

June 20, 2013

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

Control No. 168112-1

Prepared for:

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

Prepared by:

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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:

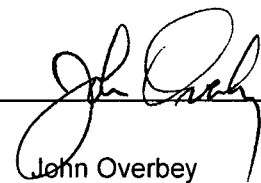
This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire 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 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.**

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: The No Observable Effects Concentration (NOEC) for survival occurred at 87 % effluent, which is above the critical dilution of 65 %. Any statistical difference with sublethal effects cannot be considered toxic due to the minimum significant difference (PMSD) calculated result being below the lower PMSD bounds. **The sample, therefore PASSED both lethal and sub-lethal effects for the *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION



John Overbey
Laboratory Director

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Table of Contents

- 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%	95.0	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.342	PASS
Control Growth CV < or = 40%	28.4	PASS
Growth Minimum Significant Difference 12 to 30%	27.2	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	25.2	PASS
Control CV < or = 40% per Surviving Female	7.67	PASS
Reproduction Minimum Significant Difference 13 to 47%	11.4	BELOW
Critical Dilution CV < or = 40%	11.4	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)	8.2	7.9	8.7
pH (standard units)	7.4	7.2	7.8
Alkalinity (mg/l as CaCO ₃)	57	54	93
Hardness (mg/l as CaCO ₃)	57	67	74
Conductivity (umhos/cm)	280	280	370
Residual Chlorine (mg/l)	0.050	<0.05	<0.05
Ammonia as N (mg/l)	0.20	0.95	4.7

2. Dilution Water Samples: Synthetic Soft Water #3998

- a. Dates Prepared: June 10 through 24, 2013
- b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.1	8.2	8.6
pH (standard units)	7.6	7.5	7.6
Alkalinity (mg/l as CaCO ₃)	30	30	30
Hardness (mg/l as CaCO ₃)	43	42	42
Conductivity (umhos/cm)	170	170	160
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 11, 2013 at 1150
Date & Time Test Terminated: June 18, 2013 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 11, 2013 at 1120
Date & Time Test Terminated: June 18, 2013 at 0940
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 and Bartlett's test. The survival data was then analyzed using Dunnett's 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 May 28, 2013 at 1645 to June 4, 2013 at 1520

The results were as follows: (Control No. 167778-1.)

Survival LC-50: 6598 mg/l
Growth IC-25: 5369 mg/l
Growth PMSD: 22.6

Ceriodaphnia dubia

Chronic reference tests are performed monthly.

A chronic reference test was performed on May 28, 2013 at 1700 to June 4, 2013 at 1500

The results were as follows: (Control No. 167778-2.)

Survival LC-50: 2236 mg/l
Growth IC-25: 1573 mg/l
Growth PMSD: 13.9

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	99.9	0.300
pH	SM 4500-H+ B	100	0.336
Conductivity	EPA 120.1	103	4.50

VI. Organism History

Pimephales promelas (Fathead minnow)

Date: June 11, 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

Ceriodaphnia dubia

Date: June 11, 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

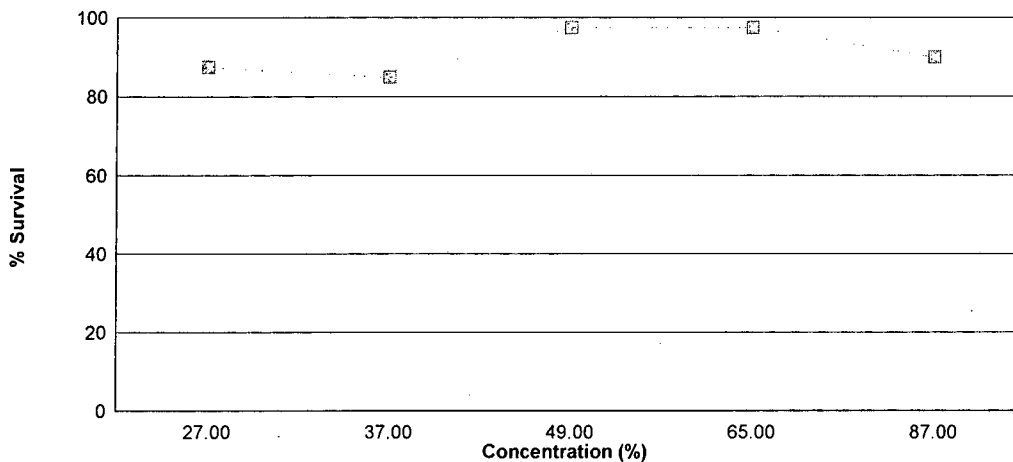
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

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 June 11, 2013 at 1150 and continued through June 18, 2013 at 1300. 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	95.0	0.325
27 %	87.5	0.288
37 %	85.0	0.276
49 %	97.5	0.326
65 %	97.5	0.403
87 %	90.0	0.329

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

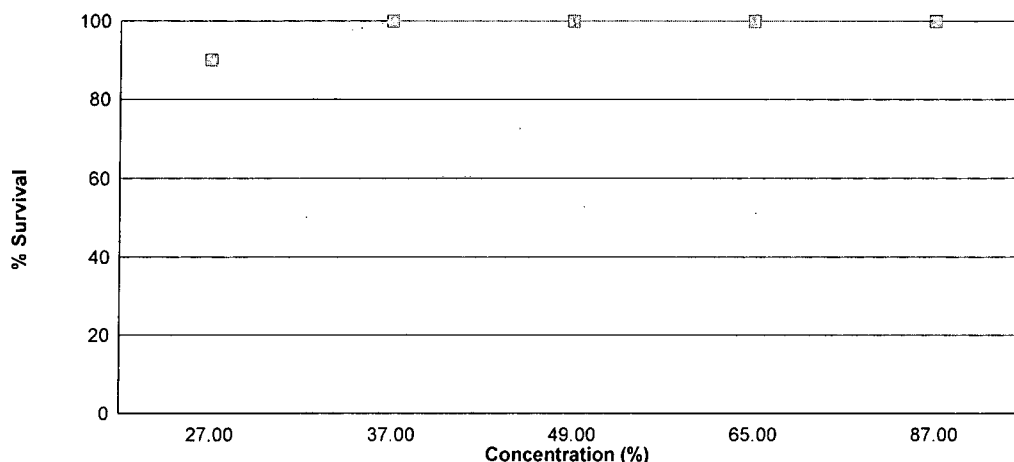
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

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 June 11, 2013 at 1120 and continued through June 18, 2013 at 0940. 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 <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	25.2
27 %	90.0	24.5
37 %	100	27.6
49 %	100	26.6
65 %	100	26.5
87 %	100	28.5

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: June 11, 2013 at 1150

Date and Time Test Terminated: June 18, 2013 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	7
	B	8	8	8	8	8	8	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
27 %	A	8	8	8	8	8	8	8
	B	8	6	6	6	6	6	6
	C	7	7	7	7	7	7	7
	D	7	7	7	7	7	7	7
	E	7	7	7	7	7	7	7
37 %	A	8	8	8	8	7	7	7
	B	8	8	8	8	8	7	7
	C	8	8	8	8	8	8	8
	D	8	7	7	7	6	6	6
	E	8	6	6	6	6	6	6
49 %	A	8	8	8	8	8	7	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
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	7
	E	8	8	8	8	8	8	8
87 %	A	8	6	6	6	6	6	6
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	7
	D	8	8	8	8	8	8	7
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: June 11, 2013 at 1150
Test Terminated: June 18, 2013 at 1300

Drying Started: June 18, 2013 at 0900
Drying Ended: June 19, 2013 at 1445

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.91318	.91501	0.00183	8	0.229
	B	.91372	.91553	0.00181	8	0.226
	C	.91335	.91629	0.00294	8	0.368
	D	.92516	.92859	0.00343	8	0.429
	E	.91943	.92242	0.00299	8	0.374
27 %	A	.92258	.92420	0.00162	8	0.202
	B	.91251	.91491	0.00240	8	0.300
	C	.91420	.91640	0.00220	8	0.275
	D	.90590	.90869	0.00279	8	0.349
	E	.90331	.90583	0.00252	8	0.315
37 %	A	.90443	.90627	0.00184	8	0.230
	B	.90748	.91004	0.00256	8	0.320
	C	.90764	.91003	0.00239	8	0.299
	D	.90889	.91060	0.00171	8	0.214
	E	.91702	.91954	0.00252	8	0.315
49 %	A	.92969	.93205	0.00236	8	0.295
	B	.91393	.91664	0.00271	8	0.339
	C	.91118	.91401	0.00283	8	0.354
	D	.91093	.91339	0.00246	8	0.308
	E	.90960	.91229	0.00269	8	0.336
65 %	A	.91779	.92040	0.00261	8	0.326
	B	.91719	.92066	0.00347	8	0.434
	C	.91774	.92110	0.00336	8	0.420
	D	.91700	.92008	0.00308	8	0.385
	E	.91636	.91995	0.00359	8	0.449
87 %	A	.92005	.92179	0.00174	8	0.218
	B	.91821	.92101	0.00280	8	0.350
	C	.91816	.92107	0.00291	8	0.364
	D	.92521	.92821	0.00300	8	0.375
	E	.93190	.93461	0.00271	8	0.339

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: June 11, 2013 at 1120

Date and Time Test Terminated: June 18, 2013 at 0940

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	4	4	0	3	0	0	3	0	0	14	10	1.40	
4	4	0	0	3	0	4	5	0	4	5	25	10	2.50	
5	8	10	10	7	9	9	10	10	10	0	83	10	8.30	
6	0	0	0	0	0	0	0	0	0	10	10	10	1.00	
7	13	12	11	11	14	10	13	13	12	11	120	10	12.0	
8														
TOTAL	25	26	25	21	26	23	28	26	26	26	252	10	25.2	

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	X	0	0	0	0	0	9	0.00	
3	0	4	2	0	0	X	2	4	0	0	12	9	1.33	
4	4	0	0	4	2	X	0	0	4	4	18	9	2.00	
5	10	12	9	11	12	X	11	8	10	9	92	9	10.2	
6	0	0	0	0	0	X	0	0	0	0	0	9	0.00	
7	13	14	14	15	13	X	11	15	14	14	123	9	13.7	
8														
TOTAL	27	30	25	30	27	0	24	27	28	27	245	10	24.5	

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	5	0	0	4	0	4	3	4	0	20	10	2.00	
4	2	0	4	4	0	4	0	0	0	3	17	10	1.70	
5	12	13	7	9	10	9	10	11	11	7	99	10	9.90	
6	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
7	14	17	16	15	13	15	13	13	14	10	140	10	14.0	
8														
TOTAL	28	35	27	28	27	28	27	27	29	20	276	10	27.6	

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: June 11, 2013 at 1120

Date and Time Test Terminated: June 18, 2013 at 0940

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	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	3	0	2	2	0	0	7	10	0.700	
4	4	5	4	4	0	4	0	0	4	5	30	10	3.00	
5	12	11	10	11	9	8	10	13	11	10	105	10	10.5	
6	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
7	11	16	14	11	12	10	14	13	11	12	124	10	12.4	
8														
TOTAL	27	32	28	26	24	22	26	28	26	27	266	10	26.6	

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	2	4	0	3	4	4	3	3	3	0	26	10	2.60	
4	0	0	4	0	0	0	0	0	0	5	9	10	0.900	
5	9	11	10	9	10	10	12	7	9	9	96	10	9.60	
6	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
7	11	18	12	16	13	13	11	13	16	11	134	10	13.4	
8														
TOTAL	22	33	26	28	27	27	26	23	28	25	265	10	26.5	

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	3	0	0	0	4	0	0	4	0	0	11	10	1.10	
4	0	4	4	5	0	4	2	0	4	4	27	10	2.70	
5	12	11	8	11	11	11	10	10	12	13	109	10	10.9	
6	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
7	14	17	13	12	13	12	12	16	14	15	138	10	13.8	
8														
TOTAL	29	32	25	28	28	27	24	30	30	32	285	10	28.5	

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	0.87500	1.20940
1	Control	2	0.87500	1.20940
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	0.75000	1.04720
2	27 %	3	0.87500	1.20940
2	27 %	4	0.87500	1.20940
2	27 %	5	0.87500	1.20940
3	37 %	1	0.87500	1.20940
3	37 %	2	0.87500	1.20940
3	37 %	3	1.00000	1.39310
3	37 %	4	0.75000	1.04720
3	37 %	5	0.75000	1.04720
4	49 %	1	0.87500	1.20940
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	0.87500	1.20940
5	65 %	5	1.00000	1.39310
6	87 %	1	0.75000	1.04720
6	87 %	2	1.00000	1.39310
6	87 %	3	0.87500	1.20940
6	87 %	4	0.87500	1.20940
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 = 0.3222 W = 0.9416 Critical W = 0.9 Critical W = 0.927	(alpha = 0.01, N = 30) (alpha = 0.05, N = 30)
Data PASS normality test (alpha = 0.01).	

