	25	25	25	25	25	
CONC: 100%									
D.O. (mg/L)	INITIAL	9.2	9.4	9.3	9.5	9.6	9.4	10	
	FINAL	7.9	8.6	8.5	8.0	7.7	7.8	7.5	
pH (s.u.)	INITIAL	7.4	7.5	7.5	7.5	7.5	7.6	7.6	
	FINAL	8.0	8.1	8.1	8.1	8.1	8.1	7.9	
temp (C)	INITIAL	26	23	24	25	24	26	25	
	FINAL	25	25	25	25	25	25	25	
CONC: 100 %		B	A	A	B	C	C	C	
ALKALINITY (mg/L)		60	58		60	66			
HARDNESS (mg/L)		42	50		42	48			
CONDUCTIVITY (umhc		868	805		868	700			
CHLORINE (mg/L)		<0.05	<0.05		<0.05	<0.05			

APPENDIX C

Fathead minnow raw data and statistics

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID K160300 1 TEST START DATE 3-4-16 TIME 1345

CLIENT DeQueen TEST END DATE 3-11-16 TIME 1145

AGE AND SOURCE OF MINNOWS <48hrs; Aquatox

SUMMARY

		DAY (NUMBER SURVIVING)								SURVIVAL		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	8	8	8	8	8	8	8	8	100	100	0
	B									100		
	C									100		
Cont	D									100		
	E									100		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	8	8	8	8	8	8	8	8	100	100	
	B									100		
	C									100		
32%	D									100		
	E									100		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	8	8	8	8	8	8	8	8	100	100	
	B									100		
	C									100		
42%	D									100		
	E									100		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	8	8	8	8	8	8	8	8	100	100	
	B									100		
	C									100		
56%	D									100		
	E									100		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	8	8	8	8	8	8	8	8	100	100	
	B									100		
	C									100		
75%	D									100		
	E									100		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	8	8	8	8	8	8	8	8	100	100	0
	B									100		
	C									100		
100%	D									100		
	E									100		
ANALYST		tb	tb	tb	tb	tb	tb	tb	mb			
DATE:		3-4-16	3-5-16	3-6-16	3-7-16	3-8-16	3-9-16	3-10-16	3-11-16			
TIME:		1345	1200	0815	1315	1100	1030	1315	1145			

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID K1603001			TEST START	DATE 3-4-16	TIME 1345								
CLIENT DeQueen			TEST END	DATE 3-11-16	TIME 1145								
A			AGE AND SOURCE OF MINNOWS										
			D A Y (NUMBER SURVIVING)								SURVIVAL		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: CONT	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: 32%	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: 42%	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: 56%	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: 75%	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: 100%	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E											
ANALYST		tb	tb	tb	tb	tb	tb	tb	mb			
DATE:		3-4-16	3-5-16	3-6-16	3-7-16	3-8-16	3-9-16	3-10-16	3-11-16			
TIME:		1345	1200	0815	1315	1100	1030	1315	1145			

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID	K1603001	TEST START DATE	3-4-16 TIME 1345
CLIENT	DeQueen	TEST END DATE	3-11-16 TIME 1145

B — AGE AND SOURCE OF MINNOWS

DAY (NUMBER SURVIVING) — SURVIVAL

	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	2	2	2	2	2	2	2	2			
	B	|	|	|	|	|	|	|	|			
	C	|	|	|	|	|	|	|	|			
CONT	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	|	|	|	|	|	|	|	|			
32%	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	|	|	|	|	|	|	|	|			
42%	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	|	|	|	|	|	|	|	|			
56%	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	|	|	|	|	|	|	|	|			
75%	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	|	|	|	|	|	|	|	|			
100%	D	|	|	|	|	|	|	|	|			
	E											
ANALYST		hb	hb	hb	hb	hb	hb	hb	mb			
DATE:		3-4	3-5	3-6	3-7	3-8	3-9	3-10	3-11			
TIME:		1345	1200	0815	1315	1100	1030	1315	1145			

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID	1603001	TEST START DATE	3-4-16 TIME 1345
CLIENT	DeQueen	TEST END DATE	3-11-16 TIME 1145
C		AGE AND SOURCE OF MINNOWS	

