Arkansas Analytical, Inc.

Toxicity Test Results

CITY of STUTTGART
NPDES PERMIT NUMBER: AR0034380
First Quarter 2016
AFIN # 01-00041

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

Ceriodaphnia dubia, Survival and Reproduction Test
Test 1002.0

Prepared for: **Tommy Lawson**
Stuttgart Municipal Water Works
516 South Main
Stuttgart, AR 72160

Prepared by: Arkansas Analytical, Inc.
8100 National Drive
Little Rock, Arkansas 72209
Lab Number K1603002

Wednesday, March 16, 2016

Introduction

This report contains test results for the toxicity testing for the City of Stuttgart, NPDES permit number AR0034380, Outfall 001. The plant is located in Stuttgart, Arkansas, on West 10th Street west of the St. Louis Railroad on the west side of town in Section 29, Township 2 South, Range 5 West in Arkansas County, Arkansas. The discharge is to receiving waters named King Bayou, thence to Bayou Meto in Segment 3B of the Arkansas River Basin.

The permit requires chronic biomonitoring testing once per quarter 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 Started	Date, Time Ended
Sample #1:	3-6-16, 0900	3-7-16, 0900
Sample #2:	3-8-16, 0900	3-9-16, 0900
Sample #3:	3-10-16, 0900	3-11-16, 0900

Samples were three composites collected at the final discharge from the City of Stuttgart Wastewater Treatment Plant, Outfall 001

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:	3-7-16, 1120	4
Sample #2:	3-9-16, 1605	4
Sample #3:	3-11-16, 1540	6 (on ice)

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 either zero flow conditions or to its earlier characterization as toxic, synthetic dilution water was substituted.

The dilution water used in the toxicity tests was synthetic moderately hard. 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 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 < 48 hour old Fathead Minnows, *Pimephales promelas*, which were purchased from Aquatox; a copy of the organism history is provided in Appendix E.

The organism 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	24.9	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	13.0%	X	

TEST ACCEPTANCE CRITERIA for *Pimephales promelas*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	96%	X	
The percent coefficient of variation between replicates must be 40% or less for survival	9.32%	X	
Minimum of 0.25 mg average dry weight of surviving controls	0.587	X	
The percent coefficient of variation between replicates must be 40% or less for growth	8.93%	X	

Reference Toxicant

The reference toxicant used was Potassium Chloride prepared in-house. The tests were performed using moderately hard water 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 Reproduction:	500 ppm KCl
LOEC Reproduction:	500 ppm KCl	LOEC Reproduction:	1000 ppm KCl

Quality Assurance charts are provided in Appendix F.

Summary of Results
City of Stuttgart

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)	31.3	%CV survival (critical dilution)	9.52%
%CV Reproduction (critical dilution)	13.5%	Mean dry weight (critical dilution) in milligrams	0.528
		%CV growth (critical dilution)	6.93%
PMSD Reproduction	17.6%	PMSD Growth	19.3%

Conclusion

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

The permit issued to the City of Stuttgart, AR0034380, specifies that the **critical dilution is 100% effluent**. The effluent samples **did not** exhibit either lethal or sublethal effects at the critical dilution, and, as such, the effluent samples **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 Stuttgart, AR0034380, 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, the effluent samples **passed** both portions of the test.

Biomonitoring Analysts:

Tracy Bounds, Hallie Freyaldenhoven, Ken Rood, Chris Turney, Shannon Turney, Zabrina Ruggles

Reviewed by:

Tracy Bounds, lab manager

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

PERMITTEE: City of Stuttgart **NPDES #: AR0034380**

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	3-6-16, 0900	3-7-16, 0900
Sample #2:	3-8-16, 0900	3-9-16, 0900
Sample #3:	3-10-16, 0900	3-11-16, 0900

Test initiated (date, time): 3-8-16, 1550 Test terminated (date, time): 3-15-16, 1000

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	80		100	98	96	9.32
32%	100	90	100	90	90		98	98	94	
42%	100	90	100	100	100		100	100	98	
56%	90	80	90	90	90		94	90	88	
75%	100	100	90	80	90		100	100	92	
100%	80	90	100	100	100		100	100	94	9.52

DATA TABLE FOR GROWTH OF FATHEAD MINNOWS

	Average Dry Weight in milligrams in replicate chambers							
Effluent Conc %	A	B	C	D	E		Mean Dry Weight	CV%
0%	0.539	0.579	0.602	0.669	0.546		0.587	8.93
32%	0.508	0.545	0.478	0.506	0.498		0.507	
42%	0.465	0.491	0.512	0.524	0.478		0.494	
56%	0.757	0.502	0.447	0.447	0.405		0.512	
75%	0.558	0.552	0.509	0.352	0.430		0.480	
100%	0.523	0.555	0.555	0.467	0.540		0.528	6.93

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) = _____**8.93**_____%

6. Enter Whole Effluent Toxicity: _____**100**____%

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING
Ceriodaphnia dubia SURVIVAL AND REPRODUCTION

Permittee: City of Stuttgart **NPDES #: AR0034380**

Sample Collection:	Date Started	Date, Time Ended
Sample #1:	3-6-16, 0900	3-7-16, 0900
Sample #2:	3-8-16, 0900	3-9-16, 0900
Sample #3:	3-10-16, 0900	3-11-16, 0900

Test initiated (date, time): 3-8-16, 1000 Test terminated (date, time): 3-15-16, 0945

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	25	28	32	31	32	30
B	30	27	30	24	39	30
C	21	25	28	28	21	29
D	24	25	32	31	32	38
E	20	19	26	20	25	25
F	26	26	33	28	32	35
G	29	32	23	33	38	26
H	26	26	24	31	28	36
I	22	25	23	32	25	31
J	26	29	31	35	31	33
Mean	24.9	26.2	28.2	29.3	30.3	31.3
Mean/surviving female	24.9	26.2	28.2	29.3	30.3	31.3
CV%*	13.0					13.5

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 Stuttgart **NPDES #: AR0034380**

PERCENT SURVIVAL

PERCENT EFFLUENT	0%	32%	42%	56%	75%	100%
Time of Reading: 24 HOURS	100	100	100	100	100	100
48 HOURS	100	100	100	100	100	100
Test termination	100	100	100	100	100	100

1. Fisher's Exact Test:
   Is the mean survival at test termination significantly different (p=0.05) than the control survival for:

   a) LOW FLOW OR CRITICAL DILUTION, (100%): YES_____ NO____**X**____

2. Dunnett's Procedure or Steel's Many One Rank Test:
   Is the mean number of young produced per female significantly different (p=0.05) than the controls number of young per female for:

   a) LOW FLOW OR CRITICAL DILUTION, (100%): YES_____ NO____**X**_____

3. If NO was answered to 1.a) enter [0] otherwise enter [1] (parameter TLP3B):_____**0**_____

4. If NO was answered to 2.a) enter [0] otherwise enter [1] (parameter TGP3B):_____**0**_____

5. Enter percentage corresponding to each parameter below:

   a) NOEC survival (parameter TOP3B)= _____**100**____ % effluent

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

   c) Coefficient of variation (parameter TQP3B)= _____**13.5**_____%

6. Enter Whole Effluent Toxicity: _____**100**____%

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 INFORMATION	Project Description	Turnaround Time
Stuttgart Municipal Water Works	Stuttgart Municipal Water Works	Chronic Toxicity	1 Day (100%)
516 South Main	P.O. Box 130		2 Day (50%)
Stuttgart, AR 72160	Stuttgart, AR 72160	Reporting Information	3 Day (25%)
		Telephone: 870-673-3246	5 Day (Routine)
Attn: Tommy Lawson		Fax: 870-673-8783	Preservative Code:
		Email: stuttgartarwater@centurytel.net, swsdept@d-c1.com	Bottle Type:

Preservation Codes:

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

Sampler(s) Signature

Sampler(s) Printed MAHDI HADDADI

Field Number	SAMPLE COLLECTION Date/s	Time/s	Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION	TEST PARAMETERS: Chronic Toxicity	Bottle Type Code
	3/6/16-3/7/16	900-900		X		water		X	A

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

Arkansas Analytical Work Order Number: K1603002

1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)
	1120 3/7/16	
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)
		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:	4 °C	
6. TEMPERATURE GUN ID:	HHT# 2	

FOR COMPLETION BY LAB ONLY

REMARKS / SAMPLE COMMENTS

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 INFORMATION	Project Description	Turnaround Time
Stuttgart Municipal Water Works	Stuttgart Municipal Water Works	Chronic Toxicity	1 Day (100%)
516 South Main	P.O. Box 130		2 Day (50%)
Stuttgart, AR 72160	Stuttgart, AR 72160	Reporting Information	3 Day (25%)
		Telephone: 870-673-3246	5 Day (Routine)
Attn: Tommy Lawson		Fax: 870-673-8783	Preservative Code:
		Email: stuttgartarwater@centurytel.net, swsdept@d-c1.com	Bottle Type:

Preservation Codes:

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

Sampler(s) Signature

Sampler(s) Printed Mahbi Hasnain

Arkansas Analytical Work Order Number: K1603002

Field Number	SAMPLE COLLECTION Date/s	Time/s	Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION	TEST PARAMETERS: Chronic Toxicity	Bottle Type Code
	3/8/16-3/9/16	900-900		X		water		X	B

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

1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)
	1605 3/9/16	
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)
		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: 4 °C
6. TEMPERATURE GUN ID: HHT# 2

FOR COMPLETION BY LAB ONLY

REMARKS / SAMPLE COMMENTS

Revision 3
7/1/15

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 INFORMATION	Project Description	Turnaround Time
Stuttgart Municipal Water Works	Stuttgart Municipal Water Works	Chronic Toxicity	1 Day (100%)
516 South Main	P.O. Box 130		2 Day (50%)
Stuttgart, AR 72160	Stuttgart, AR 72160	Reporting Information	3 Day (25%)
		Telephone: 870-673-3246	5 Day (Routine)
Attn: Tommy Lawson		Fax: 870-673-8783	Preservative Code:
		Email: stuttgartarwater@centurytel.net, swsdept@d-c1.com	Bottle Type:

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

Sampler(s) Signature

Sampler(s) Printed Mahdi Haddadi

Field Number	SAMPLE COLLECTION Date/s	Time/s	Grab	Comp	Number of Bottles	Sample Matrix	SAMPLE IDENTIFICATION/ DESCRIPTION	TEST PARAMETERS: Chronic Toxicity	Bottle Type Code (G = Glass, P = Plastic, V = Septum; A = Amber)	Arkansas Analytical Work Order Number: K1603-
	3/9/16-3/11/16	9:00-9:00		✓		water		X		002C

1. Relinquished by: (Signature)	Date/Time	2. Received by: (Signature)
	1640 3/11/16	
3. Relinquished by: (Signature)	Date/Time	4. Received by lab: (Signature)
		Sidney James

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:	6 °C	
6. TEMPERATURE GUN ID:	HHT# 2	

