Arkansas Analytical, Inc.

Toxicity Test Results
City of De Queen
NPDES PERMIT NUMBER: AR0021733
Second 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 K1606005

Thursday, June 30, 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 second 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:	6-14-16, 1000	6-15-16, 0800
Sample #2:	6-15-16, 1000	6-16-16, 0800
Sample #3:	6-19-16, 0800	6-20-16, 0600

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

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

Sample Receiving Information:	Date, Time Sample(s) Received	Temperature Upon Receipt (°C)
Sample #1:	6-16-16, 0850	2
Sample #2:	6-17-16, 0905	1
Sample #3:	6-21-16, 0900	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. There were no deviations from the reference method. The test chambers were 500 ml plastic cups, and each chamber contained ten organisms in a test solution volume of 250 mls. The test temperature was 25 degrees Centigrade. Raw data and statistics are provided in Appendix C.

EPA Method 1002.0, Cladoceran, *Ceriodaphnia dubia,* Survival and Reproduction Test, was 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	30.5	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	8.78%	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	0.521	X	
The percent coefficient of variation between replicates must be 40% or less for growth	10.8%	X	

Reference Toxicant

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

REFERENCE TOXICANT

Ceriodaphnia dubia 5/10/16-5/17/16		*Pimephales promelas* 5/10/16-5/17/16	
NOEC Survival:	500 ppm KCl	NOEC Survival:	500 ppm KCl
LOEC Survival:	1000 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)	32.7	%CV survival (critical dilution)	0%
%CV Reproduction (critical dilution)	15.8%	Mean dry weight (critical dilution) in milligrams	0.486
		%CV growth (critical dilution)	7.15%
PMSD Reproduction	21.4%	PMSD Growth	12.9%

Conclusion

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

The permit issued to the City of 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 Pigue, Shelby Chappell, Melissa Bird

Reviewed by:

Tracy Bounds

Tracy Bounds, lab manager

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

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

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	6-14-16, 1000	6-15-16, 0800
Sample #2:	6-15-16, 1000	6-16-16, 0800
Sample #3:	6-19-16, 0800	6-20-16, 0600

Test initiated (date, time): 6-16-16, 1330 Test terminated (date, time): 6-23-16, 1400

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	90		98	98	98	
75%	100	100	80	100	100		98	96	96	
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%	0.540	0.525	0.600	0.450	0.488		0.521	10.8
32%	0.560	0.490	0.439	0.529	0.568		0.517	
42%	0.487	0.530	0.483	0.555	0.488		0.509	
56%	0.456	0.489	0.472	0.446	0.563		0.485	
75%	0.512	0.540	0.459	0.557	0.478		0.509	
100%	0.500	0.482	0.438	0.476	0.533		0.486	7.15

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 dfferent (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)= _____**10.8**_____%

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING
Ceriodaphnia dubia SURVIVAL AND REPRODUCTION

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

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	6-14-16, 1000	6-15-16, 0800
Sample #2:	6-15-16, 1000	6-16-16, 0800
Sample #3:	6-19-16, 0800	6-20-16, 0600

Test initiated (date, time): 6-16-16, 1305 Test terminated (date, time): 6-22-16, 1235

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	31	33	27	27	29	31
B	29	34	22	27	29	34
C	26	38	X23	29	37	35
D	33	36	34	31	36	33
E	29	24	31	32	38	36
F	36	39	X19	39	36	37
G	31	32	34	35	33	X0
H	29	32	32	34	29	36
I	31	35	16	32	40	32
J	30	36	23	26	34	20
Mean	30.5	33.9	26.1	31.2	34.1	29.4
Mean/surviving female	30.5	33.9	27.4	31.2	34.1	32.7
CV%*	8.78					15.8

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

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING
Ceriodaphnia dubia SURVIVAL AND REPRODUCTION

PERMITTEE: City of 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	80	100	100	90

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)= _____**15.8**_____%

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	Chronic Biomonitoring	Bottle Type Code (G = Glass; P = Plastic; V = Septum; A = Amber)	Arkansas Analytical Work Order Number:
	6-14-15-16	10:15 am - 8:00 am		X	~~3~~ 48	Water	Final Discharge Outfall	X	A	K1606005

1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)
[signature]	6-15-16 11:45am	FedEx
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)
FedEx	6-16-16 850	Jeremy 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# 72750

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

Sampler(s) Printed

Field Number	SAMPLE COLLECTION Date/s	Time/s	Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION	Chronic Biomonitoring	TEST PARAMETERS	Bottle Type Code (G = Glass; P = Plastic; V = Septum; A = Amber)	Arkansas Analytical Work Order Number:
	6-15-16	10:00am-8:00am		X	~~3~~ 4-(8)	Water	Final Discharge Outfall	X		B	K1606005

1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)
	6-16-16 11:45 am	FedEx
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)
FedEx	6-17-16 905	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: ✓Yes ___No
5. TEMPERATURE ON RECEIPT: 1 °C
6. TEMPERATURE GUN ID: HHT# 2

