



REFERENCE #60186050

Pace Analytical Services, Inc.
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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

January 21, 2015

REPORT OF LABORATORY ANALYSIS

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SUMMARY

A Chronic Whole Effluent Toxicity Test using the 7-day chronic fathead minnows (Pimephales promelas), static renewal larval survival and growth test, and three brood 7-day chronic Cladoceran (Ceriodaphnia dubia), static renewal survival and reproduction test, was conducted on effluent discharge water collected at the City of Decatur effluent discharge from January 13, 2015 to January 16, 2015. All the test methods followed are as listed in EPA 821-R-02-013, "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-Kärber 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 22.8.

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 16.1.

The chronic toxicity exhibited by the fathead minnows and the Ceriodaphnia treated by the effluent sampled from January 13 to January 16 from the City of Decatur effluent discharge, is acceptable as described in EPA 821-R-02-013.

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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 Pimephales promelas at larval for survival and growth test and the Ceriodaphnia dubia survival and reproduction test described in EPA 821-R-02-013, "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 1-13-15. Subsequent samples followed by delivery on 1-14-15 and on 1-16-15. All samples were stored at $\leq 6^\circ$ Celsius. Moderately Hard Synthetic Water was used as a control and also to make the required dilutions in the test as described in EPA 821-R-02-013.

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 Pimephales and Ceriodaphnia tests were initiated on 1-13-15 and carried out until 1-20-15. 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 Ceriodaphnia 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 and/or were purchased from an external supplier. Pace maintains records of all culture techniques used in producing organisms.

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RESULTS

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TABLE 1

Permittee: City of Decatur Effluent discharge.

Date Sampled	No. 1:	1-13-15	9:00
	No. 2:	1-14-15	8:00
	No. 3:	1-16-15	7:00
Test Initiated: 14:45	Date:	1-13-15	

Dilution Water used: Moderately Hard Synthetic Water

FATHEAD MINNOW LARVAE GROWTH AND SURVIVAL
(Pimephales promelas)

DATA TABLE FOR GROWTH OF FATHEAD MINNOWS

Effluent Concentration (%)	Average Dry Weight in Milligrams in Replicate Chambers					Mean Dry Weight (mg)	CV% *
	A	B	C	D	E		
Control 0%	0.497	0.421	0.474	0.480	0.375	0.449	11.21
Dilution 1 32%	0.351	0.441	0.423	0.314	0.485	0.403	17.20
Dilution 2 42%	0.357	0.339	0.412	0.523	0.492	0.425	19.09
Dilution 3 56%	0.436	0.289	0.386	0.526	0.451	0.418	20.99
Dilution 4 75%	0.498	0.410	0.470	0.521	0.477	0.475	8.74
Dilution 5 100%	0.424	0.436	0.502	0.495	0.325	0.436	16.32

* Coefficient of Variation = Standard Deviation X 100 / Mean

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Permittee: City of Decatur Effluent discharge.

FATHEAD MINNOW SURVIVAL

Conc. %	Percent Survival in Replicate Chambers					Mean Percent Survival			CV %
	A	B	C	D	E	24hr	48hr	7 day	
Control 0%	100	100	100	100	87.5	100	100	97.5	4.79
Dilution 1 32%	87.5	100	100	87.5	100	100	100	95	5.99
Dilution 2 42%	87.5	87.5	87.5	100	100	100	100	92.5	6.12
Dilution 3 56%	100	75	100	100	100	100	100	95	9.30
Dilution 4 75%	100	87.5	100	100	100	100	100	97.5	4.79
Dilution 5 100%	100	100	100	100	87.5	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 0%	Dilution 1 32%	Dilution 2 42%	Dilution 3 56%	Dilution 4 75%	Dilution 5 100%
1	16	20	19	17	17	25
2	22	23	24	23	26	24
3	19	22	22	22	23	24
4	21	18	22	24	22	27
5	20	23	24	22	23	24
6	18	22	26	21	28	23
7	15	25	25	26	24	20
8	22	24	21	21	27	18
9	20	23	28	25	15	23
10	19	18	17	24	27	23
Mean	19.2	21.8	22.8	22.5	23.2	23.1
SD	2.348	2.394	3.293	2.550	4.315	2.514
CV %	12.23	10.98	14.44	11.33	18.60	10.88

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Permittee: City of Decatur Effluent discharge.