Bartlett's Test for Homogeneity of Variance	Transform: Arc Sin(Square Root(Y))
Calculated B1 statistic = 2.392 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) Survival

ANOVA Table			Transform: Arc Sin(Square Root(Y))		
SOURCE	DF	SS	MS	F	
Between	5	0.1412	0.02824	2.103	
Within (Error)	24	0.3223	0.01343		
Total	29	0.4635			
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				Transform: Arc Sin(Square Root(Y))	
Ho: Control < Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	1.3196	0.95		
2	27 %	1.2137	0.875	1.445	
3	37 %	1.1813	0.85	1.887	
4	49 %	1.3564	0.975	-0.5021	
5	65 %	1.3564	0.975	-0.5021	
6	87 %	1.2504	0.9	0.9441	
Dunnett's critical value = 2.36 (1 Tailed, alpha = 0.05, df = 5,24)					

Dunnett's Test - Table 2 of 2				Transform: Arc Sin(Square Root(Y))	
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	0.1076	11.5	0.075
3	37 %	5	0.1076	11.5	0.1
4	49 %	5	0.1076	11.5	-0.025
5	65 %	5	0.1076	11.5	-0.025
6	87 %	5	0.1076	11.5	0.05

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality	No Transformation
<p>D = 0.08435 W = 0.9207 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 = 5.969 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	0.04933	0.009866	2.807	
Within (Error)	24	0.08437	0.003515		
Total	29	0.1337			
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	0.3252	0.3252		
2	27 %	0.2882	0.2882	0.9868	
3	37 %	0.2756	0.2756	1.323	
4	49 %	0.3264	0.3264	-0.032	
5	65 %	0.4028	0.4028	-2.07	
6	87 %	0.3292	0.3292	-0.1067	
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	0.08849	27.2	0.037
3	37 %	5	0.08849	27.2	0.0496
4	49 %	5	0.08849	27.2	-0.0012
5	65 %	5	0.08849	27.2	-0.0776
6	87 %	5	0.08849	27.2	-0.004

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test

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

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	1	
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
<p>D = 0.2443 D* = 1.917 Critical D* = 1.035 (alpha = 0.01, N = 60)</p> <p>Data FAIL normality test (alpha = 0.01).</p>	

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 %	125.50	75.00	10.00	
3	37 %	139.50	75.00	10.00	
4	49 %	124.50	75.00	10.00	
5	65 %	120.50	75.00	10.00	
6	87 %	138.00	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	62.77	12.55	1.702	
Within (Error)	53	390.9	7.375		
Total	58	453.7			
Critical F = 3.39 (alpha = 0.01, df = 5,53)					
2.39 (alpha = 0.05, df = 5,53)					
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	25.2	25.2			
2	27 %	27.222	27.222	-1.62		
3	37 %	27.6	27.6	-1.976		
4	49 %	26.6	26.6	-1.153		
5	65 %	26.5	26.5	-1.07		
6	87 %	28.5	28.5	-2.717		
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 %	9	2.882	11.4	-2.022	
3	37 %	10	2.805	11.1	-2.4	
4	49 %	10	2.805	11.1	-1.4	
5	65 %	10	2.805	11.1	-1.3	
6	87 %	10	2.805	11.1	-3.3	

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: June 11, 2013 at 0841

Date and Time Test Terminated: June 18, 2013 at 1300

Effluent Conc.: Control	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
DO, mg/l	Initial	8.1	8.0	8.2	8.7	8.6	8.0	7.8
	Final *1	7.6	7.9	8.3	8.5	7.8	6.8	6.1
	Final *2	8.1	8.4	8.5	7.7	7.9	8.4	8.2
pH, units	Initial	7.6	7.6	7.5	7.5	7.6	7.5	7.5
	Final *1	7.5	7.5	7.4	7.6	7.7	7.3	7.0
	Final *2	7.8	8.0	7.7	7.9	7.9	7.7	7.8
Alkalinity, mg CaCO ₃ /l	30	NA	30	NA	30	NA	NA	
Hardness, mg CaCO ₃ /l	43	NA	42	NA	42	NA	NA	
Conductivity, umhos/cm	170	170	170	170	160	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	8.0	7.9	8.0	8.5	8.6	8.1	7.8
	Final *1	7.2	7.9	8.0	8.2	7.9	7.0	6.3
	Final *2	8.2	8.4	8.0	8.1	8.4	8.4	8.1
pH, units	Initial	7.5	7.5	7.5	7.4	7.7	7.8	7.6
	Final *1	7.5	7.6	7.4	7.6	7.8	7.3	7.2
	Final *2	8.1	8.0	7.8	8.0	8.0	7.9	7.9

Effluent Conc.: 37 %	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
DO, mg/l	Initial	8.1	8.0	7.9	8.6	8.6	8.1	7.9
	Final *1	7.5	7.8	8.0	8.1	7.7	7.2	6.2
	Final *2	8.2	8.5	7.9	7.9	8.5	8.1	8.0
pH, units	Initial	7.6	7.5	7.5	7.4	7.8	7.9	7.6
	Final *1	7.6	7.6	7.4	7.5	7.8	7.5	7.3
	Final *2	8.1	8.1	7.8	8.0	8.1	7.9	8.0

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: June 11, 2013 at 0841

Date and Time Test Terminated: June 18, 2013 at 1300

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.1	7.9	7.8	8.4	8.5	8.0	7.6
	Final *1	7.8	7.8	7.8	8.1	7.7	7.7	6.5
	Final *2	8.1	8.4	7.8	7.9	8.3	8.3	7.8
pH, units	Initial	7.5	7.5	7.5	7.4	7.8	7.9	7.5
	Final *1	7.6	7.6	7.4	7.6	7.9	7.6	7.4
	Final *2	8.1	8.1	7.8	8.0	8.1	8.0	8.0

Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.1	7.7	8.0	8.6	8.5	8.1	8.0
	Final *1	7.6	7.6	8.1	7.8	7.7	7.6	6.4
	Final *2	8.2	8.6	7.7	8.1	8.3	8.2	8.1
pH, units	Initial	7.5	7.5	7.5	7.4	7.8	7.8	7.5
	Final *1	7.6	7.6	7.5	7.6	7.9	7.6	7.4
	Final *2	8.2	8.2	7.9	8.1	8.1	7.9	8.0
Alkalinity, mg CaCO ₃ /l	35	NA	58	NA	42	NA	NA	NA
Hardness, mg CaCO ₃ /l	51	NA	58	NA	54	NA	NA	NA
Conductivity, umhos/cm	240	250	240	280	230	230	220	220
Res. Chlorine, mg/l	<0.05	NA	<0.05	NA	<0.05	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	8.0	7.8	8.5	8.6	8.0	7.8
	Final *1	7.7	7.7	8.1	8.2	7.6	7.1	6.4
	Final *2	8.0	8.4	7.7	8.1	8.4	8.2	7.8
pH, units	Initial	7.4	7.5	7.5	7.4	7.8	8.1	7.6
	Final *1	7.7	7.6	7.5	7.7	8.0	7.6	7.6
	Final *2	8.1	8.1	7.9	8.2	8.2	8.0	8.1

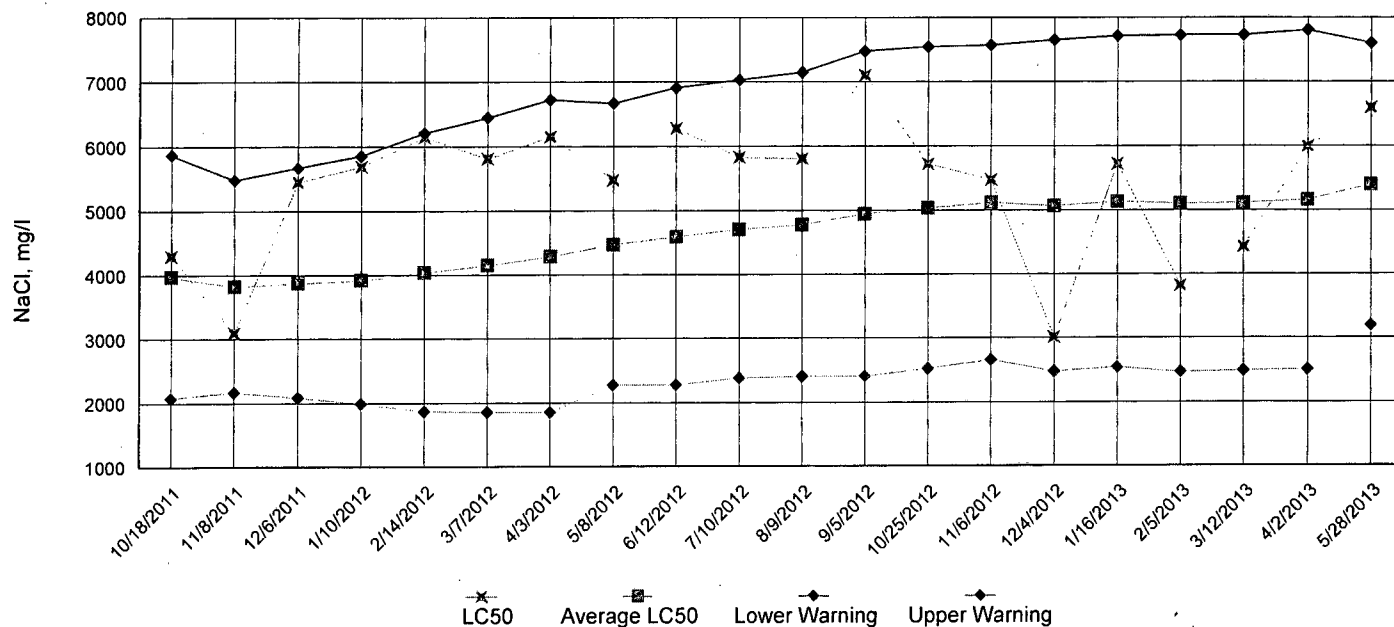
*1 = data from the *Pimephales promelas* (Fathead Minnow) test

*2 = data from the *Ceriodaphnia dubia* test

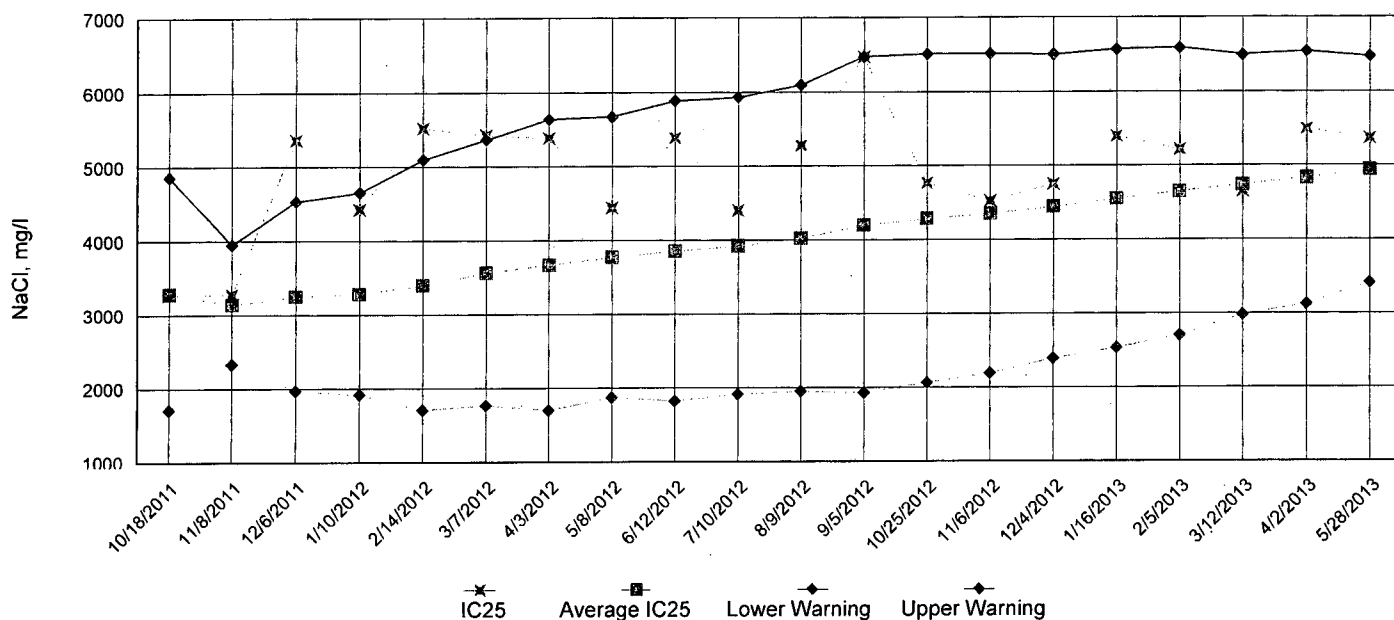
Appendix A4: Test 1000.0

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

LC50 Survival Data

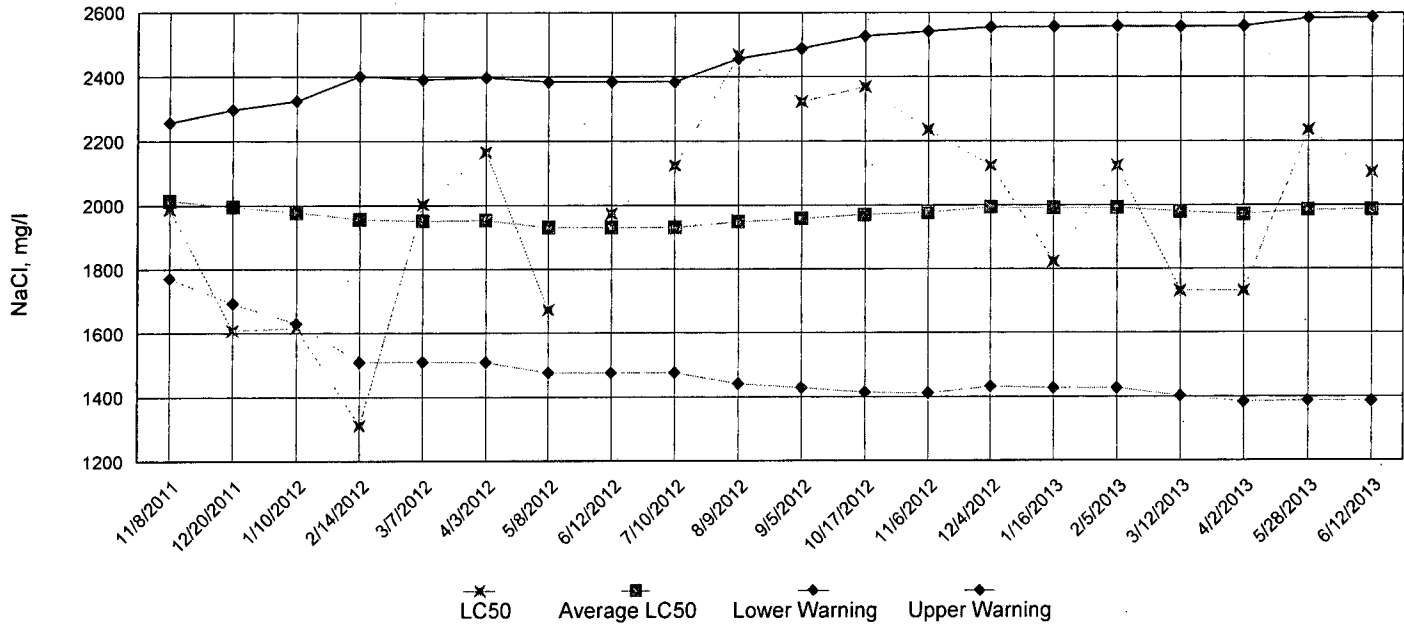


IC25 Growth Data

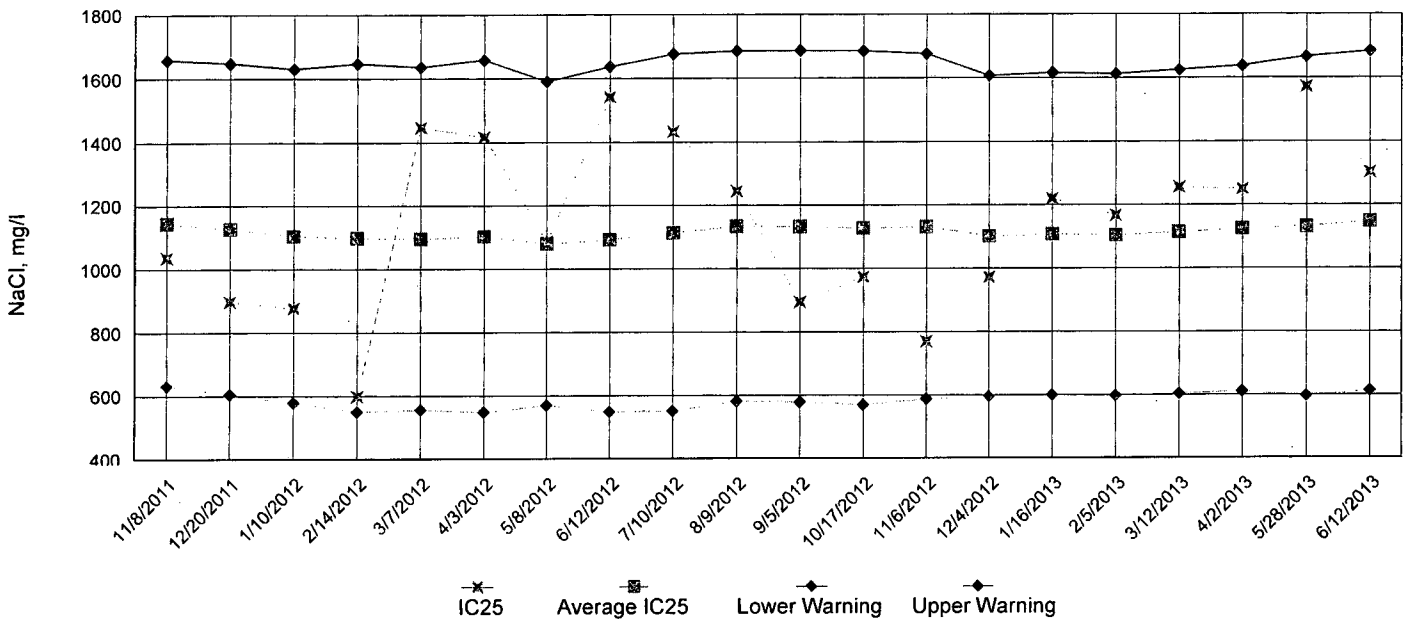


Appendix A4: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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 11, 2013 at 1150