FOR COMPLETION BY LAB ONLY

REMARKS / SAMPLE COMMENTS

~~PO#~~ PO# 72750

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

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
	6-19-20-16	8:00am-6:00am		X	~~8~~ 4-8	Water	Final Discharge Outfall	X	C

TEST PARAMETERS

G = Glass; P = Plastic
V = Septum; A = Amber

Arkansas Analytical Work Order Number: K1606005

1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)
	6-20-16 11:45am	FedEX
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)
FedEX	6-21-16 0900	Amanda Jobush

SAMPLE CONDITION UPON RECEIPT IN LAB

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

FOR COMPLETION BY LAB ONLY

REMARKS / SAMPLE COMMENTS

Po # 72150

APPENDIX B

Effluent and Dilution Water Data

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING — Fathead Minnow

Lab # / Sample ID: K16D16005 — Test Start (Date/Time): 6-16-16 / 1330

Client: DeQueen — Test End (Date/Time): 6-23-16 / 1400

		Day of Test							
		1	2	3	4	5**	6*	7	notes
Control	MHS823	6/16	6/17	6/18*	6-19	6/20	6/21	6/22	*MHS 824
D.O. (mg/L)	INITIAL	8.1	8.2	8.3	8.4	8.4	8.1	8.4	**MHS 825
	FINAL	7.5	7.7	8.0	7.4	7.2	6.6	7.6	
pH (s.u.)	INITIAL	7.8	7.7	7.9	7.9	7.8	7.8	7.7	
	FINAL	7.5	7.6	7.7	7.3	7.4	7.2	7.5	
temp (C)	INITIAL	25	24.5	24	24.2	24	24.5	24	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		52	→	46	→	40	→	→	
HARDNESS (mg/L)		68	→	66	→	86	→	→	
CONDUCTIVITY (umho		273	→	227	→	305	→	→	
CHLORINE (mg/L)		<0.05	→	<0.05	→	<0.05	→	→	
CONC: 32%									
D.O. (mg/L)	INITIAL	8.5	8.5	8.5	8.7	8.6	8.5	8.6	
	FINAL	7.5	7.7	8.2	7.6	7.2	6.9	7.5	
pH (s.u)	INITIAL	7.5	7.4	7.7	7.7	7.6	7.7	7.6	
	FINAL	7.5	7.6	7.7	7.5	7.4	7.4	7.7	
temp (C)	INITIAL	25.3	24.5	24	24.0	25.5	24.5	24.5	
	FINAL	25	25	25	25	25	25	25	
CONC: 42%									
D.O. (mg/L)	INITIAL	8.6	8.7	8.6	8.4	8.7	8.8	8.8	
	FINAL	7.3	7.8	8.2	7.6	7.3	7.0	7.5	
pH (mg/L)	INITIAL	7.5	7.4	7.6	7.7	7.5	7.6	7.6	
	FINAL	7.5	7.6	7.9	7.5	7.5	7.4	7.7	
temp (C)	INITIAL	25.5	25	24	24.3	25.5	24	25	
	FINAL	25	25	25	25	25	25	25	
CONC: 56%									
D.O. (mg/L)	INITIAL	8.8	8.9	8.7	8.5	8.8	9.0	8.9	
	FINAL	7.1	7.8	8.2	7.5	7.0	6.0	7.6	
pH (s.u.)	INITIAL	7.4	7.3	7.6	7.6	7.5	7.6	7.6	
	FINAL	7.4	7.6	7.9	7.5	7.5	7.3	7.7	
temp (C)	INITIAL	26	24.5	24	24.7	26	24	25	
	FINAL	25	25	25	25	25	25	25	
CONC: 75%									
D.O. (mg/L)	INITIAL	8.9	9.1	8.9	8.6	9.0	9.3	9.2	
	FINAL	7.1	7.7	8.1	7.1	7.2	6.3	7.8	
pH (s.u.)	INITIAL	7.3	7.3	7.5	7.6	7.4	7.6	7.5	
	FINAL	7.5	7.6	7.6	7.5	7.6	7.4	7.8	
temp (C)	INITIAL	26	25	24	24.9	26	23	25.5	
	FINAL	25	25	25	25	25	25	25	
CONC: 100%									
D.O. (mg/L)	INITIAL	9.6	9.9	9.4	8.9	9.7	9.7	9.6	
	FINAL	7.0	7.8	7.9	7.1	7.3	6.4	7.8	
pH (s.u.)	INITIAL	7.2	7.2	7.4	7.5	7.3	7.5	7.4	
	FINAL	7.6	7.6	8.1	7.6	7.6	7.4	7.8	
temp (C)	INITIAL	26.5	25	24	24.9	27	23	26	
	FINAL	25	25	25	25	25	25	25	
CONC: 100 %		A	A	B	B	B	C	C	
ALKALINITY (mg/L)		48	→	72	→	→	68	→	
HARDNESS (mg/L)		56	→	50	→	→	38	→	
CONDUCTIVITY (umho		801	→	907	→	→	861	→	
CHLORINE (mg/L)		0.05	→	<0.05	→	→	<0.05	→	

