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**REFERENCE #60130605** 

Pace Analytical Services, Inc. 9608 Loiret Blvd. Lenexa. KS 66219

> Phone: 913.599.5665 Fax: 913.599.1759

October 10, 2012

James Boston / City of Decatur P.O. Box.247 / Decatur, AR 72722

Re:

Lab Project Number: 60130605 Client Project ID: Wet Test

Dear:

Enclosed are the analytical results for sample(s) received by the laboratory. The results relate only to the samples included in this report. Results reported herein conform to the most current NELAC standards, where applicable, unless otherwise narrated in the body of the report.

If you have any question concerning this report, please feel free to contact me.

Sincerely,

Tim Harrell

Tim.Harrell@pacelabs.com

**Technical Director** 

Kansas/ NELAP Certification Number E-10116 Utah Certification Number 9135995665 Texas Certification Number T104704407-08-TX Oklahoma Certification Number 9205/9935 Louisiana Certification Number 03055 Arkansas Certification Number 05-008-0

Enclosures





Pace Analytical Services, Inc. 9608 Loiret Blvd. Lenexa. KS 66219

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## CHRONIC TOXICITY TEST FOR City of Decatur

PERMIT # AR0022292 AFIN # 04-00052

PERFORMED ON:

Pimephales promelas

and

Ceriodaphnia dubia

PREPARED FOR:

City of Decatur Attn: James Boston P.O. Box 247 Decatur, AR 72722 1-479-752-3912

PREPARED BY:
Pace Analytical Services, Inc.
808 West McKay
Frontenac, KS 66763
1-620-235-0003

October 10, 2012

## **REPORT OF LABORATORY ANALYSIS**





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## **REPORT OF LABORATORY ANALYSIS**





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#### **SUMMARY**

A Chronic Whole Effluent Toxicity Test using the 7-day chronic fathead minnows (<u>Pimephales promelas</u>), static renewal larval survival and growth test, and three brood 7-day chronic Cladoceran (<u>Ceriodaphnia dubia</u>), static renewal survival and reproduction test, was conducted on effluent discharge water collected at the City of Decatur effluent discharge from October 2, 2012 to October 5, 2012. All the test methods followed are as listed in <u>EPA 821-R-02-013</u>, "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms."

Statistically significant (p<0.05) mortality is determined by Dunnet's procedure using average percent survival of each test concentration versus the average survival of the controls. If significant mortality occurs, median lethal concentrations (LC50) are calculated using effluent concentrations and their corresponding percent mortality data. The LC50's and the 95% confidence intervals are calculated where appropriate by the Spearman-Karber method. Statistical analysis is accomplished by following steps in EPA 821-R-02-013, November 2002 and by use of Toxstat version 3.4.

In minnow section of testing, it was observed that the effluent had no significant effect on the survival of the larvae at the 100% concentration. No significant mortality was observed in the other effluent concentrations after the 7-day exposure period. The No Observed Effect Concentration (NOEC) was determined to be 100% for survival. The LC50 was estimated to be >100% effluent. No significant reduction in growth was observed in the 100% effluent concentration. The Toxic Units is <1. The IC25 is >100. The NOEC for growth in effluent was determined to be 100%. The PMSD was 10.6.

In Cladoceran section of testing, it was observed that the effluent had no significant effect on the survival of the organisms in the 100% effluent concentration. No significant mortality was observed in the other effluent concentrations after the 7-day exposure period. The No Observed Effect Concentration (NOEC) was determined to be 100% for survival. The LC50 was estimated to be >100% effluent. No significant reduction in reproduction was observed in the 100% effluent concentrations. The Toxic Units is <1. The IC25 is >100. The NOEC for reproduction in effluent was determined to be 100%. The PMSD was 13.5.

The chronic toxicity exhibited by the fathead minnows and the <u>Ceriodaphnia</u> treated by the effluent sampled from October 2 to October 5 from the City of Decatur effluent discharge, is acceptable as described in <u>EPA 821-R-02-013</u>.

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#### INTRODUCTION

Pace Analytical was contracted to perform this chronic toxicity test on effluent from the City of Decatur effluent discharge. Chronic toxicity was measured using the <u>Pimephales promelas</u> at larval for survival and growth test and the <u>Ceriodaphńia dubia</u> survival and reproduction test described in <u>EPA 821-R-02-013</u>, "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms." The raw data of the study is stored at Pace Analytical Services, INC. 808 West McKay, Frontenac, KS 66763.