	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	2	2	2	2	2	2	2	2			
	B	|	|	|	|	|	|	|	|			
CONT	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	|	|	|	|	|	|	|	|			
32%	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	|	|	|	|	|	|	|	|			
42%	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	|	|	|	|	|	|	|	|			
56%	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	|	|	|	|	|	|	|	|			
75%	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	|	|	|	|	|	|	|	|			
100%	D	|	|	|	|	|	|	|	|			
	E											
ANALYST		tb	tb	tb	tb	tb	tb	tb	mb			
DATE:		3-4	3-5	3-6	3-7	3-8	3-9	3-10	3-11			
TIME:		1345	1200	0815	1315	1100	1030	1315	1145			

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID K1603001 TEST START DATE 3-4-16 TIME 1345

CLIENT DeQueen TEST END DATE 3-11-16 TIME 1145

D — AGE AND SOURCE OF MINNOWS

		DAY (NUMBER SURVIVING)								SURVIVAL		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: Cont	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: 32%	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: 42%	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: 56%	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: 75%	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: 100%	A	2	2	2	2	2	2	2	2			
	B											
	C											
	D											
	E											
ANALYST		tb	tb	tb	tb	tb	tb	tb	mb			
DATE:		3-4	3-5	3-6	3-7	3-8	3-9	3-10	3-11			
TIME:		1345	1200	0815	1315	1100	1030	1315	1145			

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

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID K1603001 TEST START DATE 3-4-16 TIME 1345

CLIENT DeQueen TEST END DATE 3-11-16 TIME 1145

E AGE AND SOURCE OF MINNOWS

			DAY (NUMBER SURVIVING)							SURVIVAL		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	2	2	2	2	2	2	2	2			
	B											
	C											
Cont	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											
32%	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											
42%	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											
56%	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											
75%	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											
100%	D											
	E											
ANALYST		hb	hb	hb	hb	hb	hb	hb	mb			
DATE:		3-4	3-5	3-6	3-7	3-8	3-9	3-10	3-11			
TIME:		1345	1200	0815	1315	1100	1030	1315	1145			

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

Pimephales promelas **FATHEAD MINNOW** TEST 1000.0

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:	K1603001				TEST DATES (BEGIN / END):		3/4-11/16	
CLIENT:	City of DeQueen				WEIGHING DATE / TIME:		3/14/16, 1320	
ANALYSTS:	ZR, KR				DRYING TEMP (DEGREES C):		60	
SAMPLE ID:					DRYING TIME (HOURS):		24	
	REP #	FINAL DRY WEIGHT TIN+LARVAE (g)	INITIAL WEIGHT TIN (g)	TOTAL DRY WEIGHT OF LARVAE (g)	NUMBER OF LARVAE	DRY WEIGHT OF LARVAE (mg)		
CONTROL	A	0.99875	0.99089	0.00786	8	0.982	AVG DRY	
	B	1.00716	1.00029	0.00687	8	0.859	WEIGHT (mg)	
MHS	C	1.06394	1.05525	0.00869	8	1.086		0.994
	D	1.04910	1.04100	0.00810	8	1.013	CV	
	E	1.05023	1.04199	0.00824	8	1.030		8.50
CONC:	A	1.00724	0.99982	0.00742	8	0.927	AVG DRY	
	B	1.02212	1.01286	0.00926	8	1.157	WEIGHT (mg)	
32%	C	1.02101	1.01386	0.00715	8	0.894		0.995
	D	0.99605	0.98853	0.00752	8	0.940	CV	
	E	1.02704	1.01859	0.00845	8	1.056		
CONC:	A	1.04832	1.04053	0.00779	8	0.974	AVG DRY	
	B	1.05858	1.05069	0.00789	8	0.986	WEIGHT (mg)	
42%	C	1.01255	1.00396	0.00859	8	1.074		1.034
	D	1.01528	1.00738	0.00790	8	0.988	CV	
	E	0.99561	0.98643	0.00918	8	1.148		
CONC:	A	0.99643	0.98844	0.00799	8	0.999	AVG DRY	
	B	0.99147	0.98403	0.00744	8	0.930	WEIGHT (mg)	
56%	C	1.01138	1.00294	0.00844	8	1.055		1.008
	D	1.01728	1.00921	0.00807	8	1.009	CV	
	E	1.02232	1.01395	0.00837	8	1.046		
CONC:	A	1.04441	1.03687	0.00754	8	0.943	AVG DRY	
	B	0.99731	0.98812	0.00919	8	1.149	WEIGHT (mg)	
75%	C	1.00044	0.99204	0.00840	8	1.050		1.050
	D	1.02680	1.01847	0.00833	8	1.041	CV	
	E	1.01712	1.00860	0.00852	8	1.065		
CONC:	A	1.00364	0.99532	0.00832	8	1.040	AVG DRY	
	B	1.07463	1.06606	0.00857	8	1.071	WEIGHT (mg)	
100%	C	1.06215	1.05303	0.00912	8	1.140		1.093
	D	1.05404	1.04511	0.00893	8	1.116	CV	
	E	1.07557	1.06680	0.00877	8	1.096		3.56