CERIODAPHNIA MEAN PERCENT SURVIVAL

Time Elapsed	Percent Effluent (%)					
	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.)

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	Until 60% or more surviving control females have three broods or a maximum of 8 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: 1-13-15

SAMPLE NO. 2 COLLECTED: DATE: 1-14-15

SAMPLE NO. 3 COLLECTED: DATE: 1-16-15

TABLE 2 (SECTION 2)
 INITIAL WATER QUALITY
 EFFLUENT CONCENTRATION

	Control	100%
PH	7.50	7.48
D.O.	8.00	8.10
Temp	25.0	25.0
Alk	62	200
Hard	98	122
Cond	312	695
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

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TEST WATER QUALITY

24-Hour Water Quality Measurements

Effluent Concentration (%)	PH	D.O. (mg/l)	Temperature (C)
0% Control	7.73	7.30	25.1
32% Effluent	8.08	7.20	25.1
42% Effluent	8.14	7.20	25.1
56% Effluent	8.23	7.10	25.1
75% Effluent	8.27	7.10	25.1
100% Effluent	8.33	7.00	25.1

48-Hour Water Quality Measurements

Effluent Concentration (%)	PH	D.O. (mg/l)	Temperature (C)
0% Control	7.72	7.60	25.0
32% Effluent	8.10	7.00	25.0
42% Effluent	8.12	6.90	25.0
56% Effluent	8.16	6.80	25.0
75% Effluent	8.20	6.60	25.0
100% Effluent	8.25	6.40	25.0

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FINAL WATER QUALITY

EFFLUENT CONCENTRATION

	Control	100%
pH	7.69	8.08
D.O.	7.00	6.10
Temp	25.0	25.0
Alk	64	180
Hard	98	134
Cond	469	1092

- * 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 Pimephales promelas control survival rate was 97.5%. The mean dry weight (growth) of the Pimephales promelas was determined at 0.449 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 11.21. The Ceriodaphnia dubia survival rates were 100 in the control. The Ceriodaphnia in the control produced an average of 19.2 young over the seven-day exposure period. Percent CV values for Ceriodaphnia dubia control survival and reproduction was 0.00 and 12.23. Control data met or exceeded all criteria set out by EPA 821-R-02-013 for test acceptance.

CONCLUSIONS

The No Observed Effect Concentration (NOEC) for Pimephales promelas was 100% for survival and 100% for growth. The No Observed Effect Concentration (NOEC) for Ceriodaphnia dubia 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 1-13-15, 1-14-15, and 1-16-15 exhibited acceptable chronic toxicity in Pimephales promelas and in Ceriodaphnia dubia 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: 1/13/15 14:45 End: 1/20/15 13:00

Concentration of Toxicant	Avg. # of Live Organisms/replicate			
	0 hrs	24 hrs	48 hrs	7 days
10 g/l	40	7	2	0
8 g/l	40	32	22	4
6 g/l	40	38	33	26
4 g/l	40	40	40	40
2 g/l	40	40	40	39

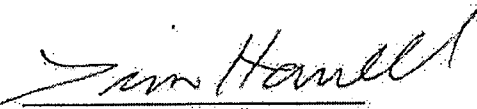
IC25 (5.28 g/l Sodium Chloride)

Survival NOEC: 4.0 g/l

Concentration of Toxicant	Avg. # of Live Organisms/replicate			
	0 hrs	24 hrs	48 hrs	7 days
2.5 g/l	10	4	0	0
2.0 g/l	10	10	9	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 (1.22 g/l Sodium Chloride)

Survival NOEC: 1.5 g/l

Submitted By: 
Timothy Harrell, Technical Director

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60186050 Decatur FATHEAD SURVIVAL
File: 6186050A 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	2.010	7.260	11.460	7.260	2.010
OBSERVED	4	5	16	5	0

Calculated Chi-Square goodness of fit test statistic = 7.1858

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

60186050 Decatur FATHEAD SURVIVAL
File: 6186050A Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.104

W = 0.815

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.