#### **TEST MATERIAL**

City of Decatur personnel collected sampling of the effluent. A sample of the effluent was delivered to Pace by commercial carrier on 10-2-12. Subsequent samples followed by delivery on 10-3-12 and on 10-5-12. All samples were stored at  $\leq$  6° Celsius. Moderately Hard Synthetic Water was used as a control and also to make the required dilutions in the test as described in <u>EPA 821-R-02-013</u>.

#### **TEST METHODS**

Pace used EPA test method 1000.0 for conducting the Fathead Minnow, Pimephales promelas, Larval Survival and Growth Test. EPA test method 1002.0 was used for conducting the Cladoceran, Ceriodaphnia dubia, Survival and Reproduction Test. The tests were conducted to estimate the LC50, NOEC, and LOEC for survival, growth, and reproduction of these test species.

The <u>Pimephales</u> and <u>Ceriodaphnia</u> tests were initiated on 10-2-12 and carried out until 10-9-12. The Pimephales tests were conducted in 500 ml plastic jars with 250 ml of test solution. Eight larvae were placed in each of at least 5 replicates to make a total of 40 larvae per sample concentration. The <u>Ceriodaphnia</u> tests were carried out in 35ml vials containing 25 ml of test solution. One Neonate was placed in each of 10 replicates to make a total of 10 neonates per sample concentration.

#### **TEST ORGANISMS**

The organisms used in these tests were cultured at Pace under controlled temperature and photoperiod conditions. Pace maintains records of all culture techniques used in producing organisms.

## **REPORT OF LABORATORY ANALYSIS**





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**RESULTS** 

## **REPORT OF LABORATORY ANALYSIS**





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#### TABLE 1

Permittee: City of Decatur Effluent discharge.

**Date Sampled** 

No. 1:

10-2-12

8:00

No. 2:

10-3-12

8:00

No. 3: 10-5-12

8:00

Test Initiated: 15:30

Date: 10-2-12

Dilution Water used: Moderately Hard Synthetic Water

## FATHEAD MINNOW LARVAE GROWTH AND SURVIVAL (Pimephales promelas)

#### DATA TABLE FOR GROWTH OF FATHEAD MINNOWS

Effluent Concentration (%)	Averag A		eight in Mi te Chamb C	lligrams in ers D	E	Mean Dry Weight (mg)	CV% *
Control 0%	0.397	0.464	0.422	0.465	0.456	0.437	4.04
Dilution 1 32%	0.408	0.421	0.448	0.440	0.482	0.429	3.10
Dilution 2 42%	0.459	0.468	0.429	0.411	0.439	0.452	4.65
Dilution 3 56%	0.452	0.410	0.486	0.458	0.477	0.456	2.89
Dilution 4 75%	0.460	0.448	0.465	0.386	0.432	0.438	4.46
Dilution 5 100%	0.484	0.471	0.460	0.372	0.455	0.448	6.10

<sup>\*</sup> Coefficient of Variation = Standard Deviation X 100 / Mean

## REPORT OF LABORATORY ANALYSIS



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Permittee: City of Decatur Effluent discharge.

### **FATHEAD MINNOW SURVIVAL**

Conc.%	Pe	Percent Survival in Replicate				Mean Percent Survival			CV %
	A	В	Chambe C	rs D	Ε	24hr	48hr	7 day	
Control 0%	87.5	100	100	100	100	100	100	97.5	4.79
Dilution 1 32%	100	100	100	100	100	100	100	100	0.00
Dilution 2 42%	100	100	100	100	100	100	100	100	0.00
Dilution 3 56%	100	100	100	100	100	100	100	100	0.00
Dilution 4 75%	100	100	100	87.5	100	100	100	97.5	4.79
Dilution 5 100%	100	100	100	87.5	100	100	100	97.5	4.79

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Permittee: City of Decatur Effluent discharge.

