Arkansas Analytical, Inc.

Toxicity Test Results

City of Wynne
NPDES PERMIT NUMBER: AR0021903
Fourth Quarter 2015

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

Ceriodaphnia dubia, Survival and Reproduction Test
Test 1002.0

Prepared for: **Harrel Williams**
Wynne Water Utilities
121 East Merriman
Wynne, AR 72396

Prepared by: Arkansas Analytical, Inc
11701 I-30, Bldg 1 Suite 115
Little Rock, Arkansas 72209
Lab Number K1510001

Thursday, October 22, 2015

Introduction

This report contains test results for the toxicity testing of Wynne facility. The NPDES permit number is AR0021903. The plant located in Wynne, Arkansas,
is authorized to discharge treated municipal wastewater from the facility located as follows: approximately 1.5 miles West of Wynne on Hwy 284 to Bowden Road, thence south on Bowden Road approximately 0.25 mile in Cross County, Arkansas. The applicant's mailing address is: 121 East Merriman Avenue, Wynne, AR 72396.

Facility Coordinates: Latitude: 35° 13' 8.81"; Longitude: 90° 49' 41.25"

The permitted outfall is located at the following coordinates:

Outfall 001: Latitude: 35° 13' *OT';* Longitude: 90° 49' 52"

The permit requires chronic biomonitoring testing semi annually 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, Time Started	Date, Time Ended
Sample #1:	10-11-15, 0700	10-12-15, 0700
Sample #2:	10-13-15, 0700	10-14-15, 0700
Sample #3:	10-15-15, 0700	10-16-15, 0700

Samples were composites collected at 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, 1620	8 (on ice)
Sample #3:	10-16-15, 1549	6

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. However, due to its earlier characterization as toxic, synthetic 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, 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 mls of test solution. The test temperature was 25 degrees Centigrade. Raw data and statistics are provided in Appendix D.

Test Organisms

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

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

Quality Assurance

Test Acceptability

TEST ACCEPTANCE CRITERIA for *Ceriodaphnia dubia*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	90%	X	
Average of 15 or more young per surviving female	16.2	X	
At least 60% of surviving females should have produced 3 broods	80%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	19.7%	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

Ceriodaphnia dubia 9/9/15 – 9/16-15		*Pimephales promelas* 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 Wynne

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)	15.5	%CV survival (critical dilution)	5.83%
%CV Reproduction (critical dilution)	22.0%	Mean dry weight (critical dilution)	0.829
PMSD Reproduction	35.4%	%CV growth (critical dilution)	12.3%
		PMSD Growth	16.5 %

Conclusion

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

The permit issued to Wynne, 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 Wynne, specifies 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.

Biomonitoring Analyst:

Ryan Hudgin / Teresa Coins / Hallie Freyaldenhoven

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 Wynne **NPDES #: AR0021903**

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	10-11-15, 0700	10-12-15, 0700
Sample #2:	10-13-15, 0700	10-14-15, 0700
Sample #3:	10-15-15, 0700	10-16-15, 0700

Test initiated (date, time): 10-13-15, 1430 Test terminated (date, time): 10-20-15, 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%	90	100	90	100	100		100	98	96	5.71
32%	90	90	80	90	100		100	96	90	
42%	100	100	100	100	100		100	100	100	
56%	100	100	100	100	90		100	100	98	
75%	100	100	100	100	100		100	100	100	
100%	100	90	90	100	90		98	98	94	5.83

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%	0.827	0.829	0.588	0.787	0.800		0.766	13.2
32%	0.812	0.860	0.907	0.704	0.923		0.841	
42%	0.913	0.975	0.982	0.952	0.867		0.938	
56%	0.828	0.818	0.948	0.998	0.789		0.876	
75%	0.850	0.849	0.820	0.819	0.977		0.863	
100%	0.868	0.790	0.684	0.960	0.844		0.829	12.3

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 Wynne **NPDES #:AR0021903**

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	10-11-15, 0700	10-12-15, 0700
Sample #2:	10-13-15, 0700	10-14-15, 0700
Sample #3:	10-15-15, 0700	10-16-15, 0700

Test initiated (date, time): 10-13-15, 1000 Test terminated (date, time): 10-20-15, 0910

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	X0	16	13	16	16	10
B	17	8	10	10	12	18
C	13	9	13	12	15	11
D	11	17	9	18	X0	15
E	19	X0	7	16	21	19
F	13	17	13	14	7	17
G	20	15	19	11	9	21
H	16	18	21	16	20	15
I	18	15	17	12	18	15
J	19	14	9	12	17	14
Mean	14.6	12.9	13.1	13.7	13.5	15.5
Mean/surviving female	16.2	14.3	13.1	13.7	15.0	15.5
CV%*	19.7					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 Wynne **NPDES #: AR0021903**