Date and Time Test Terminated: June 18, 2013 at 1300

Dilution water used: Synthetic Soft Water #3998

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	87.5	87.5	100	100	100	100	100	95.0	7.21
27 %	100	75.0	87.5	87.5	87.5	92.5	87.5	87.5	10.1
37 %	87.5	87.5	100	75.0	75.0	100	92.5	85.0	12.3
49 %	87.5	100	100	100	100	100	100	97.5	5.73
65 %	100	100	100	87.5	100	100	100	97.5	5.73
87 %	75.0	100	87.5	87.5	100	100	95.0	90.0	11.6

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.229	0.226	0.368	0.429	0.374	0.325	28.4
27 %	0.202	0.300	0.275	0.349	0.315	0.288	19.1
37 %	0.230	0.320	0.299	0.214	0.315	0.276	18.1
49 %	0.295	0.339	0.354	0.308	0.336	0.326	7.41
65 %	0.326	0.434	0.420	0.385	0.449	0.403	12.2
87 %	0.218	0.350	0.364	0.375	0.339	0.329	19.3

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. Dunnett's 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 %)	<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	(65 %)	<u> </u> YES	<u> X </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 (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: 28.4 (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: 280, 298, 304, 307

2400
2400
2400

Test Initiated: DATE: June 11, 2013 TIME: 1150
Test Terminated: DATE: June 18, 2013 TIME: 1300

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	8.1	8.0	8.2	8.7	8.6	8.0	7.8
Final	7.6	7.9	8.3	8.5	7.8	6.8	6.1
pH Initial	7.6	7.6	7.5	7.5	7.6	7.5	7.5
Final	7.5	7.5	7.4	7.6	7.7	7.3	7.0
Alkalinity	30	NA	30	NA	30	NA	NA
Hardness	43	NA	42	NA	42	NA	NA
Conductivity	170	170	170	170	160	180	170
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
27 %							
D.O. Initial	8.0	7.9	8.0	8.5	8.6	8.1	7.8
Final	7.2	7.9	8.0	8.2	7.9	7.0	6.3
pH Initial	7.5	7.5	7.5	7.4	7.7	7.8	7.6
Final	7.5	7.6	7.4	7.6	7.8	7.3	7.2
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	200	200	200	220	230	230	230
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
37 %							
D.O. Initial	8.1	8.0	7.9	8.6	8.6	8.1	7.9
Final	7.5	7.8	8.0	8.1	7.7	7.2	6.2
pH Initial	7.6	7.5	7.5	7.4	7.8	7.9	7.6
Final	7.6	7.6	7.4	7.5	7.8	7.5	7.3
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	210	210	230	250	250	250
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
49 %							
D.O. Initial	8.1	7.9	7.8	8.4	8.5	8.0	7.6
Final	7.8	7.8	7.8	8.1	7.7	7.7	6.5
pH Initial	7.5	7.5	7.5	7.4	7.8	7.9	7.5
Final	7.6	7.6	7.4	7.6	7.9	7.6	7.4
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	230	230	220	250	270	260	270
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
65 %							
D.O. Initial	8.1	7.7	8.0	8.6	8.5	8.1	8.0
Final	7.6	7.6	8.1	7.8	7.7	7.6	6.4
pH Initial	7.5	7.5	7.5	7.4	7.8	7.8	7.5
Final	7.6	7.6	7.5	7.6	7.9	7.6	7.4
Alkalinity	35	NA	58	NA	42	NA	NA
Hardness	51	NA	58	NA	54	NA	NA
Conductivity	240	250	240	280	230	230	220
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
87 %							
D.O. Initial	8.0	8.0	7.8	8.5	8.6	8.0	7.8
Final	7.7	7.7	8.1	8.2	7.6	7.1	6.4
pH Initial	7.4	7.5	7.5	7.4	7.8	8.1	7.6
Final	7.7	7.6	7.5	7.7	8.0	7.6	7.6
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	270	280	270	320	340	340	340
Chlorine	NA	NA	NA	NA	NA	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 11, 2013 at 1120

Date and Time Test Terminated: June 18, 2013 at 0940

Dilution water used: Synthetic Soft Water #3998

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent				
		27 %	37 %	49 %	65 %	87 %
24 hour	100	100	100	100	100	100
48 hour	100	90.0	100	100	100	100
7 day	100	90.0	100	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

Replicates	Control	Percent Effluent				
		27 %	37 %	49 %	65 %	87 %
A	25	27	28	27	22	29
B	26	30	35	32	33	32
C	25	25	27	28	26	25
D	21	30	28	26	28	28
E	26	27	27	24	27	28
F	23	0	28	22	27	27
G	28	24	27	26	26	24
H	26	27	27	28	23	30
I	26	28	29	26	28	30
J	26	27	20	27	25	32
Mean per Adult	25.2	24.5	27.6	26.6	26.5	28.5
Mean per Surviving Adult	25.2	27.2	27.6	26.6	26.5	28.5
CV %	7.67	7.30	13.0	9.90	11.4	9.39

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 %)	<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	(65 %)	<u> </u> YES	<u> X </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]: 0 (TGP3B)
5. NOEC Ceriodaphnia Lethality: 87 % (TOP3B)
6. LOEC Ceriodaphnia Lethality: 87 % (TXP3B)
7. NOEC Ceriodaphnia Sublethality: 87 % (TPP3B)
8. LOEC Ceriodaphnia Sublethality: 87 % (TYP3B)
9. Coefficient of variation for Ceriodaphnia Reproduction: 11.4 (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: June 11, 2013 TIME: 1120
Test Terminated: DATE: June 18, 2013 TIME: 0940

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	8.1	8.0	8.2	8.7	8.6	8.0	7.8
Final	8.1	8.4	8.5	7.7	7.9	8.4	8.2
pH Initial	7.6	7.6	7.5	7.5	7.6	7.5	7.5
Final	7.8	8.0	7.7	7.9	7.9	7.7	7.8
Alkalinity	30	NA	30	NA	30	NA	NA
Hardness	43	NA	42	NA	42	NA	NA
Conductivity	170	170	170	170	160	180	170
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
27 %							
D.O. Initial	8.0	7.9	8.0	8.5	8.6	8.1	7.8
Final	8.2	8.4	8.0	8.1	8.4	8.4	8.1
pH Initial	7.5	7.5	7.5	7.4	7.7	7.8	7.6
Final	8.1	8.0	7.8	8.0	8.0	7.9	7.9
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	200	200	200	220	230	230	230
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
37 %							
D.O. Initial	8.1	8.0	7.9	8.6	8.6	8.1	7.9
Final	8.2	8.5	7.9	7.9	8.5	8.1	8.0
pH Initial	7.6	7.5	7.5	7.4	7.8	7.9	7.6
Final	8.1	8.1	7.8	8.0	8.1	7.9	8.0
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	210	210	230	250	250	250
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
49 %							
D.O. Initial	8.1	7.9	7.8	8.4	8.5	8.0	7.6
Final	8.1	8.4	7.8	7.9	8.3	8.3	7.8
pH Initial	7.5	7.5	7.5	7.4	7.8	7.9	7.5
Final	8.1	8.1	7.8	8.0	8.1	8.0	8.0
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	230	230	220	250	270	260	270
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
65 %							
D.O. Initial	8.1	7.7	8.0	8.6	8.5	8.1	8.0
Final	8.2	8.6	7.7	8.1	8.3	8.2	8.1
pH Initial	7.5	7.5	7.5	7.4	7.8	7.8	7.5
Final	8.2	8.2	7.9	8.1	8.1	7.9	8.0
Alkalinity	35	NA	58	NA	42	NA	NA
Hardness	51	NA	58	NA	54	NA	NA
Conductivity	240	250	240	280	230	230	220
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
87 %							
D.O. Initial	8.0	8.0	7.8	8.5	8.6	8.0	7.8
Final	8.0	8.4	7.7	8.1	8.4	8.2	7.8
pH Initial	7.4	7.5	7.5	7.4	7.8	8.1	7.6
Final	8.1	8.1	7.9	8.2	8.2	8.0	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	270	280	270	320	340	340	340
Chlorine	NA	NA	NA	NA	NA	NA	NA



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs			PO No.		No of BOTTLES	Analyses Requested										AIC Control No: 168112								
Project Reference: Plant Effluent			Sample Matrix			Chronic.CD	Chronic.FH												AIC Proposal No:					
Project Manager: James Sorrells			G R A B	C O M P	W A T E R	S O I L	3	x											Carrier:					
Sampled By: <i>H MAUDIN</i>																			Received Temperature °C 8°C					
AIC No.	Sample Identification	Date/Time Collected																	Remarks					
3	PLANT EFFLUENT	6-13-13 0800-2400	X	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 = (NH4) ₂ SO4			
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS					Relinquished By: <i>A. Maudin</i>					Date/Time: <i>6/14/13</i>					Received By:					Date/Time:				
Expedited results requested by: <i>same</i>					Relinquished By:					Date/Time: <i>1225</i>					Received in Lab By: <i>[Signature]</i>					Date/Time: <i>6-14-13</i>				
Who should AIC contact with questions:					Comments:																			
Phone: <i>501-262-1125</i> Fax: <i>501-262-0339</i>																								
Report Attention to: <i>Mr. James Sorrells</i>																								
Report Address to: 320 Davidson Road Hot Springs, AR 71901																								

June 20, 2013

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

Control No. 168112-1

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:

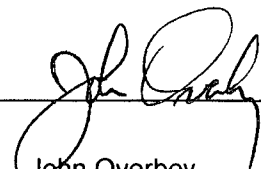
This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire 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 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.**

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: The No Observable Effects Concentration (NOEC) for survival occurred at 87 % effluent, which is above the critical dilution of 65 %. Any statistical difference with sublethal effects cannot be considered toxic due to the minimum significant difference (PMSD) calculated result being below the lower PMSD bounds. **The sample, therefore PASSED 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

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%	95.0	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.342	PASS
Control Growth CV < or = 40%	28.4	PASS
Growth Minimum Significant Difference 12 to 30%	27.2	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	25.2	PASS
Control CV < or = 40% per Surviving Female	7.67	PASS
Reproduction Minimum Significant Difference 13 to 47%	11.4	BELOW
Critical Dilution CV < or = 40%	11.4	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)	8.2	7.9	8.7
pH (standard units)	7.4	7.2	7.8
Alkalinity (mg/l as CaCO ₃)	57	54	93
Hardness (mg/l as CaCO ₃)	57	67	74
Conductivity (umhos/cm)	280	280	370
Residual Chlorine (mg/l)	0.050	<0.05	<0.05
Ammonia as N (mg/l)	0.20	0.95	4.7

2. Dilution Water Samples: Synthetic Soft Water #3998

- a. Dates Prepared: June 10 through 24, 2013
- b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.1	8.2	8.6
pH (standard units)	7.6	7.5	7.6
Alkalinity (mg/l as CaCO ₃)	30	30	30
Hardness (mg/l as CaCO ₃)	43	42	42
Conductivity (umhos/cm)	170	170	160
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 11, 2013 at 1150
Date & Time Test Terminated: June 18, 2013 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 11, 2013 at 1120
Date & Time Test Terminated: June 18, 2013 at 0940
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 and Bartlett's test. The survival data was then analyzed using Dunnett's 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 May 28, 2013 at 1645 to June 4, 2013 at 1520

The results were as follows: (Control No. 167778-1.)

Survival LC-50: 6598 mg/l

Growth IC-25: 5369 mg/l

Growth PMSD: 22.6

Ceriodaphnia dubia

Chronic reference tests are performed monthly.

A chronic reference test was performed on May 28, 2013 at 1700 to June 4, 2013 at 1500

The results were as follows: (Control No. 167778-2.)

Survival LC-50: 2236 mg/l

Growth IC-25: 1573 mg/l

Growth PMSD: 13.9

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	99.9	0.300
pH	SM 4500-H+ B	100	0.336
Conductivity	EPA 120.1	103	4.50

VI. Organism History

Pimephales promelas (Fathead minnow)

Date: June 11, 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

Ceriodaphnia dubia

Date: June 11, 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

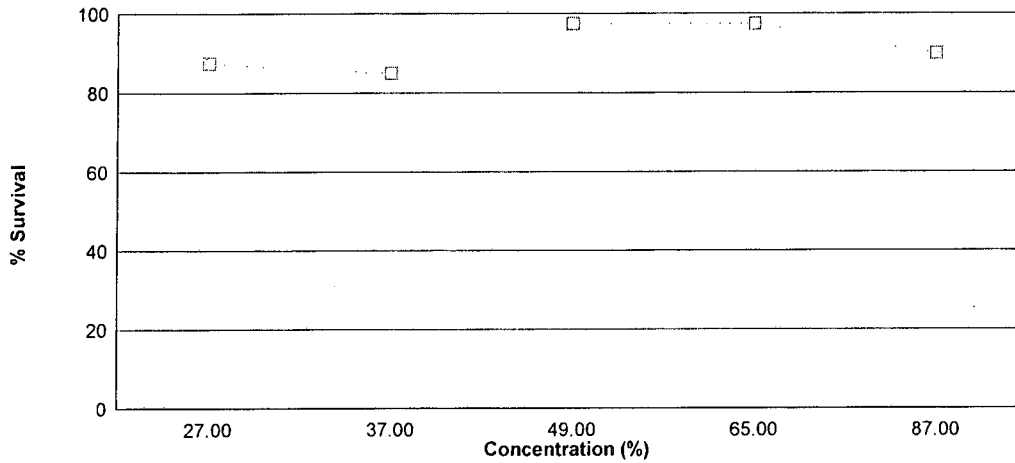
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

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 June 11, 2013 at 1150 and continued through June 18, 2013 at 1300. 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	95.0	0.325
27 %	87.5	0.288
37 %	85.0	0.276
49 %	97.5	0.326
65 %	97.5	0.403
87 %	90.0	0.329

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

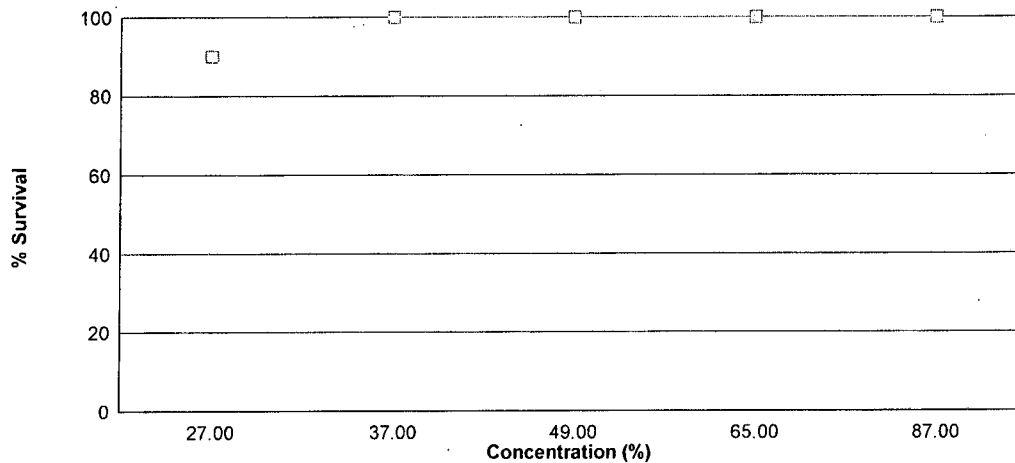
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

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 June 11, 2013 at 1120 and continued through June 18, 2013 at 0940. 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



Concentration	Percent Survival	Mean Reproduction
Control	100	25.2
27 %	90.0	24.5
37 %	100	27.6
49 %	100	26.6
65 %	100	26.5
87 %	100	28.5

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: June 11, 2013 at 1150

Date and Time Test Terminated: June 18, 2013 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	7
	B	8	8	8	8	8	8	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
27 %	A	8	8	8	8	8	8	8
	B	8	6	6	6	6	6	6
	C	7	7	7	7	7	7	7
	D	7	7	7	7	7	7	7
	E	7	7	7	7	7	7	7
37 %	A	8	8	8	8	7	7	7
	B	8	8	8	8	8	7	7
	C	8	8	8	8	8	8	8
	D	8	7	7	7	6	6	6
	E	8	6	6	6	6	6	6
49 %	A	8	8	8	8	8	7	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
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	7
	E	8	8	8	8	8	8	8
87 %	A	8	6	6	6	6	6	6
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	7
	D	8	8	8	8	8	8	7
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: June 11, 2013 at 1150
Test Terminated: June 18, 2013 at 1300

Drying Started: June 18, 2013 at 0900
Drying Ended: June 19, 2013 at 1445

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.91318	.91501	0.00183	8	0.229
	B	.91372	.91553	0.00181	8	0.226
	C	.91335	.91629	0.00294	8	0.368
	D	.92516	.92859	0.00343	8	0.429
	E	.91943	.92242	0.00299	8	0.374
27 %	A	.92258	.92420	0.00162	8	0.202
	B	.91251	.91491	0.00240	8	0.300
	C	.91420	.91640	0.00220	8	0.275
	D	.90590	.90869	0.00279	8	0.349
	E	.90331	.90583	0.00252	8	0.315
37 %	A	.90443	.90627	0.00184	8	0.230
	B	.90748	.91004	0.00256	8	0.320
	C	.90764	.91003	0.00239	8	0.299
	D	.90889	.91060	0.00171	8	0.214
	E	.91702	.91954	0.00252	8	0.315
49 %	A	.92969	.93205	0.00236	8	0.295
	B	.91393	.91664	0.00271	8	0.339
	C	.91118	.91401	0.00283	8	0.354
	D	.91093	.91339	0.00246	8	0.308
	E	.90960	.91229	0.00269	8	0.336
65 %	A	.91779	.92040	0.00261	8	0.326
	B	.91719	.92066	0.00347	8	0.434
	C	.91774	.92110	0.00336	8	0.420
	D	.91700	.92008	0.00308	8	0.385
	E	.91636	.91995	0.00359	8	0.449
87 %	A	.92005	.92179	0.00174	8	0.218
	B	.91821	.92101	0.00280	8	0.350
	C	.91816	.92107	0.00291	8	0.364
	D	.92521	.92821	0.00300	8	0.375
	E	.93190	.93461	0.00271	8	0.339

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: June 11, 2013 at 1120
Date and Time Test Terminated: June 18, 2013 at 0940

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	4	4	0	3	0	0	3	0	0	14	10	1.40	
4	4	0	0	3	0	4	5	0	4	5	25	10	2.50	
5	8	10	10	7	9	9	10	10	10	0	83	10	8.30	
6	0	0	0	0	0	0	0	0	0	10	10	10	1.00	
7	13	12	11	11	14	10	13	13	12	11	120	10	12.0	
8														
TOTAL	25	26	25	21	26	23	28	26	26	26	252	10	25.2	

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	X	0	0	0	0	0	9	0.00
3	0	4	2	0	0	X	2	4	0	0	12	9	1.33
4	4	0	0	4	2	X	0	0	4	4	18	9	2.00
5	10	12	9	11	12	X	11	8	10	9	92	9	10.2
6	0	0	0	0	0	X	0	0	0	0	0	9	0.00
7	13	14	14	15	13	X	11	15	14	14	123	9	13.7
8													
TOTAL	27	30	25	30	27	0	24	27	28	27	245	10	24.5

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	5	0	0	4	0	4	3	4	0	20	10	2.00
4	2	0	4	4	0	4	0	0	0	3	17	10	1.70
5	12	13	7	9	10	9	10	11	11	7	99	10	9.90
6	0	0	0	0	0	0	0	0	0	0	0	10	0.00
7	14	17	16	15	13	15	13	13	14	10	140	10	14.0
8													
TOTAL	28	35	27	28	27	28	27	27	29	20	276	10	27.6

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: June 11, 2013 at 1120
Date and Time Test Terminated: June 18, 2013 at 0940

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	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	3	0	2	2	0	0	7	10	0.700	
4	4	5	4	4	0	4	0	0	4	5	30	10	3.00	
5	12	11	10	11	9	8	10	13	11	10	105	10	10.5	
6	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
7	11	16	14	11	12	10	14	13	11	12	124	10	12.4	
8														
TOTAL	27	32	28	26	24	22	26	28	26	27	266	10	26.6	

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	2	4	0	3	4	4	3	3	3	0	26	10	2.60
4	0	0	4	0	0	0	0	0	0	5	9	10	0.900
5	9	11	10	9	10	10	12	7	9	9	96	10	9.60
6	0	0	0	0	0	0	0	0	0	0	0	10	0.00
7	11	18	12	16	13	13	11	13	16	11	134	10	13.4
8													
TOTAL	22	33	26	28	27	27	26	23	28	25	265	10	26.5

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	3	0	0	0	4	0	0	4	0	0	11	10	1.10
4	0	4	4	5	0	4	2	0	4	4	27	10	2.70
5	12	11	8	11	11	11	10	10	12	13	109	10	10.9
6	0	0	0	0	0	0	0	0	0	0	0	10	0.00
7	14	17	13	12	13	12	12	16	14	15	138	10	13.8
8													
TOTAL	29	32	25	28	28	27	24	30	30	32	285	10	28.5

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	0.87500	1.20940
1	Control	2	0.87500	1.20940
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	0.75000	1.04720
2	27 %	3	0.87500	1.20940
2	27 %	4	0.87500	1.20940
2	27 %	5	0.87500	1.20940
3	37 %	1	0.87500	1.20940
3	37 %	2	0.87500	1.20940
3	37 %	3	1.00000	1.39310
3	37 %	4	0.75000	1.04720
3	37 %	5	0.75000	1.04720
4	49 %	1	0.87500	1.20940
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	0.87500	1.20940
5	65 %	5	1.00000	1.39310
6	87 %	1	0.75000	1.04720
6	87 %	2	1.00000	1.39310
6	87 %	3	0.87500	1.20940
6	87 %	4	0.87500	1.20940
6	87 %	5	1.00000	1.39310

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

ANOVA Table			Transform: Arc Sin(Square Root(Y))	
SOURCE	DF	SS	MS	F
Between	5	0.1412	0.02824	2.103
Within (Error)	24	0.3223	0.01343	
Total	29	0.4635		
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				Transform: Arc Sin(Square Root(Y))	
Ho:Control<Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	1.3196	0.95		
2	27 %	1.2137	0.875	1.445	
3	37 %	1.1813	0.85	1.887	
4	49 %	1.3564	0.975	-0.5021	
5	65 %	1.3564	0.975	-0.5021	
6	87 %	1.2504	0.9	0.9441	
Dunnett's critical value = 2.36 (1 Tailed, alpha = 0.05, df = 5,24)					

Dunnett's Test - Table 2 of 2				Transform: Arc Sin(Square Root(Y))	
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	0.1076	11.5	0.075
3	37 %	5	0.1076	11.5	0.1
4	49 %	5	0.1076	11.5	-0.025
5	65 %	5	0.1076	11.5	-0.025
6	87 %	5	0.1076	11.5	0.05

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality	No Transformation
<p>D = 0.08435 W = 0.9207 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 = 5.969 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	0.04933	0.009866	2.807	
Within (Error)	24	0.08437	0.003515		
Total	29	0.1337			
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	0.3252	0.3252			
2	27 %	0.2882	0.2882	0.9868		
3	37 %	0.2756	0.2756	1.323		
4	49 %	0.3264	0.3264	-0.032		
5	65 %	0.4028	0.4028	-2.07		
6	87 %	0.3292	0.3292	-0.1067		
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	0.08849	27.2	0.037	
3	37 %	5	0.08849	27.2	0.0496	
4	49 %	5	0.08849	27.2	-0.0012	
5	65 %	5	0.08849	27.2	-0.0776	
6	87 %	5	0.08849	27.2	-0.004	

Appendix A2: Statistics

Ceriodaphnia dubia Survival

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

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	1	
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 = 0.2443
D* = 1.917
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 %	125.50	75.00	10.00	
3	37 %	139.50	75.00	10.00	
4	49 %	124.50	75.00	10.00	
5	65 %	120.50	75.00	10.00	
6	87 %	138.00	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	62.77	12.55	1.702	
Within (Error)	53	390.9	7.375		
Total	58	453.7			

Critical F = 3.39 (alpha = 0.01, df = 5,53)
2.39 (alpha = 0.05, df = 5,53)

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	25.2	25.2			
2	27 %	27.222	27.222	-1.62		
3	37 %	27.6	27.6	-1.976		
4	49 %	26.6	26.6	-1.153		
5	65 %	26.5	26.5	-1.07		
6	87 %	28.5	28.5	-2.717		

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 %	9	2.882	11.4	-2.022	
3	37 %	10	2.805	11.1	-2.4	
4	49 %	10	2.805	11.1	-1.4	
5	65 %	10	2.805	11.1	-1.3	
6	87 %	10	2.805	11.1	-3.3	

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: June 11, 2013 at 0841

Date and Time Test Terminated: June 18, 2013 at 1300

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.1	8.0	8.2	8.7	8.6	8.0	7.8
	Final *1	7.6	7.9	8.3	8.5	7.8	6.8	6.1
	Final *2	8.1	8.4	8.5	7.7	7.9	8.4	8.2
pH, units	Initial	7.6	7.6	7.5	7.5	7.6	7.5	7.5
	Final *1	7.5	7.5	7.4	7.6	7.7	7.3	7.0
	Final *2	7.8	8.0	7.7	7.9	7.9	7.7	7.8
Alkalinity, mg CaCO ₃ /l		30	NA	30	NA	30	NA	NA
Hardness, mg CaCO ₃ /l		43	NA	42	NA	42	NA	NA
Conductivity, umhos/cm		170	170	170	170	160	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	8.0	7.9	8.0	8.5	8.6	8.1	7.8
	Final *1	7.2	7.9	8.0	8.2	7.9	7.0	6.3
	Final *2	8.2	8.4	8.0	8.1	8.4	8.4	8.1
pH, units	Initial	7.5	7.5	7.5	7.4	7.7	7.8	7.6
	Final *1	7.5	7.6	7.4	7.6	7.8	7.3	7.2
	Final *2	8.1	8.0	7.8	8.0	8.0	7.9	7.9

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.1	8.0	7.9	8.6	8.6	8.1	7.9
	Final *1	7.5	7.8	8.0	8.1	7.7	7.2	6.2
	Final *2	8.2	8.5	7.9	7.9	8.5	8.1	8.0
pH, units	Initial	7.6	7.5	7.5	7.4	7.8	7.9	7.6
	Final *1	7.6	7.6	7.4	7.5	7.8	7.5	7.3
	Final *2	8.1	8.1	7.8	8.0	8.1	7.9	8.0

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: June 11, 2013 at 0841

Date and Time Test Terminated: June 18, 2013 at 1300

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.1	7.9	7.8	8.4	8.5	8.0	7.6
	Final *1	7.8	7.8	7.8	8.1	7.7	7.7	6.5
	Final *2	8.1	8.4	7.8	7.9	8.3	8.3	7.8
pH, units	Initial	7.5	7.5	7.5	7.4	7.8	7.9	7.5
	Final *1	7.6	7.6	7.4	7.6	7.9	7.6	7.4
	Final *2	8.1	8.1	7.8	8.0	8.1	8.0	8.0

Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.1	7.7	8.0	8.6	8.5	8.1	8.0
	Final *1	7.6	7.6	8.1	7.8	7.7	7.6	6.4
	Final *2	8.2	8.6	7.7	8.1	8.3	8.2	8.1
pH, units	Initial	7.5	7.5	7.5	7.4	7.8	7.8	7.5
	Final *1	7.6	7.6	7.5	7.6	7.9	7.6	7.4
	Final *2	8.2	8.2	7.9	8.1	8.1	7.9	8.0
Alkalinity, mg CaCO ₃ /l	35	NA	58	NA	42	NA	NA	NA
Hardness, mg CaCO ₃ /l	51	NA	58	NA	54	NA	NA	NA
Conductivity, umhos/cm	240	250	240	280	230	230	220	220
Res. Chlorine, mg/l	<0.05	NA	<0.05	NA	<0.05	NA	NA	NA

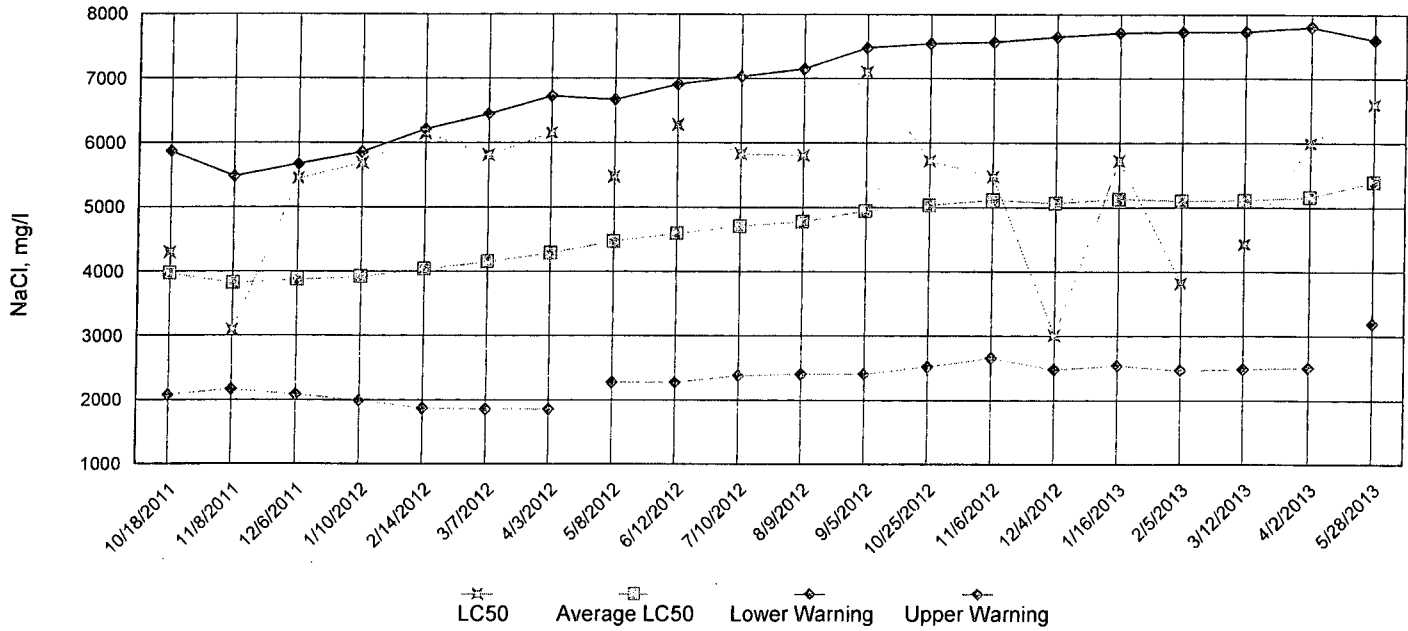
Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	8.0	7.8	8.5	8.6	8.0	7.8
	Final *1	7.7	7.7	8.1	8.2	7.6	7.1	6.4
	Final *2	8.0	8.4	7.7	8.1	8.4	8.2	7.8
pH, units	Initial	7.4	7.5	7.5	7.4	7.8	8.1	7.6
	Final *1	7.7	7.6	7.5	7.7	8.0	7.6	7.6
	Final *2	8.1	8.1	7.9	8.2	8.2	8.0	8.1

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

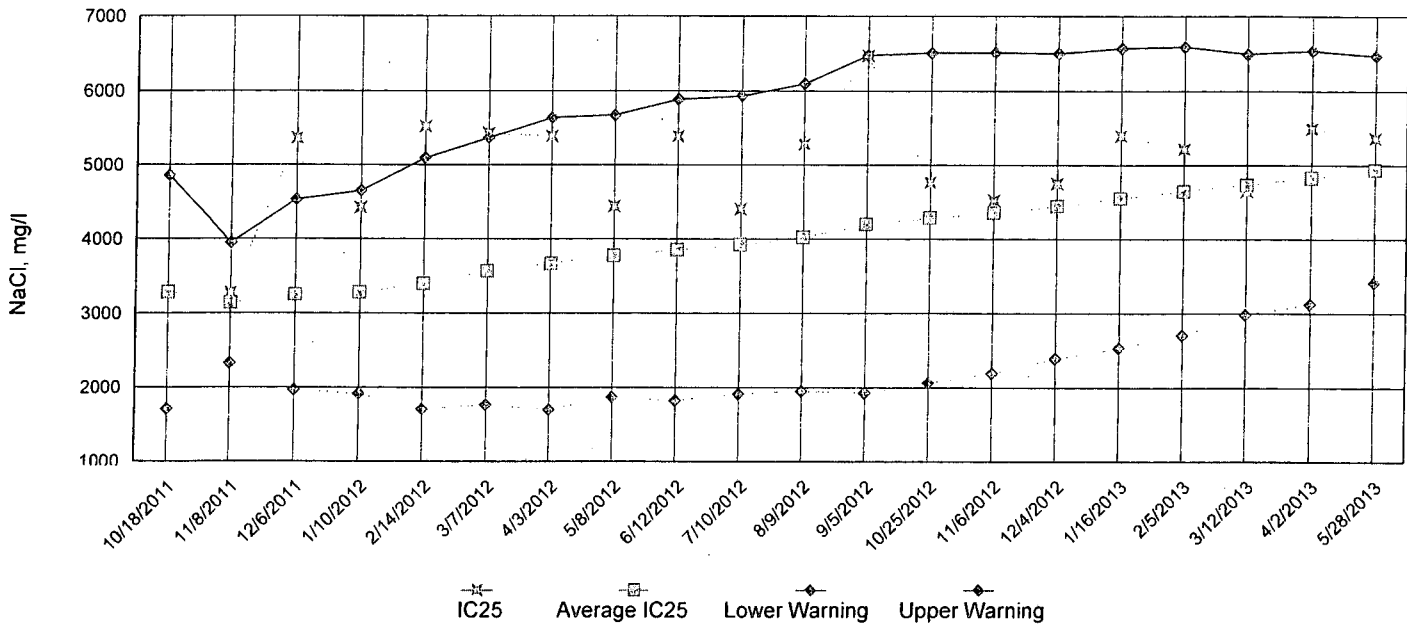
Appendix A4: Test 1000.0

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

LC50 Survival Data

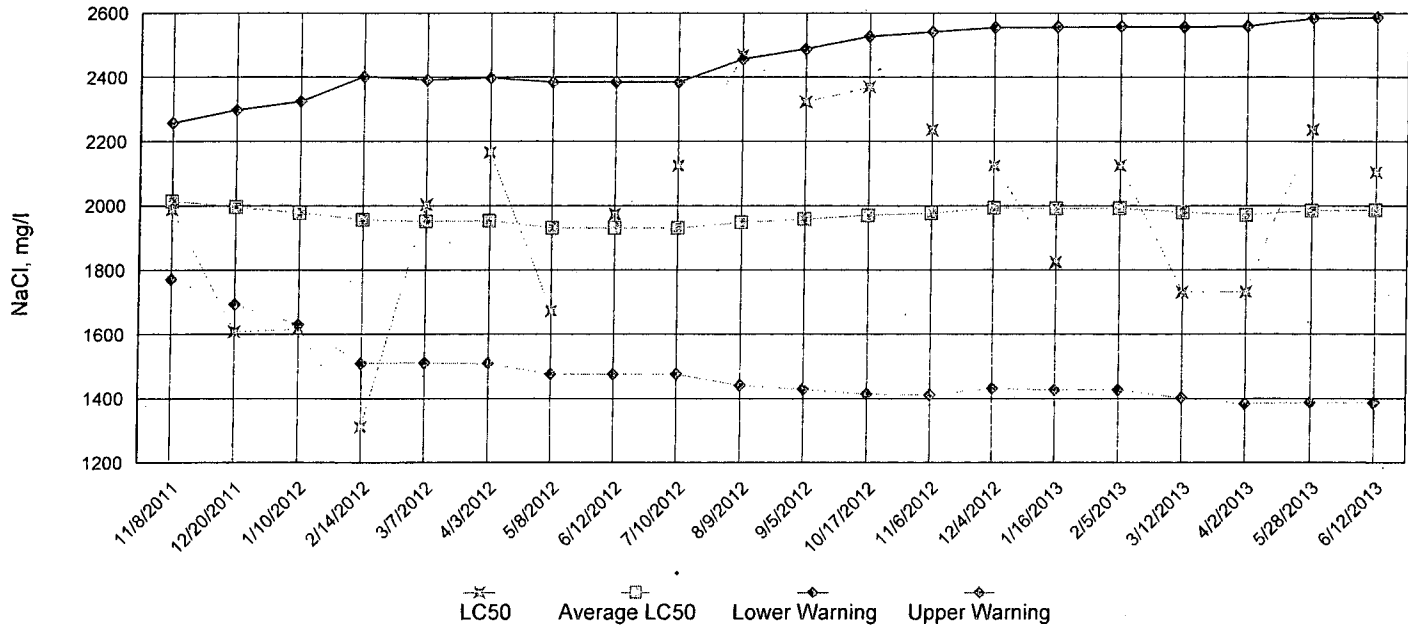


IC25 Growth Data

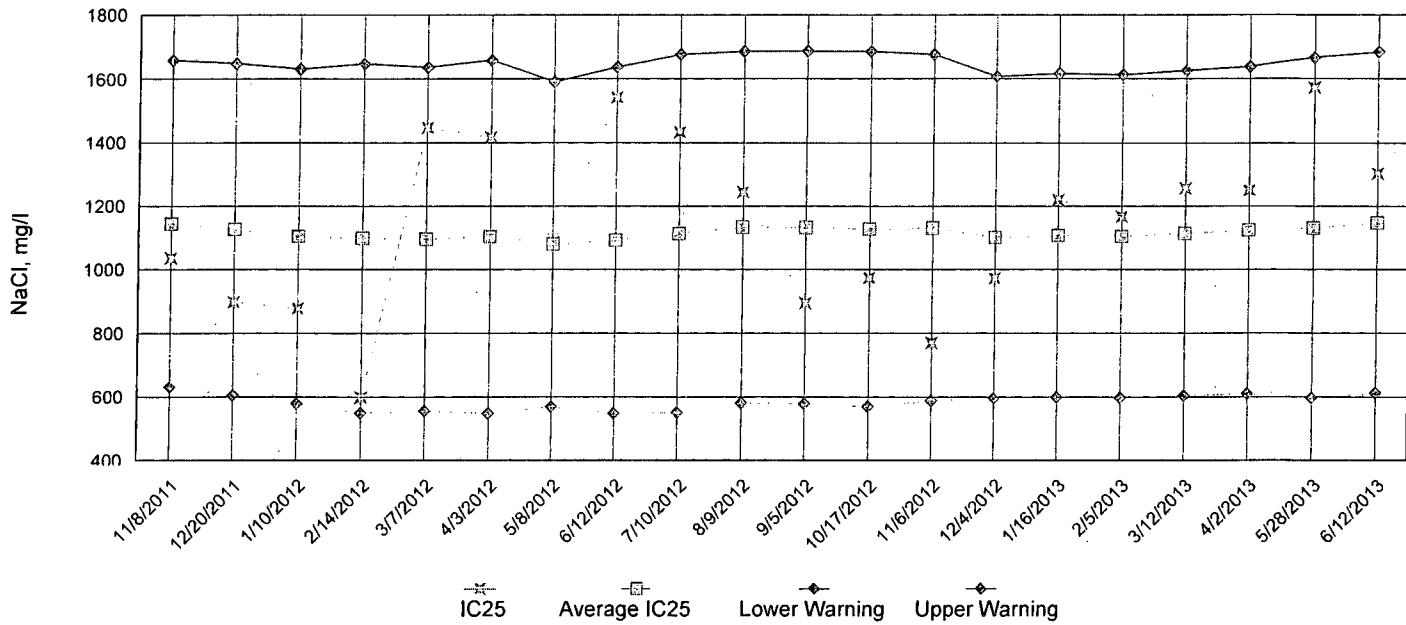


Appendix A4: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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 11, 2013 at 1150

Date and Time Test Terminated: June 18, 2013 at 1300

Dilution water used: Synthetic Soft Water #3998

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	87.5	87.5	100	100	100	100	100	95.0	7.21
27 %	100	75.0	87.5	87.5	87.5	92.5	87.5	87.5	10.1
37 %	87.5	87.5	100	75.0	75.0	100	92.5	85.0	12.3
49 %	87.5	100	100	100	100	100	100	97.5	5.73
65 %	100	100	100	87.5	100	100	100	97.5	5.73
87 %	75.0	100	87.5	87.5	100	100	95.0	90.0	11.6

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.229	0.226	0.368	0.429	0.374	0.325	28.4
27 %	0.202	0.300	0.275	0.349	0.315	0.288	19.1
37 %	0.230	0.320	0.299	0.214	0.315	0.276	18.1
49 %	0.295	0.339	0.354	0.308	0.336	0.326	7.41
65 %	0.326	0.434	0.420	0.385	0.449	0.403	12.2
87 %	0.218	0.350	0.364	0.375	0.339	0.329	19.3

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. Dunnett's 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 %)	<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	(65 %)	<u> </u> YES	<u> X </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 (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: 28.4 (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: 280, 298, 304, 307

2400
2400
2400

Test Initiated: DATE: June 11, 2013 TIME: 1150
Test Terminated: DATE: June 18, 2013 TIME: 1300

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	8.0	8.2	8.7	8.6	8.0	7.8
Final	7.6	7.9	8.3	8.5	7.8	6.8	6.1
pH Initial	7.6	7.6	7.5	7.5	7.6	7.5	7.5
Final	7.5	7.5	7.4	7.6	7.7	7.3	7.0
Alkalinity	30	NA	30	NA	30	NA	NA
Hardness	43	NA	42	NA	42	NA	NA
Conductivity	170	170	170	170	160	180	170
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 27 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.9	8.0	8.5	8.6	8.1	7.8
Final	7.2	7.9	8.0	8.2	7.9	7.0	6.3
pH Initial	7.5	7.5	7.5	7.4	7.7	7.8	7.6
Final	7.5	7.6	7.4	7.6	7.8	7.3	7.2
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	200	200	200	220	230	230	230
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 37 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	8.0	7.9	8.6	8.6	8.1	7.9
Final	7.5	7.8	8.0	8.1	7.7	7.2	6.2
pH Initial	7.6	7.5	7.5	7.4	7.8	7.9	7.6
Final	7.6	7.6	7.4	7.5	7.8	7.5	7.3
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	210	210	230	250	250	250
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 49 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	7.9	7.8	8.4	8.5	8.0	7.6
Final	7.8	7.8	7.8	8.1	7.7	7.7	6.5
pH Initial	7.5	7.5	7.5	7.4	7.8	7.9	7.5
Final	7.6	7.6	7.4	7.6	7.9	7.6	7.4
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	230	230	220	250	270	260	270
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 65 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	7.7	8.0	8.6	8.5	8.1	8.0
Final	7.6	7.6	8.1	7.8	7.7	7.6	6.4
pH Initial	7.5	7.5	7.5	7.4	7.8	7.8	7.5
Final	7.6	7.6	7.5	7.6	7.9	7.6	7.4
Alkalinity	35	NA	58	NA	42	NA	NA
Hardness	51	NA	58	NA	54	NA	NA
Conductivity	240	250	240	280	230	230	220
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	8.0	7.8	8.5	8.6	8.0	7.8
Final	7.7	7.7	8.1	8.2	7.6	7.1	6.4
pH Initial	7.4	7.5	7.5	7.4	7.8	8.1	7.6
Final	7.7	7.6	7.5	7.7	8.0	7.6	7.6
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	270	280	270	320	340	340	340
Chlorine	NA	NA	NA	NA	NA	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 11, 2013 at 1120

Date and Time Test Terminated: June 18, 2013 at 0940

Dilution water used: Synthetic Soft Water #3998

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent				
		27 %	37 %	49 %	65 %	87 %
24 hour	100	100	100	100	100	100
48 hour	100	90.0	100	100	100	100
7 day	100	90.0	100	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

Replicates	Control	Percent Effluent				
		27 %	37 %	49 %	65 %	87 %
A	25	27	28	27	22	29
B	26	30	35	32	33	32
C	25	25	27	28	26	25
D	21	30	28	26	28	28
E	26	27	27	24	27	28
F	23	0	28	22	27	27
G	28	24	27	26	26	24
H	26	27	27	28	23	30
I	26	28	29	26	28	30
J	26	27	20	27	25	32
Mean per Adult	25.2	24.5	27.6	26.6	26.5	28.5
Mean per Surviving Adult	25.2	27.2	27.6	26.6	26.5	28.5
CV %	7.67	7.30	13.0	9.90	11.4	9.39

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 %)	<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	(65 %)	<u> </u> YES	<u> X </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]: 0 (TGP3B)
5. NOEC *Ceriodaphnia* Lethality: 87 % (TOP3B)
6. LOEC *Ceriodaphnia* Lethality: 87 % (TXP3B)
7. NOEC *Ceriodaphnia* Sublethality: 87 % (TPP3B)
8. LOEC *Ceriodaphnia* Sublethality: 87 % (TYP3B)
9. Coefficient of variation for *Ceriodaphnia* Reproduction: 11.4 (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: June 11, 2013 TIME: 1120
Test Terminated: DATE: June 18, 2013 TIME: 0940

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	8.0	8.2	8.7	8.6	8.0	7.8
Final	8.1	8.4	8.5	7.7	7.9	8.4	8.2
pH Initial	7.6	7.6	7.5	7.5	7.6	7.5	7.5
Final	7.8	8.0	7.7	7.9	7.9	7.7	7.8
Alkalinity	30	NA	30	NA	30	NA	NA
Hardness	43	NA	42	NA	42	NA	NA
Conductivity	170	170	170	170	160	180	170
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 27 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.9	8.0	8.5	8.6	8.1	7.8
Final	8.2	8.4	8.0	8.1	8.4	8.4	8.1
pH Initial	7.5	7.5	7.5	7.4	7.7	7.8	7.6
Final	8.1	8.0	7.8	8.0	8.0	7.9	7.9
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	200	200	200	220	230	230	230
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 37 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	8.0	7.9	8.6	8.6	8.1	7.9
Final	8.2	8.5	7.9	7.9	8.5	8.1	8.0
pH Initial	7.6	7.5	7.5	7.4	7.8	7.9	7.6
Final	8.1	8.1	7.8	8.0	8.1	7.9	8.0
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	210	210	230	250	250	250
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 49 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	7.9	7.8	8.4	8.5	8.0	7.6
Final	8.1	8.4	7.8	7.9	8.3	8.3	7.8
pH Initial	7.5	7.5	7.5	7.4	7.8	7.9	7.5
Final	8.1	8.1	7.8	8.0	8.1	8.0	8.0
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	230	230	220	250	270	260	270
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 65 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	7.7	8.0	8.6	8.5	8.1	8.0
Final	8.2	8.6	7.7	8.1	8.3	8.2	8.1
pH Initial	7.5	7.5	7.5	7.4	7.8	7.8	7.5
Final	8.2	8.2	7.9	8.1	8.1	7.9	8.0
Alkalinity	35	NA	58	NA	42	NA	NA
Hardness	51	NA	58	NA	54	NA	NA
Conductivity	240	250	240	280	230	230	220
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	8.0	7.8	8.5	8.6	8.0	7.8
Final	8.0	8.4	7.7	8.1	8.4	8.2	7.8
pH Initial	7.4	7.5	7.5	7.4	7.8	8.1	7.6
Final	8.1	8.1	7.9	8.2	8.2	8.0	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	270	280	270	320	340	340	340
Chlorine	NA	NA	NA	NA	NA	NA	NA

May 9, 2013

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

Control No. 166946-1

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



May 9, 2013
Control No. 166946-1
Page 2 of 30

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* Survival and Growth Test: The test will need to be repeated due to a high percent minimum significant difference (PMSD). It is believed this occurred due to pathogen interference.

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: The No Observable Effects Concentration (NOEC) for survival occurred at 87 % effluent, which is above the critical dilution of 65 %. The NOEC for reproduction occurred at 87 % effluent, which is above the critical dilution of 65 %. **The sample, therefore, PASSED 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

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	0.307	PASS
Control Growth CV < or = 40%	14.5	PASS
Growth Minimum Significant Difference 12 to 30%	61.7	FAIL
Critical Dilution CV < or = 40%	73.3	FAIL

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	16.4	PASS
Control CV < or = 40% per Surviving Female	23.2	PASS
Reproduction Minimum Significant Difference 13 to 47%	26.4	PASS
Critical Dilution CV < or = 40%	21.7	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)	8.1	8.2	7.9
pH (standard units)	7.3	7.3	7.8
Alkalinity (mg/l as CaCO ₃)	57	57	66
Hardness (mg/l as CaCO ₃)	60	63	71
Conductivity (umhos/cm)	270	290	320
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.22	0.89	0.13

2. Dilution Water Samples: Synthetic Soft Water #3984
 - a. Dates Prepared: April 29 through May 13, 2013
 - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.0	7.6	8.1
pH (standard units)	7.6	7.9	8.1
Alkalinity (mg/l as CaCO ₃)	31	31	31
Hardness (mg/l as CaCO ₃)	41	43	44
Conductivity (umhos/cm)	160	170	160
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: April 30, 2013 at 0930
Date & Time Test Terminated: May 7, 2013 at 0900
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: April 30, 2013 at 1205
Date & Time Test Terminated: May 7, 2013 at 1245
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 and Bartlett's test. 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 2, 2013 at 1330 to April 9, 2013 at 1130

The results were as follows: (Control No. 166187-1.)

Survival LC-50: 5995 mg/l

Growth IC-25: 5499 mg/l

Growth PMSD: 17.2

Ceriodaphnia dubia

Chronic reference tests are performed monthly.

A chronic reference test was performed on April 2, 2013 at 1510 to April 9, 2013 at 1500

The results were as follows: (Control No. 166187-2.)

Survival LC-50: 1732 mg/l

Growth IC-25: 1250 mg/l

Growth PMSD: 8.05

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	99.8	0.151
pH	SM 4500-H+ B	100	0.134
Conductivity	EPA 120.1	103	0.647

VI. Organism History

Pimephales promelas (Fathead minnow)

Date: April 30, 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

Ceriodaphnia dubia

Date: April 30, 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

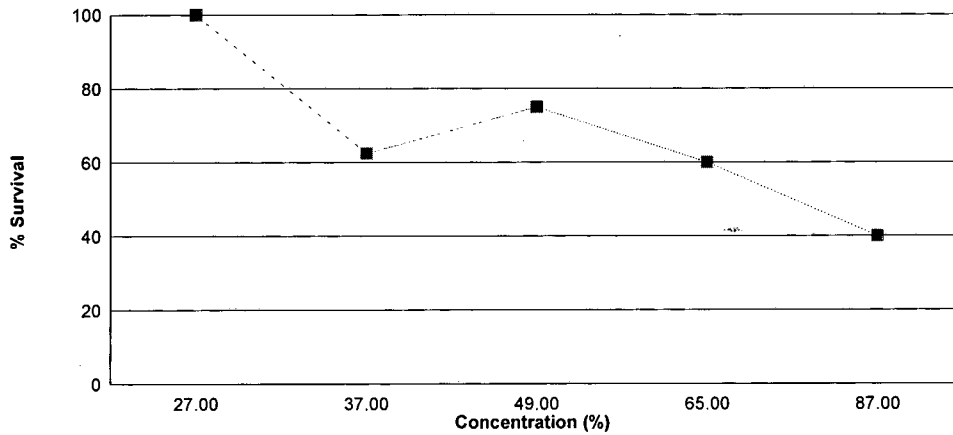
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

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 April 30, 2013 at 0930 and continued through May 7, 2013 at 0900. 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	0.307
27 %	100	0.253
37 %	62.5	0.178
49 %	75.0	0.252
65 %	60.0	0.183
87 %	40.0	0.121

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

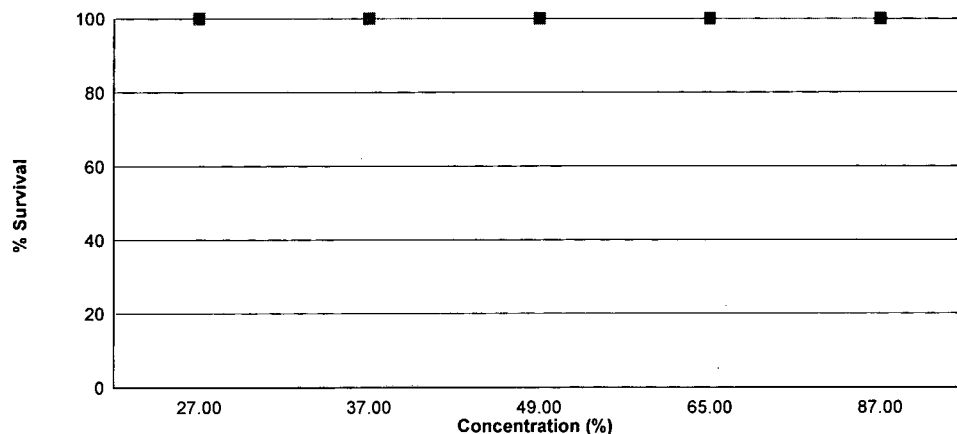
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

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 April 30, 2013 at 1205 and continued through May 7, 2013 at 1245. 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



Concentration	Percent Survival	Mean Reproduction
Control	100	16.4
27 %	100	17.4
37 %	100	17.3
49 %	100	17.1
65 %	100	16.2
87 %	100	16.4

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: April 30, 2013 at 0930

Date and Time Test Terminated: May 7, 2013 at 0900

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	6	2	1	1
	E	8	8	8	8	3	0	0
49 %	A	8	8	8	8	7	7	7
	B	8	8	8	8	8	8	8
	C	8	8	8	5	3	0	0
	D	8	8	8	8	8	8	7
	E	8	8	8	8	8	8	8
65 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	6	5	2
	C	8	8	8	8	8	8	7
	D	8	8	8	8	8	6	6
	E	8	8	8	4	1	1	1
87 %	A	8	8	7	1	0	0	0
	B	8	8	8	8	8	8	8
	C	8	8	8	4	0	0	0
	D	8	8	8	1	0	0	0
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: April 30, 2013 at 0930
Test Terminated: May 7, 2013 at 0900

Drying Started: May 2, 2013 at 1100
Drying Ended: May 8, 2013 at 1415

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.95640	.95851	0.00211	8	0.264
	B	.94833	.95082	0.00249	8	0.311
	C	.94811	.95090	0.00279	8	0.349
	D	.94889	.95170	0.00281	8	0.351
	E	.94926	.95133	0.00207	8	0.259
27 %	A	.95043	.95248	0.00205	8	0.256
	B	.95163	.95369	0.00206	8	0.258
	C	.95155	.95324	0.00169	8	0.211
	D	.95099	.95325	0.00226	8	0.282
	E	.95034	.95240	0.00206	8	0.258
37 %	A	.94903	.95126	0.00223	8	0.279
	B	.94850	.95105	0.00255	8	0.319
	C	.94767	.94996	0.00229	8	0.286
	D	.94611	.94617	0.00006	8	0.007
	E	.94537	.94537	0.00000	8	0.000
49 %	A	.94251	.94474	0.00223	8	0.279
	B	.94033	.94299	0.00266	8	0.332
	C	.93729	.93729	0.00000	8	0.000
	D	.93362	.93593	0.00231	8	0.289
	E	.93435	.93725	0.00290	8	0.362
65 %	A	.93431	.93657	0.00226	8	0.282
	B	.93524	.93586	0.00062	8	0.077
	C	.94679	.94900	0.00221	8	0.276
	D	.94774	.94997	0.00223	8	0.279
	E	.94952	.94953	0.00001	8	0.001
87 %	A	.94852	.94852	0.00000	8	0.000
	B	.94538	.94731	0.00193	8	0.241
	C	.94351	.94351	0.00000	8	0.000
	D	.94389	.94389	0.00000	8	0.000
	E	.94135	.94425	0.00290	8	0.362

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: April 30, 2013 at 1205

Date and Time Test Terminated: May 7, 2013 at 1245

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	2	2	0	4	3	0	2	0	0	4	17	10	1.70
5	6	3	5	3	4	5	3	6	6	5	46	10	4.60
6	0	0	6	0	0	5	0	4	4	0	19	10	1.90
7	9	10	0	12	9	0	10	11	9	12	82	10	8.20
8													
TOTAL	17	15	11	19	16	10	15	21	19	21	164	10	16.4

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	2	2	2	0	2	0	4	3	0	4	19	10	1.90
5	5	7	5	7	6	5	0	8	2	8	53	10	5.30
6	0	0	0	6	0	4	5	0	4	0	19	10	1.90
7	12	11	9	0	12	0	8	12	9	10	83	10	8.30
8													
TOTAL	19	20	16	13	20	9	17	23	15	22	174	10	17.4

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	2	2	4	0	0	0	2	2	0	4	16	10	1.60
5	5	8	6	3	5	5	6	6	6	8	58	10	5.80
6	0	0	0	5	4	5	0	0	4	0	18	10	1.80
7	10	9	12	0	0	9	10	12	9	10	81	10	8.10
8													
TOTAL	17	19	22	8	9	19	18	20	19	22	173	10	17.3

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: April 30, 2013 at 1205

Date and Time Test Terminated: May 7, 2013 at 1245

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	0	0	2	0	2	3	0	2	0	0	9	10	0.900
5	6	4	7	6	6	0	5	6	5	3	48	10	4.80
6	4	4	0	5	0	4	6	0	5	4	32	10	3.20
7	0	12	10	9	11	9	8	12	11	0	82	10	8.20
8													
TOTAL	10	20	19	20	19	16	19	20	21	7	171	10	17.1

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	0	2	2	0	0	2	4	2	2	2	16	10	1.60
5	4	4	6	3	4	5	0	5	4	6	41	10	4.10
6	4	0	0	6	5	0	4	0	0	0	19	10	1.90
7	0	7	12	8	9	12	8	11	9	10	86	10	8.60
8													
TOTAL	8	13	20	17	18	19	16	18	15	18	162	10	16.2

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	2	0	0	2	3	0	2	0	2	2	13	10	1.30
5	4	4	6	6	6	5	5	6	6	5	53	10	5.30
6	0	4	5	0	0	6	0	5	0	0	20	10	2.00
7	12	8	0	7	10	0	9	12	11	9	78	10	7.80
8													
TOTAL	18	16	11	15	19	11	16	23	19	16	164	10	16.4

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	0.12500	0.36137	
3	37 %	5	0.00000	0.17771	
4	49 %	1	0.87500	1.20940	
4	49 %	2	1.00000	1.39310	
4	49 %	3	0.00000	0.17771	
4	49 %	4	0.87500	1.20940	
4	49 %	5	1.00000	1.39310	
5	65 %	1	1.00000	1.39310	
5	65 %	2	0.25000	0.52360	
5	65 %	3	0.87500	1.20940	
5	65 %	4	0.75000	1.04720	
5	65 %	5	0.12500	0.36137	
6	87 %	1	0.00000	0.17771	
6	87 %	2	1.00000	1.39310	
6	87 %	3	0.00000	0.17771	
6	87 %	4	0.00000	0.17771	
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))
<p>D = 5.14 W = 0.9402 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		Transform: Arc Sin(Square Root(Y))
<p>Test can not be performed because at least one group has zero variance. Data FAIL to meet homogeneity of variance assumption.</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	37 %	22.50	16.00	5.00	
4	49 %	20.00	16.00	5.00	
5	65 %	17.50	16.00	5.00	
6	87 %	20.00	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 = 0.3857 W = 0.9626 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 = 14.00 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	0.1128	0.02256	1.404	
Within (Error)	24	0.3857	0.01607		
Total	29	0.4985			
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	0.3068	0.3068			
2	27 %	0.253	0.253	0.671		
3	37 %	0.1782	0.1782	1.604		
4	49 %	0.2524	0.2524	0.6785		
5	65 %	0.183	0.183	1.544		
6	87 %	0.1206	0.1206	2.322		
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	0.1892	61.7	0.0538	
3	37 %	5	0.1892	61.7	0.1286	
4	49 %	5	0.1892	61.7	0.0544	
5	65 %	5	0.1892	61.7	0.1238	
6	87 %	5	0.1892	61.7	0.1862	

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 = 0.1721 D* = 1.35 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 %	113.00	75.00	10.00	
3	37 %	115.50	75.00	10.00	
4	49 %	114.00	75.00	10.00	
5	65 %	103.00	75.00	10.00	
6	87 %	104.50	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	13.8	2.76	0.1569	
Within (Error)	54	949.8	17.59		
Total	59	963.6			
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	16.4	16.4			
2	27 %	17.4	17.4	-0.5332		
3	37 %	17.3	17.3	-0.4798		
4	49 %	17.1	17.1	-0.3732		
5	65 %	16.2	16.2	0.1066		
6	87 %	16.4	16.4	0		
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	4.333	26.4	-1	
3	37 %	10	4.333	26.4	-0.9	
4	49 %	10	4.333	26.4	-0.7	
5	65 %	10	4.333	26.4	0.2	
6	87 %	10	4.333	26.4	0	

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: April 30, 2013 at 0843

Date and Time Test Terminated: May 7, 2013 at 1245

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	7.8	7.6	8.2	8.1	7.6	8.0
	Final *1	7.4	7.1	7.7	7.3	7.6	7.8	6.5
	Final *2	7.6	8.0	8.2	7.8	7.7	7.9	7.5
pH, units	Initial	7.6	7.8	7.9	7.7	8.1	7.7	7.4
	Final *1	7.2	7.4	7.3	7.7	7.6	7.7	7.1
	Final *2	7.7	7.2	7.4	7.8	7.9	7.7	7.6
Alkalinity, mg CaCO ₃ /l		31	NA	31	NA	31	NA	NA
Hardness, mg CaCO ₃ /l		41	NA	43	NA	44	NA	NA
Conductivity, umhos/cm		160	160	170	190	160	210	160
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	7.9	7.6	7.6	8.1	7.8	7.7	8.2
	Final *1	6.9	6.9	7.9	7.0	7.5	7.5	6.7
	Final *2	7.6	7.8	8.1	8.0	7.2	8.0	8.0
pH, units	Initial	7.4	7.6	7.8	7.6	7.8	7.7	7.4
	Final *1	7.1	7.4	7.3	7.4	7.6	7.6	7.1
	Final *2	7.7	7.2	7.4	7.7	7.8	7.6	7.7

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.6	7.5	7.8	8.1	7.8	7.8	8.2
	Final *1	7.2	6.7	7.7	7.2	7.3	7.2	6.6
	Final *2	7.6	7.8	7.8	7.8	7.9	7.9	8.0
pH, units	Initial	7.5	7.5	7.7	7.5	7.7	7.8	7.4
	Final *1	7.1	7.4	7.3	7.4	7.6	7.6	7.2
	Final *2	7.7	7.3	7.4	7.7	7.8	7.6	7.7

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: April 30, 2013 at 0843

Date and Time Test Terminated: May 7, 2013 at 1245

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	7.8	7.8	8.2	8.0	7.6	8.1
	Final *1	7.3	6.9	7.6	7.1	7.5	7.3	6.6
	Final *2	7.7	7.6	8.1	8.0	8.0	8.1	7.4
pH, units	Initial	7.5	7.6	7.7	7.5	7.7	7.8	7.4
	Final *1	7.3	7.3	7.4	7.3	7.6	7.5	7.1
	Final *2	7.7	7.3	7.4	7.6	7.8	7.7	7.8

Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.1	7.4	7.6	8.0	7.7	7.6	8.1
	Final *1	7.2	6.6	7.6	7.4	7.4	7.5	6.3
	Final *2	7.9	7.6	8.0	7.8	7.7	8.2	7.9
pH, units	Initial	7.5	7.6	7.7	7.4	7.7	7.8	7.4
	Final *1	7.2	7.4	7.3	7.4	7.6	7.5	7.2
	Final *2	7.7	7.3	7.4	7.6	7.9	7.7	7.9
Alkalinity, mg CaCO ₃ /l	38	NA	46	NA	52	NA	NA	NA
Hardness, mg CaCO ₃ /l	54	NA	55	NA	59	NA	NA	NA
Conductivity, umhos/cm	230	240	240	260	250	290	260	260
Res. Chlorine, mg/l	<0.05	NA	<0.05	NA	<0.05	NA	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.2	7.8	8.1	7.8	NA	8.0
	Final *1	7.0	6.9	7.8	7.1	7.4	7.7	6.6
	Final *2	7.8	7.4	7.8	7.8	7.9	8.1	8.1
pH, units	Initial	7.3	7.5	7.6	7.8	7.7	NA	7.4
	Final *1	7.2	7.4	7.2	7.4	7.6	7.7	7.2
	Final *2	7.7	7.3	7.4	7.6	7.9	7.9	8.0

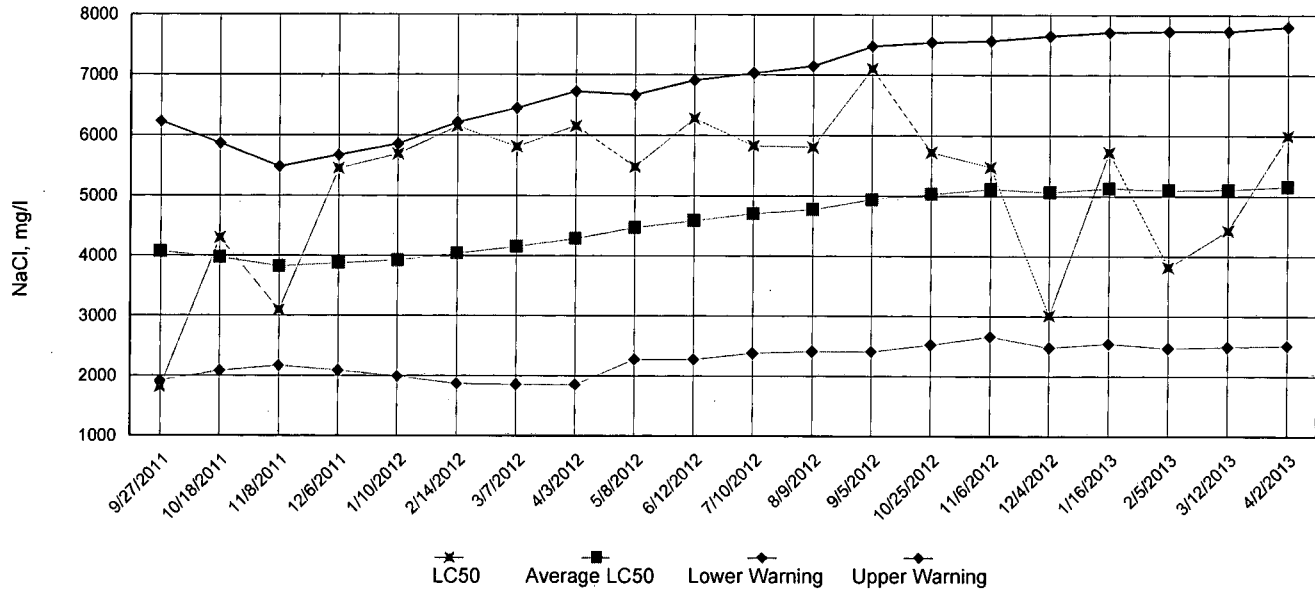
*1 = data from the *Pimephales promelas* (Fathead Minnow) test