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

Pimephales promelas **FATHEAD MINNOW** TEST 1000.0

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:	K16030001
CLIENT:	DeQueen
ANALYSTS:	ZR, KR
SAMPLE ID:	
TEST DATES (BEGIN / END):	3/4-11/16
WEIGHING DATE / TIME:	3-14-16/1320
DRYING TEMP (DEGREES C):	100°C
DRYING TIME (HOURS):	24hrs

	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 D1	0.99875	0.99089				AVG DRY
	B D2	1.00716	1.00029				WEIGHT (mg)
Control	C D3	1.06394	1.05525				
	D D4	1.04910	1.04100				CV
	E D5	1.05023	1.04199				
CONC:	A D6	1.00724	0.99982				AVG DRY
	B D7	1.02212	1.01286				WEIGHT (mg)
32%	C D8	1.02101	1.01386				
	D D9	0.99605	0.98853				CV
	E D10	1.02704	1.01859				
CONC:	A D11	1.04832	1.04053				AVG DRY
	B D12	1.05858	1.05069				WEIGHT (mg)
42%	C D13	1.01255	1.00396				
	D D14	1.01528	1.00738				CV
	E D15	0.99561	0.98643				
CONC:	A D16	0.99643	0.98844				AVG DRY
	B D17	0.99147	0.98403				WEIGHT (mg)
56%	C D18	1.01138	1.00294				
	D D19	1.01728	1.00921				CV
	E D20	1.02232	1.01395				
CONC:	A D21	1.04441	1.03687				AVG DRY
	B D22	0.99731	0.98812				WEIGHT (mg)
75%	C D23	1.00044	0.99204				
	D D24	1.02680	1.01847				CV
	E D25	1.01712	1.00860				
CONC:	A D26	1.00364	0.99532				AVG DRY
	B D27	1.07463	1.06606				WEIGHT (mg)
100%	C D28	1.06215	1.05303				
	D D29	1.05404	1.04511				CV
	E D30	1.07557	1.06680				

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA # K1603001, P. PROMELAS 7 DAY CHRONIC, 3-4-16
File: dequeenS Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.000

W = 0.000

Critical W (P = 0.05) (n = 30) = 0.927
Critical W (P = 0.01) (n = 30) = 0.900

Data FAIL normality test. Try another transformation.

Warning - The first three homogeneity tests are sensitive to non-normal data and should not be performed.

AA # K1603001, P. PROMELAS 7 DAY CHRONIC, 3-4-16
File: dequeenS Transform: ARC SINE(SQUARE ROOT(Y))

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

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

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

TITLE: AA # K1603001, P. PROMELAS 7 DAY CHRONIC, 3-4-16
FILE: dequeenS
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

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

AA # K1603001, P. PROMELAS 7 DAY CHRONIC, 3-4-16
File: dequeenS Transform: ARC SINE(SQUARE ROOT(Y))

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	1.393				
2	32 % EFFLUENT	1.393	27.50	16.00	5.00	
3	42 % EFFLUENT	1.393	27.50	16.00	5.00	
4	56 % EFFLUENT	1.393	27.50	16.00	5.00	
5	75 % EFFLUENT	1.393	27.50	16.00	5.00	
6	100 % EFFLUENT	1.393	27.50	16.00	5.00	

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

AA # K1603001, P. PROMELAS 7 DAY GROWTH, 3-4-16
File: dequeenG Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.136

W = 0.988

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 # K1603001, P. PROMELAS 7 DAY GROWTH, 3-4-16
File: dequeenG Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 4.56