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	90	90	100	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)= _____**22.0**_____%

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

ARKANSAS ANALYTICAL, INC.
8100 NATIONAL DRIVE, LITTLE ROCK, AR 72209
(501) 562-8139 (800) 331-8139
FAX # (501) 562-7025

CHAIN OF CUSTODY RECORD

PAGE ____ OF ____

TURNAROUND TIME
RUSH 24HR. 48HR.
5 DAY REG.
OTHER:

FOR LAB/OFFICE USE ONLY
LAB # 18482-001
CLIENT # ____
P. O. # ____

STANDARD METHODS PRESERVATION PER EPA 40 CFR
C 4 = COOL TO 4.0 C
S<2 = SULFURIC ACID TO PH < 2
N<2 = NITRIC ACID TO PH > 2
T = THIOSULFATE
W = AZIDE MODIFICATION (4500-0 C)
P = MEMBRANE ELECTRODE (4500-0 G)
NaOH = Ph > 12

NAME OF COMPANY, CITY, OR PROJECT: WYNNE WATER UTILITIES

PROJECT NO:

SAMPLER(S) SIGNATURE/PRINT: Harrell Williams (HARRELL WILLIAMS)

SAMPLE NO.	SAMPLE COLLECTION LOCATION	START DATE/TIME	END DATE/TIME	COMP/GRAB	FIELD ANALYSIS PH	TEMP	FLOW	CL2	D.O. (W) D.O. (P)	CONTAINER TYPE PRESERVATIVE	ANALYSIS REQUIRED
1	POST AERATION BASIN OUTFALL	10/11//15 7:00:00 AM	10/12/14 7:00 AM	COMP/24			0.713			6 - 1/2 GAL	BIO-MONITORING
											K1510001A

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

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

FIELD CALIBRATION RECORD

PH 7	
PH 4	
PH 10	
D. O.	

	Yes	No
Custody Seals:		
Containers Correct:		
COC/Labels Agree:		
Received on Ice:	✓	

Temperature on Receipt: 8°C
Temperature Gun ID: HHT # 2

FIELD ANALYSIS CONDUCTED BY: AA CLIENT

RELINQUISHED BY: Harrell Williams	DATE/TIME 10/12/15 1550	RECEIVED BY:	DATE/TIME
RELINQUISHED BY:	DATE/TIME 10/12/15	RECEIVED BY: Sydney James	DATE/TIME 10/12/15 1550

ARKANSAS ANALYTICAL, INC.
8100 NATIONAL DRIVE, LITTLE ROCK, AR 72209
(501) 562-8139 (800) 331-8139
FAX # (501) 562-7025

CHAIN OF CUSTODY RECORD

PAGE ____ OF ____

TURNAROUND TIME
RUSH 24HR. 48HR.
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 4.0 C
S<2 = SULFURIC ACID TO PH < 2
N<2 = NITRIC ACID TO PH > 2
T = THIOSULFATE
W = AZIDE MODIFICATION (4500-0 C)
P = MEMBRANE ELECTRODE (4500-0 G)
NaOH = Ph > 12

NAME OF COMPANY, CITY, OR PROJECT: WYNNE WATER UTILITIES

PROJECT NO:

SAMPLER(S) SIGNATURE/PRINT: Harrell Williams (HARRELL WILLIAMS)

SAMPLE NO.	SAMPLE COLLECTION LOCATION	START DATE/TIME	END DATE/TIME	COMP/GRAB	FIELD ANALYSIS PH	FIELD ANALYSIS TEMP	FLOW	CL2	D.O. (W) / D.O. (P)	CONTAINER TYPE / PRESERVATIVE	ANALYSIS REQUIRED
1	POST AERATION BASIN OUTFALL	10/13//15 7:00:00 AM	10/14/14 7:00 AM	COMP/24			0.689			6 - 1/2 GAL	BIO-MONITORING
											K1510-001B

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

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

FIELD CALIBRATION RECORD
PH 7
PH 4
PH 10
D. O.