60186050 Decatur FATHEAD SURVIVAL

File: 6186050A Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	CONTROL	5	0.991	1.107	1.084
2	32%	5	0.991	1.107	1.061
3	42%	5	0.991	1.107	1.038
4	56%	5	0.886	1.107	1.063
5	75%	5	0.991	1.107	1.084
6	100%	5	0.991	1.107	1.084

60186050 Decatur FATHEAD SURVIVAL

File: 6186050A 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%	0.004	0.064	0.028	5.99
3	42%	0.004	0.064	0.028	6.12
4	56%	0.010	0.099	0.044	9.30
5	75%	0.003	0.052	0.023	4.79
6	100%	0.003	0.052	0.023	4.79

60186050 Decatur FATHEAD SURVIVAL

File: 6186050A

Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.009	0.002	0.408
Within (Error)	24	0.104	0.004	
Total	29	0.112		

Critical F value = 2.62 (0.05,5,24)

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All equal

60186050 Decatur FATHEAD SURVIVAL

File: 6186050A

Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	1.084	0.780		
2	32%	1.061	0.760	0.558	
3	42%	1.038	0.740	1.116	
4	56%	1.063	0.760	0.506	
5	75%	1.084	0.780	0.000	
6	100%	1.084	0.780	0.000	

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

60186050 Decatur FATHEAD SURVIVAL

File: 6186050A

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	0.086	11.0	0.020
3	42%	5	0.086	11.0	0.040
4	56%	5	0.086	11.0	0.020
5	75%	5	0.086	11.0	0.000
6	100%	5	0.086	11.0	0.000

60186050 Decatur FATHEAD GROWTH
File: 6186050B Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.114

W = 0.969

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.

60186050 Decatur FATHEAD GROWTH
File: 6186050B 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.

60186050 Decatur FATHEAD GROWTH
File: 6186050B Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	CONTROL	5	0.375	0.497	0.449
2	32%	5	0.314	0.485	0.403
3	42%	5	0.339	0.523	0.425
4	56%	5	0.289	0.526	0.418
5	75%	5	0.410	0.521	0.475
6	100%	5	0.325	0.502	0.436

60186050 Decatur FATHEAD GROWTH
File: 6186050B Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	CONTROL	0.003	0.050	0.023	11.21
2	32%	0.005	0.069	0.031	17.20
3	42%	0.007	0.081	0.036	19.09
4	56%	0.008	0.088	0.039	20.99
5	75%	0.002	0.042	0.019	8.74
6	100%	0.005	0.071	0.032	16.32

60186050 Decatur FATHEAD GROWTH

File: 6186050B

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.016	0.003	0.691
Within (Error)	24	0.114	0.005	
Total	29	0.130		

Critical F value = 2.62 (0.05,5,24)

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All equal

60186050 Decatur FATHEAD GROWTH
 File: 6186050B 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	0.449	0.449		
2	32%	0.403	0.403	1.071	
3	42%	0.425	0.425	0.570	
4	56%	0.418	0.418	0.731	
5	75%	0.475	0.475	-0.593	
6	100%	0.436	0.436	0.299	

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

60186050 Decatur FATHEAD GROWTH
 File: 6186050B 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%	5	0.103	22.8	0.047
3	42%	5	0.103	22.8	0.025
4	56%	5	0.103	22.8	0.032
5	75%	5	0.103	22.8	-0.026
6	100%	5	0.103	22.8	0.013

FISHER'S EXACT TEST

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

NUMBER OF

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 .