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING — Ceriodaphnia Dubia

Lab # / Sample ID: K16060105 — Test Start (Date/Time): 6-16-16 / 1330

Client: DeQueen — Test End (Date/Time): 6-22-16 / 1235

		Day of Test							
		1	2	3	4	5 **	6	7	notes
Control	MHS 823	6/16	6/17	6/18*	6-19-16	6/20	6/21	6/22	*MHS 824
D.O. (mg/L)	INITIAL	8.1	8.2	8.3	8.4	8.4	8.1	8.4	**MHS 825
	FINAL	8.6	8.8	8.7	8.5	8.3	8.0	na	
pH (s.u.)	INITIAL	7.8	7.7	7.9	7.9	7.8	7.8	7.7	
	FINAL	7.9	7.9	7.9	7.8	7.7	8.0	na	
temp (C)	INITIAL	25	24.5	24	24.2	24	24	24	
	FINAL	25	25	25	25	25	25	na	
ALKALINITY (mg/L)		52	→	46	→	40	→	→	
HARDNESS (mg/L)		68	→	66	→	86	→	→	
CONDUCTIVITY (umhc		273	→	227	→	305	→	→	
CHLORINE (mg/L)		<0.05	→	<0.05	→	<0.05	→	→	
CONC: 32%									
D.O. (mg/L)	INITIAL	8.5	8.5	8.5	8.3	8.6	8.5	8.6	
	FINAL	8.6	8.6	8.7	8.6	8.4	7.7	na	
pH (s.u)	INITIAL	7.5	7.4	7.7	7.7	7.6	7.7	7.6	
	FINAL	7.8	8.0	7.9	7.9	7.8	7.9	na	
temp (C)	INITIAL	25.3	24.5	24	24.0	25.5	24	24.5	
	FINAL	25	25	25	25	25	25	na	
CONC: 42%									
D.O. (mg/L)	INITIAL	8.6	8.7	8.6	8.4	8.7	8.8	8.8	
	FINAL	8.7	8.6	8.8	8.6	8.3	7.8	na	
pH (mg/L)	INITIAL	7.5	7.4	7.6	7.7	7.5	7.6	7.6	
	FINAL	7.9	8.0	7.9	7.9	7.8	8.0	na	
temp (C)	INITIAL	25.5	25	24	24.3	25.5	24	25	
	FINAL	25	25	25	25	25	25	na	
CONC: 56%									
D.O. (mg/L)	INITIAL	8.8	8.9	8.7	8.5	8.8	9.0	8.9	
	FINAL	8.7	8.7	8.7	8.6	8.3	7.8	na	
pH (s.u.)	INITIAL	7.4	7.3	7.6	7.6	7.5	7.6	7.6	
	FINAL	7.9	8.0	8.0	8.0	7.9	8.0	na	
temp (C)	INITIAL	26	24.5	24	24.7	26	24	25	
	FINAL	25	25	25	25	25	25	na	
CONC: 75%									
D.O. (mg/L)	INITIAL	8.9	9.1	8.9	8.6	9.0	9.3	9.2	
	FINAL	8.7	8.8	8.7	8.6	8.3	7.8	na	
pH (s.u.)	INITIAL	7.3	7.3	7.5	7.6	7.4	7.6	7.5	
	FINAL	8.0	8.0	8.0	8.0	7.9	8.0	na	
temp (C)	INITIAL	26	25	24	24.9	26	23	25.5	
	FINAL	25	25	25	25	25	25	na	
CONC: 100%									
D.O. (mg/L)	INITIAL	9.6	9.9	9.4	8.9	9.7	9.7	9.6	
	FINAL	8.7	8.8	8.7	8.6	8.4	7.8	na	
pH (s.u.)	INITIAL	7.2	7.2	7.4	7.5	7.3	7.5	7.4	
	FINAL	8.0	8.0	8.0	8.0	7.9	8.0	na	
temp (C)	INITIAL	26.5	25	24	24.9	27	23	26	
	FINAL	25	25	25	25	25	25	na	
CONC: 100 %		A	A	B	B	B	C	C	
ALKALINITY (mg/L)		48	→	72	→	→	68	→	
HARDNESS (mg/L)		56	→	50	→	→	38	→	
CONDUCTIVITY (umhc		801	→	907	→	→	861	→	
CHLORINE (mg/L)		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 K16D0005 TEST START DATE 6-16-16 TIME 1330