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 # K1603001, P. PROMELAS 7 DAY GROWTH, 3-4-16
FILE: dequeenG
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.9820	0.9820
1	CONTROL	2	0.8590	0.8590
1	CONTROL	3	1.0860	1.0860
1	CONTROL	4	1.0130	1.0130
1	CONTROL	5	1.0300	1.0300
2	32 % EFFLUENT	1	0.9270	0.9270
2	32 % EFFLUENT	2	1.1570	1.1570
2	32 % EFFLUENT	3	0.8940	0.8940
2	32 % EFFLUENT	4	0.9400	0.9400
2	32 % EFFLUENT	5	1.0560	1.0560
3	42 % EFFLUENT	1	0.9740	0.9740
3	42 % EFFLUENT	2	0.9860	0.9860
3	42 % EFFLUENT	3	1.0740	1.0740
3	42 % EFFLUENT	4	0.9880	0.9880
3	42 % EFFLUENT	5	1.1480	1.1480
4	56 % EFFLUENT	1	0.9990	0.9990
4	56 % EFFLUENT	2	0.9300	0.9300
4	56 % EFFLUENT	3	1.0550	1.0550
4	56 % EFFLUENT	4	1.0090	1.0090
4	56 % EFFLUENT	5	1.0460	1.0460
5	75 % EFFLUENT	1	0.9430	0.9430
5	75 % EFFLUENT	2	1.1490	1.1490
5	75 % EFFLUENT	3	1.0500	1.0500
5	75 % EFFLUENT	4	1.0410	1.0410
5	75 % EFFLUENT	5	1.0650	1.0650
6	100 % EFFLUENT	1	1.0400	1.0400
6	100 % EFFLUENT	2	1.0710	1.0710
6	100 % EFFLUENT	3	1.1400	1.1400
6	100 % EFFLUENT	4	1.1160	1.1160
6	100 % EFFLUENT	5	1.0960	1.0960

AA # K1603001, P. PROMELAS 7 DAY GROWTH, 3-4-16
File: dequeenG Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.037	0.007	1.292
Within (Error)	24	0.136	0.006	
Total	29	0.173		

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

AA # K1603001, P. PROMELAS 7 DAY GROWTH, 3-4-16
File: dequeenG Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	0.994	0.994		
2	32 % EFFLUENT	0.995	0.995	-0.017	
3	42 % EFFLUENT	1.034	1.034	-0.839	
4	56 % EFFLUENT	1.008	1.008	-0.290	
5	75 % EFFLUENT	1.050	1.050	-1.166	
6	100 % EFFLUENT	1.093	1.093	-2.068	

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

AA # K1603001, P. PROMELAS 7 DAY GROWTH, 3-4-16
File: dequeenG Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	5			
2	32 % EFFLUENT	5	0.112	11.3	-0.001
3	42 % EFFLUENT	5	0.112	11.3	-0.040
4	56 % EFFLUENT	5	0.112	11.3	-0.014
5	75 % EFFLUENT	5	0.112	11.3	-0.056
6	100 % EFFLUENT	5	0.112	11.3	-0.099

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

SURVIVAL AND REPRODUCTION TEST

Ceriodaphnia dubia

Discharger: DeQueen | Lab Number/s: K1603001

Location: Outfall

Date Sample Collected: See COC

Analyst: tb

Test Start - Date/Time: 3-4-2016 / 0920

Test Stop - Date/Time: 3-11-2016 / 1000

CONTROL

Day	A	B	C	D	E	F	G	H	I	J	No. of Young	No. of Adult	Young /Adult	Analyst
1	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
2	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
3	0	1	0	0	0	4	2	4	0	4	15	10	1.5	tb
4	3	5	5	6	5	10	0	9	6	4	53	10	5.3	tb
5	3	0	5	8	0	0	2	0	8	0	26	10	2.6	tb
6	0	7	1	0	X0	11	10	14	0	11	54	9	5.4	tb
7	9	6	12	11	–	15	0	14	13	0	80	9	8.9	tb
8														
Total	15	19	23	25	X5	40	14	41	27	19	228	$\bar{X}$=24.8	CV=31%	
												100%	35%	brood

32%

Day	A	B	C	D	E	F	G	H	I	J	No. of Young	No. of Adult	Young /Adult	Analyst
1	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
2	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
3	0	0	0	0	0	4	4	5	0	3	16	10	1.6	tb
4	5	3	4	4	4	4	0	9	6	10	49	10	4.9	tb
5	10	X6	7	8	8	10	9	13	9	0	80	9	8.0	tb
6	0	–	0	1	9	0	13	2	0	13	38	9	4.2	tb
7	12	–	11	10	0	14	1	14	12	13	87	9	9.7	tb
8														
Total	27	X9	22	23	21	32	27	43	27	39	270			

42%

Day	A	B	C	D	E	F	G	H	I	J	No. of Young	No. of Adult	Young /Adult	Analyst
1	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
2	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
3	0	0	2	0	3	4	4	4	8	4	29	10	2.9	tb
4	3	3	0	4	0	9	8	10	10	6	53	10	5.3	tb
5	9	8	8	7	6	11	0	0	0	0	49	10	4.9	tb
6	3	0	11	0	12	4	14	14	13	11	82	10	8.2	tb
7	10	8	0	12	0	10	12	15	15	1	81	10	8.1	tb
8														
Total	25	19	21	23	21	44	38	43	46	22	302			