FOR COMPLETION BY LAB ONLY

REMARKS / SAMPLE COMMENTS

Revision 3
1/4/16

APPENDIX B

Effluent and Dilution Water Data

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING									Fathead Minnow
Lab # / Sample ID K1603002						Test Start (Date/Time) 3-8-2016 / 1550			
Client: Stuttgart						Test End (Date/Time) 3-15-16 / 1000			
		Day of Test							
		1	2	3	4	5	6812	7	notes
Control	MHS 8117	3/8	3/9	3/10	3/11	3/12	3/13	3/14	
D.O. (mg/L)	INITIAL	8.8	8.7	8.3	8.2	8.0	8.3	9.0	
	FINAL	7.5	~~8.7~~ 6.4	7.0	7.16	~~6.8.068~~	8.1	8.2	
pH (s.u.)	INITIAL	7.8	8.1	8.0	8.0	8.4	7.7	7.9	
	FINAL	~~7.6~~	~~8.1~~ 7.7	7.8	7.6	~~6.8~~ 8.0	8.1	8.1	
temp (C)	INITIAL	21	21	22	23	21	21	20	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		60					60		
HARDNESS (mg/L)		72					78		
CONDUCTIVITY (umhc		284					295		
CHLORINE (mg/L)		<0.05					<0.05		
CONC: 32%									
D.O. (mg/L)	INITIAL	8.9	8.6	8.4	8.4	8.0	8.6	9.0	
	FINAL	7.5	~~8.0~~ 6.3	7.9	7.2	6.3	7.4	8.3	
pH (s.u)	INITIAL	7.7	7.8	7.8	7.9	7.8	7.7	7.8	
	FINAL	~~7.8~~	~~8.1~~ 7.8	7.9	8.0	~~6~~ 7.9	7.9	8.1	
temp (C)	INITIAL	22	23	22	23	23	22	20	
	FINAL	25	25	25	25	25	25	25	
CONC: 42%									
D.O. (mg/L)	INITIAL	8.7	8.6	8.6	8.4	8.4	8.4	9.1	
	FINAL	7.2	~~8.0~~ 6.5	6.7	7.3	5.6	7.3	7.8	
pH (mg/L)	INITIAL	7.7	7.8	7.8	7.8	7.8	7.6	7.7	
	FINAL	7.9	~~8.3~~ 7.9	7.9	8.0	7.8	7.8	7.9	
temp (C)	INITIAL	23	23	22	24	22	24	20	
	FINAL	25	25	25	25	25	25	25	
CONC: 56%									
D.O. (mg/L)	INITIAL	8.7	8.5	8.6	8.1	8.8	8.5	8.8	
	FINAL	7.1	~~8.0~~ 6.8	7.0	7.11	4.7	7.2	8.3	
pH (s.u.)	INITIAL	7.7	7.7	7.8	7.8	7.8	7.5	7.6	
	FINAL	7.9	~~8.3~~ 8.0	8.0	8.1	7.9	7.8	7.9	
temp (C)	INITIAL	24	25	22	24	21.3	24	21	
	FINAL	25	25	25	25	25	25	25	
CONC: 75%									
D.O. (mg/L)	INITIAL	8.6	8.5	8.6	8.3	8.1	8.5	9.0	
	FINAL	7.1	6.4	7.3	7.2	4.1	7.0	7.6	
pH (s.u.)	INITIAL	7.7	7.7	7.8	7.8	7.7	7.3	7.4	
	FINAL	8.0	8.0	8.2	8.2	7.8	7.8	7.9	
temp (C)	INITIAL	25	25	22	25	24	25	20	
	FINAL	25	25	25	25	25	25	25	
CONC: 100%									
D.O. (mg/L)	INITIAL	8.5	8.4	8.6	8.2	8.8	8.7	9.1	
	FINAL	7.1	6.4	7.2	7.1	4.3	7.0	7.7	
pH (s.u.)	INITIAL	7.6	7.7	7.8	7.8	7.7	7.2	7.4	
	FINAL	8.1	8.1	8.2	8.2	8.0	8.0	7.8	
temp (C)	INITIAL	26	26	22	25	23	26	20	
	FINAL	25	25	25	25	25	25	25	
CONC: 100 %		A	A	A	B	B	C	C	
ALKALINITY (mg/L)		130			148		52		
HARDNESS (mg/L)		116			118		72		
CONDUCTIVITY (umhc		913			958		283		
CHLORINE (mg/L)		<0.05			<0.05		<0.05		