CLIENT DeQueen TEST END DATE 6-23-16 TIME 1400

AGE AND SOURCE OF MINNOWS <24 HR, Aquatox

		D A Y (NUMBER SURVIVING)								SURVIVAL		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: MHS	A	10	10	10	10	10	10	10	10	100	100%	0
	B	|	10	10	10	10	10	10	10	100		
	C	|	10	10	10	10	10	10	10	100		
	D	|	10	10	10	10	10	10	10	100		
	E	⊥	10	10	10	10	10	10	10	100		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 32	A	10	10	10	10	10	10	10	10	100	100%	0
	B	|	10	10	10	10	10	10	10	100		
	C	|	10	10	10	10	10	10	10	100		
	D	|	10	10	10	10	10	10	10	100		
	E	⊥	10	10	10	10	10	10	10	100		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 42	A	10	10	10	10	10	10	10	10	100	100%	0
	B	|	10	10	10	10	10	10	10	100		
	C	|	10	10	10	10	10	10	10	100		
	D	|	10	10	10	10	10	10	10	100		
	E	⊥	10	10	10	10	10	10	10	100		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 56	A	10	10	10	10	10	10	10	10	100	98%	4.56
	B	|	10	10	10	10	10	10	10	100		
	C	|	10	8 10	10	10	10	10	10	100		
	D	|	10	10	10	10	10	10	10	100		
	E	⊥	9	9	9	9	9	9	9	90		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 75	A	10	10	10	10	10	10	10	10	100	96%	9.32
	B	|	10	10	10	10	10	10	10	100		
	C	|	9	8	8	8	8	8	8	80		
	D	|	10	10	10	10	10	10	10	100		
	E	⊥	10	10	10	10	10	10	10	100		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 100	A	10	10	10	10	10	10	10	10	100	100%	0
	B	|	10	10	10	10	10	10	10	100		
	C	|	10	10	10	10	10	10	10	100		
	D	|	10	10	10	10	10	10	10	100		
	E	⊥	10	10	10	10	10	10	10	100		
ANALYST		SC	TB	KP	KP	SC	SC	SC	SC			
DATE:		6-16-16	6-17-16	6/18/16	6/19/16	6-20-16	6-21-16	6-22-16	6-23-16			
TIME:		1330	1310	0920		1515	1135	1240	1400			

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

AA # K1606005, P. PROMELAS 7 DAY CHRONIC, 6-16-16
File: C:\TOXSTAT\DEQUEENS. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.096

W = 0.574

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 # K1606005, P. PROMELAS 7 DAY CHRONIC, 6-16-16
File: C:\TOXSTAT\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 # K1606005, P. PROMELAS 7 DAY CHRONIC, 6-16-16
FILE: C:\TOXSTAT\DEQUEENS.
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	1.0000	1.4120
1	CONTROL	2	1.0000	1.4120
1	CONTROL	3	1.0000	1.4120
1	CONTROL	4	1.0000	1.4120
1	CONTROL	5	1.0000	1.4120
2	32 % EFFLUENT	1	1.0000	1.4120
2	32 % EFFLUENT	2	1.0000	1.4120
2	32 % EFFLUENT	3	1.0000	1.4120
2	32 % EFFLUENT	4	1.0000	1.4120
2	32 % EFFLUENT	5	1.0000	1.4120
3	42 % EFFLUENT	1	1.0000	1.4120
3	42 % EFFLUENT	2	1.0000	1.4120
3	42 % EFFLUENT	3	1.0000	1.4120
3	42 % EFFLUENT	4	1.0000	1.4120
3	42 % EFFLUENT	5	1.0000	1.4120
4	56 % EFFLUENT	1	1.0000	1.4120
4	56 % EFFLUENT	2	1.0000	1.4120
4	56 % EFFLUENT	3	1.0000	1.4120
4	56 % EFFLUENT	4	1.0000	1.4120
4	56 % EFFLUENT	5	0.9000	1.2490
5	75 % EFFLUENT	1	1.0000	1.4120
5	75 % EFFLUENT	2	1.0000	1.4120
5	75 % EFFLUENT	3	0.8000	1.1071
5	75 % EFFLUENT	4	1.0000	1.4120
5	75 % EFFLUENT	5	1.0000	1.4120
6	100 % EFFLUENT	1	1.0000	1.4120
6	100 % EFFLUENT	2	1.0000	1.4120
6	100 % EFFLUENT	3	1.0000	1.4120
6	100 % EFFLUENT	4	1.0000	1.4120
6	100 % EFFLUENT	5	1.0000	1.4120

AA # K1606005, P. PROMELAS 7 DAY CHRONIC, 6-16-16
File: C:\TOXSTAT\DEQUEENS. Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.017	0.003	0.834
Within (Error)	24	0.096	0.004	
Total	29	0.112		

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

AA # K1606005, P. PROMELAS 7 DAY CHRONIC, 6-16-16
File: C:\TOXSTAT\DEQUEENS. 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	1.412	1.000		
2	32 % EFFLUENT	1.412	1.000	0.000	
3	42 % EFFLUENT	1.412	1.000	0.000	
4	56 % EFFLUENT	1.379	0.980	0.817	
5	75 % EFFLUENT	1.351	0.960	1.527	
6	100 % EFFLUENT	1.412	1.000	0.000	

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

AA # K1606005, P. PROMELAS 7 DAY CHRONIC, 6-16-16
File: C:\TOXSTAT\DEQUEENS. 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	0.038	3.8	0.000
3	42 % EFFLUENT	5	0.038	3.8	0.000
4	56 % EFFLUENT	5	0.038	3.8	0.020
5	75 % EFFLUENT	5	0.038	3.8	0.040
6	100 % EFFLUENT	5	0.038	3.8	0.000

