AMERICAN INTERPLEX CORPORATION
LABORATORIES

August 30, 2013

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Outfall 001
City of Hot Springs

Control No. 169846-2

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Ceriodaphnia dubia*
Outfall 001 - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Chronic *Ceriodaphnia dubia* test: Due to laboratory error, the *Ceriodaphnia dubia* test was not renewed with the third sample. The test should be repeated. The data from the test is enclosed for your review.

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Table of Contents

I. Control Acceptance Criteria

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	23.6	PASS
Control CV < or = 40% per Surviving Female	13.6	PASS
Reproduction Minimum Significant Difference 13 to 47%	25.8	PASS
Critical Dilution CV < or = 40%	22.9	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly
   Test Method 1002.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point: Outfall 001
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.9	8.5	NA
pH (standard units)	7.0	7.4	NA
Alkalinity (mg/l as CaCO3)	26	34	NA
Hardness (mg/l as CaCO3)	67	71	NA
Conductivity (umhos/cm)	350	390	NA
Residual Chlorine (mg/l)	<0.05	<0.05	NA
Ammonia as N (mg/l)	0.12	0.30	NA

2. Dilution Water Samples: Synthetic Soft Water #4012
   a. Dates Prepared: August 8 through August 22, 2013
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.7	7.7	7.8
pH (standard units)	7.7	7.8	7.9
Alkalinity (mg/l as CaCO3)	32	32	NA
Hardness (mg/l as CaCO3)	47	42	NA
Conductivity (umhos/cm)	180	170	170
Residual Chlorine (mg/l)	<0.05	<0.05	NA

C. Test Methods
1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Method 1002.0, *Ceriodaphnia dubia* Survival and Reproduction.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Ceriodaphnia dubia Survival and Growth Method 1002.0

Date & Time Test Initiated:	August 22, 2013 at 1605
Date & Time Test Terminated:	August 29, 2013 at 1415
Type & Volume of Test Chamber:	30 ml disposable beaker
Volume of Sample:	15 ml
Number of Organisms per replicate:	1
Number of Replicates per dilution:	10

4. Acclimation of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms
1. Scientific Name
a. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis

The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was analyzed using Kolmogorov's Test for Normality and Bartlett's test and analyzed with Dunnett's Test to determine the No Observable Effects Concentration (NOEC) for Reproduction.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Ceriodaphnia dubia

Chronic reference tests are performed monthly.
A chronic reference test was performed on August 20, 2013 at 1505 to August 28, 2013 at 1450
The results were as follows: (Control No. 169867-2.)
Survival LC-50: 2125 mg/l
Growth IC-25: 1610 mg/l
Growth PMSD: 18.3

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	102	1.91
pH	SM 4500-H+ B	101	0.268
Conductivity	EPA 120.1	101	4.71

VI. Organism History

Ceriodaphnia dubia

Date: August 22, 2013
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

VII. Results Summary *Ceriodaphnia dubia*, Cladoceran Survival and Reproduction Test -- Method 1002.0

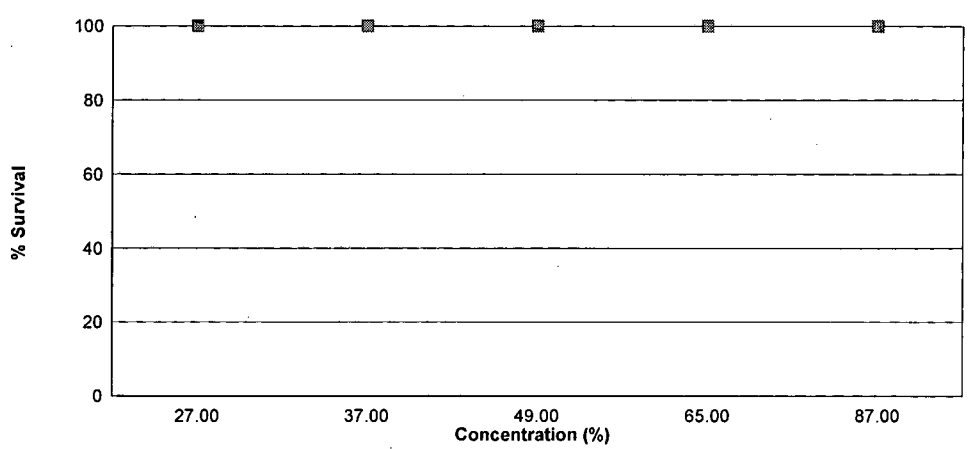
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on August 22, 2013 at 1605 and continued through August 29, 2013 at 1415. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 87 % effluent
b.) NOEC reproduction = 87 % effluent



Summary of the 7-day *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	23.6
27 %	100	24.2
37 %	100	27.6
49 %	100	26.4
65 %	100	24.2
87 %	100	25.0

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 22, 2013 at 1605
Date and Time Test Terminated: August 29, 2013 at 1415

Concentration: Control													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	4	4	2	3	4	4	3	2	33	10	3.30
5	0	0	0	0	0	1	0	0	0	6	7	10	0.700
6	9	9	7	8	7	8	7	9	8	8	80	10	8.00
7	14	14	11	13	14	11	12	14	13	0	116	10	11.6
8													
TOTAL	27	26	22	25	23	23	23	27	24	16	236	10	23.6

Concentration: 27 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	2	3	3	3	2	3	2	3	2	27	10	2.70
5	8	7	1	0	0	0	0	0	0	6	22	10	2.20
6	1	12	13	11	10	10	11	8	7	6	89	10	8.90
7	16	0	17	18	6	0	17	17	13	0	104	10	10.4
8													
TOTAL	29	21	34	32	19	12	31	27	23	14	242	10	24.2

Concentration: 37 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	3	3	3	2	3	2	2	2	27	10	2.70
5	0	0	0	0	0	1	0	0	6	6	13	10	1.30
6	12	12	12	11	10	6	8	10	13	10	104	10	10.4
7	18	16	17	18	16	15	15	17	0	0	132	10	13.2
8													
TOTAL	34	31	32	32	29	24	26	29	21	18	276	10	27.6

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 22, 2013 at 1605
Date and Time Test Terminated: August 29, 2013 at 1415

Concentration: 49 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	2	5	2	3	4	2	2	2	29	10	2.90
5	0	5	0	0	0	1	0	6	4	3	19	10	1.90
6	10	0	11	11	11	8	10	0	12	10	83	10	8.30
7	17	18	16	18	16	17	15	16	0	0	133	10	13.3
8													
TOTAL	31	26	29	34	29	29	29	24	18	15	264	10	26.4

Concentration: 65 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	2	3	5	3	3	3	2	3	2	2	28	10	2.80
5	6	0	0	0	0	1	0	0	5	4	16	10	1.60
6	13	11	11	11	10	10	8	10	0	12	96	10	9.60
7	0	15	14	16	12	0	15	16	14	0	102	10	10.2
8													
TOTAL	21	29	30	30	25	14	25	29	21	18	242	10	24.2

Concentration: 87 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	4	3	3	3	3	4	3	2	2	31	10	3.10
5	8	6	1	0	0	0	0	0	5	5	25	10	2.50
6	0	2	11	12	6	10	8	8	0	7	64	10	6.40
7	16	15	16	18	16	0	18	17	14	0	130	10	13.0
8													
TOTAL	28	27	31	33	25	13	30	28	21	14	250	10	25.0

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	10	0	10
27 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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	Alive	Dead	Total Animals
Control	10	0	10
37 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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	Alive	Dead	Total Animals
Control	10	0	10
49 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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	Alive	Dead	Total Animals
Control	10	0	10
65 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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.

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
87 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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 Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	27 %	10	0	
2	37 %	10	0	
3	49 %	10	0	
4	65 %	10	0	
5	87 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality		No Transformation
D = 0.1154 D* = 0.9054 Critical D* = 1.035	(alpha = 0.01, N = 60)	
Data PASS normality test (alpha = 0.01).		

Bartlett's Test for Homogeneity of Variance		No Transformation
Calculated B1 statistic = 6.592 Critical B = 15.086	(alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.		

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	117.9	23.58	0.6766
Within (Error)	54	1882	34.85	
Total	59	2000		

Critical F = 3.38 (alpha = 0.01, df = 5,54)
2.38 (alpha = 0.05, df = 5,54)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Dunnett's Test - Table 1 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	23.6	23.6		
2	27 %	24.2	24.2	-0.2273	
3	37 %	27.6	27.6	-1.515	
4	49 %	26.4	26.4	-1.061	
5	65 %	24.2	24.2	-0.2273	
6	87 %	25	25	-0.5303	

Dunnett's critical value = 2.31 (1 Tailed, alpha = 0.05, df [used] = 5,40) (Actual df = 5,54)

Dunnett's Test - Table 2 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	10			
2	27 %	10	6.099	25.8	-0.6
3	37 %	10	6.099	25.8	-4
4	49 %	10	6.099	25.8	-2.8
5	65 %	10	6.099	25.8	-0.6
6	87 %	10	6.099	25.8	-1.4

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 22, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.5	7.7	7.5	7.8	7.8	8.1
	Final	7.7	8.0	8.7	8.2	8.2	7.8	7.8
pH, units	Initial	7.7	7.7	7.8	8.0	7.9	8.0	7.7
	Final	8.0	8.2	8.0	8.0	8.0	7.8	7.8
Alkalinity, mg CaCO3/l		32	NA	32	NA	NA	NA	NA
Hardness, mg CaCO3/l		47	NA	42	NA	NA	NA	NA
Conductivity, umhos/cm		180	180	170	180	170	170	190
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	NA	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.4	8.0	7.5	8.0	8.0	7.5
	Final	7.5	8.2	8.6	8.2	8.2	7.7	7.7
pH, units	Initial	7.6	7.7	7.5	7.8	7.7	7.8	7.5
	Final	8.0	8.1	8.0	8.0	8.1	7.6	7.6

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.6	8.6	7.6	8.2	7.7	7.7
	Final	7.6	8.0	8.6	8.0	8.2	7.6	7.6
pH, units	Initial	7.6	7.7	7.4	7.7	7.6	7.7	7.6
	Final	8.0	8.1	8.0	8.0	8.1	7.6	7.6

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 22, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.5	8.7	7.6	8.1	7.7	7.7
	Final	7.7	8.1	8.6	8.2	8.2	7.6	7.9
pH, units	Initial	7.5	7.7	7.3	7.6	7.6	7.6	7.5
	Final	8.0	8.1	8.0	8.1	8.2	7.5	7.5

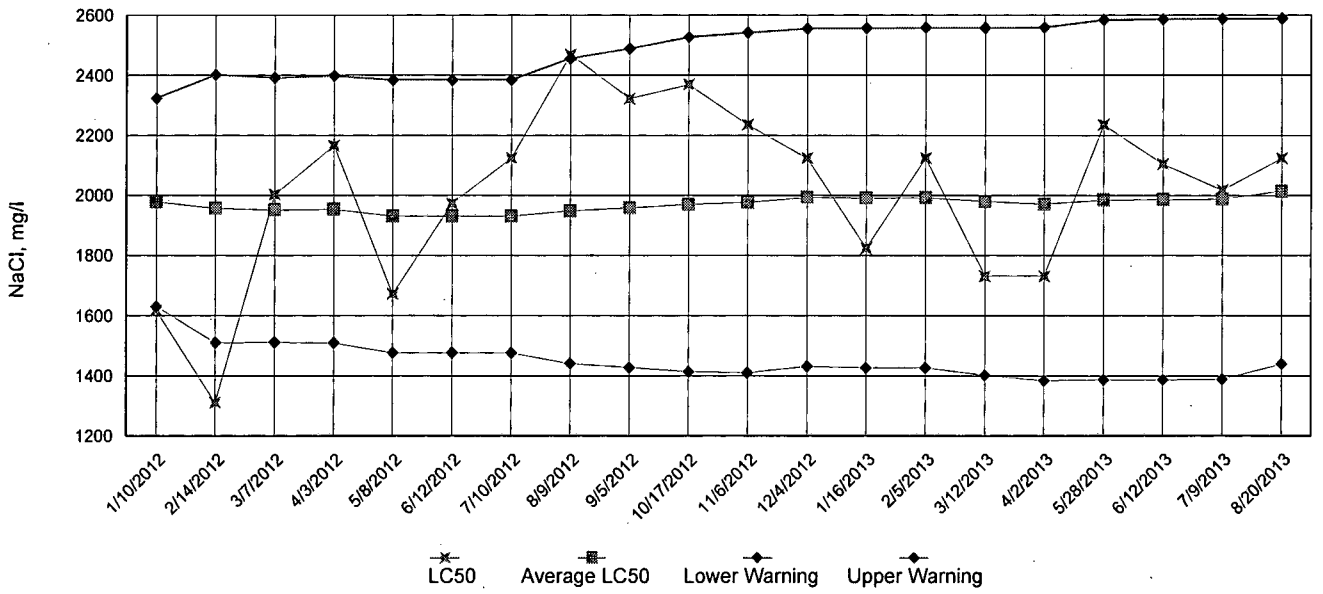
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.9	7.4	8.8	7.6	8.7	7.7	7.3
	Final	7.7	8.0	8.6	8.4	8.1	7.6	7.6
pH, units	Initial	7.5	7.7	7.2	7.5	7.5	7.6	7.5
	Final	8.0	8.0	8.0	8.1	8.1	7.5	7.5
Alkalinity, mg CaCO3/l		36	NA	30	NA	NA	NA	NA
Hardness, mg CaCO3/l		54	NA	58	NA	NA	NA	NA
Conductivity, umhos/cm		250	260	280	290	310	310	330
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	NA	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	7.4	8.9	7.6	8.3	7.9	7.4
	Final	7.4	8.1	8.7	8.2	7.7	5.4	7.2
pH, units	Initial	7.4	7.7	7.1	7.4	7.4	7.6	7.6
	Final	8.0	8.0	7.9	8.1	8.3	7.5	7.4

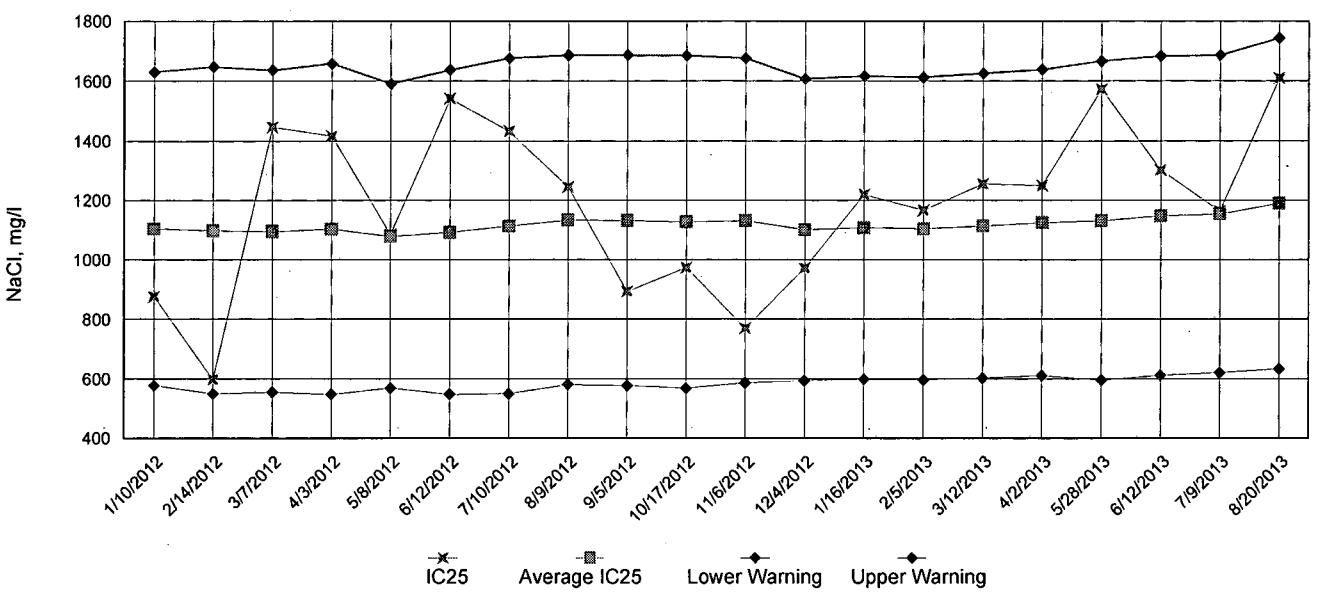
Appendix A4: Test 1002.0

Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL		No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
1	Plant Effluent	8-18-13 0900 - 2400		X	X			3	X
		Container Type							P
		Preservative							NO

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 20c
Remarks
Field pH calibration on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ___ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
A. Thomason	8-19-13 @ 1010	M. Mann	8-19-13 @ 10:10am
Relinquished By: M. Mann	Date/Time 8-19-13 @ 11:10AM	Received In Lab By: Angie Hopton	Date/Time 8-19-13 1110

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL		No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
2	Plant Effluent	8-20-13 0900-2400		X	X			3	X
		Container Type							P
		Preservative							NO

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 23C
Remarks

Field pH calibration
on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
A. Ross	8-21-13 0900	G. Mann	8-21-13 900
G. Mann	8-21-13 1130	Jimmy Day	8/21/13 1130

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By:

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
3	Plant Effluent	8/22/13 0000-2400		X			3	X
		Container Type						P
		Preservative						NO

AIC Control No: 16984b
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 2
Remarks
Field pH calibration on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	8-23-13 0955	[signature]	8-23-13 9:55
[signature]	8-23-13 11:20	[signature]	8-23-13 1120

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

September 30, 2013

Test Results of
Acute 48 hour Non-Renewal
Biomonitoring Testing
for

170741-1: Outfall 001 First Renewal
170741-2: Outfall 001 Second Renewal
170741-3: Outfall 001 Third Renewal
170741-4: Outfall 001 Original Screen

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Acute 48 hour Non-Renewal Biomonitoring utilizing *Ceriodaphnia dubia*
Outfall 001 First Renewal - City of Hot Springs
Client NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

Please find attached the results of the acute biomonitoring screens conducted as a follow up to the chronic toxicity event previously reported (AIC Control No. 170390). As indicated on the enclosed results, the sample was still acutely toxic on September 12, 2013 and exhibited no acute toxicity on September 19, 2013.

It it theorized the decrease in toxicity is directly related to the holding time which may have allowed microbial degradation, loss of volatile toxics, and/or biochemical degradation.

If I can be of further assistance, please feel free to contact me.

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Dilution Water Samples: Synthetic Soft Water #4021

Analysis	Result
Dissolved oxygen (mg/l)	7.6
pH (standard units)	7.7
Alkalinity (mg/l as CaCO3)	31
Hardness (mg/l as CaCO3)	46
Conductivity (umhos/cm)	170
Residual Chlorine (mg/l)	<0.05

Results Summary: Outfall 001 First Renewal

Ceriodaphnia dubia

The *Ceriodaphnia dubia* test was conducted from September 19, 2013 at 1615 to September 21, 2013 at 1700.

Statistical analyses:

NOEC = 100%

Concentration	24 hour % Survival	48 hour % Survival
Control	100	100
100%	100	100

Ceriodaphnia dubia
Survival Data

Number of organisms per chamber: 5
Volume of test chamber: 30 ml

Age of organisms: <24 hours
Volume of test solution: 15 ml

Effluent Concentration	Number of Survivors 24 Hours	48 Hours	% Survival	CV %
Control rep. A	5	5	100	0.00
100% rep. A	5	5	100	0.00

CV = Coefficient of variance = standard deviation X 100/mean

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	5	0	5
100%	5	0	5
Total	10	0	10

Critical Fisher's value (5,5,5) (alpha=0.05) is 1. b value is 5. Since b is greater than 1 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test

Group	Identification	Exposed	Dead	Sig 0.05
0	0	5	0	
1	100	5	0	

Chemical Data for
Ceriodaphnia dubia

Day 1	Control	100%
DO, mg/l	7.6	8.8
pH, su	7.7	7.3
Alkalinity, mg/l	31	NA
Hardness, mg/l	46	NA
Conductivity, umho/cm	170	380
Residual Chlorine, mg/l	<0.05	NA

Day 2		Control	100%
DO, mg/l	Final	7.5	8.7
pH, su	Final	8.0	7.4

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

month WR 9/19/13

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Chronic C.D.	Analyses Requested
1	Plant Effluent	9-8-13 0000-2400		X	X		3	X	
		Container Type						P	
		Preservative						NO	

No FATHEAD ANALYSIS

AIC Control No: 120390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2.1 °C
Remarks:
Field pH calibration on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z = Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-8-13 1030	M. Mann	9-9-13 10:30 AM
M. Mann	9-9-13 11:45	[signature]	9-9-13 1145

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

October 1, 2013
Control No. 171032

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

This report contains the analytical results and supporting information for samples submitted on September 27, 2013. Attached please find a copy of the Chain of Custody and/or other documents received. Note that any remaining sample will be discarded two weeks from the original report date unless other arrangements are made.

This report is intended for the sole use of the client listed above. Assessment of the data requires access to the entire document.

This report has been reviewed by the Laboratory Director or a qualified designee.

John Overbey
Laboratory Director

This document has been distributed to the following:

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

SAMPLE INFORMATION

Project Description:
Three (3) water sample(s) (AIC COntrol No. 170741-1,2,3) resubmitted September 27, 2013
P.O. No. 13-3032

Receipt Details:
A Chain of Custody was not provided with the sample(s).

Each sample container was checked for proper labeling, including date and time sampled. Sample containers were reviewed for proper type, adequate volume, integrity, temperature, preservation, and holding times. Any exceptions are noted below:

Sample Identification:

Laboratory ID	Client Sample ID	Sampled Date/Time	Notes
171032-1	Plant Effluent 9-8-13 0000-2400	08-Sep-2013 2359	
171032-2	Plant Effluent 9-10-13 0000-2400	10-Sep-2013 2359	
171032-3	Plant Effluent 9-12-13 0000-2400	12-Sep-2013 2359	

Case Narrative:
There were no qualifiers for this data and all samples met quality control criteria.

References:
"Methods for Chemical Analysis of Water and Wastes", EPA/600/4-79-020 (Mar 1983) with updates and supplements EPA/600/5-91-010 (Jun 1991), EPA/600/R-92-129 (Aug 1992) and EPA/600/R-93-100 (Aug 1993).
"Test Methods for Evaluating Solid Waste Physical/Chemical Methods (SW846)", Third Edition.
"Standard Methods for the Examination of Water and Wastewaters", 21st edition.
"American Society for Testing and Materials" (ASTM).
"Association of Analytical Chemists" (AOAC).

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

ANALYTICAL RESULTS

AIC No. 171032-1
Sample Identification: Plant Effluent 9-8-13 0000-2400

Analyte		Result	RL	Units	Qualifier
Total Organic Carbon		**6.1**	1	**mg/l**	
SM 5310 C	Prep: 27-Sep-2013 1516 by 308	Analyzed: 27-Sep-2013 1814 by 308		Batch: W45076	
Aluminum		**< 0.04**	0.04	**mg/l**	
EPA 200.7	Prep: 30-Sep-2013 0901 by 271	Analyzed: 01-Oct-2013 1242 by 305		Batch: S35491	
Sulfate		**29**	0.2	**mg/l**	
EPA 300.0	Prep: 27-Sep-2013 1628 by 07	Analyzed: 27-Sep-2013 2205 by 07		Batch: C16076	

AIC No. 171032-2
Sample Identification: Plant Effluent 9-10-13 0000-2400

Analyte		Result	RL	Units	Qualifier
Total Organic Carbon		**5.9**	1	**mg/l**	
SM 5310 C	Prep: 27-Sep-2013 1516 by 308	Analyzed: 27-Sep-2013 1828 by 308		Batch: W45076	
Aluminum		**0.041**	0.04	**mg/l**	
EPA 200.7	Prep: 30-Sep-2013 0901 by 271	Analyzed: 01-Oct-2013 1245 by 305		Batch: S35491	
Sulfate		**39**	0.2	**mg/l**	
EPA 300.0	Prep: 27-Sep-2013 1628 by 07	Analyzed: 27-Sep-2013 2232 by 07		Batch: C16076	

AIC No. 171032-3
Sample Identification: Plant Effluent 9-12-13 0000-2400

Analyte		Result	RL	Units	Qualifier
Total Organic Carbon		**5.4**	1	**mg/l**	
SM 5310 C	Prep: 27-Sep-2013 1516 by 308	Analyzed: 27-Sep-2013 1843 by 308		Batch: W45076	
Aluminum		**< 0.04**	0.04	**mg/l**	
EPA 200.7	Prep: 30-Sep-2013 0901 by 271	Analyzed: 01-Oct-2013 1248 by 305		Batch: S35491	
Sulfate		**34**	0.2	**mg/l**	
EPA 300.0	Prep: 27-Sep-2013 1628 by 07	Analyzed: 27-Sep-2013 2259 by 07		Batch: C16076	

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

LABORATORY CONTROL SAMPLE RESULTS

Analyte	Spike Amount	%	Limits	RPD	Limit	Batch	Preparation Date	Analysis Date	Dil	Qual
Total Organic Carbon	10 mg/l	96.4	80.0-120			W45076	27Sep13 1459 by 308	27Sep13 1648 by 308		
Aluminum	5 mg/l	97.6	85.0-115			S35491	30Sep13 0901 by 271	01Oct13 1152 by 305		
Sulfate	20 mg/l	108	90.0-110			C16076	27Sep13 1628 by 07	27Sep13 1710 by 07		

MATRIX SPIKE SAMPLE RESULTS

Analyte	Sample	Spike Amount	%	Limits	Batch	Preparation Date	Analysis Date	Dil	Qual
Total Organic Carbon	171006-1	10 mg/l	100	80.0-120	W45076	27Sep13 1459 by 308	27Sep13 1717 by 308		
	171006-1	10 mg/l	97.9	80.0-120	W45076	27Sep13 1459 by 308	27Sep13 1731 by 308		
	Relative Percent Difference:		1.86	25.0	W45076				
Aluminum	171022-2	5 mg/l	96.7	75.0-125	S35491	30Sep13 0901 by 271	01Oct13 1155 by 305		
	171022-2	5 mg/l	96.6	75.0-125	S35491	30Sep13 0901 by 271	01Oct13 1158 by 305		
	Relative Percent Difference:		0.142	20.0	S35491				
Sulfate	171021-1	20 mg/l	89.1	80.0-120	C16076	27Sep13 1628 by 07	27Sep13 1737 by 07		
	171021-1	20 mg/l	93.6	80.0-120	C16076	27Sep13 1628 by 07	27Sep13 1804 by 07		
	Relative Percent Difference:		4.28	10.0	C16076				

LABORATORY BLANK RESULTS

Analyte	Result	RL	PQL	QC Sample	Preparation Date	Analysis Date	Qual
Total Organic Carbon	< 1 mg/l	1	1	W45076-1	27Sep13 1459 by 308	27Sep13 1633 by 308	
Aluminum	< 0.04 mg/l	0.04	0.04	S35491-1	30Sep13 0901 by 271	01Oct13 1149 by 305	
Sulfate	< 0.2 mg/l	0.2	0.2	C16076-1	27Sep13 1628 by 07	27Sep13 1644 by 07	

AMERICAN INTERPLEX CORPORATION LABORATORIES

171032 wn 9/3/13
~~170744~~ wn 9/10/13

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client:	City of Hot Springs
Project Reference:	Plant Effluent
Project Manager:	James Sorrells
Sampled By:	A. Ross
PO No.	13-3032
AIC Control No:	~~170390~~
AIC Proposal No:	
Carrier:	Hot Springs Courier
Received Temperature °C	2.1 °C

Analyses Requested: No Fathead Analysis

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL	No of BOTTLES	Chronic CD	Remarks
1	Plant Effluent	9-8-13 0000-2400		X	X		3	X	
		Container Type					P		
		Preservative					NO		

Field pH calibration on ______ @ _____ Buffer:

G = Glass; P = Plastic; V = VOA vials; H = HCl to pH2; T = Sodium Thiosulfate
NO = none; S = Sulfuric acid pH2; N = Nitric acid pH2; B = NaOH to pH12; Z= Zinc acetate; A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: ____________
Who should AIC contact with questions: ____________
Phone: ________ Fax: ____________
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By	Date/Time	Received By	Date/Time
[signature]	9-8-13 1030	M. Mann	9-9-13 10:30 am
M. Mann	9-9-13 11:45	Received in Lab By: [signature]	9-9-13 1145

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

August 30, 2013

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Outfall 001
City of Hot Springs

Control No. 169846-1

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Pimephales promelas* (Fathead minnow)
Outfall 001 - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Method 1000.0 Chronic *Pimephales promelas* (Fathead minnow) Survival and Growth Test: The No Observable Effects Concentration (NOEC) for survival occurred at 87 % effluent, which is above the critical dilution of 65 %. The NOEC for growth occurred at 87 % effluent, which is above the critical dilution of 65 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the Fathead minnow test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Table of Contents

I. Control Acceptance Criteria

Pimephales promelas (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.296	PASS
Control Growth CV < or = 40%	7.47	PASS
Growth Minimum Significant Difference 12 to 30%	20.0	PASS
Critical Dilution CV < or = 40%	11.6	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly Test Method 1000.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point: Outfall 001
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.2	8.9	8.5
pH (standard units)	7.4	7.0	7.4
Alkalinity (mg/l as CaCO3)	41	26	34
Hardness (mg/l as CaCO3)	59	67	71
Conductivity (umhos/cm)	290	350	390
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.28	0.12	0.30

2. Dilution Water Samples: Synthetic Soft Water #4012
   a. Dates Prepared: August 8 through August 22, 2013
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.7	7.7	7.8
pH (standard units)	7.7	7.8	7.9
Alkalinity (mg/l as CaCO3)	32	32	32
Hardness (mg/l as CaCO3)	47	42	42
Conductivity (umhos/cm)	180	170	170
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05

C. Test Methods
1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Method 1000.0, Fathead Minnow Survival and Growth.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Pimephales promelas (Fathead minnow) Survival and Growth Method 1000.0

Date & Time Test Initiated:	August 20, 2013 at 1200
Date & Time Test Terminated:	August 27, 2013 at 1010
Type & Volume of Test Chamber:	500 ml disposable beaker
Volume of Sample:	250 ml
Number of Organisms per replicate:	8
Number of Replicates per dilution:	5

4. Acclimation of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms
1. Scientific Name
a. Test 1000.0 *Pimephales promelas*

III. Data Analysis

The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Pimephales promelas (Fathead minnow) survival data was transformed using the Arc Sine transformation. Normality and homogeneity of variance were checked using Shapiro-Wilk's. The survival data was then analyzed using Steel's Many-One Rank Test to determine the No Observable Effects Concentration (NOEC).

Fathead minnow growth data was analyzed for normality and homogeneity of variance using Shapiro-Wilk's and Bartlett's test. Dunnett's Test was used to determine the No Observable Effects Concentration (NOEC) for growth.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Pimephales promelas (Fathead minnow)

Chronic reference tests are performed monthly.
A chronic reference test was performed on August 20, 2013 at 1715 to August 27, 2013 at 1520
The results were as follows: (Control No. 169867-1.)
Survival LC-50: 5670.1 mg/l
Growth IC-25: 3143 mg/l
Growth PMSD: 17.3

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	102	1.91
pH	SM 4500-H+ B	101	0.268
Conductivity	EPA 120.1	101	4.71

VI. Organism History

Pimephales promelas (Fathead minnow)
Date: August 20, 2013
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

VII. Results Summary *Pimephales promelas*, Fathead minnow Larval Survival and Growth Test -- Method 1000.0

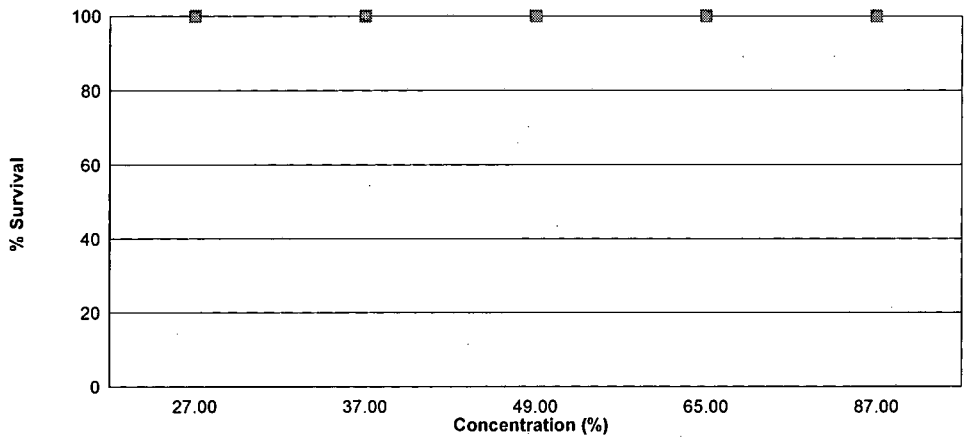
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on August 20, 2013 at 1200 and continued through August 27, 2013 at 1010. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 87 % effluent
b.) NOEC growth = 87 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.296
27 %	100	0.337
37 %	100	0.323
49 %	100	0.302
65 %	100	0.292
87 %	100	0.302

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: August 20, 2013 at 1200
Date and Time Test Terminated: August 27, 2013 at 1010

Concentration	Replicate	Number of Survivors						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
27 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
37 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
49 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
65 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
87 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: August 20, 2013 at 1200
Test Terminated: August 27, 2013 at 1010
Drying Started: August 23, 2013 at 1527
Drying Ended: August 29, 2013 at 1400

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.91827	.92042	0.00215	8	0.269
	B	.91878	.92121	0.00243	8	0.304
	C	.91659	.91922	0.00263	8	0.329
	D	.91638	.91872	0.00234	8	0.292
	E	.91455	.91685	0.00230	8	0.288
27 %	A	.91583	.91811	0.00228	8	0.285
	B	.91943	.92233	0.00290	8	0.362
	C	.92408	.92709	0.00301	8	0.376
	D	.92651	.92924	0.00273	8	0.341
	E	.92767	.93024	0.00257	8	0.321
37 %	A	.92653	.92915	0.00262	8	0.328
	B	.94872	.95160	0.00288	8	0.360
	C	.94724	.94985	0.00261	8	0.326
	D	.94532	.94750	0.00218	8	0.272
	E	.94881	.95146	0.00265	8	0.331
49 %	A	.94115	.94330	0.00215	8	0.269
	B	.92530	.92779	0.00249	8	0.311
	C	.92632	.92901	0.00269	8	0.336
	D	.93070	.93312	0.00242	8	0.302
	E	.93427	.93660	0.00233	8	0.291
65 %	A	.93862	.94107	0.00245	8	0.306
	B	.93944	.94155	0.00211	8	0.264
	C	.94132	.94377	0.00245	8	0.306
	D	.93810	.94077	0.00267	8	0.334
	E	.93899	.94100	0.00201	8	0.251
87 %	A	.93682	.93952	0.00270	8	0.338
	B	.93818	.93991	0.00173	8	0.216
	C	.93688	.93881	0.00193	8	0.241
	D	.93804	.94073	0.00269	8	0.336
	E	.95233	.95537	0.00304	8	0.380

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

		Transformation of Data		Transform: Arc Sin(Square Root(Y))
Group	Identification	Rep	Value	Transformed
1	Control	1	1.00000	1.39310
1	Control	2	1.00000	1.39310
1	Control	3	1.00000	1.39310
1	Control	4	1.00000	1.39310
1	Control	5	1.00000	1.39310
2	27 %	1	1.00000	1.39310
2	27 %	2	1.00000	1.39310
2	27 %	3	1.00000	1.39310
2	27 %	4	1.00000	1.39310
2	27 %	5	1.00000	1.39310
3	37 %	1	1.00000	1.39310
3	37 %	2	1.00000	1.39310
3	37 %	3	1.00000	1.39310
3	37 %	4	1.00000	1.39310
3	37 %	5	1.00000	1.39310
4	49 %	1	1.00000	1.39310
4	49 %	2	1.00000	1.39310
4	49 %	3	1.00000	1.39310
4	49 %	4	1.00000	1.39310
4	49 %	5	1.00000	1.39310
5	65 %	1	1.00000	1.39310
5	65 %	2	1.00000	1.39310
5	65 %	3	1.00000	1.39310
5	65 %	4	1.00000	1.39310
5	65 %	5	1.00000	1.39310
6	87 %	1	1.00000	1.39310
6	87 %	2	1.00000	1.39310
6	87 %	3	1.00000	1.39310
6	87 %	4	1.00000	1.39310
6	87 %	5	1.00000	1.39310

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Shapiro - Wilk's Test for Normality		Transform: Arc Sin(Square Root(Y))
D = 0		
W = 0		
Critical W = 0.9	(alpha = 0.01, N = 30)	
Critical W = 0.927	(alpha = 0.05, N = 30)	

Data FAIL normality test (alpha = 0.01).

Steel's Many-One Rank Test — Transform: Arc Sin(Square Root(Y))

Ho:Control<Treatment

Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	27 %	27.50	16.00	5.00	
3	37 %	27.50	16.00	5.00	
4	49 %	27.50	16.00	5.00	
5	65 %	27.50	16.00	5.00	
6	87 %	27.50	16.00	5.00	

Critical values are 1 tailed (k=5)

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality		No Transformation
D = 0.03787		
W = 0.9742		
Critical W = 0.9	(alpha = 0.01, N = 30)	
Critical W = 0.927	(alpha = 0.05, N = 30)	
Data PASS normality test (alpha = 0.01).		

Bartlett's Test for Homogeneity of Variance		No Transformation
Calculated B1 statistic = 7.076		
Critical B = 15.086	(alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.		

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	0.007651	0.00153	0.9696
Within (Error)	24	0.03787	0.001578	
Total	29	0.04552		

Critical F = 3.9 (alpha = 0.01, df = 5,24)
2.62 (alpha = 0.05, df = 5,24)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Dunnett's Test - Table 1 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	0.2964	0.2964		
2	27 %	0.337	0.337	-1.616	
3	37 %	0.3234	0.3234	-1.075	
4	49 %	0.3018	0.3018	-0.2149	
5	65 %	0.2922	0.2922	0.1672	
6	87 %	0.3022	0.3022	-0.2309	

Dunnett's critical value = 2.36 (1 Tailed, alpha = 0.05, df = 5,24)

Dunnett's Test - Table 2 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	5			
2	27 %	5	0.05929	20	-0.0406
3	37 %	5	0.05929	20	-0.027
4	49 %	5	0.05929	20	-0.0054
5	65 %	5	0.05929	20	0.0042
6	87 %	5	0.05929	20	-0.0058

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 20, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.5	7.7	7.5	7.8	7.8	8.1
	Final	7.5	7.2	7.5	7.9	7.7	7.1	7.9
pH, units	Initial	7.7	7.7	7.8	8.0	7.9	8.0	7.7
	Final	7.5	7.3	7.8	8.1	8.1	7.6	7.9
Alkalinity, mg CaCO3/l		32	NA	32	NA	32	NA	NA
Hardness, mg CaCO3/l		47	NA	42	NA	42	NA	NA
Conductivity, umhos/cm		180	180	170	180	170	170	190
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.4	8.0	7.5	8.0	8.0	7.5
	Final	7.5	7.2	7.3	7.9	7.8	8.1	7.7
pH, units	Initial	7.6	7.7	7.5	7.8	7.7	7.8	7.5
	Final	7.5	7.3	7.7	8.1	8.2	7.5	7.7

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.6	8.6	7.6	8.2	7.7	7.7
	Final	7.5	7.0	7.5	8.0	7.9	8.1	7.8
pH, units	Initial	7.6	7.7	7.4	7.7	7.6	7.7	7.6
	Final	7.5	7.3	7.7	8.1	8.2	7.5	7.6

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 20, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.5	8.7	7.6	8.1	7.7	7.7
	Final	7.5	7.4	7.6	8.0	8.1	8.2	7.8
pH, units	Initial	7.5	7.7	7.3	7.6	7.6	7.6	7.5
	Final	7.5	7.4	7.6	8.1	8.2	7.6	7.6

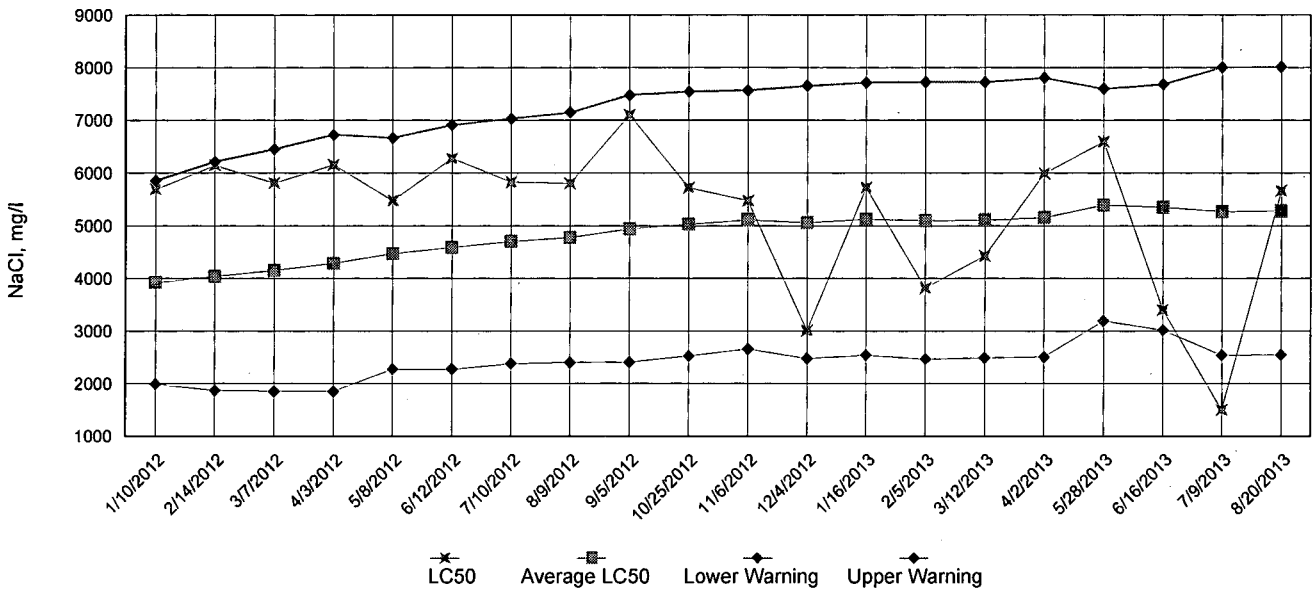
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.9	7.4	8.8	7.6	8.7	7.7	7.3
	Final	7.5	7.4	7.4	7.9	8.1	8.0	7.7
pH, units	Initial	7.5	7.7	7.2	7.5	7.5	7.6	7.5
	Final	7.5	7.4	7.6	8.1	8.3	7.6	7.5
Alkalinity, mg CaCO3/l		36	NA	30	NA	35	NA	NA
Hardness, mg CaCO3/l		54	NA	58	NA	61	NA	NA
Conductivity, umhos/cm		250	260	280	290	310	310	330
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	7.4	8.9	7.6	8.3	7.9	7.4
	Final	7.5	8.5	7.4	7.8	8.1	8.0	7.7
pH, units	Initial	7.4	7.7	7.1	7.4	7.4	7.6	7.6
	Final	7.5	7.4	7.6	8.1	8.1	7.6	7.4

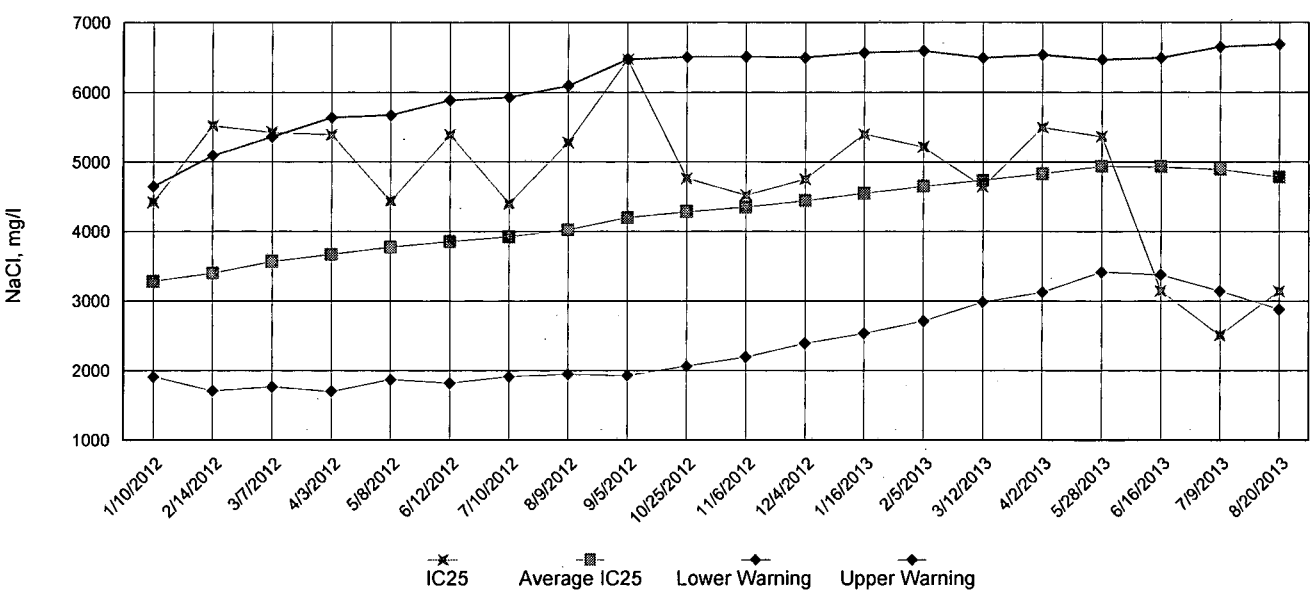
Appendix A4: Test 1000.0

Chronic Reference Toxicant, *Pimephales promelas* (Fathead Minnow)

LC50 Survival Data



IC25 Growth Data



Appendix B: Test 1000.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

Permittee: City of Hot Springs

NPDES No.: AR0033880 AFIN#26-00145

Date and Time Test Initiated: August 20, 2013 at 1200
Date and Time Test Terminated: August 27, 2013 at 1010
Dilution water used: Synthetic Soft Water #4012

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in replicate chambers A	B	C	D	E	Mean percent survival 24 hr	48 hr	7 days	CV%
Control	100	100	100	100	100	100	100	100	0.00
27 %	100	100	100	100	100	100	100	100	0.00
37 %	100	100	100	100	100	100	100	100	0.00
49 %	100	100	100	100	100	100	100	100	0.00
65 %	100	100	100	100	100	100	100	100	0.00
87 %	100	100	100	100	100	100	100	100	0.00

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers A	B	C	D	E	Mean dry weight, mg	CV%
Control	0.269	0.304	0.329	0.292	0.288	0.296	7.47
27 %	0.285	0.362	0.376	0.341	0.321	0.337	10.6
37 %	0.328	0.360	0.326	0.272	0.331	0.323	9.86
49 %	0.269	0.311	0.336	0.302	0.291	0.302	8.20
65 %	0.306	0.264	0.306	0.334	0.251	0.292	11.6
87 %	0.338	0.216	0.241	0.336	0.380	0.302	23.2

CV = Coefficient of variation = standard deviation * 100 / mean

Appendix B: Test 1000.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

1. Steel's Many-One Rank Test:

   Is the mean survival significantly different (p=0.05) than the control survival for the % effluent corresponding to (lethality):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	____YES	X NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____YES	____NO

2. Dunnett's Test:

   Is the mean dry weight (growth) significantly different (p=0.05) than the control's dry weight (growth) for the % effluent corresponding to (significant non-lethal effects):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	____YES	X NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____YES	____NO

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

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

5. NOEC Pimephales Lethality: 87 % (TOP6C)

6. LOEC Pimephales Lethality: 87 % (TXP6C)

7. NOEC Pimephales Sublethality: 87 % (TPP6C)

8. LOEC Pimephales Sublethality: 87 % (TYP6C)

9. Coefficient of variation for Pimephales growth: 11.6 (TQP6C)

AMERICAN INTERPLEX CORPORATION
LABORATORIES

Appendix B: Test 1000.0

CHRONIC TOXICITY SUMMARY FORM
Pimephales promelas (Fathead minnow)
CHEMICAL PARAMETERS CHART

PERMITTEE: City of Hot Springs 2400
NPDES NO.: AR0033880 AFIN#26-00145 2400
CONTACT: Mr. James Sorrells 2400
ANALYST: 280, 298, 304, 307

Test Initiated: DATE: August 20, 2013 TIME: 1200
Test Terminated: DATE: August 27, 2013 TIME: 1010

DILUTION Control	DAY 1	2	3	4	5	6	7
D.O. Initial	7.7	7.5	7.7	7.5	7.8	7.8	8.1
Final	7.5	7.2	7.5	7.9	7.7	7.1	7.9
pH Initial	7.7	7.7	7.8	8.0	7.9	8.0	7.7
Final	7.5	7.3	7.8	8.1	8.1	7.6	7.9
Alkalinity	32	NA	32	NA	32	NA	NA
Hardness	47	NA	42	NA	42	NA	NA
Conductivity	180	180	170	180	170	170	190
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 27 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.7	7.4	8.0	7.5	8.0	8.0	7.5
Final	7.5	7.2	7.3	7.9	7.8	8.1	7.7
pH Initial	7.6	7.7	7.5	7.8	7.7	7.8	7.5
Final	7.5	7.3	7.7	8.1	8.2	7.5	7.7
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	220	220	220	230	230	250
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 37 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.8	7.6	8.6	7.6	8.2	7.7	7.7
Final	7.5	7.0	7.5	8.0	7.9	8.1	7.8
pH Initial	7.6	7.7	7.4	7.7	7.6	7.7	7.6
Final	7.5	7.3	7.7	8.1	8.2	7.5	7.6
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	220	230	240	240	260	250	270
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 49 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.8	7.5	8.7	7.6	8.1	7.7	7.7
Final	7.5	7.4	7.6	8.0	8.1	8.2	7.8
pH Initial	7.5	7.7	7.3	7.6	7.6	7.6	7.5
Final	7.5	7.4	7.6	8.1	8.2	7.6	7.6
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	230	240	260	260	280	280	300
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 65 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.9	7.4	8.8	7.6	8.7	7.7	7.3
Final	7.5	7.4	7.4	7.9	8.1	8.0	7.7
pH Initial	7.5	7.7	7.2	7.5	7.5	7.6	7.5
Final	7.5	7.4	7.6	8.1	8.3	7.6	7.5
Alkalinity	36	NA	30	NA	35	NA	NA
Hardness	54	NA	58	NA	61	NA	NA
Conductivity	250	260	280	290	310	310	330
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 87 %	DAY 1	2	3	4	5	6	7
D.O. Initial	8.0	7.4	8.9	7.6	8.3	7.9	7.4
Final	7.5	8.5	7.4	7.8	8.1	8.0	7.7
pH Initial	7.4	7.7	7.1	7.4	7.4	7.6	7.6
Final	7.5	7.4	7.6	8.1	8.1	7.6	7.4
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	280	290	320	320	360	360	380
Chlorine	NA	NA	NA	NA	NA	NA	NA

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross
PO No.: 13-3032

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2°C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER (Sample Matrix)	SOIL (Sample Matrix)		No of BOTTLES	Chronic.CD, Chronic.FH (Analyses Requested)											Remarks
1	Plant Effluent	8-18-13 0000-2400		X	X			3	x											
																				Field pH calibration
		Container Type							P											on ______ @ ______
		Preservative							NO											Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: ______
Who should AIC contact with questions: ______
Phone: ______ Fax: ______
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By	Date/Time	Received By	Date/Time
A. Thomason	8-19-13 @ 1010	M. Mann	8-19-13 10:10 AM
M. Mann	8-19-13 @ 11:10 AM	Received In Lab By: [signature]	8-19-13 1110

Comments:

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 2.3C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH	Remarks
2	Plant Effluent	8-20-13 0000-2400		X	X		3	X	
		Container Type						P	Field pH calibration
		Preservative						NO	on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
A. Ross	8-21-13 0900	A. Man	8-21-13 9:10
A. Man	8-21-13 1130	Jimmy Day	8/21/13 1130

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By:

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL		No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
3	Plant Effluent	8/22/13 0000-2400		X				3	X
		Container Type							P
		Preservative							NO

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 2
Remarks
Field pH calibration on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	8-23-13 0955	[signature]	8-23-13 9:55
[signature]	8-23-13 11:20	[signature]	8-23-13 1120

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

September 19, 2013

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Plant Effluent
City of Hot Springs

Control No. 170390-1

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Ceriodaphnia dubia*
Plant Effluent - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: The No Observable Effects Concentration (NOEC) for survival occurred at <27 % effluent, which is below the critical dilution of 65 %. The NOEC for reproduction occurred at <27 % effluent, which is below the critical dilution of 65 %. **The sample, therefore, FAILED both lethal and sub-lethal effects for the *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Table of Contents

I. Control Acceptance Criteria

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	18.8	PASS
Control CV < or = 40% per Surviving Female	20.6	PASS
Reproduction Minimum Significant Difference 13 to 47%	NA	NA
Critical Dilution CV < or = 40%	0.00	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly Test Method 1002.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point: Plant Effluent
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.7	8.2	8.2
pH (standard units)	7.4	7.7	7.5
Alkalinity (mg/l as CaCO3)	64	58	64
Hardness (mg/l as CaCO3)	82	82	83
Conductivity (umhos/cm)	380	370	440
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	2.4	0.11

2. Dilution Water Samples: Synthetic Soft Water #4019
   a. Dates Prepared: August 28 through September 11, 2013
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.6	8.2	8.2
pH (standard units)	7.6	7.8	7.6
Alkalinity (mg/l as CaCO3)	32	32	32
Hardness (mg/l as CaCO3)	46	42	42
Conductivity (umhos/cm)	160	160	180
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05

C. Test Methods
1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Method 1002.0, *Ceriodaphnia dubia* Survival and Reproduction.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Ceriodaphnia dubia Survival and Growth Method 1002.0

Date & Time Test Initiated:	September 9, 2013 at 1235
Date & Time Test Terminated:	September 17, 2013 at 1255
Type & Volume of Test Chamber:	30 ml disposable beaker
Volume of Sample:	15 ml
Number of Organisms per replicate:	1
Number of Replicates per dilution:	10

4. Acclimation of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms
1. Scientific Name
a. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis
The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was not analyzed due to survival failure.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Ceriodaphnia dubia

Chronic reference tests are performed monthly.
A chronic reference test was performed on August 20, 2013 at 1505 to August 28, 2013 at 1450
The results were as follows: (Control No. 169867-2.)
Survival LC-50: 2125 mg/l
Growth IC-25: 1610 mg/l
Growth PMSD: 18.3

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	1.53
Hardness	EPA 200.7	101	0.450
pH	SM 4500-H+ B	101	0.267
Conductivity	EPA 120.1	103	1.97

VI. Organism History

Ceriodaphnia dubia

Date: September 9, 2013
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

VII. Results Summary *Ceriodaphnia dubia*, Cladoceran Survival and Reproduction Test -- Method 1002.0

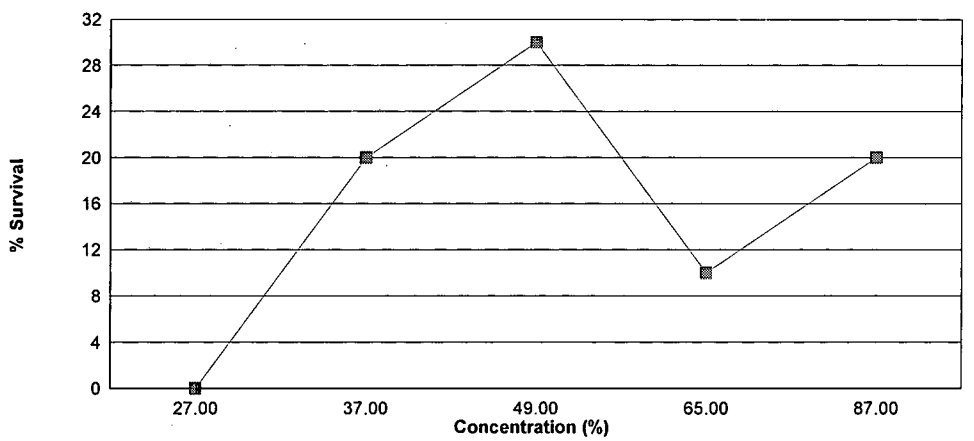
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on September 9, 2013 at 1235 and continued through September 17, 2013 at 1255. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = <27 % effluent
b.) NOEC reproduction = <27 % effluent



Summary of the 7-day *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	18.8
27 %	0.00 *	--
37 %	20.0 *	--
49 %	30.0 *	--
65 %	10.0 *	--
87 %	20.0 *	--

*Significant difference when compared to the control (p=0.05)

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 9, 2013 at 1235
Date and Time Test Terminated: September 17, 2013 at 1255

Concentration: Control													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	3	2	0	0	0	0	1	4	2	1	13	10	1.30
5	9	0	3	6	6	4	0	4	6	0	38	10	3.80
6	0	5	9	8	7	0	6	0	1	7	43	10	4.30
7	10	9	10	10	10	8	9	8	10	10	94	10	9.40
8													
TOTAL	22	16	22	24	23	12	16	16	19	18	188	10	18.8

Concentration: 27 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	0	0	0	X	0	0	0	X	0	0	7	0.00
2	X	0	0	0	X	0	0	0	X	0	0	7	0.00
3	X				X				X		0	0	0.00
4	X				X				X		0	0	0.00
5	X				X				X		0	0	0.00
6	X				X				X		0	0	0.00
7	X				X				X		0	0	0.00
8													
TOTAL	0	0	0	0	0	0	0	0	0	0	0	10	0.00

Concentration: 37 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	X	X	0	X	X	0	0	3	0.00
2	X	X	0	X	X	X	0	X	X	0	0	3	0.00
3	X	X	X	X	X	X	0	X	X	0	0	2	0.00
4	X	X	X	X	X	X	2	X	X	1	3	2	1.50
5	X	X	X	X	X	X	0	X	X	0	0	2	0.00
6	X	X	X	X	X	X	7	X	X	6	13	2	6.50
7	X	X	X	X	X	X	10	X	X	0	10	2	5.00
8													
TOTAL	0	0	0	0	0	0	19	0	0	7	26	10	2.60

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 9, 2013 at 1235
Date and Time Test Terminated: September 17, 2013 at 1255

Concentration: 49 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	X	X	0	X	X	0	0	3	0.00
2	X	X	0	X	X	X	0	X	X	0	0	3	0.00
3	X	X	0	X	X	X	0	X	X	0	0	3	0.00
4	X	X	0	X	X	X	0	X	X	0	0	3	0.00
5	X	X	4	X	X	X	5	X	X	0	9	3	3.00
6	X	X	0	X	X	X	0	X	X	6	6	3	2.00
7	X	X	6	X	X	X	8	X	X	0	14	3	4.67
8													
TOTAL	0	0	10	0	0	0	13	0	0	6	29	10	2.90

Concentration: 65 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	X	X	X	X	X	0	0	2	0.00
2	X	X	0	X	X	X	X	X	X	0	0	2	0.00
3	X	X	0	X	X	X	X	X	X	0	0	2	0.00
4	X	X	0	X	X	X	X	X	X	0	0	2	0.00
5	X	X	6	X	X	X	X	X	X	0	6	2	3.00
6	X	X	0	X	X	X	X	X	X	X	0	1	0.00
7	X	X	9	X	X	X	X	X	X	X	9	1	9.00
8													
TOTAL	0	0	15	0	0	0	0	0	0	0	15	10	1.50

Concentration: 87 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	0	X	0	X	X	0	0	4	0.00
2	X	X	0	X	0	X	X	X	X	0	0	3	0.00
3	X	X	0	X	0	X	X	X	X	0	0	3	0.00
4	X	X	0	X	3	X	X	X	X	1	4	3	1.33
5	X	X	7	X	5	X	X	X	X	0	12	3	4.00
6	X	X	0	X	X	X	X	X	X	3	3	2	1.50
7	X	X	9	X	X	X	X	X	X	0	9	2	4.50
8													
TOTAL	0	0	16	0	8	0	0	0	0	4	28	10	2.80

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
27 %	0	3	3
Total	10	3	13

Critical Fisher's value (10,3,10) (alpha=0.05) is 1. b value is 0. Since b is less than or equal to 1 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
37 %	2	8	10
Total	12	8	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 2. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
49 %	3	7	10
Total	13	7	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 3. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
65 %	1	9	10
Total	11	9	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 1. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
87 %	2	8	10
Total	12	8	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 2. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	27 %	3	3	*
2	37 %	10	8	*
3	49 %	10	7	*
4	65 %	10	9	*
5	87 %	10	8	*

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: September 9, 2013 at 0857
Date and Time Test Terminated: September 17, 2013 at 1255

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.6	7.4	8.2	8.3	8.2	8.3	8.2
	Final	7.8	8.3	8.5	8.7	8.4	8.2	7.8
pH, units	Initial	7.6	7.6	7.8	7.6	7.6	7.7	7.5
	Final	7.8	7.9	7.9	7.6	7.9	7.8	8.0
Alkalinity, mg CaCO3/l		32	NA	32	NA	32	NA	NA
Hardness, mg CaCO3/l		46	NA	42	NA	42	NA	NA
Conductivity, umhos/cm		160	170	160	180	180	180	170
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.6	7.5	8.2	8.4	8.3	8.1	8.3
	Final	6.0	8.3	8.7	8.8	8.5	8.3	8.1
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
	Final	8.0	8.1	8.2	8.0	8.3	8.0	8.1

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.6	7.4	8.2	8.4	8.1	8.0	8.1
	Final	6.4	8.3	8.6	8.8	8.6	8.2	8.2
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
	Final	8.1	8.2	8.2	8.2	8.2	7.9	7.8

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: September 9, 2013 at 0857
Date and Time Test Terminated: September 17, 2013 at 1255

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.5	7.5	8.2	8.4	8.4	8.1	8.3
	Final	6.8	8.3	7.8	9.0	8.4	8.3	8.0
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.5	7.5
	Final	8.2	8.2	8.2	8.1	8.1	8.0	8.1

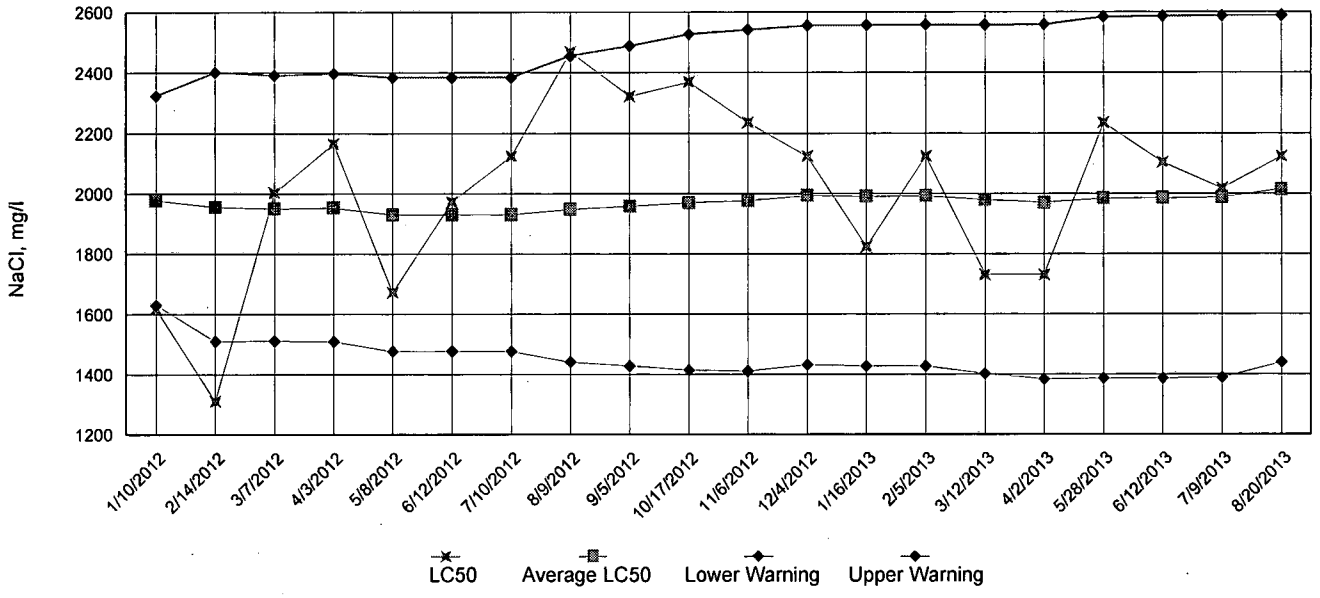
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.4	8.2	8.4	8.0	8.2	8.3
	Final	7.0	8.3	8.4	8.7	8.3	8.3	7.7
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.6
	Final	8.2	8.1	8.1	8.2	8.0	8.0	7.7
Alkalinity, mg CaCO3/l		48	NA	46	NA	47	NA	NA
Hardness, mg CaCO3/l		68	NA	68	NA	68	NA	NA
Conductivity, umhos/cm		310	310	300	340	340	340	350
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.3	7.2	8.2	8.3	8.3	8.0	7.9
	Final	7.1	8.3	8.6	8.8	7.3	8.3	8.0
pH, units	Initial	7.5	7.4	7.8	7.5	7.6	7.5	7.6
	Final	8.2	8.3	8.3	8.3	7.5	8.1	7.9

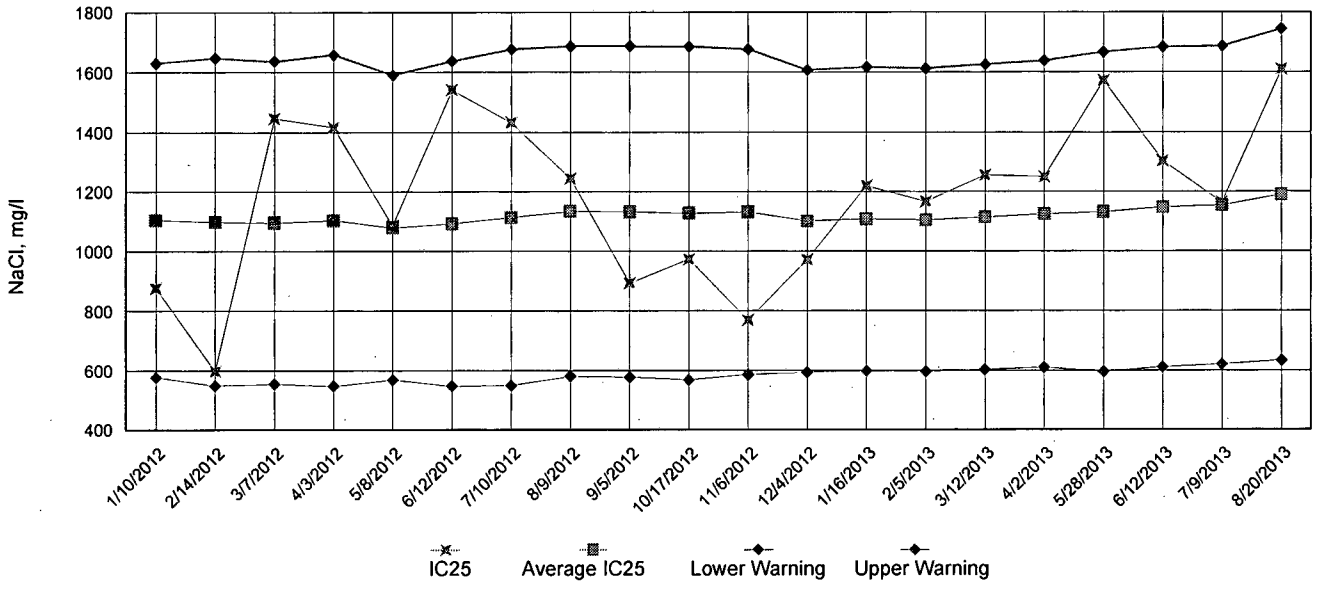
Appendix A4: Test 1002.0

Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

Permittee: City of Hot Springs

NPDES No.: AR0033880 AFIN#26-00145

Date and Time Test Initiated: September 9, 2013 at 1235
Date and Time Test Terminated: September 17, 2013 at 1255
Dilution water used: Synthetic Soft Water #4019

PERCENT SURVIVAL

		Percent Effluent				
Time of Reading	Control	27 %	37 %	49 %	65 %	87 %
24 hour	100	70.0	30.0	30.0	20.0	40.0
48 hour	100	70.0	30.0	30.0	20.0	30.0
7 day	100	0.00	20.0	30.0	10.0	20.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

		Percent Effluent				
Replicates	Control	27 %	37 %	49 %	65 %	87 %
A	22	0	0	0	0	0
B	16	0	0	0	0	0
C	22	0	0	10	15	16
D	24	0	0	0	0	0
E	23	0	0	0	0	8
F	12	0	0	0	0	0
G	16	0	19	13	0	0
H	16	0	0	0	0	0
I	19	0	0	0	0	0
J	18	0	7	6	0	4
Mean per Adult	18.8	0.00	2.60	2.90	1.50	2.80
Mean per Surviving Adult	18.8	0.00	13.0	9.67	15.0	10.0
CV %	20.6	0.00	65.3	36.3	0.00	84.9

CV = Coefficient of variation = standard deviation * 100 / mean
(calculated based on young produced by surviving females)

Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

1. Fisher's Exact Test:

   Is the mean survival significantly different (p=0.05) than the control survival for the % effluent corresponding to (lethality):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	X YES	____ NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____ YES	____ NO

2.