## **CERIODAPHNIA SURVIVAL AND REPRODUCTION**

## DATA TABLE FOR CERIODAPHNIA YOUNG PRODUCTION

Replicate	Control	Dilution 1	Dilution 2	Dilution 3	Dilution 4	Dilution 5
	0%	32%	42%	56%	75%	100%
1	22	20	25	18	22	19
2	23	21	20	23	22	28
3	27	23	23	20	18	24
4	21	28	18	22	19	29
5	26	23	28	23	22	23
6	24	27	24	24	30	24
7	27	21	22	19	22	23
8	23	25	27	23	27	20
9	25	23	29	23	24	24
10	23	30	23	28	28	25
Mean	24.1	24.1	23.9	22.3	23.4	23.9
SD	2.079	3.1315	3.479	2.830	3.864	3.071
CV %	8.63	13.75	14.55	12.69	16.51	12.85

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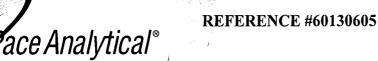
Permittee: City of Decatur Effluent discharge.

## CERIODAPHNIA MEAN PERCENT SURVIVAL

//	Percent Effluent (%)							
Time Elapsed	Control 0%	Dilution 1 32%	Dilution 2 42%	Dilution 3 56%	Dilution 4 75%	Dilution 5 100%		
24 hrs	100	100	100	100	100	100		
48 hrs	100	100	100	100	100	100		
7-day	100	100	100	100	100	100		
SD	0.000	0.000	0.000	0.000	0.000	0.000		
CV %	0.00	0.00	0.00	0.00	0.000	0.000		

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# TABLE 2 SUMMARY OF TEST CONDITIONS FOR THE FATHEAD MINNOW (Pimephales promelas) LARVAL SURVIVAL AND GROWTH TEST

1. Test type	Static renewal
2. Temperature	25 degrees Celsius
3. Light quality	Ambient laboratory light
4. Light intensity	Ambient laboratory levels
5. Photoperiod	16 hr light, 8 hr dark
6. Test chamber size	500 ml
7. Test solution volume	250 ml
8. Renewal of test concentrations	Daily
9. Age of test organism	< 24 hours
10. No. larvae/chamber	8
11. No. replicates/concentration	5
12. No. larvae/concentration	40
13. Feeding regime	Feed 0.1 ml newly hatched brine shrimp nauplii three times daily.  Larvae are not fed 12 hours prior to termination of test.
14. Cleaning	Siphon daily, immediately before test solution renewal
15. Aeration	None

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TABLE 2 (CONT.)

7	EL 2 (00111.)
16. Dilution Water	Moderately Hard Synthetic Water prepared with MILLI-Q deionized water and reagent grade chemicals
17. Effluent concentrations	0%, 32%, 42%, 56%, 75%, 100%
18. Test duration	7 days
19. Endpoints	Survival and growth
20. Test acceptability	80% or greater survival in the controls, Average dry weight in controls >0.25 mg, Coefficient of variation in the control must not exceed 40%.

## TABLE 2 (CONT.) SUMMARY OF TEST CONDITIONS FOR THE CLADOCERAN (Ceriodaphnia dubia) SURVIVAL AND REPRODUCTION TEST

1. Test type	Static renewal
2. Temperature	25 degrees Celsius
3. Light quality	Ambient laboratory light
4. Light intensity	Ambient laboratory levels
5. Photoperiod	16 hr light, 8 hr dark
6. Test chamber size	30 ml
7. Test solution volume	25 ml

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## TABLE 2 (CONT.)

8. Renewal of test concentrations	Daily
9. Age of test organism	< 24 hours
10. No. larvae/chamber	1
11. No. replicates/concentration	10
12. No. larvae/concentration	10
13. Feeding regime	Feed 0.1 ml YCT three times daily. Larvae are not fed 12 hours prior to termination of test.
14. Cleaning	Siphon daily, immediately before test solution renewal
15. Aeration	None
16. Dilution Water	Moderately Hard Synthetic Water prepared with MILLI-Q deionized water and reagent grade chemicals
17. Effluent concentrations	0%, 32%, 42%, 56%, 75%, 100%
18. Test duration	7 days - 10 days
19. Endpoints	Survival and Reproduction
20. Test acceptability	80% or greater survival in the controls, Average reproduction rate of 15 young / adult. Coefficient of variation in the control must not exceed 40%.

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## **TABLE 2 (SECTION 2)**

## BIOMONITORING CHRONIC TOXICITY REPORT FATHEAD MINNOW (Pimephales promelas) CHEMICAL PARAMETERS CHART

Permittee: City of Decatur Effluent discharge.

ANALYSTS: Pace Analytical Services, Inc.