AA # K1606005, P. PROMELAS 7 DAY CHRONIC, 6-16-16
File: C:\TOXSTAT\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	1.412				
2	32 % EFFLUENT	1.412	27.50	16.00	5.00	
3	42 % EFFLUENT	1.412	27.50	16.00	5.00	
4	56 % EFFLUENT	1.379	25.00	16.00	5.00	
5	75 % EFFLUENT	1.351	25.00	16.00	5.00	
6	100 % EFFLUENT	1.412	27.50	16.00	5.00	

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

Pimephales promelas **FATHEAD MINNOW** TEST 1000.0

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s: K16062005	TEST DATES (BEGIN / END): 6-16-16/1330/6-23-16
CLIENT: DeQueen	WEIGHING DATE / TIME: 6-24-2016/1610
ANALYSTS: tb, sc	DRYING TEMP (DEGREES C): 60
SAMPLE ID: Outfall	DRYING TIME (HOURS): 24

	REP#	FINAL DRY WEIGHT TIN+LARVAE (g)	INTIAL WEIGHT TIN (g)	TOTAL DRY WEIGHT OF LARVAE (g)	NUMBER OF LARVAE	DRY WEIGHT OF LARVAE (mg)	
CONTROL	A DQ1	1.02831	1.02291	0.00540	10	0.540	AVG DRY WEIGHT (mg) 0.5206
	B DQ2	0.99856	0.99331	0.00525	10	0.525	
MHS	C DQ3	1.04747	1.04147	0.00600	10	0.600	
	D DQ4	1.040616	1.036616	0.00450	10	0.450	CV 10.8 %
	E DQ5	1.03138	1.026050	0.00488	10	0.488	
CONC:	A DQ6	0.976663	0.971103	0.00560	10	0.560	AVG DRY WEIGHT (mg) 0.5172
	B DQ7	1.03895	1.03405	0.00490	10	0.490	
32%	C DQ8	0.99487	0.99048	0.00439	10	0.439	
	D DQ9	1.00555	1.000026	0.00529	10	0.529	CV 10.33%
	E DQ10	0.99033	0.984665	0.00568	10	0.568	
CONC:	A DQ11	1.00411	0.99924	0.00487	10	0.487	AVG DRY WEIGHT (mg) 0.5076
	B DQ12	1.001606	0.991636	0.00530	10	0.530	
42%	C DQ13	1.05097	1.046614	0.00483	10	0.483	
	D DQ14	1.00314	0.99759	0.00555	10	0.555	CV 6.0%
	E DQ15	1.00078	0.99590	0.00488	10	0.488	
CONC:	A DQ16	1.04345	1.03889	0.00456	10	0.456	AVG DRY WEIGHT (mg) 0.4852
	B DQ17	1.04624	1.04135	0.00489	10	0.489	
56%	C DQ18	1.02228	1.01756	0.00472	10	0.472	
	D DQ19	1.04912	1.044616	0.00446	10	0.446	CV 9.57%
	E DQ20	0.99743	0.99180	0.00563	10	0.563	
CONC:	A DQ21	1.05497	1.04985	0.00512	10	0.512	AVG DRY WEIGHT (mg) 0.5092
	B DQ22	1.02083	1.01543	0.00540	10	0.540	
75%	C DQ23	1.01193	1.00734	0.00459	10	0.459	
	D DQ24	1.03042	1.02485	0.00557	10	0.557	CV 8.1%
	E DQ25	1.03204	1.02726	0.00478	10	0.478	
CONC:	A DQ26	1.04314	1.03814	0.00500	10	0.500	AVG DRY WEIGHT (mg) 0.4858
	B DQ27	1.03849	1.03367	0.00482	10	0.482	
100%	C DQ28	1.04151	1.03713	0.00438	10	0.438	
	D DQ29	1.046660	1.04184	0.00476	10	0.476	CV 7.15%
	E DQ30	1.02349	1.01866	0.00533	10	0.533	

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA # K1606005, P. PROMELAS 7 DAY GROWTH, 6-16-16
File: C:\TOXSTAT\DEQUEENG. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.049

W = 0.975

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 # K1606005, P. PROMELAS 7 DAY GROWTH, 6-16-16
File: C:\TOXSTAT\DEQUEENG. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 1.83

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 # K1606005, P. PROMELAS 7 DAY GROWTH, 6-16-16
FILE: C:\TOXSTAT\DEQUEENG.
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.5400	0.8254
1	CONTROL	2	0.5250	0.8104
1	CONTROL	3	0.6000	0.8861
1	CONTROL	4	0.4500	0.7353
1	CONTROL	5	0.4880	0.7734
2	32 % EFFLUENT	1	0.5600	0.8455
2	32 % EFFLUENT	2	0.4900	0.7754
2	32 % EFFLUENT	3	0.4390	0.7242
2	32 % EFFLUENT	4	0.5290	0.8144
2	32 % EFFLUENT	5	0.5680	0.8536
3	42 % EFFLUENT	1	0.4870	0.7724
3	42 % EFFLUENT	2	0.5300	0.8154
3	42 % EFFLUENT	3	0.4830	0.7684
3	42 % EFFLUENT	4	0.5550	0.8405
3	42 % EFFLUENT	5	0.4880	0.7734
4	56 % EFFLUENT	1	0.4560	0.7413
4	56 % EFFLUENT	2	0.4890	0.7744
4	56 % EFFLUENT	3	0.4720	0.7574
4	56 % EFFLUENT	4	0.4460	0.7313
4	56 % EFFLUENT	5	0.5630	0.8486
5	75 % EFFLUENT	1	0.5120	0.7974
5	75 % EFFLUENT	2	0.5400	0.8254
5	75 % EFFLUENT	3	0.4590	0.7444
5	75 % EFFLUENT	4	0.5570	0.8425
5	75 % EFFLUENT	5	0.4780	0.7634
6	100 % EFFLUENT	1	0.5000	0.7854
6	100 % EFFLUENT	2	0.4820	0.7674
6	100 % EFFLUENT	3	0.4380	0.7232
6	100 % EFFLUENT	4	0.4760	0.7614
6	100 % EFFLUENT	5	0.5330	0.8184