   Is the mean number of young produced per female significantly different (p=0.05) than the control's number of young per female for the % effluent corresponding to (significant non-lethal effects):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	X YES	____ NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____ YES	____ NO

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 1 (TLP3B)
4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 1 (TGP3B)
5. NOEC Ceriodaphnia Lethality: <27 % (TOP3B)
6. LOEC Ceriodaphnia Lethality: 27 % (TXP3B)
7. NOEC Ceriodaphnia Sublethality: <27 % (TPP3B)
8. LOEC Ceriodaphnia Sublethality: 27 % (TYP3B)
9. Coefficient of variation for Ceriodaphnia Reproduction: 20.6 (TQP3B)

Appendix B: Test 1002.0

CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
CHEMICAL PARAMETERS CHART

PERMITTEE: City of Hot Springs
NPDES NO.: AR0033880 AFIN#26-00145
CONTACT: Mr. James Sorrells
ANALYST: 280, 298, 304, 307

2400
2400
2400

Test Initiated: DATE: September 9, 2013 TIME: 1235
Test Terminated: DATE: September 17, 2013 TIME: 1255

DILUTION	DAY						
Control	1	2	3	4	5	6	7
D.O. Initial	8.6	7.4	8.2	8.3	8.2	8.3	8.2
Final	7.8	8.3	8.5	8.7	8.4	8.2	7.8
pH Initial	7.6	7.6	7.8	7.6	7.6	7.7	7.5
Final	7.8	7.9	7.9	7.6	7.9	7.8	8.0
Alkalinity	32	NA	32	NA	32	NA	NA
Hardness	46	NA	42	NA	42	NA	NA
Conductivity	160	170	160	180	180	180	170
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
27 %	1	2	3	4	5	6	7
D.O. Initial	8.6	7.5	8.2	8.4	8.3	8.1	8.3
Final	6.0	8.3	8.7	8.8	8.5	8.3	8.1
pH Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
Final	8.0	8.1	8.2	8.0	8.3	8.0	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	220	220	220	240	250	250	250
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
37 %	1	2	3	4	5	6	7
D.O. Initial	8.6	7.4	8.2	8.4	8.1	8.0	8.1
Final	6.4	8.3	8.6	8.8	8.6	8.2	8.2
pH Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
Final	8.1	8.2	8.2	8.2	8.2	7.9	7.8
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	240	240	240	260	270	270	280
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
49 %	1	2	3	4	5	6	7
D.O. Initial	8.5	7.5	8.2	8.4	8.4	8.1	8.3
Final	6.8	8.3	7.8	9.0	8.4	8.3	8.0
pH Initial	7.6	7.5	7.8	7.5	7.6	7.5	7.5
Final	8.2	8.2	8.2	8.1	8.1	8.0	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	270	270	270	300	300	300	300
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
65 %	1	2	3	4	5	6	7
D.O. Initial	7.7	7.4	8.2	8.4	8.0	8.2	8.3
Final	7.0	8.3	8.4	8.7	8.3	8.3	7.7
pH Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.6
Final	8.2	8.1	8.1	8.2	8.0	8.0	7.7
Alkalinity	48	NA	46	NA	47	NA	NA
Hardness	68	NA	68	NA	68	NA	NA
Conductivity	310	310	300	340	340	340	350
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
87 %	1	2	3	4	5	6	7
D.O. Initial	8.3	7.2	8.2	8.3	8.3	8.0	7.9
Final	7.1	8.3	8.6	8.8	7.3	8.3	8.0
pH Initial	7.5	7.4	7.8	7.5	7.6	7.5	7.6
Final	8.2	8.3	8.3	8.3	7.5	8.1	7.9
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	350	360	350	400	440	430	440
Chlorine	NA	NA	NA	NA	NA	NA	NA

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Roys

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Chronic C.D.	Analyses Requested
1	Plant Effluent	9-8-13 2000-2400		X	X		3	X	No Fathead Analysis
		Container Type						P	
		Preservative						NO	

AIC Control No: 170390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2.1°C
Remarks:
Field pH calibration on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-8-13 1030	M. Mann	9-9-13 10:30 am
M. Mann	9-9-13 11:45	[signature]	9-9-13 1145

Comments:

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC Control No: 170390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 3°C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH	Remarks
1	Plant Effluent	9-10-13 0000-2400		X	X		3	X	
		Container Type						P	Field pH calibration
		Preservative						NO	on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-11-13 1110	M. Mann	9-11-13 1110
M. Mann	9-11-13 @ 12:05	[signature]	9-11-13 1205

Comments:

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs

Project Reference: Plant Effluent

Project Manager: James Sorrells

Sampled By: HAROLD MAULDIN

PO No.: 13-3032

AIC Control No: 170390

AIC Proposal No:

Carrier: Hot Springs Courier

Received Temperature °C: 2

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL		No of BOTTLES	Chronic.CD.	Remarks
3	Plant Effluent	9/12/13 0000-2400		X	X			3	x	
		Container Type							P	
		Preservative							NO	

Analyses Requested

Field pH calibration on ______ @ ______ Buffer:

G = Glass | P = Plastic | V = VOA vials | H = HCl to pH2 | T = Sodium Thiosulfate
NO = none | S = Sulfuric acid pH2 | N = Nitric acid pH2 | B = NaOH to pH12 | Z= Zinc acetate | A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: ____
Who should AIC contact with questions: ____
Phone: ____ Fax: ____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By	Date/Time	Received By	Date/Time
A. Thomason	9-13-13 @ 1015	M. Mann	9-13-13 @ 10:15
M. Mann	9-13-13 @ 11:20	Received in Lab By: [signature]	9-13-13 1120

Comments:

4-85

AMERICAN INTERPLEX CORPORATION
LABORATORIES

September 30, 2013

Test Results of
Acute 48 hour Non-Renewal
Biomonitoring Testing
for

170741-1: Outfall 001 First Renewal
170741-2: Outfall 001 Second Renewal
170741-3: Outfall 001 Third Renewal
170741-4: Outfall 001 Original Screen

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Acute 48 hour Non-Renewal Biomonitoring utilizing *Ceriodaphnia dubia*
Outfall 001 First Renewal - City of Hot Springs
Client NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

Please find attached the results of the acute biomonitoring screens conducted as a follow up to the chronic toxicity event previously reported (AIC Control No. 170390). As indicated on the enclosed results, the sample was still acutely toxic on September 12, 2013 and exhibited no acute toxicity on September 19, 2013.

It it theorized the decrease in toxicity is directly related to the holding time which may have allowed microbial degradation, loss of volatile toxics, and/or biochemical degradation.

If I can be of further assistance, please feel free to contact me.

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Dilution Water Samples: Synthetic Soft Water #4021

Analysis	Result
Dissolved oxygen (mg/l)	7.6
pH (standard units)	7.7
Alkalinity (mg/l as CaCO3)	31
Hardness (mg/l as CaCO3)	46
Conductivity (umhos/cm)	170
Residual Chlorine (mg/l)	<0.05

Results Summary: Outfall 001 First Renewal

Ceriodaphnia dubia

The *Ceriodaphnia dubia* test was conducted from September 19, 2013 at 1615 to September 21, 2013 at 1700.

Statistical analyses:

NOEC = 100%

Concentration	24 hour % Survival	48 hour % Survival
Control	100	100
100%	100	100

Ceriodaphnia dubia
Survival Data

Number of organisms per chamber: 5
Volume of test chamber: 30 ml

Age of organisms: <24 hours
Volume of test solution: 15 ml

Effluent Concentration	Number of Survivors 24 Hours	48 Hours	% Survival	CV %
Control rep. A	5	5	100	0.00
100% rep. A	5	5	100	0.00

CV = Coefficient of variance = standard deviation X 100/mean

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	5	0	5
100%	5	0	5
Total	10	0	10

Critical Fisher's value (5,5,5) (alpha=0.05) is 1. b value is 5. Since b is greater than 1 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test

Group	Identification	Exposed	Dead	Sig 0.05
0	0	5	0	
1	100	5	0	

Chemical Data for
Ceriodaphnia dubia

Day 1	Control	100%
DO, mg/l	7.6	8.8
pH, su	7.7	7.3
Alkalinity, mg/l	31	NA
Hardness, mg/l	46	NA
Conductivity, umho/cm	170	380
Residual Chlorine, mg/l	<0.05	NA

Day 2		Control	100%
DO, mg/l	Final	7.5	8.7
pH, su	Final	8.0	7.4

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

170744 WR 9/19/13

Client: City of Hot Springs
Project:
Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Rojs

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Chronic CD.	Analyses Requested
1	Plant Effluent	9-8-13 0000-2400		X	X		3	X	
		Container Type						P	
		Preservative						NO	

No Fathead Analysis

AIC Control No: 170390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2.1°C
Remarks:
Field pH calibration on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ___ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-9-13 1030	M. Mann	9-9-13 10:30 AM
M. Mann	9-9-13 11:45	[signature]	9-9-13 1145

Comments:

City of Hot Springs
Waste Water Treatment Plant
320 Davidson Drive
Hot Springs, AR 71901

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ADEQ
NPDES Enforcement Section
Attn: Mo Shafii
5301 Northshore Dr.
North Little Rock, AR 72118-5317

AMERICAN INTERPLEX CORPORATION
LABORATORIES

August 30, 2013

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Outfall 001
City of Hot Springs

Control No. 169846-2

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Ceriodaphnia dubia*
Outfall 001 - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Chronic *Ceriodaphnia dubia* test: Due to laboratory error, the *Ceriodaphnia dubia* test was not renewed with the third sample. The test should be repeated. The data from the test is enclosed for your review.

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Table of Contents

I. Control Acceptance Criteria

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	23.6	PASS
Control CV < or = 40% per Surviving Female	13.6	PASS
Reproduction Minimum Significant Difference 13 to 47%	25.8	PASS
Critical Dilution CV < or = 40%	22.9	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly
   Test Method 1002.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point: Outfall 001
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.9	8.5	NA
pH (standard units)	7.0	7.4	NA
Alkalinity (mg/l as CaCO3)	26	34	NA
Hardness (mg/l as CaCO3)	67	71	NA
Conductivity (umhos/cm)	350	390	NA
Residual Chlorine (mg/l)	<0.05	<0.05	NA
Ammonia as N (mg/l)	0.12	0.30	NA

2. Dilution Water Samples: Synthetic Soft Water #4012
   a. Dates Prepared: August 8 through August 22, 2013
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.7	7.7	7.8
pH (standard units)	7.7	7.8	7.9
Alkalinity (mg/l as CaCO3)	32	32	NA
Hardness (mg/l as CaCO3)	47	42	NA
Conductivity (umhos/cm)	180	170	170
Residual Chlorine (mg/l)	<0.05	<0.05	NA

C. Test Methods
1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Method 1002.0, *Ceriodaphnia dubia* Survival and Reproduction.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Ceriodaphnia dubia Survival and Growth Method 1002.0

Date & Time Test Initiated:	August 22, 2013 at 1605
Date & Time Test Terminated:	August 29, 2013 at 1415
Type & Volume of Test Chamber:	30 ml disposable beaker
Volume of Sample:	15 ml
Number of Organisms per replicate:	1
Number of Replicates per dilution:	10

4. Acclimation of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms
1. Scientific Name
a. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis

The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was analyzed using Kolmogorov's Test for Normality and Bartlett's test and analyzed with Dunnett's Test to determine the No Observable Effects Concentration (NOEC) for Reproduction.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Ceriodaphnia dubia

Chronic reference tests are performed monthly.
A chronic reference test was performed on August 20, 2013 at 1505 to August 28, 2013 at 1450
The results were as follows: (Control No. 169867-2.)
Survival LC-50: 2125 mg/l
Growth IC-25: 1610 mg/l
Growth PMSD: 18.3

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	102	1.91
pH	SM 4500-H+ B	101	0.268
Conductivity	EPA 120.1	101	4.71

VI. Organism History

Ceriodaphnia dubia

Date: August 22, 2013
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

VII. Results Summary *Ceriodaphnia dubia*, Cladoceran Survival and Reproduction Test -- Method 1002.0

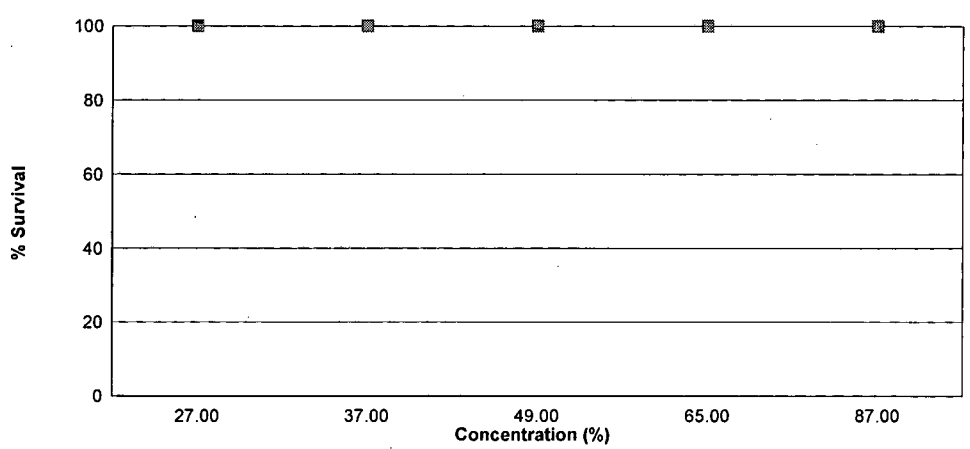
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on August 22, 2013 at 1605 and continued through August 29, 2013 at 1415. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 87 % effluent
b.) NOEC reproduction = 87 % effluent



Summary of the 7-day *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	23.6
27 %	100	24.2
37 %	100	27.6
49 %	100	26.4
65 %	100	24.2
87 %	100	25.0

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 22, 2013 at 1605
Date and Time Test Terminated: August 29, 2013 at 1415

Concentration: Control													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	4	4	2	3	4	4	3	2	33	10	3.30
5	0	0	0	0	0	1	0	0	0	6	7	10	0.700
6	9	9	7	8	7	8	7	9	8	8	80	10	8.00
7	14	14	11	13	14	11	12	14	13	0	116	10	11.6
8													
TOTAL	27	26	22	25	23	23	23	27	24	16	236	10	23.6

Concentration: 27 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	2	3	3	3	2	3	2	3	2	27	10	2.70
5	8	7	1	0	0	0	0	0	0	6	22	10	2.20
6	1	12	13	11	10	10	11	8	7	6	89	10	8.90
7	16	0	17	18	6	0	17	17	13	0	104	10	10.4
8													
TOTAL	29	21	34	32	19	12	31	27	23	14	242	10	24.2

Concentration: 37 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	3	3	3	2	3	2	2	2	27	10	2.70
5	0	0	0	0	0	1	0	0	6	6	13	10	1.30
6	12	12	12	11	10	6	8	10	13	10	104	10	10.4
7	18	16	17	18	16	15	15	17	0	0	132	10	13.2
8													
TOTAL	34	31	32	32	29	24	26	29	21	18	276	10	27.6

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 22, 2013 at 1605
Date and Time Test Terminated: August 29, 2013 at 1415

Concentration: 49 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	2	5	2	3	4	2	2	2	29	10	2.90
5	0	5	0	0	0	1	0	6	4	3	19	10	1.90
6	10	0	11	11	11	8	10	0	12	10	83	10	8.30
7	17	18	16	18	16	17	15	16	0	0	133	10	13.3
8													
TOTAL	31	26	29	34	29	29	29	24	18	15	264	10	26.4

Concentration: 65 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	2	3	5	3	3	3	2	3	2	2	28	10	2.80
5	6	0	0	0	0	1	0	0	5	4	16	10	1.60
6	13	11	11	11	10	10	8	10	0	12	96	10	9.60
7	0	15	14	16	12	0	15	16	14	0	102	10	10.2
8													
TOTAL	21	29	30	30	25	14	25	29	21	18	242	10	24.2

Concentration: 87 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	4	3	3	3	3	4	3	2	2	31	10	3.10
5	8	6	1	0	0	0	0	0	5	5	25	10	2.50
6	0	2	11	12	6	10	8	8	0	7	64	10	6.40
7	16	15	16	18	16	0	18	17	14	0	130	10	13.0
8													
TOTAL	28	27	31	33	25	13	30	28	21	14	250	10	25.0

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	10	0	10
27 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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	Alive	Dead	Total Animals
Control	10	0	10
37 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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	Alive	Dead	Total Animals
Control	10	0	10
49 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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	Alive	Dead	Total Animals
Control	10	0	10
65 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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.

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
87 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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 Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	27 %	10	0	
2	37 %	10	0	
3	49 %	10	0	
4	65 %	10	0	
5	87 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality		No Transformation
D = 0.1154 D* = 0.9054 Critical D* = 1.035	(alpha = 0.01, N = 60)	
Data PASS normality test (alpha = 0.01).		

Bartlett's Test for Homogeneity of Variance		No Transformation
Calculated B1 statistic = 6.592 Critical B = 15.086	(alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.		

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	117.9	23.58	0.6766
Within (Error)	54	1882	34.85	
Total	59	2000		

Critical F = 3.38 (alpha = 0.01, df = 5,54)
2.38 (alpha = 0.05, df = 5,54)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Dunnett's Test - Table 1 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	23.6	23.6		
2	27 %	24.2	24.2	-0.2273	
3	37 %	27.6	27.6	-1.515	
4	49 %	26.4	26.4	-1.061	
5	65 %	24.2	24.2	-0.2273	
6	87 %	25	25	-0.5303	

Dunnett's critical value = 2.31 (1 Tailed, alpha = 0.05, df [used] = 5,40) (Actual df = 5,54)

Dunnett's Test - Table 2 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	10			
2	27 %	10	6.099	25.8	-0.6
3	37 %	10	6.099	25.8	-4
4	49 %	10	6.099	25.8	-2.8
5	65 %	10	6.099	25.8	-0.6
6	87 %	10	6.099	25.8	-1.4

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 22, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.5	7.7	7.5	7.8	7.8	8.1
	Final	7.7	8.0	8.7	8.2	8.2	7.8	7.8
pH, units	Initial	7.7	7.7	7.8	8.0	7.9	8.0	7.7
	Final	8.0	8.2	8.0	8.0	8.0	7.8	7.8
Alkalinity, mg CaCO3/l		32	NA	32	NA	NA	NA	NA
Hardness, mg CaCO3/l		47	NA	42	NA	NA	NA	NA
Conductivity, umhos/cm		180	180	170	180	170	170	190
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	NA	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.4	8.0	7.5	8.0	8.0	7.5
	Final	7.5	8.2	8.6	8.2	8.2	7.7	7.7
pH, units	Initial	7.6	7.7	7.5	7.8	7.7	7.8	7.5
	Final	8.0	8.1	8.0	8.0	8.1	7.6	7.6

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.6	8.6	7.6	8.2	7.7	7.7
	Final	7.6	8.0	8.6	8.0	8.2	7.6	7.6
pH, units	Initial	7.6	7.7	7.4	7.7	7.6	7.7	7.6
	Final	8.0	8.1	8.0	8.0	8.1	7.6	7.6

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 22, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.5	8.7	7.6	8.1	7.7	7.7
	Final	7.7	8.1	8.6	8.2	8.2	7.6	7.9
pH, units	Initial	7.5	7.7	7.3	7.6	7.6	7.6	7.5
	Final	8.0	8.1	8.0	8.1	8.2	7.5	7.5

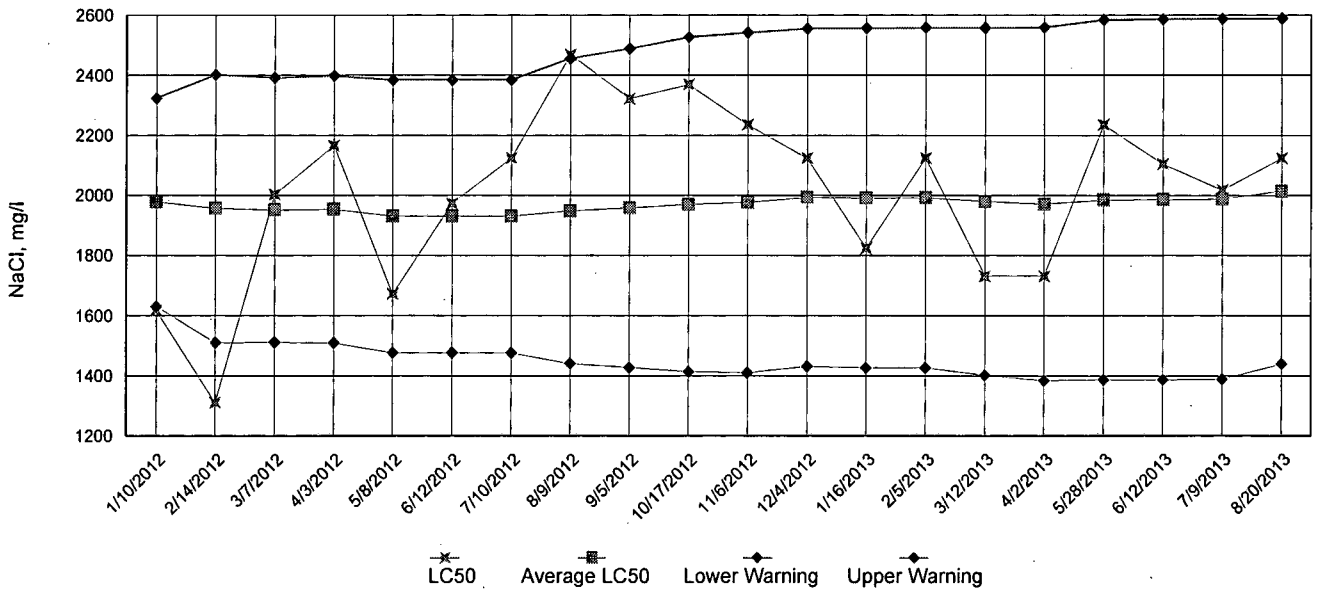
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.9	7.4	8.8	7.6	8.7	7.7	7.3
	Final	7.7	8.0	8.6	8.4	8.1	7.6	7.6
pH, units	Initial	7.5	7.7	7.2	7.5	7.5	7.6	7.5
	Final	8.0	8.0	8.0	8.1	8.1	7.5	7.5
Alkalinity, mg CaCO3/l		36	NA	30	NA	NA	NA	NA
Hardness, mg CaCO3/l		54	NA	58	NA	NA	NA	NA
Conductivity, umhos/cm		250	260	280	290	310	310	330
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	NA	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	7.4	8.9	7.6	8.3	7.9	7.4
	Final	7.4	8.1	8.7	8.2	7.7	5.4	7.2
pH, units	Initial	7.4	7.7	7.1	7.4	7.4	7.6	7.6
	Final	8.0	8.0	7.9	8.1	8.3	7.5	7.4

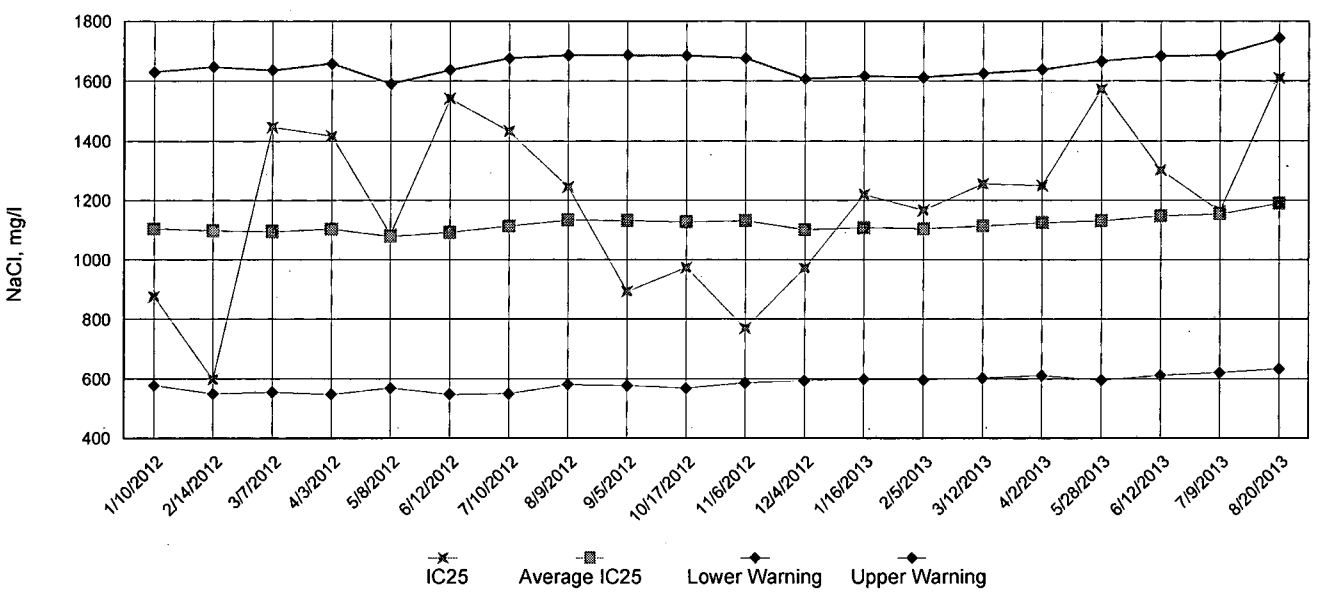
Appendix A4: Test 1002.0

Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL		No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
1	Plant Effluent	8-18-13 0900 - 2400		X	X			3	X
		Container Type							P
		Preservative							NO

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 20c
Remarks
Field pH calibration on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ___ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
A. Thomason	8-19-13 @ 1010	M. Mann	8-19-13 @ 10:10am
Relinquished By: M. Mann	Date/Time 8-19-13 @ 11:10AM	Received In Lab By: Angie Hopton	Date/Time 8-19-13 1110

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL		No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
2	Plant Effluent	8-20-13 0900-2400		X	X			3	X
		Container Type							P
		Preservative							NO

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 23C
Remarks

Field pH calibration
on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
A. Ross	8-21-13 0900	G. Mann	8-21-13 900
G. Mann	8-21-13 1130	Jimmy Day	8/21/13 1130

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By:

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
3	Plant Effluent	8/22/13 0000-2400		X			3	X
		Container Type						P
		Preservative						NO

AIC Control No: 16984b
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 2
Remarks
Field pH calibration on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	8-23-13 0955	[signature]	8-23-13 9:55
[signature]	8-23-13 11:20	[signature]	8-23-13 1120

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

September 30, 2013

Test Results of
Acute 48 hour Non-Renewal
Biomonitoring Testing
for

170741-1: Outfall 001 First Renewal
170741-2: Outfall 001 Second Renewal
170741-3: Outfall 001 Third Renewal
170741-4: Outfall 001 Original Screen

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Acute 48 hour Non-Renewal Biomonitoring utilizing *Ceriodaphnia dubia*
Outfall 001 First Renewal - City of Hot Springs
Client NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

Please find attached the results of the acute biomonitoring screens conducted as a follow up to the chronic toxicity event previously reported (AIC Control No. 170390). As indicated on the enclosed results, the sample was still acutely toxic on September 12, 2013 and exhibited no acute toxicity on September 19, 2013.

It it theorized the decrease in toxicity is directly related to the holding time which may have allowed microbial degradation, loss of volatile toxics, and/or biochemical degradation.

If I can be of further assistance, please feel free to contact me.

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Dilution Water Samples: Synthetic Soft Water #4021

Analysis	Result
Dissolved oxygen (mg/l)	7.6
pH (standard units)	7.7
Alkalinity (mg/l as CaCO3)	31
Hardness (mg/l as CaCO3)	46
Conductivity (umhos/cm)	170
Residual Chlorine (mg/l)	<0.05

Results Summary: Outfall 001 First Renewal

Ceriodaphnia dubia

The *Ceriodaphnia dubia* test was conducted from September 19, 2013 at 1615 to September 21, 2013 at 1700.

Statistical analyses:

NOEC = 100%

Concentration	24 hour % Survival	48 hour % Survival
Control	100	100
100%	100	100

Ceriodaphnia dubia
Survival Data

Number of organisms per chamber: 5
Volume of test chamber: 30 ml

Age of organisms: <24 hours
Volume of test solution: 15 ml

Effluent Concentration	Number of Survivors 24 Hours	48 Hours	% Survival	CV %
Control rep. A	5	5	100	0.00
100% rep. A	5	5	100	0.00

CV = Coefficient of variance = standard deviation X 100/mean

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	5	0	5
100%	5	0	5
Total	10	0	10

Critical Fisher's value (5,5,5) (alpha=0.05) is 1. b value is 5. Since b is greater than 1 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test

Group	Identification	Exposed	Dead	Sig 0.05
0	0	5	0	
1	100	5	0	

Chemical Data for
Ceriodaphnia dubia

Day 1	Control	100%
DO, mg/l	7.6	8.8
pH, su	7.7	7.3
Alkalinity, mg/l	31	NA
Hardness, mg/l	46	NA
Conductivity, umho/cm	170	380
Residual Chlorine, mg/l	<0.05	NA

Day 2		Control	100%
DO, mg/l	Final	7.5	8.7
pH, su	Final	8.0	7.4

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

month WR 9/19/13

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Chronic C.D.	Analyses Requested
1	Plant Effluent	9-8-13 0000-2400		X	X		3	X	
		Container Type						P	
		Preservative						NO	

No FATHEAD ANALYSIS

AIC Control No: 120390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2.1 °C
Remarks:
Field pH calibration on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z = Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-8-13 1030	M. Mann	9-9-13 10:30 AM
M. Mann	9-9-13 11:45	[signature]	9-9-13 1145

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

October 1, 2013
Control No. 171032

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

This report contains the analytical results and supporting information for samples submitted on September 27, 2013. Attached please find a copy of the Chain of Custody and/or other documents received. Note that any remaining sample will be discarded two weeks from the original report date unless other arrangements are made.

This report is intended for the sole use of the client listed above. Assessment of the data requires access to the entire document.

This report has been reviewed by the Laboratory Director or a qualified designee.

John Overbey
Laboratory Director

This document has been distributed to the following:

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

SAMPLE INFORMATION

Project Description:
Three (3) water sample(s) (AIC COntrol No. 170741-1,2,3) resubmitted September 27, 2013
P.O. No. 13-3032

Receipt Details:
A Chain of Custody was not provided with the sample(s).

Each sample container was checked for proper labeling, including date and time sampled. Sample containers were reviewed for proper type, adequate volume, integrity, temperature, preservation, and holding times. Any exceptions are noted below:

Sample Identification:

Laboratory ID	Client Sample ID	Sampled Date/Time	Notes
171032-1	Plant Effluent 9-8-13 0000-2400	08-Sep-2013 2359	
171032-2	Plant Effluent 9-10-13 0000-2400	10-Sep-2013 2359	
171032-3	Plant Effluent 9-12-13 0000-2400	12-Sep-2013 2359	

Case Narrative:
There were no qualifiers for this data and all samples met quality control criteria.

References:
"Methods for Chemical Analysis of Water and Wastes", EPA/600/4-79-020 (Mar 1983) with updates and supplements EPA/600/5-91-010 (Jun 1991), EPA/600/R-92-129 (Aug 1992) and EPA/600/R-93-100 (Aug 1993).
"Test Methods for Evaluating Solid Waste Physical/Chemical Methods (SW846)", Third Edition.
"Standard Methods for the Examination of Water and Wastewaters", 21st edition.
"American Society for Testing and Materials" (ASTM).
"Association of Analytical Chemists" (AOAC).

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

ANALYTICAL RESULTS

AIC No. 171032-1
Sample Identification: Plant Effluent 9-8-13 0000-2400

Analyte		Result	RL	Units	Qualifier
Total Organic Carbon		**6.1**	1	**mg/l**	
SM 5310 C	Prep: 27-Sep-2013 1516 by 308	Analyzed: 27-Sep-2013 1814 by 308		Batch: W45076	
Aluminum		**< 0.04**	0.04	**mg/l**	
EPA 200.7	Prep: 30-Sep-2013 0901 by 271	Analyzed: 01-Oct-2013 1242 by 305		Batch: S35491	
Sulfate		**29**	0.2	**mg/l**	
EPA 300.0	Prep: 27-Sep-2013 1628 by 07	Analyzed: 27-Sep-2013 2205 by 07		Batch: C16076	

AIC No. 171032-2
Sample Identification: Plant Effluent 9-10-13 0000-2400

Analyte		Result	RL	Units	Qualifier
Total Organic Carbon		**5.9**	1	**mg/l**	
SM 5310 C	Prep: 27-Sep-2013 1516 by 308	Analyzed: 27-Sep-2013 1828 by 308		Batch: W45076	
Aluminum		**0.041**	0.04	**mg/l**	
EPA 200.7	Prep: 30-Sep-2013 0901 by 271	Analyzed: 01-Oct-2013 1245 by 305		Batch: S35491	
Sulfate		**39**	0.2	**mg/l**	
EPA 300.0	Prep: 27-Sep-2013 1628 by 07	Analyzed: 27-Sep-2013 2232 by 07		Batch: C16076	

AIC No. 171032-3
Sample Identification: Plant Effluent 9-12-13 0000-2400

Analyte		Result	RL	Units	Qualifier
Total Organic Carbon		**5.4**	1	**mg/l**	
SM 5310 C	Prep: 27-Sep-2013 1516 by 308	Analyzed: 27-Sep-2013 1843 by 308		Batch: W45076	
Aluminum		**< 0.04**	0.04	**mg/l**	
EPA 200.7	Prep: 30-Sep-2013 0901 by 271	Analyzed: 01-Oct-2013 1248 by 305		Batch: S35491	
Sulfate		**34**	0.2	**mg/l**	
EPA 300.0	Prep: 27-Sep-2013 1628 by 07	Analyzed: 27-Sep-2013 2259 by 07		Batch: C16076	

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

LABORATORY CONTROL SAMPLE RESULTS

Analyte	Spike Amount	%	Limits	RPD	Limit	Batch	Preparation Date	Analysis Date	Dil	Qual
Total Organic Carbon	10 mg/l	96.4	80.0-120			W45076	27Sep13 1459 by 308	27Sep13 1648 by 308		
Aluminum	5 mg/l	97.6	85.0-115			S35491	30Sep13 0901 by 271	01Oct13 1152 by 305		
Sulfate	20 mg/l	108	90.0-110			C16076	27Sep13 1628 by 07	27Sep13 1710 by 07		

MATRIX SPIKE SAMPLE RESULTS

Analyte	Sample	Spike Amount	%	Limits	Batch	Preparation Date	Analysis Date	Dil	Qual
Total Organic Carbon	171006-1	10 mg/l	100	80.0-120	W45076	27Sep13 1459 by 308	27Sep13 1717 by 308		
	171006-1	10 mg/l	97.9	80.0-120	W45076	27Sep13 1459 by 308	27Sep13 1731 by 308		
	Relative Percent Difference:		1.86	25.0	W45076				
Aluminum	171022-2	5 mg/l	96.7	75.0-125	S35491	30Sep13 0901 by 271	01Oct13 1155 by 305		
	171022-2	5 mg/l	96.6	75.0-125	S35491	30Sep13 0901 by 271	01Oct13 1158 by 305		
	Relative Percent Difference:		0.142	20.0	S35491				
Sulfate	171021-1	20 mg/l	89.1	80.0-120	C16076	27Sep13 1628 by 07	27Sep13 1737 by 07		
	171021-1	20 mg/l	93.6	80.0-120	C16076	27Sep13 1628 by 07	27Sep13 1804 by 07		
	Relative Percent Difference:		4.28	10.0	C16076				

LABORATORY BLANK RESULTS

Analyte	Result	RL	PQL	QC Sample	Preparation Date	Analysis Date	Qual
Total Organic Carbon	< 1 mg/l	1	1	W45076-1	27Sep13 1459 by 308	27Sep13 1633 by 308	
Aluminum	< 0.04 mg/l	0.04	0.04	S35491-1	30Sep13 0901 by 271	01Oct13 1149 by 305	
Sulfate	< 0.2 mg/l	0.2	0.2	C16076-1	27Sep13 1628 by 07	27Sep13 1644 by 07	

AMERICAN INTERPLEX CORPORATION LABORATORIES

170 1032 WN 9/30/13
~~170741~~ WN 9/19/13

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client:	City of Hot Springs
Project Reference:	Plant Effluent
Project Manager:	James Sorrells
Sampled By:	A. Ross
PO No.	13-3032
AIC Control No:	~~170390~~
AIC Proposal No:	
Carrier:	Hot Springs Courier
Received Temperature °C	2.1 °C

Analyses Requested: No Fathead Analysis

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL	No of BOTTLES	Chronic CD	Remarks
1	Plant Effluent	9-8-13 0000-2400		X	X		3	x	
		Container Type					P		Field pH calibration
		Preservative					NO		on ____ @ ____ Buffer:

G = Glass NO = none P = Plastic S = Sulfuric acid pH2 V = VOA vials N = Nitric acid pH2 H = HCl to pH2 B = NaOH to pH12 T = Sodium Thiosulfate Z= Zinc acetate A = $(NH4)_2SO4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ___ DAYS
Expedited results requested by: ____________
Who should AIC contact with questions: ____________
Phone: ______ Fax: ______
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-8-13 1030	M. Mann	9-9-13 10:30 am
Relinquished By: M. Mann	Date/Time 9-9-13 11:45	Received In Lab By: [signature]	Date/Time 9-9-13 1145

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

August 30, 2013

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Outfall 001
City of Hot Springs

Control No. 169846-1

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Pimephales promelas* (Fathead minnow)
Outfall 001 - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Method 1000.0 Chronic *Pimephales promelas* (Fathead minnow) Survival and Growth Test: The No Observable Effects Concentration (NOEC) for survival occurred at 87 % effluent, which is above the critical dilution of 65 %. The NOEC for growth occurred at 87 % effluent, which is above the critical dilution of 65 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the Fathead minnow test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

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City of Hot Springs
ATTN: Mr. James Sorrells
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Table of Contents

I. Control Acceptance Criteria

Pimephales promelas (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.296	PASS
Control Growth CV < or = 40%	7.47	PASS
Growth Minimum Significant Difference 12 to 30%	20.0	PASS
Critical Dilution CV < or = 40%	11.6	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly Test Method 1000.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point: Outfall 001
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.2	8.9	8.5
pH (standard units)	7.4	7.0	7.4
Alkalinity (mg/l as CaCO3)	41	26	34
Hardness (mg/l as CaCO3)	59	67	71
Conductivity (umhos/cm)	290	350	390
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.28	0.12	0.30

2. Dilution Water Samples: Synthetic Soft Water #4012
   a. Dates Prepared: August 8 through August 22, 2013
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.7	7.7	7.8
pH (standard units)	7.7	7.8	7.9
Alkalinity (mg/l as CaCO3)	32	32	32
Hardness (mg/l as CaCO3)	47	42	42
Conductivity (umhos/cm)	180	170	170
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05

C. Test Methods
1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Method 1000.0, Fathead Minnow Survival and Growth.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Pimephales promelas (Fathead minnow) Survival and Growth Method 1000.0

Date & Time Test Initiated:	August 20, 2013 at 1200
Date & Time Test Terminated:	August 27, 2013 at 1010
Type & Volume of Test Chamber:	500 ml disposable beaker
Volume of Sample:	250 ml
Number of Organisms per replicate:	8
Number of Replicates per dilution:	5

4. Acclimation of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms
1. Scientific Name
a. Test 1000.0 *Pimephales promelas*

III. Data Analysis

The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Pimephales promelas (Fathead minnow) survival data was transformed using the Arc Sine transformation. Normality and homogeneity of variance were checked using Shapiro-Wilk's. The survival data was then analyzed using Steel's Many-One Rank Test to determine the No Observable Effects Concentration (NOEC).

Fathead minnow growth data was analyzed for normality and homogeneity of variance using Shapiro-Wilk's and Bartlett's test. Dunnett's Test was used to determine the No Observable Effects Concentration (NOEC) for growth.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Pimephales promelas (Fathead minnow)

Chronic reference tests are performed monthly.
A chronic reference test was performed on August 20, 2013 at 1715 to August 27, 2013 at 1520
The results were as follows: (Control No. 169867-1.)
Survival LC-50: 5670.1 mg/l
Growth IC-25: 3143 mg/l
Growth PMSD: 17.3

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	102	1.91
pH	SM 4500-H+ B	101	0.268
Conductivity	EPA 120.1	101	4.71

VI. Organism History

Pimephales promelas (Fathead minnow)
Date: August 20, 2013
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

VII. Results Summary *Pimephales promelas*, Fathead minnow Larval Survival and Growth Test -- Method 1000.0

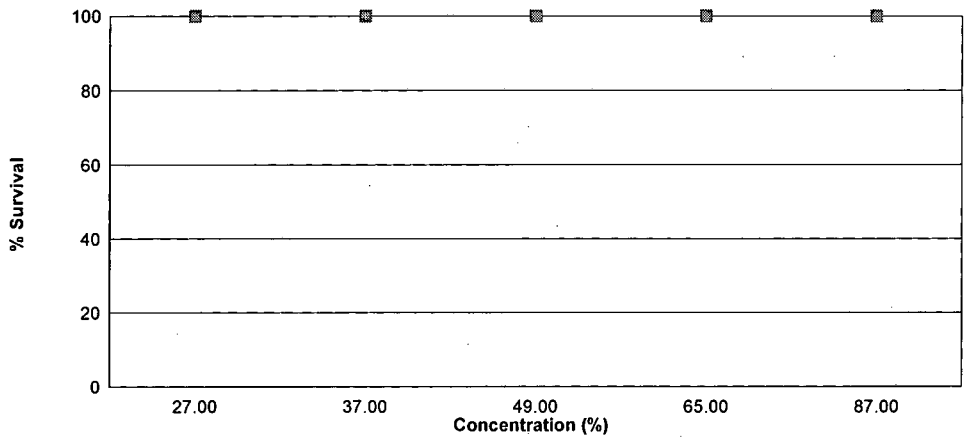
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on August 20, 2013 at 1200 and continued through August 27, 2013 at 1010. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 87 % effluent
b.) NOEC growth = 87 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.296
27 %	100	0.337
37 %	100	0.323
49 %	100	0.302
65 %	100	0.292
87 %	100	0.302

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: August 20, 2013 at 1200
Date and Time Test Terminated: August 27, 2013 at 1010

Concentration	Replicate	Number of Survivors						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
27 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
37 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
49 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
65 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
87 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: August 20, 2013 at 1200
Test Terminated: August 27, 2013 at 1010
Drying Started: August 23, 2013 at 1527
Drying Ended: August 29, 2013 at 1400

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.91827	.92042	0.00215	8	0.269
	B	.91878	.92121	0.00243	8	0.304
	C	.91659	.91922	0.00263	8	0.329
	D	.91638	.91872	0.00234	8	0.292
	E	.91455	.91685	0.00230	8	0.288
27 %	A	.91583	.91811	0.00228	8	0.285
	B	.91943	.92233	0.00290	8	0.362
	C	.92408	.92709	0.00301	8	0.376
	D	.92651	.92924	0.00273	8	0.341
	E	.92767	.93024	0.00257	8	0.321
37 %	A	.92653	.92915	0.00262	8	0.328
	B	.94872	.95160	0.00288	8	0.360
	C	.94724	.94985	0.00261	8	0.326
	D	.94532	.94750	0.00218	8	0.272
	E	.94881	.95146	0.00265	8	0.331
49 %	A	.94115	.94330	0.00215	8	0.269
	B	.92530	.92779	0.00249	8	0.311
	C	.92632	.92901	0.00269	8	0.336
	D	.93070	.93312	0.00242	8	0.302
	E	.93427	.93660	0.00233	8	0.291
65 %	A	.93862	.94107	0.00245	8	0.306
	B	.93944	.94155	0.00211	8	0.264
	C	.94132	.94377	0.00245	8	0.306
	D	.93810	.94077	0.00267	8	0.334
	E	.93899	.94100	0.00201	8	0.251
87 %	A	.93682	.93952	0.00270	8	0.338
	B	.93818	.93991	0.00173	8	0.216
	C	.93688	.93881	0.00193	8	0.241
	D	.93804	.94073	0.00269	8	0.336
	E	.95233	.95537	0.00304	8	0.380

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

		Transformation of Data		Transform: Arc Sin(Square Root(Y))
Group	Identification	Rep	Value	Transformed
1	Control	1	1.00000	1.39310
1	Control	2	1.00000	1.39310
1	Control	3	1.00000	1.39310
1	Control	4	1.00000	1.39310
1	Control	5	1.00000	1.39310
2	27 %	1	1.00000	1.39310
2	27 %	2	1.00000	1.39310
2	27 %	3	1.00000	1.39310
2	27 %	4	1.00000	1.39310
2	27 %	5	1.00000	1.39310
3	37 %	1	1.00000	1.39310
3	37 %	2	1.00000	1.39310
3	37 %	3	1.00000	1.39310
3	37 %	4	1.00000	1.39310
3	37 %	5	1.00000	1.39310
4	49 %	1	1.00000	1.39310
4	49 %	2	1.00000	1.39310
4	49 %	3	1.00000	1.39310
4	49 %	4	1.00000	1.39310
4	49 %	5	1.00000	1.39310
5	65 %	1	1.00000	1.39310
5	65 %	2	1.00000	1.39310
5	65 %	3	1.00000	1.39310
5	65 %	4	1.00000	1.39310
5	65 %	5	1.00000	1.39310
6	87 %	1	1.00000	1.39310
6	87 %	2	1.00000	1.39310
6	87 %	3	1.00000	1.39310
6	87 %	4	1.00000	1.39310
6	87 %	5	1.00000	1.39310

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Shapiro - Wilk's Test for Normality		Transform: Arc Sin(Square Root(Y))
D = 0		
W = 0		
Critical W = 0.9	(alpha = 0.01, N = 30)	
Critical W = 0.927	(alpha = 0.05, N = 30)	

Data FAIL normality test (alpha = 0.01).

Steel's Many-One Rank Test — Transform: Arc Sin(Square Root(Y))

Ho:Control<Treatment

Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	27 %	27.50	16.00	5.00	
3	37 %	27.50	16.00	5.00	
4	49 %	27.50	16.00	5.00	
5	65 %	27.50	16.00	5.00	
6	87 %	27.50	16.00	5.00	

Critical values are 1 tailed (k=5)

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality		No Transformation
D = 0.03787		
W = 0.9742		
Critical W = 0.9	(alpha = 0.01, N = 30)	
Critical W = 0.927	(alpha = 0.05, N = 30)	
Data PASS normality test (alpha = 0.01).		

Bartlett's Test for Homogeneity of Variance		No Transformation
Calculated B1 statistic = 7.076		
Critical B = 15.086	(alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.		

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	0.007651	0.00153	0.9696
Within (Error)	24	0.03787	0.001578	
Total	29	0.04552		

Critical F = 3.9 (alpha = 0.01, df = 5,24)
2.62 (alpha = 0.05, df = 5,24)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Dunnett's Test - Table 1 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	0.2964	0.2964		
2	27 %	0.337	0.337	-1.616	
3	37 %	0.3234	0.3234	-1.075	
4	49 %	0.3018	0.3018	-0.2149	
5	65 %	0.2922	0.2922	0.1672	
6	87 %	0.3022	0.3022	-0.2309	

Dunnett's critical value = 2.36 (1 Tailed, alpha = 0.05, df = 5,24)

Dunnett's Test - Table 2 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	5			
2	27 %	5	0.05929	20	-0.0406
3	37 %	5	0.05929	20	-0.027
4	49 %	5	0.05929	20	-0.0054
5	65 %	5	0.05929	20	0.0042
6	87 %	5	0.05929	20	-0.0058

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 20, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.5	7.7	7.5	7.8	7.8	8.1
	Final	7.5	7.2	7.5	7.9	7.7	7.1	7.9
pH, units	Initial	7.7	7.7	7.8	8.0	7.9	8.0	7.7
	Final	7.5	7.3	7.8	8.1	8.1	7.6	7.9
Alkalinity, mg CaCO3/l		32	NA	32	NA	32	NA	NA
Hardness, mg CaCO3/l		47	NA	42	NA	42	NA	NA
Conductivity, umhos/cm		180	180	170	180	170	170	190
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.4	8.0	7.5	8.0	8.0	7.5
	Final	7.5	7.2	7.3	7.9	7.8	8.1	7.7
pH, units	Initial	7.6	7.7	7.5	7.8	7.7	7.8	7.5
	Final	7.5	7.3	7.7	8.1	8.2	7.5	7.7

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.6	8.6	7.6	8.2	7.7	7.7
	Final	7.5	7.0	7.5	8.0	7.9	8.1	7.8
pH, units	Initial	7.6	7.7	7.4	7.7	7.6	7.7	7.6
	Final	7.5	7.3	7.7	8.1	8.2	7.5	7.6

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 20, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.5	8.7	7.6	8.1	7.7	7.7
	Final	7.5	7.4	7.6	8.0	8.1	8.2	7.8
pH, units	Initial	7.5	7.7	7.3	7.6	7.6	7.6	7.5
	Final	7.5	7.4	7.6	8.1	8.2	7.6	7.6

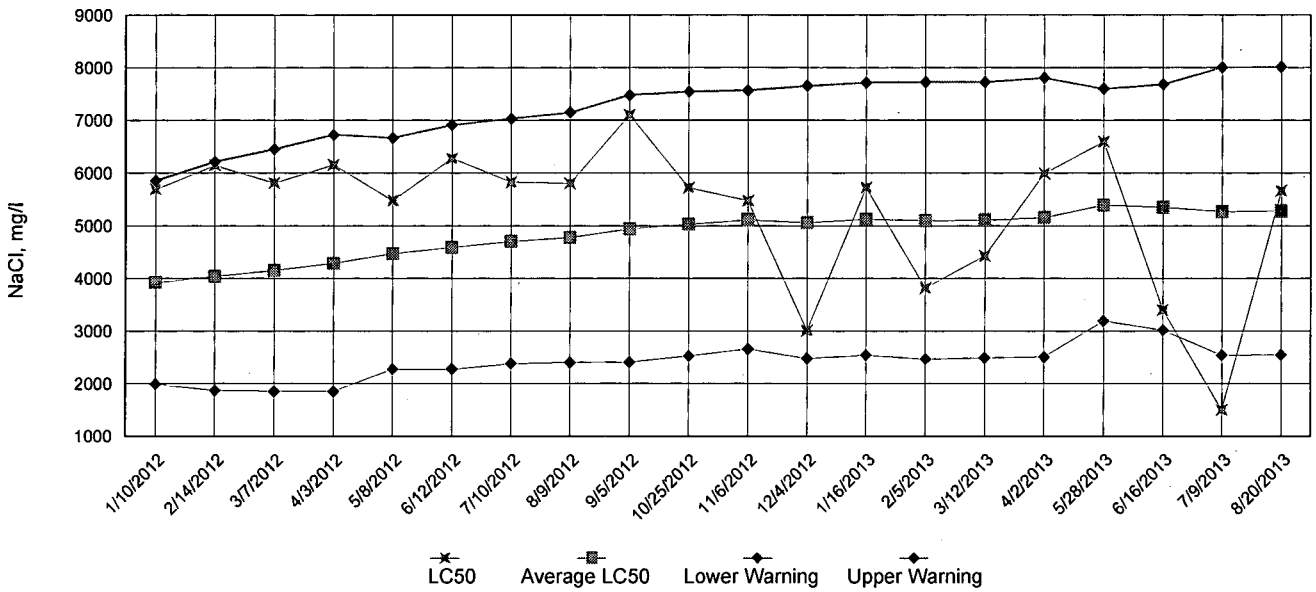
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.9	7.4	8.8	7.6	8.7	7.7	7.3
	Final	7.5	7.4	7.4	7.9	8.1	8.0	7.7
pH, units	Initial	7.5	7.7	7.2	7.5	7.5	7.6	7.5
	Final	7.5	7.4	7.6	8.1	8.3	7.6	7.5
Alkalinity, mg CaCO3/l		36	NA	30	NA	35	NA	NA
Hardness, mg CaCO3/l		54	NA	58	NA	61	NA	NA
Conductivity, umhos/cm		250	260	280	290	310	310	330
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	7.4	8.9	7.6	8.3	7.9	7.4
	Final	7.5	8.5	7.4	7.8	8.1	8.0	7.7
pH, units	Initial	7.4	7.7	7.1	7.4	7.4	7.6	7.6
	Final	7.5	7.4	7.6	8.1	8.1	7.6	7.4

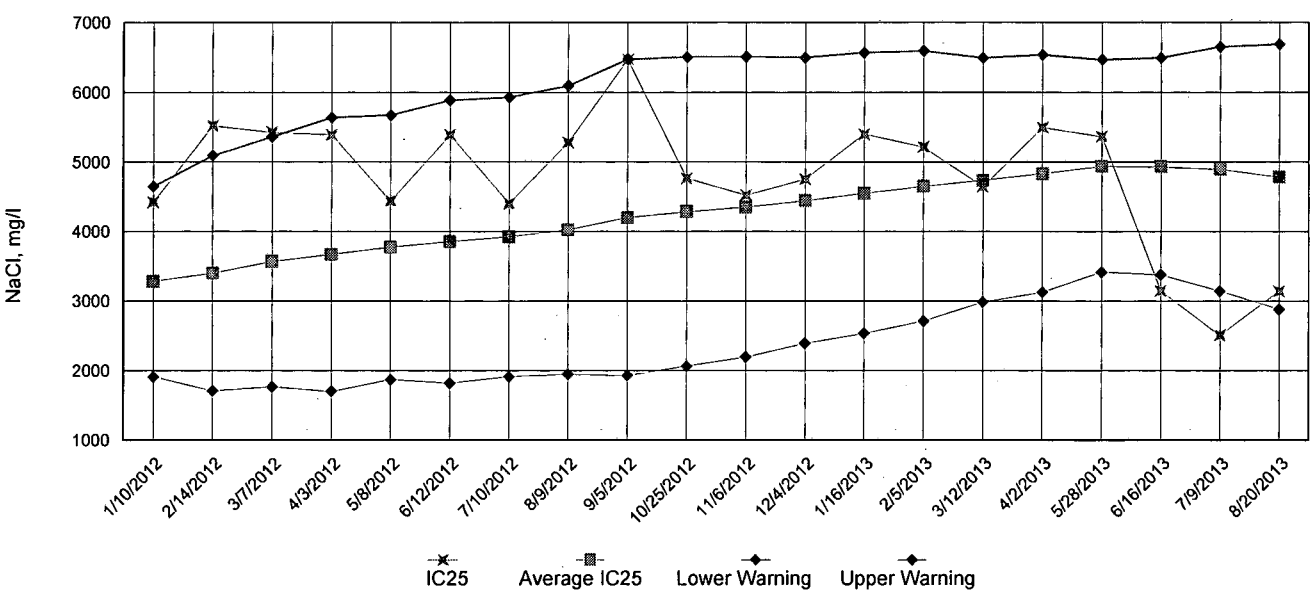
Appendix A4: Test 1000.0

Chronic Reference Toxicant, *Pimephales promelas* (Fathead Minnow)

LC50 Survival Data



IC25 Growth Data



Appendix B: Test 1000.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

Permittee: City of Hot Springs

NPDES No.: AR0033880 AFIN#26-00145

Date and Time Test Initiated: August 20, 2013 at 1200
Date and Time Test Terminated: August 27, 2013 at 1010
Dilution water used: Synthetic Soft Water #4012

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in replicate chambers A	B	C	D	E	Mean percent survival 24 hr	48 hr	7 days	CV%
Control	100	100	100	100	100	100	100	100	0.00
27 %	100	100	100	100	100	100	100	100	0.00
37 %	100	100	100	100	100	100	100	100	0.00
49 %	100	100	100	100	100	100	100	100	0.00
65 %	100	100	100	100	100	100	100	100	0.00
87 %	100	100	100	100	100	100	100	100	0.00

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers A	B	C	D	E	Mean dry weight, mg	CV%
Control	0.269	0.304	0.329	0.292	0.288	0.296	7.47
27 %	0.285	0.362	0.376	0.341	0.321	0.337	10.6
37 %	0.328	0.360	0.326	0.272	0.331	0.323	9.86
49 %	0.269	0.311	0.336	0.302	0.291	0.302	8.20
65 %	0.306	0.264	0.306	0.334	0.251	0.292	11.6
87 %	0.338	0.216	0.241	0.336	0.380	0.302	23.2

CV = Coefficient of variation = standard deviation * 100 / mean

Appendix B: Test 1000.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

1. Steel's Many-One Rank Test:

   Is the mean survival significantly different (p=0.05) than the control survival for the % effluent corresponding to (lethality):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	____YES	X NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____YES	____NO

2. Dunnett's Test:

   Is the mean dry weight (growth) significantly different (p=0.05) than the control's dry weight (growth) for the % effluent corresponding to (significant non-lethal effects):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	____YES	X NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____YES	____NO

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

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

5. NOEC Pimephales Lethality: 87 % (TOP6C)

6. LOEC Pimephales Lethality: 87 % (TXP6C)

7. NOEC Pimephales Sublethality: 87 % (TPP6C)

8. LOEC Pimephales Sublethality: 87 % (TYP6C)

9. Coefficient of variation for Pimephales growth: 11.6 (TQP6C)

AMERICAN INTERPLEX CORPORATION
LABORATORIES

Appendix B: Test 1000.0

CHRONIC TOXICITY SUMMARY FORM
Pimephales promelas (Fathead minnow)
CHEMICAL PARAMETERS CHART

PERMITTEE: City of Hot Springs 2400
NPDES NO.: AR0033880 AFIN#26-00145 2400
CONTACT: Mr. James Sorrells 2400
ANALYST: 280, 298, 304, 307

Test Initiated: DATE: August 20, 2013 TIME: 1200
Test Terminated: DATE: August 27, 2013 TIME: 1010

DILUTION Control	DAY 1	2	3	4	5	6	7
D.O. Initial	7.7	7.5	7.7	7.5	7.8	7.8	8.1
Final	7.5	7.2	7.5	7.9	7.7	7.1	7.9
pH Initial	7.7	7.7	7.8	8.0	7.9	8.0	7.7
Final	7.5	7.3	7.8	8.1	8.1	7.6	7.9
Alkalinity	32	NA	32	NA	32	NA	NA
Hardness	47	NA	42	NA	42	NA	NA
Conductivity	180	180	170	180	170	170	190
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 27 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.7	7.4	8.0	7.5	8.0	8.0	7.5
Final	7.5	7.2	7.3	7.9	7.8	8.1	7.7
pH Initial	7.6	7.7	7.5	7.8	7.7	7.8	7.5
Final	7.5	7.3	7.7	8.1	8.2	7.5	7.7
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	220	220	220	230	230	250
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 37 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.8	7.6	8.6	7.6	8.2	7.7	7.7
Final	7.5	7.0	7.5	8.0	7.9	8.1	7.8
pH Initial	7.6	7.7	7.4	7.7	7.6	7.7	7.6
Final	7.5	7.3	7.7	8.1	8.2	7.5	7.6
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	220	230	240	240	260	250	270
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 49 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.8	7.5	8.7	7.6	8.1	7.7	7.7
Final	7.5	7.4	7.6	8.0	8.1	8.2	7.8
pH Initial	7.5	7.7	7.3	7.6	7.6	7.6	7.5
Final	7.5	7.4	7.6	8.1	8.2	7.6	7.6
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	230	240	260	260	280	280	300
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 65 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.9	7.4	8.8	7.6	8.7	7.7	7.3
Final	7.5	7.4	7.4	7.9	8.1	8.0	7.7
pH Initial	7.5	7.7	7.2	7.5	7.5	7.6	7.5
Final	7.5	7.4	7.6	8.1	8.3	7.6	7.5
Alkalinity	36	NA	30	NA	35	NA	NA
Hardness	54	NA	58	NA	61	NA	NA
Conductivity	250	260	280	290	310	310	330
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 87 %	DAY 1	2	3	4	5	6	7
D.O. Initial	8.0	7.4	8.9	7.6	8.3	7.9	7.4
Final	7.5	8.5	7.4	7.8	8.1	8.0	7.7
pH Initial	7.4	7.7	7.1	7.4	7.4	7.6	7.6
Final	7.5	7.4	7.6	8.1	8.1	7.6	7.4
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	280	290	320	320	360	360	380
Chlorine	NA	NA	NA	NA	NA	NA	NA

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No.: 13-3032

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2°C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL		No of BOTTLES	Chronic.CD, Chronic.FH											Remarks
1	Plant Effluent	8-18-13 0000-2400		X	X			3	x											
																				Field pH calibration
		Container Type							P											on ______ @ ______
		Preservative							NO											Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: ________________
Who should AIC contact with questions: ______________
Phone: ______________Fax:________________
Report Attention to:__Mr. James Sorrells__
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By	Date/Time	Received By	Date/Time
A. Thomason	8-19-13 @ 1010	M. Mann	8-19-13 10:10 AM
M. Mann	8-19-13 @ 11:10 AM	Received In Lab By: [illegible signature]	8-19-13 1110

Comments:

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 2.3C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH	Remarks
2	Plant Effluent	8-20-13 0000-2400		X	X		3	X	
		Container Type						P	Field pH calibration
		Preservative						NO	on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
A. Ross	8-21-13 0900	A. Man	8-21-13 9:10
A. Man	8-21-13 1130	Jimmy Day	8/21/13 1130

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By:

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL		No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
3	Plant Effluent	8/22/13 0000-2400		X				3	X
		Container Type							P
		Preservative							NO

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 2
Remarks
Field pH calibration on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	8-23-13 0955	[signature]	8-23-13 9:55
[signature]	8-23-13 11:20	[signature]	8-23-13 1120

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

September 19, 2013

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Plant Effluent
City of Hot Springs

Control No. 170390-1

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Ceriodaphnia dubia*
Plant Effluent - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: The No Observable Effects Concentration (NOEC) for survival occurred at <27 % effluent, which is below the critical dilution of 65 %. The NOEC for reproduction occurred at <27 % effluent, which is below the critical dilution of 65 %. **The sample, therefore, FAILED both lethal and sub-lethal effects for the *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Table of Contents

I. Control Acceptance Criteria

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	18.8	PASS
Control CV < or = 40% per Surviving Female	20.6	PASS
Reproduction Minimum Significant Difference 13 to 47%	NA	NA
Critical Dilution CV < or = 40%	0.00	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly Test Method 1002.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point: Plant Effluent
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.7	8.2	8.2
pH (standard units)	7.4	7.7	7.5
Alkalinity (mg/l as CaCO3)	64	58	64
Hardness (mg/l as CaCO3)	82	82	83
Conductivity (umhos/cm)	380	370	440
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	2.4	0.11

2. Dilution Water Samples: Synthetic Soft Water #4019
   a. Dates Prepared: August 28 through September 11, 2013
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.6	8.2	8.2
pH (standard units)	7.6	7.8	7.6
Alkalinity (mg/l as CaCO3)	32	32	32
Hardness (mg/l as CaCO3)	46	42	42
Conductivity (umhos/cm)	160	160	180
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05

C. Test Methods
1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Method 1002.0, *Ceriodaphnia dubia* Survival and Reproduction.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Ceriodaphnia dubia Survival and Growth Method 1002.0

Date & Time Test Initiated:	September 9, 2013 at 1235
Date & Time Test Terminated:	September 17, 2013 at 1255
Type & Volume of Test Chamber:	30 ml disposable beaker
Volume of Sample:	15 ml
Number of Organisms per replicate:	1
Number of Replicates per dilution:	10

4. Acclimation of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms
1. Scientific Name
a. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis
The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was not analyzed due to survival failure.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Ceriodaphnia dubia

Chronic reference tests are performed monthly.
A chronic reference test was performed on August 20, 2013 at 1505 to August 28, 2013 at 1450
The results were as follows: (Control No. 169867-2.)
Survival LC-50: 2125 mg/l
Growth IC-25: 1610 mg/l
Growth PMSD: 18.3

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	1.53
Hardness	EPA 200.7	101	0.450
pH	SM 4500-H+ B	101	0.267
Conductivity	EPA 120.1	103	1.97

VI. Organism History

Ceriodaphnia dubia

Date: September 9, 2013
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

VII. Results Summary *Ceriodaphnia dubia*, Cladoceran Survival and Reproduction Test -- Method 1002.0

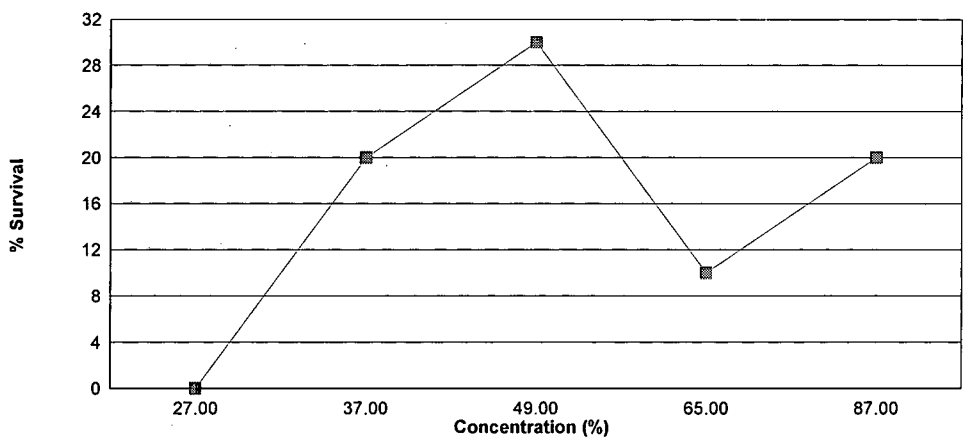
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on September 9, 2013 at 1235 and continued through September 17, 2013 at 1255. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = <27 % effluent
b.) NOEC reproduction = <27 % effluent



Summary of the 7-day *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	18.8
27 %	0.00 *	--
37 %	20.0 *	--
49 %	30.0 *	--
65 %	10.0 *	--
87 %	20.0 *	--

*Significant difference when compared to the control (p=0.05)

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 9, 2013 at 1235
Date and Time Test Terminated: September 17, 2013 at 1255

Concentration: Control													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	3	2	0	0	0	0	1	4	2	1	13	10	1.30
5	9	0	3	6	6	4	0	4	6	0	38	10	3.80
6	0	5	9	8	7	0	6	0	1	7	43	10	4.30
7	10	9	10	10	10	8	9	8	10	10	94	10	9.40
8													
TOTAL	22	16	22	24	23	12	16	16	19	18	188	10	18.8

Concentration: 27 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	0	0	0	X	0	0	0	X	0	0	7	0.00
2	X	0	0	0	X	0	0	0	X	0	0	7	0.00
3	X				X				X		0	0	0.00
4	X				X				X		0	0	0.00
5	X				X				X		0	0	0.00
6	X				X				X		0	0	0.00
7	X				X				X		0	0	0.00
8													
TOTAL	0	0	0	0	0	0	0	0	0	0	0	10	0.00

Concentration: 37 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	X	X	0	X	X	0	0	3	0.00
2	X	X	0	X	X	X	0	X	X	0	0	3	0.00
3	X	X	X	X	X	X	0	X	X	0	0	2	0.00
4	X	X	X	X	X	X	2	X	X	1	3	2	1.50
5	X	X	X	X	X	X	0	X	X	0	0	2	0.00
6	X	X	X	X	X	X	7	X	X	6	13	2	6.50
7	X	X	X	X	X	X	10	X	X	0	10	2	5.00
8													
TOTAL	0	0	0	0	0	0	19	0	0	7	26	10	2.60

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 9, 2013 at 1235
Date and Time Test Terminated: September 17, 2013 at 1255

Concentration: 49 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	X	X	0	X	X	0	0	3	0.00
2	X	X	0	X	X	X	0	X	X	0	0	3	0.00
3	X	X	0	X	X	X	0	X	X	0	0	3	0.00
4	X	X	0	X	X	X	0	X	X	0	0	3	0.00
5	X	X	4	X	X	X	5	X	X	0	9	3	3.00
6	X	X	0	X	X	X	0	X	X	6	6	3	2.00
7	X	X	6	X	X	X	8	X	X	0	14	3	4.67
8													
TOTAL	0	0	10	0	0	0	13	0	0	6	29	10	2.90

Concentration: 65 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	X	X	X	X	X	0	0	2	0.00
2	X	X	0	X	X	X	X	X	X	0	0	2	0.00
3	X	X	0	X	X	X	X	X	X	0	0	2	0.00
4	X	X	0	X	X	X	X	X	X	0	0	2	0.00
5	X	X	6	X	X	X	X	X	X	0	6	2	3.00
6	X	X	0	X	X	X	X	X	X	X	0	1	0.00
7	X	X	9	X	X	X	X	X	X	X	9	1	9.00
8													
TOTAL	0	0	15	0	0	0	0	0	0	0	15	10	1.50

Concentration: 87 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	0	X	0	X	X	0	0	4	0.00
2	X	X	0	X	0	X	X	X	X	0	0	3	0.00
3	X	X	0	X	0	X	X	X	X	0	0	3	0.00
4	X	X	0	X	3	X	X	X	X	1	4	3	1.33
5	X	X	7	X	5	X	X	X	X	0	12	3	4.00
6	X	X	0	X	X	X	X	X	X	3	3	2	1.50
7	X	X	9	X	X	X	X	X	X	0	9	2	4.50
8													
TOTAL	0	0	16	0	8	0	0	0	0	4	28	10	2.80

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
27 %	0	3	3
Total	10	3	13

Critical Fisher's value (10,3,10) (alpha=0.05) is 1. b value is 0. Since b is less than or equal to 1 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
37 %	2	8	10
Total	12	8	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 2. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
49 %	3	7	10
Total	13	7	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 3. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
65 %	1	9	10
Total	11	9	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 1. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
87 %	2	8	10
Total	12	8	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 2. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	27 %	3	3	*
2	37 %	10	8	*
3	49 %	10	7	*
4	65 %	10	9	*
5	87 %	10	8	*

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: September 9, 2013 at 0857
Date and Time Test Terminated: September 17, 2013 at 1255

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.6	7.4	8.2	8.3	8.2	8.3	8.2
	Final	7.8	8.3	8.5	8.7	8.4	8.2	7.8
pH, units	Initial	7.6	7.6	7.8	7.6	7.6	7.7	7.5
	Final	7.8	7.9	7.9	7.6	7.9	7.8	8.0
Alkalinity, mg CaCO3/l		32	NA	32	NA	32	NA	NA
Hardness, mg CaCO3/l		46	NA	42	NA	42	NA	NA
Conductivity, umhos/cm		160	170	160	180	180	180	170
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.6	7.5	8.2	8.4	8.3	8.1	8.3
	Final	6.0	8.3	8.7	8.8	8.5	8.3	8.1
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
	Final	8.0	8.1	8.2	8.0	8.3	8.0	8.1

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.6	7.4	8.2	8.4	8.1	8.0	8.1
	Final	6.4	8.3	8.6	8.8	8.6	8.2	8.2
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
	Final	8.1	8.2	8.2	8.2	8.2	7.9	7.8

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: September 9, 2013 at 0857
Date and Time Test Terminated: September 17, 2013 at 1255

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.5	7.5	8.2	8.4	8.4	8.1	8.3
	Final	6.8	8.3	7.8	9.0	8.4	8.3	8.0
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.5	7.5
	Final	8.2	8.2	8.2	8.1	8.1	8.0	8.1

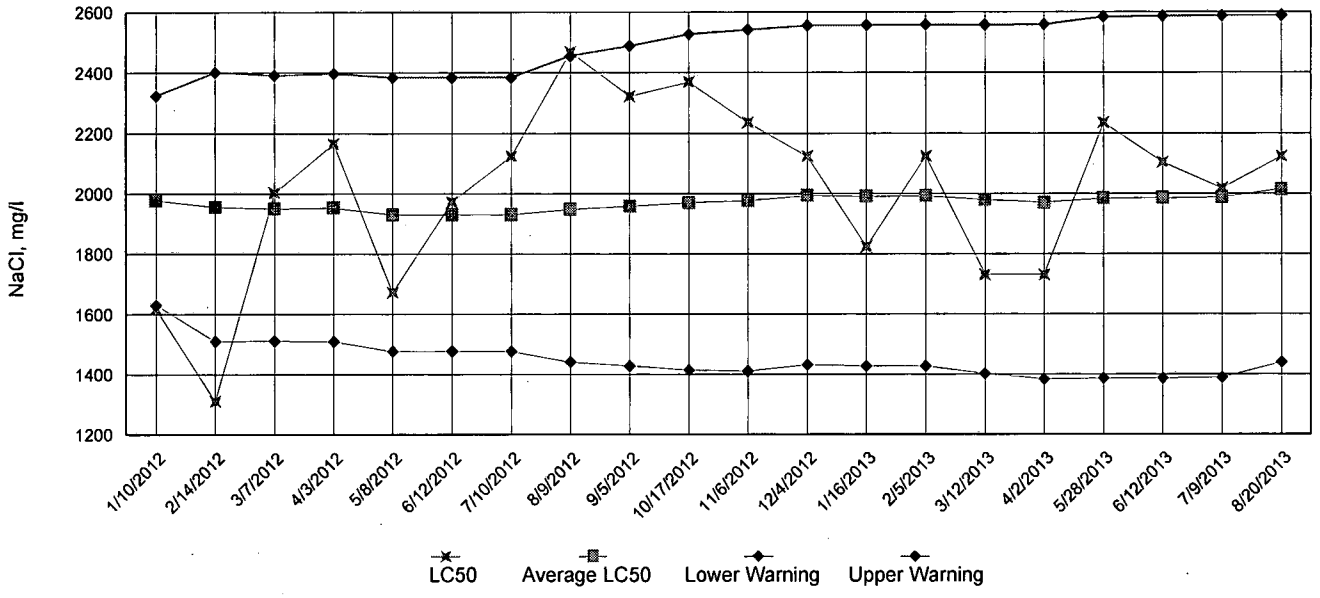
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.4	8.2	8.4	8.0	8.2	8.3
	Final	7.0	8.3	8.4	8.7	8.3	8.3	7.7
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.6
	Final	8.2	8.1	8.1	8.2	8.0	8.0	7.7
Alkalinity, mg CaCO3/l		48	NA	46	NA	47	NA	NA
Hardness, mg CaCO3/l		68	NA	68	NA	68	NA	NA
Conductivity, umhos/cm		310	310	300	340	340	340	350
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.3	7.2	8.2	8.3	8.3	8.0	7.9
	Final	7.1	8.3	8.6	8.8	7.3	8.3	8.0
pH, units	Initial	7.5	7.4	7.8	7.5	7.6	7.5	7.6
	Final	8.2	8.3	8.3	8.3	7.5	8.1	7.9

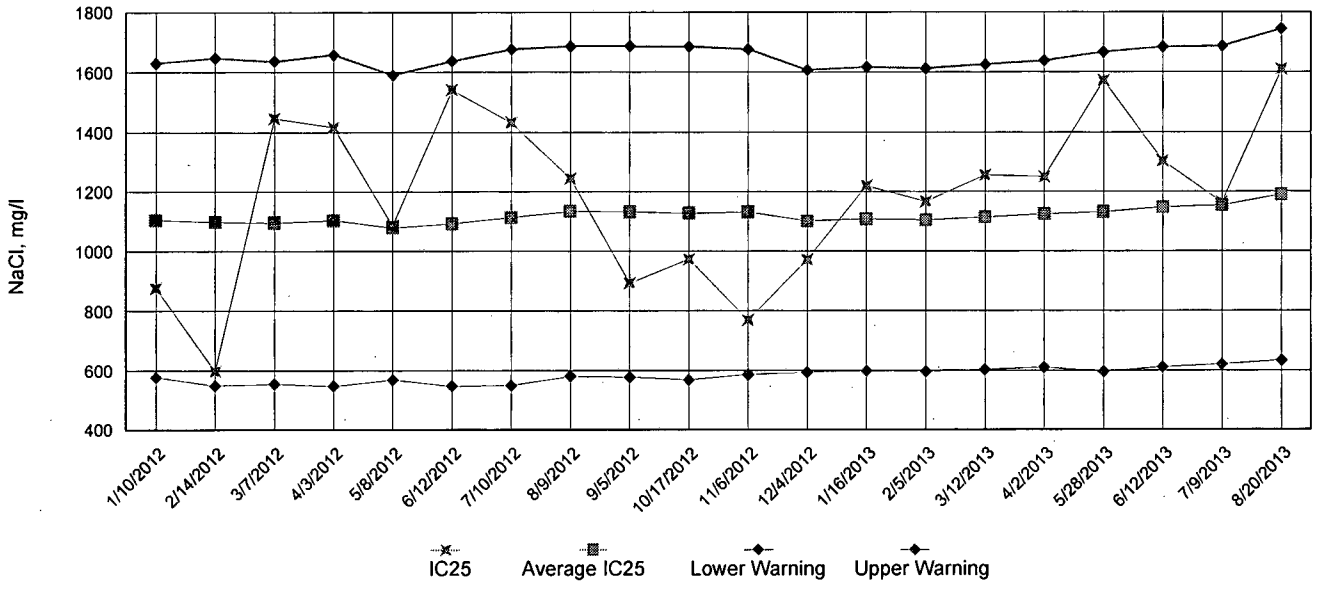
Appendix A4: Test 1002.0

Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

Permittee: City of Hot Springs

NPDES No.: AR0033880 AFIN#26-00145

Date and Time Test Initiated: September 9, 2013 at 1235
Date and Time Test Terminated: September 17, 2013 at 1255
Dilution water used: Synthetic Soft Water #4019

PERCENT SURVIVAL

		Percent Effluent				
Time of Reading	Control	27 %	37 %	49 %	65 %	87 %
24 hour	100	70.0	30.0	30.0	20.0	40.0
48 hour	100	70.0	30.0	30.0	20.0	30.0
7 day	100	0.00	20.0	30.0	10.0	20.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

		Percent Effluent				
Replicates	Control	27 %	37 %	49 %	65 %	87 %
A	22	0	0	0	0	0
B	16	0	0	0	0	0
C	22	0	0	10	15	16
D	24	0	0	0	0	0
E	23	0	0	0	0	8
F	12	0	0	0	0	0
G	16	0	19	13	0	0
H	16	0	0	0	0	0
I	19	0	0	0	0	0
J	18	0	7	6	0	4
Mean per Adult	18.8	0.00	2.60	2.90	1.50	2.80
Mean per Surviving Adult	18.8	0.00	13.0	9.67	15.0	10.0
CV %	20.6	0.00	65.3	36.3	0.00	84.9

CV = Coefficient of variation = standard deviation * 100 / mean
(calculated based on young produced by surviving females)

Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

1. Fisher's Exact Test:

   Is the mean survival significantly different (p=0.05) than the control survival for the % effluent corresponding to (lethality):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	X YES	____ NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____ YES	____ NO

2.

   Is the mean number of young produced per female significantly different (p=0.05) than the control's number of young per female for the % effluent corresponding to (significant non-lethal effects):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	X YES	____ NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____ YES	____ NO

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 1 (TLP3B)
4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 1 (TGP3B)
5. NOEC Ceriodaphnia Lethality: <27 % (TOP3B)
6. LOEC Ceriodaphnia Lethality: 27 % (TXP3B)
7. NOEC Ceriodaphnia Sublethality: <27 % (TPP3B)
8. LOEC Ceriodaphnia Sublethality: 27 % (TYP3B)
9. Coefficient of variation for Ceriodaphnia Reproduction: 20.6 (TQP3B)

Appendix B: Test 1002.0

CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
CHEMICAL PARAMETERS CHART

PERMITTEE: City of Hot Springs
NPDES NO.: AR0033880 AFIN#26-00145
CONTACT: Mr. James Sorrells
ANALYST: 280, 298, 304, 307

2400
2400
2400

Test Initiated: DATE: September 9, 2013 TIME: 1235
Test Terminated: DATE: September 17, 2013 TIME: 1255

DILUTION	DAY						
Control	1	2	3	4	5	6	7
D.O. Initial	8.6	7.4	8.2	8.3	8.2	8.3	8.2
Final	7.8	8.3	8.5	8.7	8.4	8.2	7.8
pH Initial	7.6	7.6	7.8	7.6	7.6	7.7	7.5
Final	7.8	7.9	7.9	7.6	7.9	7.8	8.0
Alkalinity	32	NA	32	NA	32	NA	NA
Hardness	46	NA	42	NA	42	NA	NA
Conductivity	160	170	160	180	180	180	170
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
27 %	1	2	3	4	5	6	7
D.O. Initial	8.6	7.5	8.2	8.4	8.3	8.1	8.3
Final	6.0	8.3	8.7	8.8	8.5	8.3	8.1
pH Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
Final	8.0	8.1	8.2	8.0	8.3	8.0	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	220	220	220	240	250	250	250
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
37 %	1	2	3	4	5	6	7
D.O. Initial	8.6	7.4	8.2	8.4	8.1	8.0	8.1
Final	6.4	8.3	8.6	8.8	8.6	8.2	8.2
pH Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
Final	8.1	8.2	8.2	8.2	8.2	7.9	7.8
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	240	240	240	260	270	270	280
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
49 %	1	2	3	4	5	6	7
D.O. Initial	8.5	7.5	8.2	8.4	8.4	8.1	8.3
Final	6.8	8.3	7.8	9.0	8.4	8.3	8.0
pH Initial	7.6	7.5	7.8	7.5	7.6	7.5	7.5
Final	8.2	8.2	8.2	8.1	8.1	8.0	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	270	270	270	300	300	300	300
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
65 %	1	2	3	4	5	6	7
D.O. Initial	7.7	7.4	8.2	8.4	8.0	8.2	8.3
Final	7.0	8.3	8.4	8.7	8.3	8.3	7.7
pH Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.6
Final	8.2	8.1	8.1	8.2	8.0	8.0	7.7
Alkalinity	48	NA	46	NA	47	NA	NA
Hardness	68	NA	68	NA	68	NA	NA
Conductivity	310	310	300	340	340	340	350
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
87 %	1	2	3	4	5	6	7
D.O. Initial	8.3	7.2	8.2	8.3	8.3	8.0	7.9
Final	7.1	8.3	8.6	8.8	7.3	8.3	8.0
pH Initial	7.5	7.4	7.8	7.5	7.6	7.5	7.6
Final	8.2	8.3	8.3	8.3	7.5	8.1	7.9
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	350	360	350	400	440	430	440
Chlorine	NA	NA	NA	NA	NA	NA	NA

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Roys

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Chronic C.D.	Analyses Requested
1	Plant Effluent	9-8-13 2000-2400		X	X		3	X	No Fathead Analysis
		Container Type						P	
		Preservative						NO	

AIC Control No: 170390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2.1°C
Remarks:
Field pH calibration on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-8-13 1030	M. Mann	9-9-13 10:30 am
M. Mann	9-9-13 11:45	[signature]	9-9-13 1145

Comments:

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC Control No: 170390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 3°C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH	Remarks
1	Plant Effluent	9-10-13 0000-2400		X	X		3	X	
		Container Type						P	Field pH calibration
		Preservative						NO	on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-11-13 1110	M. Mann	9-11-13 1110
M. Mann	9-11-13 @ 12:05	[signature]	9-11-13 1205

Comments:

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs

Project Reference: Plant Effluent

Project Manager: James Sorrells

Sampled By: Harold Mauldin

PO No.: 13-3032

AIC Control No: 170390

AIC Proposal No:

Carrier: Hot Springs Courier

Received Temperature °C: 2

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL		No of BOTTLES	Chronic.CD.	Remarks
3	Plant Effluent	9/12/13 0000-2400		X	X			3	x	
		Container Type							P	
		Preservative							NO	

Field pH calibration on ______ @ ______ Buffer:

G = Glass, P = Plastic, V = VOA vials, H = HCl to pH2, T = Sodium Thiosulfate

NO = none, S = Sulfuric acid pH2, N = Nitric acid pH2, B = NaOH to pH12, Z= Zinc acetate, A = $(NH4)_2SO4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: ______
Who should AIC contact with questions: ______
Phone: ______ Fax: ______
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By	Date/Time	Received By	Date/Time
A. Thomason	9-13-13 @ 1015	M. Mann	9-13-13 @ 10:15
M. Mann	9-13-13 @ 11:20	Received in Lab By: [signature]	9-13-13 1120

Comments:

4-85

AMERICAN INTERPLEX CORPORATION
LABORATORIES

September 30, 2013

Test Results of
Acute 48 hour Non-Renewal
Biomonitoring Testing
for

170741-1: Outfall 001 First Renewal
170741-2: Outfall 001 Second Renewal
170741-3: Outfall 001 Third Renewal
170741-4: Outfall 001 Original Screen

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Acute 48 hour Non-Renewal Biomonitoring utilizing *Ceriodaphnia dubia*
Outfall 001 First Renewal - City of Hot Springs
Client NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

Please find attached the results of the acute biomonitoring screens conducted as a follow up to the chronic toxicity event previously reported (AIC Control No. 170390). As indicated on the enclosed results, the sample was still acutely toxic on September 12, 2013 and exhibited no acute toxicity on September 19, 2013.

It it theorized the decrease in toxicity is directly related to the holding time which may have allowed microbial degradation, loss of volatile toxics, and/or biochemical degradation.

If I can be of further assistance, please feel free to contact me.

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Dilution Water Samples: Synthetic Soft Water #4021

Analysis	Result
Dissolved oxygen (mg/l)	7.6
pH (standard units)	7.7
Alkalinity (mg/l as CaCO3)	31
Hardness (mg/l as CaCO3)	46
Conductivity (umhos/cm)	170
Residual Chlorine (mg/l)	<0.05

Results Summary: Outfall 001 First Renewal

Ceriodaphnia dubia

The *Ceriodaphnia dubia* test was conducted from September 19, 2013 at 1615 to September 21, 2013 at 1700.

Statistical analyses:

NOEC = 100%

Concentration	24 hour % Survival	48 hour % Survival
Control	100	100
100%	100	100

Ceriodaphnia dubia
Survival Data

Number of organisms per chamber: 5
Volume of test chamber: 30 ml

Age of organisms: <24 hours
Volume of test solution: 15 ml

Effluent Concentration	Number of Survivors 24 Hours	48 Hours	% Survival	CV %
Control rep. A	5	5	100	0.00
100% rep. A	5	5	100	0.00

CV = Coefficient of variance = standard deviation X 100/mean

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	5	0	5
100%	5	0	5
Total	10	0	10

Critical Fisher's value (5,5,5) (alpha=0.05) is 1. b value is 5. Since b is greater than 1 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test

Group	Identification	Exposed	Dead	Sig 0.05
0	0	5	0	
1	100	5	0	

Chemical Data for
Ceriodaphnia dubia

Day 1	Control	100%
DO, mg/l	7.6	8.8
pH, su	7.7	7.3
Alkalinity, mg/l	31	NA
Hardness, mg/l	46	NA
Conductivity, umho/cm	170	380
Residual Chlorine, mg/l	<0.05	NA

Day 2		Control	100%
DO, mg/l	Final	7.5	8.7
pH, su	Final	8.0	7.4

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

170744 WR 9/19/13

Client: City of Hot Springs
Project:
Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Rojs

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Chronic CD.	Analyses Requested
1	Plant Effluent	9-8-13 0000-2400		X	X		3	X	
		Container Type						P	
		Preservative						NO	

No Fathead Analysis

AIC Control No: 170390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2.1°C
Remarks:
Field pH calibration on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ___ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-9-13 1030	M. Mann	9-9-13 10:30 AM
M. Mann	9-9-13 11:45	[signature]	9-9-13 1145

Comments:

City of Hot Springs
Waste Water Treatment Plant
320 Davidson Drive
Hot Springs, AR 71901

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NPDES Enforcement Section
Attn: Mo Shafii
5301 Northshore Dr.
North Little Rock, AR 72118-5317

AMERICAN INTERPLEX CORPORATION
LABORATORIES

August 30, 2013

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Outfall 001
City of Hot Springs

Control No. 169846-2

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Ceriodaphnia dubia*
Outfall 001 - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Chronic *Ceriodaphnia dubia* test: Due to laboratory error, the *Ceriodaphnia dubia* test was not renewed with the third sample. The test should be repeated. The data from the test is enclosed for your review.

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Table of Contents

I. Control Acceptance Criteria

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	23.6	PASS
Control CV < or = 40% per Surviving Female	13.6	PASS
Reproduction Minimum Significant Difference 13 to 47%	25.8	PASS
Critical Dilution CV < or = 40%	22.9	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly
   Test Method 1002.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point: Outfall 001
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.9	8.5	NA
pH (standard units)	7.0	7.4	NA
Alkalinity (mg/l as CaCO3)	26	34	NA
Hardness (mg/l as CaCO3)	67	71	NA
Conductivity (umhos/cm)	350	390	NA
Residual Chlorine (mg/l)	<0.05	<0.05	NA
Ammonia as N (mg/l)	0.12	0.30	NA

2. Dilution Water Samples: Synthetic Soft Water #4012
   a. Dates Prepared: August 8 through August 22, 2013
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.7	7.7	7.8
pH (standard units)	7.7	7.8	7.9
Alkalinity (mg/l as CaCO3)	32	32	NA
Hardness (mg/l as CaCO3)	47	42	NA
Conductivity (umhos/cm)	180	170	170
Residual Chlorine (mg/l)	<0.05	<0.05	NA

C. Test Methods
1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Method 1002.0, *Ceriodaphnia dubia* Survival and Reproduction.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Ceriodaphnia dubia Survival and Growth Method 1002.0

Date & Time Test Initiated:	August 22, 2013 at 1605
Date & Time Test Terminated:	August 29, 2013 at 1415
Type & Volume of Test Chamber:	30 ml disposable beaker
Volume of Sample:	15 ml
Number of Organisms per replicate:	1
Number of Replicates per dilution:	10

4. Acclimation of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms
1. Scientific Name
a. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis

The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was analyzed using Kolmogorov's Test for Normality and Bartlett's test and analyzed with Dunnett's Test to determine the No Observable Effects Concentration (NOEC) for Reproduction.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Ceriodaphnia dubia

Chronic reference tests are performed monthly.
A chronic reference test was performed on August 20, 2013 at 1505 to August 28, 2013 at 1450
The results were as follows: (Control No. 169867-2.)
Survival LC-50: 2125 mg/l
Growth IC-25: 1610 mg/l
Growth PMSD: 18.3

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	102	1.91
pH	SM 4500-H+ B	101	0.268
Conductivity	EPA 120.1	101	4.71

VI. Organism History

Ceriodaphnia dubia

Date: August 22, 2013
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

VII. Results Summary *Ceriodaphnia dubia*, Cladoceran Survival and Reproduction Test -- Method 1002.0

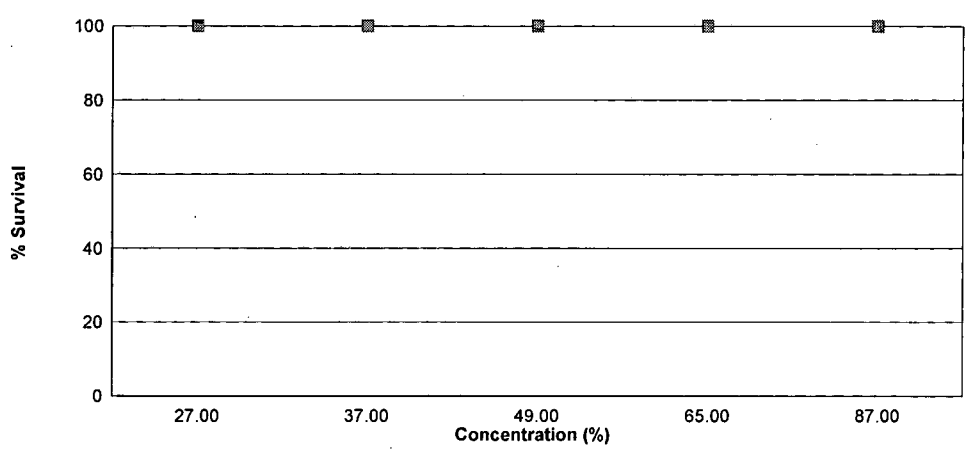
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on August 22, 2013 at 1605 and continued through August 29, 2013 at 1415. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 87 % effluent
b.) NOEC reproduction = 87 % effluent



Summary of the 7-day *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	23.6
27 %	100	24.2
37 %	100	27.6
49 %	100	26.4
65 %	100	24.2
87 %	100	25.0

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 22, 2013 at 1605
Date and Time Test Terminated: August 29, 2013 at 1415

Concentration: Control													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	4	4	2	3	4	4	3	2	33	10	3.30
5	0	0	0	0	0	1	0	0	0	6	7	10	0.700
6	9	9	7	8	7	8	7	9	8	8	80	10	8.00
7	14	14	11	13	14	11	12	14	13	0	116	10	11.6
8													
TOTAL	27	26	22	25	23	23	23	27	24	16	236	10	23.6

Concentration: 27 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	2	3	3	3	2	3	2	3	2	27	10	2.70
5	8	7	1	0	0	0	0	0	0	6	22	10	2.20
6	1	12	13	11	10	10	11	8	7	6	89	10	8.90
7	16	0	17	18	6	0	17	17	13	0	104	10	10.4
8													
TOTAL	29	21	34	32	19	12	31	27	23	14	242	10	24.2

Concentration: 37 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	3	3	3	2	3	2	2	2	27	10	2.70
5	0	0	0	0	0	1	0	0	6	6	13	10	1.30
6	12	12	12	11	10	6	8	10	13	10	104	10	10.4
7	18	16	17	18	16	15	15	17	0	0	132	10	13.2
8													
TOTAL	34	31	32	32	29	24	26	29	21	18	276	10	27.6

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 22, 2013 at 1605
Date and Time Test Terminated: August 29, 2013 at 1415

Concentration: 49 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	2	5	2	3	4	2	2	2	29	10	2.90
5	0	5	0	0	0	1	0	6	4	3	19	10	1.90
6	10	0	11	11	11	8	10	0	12	10	83	10	8.30
7	17	18	16	18	16	17	15	16	0	0	133	10	13.3
8													
TOTAL	31	26	29	34	29	29	29	24	18	15	264	10	26.4

Concentration: 65 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	2	3	5	3	3	3	2	3	2	2	28	10	2.80
5	6	0	0	0	0	1	0	0	5	4	16	10	1.60
6	13	11	11	11	10	10	8	10	0	12	96	10	9.60
7	0	15	14	16	12	0	15	16	14	0	102	10	10.2
8													
TOTAL	21	29	30	30	25	14	25	29	21	18	242	10	24.2

Concentration: 87 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	4	3	3	3	3	4	3	2	2	31	10	3.10
5	8	6	1	0	0	0	0	0	5	5	25	10	2.50
6	0	2	11	12	6	10	8	8	0	7	64	10	6.40
7	16	15	16	18	16	0	18	17	14	0	130	10	13.0
8													
TOTAL	28	27	31	33	25	13	30	28	21	14	250	10	25.0

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	10	0	10
27 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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	Alive	Dead	Total Animals
Control	10	0	10
37 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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	Alive	Dead	Total Animals
Control	10	0	10
49 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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	Alive	Dead	Total Animals
Control	10	0	10
65 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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.

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
87 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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 Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	27 %	10	0	
2	37 %	10	0	
3	49 %	10	0	
4	65 %	10	0	
5	87 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality		No Transformation
D = 0.1154 D* = 0.9054 Critical D* = 1.035	(alpha = 0.01, N = 60)	
Data PASS normality test (alpha = 0.01).		

Bartlett's Test for Homogeneity of Variance		No Transformation
Calculated B1 statistic = 6.592 Critical B = 15.086	(alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.		

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	117.9	23.58	0.6766
Within (Error)	54	1882	34.85	
Total	59	2000		

Critical F = 3.38 (alpha = 0.01, df = 5,54)
2.38 (alpha = 0.05, df = 5,54)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Dunnett's Test - Table 1 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	23.6	23.6		
2	27 %	24.2	24.2	-0.2273	
3	37 %	27.6	27.6	-1.515	
4	49 %	26.4	26.4	-1.061	
5	65 %	24.2	24.2	-0.2273	
6	87 %	25	25	-0.5303	

Dunnett's critical value = 2.31 (1 Tailed, alpha = 0.05, df [used] = 5,40) (Actual df = 5,54)

Dunnett's Test - Table 2 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	10			
2	27 %	10	6.099	25.8	-0.6
3	37 %	10	6.099	25.8	-4
4	49 %	10	6.099	25.8	-2.8
5	65 %	10	6.099	25.8	-0.6
6	87 %	10	6.099	25.8	-1.4

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 22, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.5	7.7	7.5	7.8	7.8	8.1
	Final	7.7	8.0	8.7	8.2	8.2	7.8	7.8
pH, units	Initial	7.7	7.7	7.8	8.0	7.9	8.0	7.7
	Final	8.0	8.2	8.0	8.0	8.0	7.8	7.8
Alkalinity, mg CaCO3/l		32	NA	32	NA	NA	NA	NA
Hardness, mg CaCO3/l		47	NA	42	NA	NA	NA	NA
Conductivity, umhos/cm		180	180	170	180	170	170	190
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	NA	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.4	8.0	7.5	8.0	8.0	7.5
	Final	7.5	8.2	8.6	8.2	8.2	7.7	7.7
pH, units	Initial	7.6	7.7	7.5	7.8	7.7	7.8	7.5
	Final	8.0	8.1	8.0	8.0	8.1	7.6	7.6

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.6	8.6	7.6	8.2	7.7	7.7
	Final	7.6	8.0	8.6	8.0	8.2	7.6	7.6
pH, units	Initial	7.6	7.7	7.4	7.7	7.6	7.7	7.6
	Final	8.0	8.1	8.0	8.0	8.1	7.6	7.6

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 22, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.5	8.7	7.6	8.1	7.7	7.7
	Final	7.7	8.1	8.6	8.2	8.2	7.6	7.9
pH, units	Initial	7.5	7.7	7.3	7.6	7.6	7.6	7.5
	Final	8.0	8.1	8.0	8.1	8.2	7.5	7.5

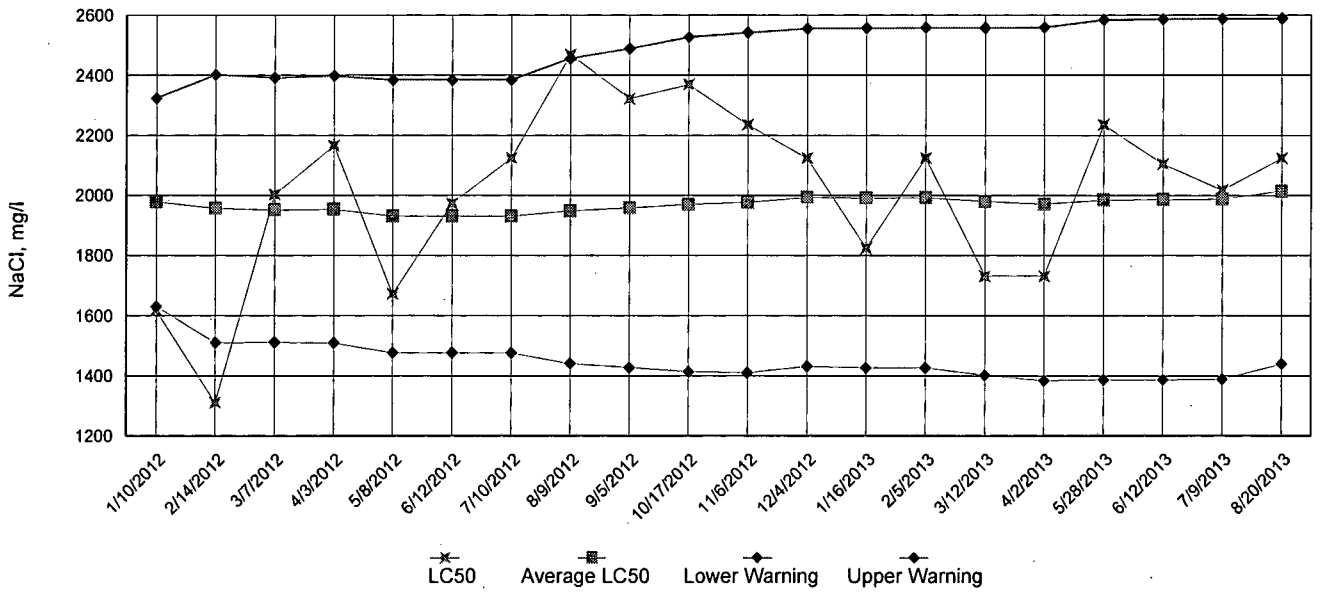
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.9	7.4	8.8	7.6	8.7	7.7	7.3
	Final	7.7	8.0	8.6	8.4	8.1	7.6	7.6
pH, units	Initial	7.5	7.7	7.2	7.5	7.5	7.6	7.5
	Final	8.0	8.0	8.0	8.1	8.1	7.5	7.5
Alkalinity, mg CaCO3/l		36	NA	30	NA	NA	NA	NA
Hardness, mg CaCO3/l		54	NA	58	NA	NA	NA	NA
Conductivity, umhos/cm		250	260	280	290	310	310	330
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	NA	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	7.4	8.9	7.6	8.3	7.9	7.4
	Final	7.4	8.1	8.7	8.2	7.7	5.4	7.2
pH, units	Initial	7.4	7.7	7.1	7.4	7.4	7.6	7.6
	Final	8.0	8.0	7.9	8.1	8.3	7.5	7.4

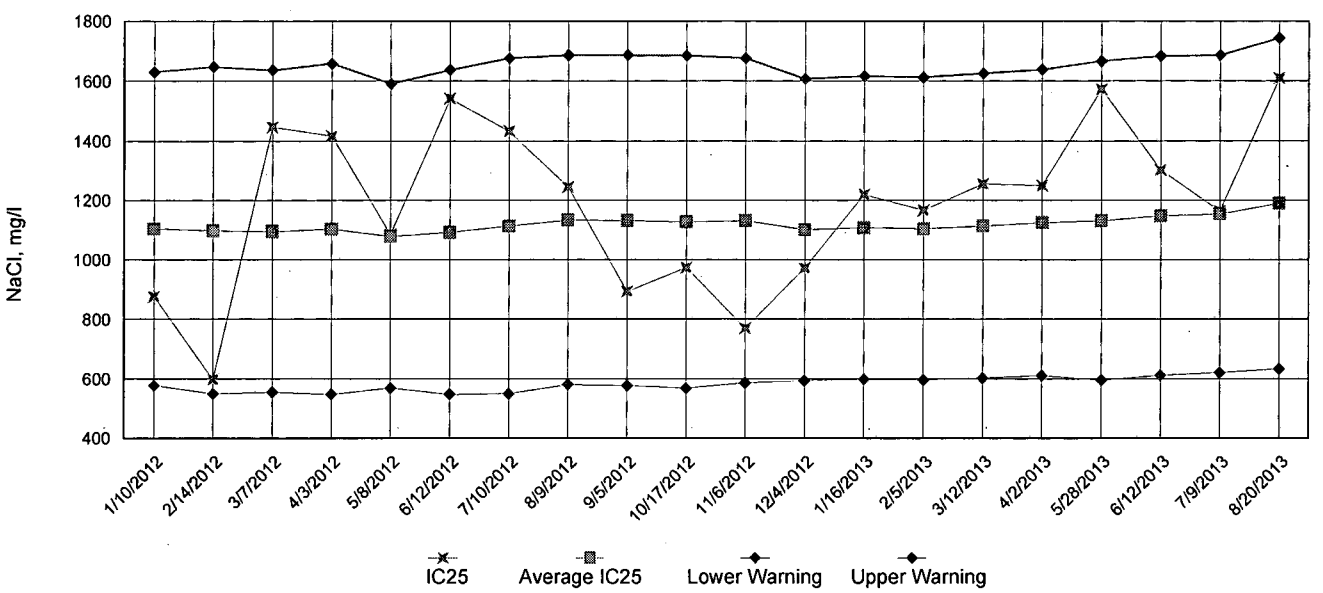
Appendix A4: Test 1002.0

Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL		No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
1	Plant Effluent	8-18-13 0900 - 2400		X	X			3	X
		Container Type							P
		Preservative							NO

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 20c
Remarks
Field pH calibration on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ___ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
A. Thomason	8-19-13 @ 1010	M. Mann	8-19-13 @ 10:10am
Relinquished By: M. Mann	Date/Time 8-19-13 @ 11:10AM	Received In Lab By: Angie Hopton	Date/Time 8-19-13 1110

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL		No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
2	Plant Effluent	8-20-13 0900-2400		X	X			3	X
		Container Type							P
		Preservative							NO

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 23C
Remarks

Field pH calibration
on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
A. Ross	8-21-13 0900	G. Mann	8-21-13 900
G. Mann	8-21-13 1130	Jimmy Day	8/21/13 1130

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By:

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
3	Plant Effluent	8/22/13 0000-2400		X			3	X
		Container Type						P
		Preservative						NO

AIC Control No: 16984b
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 2
Remarks
Field pH calibration on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	8-23-13 0955	[signature]	8-23-13 9:55
[signature]	8-23-13 11:20	[signature]	8-23-13 1120

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

September 30, 2013

Test Results of
Acute 48 hour Non-Renewal
Biomonitoring Testing
for

170741-1: Outfall 001 First Renewal
170741-2: Outfall 001 Second Renewal
170741-3: Outfall 001 Third Renewal
170741-4: Outfall 001 Original Screen

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Acute 48 hour Non-Renewal Biomonitoring utilizing *Ceriodaphnia dubia*
Outfall 001 First Renewal - City of Hot Springs
Client NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

Please find attached the results of the acute biomonitoring screens conducted as a follow up to the chronic toxicity event previously reported (AIC Control No. 170390). As indicated on the enclosed results, the sample was still acutely toxic on September 12, 2013 and exhibited no acute toxicity on September 19, 2013.

It it theorized the decrease in toxicity is directly related to the holding time which may have allowed microbial degradation, loss of volatile toxics, and/or biochemical degradation.

If I can be of further assistance, please feel free to contact me.

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Dilution Water Samples: Synthetic Soft Water #4021

Analysis	Result
Dissolved oxygen (mg/l)	7.6
pH (standard units)	7.7
Alkalinity (mg/l as CaCO3)	31
Hardness (mg/l as CaCO3)	46
Conductivity (umhos/cm)	170
Residual Chlorine (mg/l)	<0.05

Results Summary: Outfall 001 First Renewal

Ceriodaphnia dubia

The *Ceriodaphnia dubia* test was conducted from September 19, 2013 at 1615 to September 21, 2013 at 1700.

Statistical analyses:

NOEC = 100%

Concentration	24 hour % Survival	48 hour % Survival
Control	100	100
100%	100	100

Ceriodaphnia dubia
Survival Data

Number of organisms per chamber: 5
Volume of test chamber: 30 ml

Age of organisms: <24 hours
Volume of test solution: 15 ml

Effluent Concentration	Number of Survivors 24 Hours	48 Hours	% Survival	CV %
Control rep. A	5	5	100	0.00
100% rep. A	5	5	100	0.00

CV = Coefficient of variance = standard deviation X 100/mean

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	5	0	5
100%	5	0	5
Total	10	0	10

Critical Fisher's value (5,5,5) (alpha=0.05) is 1. b value is 5. Since b is greater than 1 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test

Group	Identification	Exposed	Dead	Sig 0.05
0	0	5	0	
1	100	5	0	

Chemical Data for
Ceriodaphnia dubia

Day 1	Control	100%
DO, mg/l	7.6	8.8
pH, su	7.7	7.3
Alkalinity, mg/l	31	NA
Hardness, mg/l	46	NA
Conductivity, umho/cm	170	380
Residual Chlorine, mg/l	<0.05	NA

Day 2		Control	100%
DO, mg/l	Final	7.5	8.7
pH, su	Final	8.0	7.4

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

month WR 9/19/13

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Chronic C.D.	Analyses Requested
1	Plant Effluent	9-8-13 0000-2400		X	X		3	X	
		Container Type						P	
		Preservative						NO	

No FATHEAD ANALYSIS

AIC Control No: 120390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2.1 °C
Remarks:
Field pH calibration on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z = Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-8-13 1030	M. Mann	9-9-13 10:30 AM
M. Mann	9-9-13 11:45	[signature]	9-9-13 1145

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

October 1, 2013
Control No. 171032

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

This report contains the analytical results and supporting information for samples submitted on September 27, 2013. Attached please find a copy of the Chain of Custody and/or other documents received. Note that any remaining sample will be discarded two weeks from the original report date unless other arrangements are made.

This report is intended for the sole use of the client listed above. Assessment of the data requires access to the entire document.

This report has been reviewed by the Laboratory Director or a qualified designee.

John Overbey
Laboratory Director

This document has been distributed to the following:

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

SAMPLE INFORMATION

Project Description:
Three (3) water sample(s) (AIC COntrol No. 170741-1,2,3) resubmitted September 27, 2013
P.O. No. 13-3032

Receipt Details:
A Chain of Custody was not provided with the sample(s).

Each sample container was checked for proper labeling, including date and time sampled. Sample containers were reviewed for proper type, adequate volume, integrity, temperature, preservation, and holding times. Any exceptions are noted below:

Sample Identification:

Laboratory ID	Client Sample ID	Sampled Date/Time	Notes
171032-1	Plant Effluent 9-8-13 0000-2400	08-Sep-2013 2359	
171032-2	Plant Effluent 9-10-13 0000-2400	10-Sep-2013 2359	
171032-3	Plant Effluent 9-12-13 0000-2400	12-Sep-2013 2359	

Case Narrative:
There were no qualifiers for this data and all samples met quality control criteria.

References:
"Methods for Chemical Analysis of Water and Wastes", EPA/600/4-79-020 (Mar 1983) with updates and supplements EPA/600/5-91-010 (Jun 1991), EPA/600/R-92-129 (Aug 1992) and EPA/600/R-93-100 (Aug 1993).
"Test Methods for Evaluating Solid Waste Physical/Chemical Methods (SW846)", Third Edition.
"Standard Methods for the Examination of Water and Wastewaters", 21st edition.
"American Society for Testing and Materials" (ASTM).
"Association of Analytical Chemists" (AOAC).

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

ANALYTICAL RESULTS

AIC No. 171032-1
Sample Identification: Plant Effluent 9-8-13 0000-2400

Analyte		Result	RL	Units	Qualifier
Total Organic Carbon		**6.1**	1	**mg/l**	
SM 5310 C	Prep: 27-Sep-2013 1516 by 308	Analyzed: 27-Sep-2013 1814 by 308		Batch: W45076	
Aluminum		**< 0.04**	0.04	**mg/l**	
EPA 200.7	Prep: 30-Sep-2013 0901 by 271	Analyzed: 01-Oct-2013 1242 by 305		Batch: S35491	
Sulfate		**29**	0.2	**mg/l**	
EPA 300.0	Prep: 27-Sep-2013 1628 by 07	Analyzed: 27-Sep-2013 2205 by 07		Batch: C16076	

AIC No. 171032-2
Sample Identification: Plant Effluent 9-10-13 0000-2400

Analyte		Result	RL	Units	Qualifier
Total Organic Carbon		**5.9**	1	**mg/l**	
SM 5310 C	Prep: 27-Sep-2013 1516 by 308	Analyzed: 27-Sep-2013 1828 by 308		Batch: W45076	
Aluminum		**0.041**	0.04	**mg/l**	
EPA 200.7	Prep: 30-Sep-2013 0901 by 271	Analyzed: 01-Oct-2013 1245 by 305		Batch: S35491	
Sulfate		**39**	0.2	**mg/l**	
EPA 300.0	Prep: 27-Sep-2013 1628 by 07	Analyzed: 27-Sep-2013 2232 by 07		Batch: C16076	

AIC No. 171032-3
Sample Identification: Plant Effluent 9-12-13 0000-2400

Analyte		Result	RL	Units	Qualifier
Total Organic Carbon		**5.4**	1	**mg/l**	
SM 5310 C	Prep: 27-Sep-2013 1516 by 308	Analyzed: 27-Sep-2013 1843 by 308		Batch: W45076	
Aluminum		**< 0.04**	0.04	**mg/l**	
EPA 200.7	Prep: 30-Sep-2013 0901 by 271	Analyzed: 01-Oct-2013 1248 by 305		Batch: S35491	
Sulfate		**34**	0.2	**mg/l**	
EPA 300.0	Prep: 27-Sep-2013 1628 by 07	Analyzed: 27-Sep-2013 2259 by 07		Batch: C16076	

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

LABORATORY CONTROL SAMPLE RESULTS

Analyte	Spike Amount	%	Limits	RPD	Limit	Batch	Preparation Date	Analysis Date	Dil	Qual
Total Organic Carbon	10 mg/l	96.4	80.0-120			W45076	27Sep13 1459 by 308	27Sep13 1648 by 308		
Aluminum	5 mg/l	97.6	85.0-115			S35491	30Sep13 0901 by 271	01Oct13 1152 by 305		
Sulfate	20 mg/l	108	90.0-110			C16076	27Sep13 1628 by 07	27Sep13 1710 by 07		

MATRIX SPIKE SAMPLE RESULTS

Analyte	Sample	Spike Amount	%	Limits	Batch	Preparation Date	Analysis Date	Dil	Qual
Total Organic Carbon	171006-1	10 mg/l	100	80.0-120	W45076	27Sep13 1459 by 308	27Sep13 1717 by 308		
	171006-1	10 mg/l	97.9	80.0-120	W45076	27Sep13 1459 by 308	27Sep13 1731 by 308		
	Relative Percent Difference:		1.86	25.0	W45076				
Aluminum	171022-2	5 mg/l	96.7	75.0-125	S35491	30Sep13 0901 by 271	01Oct13 1155 by 305		
	171022-2	5 mg/l	96.6	75.0-125	S35491	30Sep13 0901 by 271	01Oct13 1158 by 305		
	Relative Percent Difference:		0.142	20.0	S35491				
Sulfate	171021-1	20 mg/l	89.1	80.0-120	C16076	27Sep13 1628 by 07	27Sep13 1737 by 07		
	171021-1	20 mg/l	93.6	80.0-120	C16076	27Sep13 1628 by 07	27Sep13 1804 by 07		
	Relative Percent Difference:		4.28	10.0	C16076				

LABORATORY BLANK RESULTS

Analyte	Result	RL	PQL	QC Sample	Preparation Date	Analysis Date	Qual
Total Organic Carbon	< 1 mg/l	1	1	W45076-1	27Sep13 1459 by 308	27Sep13 1633 by 308	
Aluminum	< 0.04 mg/l	0.04	0.04	S35491-1	30Sep13 0901 by 271	01Oct13 1149 by 305	
Sulfate	< 0.2 mg/l	0.2	0.2	C16076-1	27Sep13 1628 by 07	27Sep13 1644 by 07	

AMERICAN INTERPLEX CORPORATION LABORATORIES

171032 wn 9/13/13
~~170741~~ wn 9/16/13

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross
PO No.: 13-3032
AIC Control No: ~~170390~~
AIC Proposal No:
Carrier: Hot Springs Couriers
Received Temperature °C: 2.1 °C

Analyses Requested: No Fathead Analysis

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL	No of BOTTLES	Chronic CD.	Remarks
1	Plant Effluent	9-8-13 0000-2400		X	X		3	X	
		Container Type					P		
		Preservative					NO		

Field pH calibration on ______ @ ______ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: ______
Who should AIC contact with questions: ______
Phone: ______ Fax: ______
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By	Date/Time	Received By	Date/Time
[signature]	9-8-13 1030	M. Mann	9-9-13 10:30 am
M. Mann	9-9-13 11:45	Received in Lab By: [signature]	9-9-13 1145

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

August 30, 2013

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Outfall 001
City of Hot Springs

Control No. 169846-1

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Pimephales promelas* (Fathead minnow)
Outfall 001 - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Method 1000.0 Chronic *Pimephales promelas* (Fathead minnow) Survival and Growth Test: The No Observable Effects Concentration (NOEC) for survival occurred at 87 % effluent, which is above the critical dilution of 65 %. The NOEC for growth occurred at 87 % effluent, which is above the critical dilution of 65 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the Fathead minnow test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Table of Contents

I. Control Acceptance Criteria

Pimephales promelas (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.296	PASS
Control Growth CV < or = 40%	7.47	PASS
Growth Minimum Significant Difference 12 to 30%	20.0	PASS
Critical Dilution CV < or = 40%	11.6	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly Test Method 1000.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point: Outfall 001
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.2	8.9	8.5
pH (standard units)	7.4	7.0	7.4
Alkalinity (mg/l as CaCO3)	41	26	34
Hardness (mg/l as CaCO3)	59	67	71
Conductivity (umhos/cm)	290	350	390
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.28	0.12	0.30

2. Dilution Water Samples: Synthetic Soft Water #4012
   a. Dates Prepared: August 8 through August 22, 2013
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.7	7.7	7.8
pH (standard units)	7.7	7.8	7.9
Alkalinity (mg/l as CaCO3)	32	32	32
Hardness (mg/l as CaCO3)	47	42	42
Conductivity (umhos/cm)	180	170	170
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05

C. Test Methods
1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Method 1000.0, Fathead Minnow Survival and Growth.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Pimephales promelas (Fathead minnow) Survival and Growth Method 1000.0

Date & Time Test Initiated:	August 20, 2013 at 1200
Date & Time Test Terminated:	August 27, 2013 at 1010
Type & Volume of Test Chamber:	500 ml disposable beaker
Volume of Sample:	250 ml
Number of Organisms per replicate:	8
Number of Replicates per dilution:	5

4. Acclimation of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms
1. Scientific Name
a. Test 1000.0 *Pimephales promelas*

III. Data Analysis

The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Pimephales promelas (Fathead minnow) survival data was transformed using the Arc Sine transformation. Normality and homogeneity of variance were checked using Shapiro-Wilk's. The survival data was then analyzed using Steel's Many-One Rank Test to determine the No Observable Effects Concentration (NOEC).

Fathead minnow growth data was analyzed for normality and homogeneity of variance using Shapiro-Wilk's and Bartlett's test. Dunnett's Test was used to determine the No Observable Effects Concentration (NOEC) for growth.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Pimephales promelas (Fathead minnow)

Chronic reference tests are performed monthly.
A chronic reference test was performed on August 20, 2013 at 1715 to August 27, 2013 at 1520
The results were as follows: (Control No. 169867-1.)
Survival LC-50: 5670.1 mg/l
Growth IC-25: 3143 mg/l
Growth PMSD: 17.3

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	102	1.91
pH	SM 4500-H+ B	101	0.268
Conductivity	EPA 120.1	101	4.71

VI. Organism History

Pimephales promelas (Fathead minnow)
Date: August 20, 2013
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

VII. Results Summary *Pimephales promelas*, Fathead minnow Larval Survival and Growth Test -- Method 1000.0

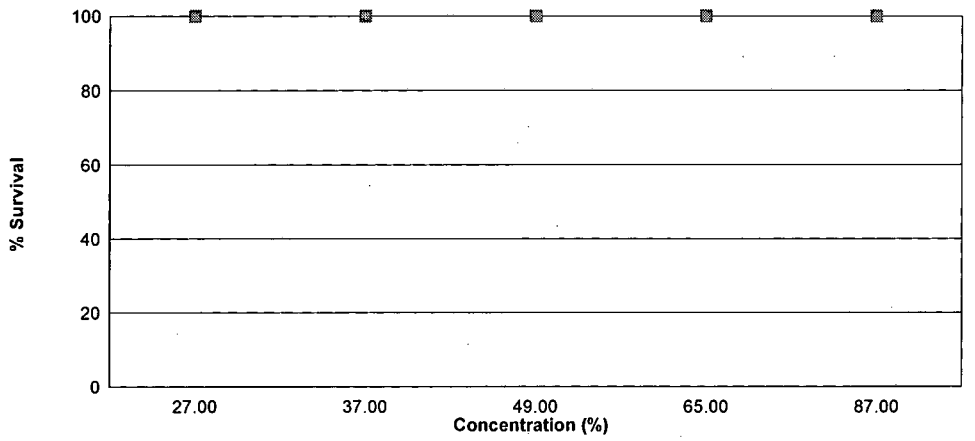
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on August 20, 2013 at 1200 and continued through August 27, 2013 at 1010. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 87 % effluent
b.) NOEC growth = 87 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.296
27 %	100	0.337
37 %	100	0.323
49 %	100	0.302
65 %	100	0.292
87 %	100	0.302

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: August 20, 2013 at 1200
Date and Time Test Terminated: August 27, 2013 at 1010

Concentration	Replicate	Number of Survivors						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
27 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
37 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
49 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
65 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
87 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: August 20, 2013 at 1200
Test Terminated: August 27, 2013 at 1010
Drying Started: August 23, 2013 at 1527
Drying Ended: August 29, 2013 at 1400

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.91827	.92042	0.00215	8	0.269
	B	.91878	.92121	0.00243	8	0.304
	C	.91659	.91922	0.00263	8	0.329
	D	.91638	.91872	0.00234	8	0.292
	E	.91455	.91685	0.00230	8	0.288
27 %	A	.91583	.91811	0.00228	8	0.285
	B	.91943	.92233	0.00290	8	0.362
	C	.92408	.92709	0.00301	8	0.376
	D	.92651	.92924	0.00273	8	0.341
	E	.92767	.93024	0.00257	8	0.321
37 %	A	.92653	.92915	0.00262	8	0.328
	B	.94872	.95160	0.00288	8	0.360
	C	.94724	.94985	0.00261	8	0.326
	D	.94532	.94750	0.00218	8	0.272
	E	.94881	.95146	0.00265	8	0.331
49 %	A	.94115	.94330	0.00215	8	0.269
	B	.92530	.92779	0.00249	8	0.311
	C	.92632	.92901	0.00269	8	0.336
	D	.93070	.93312	0.00242	8	0.302
	E	.93427	.93660	0.00233	8	0.291
65 %	A	.93862	.94107	0.00245	8	0.306
	B	.93944	.94155	0.00211	8	0.264
	C	.94132	.94377	0.00245	8	0.306
	D	.93810	.94077	0.00267	8	0.334
	E	.93899	.94100	0.00201	8	0.251
87 %	A	.93682	.93952	0.00270	8	0.338
	B	.93818	.93991	0.00173	8	0.216
	C	.93688	.93881	0.00193	8	0.241
	D	.93804	.94073	0.00269	8	0.336
	E	.95233	.95537	0.00304	8	0.380

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

		Transformation of Data		Transform: Arc Sin(Square Root(Y))
Group	Identification	Rep	Value	Transformed
1	Control	1	1.00000	1.39310
1	Control	2	1.00000	1.39310
1	Control	3	1.00000	1.39310
1	Control	4	1.00000	1.39310
1	Control	5	1.00000	1.39310
2	27 %	1	1.00000	1.39310
2	27 %	2	1.00000	1.39310
2	27 %	3	1.00000	1.39310
2	27 %	4	1.00000	1.39310
2	27 %	5	1.00000	1.39310
3	37 %	1	1.00000	1.39310
3	37 %	2	1.00000	1.39310
3	37 %	3	1.00000	1.39310
3	37 %	4	1.00000	1.39310
3	37 %	5	1.00000	1.39310
4	49 %	1	1.00000	1.39310
4	49 %	2	1.00000	1.39310
4	49 %	3	1.00000	1.39310
4	49 %	4	1.00000	1.39310
4	49 %	5	1.00000	1.39310
5	65 %	1	1.00000	1.39310
5	65 %	2	1.00000	1.39310
5	65 %	3	1.00000	1.39310
5	65 %	4	1.00000	1.39310
5	65 %	5	1.00000	1.39310
6	87 %	1	1.00000	1.39310
6	87 %	2	1.00000	1.39310
6	87 %	3	1.00000	1.39310
6	87 %	4	1.00000	1.39310
6	87 %	5	1.00000	1.39310

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Shapiro - Wilk's Test for Normality		Transform: Arc Sin(Square Root(Y))
D = 0		
W = 0		
Critical W = 0.9	(alpha = 0.01, N = 30)	
Critical W = 0.927	(alpha = 0.05, N = 30)	

Data FAIL normality test (alpha = 0.01).

Steel's Many-One Rank Test — Transform: Arc Sin(Square Root(Y))

Ho:Control<Treatment

Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	27 %	27.50	16.00	5.00	
3	37 %	27.50	16.00	5.00	
4	49 %	27.50	16.00	5.00	
5	65 %	27.50	16.00	5.00	
6	87 %	27.50	16.00	5.00	

Critical values are 1 tailed (k=5)

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality		No Transformation
D = 0.03787		
W = 0.9742		
Critical W = 0.9	(alpha = 0.01, N = 30)	
Critical W = 0.927	(alpha = 0.05, N = 30)	
Data PASS normality test (alpha = 0.01).		

Bartlett's Test for Homogeneity of Variance		No Transformation
Calculated B1 statistic = 7.076		
Critical B = 15.086	(alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.		

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	0.007651	0.00153	0.9696
Within (Error)	24	0.03787	0.001578	
Total	29	0.04552		

Critical F = 3.9 (alpha = 0.01, df = 5,24)
2.62 (alpha = 0.05, df = 5,24)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Dunnett's Test - Table 1 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	0.2964	0.2964		
2	27 %	0.337	0.337	-1.616	
3	37 %	0.3234	0.3234	-1.075	
4	49 %	0.3018	0.3018	-0.2149	
5	65 %	0.2922	0.2922	0.1672	
6	87 %	0.3022	0.3022	-0.2309	

Dunnett's critical value = 2.36 (1 Tailed, alpha = 0.05, df = 5,24)

Dunnett's Test - Table 2 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	5			
2	27 %	5	0.05929	20	-0.0406
3	37 %	5	0.05929	20	-0.027
4	49 %	5	0.05929	20	-0.0054
5	65 %	5	0.05929	20	0.0042
6	87 %	5	0.05929	20	-0.0058

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 20, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.5	7.7	7.5	7.8	7.8	8.1
	Final	7.5	7.2	7.5	7.9	7.7	7.1	7.9
pH, units	Initial	7.7	7.7	7.8	8.0	7.9	8.0	7.7
	Final	7.5	7.3	7.8	8.1	8.1	7.6	7.9
Alkalinity, mg CaCO3/l		32	NA	32	NA	32	NA	NA
Hardness, mg CaCO3/l		47	NA	42	NA	42	NA	NA
Conductivity, umhos/cm		180	180	170	180	170	170	190
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.4	8.0	7.5	8.0	8.0	7.5
	Final	7.5	7.2	7.3	7.9	7.8	8.1	7.7
pH, units	Initial	7.6	7.7	7.5	7.8	7.7	7.8	7.5
	Final	7.5	7.3	7.7	8.1	8.2	7.5	7.7

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.6	8.6	7.6	8.2	7.7	7.7
	Final	7.5	7.0	7.5	8.0	7.9	8.1	7.8
pH, units	Initial	7.6	7.7	7.4	7.7	7.6	7.7	7.6
	Final	7.5	7.3	7.7	8.1	8.2	7.5	7.6

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 20, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.5	8.7	7.6	8.1	7.7	7.7
	Final	7.5	7.4	7.6	8.0	8.1	8.2	7.8
pH, units	Initial	7.5	7.7	7.3	7.6	7.6	7.6	7.5
	Final	7.5	7.4	7.6	8.1	8.2	7.6	7.6

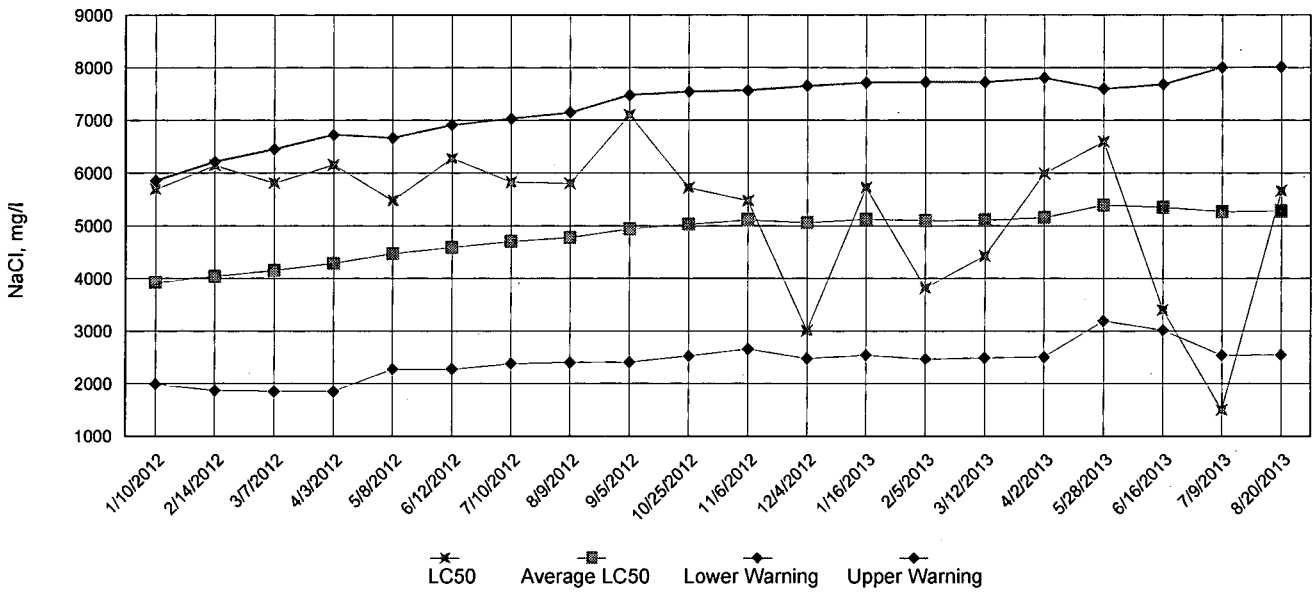
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.9	7.4	8.8	7.6	8.7	7.7	7.3
	Final	7.5	7.4	7.4	7.9	8.1	8.0	7.7
pH, units	Initial	7.5	7.7	7.2	7.5	7.5	7.6	7.5
	Final	7.5	7.4	7.6	8.1	8.3	7.6	7.5
Alkalinity, mg CaCO3/l		36	NA	30	NA	35	NA	NA
Hardness, mg CaCO3/l		54	NA	58	NA	61	NA	NA
Conductivity, umhos/cm		250	260	280	290	310	310	330
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	7.4	8.9	7.6	8.3	7.9	7.4
	Final	7.5	8.5	7.4	7.8	8.1	8.0	7.7
pH, units	Initial	7.4	7.7	7.1	7.4	7.4	7.6	7.6
	Final	7.5	7.4	7.6	8.1	8.1	7.6	7.4

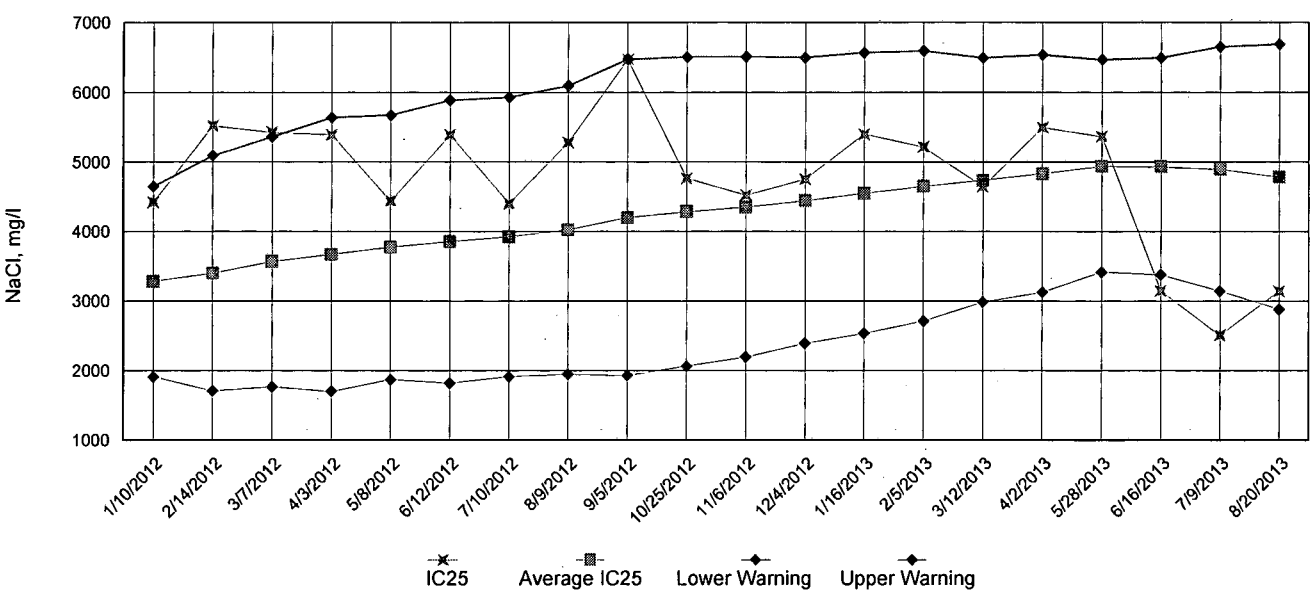
Appendix A4: Test 1000.0

Chronic Reference Toxicant, *Pimephales promelas* (Fathead Minnow)

LC50 Survival Data



IC25 Growth Data



Appendix B: Test 1000.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

Permittee: City of Hot Springs

NPDES No.: AR0033880 AFIN#26-00145

Date and Time Test Initiated: August 20, 2013 at 1200
Date and Time Test Terminated: August 27, 2013 at 1010
Dilution water used: Synthetic Soft Water #4012

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in replicate chambers A	B	C	D	E	Mean percent survival 24 hr	48 hr	7 days	CV%
Control	100	100	100	100	100	100	100	100	0.00
27 %	100	100	100	100	100	100	100	100	0.00
37 %	100	100	100	100	100	100	100	100	0.00
49 %	100	100	100	100	100	100	100	100	0.00
65 %	100	100	100	100	100	100	100	100	0.00
87 %	100	100	100	100	100	100	100	100	0.00

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers A	B	C	D	E	Mean dry weight, mg	CV%
Control	0.269	0.304	0.329	0.292	0.288	0.296	7.47
27 %	0.285	0.362	0.376	0.341	0.321	0.337	10.6
37 %	0.328	0.360	0.326	0.272	0.331	0.323	9.86
49 %	0.269	0.311	0.336	0.302	0.291	0.302	8.20
65 %	0.306	0.264	0.306	0.334	0.251	0.292	11.6
87 %	0.338	0.216	0.241	0.336	0.380	0.302	23.2

CV = Coefficient of variation = standard deviation * 100 / mean

Appendix B: Test 1000.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

1. Steel's Many-One Rank Test:

   Is the mean survival significantly different (p=0.05) than the control survival for the % effluent corresponding to (lethality):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	____YES	X NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____YES	____NO

2. Dunnett's Test:

   Is the mean dry weight (growth) significantly different (p=0.05) than the control's dry weight (growth) for the % effluent corresponding to (significant non-lethal effects):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	____YES	X NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____YES	____NO

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

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

5. NOEC Pimephales Lethality: 87 % (TOP6C)

6. LOEC Pimephales Lethality: 87 % (TXP6C)

7. NOEC Pimephales Sublethality: 87 % (TPP6C)

8. LOEC Pimephales Sublethality: 87 % (TYP6C)

9. Coefficient of variation for Pimephales growth: 11.6 (TQP6C)

AMERICAN INTERPLEX CORPORATION
LABORATORIES

Appendix B: Test 1000.0

CHRONIC TOXICITY SUMMARY FORM
Pimephales promelas (Fathead minnow)
CHEMICAL PARAMETERS CHART

PERMITTEE: City of Hot Springs 2400
NPDES NO.: AR0033880 AFIN#26-00145 2400
CONTACT: Mr. James Sorrells 2400
ANALYST: 280, 298, 304, 307

Test Initiated: DATE: August 20, 2013 TIME: 1200
Test Terminated: DATE: August 27, 2013 TIME: 1010

DILUTION Control	DAY 1	2	3	4	5	6	7
D.O. Initial	7.7	7.5	7.7	7.5	7.8	7.8	8.1
Final	7.5	7.2	7.5	7.9	7.7	7.1	7.9
pH Initial	7.7	7.7	7.8	8.0	7.9	8.0	7.7
Final	7.5	7.3	7.8	8.1	8.1	7.6	7.9
Alkalinity	32	NA	32	NA	32	NA	NA
Hardness	47	NA	42	NA	42	NA	NA
Conductivity	180	180	170	180	170	170	190
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 27 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.7	7.4	8.0	7.5	8.0	8.0	7.5
Final	7.5	7.2	7.3	7.9	7.8	8.1	7.7
pH Initial	7.6	7.7	7.5	7.8	7.7	7.8	7.5
Final	7.5	7.3	7.7	8.1	8.2	7.5	7.7
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	220	220	220	230	230	250
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 37 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.8	7.6	8.6	7.6	8.2	7.7	7.7
Final	7.5	7.0	7.5	8.0	7.9	8.1	7.8
pH Initial	7.6	7.7	7.4	7.7	7.6	7.7	7.6
Final	7.5	7.3	7.7	8.1	8.2	7.5	7.6
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	220	230	240	240	260	250	270
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 49 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.8	7.5	8.7	7.6	8.1	7.7	7.7
Final	7.5	7.4	7.6	8.0	8.1	8.2	7.8
pH Initial	7.5	7.7	7.3	7.6	7.6	7.6	7.5
Final	7.5	7.4	7.6	8.1	8.2	7.6	7.6
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	230	240	260	260	280	280	300
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 65 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.9	7.4	8.8	7.6	8.7	7.7	7.3
Final	7.5	7.4	7.4	7.9	8.1	8.0	7.7
pH Initial	7.5	7.7	7.2	7.5	7.5	7.6	7.5
Final	7.5	7.4	7.6	8.1	8.3	7.6	7.5
Alkalinity	36	NA	30	NA	35	NA	NA
Hardness	54	NA	58	NA	61	NA	NA
Conductivity	250	260	280	290	310	310	330
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 87 %	DAY 1	2	3	4	5	6	7
D.O. Initial	8.0	7.4	8.9	7.6	8.3	7.9	7.4
Final	7.5	8.5	7.4	7.8	8.1	8.0	7.7
pH Initial	7.4	7.7	7.1	7.4	7.4	7.6	7.6
Final	7.5	7.4	7.6	8.1	8.1	7.6	7.4
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	280	290	320	320	360	360	380
Chlorine	NA	NA	NA	NA	NA	NA	NA

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No.: 13-3032

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2°C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL		No of BOTTLES	Chronic.CD, Chronic.FH	Remarks
1	Plant Effluent	8-18-13 0000-2400		X	X			3	x	
		Container Type							P	
		Preservative							NO	

Field pH calibration on ________ @ ______
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: ____________
Who should AIC contact with questions: ____________
Phone: ____________ Fax: ____________
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By	Date/Time	Received By	Date/Time
A. Thomason	8-19-13 @ 1010	M. Mann	8-19-13 10:10 AM
M. Mann	8-19-13 @ 11:10 AM	Received In Lab By: [illegible signature]	8-19-13 1110

Comments:

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 2.3C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH	Remarks
2	Plant Effluent	8-20-13 0000-2400		X	X		3	X	
		Container Type						P	Field pH calibration
		Preservative						NO	on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
A. Ross	8-21-13 0900	A. Man	8-21-13 9:10
A. Man	8-21-13 1130	Jimmy Day	8/21/13 1130

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By:

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL		No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
3	Plant Effluent	8/22/13 0000-2400		X				3	X
		Container Type							P
		Preservative							NO

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 2
Remarks
Field pH calibration on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	8-23-13 0955	[signature]	8-23-13 9:55
[signature]	8-23-13 11:20	[signature]	8-23-13 1120

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

September 19, 2013

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Plant Effluent
City of Hot Springs

Control No. 170390-1

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Ceriodaphnia dubia*
Plant Effluent - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: The No Observable Effects Concentration (NOEC) for survival occurred at <27 % effluent, which is below the critical dilution of 65 %. The NOEC for reproduction occurred at <27 % effluent, which is below the critical dilution of 65 %. **The sample, therefore, FAILED both lethal and sub-lethal effects for the *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Table of Contents

I. Control Acceptance Criteria

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	18.8	PASS
Control CV < or = 40% per Surviving Female	20.6	PASS
Reproduction Minimum Significant Difference 13 to 47%	NA	NA
Critical Dilution CV < or = 40%	0.00	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly Test Method 1002.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point: Plant Effluent
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.7	8.2	8.2
pH (standard units)	7.4	7.7	7.5
Alkalinity (mg/l as CaCO3)	64	58	64
Hardness (mg/l as CaCO3)	82	82	83
Conductivity (umhos/cm)	380	370	440
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	2.4	0.11

2. Dilution Water Samples: Synthetic Soft Water #4019
   a. Dates Prepared: August 28 through September 11, 2013
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.6	8.2	8.2
pH (standard units)	7.6	7.8	7.6
Alkalinity (mg/l as CaCO3)	32	32	32
Hardness (mg/l as CaCO3)	46	42	42
Conductivity (umhos/cm)	160	160	180
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05

C. Test Methods
1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Method 1002.0, *Ceriodaphnia dubia* Survival and Reproduction.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Ceriodaphnia dubia Survival and Growth Method 1002.0

Date & Time Test Initiated:	September 9, 2013 at 1235
Date & Time Test Terminated:	September 17, 2013 at 1255
Type & Volume of Test Chamber:	30 ml disposable beaker
Volume of Sample:	15 ml
Number of Organisms per replicate:	1
Number of Replicates per dilution:	10

4. Acclimation of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms
1. Scientific Name
a. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis
The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was not analyzed due to survival failure.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Ceriodaphnia dubia

Chronic reference tests are performed monthly.
A chronic reference test was performed on August 20, 2013 at 1505 to August 28, 2013 at 1450
The results were as follows: (Control No. 169867-2.)
Survival LC-50: 2125 mg/l
Growth IC-25: 1610 mg/l
Growth PMSD: 18.3

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	1.53
Hardness	EPA 200.7	101	0.450
pH	SM 4500-H+ B	101	0.267
Conductivity	EPA 120.1	103	1.97

VI. Organism History

Ceriodaphnia dubia

Date: September 9, 2013
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

VII. Results Summary *Ceriodaphnia dubia*, Cladoceran Survival and Reproduction Test -- Method 1002.0

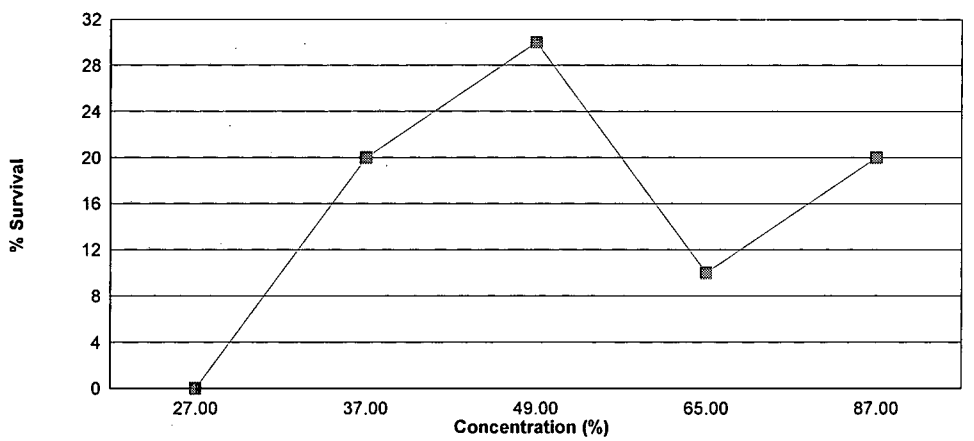
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on September 9, 2013 at 1235 and continued through September 17, 2013 at 1255. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = <27 % effluent
b.) NOEC reproduction = <27 % effluent



Summary of the 7-day *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	18.8
27 %	0.00 *	--
37 %	20.0 *	--
49 %	30.0 *	--
65 %	10.0 *	--
87 %	20.0 *	--

*Significant difference when compared to the control (p=0.05)

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 9, 2013 at 1235
Date and Time Test Terminated: September 17, 2013 at 1255

Concentration: Control													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	3	2	0	0	0	0	1	4	2	1	13	10	1.30
5	9	0	3	6	6	4	0	4	6	0	38	10	3.80
6	0	5	9	8	7	0	6	0	1	7	43	10	4.30
7	10	9	10	10	10	8	9	8	10	10	94	10	9.40
8													
TOTAL	22	16	22	24	23	12	16	16	19	18	188	10	18.8

Concentration: 27 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	0	0	0	X	0	0	0	X	0	0	7	0.00
2	X	0	0	0	X	0	0	0	X	0	0	7	0.00
3	X				X				X		0	0	0.00
4	X				X				X		0	0	0.00
5	X				X				X		0	0	0.00
6	X				X				X		0	0	0.00
7	X				X				X		0	0	0.00
8													
TOTAL	0	0	0	0	0	0	0	0	0	0	0	10	0.00

Concentration: 37 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	X	X	0	X	X	0	0	3	0.00
2	X	X	0	X	X	X	0	X	X	0	0	3	0.00
3	X	X	X	X	X	X	0	X	X	0	0	2	0.00
4	X	X	X	X	X	X	2	X	X	1	3	2	1.50
5	X	X	X	X	X	X	0	X	X	0	0	2	0.00
6	X	X	X	X	X	X	7	X	X	6	13	2	6.50
7	X	X	X	X	X	X	10	X	X	0	10	2	5.00
8													
TOTAL	0	0	0	0	0	0	19	0	0	7	26	10	2.60

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 9, 2013 at 1235
Date and Time Test Terminated: September 17, 2013 at 1255

Concentration: 49 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	X	X	0	X	X	0	0	3	0.00
2	X	X	0	X	X	X	0	X	X	0	0	3	0.00
3	X	X	0	X	X	X	0	X	X	0	0	3	0.00
4	X	X	0	X	X	X	0	X	X	0	0	3	0.00
5	X	X	4	X	X	X	5	X	X	0	9	3	3.00
6	X	X	0	X	X	X	0	X	X	6	6	3	2.00
7	X	X	6	X	X	X	8	X	X	0	14	3	4.67
8													
TOTAL	0	0	10	0	0	0	13	0	0	6	29	10	2.90

Concentration: 65 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	X	X	X	X	X	0	0	2	0.00
2	X	X	0	X	X	X	X	X	X	0	0	2	0.00
3	X	X	0	X	X	X	X	X	X	0	0	2	0.00
4	X	X	0	X	X	X	X	X	X	0	0	2	0.00
5	X	X	6	X	X	X	X	X	X	0	6	2	3.00
6	X	X	0	X	X	X	X	X	X	X	0	1	0.00
7	X	X	9	X	X	X	X	X	X	X	9	1	9.00
8													
TOTAL	0	0	15	0	0	0	0	0	0	0	15	10	1.50

Concentration: 87 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	0	X	0	X	X	0	0	4	0.00
2	X	X	0	X	0	X	X	X	X	0	0	3	0.00
3	X	X	0	X	0	X	X	X	X	0	0	3	0.00
4	X	X	0	X	3	X	X	X	X	1	4	3	1.33
5	X	X	7	X	5	X	X	X	X	0	12	3	4.00
6	X	X	0	X	X	X	X	X	X	3	3	2	1.50
7	X	X	9	X	X	X	X	X	X	0	9	2	4.50
8													
TOTAL	0	0	16	0	8	0	0	0	0	4	28	10	2.80

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
27 %	0	3	3
Total	10	3	13

Critical Fisher's value (10,3,10) (alpha=0.05) is 1. b value is 0. Since b is less than or equal to 1 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
37 %	2	8	10
Total	12	8	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 2. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
49 %	3	7	10
Total	13	7	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 3. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
65 %	1	9	10
Total	11	9	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 1. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
87 %	2	8	10
Total	12	8	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 2. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	27 %	3	3	*
2	37 %	10	8	*
3	49 %	10	7	*
4	65 %	10	9	*
5	87 %	10	8	*

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: September 9, 2013 at 0857
Date and Time Test Terminated: September 17, 2013 at 1255

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.6	7.4	8.2	8.3	8.2	8.3	8.2
	Final	7.8	8.3	8.5	8.7	8.4	8.2	7.8
pH, units	Initial	7.6	7.6	7.8	7.6	7.6	7.7	7.5
	Final	7.8	7.9	7.9	7.6	7.9	7.8	8.0
Alkalinity, mg CaCO3/l		32	NA	32	NA	32	NA	NA
Hardness, mg CaCO3/l		46	NA	42	NA	42	NA	NA
Conductivity, umhos/cm		160	170	160	180	180	180	170
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.6	7.5	8.2	8.4	8.3	8.1	8.3
	Final	6.0	8.3	8.7	8.8	8.5	8.3	8.1
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
	Final	8.0	8.1	8.2	8.0	8.3	8.0	8.1

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.6	7.4	8.2	8.4	8.1	8.0	8.1
	Final	6.4	8.3	8.6	8.8	8.6	8.2	8.2
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
	Final	8.1	8.2	8.2	8.2	8.2	7.9	7.8

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: September 9, 2013 at 0857
Date and Time Test Terminated: September 17, 2013 at 1255

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.5	7.5	8.2	8.4	8.4	8.1	8.3
	Final	6.8	8.3	7.8	9.0	8.4	8.3	8.0
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.5	7.5
	Final	8.2	8.2	8.2	8.1	8.1	8.0	8.1

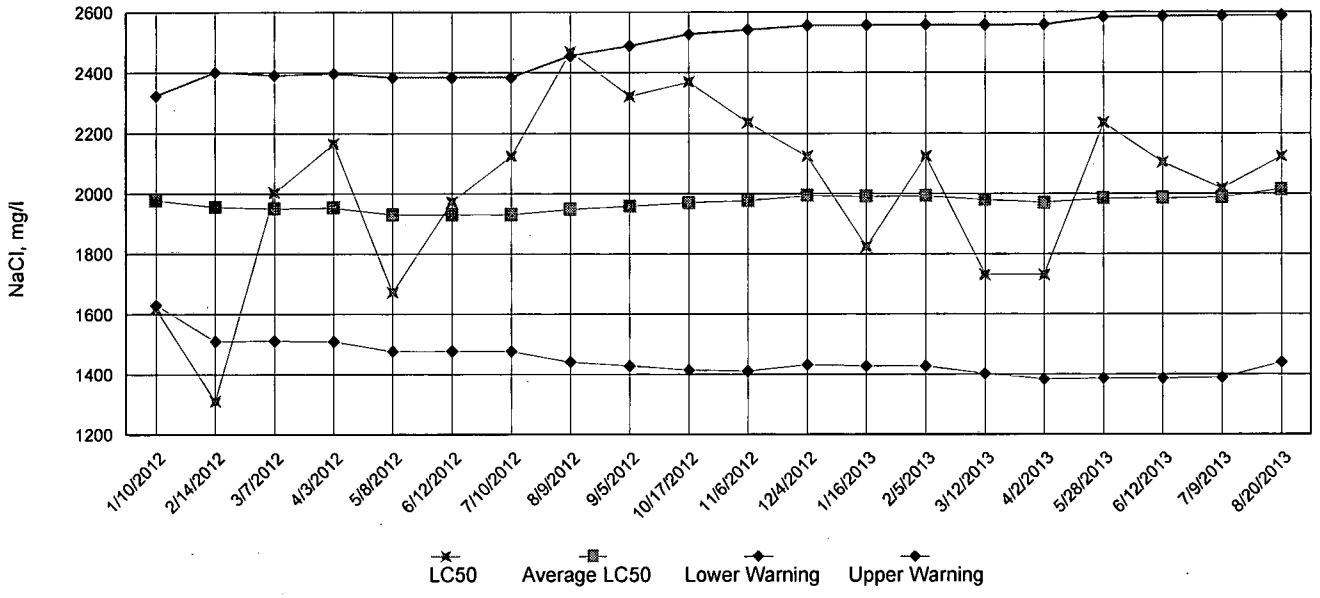
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.4	8.2	8.4	8.0	8.2	8.3
	Final	7.0	8.3	8.4	8.7	8.3	8.3	7.7
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.6
	Final	8.2	8.1	8.1	8.2	8.0	8.0	7.7
Alkalinity, mg CaCO3/l		48	NA	46	NA	47	NA	NA
Hardness, mg CaCO3/l		68	NA	68	NA	68	NA	NA
Conductivity, umhos/cm		310	310	300	340	340	340	350
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.3	7.2	8.2	8.3	8.3	8.0	7.9
	Final	7.1	8.3	8.6	8.8	7.3	8.3	8.0
pH, units	Initial	7.5	7.4	7.8	7.5	7.6	7.5	7.6
	Final	8.2	8.3	8.3	8.3	7.5	8.1	7.9

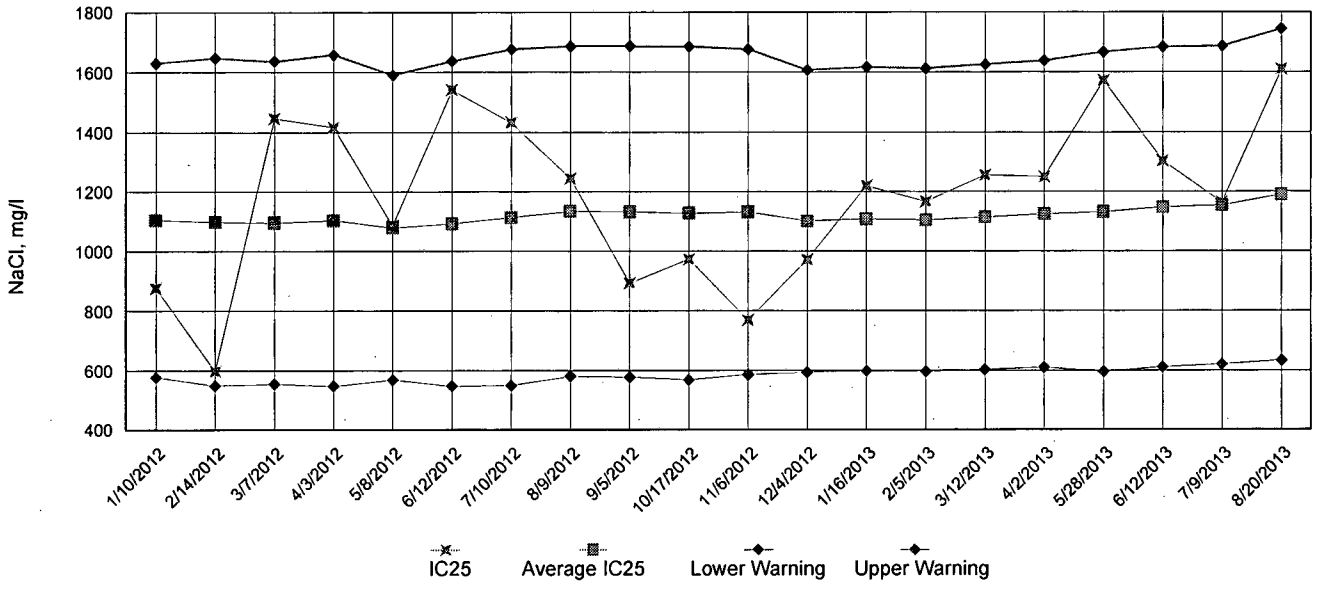
Appendix A4: Test 1002.0

Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

Permittee: City of Hot Springs

NPDES No.: AR0033880 AFIN#26-00145

Date and Time Test Initiated: September 9, 2013 at 1235
Date and Time Test Terminated: September 17, 2013 at 1255
Dilution water used: Synthetic Soft Water #4019

PERCENT SURVIVAL

		Percent Effluent				
Time of Reading	Control	27 %	37 %	49 %	65 %	87 %
24 hour	100	70.0	30.0	30.0	20.0	40.0
48 hour	100	70.0	30.0	30.0	20.0	30.0
7 day	100	0.00	20.0	30.0	10.0	20.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

		Percent Effluent				
Replicates	Control	27 %	37 %	49 %	65 %	87 %
A	22	0	0	0	0	0
B	16	0	0	0	0	0
C	22	0	0	10	15	16
D	24	0	0	0	0	0
E	23	0	0	0	0	8
F	12	0	0	0	0	0
G	16	0	19	13	0	0
H	16	0	0	0	0	0
I	19	0	0	0	0	0
J	18	0	7	6	0	4
Mean per Adult	18.8	0.00	2.60	2.90	1.50	2.80
Mean per Surviving Adult	18.8	0.00	13.0	9.67	15.0	10.0
CV %	20.6	0.00	65.3	36.3	0.00	84.9

CV = Coefficient of variation = standard deviation * 100 / mean
(calculated based on young produced by surviving females)

Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

1. Fisher's Exact Test:

   Is the mean survival significantly different (p=0.05) than the control survival for the % effluent corresponding to (lethality):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	X YES	____ NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____ YES	____ NO

2.

   Is the mean number of young produced per female significantly different (p=0.05) than the control's number of young per female for the % effluent corresponding to (significant non-lethal effects):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	X YES	____ NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____ YES	____ NO

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 1 (TLP3B)
4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 1 (TGP3B)
5. NOEC Ceriodaphnia Lethality: <27 % (TOP3B)
6. LOEC Ceriodaphnia Lethality: 27 % (TXP3B)
7. NOEC Ceriodaphnia Sublethality: <27 % (TPP3B)
8. LOEC Ceriodaphnia Sublethality: 27 % (TYP3B)
9. Coefficient of variation for Ceriodaphnia Reproduction: 20.6 (TQP3B)

Appendix B: Test 1002.0

CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
CHEMICAL PARAMETERS CHART

PERMITTEE: City of Hot Springs
NPDES NO.: AR0033880 AFIN#26-00145
CONTACT: Mr. James Sorrells
ANALYST: 280, 298, 304, 307

2400
2400
2400

Test Initiated: DATE: September 9, 2013 TIME: 1235
Test Terminated: DATE: September 17, 2013 TIME: 1255

DILUTION	DAY						
Control	1	2	3	4	5	6	7
D.O. Initial	8.6	7.4	8.2	8.3	8.2	8.3	8.2
Final	7.8	8.3	8.5	8.7	8.4	8.2	7.8
pH Initial	7.6	7.6	7.8	7.6	7.6	7.7	7.5
Final	7.8	7.9	7.9	7.6	7.9	7.8	8.0
Alkalinity	32	NA	32	NA	32	NA	NA
Hardness	46	NA	42	NA	42	NA	NA
Conductivity	160	170	160	180	180	180	170
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
27 %	1	2	3	4	5	6	7
D.O. Initial	8.6	7.5	8.2	8.4	8.3	8.1	8.3
Final	6.0	8.3	8.7	8.8	8.5	8.3	8.1
pH Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
Final	8.0	8.1	8.2	8.0	8.3	8.0	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	220	220	220	240	250	250	250
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
37 %	1	2	3	4	5	6	7
D.O. Initial	8.6	7.4	8.2	8.4	8.1	8.0	8.1
Final	6.4	8.3	8.6	8.8	8.6	8.2	8.2
pH Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
Final	8.1	8.2	8.2	8.2	8.2	7.9	7.8
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	240	240	240	260	270	270	280
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
49 %	1	2	3	4	5	6	7
D.O. Initial	8.5	7.5	8.2	8.4	8.4	8.1	8.3
Final	6.8	8.3	7.8	9.0	8.4	8.3	8.0
pH Initial	7.6	7.5	7.8	7.5	7.6	7.5	7.5
Final	8.2	8.2	8.2	8.1	8.1	8.0	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	270	270	270	300	300	300	300
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
65 %	1	2	3	4	5	6	7
D.O. Initial	7.7	7.4	8.2	8.4	8.0	8.2	8.3
Final	7.0	8.3	8.4	8.7	8.3	8.3	7.7
pH Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.6
Final	8.2	8.1	8.1	8.2	8.0	8.0	7.7
Alkalinity	48	NA	46	NA	47	NA	NA
Hardness	68	NA	68	NA	68	NA	NA
Conductivity	310	310	300	340	340	340	350
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
87 %	1	2	3	4	5	6	7
D.O. Initial	8.3	7.2	8.2	8.3	8.3	8.0	7.9
Final	7.1	8.3	8.6	8.8	7.3	8.3	8.0
pH Initial	7.5	7.4	7.8	7.5	7.6	7.5	7.6
Final	8.2	8.3	8.3	8.3	7.5	8.1	7.9
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	350	360	350	400	440	430	440
Chlorine	NA	NA	NA	NA	NA	NA	NA

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Roys

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Chronic C.D.	Analyses Requested
1	Plant Effluent	9-8-13 2000-2400		X	X		3	X	No Fathead Analysis
		Container Type						P	
		Preservative						NO	

AIC Control No: 170390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2.1°C
Remarks:
Field pH calibration on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-8-13 1030	M. Mann	9-9-13 10:30 am
M. Mann	9-9-13 11:45	[signature]	9-9-13 1145

Comments:

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC Control No: 170390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 3°C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH	Remarks
1	Plant Effluent	9-10-13 0000-2400		X	X		3	X	
		Container Type						P	Field pH calibration
		Preservative						NO	on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-11-13 1110	M. Mann	9-11-13 1110
M. Mann	9-11-13 @ 12:05	[signature]	9-11-13 1205

Comments:

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs

Project Reference: Plant Effluent

Project Manager: James Sorrells

Sampled By: HAROLD MAULDIN

PO No.: 13-3032

AIC Control No: 170390

AIC Proposal No:

Carrier: Hot Springs Courier

Received Temperature °C: 2

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL		No of BOTTLES	Chronic.CD.	Remarks
3	Plant Effluent	9/12/13 0000-2400		X	X			3	x	

Container Type: | P

Preservative: | NO

Field pH calibration on ______ @ ______

Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate

NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by:
Who should AIC contact with questions:
Phone: Fax:
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By	Date/Time	Received By	Date/Time
A. Thomason	9-13-13 @ 1015	M. Mann	9-13-13 @ 10:15
M. Mann	9-13-13 @ 11:20	(Received in Lab) [signature]	9-13-13 1120

Comments:

4-85

AMERICAN INTERPLEX CORPORATION
LABORATORIES

September 30, 2013

Test Results of
Acute 48 hour Non-Renewal
Biomonitoring Testing
for

170741-1: Outfall 001 First Renewal
170741-2: Outfall 001 Second Renewal
170741-3: Outfall 001 Third Renewal
170741-4: Outfall 001 Original Screen

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Acute 48 hour Non-Renewal Biomonitoring utilizing *Ceriodaphnia dubia*
Outfall 001 First Renewal - City of Hot Springs
Client NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

Please find attached the results of the acute biomonitoring screens conducted as a follow up to the chronic toxicity event previously reported (AIC Control No. 170390). As indicated on the enclosed results, the sample was still acutely toxic on September 12, 2013 and exhibited no acute toxicity on September 19, 2013.

It it theorized the decrease in toxicity is directly related to the holding time which may have allowed microbial degradation, loss of volatile toxics, and/or biochemical degradation.

If I can be of further assistance, please feel free to contact me.

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Dilution Water Samples: Synthetic Soft Water #4021

Analysis	Result
Dissolved oxygen (mg/l)	7.6
pH (standard units)	7.7
Alkalinity (mg/l as CaCO3)	31
Hardness (mg/l as CaCO3)	46
Conductivity (umhos/cm)	170
Residual Chlorine (mg/l)	<0.05

Results Summary: Outfall 001 First Renewal

Ceriodaphnia dubia

The *Ceriodaphnia dubia* test was conducted from September 19, 2013 at 1615 to September 21, 2013 at 1700.

Statistical analyses:

NOEC = 100%

Concentration	24 hour % Survival	48 hour % Survival
Control	100	100
100%	100	100

Ceriodaphnia dubia
Survival Data

Number of organisms per chamber: 5
Volume of test chamber: 30 ml

Age of organisms: <24 hours
Volume of test solution: 15 ml

Effluent Concentration	Number of Survivors 24 Hours	48 Hours	% Survival	CV %
Control rep. A	5	5	100	0.00
100% rep. A	5	5	100	0.00

CV = Coefficient of variance = standard deviation X 100/mean

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	5	0	5
100%	5	0	5
Total	10	0	10

Critical Fisher's value (5,5,5) (alpha=0.05) is 1. b value is 5. Since b is greater than 1 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test

Group	Identification	Exposed	Dead	Sig 0.05
0	0	5	0	
1	100	5	0	

Chemical Data for
Ceriodaphnia dubia

Day 1	Control	100%
DO, mg/l	7.6	8.8
pH, su	7.7	7.3
Alkalinity, mg/l	31	NA
Hardness, mg/l	46	NA
Conductivity, umho/cm	170	380
Residual Chlorine, mg/l	<0.05	NA

Day 2		Control	100%
DO, mg/l	Final	7.5	8.7
pH, su	Final	8.0	7.4

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

170744 WR 9/19/13

Client: City of Hot Springs
Project:
Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Rojs

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Chronic CD.	Analyses Requested
1	Plant Effluent	9-8-13 0000-2400		X	X		3	X	
		Container Type						P	
		Preservative						NO	

No Fathead Analysis

AIC Control No: 170390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2.1°C
Remarks:
Field pH calibration on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ___ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-9-13 1030	M. Mann	9-9-13 10:30 AM
M. Mann	9-9-13 11:45	[signature]	9-9-13 1145

Comments:

City of Hot Springs
Waste Water Treatment Plant
320 Davidson Drive
Hot Springs, AR 71901

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NPDES Enforcement Section
Attn: Mo Shafii
5301 Northshore Dr.
North Little Rock, AR 72118-5317

AMERICAN INTERPLEX CORPORATION
LABORATORIES

August 30, 2013

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Outfall 001
City of Hot Springs

Control No. 169846-2

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Ceriodaphnia dubia*
Outfall 001 - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Chronic *Ceriodaphnia dubia* test: Due to laboratory error, the *Ceriodaphnia dubia* test was not renewed with the third sample. The test should be repeated. The data from the test is enclosed for your review.

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Table of Contents

I. Control Acceptance Criteria

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	23.6	PASS
Control CV < or = 40% per Surviving Female	13.6	PASS
Reproduction Minimum Significant Difference 13 to 47%	25.8	PASS
Critical Dilution CV < or = 40%	22.9	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly
   Test Method 1002.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point: Outfall 001
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.9	8.5	NA
pH (standard units)	7.0	7.4	NA
Alkalinity (mg/l as CaCO3)	26	34	NA
Hardness (mg/l as CaCO3)	67	71	NA
Conductivity (umhos/cm)	350	390	NA
Residual Chlorine (mg/l)	<0.05	<0.05	NA
Ammonia as N (mg/l)	0.12	0.30	NA

2. Dilution Water Samples: Synthetic Soft Water #4012
   a. Dates Prepared: August 8 through August 22, 2013
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.7	7.7	7.8
pH (standard units)	7.7	7.8	7.9
Alkalinity (mg/l as CaCO3)	32	32	NA
Hardness (mg/l as CaCO3)	47	42	NA
Conductivity (umhos/cm)	180	170	170
Residual Chlorine (mg/l)	<0.05	<0.05	NA

C. Test Methods
1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Method 1002.0, *Ceriodaphnia dubia* Survival and Reproduction.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Ceriodaphnia dubia Survival and Growth Method 1002.0

Date & Time Test Initiated:	August 22, 2013 at 1605
Date & Time Test Terminated:	August 29, 2013 at 1415
Type & Volume of Test Chamber:	30 ml disposable beaker
Volume of Sample:	15 ml
Number of Organisms per replicate:	1
Number of Replicates per dilution:	10

4. Acclimation of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms
1. Scientific Name
a. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis

The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was analyzed using Kolmogorov's Test for Normality and Bartlett's test and analyzed with Dunnett's Test to determine the No Observable Effects Concentration (NOEC) for Reproduction.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Ceriodaphnia dubia

Chronic reference tests are performed monthly.
A chronic reference test was performed on August 20, 2013 at 1505 to August 28, 2013 at 1450
The results were as follows: (Control No. 169867-2.)
Survival LC-50: 2125 mg/l
Growth IC-25: 1610 mg/l
Growth PMSD: 18.3

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	102	1.91
pH	SM 4500-H+ B	101	0.268
Conductivity	EPA 120.1	101	4.71

VI. Organism History

Ceriodaphnia dubia

Date: August 22, 2013
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

VII. Results Summary *Ceriodaphnia dubia*, Cladoceran Survival and Reproduction Test -- Method 1002.0

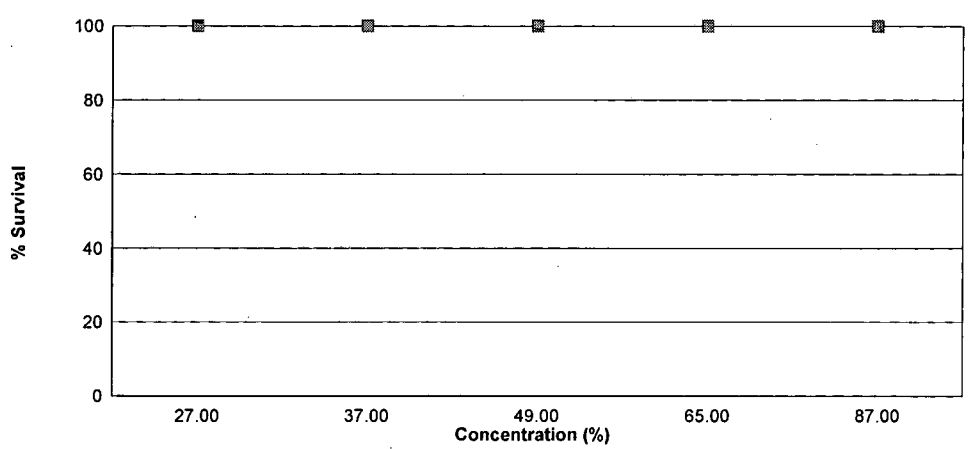
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on August 22, 2013 at 1605 and continued through August 29, 2013 at 1415. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 87 % effluent
b.) NOEC reproduction = 87 % effluent



Summary of the 7-day *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	23.6
27 %	100	24.2
37 %	100	27.6
49 %	100	26.4
65 %	100	24.2
87 %	100	25.0

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 22, 2013 at 1605
Date and Time Test Terminated: August 29, 2013 at 1415

Concentration: Control													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	4	4	2	3	4	4	3	2	33	10	3.30
5	0	0	0	0	0	1	0	0	0	6	7	10	0.700
6	9	9	7	8	7	8	7	9	8	8	80	10	8.00
7	14	14	11	13	14	11	12	14	13	0	116	10	11.6
8													
TOTAL	27	26	22	25	23	23	23	27	24	16	236	10	23.6

Concentration: 27 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	2	3	3	3	2	3	2	3	2	27	10	2.70
5	8	7	1	0	0	0	0	0	0	6	22	10	2.20
6	1	12	13	11	10	10	11	8	7	6	89	10	8.90
7	16	0	17	18	6	0	17	17	13	0	104	10	10.4
8													
TOTAL	29	21	34	32	19	12	31	27	23	14	242	10	24.2

Concentration: 37 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	3	3	3	2	3	2	2	2	27	10	2.70
5	0	0	0	0	0	1	0	0	6	6	13	10	1.30
6	12	12	12	11	10	6	8	10	13	10	104	10	10.4
7	18	16	17	18	16	15	15	17	0	0	132	10	13.2
8													
TOTAL	34	31	32	32	29	24	26	29	21	18	276	10	27.6

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 22, 2013 at 1605
Date and Time Test Terminated: August 29, 2013 at 1415

Concentration: 49 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	2	5	2	3	4	2	2	2	29	10	2.90
5	0	5	0	0	0	1	0	6	4	3	19	10	1.90
6	10	0	11	11	11	8	10	0	12	10	83	10	8.30
7	17	18	16	18	16	17	15	16	0	0	133	10	13.3
8													
TOTAL	31	26	29	34	29	29	29	24	18	15	264	10	26.4

Concentration: 65 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	2	3	5	3	3	3	2	3	2	2	28	10	2.80
5	6	0	0	0	0	1	0	0	5	4	16	10	1.60
6	13	11	11	11	10	10	8	10	0	12	96	10	9.60
7	0	15	14	16	12	0	15	16	14	0	102	10	10.2
8													
TOTAL	21	29	30	30	25	14	25	29	21	18	242	10	24.2

Concentration: 87 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	4	3	3	3	3	4	3	2	2	31	10	3.10
5	8	6	1	0	0	0	0	0	5	5	25	10	2.50
6	0	2	11	12	6	10	8	8	0	7	64	10	6.40
7	16	15	16	18	16	0	18	17	14	0	130	10	13.0
8													
TOTAL	28	27	31	33	25	13	30	28	21	14	250	10	25.0

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	10	0	10
27 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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	Alive	Dead	Total Animals
Control	10	0	10
37 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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	Alive	Dead	Total Animals
Control	10	0	10
49 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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	Alive	Dead	Total Animals
Control	10	0	10
65 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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.

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
87 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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 Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	27 %	10	0	
2	37 %	10	0	
3	49 %	10	0	
4	65 %	10	0	
5	87 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality		No Transformation
D = 0.1154 D* = 0.9054 Critical D* = 1.035	(alpha = 0.01, N = 60)	
Data PASS normality test (alpha = 0.01).		

Bartlett's Test for Homogeneity of Variance		No Transformation
Calculated B1 statistic = 6.592 Critical B = 15.086	(alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.		

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	117.9	23.58	0.6766
Within (Error)	54	1882	34.85	
Total	59	2000		

Critical F = 3.38 (alpha = 0.01, df = 5,54)
2.38 (alpha = 0.05, df = 5,54)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Dunnett's Test - Table 1 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	23.6	23.6		
2	27 %	24.2	24.2	-0.2273	
3	37 %	27.6	27.6	-1.515	
4	49 %	26.4	26.4	-1.061	
5	65 %	24.2	24.2	-0.2273	
6	87 %	25	25	-0.5303	

Dunnett's critical value = 2.31 (1 Tailed, alpha = 0.05, df [used] = 5,40) (Actual df = 5,54)

Dunnett's Test - Table 2 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	10			
2	27 %	10	6.099	25.8	-0.6
3	37 %	10	6.099	25.8	-4
4	49 %	10	6.099	25.8	-2.8
5	65 %	10	6.099	25.8	-0.6
6	87 %	10	6.099	25.8	-1.4

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 22, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.5	7.7	7.5	7.8	7.8	8.1
	Final	7.7	8.0	8.7	8.2	8.2	7.8	7.8
pH, units	Initial	7.7	7.7	7.8	8.0	7.9	8.0	7.7
	Final	8.0	8.2	8.0	8.0	8.0	7.8	7.8
Alkalinity, mg CaCO3/l		32	NA	32	NA	NA	NA	NA
Hardness, mg CaCO3/l		47	NA	42	NA	NA	NA	NA
Conductivity, umhos/cm		180	180	170	180	170	170	190
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	NA	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.4	8.0	7.5	8.0	8.0	7.5
	Final	7.5	8.2	8.6	8.2	8.2	7.7	7.7
pH, units	Initial	7.6	7.7	7.5	7.8	7.7	7.8	7.5
	Final	8.0	8.1	8.0	8.0	8.1	7.6	7.6

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.6	8.6	7.6	8.2	7.7	7.7
	Final	7.6	8.0	8.6	8.0	8.2	7.6	7.6
pH, units	Initial	7.6	7.7	7.4	7.7	7.6	7.7	7.6
	Final	8.0	8.1	8.0	8.0	8.1	7.6	7.6

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 22, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.5	8.7	7.6	8.1	7.7	7.7
	Final	7.7	8.1	8.6	8.2	8.2	7.6	7.9
pH, units	Initial	7.5	7.7	7.3	7.6	7.6	7.6	7.5
	Final	8.0	8.1	8.0	8.1	8.2	7.5	7.5

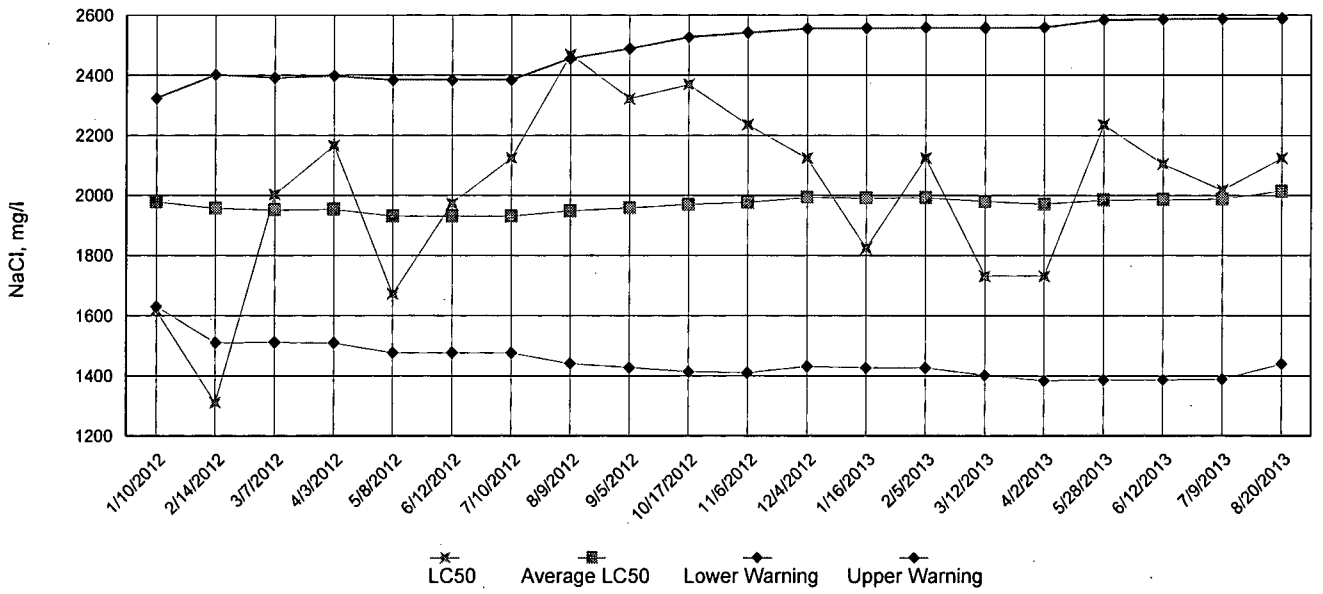
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.9	7.4	8.8	7.6	8.7	7.7	7.3
	Final	7.7	8.0	8.6	8.4	8.1	7.6	7.6
pH, units	Initial	7.5	7.7	7.2	7.5	7.5	7.6	7.5
	Final	8.0	8.0	8.0	8.1	8.1	7.5	7.5
Alkalinity, mg CaCO3/l		36	NA	30	NA	NA	NA	NA
Hardness, mg CaCO3/l		54	NA	58	NA	NA	NA	NA
Conductivity, umhos/cm		250	260	280	290	310	310	330
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	NA	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	7.4	8.9	7.6	8.3	7.9	7.4
	Final	7.4	8.1	8.7	8.2	7.7	5.4	7.2
pH, units	Initial	7.4	7.7	7.1	7.4	7.4	7.6	7.6
	Final	8.0	8.0	7.9	8.1	8.3	7.5	7.4

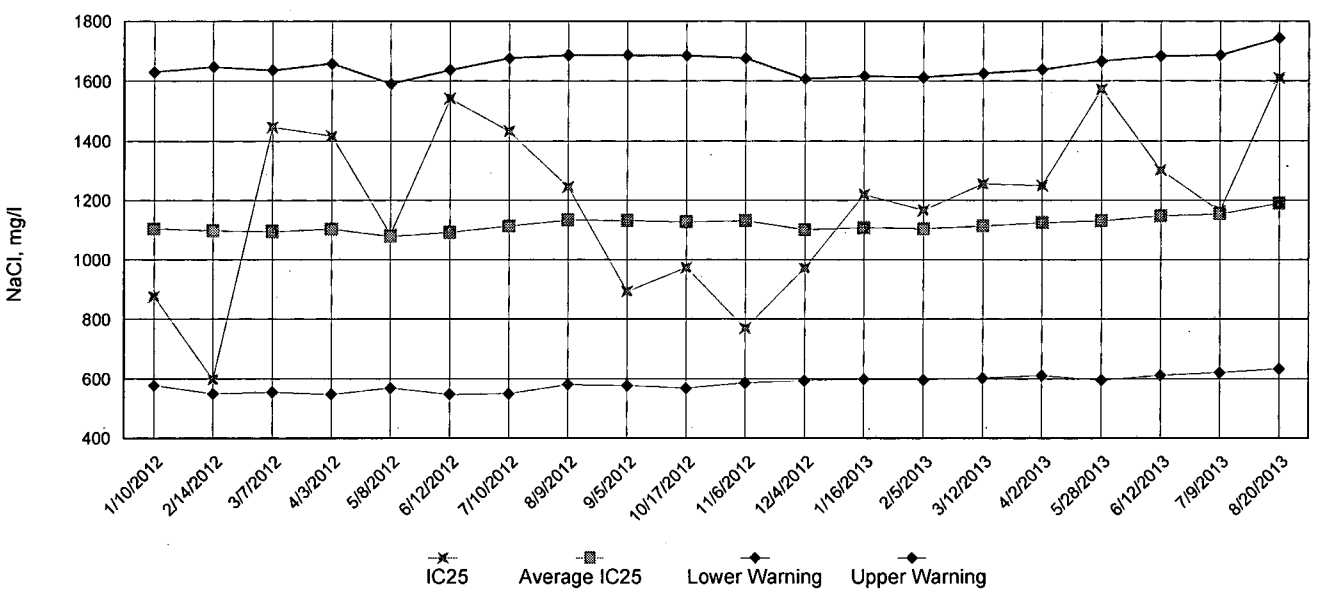
Appendix A4: Test 1002.0

Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL		No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
1	Plant Effluent	8-18-13 0900 - 2400		X	X			3	X
		Container Type							P
		Preservative							NO

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 20c
Remarks
Field pH calibration on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ___ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
A. Thomason	8-19-13 @ 1010	M. Mann	8-19-13 @ 10:10am
Relinquished By: M. Mann	Date/Time 8-19-13 @ 11:10AM	Received In Lab By: Angie Hopton	Date/Time 8-19-13 1110

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL		No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
2	Plant Effluent	8-20-13 0900-2400		X	X			3	X
		Container Type							P
		Preservative							NO

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 23C
Remarks

Field pH calibration
on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
A. Ross	8-21-13 0900	G. Mann	8-21-13 900
G. Mann	8-21-13 1130	Jimmy Day	8/21/13 1130

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By:

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
3	Plant Effluent	8/22/13 0000-2400		X			3	X
		Container Type						P
		Preservative						NO

AIC Control No: 16984b
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 2
Remarks
Field pH calibration on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	8-23-13 0955	[signature]	8-23-13 9:55
[signature]	8-23-13 11:20	[signature]	8-23-13 1120

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

September 30, 2013

Test Results of
Acute 48 hour Non-Renewal
Biomonitoring Testing
for

170741-1: Outfall 001 First Renewal
170741-2: Outfall 001 Second Renewal
170741-3: Outfall 001 Third Renewal
170741-4: Outfall 001 Original Screen

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Acute 48 hour Non-Renewal Biomonitoring utilizing *Ceriodaphnia dubia*
Outfall 001 First Renewal - City of Hot Springs
Client NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

Please find attached the results of the acute biomonitoring screens conducted as a follow up to the chronic toxicity event previously reported (AIC Control No. 170390). As indicated on the enclosed results, the sample was still acutely toxic on September 12, 2013 and exhibited no acute toxicity on September 19, 2013.

It it theorized the decrease in toxicity is directly related to the holding time which may have allowed microbial degradation, loss of volatile toxics, and/or biochemical degradation.

If I can be of further assistance, please feel free to contact me.

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Dilution Water Samples: Synthetic Soft Water #4021

Analysis	Result
Dissolved oxygen (mg/l)	7.6
pH (standard units)	7.7
Alkalinity (mg/l as CaCO3)	31
Hardness (mg/l as CaCO3)	46
Conductivity (umhos/cm)	170
Residual Chlorine (mg/l)	<0.05

Results Summary: Outfall 001 First Renewal

Ceriodaphnia dubia

The *Ceriodaphnia dubia* test was conducted from September 19, 2013 at 1615 to September 21, 2013 at 1700.

Statistical analyses:

NOEC = 100%

Concentration	24 hour % Survival	48 hour % Survival
Control	100	100
100%	100	100

Ceriodaphnia dubia
Survival Data

Number of organisms per chamber: 5
Volume of test chamber: 30 ml

Age of organisms: <24 hours
Volume of test solution: 15 ml

Effluent Concentration	Number of Survivors 24 Hours	48 Hours	% Survival	CV %
Control rep. A	5	5	100	0.00
100% rep. A	5	5	100	0.00

CV = Coefficient of variance = standard deviation X 100/mean

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	5	0	5
100%	5	0	5
Total	10	0	10

Critical Fisher's value (5,5,5) (alpha=0.05) is 1. b value is 5. Since b is greater than 1 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test

Group	Identification	Exposed	Dead	Sig 0.05
0	0	5	0	
1	100	5	0	

Chemical Data for
Ceriodaphnia dubia

Day 1	Control	100%
DO, mg/l	7.6	8.8
pH, su	7.7	7.3
Alkalinity, mg/l	31	NA
Hardness, mg/l	46	NA
Conductivity, umho/cm	170	380
Residual Chlorine, mg/l	<0.05	NA

Day 2		Control	100%
DO, mg/l	Final	7.5	8.7
pH, su	Final	8.0	7.4

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

month WR 9/19/13

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Chronic C.D.	Analyses Requested
1	Plant Effluent	9-8-13 0000-2400		X	X		3	X	
		Container Type						P	
		Preservative						NO	

No FATHEAD ANALYSIS

AIC Control No: 120390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2.1 °C
Remarks:
Field pH calibration on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z = Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-8-13 1030	M. Mann	9-9-13 10:30 AM
M. Mann	9-9-13 11:45	[signature]	9-9-13 1145

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

October 1, 2013
Control No. 171032

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

This report contains the analytical results and supporting information for samples submitted on September 27, 2013. Attached please find a copy of the Chain of Custody and/or other documents received. Note that any remaining sample will be discarded two weeks from the original report date unless other arrangements are made.

This report is intended for the sole use of the client listed above. Assessment of the data requires access to the entire document.

This report has been reviewed by the Laboratory Director or a qualified designee.

John Overbey
Laboratory Director

This document has been distributed to the following:

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

SAMPLE INFORMATION

Project Description:
Three (3) water sample(s) (AIC COntrol No. 170741-1,2,3) resubmitted September 27, 2013
P.O. No. 13-3032

Receipt Details:
A Chain of Custody was not provided with the sample(s).

Each sample container was checked for proper labeling, including date and time sampled. Sample containers were reviewed for proper type, adequate volume, integrity, temperature, preservation, and holding times. Any exceptions are noted below:

Sample Identification:

Laboratory ID	Client Sample ID	Sampled Date/Time	Notes
171032-1	Plant Effluent 9-8-13 0000-2400	08-Sep-2013 2359	
171032-2	Plant Effluent 9-10-13 0000-2400	10-Sep-2013 2359	
171032-3	Plant Effluent 9-12-13 0000-2400	12-Sep-2013 2359	

Case Narrative:
There were no qualifiers for this data and all samples met quality control criteria.

References:
"Methods for Chemical Analysis of Water and Wastes", EPA/600/4-79-020 (Mar 1983) with updates and supplements EPA/600/5-91-010 (Jun 1991), EPA/600/R-92-129 (Aug 1992) and EPA/600/R-93-100 (Aug 1993).
"Test Methods for Evaluating Solid Waste Physical/Chemical Methods (SW846)", Third Edition.
"Standard Methods for the Examination of Water and Wastewaters", 21st edition.
"American Society for Testing and Materials" (ASTM).
"Association of Analytical Chemists" (AOAC).

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

ANALYTICAL RESULTS

AIC No. 171032-1
Sample Identification: Plant Effluent 9-8-13 0000-2400

Analyte		Result	RL	Units	Qualifier
Total Organic Carbon		**6.1**	1	**mg/l**	
SM 5310 C	Prep: 27-Sep-2013 1516 by 308	Analyzed: 27-Sep-2013 1814 by 308		Batch: W45076	
Aluminum		**< 0.04**	0.04	**mg/l**	
EPA 200.7	Prep: 30-Sep-2013 0901 by 271	Analyzed: 01-Oct-2013 1242 by 305		Batch: S35491	
Sulfate		**29**	0.2	**mg/l**	
EPA 300.0	Prep: 27-Sep-2013 1628 by 07	Analyzed: 27-Sep-2013 2205 by 07		Batch: C16076	

AIC No. 171032-2
Sample Identification: Plant Effluent 9-10-13 0000-2400

Analyte		Result	RL	Units	Qualifier
Total Organic Carbon		**5.9**	1	**mg/l**	
SM 5310 C	Prep: 27-Sep-2013 1516 by 308	Analyzed: 27-Sep-2013 1828 by 308		Batch: W45076	
Aluminum		**0.041**	0.04	**mg/l**	
EPA 200.7	Prep: 30-Sep-2013 0901 by 271	Analyzed: 01-Oct-2013 1245 by 305		Batch: S35491	
Sulfate		**39**	0.2	**mg/l**	
EPA 300.0	Prep: 27-Sep-2013 1628 by 07	Analyzed: 27-Sep-2013 2232 by 07		Batch: C16076	

AIC No. 171032-3
Sample Identification: Plant Effluent 9-12-13 0000-2400

Analyte		Result	RL	Units	Qualifier
Total Organic Carbon		**5.4**	1	**mg/l**	
SM 5310 C	Prep: 27-Sep-2013 1516 by 308	Analyzed: 27-Sep-2013 1843 by 308		Batch: W45076	
Aluminum		**< 0.04**	0.04	**mg/l**	
EPA 200.7	Prep: 30-Sep-2013 0901 by 271	Analyzed: 01-Oct-2013 1248 by 305		Batch: S35491	
Sulfate		**34**	0.2	**mg/l**	
EPA 300.0	Prep: 27-Sep-2013 1628 by 07	Analyzed: 27-Sep-2013 2259 by 07		Batch: C16076	

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

LABORATORY CONTROL SAMPLE RESULTS

Analyte	Spike Amount	%	Limits	RPD	Limit	Batch	Preparation Date	Analysis Date	Dil	Qual
Total Organic Carbon	10 mg/l	96.4	80.0-120			W45076	27Sep13 1459 by 308	27Sep13 1648 by 308		
Aluminum	5 mg/l	97.6	85.0-115			S35491	30Sep13 0901 by 271	01Oct13 1152 by 305		
Sulfate	20 mg/l	108	90.0-110			C16076	27Sep13 1628 by 07	27Sep13 1710 by 07		

MATRIX SPIKE SAMPLE RESULTS

Analyte	Sample	Spike Amount	%	Limits	Batch	Preparation Date	Analysis Date	Dil	Qual
Total Organic Carbon	171006-1	10 mg/l	100	80.0-120	W45076	27Sep13 1459 by 308	27Sep13 1717 by 308		
	171006-1	10 mg/l	97.9	80.0-120	W45076	27Sep13 1459 by 308	27Sep13 1731 by 308		
	Relative Percent Difference:		1.86	25.0	W45076				
Aluminum	171022-2	5 mg/l	96.7	75.0-125	S35491	30Sep13 0901 by 271	01Oct13 1155 by 305		
	171022-2	5 mg/l	96.6	75.0-125	S35491	30Sep13 0901 by 271	01Oct13 1158 by 305		
	Relative Percent Difference:		0.142	20.0	S35491				
Sulfate	171021-1	20 mg/l	89.1	80.0-120	C16076	27Sep13 1628 by 07	27Sep13 1737 by 07		
	171021-1	20 mg/l	93.6	80.0-120	C16076	27Sep13 1628 by 07	27Sep13 1804 by 07		
	Relative Percent Difference:		4.28	10.0	C16076				

LABORATORY BLANK RESULTS

Analyte	Result	RL	PQL	QC Sample	Preparation Date	Analysis Date	Qual
Total Organic Carbon	< 1 mg/l	1	1	W45076-1	27Sep13 1459 by 308	27Sep13 1633 by 308	
Aluminum	< 0.04 mg/l	0.04	0.04	S35491-1	30Sep13 0901 by 271	01Oct13 1149 by 305	
Sulfate	< 0.2 mg/l	0.2	0.2	C16076-1	27Sep13 1628 by 07	27Sep13 1644 by 07	

AMERICAN INTERPLEX CORPORATION LABORATORIES

171032 wn 9/3/13
~~170741~~ wn 9/16/13

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No.: 13-3032

AIC Control No: ~~170390~~
AIC Proposal No:
Carrier: Hot Springs Couriers
Received Temperature °C: 2.1 °C

Analyses Requested: No Fathead Analysis

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL	No of BOTTLES	Chronic CD	Remarks
1	Plant Effluent	9-8-13 0000-2400		X	X		3	x	
		Container Type					P		
		Preservative					NO		

Field pH calibration
on ______ @ ______
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: ____________
Who should AIC contact with questions: ____________
Phone: ________ Fax: ____________
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By	Date/Time	Received By	Date/Time
[signature]	9-8-13 1030	M. Mann	9-9-13 10:30 am
M. Mann	9-9-13 11:45	Received in Lab By: [signature]	9-9-13 1145

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

August 30, 2013

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Outfall 001
City of Hot Springs

Control No. 169846-1

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Pimephales promelas* (Fathead minnow)
Outfall 001 - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Method 1000.0 Chronic *Pimephales promelas* (Fathead minnow) Survival and Growth Test: The No Observable Effects Concentration (NOEC) for survival occurred at 87 % effluent, which is above the critical dilution of 65 %. The NOEC for growth occurred at 87 % effluent, which is above the critical dilution of 65 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the Fathead minnow test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Table of Contents

I. Control Acceptance Criteria

Pimephales promelas (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.296	PASS
Control Growth CV < or = 40%	7.47	PASS
Growth Minimum Significant Difference 12 to 30%	20.0	PASS
Critical Dilution CV < or = 40%	11.6	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly Test Method 1000.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point: Outfall 001
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.2	8.9	8.5
pH (standard units)	7.4	7.0	7.4
Alkalinity (mg/l as CaCO3)	41	26	34
Hardness (mg/l as CaCO3)	59	67	71
Conductivity (umhos/cm)	290	350	390
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.28	0.12	0.30

2. Dilution Water Samples: Synthetic Soft Water #4012
   a. Dates Prepared: August 8 through August 22, 2013
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.7	7.7	7.8
pH (standard units)	7.7	7.8	7.9
Alkalinity (mg/l as CaCO3)	32	32	32
Hardness (mg/l as CaCO3)	47	42	42
Conductivity (umhos/cm)	180	170	170
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05

C. Test Methods
1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Method 1000.0, Fathead Minnow Survival and Growth.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Pimephales promelas (Fathead minnow) Survival and Growth Method 1000.0

Date & Time Test Initiated:	August 20, 2013 at 1200
Date & Time Test Terminated:	August 27, 2013 at 1010
Type & Volume of Test Chamber:	500 ml disposable beaker
Volume of Sample:	250 ml
Number of Organisms per replicate:	8
Number of Replicates per dilution:	5

4. Acclimation of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms
1. Scientific Name
a. Test 1000.0 *Pimephales promelas*

III. Data Analysis

The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Pimephales promelas (Fathead minnow) survival data was transformed using the Arc Sine transformation. Normality and homogeneity of variance were checked using Shapiro-Wilk's. The survival data was then analyzed using Steel's Many-One Rank Test to determine the No Observable Effects Concentration (NOEC).

Fathead minnow growth data was analyzed for normality and homogeneity of variance using Shapiro-Wilk's and Bartlett's test. Dunnett's Test was used to determine the No Observable Effects Concentration (NOEC) for growth.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Pimephales promelas (Fathead minnow)

Chronic reference tests are performed monthly.
A chronic reference test was performed on August 20, 2013 at 1715 to August 27, 2013 at 1520
The results were as follows: (Control No. 169867-1.)
Survival LC-50: 5670.1 mg/l
Growth IC-25: 3143 mg/l
Growth PMSD: 17.3

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	102	1.91
pH	SM 4500-H+ B	101	0.268
Conductivity	EPA 120.1	101	4.71

VI. Organism History

Pimephales promelas (Fathead minnow)
Date: August 20, 2013
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

VII. Results Summary *Pimephales promelas*, Fathead minnow Larval Survival and Growth Test -- Method 1000.0

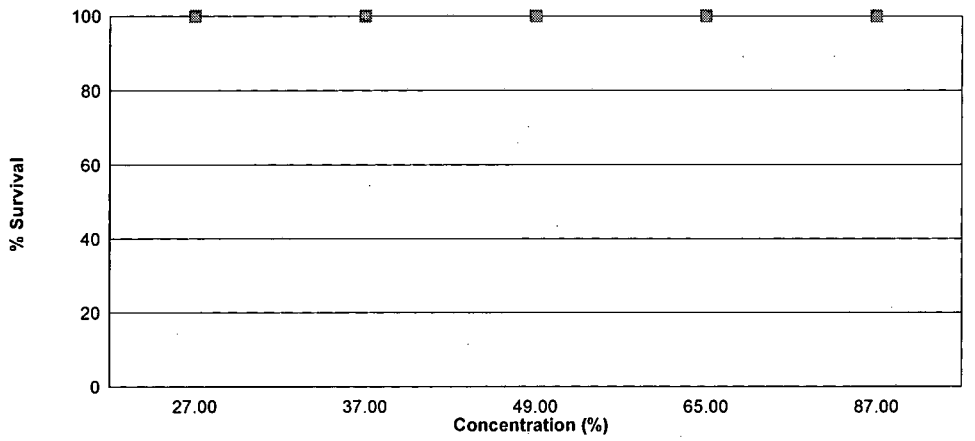
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on August 20, 2013 at 1200 and continued through August 27, 2013 at 1010. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 87 % effluent
b.) NOEC growth = 87 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.296
27 %	100	0.337
37 %	100	0.323
49 %	100	0.302
65 %	100	0.292
87 %	100	0.302

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: August 20, 2013 at 1200
Date and Time Test Terminated: August 27, 2013 at 1010

Concentration	Replicate	Number of Survivors						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
27 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
37 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
49 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
65 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
87 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: August 20, 2013 at 1200
Test Terminated: August 27, 2013 at 1010
Drying Started: August 23, 2013 at 1527
Drying Ended: August 29, 2013 at 1400

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.91827	.92042	0.00215	8	0.269
	B	.91878	.92121	0.00243	8	0.304
	C	.91659	.91922	0.00263	8	0.329
	D	.91638	.91872	0.00234	8	0.292
	E	.91455	.91685	0.00230	8	0.288
27 %	A	.91583	.91811	0.00228	8	0.285
	B	.91943	.92233	0.00290	8	0.362
	C	.92408	.92709	0.00301	8	0.376
	D	.92651	.92924	0.00273	8	0.341
	E	.92767	.93024	0.00257	8	0.321
37 %	A	.92653	.92915	0.00262	8	0.328
	B	.94872	.95160	0.00288	8	0.360
	C	.94724	.94985	0.00261	8	0.326
	D	.94532	.94750	0.00218	8	0.272
	E	.94881	.95146	0.00265	8	0.331
49 %	A	.94115	.94330	0.00215	8	0.269
	B	.92530	.92779	0.00249	8	0.311
	C	.92632	.92901	0.00269	8	0.336
	D	.93070	.93312	0.00242	8	0.302
	E	.93427	.93660	0.00233	8	0.291
65 %	A	.93862	.94107	0.00245	8	0.306
	B	.93944	.94155	0.00211	8	0.264
	C	.94132	.94377	0.00245	8	0.306
	D	.93810	.94077	0.00267	8	0.334
	E	.93899	.94100	0.00201	8	0.251
87 %	A	.93682	.93952	0.00270	8	0.338
	B	.93818	.93991	0.00173	8	0.216
	C	.93688	.93881	0.00193	8	0.241
	D	.93804	.94073	0.00269	8	0.336
	E	.95233	.95537	0.00304	8	0.380

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

		Transformation of Data		Transform: Arc Sin(Square Root(Y))
Group	Identification	Rep	Value	Transformed
1	Control	1	1.00000	1.39310
1	Control	2	1.00000	1.39310
1	Control	3	1.00000	1.39310
1	Control	4	1.00000	1.39310
1	Control	5	1.00000	1.39310
2	27 %	1	1.00000	1.39310
2	27 %	2	1.00000	1.39310
2	27 %	3	1.00000	1.39310
2	27 %	4	1.00000	1.39310
2	27 %	5	1.00000	1.39310
3	37 %	1	1.00000	1.39310
3	37 %	2	1.00000	1.39310
3	37 %	3	1.00000	1.39310
3	37 %	4	1.00000	1.39310
3	37 %	5	1.00000	1.39310
4	49 %	1	1.00000	1.39310
4	49 %	2	1.00000	1.39310
4	49 %	3	1.00000	1.39310
4	49 %	4	1.00000	1.39310
4	49 %	5	1.00000	1.39310
5	65 %	1	1.00000	1.39310
5	65 %	2	1.00000	1.39310
5	65 %	3	1.00000	1.39310
5	65 %	4	1.00000	1.39310
5	65 %	5	1.00000	1.39310
6	87 %	1	1.00000	1.39310
6	87 %	2	1.00000	1.39310
6	87 %	3	1.00000	1.39310
6	87 %	4	1.00000	1.39310
6	87 %	5	1.00000	1.39310

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Shapiro - Wilk's Test for Normality		Transform: Arc Sin(Square Root(Y))
D = 0		
W = 0		
Critical W = 0.9	(alpha = 0.01, N = 30)	
Critical W = 0.927	(alpha = 0.05, N = 30)	

Data FAIL normality test (alpha = 0.01).

Steel's Many-One Rank Test — Transform: Arc Sin(Square Root(Y))

Ho:Control<Treatment

Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	27 %	27.50	16.00	5.00	
3	37 %	27.50	16.00	5.00	
4	49 %	27.50	16.00	5.00	
5	65 %	27.50	16.00	5.00	
6	87 %	27.50	16.00	5.00	

Critical values are 1 tailed (k=5)

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality		No Transformation
D = 0.03787		
W = 0.9742		
Critical W = 0.9	(alpha = 0.01, N = 30)	
Critical W = 0.927	(alpha = 0.05, N = 30)	
Data PASS normality test (alpha = 0.01).		

Bartlett's Test for Homogeneity of Variance		No Transformation
Calculated B1 statistic = 7.076		
Critical B = 15.086	(alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.		

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	0.007651	0.00153	0.9696
Within (Error)	24	0.03787	0.001578	
Total	29	0.04552		

Critical F = 3.9 (alpha = 0.01, df = 5,24)
2.62 (alpha = 0.05, df = 5,24)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Dunnett's Test - Table 1 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	0.2964	0.2964		
2	27 %	0.337	0.337	-1.616	
3	37 %	0.3234	0.3234	-1.075	
4	49 %	0.3018	0.3018	-0.2149	
5	65 %	0.2922	0.2922	0.1672	
6	87 %	0.3022	0.3022	-0.2309	

Dunnett's critical value = 2.36 (1 Tailed, alpha = 0.05, df = 5,24)

Dunnett's Test - Table 2 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	5			
2	27 %	5	0.05929	20	-0.0406
3	37 %	5	0.05929	20	-0.027
4	49 %	5	0.05929	20	-0.0054
5	65 %	5	0.05929	20	0.0042
6	87 %	5	0.05929	20	-0.0058

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 20, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.5	7.7	7.5	7.8	7.8	8.1
	Final	7.5	7.2	7.5	7.9	7.7	7.1	7.9
pH, units	Initial	7.7	7.7	7.8	8.0	7.9	8.0	7.7
	Final	7.5	7.3	7.8	8.1	8.1	7.6	7.9
Alkalinity, mg CaCO3/l		32	NA	32	NA	32	NA	NA
Hardness, mg CaCO3/l		47	NA	42	NA	42	NA	NA
Conductivity, umhos/cm		180	180	170	180	170	170	190
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.4	8.0	7.5	8.0	8.0	7.5
	Final	7.5	7.2	7.3	7.9	7.8	8.1	7.7
pH, units	Initial	7.6	7.7	7.5	7.8	7.7	7.8	7.5
	Final	7.5	7.3	7.7	8.1	8.2	7.5	7.7

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.6	8.6	7.6	8.2	7.7	7.7
	Final	7.5	7.0	7.5	8.0	7.9	8.1	7.8
pH, units	Initial	7.6	7.7	7.4	7.7	7.6	7.7	7.6
	Final	7.5	7.3	7.7	8.1	8.2	7.5	7.6

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 20, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.5	8.7	7.6	8.1	7.7	7.7
	Final	7.5	7.4	7.6	8.0	8.1	8.2	7.8
pH, units	Initial	7.5	7.7	7.3	7.6	7.6	7.6	7.5
	Final	7.5	7.4	7.6	8.1	8.2	7.6	7.6

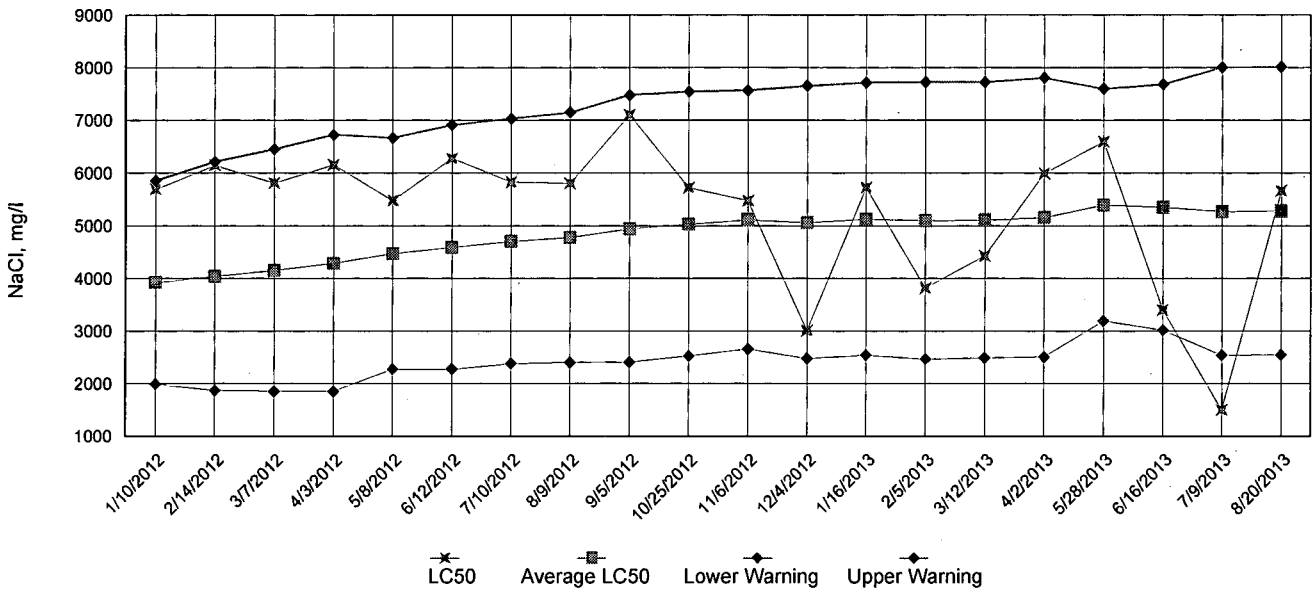
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.9	7.4	8.8	7.6	8.7	7.7	7.3
	Final	7.5	7.4	7.4	7.9	8.1	8.0	7.7
pH, units	Initial	7.5	7.7	7.2	7.5	7.5	7.6	7.5
	Final	7.5	7.4	7.6	8.1	8.3	7.6	7.5
Alkalinity, mg CaCO3/l		36	NA	30	NA	35	NA	NA
Hardness, mg CaCO3/l		54	NA	58	NA	61	NA	NA
Conductivity, umhos/cm		250	260	280	290	310	310	330
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	7.4	8.9	7.6	8.3	7.9	7.4
	Final	7.5	8.5	7.4	7.8	8.1	8.0	7.7
pH, units	Initial	7.4	7.7	7.1	7.4	7.4	7.6	7.6
	Final	7.5	7.4	7.6	8.1	8.1	7.6	7.4

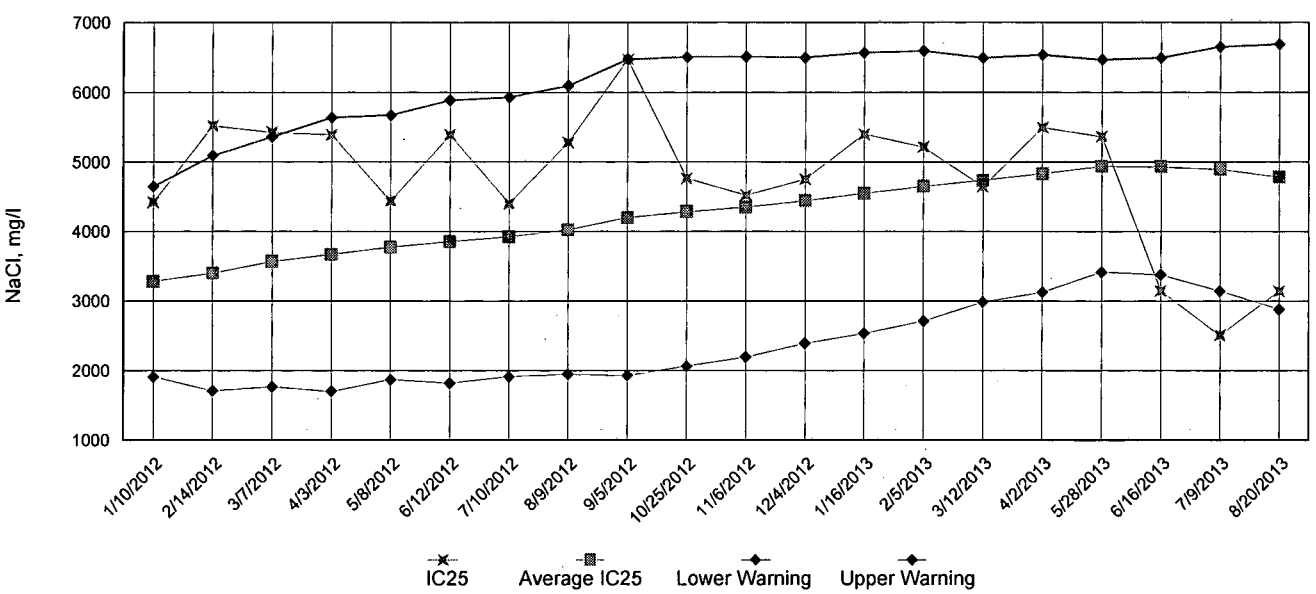
Appendix A4: Test 1000.0

Chronic Reference Toxicant, *Pimephales promelas* (Fathead Minnow)

LC50 Survival Data



IC25 Growth Data



Appendix B: Test 1000.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

Permittee: City of Hot Springs

NPDES No.: AR0033880 AFIN#26-00145

Date and Time Test Initiated: August 20, 2013 at 1200
Date and Time Test Terminated: August 27, 2013 at 1010
Dilution water used: Synthetic Soft Water #4012

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in replicate chambers A	B	C	D	E	Mean percent survival 24 hr	48 hr	7 days	CV%
Control	100	100	100	100	100	100	100	100	0.00
27 %	100	100	100	100	100	100	100	100	0.00
37 %	100	100	100	100	100	100	100	100	0.00
49 %	100	100	100	100	100	100	100	100	0.00
65 %	100	100	100	100	100	100	100	100	0.00
87 %	100	100	100	100	100	100	100	100	0.00

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers A	B	C	D	E	Mean dry weight, mg	CV%
Control	0.269	0.304	0.329	0.292	0.288	0.296	7.47
27 %	0.285	0.362	0.376	0.341	0.321	0.337	10.6
37 %	0.328	0.360	0.326	0.272	0.331	0.323	9.86
49 %	0.269	0.311	0.336	0.302	0.291	0.302	8.20
65 %	0.306	0.264	0.306	0.334	0.251	0.292	11.6
87 %	0.338	0.216	0.241	0.336	0.380	0.302	23.2

CV = Coefficient of variation = standard deviation * 100 / mean

Appendix B: Test 1000.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

1. Steel's Many-One Rank Test:

   Is the mean survival significantly different (p=0.05) than the control survival for the % effluent corresponding to (lethality):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	____YES	X NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____YES	____NO

2. Dunnett's Test:

   Is the mean dry weight (growth) significantly different (p=0.05) than the control's dry weight (growth) for the % effluent corresponding to (significant non-lethal effects):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	____YES	X NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____YES	____NO

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

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

5. NOEC Pimephales Lethality: 87 % (TOP6C)

6. LOEC Pimephales Lethality: 87 % (TXP6C)

7. NOEC Pimephales Sublethality: 87 % (TPP6C)

8. LOEC Pimephales Sublethality: 87 % (TYP6C)

9. Coefficient of variation for Pimephales growth: 11.6 (TQP6C)

AMERICAN INTERPLEX CORPORATION
LABORATORIES

Appendix B: Test 1000.0

CHRONIC TOXICITY SUMMARY FORM
Pimephales promelas (Fathead minnow)
CHEMICAL PARAMETERS CHART

PERMITTEE: City of Hot Springs 2400
NPDES NO.: AR0033880 AFIN#26-00145 2400
CONTACT: Mr. James Sorrells 2400
ANALYST: 280, 298, 304, 307

Test Initiated: DATE: August 20, 2013 TIME: 1200
Test Terminated: DATE: August 27, 2013 TIME: 1010

DILUTION Control	DAY 1	2	3	4	5	6	7
D.O. Initial	7.7	7.5	7.7	7.5	7.8	7.8	8.1
Final	7.5	7.2	7.5	7.9	7.7	7.1	7.9
pH Initial	7.7	7.7	7.8	8.0	7.9	8.0	7.7
Final	7.5	7.3	7.8	8.1	8.1	7.6	7.9
Alkalinity	32	NA	32	NA	32	NA	NA
Hardness	47	NA	42	NA	42	NA	NA
Conductivity	180	180	170	180	170	170	190
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 27 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.7	7.4	8.0	7.5	8.0	8.0	7.5
Final	7.5	7.2	7.3	7.9	7.8	8.1	7.7
pH Initial	7.6	7.7	7.5	7.8	7.7	7.8	7.5
Final	7.5	7.3	7.7	8.1	8.2	7.5	7.7
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	220	220	220	230	230	250
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 37 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.8	7.6	8.6	7.6	8.2	7.7	7.7
Final	7.5	7.0	7.5	8.0	7.9	8.1	7.8
pH Initial	7.6	7.7	7.4	7.7	7.6	7.7	7.6
Final	7.5	7.3	7.7	8.1	8.2	7.5	7.6
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	220	230	240	240	260	250	270
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 49 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.8	7.5	8.7	7.6	8.1	7.7	7.7
Final	7.5	7.4	7.6	8.0	8.1	8.2	7.8
pH Initial	7.5	7.7	7.3	7.6	7.6	7.6	7.5
Final	7.5	7.4	7.6	8.1	8.2	7.6	7.6
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	230	240	260	260	280	280	300
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 65 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.9	7.4	8.8	7.6	8.7	7.7	7.3
Final	7.5	7.4	7.4	7.9	8.1	8.0	7.7
pH Initial	7.5	7.7	7.2	7.5	7.5	7.6	7.5
Final	7.5	7.4	7.6	8.1	8.3	7.6	7.5
Alkalinity	36	NA	30	NA	35	NA	NA
Hardness	54	NA	58	NA	61	NA	NA
Conductivity	250	260	280	290	310	310	330
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 87 %	DAY 1	2	3	4	5	6	7
D.O. Initial	8.0	7.4	8.9	7.6	8.3	7.9	7.4
Final	7.5	8.5	7.4	7.8	8.1	8.0	7.7
pH Initial	7.4	7.7	7.1	7.4	7.4	7.6	7.6
Final	7.5	7.4	7.6	8.1	8.1	7.6	7.4
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	280	290	320	320	360	360	380
Chlorine	NA	NA	NA	NA	NA	NA	NA

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client:	City of Hot Springs
Project Reference:	Plant Effluent
Project Manager:	James Sorrells
Sampled By:	A. Ross
PO No.	13-3032
AIC Control No:	169846
AIC Proposal No:	
Carrier:	Hot Springs Courier
Received Temperature °C	2°C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL		No of BOTTLES	Chronic.CD, Chronic.FH											Remarks
1	Plant Effluent	8-18-13 0000-2400		X	X			3	x											
		Container Type							P											Field pH calibration on ____ @ ____
		Preservative							NO											Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate

NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: ____
Who should AIC contact with questions: ____
Phone: ____ Fax: ____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By	Date/Time	Received By	Date/Time
A. Thomason	8-19-13 @ 1010	M. Mann	8-19-13 10:10am

Relinquished By	Date/Time	Received In Lab By	Date/Time
M. Mann	8-19-13 @ 11:10AM	[signature]	8-19-13 1110

Comments:

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 2.3C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH	Remarks
2	Plant Effluent	8-20-13 0000-2400		X	X		3	X	
		Container Type						P	Field pH calibration
		Preservative						NO	on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
A. Ross	8-21-13 0900	A. Man	8-21-13 9:10
A. Man	8-21-13 1130	Jimmy Day	8/21/13 1130

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By:

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL		No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
3	Plant Effluent	8/22/13 0000-2400		X				3	X
		Container Type							P
		Preservative							NO

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 2
Remarks
Field pH calibration on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	8-23-13 0955	[signature]	8-23-13 9:55
[signature]	8-23-13 11:20	[signature]	8-23-13 1120

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

September 19, 2013

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Plant Effluent
City of Hot Springs

Control No. 170390-1

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Ceriodaphnia dubia*
Plant Effluent - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: The No Observable Effects Concentration (NOEC) for survival occurred at <27 % effluent, which is below the critical dilution of 65 %. The NOEC for reproduction occurred at <27 % effluent, which is below the critical dilution of 65 %. **The sample, therefore, FAILED both lethal and sub-lethal effects for the *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Table of Contents

I. Control Acceptance Criteria

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	18.8	PASS
Control CV < or = 40% per Surviving Female	20.6	PASS
Reproduction Minimum Significant Difference 13 to 47%	NA	NA
Critical Dilution CV < or = 40%	0.00	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly Test Method 1002.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point: Plant Effluent
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.7	8.2	8.2
pH (standard units)	7.4	7.7	7.5
Alkalinity (mg/l as CaCO3)	64	58	64
Hardness (mg/l as CaCO3)	82	82	83
Conductivity (umhos/cm)	380	370	440
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	2.4	0.11

2. Dilution Water Samples: Synthetic Soft Water #4019
   a. Dates Prepared: August 28 through September 11, 2013
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.6	8.2	8.2
pH (standard units)	7.6	7.8	7.6
Alkalinity (mg/l as CaCO3)	32	32	32
Hardness (mg/l as CaCO3)	46	42	42
Conductivity (umhos/cm)	160	160	180
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05

C. Test Methods
1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Method 1002.0, *Ceriodaphnia dubia* Survival and Reproduction.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Ceriodaphnia dubia Survival and Growth Method 1002.0

Date & Time Test Initiated:	September 9, 2013 at 1235
Date & Time Test Terminated:	September 17, 2013 at 1255
Type & Volume of Test Chamber:	30 ml disposable beaker
Volume of Sample:	15 ml
Number of Organisms per replicate:	1
Number of Replicates per dilution:	10

4. Acclimation of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms
1. Scientific Name
a. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis
The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was not analyzed due to survival failure.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Ceriodaphnia dubia

Chronic reference tests are performed monthly.
A chronic reference test was performed on August 20, 2013 at 1505 to August 28, 2013 at 1450
The results were as follows: (Control No. 169867-2.)
Survival LC-50: 2125 mg/l
Growth IC-25: 1610 mg/l
Growth PMSD: 18.3

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	1.53
Hardness	EPA 200.7	101	0.450
pH	SM 4500-H+ B	101	0.267
Conductivity	EPA 120.1	103	1.97

VI. Organism History

Ceriodaphnia dubia

Date: September 9, 2013
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

VII. Results Summary *Ceriodaphnia dubia*, Cladoceran Survival and Reproduction Test -- Method 1002.0

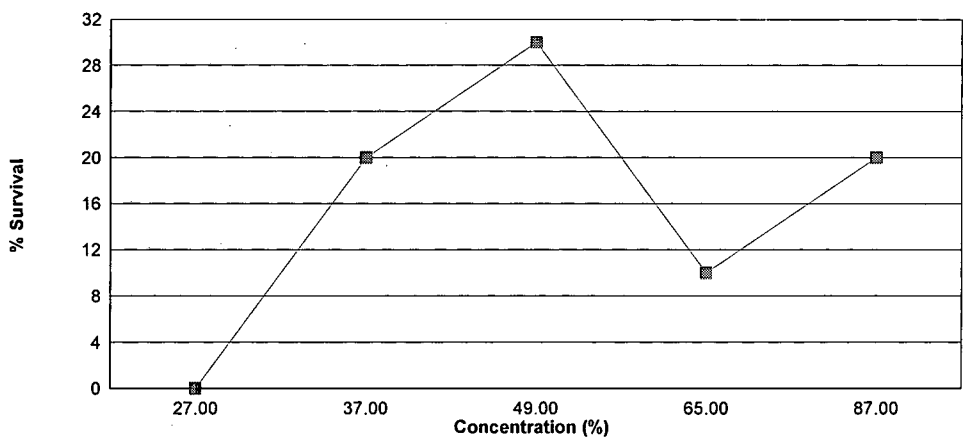
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on September 9, 2013 at 1235 and continued through September 17, 2013 at 1255. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = <27 % effluent
b.) NOEC reproduction = <27 % effluent



Summary of the 7-day *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	18.8
27 %	0.00 *	--
37 %	20.0 *	--
49 %	30.0 *	--
65 %	10.0 *	--
87 %	20.0 *	--

*Significant difference when compared to the control (p=0.05)

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 9, 2013 at 1235
Date and Time Test Terminated: September 17, 2013 at 1255

Concentration: Control													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	3	2	0	0	0	0	1	4	2	1	13	10	1.30
5	9	0	3	6	6	4	0	4	6	0	38	10	3.80
6	0	5	9	8	7	0	6	0	1	7	43	10	4.30
7	10	9	10	10	10	8	9	8	10	10	94	10	9.40
8													
TOTAL	22	16	22	24	23	12	16	16	19	18	188	10	18.8

Concentration: 27 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	0	0	0	X	0	0	0	X	0	0	7	0.00
2	X	0	0	0	X	0	0	0	X	0	0	7	0.00
3	X				X				X		0	0	0.00
4	X				X				X		0	0	0.00
5	X				X				X		0	0	0.00
6	X				X				X		0	0	0.00
7	X				X				X		0	0	0.00
8													
TOTAL	0	0	0	0	0	0	0	0	0	0	0	10	0.00

Concentration: 37 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	X	X	0	X	X	0	0	3	0.00
2	X	X	0	X	X	X	0	X	X	0	0	3	0.00
3	X	X	X	X	X	X	0	X	X	0	0	2	0.00
4	X	X	X	X	X	X	2	X	X	1	3	2	1.50
5	X	X	X	X	X	X	0	X	X	0	0	2	0.00
6	X	X	X	X	X	X	7	X	X	6	13	2	6.50
7	X	X	X	X	X	X	10	X	X	0	10	2	5.00
8													
TOTAL	0	0	0	0	0	0	19	0	0	7	26	10	2.60

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 9, 2013 at 1235
Date and Time Test Terminated: September 17, 2013 at 1255

Concentration: 49 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	X	X	0	X	X	0	0	3	0.00
2	X	X	0	X	X	X	0	X	X	0	0	3	0.00
3	X	X	0	X	X	X	0	X	X	0	0	3	0.00
4	X	X	0	X	X	X	0	X	X	0	0	3	0.00
5	X	X	4	X	X	X	5	X	X	0	9	3	3.00
6	X	X	0	X	X	X	0	X	X	6	6	3	2.00
7	X	X	6	X	X	X	8	X	X	0	14	3	4.67
8													
TOTAL	0	0	10	0	0	0	13	0	0	6	29	10	2.90

Concentration: 65 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	X	X	X	X	X	0	0	2	0.00
2	X	X	0	X	X	X	X	X	X	0	0	2	0.00
3	X	X	0	X	X	X	X	X	X	0	0	2	0.00
4	X	X	0	X	X	X	X	X	X	0	0	2	0.00
5	X	X	6	X	X	X	X	X	X	0	6	2	3.00
6	X	X	0	X	X	X	X	X	X	X	0	1	0.00
7	X	X	9	X	X	X	X	X	X	X	9	1	9.00
8													
TOTAL	0	0	15	0	0	0	0	0	0	0	15	10	1.50

Concentration: 87 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	0	X	0	X	X	0	0	4	0.00
2	X	X	0	X	0	X	X	X	X	0	0	3	0.00
3	X	X	0	X	0	X	X	X	X	0	0	3	0.00
4	X	X	0	X	3	X	X	X	X	1	4	3	1.33
5	X	X	7	X	5	X	X	X	X	0	12	3	4.00
6	X	X	0	X	X	X	X	X	X	3	3	2	1.50
7	X	X	9	X	X	X	X	X	X	0	9	2	4.50
8													
TOTAL	0	0	16	0	8	0	0	0	0	4	28	10	2.80

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
27 %	0	3	3
Total	10	3	13

Critical Fisher's value (10,3,10) (alpha=0.05) is 1. b value is 0. Since b is less than or equal to 1 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
37 %	2	8	10
Total	12	8	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 2. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
49 %	3	7	10
Total	13	7	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 3. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
65 %	1	9	10
Total	11	9	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 1. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
87 %	2	8	10
Total	12	8	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 2. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	27 %	3	3	*
2	37 %	10	8	*
3	49 %	10	7	*
4	65 %	10	9	*
5	87 %	10	8	*

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: September 9, 2013 at 0857
Date and Time Test Terminated: September 17, 2013 at 1255

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.6	7.4	8.2	8.3	8.2	8.3	8.2
	Final	7.8	8.3	8.5	8.7	8.4	8.2	7.8
pH, units	Initial	7.6	7.6	7.8	7.6	7.6	7.7	7.5
	Final	7.8	7.9	7.9	7.6	7.9	7.8	8.0
Alkalinity, mg CaCO3/l		32	NA	32	NA	32	NA	NA
Hardness, mg CaCO3/l		46	NA	42	NA	42	NA	NA
Conductivity, umhos/cm		160	170	160	180	180	180	170
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.6	7.5	8.2	8.4	8.3	8.1	8.3
	Final	6.0	8.3	8.7	8.8	8.5	8.3	8.1
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
	Final	8.0	8.1	8.2	8.0	8.3	8.0	8.1

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.6	7.4	8.2	8.4	8.1	8.0	8.1
	Final	6.4	8.3	8.6	8.8	8.6	8.2	8.2
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
	Final	8.1	8.2	8.2	8.2	8.2	7.9	7.8

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: September 9, 2013 at 0857
Date and Time Test Terminated: September 17, 2013 at 1255

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.5	7.5	8.2	8.4	8.4	8.1	8.3
	Final	6.8	8.3	7.8	9.0	8.4	8.3	8.0
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.5	7.5
	Final	8.2	8.2	8.2	8.1	8.1	8.0	8.1

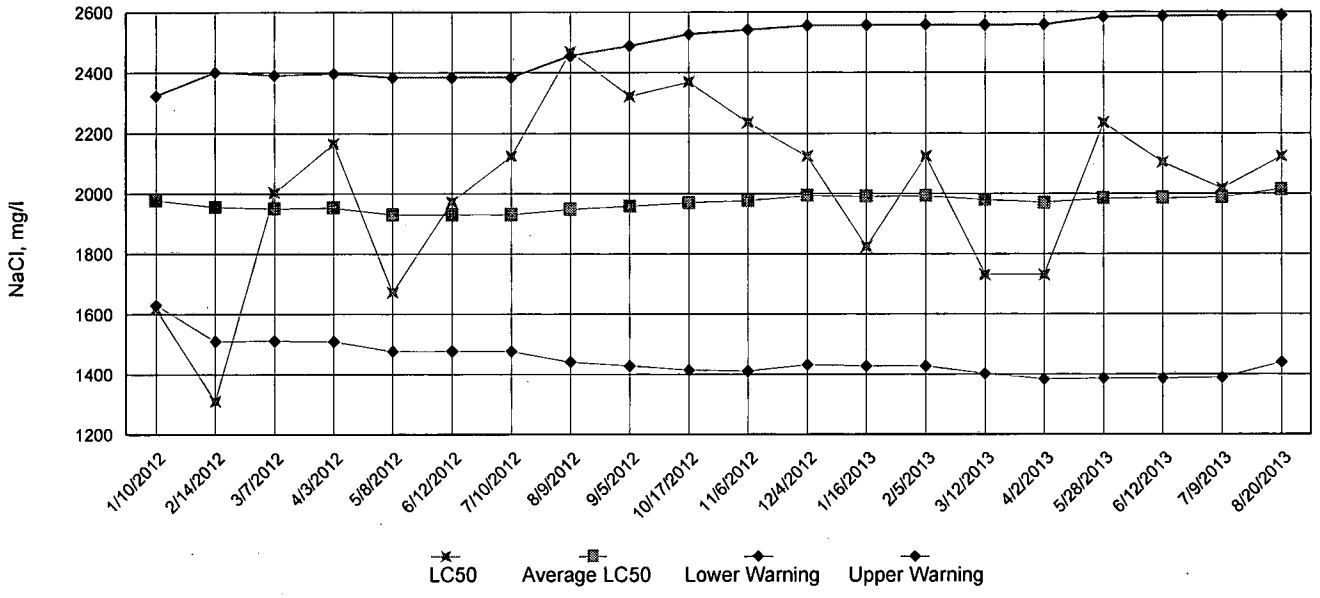
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.4	8.2	8.4	8.0	8.2	8.3
	Final	7.0	8.3	8.4	8.7	8.3	8.3	7.7
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.6
	Final	8.2	8.1	8.1	8.2	8.0	8.0	7.7
Alkalinity, mg CaCO3/l		48	NA	46	NA	47	NA	NA
Hardness, mg CaCO3/l		68	NA	68	NA	68	NA	NA
Conductivity, umhos/cm		310	310	300	340	340	340	350
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.3	7.2	8.2	8.3	8.3	8.0	7.9
	Final	7.1	8.3	8.6	8.8	7.3	8.3	8.0
pH, units	Initial	7.5	7.4	7.8	7.5	7.6	7.5	7.6
	Final	8.2	8.3	8.3	8.3	7.5	8.1	7.9

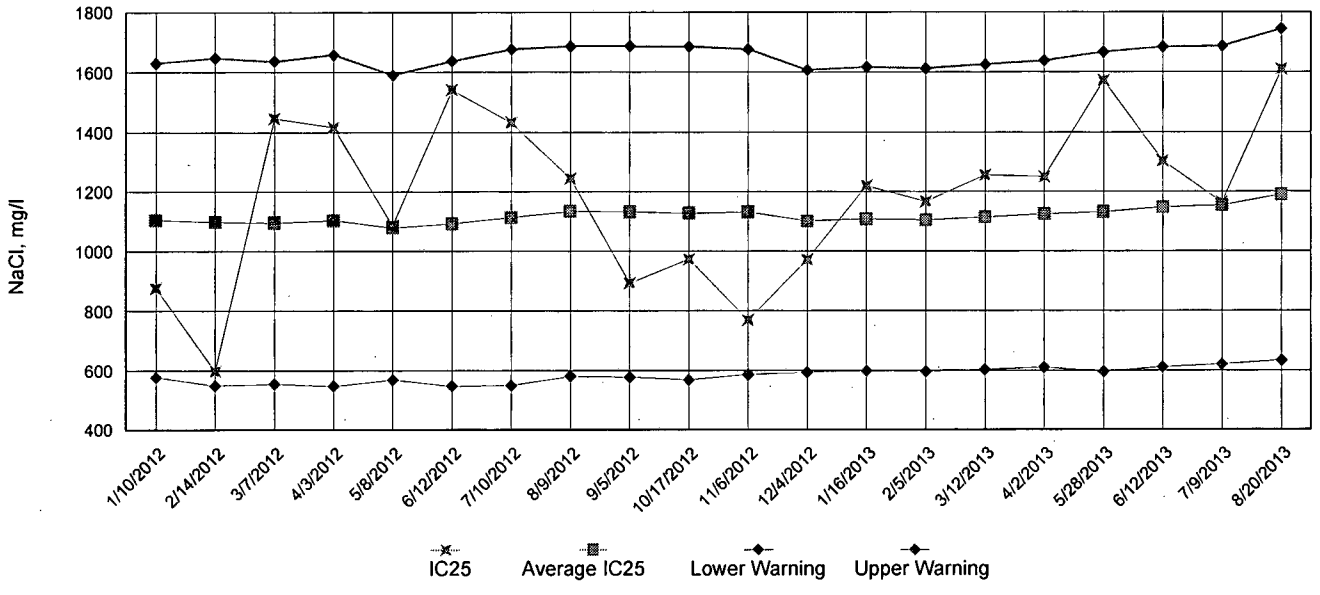
Appendix A4: Test 1002.0

Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

Permittee: City of Hot Springs

NPDES No.: AR0033880 AFIN#26-00145

Date and Time Test Initiated: September 9, 2013 at 1235
Date and Time Test Terminated: September 17, 2013 at 1255
Dilution water used: Synthetic Soft Water #4019

PERCENT SURVIVAL

		Percent Effluent				
Time of Reading	Control	27 %	37 %	49 %	65 %	87 %
24 hour	100	70.0	30.0	30.0	20.0	40.0
48 hour	100	70.0	30.0	30.0	20.0	30.0
7 day	100	0.00	20.0	30.0	10.0	20.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

		Percent Effluent				
Replicates	Control	27 %	37 %	49 %	65 %	87 %
A	22	0	0	0	0	0
B	16	0	0	0	0	0
C	22	0	0	10	15	16
D	24	0	0	0	0	0
E	23	0	0	0	0	8
F	12	0	0	0	0	0
G	16	0	19	13	0	0
H	16	0	0	0	0	0
I	19	0	0	0	0	0
J	18	0	7	6	0	4
Mean per Adult	18.8	0.00	2.60	2.90	1.50	2.80
Mean per Surviving Adult	18.8	0.00	13.0	9.67	15.0	10.0
CV %	20.6	0.00	65.3	36.3	0.00	84.9

CV = Coefficient of variation = standard deviation * 100 / mean
(calculated based on young produced by surviving females)

Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

1. Fisher's Exact Test:

   Is the mean survival significantly different (p=0.05) than the control survival for the % effluent corresponding to (lethality):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	X YES	____ NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____ YES	____ NO

2.

   Is the mean number of young produced per female significantly different (p=0.05) than the control's number of young per female for the % effluent corresponding to (significant non-lethal effects):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	X YES	____ NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____ YES	____ NO

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 1 (TLP3B)
4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 1 (TGP3B)
5. NOEC Ceriodaphnia Lethality: <27 % (TOP3B)
6. LOEC Ceriodaphnia Lethality: 27 % (TXP3B)
7. NOEC Ceriodaphnia Sublethality: <27 % (TPP3B)
8. LOEC Ceriodaphnia Sublethality: 27 % (TYP3B)
9. Coefficient of variation for Ceriodaphnia Reproduction: 20.6 (TQP3B)

Appendix B: Test 1002.0

CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
CHEMICAL PARAMETERS CHART

PERMITTEE: City of Hot Springs
NPDES NO.: AR0033880 AFIN#26-00145
CONTACT: Mr. James Sorrells
ANALYST: 280, 298, 304, 307

2400
2400
2400

Test Initiated: DATE: September 9, 2013 TIME: 1235
Test Terminated: DATE: September 17, 2013 TIME: 1255

DILUTION	DAY						
Control	1	2	3	4	5	6	7
D.O. Initial	8.6	7.4	8.2	8.3	8.2	8.3	8.2
Final	7.8	8.3	8.5	8.7	8.4	8.2	7.8
pH Initial	7.6	7.6	7.8	7.6	7.6	7.7	7.5
Final	7.8	7.9	7.9	7.6	7.9	7.8	8.0
Alkalinity	32	NA	32	NA	32	NA	NA
Hardness	46	NA	42	NA	42	NA	NA
Conductivity	160	170	160	180	180	180	170
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
27 %	1	2	3	4	5	6	7
D.O. Initial	8.6	7.5	8.2	8.4	8.3	8.1	8.3
Final	6.0	8.3	8.7	8.8	8.5	8.3	8.1
pH Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
Final	8.0	8.1	8.2	8.0	8.3	8.0	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	220	220	220	240	250	250	250
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
37 %	1	2	3	4	5	6	7
D.O. Initial	8.6	7.4	8.2	8.4	8.1	8.0	8.1
Final	6.4	8.3	8.6	8.8	8.6	8.2	8.2
pH Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
Final	8.1	8.2	8.2	8.2	8.2	7.9	7.8
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	240	240	240	260	270	270	280
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
49 %	1	2	3	4	5	6	7
D.O. Initial	8.5	7.5	8.2	8.4	8.4	8.1	8.3
Final	6.8	8.3	7.8	9.0	8.4	8.3	8.0
pH Initial	7.6	7.5	7.8	7.5	7.6	7.5	7.5
Final	8.2	8.2	8.2	8.1	8.1	8.0	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	270	270	270	300	300	300	300
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
65 %	1	2	3	4	5	6	7
D.O. Initial	7.7	7.4	8.2	8.4	8.0	8.2	8.3
Final	7.0	8.3	8.4	8.7	8.3	8.3	7.7
pH Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.6
Final	8.2	8.1	8.1	8.2	8.0	8.0	7.7
Alkalinity	48	NA	46	NA	47	NA	NA
Hardness	68	NA	68	NA	68	NA	NA
Conductivity	310	310	300	340	340	340	350
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
87 %	1	2	3	4	5	6	7
D.O. Initial	8.3	7.2	8.2	8.3	8.3	8.0	7.9
Final	7.1	8.3	8.6	8.8	7.3	8.3	8.0
pH Initial	7.5	7.4	7.8	7.5	7.6	7.5	7.6
Final	8.2	8.3	8.3	8.3	7.5	8.1	7.9
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	350	360	350	400	440	430	440
Chlorine	NA	NA	NA	NA	NA	NA	NA

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Roys

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Chronic C.D.	Analyses Requested
1	Plant Effluent	9-8-13 2000-2400		X	X		3	X	No Fathead Analysis
		Container Type						P	
		Preservative						NO	

AIC Control No: 170390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2.1°C
Remarks:
Field pH calibration on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-8-13 1030	M. Mann	9-9-13 10:30 am
M. Mann	9-9-13 11:45	[signature]	9-9-13 1145

Comments:

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC Control No: 170390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 3°C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH	Remarks
1	Plant Effluent	9-10-13 0000-2400		X	X		3	X	
		Container Type						P	Field pH calibration
		Preservative						NO	on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-11-13 1110	M. Mann	9-11-13 1110
M. Mann	9-11-13 @ 12:05	[signature]	9-11-13 1205

Comments:

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client	City of Hot Springs
Project Reference:	Plant Effluent
Project Manager:	James Sorrells
Sampled By:	HAROLD MAULDIN
PO No.	13-3032
AIC Control No:	170390
AIC Proposal No:	
Carrier:	Hot Springs Courier
Received Temperature °C	2

Analyses Requested

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER (Sample Matrix)	SOIL (Sample Matrix)		No of BOTTLES	Chronic.CD.												Remarks
3	PLANT Effluent	9/12/13 0000-2400		X	X			3	x												
		Container Type							P												Field pH calibration
		Preservative							NO												on ______ @ _____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate

NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: ____
Who should AIC contact with questions: ____
Phone: ____ Fax: ____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
A. Thomason	9-13-13 @ 1015	M. Mann	9-13-13 @ 10:15
Relinquished By: M. Mann	9-13-13 @ 11:20	Received in Lab By: [signature]	9-13-13 1120

Comments:

4-85

AMERICAN INTERPLEX CORPORATION
LABORATORIES

September 30, 2013

Test Results of
Acute 48 hour Non-Renewal
Biomonitoring Testing
for

170741-1: Outfall 001 First Renewal
170741-2: Outfall 001 Second Renewal
170741-3: Outfall 001 Third Renewal
170741-4: Outfall 001 Original Screen

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Acute 48 hour Non-Renewal Biomonitoring utilizing *Ceriodaphnia dubia*
Outfall 001 First Renewal - City of Hot Springs
Client NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

Please find attached the results of the acute biomonitoring screens conducted as a follow up to the chronic toxicity event previously reported (AIC Control No. 170390). As indicated on the enclosed results, the sample was still acutely toxic on September 12, 2013 and exhibited no acute toxicity on September 19, 2013.

It it theorized the decrease in toxicity is directly related to the holding time which may have allowed microbial degradation, loss of volatile toxics, and/or biochemical degradation.

If I can be of further assistance, please feel free to contact me.

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Dilution Water Samples: Synthetic Soft Water #4021

Analysis	Result
Dissolved oxygen (mg/l)	7.6
pH (standard units)	7.7
Alkalinity (mg/l as CaCO3)	31
Hardness (mg/l as CaCO3)	46
Conductivity (umhos/cm)	170
Residual Chlorine (mg/l)	<0.05

Results Summary: Outfall 001 First Renewal

Ceriodaphnia dubia

The *Ceriodaphnia dubia* test was conducted from September 19, 2013 at 1615 to September 21, 2013 at 1700.

Statistical analyses:

NOEC = 100%

Concentration	24 hour % Survival	48 hour % Survival
Control	100	100
100%	100	100

Ceriodaphnia dubia
Survival Data

Number of organisms per chamber: 5
Volume of test chamber: 30 ml

Age of organisms: <24 hours
Volume of test solution: 15 ml

Effluent Concentration	Number of Survivors 24 Hours	48 Hours	% Survival	CV %
Control rep. A	5	5	100	0.00
100% rep. A	5	5	100	0.00

CV = Coefficient of variance = standard deviation X 100/mean

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	5	0	5
100%	5	0	5
Total	10	0	10

Critical Fisher's value (5,5,5) (alpha=0.05) is 1. b value is 5. Since b is greater than 1 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test

Group	Identification	Exposed	Dead	Sig 0.05
0	0	5	0	
1	100	5	0	

Chemical Data for
Ceriodaphnia dubia

Day 1	Control	100%
DO, mg/l	7.6	8.8
pH, su	7.7	7.3
Alkalinity, mg/l	31	NA
Hardness, mg/l	46	NA
Conductivity, umho/cm	170	380
Residual Chlorine, mg/l	<0.05	NA

Day 2		Control	100%
DO, mg/l	Final	7.5	8.7
pH, su	Final	8.0	7.4

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

170744 WR 9/19/13

Client: City of Hot Springs
Project:
Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Rojs

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Chronic CD.	Analyses Requested
1	Plant Effluent	9-8-13 0000-2400		X	X		3	X	
		Container Type						P	
		Preservative						NO	

No Fathead Analysis

AIC Control No: 170390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2.1°C
Remarks:
Field pH calibration on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ___ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-9-13 1030	M. Mann	9-9-13 10:30 AM
M. Mann	9-9-13 11:45	[signature]	9-9-13 1145

Comments:

City of Hot Springs
Waste Water Treatment Plant
320 Davidson Drive
Hot Springs, AR 71901

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ADEQ
NPDES Enforcement Section
Attn: Mo Shafii
5301 Northshore Dr.
North Little Rock, AR 72118-5317

AMERICAN INTERPLEX CORPORATION
LABORATORIES

August 30, 2013

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Outfall 001
City of Hot Springs

Control No. 169846-2

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Ceriodaphnia dubia*
Outfall 001 - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Chronic *Ceriodaphnia dubia* test: Due to laboratory error, the *Ceriodaphnia dubia* test was not renewed with the third sample. The test should be repeated. The data from the test is enclosed for your review.

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Table of Contents

I. Control Acceptance Criteria

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	23.6	PASS
Control CV < or = 40% per Surviving Female	13.6	PASS
Reproduction Minimum Significant Difference 13 to 47%	25.8	PASS
Critical Dilution CV < or = 40%	22.9	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly
   Test Method 1002.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point: Outfall 001
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.9	8.5	NA
pH (standard units)	7.0	7.4	NA
Alkalinity (mg/l as CaCO3)	26	34	NA
Hardness (mg/l as CaCO3)	67	71	NA
Conductivity (umhos/cm)	350	390	NA
Residual Chlorine (mg/l)	<0.05	<0.05	NA
Ammonia as N (mg/l)	0.12	0.30	NA

2. Dilution Water Samples: Synthetic Soft Water #4012
   a. Dates Prepared: August 8 through August 22, 2013
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.7	7.7	7.8
pH (standard units)	7.7	7.8	7.9
Alkalinity (mg/l as CaCO3)	32	32	NA
Hardness (mg/l as CaCO3)	47	42	NA
Conductivity (umhos/cm)	180	170	170
Residual Chlorine (mg/l)	<0.05	<0.05	NA

C. Test Methods
1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Method 1002.0, *Ceriodaphnia dubia* Survival and Reproduction.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Ceriodaphnia dubia Survival and Growth Method 1002.0

Date & Time Test Initiated:	August 22, 2013 at 1605
Date & Time Test Terminated:	August 29, 2013 at 1415
Type & Volume of Test Chamber:	30 ml disposable beaker
Volume of Sample:	15 ml
Number of Organisms per replicate:	1
Number of Replicates per dilution:	10

4. Acclimation of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms
1. Scientific Name
a. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis

The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was analyzed using Kolmogorov's Test for Normality and Bartlett's test and analyzed with Dunnett's Test to determine the No Observable Effects Concentration (NOEC) for Reproduction.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Ceriodaphnia dubia

Chronic reference tests are performed monthly.
A chronic reference test was performed on August 20, 2013 at 1505 to August 28, 2013 at 1450
The results were as follows: (Control No. 169867-2.)
Survival LC-50: 2125 mg/l
Growth IC-25: 1610 mg/l
Growth PMSD: 18.3

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	102	1.91
pH	SM 4500-H+ B	101	0.268
Conductivity	EPA 120.1	101	4.71

VI. Organism History

Ceriodaphnia dubia

Date: August 22, 2013
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

VII. Results Summary *Ceriodaphnia dubia*, Cladoceran Survival and Reproduction Test -- Method 1002.0

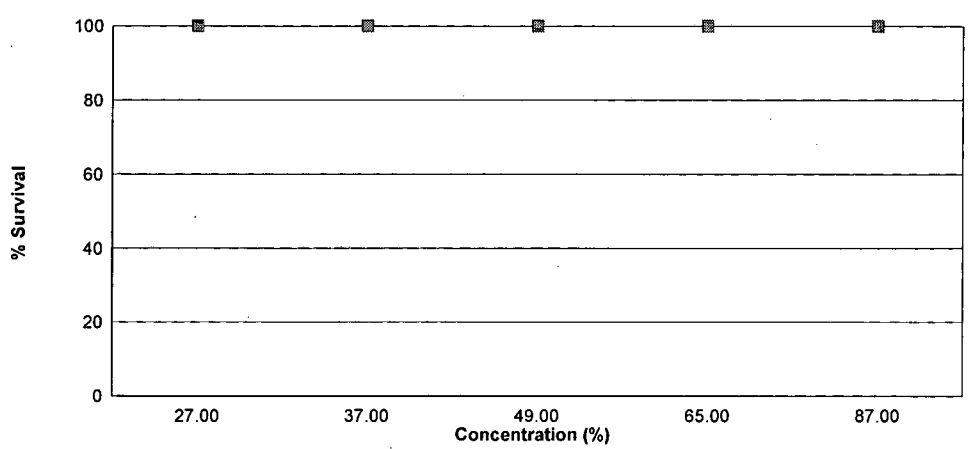
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on August 22, 2013 at 1605 and continued through August 29, 2013 at 1415. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 87 % effluent
b.) NOEC reproduction = 87 % effluent



Summary of the 7-day *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	23.6
27 %	100	24.2
37 %	100	27.6
49 %	100	26.4
65 %	100	24.2
87 %	100	25.0

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 22, 2013 at 1605
Date and Time Test Terminated: August 29, 2013 at 1415

Concentration: Control													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	4	4	2	3	4	4	3	2	33	10	3.30
5	0	0	0	0	0	1	0	0	0	6	7	10	0.700
6	9	9	7	8	7	8	7	9	8	8	80	10	8.00
7	14	14	11	13	14	11	12	14	13	0	116	10	11.6
8													
TOTAL	27	26	22	25	23	23	23	27	24	16	236	10	23.6

Concentration: 27 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	2	3	3	3	2	3	2	3	2	27	10	2.70
5	8	7	1	0	0	0	0	0	0	6	22	10	2.20
6	1	12	13	11	10	10	11	8	7	6	89	10	8.90
7	16	0	17	18	6	0	17	17	13	0	104	10	10.4
8													
TOTAL	29	21	34	32	19	12	31	27	23	14	242	10	24.2

Concentration: 37 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	3	3	3	2	3	2	2	2	27	10	2.70
5	0	0	0	0	0	1	0	0	6	6	13	10	1.30
6	12	12	12	11	10	6	8	10	13	10	104	10	10.4
7	18	16	17	18	16	15	15	17	0	0	132	10	13.2
8													
TOTAL	34	31	32	32	29	24	26	29	21	18	276	10	27.6

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 22, 2013 at 1605
Date and Time Test Terminated: August 29, 2013 at 1415

Concentration: 49 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	2	5	2	3	4	2	2	2	29	10	2.90
5	0	5	0	0	0	1	0	6	4	3	19	10	1.90
6	10	0	11	11	11	8	10	0	12	10	83	10	8.30
7	17	18	16	18	16	17	15	16	0	0	133	10	13.3
8													
TOTAL	31	26	29	34	29	29	29	24	18	15	264	10	26.4

Concentration: 65 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	2	3	5	3	3	3	2	3	2	2	28	10	2.80
5	6	0	0	0	0	1	0	0	5	4	16	10	1.60
6	13	11	11	11	10	10	8	10	0	12	96	10	9.60
7	0	15	14	16	12	0	15	16	14	0	102	10	10.2
8													
TOTAL	21	29	30	30	25	14	25	29	21	18	242	10	24.2

Concentration: 87 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	4	3	3	3	3	4	3	2	2	31	10	3.10
5	8	6	1	0	0	0	0	0	5	5	25	10	2.50
6	0	2	11	12	6	10	8	8	0	7	64	10	6.40
7	16	15	16	18	16	0	18	17	14	0	130	10	13.0
8													
TOTAL	28	27	31	33	25	13	30	28	21	14	250	10	25.0

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	10	0	10
27 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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	Alive	Dead	Total Animals
Control	10	0	10
37 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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	Alive	Dead	Total Animals
Control	10	0	10
49 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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	Alive	Dead	Total Animals
Control	10	0	10
65 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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.

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
87 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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 Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	27 %	10	0	
2	37 %	10	0	
3	49 %	10	0	
4	65 %	10	0	
5	87 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality		No Transformation
D = 0.1154 D* = 0.9054 Critical D* = 1.035	(alpha = 0.01, N = 60)	
Data PASS normality test (alpha = 0.01).		

Bartlett's Test for Homogeneity of Variance		No Transformation
Calculated B1 statistic = 6.592 Critical B = 15.086	(alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.		

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	117.9	23.58	0.6766
Within (Error)	54	1882	34.85	
Total	59	2000		

Critical F = 3.38 (alpha = 0.01, df = 5,54)
2.38 (alpha = 0.05, df = 5,54)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Dunnett's Test - Table 1 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	23.6	23.6		
2	27 %	24.2	24.2	-0.2273	
3	37 %	27.6	27.6	-1.515	
4	49 %	26.4	26.4	-1.061	
5	65 %	24.2	24.2	-0.2273	
6	87 %	25	25	-0.5303	

Dunnett's critical value = 2.31 (1 Tailed, alpha = 0.05, df [used] = 5,40) (Actual df = 5,54)

Dunnett's Test - Table 2 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	10			
2	27 %	10	6.099	25.8	-0.6
3	37 %	10	6.099	25.8	-4
4	49 %	10	6.099	25.8	-2.8
5	65 %	10	6.099	25.8	-0.6
6	87 %	10	6.099	25.8	-1.4

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 22, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.5	7.7	7.5	7.8	7.8	8.1
	Final	7.7	8.0	8.7	8.2	8.2	7.8	7.8
pH, units	Initial	7.7	7.7	7.8	8.0	7.9	8.0	7.7
	Final	8.0	8.2	8.0	8.0	8.0	7.8	7.8
Alkalinity, mg CaCO3/l		32	NA	32	NA	NA	NA	NA
Hardness, mg CaCO3/l		47	NA	42	NA	NA	NA	NA
Conductivity, umhos/cm		180	180	170	180	170	170	190
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	NA	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.4	8.0	7.5	8.0	8.0	7.5
	Final	7.5	8.2	8.6	8.2	8.2	7.7	7.7
pH, units	Initial	7.6	7.7	7.5	7.8	7.7	7.8	7.5
	Final	8.0	8.1	8.0	8.0	8.1	7.6	7.6

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.6	8.6	7.6	8.2	7.7	7.7
	Final	7.6	8.0	8.6	8.0	8.2	7.6	7.6
pH, units	Initial	7.6	7.7	7.4	7.7	7.6	7.7	7.6
	Final	8.0	8.1	8.0	8.0	8.1	7.6	7.6

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 22, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.5	8.7	7.6	8.1	7.7	7.7
	Final	7.7	8.1	8.6	8.2	8.2	7.6	7.9
pH, units	Initial	7.5	7.7	7.3	7.6	7.6	7.6	7.5
	Final	8.0	8.1	8.0	8.1	8.2	7.5	7.5

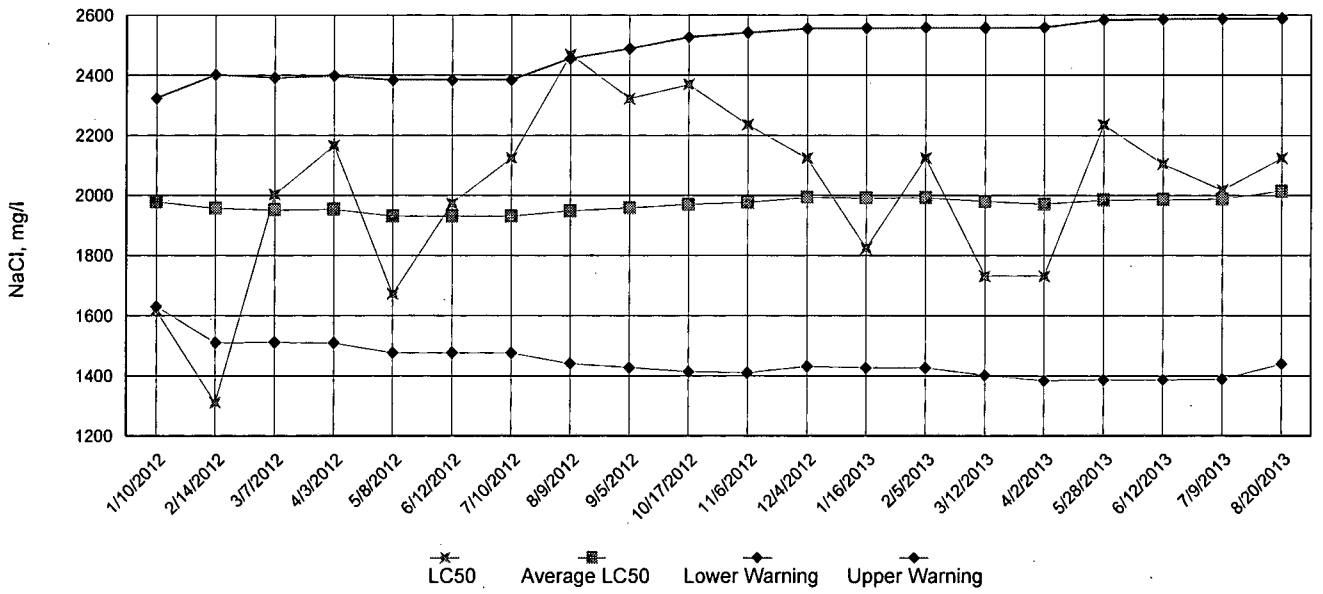
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.9	7.4	8.8	7.6	8.7	7.7	7.3
	Final	7.7	8.0	8.6	8.4	8.1	7.6	7.6
pH, units	Initial	7.5	7.7	7.2	7.5	7.5	7.6	7.5
	Final	8.0	8.0	8.0	8.1	8.1	7.5	7.5
Alkalinity, mg CaCO3/l		36	NA	30	NA	NA	NA	NA
Hardness, mg CaCO3/l		54	NA	58	NA	NA	NA	NA
Conductivity, umhos/cm		250	260	280	290	310	310	330
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	NA	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	7.4	8.9	7.6	8.3	7.9	7.4
	Final	7.4	8.1	8.7	8.2	7.7	5.4	7.2
pH, units	Initial	7.4	7.7	7.1	7.4	7.4	7.6	7.6
	Final	8.0	8.0	7.9	8.1	8.3	7.5	7.4

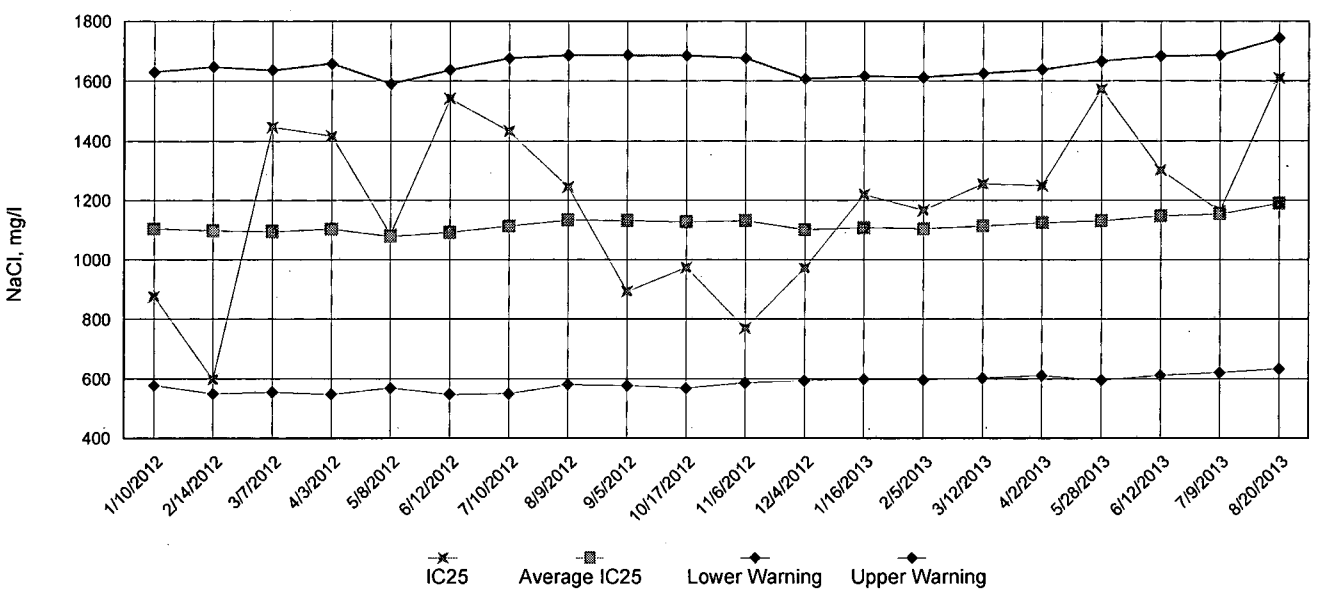
Appendix A4: Test 1002.0

Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL		No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
1	Plant Effluent	8-18-13 0900 - 2400		X	X			3	X
		Container Type							P
		Preservative							NO

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 20c
Remarks
Field pH calibration on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ___ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
A. Thomason	8-19-13 @ 1010	M. Mann	8-19-13 @ 10:10am
Relinquished By: M. Mann	Date/Time 8-19-13 @ 11:10AM	Received In Lab By: Angie Hopton	Date/Time 8-19-13 1110

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL		No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
2	Plant Effluent	8-20-13 0900-2400		X	X			3	X
		Container Type							P
		Preservative							NO

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 23C
Remarks

Field pH calibration
on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
A. Ross	8-21-13 0900	G. Mann	8-21-13 900
G. Mann	8-21-13 1130	Jimmy Day	8/21/13 1130

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By:

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
3	Plant Effluent	8/22/13 0000-2400		X			3	X
		Container Type						P
		Preservative						NO

AIC Control No: 16984b
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 2
Remarks
Field pH calibration on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	8-23-13 0955	[signature]	8-23-13 9:55
[signature]	8-23-13 11:20	[signature]	8-23-13 1120

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

September 30, 2013

Test Results of
Acute 48 hour Non-Renewal
Biomonitoring Testing
for

170741-1: Outfall 001 First Renewal
170741-2: Outfall 001 Second Renewal
170741-3: Outfall 001 Third Renewal
170741-4: Outfall 001 Original Screen

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Acute 48 hour Non-Renewal Biomonitoring utilizing *Ceriodaphnia dubia*
Outfall 001 First Renewal - City of Hot Springs
Client NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

Please find attached the results of the acute biomonitoring screens conducted as a follow up to the chronic toxicity event previously reported (AIC Control No. 170390). As indicated on the enclosed results, the sample was still acutely toxic on September 12, 2013 and exhibited no acute toxicity on September 19, 2013.

It it theorized the decrease in toxicity is directly related to the holding time which may have allowed microbial degradation, loss of volatile toxics, and/or biochemical degradation.

If I can be of further assistance, please feel free to contact me.

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Dilution Water Samples: Synthetic Soft Water #4021

Analysis	Result
Dissolved oxygen (mg/l)	7.6
pH (standard units)	7.7
Alkalinity (mg/l as CaCO3)	31
Hardness (mg/l as CaCO3)	46
Conductivity (umhos/cm)	170
Residual Chlorine (mg/l)	<0.05

Results Summary: Outfall 001 First Renewal

Ceriodaphnia dubia

The *Ceriodaphnia dubia* test was conducted from September 19, 2013 at 1615 to September 21, 2013 at 1700.

Statistical analyses:

NOEC = 100%

Concentration	24 hour % Survival	48 hour % Survival
Control	100	100
100%	100	100

Ceriodaphnia dubia
Survival Data

Number of organisms per chamber: 5
Volume of test chamber: 30 ml

Age of organisms: <24 hours
Volume of test solution: 15 ml

Effluent Concentration	Number of Survivors 24 Hours	48 Hours	% Survival	CV %
Control rep. A	5	5	100	0.00
100% rep. A	5	5	100	0.00

CV = Coefficient of variance = standard deviation X 100/mean

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	5	0	5
100%	5	0	5
Total	10	0	10

Critical Fisher's value (5,5,5) (alpha=0.05) is 1. b value is 5. Since b is greater than 1 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test

Group	Identification	Exposed	Dead	Sig 0.05
0	0	5	0	
1	100	5	0	

Chemical Data for
Ceriodaphnia dubia

Day 1	Control	100%
DO, mg/l	7.6	8.8
pH, su	7.7	7.3
Alkalinity, mg/l	31	NA
Hardness, mg/l	46	NA
Conductivity, umho/cm	170	380
Residual Chlorine, mg/l	<0.05	NA

Day 2		Control	100%
DO, mg/l	Final	7.5	8.7
pH, su	Final	8.0	7.4

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

month WR 9/19/13

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Chronic C.D.	Analyses Requested
1	Plant Effluent	9-8-13 0000-2400		X	X		3	X	
		Container Type						P	
		Preservative						NO	

No FATHEAD ANALYSIS

AIC Control No: 120390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2.1 °C
Remarks:
Field pH calibration on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z = Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-8-13 1030	M. Mann	9-9-13 10:30 AM
M. Mann	9-9-13 11:45	[signature]	9-9-13 1145

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

October 1, 2013
Control No. 171032

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

This report contains the analytical results and supporting information for samples submitted on September 27, 2013. Attached please find a copy of the Chain of Custody and/or other documents received. Note that any remaining sample will be discarded two weeks from the original report date unless other arrangements are made.

This report is intended for the sole use of the client listed above. Assessment of the data requires access to the entire document.

This report has been reviewed by the Laboratory Director or a qualified designee.

John Overbey
Laboratory Director

This document has been distributed to the following:

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

SAMPLE INFORMATION

Project Description:
Three (3) water sample(s) (AIC COntrol No. 170741-1,2,3) resubmitted September 27, 2013
P.O. No. 13-3032

Receipt Details:
A Chain of Custody was not provided with the sample(s).

Each sample container was checked for proper labeling, including date and time sampled. Sample containers were reviewed for proper type, adequate volume, integrity, temperature, preservation, and holding times. Any exceptions are noted below:

Sample Identification:

Laboratory ID	Client Sample ID	Sampled Date/Time	Notes
171032-1	Plant Effluent 9-8-13 0000-2400	08-Sep-2013 2359	
171032-2	Plant Effluent 9-10-13 0000-2400	10-Sep-2013 2359	
171032-3	Plant Effluent 9-12-13 0000-2400	12-Sep-2013 2359	

Case Narrative:
There were no qualifiers for this data and all samples met quality control criteria.

References:
"Methods for Chemical Analysis of Water and Wastes", EPA/600/4-79-020 (Mar 1983) with updates and supplements EPA/600/5-91-010 (Jun 1991), EPA/600/R-92-129 (Aug 1992) and EPA/600/R-93-100 (Aug 1993).
"Test Methods for Evaluating Solid Waste Physical/Chemical Methods (SW846)", Third Edition.
"Standard Methods for the Examination of Water and Wastewaters", 21st edition.
"American Society for Testing and Materials" (ASTM).
"Association of Analytical Chemists" (AOAC).

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

ANALYTICAL RESULTS

AIC No. 171032-1
Sample Identification: Plant Effluent 9-8-13 0000-2400

Analyte		Result	RL	Units	Qualifier
Total Organic Carbon		**6.1**	1	**mg/l**	
SM 5310 C	Prep: 27-Sep-2013 1516 by 308	Analyzed: 27-Sep-2013 1814 by 308		Batch: W45076	
Aluminum		**< 0.04**	0.04	**mg/l**	
EPA 200.7	Prep: 30-Sep-2013 0901 by 271	Analyzed: 01-Oct-2013 1242 by 305		Batch: S35491	
Sulfate		**29**	0.2	**mg/l**	
EPA 300.0	Prep: 27-Sep-2013 1628 by 07	Analyzed: 27-Sep-2013 2205 by 07		Batch: C16076	

AIC No. 171032-2
Sample Identification: Plant Effluent 9-10-13 0000-2400

Analyte		Result	RL	Units	Qualifier
Total Organic Carbon		**5.9**	1	**mg/l**	
SM 5310 C	Prep: 27-Sep-2013 1516 by 308	Analyzed: 27-Sep-2013 1828 by 308		Batch: W45076	
Aluminum		**0.041**	0.04	**mg/l**	
EPA 200.7	Prep: 30-Sep-2013 0901 by 271	Analyzed: 01-Oct-2013 1245 by 305		Batch: S35491	
Sulfate		**39**	0.2	**mg/l**	
EPA 300.0	Prep: 27-Sep-2013 1628 by 07	Analyzed: 27-Sep-2013 2232 by 07		Batch: C16076	

AIC No. 171032-3
Sample Identification: Plant Effluent 9-12-13 0000-2400

Analyte		Result	RL	Units	Qualifier
Total Organic Carbon		**5.4**	1	**mg/l**	
SM 5310 C	Prep: 27-Sep-2013 1516 by 308	Analyzed: 27-Sep-2013 1843 by 308		Batch: W45076	
Aluminum		**< 0.04**	0.04	**mg/l**	
EPA 200.7	Prep: 30-Sep-2013 0901 by 271	Analyzed: 01-Oct-2013 1248 by 305		Batch: S35491	
Sulfate		**34**	0.2	**mg/l**	
EPA 300.0	Prep: 27-Sep-2013 1628 by 07	Analyzed: 27-Sep-2013 2259 by 07		Batch: C16076	

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

LABORATORY CONTROL SAMPLE RESULTS

Analyte	Spike Amount	%	Limits	RPD	Limit	Batch	Preparation Date	Analysis Date	Dil	Qual
Total Organic Carbon	10 mg/l	96.4	80.0-120			W45076	27Sep13 1459 by 308	27Sep13 1648 by 308		
Aluminum	5 mg/l	97.6	85.0-115			S35491	30Sep13 0901 by 271	01Oct13 1152 by 305		
Sulfate	20 mg/l	108	90.0-110			C16076	27Sep13 1628 by 07	27Sep13 1710 by 07		

MATRIX SPIKE SAMPLE RESULTS

Analyte	Sample	Spike Amount	%	Limits	Batch	Preparation Date	Analysis Date	Dil	Qual
Total Organic Carbon	171006-1	10 mg/l	100	80.0-120	W45076	27Sep13 1459 by 308	27Sep13 1717 by 308		
	171006-1	10 mg/l	97.9	80.0-120	W45076	27Sep13 1459 by 308	27Sep13 1731 by 308		
	Relative Percent Difference:		1.86	25.0	W45076				
Aluminum	171022-2	5 mg/l	96.7	75.0-125	S35491	30Sep13 0901 by 271	01Oct13 1155 by 305		
	171022-2	5 mg/l	96.6	75.0-125	S35491	30Sep13 0901 by 271	01Oct13 1158 by 305		
	Relative Percent Difference:		0.142	20.0	S35491				
Sulfate	171021-1	20 mg/l	89.1	80.0-120	C16076	27Sep13 1628 by 07	27Sep13 1737 by 07		
	171021-1	20 mg/l	93.6	80.0-120	C16076	27Sep13 1628 by 07	27Sep13 1804 by 07		
	Relative Percent Difference:		4.28	10.0	C16076				

LABORATORY BLANK RESULTS

Analyte	Result	RL	PQL	QC Sample	Preparation Date	Analysis Date	Qual
Total Organic Carbon	< 1 mg/l	1	1	W45076-1	27Sep13 1459 by 308	27Sep13 1633 by 308	
Aluminum	< 0.04 mg/l	0.04	0.04	S35491-1	30Sep13 0901 by 271	01Oct13 1149 by 305	
Sulfate	< 0.2 mg/l	0.2	0.2	C16076-1	27Sep13 1628 by 07	27Sep13 1644 by 07	

AMERICAN INTERPLEX CORPORATION LABORATORIES

171032 MM 9/3/13
~~170741~~ MM 9/16/13

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client:	City of Hot Springs
Project Reference:	Plant Effluent
Project Manager:	James Sorrells
Sampled By:	A. Ross
PO No.	13-3032
AIC Control No:	~~170390~~
AIC Proposal No:	
Carrier:	Hot Springs Courier
Received Temperature °C	2.1 °C

Analyses Requested: No Fathead Analysis

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL	No of BOTTLES	Chronic CD	Remarks
1	Plant Effluent	9-8-13 0000-2400		X	X		3	X	
		Container Type					P		
		Preservative					NO		

Field pH calibration on ______ @ _____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH4)_2SO4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: ____________
Who should AIC contact with questions: ____________
Phone: ________ Fax: ____________
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By	Date/Time	Received By	Date/Time
[signature]	9-8-13 1030	M. Mann	9-9-13 10:30 am
M. Mann	9-9-13 11:45	Received In Lab By: [signature]	9-9-13 1145

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

August 30, 2013

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Outfall 001
City of Hot Springs

Control No. 169846-1

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Pimephales promelas* (Fathead minnow)
Outfall 001 - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Method 1000.0 Chronic *Pimephales promelas* (Fathead minnow) Survival and Growth Test: The No Observable Effects Concentration (NOEC) for survival occurred at 87 % effluent, which is above the critical dilution of 65 %. The NOEC for growth occurred at 87 % effluent, which is above the critical dilution of 65 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the Fathead minnow test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Table of Contents

I. Control Acceptance Criteria

Pimephales promelas (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.296	PASS
Control Growth CV < or = 40%	7.47	PASS
Growth Minimum Significant Difference 12 to 30%	20.0	PASS
Critical Dilution CV < or = 40%	11.6	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly Test Method 1000.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point: Outfall 001
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.2	8.9	8.5
pH (standard units)	7.4	7.0	7.4
Alkalinity (mg/l as CaCO3)	41	26	34
Hardness (mg/l as CaCO3)	59	67	71
Conductivity (umhos/cm)	290	350	390
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.28	0.12	0.30

2. Dilution Water Samples: Synthetic Soft Water #4012
   a. Dates Prepared: August 8 through August 22, 2013
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.7	7.7	7.8
pH (standard units)	7.7	7.8	7.9
Alkalinity (mg/l as CaCO3)	32	32	32
Hardness (mg/l as CaCO3)	47	42	42
Conductivity (umhos/cm)	180	170	170
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05

C. Test Methods
1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Method 1000.0, Fathead Minnow Survival and Growth.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Pimephales promelas (Fathead minnow) Survival and Growth Method 1000.0

Date & Time Test Initiated:	August 20, 2013 at 1200
Date & Time Test Terminated:	August 27, 2013 at 1010
Type & Volume of Test Chamber:	500 ml disposable beaker
Volume of Sample:	250 ml
Number of Organisms per replicate:	8
Number of Replicates per dilution:	5

4. Acclimation of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms
1. Scientific Name
a. Test 1000.0 *Pimephales promelas*

III. Data Analysis

The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Pimephales promelas (Fathead minnow) survival data was transformed using the Arc Sine transformation. Normality and homogeneity of variance were checked using Shapiro-Wilk's. The survival data was then analyzed using Steel's Many-One Rank Test to determine the No Observable Effects Concentration (NOEC).

Fathead minnow growth data was analyzed for normality and homogeneity of variance using Shapiro-Wilk's and Bartlett's test. Dunnett's Test was used to determine the No Observable Effects Concentration (NOEC) for growth.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Pimephales promelas (Fathead minnow)

Chronic reference tests are performed monthly.
A chronic reference test was performed on August 20, 2013 at 1715 to August 27, 2013 at 1520
The results were as follows: (Control No. 169867-1.)
Survival LC-50: 5670.1 mg/l
Growth IC-25: 3143 mg/l
Growth PMSD: 17.3

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	102	1.91
pH	SM 4500-H+ B	101	0.268
Conductivity	EPA 120.1	101	4.71

VI. Organism History

Pimephales promelas (Fathead minnow)
Date: August 20, 2013
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

VII. Results Summary *Pimephales promelas*, Fathead minnow Larval Survival and Growth Test -- Method 1000.0

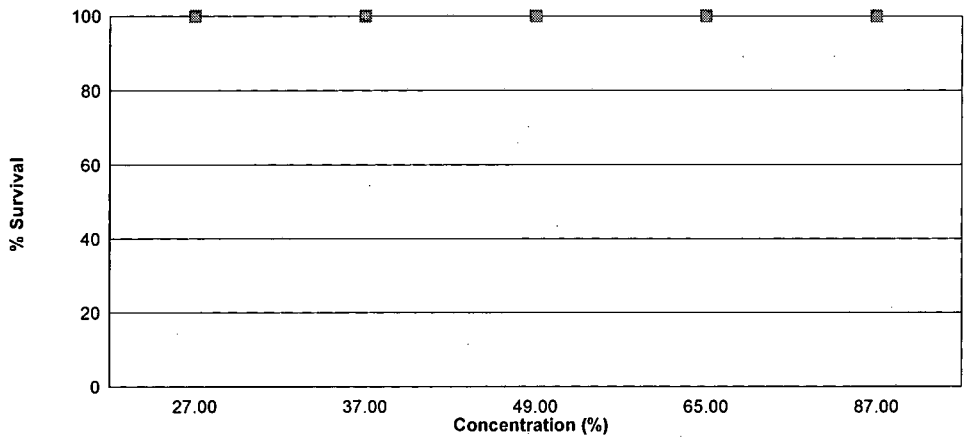
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on August 20, 2013 at 1200 and continued through August 27, 2013 at 1010. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 87 % effluent
b.) NOEC growth = 87 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.296
27 %	100	0.337
37 %	100	0.323
49 %	100	0.302
65 %	100	0.292
87 %	100	0.302

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: August 20, 2013 at 1200
Date and Time Test Terminated: August 27, 2013 at 1010

Concentration	Replicate	Number of Survivors						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
27 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
37 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
49 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
65 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
87 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: August 20, 2013 at 1200
Test Terminated: August 27, 2013 at 1010
Drying Started: August 23, 2013 at 1527
Drying Ended: August 29, 2013 at 1400

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.91827	.92042	0.00215	8	0.269
	B	.91878	.92121	0.00243	8	0.304
	C	.91659	.91922	0.00263	8	0.329
	D	.91638	.91872	0.00234	8	0.292
	E	.91455	.91685	0.00230	8	0.288
27 %	A	.91583	.91811	0.00228	8	0.285
	B	.91943	.92233	0.00290	8	0.362
	C	.92408	.92709	0.00301	8	0.376
	D	.92651	.92924	0.00273	8	0.341
	E	.92767	.93024	0.00257	8	0.321
37 %	A	.92653	.92915	0.00262	8	0.328
	B	.94872	.95160	0.00288	8	0.360
	C	.94724	.94985	0.00261	8	0.326
	D	.94532	.94750	0.00218	8	0.272
	E	.94881	.95146	0.00265	8	0.331
49 %	A	.94115	.94330	0.00215	8	0.269
	B	.92530	.92779	0.00249	8	0.311
	C	.92632	.92901	0.00269	8	0.336
	D	.93070	.93312	0.00242	8	0.302
	E	.93427	.93660	0.00233	8	0.291
65 %	A	.93862	.94107	0.00245	8	0.306
	B	.93944	.94155	0.00211	8	0.264
	C	.94132	.94377	0.00245	8	0.306
	D	.93810	.94077	0.00267	8	0.334
	E	.93899	.94100	0.00201	8	0.251
87 %	A	.93682	.93952	0.00270	8	0.338
	B	.93818	.93991	0.00173	8	0.216
	C	.93688	.93881	0.00193	8	0.241
	D	.93804	.94073	0.00269	8	0.336
	E	.95233	.95537	0.00304	8	0.380

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

		Transformation of Data		Transform: Arc Sin(Square Root(Y))
Group	Identification	Rep	Value	Transformed
1	Control	1	1.00000	1.39310
1	Control	2	1.00000	1.39310
1	Control	3	1.00000	1.39310
1	Control	4	1.00000	1.39310
1	Control	5	1.00000	1.39310
2	27 %	1	1.00000	1.39310
2	27 %	2	1.00000	1.39310
2	27 %	3	1.00000	1.39310
2	27 %	4	1.00000	1.39310
2	27 %	5	1.00000	1.39310
3	37 %	1	1.00000	1.39310
3	37 %	2	1.00000	1.39310
3	37 %	3	1.00000	1.39310
3	37 %	4	1.00000	1.39310
3	37 %	5	1.00000	1.39310
4	49 %	1	1.00000	1.39310
4	49 %	2	1.00000	1.39310
4	49 %	3	1.00000	1.39310
4	49 %	4	1.00000	1.39310
4	49 %	5	1.00000	1.39310
5	65 %	1	1.00000	1.39310
5	65 %	2	1.00000	1.39310
5	65 %	3	1.00000	1.39310
5	65 %	4	1.00000	1.39310
5	65 %	5	1.00000	1.39310
6	87 %	1	1.00000	1.39310
6	87 %	2	1.00000	1.39310
6	87 %	3	1.00000	1.39310
6	87 %	4	1.00000	1.39310
6	87 %	5	1.00000	1.39310

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Shapiro - Wilk's Test for Normality		Transform: Arc Sin(Square Root(Y))
D = 0		
W = 0		
Critical W = 0.9	(alpha = 0.01, N = 30)	
Critical W = 0.927	(alpha = 0.05, N = 30)	

Data FAIL normality test (alpha = 0.01).

Steel's Many-One Rank Test — Transform: Arc Sin(Square Root(Y))

Ho:Control<Treatment

Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	27 %	27.50	16.00	5.00	
3	37 %	27.50	16.00	5.00	
4	49 %	27.50	16.00	5.00	
5	65 %	27.50	16.00	5.00	
6	87 %	27.50	16.00	5.00	

Critical values are 1 tailed (k=5)

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality		No Transformation
D = 0.03787		
W = 0.9742		
Critical W = 0.9	(alpha = 0.01, N = 30)	
Critical W = 0.927	(alpha = 0.05, N = 30)	
Data PASS normality test (alpha = 0.01).		

Bartlett's Test for Homogeneity of Variance		No Transformation
Calculated B1 statistic = 7.076		
Critical B = 15.086	(alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.		

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	0.007651	0.00153	0.9696
Within (Error)	24	0.03787	0.001578	
Total	29	0.04552		

Critical F = 3.9 (alpha = 0.01, df = 5,24)
2.62 (alpha = 0.05, df = 5,24)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Dunnett's Test - Table 1 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	0.2964	0.2964		
2	27 %	0.337	0.337	-1.616	
3	37 %	0.3234	0.3234	-1.075	
4	49 %	0.3018	0.3018	-0.2149	
5	65 %	0.2922	0.2922	0.1672	
6	87 %	0.3022	0.3022	-0.2309	

Dunnett's critical value = 2.36 (1 Tailed, alpha = 0.05, df = 5,24)

Dunnett's Test - Table 2 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	5			
2	27 %	5	0.05929	20	-0.0406
3	37 %	5	0.05929	20	-0.027
4	49 %	5	0.05929	20	-0.0054
5	65 %	5	0.05929	20	0.0042
6	87 %	5	0.05929	20	-0.0058

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 20, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.5	7.7	7.5	7.8	7.8	8.1
	Final	7.5	7.2	7.5	7.9	7.7	7.1	7.9
pH, units	Initial	7.7	7.7	7.8	8.0	7.9	8.0	7.7
	Final	7.5	7.3	7.8	8.1	8.1	7.6	7.9
Alkalinity, mg CaCO3/l		32	NA	32	NA	32	NA	NA
Hardness, mg CaCO3/l		47	NA	42	NA	42	NA	NA
Conductivity, umhos/cm		180	180	170	180	170	170	190
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.4	8.0	7.5	8.0	8.0	7.5
	Final	7.5	7.2	7.3	7.9	7.8	8.1	7.7
pH, units	Initial	7.6	7.7	7.5	7.8	7.7	7.8	7.5
	Final	7.5	7.3	7.7	8.1	8.2	7.5	7.7

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.6	8.6	7.6	8.2	7.7	7.7
	Final	7.5	7.0	7.5	8.0	7.9	8.1	7.8
pH, units	Initial	7.6	7.7	7.4	7.7	7.6	7.7	7.6
	Final	7.5	7.3	7.7	8.1	8.2	7.5	7.6

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 20, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.5	8.7	7.6	8.1	7.7	7.7
	Final	7.5	7.4	7.6	8.0	8.1	8.2	7.8
pH, units	Initial	7.5	7.7	7.3	7.6	7.6	7.6	7.5
	Final	7.5	7.4	7.6	8.1	8.2	7.6	7.6

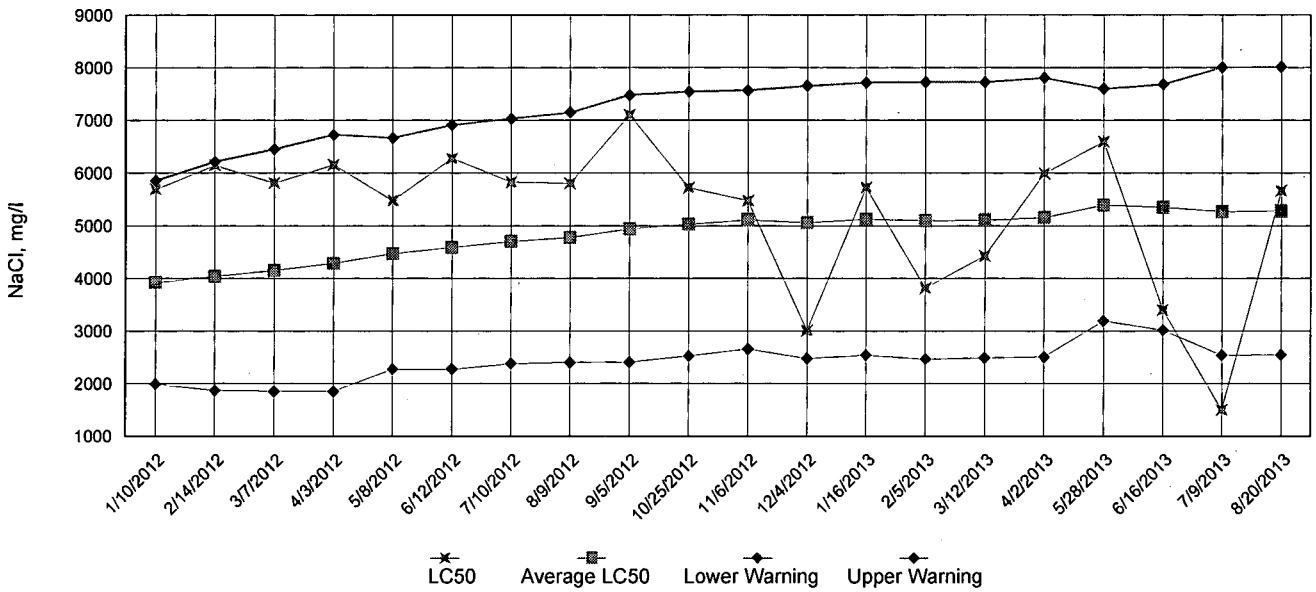
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.9	7.4	8.8	7.6	8.7	7.7	7.3
	Final	7.5	7.4	7.4	7.9	8.1	8.0	7.7
pH, units	Initial	7.5	7.7	7.2	7.5	7.5	7.6	7.5
	Final	7.5	7.4	7.6	8.1	8.3	7.6	7.5
Alkalinity, mg CaCO3/l		36	NA	30	NA	35	NA	NA
Hardness, mg CaCO3/l		54	NA	58	NA	61	NA	NA
Conductivity, umhos/cm		250	260	280	290	310	310	330
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	7.4	8.9	7.6	8.3	7.9	7.4
	Final	7.5	8.5	7.4	7.8	8.1	8.0	7.7
pH, units	Initial	7.4	7.7	7.1	7.4	7.4	7.6	7.6
	Final	7.5	7.4	7.6	8.1	8.1	7.6	7.4

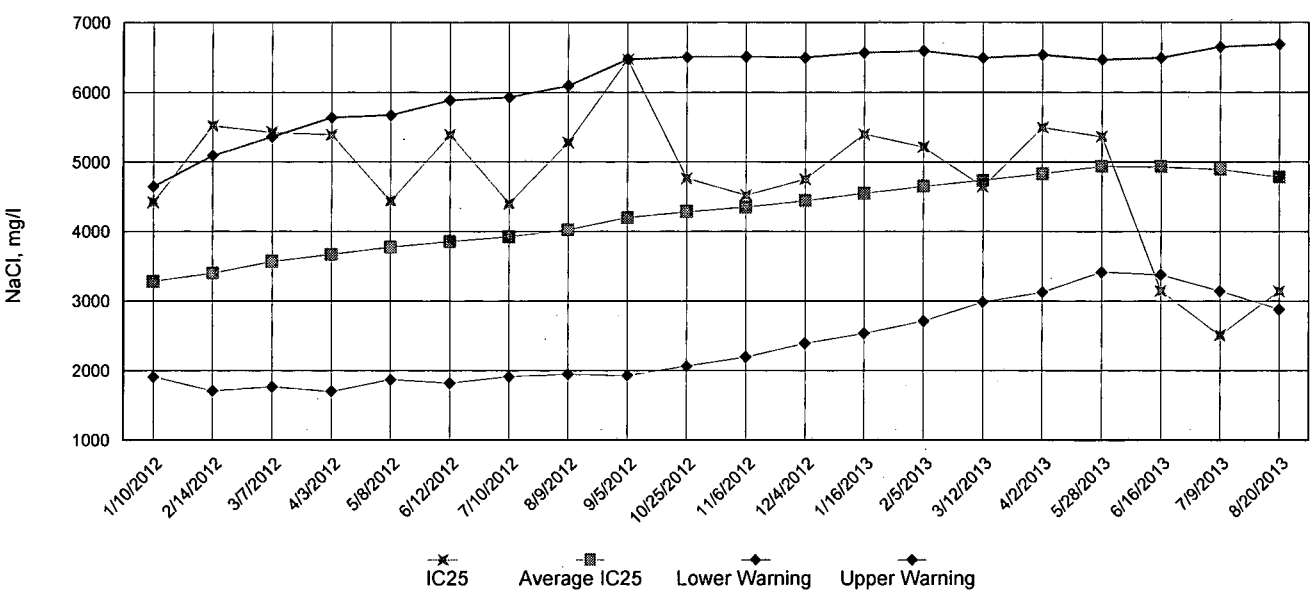
Appendix A4: Test 1000.0

Chronic Reference Toxicant, *Pimephales promelas* (Fathead Minnow)

LC50 Survival Data



IC25 Growth Data



Appendix B: Test 1000.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

Permittee: City of Hot Springs

NPDES No.: AR0033880 AFIN#26-00145

Date and Time Test Initiated: August 20, 2013 at 1200
Date and Time Test Terminated: August 27, 2013 at 1010
Dilution water used: Synthetic Soft Water #4012

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in replicate chambers A	B	C	D	E	Mean percent survival 24 hr	48 hr	7 days	CV%
Control	100	100	100	100	100	100	100	100	0.00
27 %	100	100	100	100	100	100	100	100	0.00
37 %	100	100	100	100	100	100	100	100	0.00
49 %	100	100	100	100	100	100	100	100	0.00
65 %	100	100	100	100	100	100	100	100	0.00
87 %	100	100	100	100	100	100	100	100	0.00

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers A	B	C	D	E	Mean dry weight, mg	CV%
Control	0.269	0.304	0.329	0.292	0.288	0.296	7.47
27 %	0.285	0.362	0.376	0.341	0.321	0.337	10.6
37 %	0.328	0.360	0.326	0.272	0.331	0.323	9.86
49 %	0.269	0.311	0.336	0.302	0.291	0.302	8.20
65 %	0.306	0.264	0.306	0.334	0.251	0.292	11.6
87 %	0.338	0.216	0.241	0.336	0.380	0.302	23.2

CV = Coefficient of variation = standard deviation * 100 / mean

Appendix B: Test 1000.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

1. Steel's Many-One Rank Test:

   Is the mean survival significantly different (p=0.05) than the control survival for the % effluent corresponding to (lethality):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	____YES	X NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____YES	____NO

2. Dunnett's Test:

   Is the mean dry weight (growth) significantly different (p=0.05) than the control's dry weight (growth) for the % effluent corresponding to (significant non-lethal effects):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	____YES	X NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____YES	____NO

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

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

5. NOEC Pimephales Lethality: 87 % (TOP6C)

6. LOEC Pimephales Lethality: 87 % (TXP6C)

7. NOEC Pimephales Sublethality: 87 % (TPP6C)

8. LOEC Pimephales Sublethality: 87 % (TYP6C)

9. Coefficient of variation for Pimephales growth: 11.6 (TQP6C)

AMERICAN INTERPLEX CORPORATION
LABORATORIES

Appendix B: Test 1000.0

CHRONIC TOXICITY SUMMARY FORM
Pimephales promelas (Fathead minnow)
CHEMICAL PARAMETERS CHART

PERMITTEE: City of Hot Springs 2400
NPDES NO.: AR0033880 AFIN#26-00145 2400
CONTACT: Mr. James Sorrells 2400
ANALYST: 280, 298, 304, 307

Test Initiated: DATE: August 20, 2013 TIME: 1200
Test Terminated: DATE: August 27, 2013 TIME: 1010

DILUTION Control	DAY 1	2	3	4	5	6	7
D.O. Initial	7.7	7.5	7.7	7.5	7.8	7.8	8.1
Final	7.5	7.2	7.5	7.9	7.7	7.1	7.9
pH Initial	7.7	7.7	7.8	8.0	7.9	8.0	7.7
Final	7.5	7.3	7.8	8.1	8.1	7.6	7.9
Alkalinity	32	NA	32	NA	32	NA	NA
Hardness	47	NA	42	NA	42	NA	NA
Conductivity	180	180	170	180	170	170	190
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 27 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.7	7.4	8.0	7.5	8.0	8.0	7.5
Final	7.5	7.2	7.3	7.9	7.8	8.1	7.7
pH Initial	7.6	7.7	7.5	7.8	7.7	7.8	7.5
Final	7.5	7.3	7.7	8.1	8.2	7.5	7.7
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	220	220	220	230	230	250
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 37 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.8	7.6	8.6	7.6	8.2	7.7	7.7
Final	7.5	7.0	7.5	8.0	7.9	8.1	7.8
pH Initial	7.6	7.7	7.4	7.7	7.6	7.7	7.6
Final	7.5	7.3	7.7	8.1	8.2	7.5	7.6
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	220	230	240	240	260	250	270
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 49 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.8	7.5	8.7	7.6	8.1	7.7	7.7
Final	7.5	7.4	7.6	8.0	8.1	8.2	7.8
pH Initial	7.5	7.7	7.3	7.6	7.6	7.6	7.5
Final	7.5	7.4	7.6	8.1	8.2	7.6	7.6
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	230	240	260	260	280	280	300
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 65 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.9	7.4	8.8	7.6	8.7	7.7	7.3
Final	7.5	7.4	7.4	7.9	8.1	8.0	7.7
pH Initial	7.5	7.7	7.2	7.5	7.5	7.6	7.5
Final	7.5	7.4	7.6	8.1	8.3	7.6	7.5
Alkalinity	36	NA	30	NA	35	NA	NA
Hardness	54	NA	58	NA	61	NA	NA
Conductivity	250	260	280	290	310	310	330
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 87 %	DAY 1	2	3	4	5	6	7
D.O. Initial	8.0	7.4	8.9	7.6	8.3	7.9	7.4
Final	7.5	8.5	7.4	7.8	8.1	8.0	7.7
pH Initial	7.4	7.7	7.1	7.4	7.4	7.6	7.6
Final	7.5	7.4	7.6	8.1	8.1	7.6	7.4
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	280	290	320	320	360	360	380
Chlorine	NA	NA	NA	NA	NA	NA	NA

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs

Project Reference: Plant Effluent

Project Manager: James Sorrells

Sampled By: A. Ross

PO No.: 13-3032

AIC Control No: 169846

AIC Proposal No:

Carrier: Hot Springs Courier

Received Temperature °C: 2°C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL		No of BOTTLES	Chronic.CD, Chronic.FH												Remarks
1	Plant Effluent	8-18-13 0000-2400		X	X			3	X												
																					Field pH calibration
		Container Type							P												on ______ @ ______
		Preservative							NO												Buffer:

G = Glass   P = Plastic   V = VOA vials   H = HCl to pH2   T = Sodium Thiosulfate

NO = none   S = Sulfuric acid pH2   N = Nitric acid pH2   B = NaOH to pH12   Z= Zinc acetate   A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)

NORMAL or EXPEDITED IN ____ DAYS

Expedited results requested by: ____________

Who should AIC contact with questions: ____________

Phone: ____________ Fax: ____________

Report Attention to: Mr. James Sorrells

Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By	Date/Time	Received By	Date/Time
A. Thomason	8-19-13 @ 1010	M. Mann	8-19-13 10:10am

Relinquished By	Date/Time	Received In Lab By	Date/Time
M. Mann	8-19-13 @ 11:10AM	[signature]	8-19-13 1110

Comments:

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 2.3C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH	Remarks
2	Plant Effluent	8-20-13 0000-2400		X	X		3	X	
		Container Type						P	Field pH calibration
		Preservative						NO	on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
A. Ross	8-21-13 0900	A. Man	8-21-13 9:10
A. Man	8-21-13 1130	Jimmy Day	8/21/13 1130

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By:

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL		No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
3	Plant Effluent	8/22/13 0000-2400		X				3	X
		Container Type							P
		Preservative							NO

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 2
Remarks
Field pH calibration on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	8-23-13 0955	[signature]	8-23-13 9:55
[signature]	8-23-13 11:20	[signature]	8-23-13 1120

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

September 19, 2013

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Plant Effluent
City of Hot Springs

Control No. 170390-1

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Ceriodaphnia dubia*
Plant Effluent - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: The No Observable Effects Concentration (NOEC) for survival occurred at <27 % effluent, which is below the critical dilution of 65 %. The NOEC for reproduction occurred at <27 % effluent, which is below the critical dilution of 65 %. **The sample, therefore, FAILED both lethal and sub-lethal effects for the *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Table of Contents

I. Control Acceptance Criteria

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	18.8	PASS
Control CV < or = 40% per Surviving Female	20.6	PASS
Reproduction Minimum Significant Difference 13 to 47%	NA	NA
Critical Dilution CV < or = 40%	0.00	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly Test Method 1002.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point: Plant Effluent
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.7	8.2	8.2
pH (standard units)	7.4	7.7	7.5
Alkalinity (mg/l as CaCO3)	64	58	64
Hardness (mg/l as CaCO3)	82	82	83
Conductivity (umhos/cm)	380	370	440
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	2.4	0.11

2. Dilution Water Samples: Synthetic Soft Water #4019
   a. Dates Prepared: August 28 through September 11, 2013
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.6	8.2	8.2
pH (standard units)	7.6	7.8	7.6
Alkalinity (mg/l as CaCO3)	32	32	32
Hardness (mg/l as CaCO3)	46	42	42
Conductivity (umhos/cm)	160	160	180
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05

C. Test Methods
1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Method 1002.0, *Ceriodaphnia dubia* Survival and Reproduction.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Ceriodaphnia dubia Survival and Growth Method 1002.0

Date & Time Test Initiated:	September 9, 2013 at 1235
Date & Time Test Terminated:	September 17, 2013 at 1255
Type & Volume of Test Chamber:	30 ml disposable beaker
Volume of Sample:	15 ml
Number of Organisms per replicate:	1
Number of Replicates per dilution:	10

4. Acclimation of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms
1. Scientific Name
a. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis
The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was not analyzed due to survival failure.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Ceriodaphnia dubia

Chronic reference tests are performed monthly.
A chronic reference test was performed on August 20, 2013 at 1505 to August 28, 2013 at 1450
The results were as follows: (Control No. 169867-2.)
Survival LC-50: 2125 mg/l
Growth IC-25: 1610 mg/l
Growth PMSD: 18.3

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	1.53
Hardness	EPA 200.7	101	0.450
pH	SM 4500-H+ B	101	0.267
Conductivity	EPA 120.1	103	1.97

VI. Organism History

Ceriodaphnia dubia

Date: September 9, 2013
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

VII. Results Summary *Ceriodaphnia dubia*, Cladoceran Survival and Reproduction Test -- Method 1002.0

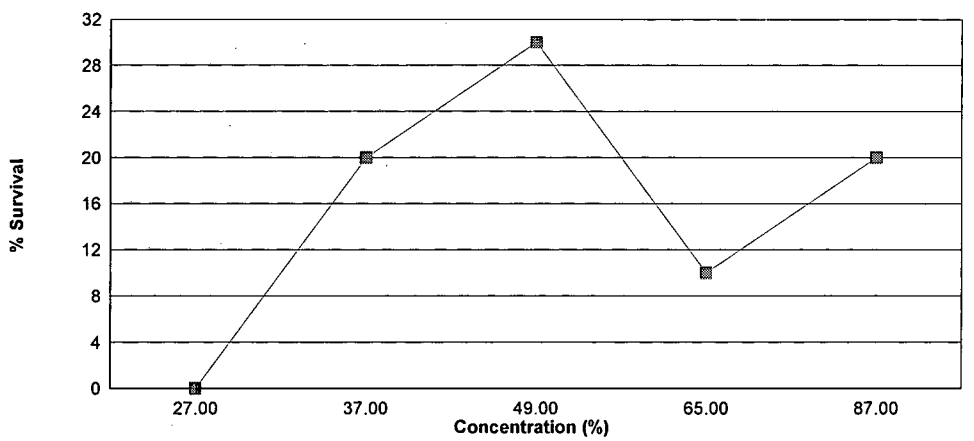
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on September 9, 2013 at 1235 and continued through September 17, 2013 at 1255. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = <27 % effluent
b.) NOEC reproduction = <27 % effluent



Summary of the 7-day *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	18.8
27 %	0.00 *	--
37 %	20.0 *	--
49 %	30.0 *	--
65 %	10.0 *	--
87 %	20.0 *	--

*Significant difference when compared to the control (p=0.05)

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 9, 2013 at 1235
Date and Time Test Terminated: September 17, 2013 at 1255

Concentration: Control													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	3	2	0	0	0	0	1	4	2	1	13	10	1.30
5	9	0	3	6	6	4	0	4	6	0	38	10	3.80
6	0	5	9	8	7	0	6	0	1	7	43	10	4.30
7	10	9	10	10	10	8	9	8	10	10	94	10	9.40
8													
TOTAL	22	16	22	24	23	12	16	16	19	18	188	10	18.8

Concentration: 27 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	0	0	0	X	0	0	0	X	0	0	7	0.00
2	X	0	0	0	X	0	0	0	X	0	0	7	0.00
3	X				X				X		0	0	0.00
4	X				X				X		0	0	0.00
5	X				X				X		0	0	0.00
6	X				X				X		0	0	0.00
7	X				X				X		0	0	0.00
8													
TOTAL	0	0	0	0	0	0	0	0	0	0	0	10	0.00

Concentration: 37 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	X	X	0	X	X	0	0	3	0.00
2	X	X	0	X	X	X	0	X	X	0	0	3	0.00
3	X	X	X	X	X	X	0	X	X	0	0	2	0.00
4	X	X	X	X	X	X	2	X	X	1	3	2	1.50
5	X	X	X	X	X	X	0	X	X	0	0	2	0.00
6	X	X	X	X	X	X	7	X	X	6	13	2	6.50
7	X	X	X	X	X	X	10	X	X	0	10	2	5.00
8													
TOTAL	0	0	0	0	0	0	19	0	0	7	26	10	2.60

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 9, 2013 at 1235
Date and Time Test Terminated: September 17, 2013 at 1255

Concentration: 49 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	X	X	0	X	X	0	0	3	0.00
2	X	X	0	X	X	X	0	X	X	0	0	3	0.00
3	X	X	0	X	X	X	0	X	X	0	0	3	0.00
4	X	X	0	X	X	X	0	X	X	0	0	3	0.00
5	X	X	4	X	X	X	5	X	X	0	9	3	3.00
6	X	X	0	X	X	X	0	X	X	6	6	3	2.00
7	X	X	6	X	X	X	8	X	X	0	14	3	4.67
8													
TOTAL	0	0	10	0	0	0	13	0	0	6	29	10	2.90

Concentration: 65 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	X	X	X	X	X	0	0	2	0.00
2	X	X	0	X	X	X	X	X	X	0	0	2	0.00
3	X	X	0	X	X	X	X	X	X	0	0	2	0.00
4	X	X	0	X	X	X	X	X	X	0	0	2	0.00
5	X	X	6	X	X	X	X	X	X	0	6	2	3.00
6	X	X	0	X	X	X	X	X	X	X	0	1	0.00
7	X	X	9	X	X	X	X	X	X	X	9	1	9.00
8													
TOTAL	0	0	15	0	0	0	0	0	0	0	15	10	1.50

Concentration: 87 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	0	X	0	X	X	0	0	4	0.00
2	X	X	0	X	0	X	X	X	X	0	0	3	0.00
3	X	X	0	X	0	X	X	X	X	0	0	3	0.00
4	X	X	0	X	3	X	X	X	X	1	4	3	1.33
5	X	X	7	X	5	X	X	X	X	0	12	3	4.00
6	X	X	0	X	X	X	X	X	X	3	3	2	1.50
7	X	X	9	X	X	X	X	X	X	0	9	2	4.50
8													
TOTAL	0	0	16	0	8	0	0	0	0	4	28	10	2.80

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
27 %	0	3	3
Total	10	3	13

Critical Fisher's value (10,3,10) (alpha=0.05) is 1. b value is 0. Since b is less than or equal to 1 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
37 %	2	8	10
Total	12	8	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 2. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
49 %	3	7	10
Total	13	7	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 3. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
65 %	1	9	10
Total	11	9	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 1. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
87 %	2	8	10
Total	12	8	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 2. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	27 %	3	3	*
2	37 %	10	8	*
3	49 %	10	7	*
4	65 %	10	9	*
5	87 %	10	8	*

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: September 9, 2013 at 0857
Date and Time Test Terminated: September 17, 2013 at 1255

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.6	7.4	8.2	8.3	8.2	8.3	8.2
	Final	7.8	8.3	8.5	8.7	8.4	8.2	7.8
pH, units	Initial	7.6	7.6	7.8	7.6	7.6	7.7	7.5
	Final	7.8	7.9	7.9	7.6	7.9	7.8	8.0
Alkalinity, mg CaCO3/l		32	NA	32	NA	32	NA	NA
Hardness, mg CaCO3/l		46	NA	42	NA	42	NA	NA
Conductivity, umhos/cm		160	170	160	180	180	180	170
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.6	7.5	8.2	8.4	8.3	8.1	8.3
	Final	6.0	8.3	8.7	8.8	8.5	8.3	8.1
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
	Final	8.0	8.1	8.2	8.0	8.3	8.0	8.1

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.6	7.4	8.2	8.4	8.1	8.0	8.1
	Final	6.4	8.3	8.6	8.8	8.6	8.2	8.2
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
	Final	8.1	8.2	8.2	8.2	8.2	7.9	7.8

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: September 9, 2013 at 0857
Date and Time Test Terminated: September 17, 2013 at 1255

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.5	7.5	8.2	8.4	8.4	8.1	8.3
	Final	6.8	8.3	7.8	9.0	8.4	8.3	8.0
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.5	7.5
	Final	8.2	8.2	8.2	8.1	8.1	8.0	8.1

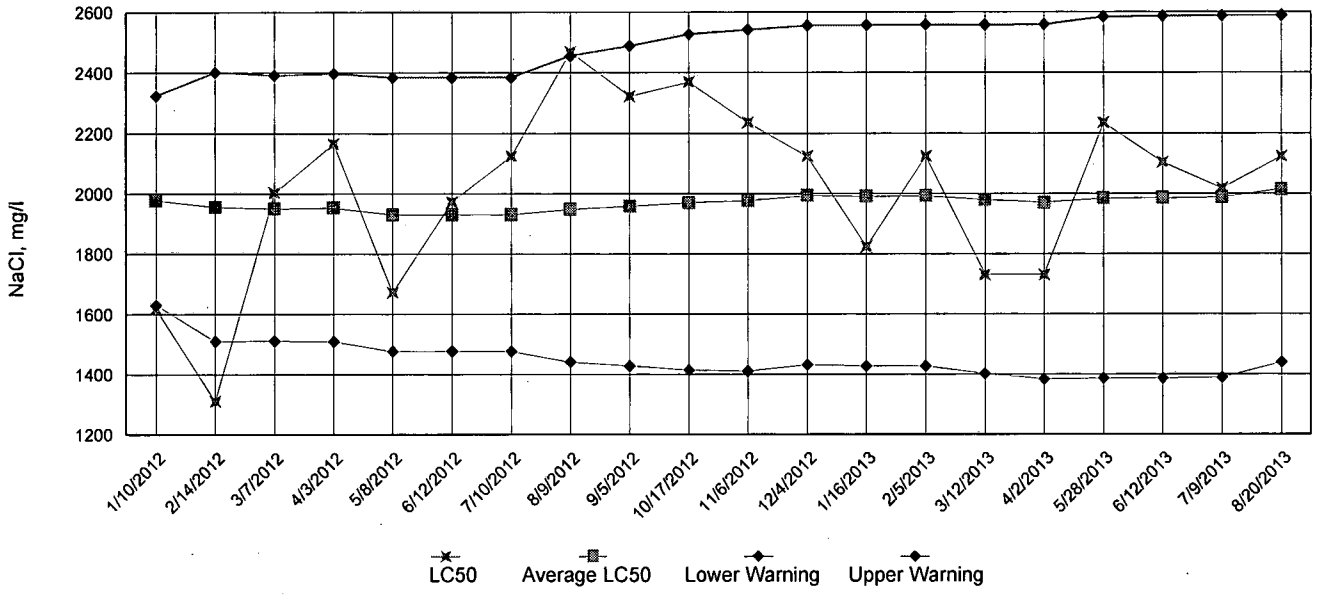
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.4	8.2	8.4	8.0	8.2	8.3
	Final	7.0	8.3	8.4	8.7	8.3	8.3	7.7
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.6
	Final	8.2	8.1	8.1	8.2	8.0	8.0	7.7
Alkalinity, mg CaCO3/l		48	NA	46	NA	47	NA	NA
Hardness, mg CaCO3/l		68	NA	68	NA	68	NA	NA
Conductivity, umhos/cm		310	310	300	340	340	340	350
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.3	7.2	8.2	8.3	8.3	8.0	7.9
	Final	7.1	8.3	8.6	8.8	7.3	8.3	8.0
pH, units	Initial	7.5	7.4	7.8	7.5	7.6	7.5	7.6
	Final	8.2	8.3	8.3	8.3	7.5	8.1	7.9

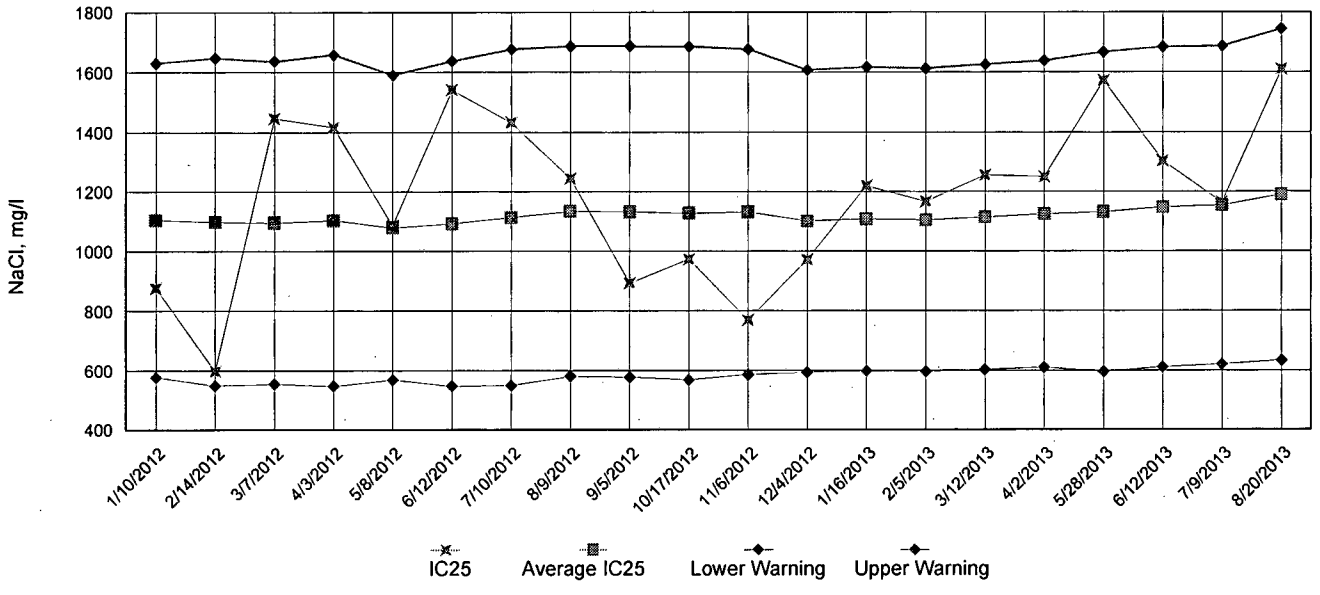
Appendix A4: Test 1002.0

Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

Permittee: City of Hot Springs

NPDES No.: AR0033880 AFIN#26-00145

Date and Time Test Initiated: September 9, 2013 at 1235
Date and Time Test Terminated: September 17, 2013 at 1255
Dilution water used: Synthetic Soft Water #4019

PERCENT SURVIVAL

		Percent Effluent				
Time of Reading	Control	27 %	37 %	49 %	65 %	87 %
24 hour	100	70.0	30.0	30.0	20.0	40.0
48 hour	100	70.0	30.0	30.0	20.0	30.0
7 day	100	0.00	20.0	30.0	10.0	20.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

		Percent Effluent				
Replicates	Control	27 %	37 %	49 %	65 %	87 %
A	22	0	0	0	0	0
B	16	0	0	0	0	0
C	22	0	0	10	15	16
D	24	0	0	0	0	0
E	23	0	0	0	0	8
F	12	0	0	0	0	0
G	16	0	19	13	0	0
H	16	0	0	0	0	0
I	19	0	0	0	0	0
J	18	0	7	6	0	4
Mean per Adult	18.8	0.00	2.60	2.90	1.50	2.80
Mean per Surviving Adult	18.8	0.00	13.0	9.67	15.0	10.0
CV %	20.6	0.00	65.3	36.3	0.00	84.9

CV = Coefficient of variation = standard deviation * 100 / mean
(calculated based on young produced by surviving females)

Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

1. Fisher's Exact Test:

   Is the mean survival significantly different (p=0.05) than the control survival for the % effluent corresponding to (lethality):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	X YES	____ NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____ YES	____ NO

2.

   Is the mean number of young produced per female significantly different (p=0.05) than the control's number of young per female for the % effluent corresponding to (significant non-lethal effects):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	X YES	____ NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____ YES	____ NO

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 1 (TLP3B)
4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 1 (TGP3B)
5. NOEC Ceriodaphnia Lethality: <27 % (TOP3B)
6. LOEC Ceriodaphnia Lethality: 27 % (TXP3B)
7. NOEC Ceriodaphnia Sublethality: <27 % (TPP3B)
8. LOEC Ceriodaphnia Sublethality: 27 % (TYP3B)
9. Coefficient of variation for Ceriodaphnia Reproduction: 20.6 (TQP3B)

Appendix B: Test 1002.0

CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
CHEMICAL PARAMETERS CHART

PERMITTEE: City of Hot Springs
NPDES NO.: AR0033880 AFIN#26-00145
CONTACT: Mr. James Sorrells
ANALYST: 280, 298, 304, 307

2400
2400
2400

Test Initiated: DATE: September 9, 2013 TIME: 1235
Test Terminated: DATE: September 17, 2013 TIME: 1255

DILUTION	DAY						
Control	1	2	3	4	5	6	7
D.O. Initial	8.6	7.4	8.2	8.3	8.2	8.3	8.2
Final	7.8	8.3	8.5	8.7	8.4	8.2	7.8
pH Initial	7.6	7.6	7.8	7.6	7.6	7.7	7.5
Final	7.8	7.9	7.9	7.6	7.9	7.8	8.0
Alkalinity	32	NA	32	NA	32	NA	NA
Hardness	46	NA	42	NA	42	NA	NA
Conductivity	160	170	160	180	180	180	170
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
27 %	1	2	3	4	5	6	7
D.O. Initial	8.6	7.5	8.2	8.4	8.3	8.1	8.3
Final	6.0	8.3	8.7	8.8	8.5	8.3	8.1
pH Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
Final	8.0	8.1	8.2	8.0	8.3	8.0	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	220	220	220	240	250	250	250
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
37 %	1	2	3	4	5	6	7
D.O. Initial	8.6	7.4	8.2	8.4	8.1	8.0	8.1
Final	6.4	8.3	8.6	8.8	8.6	8.2	8.2
pH Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
Final	8.1	8.2	8.2	8.2	8.2	7.9	7.8
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	240	240	240	260	270	270	280
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
49 %	1	2	3	4	5	6	7
D.O. Initial	8.5	7.5	8.2	8.4	8.4	8.1	8.3
Final	6.8	8.3	7.8	9.0	8.4	8.3	8.0
pH Initial	7.6	7.5	7.8	7.5	7.6	7.5	7.5
Final	8.2	8.2	8.2	8.1	8.1	8.0	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	270	270	270	300	300	300	300
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
65 %	1	2	3	4	5	6	7
D.O. Initial	7.7	7.4	8.2	8.4	8.0	8.2	8.3
Final	7.0	8.3	8.4	8.7	8.3	8.3	7.7
pH Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.6
Final	8.2	8.1	8.1	8.2	8.0	8.0	7.7
Alkalinity	48	NA	46	NA	47	NA	NA
Hardness	68	NA	68	NA	68	NA	NA
Conductivity	310	310	300	340	340	340	350
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
87 %	1	2	3	4	5	6	7
D.O. Initial	8.3	7.2	8.2	8.3	8.3	8.0	7.9
Final	7.1	8.3	8.6	8.8	7.3	8.3	8.0
pH Initial	7.5	7.4	7.8	7.5	7.6	7.5	7.6
Final	8.2	8.3	8.3	8.3	7.5	8.1	7.9
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	350	360	350	400	440	430	440
Chlorine	NA	NA	NA	NA	NA	NA	NA

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Roys

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Chronic C.D.	Analyses Requested
1	Plant Effluent	9-8-13 2000-2400		X	X		3	X	No Fathead Analysis
		Container Type						P	
		Preservative						NO	

AIC Control No: 170390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2.1°C
Remarks:
Field pH calibration on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-8-13 1030	M. Mann	9-9-13 10:30 am
M. Mann	9-9-13 11:45	[signature]	9-9-13 1145

Comments:

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC Control No: 170390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 3°C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH	Remarks
1	Plant Effluent	9-10-13 0000-2400		X	X		3	X	
		Container Type						P	Field pH calibration
		Preservative						NO	on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-11-13 1110	M. Mann	9-11-13 1110
M. Mann	9-11-13 @ 12:05	[signature]	9-11-13 1205

Comments:

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs

Project Reference: Plant Effluent

Project Manager: James Sorrells

Sampled By: HAROLD MAULDIN

PO No.: 13-3032

AIC Control No: 170390

AIC Proposal No:

Carrier: Hot Springs Courier

Received Temperature °C: 2

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL		No of BOTTLES	Chronic CD												Remarks
3	Plant Effluent	9/12/13 0000-2400		X	X			3	X												
		Container Type							P												
		Preservative							NO												

Field pH calibration on ______ @ ______ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate

NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)

NORMAL or EXPEDITED IN ____ DAYS

Expedited results requested by: ____________

Who should AIC contact with questions: ____________

Phone: ____________ Fax: ____________

Report Attention to: Mr. James Sorrells

Report Address to: 320 Davidson Road Hot Springs, AR 71901

Relinquished By	Date/Time	Received By	Date/Time
A. Thomason	9-13-13 @ 1015	M. Mann	9-13-13 @ 10:15
M. Mann	9-13-13 @ 11:20	Received in Lab By: [signature]	9-13-13 1120

Comments:

4-85

AMERICAN INTERPLEX CORPORATION
LABORATORIES

September 30, 2013

Test Results of
Acute 48 hour Non-Renewal
Biomonitoring Testing
for

170741-1: Outfall 001 First Renewal
170741-2: Outfall 001 Second Renewal
170741-3: Outfall 001 Third Renewal
170741-4: Outfall 001 Original Screen

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Acute 48 hour Non-Renewal Biomonitoring utilizing *Ceriodaphnia dubia*
Outfall 001 First Renewal - City of Hot Springs
Client NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

Please find attached the results of the acute biomonitoring screens conducted as a follow up to the chronic toxicity event previously reported (AIC Control No. 170390). As indicated on the enclosed results, the sample was still acutely toxic on September 12, 2013 and exhibited no acute toxicity on September 19, 2013.

It it theorized the decrease in toxicity is directly related to the holding time which may have allowed microbial degradation, loss of volatile toxics, and/or biochemical degradation.

If I can be of further assistance, please feel free to contact me.

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Dilution Water Samples: Synthetic Soft Water #4021

Analysis	Result
Dissolved oxygen (mg/l)	7.6
pH (standard units)	7.7
Alkalinity (mg/l as CaCO3)	31
Hardness (mg/l as CaCO3)	46
Conductivity (umhos/cm)	170
Residual Chlorine (mg/l)	<0.05

Results Summary: Outfall 001 First Renewal

Ceriodaphnia dubia

The *Ceriodaphnia dubia* test was conducted from September 19, 2013 at 1615 to September 21, 2013 at 1700.

Statistical analyses:

NOEC = 100%

Concentration	24 hour % Survival	48 hour % Survival
Control	100	100
100%	100	100

Ceriodaphnia dubia
Survival Data

Number of organisms per chamber: 5
Volume of test chamber: 30 ml

Age of organisms: <24 hours
Volume of test solution: 15 ml

Effluent Concentration	Number of Survivors 24 Hours	48 Hours	% Survival	CV %
Control rep. A	5	5	100	0.00
100% rep. A	5	5	100	0.00

CV = Coefficient of variance = standard deviation X 100/mean

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	5	0	5
100%	5	0	5
Total	10	0	10

Critical Fisher's value (5,5,5) (alpha=0.05) is 1. b value is 5. Since b is greater than 1 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test

Group	Identification	Exposed	Dead	Sig 0.05
0	0	5	0	
1	100	5	0	

Chemical Data for
Ceriodaphnia dubia

Day 1	Control	100%
DO, mg/l	7.6	8.8
pH, su	7.7	7.3
Alkalinity, mg/l	31	NA
Hardness, mg/l	46	NA
Conductivity, umho/cm	170	380
Residual Chlorine, mg/l	<0.05	NA

Day 2		Control	100%
DO, mg/l	Final	7.5	8.7
pH, su	Final	8.0	7.4

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

170744 WR 9/19/13

Client: City of Hot Springs
Project:
Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Rojs

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Chronic CD.	Analyses Requested
1	Plant Effluent	9-8-13 0000-2400		X	X		3	X	
		Container Type						P	
		Preservative						NO	

No Fathead Analysis

AIC Control No: 170390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2.1°C
Remarks:
Field pH calibration on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ___ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-9-13 1030	M. Mann	9-9-13 10:30 AM
M. Mann	9-9-13 11:45	[signature]	9-9-13 1145

Comments:

City of Hot Springs
Waste Water Treatment Plant
320 Davidson Drive
Hot Springs, AR 71901

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Attn: Mo Shafii
5301 Northshore Dr.
North Little Rock, AR 72118-5317

AMERICAN INTERPLEX CORPORATION
LABORATORIES

August 30, 2013

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Outfall 001
City of Hot Springs

Control No. 169846-2

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Ceriodaphnia dubia*
Outfall 001 - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Chronic *Ceriodaphnia dubia* test: Due to laboratory error, the *Ceriodaphnia dubia* test was not renewed with the third sample. The test should be repeated. The data from the test is enclosed for your review.

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Table of Contents

I. Control Acceptance Criteria

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	23.6	PASS
Control CV < or = 40% per Surviving Female	13.6	PASS
Reproduction Minimum Significant Difference 13 to 47%	25.8	PASS
Critical Dilution CV < or = 40%	22.9	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly
   Test Method 1002.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point: Outfall 001
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.9	8.5	NA
pH (standard units)	7.0	7.4	NA
Alkalinity (mg/l as CaCO3)	26	34	NA
Hardness (mg/l as CaCO3)	67	71	NA
Conductivity (umhos/cm)	350	390	NA
Residual Chlorine (mg/l)	<0.05	<0.05	NA
Ammonia as N (mg/l)	0.12	0.30	NA

2. Dilution Water Samples: Synthetic Soft Water #4012
   a. Dates Prepared: August 8 through August 22, 2013
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.7	7.7	7.8
pH (standard units)	7.7	7.8	7.9
Alkalinity (mg/l as CaCO3)	32	32	NA
Hardness (mg/l as CaCO3)	47	42	NA
Conductivity (umhos/cm)	180	170	170
Residual Chlorine (mg/l)	<0.05	<0.05	NA

C. Test Methods
1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Method 1002.0, *Ceriodaphnia dubia* Survival and Reproduction.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Ceriodaphnia dubia Survival and Growth Method 1002.0

Date & Time Test Initiated:	August 22, 2013 at 1605
Date & Time Test Terminated:	August 29, 2013 at 1415
Type & Volume of Test Chamber:	30 ml disposable beaker
Volume of Sample:	15 ml
Number of Organisms per replicate:	1
Number of Replicates per dilution:	10

4. Acclimation of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms
1. Scientific Name
a. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis

The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was analyzed using Kolmogorov's Test for Normality and Bartlett's test and analyzed with Dunnett's Test to determine the No Observable Effects Concentration (NOEC) for Reproduction.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Ceriodaphnia dubia

Chronic reference tests are performed monthly.
A chronic reference test was performed on August 20, 2013 at 1505 to August 28, 2013 at 1450
The results were as follows: (Control No. 169867-2.)
Survival LC-50: 2125 mg/l
Growth IC-25: 1610 mg/l
Growth PMSD: 18.3

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	102	1.91
pH	SM 4500-H+ B	101	0.268
Conductivity	EPA 120.1	101	4.71

VI. Organism History

Ceriodaphnia dubia

Date: August 22, 2013
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

VII. Results Summary *Ceriodaphnia dubia*, Cladoceran Survival and Reproduction Test -- Method 1002.0

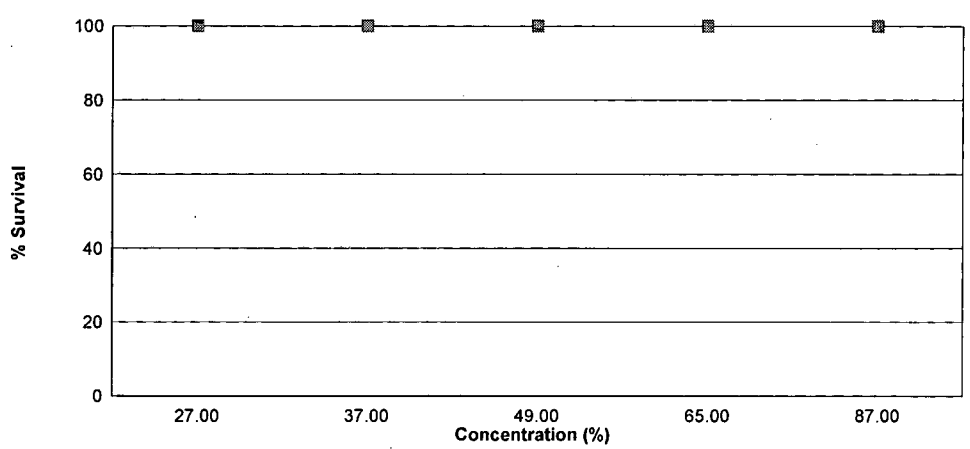
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on August 22, 2013 at 1605 and continued through August 29, 2013 at 1415. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 87 % effluent
b.) NOEC reproduction = 87 % effluent



Summary of the 7-day *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	23.6
27 %	100	24.2
37 %	100	27.6
49 %	100	26.4
65 %	100	24.2
87 %	100	25.0

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 22, 2013 at 1605
Date and Time Test Terminated: August 29, 2013 at 1415

Concentration: Control													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	4	4	2	3	4	4	3	2	33	10	3.30
5	0	0	0	0	0	1	0	0	0	6	7	10	0.700
6	9	9	7	8	7	8	7	9	8	8	80	10	8.00
7	14	14	11	13	14	11	12	14	13	0	116	10	11.6
8													
TOTAL	27	26	22	25	23	23	23	27	24	16	236	10	23.6

Concentration: 27 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	2	3	3	3	2	3	2	3	2	27	10	2.70
5	8	7	1	0	0	0	0	0	0	6	22	10	2.20
6	1	12	13	11	10	10	11	8	7	6	89	10	8.90
7	16	0	17	18	6	0	17	17	13	0	104	10	10.4
8													
TOTAL	29	21	34	32	19	12	31	27	23	14	242	10	24.2

Concentration: 37 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	3	3	3	2	3	2	2	2	27	10	2.70
5	0	0	0	0	0	1	0	0	6	6	13	10	1.30
6	12	12	12	11	10	6	8	10	13	10	104	10	10.4
7	18	16	17	18	16	15	15	17	0	0	132	10	13.2
8													
TOTAL	34	31	32	32	29	24	26	29	21	18	276	10	27.6

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 22, 2013 at 1605
Date and Time Test Terminated: August 29, 2013 at 1415

Concentration: 49 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	2	5	2	3	4	2	2	2	29	10	2.90
5	0	5	0	0	0	1	0	6	4	3	19	10	1.90
6	10	0	11	11	11	8	10	0	12	10	83	10	8.30
7	17	18	16	18	16	17	15	16	0	0	133	10	13.3
8													
TOTAL	31	26	29	34	29	29	29	24	18	15	264	10	26.4

Concentration: 65 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	2	3	5	3	3	3	2	3	2	2	28	10	2.80
5	6	0	0	0	0	1	0	0	5	4	16	10	1.60
6	13	11	11	11	10	10	8	10	0	12	96	10	9.60
7	0	15	14	16	12	0	15	16	14	0	102	10	10.2
8													
TOTAL	21	29	30	30	25	14	25	29	21	18	242	10	24.2

Concentration: 87 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	4	3	3	3	3	4	3	2	2	31	10	3.10
5	8	6	1	0	0	0	0	0	5	5	25	10	2.50
6	0	2	11	12	6	10	8	8	0	7	64	10	6.40
7	16	15	16	18	16	0	18	17	14	0	130	10	13.0
8													
TOTAL	28	27	31	33	25	13	30	28	21	14	250	10	25.0

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	10	0	10
27 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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	Alive	Dead	Total Animals
Control	10	0	10
37 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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	Alive	Dead	Total Animals
Control	10	0	10
49 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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	Alive	Dead	Total Animals
Control	10	0	10
65 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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.

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
87 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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 Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	27 %	10	0	
2	37 %	10	0	
3	49 %	10	0	
4	65 %	10	0	
5	87 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality		No Transformation
D = 0.1154 D* = 0.9054 Critical D* = 1.035	(alpha = 0.01, N = 60)	
Data PASS normality test (alpha = 0.01).		

Bartlett's Test for Homogeneity of Variance		No Transformation
Calculated B1 statistic = 6.592 Critical B = 15.086	(alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.		

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	117.9	23.58	0.6766
Within (Error)	54	1882	34.85	
Total	59	2000		

Critical F = 3.38 (alpha = 0.01, df = 5,54)
2.38 (alpha = 0.05, df = 5,54)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Dunnett's Test - Table 1 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	23.6	23.6		
2	27 %	24.2	24.2	-0.2273	
3	37 %	27.6	27.6	-1.515	
4	49 %	26.4	26.4	-1.061	
5	65 %	24.2	24.2	-0.2273	
6	87 %	25	25	-0.5303	

Dunnett's critical value = 2.31 (1 Tailed, alpha = 0.05, df [used] = 5,40) (Actual df = 5,54)

Dunnett's Test - Table 2 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	10			
2	27 %	10	6.099	25.8	-0.6
3	37 %	10	6.099	25.8	-4
4	49 %	10	6.099	25.8	-2.8
5	65 %	10	6.099	25.8	-0.6
6	87 %	10	6.099	25.8	-1.4

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 22, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.5	7.7	7.5	7.8	7.8	8.1
	Final	7.7	8.0	8.7	8.2	8.2	7.8	7.8
pH, units	Initial	7.7	7.7	7.8	8.0	7.9	8.0	7.7
	Final	8.0	8.2	8.0	8.0	8.0	7.8	7.8
Alkalinity, mg CaCO3/l		32	NA	32	NA	NA	NA	NA
Hardness, mg CaCO3/l		47	NA	42	NA	NA	NA	NA
Conductivity, umhos/cm		180	180	170	180	170	170	190
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	NA	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.4	8.0	7.5	8.0	8.0	7.5
	Final	7.5	8.2	8.6	8.2	8.2	7.7	7.7
pH, units	Initial	7.6	7.7	7.5	7.8	7.7	7.8	7.5
	Final	8.0	8.1	8.0	8.0	8.1	7.6	7.6

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.6	8.6	7.6	8.2	7.7	7.7
	Final	7.6	8.0	8.6	8.0	8.2	7.6	7.6
pH, units	Initial	7.6	7.7	7.4	7.7	7.6	7.7	7.6
	Final	8.0	8.1	8.0	8.0	8.1	7.6	7.6

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 22, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.5	8.7	7.6	8.1	7.7	7.7
	Final	7.7	8.1	8.6	8.2	8.2	7.6	7.9
pH, units	Initial	7.5	7.7	7.3	7.6	7.6	7.6	7.5
	Final	8.0	8.1	8.0	8.1	8.2	7.5	7.5

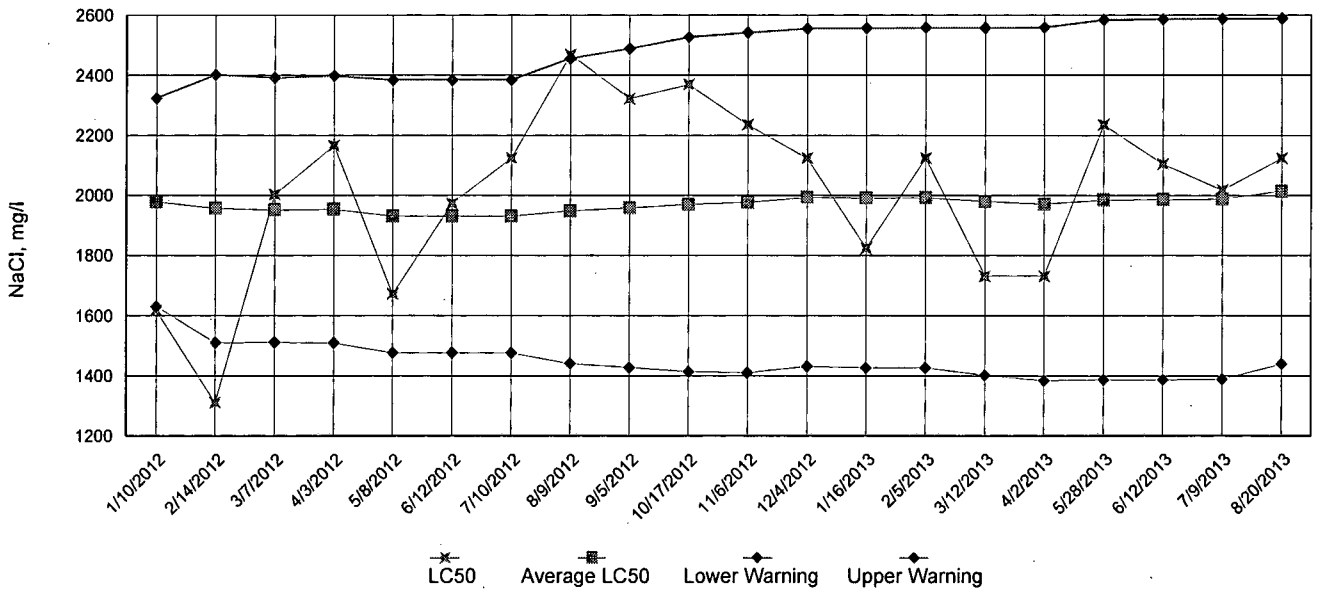
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.9	7.4	8.8	7.6	8.7	7.7	7.3
	Final	7.7	8.0	8.6	8.4	8.1	7.6	7.6
pH, units	Initial	7.5	7.7	7.2	7.5	7.5	7.6	7.5
	Final	8.0	8.0	8.0	8.1	8.1	7.5	7.5
Alkalinity, mg CaCO3/l		36	NA	30	NA	NA	NA	NA
Hardness, mg CaCO3/l		54	NA	58	NA	NA	NA	NA
Conductivity, umhos/cm		250	260	280	290	310	310	330
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	NA	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	7.4	8.9	7.6	8.3	7.9	7.4
	Final	7.4	8.1	8.7	8.2	7.7	5.4	7.2
pH, units	Initial	7.4	7.7	7.1	7.4	7.4	7.6	7.6
	Final	8.0	8.0	7.9	8.1	8.3	7.5	7.4

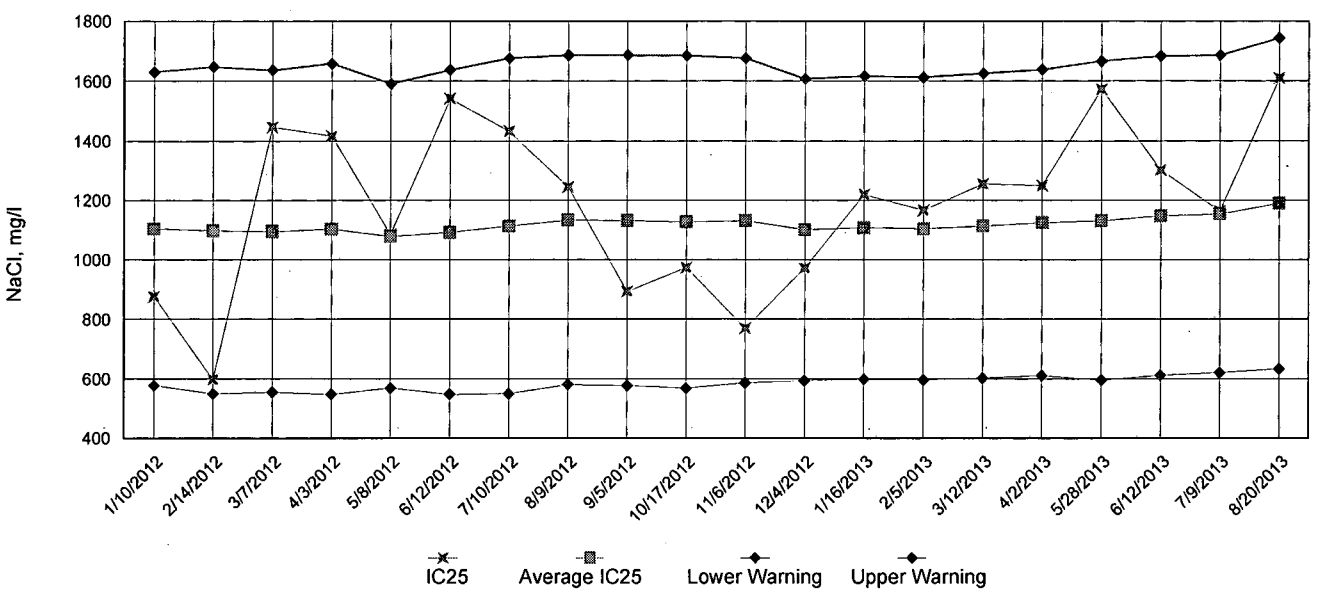
Appendix A4: Test 1002.0

Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL		No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
1	Plant Effluent	8-18-13 0900 - 2400		X	X			3	X
		Container Type							P
		Preservative							NO

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 20c
Remarks
Field pH calibration on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ___ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
A. Thomason	8-19-13 @ 1010	M. Mann	8-19-13 @ 10:10am
Relinquished By: M. Mann	Date/Time 8-19-13 @ 11:10AM	Received In Lab By: Angie Hopton	Date/Time 8-19-13 1110

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL		No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
2	Plant Effluent	8-20-13 0900-2400		X	X			3	X
		Container Type							P
		Preservative							NO

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 23C
Remarks

Field pH calibration
on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
A. Ross	8-21-13 0900	G. Mann	8-21-13 900
G. Mann	8-21-13 1130	Jimmy Day	8/21/13 1130

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By:

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
3	Plant Effluent	8/22/13 0000-2400		X			3	X
		Container Type						P
		Preservative						NO

AIC Control No: 16984b
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 2
Remarks
Field pH calibration on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	8-23-13 0955	[signature]	8-23-13 9:55
[signature]	8-23-13 11:20	[signature]	8-23-13 1120

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

September 30, 2013

Test Results of
Acute 48 hour Non-Renewal
Biomonitoring Testing
for

170741-1: Outfall 001 First Renewal
170741-2: Outfall 001 Second Renewal
170741-3: Outfall 001 Third Renewal
170741-4: Outfall 001 Original Screen

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Acute 48 hour Non-Renewal Biomonitoring utilizing *Ceriodaphnia dubia*
Outfall 001 First Renewal - City of Hot Springs
Client NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

Please find attached the results of the acute biomonitoring screens conducted as a follow up to the chronic toxicity event previously reported (AIC Control No. 170390). As indicated on the enclosed results, the sample was still acutely toxic on September 12, 2013 and exhibited no acute toxicity on September 19, 2013.

It it theorized the decrease in toxicity is directly related to the holding time which may have allowed microbial degradation, loss of volatile toxics, and/or biochemical degradation.

If I can be of further assistance, please feel free to contact me.

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Dilution Water Samples: Synthetic Soft Water #4021

Analysis	Result
Dissolved oxygen (mg/l)	7.6
pH (standard units)	7.7
Alkalinity (mg/l as CaCO3)	31
Hardness (mg/l as CaCO3)	46
Conductivity (umhos/cm)	170
Residual Chlorine (mg/l)	<0.05

Results Summary: Outfall 001 First Renewal

Ceriodaphnia dubia

The *Ceriodaphnia dubia* test was conducted from September 19, 2013 at 1615 to September 21, 2013 at 1700.

Statistical analyses:

NOEC = 100%

Concentration	24 hour % Survival	48 hour % Survival
Control	100	100
100%	100	100

Ceriodaphnia dubia
Survival Data

Number of organisms per chamber: 5
Volume of test chamber: 30 ml

Age of organisms: <24 hours
Volume of test solution: 15 ml

Effluent Concentration	Number of Survivors 24 Hours	48 Hours	% Survival	CV %
Control rep. A	5	5	100	0.00
100% rep. A	5	5	100	0.00

CV = Coefficient of variance = standard deviation X 100/mean

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	5	0	5
100%	5	0	5
Total	10	0	10

Critical Fisher's value (5,5,5) (alpha=0.05) is 1. b value is 5. Since b is greater than 1 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test

Group	Identification	Exposed	Dead	Sig 0.05
0	0	5	0	
1	100	5	0	

Chemical Data for
Ceriodaphnia dubia

Day 1	Control	100%
DO, mg/l	7.6	8.8
pH, su	7.7	7.3
Alkalinity, mg/l	31	NA
Hardness, mg/l	46	NA
Conductivity, umho/cm	170	380
Residual Chlorine, mg/l	<0.05	NA

Day 2		Control	100%
DO, mg/l	Final	7.5	8.7
pH, su	Final	8.0	7.4

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

month WR 9/19/13

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Chronic C.D.	Analyses Requested
1	Plant Effluent	9-8-13 0000-2400		X	X		3	X	
		Container Type						P	
		Preservative						NO	

No FATHEAD ANALYSIS

AIC Control No: 120390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2.1 °C
Remarks:
Field pH calibration on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z = Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-8-13 1030	M. Mann	9-9-13 10:30 AM
M. Mann	9-9-13 11:45	[signature]	9-9-13 1145

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

October 1, 2013
Control No. 171032

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

This report contains the analytical results and supporting information for samples submitted on September 27, 2013. Attached please find a copy of the Chain of Custody and/or other documents received. Note that any remaining sample will be discarded two weeks from the original report date unless other arrangements are made.

This report is intended for the sole use of the client listed above. Assessment of the data requires access to the entire document.

This report has been reviewed by the Laboratory Director or a qualified designee.

John Overbey
Laboratory Director

This document has been distributed to the following:

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

SAMPLE INFORMATION

Project Description:
Three (3) water sample(s) (AIC COntrol No. 170741-1,2,3) resubmitted September 27, 2013
P.O. No. 13-3032

Receipt Details:
A Chain of Custody was not provided with the sample(s).

Each sample container was checked for proper labeling, including date and time sampled. Sample containers were reviewed for proper type, adequate volume, integrity, temperature, preservation, and holding times. Any exceptions are noted below:

Sample Identification:

Laboratory ID	Client Sample ID	Sampled Date/Time	Notes
171032-1	Plant Effluent 9-8-13 0000-2400	08-Sep-2013 2359	
171032-2	Plant Effluent 9-10-13 0000-2400	10-Sep-2013 2359	
171032-3	Plant Effluent 9-12-13 0000-2400	12-Sep-2013 2359	

Case Narrative:
There were no qualifiers for this data and all samples met quality control criteria.

References:
"Methods for Chemical Analysis of Water and Wastes", EPA/600/4-79-020 (Mar 1983) with updates and supplements EPA/600/5-91-010 (Jun 1991), EPA/600/R-92-129 (Aug 1992) and EPA/600/R-93-100 (Aug 1993).
"Test Methods for Evaluating Solid Waste Physical/Chemical Methods (SW846)", Third Edition.
"Standard Methods for the Examination of Water and Wastewaters", 21st edition.
"American Society for Testing and Materials" (ASTM).
"Association of Analytical Chemists" (AOAC).

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

ANALYTICAL RESULTS

AIC No. 171032-1
Sample Identification: Plant Effluent 9-8-13 0000-2400

Analyte		Result	RL	Units	Qualifier
Total Organic Carbon		**6.1**	1	**mg/l**	
SM 5310 C	Prep: 27-Sep-2013 1516 by 308	Analyzed: 27-Sep-2013 1814 by 308		Batch: W45076	
Aluminum		**< 0.04**	0.04	**mg/l**	
EPA 200.7	Prep: 30-Sep-2013 0901 by 271	Analyzed: 01-Oct-2013 1242 by 305		Batch: S35491	
Sulfate		**29**	0.2	**mg/l**	
EPA 300.0	Prep: 27-Sep-2013 1628 by 07	Analyzed: 27-Sep-2013 2205 by 07		Batch: C16076	

AIC No. 171032-2
Sample Identification: Plant Effluent 9-10-13 0000-2400

Analyte		Result	RL	Units	Qualifier
Total Organic Carbon		**5.9**	1	**mg/l**	
SM 5310 C	Prep: 27-Sep-2013 1516 by 308	Analyzed: 27-Sep-2013 1828 by 308		Batch: W45076	
Aluminum		**0.041**	0.04	**mg/l**	
EPA 200.7	Prep: 30-Sep-2013 0901 by 271	Analyzed: 01-Oct-2013 1245 by 305		Batch: S35491	
Sulfate		**39**	0.2	**mg/l**	
EPA 300.0	Prep: 27-Sep-2013 1628 by 07	Analyzed: 27-Sep-2013 2232 by 07		Batch: C16076	

AIC No. 171032-3
Sample Identification: Plant Effluent 9-12-13 0000-2400

Analyte		Result	RL	Units	Qualifier
Total Organic Carbon		**5.4**	1	**mg/l**	
SM 5310 C	Prep: 27-Sep-2013 1516 by 308	Analyzed: 27-Sep-2013 1843 by 308		Batch: W45076	
Aluminum		**< 0.04**	0.04	**mg/l**	
EPA 200.7	Prep: 30-Sep-2013 0901 by 271	Analyzed: 01-Oct-2013 1248 by 305		Batch: S35491	
Sulfate		**34**	0.2	**mg/l**	
EPA 300.0	Prep: 27-Sep-2013 1628 by 07	Analyzed: 27-Sep-2013 2259 by 07		Batch: C16076	

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

LABORATORY CONTROL SAMPLE RESULTS

Analyte	Spike Amount	%	Limits	RPD	Limit	Batch	Preparation Date	Analysis Date	Dil	Qual
Total Organic Carbon	10 mg/l	96.4	80.0-120			W45076	27Sep13 1459 by 308	27Sep13 1648 by 308		
Aluminum	5 mg/l	97.6	85.0-115			S35491	30Sep13 0901 by 271	01Oct13 1152 by 305		
Sulfate	20 mg/l	108	90.0-110			C16076	27Sep13 1628 by 07	27Sep13 1710 by 07		

MATRIX SPIKE SAMPLE RESULTS

Analyte	Sample	Spike Amount	%	Limits	Batch	Preparation Date	Analysis Date	Dil	Qual
Total Organic Carbon	171006-1	10 mg/l	100	80.0-120	W45076	27Sep13 1459 by 308	27Sep13 1717 by 308		
	171006-1	10 mg/l	97.9	80.0-120	W45076	27Sep13 1459 by 308	27Sep13 1731 by 308		
	Relative Percent Difference:		1.86	25.0	W45076				
Aluminum	171022-2	5 mg/l	96.7	75.0-125	S35491	30Sep13 0901 by 271	01Oct13 1155 by 305		
	171022-2	5 mg/l	96.6	75.0-125	S35491	30Sep13 0901 by 271	01Oct13 1158 by 305		
	Relative Percent Difference:		0.142	20.0	S35491				
Sulfate	171021-1	20 mg/l	89.1	80.0-120	C16076	27Sep13 1628 by 07	27Sep13 1737 by 07		
	171021-1	20 mg/l	93.6	80.0-120	C16076	27Sep13 1628 by 07	27Sep13 1804 by 07		
	Relative Percent Difference:		4.28	10.0	C16076				

LABORATORY BLANK RESULTS

Analyte	Result	RL	PQL	QC Sample	Preparation Date	Analysis Date	Qual
Total Organic Carbon	< 1 mg/l	1	1	W45076-1	27Sep13 1459 by 308	27Sep13 1633 by 308	
Aluminum	< 0.04 mg/l	0.04	0.04	S35491-1	30Sep13 0901 by 271	01Oct13 1149 by 305	
Sulfate	< 0.2 mg/l	0.2	0.2	C16076-1	27Sep13 1628 by 07	27Sep13 1644 by 07	

AMERICAN INTERPLEX CORPORATION LABORATORIES

171032 wn 9/13/13

~~170744~~ wn 9/10/13

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs

Project Reference: Plant Effluent

Project Manager: James Sorrells

Sampled By: A. Ross

PO No.: 13-3032

AIC Control No: ~~170390~~

AIC Proposal No:

Carrier: Hot Springs Couriers

Received Temperature °C: 2.1 °C

Analyses Requested: Chronic CD

No Fathead Analysis

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL	No of BOTTLES	Chronic CD	Remarks
1	Plant Effluent	9-8-13 0000-2400		X	X		3	x	
		Container Type					P		
		Preservative					NO		

Field pH calibration on ______ @ ______ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate

NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH4)_2SO4$

Turnaround Time Requested: (Please circle)

NORMAL or EXPEDITED IN ____ DAYS

Expedited results requested by: ____________

Who should AIC contact with questions: ____________

Phone: ______ Fax: ______

Report Attention to: Mr. James Sorrells

Report Address to: 320 Davidson Road, Hot Springs, AR 71901

Relinquished By	Date/Time	Received By	Date/Time
[signature]	9-8-13 1030	M. Mann	9-9-13 10:30 am
M. Mann	9-9-13 11:45	Received in Lab By: [signature]	9-9-13 1145

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

August 30, 2013

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Outfall 001
City of Hot Springs

Control No. 169846-1

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Pimephales promelas* (Fathead minnow)
Outfall 001 - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Method 1000.0 Chronic *Pimephales promelas* (Fathead minnow) Survival and Growth Test: The No Observable Effects Concentration (NOEC) for survival occurred at 87 % effluent, which is above the critical dilution of 65 %. The NOEC for growth occurred at 87 % effluent, which is above the critical dilution of 65 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the Fathead minnow test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Table of Contents

I. Control Acceptance Criteria

Pimephales promelas (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.296	PASS
Control Growth CV < or = 40%	7.47	PASS
Growth Minimum Significant Difference 12 to 30%	20.0	PASS
Critical Dilution CV < or = 40%	11.6	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly Test Method 1000.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point: Outfall 001
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.2	8.9	8.5
pH (standard units)	7.4	7.0	7.4
Alkalinity (mg/l as CaCO3)	41	26	34
Hardness (mg/l as CaCO3)	59	67	71
Conductivity (umhos/cm)	290	350	390
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.28	0.12	0.30

2. Dilution Water Samples: Synthetic Soft Water #4012
   a. Dates Prepared: August 8 through August 22, 2013
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.7	7.7	7.8
pH (standard units)	7.7	7.8	7.9
Alkalinity (mg/l as CaCO3)	32	32	32
Hardness (mg/l as CaCO3)	47	42	42
Conductivity (umhos/cm)	180	170	170
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05

C. Test Methods
1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Method 1000.0, Fathead Minnow Survival and Growth.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Pimephales promelas (Fathead minnow) Survival and Growth Method 1000.0

Date & Time Test Initiated:	August 20, 2013 at 1200
Date & Time Test Terminated:	August 27, 2013 at 1010
Type & Volume of Test Chamber:	500 ml disposable beaker
Volume of Sample:	250 ml
Number of Organisms per replicate:	8
Number of Replicates per dilution:	5

4. Acclimation of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms
1. Scientific Name
a. Test 1000.0 *Pimephales promelas*

III. Data Analysis

The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Pimephales promelas (Fathead minnow) survival data was transformed using the Arc Sine transformation. Normality and homogeneity of variance were checked using Shapiro-Wilk's. The survival data was then analyzed using Steel's Many-One Rank Test to determine the No Observable Effects Concentration (NOEC).

Fathead minnow growth data was analyzed for normality and homogeneity of variance using Shapiro-Wilk's and Bartlett's test. Dunnett's Test was used to determine the No Observable Effects Concentration (NOEC) for growth.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Pimephales promelas (Fathead minnow)

Chronic reference tests are performed monthly.
A chronic reference test was performed on August 20, 2013 at 1715 to August 27, 2013 at 1520
The results were as follows: (Control No. 169867-1.)
Survival LC-50: 5670.1 mg/l
Growth IC-25: 3143 mg/l
Growth PMSD: 17.3

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	102	1.91
pH	SM 4500-H+ B	101	0.268
Conductivity	EPA 120.1	101	4.71

VI. Organism History

Pimephales promelas (Fathead minnow)
Date: August 20, 2013
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

VII. Results Summary *Pimephales promelas*, Fathead minnow Larval Survival and Growth Test -- Method 1000.0

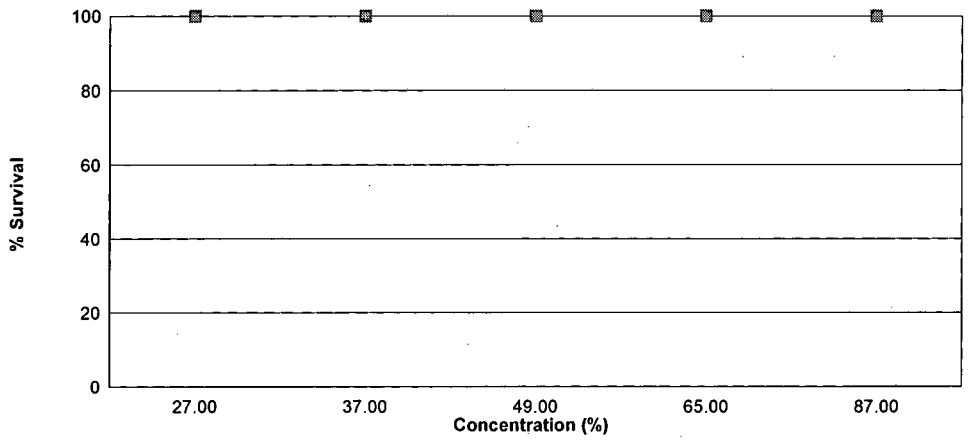
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on August 20, 2013 at 1200 and continued through August 27, 2013 at 1010. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 87 % effluent
b.) NOEC growth = 87 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.296
27 %	100	0.337
37 %	100	0.323
49 %	100	0.302
65 %	100	0.292
87 %	100	0.302

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: August 20, 2013 at 1200
Date and Time Test Terminated: August 27, 2013 at 1010

Concentration	Replicate	Number of Survivors						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
27 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
37 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
49 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
65 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
87 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: August 20, 2013 at 1200
Test Terminated: August 27, 2013 at 1010
Drying Started: August 23, 2013 at 1527
Drying Ended: August 29, 2013 at 1400

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.91827	.92042	0.00215	8	0.269
	B	.91878	.92121	0.00243	8	0.304
	C	.91659	.91922	0.00263	8	0.329
	D	.91638	.91872	0.00234	8	0.292
	E	.91455	.91685	0.00230	8	0.288
27 %	A	.91583	.91811	0.00228	8	0.285
	B	.91943	.92233	0.00290	8	0.362
	C	.92408	.92709	0.00301	8	0.376
	D	.92651	.92924	0.00273	8	0.341
	E	.92767	.93024	0.00257	8	0.321
37 %	A	.92653	.92915	0.00262	8	0.328
	B	.94872	.95160	0.00288	8	0.360
	C	.94724	.94985	0.00261	8	0.326
	D	.94532	.94750	0.00218	8	0.272
	E	.94881	.95146	0.00265	8	0.331
49 %	A	.94115	.94330	0.00215	8	0.269
	B	.92530	.92779	0.00249	8	0.311
	C	.92632	.92901	0.00269	8	0.336
	D	.93070	.93312	0.00242	8	0.302
	E	.93427	.93660	0.00233	8	0.291
65 %	A	.93862	.94107	0.00245	8	0.306
	B	.93944	.94155	0.00211	8	0.264
	C	.94132	.94377	0.00245	8	0.306
	D	.93810	.94077	0.00267	8	0.334
	E	.93899	.94100	0.00201	8	0.251
87 %	A	.93682	.93952	0.00270	8	0.338
	B	.93818	.93991	0.00173	8	0.216
	C	.93688	.93881	0.00193	8	0.241
	D	.93804	.94073	0.00269	8	0.336
	E	.95233	.95537	0.00304	8	0.380

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

		Transformation of Data		Transform: Arc Sin(Square Root(Y))
Group	Identification	Rep	Value	Transformed
1	Control	1	1.00000	1.39310
1	Control	2	1.00000	1.39310
1	Control	3	1.00000	1.39310
1	Control	4	1.00000	1.39310
1	Control	5	1.00000	1.39310
2	27 %	1	1.00000	1.39310
2	27 %	2	1.00000	1.39310
2	27 %	3	1.00000	1.39310
2	27 %	4	1.00000	1.39310
2	27 %	5	1.00000	1.39310
3	37 %	1	1.00000	1.39310
3	37 %	2	1.00000	1.39310
3	37 %	3	1.00000	1.39310
3	37 %	4	1.00000	1.39310
3	37 %	5	1.00000	1.39310
4	49 %	1	1.00000	1.39310
4	49 %	2	1.00000	1.39310
4	49 %	3	1.00000	1.39310
4	49 %	4	1.00000	1.39310
4	49 %	5	1.00000	1.39310
5	65 %	1	1.00000	1.39310
5	65 %	2	1.00000	1.39310
5	65 %	3	1.00000	1.39310
5	65 %	4	1.00000	1.39310
5	65 %	5	1.00000	1.39310
6	87 %	1	1.00000	1.39310
6	87 %	2	1.00000	1.39310
6	87 %	3	1.00000	1.39310
6	87 %	4	1.00000	1.39310
6	87 %	5	1.00000	1.39310

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Shapiro - Wilk's Test for Normality		Transform: Arc Sin(Square Root(Y))
D = 0		
W = 0		
Critical W = 0.9	(alpha = 0.01, N = 30)	
Critical W = 0.927	(alpha = 0.05, N = 30)	

Data FAIL normality test (alpha = 0.01).

Steel's Many-One Rank Test — Transform: Arc Sin(Square Root(Y))

Ho:Control<Treatment

Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	27 %	27.50	16.00	5.00	
3	37 %	27.50	16.00	5.00	
4	49 %	27.50	16.00	5.00	
5	65 %	27.50	16.00	5.00	
6	87 %	27.50	16.00	5.00	

Critical values are 1 tailed (k=5)

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality		No Transformation
D = 0.03787		
W = 0.9742		
Critical W = 0.9	(alpha = 0.01, N = 30)	
Critical W = 0.927	(alpha = 0.05, N = 30)	
Data PASS normality test (alpha = 0.01).		

Bartlett's Test for Homogeneity of Variance		No Transformation
Calculated B1 statistic = 7.076		
Critical B = 15.086	(alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.		

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	0.007651	0.00153	0.9696
Within (Error)	24	0.03787	0.001578	
Total	29	0.04552		

Critical F = 3.9 (alpha = 0.01, df = 5,24)
2.62 (alpha = 0.05, df = 5,24)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Dunnett's Test - Table 1 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	0.2964	0.2964		
2	27 %	0.337	0.337	-1.616	
3	37 %	0.3234	0.3234	-1.075	
4	49 %	0.3018	0.3018	-0.2149	
5	65 %	0.2922	0.2922	0.1672	
6	87 %	0.3022	0.3022	-0.2309	

Dunnett's critical value = 2.36 (1 Tailed, alpha = 0.05, df = 5,24)

Dunnett's Test - Table 2 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	5			
2	27 %	5	0.05929	20	-0.0406
3	37 %	5	0.05929	20	-0.027
4	49 %	5	0.05929	20	-0.0054
5	65 %	5	0.05929	20	0.0042
6	87 %	5	0.05929	20	-0.0058

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 20, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.5	7.7	7.5	7.8	7.8	8.1
	Final	7.5	7.2	7.5	7.9	7.7	7.1	7.9
pH, units	Initial	7.7	7.7	7.8	8.0	7.9	8.0	7.7
	Final	7.5	7.3	7.8	8.1	8.1	7.6	7.9
Alkalinity, mg CaCO3/l		32	NA	32	NA	32	NA	NA
Hardness, mg CaCO3/l		47	NA	42	NA	42	NA	NA
Conductivity, umhos/cm		180	180	170	180	170	170	190
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.4	8.0	7.5	8.0	8.0	7.5
	Final	7.5	7.2	7.3	7.9	7.8	8.1	7.7
pH, units	Initial	7.6	7.7	7.5	7.8	7.7	7.8	7.5
	Final	7.5	7.3	7.7	8.1	8.2	7.5	7.7

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.6	8.6	7.6	8.2	7.7	7.7
	Final	7.5	7.0	7.5	8.0	7.9	8.1	7.8
pH, units	Initial	7.6	7.7	7.4	7.7	7.6	7.7	7.6
	Final	7.5	7.3	7.7	8.1	8.2	7.5	7.6

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 20, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.5	8.7	7.6	8.1	7.7	7.7
	Final	7.5	7.4	7.6	8.0	8.1	8.2	7.8
pH, units	Initial	7.5	7.7	7.3	7.6	7.6	7.6	7.5
	Final	7.5	7.4	7.6	8.1	8.2	7.6	7.6

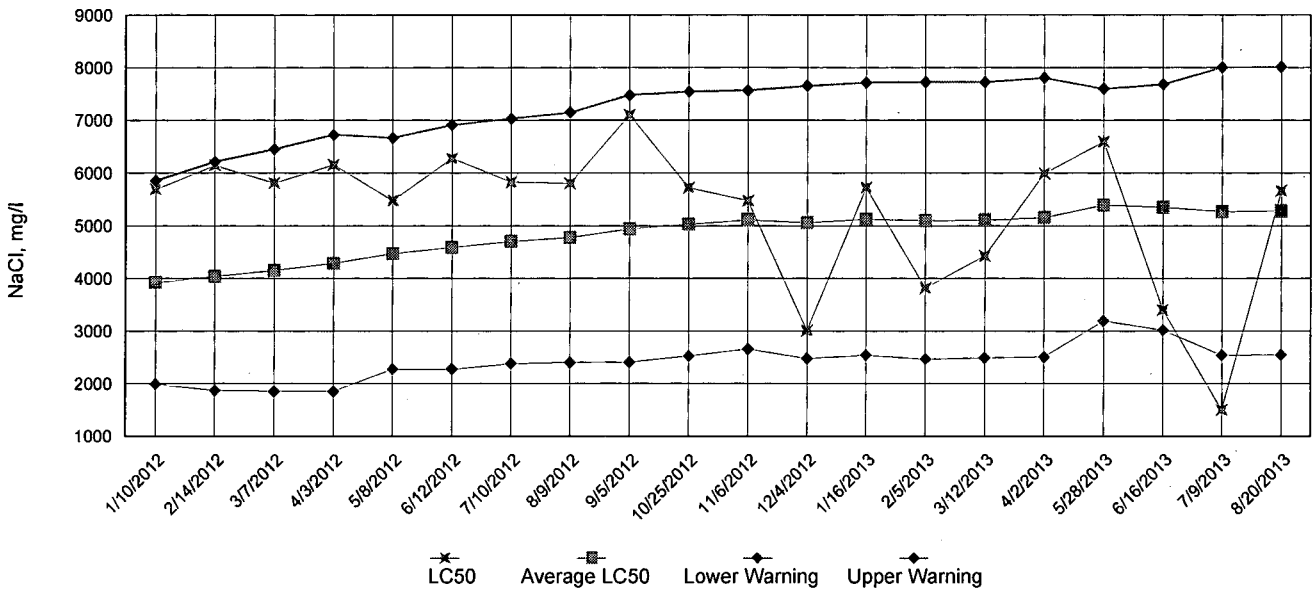
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.9	7.4	8.8	7.6	8.7	7.7	7.3
	Final	7.5	7.4	7.4	7.9	8.1	8.0	7.7
pH, units	Initial	7.5	7.7	7.2	7.5	7.5	7.6	7.5
	Final	7.5	7.4	7.6	8.1	8.3	7.6	7.5
Alkalinity, mg CaCO3/l		36	NA	30	NA	35	NA	NA
Hardness, mg CaCO3/l		54	NA	58	NA	61	NA	NA
Conductivity, umhos/cm		250	260	280	290	310	310	330
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	7.4	8.9	7.6	8.3	7.9	7.4
	Final	7.5	8.5	7.4	7.8	8.1	8.0	7.7
pH, units	Initial	7.4	7.7	7.1	7.4	7.4	7.6	7.6
	Final	7.5	7.4	7.6	8.1	8.1	7.6	7.4

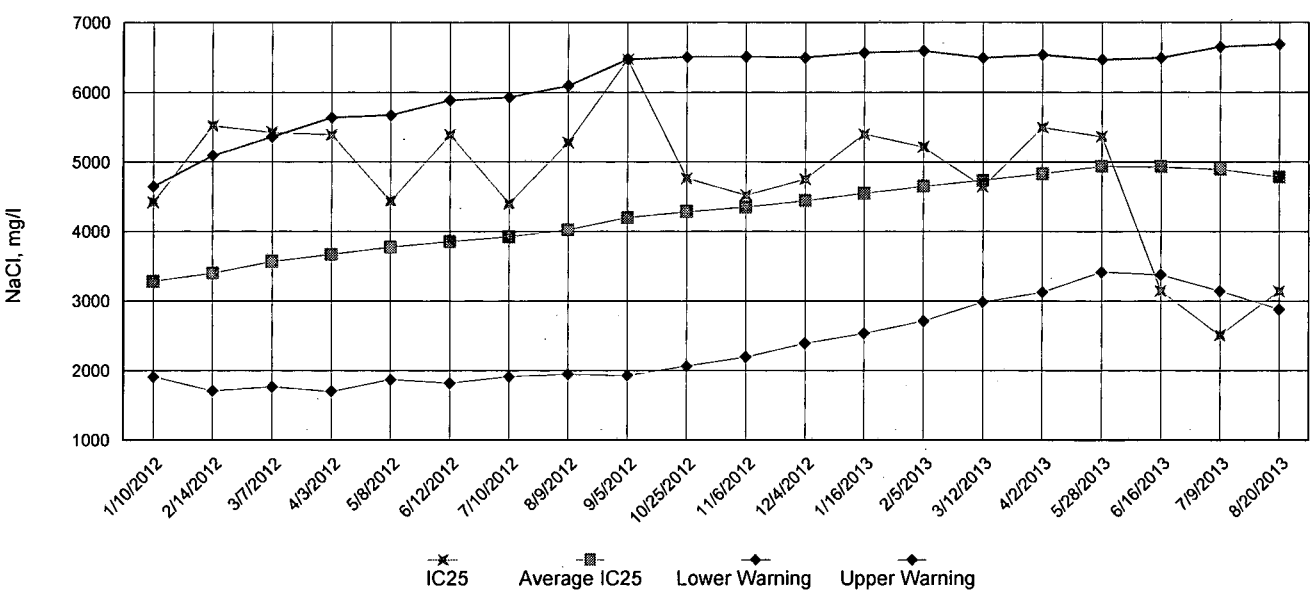
Appendix A4: Test 1000.0

Chronic Reference Toxicant, *Pimephales promelas* (Fathead Minnow)

LC50 Survival Data



IC25 Growth Data



Appendix B: Test 1000.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

Permittee: City of Hot Springs

NPDES No.: AR0033880 AFIN#26-00145

Date and Time Test Initiated: August 20, 2013 at 1200
Date and Time Test Terminated: August 27, 2013 at 1010
Dilution water used: Synthetic Soft Water #4012

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in replicate chambers A	B	C	D	E	Mean percent survival 24 hr	48 hr	7 days	CV%
Control	100	100	100	100	100	100	100	100	0.00
27 %	100	100	100	100	100	100	100	100	0.00
37 %	100	100	100	100	100	100	100	100	0.00
49 %	100	100	100	100	100	100	100	100	0.00
65 %	100	100	100	100	100	100	100	100	0.00
87 %	100	100	100	100	100	100	100	100	0.00

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers A	B	C	D	E	Mean dry weight, mg	CV%
Control	0.269	0.304	0.329	0.292	0.288	0.296	7.47
27 %	0.285	0.362	0.376	0.341	0.321	0.337	10.6
37 %	0.328	0.360	0.326	0.272	0.331	0.323	9.86
49 %	0.269	0.311	0.336	0.302	0.291	0.302	8.20
65 %	0.306	0.264	0.306	0.334	0.251	0.292	11.6
87 %	0.338	0.216	0.241	0.336	0.380	0.302	23.2

CV = Coefficient of variation = standard deviation * 100 / mean

Appendix B: Test 1000.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

1. Steel's Many-One Rank Test:

   Is the mean survival significantly different (p=0.05) than the control survival for the % effluent corresponding to (lethality):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	____YES	X NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____YES	____NO

2. Dunnett's Test:

   Is the mean dry weight (growth) significantly different (p=0.05) than the control's dry weight (growth) for the % effluent corresponding to (significant non-lethal effects):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	____YES	X NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____YES	____NO

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

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

5. NOEC Pimephales Lethality: 87 % (TOP6C)

6. LOEC Pimephales Lethality: 87 % (TXP6C)

7. NOEC Pimephales Sublethality: 87 % (TPP6C)

8. LOEC Pimephales Sublethality: 87 % (TYP6C)

9. Coefficient of variation for Pimephales growth: 11.6 (TQP6C)

AMERICAN INTERPLEX CORPORATION
LABORATORIES

Appendix B: Test 1000.0

CHRONIC TOXICITY SUMMARY FORM
Pimephales promelas (Fathead minnow)
CHEMICAL PARAMETERS CHART

PERMITTEE: City of Hot Springs 2400
NPDES NO.: AR0033880 AFIN#26-00145 2400
CONTACT: Mr. James Sorrells 2400
ANALYST: 280, 298, 304, 307

Test Initiated: DATE: August 20, 2013 TIME: 1200
Test Terminated: DATE: August 27, 2013 TIME: 1010

DILUTION Control	DAY 1	2	3	4	5	6	7
D.O. Initial	7.7	7.5	7.7	7.5	7.8	7.8	8.1
Final	7.5	7.2	7.5	7.9	7.7	7.1	7.9
pH Initial	7.7	7.7	7.8	8.0	7.9	8.0	7.7
Final	7.5	7.3	7.8	8.1	8.1	7.6	7.9
Alkalinity	32	NA	32	NA	32	NA	NA
Hardness	47	NA	42	NA	42	NA	NA
Conductivity	180	180	170	180	170	170	190
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 27 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.7	7.4	8.0	7.5	8.0	8.0	7.5
Final	7.5	7.2	7.3	7.9	7.8	8.1	7.7
pH Initial	7.6	7.7	7.5	7.8	7.7	7.8	7.5
Final	7.5	7.3	7.7	8.1	8.2	7.5	7.7
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	220	220	220	230	230	250
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 37 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.8	7.6	8.6	7.6	8.2	7.7	7.7
Final	7.5	7.0	7.5	8.0	7.9	8.1	7.8
pH Initial	7.6	7.7	7.4	7.7	7.6	7.7	7.6
Final	7.5	7.3	7.7	8.1	8.2	7.5	7.6
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	220	230	240	240	260	250	270
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 49 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.8	7.5	8.7	7.6	8.1	7.7	7.7
Final	7.5	7.4	7.6	8.0	8.1	8.2	7.8
pH Initial	7.5	7.7	7.3	7.6	7.6	7.6	7.5
Final	7.5	7.4	7.6	8.1	8.2	7.6	7.6
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	230	240	260	260	280	280	300
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 65 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.9	7.4	8.8	7.6	8.7	7.7	7.3
Final	7.5	7.4	7.4	7.9	8.1	8.0	7.7
pH Initial	7.5	7.7	7.2	7.5	7.5	7.6	7.5
Final	7.5	7.4	7.6	8.1	8.3	7.6	7.5
Alkalinity	36	NA	30	NA	35	NA	NA
Hardness	54	NA	58	NA	61	NA	NA
Conductivity	250	260	280	290	310	310	330
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 87 %	DAY 1	2	3	4	5	6	7
D.O. Initial	8.0	7.4	8.9	7.6	8.3	7.9	7.4
Final	7.5	8.5	7.4	7.8	8.1	8.0	7.7
pH Initial	7.4	7.7	7.1	7.4	7.4	7.6	7.6
Final	7.5	7.4	7.6	8.1	8.1	7.6	7.4
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	280	290	320	320	360	360	380
Chlorine	NA	NA	NA	NA	NA	NA	NA

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs

Project Reference: Plant Effluent

Project Manager: James Sorrells

Sampled By: A. Ross

PO No.: 13-3032

AIC Control No: 169846

AIC Proposal No:

Carrier: Hot Springs Courier

Received Temperature °C: 2°C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL		No of BOTTLES	Chronic.CD, Chronic.FH	Remarks
1	Plant Effluent	8-18-13 0800-2400		X	X			3	X	
		Container Type							P	
		Preservative							NO	

Field pH calibration on ________ @ ______ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate

NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: ________________
Who should AIC contact with questions: ____________
Phone: ____________ Fax: ______________
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By	Date/Time	Received By	Date/Time
A. Thomason	8-19-13 @ 1010	M. Mann	8-19-13 10:10 AM
M. Mann	8-19-13 @ 11:10 AM	Received In Lab By: [signature]	8-19-13 1110

Comments:

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 2.3C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH	Remarks
2	Plant Effluent	8-20-13 0000-2400		X	X		3	X	
		Container Type						P	Field pH calibration
		Preservative						NO	on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
A. Ross	8-21-13 0900	A. Man	8-21-13 9:10
A. Man	8-21-13 1130	Jimmy Day	8/21/13 1130

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By:

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL		No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
3	Plant Effluent	8/22/13 0000-2400		X				3	X
		Container Type							P
		Preservative							NO

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 2
Remarks
Field pH calibration on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	8-23-13 0955	[signature]	8-23-13 9:55
[signature]	8-23-13 11:20	[signature]	8-23-13 1120

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

September 19, 2013

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Plant Effluent
City of Hot Springs

Control No. 170390-1

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Ceriodaphnia dubia*
Plant Effluent - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: The No Observable Effects Concentration (NOEC) for survival occurred at <27 % effluent, which is below the critical dilution of 65 %. The NOEC for reproduction occurred at <27 % effluent, which is below the critical dilution of 65 %. **The sample, therefore, FAILED both lethal and sub-lethal effects for the *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Table of Contents

I. Control Acceptance Criteria

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	18.8	PASS
Control CV < or = 40% per Surviving Female	20.6	PASS
Reproduction Minimum Significant Difference 13 to 47%	NA	NA
Critical Dilution CV < or = 40%	0.00	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly Test Method 1002.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point: Plant Effluent
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.7	8.2	8.2
pH (standard units)	7.4	7.7	7.5
Alkalinity (mg/l as CaCO3)	64	58	64
Hardness (mg/l as CaCO3)	82	82	83
Conductivity (umhos/cm)	380	370	440
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	2.4	0.11

2. Dilution Water Samples: Synthetic Soft Water #4019
   a. Dates Prepared: August 28 through September 11, 2013
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.6	8.2	8.2
pH (standard units)	7.6	7.8	7.6
Alkalinity (mg/l as CaCO3)	32	32	32
Hardness (mg/l as CaCO3)	46	42	42
Conductivity (umhos/cm)	160	160	180
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05

C. Test Methods
1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Method 1002.0, *Ceriodaphnia dubia* Survival and Reproduction.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Ceriodaphnia dubia Survival and Growth Method 1002.0

Date & Time Test Initiated:	September 9, 2013 at 1235
Date & Time Test Terminated:	September 17, 2013 at 1255
Type & Volume of Test Chamber:	30 ml disposable beaker
Volume of Sample:	15 ml
Number of Organisms per replicate:	1
Number of Replicates per dilution:	10

4. Acclimation of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms
1. Scientific Name
a. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis
The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was not analyzed due to survival failure.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Ceriodaphnia dubia

Chronic reference tests are performed monthly.
A chronic reference test was performed on August 20, 2013 at 1505 to August 28, 2013 at 1450
The results were as follows: (Control No. 169867-2.)
Survival LC-50: 2125 mg/l
Growth IC-25: 1610 mg/l
Growth PMSD: 18.3

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	1.53
Hardness	EPA 200.7	101	0.450
pH	SM 4500-H+ B	101	0.267
Conductivity	EPA 120.1	103	1.97

VI. Organism History

Ceriodaphnia dubia

Date: September 9, 2013
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

VII. Results Summary *Ceriodaphnia dubia*, Cladoceran Survival and Reproduction Test -- Method 1002.0

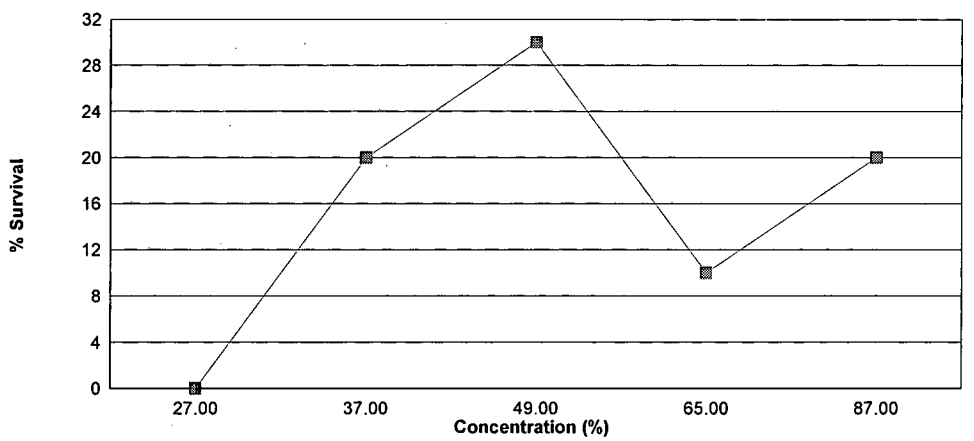
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on September 9, 2013 at 1235 and continued through September 17, 2013 at 1255. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = <27 % effluent
b.) NOEC reproduction = <27 % effluent



Summary of the 7-day *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	18.8
27 %	0.00 *	--
37 %	20.0 *	--
49 %	30.0 *	--
65 %	10.0 *	--
87 %	20.0 *	--

*Significant difference when compared to the control (p=0.05)

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 9, 2013 at 1235
Date and Time Test Terminated: September 17, 2013 at 1255

Concentration: Control													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	3	2	0	0	0	0	1	4	2	1	13	10	1.30
5	9	0	3	6	6	4	0	4	6	0	38	10	3.80
6	0	5	9	8	7	0	6	0	1	7	43	10	4.30
7	10	9	10	10	10	8	9	8	10	10	94	10	9.40
8													
TOTAL	22	16	22	24	23	12	16	16	19	18	188	10	18.8

Concentration: 27 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	0	0	0	X	0	0	0	X	0	0	7	0.00
2	X	0	0	0	X	0	0	0	X	0	0	7	0.00
3	X				X				X		0	0	0.00
4	X				X				X		0	0	0.00
5	X				X				X		0	0	0.00
6	X				X				X		0	0	0.00
7	X				X				X		0	0	0.00
8													
TOTAL	0	0	0	0	0	0	0	0	0	0	0	10	0.00

Concentration: 37 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	X	X	0	X	X	0	0	3	0.00
2	X	X	0	X	X	X	0	X	X	0	0	3	0.00
3	X	X	X	X	X	X	0	X	X	0	0	2	0.00
4	X	X	X	X	X	X	2	X	X	1	3	2	1.50
5	X	X	X	X	X	X	0	X	X	0	0	2	0.00
6	X	X	X	X	X	X	7	X	X	6	13	2	6.50
7	X	X	X	X	X	X	10	X	X	0	10	2	5.00
8													
TOTAL	0	0	0	0	0	0	19	0	0	7	26	10	2.60

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 9, 2013 at 1235
Date and Time Test Terminated: September 17, 2013 at 1255

Concentration: 49 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	X	X	0	X	X	0	0	3	0.00
2	X	X	0	X	X	X	0	X	X	0	0	3	0.00
3	X	X	0	X	X	X	0	X	X	0	0	3	0.00
4	X	X	0	X	X	X	0	X	X	0	0	3	0.00
5	X	X	4	X	X	X	5	X	X	0	9	3	3.00
6	X	X	0	X	X	X	0	X	X	6	6	3	2.00
7	X	X	6	X	X	X	8	X	X	0	14	3	4.67
8													
TOTAL	0	0	10	0	0	0	13	0	0	6	29	10	2.90

Concentration: 65 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	X	X	X	X	X	0	0	2	0.00
2	X	X	0	X	X	X	X	X	X	0	0	2	0.00
3	X	X	0	X	X	X	X	X	X	0	0	2	0.00
4	X	X	0	X	X	X	X	X	X	0	0	2	0.00
5	X	X	6	X	X	X	X	X	X	0	6	2	3.00
6	X	X	0	X	X	X	X	X	X	X	0	1	0.00
7	X	X	9	X	X	X	X	X	X	X	9	1	9.00
8													
TOTAL	0	0	15	0	0	0	0	0	0	0	15	10	1.50

Concentration: 87 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	0	X	0	X	X	0	0	4	0.00
2	X	X	0	X	0	X	X	X	X	0	0	3	0.00
3	X	X	0	X	0	X	X	X	X	0	0	3	0.00
4	X	X	0	X	3	X	X	X	X	1	4	3	1.33
5	X	X	7	X	5	X	X	X	X	0	12	3	4.00
6	X	X	0	X	X	X	X	X	X	3	3	2	1.50
7	X	X	9	X	X	X	X	X	X	0	9	2	4.50
8													
TOTAL	0	0	16	0	8	0	0	0	0	4	28	10	2.80

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
27 %	0	3	3
Total	10	3	13

Critical Fisher's value (10,3,10) (alpha=0.05) is 1. b value is 0. Since b is less than or equal to 1 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
37 %	2	8	10
Total	12	8	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 2. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
49 %	3	7	10
Total	13	7	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 3. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
65 %	1	9	10
Total	11	9	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 1. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
87 %	2	8	10
Total	12	8	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 2. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	27 %	3	3	*
2	37 %	10	8	*
3	49 %	10	7	*
4	65 %	10	9	*
5	87 %	10	8	*

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: September 9, 2013 at 0857
Date and Time Test Terminated: September 17, 2013 at 1255

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.6	7.4	8.2	8.3	8.2	8.3	8.2
	Final	7.8	8.3	8.5	8.7	8.4	8.2	7.8
pH, units	Initial	7.6	7.6	7.8	7.6	7.6	7.7	7.5
	Final	7.8	7.9	7.9	7.6	7.9	7.8	8.0
Alkalinity, mg CaCO3/l		32	NA	32	NA	32	NA	NA
Hardness, mg CaCO3/l		46	NA	42	NA	42	NA	NA
Conductivity, umhos/cm		160	170	160	180	180	180	170
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.6	7.5	8.2	8.4	8.3	8.1	8.3
	Final	6.0	8.3	8.7	8.8	8.5	8.3	8.1
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
	Final	8.0	8.1	8.2	8.0	8.3	8.0	8.1

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.6	7.4	8.2	8.4	8.1	8.0	8.1
	Final	6.4	8.3	8.6	8.8	8.6	8.2	8.2
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
	Final	8.1	8.2	8.2	8.2	8.2	7.9	7.8

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: September 9, 2013 at 0857
Date and Time Test Terminated: September 17, 2013 at 1255

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.5	7.5	8.2	8.4	8.4	8.1	8.3
	Final	6.8	8.3	7.8	9.0	8.4	8.3	8.0
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.5	7.5
	Final	8.2	8.2	8.2	8.1	8.1	8.0	8.1

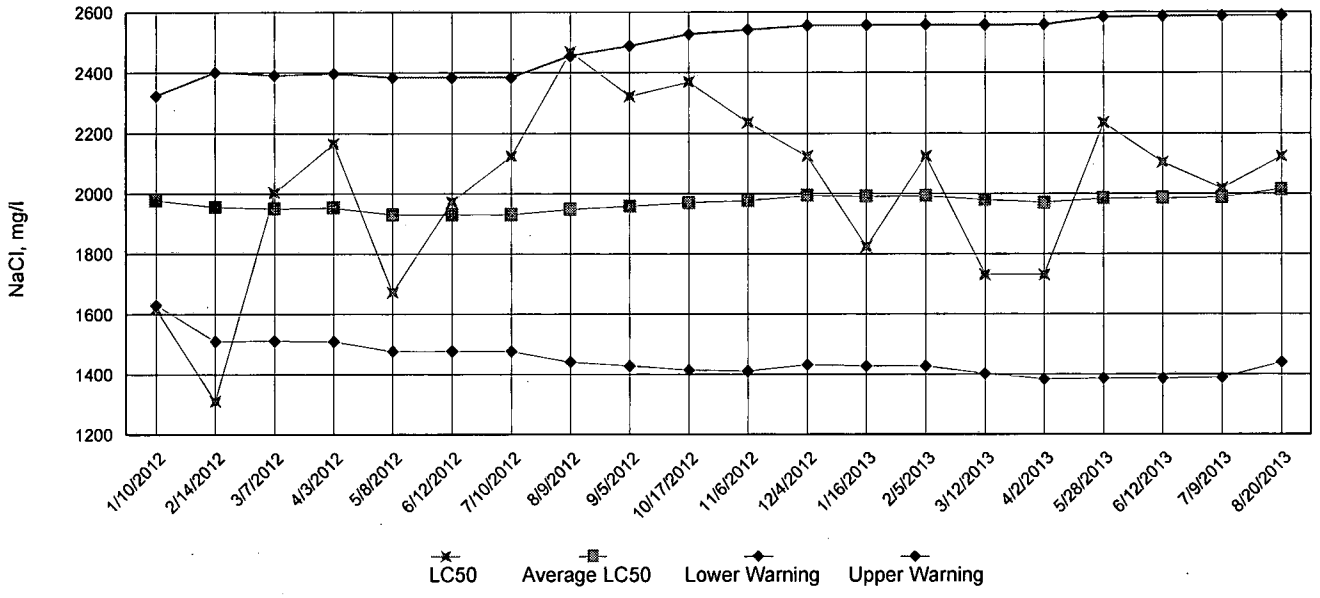
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.4	8.2	8.4	8.0	8.2	8.3
	Final	7.0	8.3	8.4	8.7	8.3	8.3	7.7
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.6
	Final	8.2	8.1	8.1	8.2	8.0	8.0	7.7
Alkalinity, mg CaCO3/l		48	NA	46	NA	47	NA	NA
Hardness, mg CaCO3/l		68	NA	68	NA	68	NA	NA
Conductivity, umhos/cm		310	310	300	340	340	340	350
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.3	7.2	8.2	8.3	8.3	8.0	7.9
	Final	7.1	8.3	8.6	8.8	7.3	8.3	8.0
pH, units	Initial	7.5	7.4	7.8	7.5	7.6	7.5	7.6
	Final	8.2	8.3	8.3	8.3	7.5	8.1	7.9

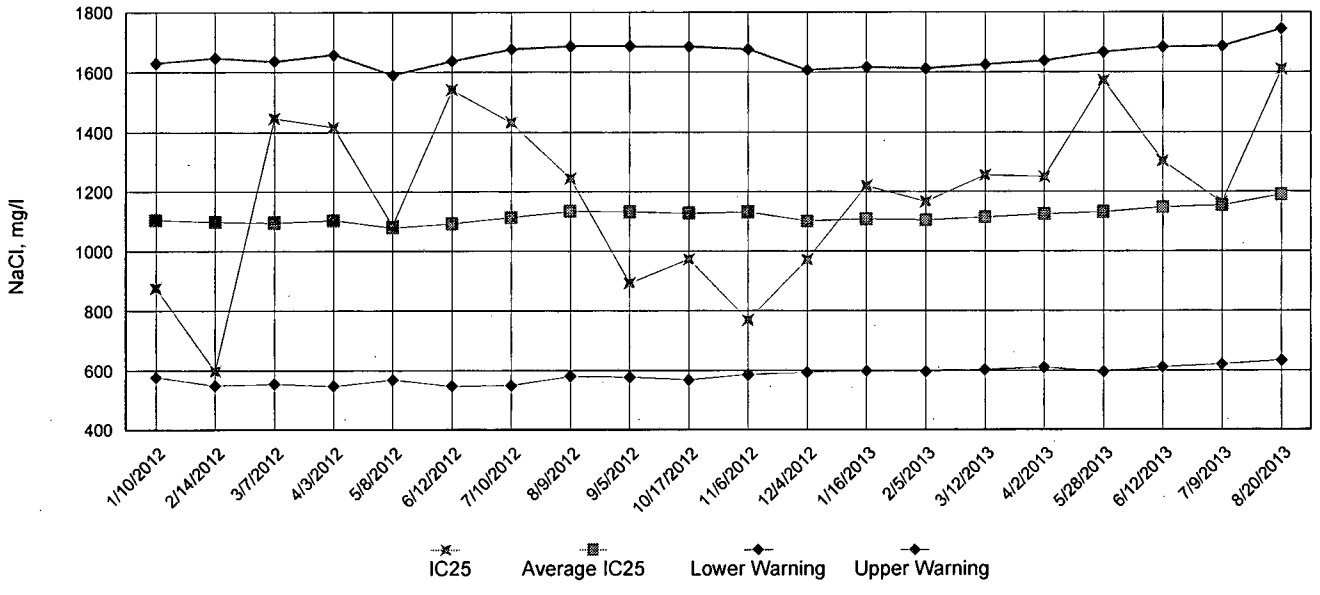
Appendix A4: Test 1002.0

Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

Permittee: City of Hot Springs

NPDES No.: AR0033880 AFIN#26-00145

Date and Time Test Initiated: September 9, 2013 at 1235
Date and Time Test Terminated: September 17, 2013 at 1255
Dilution water used: Synthetic Soft Water #4019

PERCENT SURVIVAL

		Percent Effluent				
Time of Reading	Control	27 %	37 %	49 %	65 %	87 %
24 hour	100	70.0	30.0	30.0	20.0	40.0
48 hour	100	70.0	30.0	30.0	20.0	30.0
7 day	100	0.00	20.0	30.0	10.0	20.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

		Percent Effluent				
Replicates	Control	27 %	37 %	49 %	65 %	87 %
A	22	0	0	0	0	0
B	16	0	0	0	0	0
C	22	0	0	10	15	16
D	24	0	0	0	0	0
E	23	0	0	0	0	8
F	12	0	0	0	0	0
G	16	0	19	13	0	0
H	16	0	0	0	0	0
I	19	0	0	0	0	0
J	18	0	7	6	0	4
Mean per Adult	18.8	0.00	2.60	2.90	1.50	2.80
Mean per Surviving Adult	18.8	0.00	13.0	9.67	15.0	10.0
CV %	20.6	0.00	65.3	36.3	0.00	84.9

CV = Coefficient of variation = standard deviation * 100 / mean
(calculated based on young produced by surviving females)

Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

1. Fisher's Exact Test:

   Is the mean survival significantly different (p=0.05) than the control survival for the % effluent corresponding to (lethality):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	X YES	____ NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____ YES	____ NO

2.

   Is the mean number of young produced per female significantly different (p=0.05) than the control's number of young per female for the % effluent corresponding to (significant non-lethal effects):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	X YES	____ NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____ YES	____ NO

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 1 (TLP3B)
4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 1 (TGP3B)
5. NOEC Ceriodaphnia Lethality: <27 % (TOP3B)
6. LOEC Ceriodaphnia Lethality: 27 % (TXP3B)
7. NOEC Ceriodaphnia Sublethality: <27 % (TPP3B)
8. LOEC Ceriodaphnia Sublethality: 27 % (TYP3B)
9. Coefficient of variation for Ceriodaphnia Reproduction: 20.6 (TQP3B)

Appendix B: Test 1002.0

CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
CHEMICAL PARAMETERS CHART

PERMITTEE: City of Hot Springs
NPDES NO.: AR0033880 AFIN#26-00145
CONTACT: Mr. James Sorrells
ANALYST: 280, 298, 304, 307

2400
2400
2400

Test Initiated: DATE: September 9, 2013 TIME: 1235
Test Terminated: DATE: September 17, 2013 TIME: 1255

DILUTION	DAY						
Control	1	2	3	4	5	6	7
D.O. Initial	8.6	7.4	8.2	8.3	8.2	8.3	8.2
Final	7.8	8.3	8.5	8.7	8.4	8.2	7.8
pH Initial	7.6	7.6	7.8	7.6	7.6	7.7	7.5
Final	7.8	7.9	7.9	7.6	7.9	7.8	8.0
Alkalinity	32	NA	32	NA	32	NA	NA
Hardness	46	NA	42	NA	42	NA	NA
Conductivity	160	170	160	180	180	180	170
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
27 %	1	2	3	4	5	6	7
D.O. Initial	8.6	7.5	8.2	8.4	8.3	8.1	8.3
Final	6.0	8.3	8.7	8.8	8.5	8.3	8.1
pH Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
Final	8.0	8.1	8.2	8.0	8.3	8.0	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	220	220	220	240	250	250	250
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
37 %	1	2	3	4	5	6	7
D.O. Initial	8.6	7.4	8.2	8.4	8.1	8.0	8.1
Final	6.4	8.3	8.6	8.8	8.6	8.2	8.2
pH Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
Final	8.1	8.2	8.2	8.2	8.2	7.9	7.8
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	240	240	240	260	270	270	280
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
49 %	1	2	3	4	5	6	7
D.O. Initial	8.5	7.5	8.2	8.4	8.4	8.1	8.3
Final	6.8	8.3	7.8	9.0	8.4	8.3	8.0
pH Initial	7.6	7.5	7.8	7.5	7.6	7.5	7.5
Final	8.2	8.2	8.2	8.1	8.1	8.0	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	270	270	270	300	300	300	300
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
65 %	1	2	3	4	5	6	7
D.O. Initial	7.7	7.4	8.2	8.4	8.0	8.2	8.3
Final	7.0	8.3	8.4	8.7	8.3	8.3	7.7
pH Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.6
Final	8.2	8.1	8.1	8.2	8.0	8.0	7.7
Alkalinity	48	NA	46	NA	47	NA	NA
Hardness	68	NA	68	NA	68	NA	NA
Conductivity	310	310	300	340	340	340	350
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
87 %	1	2	3	4	5	6	7
D.O. Initial	8.3	7.2	8.2	8.3	8.3	8.0	7.9
Final	7.1	8.3	8.6	8.8	7.3	8.3	8.0
pH Initial	7.5	7.4	7.8	7.5	7.6	7.5	7.6
Final	8.2	8.3	8.3	8.3	7.5	8.1	7.9
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	350	360	350	400	440	430	440
Chlorine	NA	NA	NA	NA	NA	NA	NA

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Roys

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Chronic C.D.	Analyses Requested
1	Plant Effluent	9-8-13 2000-2400		X	X		3	X	No Fathead Analysis
		Container Type						P	
		Preservative						NO	

AIC Control No: 170390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2.1°C
Remarks:
Field pH calibration on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-8-13 1030	M. Mann	9-9-13 10:30 am
M. Mann	9-9-13 11:45	[signature]	9-9-13 1145

Comments:

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC Control No: 170390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 3°C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH	Remarks
1	Plant Effluent	9-10-13 0000-2400		X	X		3	X	
		Container Type						P	Field pH calibration
		Preservative						NO	on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-11-13 1110	M. Mann	9-11-13 1110
M. Mann	9-11-13 @ 12:05	[signature]	9-11-13 1205

Comments:

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs

Project Reference: Plant Effluent

Project Manager: James Sorrells

Sampled By: HAROLD MAULDIN

PO No.: 13-3032

AIC Control No: 170390

AIC Proposal No:

Carrier: Hot Springs Courier

Received Temperature °C: 2

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL		No of BOTTLES	Chronic.CD.	Remarks
3	PLANT Effluent	9/12/13 0000-2400		X	X			3	x	
		Container Type							P	
		Preservative							NO	

Field pH calibration on ______ @ ______ Buffer:

G = Glass; P = Plastic; V = VOA vials; H = HCl to pH2; T = Sodium Thiosulfate; NO = none; S = Sulfuric acid pH2; N = Nitric acid pH2; B = NaOH to pH12; Z = Zinc acetate; A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN ____ DAYS

Expedited results requested by:

Who should AIC contact with questions:

Phone: Fax:

Report Attention to: Mr. James Sorrells

Report Address to: 320 Davidson Road, Hot Springs, AR 71901

Relinquished By	Date/Time	Received By	Date/Time
A. Thomason	9-13-13 @ 1015	M. Mann	9-13-13 @ 10:15
M. Mann	9-13-13 @ 11:20	Received in Lab By: [signature]	9-13-13 1120

Comments:

4-85

AMERICAN INTERPLEX CORPORATION
LABORATORIES

September 30, 2013

Test Results of
Acute 48 hour Non-Renewal
Biomonitoring Testing
for

170741-1: Outfall 001 First Renewal
170741-2: Outfall 001 Second Renewal
170741-3: Outfall 001 Third Renewal
170741-4: Outfall 001 Original Screen

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Acute 48 hour Non-Renewal Biomonitoring utilizing *Ceriodaphnia dubia*
Outfall 001 First Renewal - City of Hot Springs
Client NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

Please find attached the results of the acute biomonitoring screens conducted as a follow up to the chronic toxicity event previously reported (AIC Control No. 170390). As indicated on the enclosed results, the sample was still acutely toxic on September 12, 2013 and exhibited no acute toxicity on September 19, 2013.

It it theorized the decrease in toxicity is directly related to the holding time which may have allowed microbial degradation, loss of volatile toxics, and/or biochemical degradation.

If I can be of further assistance, please feel free to contact me.

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Dilution Water Samples: Synthetic Soft Water #4021

Analysis	Result
Dissolved oxygen (mg/l)	7.6
pH (standard units)	7.7
Alkalinity (mg/l as CaCO3)	31
Hardness (mg/l as CaCO3)	46
Conductivity (umhos/cm)	170
Residual Chlorine (mg/l)	<0.05

Results Summary: Outfall 001 First Renewal

Ceriodaphnia dubia

The *Ceriodaphnia dubia* test was conducted from September 19, 2013 at 1615 to September 21, 2013 at 1700.

Statistical analyses:

NOEC = 100%

Concentration	24 hour % Survival	48 hour % Survival
Control	100	100
100%	100	100

Ceriodaphnia dubia
Survival Data

Number of organisms per chamber: 5
Volume of test chamber: 30 ml

Age of organisms: <24 hours
Volume of test solution: 15 ml

Effluent Concentration	Number of Survivors 24 Hours	48 Hours	% Survival	CV %
Control rep. A	5	5	100	0.00
100% rep. A	5	5	100	0.00

CV = Coefficient of variance = standard deviation X 100/mean

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	5	0	5
100%	5	0	5
Total	10	0	10

Critical Fisher's value (5,5,5) (alpha=0.05) is 1. b value is 5. Since b is greater than 1 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test

Group	Identification	Exposed	Dead	Sig 0.05
0	0	5	0	
1	100	5	0	

Chemical Data for
Ceriodaphnia dubia

Day 1	Control	100%
DO, mg/l	7.6	8.8
pH, su	7.7	7.3
Alkalinity, mg/l	31	NA
Hardness, mg/l	46	NA
Conductivity, umho/cm	170	380
Residual Chlorine, mg/l	<0.05	NA

Day 2		Control	100%
DO, mg/l	Final	7.5	8.7
pH, su	Final	8.0	7.4

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

170744 WR 9/19/13

Client: City of Hot Springs
Project:
Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Rojs

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Chronic CD.	Analyses Requested
1	Plant Effluent	9-8-13 0000-2400		X	X		3	X	
		Container Type						P	
		Preservative						NO	

No Fathead Analysis

AIC Control No: 170390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2.1°C
Remarks:
Field pH calibration on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ___ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-9-13 1030	M. Mann	9-9-13 10:30 AM
M. Mann	9-9-13 11:45	[signature]	9-9-13 1145

Comments:

City of Hot Springs
Waste Water Treatment Plant
320 Davidson Drive
Hot Springs, AR 71901

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NPDES Enforcement Section
Attn: Mo Shafii
5301 Northshore Dr.
North Little Rock, AR 72118-5317

AMERICAN INTERPLEX CORPORATION
LABORATORIES

August 30, 2013

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Outfall 001
City of Hot Springs

Control No. 169846-2

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Ceriodaphnia dubia*
Outfall 001 - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Chronic *Ceriodaphnia dubia* test: Due to laboratory error, the *Ceriodaphnia dubia* test was not renewed with the third sample. The test should be repeated. The data from the test is enclosed for your review.

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Table of Contents

I. Control Acceptance Criteria

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	23.6	PASS
Control CV < or = 40% per Surviving Female	13.6	PASS
Reproduction Minimum Significant Difference 13 to 47%	25.8	PASS
Critical Dilution CV < or = 40%	22.9	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly
   Test Method 1002.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point: Outfall 001
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.9	8.5	NA
pH (standard units)	7.0	7.4	NA
Alkalinity (mg/l as CaCO3)	26	34	NA
Hardness (mg/l as CaCO3)	67	71	NA
Conductivity (umhos/cm)	350	390	NA
Residual Chlorine (mg/l)	<0.05	<0.05	NA
Ammonia as N (mg/l)	0.12	0.30	NA

2. Dilution Water Samples: Synthetic Soft Water #4012
   a. Dates Prepared: August 8 through August 22, 2013
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.7	7.7	7.8
pH (standard units)	7.7	7.8	7.9
Alkalinity (mg/l as CaCO3)	32	32	NA
Hardness (mg/l as CaCO3)	47	42	NA
Conductivity (umhos/cm)	180	170	170
Residual Chlorine (mg/l)	<0.05	<0.05	NA

C. Test Methods
1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Method 1002.0, *Ceriodaphnia dubia* Survival and Reproduction.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Ceriodaphnia dubia Survival and Growth Method 1002.0

Date & Time Test Initiated:	August 22, 2013 at 1605
Date & Time Test Terminated:	August 29, 2013 at 1415
Type & Volume of Test Chamber:	30 ml disposable beaker
Volume of Sample:	15 ml
Number of Organisms per replicate:	1
Number of Replicates per dilution:	10

4. Acclimation of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms
1. Scientific Name
a. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis

The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was analyzed using Kolmogorov's Test for Normality and Bartlett's test and analyzed with Dunnett's Test to determine the No Observable Effects Concentration (NOEC) for Reproduction.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Ceriodaphnia dubia

Chronic reference tests are performed monthly.
A chronic reference test was performed on August 20, 2013 at 1505 to August 28, 2013 at 1450
The results were as follows: (Control No. 169867-2.)
Survival LC-50: 2125 mg/l
Growth IC-25: 1610 mg/l
Growth PMSD: 18.3

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	102	1.91
pH	SM 4500-H+ B	101	0.268
Conductivity	EPA 120.1	101	4.71

VI. Organism History

Ceriodaphnia dubia

Date: August 22, 2013
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

VII. Results Summary *Ceriodaphnia dubia*, Cladoceran Survival and Reproduction Test -- Method 1002.0

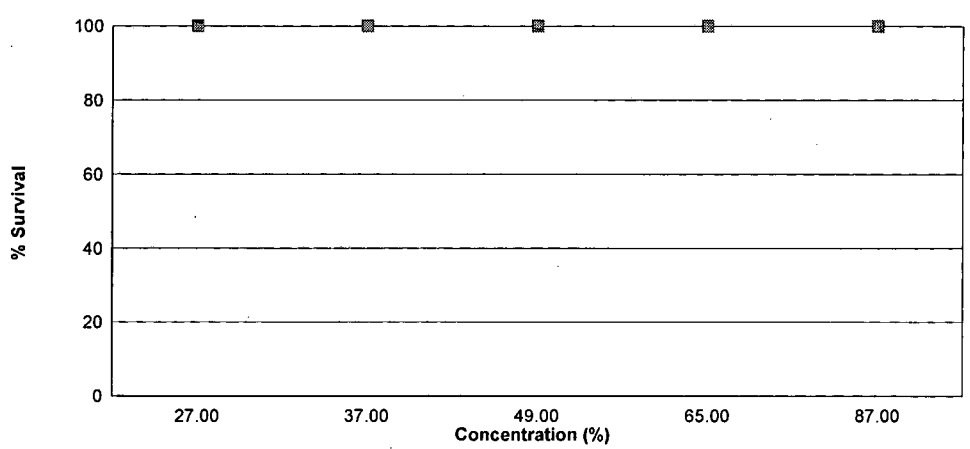
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on August 22, 2013 at 1605 and continued through August 29, 2013 at 1415. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 87 % effluent
b.) NOEC reproduction = 87 % effluent



Summary of the 7-day *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	23.6
27 %	100	24.2
37 %	100	27.6
49 %	100	26.4
65 %	100	24.2
87 %	100	25.0

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 22, 2013 at 1605
Date and Time Test Terminated: August 29, 2013 at 1415

Concentration: Control													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	4	4	2	3	4	4	3	2	33	10	3.30
5	0	0	0	0	0	1	0	0	0	6	7	10	0.700
6	9	9	7	8	7	8	7	9	8	8	80	10	8.00
7	14	14	11	13	14	11	12	14	13	0	116	10	11.6
8													
TOTAL	27	26	22	25	23	23	23	27	24	16	236	10	23.6

Concentration: 27 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	2	3	3	3	2	3	2	3	2	27	10	2.70
5	8	7	1	0	0	0	0	0	0	6	22	10	2.20
6	1	12	13	11	10	10	11	8	7	6	89	10	8.90
7	16	0	17	18	6	0	17	17	13	0	104	10	10.4
8													
TOTAL	29	21	34	32	19	12	31	27	23	14	242	10	24.2

Concentration: 37 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	3	3	3	2	3	2	2	2	27	10	2.70
5	0	0	0	0	0	1	0	0	6	6	13	10	1.30
6	12	12	12	11	10	6	8	10	13	10	104	10	10.4
7	18	16	17	18	16	15	15	17	0	0	132	10	13.2
8													
TOTAL	34	31	32	32	29	24	26	29	21	18	276	10	27.6

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 22, 2013 at 1605
Date and Time Test Terminated: August 29, 2013 at 1415

Concentration: 49 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	2	5	2	3	4	2	2	2	29	10	2.90
5	0	5	0	0	0	1	0	6	4	3	19	10	1.90
6	10	0	11	11	11	8	10	0	12	10	83	10	8.30
7	17	18	16	18	16	17	15	16	0	0	133	10	13.3
8													
TOTAL	31	26	29	34	29	29	29	24	18	15	264	10	26.4

Concentration: 65 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	2	3	5	3	3	3	2	3	2	2	28	10	2.80
5	6	0	0	0	0	1	0	0	5	4	16	10	1.60
6	13	11	11	11	10	10	8	10	0	12	96	10	9.60
7	0	15	14	16	12	0	15	16	14	0	102	10	10.2
8													
TOTAL	21	29	30	30	25	14	25	29	21	18	242	10	24.2

Concentration: 87 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	4	3	3	3	3	4	3	2	2	31	10	3.10
5	8	6	1	0	0	0	0	0	5	5	25	10	2.50
6	0	2	11	12	6	10	8	8	0	7	64	10	6.40
7	16	15	16	18	16	0	18	17	14	0	130	10	13.0
8													
TOTAL	28	27	31	33	25	13	30	28	21	14	250	10	25.0

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	10	0	10
27 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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	Alive	Dead	Total Animals
Control	10	0	10
37 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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	Alive	Dead	Total Animals
Control	10	0	10
49 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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	Alive	Dead	Total Animals
Control	10	0	10
65 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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.

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
87 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=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 Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	27 %	10	0	
2	37 %	10	0	
3	49 %	10	0	
4	65 %	10	0	
5	87 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality		No Transformation
D = 0.1154 D* = 0.9054 Critical D* = 1.035	(alpha = 0.01, N = 60)	
Data PASS normality test (alpha = 0.01).		

Bartlett's Test for Homogeneity of Variance		No Transformation
Calculated B1 statistic = 6.592 Critical B = 15.086	(alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.		

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	117.9	23.58	0.6766
Within (Error)	54	1882	34.85	
Total	59	2000		

Critical F = 3.38 (alpha = 0.01, df = 5,54)
2.38 (alpha = 0.05, df = 5,54)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Dunnett's Test - Table 1 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	23.6	23.6		
2	27 %	24.2	24.2	-0.2273	
3	37 %	27.6	27.6	-1.515	
4	49 %	26.4	26.4	-1.061	
5	65 %	24.2	24.2	-0.2273	
6	87 %	25	25	-0.5303	

Dunnett's critical value = 2.31 (1 Tailed, alpha = 0.05, df [used] = 5,40) (Actual df = 5,54)

Dunnett's Test - Table 2 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	10			
2	27 %	10	6.099	25.8	-0.6
3	37 %	10	6.099	25.8	-4
4	49 %	10	6.099	25.8	-2.8
5	65 %	10	6.099	25.8	-0.6
6	87 %	10	6.099	25.8	-1.4

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 22, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.5	7.7	7.5	7.8	7.8	8.1
	Final	7.7	8.0	8.7	8.2	8.2	7.8	7.8
pH, units	Initial	7.7	7.7	7.8	8.0	7.9	8.0	7.7
	Final	8.0	8.2	8.0	8.0	8.0	7.8	7.8
Alkalinity, mg CaCO3/l		32	NA	32	NA	NA	NA	NA
Hardness, mg CaCO3/l		47	NA	42	NA	NA	NA	NA
Conductivity, umhos/cm		180	180	170	180	170	170	190
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	NA	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.4	8.0	7.5	8.0	8.0	7.5
	Final	7.5	8.2	8.6	8.2	8.2	7.7	7.7
pH, units	Initial	7.6	7.7	7.5	7.8	7.7	7.8	7.5
	Final	8.0	8.1	8.0	8.0	8.1	7.6	7.6

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.6	8.6	7.6	8.2	7.7	7.7
	Final	7.6	8.0	8.6	8.0	8.2	7.6	7.6
pH, units	Initial	7.6	7.7	7.4	7.7	7.6	7.7	7.6
	Final	8.0	8.1	8.0	8.0	8.1	7.6	7.6

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 22, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.5	8.7	7.6	8.1	7.7	7.7
	Final	7.7	8.1	8.6	8.2	8.2	7.6	7.9
pH, units	Initial	7.5	7.7	7.3	7.6	7.6	7.6	7.5
	Final	8.0	8.1	8.0	8.1	8.2	7.5	7.5

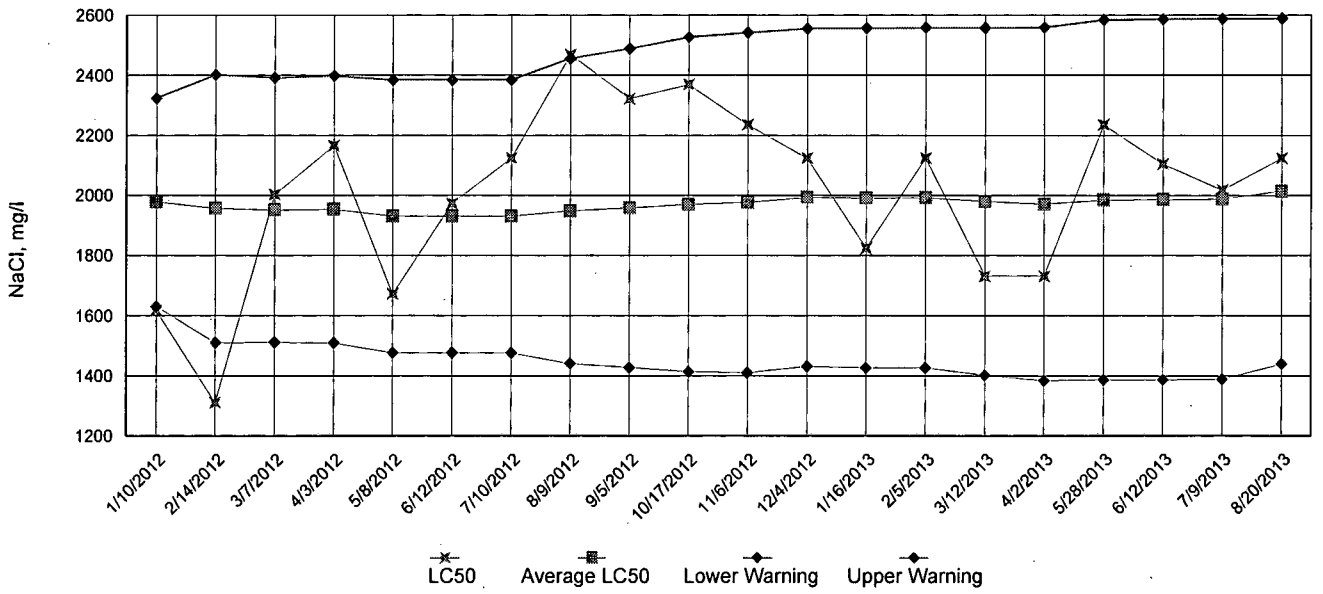
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.9	7.4	8.8	7.6	8.7	7.7	7.3
	Final	7.7	8.0	8.6	8.4	8.1	7.6	7.6
pH, units	Initial	7.5	7.7	7.2	7.5	7.5	7.6	7.5
	Final	8.0	8.0	8.0	8.1	8.1	7.5	7.5
Alkalinity, mg CaCO3/l		36	NA	30	NA	NA	NA	NA
Hardness, mg CaCO3/l		54	NA	58	NA	NA	NA	NA
Conductivity, umhos/cm		250	260	280	290	310	310	330
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	NA	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	7.4	8.9	7.6	8.3	7.9	7.4
	Final	7.4	8.1	8.7	8.2	7.7	5.4	7.2
pH, units	Initial	7.4	7.7	7.1	7.4	7.4	7.6	7.6
	Final	8.0	8.0	7.9	8.1	8.3	7.5	7.4

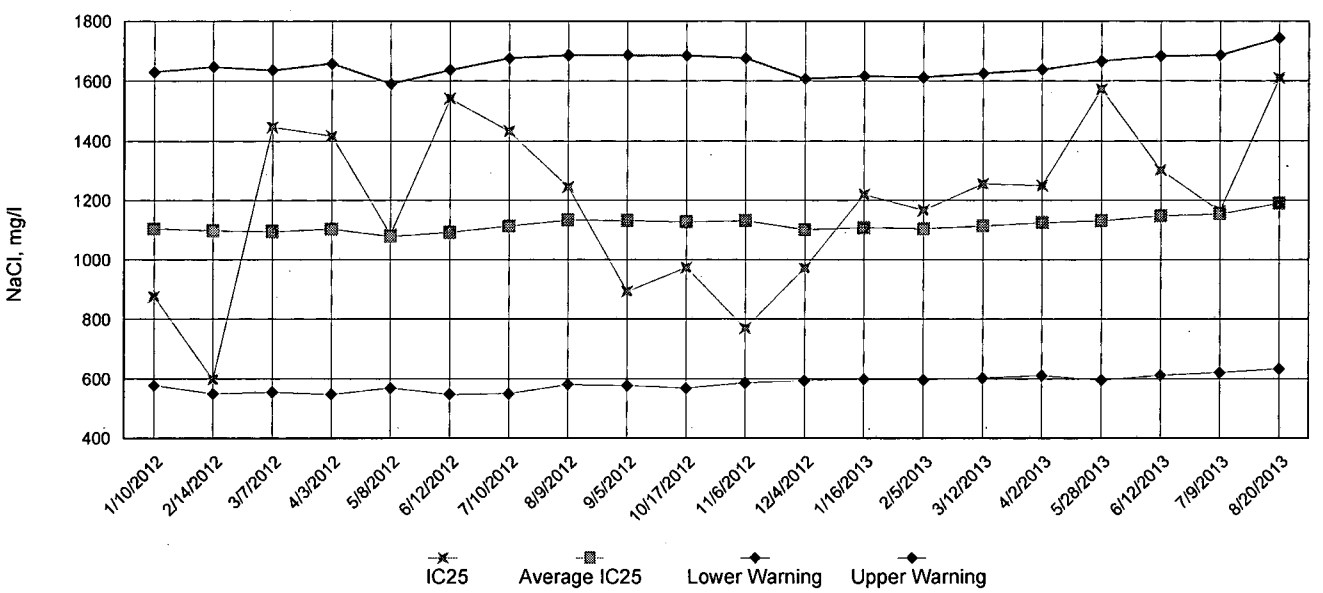
Appendix A4: Test 1002.0

Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL		No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
1	Plant Effluent	8-18-13 0900 - 2400		X	X			3	X
		Container Type							P
		Preservative							NO

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 20c
Remarks
Field pH calibration on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ___ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
A. Thomason	8-19-13 @ 1010	M. Mann	8-19-13 @ 10:10am
Relinquished By: M. Mann	Date/Time 8-19-13 @ 11:10AM	Received In Lab By: Angie Hopton	Date/Time 8-19-13 1110

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL		No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
2	Plant Effluent	8-20-13 0900-2400		X	X			3	X
		Container Type							P
		Preservative							NO

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 23C
Remarks

Field pH calibration
on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
A. Ross	8-21-13 0900	G. Mann	8-21-13 900
G. Mann	8-21-13 1130	Jimmy Day	8/21/13 1130

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By:

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
3	Plant Effluent	8/22/13 0000-2400		X			3	X
		Container Type						P
		Preservative						NO

AIC Control No: 16984b
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 2
Remarks
Field pH calibration on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	8-23-13 0955	[signature]	8-23-13 9:55
[signature]	8-23-13 11:20	[signature]	8-23-13 1120

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

September 30, 2013

Test Results of
Acute 48 hour Non-Renewal
Biomonitoring Testing
for

170741-1: Outfall 001 First Renewal
170741-2: Outfall 001 Second Renewal
170741-3: Outfall 001 Third Renewal
170741-4: Outfall 001 Original Screen

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Acute 48 hour Non-Renewal Biomonitoring utilizing *Ceriodaphnia dubia*
Outfall 001 First Renewal - City of Hot Springs
Client NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

Please find attached the results of the acute biomonitoring screens conducted as a follow up to the chronic toxicity event previously reported (AIC Control No. 170390). As indicated on the enclosed results, the sample was still acutely toxic on September 12, 2013 and exhibited no acute toxicity on September 19, 2013.

It it theorized the decrease in toxicity is directly related to the holding time which may have allowed microbial degradation, loss of volatile toxics, and/or biochemical degradation.

If I can be of further assistance, please feel free to contact me.

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Dilution Water Samples: Synthetic Soft Water #4021

Analysis	Result
Dissolved oxygen (mg/l)	7.6
pH (standard units)	7.7
Alkalinity (mg/l as CaCO3)	31
Hardness (mg/l as CaCO3)	46
Conductivity (umhos/cm)	170
Residual Chlorine (mg/l)	<0.05

Results Summary: Outfall 001 First Renewal

Ceriodaphnia dubia

The *Ceriodaphnia dubia* test was conducted from September 19, 2013 at 1615 to September 21, 2013 at 1700.

Statistical analyses:

NOEC = 100%

Concentration	24 hour % Survival	48 hour % Survival
Control	100	100
100%	100	100

Ceriodaphnia dubia
Survival Data

Number of organisms per chamber: 5
Volume of test chamber: 30 ml

Age of organisms: <24 hours
Volume of test solution: 15 ml

Effluent Concentration	Number of Survivors 24 Hours	48 Hours	% Survival	CV %
Control rep. A	5	5	100	0.00
100% rep. A	5	5	100	0.00

CV = Coefficient of variance = standard deviation X 100/mean

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	5	0	5
100%	5	0	5
Total	10	0	10

Critical Fisher's value (5,5,5) (alpha=0.05) is 1. b value is 5. Since b is greater than 1 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test

Group	Identification	Exposed	Dead	Sig 0.05
0	0	5	0	
1	100	5	0	

Chemical Data for
Ceriodaphnia dubia

Day 1	Control	100%
DO, mg/l	7.6	8.8
pH, su	7.7	7.3
Alkalinity, mg/l	31	NA
Hardness, mg/l	46	NA
Conductivity, umho/cm	170	380
Residual Chlorine, mg/l	<0.05	NA

Day 2		Control	100%
DO, mg/l	Final	7.5	8.7
pH, su	Final	8.0	7.4

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

month WR 9/19/13

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Chronic C.D.	Analyses Requested
1	Plant Effluent	9-8-13 0000-2400		X	X		3	X	
		Container Type						P	
		Preservative						NO	

No FATHEAD ANALYSIS

AIC Control No: 120390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2.1 °C
Remarks:
Field pH calibration on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z = Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-8-13 1030	M. Mann	9-9-13 10:30 AM
M. Mann	9-9-13 11:45	[signature]	9-9-13 1145

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

October 1, 2013
Control No. 171032

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

This report contains the analytical results and supporting information for samples submitted on September 27, 2013. Attached please find a copy of the Chain of Custody and/or other documents received. Note that any remaining sample will be discarded two weeks from the original report date unless other arrangements are made.

This report is intended for the sole use of the client listed above. Assessment of the data requires access to the entire document.

This report has been reviewed by the Laboratory Director or a qualified designee.

John Overbey
Laboratory Director

This document has been distributed to the following:

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

SAMPLE INFORMATION

Project Description:
Three (3) water sample(s) (AIC COntrol No. 170741-1,2,3) resubmitted September 27, 2013
P.O. No. 13-3032

Receipt Details:
A Chain of Custody was not provided with the sample(s).

Each sample container was checked for proper labeling, including date and time sampled. Sample containers were reviewed for proper type, adequate volume, integrity, temperature, preservation, and holding times. Any exceptions are noted below:

Sample Identification:

Laboratory ID	Client Sample ID	Sampled Date/Time	Notes
171032-1	Plant Effluent 9-8-13 0000-2400	08-Sep-2013 2359	
171032-2	Plant Effluent 9-10-13 0000-2400	10-Sep-2013 2359	
171032-3	Plant Effluent 9-12-13 0000-2400	12-Sep-2013 2359	

Case Narrative:
There were no qualifiers for this data and all samples met quality control criteria.

References:
"Methods for Chemical Analysis of Water and Wastes", EPA/600/4-79-020 (Mar 1983) with updates and supplements EPA/600/5-91-010 (Jun 1991), EPA/600/R-92-129 (Aug 1992) and EPA/600/R-93-100 (Aug 1993).
"Test Methods for Evaluating Solid Waste Physical/Chemical Methods (SW846)", Third Edition.
"Standard Methods for the Examination of Water and Wastewaters", 21st edition.
"American Society for Testing and Materials" (ASTM).
"Association of Analytical Chemists" (AOAC).

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

ANALYTICAL RESULTS

AIC No. 171032-1
Sample Identification: Plant Effluent 9-8-13 0000-2400

Analyte		Result	RL	Units	Qualifier
Total Organic Carbon		**6.1**	1	**mg/l**	
SM 5310 C	Prep: 27-Sep-2013 1516 by 308	Analyzed: 27-Sep-2013 1814 by 308		Batch: W45076	
Aluminum		**< 0.04**	0.04	**mg/l**	
EPA 200.7	Prep: 30-Sep-2013 0901 by 271	Analyzed: 01-Oct-2013 1242 by 305		Batch: S35491	
Sulfate		**29**	0.2	**mg/l**	
EPA 300.0	Prep: 27-Sep-2013 1628 by 07	Analyzed: 27-Sep-2013 2205 by 07		Batch: C16076	

AIC No. 171032-2
Sample Identification: Plant Effluent 9-10-13 0000-2400

Analyte		Result	RL	Units	Qualifier
Total Organic Carbon		**5.9**	1	**mg/l**	
SM 5310 C	Prep: 27-Sep-2013 1516 by 308	Analyzed: 27-Sep-2013 1828 by 308		Batch: W45076	
Aluminum		**0.041**	0.04	**mg/l**	
EPA 200.7	Prep: 30-Sep-2013 0901 by 271	Analyzed: 01-Oct-2013 1245 by 305		Batch: S35491	
Sulfate		**39**	0.2	**mg/l**	
EPA 300.0	Prep: 27-Sep-2013 1628 by 07	Analyzed: 27-Sep-2013 2232 by 07		Batch: C16076	

AIC No. 171032-3
Sample Identification: Plant Effluent 9-12-13 0000-2400

Analyte		Result	RL	Units	Qualifier
Total Organic Carbon		**5.4**	1	**mg/l**	
SM 5310 C	Prep: 27-Sep-2013 1516 by 308	Analyzed: 27-Sep-2013 1843 by 308		Batch: W45076	
Aluminum		**< 0.04**	0.04	**mg/l**	
EPA 200.7	Prep: 30-Sep-2013 0901 by 271	Analyzed: 01-Oct-2013 1248 by 305		Batch: S35491	
Sulfate		**34**	0.2	**mg/l**	
EPA 300.0	Prep: 27-Sep-2013 1628 by 07	Analyzed: 27-Sep-2013 2259 by 07		Batch: C16076	

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

LABORATORY CONTROL SAMPLE RESULTS

Analyte	Spike Amount	%	Limits	RPD	Limit	Batch	Preparation Date	Analysis Date	Dil	Qual
Total Organic Carbon	10 mg/l	96.4	80.0-120			W45076	27Sep13 1459 by 308	27Sep13 1648 by 308		
Aluminum	5 mg/l	97.6	85.0-115			S35491	30Sep13 0901 by 271	01Oct13 1152 by 305		
Sulfate	20 mg/l	108	90.0-110			C16076	27Sep13 1628 by 07	27Sep13 1710 by 07		

MATRIX SPIKE SAMPLE RESULTS

Analyte	Sample	Spike Amount	%	Limits	Batch	Preparation Date	Analysis Date	Dil	Qual
Total Organic Carbon	171006-1	10 mg/l	100	80.0-120	W45076	27Sep13 1459 by 308	27Sep13 1717 by 308		
	171006-1	10 mg/l	97.9	80.0-120	W45076	27Sep13 1459 by 308	27Sep13 1731 by 308		
	Relative Percent Difference:		1.86	25.0	W45076				
Aluminum	171022-2	5 mg/l	96.7	75.0-125	S35491	30Sep13 0901 by 271	01Oct13 1155 by 305		
	171022-2	5 mg/l	96.6	75.0-125	S35491	30Sep13 0901 by 271	01Oct13 1158 by 305		
	Relative Percent Difference:		0.142	20.0	S35491				
Sulfate	171021-1	20 mg/l	89.1	80.0-120	C16076	27Sep13 1628 by 07	27Sep13 1737 by 07		
	171021-1	20 mg/l	93.6	80.0-120	C16076	27Sep13 1628 by 07	27Sep13 1804 by 07		
	Relative Percent Difference:		4.28	10.0	C16076				

LABORATORY BLANK RESULTS

Analyte	Result	RL	PQL	QC Sample	Preparation Date	Analysis Date	Qual
Total Organic Carbon	< 1 mg/l	1	1	W45076-1	27Sep13 1459 by 308	27Sep13 1633 by 308	
Aluminum	< 0.04 mg/l	0.04	0.04	S35491-1	30Sep13 0901 by 271	01Oct13 1149 by 305	
Sulfate	< 0.2 mg/l	0.2	0.2	C16076-1	27Sep13 1628 by 07	27Sep13 1644 by 07	

AMERICAN INTERPLEX CORPORATION LABORATORIES

171032 wn 9/13/13
~~170741~~ wn 9/10/13

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No.: 13-3032

AIC Control No: ~~170390~~
AIC Proposal No:
Carrier: Hot Springs Couriers
Received Temperature °C: 2.1 °C

Analyses Requested: Chronic CD — No Fathead Analysis

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL	No of BOTTLES	Chronic CD	Remarks
1	Plant Effluent	9-8-13 0000-2400		X	X		3	X	
		Container Type					P		Field pH calibration
		Preservative					NO		on ____ @ ____ Buffer:

G = Glass NO = none P = Plastic S = Sulfuric acid pH2 V = VOA vials N = Nitric acid pH2 H = HCl to pH2 B = NaOH to pH12 T = Sodium Thiosulfate Z= Zinc acetate A = $(NH4)_2SO4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: ____
Who should AIC contact with questions: ____
Phone: ____ Fax: ____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By	Date/Time	Received By	Date/Time
[signature]	9-8-13 1030	M. Mann	9-9-13 10:30 am
M. Mann	9-9-13 11:45	Received In Lab By: [signature]	9-9-13 1145

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

August 30, 2013

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Outfall 001
City of Hot Springs

Control No. 169846-1

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Pimephales promelas* (Fathead minnow)
Outfall 001 - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Method 1000.0 Chronic *Pimephales promelas* (Fathead minnow) Survival and Growth Test: The No Observable Effects Concentration (NOEC) for survival occurred at 87 % effluent, which is above the critical dilution of 65 %. The NOEC for growth occurred at 87 % effluent, which is above the critical dilution of 65 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the Fathead minnow test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Table of Contents

I. Control Acceptance Criteria

Pimephales promelas (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.296	PASS
Control Growth CV < or = 40%	7.47	PASS
Growth Minimum Significant Difference 12 to 30%	20.0	PASS
Critical Dilution CV < or = 40%	11.6	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly Test Method 1000.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point: Outfall 001
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.2	8.9	8.5
pH (standard units)	7.4	7.0	7.4
Alkalinity (mg/l as CaCO3)	41	26	34
Hardness (mg/l as CaCO3)	59	67	71
Conductivity (umhos/cm)	290	350	390
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.28	0.12	0.30

2. Dilution Water Samples: Synthetic Soft Water #4012
   a. Dates Prepared: August 8 through August 22, 2013
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.7	7.7	7.8
pH (standard units)	7.7	7.8	7.9
Alkalinity (mg/l as CaCO3)	32	32	32
Hardness (mg/l as CaCO3)	47	42	42
Conductivity (umhos/cm)	180	170	170
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05

C. Test Methods
1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Method 1000.0, Fathead Minnow Survival and Growth.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Pimephales promelas (Fathead minnow) Survival and Growth Method 1000.0

Date & Time Test Initiated:	August 20, 2013 at 1200
Date & Time Test Terminated:	August 27, 2013 at 1010
Type & Volume of Test Chamber:	500 ml disposable beaker
Volume of Sample:	250 ml
Number of Organisms per replicate:	8
Number of Replicates per dilution:	5

4. Acclimation of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms
1. Scientific Name
a. Test 1000.0 *Pimephales promelas*

III. Data Analysis

The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Pimephales promelas (Fathead minnow) survival data was transformed using the Arc Sine transformation. Normality and homogeneity of variance were checked using Shapiro-Wilk's. The survival data was then analyzed using Steel's Many-One Rank Test to determine the No Observable Effects Concentration (NOEC).

Fathead minnow growth data was analyzed for normality and homogeneity of variance using Shapiro-Wilk's and Bartlett's test. Dunnett's Test was used to determine the No Observable Effects Concentration (NOEC) for growth.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Pimephales promelas (Fathead minnow)

Chronic reference tests are performed monthly.
A chronic reference test was performed on August 20, 2013 at 1715 to August 27, 2013 at 1520
The results were as follows: (Control No. 169867-1.)
Survival LC-50: 5670.1 mg/l
Growth IC-25: 3143 mg/l
Growth PMSD: 17.3

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	102	1.91
pH	SM 4500-H+ B	101	0.268
Conductivity	EPA 120.1	101	4.71

VI. Organism History

Pimephales promelas (Fathead minnow)
Date: August 20, 2013
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

VII. Results Summary *Pimephales promelas*, Fathead minnow Larval Survival and Growth Test -- Method 1000.0

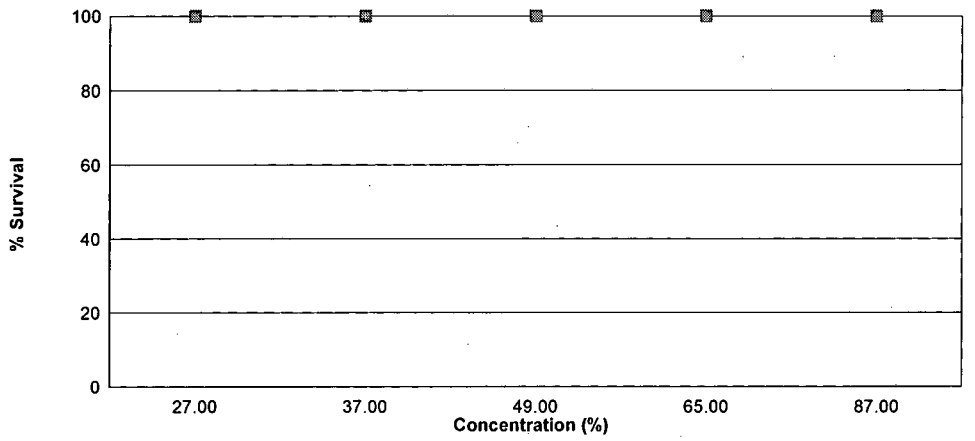
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on August 20, 2013 at 1200 and continued through August 27, 2013 at 1010. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 87 % effluent
b.) NOEC growth = 87 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.296
27 %	100	0.337
37 %	100	0.323
49 %	100	0.302
65 %	100	0.292
87 %	100	0.302

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: August 20, 2013 at 1200
Date and Time Test Terminated: August 27, 2013 at 1010

Concentration	Replicate	Number of Survivors						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
27 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
37 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
49 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
65 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
87 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: August 20, 2013 at 1200
Test Terminated: August 27, 2013 at 1010
Drying Started: August 23, 2013 at 1527
Drying Ended: August 29, 2013 at 1400

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.91827	.92042	0.00215	8	0.269
	B	.91878	.92121	0.00243	8	0.304
	C	.91659	.91922	0.00263	8	0.329
	D	.91638	.91872	0.00234	8	0.292
	E	.91455	.91685	0.00230	8	0.288
27 %	A	.91583	.91811	0.00228	8	0.285
	B	.91943	.92233	0.00290	8	0.362
	C	.92408	.92709	0.00301	8	0.376
	D	.92651	.92924	0.00273	8	0.341
	E	.92767	.93024	0.00257	8	0.321
37 %	A	.92653	.92915	0.00262	8	0.328
	B	.94872	.95160	0.00288	8	0.360
	C	.94724	.94985	0.00261	8	0.326
	D	.94532	.94750	0.00218	8	0.272
	E	.94881	.95146	0.00265	8	0.331
49 %	A	.94115	.94330	0.00215	8	0.269
	B	.92530	.92779	0.00249	8	0.311
	C	.92632	.92901	0.00269	8	0.336
	D	.93070	.93312	0.00242	8	0.302
	E	.93427	.93660	0.00233	8	0.291
65 %	A	.93862	.94107	0.00245	8	0.306
	B	.93944	.94155	0.00211	8	0.264
	C	.94132	.94377	0.00245	8	0.306
	D	.93810	.94077	0.00267	8	0.334
	E	.93899	.94100	0.00201	8	0.251
87 %	A	.93682	.93952	0.00270	8	0.338
	B	.93818	.93991	0.00173	8	0.216
	C	.93688	.93881	0.00193	8	0.241
	D	.93804	.94073	0.00269	8	0.336
	E	.95233	.95537	0.00304	8	0.380

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

		Transformation of Data		Transform: Arc Sin(Square Root(Y))
Group	Identification	Rep	Value	Transformed
1	Control	1	1.00000	1.39310
1	Control	2	1.00000	1.39310
1	Control	3	1.00000	1.39310
1	Control	4	1.00000	1.39310
1	Control	5	1.00000	1.39310
2	27 %	1	1.00000	1.39310
2	27 %	2	1.00000	1.39310
2	27 %	3	1.00000	1.39310
2	27 %	4	1.00000	1.39310
2	27 %	5	1.00000	1.39310
3	37 %	1	1.00000	1.39310
3	37 %	2	1.00000	1.39310
3	37 %	3	1.00000	1.39310
3	37 %	4	1.00000	1.39310
3	37 %	5	1.00000	1.39310
4	49 %	1	1.00000	1.39310
4	49 %	2	1.00000	1.39310
4	49 %	3	1.00000	1.39310
4	49 %	4	1.00000	1.39310
4	49 %	5	1.00000	1.39310
5	65 %	1	1.00000	1.39310
5	65 %	2	1.00000	1.39310
5	65 %	3	1.00000	1.39310
5	65 %	4	1.00000	1.39310
5	65 %	5	1.00000	1.39310
6	87 %	1	1.00000	1.39310
6	87 %	2	1.00000	1.39310
6	87 %	3	1.00000	1.39310
6	87 %	4	1.00000	1.39310
6	87 %	5	1.00000	1.39310

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Shapiro - Wilk's Test for Normality		Transform: Arc Sin(Square Root(Y))
D = 0		
W = 0		
Critical W = 0.9	(alpha = 0.01, N = 30)	
Critical W = 0.927	(alpha = 0.05, N = 30)	

Data FAIL normality test (alpha = 0.01).

Steel's Many-One Rank Test — Transform: Arc Sin(Square Root(Y))

Ho:Control<Treatment

Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	27 %	27.50	16.00	5.00	
3	37 %	27.50	16.00	5.00	
4	49 %	27.50	16.00	5.00	
5	65 %	27.50	16.00	5.00	
6	87 %	27.50	16.00	5.00	

Critical values are 1 tailed (k=5)

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality		No Transformation
D = 0.03787		
W = 0.9742		
Critical W = 0.9	(alpha = 0.01, N = 30)	
Critical W = 0.927	(alpha = 0.05, N = 30)	
Data PASS normality test (alpha = 0.01).		

Bartlett's Test for Homogeneity of Variance		No Transformation
Calculated B1 statistic = 7.076		
Critical B = 15.086	(alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.		

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	0.007651	0.00153	0.9696
Within (Error)	24	0.03787	0.001578	
Total	29	0.04552		

Critical F = 3.9 (alpha = 0.01, df = 5,24)
2.62 (alpha = 0.05, df = 5,24)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Dunnett's Test - Table 1 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	0.2964	0.2964		
2	27 %	0.337	0.337	-1.616	
3	37 %	0.3234	0.3234	-1.075	
4	49 %	0.3018	0.3018	-0.2149	
5	65 %	0.2922	0.2922	0.1672	
6	87 %	0.3022	0.3022	-0.2309	

Dunnett's critical value = 2.36 (1 Tailed, alpha = 0.05, df = 5,24)

Dunnett's Test - Table 2 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	5			
2	27 %	5	0.05929	20	-0.0406
3	37 %	5	0.05929	20	-0.027
4	49 %	5	0.05929	20	-0.0054
5	65 %	5	0.05929	20	0.0042
6	87 %	5	0.05929	20	-0.0058

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 20, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.5	7.7	7.5	7.8	7.8	8.1
	Final	7.5	7.2	7.5	7.9	7.7	7.1	7.9
pH, units	Initial	7.7	7.7	7.8	8.0	7.9	8.0	7.7
	Final	7.5	7.3	7.8	8.1	8.1	7.6	7.9
Alkalinity, mg CaCO3/l		32	NA	32	NA	32	NA	NA
Hardness, mg CaCO3/l		47	NA	42	NA	42	NA	NA
Conductivity, umhos/cm		180	180	170	180	170	170	190
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.4	8.0	7.5	8.0	8.0	7.5
	Final	7.5	7.2	7.3	7.9	7.8	8.1	7.7
pH, units	Initial	7.6	7.7	7.5	7.8	7.7	7.8	7.5
	Final	7.5	7.3	7.7	8.1	8.2	7.5	7.7

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.6	8.6	7.6	8.2	7.7	7.7
	Final	7.5	7.0	7.5	8.0	7.9	8.1	7.8
pH, units	Initial	7.6	7.7	7.4	7.7	7.6	7.7	7.6
	Final	7.5	7.3	7.7	8.1	8.2	7.5	7.6

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 20, 2013 at 0821
Date and Time Test Terminated: August 29, 2013 at 1415

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.5	8.7	7.6	8.1	7.7	7.7
	Final	7.5	7.4	7.6	8.0	8.1	8.2	7.8
pH, units	Initial	7.5	7.7	7.3	7.6	7.6	7.6	7.5
	Final	7.5	7.4	7.6	8.1	8.2	7.6	7.6

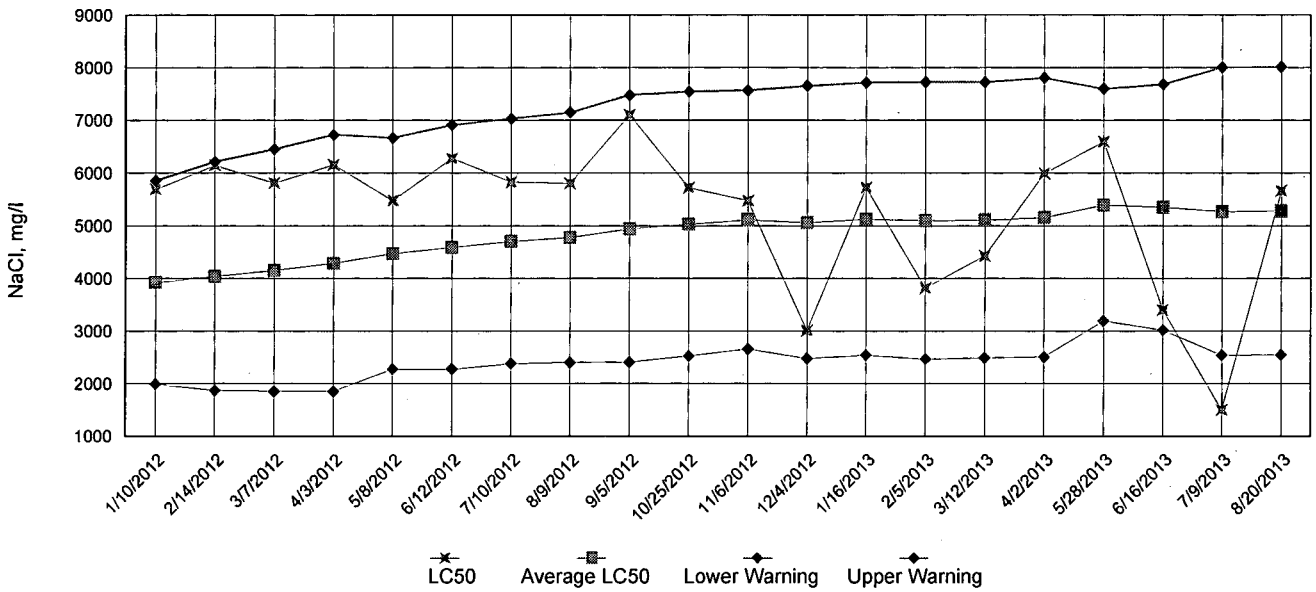
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.9	7.4	8.8	7.6	8.7	7.7	7.3
	Final	7.5	7.4	7.4	7.9	8.1	8.0	7.7
pH, units	Initial	7.5	7.7	7.2	7.5	7.5	7.6	7.5
	Final	7.5	7.4	7.6	8.1	8.3	7.6	7.5
Alkalinity, mg CaCO3/l		36	NA	30	NA	35	NA	NA
Hardness, mg CaCO3/l		54	NA	58	NA	61	NA	NA
Conductivity, umhos/cm		250	260	280	290	310	310	330
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	7.4	8.9	7.6	8.3	7.9	7.4
	Final	7.5	8.5	7.4	7.8	8.1	8.0	7.7
pH, units	Initial	7.4	7.7	7.1	7.4	7.4	7.6	7.6
	Final	7.5	7.4	7.6	8.1	8.1	7.6	7.4

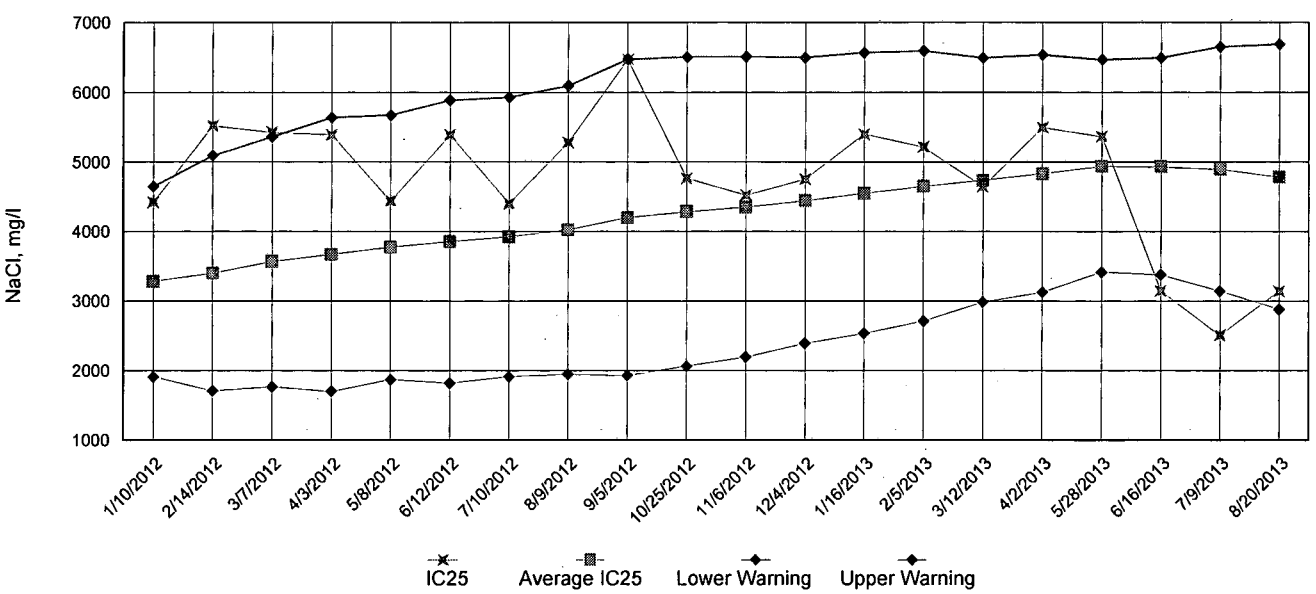
Appendix A4: Test 1000.0

Chronic Reference Toxicant, *Pimephales promelas* (Fathead Minnow)

LC50 Survival Data



IC25 Growth Data



Appendix B: Test 1000.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

Permittee: City of Hot Springs

NPDES No.: AR0033880 AFIN#26-00145

Date and Time Test Initiated: August 20, 2013 at 1200
Date and Time Test Terminated: August 27, 2013 at 1010
Dilution water used: Synthetic Soft Water #4012

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in replicate chambers A	B	C	D	E	Mean percent survival 24 hr	48 hr	7 days	CV%
Control	100	100	100	100	100	100	100	100	0.00
27 %	100	100	100	100	100	100	100	100	0.00
37 %	100	100	100	100	100	100	100	100	0.00
49 %	100	100	100	100	100	100	100	100	0.00
65 %	100	100	100	100	100	100	100	100	0.00
87 %	100	100	100	100	100	100	100	100	0.00

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers A	B	C	D	E	Mean dry weight, mg	CV%
Control	0.269	0.304	0.329	0.292	0.288	0.296	7.47
27 %	0.285	0.362	0.376	0.341	0.321	0.337	10.6
37 %	0.328	0.360	0.326	0.272	0.331	0.323	9.86
49 %	0.269	0.311	0.336	0.302	0.291	0.302	8.20
65 %	0.306	0.264	0.306	0.334	0.251	0.292	11.6
87 %	0.338	0.216	0.241	0.336	0.380	0.302	23.2

CV = Coefficient of variation = standard deviation * 100 / mean

Appendix B: Test 1000.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

1. Steel's Many-One Rank Test:

   Is the mean survival significantly different (p=0.05) than the control survival for the % effluent corresponding to (lethality):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	____YES	X NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____YES	____NO

2. Dunnett's Test:

   Is the mean dry weight (growth) significantly different (p=0.05) than the control's dry weight (growth) for the % effluent corresponding to (significant non-lethal effects):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	____YES	X NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____YES	____NO

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

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

5. NOEC Pimephales Lethality: 87 % (TOP6C)

6. LOEC Pimephales Lethality: 87 % (TXP6C)

7. NOEC Pimephales Sublethality: 87 % (TPP6C)

8. LOEC Pimephales Sublethality: 87 % (TYP6C)

9. Coefficient of variation for Pimephales growth: 11.6 (TQP6C)

AMERICAN INTERPLEX CORPORATION
LABORATORIES

Appendix B: Test 1000.0

CHRONIC TOXICITY SUMMARY FORM
Pimephales promelas (Fathead minnow)
CHEMICAL PARAMETERS CHART

PERMITTEE: City of Hot Springs 2400
NPDES NO.: AR0033880 AFIN#26-00145 2400
CONTACT: Mr. James Sorrells 2400
ANALYST: 280, 298, 304, 307

Test Initiated: DATE: August 20, 2013 TIME: 1200
Test Terminated: DATE: August 27, 2013 TIME: 1010

DILUTION Control	DAY 1	2	3	4	5	6	7
D.O. Initial	7.7	7.5	7.7	7.5	7.8	7.8	8.1
Final	7.5	7.2	7.5	7.9	7.7	7.1	7.9
pH Initial	7.7	7.7	7.8	8.0	7.9	8.0	7.7
Final	7.5	7.3	7.8	8.1	8.1	7.6	7.9
Alkalinity	32	NA	32	NA	32	NA	NA
Hardness	47	NA	42	NA	42	NA	NA
Conductivity	180	180	170	180	170	170	190
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 27 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.7	7.4	8.0	7.5	8.0	8.0	7.5
Final	7.5	7.2	7.3	7.9	7.8	8.1	7.7
pH Initial	7.6	7.7	7.5	7.8	7.7	7.8	7.5
Final	7.5	7.3	7.7	8.1	8.2	7.5	7.7
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	220	220	220	230	230	250
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 37 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.8	7.6	8.6	7.6	8.2	7.7	7.7
Final	7.5	7.0	7.5	8.0	7.9	8.1	7.8
pH Initial	7.6	7.7	7.4	7.7	7.6	7.7	7.6
Final	7.5	7.3	7.7	8.1	8.2	7.5	7.6
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	220	230	240	240	260	250	270
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 49 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.8	7.5	8.7	7.6	8.1	7.7	7.7
Final	7.5	7.4	7.6	8.0	8.1	8.2	7.8
pH Initial	7.5	7.7	7.3	7.6	7.6	7.6	7.5
Final	7.5	7.4	7.6	8.1	8.2	7.6	7.6
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	230	240	260	260	280	280	300
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 65 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.9	7.4	8.8	7.6	8.7	7.7	7.3
Final	7.5	7.4	7.4	7.9	8.1	8.0	7.7
pH Initial	7.5	7.7	7.2	7.5	7.5	7.6	7.5
Final	7.5	7.4	7.6	8.1	8.3	7.6	7.5
Alkalinity	36	NA	30	NA	35	NA	NA
Hardness	54	NA	58	NA	61	NA	NA
Conductivity	250	260	280	290	310	310	330
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 87 %	DAY 1	2	3	4	5	6	7
D.O. Initial	8.0	7.4	8.9	7.6	8.3	7.9	7.4
Final	7.5	8.5	7.4	7.8	8.1	8.0	7.7
pH Initial	7.4	7.7	7.1	7.4	7.4	7.6	7.6
Final	7.5	7.4	7.6	8.1	8.1	7.6	7.4
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	280	290	320	320	360	360	380
Chlorine	NA	NA	NA	NA	NA	NA	NA

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs

Project Reference: Plant Effluent

Project Manager: James Sorrells

Sampled By: A. Ross

PO No.: 13-3032

AIC Control No: 169846

AIC Proposal No:

Carrier: Hot Springs Courier

Received Temperature °C: 2°C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER (Sample Matrix)	SOIL (Sample Matrix)		No of BOTTLES	Chronic.CD, Chronic.FH												Remarks
1	Plant Effluent	8-18-13 0000-2400		X	X			3	X												
		Container Type							P												Field pH calibration
		Preservative							NO												on ______ @ ______ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate

NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: ____________
Who should AIC contact with questions: ____________
Phone: ____________ Fax: ____________
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By	Date/Time	Received By	Date/Time
A. Thomason	8-19-13 @ 1010	M. Mann	8-19-13 10:10 AM
M. Mann	8-19-13 @ 11:10 AM	Received In Lab By: [signature]	8-19-13 1110

Comments:

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 2.3C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH	Remarks
2	Plant Effluent	8-20-13 0000-2400		X	X		3	X	
		Container Type						P	Field pH calibration
		Preservative						NO	on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
A. Ross	8-21-13 0900	A. Man	8-21-13 9:10
A. Man	8-21-13 1130	Jimmy Day	8/21/13 1130

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By:

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL		No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH
3	Plant Effluent	8/22/13 0000-2400		X				3	X
		Container Type							P
		Preservative							NO

AIC Control No: 169846
AIC Proposal No:
Carrier: Hot Springs Shuttle
Received Temperature °C: 2
Remarks
Field pH calibration on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	8-23-13 0955	[signature]	8-23-13 9:55
[signature]	8-23-13 11:20	[signature]	8-23-13 1120

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

September 19, 2013

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Plant Effluent
City of Hot Springs

Control No. 170390-1

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Ceriodaphnia dubia*
Plant Effluent - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: The No Observable Effects Concentration (NOEC) for survival occurred at <27 % effluent, which is below the critical dilution of 65 %. The NOEC for reproduction occurred at <27 % effluent, which is below the critical dilution of 65 %. **The sample, therefore, FAILED both lethal and sub-lethal effects for the *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Table of Contents

I. Control Acceptance Criteria

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	18.8	PASS
Control CV < or = 40% per Surviving Female	20.6	PASS
Reproduction Minimum Significant Difference 13 to 47%	NA	NA
Critical Dilution CV < or = 40%	0.00	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly Test Method 1002.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point: Plant Effluent
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.7	8.2	8.2
pH (standard units)	7.4	7.7	7.5
Alkalinity (mg/l as CaCO3)	64	58	64
Hardness (mg/l as CaCO3)	82	82	83
Conductivity (umhos/cm)	380	370	440
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	2.4	0.11

2. Dilution Water Samples: Synthetic Soft Water #4019
   a. Dates Prepared: August 28 through September 11, 2013
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.6	8.2	8.2
pH (standard units)	7.6	7.8	7.6
Alkalinity (mg/l as CaCO3)	32	32	32
Hardness (mg/l as CaCO3)	46	42	42
Conductivity (umhos/cm)	160	160	180
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05

C. Test Methods
1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Method 1002.0, *Ceriodaphnia dubia* Survival and Reproduction.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Ceriodaphnia dubia Survival and Growth Method 1002.0

Date & Time Test Initiated:	September 9, 2013 at 1235
Date & Time Test Terminated:	September 17, 2013 at 1255
Type & Volume of Test Chamber:	30 ml disposable beaker
Volume of Sample:	15 ml
Number of Organisms per replicate:	1
Number of Replicates per dilution:	10

4. Acclimation of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms
1. Scientific Name
a. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis
The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was not analyzed due to survival failure.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Ceriodaphnia dubia

Chronic reference tests are performed monthly.
A chronic reference test was performed on August 20, 2013 at 1505 to August 28, 2013 at 1450
The results were as follows: (Control No. 169867-2.)
Survival LC-50: 2125 mg/l
Growth IC-25: 1610 mg/l
Growth PMSD: 18.3

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	1.53
Hardness	EPA 200.7	101	0.450
pH	SM 4500-H+ B	101	0.267
Conductivity	EPA 120.1	103	1.97

VI. Organism History

Ceriodaphnia dubia

Date: September 9, 2013
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

VII. Results Summary *Ceriodaphnia dubia*, Cladoceran Survival and Reproduction Test -- Method 1002.0

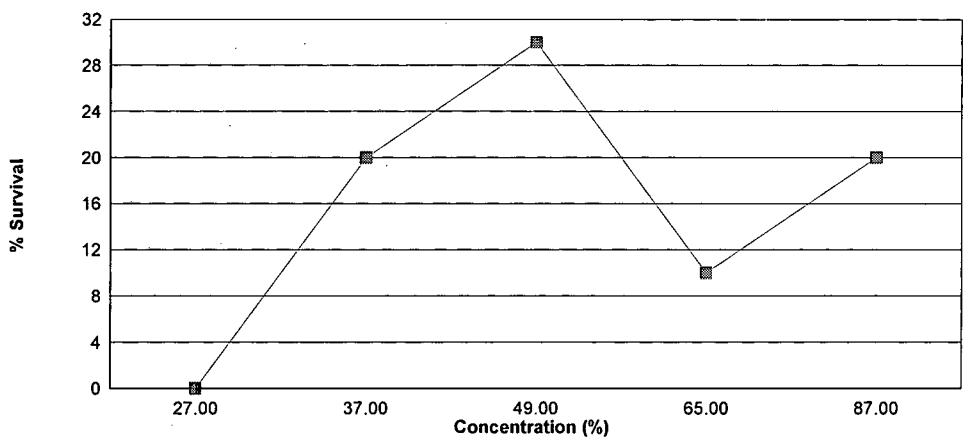
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on September 9, 2013 at 1235 and continued through September 17, 2013 at 1255. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = <27 % effluent
b.) NOEC reproduction = <27 % effluent



Summary of the 7-day *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	18.8
27 %	0.00 *	--
37 %	20.0 *	--
49 %	30.0 *	--
65 %	10.0 *	--
87 %	20.0 *	--

*Significant difference when compared to the control (p=0.05)

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 9, 2013 at 1235
Date and Time Test Terminated: September 17, 2013 at 1255

Concentration: Control													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	3	2	0	0	0	0	1	4	2	1	13	10	1.30
5	9	0	3	6	6	4	0	4	6	0	38	10	3.80
6	0	5	9	8	7	0	6	0	1	7	43	10	4.30
7	10	9	10	10	10	8	9	8	10	10	94	10	9.40
8													
TOTAL	22	16	22	24	23	12	16	16	19	18	188	10	18.8

Concentration: 27 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	0	0	0	X	0	0	0	X	0	0	7	0.00
2	X	0	0	0	X	0	0	0	X	0	0	7	0.00
3	X				X				X		0	0	0.00
4	X				X				X		0	0	0.00
5	X				X				X		0	0	0.00
6	X				X				X		0	0	0.00
7	X				X				X		0	0	0.00
8													
TOTAL	0	0	0	0	0	0	0	0	0	0	0	10	0.00

Concentration: 37 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	X	X	0	X	X	0	0	3	0.00
2	X	X	0	X	X	X	0	X	X	0	0	3	0.00
3	X	X	X	X	X	X	0	X	X	0	0	2	0.00
4	X	X	X	X	X	X	2	X	X	1	3	2	1.50
5	X	X	X	X	X	X	0	X	X	0	0	2	0.00
6	X	X	X	X	X	X	7	X	X	6	13	2	6.50
7	X	X	X	X	X	X	10	X	X	0	10	2	5.00
8													
TOTAL	0	0	0	0	0	0	19	0	0	7	26	10	2.60

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 9, 2013 at 1235
Date and Time Test Terminated: September 17, 2013 at 1255

Concentration: 49 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	X	X	0	X	X	0	0	3	0.00
2	X	X	0	X	X	X	0	X	X	0	0	3	0.00
3	X	X	0	X	X	X	0	X	X	0	0	3	0.00
4	X	X	0	X	X	X	0	X	X	0	0	3	0.00
5	X	X	4	X	X	X	5	X	X	0	9	3	3.00
6	X	X	0	X	X	X	0	X	X	6	6	3	2.00
7	X	X	6	X	X	X	8	X	X	0	14	3	4.67
8													
TOTAL	0	0	10	0	0	0	13	0	0	6	29	10	2.90

Concentration: 65 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	X	X	X	X	X	0	0	2	0.00
2	X	X	0	X	X	X	X	X	X	0	0	2	0.00
3	X	X	0	X	X	X	X	X	X	0	0	2	0.00
4	X	X	0	X	X	X	X	X	X	0	0	2	0.00
5	X	X	6	X	X	X	X	X	X	0	6	2	3.00
6	X	X	0	X	X	X	X	X	X	X	0	1	0.00
7	X	X	9	X	X	X	X	X	X	X	9	1	9.00
8													
TOTAL	0	0	15	0	0	0	0	0	0	0	15	10	1.50

Concentration: 87 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	X	X	0	X	0	X	0	X	X	0	0	4	0.00
2	X	X	0	X	0	X	X	X	X	0	0	3	0.00
3	X	X	0	X	0	X	X	X	X	0	0	3	0.00
4	X	X	0	X	3	X	X	X	X	1	4	3	1.33
5	X	X	7	X	5	X	X	X	X	0	12	3	4.00
6	X	X	0	X	X	X	X	X	X	3	3	2	1.50
7	X	X	9	X	X	X	X	X	X	0	9	2	4.50
8													
TOTAL	0	0	16	0	8	0	0	0	0	4	28	10	2.80

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
27 %	0	3	3
Total	10	3	13

Critical Fisher's value (10,3,10) (alpha=0.05) is 1. b value is 0. Since b is less than or equal to 1 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
37 %	2	8	10
Total	12	8	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 2. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
49 %	3	7	10
Total	13	7	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 3. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
65 %	1	9	10
Total	11	9	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 1. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
87 %	2	8	10
Total	12	8	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 2. Since b is less than or equal to 6 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	27 %	3	3	*
2	37 %	10	8	*
3	49 %	10	7	*
4	65 %	10	9	*
5	87 %	10	8	*

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: September 9, 2013 at 0857
Date and Time Test Terminated: September 17, 2013 at 1255

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.6	7.4	8.2	8.3	8.2	8.3	8.2
	Final	7.8	8.3	8.5	8.7	8.4	8.2	7.8
pH, units	Initial	7.6	7.6	7.8	7.6	7.6	7.7	7.5
	Final	7.8	7.9	7.9	7.6	7.9	7.8	8.0
Alkalinity, mg CaCO3/l		32	NA	32	NA	32	NA	NA
Hardness, mg CaCO3/l		46	NA	42	NA	42	NA	NA
Conductivity, umhos/cm		160	170	160	180	180	180	170
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.6	7.5	8.2	8.4	8.3	8.1	8.3
	Final	6.0	8.3	8.7	8.8	8.5	8.3	8.1
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
	Final	8.0	8.1	8.2	8.0	8.3	8.0	8.1

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.6	7.4	8.2	8.4	8.1	8.0	8.1
	Final	6.4	8.3	8.6	8.8	8.6	8.2	8.2
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
	Final	8.1	8.2	8.2	8.2	8.2	7.9	7.8

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: September 9, 2013 at 0857
Date and Time Test Terminated: September 17, 2013 at 1255

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.5	7.5	8.2	8.4	8.4	8.1	8.3
	Final	6.8	8.3	7.8	9.0	8.4	8.3	8.0
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.5	7.5
	Final	8.2	8.2	8.2	8.1	8.1	8.0	8.1

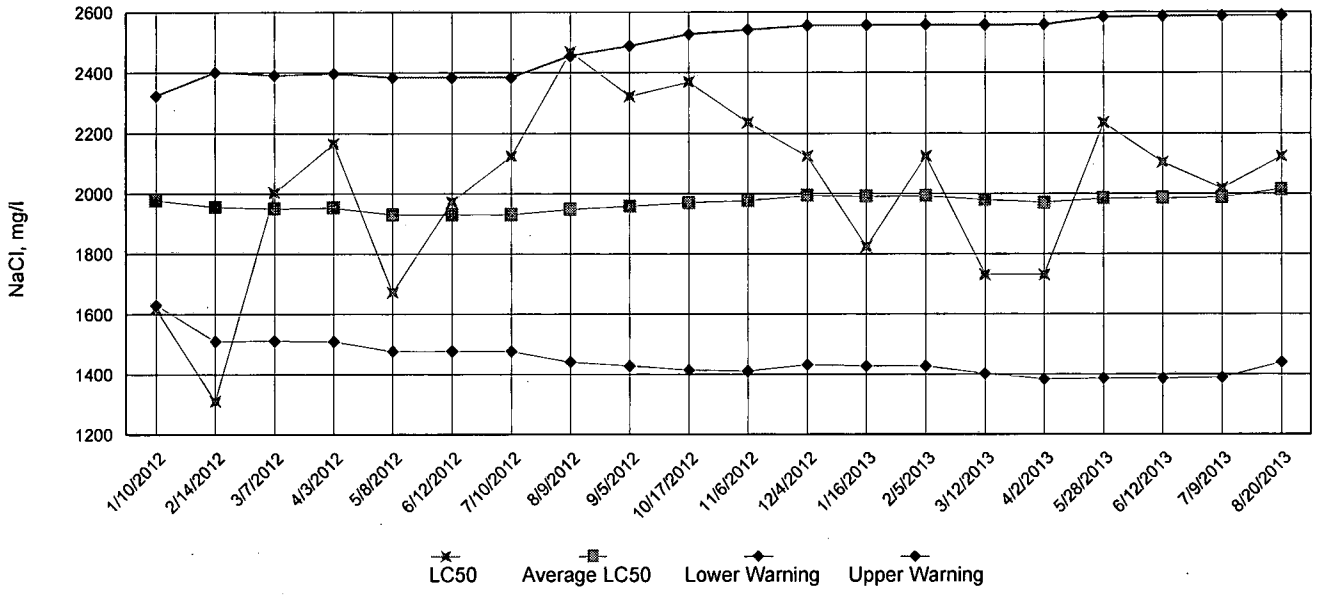
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.4	8.2	8.4	8.0	8.2	8.3
	Final	7.0	8.3	8.4	8.7	8.3	8.3	7.7
pH, units	Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.6
	Final	8.2	8.1	8.1	8.2	8.0	8.0	7.7
Alkalinity, mg CaCO3/l		48	NA	46	NA	47	NA	NA
Hardness, mg CaCO3/l		68	NA	68	NA	68	NA	NA
Conductivity, umhos/cm		310	310	300	340	340	340	350
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.3	7.2	8.2	8.3	8.3	8.0	7.9
	Final	7.1	8.3	8.6	8.8	7.3	8.3	8.0
pH, units	Initial	7.5	7.4	7.8	7.5	7.6	7.5	7.6
	Final	8.2	8.3	8.3	8.3	7.5	8.1	7.9

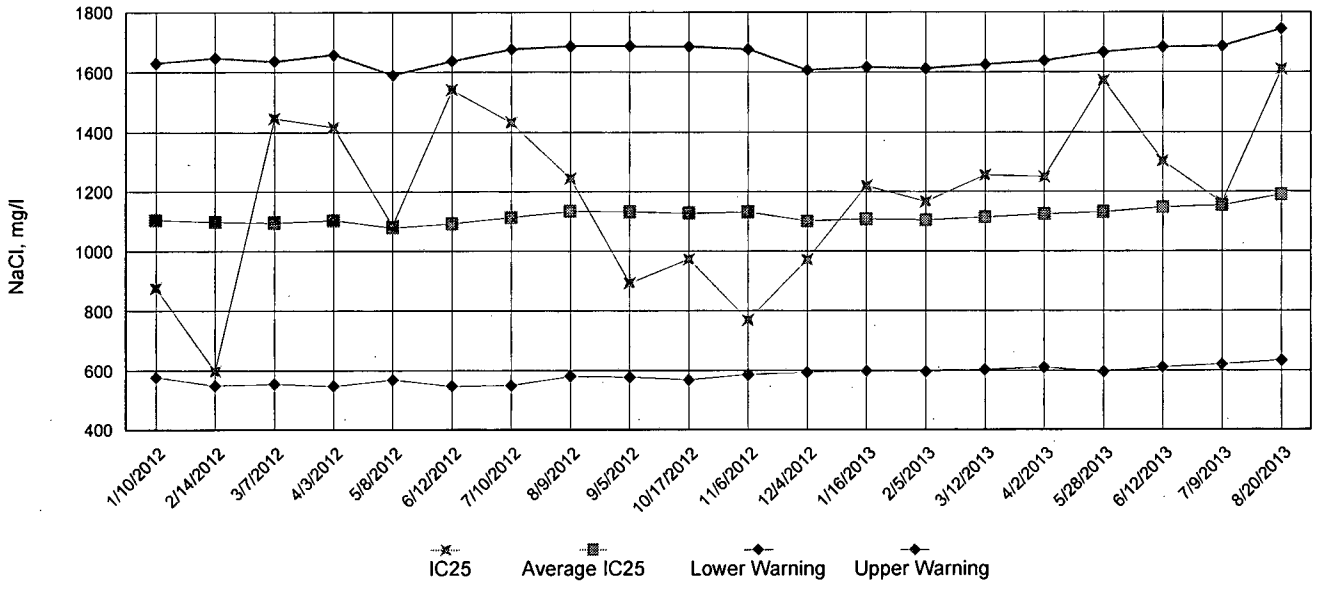
Appendix A4: Test 1002.0

Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

Permittee: City of Hot Springs

NPDES No.: AR0033880 AFIN#26-00145

Date and Time Test Initiated: September 9, 2013 at 1235
Date and Time Test Terminated: September 17, 2013 at 1255
Dilution water used: Synthetic Soft Water #4019

PERCENT SURVIVAL

		Percent Effluent				
Time of Reading	Control	27 %	37 %	49 %	65 %	87 %
24 hour	100	70.0	30.0	30.0	20.0	40.0
48 hour	100	70.0	30.0	30.0	20.0	30.0
7 day	100	0.00	20.0	30.0	10.0	20.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

		Percent Effluent				
Replicates	Control	27 %	37 %	49 %	65 %	87 %
A	22	0	0	0	0	0
B	16	0	0	0	0	0
C	22	0	0	10	15	16
D	24	0	0	0	0	0
E	23	0	0	0	0	8
F	12	0	0	0	0	0
G	16	0	19	13	0	0
H	16	0	0	0	0	0
I	19	0	0	0	0	0
J	18	0	7	6	0	4
Mean per Adult	18.8	0.00	2.60	2.90	1.50	2.80
Mean per Surviving Adult	18.8	0.00	13.0	9.67	15.0	10.0
CV %	20.6	0.00	65.3	36.3	0.00	84.9

CV = Coefficient of variation = standard deviation * 100 / mean
(calculated based on young produced by surviving females)

Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

1. Fisher's Exact Test:

   Is the mean survival significantly different (p=0.05) than the control survival for the % effluent corresponding to (lethality):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	X YES	____ NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____ YES	____ NO

2.

   Is the mean number of young produced per female significantly different (p=0.05) than the control's number of young per female for the % effluent corresponding to (significant non-lethal effects):

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	X YES	____ NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____ YES	____ NO

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 1 (TLP3B)
4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 1 (TGP3B)
5. NOEC Ceriodaphnia Lethality: <27 % (TOP3B)
6. LOEC Ceriodaphnia Lethality: 27 % (TXP3B)
7. NOEC Ceriodaphnia Sublethality: <27 % (TPP3B)
8. LOEC Ceriodaphnia Sublethality: 27 % (TYP3B)
9. Coefficient of variation for Ceriodaphnia Reproduction: 20.6 (TQP3B)

Appendix B: Test 1002.0

CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
CHEMICAL PARAMETERS CHART

PERMITTEE: City of Hot Springs
NPDES NO.: AR0033880 AFIN#26-00145
CONTACT: Mr. James Sorrells
ANALYST: 280, 298, 304, 307

2400
2400
2400

Test Initiated: DATE: September 9, 2013 TIME: 1235
Test Terminated: DATE: September 17, 2013 TIME: 1255

DILUTION	DAY						
Control	1	2	3	4	5	6	7
D.O. Initial	8.6	7.4	8.2	8.3	8.2	8.3	8.2
Final	7.8	8.3	8.5	8.7	8.4	8.2	7.8
pH Initial	7.6	7.6	7.8	7.6	7.6	7.7	7.5
Final	7.8	7.9	7.9	7.6	7.9	7.8	8.0
Alkalinity	32	NA	32	NA	32	NA	NA
Hardness	46	NA	42	NA	42	NA	NA
Conductivity	160	170	160	180	180	180	170
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
27 %	1	2	3	4	5	6	7
D.O. Initial	8.6	7.5	8.2	8.4	8.3	8.1	8.3
Final	6.0	8.3	8.7	8.8	8.5	8.3	8.1
pH Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
Final	8.0	8.1	8.2	8.0	8.3	8.0	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	220	220	220	240	250	250	250
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
37 %	1	2	3	4	5	6	7
D.O. Initial	8.6	7.4	8.2	8.4	8.1	8.0	8.1
Final	6.4	8.3	8.6	8.8	8.6	8.2	8.2
pH Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.5
Final	8.1	8.2	8.2	8.2	8.2	7.9	7.8
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	240	240	240	260	270	270	280
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
49 %	1	2	3	4	5	6	7
D.O. Initial	8.5	7.5	8.2	8.4	8.4	8.1	8.3
Final	6.8	8.3	7.8	9.0	8.4	8.3	8.0
pH Initial	7.6	7.5	7.8	7.5	7.6	7.5	7.5
Final	8.2	8.2	8.2	8.1	8.1	8.0	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	270	270	270	300	300	300	300
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
65 %	1	2	3	4	5	6	7
D.O. Initial	7.7	7.4	8.2	8.4	8.0	8.2	8.3
Final	7.0	8.3	8.4	8.7	8.3	8.3	7.7
pH Initial	7.6	7.5	7.8	7.5	7.6	7.6	7.6
Final	8.2	8.1	8.1	8.2	8.0	8.0	7.7
Alkalinity	48	NA	46	NA	47	NA	NA
Hardness	68	NA	68	NA	68	NA	NA
Conductivity	310	310	300	340	340	340	350
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
87 %	1	2	3	4	5	6	7
D.O. Initial	8.3	7.2	8.2	8.3	8.3	8.0	7.9
Final	7.1	8.3	8.6	8.8	7.3	8.3	8.0
pH Initial	7.5	7.4	7.8	7.5	7.6	7.5	7.6
Final	8.2	8.3	8.3	8.3	7.5	8.1	7.9
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	350	360	350	400	440	430	440
Chlorine	NA	NA	NA	NA	NA	NA	NA

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Roys

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Chronic C.D.	Analyses Requested
1	Plant Effluent	9-8-13 2000-2400		X	X		3	X	No Fathead Analysis
		Container Type						P	
		Preservative						NO	

AIC Control No: 170390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2.1°C
Remarks:
Field pH calibration on _____ @ _____
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-8-13 1030	M. Mann	9-9-13 10:30 am
M. Mann	9-9-13 11:45	[signature]	9-9-13 1145

Comments:

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Ross

PO No. 13-3032

AIC Control No: 170390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 3°C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Analyses Requested: Chronic.CD, Chronic.FH	Remarks
1	Plant Effluent	9-10-13 0000-2400		X	X		3	X	
		Container Type						P	Field pH calibration
		Preservative						NO	on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-11-13 1110	M. Mann	9-11-13 1110
M. Mann	9-11-13 @ 12:05	[signature]	9-11-13 1205

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs
Project Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: HAROLD MAULDIN

PO No.: 13-3032

AIC Control No: 170390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL		No of BOTTLES	Chronic.CD.	Remarks
3	Plant Effluent	9/12/13 0000-2400		X	X			3	x	
Container Type									P	
Preservative									NO	

Field pH calibration on ______ @ ______
Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH4)_2SO4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: ____________
Who should AIC contact with questions: ____________
Phone: ____________ Fax: ____________
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By	Date/Time	Received By	Date/Time
A. Thomason	9-13-13 @ 1015	M. Mann	9-13-13 @ 10:15
M. Mann	9-13-13 @ 11:20	Received in Lab By: [signature]	9-13-13 1120

Comments:

4-85

AMERICAN INTERPLEX CORPORATION
LABORATORIES

September 30, 2013

Test Results of
Acute 48 hour Non-Renewal
Biomonitoring Testing
for

170741-1: Outfall 001 First Renewal
170741-2: Outfall 001 Second Renewal
170741-3: Outfall 001 Third Renewal
170741-4: Outfall 001 Original Screen

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

AMERICAN INTERPLEX CORPORATION
LABORATORIES

City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Acute 48 hour Non-Renewal Biomonitoring utilizing *Ceriodaphnia dubia*
Outfall 001 First Renewal - City of Hot Springs
Client NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

Please find attached the results of the acute biomonitoring screens conducted as a follow up to the chronic toxicity event previously reported (AIC Control No. 170390). As indicated on the enclosed results, the sample was still acutely toxic on September 12, 2013 and exhibited no acute toxicity on September 19, 2013.

It it theorized the decrease in toxicity is directly related to the holding time which may have allowed microbial degradation, loss of volatile toxics, and/or biochemical degradation.

If I can be of further assistance, please feel free to contact me.

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

Dilution Water Samples: Synthetic Soft Water #4021

Analysis	Result
Dissolved oxygen (mg/l)	7.6
pH (standard units)	7.7
Alkalinity (mg/l as CaCO3)	31
Hardness (mg/l as CaCO3)	46
Conductivity (umhos/cm)	170
Residual Chlorine (mg/l)	<0.05

Results Summary: Outfall 001 First Renewal

Ceriodaphnia dubia

The *Ceriodaphnia dubia* test was conducted from September 19, 2013 at 1615 to September 21, 2013 at 1700.

Statistical analyses:

NOEC = 100%

Concentration	24 hour % Survival	48 hour % Survival
Control	100	100
100%	100	100

Ceriodaphnia dubia
Survival Data

Number of organisms per chamber: 5
Volume of test chamber: 30 ml

Age of organisms: <24 hours
Volume of test solution: 15 ml

Effluent Concentration	Number of Survivors 24 Hours	48 Hours	% Survival	CV %
Control rep. A	5	5	100	0.00
100% rep. A	5	5	100	0.00

CV = Coefficient of variance = standard deviation X 100/mean

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	5	0	5
100%	5	0	5
Total	10	0	10

Critical Fisher's value (5,5,5) (alpha=0.05) is 1. b value is 5. Since b is greater than 1 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test

Group	Identification	Exposed	Dead	Sig 0.05
0	0	5	0	
1	100	5	0	

Chemical Data for
Ceriodaphnia dubia

Day 1	Control	100%
DO, mg/l	7.6	8.8
pH, su	7.7	7.3
Alkalinity, mg/l	31	NA
Hardness, mg/l	46	NA
Conductivity, umho/cm	170	380
Residual Chlorine, mg/l	<0.05	NA

Day 2		Control	100%
DO, mg/l	Final	7.5	8.7
pH, su	Final	8.0	7.4

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

170744 WR 9/19/13

Client: City of Hot Springs
Project:
Reference: Plant Effluent
Project Manager: James Sorrells
Sampled By: A. Rojs

PO No. 13-3032

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	Sample Matrix: WATER	Sample Matrix: SOIL	No of BOTTLES	Chronic CD.	Analyses Requested
1	Plant Effluent	9-8-13 0000-2400		X	X		3	X	
		Container Type						P	
		Preservative						NO	

No Fathead Analysis

AIC Control No: 170390
AIC Proposal No:
Carrier: Hot Springs Courier
Received Temperature °C: 2.1°C
Remarks:
Field pH calibration on ____ @ ____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z= Zinc acetate A = $(NH_4)_2SO_4$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ___ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Phone: _____ Fax: _____
Report Attention to: Mr. James Sorrells
Report Address to: 320 Davidson Road
Hot Springs, AR 71901

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	9-9-13 1030	M. Mann	9-9-13 10:30 AM
M. Mann	9-9-13 11:45	[signature]	9-9-13 1145

Comments:

City of Hot Springs
Waste Water Treatment Plant
320 Davidson Drive
Hot Springs, AR 71901

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