Timothy Harrell Mike Bollin

SAMPLE NO. 1 COLLECTED: DATE: 10-2-12

SAMPLE NO. 2 COLLECTED: DATE: 10-3-12

SAMPLE NO. 3 COLLECTED: DATE: 10-5-12

## TABLE 2 (SECTION 2) INITIAL WATER QUALITY EFFLUENT CONCENTRATION

	Control	100%
PH	7.57	7.81
D.O.	8.25	8.20
Temp	25	- 25
Alk	62	130
Hard	98	126
Cond	367	734
Chlorine	<0.1	<0.1

\* D.O. is reported as mg/L
Alkalinity is reported as mg/L CaCO3
Hardness is reported as mg/L CaCO3
Conductance is reported as umhos
Ammonia is reported as mg/L
Chlorine is reported as mg/L

## **REPORT OF LABORATORY ANALYSIS**





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### **TEST WATER QUALITY**

24-Hour Water Quality Measurements

Ziriodi Trato, dadity	modeuromente		
Effluent	PH	D.O.	Temperature
Concentration (%)		(mg/l)	(C)
0% Control	7.67	6.90	25
32% Effluent	7.87	7.10	25
742% Effluent	8.01	7.10	25
56% Effluent	8.13	7.10	25
75% Effluent	8.21	7.10	25
100% Effluent	8.30	7.10	25

48-Hour Water Quality Measurements

40-110ul Watel Qual	ity Measurements		
Effluent	PH	D.O.	Temperature
Concentration (%)		(mg/l)	(C)
0% Control	7.79	7.40	25
32% Effluent	7.82	7.30	25
42% Effluent	7.96	7.20	25
56% Effluent	8.16	7.20	25
75% Effluent	8.19	7.10	25
100% Effluent	8.23	7.00	25

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### FINAL WATER QUALITY

## **EFFLUENT CONCENTRATION**

,	Cóntrol	100%
pH /	7.67	8.39
D.O./	7.30	7.40
Temp′	25	25
/ Alk	64	126
∠ Hard	96	118
Cond	368	815

\* D.O. is reported as mg/L
Alkalinity is reported as mg/L CaCO3
Hardness is reported as mg/L CaCO3
Conductance is reported as umhos

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#### **TEST VALIDITY**

The <u>Pimephales promelas</u> control survival rate was 97.5%. The mean dry weight (growth) of the <u>Pimephales promelas</u> was determined at 0.437 mg/organism in the controls. The percent coefficient of variation (%CV) values for the fathead minnow control for survival and growth were 4.79 and 4.04. The <u>Ceriodaphnia dubia</u> survival rates were 100 in the control. The <u>Ceriodaphnia in the control produced an average of 24.1 young over the seven-day exposure period. Percent CV values for <u>Ceriodaphnia dubia</u> control survival and reproduction was 0.00 and 8.63. Control data met or exceeded all criteria set out by EPA 821-R-02-013 for test acceptance.</u>

#### **CONCLUSIONS**

The No Observed Effect Concentration (NOEC) for <u>Pimephales promelas</u> was 100% for survival and 100% for growth. The No Observed Effect Concentration (NOEC) for <u>Ceriodaphnia dubia</u> was 100% for Survival and 100% for Reproduction. The tests were ran using a synthetic control against effluent concentrations of 32%, 42%, 56%, 75%, and 100%. The effluent sampled on 10-2-12, 10-3-12, and 10-5-12 exhibited acceptable chronic toxicity in <u>Pimephales promelas</u> and in <u>Ceriodaphnia dubia</u> during the exposure period as described in EPA 821-R-02-013.

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#### **APPENDIX C**

#### REFERENCE TOXICANTS

The absence of significant control mortality during this test indicated the health of the organisms and indicated that any significant mortality in the test concentrations was not due to contaminants or variations in testing conditions.

Reference toxicity testing is routinely performed by staff members in our biomonitoring - bioassay laboratory.

Start: 9/18/12 14:00

End: 9/25/12 12:00

Reference Toxicant (NaCl) <u>Pimephales promelas</u>

Concentration of Toxicant	A	Avg. # of Live Organisms/replicate					
•	0 hrs	24 hrs	48 hrs	7 days			
10 g/l	40	6	1	0			
8 g/l	40	37	30	5			
6 g/l	40	39	35	26			
4 g/l	40	40	40	39			
2 g/l	40	40	40	40			

IC25 (5.16 g/l Sodium Chloride)