AA # K1606005, P. PROMELAS 7 DAY GROWTH, 6-16-16
File: C:\TOXSTAT\DEQUEENG. Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.006	0.001	0.585
Within (Error)	24	0.049	0.002	
Total	29	0.055		

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

AA # K1606005, P. PROMELAS 7 DAY GROWTH, 6-16-16
File: C:\TOXSTAT\DEQUEENG. 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	0.806	0.521		
2	32 % EFFLUENT	0.803	0.517	0.122	
3	42 % EFFLUENT	0.794	0.509	0.425	
4	56 % EFFLUENT	0.771	0.485	1.246	
5	75 % EFFLUENT	0.795	0.509	0.404	
6	100 % EFFLUENT	0.771	0.486	1.226	

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

AA # K1606005, P. PROMELAS 7 DAY GROWTH, 6-16-16
File: C:\TOXSTAT\DEQUEENG. 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	0.067	12.9	0.003
3	42 % EFFLUENT	5	0.067	12.9	0.012
4	56 % EFFLUENT	5	0.067	12.9	0.035
5	75 % EFFLUENT	5	0.067	12.9	0.011
6	100 % EFFLUENT	5	0.067	12.9	0.035

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

SURVIVAL AND REPRODUCTION TEST

Ceriodaphnia dubia

Discharger: DeSoto
Location: Outfall
Date Sample Collected: See COC

Lab Number/s: K1606005

Analyst: tb, SC
Test Start - Date/Time: 6-16-16 / 1305
Test Stop - Date/Time: 6-22-16 / 1235

Conc 1 — MHS

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	6	0	0	8	0	10	0	tb
3	3	0	0	0	0	0	0	0	0	0	3	10	0.3	tb
4	2	6	4	6	6	5	6	5	6	5	51	10	5.1	tb
5	11	9	8	11	10	14	9	10	9	10	101	10	10.1	tb
6	15	14	14	16	13	17	16	14	16	15	150	10	15.0	tb
7														
8														
Total	31	29	26	33	29	36	31	29	31	30	305	CV = 8.8%		
												$\bar{X}$ = 30.5		

Conc 2 — 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	6	0	0	0	0	0	0	0	0	0	6	10	0.6	tb
4	12	6	6	6	10	6	4	6	6	6	68	10	6.8	tb
5	0	12	12	12	0	12	12	11	13	13	97	10	9.7	tb
6	15	16	20	18	14	21	16	15	16	17	168	10	16.8	tb
7														
8														
Total	33	34	38	36	24	39	32	32	35	36	339	CV = 12.4%		
												$\bar{X}$ = 33.9		

Conc 3 — 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	4	0	0	0	0	0	0	0	0	0	4	10	0.4	tb
4	13	6	6	5	6	7	6	4	6	4	53	10	5.3	tb
5	0	10	11	11	10	X12	10	12	10	9	95	9	9.5	tb
6	10	16	X6	18	15	–	18	16	0	10	109	8	10.9	tb
7														
8														
Total	27	22	X23	34	31	X19	34	32	16	23	261	CV = 24.7%		
												$\bar{X}$ = 26.1		

X = Dead

Conc 4 — 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	5	0	0	0	5	0	0	0	0	0	10	10	1.0	tb
4	0	5	4	6	1	5	6	6	6	6	45	10	4.5	tb
5	12	10	10	11	9	15	14	11	13	9	114	10	11.4	tb
6	10	12	15	14	17	19	15	17	13	11	143	10	14.3	tb
7														
8														
Total	27	27	29	31	32	39	35	34	32	26	312	CV = 13.2%		
												$\bar{X}$ = 31.2		

Conc 5 — 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	5	0	0	0	6	0	0	0	0	3	14	10	1.4	tb
4	10	5	5	5	10	7	6	6	6	6	66	10	6.6	tb
5	0	10	12	14	0	12	12	11	15	12	98	10	9.8	tb
6	14	14	20	17	22	17	15	12	19	13	163	10	16.3	tb
7														
8														
Total	29	29	37	36	38	36	33	29	40	34	341	CV = 11.8%		
												$\bar{X}$ = 34.1		