NUMBER OF

IDENTIFICATION	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%	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
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	0	
2	42%	10	0	
3	56%	10	0	
4	75%	10	0	
5	100%	10	0	

60186050 Decatur CERIODAPHNIA DUBIA SURVIVAL
 File: 6186050D 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

60186050 Decatur CERIODAPHNIA DUBIA SURVIVAL
 File: 6186050D 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

60186050 Decatur CERIODAPHNIA DUBIA REPRODU
File: 6186050E 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
EXPECTED	4.020	14.520	22.920	14.520	4.020
OBSERVED	7	9	23	19	2

Calculated Chi-Square goodness of fit test statistic = 6.7051

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

60186050 Decatur CERIODAPHNIA DUBIA REPRODU
File: 6186050E Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 5.65

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.

60186050 Decatur CERIODAPHNIA DUBIA REPRODU
File: 6186050E Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	CONTROL	10	15.000	22.000	19.200
2	32%	10	18.000	25.000	21.800
3	42%	10	17.000	28.000	22.800
4	56%	10	17.000	26.000	22.500
5	75%	10	15.000	28.000	23.200
6	100%	10	18.000	27.000	23.100

60186050 Decatur CERIODAPHNIA DUBIA REPRODU
File: 6186050E Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	CONTROL	5.511	2.348	0.742	12.23
2	32%	5.733	2.394	0.757	10.98
3	42%	10.844	3.293	1.041	14.44
4	56%	6.500	2.550	0.806	11.33
5	75%	18.622	4.315	1.365	18.60
6	100%	6.322	2.514	0.795	10.88

60186050 Decatur CERIODAPHNIA DUBIA REPRODU
File: 6186050E Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	113.600	22.720	2.546
Within (Error)	54	481.800	8.922	
Total	59	595.400		

Critical F value = 2.45 (0.05, 5, 40)
Since $F > \text{Critical } F$ REJECT H_0 : All equal

60186050 Decatur CERIODAPHNIA DUBIA REPRODU
 File: 6186050E 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	19.200	19.200		
2	32%	21.800	21.800	-1.946	
3	42%	22.800	22.800	-2.695	
4	56%	22.500	22.500	-2.470	
5	75%	23.200	23.200	-2.994	
6	100%	23.100	23.100	-2.920	

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

60186050 Decatur CERIODAPHNIA DUBIA REPRODU
 File: 6186050E 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%	10	3.086	16.1	-2.600
3	42%	10	3.086	16.1	-3.600
4	56%	10	3.086	16.1	-3.300
5	75%	10	3.086	16.1	-4.000
6	100%	10	3.086	16.1	-3.900

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	32	42	56	75	100
Response 1	.497	.351	.357	.436	.498	.424
Response 2	.421	.441	.339	.289	.410	.436
Response 3	.474	.423	.412	.386	.470	.502
Response 4	.480	.314	.523	.526	.521	.495
Response 5	.375	.485	.492	.451	.477	.325

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: Decatur

Test Start Date: 1/13/15 Test Ending Date: 1/20/15

Test Species: Fathead

Test Duration: 7 Day

DATA FILE:

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	5	0.000	0.449	0.050	0.449
2	5	32.000	0.403	0.069	0.431
3	5	42.000	0.425	0.081	0.431
4	5	56.000	0.418	0.088	0.431
5	5	75.000	0.475	0.042	0.431
6	5	100.000	0.436	0.071	0.431

*** 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. Tested	0	32	42	56	75	100
Response 1	16	20	19	17	17	25
Response 2	22	23	24	23	26	24
Response 3	19	22	22	22	23	24
Response 4	21	18	22	24	22	27
Response 5	20	23	24	22	23	24
Response 6	18	22	26	21	28	23
Response 7	15	25	25	26	24	20
Response 8	22	24	21	21	27	18
Response 9	20	23	28	25	15	23
Response 10	19	18	17	24	27	23

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: Decatur

Test Start Date: 1/13/15 Test Ending Date: 1/20/15

Test Species: Dubia

Test Duration: 7 Day

DATA FILE:

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	10	0.000	19.200	2.348	22.100
2	10	32.000	21.800	2.394	22.100
3	10	42.000	22.800	3.293	22.100
4	10	56.000	22.500	2.550	22.100
5	10	75.000	23.200	4.315	22.100
6	10	100.000	23.100	2.514	22.100