Survival NOEC: 4.0 g/l

Reference Toxicant (NaCl) Ceriodaphnia Dubia

Concentration of Toxicant	Avg. # of Live Organisms/replicate					
	0 hrs	24 hrs	48 hrs	7 days		
2.5 g/l	10	6	0	0		
2.0 g/l	10	10	8	2		
1.5 g/l	10	10	10	10		
1.0 g/l	10	10	10	10		
0.5 g/l	10	10	10	10		

IC25 (1.21g/l Sodium Chloride)

Survival NOEC: 1.5 g/l

Submitted By:

Timothy Harrell, Technical Director

## **REPORT OF LABORATORY ANALYSIS**



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File: 6130605A Transform: ARC SINE(SQUARE ROOT(Y))

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED OBSERVED	2.010	7.260 0	11.460 27	7.260 0	2.010

Calculated Chi-Square goodness of fit test statistic = 38.0902 Table Chi-Square value (alpha = 0.01) = 13.277

Dată FAIL normality test. Try another transformation.

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

Data FAIL normality test. Try another transformation.

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

60130605 Decatur FATHEAD SURVIVAL
File: 6130605A Transform: ARC SINE(SQUARE ROOT(Y))

## SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	Ń	MIN	MAX	MEAN
1	CONTROL	5	0.991	1.107	1.084
2	32%	5	1.107	1.107	1.107
3	42%	5	1.107	1.107	1.107
4	56%	5	1.107	1.107	1.107
5	75%	5	0.991	1.107	1.084
6	100%	5	0.991	1.107	1.084

60130605 Decatur FATHEAD SURVIVAL

File: 6130605A Transform: ARC SINE(SQUARE ROOT(Y))

## SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	CONTROL	0.003	0.052	0.023	4.79
2	32%	0.000	0.000	0.000	0.00
3 ,	42%	0.000	0.000	0.000	0.00
4	56%	0.000	0.000	0.000	0.00
5	75%	0.003	0.052	0.023	4.79
6	100%	0.003	0.052	0.023	4.79

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File: 6130605A Transform: ARC SINE(SQUARE ROOT(Y))

### ANOVA TABLE

SOURCE	DF	SS	MS	F	
Between	5	0.004	0.001	0.600	•
Within (Error)	24	0.032	0.001		
Total	29	0.036			•

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

3

60130605 Decatur FATHEAD SURVIVAL File: 6130605A Transform: ARC SINE(SQUARE ROOT(Y))

ļ	DUNNETT'S TEST -	TABLE 1 OF 2	Ho:Control <treatment< th=""><th></th></treatment<>		
GROUI	P IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1 2 3 4 5	CONTROL 32% 42% 56% 75%	1.084 1.107 1.107 1.107 1.084 1.084	0.780 0.800 0.800 0.800 0.780 0.780	-1.000 -1.000 -1.000 0.000	