Conc 6 — 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	7	0	0	2	4	0	0	0	0	0	13	10	1.3	tb
4	0	5	5	4	2	5	X	5	5	8	39	9	3.9	tb
5	10	12	13	9	12	11	–	10	10	7	94	9	9.4	tb
6	14	17	17	18	18	21	–	21	17	5	148	9	14.8	tb
7														
8														
Total	31	34	35	33	36	37	X0	36	32	20	294	CV = 13.8%		
												$\bar{X}$ = 32.67		

$\bar{X}$/SA = 27.4

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
32% Effluent	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% Effluent	8	2	10
TOTAL	18	2	20

CRITICAL FISHER'S VALUE (10,10,10) (p=0.05) IS 6. b VALUE IS 8.
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% Effluent	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	NUMBER OF DEAD	TOTAL ANIMALS
CONTROL	10	0	10
75% Effluent	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	NUMBER OF DEAD	TOTAL ANIMALS
CONTROL	10	0	10
100% Effluent	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.

SUMMARY OF FISHER'S EXACT TESTS

	NUMBER	NUMBER	SIG

GROUP	IDENTIFICATION	EXPOSED	DEAD	(P=.05)
	CONTROL	10	0	
1	32% Effluent	10	0	
2	42% Effluent	10	2	
3	56% Effluent	10	0	
4	75% Effluent	10	0	
5	100% Effluent	10	1	

AA # K1606005, CERIODAPHNIA DUBIA REPRODUCTION, 6-16-16
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 # K1606005, CERIODAPHNIA DUBIA REPRODUCTION, 6-16-16
File: C:\toxstat\DEQUEENC. Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 24.39

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

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

TITLE: AA # K1606005, CERIODAPHNIA DUBIA REPRODUCTION, 6-16-16
FILE: C:\toxstat\DEQUEENC.
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	31.0000	31.0000
1	CONTROL	2	29.0000	29.0000
1	CONTROL	3	26.0000	26.0000
1	CONTROL	4	33.0000	33.0000
1	CONTROL	5	29.0000	29.0000
1	CONTROL	6	36.0000	36.0000
1	CONTROL	7	31.0000	31.0000
1	CONTROL	8	29.0000	29.0000
1	CONTROL	9	31.0000	31.0000
1	CONTROL	10	30.0000	30.0000
2	32 % EFFLUENT	1	33.0000	33.0000
2	32 % EFFLUENT	2	34.0000	34.0000
2	32 % EFFLUENT	3	38.0000	38.0000
2	32 % EFFLUENT	4	36.0000	36.0000
2	32 % EFFLUENT	5	24.0000	24.0000
2	32 % EFFLUENT	6	39.0000	39.0000
2	32 % EFFLUENT	7	32.0000	32.0000
2	32 % EFFLUENT	8	32.0000	32.0000
2	32 % EFFLUENT	9	35.0000	35.0000
2	32 % EFFLUENT	10	36.0000	36.0000
3	42 % EFFLUENT	1	27.0000	27.0000
3	42 % EFFLUENT	2	22.0000	22.0000
3	42 % EFFLUENT	3	23.0000	23.0000
3	42 % EFFLUENT	4	34.0000	34.0000
3	42 % EFFLUENT	5	31.0000	31.0000
3	42 % EFFLUENT	6	19.0000	19.0000
3	42 % EFFLUENT	7	34.0000	34.0000
3	42 % EFFLUENT	8	32.0000	32.0000
3	42 % EFFLUENT	9	16.0000	16.0000
3	42 % EFFLUENT	10	23.0000	23.0000
4	56 % EFFLUENT	1	27.0000	27.0000
4	56 % EFFLUENT	2	27.0000	27.0000
4	56 % EFFLUENT	3	29.0000	29.0000
4	56 % EFFLUENT	4	31.0000	31.0000
4	56 % EFFLUENT	5	32.0000	32.0000
4	56 % EFFLUENT	6	39.0000	39.0000
4	56 % EFFLUENT	7	35.0000	35.0000
4	56 % EFFLUENT	8	34.0000	34.0000
4	56 % EFFLUENT	9	32.0000	32.0000
4	56 % EFFLUENT	10	26.0000	26.0000
5	75 % EFFLUENT	1	29.0000	29.0000
5	75 % EFFLUENT	2	29.0000	29.0000
5	75 % EFFLUENT	3	37.0000	37.0000
5	75 % EFFLUENT	4	36.0000	36.0000
5	75 % EFFLUENT	5	38.0000	38.0000
5	75 % EFFLUENT	6	36.0000	36.0000
5	75 % EFFLUENT	7	33.0000	33.0000
5	75 % EFFLUENT	8	29.0000	29.0000
5	75 % EFFLUENT	9	40.0000	40.0000
5	75 % EFFLUENT	10	34.0000	34.0000

6	100 % EFFLUENT	1	31.0000	31.0000
6	100 % EFFLUENT	2	34.0000	34.0000
6	100 % EFFLUENT	3	35.0000	35.0000
6	100 % EFFLUENT	4	33.0000	33.0000
6	100 % EFFLUENT	5	36.0000	36.0000
6	100 % EFFLUENT	6	37.0000	37.0000
6	100 % EFFLUENT	7	0.0000	0.0000
6	100 % EFFLUENT	8	36.0000	36.0000
6	100 % EFFLUENT	9	32.0000	32.0000
6	100 % EFFLUENT	10	20.0000	20.0000