NOTES/COMMENTS/OBSERVATIONS

	Yes	No
Custody Seals:	✓	
Containers Correct:		
COC/Labels Agree:		
Received on Ice:		

Temperature on Receipt: 8°C
Temperature Gun ID: HHT # 2

FIELD ANALYSIS CONDUCTED BY: (AA) / CLIENT

RELINQUISHED BY: Harrell Williams DATE/TIME

RECEIVED BY: DATE/TIME

RELINQUISHED BY: DATE/TIME 10/14/15 1600

RECEIVED BY: Sydney James DATE/TIME 10/14/15 1620

ARKANSAS ANALYTICAL, INC.
8100 NATIONAL DRIVE, LITTLE ROCK, AR 72209
(501) 562-8139 (800) 331-8139
FAX # (501) 562-7025

CHAIN OF CUSTODY RECORD

PAGE ____ OF ____

TURNAROUND TIME
RUSH 24HR. 48HR.
5 DAY REG.
OTHER:

FOR LAB/OFFICE USE ONLY
LAB # 18482.0003
CLIENT # ____
P. O. # ____

STANDARD METHODS PRESERVATION PER EPA 40 CFR
C 4 = COOL TO 4.0 C
S<2 = SULFURIC ACID TO PH < 2
N<2 = NITRIC ACID TO PH > 2
T = THIOSULFATE
W = AZIDE MODIFICATION (4500-0 C)
P = MEMBRANE ELECTRODE (4500-0 G)
NaOH = Ph > 12

NAME OF COMPANY, CITY, OR PROJECT: WYNNE WATER UTILITIES

PROJECT NO:

SAMPLER(S) SIGNATURE/PRINT: Harrell Williams (HARRELL WILLIAMS)

SAMPLE NO.	SAMPLE COLLECTION LOCATION	START DATE/TIME	END DATE/TIME	COMP/GRAB	FIELD ANALYSIS PH	TEMP	FLOW	CL2	D.O. (W) D.O. (P)	CONTAINER TYPE PRESERVATIVE	ANALYSIS REQUIRED
1	POST AERATION BASIN OUTFALL	10/15//15 7:00:00 AM	10/16/14 7:00 AM	COMP/24			0.661			6 - 1/2 GAL	BIO-MONITORING
											K1510001 C

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

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

FIELD CALIBRATION RECORD
PH 7
PH 4
PH 10
D. O.

NOTES/COMMENTS/OBSERVATIONS

	Yes	No
Custody Seals:		
Containers Correct:	√	
COC/Labels Agree:	√	
Received on Ice:	√	

Temperature on Receipt: 6'C
Temperature Gun ID: HHT # 2

FIELD ANALYSIS CONDUCTED BY: (AA) CLIENT

RELINQUISHED BY: Harrell Williams	DATE/TIME 1545	RECEIVED BY:	DATE/TIME 10-16-15 1330
RELINQUISHED BY:	DATE/TIME 10-16-15	RECEIVED BY: Sydney James	DATE/TIME 10-16-15, 1349

APPENDIX B

Effluent and Dilution Water Data

Biomonitoring Quality Control Benchsheet

Analyst	RH	JB	RH	RH	RH	RH	RH	RH
Date	9-26-15	27SEP15	9-28-15	9-29-15	9-30-15	10-13-15	10-14-15	10-15-15
pH Meter ID	AR60	X60						
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	DO 1305							
Meter Reading	8.59	8.54	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	8.418	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	X60						
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	Cond 2							
Blank (<1)			<1					
STD Result			1438					
T.V. / %REC			1412	102%				
Acceptance Criteria	99.2-104.0% Recovery							

Chlorine Meter ID	DR890							
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

Biomonitoring Quality Control Benchsheet

Analyst	RH	HF	RH	RH	RH	RH	RH	
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	DO 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	23	
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					99			
T.V. / %REC					100/99%			
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					1431			
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							