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

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File: 6130605A 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%	5	0.047	6.0	-0.020
3 ,	42%	5	0.047	6.0	-0.020
4	56%	5	0.047	6.0	-0.020
5 ;	75%	5	0.047	6.0	-0.000
6	100%	5	0.047	6.0	-0.000

```
60130605 Decatur FATHEAD GROWTH
File: C:\TOXSTAT\6130605B. Transform: ARC SINE(SQUARE ROOT(Y))
Shapiro - Wilk's test for normality

D = 0.024

W = 0.924

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.

60130605 Decatur FATHEAD GROWTH
File: C:\TOXSTAT\6130605B. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 2.84

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.

60130605 Decatur FATHEAD GROWTH
File: C:\TOXSTAT\6130605B. Transform: ARC SINE(SQUARE ROOT(Y))

## SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	XAM	MEAN
1	CONTROL	5	0.682	0.750	0.723
2	32%	5	0.693	0.741	0.714
3	42%	5	0.695	0.771	0.738
4	56%	5	0.714	0.767	0.741
5	75%	5	0.670	0.750	0.723
6	100%	5	0.656	0.769	0.733

60130605 Decatur FATHEAD GROWTH

File: C:\TOXSTAT\6130605B. Transform: ARC SINE(SQUARE ROOT(Y))

## SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	CONTROL	0.001	0.029	0.013	4.04
2 ;	32%	0.000	0.022	0.010	3.10
3 .	42%	0.001	0.034	0.015	4.65
4	56%	0.000	0.021	0.010	2.89
5 :	75%	0.001	0.032	0.014	4.46
6 .	100%	0.002	0.045	0.020	6.10

60130605 Decatur FATHEAD GROWTH
File: C:\TOXSTAT\6130605B. Transform: ARC SINE(SQUARE ROOT(Y))

### ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.003	0.001	0.536
Within (Error)	24	0.024	0.001	
Total	29	0.027		

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

60130605 Decatur FATHEAD GROWTH
File: C:\TOXSTAT\6130605B. 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 2 3 4 5	CONTROL 32% 42% 56% 75% 100%	0.723 0.714 0.738 0.741 0.723 0.733	0.437 0.429 0.452 0.456 0.438 0.448	0.432 -0.753 -0.917 -0.039 -0.546	

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

60130605 Decatur FATHEAD GROWTH

File: C:\TOXSTAT\6130605B. Transform: ARC SINE(SQUARE ROOT(Y))

**************************************	DUNNETT'S TEST -	TABLE 2	OF 2 Ho	:Control<	Treatment
GROŲ	P IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1,	CONTROL	5			
2	32%	5	0.047	10.6	0.009
3 /	42%	5	0.047	10.6	-0.015
4 ,	56%	5	0.047	10.6	-0.018
5 ,	75%	5	0.047	10.6	-0.001
6 '	100%	5	0.047	10.6	-0.011

#### FISHER'S EXACT TEST

NUMBER OF

NUMBER OF

IDENTIFICATION	ALIVE	DEAD 	TOTAL ANIMALS
CONTROL	10	0	10
32%	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

ļ t				
1	IDENTIFICATION	ALIVE	DEAD 	TOTAL ANIMALS
	CONTROL	10	0	10
	42%	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

		NUMB:	ER OF 
IDENTIFICATION	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
56%	10	0	10

3 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

NUMBER OF

IDENTI	FICATION	ALIVE	DEAD	TOTAL ANIMALS
I	CONTROL	10	0	10
	75%	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

	•		NU:	MBER OF
	DENTIFICATION	ALIVE	DEAD 	TOTAL ANIMALS
	CONTROL	10	0	10
	100%	10	0	10
	TOTAL	20	0	20
i	•			

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

.

60130605 Decatur CERIODAPHNIA DUBIA SURVIVAL File: 6130605D Transform: NO TRANSFORM

### SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	CONTROL	10	1.000	1.000	1.000
2	32%	10	1.000	1.000	1.000
3 ,	42%	10	1.000	1.000	1.000
4	56%	10	1.000	1.000	1.000
5	75%	10	1.000	1.000	1.000
6	100%	10	1.000	1.000	1.000

60130605 Decatur CERIODAPHNIA DUBIA SURVIVAl File: 6130605D Transform: NO TRANSFORM

## SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

,					
GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	CONTROL	0.000	0.000	0.000	0.00
2	32%	0.000	0.000	0.000	0.00
3	42%	. 0.000	0.000	0.000	0.00
4	56%	0.000	0.000	0.000	0.00
5	75%	0.000	0.000	0.000	0.00
6	100%	0.000	0.000	0.000	0.00
_				•	

 $60130605_{2}$ Decatur CERIODAPHNIA DUBIA REPRODU

File: 6130605E Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
	4 000	14 500			
EXPECTED OBSERVED	4.020 3	14.520 15	22.920 26	14.520 12	4.020 4

Calculated Chi-Square goodness of fit test statistic = 1.1260 Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

60130605 Decatur CERIODAPHNIA DUBIA REPRODU
File: 6130605E Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 3.55

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.