56%

Day	A	B	C	D	E	F	G	H	I	J	No. of Young	No. of Adult	Young /Adult	Analyst
1	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
2	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
3	0	0	2	0	1	0	4	4	0	3	14	10	1.4	tb
4	6	3	1	3	2	6	8	8	6	7	50	10	5.0	tb
5	12	0	7	9	6	8	0	0	9	0	51	10	5.1	tb
6	15	7	9	2	7	8	11	12	0	X2	73	9	7.3	tb
7	0	10	0	9	0	14	14	13	11	–	71	9	7.9	tb
8														
Total	33	20	19	23	16	36	37	37	26	X12	259			

75%

Day	A	B	C	D	E	F	G	H	I	J	No. of Young	No. of Adult	Young /Adult	Analyst
1	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
2	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
3	0	0	0	0	0	0	4	4	4	4	16	10	1.6	tb
4	5	3	5	3	3	5	11	0	9	10	54	10	5.4	tb
5	9	6	7	7	1	9	0	8	0	0	42	10	4.2	tb
6	0	9	0	11	10	2	15	0	14	16	77	10	7.7	tb
7	11	8	10	0	9	12	18	13	11	17	109	10	10.9	tb
8														
Total	25	21	22	21	23	28	48	25	38	47	298			

100%

Day	A	B	C	D	E	F	G	H	I	J	No. of Young	No. of Adult	Young /Adult	Analyst
1	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
2	0	0	0	0	0	0	0	0	0	0	0	10	0	tb
3	0	0	0	0	0	0	4	5	4	4	17	10	1.7	tb
4	6	4	4	4	2	6	7	10	8	8	59	10	5.9	tb
5	10	9	9	9	0	8	2	0	0	0	47	10	4.7	tb
6	0	0	13	0	8	10	13	14	15	14	87	10	8.7	tb
7	11	14	0	11	9	13	0	13	0	9	78	10	7.8	tb
8														
Total	27	27	26	24	17	37	26	42	27	35	288	$\bar{X}$=28.8		
													CV=25.0%	

X = Dead

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF ALIVE	DEAD	TOTAL ANIMALS
CONTROL	9	1	10
32% effluent	9	1	10
TOTAL	18	2	20

CRITICAL FISHER'S VALUE (10,10,9) (p=0.05) IS 4. b VALUE IS 9.
Since b is greater than 4 there is no significant difference between CONTROL and TREATMENT at the 0.05 level.

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF DEAD	ALIVE	TOTAL ANIMALS
CONTROL	1	9	10
42% effluent	0	10	10
TOTAL	1	19	20

CRITICAL FISHER'S VALUE (10,10,1) (p=0.05) IS LESS THAN 0. b VALUE IS 0.
NO SIGNIFICANT DIFFERENCE

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF ALIVE	DEAD	TOTAL ANIMALS
CONTROL	9	1	10
56% effluent	9	1	10

TOTAL	18	2	20

CRITICAL FISHER'S VALUE (10,10,9) (p=0.05) IS 4. b VALUE IS 9.
Since b is greater than 4 there is no significant difference between CONTROL and TREATMENT at the 0.05 level.

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF DEAD	NUMBER OF ALIVE	TOTAL ANIMALS
CONTROL	1	9	10
75% effluent	0	10	10
TOTAL	1	19	20

CRITICAL FISHER'S VALUE (10,10,1) (p=0.05) IS LESS THAN 0. b VALUE IS 0.
NO SIGNIFICANT DIFFERENCE

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF DEAD	NUMBER OF ALIVE	TOTAL ANIMALS
CONTROL	1	9	10
100% effluent	0	10	10
TOTAL	1	19	20

CRITICAL FISHER'S VALUE (10,10,1) (p=0.05) IS LESS THAN 0. b VALUE IS 0.
NO SIGNIFICANT DIFFERENCE

SUMMARY OF FISHER'S EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)

	CONTROL	10	1
1	32% effluent	10	1
2	42% effluent	10	0
3	56% effluent	10	1
4	75% effluent	10	0
5	100% effluent	10	0

AA # K1603001, CERIODAPHNIA DUBIA REPRODUCTION, 3-4-16
File: C:\TOXSTAT\DEQUEENC. Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

