

Arkansas Analytical, Inc.

Toxicity Test Results

CITY of STUTTGART
NPDES PERMIT NUMBER: AR0034380
Fourth Quarter 2015
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**
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Stuttgart, AR 72160

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Lab Number K1510002

Thursday, October 22, 2015

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 fourth quarter of 2015.

Plant Operations

To be provided by permittee.

Source of Effluent and Dilution Water

Effluent samples were collected as follows:

Sample Collection:	Date Started	Date, Time Ended
Sample #1:	10-11-15, 0900	10-12-15, 0900
Sample #2:	10-13-15, 0900	10-14-15, 0900
Sample #3:	10-15-15, 0900	10-16-15, 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:	10-12-15, 1550	8 (on ice)
Sample #2:	10-14-15, 1600	8 (on ice)
Sample #3:	10-16-15, 1549	7 (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	15.9	X	
At least 60% of surviving females should have produced 3 broods	70%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	24.0%	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	5.71 %	X	
Minimum of 0.25 mg average dry weight of surviving controls	0.766	X	
The percent coefficient of variation between replicates must be 40% or less for growth	13.2%	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

<i>Ceriodaphnia dubia</i> 9/9/15 – 9/16/15		<i>Pimephales promelas</i> 9/9/15 – 9/16/15	
NOEC Survival:	250 ppm KCl	NOEC Survival:	500 ppm KCl
LOEC Survival:	500 ppm KCl	LOEC Survival:	1000 ppm KCl
NOEC Reproduction:	250 ppm KCl	NOEC 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

<i>Ceriodaphnia dubia</i>		<i>Pimephales promelas</i>	
NOEC / LOEC Survival	100% / NA	NOEC / LOEC survival	100%/ NA
NOEC / LOEC Reproduction	100% / NA	NOEC / LOEC growth	100%/ NA
Mean number of neonates (critical dilution)	15.6	%CV survival (critical dilution)	0.00%
%CV Reproduction (critical dilution)	22.0%	Mean dry weight (critical dilution) in milligrams	0.987
		%CV growth (critical dilution)	6.63%
PMSD Reproduction	33.4%	PMSD Growth	17.9%

Conclusion

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

The permit issued to the City of 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.

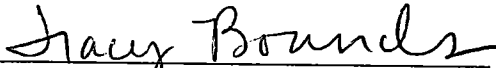
Chronic static renewal survival and reproduction test using *Ceriodaphnia dubia*, (Method 1002.0)

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:

Ryan Hudgin / Tracy Bounds

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:	10-11-15, 0900	10-12-15, 0900
Sample #2:	10-13-15, 0900	10-14-15, 0900
Sample #3:	10-15-15, 0900	10-16-15, 0900

Test initiated (date, time): 10-13-15, 1410 Test terminated (date, time): 10-21-15, 0930

Dilution water used: Moderately Hard Synthetic

DATA TABLE FOR FATHEAD MINNOW SURVIVAL

Effluent Conc %	Percent Survival in Replicate Chambers					Mean Percent Survival				CV %
	A	B	C	D	E	24 hours	48 hours	7 days		
0%	90	100	90	100	100	100	98	96	96	5.71
32%	100	100	100	90	90	98	96	96		
42%	100	100	100	80	100	100	96	96		
56%	100	90	100	100	100	100	100	98		
75%	100	100	100	100	100	100	100	100		
100%	100	100	100	100	100	100	100	100	100	0.00

DATA TABLE FOR GROWTH OF FATHEAD MINNOWS

Effluent Conc %	Average Dry Weight in milligrams in replicate chambers					Mean Dry Weight	CV%
	A	B	C	D	E		
0%	0.827	0.829	0.588	0.787	0.800	0.766	13.2
32%	0.847	0.716	0.980	0.618	0.757	0.784	
42%	0.780	0.737	0.823	0.701	0.671	0.742	
56%	0.866	0.871	0.827	0.910	0.814	0.858	
75%	0.790	0.752	0.717	0.989	0.738	0.797	
100%	0.977	1.094	0.988	0.920	0.954	0.987	6.63

Coefficient of Variation = standard deviation / mean * 100

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

1. Dunnett's procedure or Steel's Many-One Rank Test as appropriate:
Is the mean survival at 7 days significantly different ($p=0.05$) than the control survival for:
a) LOW FLOW OR CRITICAL DILUTION, (100%) YES _____ NO X

2. Dunnett's Procedure
Is the mean dry weight (growth) at 7 days significantly different ($p=0.05$) than the control's dry weight (growth) for:
a) LOW FLOW OR CRITICAL DILUTION, (100%) YES _____ NO X

3. If NO was answered to 1.a) enter [0] otherwise enter [1] (parameter TLP6C): 0

4. If NO was answered to 2.a) enter [0] otherwise enter [1] (parameter TGP6C): 0

5. Enter percentage corresponding to each parameter below:
a) NOEC survival (parameter TOP6C)= 100 % effluent
b) NOEC growth (parameter TPP6C)= 100 % effluent
c) Coefficient of variation (parameter TQP6C)= 13.2 %

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:	10-11-15, 0900	10-12-15, 0900
Sample #2:	10-13-15, 0900	10-14-15, 0900
Sample #3:	10-15-15, 0900	10-16-15, 0900

Test initiated (date, time): 10-13-15, 1030 Test terminated (date, time): 10-20-15, 0835

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	13	12	10	19	14	15
B	16	10	11	8	20	10
C	9	7	8	17	15	20
D	16	X0	20	10	17	14
E	18	14	X0	9	X0	13
F	17	12	13	14	11	13
G	21	18	13	16	11	18
H	11	16	25	15	14	20
I	19	18	10	8	20	14
J	19	14	13	20	20	19
Mean	14.9	12.1	12.3	13.6	14.2	15.6
Mean/surviving female	15.9	13.4	13.7	13.6	15.8	15.6
CV%*	24.0					22.0

X= Dead Adult; M= Male (Not considered in statistics)

*Coefficient of Variation = standard deviation/ mean * 100; CV% calculation based on young per surviving female

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	90	90	100	90	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)= 24.0 %

6. Enter Whole Effluent Toxicity: 100 %

APPENDIX A

Chain of Custody Forms

SORRELLS RESEARCH ASSOCIATES, INC

8100 NATIONAL DRIVE, LITTLE ROCK, AR 72209

501-562-8139 800-331-8139

FAX 501-562-7025

CHAIN OF CUSTODY RECORD

TURN AROUND TIME
RUSH 24HR. 48 HR.
5 DAY REG
OTHER _____

FOR LAB/OFFICE USE ONLY

LAB # 18481.0001

CLIENT # _____

P.O.# _____

STANDARD METHODS PRESERVATION PER EPA 40 CFR

C 4= COOL TO 4.C

S<2= SULFURIC ACID TO pH<2

N<2= NITRIC ACID TO pH<2

T= THIOSULFATE FOR DECHLORINATION

W= WINKLER AZIDE MODIFICATION

P= MEMBRANE ELECTRODE

NaOH= pH >12

110913K2

NAME OF COMPANY, CITY, OR PROJECT

PROJECT NO:

SAMPLER(S) NAME: (PRINT)

Stuttgart

SAMPLE NO:	SAMPLE ID AND/ OR COLLECTION LOCATION	START	END	COMP	FIELD ANALYSIS				D,O (W)	CONTAINER TYPE	ANALYSIS REQUIRED
		DATE/TIME	DATE/TIME	GRAB	pH	TEMP	FLOW	CL2	D.O(P)	PRESERVATIVE	
	Outfall 001	0900 10-11-15	0900 10-12-15	C						6 1/2 Gal P C4	K1510002A WET/BIO

METHOD OF SHIPMENT (CIRCLE)
FED EX WALK IN SRA UPS OTHER

FIELD CALIBRATION RECORD

pH 7	7.00	
pH 4	4.01	
pH 10	10.00	
D.O		

Custody Seals: Yes No
Containers Correct: Yes No
COC/Labels Agree: Yes No
Received on Ice: Yes No
Temperature on Receipt: 8°C
Temperature Gun ID: HHT # 2

TYPE OF SAMPLE(S): (CIRCLE)
WATER SOIL W/W SLUDGE OTHER

FIELD ANALYSIS CONDUCTED BY: (CIRCLE) SRA CLIENT

RELINQUISHED BY: _____	DATE/TIME: <u>1550</u>	RECEIVED BY: _____	DATE/TIME: _____
RELINQUISHED BY: _____	DATE/TIME: <u>10/12/15</u>	RECEIVED BY: <u>Sydney James</u>	DATE/TIME: <u>10/12/15 1550</u>
RELINQUISHED BY: _____	DATE/TIME: _____	RECEIVED BY(LAB): _____	DATE/TIME: _____

SORRELLS RESEARCH ASSOCIATES, INC

8100 NATIONAL DRIVE, LITTLE ROCK, AR 72209

501-562-8139 800-331-8139

FAX 501-562-7025

CHAIN OF CUSTODY RECORD

TURN AROUND TIME

RUSH 24HR. 48 HR.

5 DAY REG

OTHER _____

FOR LAB/OFFICE USE ONLY

LAB # 18481-0002

CLIENT # _____

P.O.# _____

STANDARD METHODS PRESERVATION PER EPA 40 CFR

C 4= COOL TO 4.C

S<2= SULFURIC ACID TO pH<2

N<2= NITRIC ACID TO pH<2

T= THIOSULFATE FOR DECHLORINATION

W= WINKLER AZIDE MODIFICATION

P= MEMBRANE ELECTRODE

NaOH= pH >12

110913K2

NAME OF COMPANY, CITY, OR PROJECT

PROJECT NO:

SAMPLER(S) NAME: (PRINT)

Stuttgart

SAMPLE NO:	SAMPLE ID AND/OR COLLECTION LOCATION	START	END	COMP	FIELD ANALYSIS				D,O (W)	CONTAINER TYPE	ANALYSIS REQUIRED
		DATE/TIME	DATE/TIME	GRAB	pH	TEMP	FLOW	CL2	D.O(P)	PRESERVATIVE	
	Outfall 001	0900 12,13,15	0900 14,15 1045	C						6 1/2 Gal P C4	11510 - WET/BIO 002B

METHOD OF SHIPMENT (CIRCLE)
 FED EX WALK IN SRA UPS OTHER

FIELD CALIBRATION RECORD

pH 7 7.00
 pH 4 4.01
 pH 10 10.00
 D.O

	Yes	No
Custody Seals:	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Containers Correct:	<input checked="" type="checkbox"/>	<input type="checkbox"/>
COC/Labels Agree:	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Received on Ice:	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Temperature on Receipt: 8°C		
Temperature Gun ID: HHT # 2		

TYPE OF SAMPLE(S) (CIRCLE)
 WATER SOIL W/W SLUDGE OTHER

FIELD ANALYSIS CONDUCTED BY: (CIRCLE) SRA CLIENT

RELINQUISHED BY:

DATE/TIME:

RELINQUISHED BY:

DATE/TIME:

RELINQUISHED BY:

DATE/TIME:

RECEIVED BY:

RECEIVED BY:

RECEIVED BY(LAB):

DATE/TIME:

DATE/TIME:

DATE/TIME:

[Signature]

1600
10/14/15

[Signature: Sydney James]

10/14/15, 1620

SORRELLS RESEARCH ASSOCIATES, INC

8100 NATIONAL DRIVE, LITTLE ROCK, AR 72209

501-562-8139 800-331-8139

FAX 501-562-7025

CHAIN OF CUSTODY RECORD

TURN AROUND TIME

RUSH 24HR. 48 HR.

5 DAY REG

OTHER _____

FOR LAB/OFFICE USE ONLY

LAB # 18481-0003

CLIENT # _____

P.O.# _____

STANDARD METHODS PRESERVATION PER EPA 40 CFR

C 4= COOL TO 4.C

S<2= SULFURIC ACID TO pH<2

N<2= NITRIC ACID TO pH<2

T= THIOSULFATE FOR DECHLORINATION

W= WINKLER AZIDE MODIFICATION

P= MEMBRANE ELECTRODE

NaOH= pH >12

110913K2

NAME OF COMPANY, CITY, OR PROJECT

PROJECT NO:

SAMPLER(S) NAME: (PRINT)

Stuttgart

SAMPLE NO:	SAMPLE ID AND/OR COLLECTION LOCATION	START	END	COMP	FIELD ANALYSIS				D.O (W)	CONTAINER TYPE	ANALYSIS REQUIRED
		DATE/TIME	DATE/TIME	GRAB	pH	TEMP	FLOW	CL2	D.O(P)	PRESERVATIVE	
	Outfall 001	0900 <u>10-15-15</u>	0900 <u>10-16-15</u>	C						6 1/2 Gal P C4	K1510002-C WET/BIO
METHOD OF SHIPMENT (CIRCLE)		FIELD CALIBRATION RECORD				Custody Seals:		Yes	No		
FED EX WALK IN <u>SRA</u> UPS OTHER		pH 7	7.00								
		pH 4	4.01								
		pH 10	10.00								
		D.O									
TYPE OF SAMPLE(S): (CIRCLE)						Received on Ice:					
WATER SOIL <u>W/W</u> SLUDGE OTHER						Temperature on Receipt:		7°C			
						Temperature Gun ID:		HHT # 2			
						FIELD ANALYSIS CONDUCTED BY: (CIRCLE) <u>SRA</u> CLIENT					

RELINQUISHED BY:

DATE/TIME:

1545

RECEIVED BY:

[Signature]

DATE/TIME:

1050

10-16-15

RELINQUISHED BY:

DATE/TIME:

10-16-15

RECEIVED BY:

Sydney James

DATE/TIME:

10-16-15, 1919

RELINQUISHED BY:

[Signature]

APPENDIX B

Effluent and Dilution Water Data

Biomonitoring Quality Control Benchsheet

Analyst	RH	JB	RH	RH	RH	RH	RH	RH
Date	9-26-15	27 SEP 15	9-28-15	9-29-15	9-30-15	10-13-15	10-14-15	10-15-15
pH Meter ID	AR60	XL60						
LIN pH 4 Buffer	1501243	1500708	1501243					
LIN pH 7 Buffer	1501244	1500707	1501244					
LIN pH 10 Buffer	1501245	1500706	1501245					
Slope (>90%)	97.2%	102.6%	96%	98%	97.1%	97.9%	95.7	97.2%

Dissolved O ₂ Meter	AR60	XL60						
Meter Reading	8.59	8.59	8.55	8.58	8.58	8.32	8.27	8.34
Temp.	22	22	23	22	22	24	24	24
Chart Value at Temp.	8.743	8.743	8.578	8.743	8.743	8.718	8.418	8.418
Difference	0.153	0.153	0.028	0.163	0.163	0.098	0.148	0.078
Acceptance Criteria	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L

Temp. Meter ID	AR60	XL60						
Meter Reading	22	22	23	22	22	24	22	24
Thermometer Reading	22	22	22	23	22	23	22	
Thermometer ID	PB	PB						
Acceptance Criteria	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C

Alkalinity								
Blank (<5mg/L)			<5mg/L					
STD Result			102					
T.V. / %REC			100/102%					
Acceptance Criteria			93.5-108.5% Recovery					

Hardness								
Blank (<2mg/L)			<2mg/L					
STD. Result			96					
T.V. / %REC			100/96%					
Acceptance Criteria			90.0-105.5% Recovery					

Conductivity Meter ID	Con 02							
Blank (<1)			<1					
STD Result			1438					
T.V. / %REC			1412	102%				
Acceptance Criteria			99.2-104.0% Recovery					

Chlorine Meter ID	DR820							
Blank (<0.05mg/L)			<0.05mg/L					
STD Result			0.21					
T.V. / % REC			0.21/0.21%					
Acceptance Criteria			100.0-120% Recovery					

Revision 0
Effective Date 01APR15

Biomonitoring Quality Control Benchsheet

Analyst	RT	HE	SH	RT	RT	RT	RT	
Date	10-16-15	10-17-15	10-18-15	10-19-15	10-20-15	10-21-15	10-22-15	
pH Meter ID	AR60							
LIN pH 4 Buffer	1501243							
LIN pH 7 Buffer	1501244							
LIN pH 10 Buffer	1501245							
Slope (>90%)	96.3%	99.4	97.8%	98.0%	98.5%	98.1%	97.6%	

Dissolved O ₂ Meter	PO 1305							
Meter Reading	8.65	8.90	8.64	8.63	8.72	8.70	8.52	
Temp.	23	21.1	23	23	22	22	23	
Chart Value at Temp.	8.578	8.743	8.578	8.578	8.743	8.743	8.578	
Difference	0.088	0.157	0.078	0.063	0.023	0.043	0.058	
Acceptance Criteria	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L	<0.2mg/L

Temp. Meter ID	AR60							
Meter Reading	22	23	23	23	22	22	22	
Thermometer Reading	23	23	23	22	22	21	22	
Thermometer ID	PB							
Acceptance Criteria	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C	±1°C

Alkalinity								
Blank (<5mg/L)					<5mg/L			
STD Result					94'			
T.V. / %REC					100/94%			
Acceptance Criteria	93.5-108.5% Recovery							

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

Conductivity Meter ID								
Blank (<1)					<1			
STD Result					1941			
T.V. / %REC					1412/102%			
Acceptance Criteria	99.2-104.0% Recovery							

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

Revision 0
Effective Date 01APR15

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Fathead Minnow

Lab # / Sample ID K1510002

Test Start (Date/Time) 10-13-15 1410

Client: Stv Hgart

Test End (Date/Time) 10-20-15 0930

Day of Test

		1	2	3	4	5	6	7	notes
Control	mHS	10-13	10-14	10-15	10-16	10-17	10-18	10-19	
D.O. (mg/L)	INITIAL	8.9	8.4	8.4	8.7	8.6	8.8	8.7	
	FINAL	7.5	7.2	7.3	6.9	7.5	7.3	7.3	
pH (s.u.)	INITIAL	7.9	7.7	7.7	7.8	7.7	8.0	8.2	
	FINAL	7.1	7.0	7.4	7.4	7.6	8.0	7.6	
temp (C)	INITIAL	23	22	22.9	23	24	20	23	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		62						60	
HARDNESS (mg/L)		96						82	
CONDUCTIVITY (umhd)		471						429	
CHLORINE (mg/L)		10.05							
CONC:	32								
D.O. (mg/L)	INITIAL	8.0	8.6	8.6	8.7	8.8	8.7	8.5	
	FINAL	7.3	7.8	7.3	6.4	7.3	7.1	7.5	
pH (s.u.)	INITIAL	7.7	7.4	7.3	7.3	7.7	7.8	7.3	
	FINAL	7.5	7.3	7.6	7.5	7.6	7.7	7.6	
temp (C)	INITIAL	22	22	22.5	23	22	22	23	
	FINAL	25	25	25	25	25	25	25	
CONC:	42								
D.O. (mg/L)	INITIAL	8.7	8.1	8.8	8.7	8.7	8.8	8.6	
	FINAL	7.4	7.5	7.2	6.9	8.0	7.7	7.9	
pH (mg/L)	INITIAL	7.7	7.3	7.9	7.5	7.7	7.7	7.4	
	FINAL	7.7	7.4	7.6	7.6	7.7	7.7	7.7	
temp (C)	INITIAL	21	22	22.4	23	22	23	24	
	FINAL	25	25	25	25	25	25	25	
CONC:	56								
D.O. (mg/L)	INITIAL	8.1	8.6	8.7	8.6	8.7	8.8	8.9	
	FINAL	7.1	7.4	7.4	6.2	8.2	7.8	7.4	
pH (s.u.)	INITIAL	7.6	7.4	7.4	7.5	7.7	7.7	7.5	
	FINAL	7.7	7.6	7.9	7.8	7.8	7.9	7.7	
temp (C)	INITIAL	21	22	22.2	23	23	24	24	
	FINAL	25	25	25	25	25	25	25	
CONC:	75								
D.O. (mg/L)	INITIAL	8.9	8.6	8.7	8.6	8.6	8.6	9.1	
	FINAL	7.0	6.2	7.5	6.1	7.6	7.7	7.3	
pH (s.u.)	INITIAL	7.6	7.4	7.9	7.6	7.7	7.7	7.5	
	FINAL	7.8	8.1	8.0	7.9	7.9	8.0	7.9	
temp (C)	INITIAL	20	22	22.2	23	23	25	25	
	FINAL	25	25	25	25	25	25	25	
CONC:	100								
D.O. (mg/L)	INITIAL	8.3	8.7	8.0	8.7	8.7	8.7	9.1	
	FINAL	7.4	6.2	7.4	8.1	7.8	7.7	7.3	
pH (s.u.)	INITIAL	7.6	7.4	7.3	7.6	7.7	7.6	7.5	
	FINAL	8.0	8.2	8.1	8.0	8.0	8.1	8.0	
temp (C)	INITIAL	20	20	22.3	22	23	25	25	
	FINAL	25	25	25	25	25	25	25	
CONC:	100 %	A	A	A	B	B	C	C	
ALKALINITY (mg/L)		230			208		230		
HARDNESS (mg/L)		208	168		134		144		
CONDUCTIVITY (umhd)		235	1291		1234		1188		
CHLORINE (mg/L)		10.05							

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Ceriodaphnia Dubia

Lab # / Sample ID K1510002

Test Start (Date/Time)

10-13-15 1030

Client: SNAgent

Test End (Date/Time)

10-20-15 0835

Day of Test

		1	2	3	4	5	6	7	notes
Control	mg HS	10-13	10-14	10-15	10-16	10-17	10-18	10-19	
D.O. (mg/L)	INITIAL	8.4	8.4	8.3	8.7	8.6	8.8	8.7	
	FINAL	8.2	8.1	8.2 8.2	8.0 8.2	7.7	8.5	8.0	
pH (s.u.)	INITIAL	7.9	7.7	7.2	7.2	7.7	8.0	8.2	
	FINAL	7.8	7.5	7.5 7.4	7.4 7.6	7.8	8.0	8.1	
temp (C)	INITIAL	23	23	22.9	23	21	20	25	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		62						60	
HARDNESS (mg/L)		96						82	
CONDUCTIVITY (umhc)		471						429	
CHLORINE (mg/L)		0.05							
CONC:	32								
D.O. (mg/L)	INITIAL	8.0	8.6	8.6	8.7	8.8	8.7	8.5	
	FINAL	8.3	8.2	8.0 8.0	8.0 8.0	7.9	8.5	8.1	
pH (s.u.)	INITIAL	7.7	7.4	7.3	7.3	7.7	7.8	7.3	
	FINAL	7.0	7.7	7.5 7.9	7.9 7.7	7.9	8.0	7.7	
temp (C)	INITIAL	22	22	22.5	23	22	22	23	
	FINAL	25	25	25	25	25	25	25	
CONC:	42								
D.O. (mg/L)	INITIAL	8.7	8.6	8.8	8.7	8.7	8.8	8.6	
	FINAL	8.3	8.2	8.3 8.3	8.3 8.3	7.9	8.4	8.2	
pH (mg/L)	INITIAL	7.7	7.3	7.4	7.5	7.7	7.7	7.4	
	FINAL	7.1	7.4	7.4 8.0	8.0 7.8	7.7	7.9	7.7	
temp (C)	INITIAL	21	22	22.4	23	22	23	24	
	FINAL	25	25	25	25	25	25	25	
CONC:	56								
D.O. (mg/L)	INITIAL	8.1	8.6 8.6	8.7	8.6	8.7	8.8	8.9	
	FINAL	8.3	8.3	8.4 8.4	8.5 8.4	8.0	8.5	8.2	
pH (s.u.)	INITIAL	7.6	7.4	7.4	7.5	7.7	7.7	7.5	
	FINAL	8.2	8.0	8.6 8.1	8.1 7.5	7.6	7.7	7.7	
temp (C)	INITIAL	21	22 22	22.4	23	23	24	24	
	FINAL	25	25	25	25	25	25	25	
CONC:	75								
D.O. (mg/L)	INITIAL	8.9	8.6	8.7	8.6	8.6	8.6	9.1	
	FINAL	8.3	8.2	8.3 8.3	8.3 8.3	7.9	8.4	8.3	
pH (s.u.)	INITIAL	7.6	7.4	7.4	7.6	7.7	7.7	7.5	
	FINAL	8.3	8.1	8.3 8.3	8.3 7.7	7.6	7.9	7.6	
temp (C)	INITIAL	20	22	22.2	23	23	25 25	25	
	FINAL	25	25	25	25	25	25	25	
CONC:	100								
D.O. (mg/L)	INITIAL	8.3	8.7	8.0	8.7	8.7	8.7	9.1	
	FINAL	8.3	8.2	8.2 8.2	8.1 8.1	8.0	8.4	8.3	
pH (s.u.)	INITIAL	7.6	7.4	7.3	7.6	7.7	7.6	7.5	
	FINAL	8.4	8.2	8.4 7.9	8.3 7.6	7.5	7.6	7.6	
temp (C)	INITIAL	20	22	22.3	22	23	25	25	
	FINAL	25	25	25	25	25	25	25	
CONC:	100 %	A	A	A	B	B	C	C	
ALKALINITY (mg/L)		230			208		230		
HARDNESS (mg/L)		168			134		144		
CONDUCTIVITY (umhc)		1291			1234		1188		
CHLORINE (mg/L)		0.05							

APPENDIX C

Fathead minnow raw data and statistics

SURVIVAL DATA FOR LARVAL SURVIVAL AND GROWTH TEST (CHRONIC)

LAB #: K1510002			TEST START		DATE	10/13/15	TIME	1410				
CLIENT: Stuttgart			TEST END		DATE	10/20/13	TIME	0930				
ANALYST: RH/ JB			AGE AND SOURCE OF MINNOWS		< 48 hrs old, Aquatox							
DAY(NUMBER SURVIVING)												
SURVIVAL												
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONTROL	A	10	10	10	10	10	9	9	9	90%	96.0%	5.71
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	9	9	9	9	9	9	90%		
	D	10	10	10	10	10	10	10	10	100%		
	E	10	10	10	10	10	10	10	10	100%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	10	10	10	10	10	10	100%	96.0%	
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	10	10	10	10	10	10	100%		
	D	10	10	9	9	9	9	9	9	90%		
	E	10	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%	96.0%	
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	10	10	10	10	10	10	100%		
	D	10	10	8	8	8	8	8	8	80%		
	E	10	10	10	10	10	10	10	10	100%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	10	10	10	10	10	10	100%	98.0%	
	B	10	10	10	10	10	10	10	9	90%		
	C	10	10	10	10	10	10	10	10	100%		
	D	10	10	10	10	10	10	10	10	100%		
	E	10	10	10	10	10	10	10	10	100%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	10	10	10	10	10	10	100%	100.0%	
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	10	10	10	10	10	10	100%		
	D	10	10	10	10	10	10	10	10	100%		
	E	10	10	10	10	10	10	10	10	100%		
	REP #	START	1	2	3	4	5	6	7	%	MEAN %	CV
CONC:	A	10	10	10	10	10	10	10	10	100%	100.0%	0.00
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	10	10	10	10	10	10	100%		
	D	10	10	10	10	10	10	10	10	100%		
	E	10	10	10	10	10	10	10	10	100%		
ANALYST:		RH	TB	TB	RH	RH	RH	RH	RH			
DATE:		10/13/15	10/14/15	10/15/15	10/16/15	10/17/15	10/18/15	10/19/15	10/20/15			
TIME:		1410	1500	1600	1050	1005	0915	09030	0930			

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

REMARKS:

AA# K1510002, FATHEAD MINNOW SURV.,CHRONIC, 10-13-15

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

Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.159

W = 0.792

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# K1510002, FATHEAD MINNOW SURV.,CHRONIC, 10-13-15

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

Transform: ARC SINE(SQUARE ROOT(Y))

Hartley's test for homogeneity of variance

Bartlett's test for homogeneity of variance

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

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

TITLE: AA# K1510002, FATHEAD MINNOW SURV.,CHRONIC, 10-13-15
 FILE: C:\COPYTO~1\TOXSTAT\FHSURV~1.
 TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.9000	1.2490
1	CONTROL	2	1.0000	1.4120
1	CONTROL	3	0.9000	1.2490
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	0.9000	1.2490
2	32 % EFFLUENT	5	0.9000	1.2490
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	0.8000	1.1071
3	42 % EFFLUENT	5	1.0000	1.4120
4	56 % EFFLUENT	1	1.0000	1.4120
4	56 % EFFLUENT	2	0.9000	1.2490
4	56 % EFFLUENT	3	1.0000	1.4120
4	56 % EFFLUENT	4	1.0000	1.4120
4	56 % EFFLUENT	5	1.0000	1.4120
5	75 % EFFLUENT	1	1.0000	1.4120
5	75 % EFFLUENT	2	1.0000	1.4120
5	75 % EFFLUENT	3	1.0000	1.4120
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# K1510002, FATHEAD MINNOW SURV.,CHRONIC, 10-13-15
 File: C:\COPYTO~1\TOXSTAT\FHSURV~1. Transform: ARC SINE(SQUARE ROOT(Y))

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	1.347				
2	32 % EFFLUENT	1.347	27.50	16.00	5.00	
3	42 % EFFLUENT	1.351	29.00	16.00	5.00	
4	56 % EFFLUENT	1.379	30.00	16.00	5.00	
5	75 % EFFLUENT	1.412	32.50	16.00	5.00	
6	100 % EFFLUENT	1.412	32.50	16.00	5.00	

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

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:		K1510002		TEST DATES (BEGIN / END):		10/13/15 - 10/20/15	
CLIENT:		Stuttgart		WEIGHING DATE / TIME:		10/21/2015 1330	
ANALYSTS:		RH/JB		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	0.99754	0.98927	0.00827	10	0.827	AVG DRY
	B	0.97685	0.96856	0.00829	10	0.829	WEIGHT (mg)
	C	0.98321	0.97733	0.00588	10	0.588	0.766
	D	0.99902	0.99115	0.00787	10	0.787	CV
	E	1.00770	0.99970	0.00800	10	0.800	13.2
32%	A	1.00838	0.99991	0.00847	10	0.847	AVG DRY
	B	1.04746	1.04030	0.00716	10	0.716	WEIGHT (mg)
	C	1.03063	1.02083	0.00980	10	0.980	0.784
	D	1.02513	1.01895	0.00618	10	0.618	CV
	E	1.00048	0.99291	0.00757	10	0.757	
42%	A	1.00940	1.00160	0.00780	10	0.780	AVG DRY
	B	1.04169	1.03432	0.00737	10	0.737	WEIGHT (mg)
	C	1.00066	0.99243	0.00823	10	0.823	0.742
	D	0.98727	0.98026	0.00701	10	0.701	CV
	E	0.97970	0.97299	0.00671	10	0.671	
56%	A	1.04317	1.03451	0.00866	10	0.866	AVG DRY
	B	0.99807	0.98936	0.00871	10	0.871	WEIGHT (mg)
	C	1.00310	0.99483	0.00827	10	0.827	0.858
	D	1.02042	1.01132	0.00910	10	0.910	CV
	E	0.98839	0.98025	0.00814	10	0.814	
75%	A	0.97997	0.97207	0.00790	10	0.790	AVG DRY
	B	1.01130	1.00378	0.00752	10	0.752	WEIGHT (mg)
	C	1.01943	1.01226	0.00717	10	0.717	0.797
	D	0.96940	0.95951	0.00989	10	0.989	CV
	E	0.97624	0.96886	0.00738	10	0.738	
100%	A	1.00919	0.99942	0.00977	10	0.977	AVG DRY
	B	0.97774	0.96680	0.01094	10	1.094	WEIGHT (mg)
	C	1.00308	0.99320	0.00988	10	0.988	0.987
	D	0.97362	0.96442	0.00920	10	0.920	CV
	E	0.99155	0.98201	0.00954	10	0.954	6.63

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA# K1510002, FATHEAD MINNOW GROWTH CHRONIC, 10-13-15
File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.203

W = 0.959

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# K1510002, FATHEAD MINNOW GROWTH CHRONIC, 10-13-15
File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 6.94

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# K1510002, FATHEAD MINNOW GROWTH CHRONIC, 10-13-15
FILE: C:\COPYTO~1\TOXSTAT\FHGROWTH.
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.8270	0.8270
1	CONTROL	2	0.8290	0.8290
1	CONTROL	3	0.5880	0.5880
1	CONTROL	4	0.7870	0.7870
1	CONTROL	5	0.8000	0.8000
2	32 % EFFLUENT	1	0.8470	0.8470
2	32 % EFFLUENT	2	0.7160	0.7160
2	32 % EFFLUENT	3	0.9800	0.9800
2	32 % EFFLUENT	4	0.6180	0.6180
2	32 % EFFLUENT	5	0.7570	0.7570
3	42 % EFFLUENT	1	0.7800	0.7800
3	42 % EFFLUENT	2	0.7370	0.7370
3	42 % EFFLUENT	3	0.8230	0.8230
3	42 % EFFLUENT	4	0.7010	0.7010

3	42 %	EFFLUENT	5	0.6710	0.6710
4	56 %	EFFLUENT	1	0.8660	0.8660
4	56 %	EFFLUENT	2	0.8710	0.8710
4	56 %	EFFLUENT	3	0.8270	0.8270
4	56 %	EFFLUENT	4	0.9100	0.9100
4	56 %	EFFLUENT	5	0.8140	0.8140
5	75 %	EFFLUENT	1	0.7900	0.7900
5	75 %	EFFLUENT	2	0.7520	0.7520
5	75 %	EFFLUENT	3	0.7170	0.7170
5	75 %	EFFLUENT	4	0.9890	0.9890
5	75 %	EFFLUENT	5	0.7380	0.7380
6	100 %	EFFLUENT	1	0.9770	0.9770
6	100 %	EFFLUENT	2	1.0940	1.0940
6	100 %	EFFLUENT	3	0.9880	0.9880
6	100 %	EFFLUENT	4	0.9200	0.9200
6	100 %	EFFLUENT	5	0.9540	0.9540

AA# K1510002, FATHEAD MINNOW GROWTH CHRONIC, 10-13-15
 File: C:\COPYTO~1\TOXSTAT\FHGGROWTH. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.199	0.040	4.722
Within (Error)	24	0.203	0.008	
Total	29	0.402		

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

AA# K1510002, FATHEAD MINNOW GROWTH CHRONIC, 10-13-15
 File: C:\COPYTO~1\TOXSTAT\FHGGROWTH. 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	0.766	0.766		
2	32 % EFFLUENT	0.784	0.784	-0.299	
3	42 % EFFLUENT	0.742	0.742	0.409	
4	56 % EFFLUENT	0.858	0.858	-1.572	
5	75 % EFFLUENT	0.797	0.797	-0.533	
6	100 % EFFLUENT	0.987	0.987	-3.791	

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

AA# K1510002, FATHEAD MINNOW GROWTH CHRONIC, 10-13-15
 File: C:\COPYTO~1\TOXSTAT\FHGGROWTH. 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	0.137	17.9	-0.017
3	42 % EFFLUENT	5	0.137	17.9	0.024
4	56 % EFFLUENT	5	0.137	17.9	-0.091
5	75 % EFFLUENT	5	0.137	17.9	-0.031
6	100 % EFFLUENT	5	0.137	17.9	-0.220

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

SURVIVAL AND REPRODUCTION TEST

Ceriodaphnia dubia												Lab Number/s				Analyst: RH															
Discharger: Stuttgart												K1510002				Test Start - Date/Time: 10-13-15, 1030															
Location: Outfall 001																Test Stop - Date/Time: 10-20-15, 0835															
Date Sample Collected: 10 - 12/14/16 - 15																															
Conc	1	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	Conc	4	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J					%	Day	A	B	C	D	E	F	G	H	I	J				
MHS	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH	56%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH		2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	3	0	0	0	0	0	0	0	0	0	1	0	1	0.1	RH		3	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	4	6	5	0	3	0	7	0	1	6	4	32	10	3.2	RH		4	8	0	1	5	0	7	2	2	0	0	25	10	2.5	RH
	5	0	1	2	2	6	0	8	5	6	8	38	10	3.8	RH		5	3	8	2	0	0	1	8	5	6	9	42	10	4.2	RH
	6	7	10	0	8	0	9	8	5	1	7	55	10	5.5	RH		6	8	0	13	5	0	1	6	0	0	10	43	10	4.3	RH
	7	0	0	7	3	12	1	5	0	5	0	33	10	3.3	RH		7	0	0	1	0	9	5	0	8	2	1	26	10	2.6	RH
	8																8														
	Total	13	16	9	16	18	17	21	11	19	19	159		Avg. = 15.9		Total	19	8	17	10	9	14	16	15	8	20	136		Avg. = 13.6		
														C.V. = 24.0																C.V. = 33.5	
Conc	2	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	Conc	5	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J					%	Day	A	B	C	D	E	F	G	H	I	J				
32%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH	75%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH		2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	3	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH		3	0	1	0	0	0	0	1	0	0	0	2	10	0.2	RH
	4	2	0	3	X	0	0	1	6	5	2	19	10	1.9	RH		4	7	9	1	8	0	5	2	0	6	3	41	10	4.1	RH
	5	9	10	0		4	0	7	2	5	3	40	10	4.0	RH		5	7	8	8	0	X	0	8	10	9	5	55	10	5.5	RH
	6	0	0	0		5	12	5	8	7	0	37	10	3.7	RH		6	0	0	3	8		0	0	0	0	12	23	10	2.3	RH
	7	1	0	4		5	0	5	0	1	9	25	10	2.5	RH		7	0	2	3	1		6	0	4	5	0	21	10	2.1	RH
	8																8														
	Total	12	10	7	0	14	12	18	16	18	14	121		Avg. = 13.4		Total	14	20	15	17	0	11	11	14	20	20	142		Avg. = 15.8		
					X									C.V. = 27.1							X									C.V. = 23.2	
Conc	3	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst	Conc	6	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J					%	Day	A	B	C	D	E	F	G	H	I	J				
42%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH	100%	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH		2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	3	0	1	0	0	0	0	0	0	0	0	1	10	0.1	RH		3	1	0	0	0	0	0	0	0	0	1	2	10	0.2	RH
	4	3	4	0	2	0	1	0	5	6	7	28	10	2.8	RH		4	7	1	8	2	5	1	8	0	5	6	43	10	4.3	RH
	5	7	6	8	0	X	0	12	9	0	3	45	10	4.5	RH		5	6	4	0	9	8	1	1	5	9	7	50	10	5.0	RH
	6	0	0	0	12		7	0	6	2	3	30	10	3.0	RH		6	1	0	12	0	0	11	0	10	0	0	34	10	3.4	RH
	7	0	0	0	6		5	1	5	2	0	19	10	1.9	RH		7	0	5	0	3	0	0	9	5	0	5	27	10	2.7	RH
	8																8														
	Total	10	11	8	20	0	13	13	25	10	13	123		Avg. = 13.7		Total	15	10	20	14	13	13	18	20	14	19	156		Avg. = 15.6		
						X								C.V. = 39.7																C.V. = 22.0	

AA # K1510002, C.DUBIA CHRONIC, REPRODUCCION, 10-13-15
File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

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

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

Total number of replicates = 60

AA # K1510002, C.DUBIA CHRONIC, REPRODUCCION, 10-13-15
File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 5.74

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

Data PASS B1 homogeneity test at 0.01 level. Continue analysis.

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
32	9	1	10
TOTAL	19	1	20

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

FISHER'S EXACT TEST

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

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

FISHER'S EXACT TEST

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

TOTAL 20 0 20

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

FISHER'S EXACT TEST

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

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

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
100	10	0	10
TOTAL	20	0	20

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

SUMMARY OF FISHER'S EXACT TESTS

NUMBER	NUMBER	SIG
--------	--------	-----

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

TITLE: AA # K1510002, C.DUBIA CHRONIC, REPRODUCTION, 10-13-15
FILE: C:\COPYTO~1\TOXSTAT\C.DUB
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	13.0000	13.0000
1	CONTROL	2	16.0000	16.0000
1	CONTROL	3	9.0000	9.0000
1	CONTROL	4	16.0000	16.0000
1	CONTROL	5	18.0000	18.0000
1	CONTROL	6	17.0000	17.0000
1	CONTROL	7	21.0000	21.0000
1	CONTROL	8	11.0000	11.0000
1	CONTROL	9	19.0000	19.0000
1	CONTROL	10	19.0000	19.0000
2	32 % EFFLUENT	1	12.0000	12.0000
2	32 % EFFLUENT	2	10.0000	10.0000
2	32 % EFFLUENT	3	7.0000	7.0000
2	32 % EFFLUENT	4	0.0000	0.0000
2	32 % EFFLUENT	5	14.0000	14.0000
2	32 % EFFLUENT	6	12.0000	12.0000
2	32 % EFFLUENT	7	18.0000	18.0000
2	32 % EFFLUENT	8	16.0000	16.0000
2	32 % EFFLUENT	9	18.0000	18.0000
2	32 % EFFLUENT	10	14.0000	14.0000
3	42 % EFFLUENT	1	10.0000	10.0000
3	42 % EFFLUENT	2	11.0000	11.0000
3	42 % EFFLUENT	3	8.0000	8.0000
3	42 % EFFLUENT	4	20.0000	20.0000
3	42 % EFFLUENT	5	0.0000	0.0000
3	42 % EFFLUENT	6	13.0000	13.0000
3	42 % EFFLUENT	7	13.0000	13.0000
3	42 % EFFLUENT	8	25.0000	25.0000
3	42 % EFFLUENT	9	10.0000	10.0000
3	42 % EFFLUENT	10	13.0000	13.0000
4	56 % EFFLUENT	1	19.0000	19.0000
4	56 % EFFLUENT	2	8.0000	8.0000
4	56 % EFFLUENT	3	17.0000	17.0000
4	56 % EFFLUENT	4	10.0000	10.0000
4	56 % EFFLUENT	5	9.0000	9.0000
4	56 % EFFLUENT	6	14.0000	14.0000
4	56 % EFFLUENT	7	16.0000	16.0000
4	56 % EFFLUENT	8	15.0000	15.0000

4	56 %	EFFLUENT	9	8.0000	8.0000
4	56 %	EFFLUENT	10	20.0000	20.0000
5	75 %	EFFLUENT	1	14.0000	14.0000
5	75 %	EFFLUENT	2	20.0000	20.0000
5	75 %	EFFLUENT	3	15.0000	15.0000
5	75 %	EFFLUENT	4	17.0000	17.0000
5	75 %	EFFLUENT	5	0.0000	0.0000
5	75 %	EFFLUENT	6	11.0000	11.0000
5	75 %	EFFLUENT	7	11.0000	11.0000
5	75 %	EFFLUENT	8	14.0000	14.0000
5	75 %	EFFLUENT	9	20.0000	20.0000
5	75 %	EFFLUENT	10	20.0000	20.0000
6	100 %	EFFLUENT	1	15.0000	15.0000
6	100 %	EFFLUENT	2	10.0000	10.0000
6	100 %	EFFLUENT	3	20.0000	20.0000
6	100 %	EFFLUENT	4	14.0000	14.0000
6	100 %	EFFLUENT	5	13.0000	13.0000
6	100 %	EFFLUENT	6	13.0000	13.0000
6	100 %	EFFLUENT	7	18.0000	18.0000
6	100 %	EFFLUENT	8	20.0000	20.0000
6	100 %	EFFLUENT	9	14.0000	14.0000
6	100 %	EFFLUENT	10	19.0000	19.0000

AA # K1510002, C.DUBIA CHRONIC, REPRODUCCION, 10-13-15
 File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	128.550	25.710	0.972
Within (Error)	54	1428.300	26.450	
Total	59	1556.850		

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

AA # K1510002, C.DUBIA CHRONIC, REPRODUCCION, 10-13-15
 File: C:\COPYTO~1\TOXSTAT\C.DUB Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	15.900	15.900		
2	32 % EFFLUENT	12.100	12.100	1.652	
3	42 % EFFLUENT	12.300	12.300	1.565	
4	56 % EFFLUENT	13.600	13.600	1.000	
5	75 % EFFLUENT	14.200	14.200	0.739	
6	100 % EFFLUENT	15.600	15.600	0.130	

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

AA # K1510002, C.DUBIA CHRONIC, REPRODUCTION, 10-13-15

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

Transform: NO TRANSFORMATION

DUNNETT'S TEST

TABLE 2 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	10			
2	32 % EFFLUENT	10	5.313	33.4	3.800
3	42 % EFFLUENT	10	5.313	33.4	3.600
4	56 % EFFLUENT	10	5.313	33.4	2.300
5	75 % EFFLUENT	10	5.313	33.4	1.700
6	100 % EFFLUENT	10	5.313	33.4	0.300

APPENDIX E

Organism History

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

TEST ORGANISM HISTORY

DATE SHIPPED 10/13/15 CLIENT ARV ANALYTICAL

Purchase Order #: _____

SPECIES: Pimephales promelas

Quantity Shipped: 550^T 15-1600
EST

Age: HATCHED 10/11/15

Brood Stock Source: Anderson Farms, AR

Culture Water: Groundwater

Hardness (Mg/l CaCO₃): =160

Dissolved Oxygen (Mg/l): 8.5

Temperature (°C): 25.4

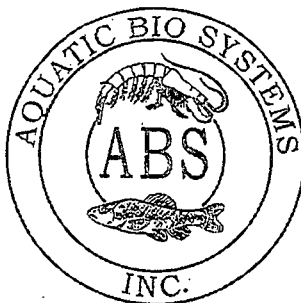
Feeding: ARTEMIA

Comments: _____

Shipped Via: Federal Express UPS Overnight Shuttle

Packaged By: _____

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



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

ORGANISM HISTORY

DATE: 11/25/2013

SPECIES: Ceriodaphnia dubia

AGE: > 3 day

LIFE STAGE: Adult

HATCH DATE: Variable

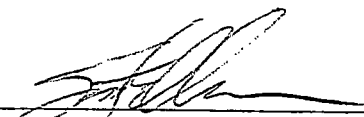
BEGAN FEEDING: Immediately

FOOD: YTC, Selenastrum sp.

Water Chemistry Record:

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

Comments:

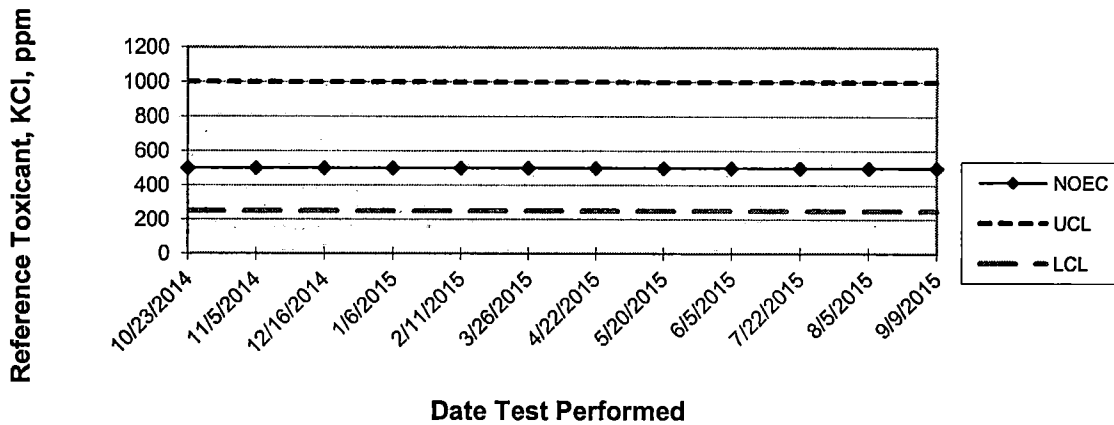


Facility Supervisor

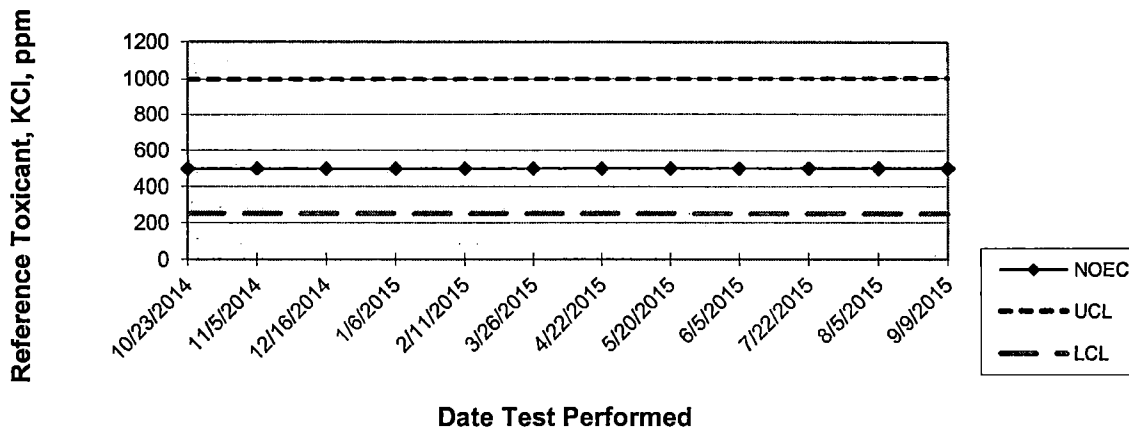
APPENDIX F

Quality Assurance Charts

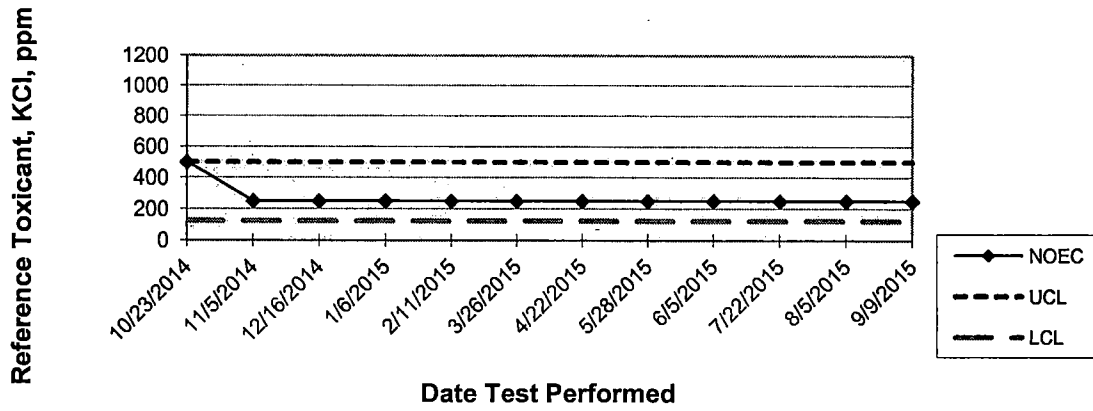
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW SURVIVAL 7 Day
QUALITY ASSURANCE



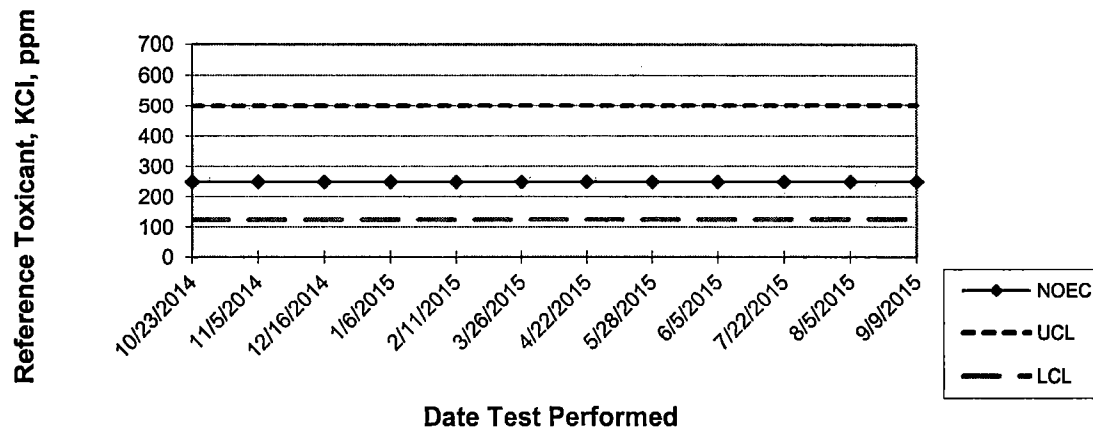
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW GROWTH 7 Day
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE



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

Hasler

FIRST-CLASS MAIL

11/12/2015

US POSTAGE \$002.52⁰



ZIP 72160
011E10672532

ARKANSAS DEPARTMENT OF ENVIRONMENTAL QUALITY
MARY BARNETT, WATER DIVISION
5301 NORTSHORE DRIVE
NORTH LITTLE ROCK, AR 72118-5317