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING									Ceriodaphnia Dubia
Lab # / Sample ID K1603002						Test Start (Date/Time) 3-8-2016 / 1000			
Client: Stuttgart						Test End (Date/Time) 3-15-16 / 0945			
		Day of Test							
		1	2	3	4	5	6 & 12	7	notes
Control	MHS811	3/8	3/9	3/10	3/11	3/12	3/13	3/14	
D.O. (mg/L)	INITIAL	8.8	8.7	8.3	8.2	8.0	8.3	9.0	
	FINAL	7.8	8.1	7.9	8.7	8.6	7.7	7.1	
pH (s.u.)	INITIAL	7.8	8.1	8.0	8.0	8.4	7.7	7.9	
	FINAL	7.9	8.1	7.8	8.0	8.0	8.0	7.7	
temp (C)	INITIAL	21	21	22	23	21	21	20	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		60					60		
HARDNESS (mg/L)		72					78		
CONDUCTIVITY (umhc		284					295		
CHLORINE (mg/L)		<0.05					<0.05		
CONC: 32%									
D.O. (mg/L)	INITIAL	8.9	8.6	8.4	8.4	8.0	8.6	9.0	
	FINAL	7.9	8.0	7.3	8.7	8.6	7.9	7.5	
pH (s.u)	INITIAL	7.7	7.8	7.8	7.9	7.8	7.7	7.8	
	FINAL	8.1	8.1	7.9	8.1	8.1	8.1	7.8	
temp (C)	INITIAL	22	23	22	23	23	22	20	
	FINAL	25	25	25	25	25	25	25	
CONC: 42%									
D.O. (mg/L)	INITIAL	8.7	8.6	8.6	8.4	8.4	8.4	9.1	
	FINAL	7.9	8.0	7.2	8.8	8.6	8.0	7.2	
pH (mg/L)	INITIAL	7.7	7.8	7.8	7.8	7.8	7.6	7.7	
	FINAL	8.2	8.3	8.0	8.3	8.2	8.1	7.8	
temp (C)	INITIAL	23	23	22	24	22	24	20	
	FINAL	25	25	25	25	25	25	25	
CONC: 56%									
D.O. (mg/L)	INITIAL	8.7	8.5	8.6	8.3	8.8	8.5	8.8	
	FINAL	7.9	8.0	7.0	8.8	8.6	7.9	6.9	
pH (s.u.)	INITIAL	7.7	7.7	7.8	7.8	7.8	7.5	7.6	
	FINAL	8.2	8.3	8.0	8.3	8.3	8.1	7.8	
temp (C)	INITIAL	24	25	22	24	21.3	24	20	
	FINAL	25	25	25	25	25	25	25	
CONC: 75%									
D.O. (mg/L)	INITIAL	8.6	8.5	8.6	8.3	8.1	8.5	9.2	
	FINAL	7.9	8.0	7.1	8.7	8.5	7.9	7.3	
pH (s.u.)	INITIAL	7.7	7.7	7.8	7.8	7.87	7.3	7.4	
	FINAL	8.3	8.4	8.1	8.4	8.3	8.0	7.7	
temp (C)	INITIAL	25	25	22	25	21.3 24	25	20	
	FINAL	25	25	25	25	25	25	25	
CONC: 100%									
D.O. (mg/L)	INITIAL	8.5	8.4	8.6	8.2	8.8	8.7	9.1	
	FINAL	7.8	8.0	7.2	8.7	8.6	8.0	7.0	
pH (s.u.)	INITIAL	7.6	7.7	7.8	7.8	7.1	7.2	7.4	
	FINAL	8.4	8.4	8.3	8.5	8.4	8.0	7.7	
temp (C)	INITIAL	26	26	22	25	23	26	20	
	FINAL	25	25	25	25	25	25	25	
CONC: 100 %		A	A	A	B	B	C	C	
ALKALINITY (mg/L)		130			148		52		
HARDNESS (mg/L)		116			118		72		
CONDUCTIVITY (umhc		913			458		283		
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 K160300Z TEST START DATE 3-8-2016 TIME 1550

CLIENT Stuttgart TEST END DATE 3-15-16 TIME 1000

AGE AND SOURCE OF MINNOWS Aquatox <48hrs.

		DAY (NUMBER SURVIVING)								SURVIVAL		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	10	10	10	10	10	10	100	96%	9.32%
	B			10		10	10		10	100		
	C			10		10	10		10	100		
CONT	D			10		10	10		10	100		
	E			9	8	8	9	8	8	80		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	10	10	10	10	10	10	100	94%	
	B					10	10		9	90		
	C					10	10		10	100		
32%	D				9	9	9	9	9	90		
	E		9	9	9	9	9	9	9	90		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	10	10	10	10	10	10	100	98%	
	B					9	9	9	9	90		
	C					10	10	10	10	100		
42%	D					10	10		10	100		
	E					10	10		10	100		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	10	10	9	9	9	9	90	88%	
	B		9	8	8	8	8	8	8	80		
	C		10	9	9	9	9	9	9	90		
56%	D		9	9	9	9	9		9	90		
	E		9	9	9	9	9		9	90		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	10	10	10	10	10	10	100	92%	
	B				10	10	10		10	100		
	C				9	9	9	9	9	90		
75%	D				9	9	9		8	80		
	E				9	9	9		9	90		
	REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	10	9	9	9	8	8	80	94%	9.52%
	B				10	10	10	10	9	90		
	C					10	10		10	100		
100%	D					10	10		10	100		
	E					10	10		10	100		
ANALYST		HB/KR	KR	KR	KR	SS	CA	KR	ER			
DATE:		3-8-16	3-9-16	3-10-16	3-11-16	3-12-16	3-13-16	3-14-16	15Mar16			
TIME:		1550	1230	1345	1115	0950	1015	1000	1000			

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: K1603002
CLIENT: Stuttgart
ANALYSTS: ZR
SAMPLE ID: Outfall

TEST DATES (BEGIN / END): 3-8-2016 / 3-15-2016
WEIGHING DATE / TIME: 15Mar16 1615
DRYING TEMP (DEGREES C): 60
DRYING TIME (HOURS): 24