*** 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.

Section A Required Client Information:	Section B Required Project Information:	Section C Invoice Information:	Page: of 1830889
Company: <u>City of Decatur</u>	Report To: <u>James Boston</u>	Attention:	
Address:	Copy To:	Company Name:	REGULATORY AGENCY
Email To:	Purchase Order No.:	Address:	<input type="checkbox"/> NPDES <input type="checkbox"/> GROUND WATER <input type="checkbox"/> DRINKING WATER <input type="checkbox"/> UST <input type="checkbox"/> RCRA <input type="checkbox"/> OTHER _____
Phone: Fax:	Project Name:	Pace Quote Reference:	Site Location: STATE:
Requested Due Date/TAT:	Project Number:	Pace Project Manager: Pace Profile #:	

ITEM #	Section D Required Client Information	Matrix Codes MATRIX / CODE	MATRIX CODE (see valid codes to left)	SAMPLE TYPE (G=GRAB C=COMP)	COLLECTED		SAMPLE TEMP AT COLLECTION	# OF CONTAINERS	Preservatives							Requested Analysis Filtered (Y/N)		Residual Chlorine (Y/N)		
					COMPOSITE START	COMPOSITE END/GRAB			Unpreserved	H ₂ SO ₄	HNO ₃	HCl	NaOH	Na ₂ S ₂ O ₃	Methanol	Other	Analysis Test ↓			
	SAMPLE ID (A-Z, 0-9 / .-) Sample IDs MUST BE UNIQUE	Drinking Water DW Water WT Waste Water WW Product P Soil/Solid SL Oil OL Wipe WP Air AR Tissue TS Other OT			DATE	TIME														
1	Chronic Bio (1)				1/13	10am	1/13	9am	1	X								X	Chronic Wet Test	60/86050
2																				
3																				
4																				
5																				
6																				
7																				
8																				
9																				
10																				
11																				
12																				

ADDITIONAL COMMENTS	RELINQUISHED BY / AFFILIATION	DATE	TIME	ACCEPTED BY / AFFILIATION	DATE	TIME	SAMPLE CONDITIONS			
	<u>ml [signature]</u>	<u>1/13/15</u>	<u>9:20am</u>	<u>[signature]</u>	<u>1/13/15</u>	<u>14:30</u>	<u>3.4</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>

CHAIN-OF-CUSTODY / Analytical Request Document

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

Section A Required Client Information:	Section B Required Project Information:	Section C Invoice Information:	Page: _____ of _____
Company: CITY OF DECATUR	Report To: JAMES BOSTON	Attention:	1830868
Address:	Copy To:	Company Name:	REGULATORY AGENCY
Email To:	Purchase Order No.:	Address:	
Phone: _____ Fax: _____	Project Name:	Pace Quote Reference:	
Requested Due Date/TAT:	Project Number:	Pace Project Manager:	<input type="checkbox"/> NPDES <input type="checkbox"/> GROUND WATER <input type="checkbox"/> DRINKING WATER
		Pace Profile #:	<input type="checkbox"/> UST <input type="checkbox"/> RCRA <input type="checkbox"/> OTHER _____
			Site Location STATE: _____

ITEM #	SAMPLE ID (A-Z, 0-9 / -) Sample IDs MUST BE UNIQUE	Matrix Codes MATRIX / CODE		COLLECTED				SAMPLE TEMP AT COLLECTION	# OF CONTAINERS	Preservatives								Analysis Test Y/N	Requested Analysis Filtered (Y/N)	Residual Chlorine (Y/N)	Pace Project No./ Lab I.D.													
		DW	WT	COMPOSITE START	COMPOSITE END/GRAB	Unpreserved	H ₂ SO ₄			HNO ₃	HCl	NaOH	Na ₂ S ₂ O ₃	Methanol	Other																			
																WW	P					SL	OL	WP	AR	TS	OT							
1	Chronic BID(2)	NA	C			1/14/15	9:00 AM		1	X									X															
2																																		
3																																		
4																																		
5																																		
6																																		
7																																		
8																																		
9																																		
10																																		
11																																		
12																																		