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING									Fathead Minnow
Lab # / Sample ID: K15 10001					Test Start (Date/Time):		10-13-15		1430
Client: Wynne					Test End (Date/Time):		10-20-15		1000
		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	8.4	8.4	8.2	8.7	8.6	8.8	8.7	
	FINAL	7.2	7.8	7.3	8.5	8.0	7.5	7.3	
pH (s.u.)	INITIAL	7.9	7.7	7.2	7.2	7.7	8.0	8.2	
	FINAL	7.1	7.0	7.4	7.4	7.7	8.0	7.6	
temp (C)	INITIAL	23	23	22.9	23	21	20	23	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		62						60	
HARDNESS (mg/L)		96						82	
CONDUCTIVITY (umho		471						429	
CHLORINE (mg/L)		<0.05							
CONC:	32								
D.O. (mg/L)	INITIAL	8.6	8.6	8.6	8.7	8.8	8.8	8.4	
	FINAL	7.4	7.3	7.8	7.8	8.0	7.8	7.5	
pH (s.u)	INITIAL	7.6	7.5	7.2	7.1	7.7	7.6	7.6	
	FINAL	7.2	7.7	7.5	7.5	7.7	7.9	7.8	
temp (C)	INITIAL	21	23	22.9	23	23	23	23	
	FINAL	25	25	25	25	25	25	25	
CONC:	42								
D.O. (mg/L)	INITIAL	8.7	8.7	8.6	8.8	8.8	8.8	8.6	
	FINAL	7.3	7.3	8.0	8.0	7.9	8.0	7.7	
pH (mg/L)	INITIAL	7.5	7.5	7.2	7.0	7.6	7.5	7.4	
	FINAL	7.2	7.6	7.4	7.5	7.6	7.7	7.6	
temp (C)	INITIAL	22	21	22.1	23	22	23	24	
	FINAL	25	25	25	25	25	25	25	
CONC:	56								
D.O. (mg/L)	INITIAL	8.9	8.9	8.6	9.0	8.9	8.8	8.8	
	FINAL	7.3	7.5	7.9	8.1	7.9	7.6	7.2	
pH (s.u.)	INITIAL	7.4	7.2	7.2	7.1	7.5	7.5	7.4	
	FINAL	7.2	7.25	7.4	7.5	7.6	7.6	7.5	
temp (C)	INITIAL	21	22	22.1	23	23	24	25	
	FINAL	25	25	25	25	25	25	25	
CONC:	75								
D.O. (mg/L)	INITIAL	9.1	9.0	9.1	9.0	8.9	8.9	9.0	
	FINAL	7.3	7.3	7.9	8.2	7.9	7.9	7.5	
pH (s.u.)	INITIAL	7.4	7.3	7.1	7.0	7.5	7.5	7.3	
	FINAL	7.2	7.4	7.3	7.4	7.6	7.5	7.5	
temp (C)	INITIAL	20	21	22.1	23	23	24	25	
	FINAL	25	25	25	25	25	25	25	
CONC:	100								
D.O. (mg/L)	INITIAL	9.1	9.1	9.1	8.6	9.1	8.9	9.2	
	FINAL	7.3	7.2	7.8	7.9	7.9	8.1	7.2	
pH (s.u.)	INITIAL	7.3	7.2	7.1	6.9	7.3	7.4	7.2	
	FINAL	7.2	7.3	7.4	7.4	7.4	7.4	7.4	
temp (C)	INITIAL	20	21	22.3	23	23	25	26	
	FINAL	25	25	25	25	25	25	25	
CONC:	100 %	A	A	A	B	B	C	C	
ALKALINITY (mg/L)		44			46		48		
HARDNESS (mg/L)		146			158		160		
CONDUCTIVITY (umho		684			704		713		
CHLORINE (mg/L)		<0.05							

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING									Ceriodaphnia Dubia
Lab # / Sample ID		K1510001			Test Start (Date/Time)		10-13-15		1000
Client:		Wynne			Test End (Date/Time)		10-20-15		0910
		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	8.4	8.4	9.1	8.7	8.4	8.8	8.7	
	FINAL	8.1	8.1	8.0	7.9	8.1	8.2	8.3	
pH (s.u.)	INITIAL	7.9	7.7	7.5 7.2	7.2	7.7	8.0	8.2	
	FINAL	7.6	7.5	7.3	7.6	7.6	8.0	8.1	
temp (C)	INITIAL	23	23	22.9	23	23	23	23	
	FINAL	25	25	25	25	25	25	25	
ALKALINITY (mg/L)		62						60	
HARDNESS (mg/L)		96						80	
CONDUCTIVITY (umhc		471						429	
CHLORINE (mg/L)		<0.05							
CONC:	32								
D.O. (mg/L)	INITIAL	8.6	8.6	8.4 8.6	8.7	8.8	8.8	8.6	
	FINAL	8.2	8.2	7.5	7.9	8.1	8.2	8.3	
pH (s.u)	INITIAL	7.6	7.5	7.2 7.2	7.1	7.7	7.6	7.6	
	FINAL	7.7	7.3	7.4	7.7	7.6	8.0	8.0	
temp (C)	INITIAL	21	23	22 23.3	23	23	23	23	
	FINAL	25	25	25	25	25	25	25	
CONC:	42								
D.O. (mg/L)	INITIAL	9.7	9.0	8.6	8.8	8.8	8.8	8.6	
	FINAL	8.2	8.2	8.3	8.2	8.2	8.3	8.3	
pH (mg/L)	INITIAL	7.5	7.5	7.2	7.0	7.6	7.5	7.4	
	FINAL	7.7	7.4	7.4	7.8	7.7	7.6	7.9	
temp (C)	INITIAL	22	21	22.1	23	22	23	24	
	FINAL	25	25	25	25	25	25	25	
CONC:	56								
D.O. (mg/L)	INITIAL	8.7	8.9	8.7	9.0	8.9	8.8	8.8	
	FINAL	8.2	8.3	8.2	8.2	8.2	8.3	8.3	
pH (s.u.)	INITIAL	7.4	7.2	7.2	7.1	7.5	7.5	7.4	
	FINAL	7.7	7.4	7.4	7.9	7.7	7.7	7.8	
temp (C)	INITIAL	21	22	22.1	23	23	24	25	
	FINAL	25	25	25	25	25	25	25	
CONC:	75								
D.O. (mg/L)	INITIAL	9.1	9.0	9.1	9.0	8.9	8.8	9.0	
	FINAL	8.2	8.2	8.0	8.4	8.2	8.3	8.4	
pH (s.u.)	INITIAL	7.4	7.0	7.1	7.6	7.5	7.5	7.3	
	FINAL	7.6	7.4	7.3	7.6	7.7	7.6	7.6	
temp (C)	INITIAL	20	21	22.	23	23	24	25	
	FINAL	25	25	25	25	25	25	25	
CONC:	100								
D.O. (mg/L)	INITIAL	9.1	9.1	9.1	8.6	9.1	8.9	9.2	
	FINAL	8.2	8.2	8.1	8.4	8.3	8.3	8.5	
pH (s.u.)	INITIAL	7.3	7.0	7.1	6.9	7.8	7.4	7.2	
	FINAL	7.6	7.4	7.3	7.6	7.5	7.7	7.5	
temp (C)	INITIAL	20	21	22.0	23	23	23	24	
	FINAL	25	25	25	25	25	25	25	
CONC:	100 %	A	A	A	B	B	C	C	
ALKALINITY (mg/L)		44			46		48		
HARDNESS (mg/L)		146			158		160		
CONDUCTIVITY (umhc		684			704		713		
CHLORINE (mg/L)		<0.05							