	REP #	FINAL DRY WEIGHT TIN+LARVAE (g)	INITIAL WEIGHT TIN (g)	TOTAL DRY WEIGHT OF LARVAE	NUMBER OF LARVAE	DRY WEIGHT OF LARVAE (mg)	
CONTROL	A S1	1.03858	1.03319	~~1.03858~~ 0.00539	10	0.539	AVG DRY
	B S2	1.04035	1.03456	~~1.04035~~ 0.00579	|	0.579	WEIGHT (mg)
control	C S3	1.00045	0.99443	~~1.00045~~ 0.00602	|	0.602	0.587
	D S4	1.01947	1.01278	~~1.01947~~ 0.00669	|	0.669	CV
	E S5	1.05829	1.05283	~~1.05829~~ 0.00546	1	0.546	8.93%
CONC:	A S6	1.02606	1.02098	0.00508	10	0.508	AVG DRY
	B S7	1.01395	1.00850	0.00545	|	0.545	WEIGHT (mg)
32%	C S8	1.00616	1.00138	0.00478	|	0.478	0.507
	D S9	1.01148	1.00642	0.00506	|	0.506	CV
	E S10	1.03201	1.02703	0.00498	1	0.498	
CONC:	A S11	1.00130	0.99665	0.00465	10	0.465	AVG DRY
	B S12	1.01948	1.01457	0.00491	|	0.491	WEIGHT (mg)
42%	C S13	1.02149	1.01637	0.00512	|	0.512	0.494
	D S14	0.99754	0.99230	0.00524	|	0.524	CV
	E S15	0.98686	0.98208	0.00478	1	0.478	
CONC:	A S16	1.00261	0.99504	0.00757	10	0.757	AVG DRY
	B S17	1.01014	1.00512	0.00502	|	0.502	WEIGHT (mg)
56%	C S18	1.07251	1.06804	0.00447	|	0.447	0.512
	D S19	1.07065	1.06618	0.00447	|	0.447	CV
	E S20	1.07314	1.06909	0.00405	1	0.405	
CONC:	A S21	1.02389	1.01831	0.00558	10	0.558	AVG DRY
	B S22	1.02260	1.01708	0.00552	|	0.552	WEIGHT (mg)
75%	C S23	1.01579	1.01070	0.00509	|	0.509	0.480
	D S24	1.01811	1.01459	0.00352	|	0.352	CV
	E S25	1.03181	1.02751	0.00430	1	0.430	
CONC:	A S26	1.02157	1.01634	0.00523	10	0.523	AVG DRY
	B S27	1.02396	1.01841	0.00555	|	0.555	WEIGHT (mg)
100%	C S28	0.99964	0.99409	0.00555	|	0.555	0.528
	D S29	1.04425	1.03958	0.00467	|	0.467	CV
	E S30	1.01973	1.01433	0.00540	1	0.540	6.93%

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA # K1603002, FATHEAD MINNOW SURVIVAL, 3-8-16
File: c:\toxstat\STUTTS. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.286

W = 0.898

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 # K1603002, FATHEAD MINNOW SURVIVAL, 3-8-16
File: c:\toxstat\STUTTS. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 3.83

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 # K1603002, FATHEAD MINNOW SURVIVAL, 3-8-16
FILE: c:\toxstat\STUTTS.
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	0.8000	1.1071
2	32 % EFFLUENT	1	1.0000	1.4120
2	32 % EFFLUENT	2	0.9000	1.2490
2	32 % EFFLUENT	3	1.0000	1.4120
2	32 % EFFLUENT	4	0.9000	1.2490
2	32 % EFFLUENT	5	0.9000	1.2490
3	42 % EFFLUENT	1	1.0000	1.4120
3	42 % EFFLUENT	2	0.9000	1.2490
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	0.9000	1.2490
4	56 % EFFLUENT	2	0.8000	1.1071
4	56 % EFFLUENT	3	0.9000	1.2490
4	56 % EFFLUENT	4	0.9000	1.2490
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	0.9000	1.2490
5	75 % EFFLUENT	4	0.8000	1.1071
5	75 % EFFLUENT	5	0.9000	1.2490
6	100 % EFFLUENT	1	0.8000	1.1071
6	100 % EFFLUENT	2	0.9000	1.2490
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 # K1603002, FATHEAD MINNOW SURVIVAL, 3-8-16
File: c:\toxstat\STUTTS. 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	1.351				
2	32 % EFFLUENT	1.314	24.00	16.00	5.00	
3	42 % EFFLUENT	1.379	28.00	16.00	5.00	
4	56 % EFFLUENT	1.221	19.50	16.00	5.00	
5	75 % EFFLUENT	1.286	23.50	16.00	5.00	
6	100 % EFFLUENT	1.318	25.50	16.00	5.00	

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

AA # K1603002, FATHEAD MINNOW GROWTH, 3-8-16
File: C:\TOXSTAT\STUTTG. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.140

W = 0.887

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 # K1603002, FATHEAD MINNOW GROWTH, 3-8-16
File: C:\TOXSTAT\STUTTG. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 18.74

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 # K1603002, FATHEAD MINNOW GROWTH, 3-8-16
FILE: C:\TOXSTAT\STUTTG.
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.5390	0.8244
1	CONTROL	2	0.5790	0.8647
1	CONTROL	3	0.6020	0.8881
1	CONTROL	4	0.6690	0.9578
1	CONTROL	5	0.5460	0.8315
2	32 % EFFLUENT	1	0.5080	0.7934
2	32 % EFFLUENT	2	0.5450	0.8305
2	32 % EFFLUENT	3	0.4780	0.7634
2	32 % EFFLUENT	4	0.5060	0.7914
2	32 % EFFLUENT	5	0.4980	0.7834
3	42 % EFFLUENT	1	0.4650	0.7504
3	42 % EFFLUENT	2	0.4910	0.7764
3	42 % EFFLUENT	3	0.5120	0.7974
3	42 % EFFLUENT	4	0.5240	0.8094
3	42 % EFFLUENT	5	0.4780	0.7634
4	56 % EFFLUENT	1	0.7570	1.0553
4	56 % EFFLUENT	2	0.5020	0.7874
4	56 % EFFLUENT	3	0.4470	0.7323
4	56 % EFFLUENT	4	0.4470	0.7323
4	56 % EFFLUENT	5	0.4050	0.6898
5	75 % EFFLUENT	1	0.5580	0.8435
5	75 % EFFLUENT	2	0.5520	0.8375
5	75 % EFFLUENT	3	0.5090	0.7944
5	75 % EFFLUENT	4	0.3520	0.6351
5	75 % EFFLUENT	5	0.4300	0.7152
6	100 % EFFLUENT	1	0.5230	0.8084
6	100 % EFFLUENT	2	0.5550	0.8405
6	100 % EFFLUENT	3	0.5550	0.8405
6	100 % EFFLUENT	4	0.4670	0.7524
6	100 % EFFLUENT	5	0.5400	0.8254