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

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

Total number of replicates = 60

AA # K1603001, CERIODAPHNIA DUBIA REPRODUCTION, 3-4-16
File: C:\TOXSTAT\DEQUEENC. Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 2.11

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 # K1603001, CERIODAPHNIA DUBIA REPRODUCTION, 3-4-16
FILE: C:\TOXSTAT\DEQUEENC.
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	15.0000	15.0000
1	CONTROL	2	19.0000	19.0000
1	CONTROL	3	23.0000	23.0000
1	CONTROL	4	25.0000	25.0000
1	CONTROL	5	5.0000	5.0000
1	CONTROL	6	40.0000	40.0000
1	CONTROL	7	14.0000	14.0000
1	CONTROL	8	41.0000	41.0000
1	CONTROL	9	27.0000	27.0000
1	CONTROL	10	19.0000	19.0000
2	32 % EFFLUENT	1	27.0000	27.0000
2	32 % EFFLUENT	2	9.0000	9.0000
2	32 % EFFLUENT	3	22.0000	22.0000
2	32 % EFFLUENT	4	23.0000	23.0000
2	32 % EFFLUENT	5	21.0000	21.0000
2	32 % EFFLUENT	6	32.0000	32.0000
2	32 % EFFLUENT	7	27.0000	27.0000
2	32 % EFFLUENT	8	43.0000	43.0000
2	32 % EFFLUENT	9	27.0000	27.0000
2	32 % EFFLUENT	10	39.0000	39.0000
3	42 % EFFLUENT	1	25.0000	25.0000
3	42 % EFFLUENT	2	19.0000	19.0000
3	42 % EFFLUENT	3	21.0000	21.0000
3	42 % EFFLUENT	4	23.0000	23.0000
3	42 % EFFLUENT	5	21.0000	21.0000
3	42 % EFFLUENT	6	44.0000	44.0000
3	42 % EFFLUENT	7	38.0000	38.0000
3	42 % EFFLUENT	8	43.0000	43.0000
3	42 % EFFLUENT	9	46.0000	46.0000
3	42 % EFFLUENT	10	22.0000	22.0000
4	56 % EFFLUENT	1	33.0000	33.0000
4	56 % EFFLUENT	2	20.0000	20.0000
4	56 % EFFLUENT	3	19.0000	19.0000
4	56 % EFFLUENT	4	23.0000	23.0000
4	56 % EFFLUENT	5	16.0000	16.0000
4	56 % EFFLUENT	6	36.0000	36.0000
4	56 % EFFLUENT	7	37.0000	37.0000
4	56 % EFFLUENT	8	37.0000	37.0000
4	56 % EFFLUENT	9	26.0000	26.0000
4	56 % EFFLUENT	10	12.0000	12.0000
5	75 % EFFLUENT	1	25.0000	25.0000
5	75 % EFFLUENT	2	21.0000	21.0000
5	75 % EFFLUENT	3	22.0000	22.0000
5	75 % EFFLUENT	4	21.0000	21.0000
5	75 % EFFLUENT	5	23.0000	23.0000
5	75 % EFFLUENT	6	28.0000	28.0000
5	75 % EFFLUENT	7	48.0000	48.0000
5	75 % EFFLUENT	8	25.0000	25.0000
5	75 % EFFLUENT	9	38.0000	38.0000

5	75	%	EFFLUENT	10	47.0000	47.0000
6	100	%	EFFLUENT	1	27.0000	27.0000
6	100	%	EFFLUENT	2	27.0000	27.0000
6	100	%	EFFLUENT	3	26.0000	26.0000
6	100	%	EFFLUENT	4	24.0000	24.0000
6	100	%	EFFLUENT	5	17.0000	17.0000
6	100	%	EFFLUENT	6	37.0000	37.0000
6	100	%	EFFLUENT	7	26.0000	26.0000
6	100	%	EFFLUENT	8	42.0000	42.0000
6	100	%	EFFLUENT	9	27.0000	27.0000
6	100	%	EFFLUENT	10	35.0000	35.0000

AA # K1603001, CERIODAPHNIA DUBIA REPRODUCTION, 3-4-16
File: C:\TOXSTAT\DEQUEENC. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	391.283	78.257	0.794
Within (Error)	54	5319.300	98.506	
Total	59	5710.583		