APPENDIX C

Fathead minnow raw data and statistics

Pimephales promelas FATHEAD MINNOW

SURVIVAL DATA FOR LARVAL SURVIVAL AND GROWTH TEST (CHRONIC)

LAB #: K1510001						TEST START		DATE	10/13/15	TIME	1430	
CLIENT: Wynne						TEST END		DATE	10/20/15	TIME	1000	
ANALYST: RH						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	9	9	9	9	90%		
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	9	9	9	9	9	9	90%	*96.0%*	*5.71*
MHS	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	9	9	9	90%		
	B	10	10	9	9	9	9	9	9	90%		
	C	10	10	10	9	9	8	8	8	80%	*90.0%*	
32%	D	10	10	9	9	9	9	9	9	90%		
	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%		
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	10	10	10	10	10	10	100%	*100.0%*	
42%	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%		
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	10	10	10	10	10	10	100%	*98.0%*	
56%	D	10	10	10	10	10	10	10	10	100%		
	E	10	10	10	10	10	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%		
	B	10	10	10	10	10	10	10	10	100%		
	C	10	10	10	10	10	10	10	10	100%	*100.0%*	
75%	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%		
	B	10	10	10	10	10	9	9	9	90%		
	C	10	9	9	9	9	9	9	9	90%	*94.0%*	*5.83*
100%	D	10	10	10	10	10	10	10	10	100%		
	E	10	10	10	10	10	9	9	9	90%		
ANALYST:		RH	RH	HF	RH	RH	TC	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:		1430	1545	1600	1120	1020	1249	1000	1000			

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

REMARKS:

Revision 0

5/1/2014

TITLE: AA# K1510001, 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	0.9000	1.2490
2	32 % EFFLUENT	2	0.9000	1.2490
2	32 % EFFLUENT	3	0.8000	1.1071
2	32 % EFFLUENT	4	0.9000	1.2490
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	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	0.9000	1.2490
6	100 % EFFLUENT	3	0.9000	1.2490
6	100 % EFFLUENT	4	1.0000	1.4120
6	100 % EFFLUENT	5	0.9000	1.2490

AA# K1510001, 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	1.253	21.50	16.00	5.00	
3	42 % EFFLUENT	1.412	32.50	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	1.314	25.00	16.00	5.00	

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

AA# K1510001, 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 = 0.132

W = 0.939

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# K1510001, 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.

Pimephales promelas **FATHEAD MINNOW** TEST 1000.0

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:	K151001				TEST DATES (BEGIN / END):		10/13/15 - 10/20/15	
CLIENT:	Wynne				WEIGHING DATE / TIME:		10/21/2015	
ANALYSTS:	RH				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)	
MHS	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
CONC:	A	0.98393	0.97581	0.00812	10	0.812	AVG DRY	
	B	0.98357	0.97497	0.00860	10	0.860	WEIGHT (mg)	
32%	C	0.97755	0.96848	0.00907	10	0.907		0.841
	D	1.01321	1.00617	0.00704	10	0.704	CV	
	E	0.99954	0.99031	0.00923	10	0.923		
CONC:	A	0.96165	0.95252	0.00913	10	0.913	AVG DRY	
	B	0.95775	0.94800	0.00975	10	0.975	WEIGHT (mg)	
42%	C	1.01257	1.00275	0.00982	10	0.982		0.938
	D	0.97474	0.96522	0.00952	10	0.952	CV	
	E	1.01873	1.01006	0.00867	10	0.867		
CONC:	A	0.98109	0.97281	0.00828	10	0.828	AVG DRY	
	B	0.97978	0.97160	0.00818	10	0.818	WEIGHT (mg)	
56%	C	1.02200	1.01252	0.00948	10	0.948		0.876
	D	1.01310	1.00312	0.00998	10	0.998	CV	
	E	1.01354	1.00565	0.00789	10	0.789		
CONC:	A	1.03019	1.02169	0.00850	10	0.850	AVG DRY	
	B	1.04445	1.03596	0.00849	10	0.849	WEIGHT (mg)	
75%	C	0.99543	0.98723	0.00820	10	0.820		0.863
	D	1.01263	1.00444	0.00819	10	0.819	CV	
	E	1.04297	1.03320	0.00977	10	0.977		
CONC:	A	0.99664	0.98796	0.00868	10	0.868	AVG DRY	
	B	1.04603	1.03813	0.00790	10	0.790	WEIGHT (mg)	
100%	C	1.00930	1.00246	0.00684	10	0.684		0.829
	D	1.01103	1.00143	0.00960	10	0.960	CV	
	E	1.03233	1.02389	0.00844	10	0.844		12.3

CV = (STANDARD DEVIATION/MEAN)*100

REMARKS:

AA# K1510001, FATHEAD MINNOW GROWTH CHRONIC, 10-13-15
File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.173

W = 0.970

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# K1510001, 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 = 2.68

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# K1510001, 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	0.8120	0.8120
2	32 % EFFLUENT	2	0.8600	0.8600
2	32 % EFFLUENT	3	0.9070	0.9070
2	32 % EFFLUENT	4	0.7040	0.7040
2	32 % EFFLUENT	5	0.9230	0.9230
3	42 % EFFLUENT	1	0.9130	0.9130
3	42 % EFFLUENT	2	0.9750	0.9750
3	42 % EFFLUENT	3	0.9820	0.9820
3	42 % EFFLUENT	4	0.9520	0.9520
3	42 % EFFLUENT	5	0.8670	0.8670
4	56 % EFFLUENT	1	0.8280	0.8280
4	56 % EFFLUENT	2	0.8180	0.8180
4	56 % EFFLUENT	3	0.9480	0.9480
4	56 % EFFLUENT	4	0.9980	0.9980
4	56 % EFFLUENT	5	0.7890	0.7890
5	75 % EFFLUENT	1	0.8500	0.8500
5	75 % EFFLUENT	2	0.8490	0.8490
5	75 % EFFLUENT	3	0.8200	0.8200
5	75 % EFFLUENT	4	0.8190	0.8190
5	75 % EFFLUENT	5	0.9770	0.9770
6	100 % EFFLUENT	1	0.8680	0.8680
6	100 % EFFLUENT	2	0.7900	0.7900
6	100 % EFFLUENT	3	0.6840	0.6840
6	100 % EFFLUENT	4	0.9600	0.9600
6	100 % EFFLUENT	5	0.8440	0.8440

AA# K1510001, FATHEAD MINNOW GROWTH CHRONIC, 10-13-15
File: C:\COPYTO~1\TOXSTAT\FHGROWTH. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.080	0.016	2.228
Within (Error)	24	0.173	0.007	
Total	29	0.253		

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

AA# K1510001, FATHEAD MINNOW GROWTH CHRONIC, 10-13-15
File: C:\COPYTO~1\TOXSTAT\FHGROWTH. 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	0.841	0.841	-1.396	
3	42 % EFFLUENT	0.938	0.938	-3.195	
4	56 % EFFLUENT	0.876	0.876	-2.048	
5	75 % EFFLUENT	0.863	0.863	-1.802	
6	100 % EFFLUENT	0.829	0.829	-1.173	

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

AA# K1510001, FATHEAD MINNOW GROWTH CHRONIC, 10-13-15
File: C:\COPYTO~1\TOXSTAT\FHGROWTH. 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	0.127	16.5	-0.075
3	42 % EFFLUENT	5	0.127	16.5	-0.172
4	56 % EFFLUENT	5	0.127	16.5	-0.110
5	75 % EFFLUENT	5	0.127	16.5	-0.097
6	100 % EFFLUENT	5	0.127	16.5	-0.063

APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

SURVIVAL AND REPRODUCTION TEST

Ceriodaphnia dubia

Discharger: Wynne	Lab Number/s	Analyst: RH
Location: Outfall 001	*K1510001*	Test Start - Date/Time: 10-13-15, 1000
Date Sample Collected: 10 - 12/14/16 - 15		Test Stop - Date/Time: 10-20-15, 0910

Conc	1	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	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
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	3	X	3	0	0	0	0	0	4	4	2	13	10	1.3	RH
	4		0	5	2	6	8	0	1	5	2	29	10	2.9	RH
	5		6	8	9	0	0	11	5	2	0	41	10	4.1	RH
	6		0	0	0	12	1	7	0	0	10	30	10	3.0	RH
	7		8	0	0	1	4	2	6	7	5	33	10	3.3	RH
	8														
	Total	0	17	13	11	19	13	20	16	18	19	146		Avg. =	16.2
		X												C.V. =	19.7

Conc	2	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	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
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0	RH
	3	2	0	0	0	0	4	3	4	3	2	18	10	1.8	RH
	4	7	6	1	0	0	4	8	7	6	0	39	10	3.9	RH
	5	0	0	8	5	X	0	3	1	0	8	25	10	2.5	RH
	6	0	0	0	10		0	0	6	5	3	24	10	2.4	RH
	7	7	2	0	2		9	1	0	1	1	23	10	2.3	RH
	8														
	Total	16	8	9	17	0	17	15	18	15	14	129		Avg. =	14.3
						X								C.V. =	24.7

Conc	3	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	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
	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	3	0	3	10	0.3	RH
	4	2	2	2	0	0	6	2	8	6	1	29	10	2.9	RH
	5	5	6	5	1	0	0	9	7	0	4	37	10	3.7	RH
	6	5	1	6	0	0	0	0	0	5	2	19	10	1.9	RH
	7	1	1	0	8	7	7	8	6	3	2	43	10	4.3	RH
	8														
	Total	13	10	13	9	7	13	19	21	17	9	131		Avg. =	13.1
														C.V. =	35.3

Conc	4	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
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
	3	5	0	0	6	0	0	6	2	0	5	24	10	2.4	RH
	4	5	1	0	2	1	0	5	6	3	2	25	10	2.5	RH
	5	0	9	12	2	8	9	0	0	0	5	45	10	4.5	RH
	6	5	0	0	7	1	2	0	8	0	0	23	10	2.3	RH
	7	1	0	0	1	6	3	0	0	9	0	20	10	2.0	RH
	8														
	Total	16	10	12	18	16	14	11	16	12	12	137		Avg. =	13.7
														C.V. =	19.5

Conc	5	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
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
	3	0	0	0	0	0	4	0	3	0	4	11	10	1.1	RH
	4	9	6	2	0	0	2	7	1	8	0	35	10	3.5	RH
	5	4	0	5	X	6	0	0	9	8	10	42	10	4.2	RH
	6	0	0	7		12	0	1	7	0	0	27	10	2.7	RH
	7	3	6	1		3	1	1	0	2	3	20	10	2.0	RH
	8														
	Total	16	12	15	0	21	7	9	20	18	17	135		Avg. =	15.0
					X									C.V. =	32.0

Conc	6	Replicate										No. of Young	No. of Adult	Young /Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
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
	3	0	0	0	0	3	0	0	0	0	4	7	10	0.7	RH
	4	5	0	5	8	1	6	7	8	8	4	52	10	5.2	RH
	5	0	10	0	5	0	4	8	0	5	6	38	10	3.8	RH
	6	0	0	0	2	12	0	0	1	2	0	17	10	1.7	RH
	7	5	8	6	0	3	7	6	6	0	0	41	10	4.1	RH
	8														
	Total	10	18	11	15	19	17	21	15	15	14	155		Avg. =	15.5
														C.V. =	22.0

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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 # K1510001, C.DUBIA CHRONIC, REPRODUCION, 10-13-15
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Bartlett's test for homogeneity of variance
Calculated B1 statistic = 9.13

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	9	1	10
32	9	1	10
TOTAL	18	2	20

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

FISHER'S EXACT TEST

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

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

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF DEAD	ALIVE	TOTAL ANIMALS
CONTROL	1	9	10
56	0	10	10

TOTAL	1	19	20

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

FISHER'S EXACT TEST

	NUMBER OF		
IDENTIFICATION	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	9	1	10
75	9	1	10
TOTAL	18	2	20

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

FISHER'S EXACT TEST

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

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

SUMMARY OF FISHER'S EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	10	1	

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

TITLE: AA # K1510001, C.DUBIA CHRONIC, REPRODUCION, 10-13-15
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TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.0000	0.0000
1	CONTROL	2	17.0000	17.0000
1	CONTROL	3	13.0000	13.0000
1	CONTROL	4	11.0000	11.0000
1	CONTROL	5	19.0000	19.0000
1	CONTROL	6	13.0000	13.0000
1	CONTROL	7	20.0000	20.0000
1	CONTROL	8	16.0000	16.0000
1	CONTROL	9	18.0000	18.0000
1	CONTROL	10	19.0000	19.0000
2	32 % EFFLUENT	1	16.0000	16.0000
2	32 % EFFLUENT	2	8.0000	8.0000
2	32 % EFFLUENT	3	9.0000	9.0000
2	32 % EFFLUENT	4	17.0000	17.0000
2	32 % EFFLUENT	5	0.0000	0.0000
2	32 % EFFLUENT	6	17.0000	17.0000
2	32 % EFFLUENT	7	15.0000	15.0000
2	32 % EFFLUENT	8	18.0000	18.0000
2	32 % EFFLUENT	9	15.0000	15.0000
2	32 % EFFLUENT	10	14.0000	14.0000
3	42 % EFFLUENT	1	13.0000	13.0000
3	42 % EFFLUENT	2	10.0000	10.0000
3	42 % EFFLUENT	3	13.0000	13.0000
3	42 % EFFLUENT	4	9.0000	9.0000
3	42 % EFFLUENT	5	7.0000	7.0000
3	42 % EFFLUENT	6	13.0000	13.0000
3	42 % EFFLUENT	7	19.0000	19.0000
3	42 % EFFLUENT	8	21.0000	21.0000
3	42 % EFFLUENT	9	17.0000	17.0000
3	42 % EFFLUENT	10	9.0000	9.0000
4	56 % EFFLUENT	1	16.0000	16.0000
4	56 % EFFLUENT	2	10.0000	10.0000
4	56 % EFFLUENT	3	12.0000	12.0000
4	56 % EFFLUENT	4	18.0000	18.0000
4	56 % EFFLUENT	5	16.0000	16.0000
4	56 % EFFLUENT	6	14.0000	14.0000
4	56 % EFFLUENT	7	11.0000	11.0000
4	56 % EFFLUENT	8	16.0000	16.0000
4	56 % EFFLUENT	9	12.0000	12.0000
4	56 % EFFLUENT	10	12.0000	12.0000
5	75 % EFFLUENT	1	16.0000	16.0000

5	75 % EFFLUENT	2	12.0000	12.0000
5	75 % EFFLUENT	3	15.0000	15.0000
5	75 % EFFLUENT	4	0.0000	0.0000
5	75 % EFFLUENT	5	21.0000	21.0000
5	75 % EFFLUENT	6	7.0000	7.0000
5	75 % EFFLUENT	7	9.0000	9.0000
5	75 % EFFLUENT	8	20.0000	20.0000
5	75 % EFFLUENT	9	18.0000	18.0000
5	75 % EFFLUENT	10	17.0000	17.0000
6	100 % EFFLUENT	1	10.0000	10.0000
6	100 % EFFLUENT	2	18.0000	18.0000
6	100 % EFFLUENT	3	11.0000	11.0000
6	100 % EFFLUENT	4	15.0000	15.0000
6	100 % EFFLUENT	5	19.0000	19.0000
6	100 % EFFLUENT	6	17.0000	17.0000
6	100 % EFFLUENT	7	21.0000	21.0000
6	100 % EFFLUENT	8	15.0000	15.0000
6	100 % EFFLUENT	9	15.0000	15.0000
6	100 % EFFLUENT	10	14.0000	14.0000

AA # K1510001, C.DUBIA CHRONIC, REPRODUCION, 10-13-15
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	48.883	9.777	0.391
Within (Error)	54	1351.300	25.024	
Total	59	1400.183		

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

AA # K1510001, C.DUBIA CHRONIC, REPRODUCION, 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	14.600	14.600		
2	32 % EFFLUENT	12.900	12.900	0.760	
3	42 % EFFLUENT	13.100	13.100	0.670	
4	56 % EFFLUENT	13.700	13.700	0.402	
5	75 % EFFLUENT	13.500	13.500	0.492	
6	100 % EFFLUENT	15.500	15.500	-0.402	

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

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