AA # K1603002, FATHEAD MINNOW GROWTH, 3-8-16
File: C:\TOXSTAT\STUTTG. Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.036	0.007	1.229
Within (Error)	24	0.140	0.006	
Total	29	0.176		

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

AA # K1603002, FATHEAD MINNOW GROWTH, 3-8-16
File: C:\TOXSTAT\STUTTG. 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	0.873	0.587		
2	32 % EFFLUENT	0.792	0.507	1.675	
3	42 % EFFLUENT	0.779	0.494	1.945	
4	56 % EFFLUENT	0.799	0.512	1.530	
5	75 % EFFLUENT	0.765	0.480	2.240	
6	100 % EFFLUENT	0.813	0.528	1.240	

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

AA # K1603002, FATHEAD MINNOW GROWTH, 3-8-16
File: C:\TOXSTAT\STUTTG. 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	0.114	19.3	0.080
3	42 % EFFLUENT	5	0.114	19.3	0.093
4	56 % EFFLUENT	5	0.114	19.3	0.075
5	75 % EFFLUENT	5	0.114	19.3	0.107
6	100 % EFFLUENT	5	0.114	19.3	0.059

AA # K1603002, FATHEAD MINNOW GROWTH, 3-8-16
File: C:\TOXSTAT\STUTTG. 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	0.873				
2	32 % EFFLUENT	0.792	16.00	16.00	5.00	*
3	42 % EFFLUENT	0.779	15.00	16.00	5.00	*
4	56 % EFFLUENT	0.799	20.00	16.00	5.00	
5	75 % EFFLUENT	0.765	19.00	16.00	5.00	
6	100 % EFFLUENT	0.813	20.00	16.00	5.00	

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

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

SURVIVAL AND REPRODUCTION TEST

Ceriodaphnia dubia

Discharger: Stuttgart | Lab Number/s: K1603002

Location: Outfall | Analyst: tb, HF

Date Sample Collected: See COC | Test Start - Date/Time: 3-8-2016/1000

Test Stop - Date/Time: 3-15-2016/0945

Conc % 1 (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	5	0	5	0	0	6	0	0	0	16	10	1.6	tb
4	5	0	3	0	4	3	0	4	3	3	25	10	2.5	HF
5	8	8	6	7	0	9	9	8	6	8	69	10	6.9	tb
6	1	17	2	12	7	14	13	14	3	2	85	10	8.5	tb
7	11	0	10	0	9	0	1	0	10	13	54	10	5.4	tb
8														
Total	25	30	21	24	20	26	29	26	22	26	249	$\bar{X}$= 24.9	CV= 13.0%	

100% 3rd brood

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	0	5	0	0	1	4	4	0	0	4	18	10	1.8	tb
4	4	0	4	4	5	0	0	2	4	0	23	10	2.3	HF
5	6	7	7	8	4	6	9	9	6	8	70	10	7.0	tb
6	9	15	4	13	1	16	19	15	2	17	111	10	11.1	tb
7	9	0	10	0	8	0	0	0	13	0	40	10	4.0	tb
8														
Total	28	27	25	25	19	26	32	26	25	29	262			

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	0	5	0	5	0	0	4	0	0	0	14	10	1.4	tb
4	4	0	3	0	5	5	0	3	4	5	29	10	2.9	HF
5	10	10	8	11	2	9	7	6	8	12	83	10	8.3	tb
6	3	15	3	16	7	15	12	5	3	14	93	10	9.3	tb
7	15	0	14	0	12	4	0	10	8	0	63	10	6.3	tb
8														
Total	32	30	28	32	26	33	23	24	23	31	282			

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	0	0	0	6	0	0	5	4	0	5	20	10	2.0	tb
4	4	4	3	0	4	4	0	0	6	0	25	10	2.5	HF
5	10	7	6	9	8	8	9	9	9	9	84	10	8.4	tb
6	2	13	5	16	1	13	19	17	4	21	111	10	11.1	tb
7	15	0	14	0	7	3	0	1	13	0	53	10	5.3	tb
8														
Total	31	24	28	31	20	28	33	31	32	35	293			

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	0	6	0	6	0	7	7	0	0	5	31	10	3.1	tb
4	4	0	4	0	5	0	0	4	3	0	20	10	2.0	HF
5	8	10	7	8	8	9	9	10	7	9	85	10	8.5	tb
6	4	23	1	18	3	16	22	14	3	17	121	10	12.1	tb
7	16	0	9	0	9	0	0	0	12	0	46	10	4.6	tb
8														
Total	32	39	21	32	25	32	38	28	25	31	303			

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	0	4	0	5	0	5	4	5	0	6	29	10	2.9	tb
4	6	0	4	2	5	0	0	0	5	0	22	10	2.2	HF
5	7	9	5	11	2	8	8	12	7	6	75	10	7.5	tb
6	6	17	4	20	12	22	14	19	5	21	140	10	14.0	tb
7	11	0	16	6	6	0	0	0	14	0	47	10	4.7	tb
8														
Total	30	30	29	38	25	35	26	36	31	33	313	$\bar{X}$= 31.3		
												CV= 13.5%		

X = Dead

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	10	0	10
TOTAL	20	0	20

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

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
56% 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	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

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

AA # K1603002, CERIODAPHNIA DUBIA REPRODUCTION, 3-8-16
File: C:\TOXSTAT\STUTTC. 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 # K1603002, CERIODAPHNIA DUBIA REPRODUCTION, 3-8-16
File: C:\TOXSTAT\STUTTC. Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 3.83

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 # K1603002, CERIODAPHNIA DUBIA REPRODUCTION, 3-8-16
FILE: C:\TOXSTAT\STUTTC.
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	25.0000	25.0000
1	CONTROL	2	30.0000	30.0000
1	CONTROL	3	21.0000	21.0000
1	CONTROL	4	24.0000	24.0000
1	CONTROL	5	20.0000	20.0000
1	CONTROL	6	26.0000	26.0000
1	CONTROL	7	29.0000	29.0000
1	CONTROL	8	26.0000	26.0000
1	CONTROL	9	22.0000	22.0000
1	CONTROL	10	26.0000	26.0000
2	32 % EFFLUENT	1	28.0000	28.0000
2	32 % EFFLUENT	2	27.0000	27.0000
2	32 % EFFLUENT	3	25.0000	25.0000
2	32 % EFFLUENT	4	25.0000	25.0000
2	32 % EFFLUENT	5	19.0000	19.0000
2	32 % EFFLUENT	6	26.0000	26.0000
2	32 % EFFLUENT	7	32.0000	32.0000
2	32 % EFFLUENT	8	26.0000	26.0000
2	32 % EFFLUENT	9	25.0000	25.0000
2	32 % EFFLUENT	10	29.0000	29.0000
3	42 % EFFLUENT	1	32.0000	32.0000
3	42 % EFFLUENT	2	30.0000	30.0000
3	42 % EFFLUENT	3	28.0000	28.0000
3	42 % EFFLUENT	4	32.0000	32.0000
3	42 % EFFLUENT	5	26.0000	26.0000
3	42 % EFFLUENT	6	33.0000	33.0000
3	42 % EFFLUENT	7	23.0000	23.0000
3	42 % EFFLUENT	8	24.0000	24.0000
3	42 % EFFLUENT	9	23.0000	23.0000
3	42 % EFFLUENT	10	31.0000	31.0000
4	56 % EFFLUENT	1	31.0000	31.0000
4	56 % EFFLUENT	2	24.0000	24.0000
4	56 % EFFLUENT	3	28.0000	28.0000
4	56 % EFFLUENT	4	31.0000	31.0000
4	56 % EFFLUENT	5	20.0000	20.0000
4	56 % EFFLUENT	6	28.0000	28.0000
4	56 % EFFLUENT	7	33.0000	33.0000
4	56 % EFFLUENT	8	31.0000	31.0000
4	56 % EFFLUENT	9	32.0000	32.0000
4	56 % EFFLUENT	10	35.0000	35.0000
5	75 % EFFLUENT	1	32.0000	32.0000
5	75 % EFFLUENT	2	39.0000	39.0000
5	75 % EFFLUENT	3	21.0000	21.0000
5	75 % EFFLUENT	4	32.0000	32.0000
5	75 % EFFLUENT	5	25.0000	25.0000
5	75 % EFFLUENT	6	32.0000	32.0000
5	75 % EFFLUENT	7	38.0000	38.0000
5	75 % EFFLUENT	8	28.0000	28.0000
5	75 % EFFLUENT	9	25.0000	25.0000

5	75	%	EFFLUENT	10	31.0000	31.0000
6	100	%	EFFLUENT	1	30.0000	30.0000
6	100	%	EFFLUENT	2	30.0000	30.0000
6	100	%	EFFLUENT	3	29.0000	29.0000
6	100	%	EFFLUENT	4	38.0000	38.0000
6	100	%	EFFLUENT	5	25.0000	25.0000
6	100	%	EFFLUENT	6	35.0000	35.0000
6	100	%	EFFLUENT	7	26.0000	26.0000
6	100	%	EFFLUENT	8	36.0000	36.0000
6	100	%	EFFLUENT	9	31.0000	31.0000
6	100	%	EFFLUENT	10	33.0000	33.0000

AA # K1603002, CERIODAPHNIA DUBIA REPRODUCTION, 3-8-16
File: C:\TOXSTAT\STUTTC. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	299.533	59.907	3.341
Within (Error)	54	968.400	17.933	
Total	59	1267.933		

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

AA # K1603002, CERIODAPHNIA DUBIA REPRODUCTION, 3-8-16
File: C:\TOXSTAT\STUTTC. 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	24.900	24.900		
2	32 % EFFLUENT	26.200	26.200	-0.686	
3	42 % EFFLUENT	28.200	28.200	-1.742	
4	56 % EFFLUENT	29.300	29.300	-2.323	
5	75 % EFFLUENT	30.300	30.300	-2.851	
6	100 % EFFLUENT	31.300	31.300	-3.379	

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

AA # K1603002, CERIODAPHNIA DUBIA REPRODUCTION, 3-8-16
File: C:\TOXSTAT\STUTTC. 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	4.375	17.6	-1.300
3	42 % EFFLUENT	10	4.375	17.6	-3.300
4	56 % EFFLUENT	10	4.375	17.6	-4.400
5	75 % EFFLUENT	10	4.375	17.6	-5.400
6	100 % EFFLUENT	10	4.375	17.6	-6.400

AA # K1603002, CERIODAPHNIA DUBIA REPRODUCTION, 3-8-16
File: C:\TOXSTAT\STUTTC. Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	24.900				
2	32 % EFFLUENT	26.200	116.00	75.00	10.00	
3	42 % EFFLUENT	28.200	128.50	75.00	10.00	
4	56 % EFFLUENT	29.300	135.00	75.00	10.00	
5	75 % EFFLUENT	30.300	133.50	75.00	10.00	
6	100 % EFFLUENT	31.300	143.50	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 3/8/16 **CLIENT** ARK ANALYTICAL

Purchase Order #: _____

SPECIES: Pimephales promelas

Quantity Shipped: 300+ 15-1600 CST

Age: HATCHED 3/6/16

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.

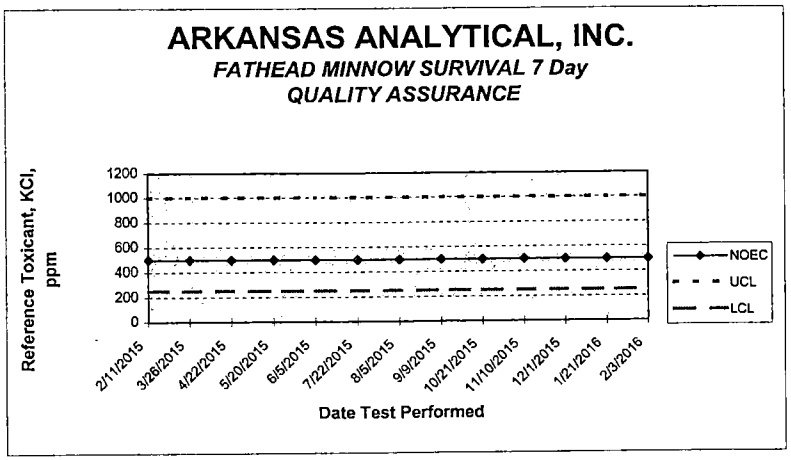
Water Chemistry Record:	Current	Range
TEMPERATURE:	22°C	22-26°C
SALINITY/CONDUCTIVITY:	--	--
TOTAL HARDNESS (as $CaCO_3$):	94 mg/l	76-130 mg/l
TOTAL ALKALINITY (as $CaCO_3$):	65 mg/l	65-100 mg/l
pH:	7.98	7.50-8.20

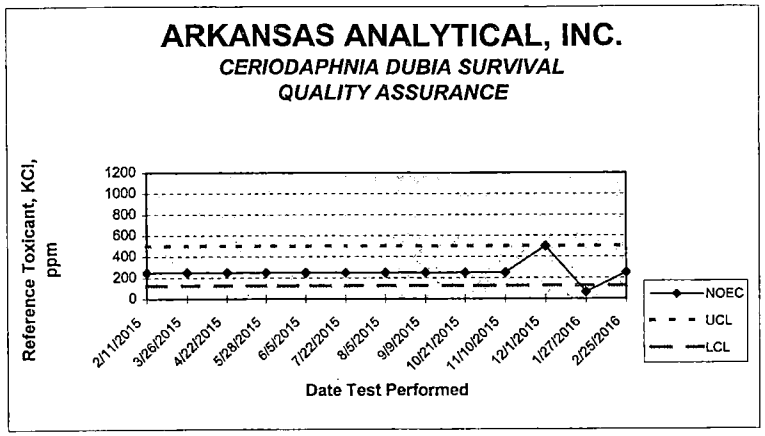
Comments:

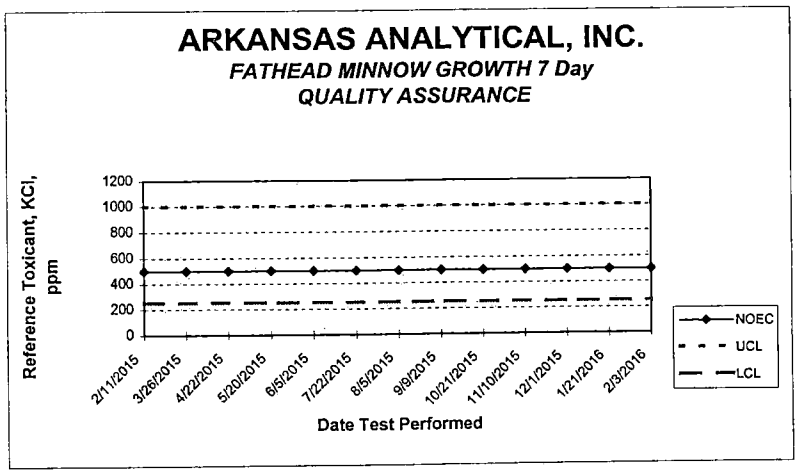
Facility Supervisor

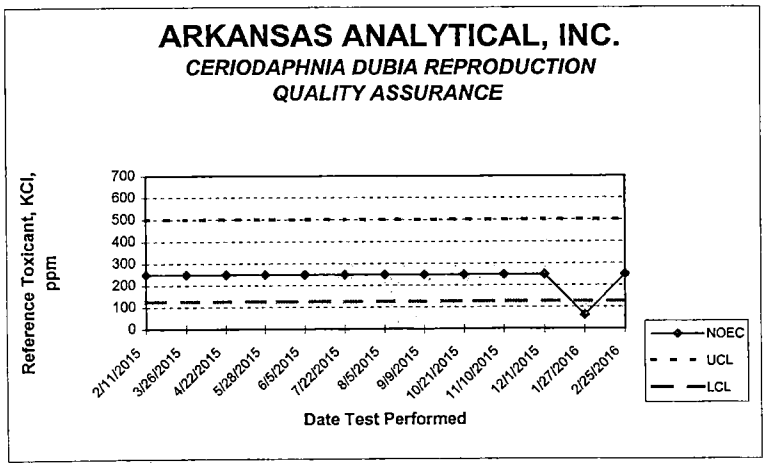
APPENDIX F

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

STUTTGART MUNICIPAL WATER WORKS
P.O. BOX 130
STUTTGART, AR 72160
PHONE: 870-673-3246

Hasler FIRST-CLASS MAIL
05/02/2016
US POSTAGE $002.83⁰
ZIP 72160
011E10672532

Arkansas Dept. of Environmental Quality
ATTN: Mary Barnett-Water Division
5301 Northshore Drive
North Little Rock, AR 72118-5317