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

AA # K1603001, CERIODAPHNIA DUBIA REPRODUCTION, 3-4-16
File: C:\TOXSTAT\DEQUEENC. 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	22.800	22.800		
2	32 % EFFLUENT	27.000	27.000	-0.946	
3	42 % EFFLUENT	30.200	30.200	-1.667	
4	56 % EFFLUENT	25.900	25.900	-0.698	
5	75 % EFFLUENT	29.800	29.800	-1.577	
6	100 % EFFLUENT	28.800	28.800	-1.352	

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

AA # K1603001, CERIODAPHNIA DUBIA REPRODUCTION, 3-4-16
File: C:\TOXSTAT\DEQUEENC. 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	10.253	45.0	-4.200
3	42 % EFFLUENT	10	10.253	45.0	-7.400
4	56 % EFFLUENT	10	10.253	45.0	-3.100
5	75 % EFFLUENT	10	10.253	45.0	-7.000
6	100 % EFFLUENT	10	10.253	45.0	-6.000

AA # K1603001, CERIODAPHNIA DUBIA REPRODUCTION, 3-4-16
File: C:\TOXSTAT\DEQUEENC. Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	22.800				
2	32 % EFFLUENT	27.000	120.00	75.00	10.00	
3	42 % EFFLUENT	30.200	124.00	75.00	10.00	
4	56 % EFFLUENT	25.900	112.50	75.00	10.00	
5	75 % EFFLUENT	29.800	124.50	75.00	10.00	
6	100 % EFFLUENT	28.800	126.50	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 3/3/16 **CLIENT** ARK ANALYTICAL

Purchase Order #: _____

SPECIES: Pimephales promelas

Quantity Shipped: 300+ 15-1600 CST

Age: HATCHED 3/2/16

Brood Stock Source: Anderson Farms, AR

Culture Water: Groundwater

Hardness (Mg/l CaCO3): =160

Dissolved Oxygen (Mg/1): 8.5

Temperature (°C): 25.1

Feeding: ARTEMIA

Comments: _____

Shipped Via: Federal Express UPS Overnight (Shuttle)

Packaged By: _____

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

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

ORGANISM HISTORY

DATE: 11/25/2013

SPECIES: *Ceriodaphnia dubia*

AGE: > 3 day

LIFE STAGE: Adult

HATCH DATE: Variable

BEGAN FEEDING: Immediately

FOOD: YTC, *Selenastrum* sp.

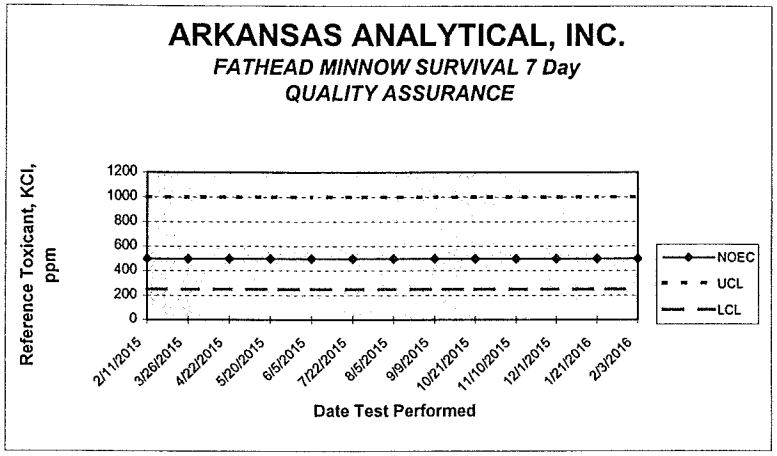
Water Chemistry Record:	Current	Range
TEMPERATURE:	22°C	22-26°C
SALINITY/CONDUCTIVITY:	--	--
TOTAL HARDNESS (as $CaCO_3$):	94 mg/l	76-130 mg/l
TOTAL ALKALINITY (as $CaCO_3$):	65 mg/l	65-100 mg/l
pH:	7.98	7.50-8.20

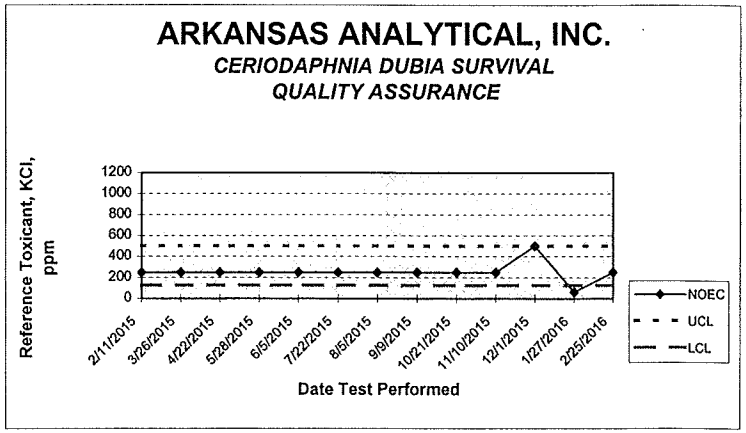
Comments:

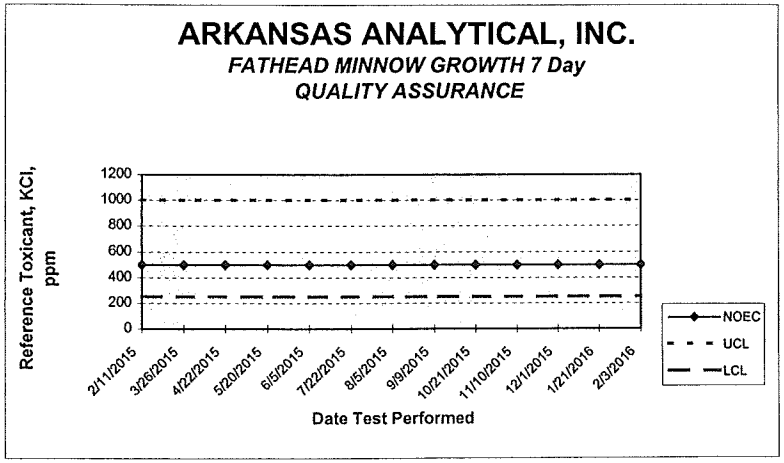
Facility Supervisor

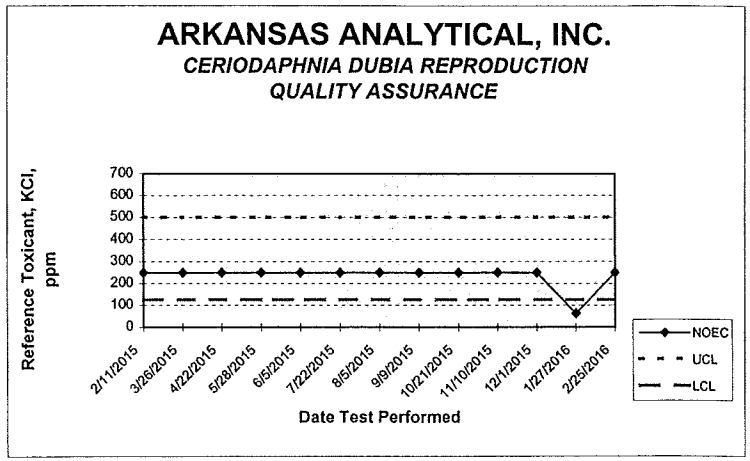
APPENDIX F

Quality Assurance Charts


ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW SURVIVAL 7 Day
QUALITY ASSURANCE
Reference Toxicant, KCl, ppm
1200
1000
800
600
400
200
0
2/11/2015
3/26/2015
4/22/2015
5/20/2015
6/5/2015
7/22/2015
8/5/2015
9/9/2015
10/21/2015
11/10/2015
12/1/2015
1/21/2016
2/3/2016
Date Test Performed
NOEC
UCL
LCL


ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE
Reference Toxicant, KCl, ppm
1200
1000
800
600
400
200
0
2/11/2015
3/26/2015
4/22/2015
5/28/2015
6/5/2015
7/22/2015
8/5/2015
9/9/2015
10/21/2015
11/10/2015
12/1/2015
1/27/2016
2/25/2016
Date Test Performed
NOEC
UCL
LCL


ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW GROWTH 7 Day
QUALITY ASSURANCE
Reference Toxicant, KCl, ppm
1200
1000
800
600
400
200
0
2/11/2015
3/26/2015
4/22/2015
5/20/2015
6/5/2015
7/22/2015
8/5/2015
9/9/2015
10/21/2015
11/10/2015
12/1/2015
1/21/2016
2/3/2016
Date Test Performed
NOEC
UCL
LCL


ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE
Reference Toxicant, KCl, ppm
700
600
500
400
300
200
100
0
2/11/2015
3/26/2015
4/22/2015
5/28/2015
6/5/2015
7/22/2015
8/5/2015
9/9/2015
10/21/2015
11/10/2015
12/1/2015
1/27/2016
2/25/2016
Date Test Performed
NOEC
UCL
LCL