ADDITIONAL COMMENTS	RELINQUISHED BY / AFFILIATION	DATE	TIME	ACCEPTED BY / AFFILIATION	DATE	TIME	SAMPLE CONDITIONS
	Alton Verser	1/14/15	9:00 AM	[Signature]	1/14/15	1530	3.6 Y Y Y

Page 50 of 55

ORIGINAL

SAMPLER NAME AND SIGNATURE			
PRINT Name of SAMPLER: Alton Verser			
SIGNATURE of SAMPLER: [Signature]			DATE Signed (MM/DD/YY): 1/14/15
Temp in °C	Received on Ice (Y/N)	Custody Sealed Cooler (Y/N)	Samples Intact (Y/N)

*Important Note: By signing this form you are accepting Pace's NET 30 day payment terms and agreeing to late charges of 1.5% per month for any invoices not paid within 30 days.



Sample Condition Upon Receipt

Client Name: DeCatur

Courier: Fed Ex UPS USPS Client Commercial Pace Other

Tracking #: _____ Pace Shipping Label Used? Yes No

Custody Seal on Cooler/Box Present: Yes No Seals intact: Yes No

Packing Material: Bubble Wrap Bubble Bags Foam None Other

Thermometer Used: T-243 Type of Ice: Wet Blue None Samples received on ice, cooling process has begun.
(circle one)

Cooler Temperature: 3.6

Temperature should be above freezing to 6°C

Optional
Proj Due Date:
Proj Name:

Date and initials of person examining contents: 1/14/15 1530 MB

Chain of Custody present:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	1.
Chain of Custody filled out:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	2.
Chain of Custody relinquished:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	3.
Sampler name & signature on COC:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	4.
Samples arrived within holding time:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	5.
Short Hold Time analyses (<72hr):	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	6.
Rush Turn Around Time requested:	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	7.
Sufficient volume:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	8.
Correct containers used:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	
Pace containers used:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	9.
Containers intact:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	10.
Unpreserved 5035A soils frozen w/in 48hrs?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A	11.
Filtered volume received for dissolved tests?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A	12.
Sample labels match COC:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	
Includes date/time/ID/analyses: Matrix: <u>WT</u>		13.
All containers needing preservation have been checked.	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A	
All containers needing preservation are found to be in compliance with EPA recommendation.	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A	14.
Exceptions: VOA, coliform, TOC, O&G, WI-DRO (water), Phenolics	<input checked="" type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Initial when completed
Trip Blank present: <u>MB 1/14/15</u>	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A	Lot # of added preservative
Pace Trip Blank lot # (if purchased):		15.
Headspace in VOA vials (>6mm):	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A	
		16.
Project sampled in USDA Regulated Area:	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A	17. List State:

Client Notification/ Resolution: Copy COC to Client? Y / N Field Data Required? Y / N

Person Contacted: _____ Date/Time: _____

Comments/ Resolution: _____

Project Manager Review: _____ Date: _____



CHAIN-OF-CUSTODY / Analytical Request Document

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

Section A Required Client Information:		Section B Required Project Information:		Section C Invoice Information:		Page: _____ of _____	
Company: <u>City of Decatur</u>		Report To: <u>James Boston</u>		Attention:		1830870	
Address:		Copy To:		Company Name:		REGULATORY AGENCY:	
Email To:		Purchase Order No.:		Address:		<input type="checkbox"/> NPDES <input type="checkbox"/> GROUND WATER <input type="checkbox"/> DRINKING WATER <input type="checkbox"/> UST <input type="checkbox"/> RCRA <input type="checkbox"/> OTHER _____	
Phone:	Fax:	Project Name:		Pace Quote Reference:		Site Location	
Requested Due Date/TAT:		Project Number:		Pace Project Manager:		STATE:	
				Pace Profile #:			