AA # K1606005, CERIODAPHNIA DUBIA REPRODUCTION, 6-16-16
File: C:\toxstat\DEQUEENC. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	447.733	89.547	2.341
Within (Error)	54	2065.200	38.244	
Total	59	2512.933		

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

AA # K1606005, CERIODAPHNIA DUBIA REPRODUCTION, 6-16-16
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	30.500	30.500		
2	32 % EFFLUENT	33.900	33.900	-1.229	
3	42 % EFFLUENT	26.100	26.100	1.591	
4	56 % EFFLUENT	31.200	31.200	-0.253	
5	75 % EFFLUENT	34.100	34.100	-1.302	
6	100 % EFFLUENT	29.400	29.400	0.398	

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

AA # K1606005, CERIODAPHNIA DUBIA REPRODUCTION, 6-16-16
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	6.389	20.9	-3.400
3	42 % EFFLUENT	10	6.389	20.9	4.400
4	56 % EFFLUENT	10	6.389	20.9	-0.700
5	75 % EFFLUENT	10	6.389	20.9	-3.600
6	100 % EFFLUENT	10	6.389	20.9	1.100

APPENDIX E

Organism History

AQUATOX, INC.

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

TEST ORGANISM HISTORY

DATE SHIPPED 6/16/16 **CLIENT** Ar Analytical Tracy

Purchase Order #: _____

SPECIES: Pimephales promelas

Quantity Shipped: 300

Age: Hatched 6/15/16 @ 15-1600 CST

Brood Stock Source: Anderson Farms, AR

Culture Water: Groundwater

Hardness (Mg/l CaCO3): 160

Dissolved Oxygen (Mg/1): 8.2

Temperature (°C): 25.1°C

Feeding: Artm

Comments: _____

Shipped Via: Federal Express UPS Overnight Shuttle

Packaged By: _____

Bill Hall Printers 3171

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

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

ORGANISM HISTORY

DATE: 11/25/2013

SPECIES: *Ceriodaphnia dubia*

AGE: > 3 day

LIFE STAGE: Adult

HATCH DATE: Variable

BEGAN FEEDING: Immediately

FOOD: YTC, *Selenastrum* sp.

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

Comments:

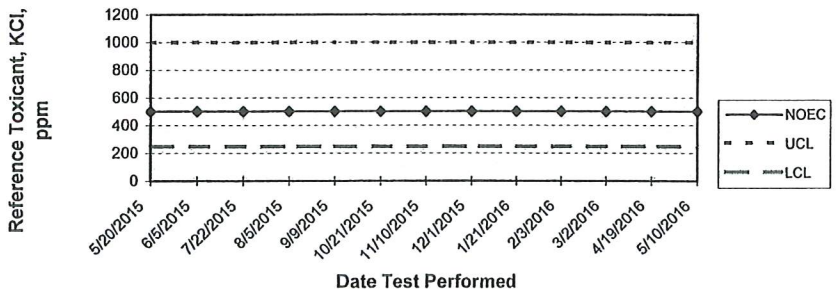
Facility Supervisor

APPENDIX F

Quality Assurance Charts

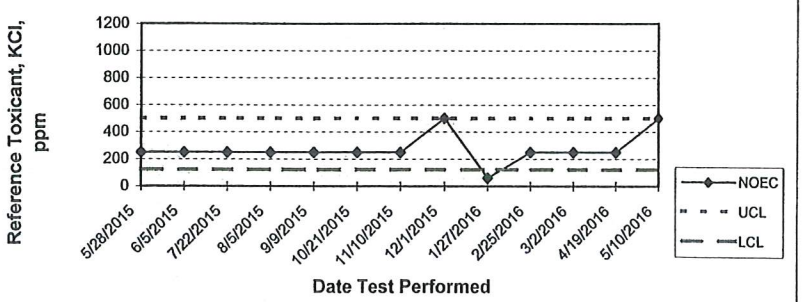
ARKANSAS ANALYTICAL, INC.

FATHEAD MINNOW SURVIVAL 7 Day
QUALITY ASSURANCE



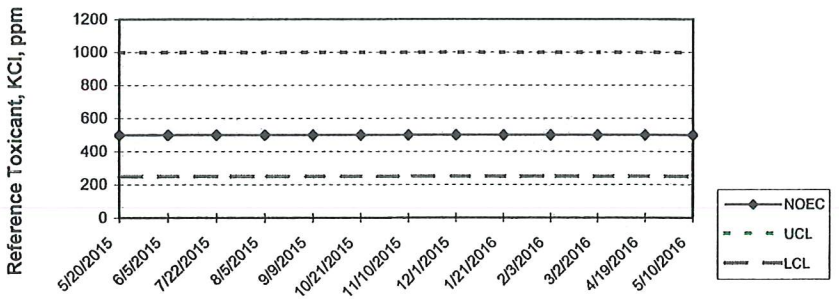
ARKANSAS ANALYTICAL, INC.

CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.

FATHEAD MINNOW GROWTH 7 Day
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.

CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE