

May 22, 2012

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

Control No. 157538-1

Prepared for:

Mr. Ron Wacaster
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
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City of Hot Springs
ATTN: Mr. Ron Wacaster
320 Davidson Drive
Hot Springs, AR 71901

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

Dear Mr. Ron Wacaster:

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 85 % effluent, which is equal to the critical dilution of 85 %. The NOEC for growth occurred at 27 % effluent, which is below the critical dilution of 85 %. **The sample PASSED lethal effects, however, FAILED sub-lethal effects for the Fathead minnow test.**

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: The No Observable Effects Concentration (NOEC) for survival occurred at 85 % effluent, which is equal to the critical dilution of 85 %. The NOEC for reproduction occurred at 64 % effluent, which is below the critical dilution of 85 %. **The sample PASSED lethal effects, however, FAILED sub-lethal effects for the *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
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I. Control Acceptance Criteria

Pimephales promelas (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	97.5	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.537	PASS
Control Growth CV < or = 40%	13.1	PASS
Growth Minimum Significant Difference 12 to 30%	17.7	PASS
Critical Dilution CV < or = 40%	27.6	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	23.5	PASS
Control CV < or = 40% per Surviving Female	18.2	PASS
Reproduction Minimum Significant Difference 13 to 47%	18.1	PASS
Critical Dilution CV < or = 40%	21.5	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly
Test Methods 1000.0 and 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)	7.9	8.2	8.1
pH (standard units)	7.2	7.8	7.6
Alkalinity (mg/l as CaCO ₃)	69	68	67
Hardness (mg/l as CaCO ₃)	80	82	86
Conductivity (umhos/cm)	220	250	380
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	<0.1	0.20

2. Dilution Water Samples: Synthetic Laboratory Soft Water #4225

- a. Dates Prepared: April 26 through May 10, 2012
- b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.9	7.6	8.5
pH (standard units)	7.5	7.9	7.8
Alkalinity (mg/l as CaCO ₃)	31	31	31
Hardness (mg/l as CaCO ₃)	41	45	41
Conductivity (umhos/cm)	85	94	140
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 Methods 1000.0 and 1002.0, Fathead Minnow Survival and Growth and *Ceriodaphnia dubia* Survival and Reproduction.

2. Endpoint: No Observable Effects Concentration (NOEC)

3. Test Conditions:

Pimephales promelas (Fathead minnow) Survival and Growth Method 1000.0

Date & Time Test Initiated: May 8, 2012 at 1545
Date & Time Test Terminated: May 15, 2012 at 1400
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

Ceriodaphnia dubia Survival and Growth Method 1002.0

Date & Time Test Initiated: May 8, 2012 at 1355
Date & Time Test Terminated: May 15, 2012 at 1450
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 1000.0 *Pimephales promelas*
- b. Test 1002.0 *Ceriodaphnia dubia*

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.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was analyzed using Kolmogorov's Test for Normality and analyzed with Steel's Many-One Rank Test to determine the No Observable Effects Concentration (NOEC) for Reproduction. Dunnett's Test was used to calculate the PMSD.

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 April 3, 2012 at 1600 to April 10, 2012 at 1500

The results were as follows: (Control No. 156622-2.)

Survival LC-50: 6157 mg/l

Growth IC-25: 5394 mg/l

Growth PMSD: 27.4

Ceriodaphnia dubia

Chronic reference tests are performed monthly.

A chronic reference test was performed on April 3, 2012 at 1425 to April 10, 2012 at 1410

The results were as follows: (Control No. 156622-1.)

Survival LC-50: 2166 mg/l

Growth IC-25: 1417 mg/l

Growth PMSD: 20.1

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	101	1.06
pH	SM 4500-H+ B	100	0.135
Conductivity	EPA 120.1	99.4	1.22

VI. Organism History

Pimephales promelas (Fathead minnow)

Date: May 8, 2012

Age: <24 hours

Source: In-house culture

Water Chemistry Record:

Alkalinity: 57-64 mg/l

Hardness: 80-100 mg/l

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: May 8, 2012

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

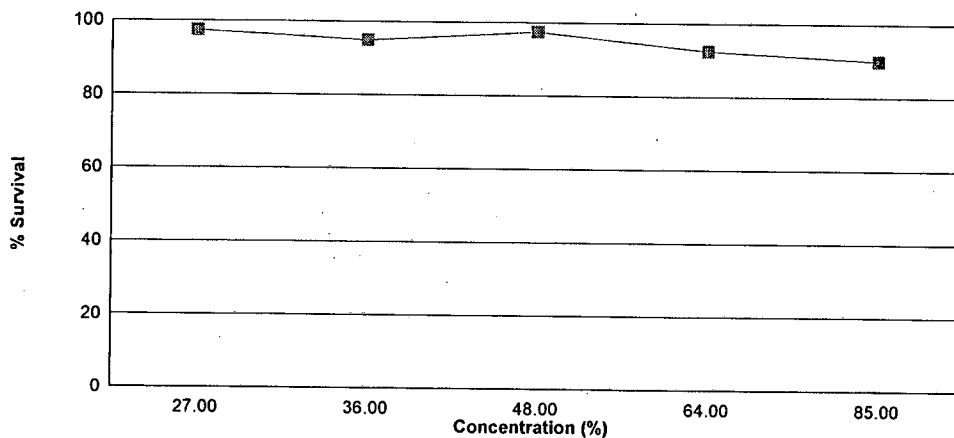
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

Effluent dilutions for this test were 27 %, 36 %, 48 %, 64 %, 85 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 85 % effluent.

The test was initiated on May 8, 2012 at 1545 and continued through May 15, 2012 at 1400. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 85 % effluent
- b.) NOEC growth = 27 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	97.5	0.524
27 %	97.5	0.442
36 %	95.0	0.408 *
48 %	97.5	0.408 *
64 %	92.5	0.382 *
85 %	90.0	0.349 *

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

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

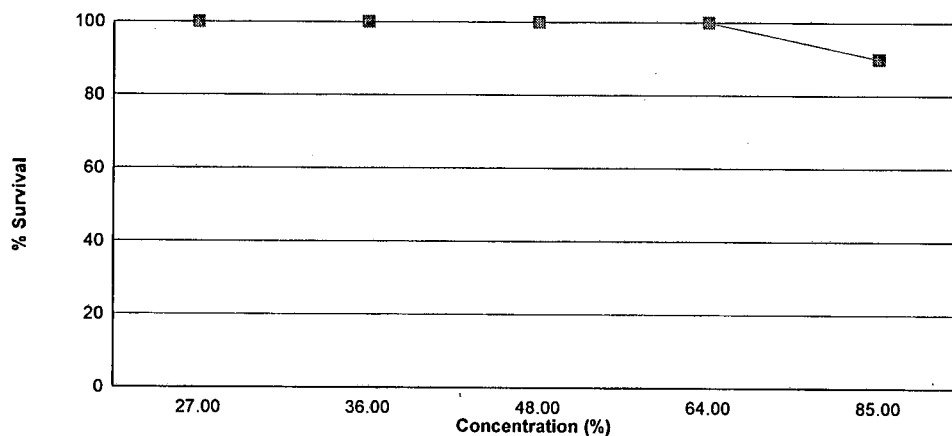
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 27 %, 36 %, 48 %, 64 %, 85 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 85 % effluent.

The test was initiated on May 8, 2012 at 1355 and continued through May 15, 2012 at 1450. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 85 % effluent
- b.) NOEC reproduction = 64 % effluent



Concentration	Percent Survival	Mean Reproduction
Control	100	23.5
27 %	100	24.2
36 %	100	24.2
48 %	100	20.8
64 %	100	20.5
85 %	90.0	17.2 *

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

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: May 8, 2012 at 1545

Date and Time Test Terminated: May 15, 2012 at 1400

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	7
	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	7	7
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
36 %	A	8	8	8	8	8	8	8
	B	8	7	7	7	7	7	7
	C	8	8	8	8	8	8	8
	D	8	7	7	7	7	7	7
	E	8	8	8	8	8	8	8
48 %	A	8	8	8	8	8	8	7
	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
64 %	A	8	8	8	8	8	7	7
	B	8	8	8	8	7	7	7
	C	8	8	8	8	8	8	8
	D	8	7	7	7	7	7	7
	E	8	8	8	8	8	8	8
85 %	A	8	8	8	8	7	6	6
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	6	6	6	6	6	6
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

 Test Initiated: May 8, 2012 at 1545
 Test Terminated: May 15, 2012 at 1400

 Drying Started: May 14, 2012 at 1515
 Drying Ended: May 16, 2012 at 1341

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.95835	.96297	0.00462	8	0.578
	B	.95783	.96222	0.00439	8	0.549
	C	.95772	.96101	0.00329	8	0.411
	D	.95526	.95983	0.00457	8	0.571
	E	.95311	.95718	0.00407	8	0.509
27 %	A	.95265	.95626	0.00361	8	0.451
	B	.95040	.95402	0.00362	8	0.452
	C	.94763	.95088	0.00325	8	0.406
	D	.94279	.94651	0.00372	8	0.465
	E	.93817	.94166	0.00349	8	0.436
36 %	A	.93495	.93849	0.00354	8	0.442
	B	.93355	.93683	0.00328	8	0.410
	C	.93538	.93907	0.00369	8	0.461
	D	.93999	.94247	0.00248	8	0.310
	E	.94211	.94543	0.00332	8	0.415
48 %	A	.94601	.94880	0.00279	8	0.349
	B	.94938	.95230	0.00292	8	0.365
	C	.95378	.95769	0.00391	8	0.489
	D	.95568	.95903	0.00335	8	0.419
	E	.95912	.96247	0.00335	8	0.419
64 %	A	.95942	.96241	0.00299	8	0.374
	B	.95823	.96109	0.00286	8	0.358
	C	.96001	.96268	0.00267	8	0.334
	D	.96016	.96324	0.00308	8	0.385
	E	.96064	.96433	0.00369	8	0.461
85 %	A	.95930	.96148	0.00218	8	0.272
	B	.95820	.96178	0.00358	8	0.448
	C	.95741	.96013	0.00272	8	0.340
	D	.95411	.95602	0.00191	8	0.239
	E	.95155	.95511	0.00356	8	0.445

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: May 8, 2012 at 1355
Date and Time Test Terminated: May 15, 2012 at 1450

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	0	10	0.00
2	0	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	0	10	0.00
4	0	4	3	3	4	2	4	4	2	4	30	10	3.00	
5	6	8	11	9	9	10	9	10	9	0	81	10	8.10	
6	10	0	0	0	0	0	0	0	0	10	20	10	2.00	
7	0	12	12	12	16	4	12	13	13	10	104	10	10.4	
8														
TOTAL	16	24	26	24	29	16	25	27	24	24	235	10	23.5	

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	0	2	4	5	4	3	2	4	4	2	30	10	3.00	
5	9	8	9	0	10	10	9	8	0	10	73	10	7.30	
6	9	0	0	11	0	0	0	0	8	0	28	10	2.80	
7	0	13	12	15	16	4	9	13	15	14	111	10	11.1	
8														
TOTAL	18	23	25	31	30	17	20	25	27	26	242	10	24.2	

Concentration: 36 %														
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	2	0	0	0	0	0	0	0	0	0	2	10	0.200	
4	0	4	4	2	3	4	0	3	2	4	26	10	2.60	
5	11	8	7	9	9	10	10	9	9	11	93	10	9.30	
6	10	0	0	0	0	0	0	0	0	0	10	10	1.00	
7	1	12	14	10	14	14	15	10	12	9	111	10	11.1	
8														
TOTAL	24	24	25	21	26	28	25	22	23	24	242	10	24.2	

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: May 8, 2012 at 1355
Date and Time Test Terminated: May 15, 2012 at 1450

Concentration: 48 %														
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	0	10	0.00
2	0	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	0	10	0.00
4	0	2	4	2	4	2	3	2	4	3	26	10	2.60	
5	8	0	10	8	11	10	8	8	0	9	72	10	7.20	
6	8	0	0	11	0	0	0	0	12	0	31	10	3.10	
7	0	6	10	0	11	11	12	10	9	10	79	10	7.90	
8														
TOTAL	16	8	24	21	26	23	23	20	25	22	208	10	20.8	

Concentration: 64 %														
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	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	2	0	0	0	0	0	0	0	0	0	2	10	0.200	
4	0	3	3	2	3	2	2	0	4	4	23	10	2.30	
5	8	8	8	0	0	8	9	8	0	0	49	10	4.90	
6	8	0	0	8	10	0	0	0	10	9	45	10	4.50	
7	0	8	11	8	11	12	7	10	10	9	86	10	8.60	
8														
TOTAL	18	19	22	18	24	22	18	18	24	22	205	10	20.5	

Concentration: 85 %														
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	X	0	0	0	0	0	9	0.00	
4	5	3	3	3	3	X	2	2	2	3	26	9	2.89	
5	0	5	9	9	10	X	4	10	9	8	64	9	7.11	
6	10	0	0	0	0	X	0	0	0	0	10	9	1.11	
7	10	4	9	10	9	X	8	6	9	7	72	9	8.00	
8														
TOTAL	25	12	21	22	22	0	14	18	20	18	172	10	17.2	

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	0.87500	1.20940
1	Control	4	1.00000	1.39310
1	Control	5	1.00000	1.39310
2	27 %	1	1.00000	1.39310
2	27 %	2	0.87500	1.20940
2	27 %	3	1.00000	1.39310
2	27 %	4	1.00000	1.39310
2	27 %	5	1.00000	1.39310
3	36 %	1	1.00000	1.39310
3	36 %	2	0.87500	1.20940
3	36 %	3	1.00000	1.39310
3	36 %	4	0.87500	1.20940
3	36 %	5	1.00000	1.39310
4	48 %	1	0.87500	1.20940
4	48 %	2	1.00000	1.39310
4	48 %	3	1.00000	1.39310
4	48 %	4	1.00000	1.39310
4	48 %	5	1.00000	1.39310
5	64 %	1	0.87500	1.20940
5	64 %	2	0.87500	1.20940
5	64 %	3	1.00000	1.39310
5	64 %	4	0.87500	1.20940
5	64 %	5	1.00000	1.39310
6	85 %	1	0.75000	1.04720
6	85 %	2	1.00000	1.39310
6	85 %	3	1.00000	1.39310
6	85 %	4	0.75000	1.04720
6	85 %	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 = 0.3056		
W = 0.8783		
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	36 %	25.00	16.00	5.00	
4	48 %	27.50	16.00	5.00	
5	64 %	22.50	16.00	5.00	
6	85 %	24.00	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
<p>D = 0.09286 W = 0.9649 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30)</p> <p>Data PASS normality test (alpha = 0.01).</p>	

Bartlett's Test for Homogeneity of Variance	No Transformation
<p>Calculated B1 statistic = 6.748 Critical B = 15.086 (alpha = 0.01, df = 5)</p> <p>Data PASS B1 homogeneity test at 0.01 level.</p>	

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	0.08992	0.01798	4.646	
Within (Error)	24	0.09288	0.00387		
Total	29	0.1828			
Critical F = 3.9 (alpha = 0.01, df = 5,24)					
2.62 (alpha = 0.05, df = 5,24)					
Since F > Critical F 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	0.5236	0.5236			
2	27 %	0.442	0.442	2.074		
3	36 %	0.4076	0.4076	2.948	*	
4	48 %	0.4082	0.4082	2.933	*	
5	64 %	0.3824	0.3824	3.589	*	
6	85 %	0.3488	0.3488	4.443	*	
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	0.09285	17.7	0.0816	
3	36 %	5	0.09285	17.7	0.116	
4	48 %	5	0.09285	17.7	0.1154	
5	64 %	5	0.09285	17.7	0.1412	
6	85 %	5	0.09285	17.7	0.1748	

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
36 %	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
48 %	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
64 %	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
85 %	9	1	10
Total	19	1	20

Critical Fisher's value (10,10,10) ($\alpha=0.05$) is 6. b value is 9. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	27 %	10	0	
2	36 %	10	0	
3	48 %	10	0	
4	64 %	10	0	
5	85 %	10	1	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Chi-Square Test for Normality	No Transformation
Chi-Square = 5.4799 Critical Chi-Square = 13.28	(alpha = 0.01, df = 4)
Data PASS normality test (alpha = 0.01).	

Kolmogorov Test for Normality	No Transformation
D = 0.1507 D* = 1.182 Critical D* = 1.035	(alpha = 0.01, N = 60)
Data FAIL normality test (alpha = 0.01).	

Steel's Many-One Rank Test					No Transformation
Ho:Control<Treatment					
Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	27 %	112.00	75.00	10.00	
3	36 %	102.50	75.00	10.00	
4	48 %	84.00	75.00	10.00	
5	64 %	79.00	75.00	10.00	
6	85 %	73.50	75.00	10.00	*

Critical values are 1 tailed (k=5)

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Dunnett's Test for PMSD Calculation (excluding deaths if applicable)

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	230.7	46.14	2.882	
Within (Error)	53	848.3	16.01		
Total	58	1079			
Critical F = 3.39 (alpha = 0.01, df = 5,53)					
2.39 (alpha = 0.05, df = 5,53)					
Since F > Critical F 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	23.5	23.5			
2	27 %	24.2	24.2	-0.3912		
3	36 %	24.2	24.2	-0.3912		
4	48 %	20.8	20.8	1.509		
5	64 %	20.5	20.5	1.677		
6	85 %	19.111	19.111	2.387	*	
Dunnett's critical value = 2.31 (1 Tailed, alpha = 0.05, df [used] = 5,40) (Actual df = 5,53)						
WARNING - Unequal replicate sizes. Critical values assuming equal replicate sizes have been used.						

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	4.134	17.6	-0.7	
3	36 %	10	4.134	17.6	-0.7	
4	48 %	10	4.134	17.6	2.7	
5	64 %	10	4.134	17.6	3	
6	85 %	9	4.247	18.1	4.389	

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: May 8, 2012 at 1811
Date and Time Test Terminated: May 15, 2012 at 1450

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.9	8.1	7.6	7.8	8.5	7.7	7.7
	Final *1	7.3	5.7	7.3	6.9	7.1	7.4	6.2
	Final *2	7.9	7.7	8.4	7.7	7.9	7.8	7.9
pH, units	Initial	7.5	7.8	7.9	8.0	7.8	7.7	7.8
	Final *1	7.5	7.1	7.5	7.4	7.3	7.6	7.4
	Final *2	8.3	7.8	8.0	8.0	8.2	8.0	8.0
Alkalinity, mg CaCO ₃ /l		31	NA	31	NA	31	NA	NA
Hardness, mg CaCO ₃ /l		41	NA	45	NA	41	NA	NA
Conductivity, umhos/cm		85	100	94	90	140	140	130
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	7.8	7.7	7.7	7.7	8.5	8.0	7.8
	Final *1	6.8	6.3	7.4	6.7	6.9	7.2	6.3
	Final *2	8.2	7.5	8.5	7.7	8.1	8.1	7.9
pH, units	Initial	7.3	7.8	7.9	7.8	7.6	7.6	7.6
	Final *1	7.5	7.1	7.5	7.4	7.4	7.6	7.5
	Final *2	8.2	8.0	8.1	7.9	8.0	7.9	8.1

Effluent Conc.: 36 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.9	7.7	7.8	7.6	8.4	7.8	7.8
	Final *1	7.6	5.5	7.5	6.8	7.1	7.2	6.7
	Final *2	8.4	7.7	8.4	8.0	8.0	8.0	7.7
pH, units	Initial	7.3	7.8	7.9	7.7	7.6	7.6	7.6
	Final *1	7.7	7.1	7.5	7.5	7.4	7.6	7.5
	Final *2	8.4	8.1	8.1	7.9	8.0	7.9	8.1

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: May 8, 2012 at 1811
Date and Time Test Terminated: May 15, 2012 at 1450

Effluent Conc.: 48 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.9	7.9	7.6	7.4	8.4	8.3	7.7
	Final *1	7.6	6.2	8.6	7.4	7.4	7.1	6.7
	Final *2	8.0	7.9	8.5	7.7	7.5	8.0	7.9
pH, units	Initial	7.3	7.8	7.9	7.6	7.6	7.6	7.6
	Final *1	7.7	7.3	7.9	7.6	7.5	7.7	7.6
	Final *2	8.3	8.1	8.1	8.0	8.0	7.9	8.1

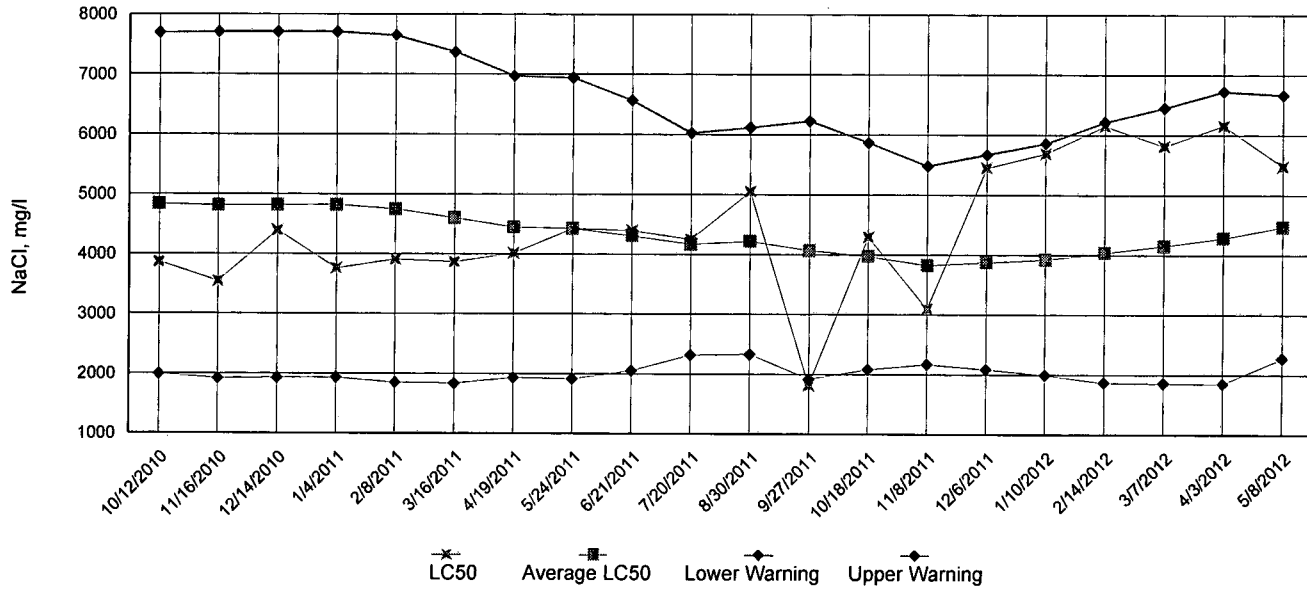
Effluent Conc.: 64 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	8.0	7.6	7.9	8.4	8.3	7.7
	Final *1	7.6	6.1	8.6	6.5	7.4	7.2	6.2
	Final *2	8.0	7.9	8.4	7.8	7.7	7.9	7.9
pH, units	Initial	7.3	7.8	7.9	7.6	7.6	7.5	7.6
	Final *1	7.7	7.2	8.0	7.5	7.5	7.7	7.5
	Final *2	8.3	8.2	8.1	8.0	8.0	8.0	8.1

Effluent Conc.: 85 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.6	7.8	7.5	8.4	8.3	7.8
	Final *1	7.4	7.9	8.9	6.6	7.0	7.2	6.2
	Final *2	7.8	8.0	8.5	7.8	7.8	7.8	7.8
pH, units	Initial	7.3	7.8	7.9	7.6	7.6	7.5	7.6
	Final *1	7.7	7.4	7.7	7.6	7.5	7.8	7.6
	Final *2	8.4	8.3	8.2	8.0	8.0	8.0	8.2
Alkalinity, mg CaCO ₃ /l	50	NA	50	NA	50	NA	NA	NA
Hardness, mg CaCO ₃ /l	95	NA	83	NA	72	NA	NA	NA
Conductivity, umhos/cm	200	240	220	220	340	340	320	
Res. Chlorine, mg/l	<0.05	NA	<0.05	NA	<0.05	NA	NA	NA

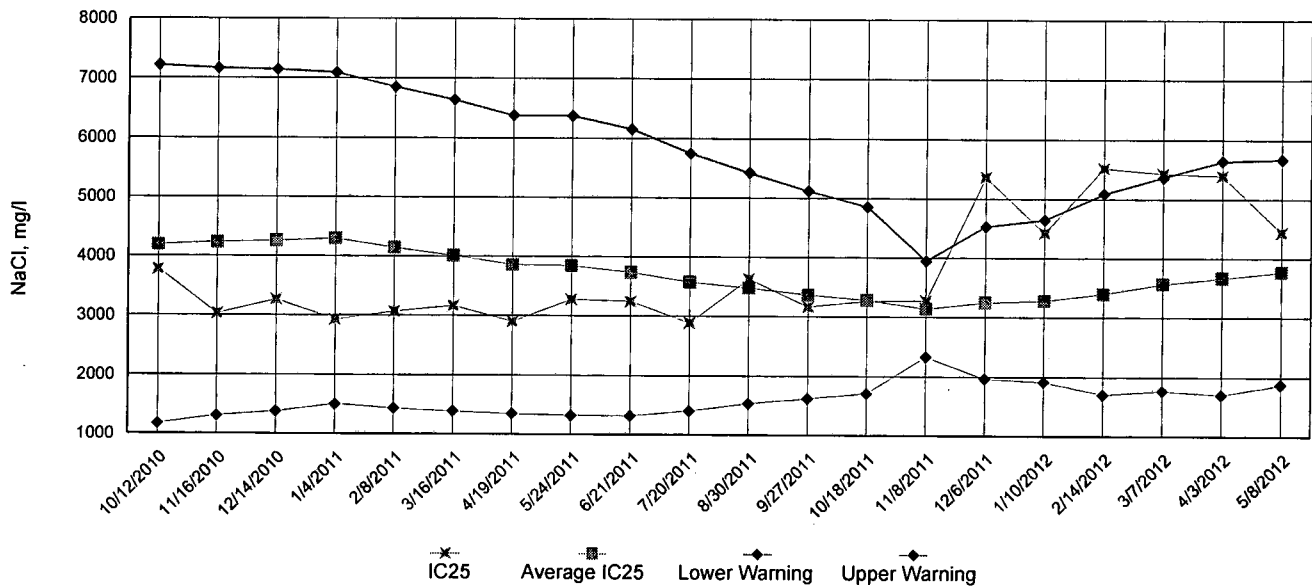
*1 = data from the *Pimephales promelas* (Fathead Minnow) test *2 = data from the *Ceriodaphnia dubia* test

Appendix A4: Test 1000.0
Chronic Reference Toxicant, *Pimephales promelas* (Fathead Minnow)

LC50 Survival Data

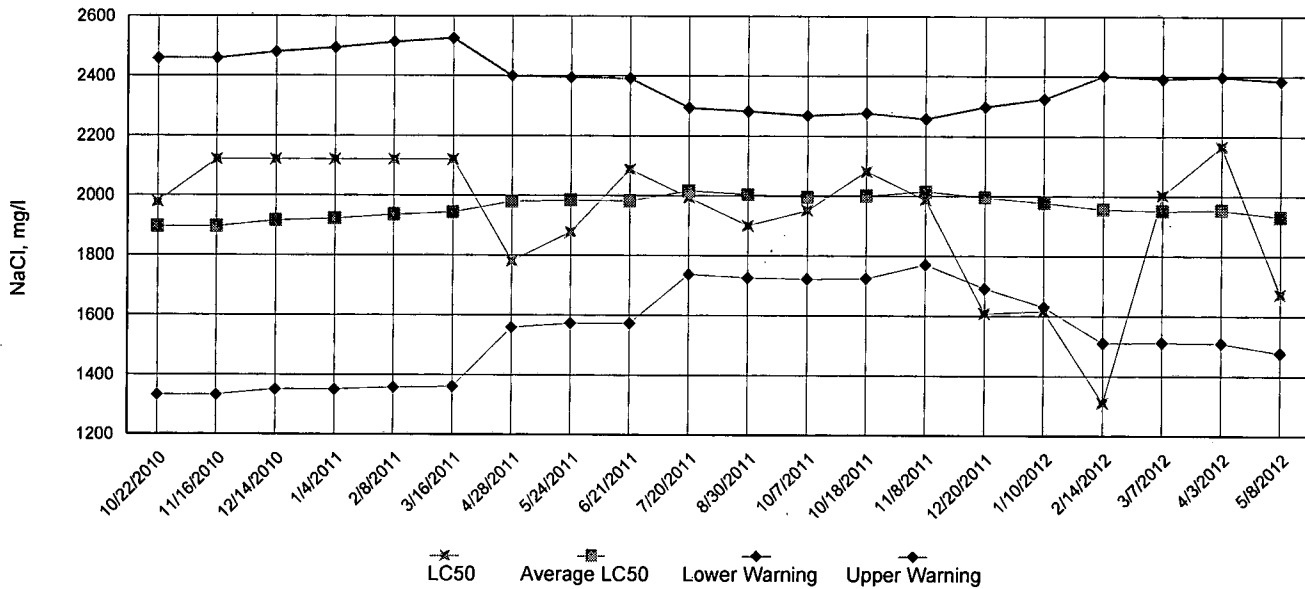


IC25 Growth Data

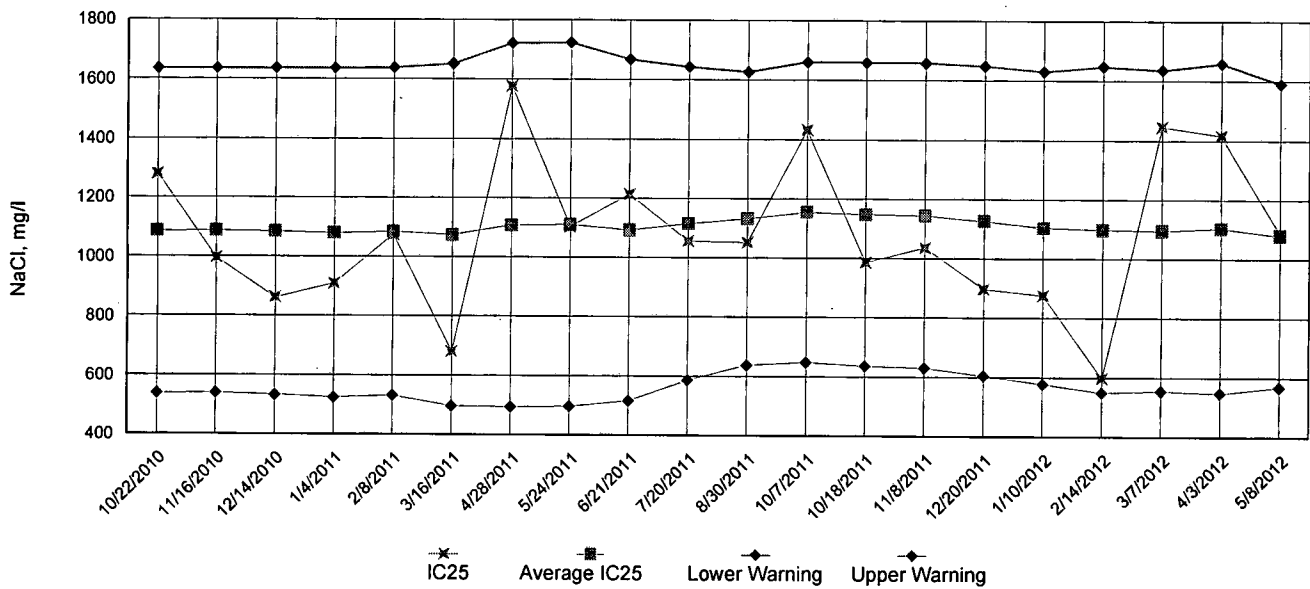


Appendix A4: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction 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: May 8, 2012 at 1545

Date and Time Test Terminated: May 15, 2012 at 1400

Dilution water used: Synthetic Laboratory Soft Water #4225

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in replicate chambers					Mean percent survival			CV%
	A	B	C	D	E	24 hr	48 hr	7 days	
Control	100	100	87.5	100	100	100	100	97.5	5.73
27 %	100	87.5	100	100	100	100	100	97.5	5.73
36 %	100	87.5	100	87.5	100	100	95.0	95.0	7.21
48 %	87.5	100	100	100	100	100	100	97.5	5.73
64 %	87.5	87.5	100	87.5	100	100	97.5	92.5	7.40
85 %	75.0	100	100	75.0	100	100	95.0	90.0	15.2

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.578	0.549	0.411	0.571	0.509	0.524	13.1
27 %	0.451	0.452	0.406	0.465	0.436	0.442	5.11
36 %	0.442	0.410	0.461	0.310	0.415	0.408	14.3
48 %	0.349	0.365	0.489	0.419	0.419	0.408	13.5
64 %	0.374	0.358	0.334	0.385	0.461	0.382	12.5
85 %	0.272	0.448	0.340	0.239	0.445	0.349	27.6

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	(85 %)	<u> </u> YES	<u> X </u> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<u> </u> YES	<u> </u> 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	(85 %)	<u> X </u> YES	<u> </u> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<u> </u> YES	<u> </u> NO

- | | | |
|--|-----------------|---------|
| 3. If you answered NO to 1.a) enter [0] otherwise enter [1]: | <u> 0 </u> | (TLP6C) |
| 4. If you answered NO to 2.a) enter [0] otherwise enter [1]: | <u> 1 </u> | (TGP6C) |
| 5. NOEC Pimephales Lethality: | <u> 85 % </u> | (TOP6C) |
| 6. LOEC Pimephales Lethality: | <u> 85 % </u> | (TXP6C) |
| 7. NOEC Pimephales Sublethality: | <u> 27 % </u> | (TPP6C) |
| 8. LOEC Pimephales Sublethality: | <u> 36 % </u> | (TYP6C) |
| 9. Coefficient of variation for Pimephales growth: | <u> 27.6 </u> | (TQP6C) |

Appendix B: Test 1000.0

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

PERMITTEE: City of Hot Springs
NPDES NO.: AR0033880 AFIN#26-00145
CONTACT: Mr. Ron Wacaster
ANALYST: 275, 280, 298, 304

2400
2400
2400

Test Initiated: DATE: May 8, 2012 TIME: 1545
Test Terminated: DATE: May 15, 2012 TIME: 1400

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.9	8.1	7.6	7.8	8.5	7.7	7.7
Final	7.3	5.7	7.3	6.9	7.1	7.4	6.2
pH Initial	7.5	7.8	7.9	8.0	7.8	7.7	7.8
Final	7.5	7.1	7.5	7.4	7.3	7.6	7.4
Alkalinity	31	NA	31	NA	31	NA	NA
Hardness	41	NA	45	NA	41	NA	NA
Conductivity	85	100	94	90	140	140	130
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 27 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.7	7.7	7.7	8.5	8.0	7.8
Final	6.8	6.3	7.4	6.7	6.9	7.2	6.3
pH Initial	7.3	7.8	7.9	7.8	7.6	7.6	7.6
Final	7.5	7.1	7.5	7.4	7.4	7.6	7.5
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	120	150	130	130	200	200	190
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 36 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.9	7.7	7.8	7.6	8.4	7.8	7.8
Final	7.6	5.5	7.5	6.8	7.1	7.2	6.7
pH Initial	7.3	7.8	7.9	7.7	7.6	7.6	7.6
Final	7.7	7.1	7.5	7.5	7.4	7.6	7.5
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	130	160	150	140	220	220	200
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 48 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.9	7.9	7.6	7.4	8.4	8.3	7.7
Final	7.6	6.2	8.6	7.4	7.4	7.1	6.7
pH Initial	7.3	7.8	7.9	7.6	7.6	7.6	7.6
Final	7.7	7.3	7.9	7.6	7.5	7.7	7.6
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	150	180	160	160	250	250	240
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 64 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	8.0	7.6	7.9	8.4	8.3	7.7
Final	7.6	6.1	8.6	6.5	7.4	7.2	6.2
pH Initial	7.3	7.8	7.9	7.6	7.6	7.5	7.6
Final	7.7	7.2	8.0	7.5	7.5	7.7	7.5
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	170	210	190	190	290	280	270
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 85 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.6	7.8	7.5	8.4	8.3	7.8
Final	7.4	7.9	8.9	6.6	7.0	7.2	6.2
pH Initial	7.3	7.8	7.9	7.6	7.6	7.5	7.6
Final	7.7	7.4	7.7	7.6	7.5	7.8	7.6
Alkalinity	50	NA	50	NA	50	NA	NA
Hardness	95	NA	83	NA	72	NA	NA
Conductivity	200	240	220	220	340	340	320
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

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: May 8, 2012 at 1355

Date and Time Test Terminated: May 15, 2012 at 1450

Dilution water used: Synthetic Laboratory Soft Water #4225

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent				
		27 %	36 %	48 %	64 %	85 %
24 hour	100	100	100	100	100	100
48 hour	100	100	100	100	100	100
7 day	100	100	100	100	100	90.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

Replicates	Control	Percent Effluent				
		27 %	36 %	48 %	64 %	85 %
A	16	18	24	16	18	25
B	24	23	24	8	19	12
C	26	25	25	24	22	21
D	24	31	21	21	18	22
E	29	30	26	26	24	22
F	16	17	28	23	22	0
G	25	20	25	23	18	14
H	27	25	22	20	18	18
I	24	27	23	25	24	20
J	24	26	24	22	22	18
Mean per Adult	23.5	24.2	24.2	20.8	20.5	17.2
Mean per Surviving Adult	23.5	24.2	24.2	20.8	20.5	19.1
CV %	18.2	19.6	8.22	25.5	12.4	21.5

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	(85 %)	<u> </u> YES	<u> X </u> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<u> </u> YES	<u> </u> NO

2. Steel's Many-One Rank Test:

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	(85 %)	<u> X </u> YES	<u> </u> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<u> </u> YES	<u> </u> NO

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 0 (TLP3B)
4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 1 (TGP3B)
5. NOEC Ceriodaphnia Lethality: 85 % (TOP3B)
6. LOEC Ceriodaphnia Lethality: 85 % (TXP3B)
7. NOEC Ceriodaphnia Sublethality: 64 % (TPP3B)
8. LOEC Ceriodaphnia Sublethality: 85 % (TYP3B)
9. Coefficient of variation for Ceriodaphnia Reproduction: 21.5 (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. Ron Wacaster
ANALYST: 275, 280, 298, 304

2400
2400
2400

Test Initiated: DATE: May 8, 2012 TIME: 1355
Test Terminated: DATE: May 15, 2012 TIME: 1450

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.9	8.1	7.6	7.8	8.5	7.7	7.7
Final	7.9	7.7	8.4	7.7	7.9	7.8	7.9
pH Initial	7.5	7.8	7.9	8.0	7.8	7.7	7.8
Final	8.3	7.8	8.0	8.0	8.2	8.0	8.0
Alkalinity	31	NA	31	NA	31	NA	NA
Hardness	41	NA	45	NA	41	NA	NA
Conductivity	85	100	94	90	140	140	130
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 27 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.7	7.7	7.7	8.5	8.0	7.8
Final	8.2	7.5	8.5	7.7	8.1	8.1	7.9
pH Initial	7.3	7.8	7.9	7.8	7.6	7.6	7.6
Final	8.2	8.0	8.1	7.9	8.0	7.9	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	120	150	130	130	200	200	190
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 36 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.9	7.7	7.8	7.6	8.4	7.8	7.8
Final	8.4	7.7	8.4	8.0	8.0	8.0	7.7
pH Initial	7.3	7.8	7.9	7.7	7.6	7.6	7.6
Final	8.4	8.1	8.1	7.9	8.0	7.9	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	130	160	150	140	220	220	200
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 48 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.9	7.9	7.6	7.4	8.4	8.3	7.7
Final	8.0	7.9	8.5	7.7	7.5	8.0	7.9
pH Initial	7.3	7.8	7.9	7.6	7.6	7.6	7.6
Final	8.3	8.1	8.1	8.0	8.0	7.9	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	150	180	160	160	250	250	240
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 64 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	8.0	7.6	7.9	8.4	8.3	7.7
Final	8.0	7.9	8.4	7.8	7.7	7.9	7.9
pH Initial	7.3	7.8	7.9	7.6	7.6	7.5	7.6
Final	8.3	8.2	8.1	8.0	8.0	8.0	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	170	210	190	190	290	280	270
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 85 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.6	7.8	7.5	8.4	8.3	7.8
Final	7.8	8.0	8.5	7.8	7.8	7.8	7.8
pH Initial	7.3	7.8	7.9	7.6	7.6	7.5	7.6
Final	8.4	8.3	8.2	8.0	8.0	8.0	8.2
Alkalinity	50	NA	50	NA	50	NA	NA
Hardness	95	NA	83	NA	72	NA	NA
Conductivity	200	240	220	220	340	340	320
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE OF

Client: <u>Hot Springs Wastewater</u>			PO No. <u>12-2517</u>		NO OF BOTTLES	ANALYSES REQUESTED ¹										AIC CONTROL NO: <u>157538</u>									
Project Reference: <u>Bio-Monitoring</u>			SAMPLE MATRIX			BIO MONITORING											AIC PROPOSAL NO:								
Project Manager: <u>JAMES SORRELLS</u>			WATER														Carrier/Tracking No. <u>Hot Springs Delivery</u>								
Sampled By: <u>H MAULDIN</u>			G	C	A	S																	Received Temperature C <u>2</u>		
AIC No.	Sample Identification	Date/Time Collected	GRA	COMP	WATER	SOIL																		Remarks	
<u>1</u>	<u>PLANT EFFLUENT</u>	<u>5/6-12</u> <u>0000-2400</u>			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>																			
Container Type			Preservative			Field pH calibration 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													
Turnaround Time Requested: (Please circle) <u>NORMAL</u> or EXPEDITED IN _____ DAYS Expedited results requested by: <u>SAME</u>					Relinquished By: <u>H Mauldin</u>					Date/Time <u>5/7/12</u> <u>0955</u>					Received By: <u>M. Mann</u>					Date/Time <u>5-7-12 9:55</u>					
Who should AIC contact with questions: <u>SAME</u>					Relinquished By: <u>M. MANN</u>					Date/Time <u>5/7-12</u> <u>1200</u>					Received in Lab By: <u>Wade Hopton</u>					Date/Time <u>5-7-12</u> <u>1200</u>					
Phone: <u>501-262-0881</u> Fax: <u>501-262-0339</u>					Comments:																				
Report Attention to:																									
Report Address to:																									



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE OF

Client: <u>Hot Springs Wastewater</u>			PO No. <u>12-2517</u>		NO OF BOTTLES	ANALYSES REQUESTED ¹										AIC CONTROL NO: <u>157538</u>				
Project Reference:			SAMPLE MATRIX			Bio Monitoring										AIC PROPOSAL NO:				
Project Manager: <u>Jim Sorrells</u>			WATER													Received Temperature C <u>22</u>				
Sampled By: <u>A. Ross</u>			G	C	A	S	3											Remarks		
AIC No.	Sample Identification	Date/Time Collected	R	O	T	E														
<u>2</u>	<u>Plant Effluent</u>	<u>5-8-12 0000-2400</u>					<u>3</u>													
Container Type			Preservative			P													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								
Turnaround Time Requested: (Please circle) <u>NORMAL</u> or EXPEDITED IN _____ DAYS						Relinquished By: <u>A. Ross</u>		Date/Time <u>5-9-12 0910</u>		Received By: <u>M. Mann</u>		Date/Time <u>5-9-12 9:10</u>								
Expedited results requested by: _____						Relinquished By: <u>M. Mann</u>		Date/Time <u>5-9-12 11:24</u>		Received in Lab By: <u>Joe P...</u>		Date/Time <u>5-9-12 11:24am</u>								
Who should AIC contact with questions: _____						Comments:														
Phone: _____ Fax: _____																				
Report Attention to: _____																				
Report Address to: _____																				

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>Hot Springs Wastewater</u>			PO No. <u>12-2517</u>		No of BOTTLES	Analyses Requested <u>Bio Monitoring</u>										AIC Control No: <u>157538</u>		
Project Reference: <u>Bio Monitoring</u>			Sample Matrix													AIC Proposal No:		
Project Manager: <u>JAMES BORRELLS</u>			WATER SOIL													Carrier: <u>Hot Springs Shuttle</u>		
Sampled By: <u>A MAULDIN</u>			G	C	BOTTLES											Received Temperature °C <u>2°C</u>		
AIC No.	Sample Identification	Date/Time Collected	R	A												Remarks		
<u>3</u>	<u>Plant Effluent</u>	<u>5-10-12</u> <u>0000-2400</u>		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<u>3</u>												
			Container Type <u>P</u>												Field pH calibration on _____ @ _____			
			Preservative <u>NO</u>												Buffer:			
			G = Glass <u>NO = none</u>		P = Plastic <u>S = Sulfuric acid pH2</u>		V = VOA vials N = Nitric acid pH2		H = HCl to pH2 B = NaOH to pH12		T = Sodium Thiosulfate Z = Zinc acetate							
Turnaround Time Requested: (Please circle) <u>NORMAL</u> or EXPEDITED IN _____ DAYS						Relinquished By: <u>A. Mauldin</u>		Date/Time <u>10:35</u> <u>5-11-12</u>		Received By: <u>M. Mann</u>		Date/Time <u>10:35</u> <u>5-11-12</u>						
Expedited results requested by: _____						Relinquished By: <u>m. mann</u>		Date/Time <u>11:30</u> <u>5-11-12</u>		Received in Lab By: <u>Joanna White</u>		Date/Time <u>5-11-12</u> <u>11:30</u>						
Who should AIC contact with questions: <u>JAMES BORRELLS</u>						Comments:												
Phone: <u>501-262-1125</u> Fax: <u>501-262-0339</u>																		
Report Attention to: <u>SAME</u>																		
Report Address to: _____																		

NON-COMPLIANCE REPORT

Arkansas Department of Environmental Quality
 NPDES Enforcement Section
 5301 Northshore Drive
 North Little Rock, AR 72118

RE: NPDES Permit No: AR0033880 Discharge Number: TX1-Q
 Facility: Hot Springs Regional WWTP
 Address: 320 Davidson Dr ~~05500~~
 City: Hot Springs State: AR Zip: 71901
 Contact: James Sorrells Phone: 501-262-1125 EXT 10

Date of Non-Compliance	Parameter Exceeded	Quantity or Loading	Quality or Concentration	Permit Limits
<u>MAY 22-2012</u>	<u>7 Day Chronic</u>	<u>Cerrodaphnia</u>	<u>306 cotech</u>	
<u>MAY 27-2012</u>	<u>7 Day Chronic</u>	<u>Pimephales P.</u>	<u>306 Lotech</u>	
<u>June 18, 2012</u>	<u>7-Day Chronic</u>	<u>Pimephales P.</u>	<u>306 Lotech</u>	

We feel this problem was due to:

i really don't know at this time -
We have cleaned everything - possibly due to
Heat - drought conditions, no rain here several months

We plan on correcting the problem in this manner:

Look at everything -

Time estimated that it will take to correct problem:

? Due to test again next week.
7-22-12

[Signature] 7-19-12

Sincerely,

[Signature]

Authorized Signature

7/19/12
 Date



July 6, 2012
Control No. 158685-1
Page 1 of 31

July 6, 2012

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

Control No. 158685-1

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 *Pimephales promelas* (Fathead minnow) and *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 1000.0 Chronic *Pimephales promelas* (Fathead minnow) Survival and Growth Test: The No Observable Effects Concentration (NOEC) for survival occurred at 85 % effluent, which is equal to the critical dilution of 85 %. The NOEC for growth occurred at 85 % effluent, which is equal to the critical dilution of 85 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the Fathead minnow test.**

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: The No Observable Effects Concentration (NOEC) for survival occurred at 85 % effluent, which is equal to the critical dilution of 85 %. The NOEC for reproduction occurred at 48 % effluent, which is below the critical dilution of 85 %. **The sample PASSED lethal effects, however, FAILED sub-lethal effects for the *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

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

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I. Control Acceptance Criteria

II. Outlined Report

III. Data Analysis

IV. Standard Reference Toxicants

V. Chemical Analysis/Quality Control

VI. Organism History

VII. Results Summary

Pimephales promelas (Fathead minnow)
Ceriodaphnia dubia

Appendix A: Raw Data

A1: Test 1000.0

Pimephales promelas (Fathead minnow) Survival and Growth

Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

A2: Statistics

A3: Water Chemistry

A4: Reference Toxicant

Appendix B: Chains of Custody

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	0.478	PASS
Control Growth CV < or = 40%	16.5	PASS
Growth Minimum Significant Difference 12 to 30%	20.0	PASS
Critical Dilution CV < or = 40%	12.2	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	21.8	PASS
Control CV < or = 40% per Surviving Female	17.8	PASS
Reproduction Minimum Significant Difference 13 to 47%	21.3	PASS
Critical Dilution CV < or = 40%	20.7	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly Test Methods 1000.0 and 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)	7.0	7.4	7.7
pH (standard units)	7.4	7.5	7.8
Alkalinity (mg/l as CaCO ₃)	57	64	67
Hardness (mg/l as CaCO ₃)	96	160	170
Conductivity (umhos/cm)	290	220	380
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	0.63	1.6

2. Dilution Water Samples: Synthetic Laboratory Soft Water #3882

- a. Dates Prepared: June 8 through June 22, 2012
- b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	5.6	7.6	7.8
pH (standard units)	7.0	7.4	7.8
Alkalinity (mg/l as CaCO ₃)	30	30	30
Hardness (mg/l as CaCO ₃)	44	46	46
Conductivity (umhos/cm)	100	83	86
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 Methods 1000.0 and 1002.0, Fathead Minnow Survival and Growth and *Ceriodaphnia dubia* Survival and Reproduction.

2. Endpoint: No Observable Effects Concentration (NOEC)

3. Test Conditions:

Pimephales promelas (Fathead minnow) Survival and Growth Method 1000.0

Date & Time Test Initiated: June 19, 2012 at 1350
Date & Time Test Terminated: June 26, 2012 at 1300
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

Ceriodaphnia dubia Survival and Growth Method 1002.0

Date & Time Test Initiated: June 19, 2012 at 1455
Date & Time Test Terminated: June 26, 2012 at 1400
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 1000.0 *Pimephales promelas*
- b. Test 1002.0 *Ceriodaphnia dubia*

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.

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. Dunnett's Test was used to calculate the PMSD.

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 June 12, 2012 at 1410 to June 19, 2012 at 1305

The results were as follows: (Control No. 158523-2.)

Survival LC-50: 6282 mg/l

Growth IC-25: 5392 mg/l

Growth PMSD: 13.5

Ceriodaphnia dubia

Chronic reference tests are performed monthly.

A chronic reference test was performed on June 12, 2012 at 1435 to June 19, 2012 at 1520

The results were as follows: (Control No. 158523-1.)

Survival LC-50: 1974 mg/l

Growth IC-25: 1542 mg/l

Growth PMSD: 25.6

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	98.9	1.86
pH	SM 4500-H+ B	101	0.268
Conductivity	EPA 120.1	97.8	1.45

VI. Organism History

Pimephales promelas (Fathead minnow)

Date: June 19, 2012

Age: <24 hours

Source: In-house culture

Water Chemistry Record:

Alkalinity: 57-64 mg/l

Hardness: 80-100 mg/l

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: June 19, 2012

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

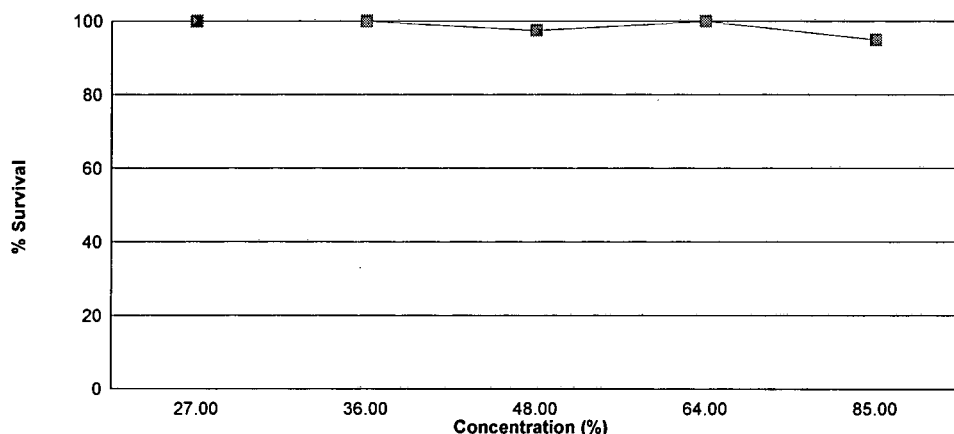
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

Effluent dilutions for this test were 27 %, 36 %, 48 %, 64 %, 85 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 85 % effluent.

The test was initiated on June 19, 2012 at 1350 and continued through June 26, 2012 at 1300. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 85 % effluent
- b.) NOEC growth = 85 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.478
27 %	100	0.505
36 %	100	0.497
48 %	97.5	0.470
64 %	100	0.462
85 %	95.0	0.416

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

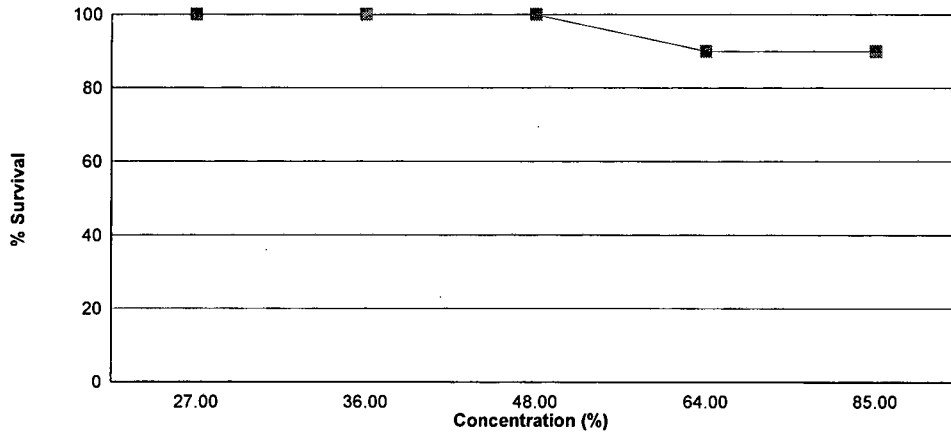
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 27 %, 36 %, 48 %, 64 %, 85 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 85 % effluent.

The test was initiated on June 19, 2012 at 1455 and continued through June 26, 2012 at 1400. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 85 % effluent
- b.) NOEC reproduction = 48 % effluent



Concentration	Percent Survival	Mean Reproduction
Control	100	21.8
27 %	100	19.9
36 %	100	17.6
48 %	100	17.8
64 %	90.0	16.7 *
85 %	90.0	15.7 *

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

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: June 19, 2012 at 1350

Date and Time Test Terminated: June 26, 2012 at 1300

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
36 %	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
48 %	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	7	7	7	7	7	7	7
64 %	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
85 %	A	8	8	8	8	8	8	8
	B	8	7	7	6	6	6	6
	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: June 19, 2012 at 1350
Test Terminated: June 26, 2012 at 1300

Drying Started: June 25, 2012 at 1229
Drying Ended: June 27, 2012 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	.93215	.93575	0.00360	8	0.450
	B	.93245	.93732	0.00487	8	0.609
	C	.94305	.94658	0.00353	8	0.441
	D	.93408	.93797	0.00389	8	0.486
	E	.94448	.94771	0.00323	8	0.404
27 %	A	.93174	.93472	0.00298	8	0.372
	B	.93281	.93732	0.00451	8	0.564
	C	.93405	.93811	0.00406	8	0.508
	D	.93371	.93787	0.00416	8	0.520
	E	.93354	.93804	0.00450	8	0.562
36 %	A	.93253	.93685	0.00432	8	0.540
	B	.93162	.93618	0.00456	8	0.570
	C	.93642	.94061	0.00419	8	0.524
	D	.93624	.93946	0.00322	8	0.402
	E	.93293	.93654	0.00361	8	0.451
48 %	A	.93300	.93680	0.00380	8	0.475
	B	.93114	.93492	0.00378	8	0.472
	C	.93405	.93775	0.00370	8	0.462
	D	.93130	.93555	0.00425	8	0.531
	E	.93131	.93461	0.00330	8	0.412
64 %	A	.93243	.93612	0.00369	8	0.461
	B	.93327	.93717	0.00390	8	0.488
	C	.93362	.93787	0.00425	8	0.531
	D	.93494	.93857	0.00363	8	0.454
	E	.93435	.93737	0.00302	8	0.378
85 %	A	.93495	.93834	0.00339	8	0.424
	B	.93469	.93735	0.00266	8	0.332
	C	.93440	.93789	0.00349	8	0.436
	D	.93548	.93923	0.00375	8	0.469
	E	.93137	.93472	0.00335	8	0.419

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: June 19, 2012 at 1455
Date and Time Test Terminated: June 26, 2012 at 1400

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	0	10	0.00
2	0	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	0	10	0.00
4	0	5	4	0	5	4	2	3	0	2	25	10	2.50	
5	6	0	0	3	1	0	8	6	5	7	36	10	3.60	
6	8	10	10	11	9	7	0	0	8	0	63	10	6.30	
7	9	11	12	0	11	12	10	10	9	10	94	10	9.40	
8														
TOTAL	23	26	26	14	26	23	20	19	22	19	218	10	21.8	

Concentration: 27 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	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	0	5	0	3	5	4	2	1	2	26	10	2.60
5	7	4	0	5	0	0	9	5	7	6	43	10	4.30
6	0	8	7	8	6	8	0	0	0	0	37	10	3.70
7	8	2	14	0	13	12	12	10	10	12	93	10	9.30
8													
TOTAL	19	14	26	13	22	25	25	17	18	20	199	10	19.9

Concentration: 36 %													
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	0	0	2	4	4	3	2	0	2	20	10	2.00
5	5	4	3	6	6	0	8	5	4	5	46	10	4.60
6	0	7	7	1	0	11	0	1	7	0	34	10	3.40
7	12	2	0	10	10	10	11	11	1	9	76	10	7.60
8													
TOTAL	20	13	10	19	20	25	22	19	12	16	176	10	17.6

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: June 19, 2012 at 1455

Date and Time Test Terminated: June 26, 2012 at 1400

Concentration: 48 %														
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	0	10	0.00
2	0	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	0	10	0.00
4	4	0	0	0	4	3	0	4	2	0	17	10	1.70	
5	0	6	5	4	0	0	6	7	6	6	40	10	4.00	
6	10	9	7	7	8	8	8	0	0	8	65	10	6.50	
7	10	0	0	0	12	13	0	11	10	0	56	10	5.60	
8														
TOTAL	24	15	12	11	24	24	14	22	18	14	178	10	17.8	

Concentration: 64 %														
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	0	0	0	4	2	4	2	0	2	18	10	1.80	
5	6	5	6	6	0	6	0	8	4	6	47	10	4.70	
6	0	8	5X	9	9	0	8	0	0	0	39	9	4.33	
7	10	0	X	1	10	10	0	11	10	11	63	9	7.00	
8														
TOTAL	20	13	11	16	23	18	12	21	14	19	167	10	16.7	

Concentration: 85 %														
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	X	0	0	0	0	0	0	0	0	9	0.00	
4	3	2	X	0	2	4	2	2	2	2	19	9	2.11	
5	6	1	X	6	4	0	7	6	6	6	42	9	4.67	
6	0	8	X	8	8	10	0	0	0	0	34	9	3.78	
7	9	0	X	0	1	9	11	10	10	12	62	9	6.89	
8														
TOTAL	18	11	0	14	15	23	20	18	18	20	157	10	15.7	

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	36 %	1	1.00000	1.39310
3	36 %	2	1.00000	1.39310
3	36 %	3	1.00000	1.39310
3	36 %	4	1.00000	1.39310
3	36 %	5	1.00000	1.39310
4	48 %	1	1.00000	1.39310
4	48 %	2	1.00000	1.39310
4	48 %	3	1.00000	1.39310
4	48 %	4	1.00000	1.39310
4	48 %	5	0.87500	1.20940
5	64 %	1	1.00000	1.39310
5	64 %	2	1.00000	1.39310
5	64 %	3	1.00000	1.39310
5	64 %	4	1.00000	1.39310
5	64 %	5	1.00000	1.39310
6	85 %	1	1.00000	1.39310
6	85 %	2	0.75000	1.04720
6	85 %	3	1.00000	1.39310
6	85 %	4	1.00000	1.39310
6	85 %	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))
<p>D = 0.1227 W = 0.5739 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30)</p> <p>Data FAIL normality test (alpha = 0.01).</p>		

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	36 %	27.50	16.00	5.00	
4	48 %	25.00	16.00	5.00	
5	64 %	27.50	16.00	5.00	
6	85 %	25.00	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
<p>D = 0.09863 W = 0.9753 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30)</p> <p>Data PASS normality test (alpha = 0.01).</p>	

Bartlett's Test for Homogeneity of Variance	No Transformation
<p>Calculated B1 statistic = 2.171 Critical B = 15.086 (alpha = 0.01, df = 5)</p> <p>Data PASS B1 homogeneity test at 0.01 level.</p>	

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	0.02506	0.005012	1.219	
Within (Error)	24	0.09864	0.00411		
Total	29	0.1237			
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	0.478	0.478			
2	27 %	0.5052	0.5052	-0.6708		
3	36 %	0.4974	0.4974	-0.4785		
4	48 %	0.4704	0.4704	0.1874		
5	64 %	0.4624	0.4624	0.3847		
6	85 %	0.416	0.416	1.529		
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	0.09569	20	-0.0272	
3	36 %	5	0.09569	20	-0.0194	
4	48 %	5	0.09569	20	0.0076	
5	64 %	5	0.09569	20	0.0156	
6	85 %	5	0.09569	20	0.062	

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
36 %	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
48 %	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
64 %	9	1	10
Total	19	1	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 9. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
85 %	9	1	10
Total	19	1	20

Critical Fisher's value (10,10,10) ($\alpha=0.05$) is 6. b value is 9. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	27 %	10	0	
2	36 %	10	0	
3	48 %	10	0	
4	64 %	10	1	
5	85 %	10	1	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Chi-Square Test for Normality	No Transformation
Chi-Square = 3.2187 Critical Chi-Square = 13.28 (alpha = 0.01, df = 4) Data PASS normality test (alpha = 0.01).	

Kolmogorov Test for Normality	No Transformation
D = 0.0987 D* = 0.7744 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 = 3.108 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	248.6	49.72	2.055	
Within (Error)	54	1306	24.19		
Total	59	1555			
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	21.8	21.8			
2	27 %	19.9	19.9	0.8638		
3	36 %	17.6	17.6	1.909		
4	48 %	17.8	17.8	1.819		
5	64 %	16.7	16.7	2.319	*	
6	85 %	15.7	15.7	2.773	*	
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	5.081	23.3	1.9	
3	36 %	10	5.081	23.3	4.2	
4	48 %	10	5.081	23.3	4	
5	64 %	10	5.081	23.3	5.1	
6	85 %	10	5.081	23.3	6.1	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Dunnett's Test for PMSD Calculation (excluding deaths if applicable)

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	161.7	32.34	1.688	
Within (Error)	52	996.3	19.16		
Total	57	1158			
Critical F = 3.39 (alpha = 0.01, df = 5,52) 2.39 (alpha = 0.05, df = 5,52)					
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	21.8	21.8			
2	27 %	19.9	19.9	0.9706		
3	36 %	17.6	17.6	2.146		
4	48 %	17.8	17.8	2.043		
5	64 %	17.333	17.333	2.221		
6	85 %	17.444	17.444	2.166		
Dunnett's critical value = 2.31 (1 Tailed, alpha = 0.05, df [used] = 5,40) (Actual df = 5,52) WARNING - Unequal replicate sizes. Critical values assuming equal replicate sizes have been used.						

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	4.522	20.7	1.9	
3	36 %	10	4.522	20.7	4.2	
4	48 %	10	4.522	20.7	4	
5	64 %	9	4.646	21.3	4.467	
6	85 %	9	4.646	21.3	4.356	

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: June 19, 2012 at 0819

Date and Time Test Terminated: June 26, 2012 at 1400

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	5.6	7.6	7.6	7.7	7.8	7.2	7.5
	Final *1	5.8	6.7	7.0	6.9	6.6	6.9	5.9
	Final *2	7.5	7.6	8.0	8.1	8.2	7.6	7.3
pH, units	Initial	7.0	7.9	7.4	7.8	7.8	7.4	7.9
	Final *1	7.5	7.4	7.6	7.6	7.7	7.4	7.2
	Final *2	7.4	7.6	8.2	8.2	8.0	8.1	7.8
Alkalinity, mg CaCO ₃ /l		30	NA	30	NA	30	NA	NA
Hardness, mg CaCO ₃ /l		44	NA	46	NA	46	NA	NA
Conductivity, umhos/cm		100	110	83	110	86	120	120
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	5.5	7.5	7.8	7.7	7.7	7.2	7.4
	Final *1	5.5	6.0	6.4	6.8	6.5	6.9	5.7
	Final *2	7.6	8.0	7.9	8.1	8.2	7.9	7.4
pH, units	Initial	7.2	7.8	7.5	7.8	7.8	7.8	7.8
	Final *1	7.4	7.3	7.5	7.6	7.7	7.5	7.4
	Final *2	7.5	7.9	8.3	8.3	8.2	8.2	7.8

Effluent Conc.: 36 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	5.5	7.6	7.5	7.6	7.5	7.2	7.7
	Final *1	5.6	6.3	6.4	6.3	6.4	6.7	6.1
	Final *2	7.6	8.0	7.8	8.1	7.9	7.8	7.4
pH, units	Initial	7.3	7.8	7.5	7.8	7.8	7.9	7.9
	Final *1	7.5	7.3	7.5	7.5	7.7	7.6	7.4
	Final *2	7.5	8.0	8.3	8.3	8.3	8.2	7.9

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: June 19, 2012 at 0819

Date and Time Test Terminated: June 26, 2012 at 1400

Effluent Conc.: 48 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	6.4	7.4	7.5	7.7	7.6	7.4	7.6
	Final *1	5.8	5.9	6.8	6.8	6.8	6.7	6.5
	Final *2	7.5	7.9	8.1	8.0	8.2	7.9	7.5
pH, units	Initial	7.3	7.8	7.5	7.8	7.8	8.0	7.9
	Final *1	7.5	7.2	7.5	7.6	7.7	7.6	7.7
	Final *2	7.6	7.9	8.4	8.4	8.4	8.3	8.0

Effluent Conc.: 64 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	6.9	7.4	7.6	7.6	7.6	7.2	7.2
	Final *1	6.1	6.0	6.7	6.5	6.6	6.8	6.5
	Final *2	7.5	8.0	8.1	8.0	8.3	7.8	7.4
pH, units	Initial	7.4	7.8	7.5	7.8	7.8	8.2	8.0
	Final *1	7.6	7.2	7.5	7.6	7.7	7.7	7.7
	Final *2	7.6	8.0	8.4	8.5	8.5	8.4	8.2

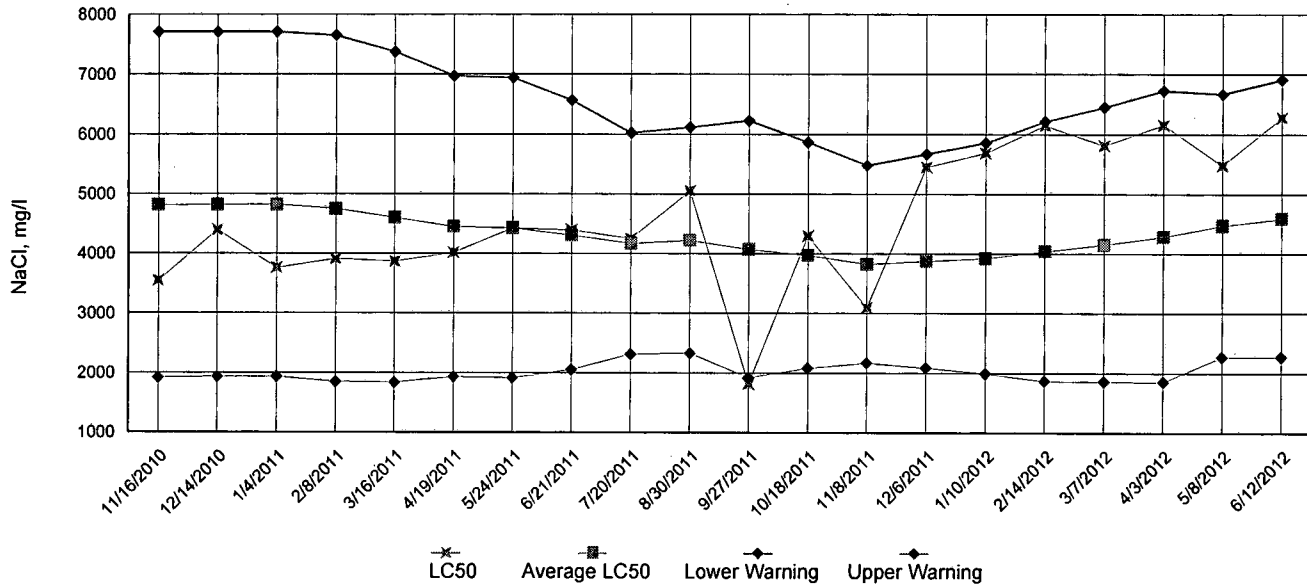
Effluent Conc.: 85 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.1	7.2	7.6	7.7	7.6	7.3	7.6
	Final *1	6.1	6.5	6.6	6.8	6.7	6.7	6.4
	Final *2	7.6	7.9	8.1	8.1	8.3	8.0	7.2
pH, units	Initial	7.4	7.8	7.6	7.9	7.9	8.2	8.0
	Final *1	7.6	7.3	7.5	7.7	7.8	7.7	7.6
	Final *2	7.7	8.1	8.4	8.5	8.5	8.4	8.3
Alkalinity, mg CaCO ₃ /l	55	NA	62	NA	65	NA	NA	
Hardness, mg CaCO ₃ /l	74	NA	140	NA	160	NA	NA	
Conductivity, umhos/cm	250	260	190	320	300	400	400	
Res. Chlorine, mg/l	<0.05	NA	<0.05	NA	<0.05	NA	NA	

*1 = data from the *Pimephales promelas* (Fathead Minnow) test

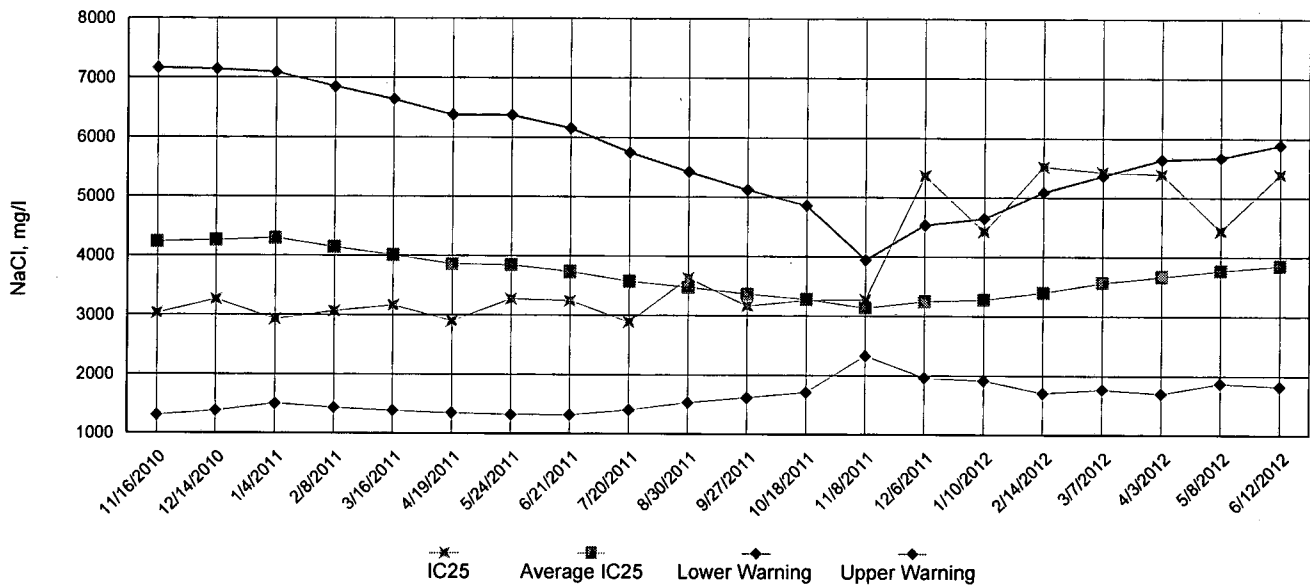
*2 = data from the *Ceriodaphnia dubia* test

Appendix A4: Test 1000.0
Chronic Reference Toxicant, *Pimephales promelas* (Fathead Minnow)

LC50 Survival Data

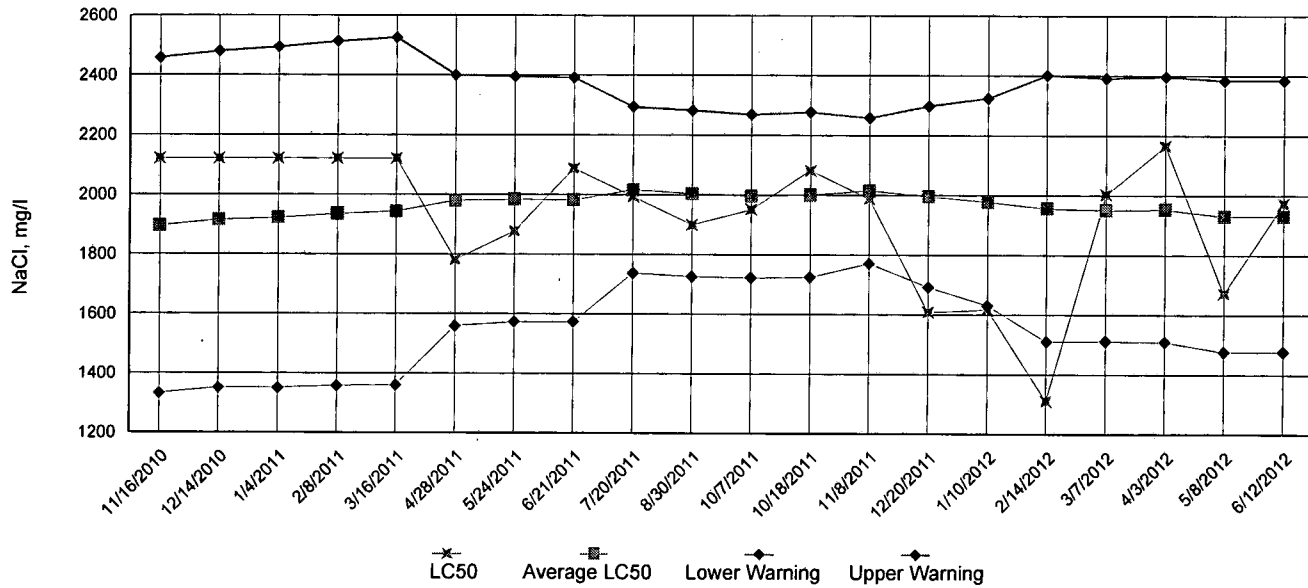


IC25 Growth Data

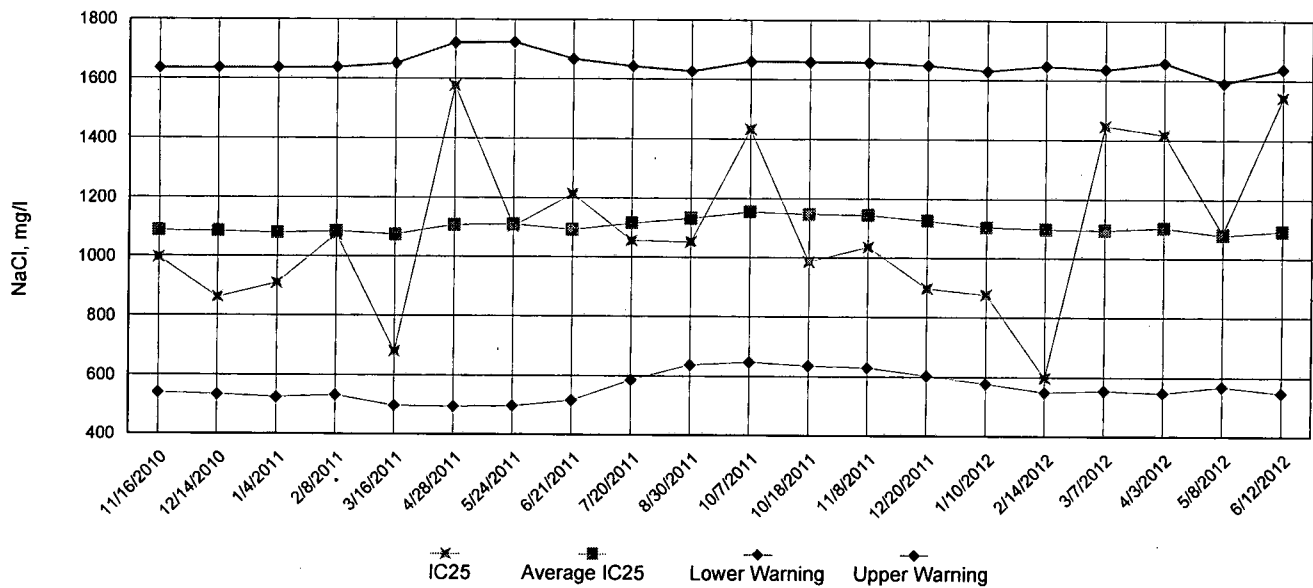


Appendix A4: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction 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: June 19, 2012 at 1350

Date and Time Test Terminated: June 26, 2012 at 1300

Dilution water used: Synthetic Laboratory Soft Water #3882

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in replicate chambers					Mean percent survival			CV%
	A	B	C	D	E	24 hr	48 hr	7 days	
Control	100	100	100	100	100	100	100	100	0.00
27 %	100	100	100	100	100	100	100	100	0.00
36 %	100	100	100	100	100	100	100	100	0.00
48 %	100	100	100	100	87.5	97.5	97.5	97.5	5.73
64 %	100	100	100	100	100	100	100	100	0.00
85 %	100	75.0	100	100	100	100	97.5	95.0	11.8

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.450	0.609	0.441	0.486	0.404	0.478	16.5
27 %	0.372	0.564	0.508	0.520	0.562	0.505	15.5
36 %	0.540	0.570	0.524	0.402	0.451	0.497	13.9
48 %	0.475	0.472	0.462	0.531	0.412	0.47	9.00
64 %	0.461	0.488	0.531	0.454	0.378	0.462	12.1
85 %	0.424	0.332	0.436	0.469	0.419	0.416	12.2

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	(85 %)	_____ 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	(85 %)	_____ 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]: | <u> 0 </u> | (TLP6C) |
| 4. If you answered NO to 2.a) enter [0] otherwise enter [1]: | <u> 0 </u> | (TGP6C) |
| 5. NOEC Pimephales Lethality: | <u> 85 % </u> | (TOP6C) |
| 6. LOEC Pimephales Lethality: | <u> 85 % </u> | (TXP6C) |
| 7. NOEC Pimephales Sublethality: | <u> 85 % </u> | (TPP6C) |
| 8. LOEC Pimephales Sublethality: | <u> 85 % </u> | (TYP6C) |
| 9. Coefficient of variation for Pimephales growth: | <u> 16.5 </u> | (TQP6C) |

Appendix B: Test 1000.0

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

PERMITTEE: City of Hot Springs
NPDES NO.: AR0033880 AFIN#26-00145
CONTACT: Mr. James Sorrells
ANALYST: 275, 280, 298, 304

2400
2400
2400

Test Initiated: DATE: June 19, 2012 TIME: 1350
Test Terminated: DATE: June 26, 2012 TIME: 1300

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	5.6	7.6	7.6	7.7	7.8	7.2	7.5
Final	5.8	6.7	7.0	6.9	6.6	6.9	5.9
pH Initial	7.0	7.9	7.4	7.8	7.8	7.4	7.9
Final	7.5	7.4	7.6	7.6	7.7	7.4	7.2
Alkalinity	30	NA	30	NA	30	NA	NA
Hardness	44	NA	46	NA	46	NA	NA
Conductivity	100	110	83	110	86	120	120
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 27 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	5.5	7.5	7.8	7.7	7.7	7.2	7.4
Final	5.5	6.0	6.4	6.8	6.5	6.9	5.7
pH Initial	7.2	7.8	7.5	7.8	7.8	7.8	7.8
Final	7.4	7.3	7.5	7.6	7.7	7.5	7.4
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	150	160	120	170	160	210	200
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 36 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	5.5	7.6	7.5	7.6	7.5	7.2	7.7
Final	5.6	6.3	6.4	6.3	6.4	6.7	6.1
pH Initial	7.3	7.8	7.5	7.8	7.8	7.9	7.9
Final	7.5	7.3	7.5	7.5	7.7	7.6	7.4
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	160	180	130	200	180	250	230
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 48 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.4	7.4	7.5	7.7	7.6	7.4	7.6
Final	5.8	5.9	6.8	6.8	6.8	6.7	6.5
pH Initial	7.3	7.8	7.5	7.8	7.8	8.0	7.9
Final	7.5	7.2	7.5	7.6	7.7	7.6	7.7
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	190	200	150	220	210	280	280
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 64 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.9	7.4	7.6	7.6	7.6	7.2	7.2
Final	6.1	6.0	6.7	6.5	6.6	6.8	6.5
pH Initial	7.4	7.8	7.5	7.8	7.8	8.2	8.0
Final	7.6	7.2	7.5	7.6	7.7	7.7	7.7
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	220	170	260	250	340	330
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 85 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.1	7.2	7.6	7.7	7.6	7.3	7.6
Final	6.1	6.5	6.6	6.8	6.7	6.7	6.4
pH Initial	7.4	7.8	7.6	7.9	7.9	8.2	8.0
Final	7.6	7.3	7.5	7.7	7.8	7.7	7.6
Alkalinity	55	NA	62	NA	65	NA	NA
Hardness	74	NA	140	NA	160	NA	NA
Conductivity	250	260	190	320	300	400	400
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

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: June 19, 2012 at 1455

Date and Time Test Terminated: June 26, 2012 at 1400

Dilution water used: Synthetic Laboratory Soft Water #3882

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent				
		27 %	36 %	48 %	64 %	85 %
24 hour	100	100	100	100	100	100
48 hour	100	100	100	100	100	100
7 day	100	100	100	100	90.0	90.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

Replicates	Control	Percent Effluent				
		27 %	36 %	48 %	64 %	85 %
A	23	19	20	24	20	18
B	26	14	13	15	13	11
C	26	26	10	12	11	0
D	14	13	19	11	16	14
E	26	22	20	24	23	15
F	23	25	25	24	18	23
G	20	25	22	14	12	20
H	19	17	19	22	21	18
I	22	18	12	18	14	18
J	19	20	16	14	19	20
Mean per Adult	21.8	19.9	17.6	17.8	16.7	15.7
Mean per Surviving Adult	21.8	19.9	17.6	17.8	17.3	17.4
CV %	17.8	23.0	26.9	29.6	22.0	20.7

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	(85 %)	<input type="checkbox"/> YES	<input checked="" type="checkbox"/> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<input type="checkbox"/> YES	<input type="checkbox"/> NO

2. Dunnett's Test:

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	(85 %)	<input checked="" type="checkbox"/> YES	<input type="checkbox"/> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<input type="checkbox"/> YES	<input type="checkbox"/> NO

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 0 (TLP3B)
4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 1 (TGP3B)
5. NOEC *Ceriodaphnia* Lethality: 85 % (TOP3B)
6. LOEC *Ceriodaphnia* Lethality: 85 % (TXP3B)
7. NOEC *Ceriodaphnia* Sublethality: 48 % (TPP3B)
8. LOEC *Ceriodaphnia* Sublethality: 64 % (TYP3B)
9. Coefficient of variation for *Ceriodaphnia* Reproduction: 20.7 (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: 275, 280, 298, 304

2400
2400
2400

Test Initiated: DATE: June 19, 2012 TIME: 1455
Test Terminated: DATE: June 26, 2012 TIME: 1400

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	5.6	7.6	7.6	7.7	7.8	7.2	7.5
Final	7.5	7.6	8.0	8.1	8.2	7.6	7.3
pH Initial	7.0	7.9	7.4	7.8	7.8	7.4	7.9
Final	7.4	7.6	8.2	8.2	8.0	8.1	7.8
Alkalinity	30	NA	30	NA	30	NA	NA
Hardness	44	NA	46	NA	46	NA	NA
Conductivity	100	110	83	110	86	120	120
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 27 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	5.5	7.5	7.8	7.7	7.7	7.2	7.4
Final	7.6	8.0	7.9	8.1	8.2	7.9	7.4
pH Initial	7.2	7.8	7.5	7.8	7.8	7.8	7.8
Final	7.5	7.9	8.3	8.3	8.2	8.2	7.8
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	150	160	120	170	160	210	200
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 36 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	5.5	7.6	7.5	7.6	7.5	7.2	7.7
Final	7.6	8.0	7.8	8.1	7.9	7.8	7.4
pH Initial	7.3	7.8	7.5	7.8	7.8	7.9	7.9
Final	7.5	8.0	8.3	8.3	8.3	8.2	7.9
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	160	180	130	200	180	250	230
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 48 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.4	7.4	7.5	7.7	7.6	7.4	7.6
Final	7.5	7.9	8.1	8.0	8.2	7.9	7.5
pH Initial	7.3	7.8	7.5	7.8	7.8	8.0	7.9
Final	7.6	7.9	8.4	8.4	8.4	8.3	8.0
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	190	200	150	220	210	280	280
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 64 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.9	7.4	7.6	7.6	7.6	7.2	7.2
Final	7.5	8.0	8.1	8.0	8.3	7.8	7.4
pH Initial	7.4	7.8	7.5	7.8	7.8	8.2	8.0
Final	7.6	8.0	8.4	8.5	8.5	8.4	8.2
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	220	170	260	250	340	330
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 85 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.1	7.2	7.6	7.7	7.6	7.3	7.6
Final	7.6	7.9	8.1	8.1	8.3	8.0	7.2
pH Initial	7.4	7.8	7.6	7.9	7.9	8.2	8.0
Final	7.7	8.1	8.4	8.5	8.5	8.4	8.3
Alkalinity	55	NA	62	NA	65	NA	NA
Hardness	74	NA	140	NA	160	NA	NA
Conductivity	250	260	190	320	300	400	400
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <i>Hot Springs Waste water</i>			PO No. <i>12-2862</i>		No of BOTTLES	Analyses Requested <i>Bio Monitoring</i>										AIC Control No: <i>158685</i>			
Project Reference:			Sample Matrix													AIC Proposal No:			
Project Manager: <i>Jim Sorrells</i>			WATER													Carrier:			
Sampled By: <i>A. Ross</i>			G R A B	C O M P	A T E R	S O I L	No of BOTTLES											Received Temperature °C <i>20C</i>	
AIC No.	Sample Identification	Date/Time Collected																Remarks	
<i>(1)</i>	<i>Plant Effluent</i>	<i>6-17-12 0000-2400</i>		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<i>3</i>	<input checked="" type="checkbox"/>												
															Field pH calibration				
			Container Type			<i>P</i>						on _____ @ _____							
			Preservative			<i>NO</i>						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				
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS						Relinquished By: <i>A. Ross</i>		Date/Time <i>6-18-12 10:10</i>		Received By: <i>m. mann</i>		Date/Time <i>6-18-12 10:10</i>							
Expedited results requested by: _____						Relinquished By: <i>m. mann</i>		Date/Time <i>6-18-12 12:00</i>		Received in Lab By: <i>[Signature]</i>		Date/Time <i>6-18-12 12:00 P</i>							
Who should AIC contact with questions: _____						Comments:													
Phone: _____ Fax: _____																			
Report Attention to: _____																			
Report Address to: _____																			

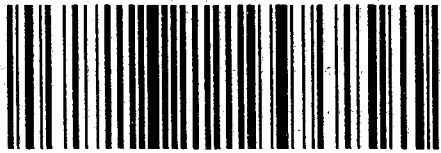
CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>Hot Springs Wastewater</u>			PO No. <u>12-2862</u>		No of BOTTLES	Analyses Requested <u>Bio-Monitoring</u>										AIC Control No: <u>158685</u>					
Project Reference:			Sample Matrix													AIC Proposal No:					
Project Manager: <u>Jim Sorrells</u>			WATER	SOIL												Carrier: <u>Hot Springs Delivery</u>					
By: <u>A. Ross</u>																Received Temperature °C <u>2</u>					
AIC No.	Sample Identification	Date/Time Collected	G R A B	C O M P	W A T E R	S O I L	No of BOTTLES											Remarks			
<u>2</u>	<u>Plant Effluent</u>	<u>6-19-12 0000-2400</u>		✓	✓		3														
			Container Type				P											Field pH calibration on _____ @ _____			
			Preservative				NO											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										
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS							Relinquished By: <u>A. Hammond</u>		Date/Time <u>6-20-12 @ 0955</u>		Received By: <u>M. Mann</u>		Date/Time <u>6-20-12 9:55AM</u>								
Expedited results requested by: _____							Relinquished By: <u>M. Mann</u>		Date/Time <u>6-20-12 @ 10:50AM</u>		Received in Lab By: <u>Lugge Heston</u>		Date/Time <u>6-20-12 1050</u>								
Who should AIC contact with questions: _____							Comments:														
Phone: _____ Fax: _____																					
Report Attention to: _____																					
Report Address to: _____																					

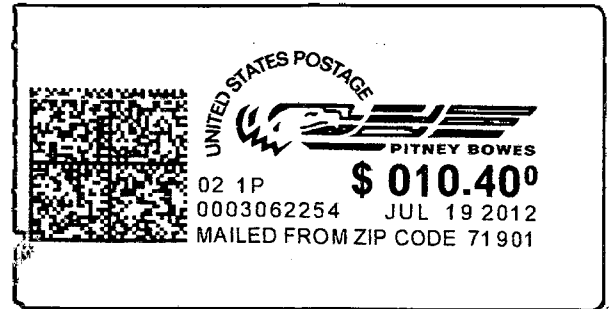
CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>Hot Springs Wastewater</u>		PO No: <u>12-2862</u>		No of		Bio Monitoring						A/C Control No: <u>158685</u>	
Project Reference:		Sample Matrix		BOTTLES								A/C Proposal No:	
Project Manager: <u>Jim Sorcells</u>		WATER		SOIL								Carrier: <u>Hot Springs Delivery</u>	
Sampled By: <u>AT</u>		G R A B		C O M P								Received Temperature °C <u>2</u>	
AIC No. <u>3</u>		Date/Time Collected <u>6-21-12 @ 0000-2400</u>		Container Type <u>P</u>								Preservative <u>N</u>	
Sample Identification <u>Plant Effluent</u>		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			
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN <u> </u> DAYS				Relinquished By: <u>J. Johnson</u>		Date/Time <u>6-22-12 @ 0900</u>		Received By: <u>A. Mann</u>		Date/Time <u>6-22-12 9:00</u>			
Expedited results requested by: <u>Same</u>				Relinquished By: <u>w. mann</u>		Date/Time <u>6-22 @ 11:10</u>		Received in Lab By: <u>Luzer Hyden</u>		Date/Time <u>6-22-12 1110</u>			
Who should AIC contact with questions: <u>J. Sorcells</u>				Comments:									
Phone: <u>501-262-1125</u> Fax: <u>501-262-0339</u>													
Report Attention to:													
Report Address to:													

CERTIFIED MAIL™



7010 0290 0000 5195 6506



City of Hot Springs Municipal Utilities
780 Adams St.
Hot Springs, AR 71901

ADEQ
NPDES Enforcement Section
Attn: Mo Shafii
5301 Northshore Dr.
No. Little Rock, AR 72118-5317