ITEM #	Section D Required Client Information	Matrix Codes MATRIX / CODE	MATRIX CODE (see valid codes to left)	SAMPLE TYPE (G=GRAB C=COMP)	COLLECTED				SAMPLE TEMP AT COLLECTION	# OF CONTAINERS	Preservatives								Analysis Test ↓	Y/N	Requested Analysis Filtered (Y/N)	Residual Chlorine (Y/N)	Pace Project No./ Lab I.D.						
					COMPOSITE START		COMPOSITE END/GRAB				Unpreserved	H ₂ SO ₄	HNO ₃	HCl	NaOH	Nap ₂ S ₂ O ₃	Methanol	Other											
					DATE	TIME	DATE	TIME																					
1	Chlorine Bio (3)		C	C	1/15/15	8Am	1/16/15	7Am	10	2	X																		
2																													
3																													
4																													
5																													
6																													
7																													
8																													
9																													
10																													
11																													
12																													

ADDITIONAL COMMENTS	RELINQUISHED BY / AFFILIATION	DATE	TIME	ACCEPTED BY / AFFILIATION	DATE	TIME	SAMPLE CONDITIONS
	ml 23	1/16/15	8Am	Bill Pace	1/16/15	1500 2.0	Y Y Y

ORIGINAL	SAMPLER NAME AND SIGNATURE		Temp in °C	Received on ice (Y/N)	Custody Sealed Cooler (Y/N)	Samples Intact (Y/N)
	PRINT Name of SAMPLER: <u>Mike J. [Signature]</u>					
	SIGNATURE of SAMPLER: <u>[Signature]</u>					
		DATE Signed (MM/DD/YY): <u>1/16/15</u>				

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Sample Condition Upon Receipt

Client Name: Decatur

Courier: Fed Ex UPS USPS Client Commercial Pace Other

Tracking #: _____ Pace Shipping Label Used? Yes No

Custody Seal on Cooler/Box Present: Yes No Seals intact: Yes No

Packing Material: Bubble Wrap Bubble Bags Foam None Other

Thermometer Used: T-243

Type of Ice: Wet Blue None Samples received on ice, cooling process has begun.
(circle one)

Cooler Temperature: 2.0

Temperature should be above freezing to 6°C

Date and initials of person examining contents: 1/16/15 1500 MS

Chain of Custody present:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	1.
Chain of Custody filled out:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	2.
Chain of Custody relinquished:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	3.
Sampler name & signature on COC:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	4.
Samples arrived within holding time:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	5.
Short Hold Time analyses (<72hr):	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	6.
Rush Turn Around Time requested:	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No <input type="checkbox"/> N/A	7.
Sufficient volume:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	8.
Correct containers used:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	
Pace containers used:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	9.
Containers intact:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	10.
Unpreserved 5035A soils frozen w/in 48hrs?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A	11.
Filtered volume received for dissolved tests?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A	12.
Sample labels match COC:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	
Includes date/time/ID/analyses Matrix: <u>WT</u>		13.
All containers needing preservation have been checked.	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A	
All containers needing preservation are found to be in compliance with EPA recommendation.	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A	14.
Exceptions: VOA, coliform, TOC, O&G, W/DRO (water), Phenolics.	<input checked="" type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Initial when completed
Trip Blank present: <u>MS 1/16/15</u>	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A	Lot # of added preservative
Pace Trip Blank lot # (if purchased):		15.
Headspace in VOA vials (>6mm):	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A	16.
Project sampled in USDA Regulated Area:	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A	17. List State:

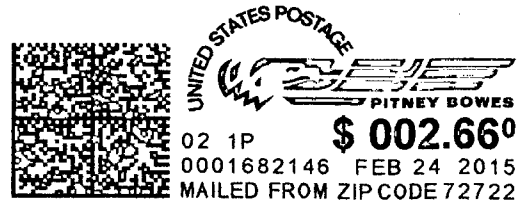
Client Notification/ Resolution: Copy COC to Client? Y / N Field Data Required? Y / N

Person Contacted _____ Date/Time: _____

Comments/ Resolution: _____

Project Manager Review: _____ Date: _____

Cit 310
Decatur AR 72722



Arkansas Dept. of Environmental Quality
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