60130605 Decatur CERIODAPHNIA DUBIA REPRODU

File: 6130605E Transform: NO TRANSFORMATION

## SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	CONTROL	10	21.000	27.000	24.100
2	32%	10	20.000	30.000	24.100
3	42%	10	18.000	29.000	23.900
4	56%	10	18.000	28.000	22.300
5	75%	10	18.000	30.000	23.400
6	100%	10	19.000	29.000	23.900

60130605 Decatur CERIODAPHNIA DUBIA REPRODU

File: 6130605E Transform: NO TRANSFORMATION

## SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	CONTROL	4.322	2.079	0.657	8.63
2	32%	10.989	3.315	1.048	13.75
3	42%	12.100	3.479	1.100	14.55
4	56%	8.011	2.830	0.895	12.69
5	75%	14.933	3.864	1.222	16.51
6	100%	9.433	3.071	0.971	12.85

60130605 Decatur CERIODAPHNIA DUBIA REPRODU

File: 6130605E Transform: NO TRANSFORMATION

## ANOVA TABLE

1				
SOURCE	DF	SS	MS	F
Between	5	24.083	4.817	0.483
Within (Error)	54	538.100	9.965	
Total	59	562.183		

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

60130605 Decatur CERIODAPHNIA DUBIA REPRODU

File: 6130605E Transform: NO TRANSFORMATION

	DUNNETT'S TEST -	TABLE 1 OF 2	Ho:Control <t< th=""><th>reatment</th></t<>	reatment
GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT SIG
1 2 3 4	CONTROL 32% 42% 56% 75%	24.100 24.100 23.900 22.300 23.400	24.100 24.100 23.900 22.300 23.400	0.000 0.142 1.275 0.496
6 <u>.</u>	100%	23.900	23.900	0.142

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

60130605 Decatur CERIODAPHNIA DUBIA REPRODU

File: 6130605E Transform: NO TRANSFORMATION

•	DUNNETT'S TEST -	TABLE 2	OF 2 Ho	:Control<	Treatment
GROUF	· identification	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	10			
2 .	32%	10	3.261	13.5	0.000
3	42%	10	3.261	13.5	0.200
4 '	56%	10	3.261	13.5	1.800
5 -	75%	10	3.261	13.5	0.700
6	100%	10	3.261	13.5	0.200

Conc. ID		1	2	3	4	5	6
Conc. Tes	ted	0	32	42 	56 	75	100
Response Response Response Response	1 2 3 4	.397 .464 .422 .465	.408 .421 .448 .440	.459 .468 .429 .411	.452 .410 .486 .458	.460 .448 .465 .386	.484 .471 .460 .372
Response	5	.456	.482	.439	.477	.432	.455

\*\*\* Inhibition Concentration Percentage Estimate \*\*\*

Toxicant/Effluent: Decatur

Test Start Date: 10/2/12 Test Ending Date: 10/9/12 Test Species: Fathead

Test Duration: 7 Day

DATA FILE:

Conc.	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1,	5	0.000	0.441	0.030	0.445
2	5	32.000	0.440	0.028	0.445
3	5	42.000	0.441	0.023	0.445
4	5	56.000	0.457	0.029	0.445
5	5	75.000	0.438	0.032	0.443
6	5	100.000	0.448	0.044	0.443

<sup>\*\*\*</sup> No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 75% of the control response mean.

Conc. ID		1	2	3	4	5	6
Conc. Res	ted	0	32	42	56	75	100
Response	1	22	20	25	18	22	19
Response	2	23	21	20	23	22	28
Response	3	27	23	23	20	18	24
Response	4	21	28	18	22	19	29
Response	5	26	23	28	23	22	23
Response	6	24	27	24	24	30	24
Response	7	27	21	22	19	22	23
Response	8	23	25	27	23	27	20
Response	9	25	23	29	23	24	24
Response	10	23	30	23	28	28	25

\*\*\* Inhibition Concentration Percentage Estimate \*\*\*

Toxicant/Effluent: Decatur

Test Start Date: 10/2/12 Test Ending Date: 10/9/12

Test Species: Dubia

Test Duration:

7 Day

DATA FILE:

Conc.	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1 2 3	10 10 10	0.000 32.000 42.000	24.100 24.100 23.900	2.079 3.315 3.479	24.100 24.100 23.900
3 · 4 <sup>*</sup> 5	10 10 10	56.000 75.000 100.000	22.300 23.400 23.900	2.830 3.864 3.071	23.200 23.200 23.200 23.200

<sup>\*\*\*</sup> No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 75% of the control response mean.



## **CHAIN-OF-CUSTODY / Analytical Request Document**

The Chain-of-Custody is a LEGAL DOCUMENT. All relevant fields must be completed accurately.

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## **CHAIN-OF-CUSTODY / Analytical Request Document**

The Chain-of-Custody is a LEGAL DOCUMENT. All relevant fields must be completed accurately.

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rtant Note:	By signing this	form you are accep	oting Pace's I	NET 30	0 day pa	ayment terms	and agreeing	to late charge	es of 1.5% pe	r mont	h for a	any invo	oices no	ot paid	w <b>j</b> thir	n 30 day:	s.									F	r-ALL	-Q-020re\	.07, 15-Ma	iy-2007	
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Arkansas Dept of Environmental Quality Water Division-Enforcement Branch 5301 Northshore Drive North Little Rock AR 72118-5317