*2 = data from the *Ceriodaphnia dubia* test

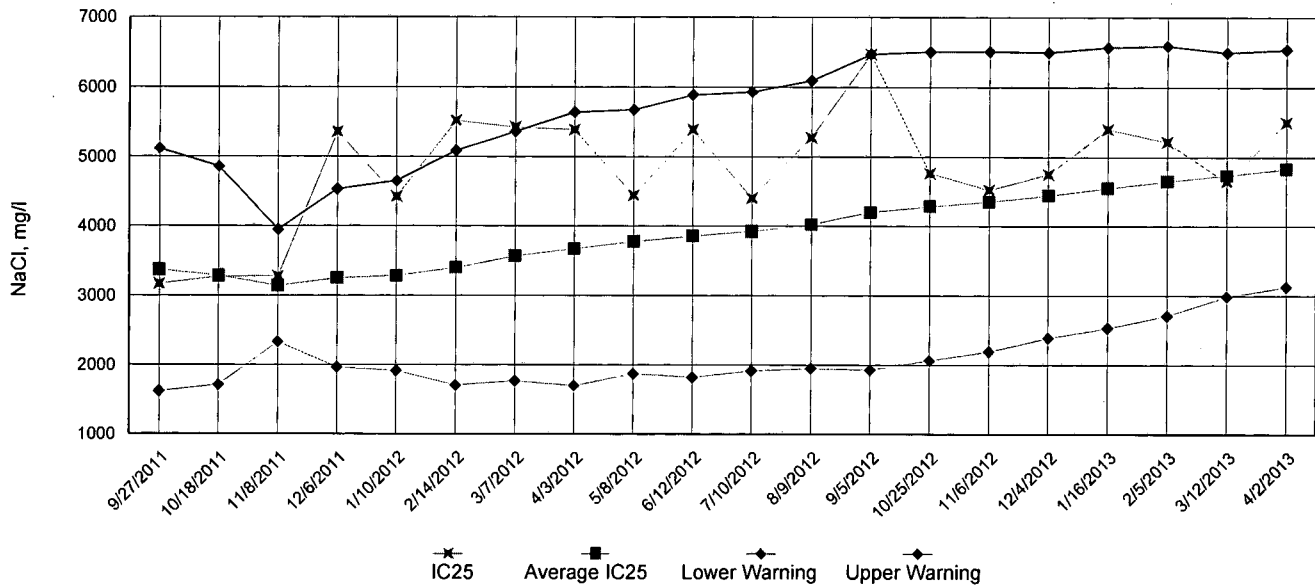
Appendix A4: Test 1000.0

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

LC50 Survival Data

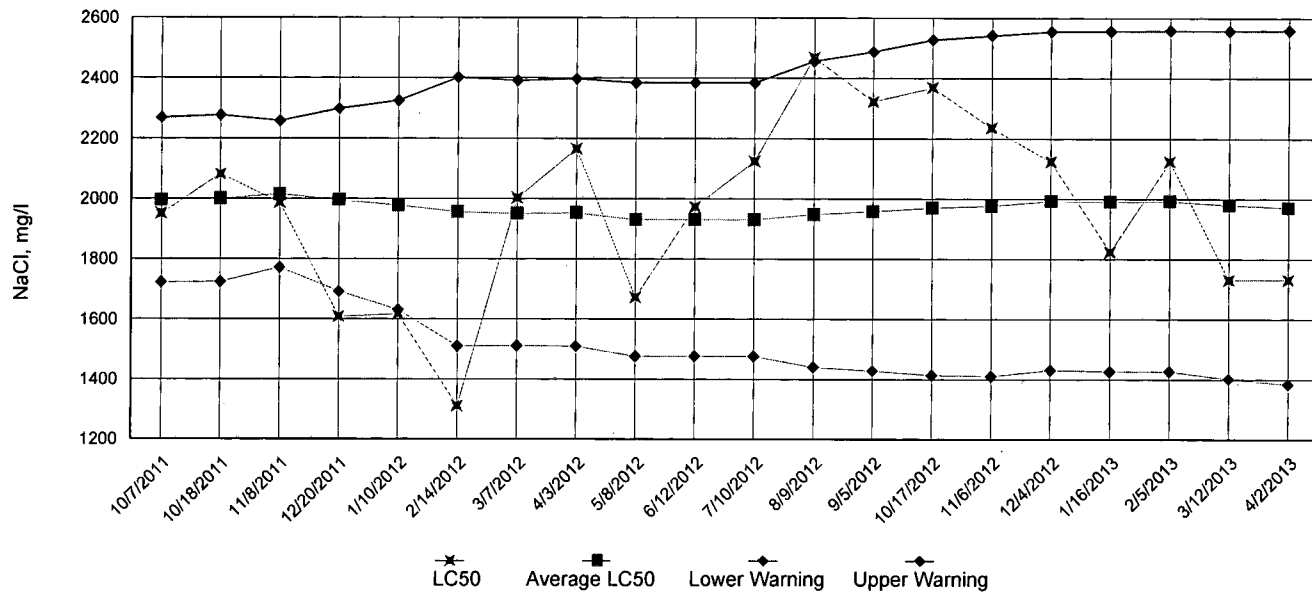


IC25 Growth Data

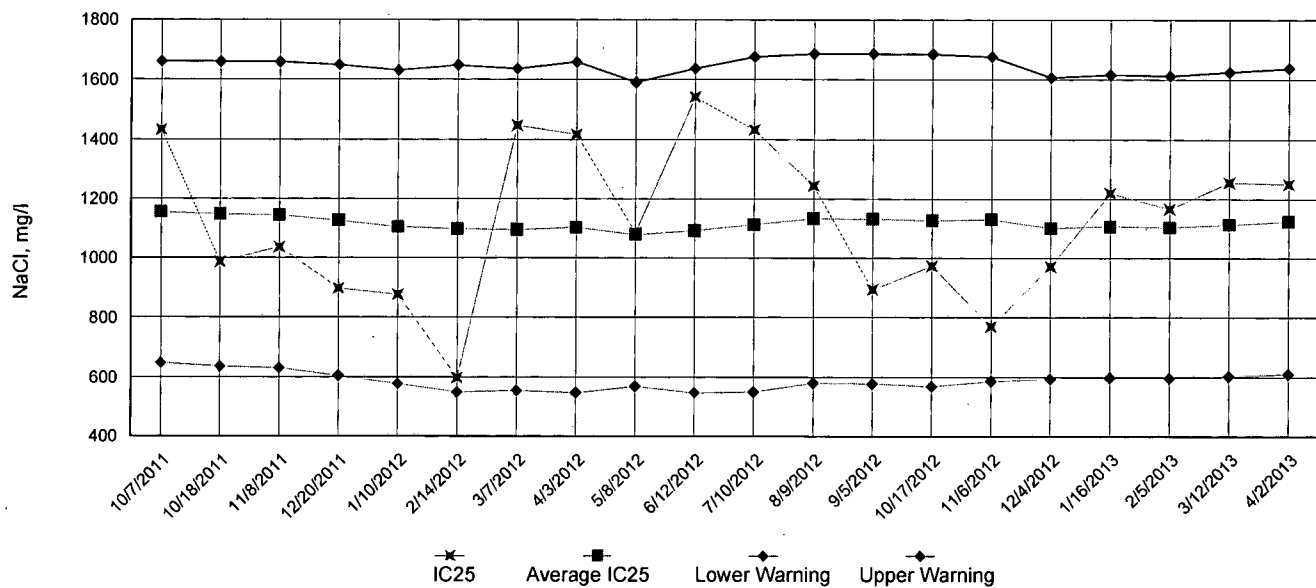


Appendix A4: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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: April 30, 2013 at 0930

Date and Time Test Terminated: May 7, 2013 at 0900

Dilution water used: Synthetic Soft Water #3984

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
37 %	100	100	100	12.5	0.00	100	100	62.5	82.5
49 %	87.5	100	0.00	87.5	100	100	100	75.0	56.5
65 %	100	25.0	87.5	75.0	12.5	100	100	60.0	64.9
87 %	0.00	100	0.00	0.00	100	100	100	40.0	137

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.264	0.311	0.349	0.351	0.259	0.307	14.5
27 %	0.256	0.258	0.211	0.282	0.258	0.253	10.2
37 %	0.279	0.319	0.286	0.007	0.000	0.178	89.9
49 %	0.279	0.332	0.000	0.289	0.362	0.252	57.4
65 %	0.282	0.077	0.276	0.279	0.001	0.183	73.3
87 %	0.000	0.241	0.000	0.000	0.362	0.121	141

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

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: 280, 298, 304, 307

2400
2400
2400

Test Initiated: DATE: April 30, 2013 TIME: 0930
Test Terminated: DATE: May 7, 2013 TIME: 0900

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.8	7.6	8.2	8.1	7.6	8.0
Final	7.4	7.1	7.7	7.3	7.6	7.8	6.5
pH Initial	7.6	7.8	7.9	7.7	8.1	7.7	7.4
Final	7.2	7.4	7.3	7.7	7.6	7.7	7.1
Alkalinity	31	NA	31	NA	31	NA	NA
Hardness	41	NA	43	NA	44	NA	NA
Conductivity	160	160	170	190	160	210	160
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 27 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.9	7.6	7.6	8.1	7.8	7.7	8.2
Final	6.9	6.9	7.9	7.0	7.5	7.5	6.7
pH Initial	7.4	7.6	7.8	7.6	7.8	7.7	7.4
Final	7.1	7.4	7.3	7.4	7.6	7.6	7.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	180	190	190	200	200	220	200
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 37 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.6	7.5	7.8	8.1	7.8	7.8	8.2
Final	7.2	6.7	7.7	7.2	7.3	7.2	6.6
pH Initial	7.5	7.5	7.7	7.5	7.7	7.8	7.4
Final	7.1	7.4	7.3	7.4	7.6	7.6	7.2
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	200	200	200	210	210	240	220
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 49 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.8	7.8	8.2	8.0	7.6	8.1
Final	7.3	6.9	7.6	7.1	7.5	7.3	6.6
pH Initial	7.5	7.6	7.7	7.5	7.7	7.8	7.4
Final	7.3	7.3	7.4	7.3	7.6	7.5	7.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	220	210	230	220	260	240
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 65 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	7.4	7.6	8.0	7.7	7.6	8.1
Final	7.2	6.6	7.6	7.4	7.4	7.5	6.3
pH Initial	7.5	7.6	7.7	7.4	7.7	7.8	7.4
Final	7.2	7.4	7.3	7.4	7.6	7.5	7.2
Alkalinity	38	NA	46	NA	52	NA	NA
Hardness	54	NA	55	NA	59	NA	NA
Conductivity	230	240	240	260	250	290	260
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 87 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.7	7.2	7.8	8.1	7.8	NA	8.0
Final	7.0	6.9	7.8	7.1	7.4	7.7	6.6
pH Initial	7.3	7.5	7.6	7.8	7.7	NA	7.4
Final	7.2	7.4	7.2	7.4	7.6	7.7	7.2
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	250	260	260	290	280	NA	300
Chlorine	NA	NA	NA	NA	NA	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: April 30, 2013 at 1205

Date and Time Test Terminated: May 7, 2013 at 1245

Dilution water used: Synthetic Soft Water #3984

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent				
		27 %	37 %	49 %	65 %	87 %
24 hour	100	100	100	100	100	100
48 hour	100	100	100	100	100	100
7 day	100	100	100	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

Replicates	Control	Percent Effluent				
		27 %	37 %	49 %	65 %	87 %
A	17	19	17	10	8	18
B	15	20	19	20	13	16
C	11	16	22	19	20	11
D	19	13	8	20	17	15
E	16	20	9	19	18	19
F	10	9	19	16	19	11
G	15	17	18	19	16	16
H	21	23	20	20	18	23
I	19	15	19	21	15	19
J	21	22	22	7	18	16
Mean per Adult	16.4	17.4	17.3	17.1	16.2	16.4
Mean per Surviving Adult	16.4	17.4	17.3	17.1	16.2	16.4
CV %	23.2	24.7	28.3	27.9	21.7	22.3

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 %)	<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	(65 %)	<u> </u> YES	<u> X </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]: 0 (TGP3B)
5. NOEC *Ceriodaphnia* Lethality: 87 % (TOP3B)
6. LOEC *Ceriodaphnia* Lethality: 87 % (TXP3B)
7. NOEC *Ceriodaphnia* Sublethality: 87 % (TPP3B)
8. LOEC *Ceriodaphnia* Sublethality: 87 % (TYP3B)
9. Coefficient of variation for *Ceriodaphnia* Reproduction: 23.2 (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: April 30, 2013 TIME: 1205
Test Terminated: DATE: May 7, 2013 TIME: 1245

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.8	7.6	8.2	8.1	7.6	8.0
Final	7.6	8.0	8.2	7.8	7.7	7.9	7.5
pH Initial	7.6	7.8	7.9	7.7	8.1	7.7	7.4
Final	7.7	7.2	7.4	7.8	7.9	7.7	7.6
Alkalinity	31	NA	31	NA	31	NA	NA
Hardness	41	NA	43	NA	44	NA	NA
Conductivity	160	160	170	190	160	210	160
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 27 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.9	7.6	7.6	8.1	7.8	7.7	8.2
Final	7.6	7.8	8.1	8.0	7.2	8.0	8.0
pH Initial	7.4	7.6	7.8	7.6	7.8	7.7	7.4
Final	7.7	7.2	7.4	7.7	7.8	7.6	7.7
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	180	190	190	200	200	220	200
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 37 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.6	7.5	7.8	8.1	7.8	7.8	8.2
Final	7.6	7.8	7.8	7.8	7.9	7.9	8.0
pH Initial	7.5	7.5	7.7	7.5	7.7	7.8	7.4
Final	7.7	7.3	7.4	7.7	7.8	7.6	7.7
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	200	200	200	210	210	240	220
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 49 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.8	7.8	8.2	8.0	7.6	8.1
Final	7.7	7.6	8.1	8.0	8.0	8.1	7.4
pH Initial	7.5	7.6	7.7	7.5	7.7	7.8	7.4
Final	7.7	7.3	7.4	7.6	7.8	7.7	7.8
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	220	210	230	220	260	240
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 65 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	7.4	7.6	8.0	7.7	7.6	8.1
Final	7.9	7.6	8.0	7.8	7.7	8.2	7.9
pH Initial	7.5	7.6	7.7	7.4	7.7	7.8	7.4
Final	7.7	7.3	7.4	7.6	7.9	7.7	7.9
Alkalinity	38	NA	46	NA	52	NA	NA
Hardness	54	NA	55	NA	59	NA	NA
Conductivity	230	240	240	260	250	290	260
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 87 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.7	7.2	7.8	8.1	7.8	NA	8.0
Final	7.8	7.4	7.8	7.8	7.9	8.1	8.1
pH Initial	7.3	7.5	7.6	7.8	7.7	NA	7.4
Final	7.7	7.3	7.4	7.6	7.9	7.9	8.0
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	250	260	260	290	280	NA	300
Chlorine	NA	NA	NA	NA	NA	NA	NA



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs			PO No. 13-2030		No of BOTTLES	Analyses Requested										AIC Control No: 166946				
Project Reference: Plant Effluent			Sample Matrix			Chronic.CD	Chronic.FH												AIC Proposal No:	
Project Manager: James Sorrells			GRA B	COM P	WATER	SOIL	BOTTLES	Chronic.CD	Chronic.FH										Carrier: Hot Springs SHARK	
Sampled By: A. Ross																				
AIC No.	Sample Identification	Date/Time Collected																		Remarks
2	Plant Effluent	4-30-13 0600-2400		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 = (NH4)2SO4					
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS					Relinquished By: <i>A. Thomas</i>			Date/Time: 5-1-13 @ 0915			Received By: <i>Allen Man</i>			Date/Time: 5-1-13 9:15						
Expedited results requested by: _____					Relinquished By: <i>Allen Man</i>			Date/Time: 5-1-13 1120			Received in Lab By: <i>Greg Hopton</i>			Date/Time: 5-1-13 1120						
Who should AIC contact with questions: _____					Comments:															
Phone: _____ Fax: _____																				
Report Attention to: Mr. James Sorrells																				
Report Address to: 320 Davidson Road Hot Springs, AR 71901																				



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs			PO No. 13-2030		No of BOTTLES	Analyses Requested										AIC Control No: 116946						
Project Reference: Plant Effluent			Sample Matrix			Chronic CD Chronic FH											AIC Proposal No:					
Project Manager: James Sorrells			W	A			S	O	I	L											Carrier: Hot Springs Shuttle	
Sampled By: H. MAULDIN			G	C						3	x											Received Temperature °C: 2
AIC No.	Sample Identification	Date/Time Collected	A	M	P																	Remarks
3	PLANT EFFLUENT @ 0000-2100	5/2/13	X	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 = (NH4)2SO4		
Turnaround Time Requested: (Please circle) <u>NORMAL</u> or EXPEDITED IN _____ DAYS						Relinquished By: <u>H. Mauldin</u>		Date/Time: <u>5/3/13 @ 11:15</u>		Received By: <u>Mr. Mann</u>		Date/Time: <u>5/3/13 @ 11:15</u>										
Expedited results requested by: <u>SORRELLS</u>						Relinquished By: <u>M. Mann</u>		Date/Time: <u>5/3/13 @ 12:20</u>		Received in Lab By: <u>Steve Pulane</u>		Date/Time: <u>5-3-13 12:20</u>										
Who should AIC contact with questions: <u>SORRELLS</u>						Comments:																
Phone: <u>501-261-1125</u> Fax: <u>501-262-0339</u>																						
Report Attention to: <u>Mr. James Sorrells</u>																						
Report Address to: 320 Davidson Road Hot Springs, AR 71901																						



May 9, 2013
Control No. 166946-1
Page 1 of 30

May 9, 2013

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

Control No. 166946-1

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:

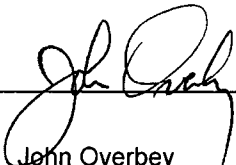
This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire 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* Survival and Growth Test: The test will need to be repeated due to a high percent minimum significant difference (PMSD). It is believed this occurred due to pathogen interference.

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: The No Observable Effects Concentration (NOEC) for survival occurred at 87 % effluent, which is above the critical dilution of 65 %. The NOEC for reproduction occurred at 87 % effluent, which is above the critical dilution of 65 %. **The sample, therefore, PASSED 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

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	0.307	PASS
Control Growth CV < or = 40%	14.5	PASS
Growth Minimum Significant Difference 12 to 30%	61.7	FAIL
Critical Dilution CV < or = 40%	73.3	FAIL

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	16.4	PASS
Control CV < or = 40% per Surviving Female	23.2	PASS
Reproduction Minimum Significant Difference 13 to 47%	26.4	PASS
Critical Dilution CV < or = 40%	21.7	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)	8.1	8.2	7.9
pH (standard units)	7.3	7.3	7.8
Alkalinity (mg/l as CaCO ₃)	57	57	66
Hardness (mg/l as CaCO ₃)	60	63	71
Conductivity (umhos/cm)	270	290	320
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.22	0.89	0.13

2. Dilution Water Samples: Synthetic Soft Water #3984
 - a. Dates Prepared: April 29 through May 13, 2013
 - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.0	7.6	8.1
pH (standard units)	7.6	7.9	8.1
Alkalinity (mg/l as CaCO ₃)	31	31	31
Hardness (mg/l as CaCO ₃)	41	43	44
Conductivity (umhos/cm)	160	170	160
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: April 30, 2013 at 0930
Date & Time Test Terminated: May 7, 2013 at 0900
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: April 30, 2013 at 1205
Date & Time Test Terminated: May 7, 2013 at 1245
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 and Bartlett's test. 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 2, 2013 at 1330 to April 9, 2013 at 1130

The results were as follows: (Control No. 166187-1.)

Survival LC-50: 5995 mg/l

Growth IC-25: 5499 mg/l

Growth PMSD: 17.2

Ceriodaphnia dubia

Chronic reference tests are performed monthly.

A chronic reference test was performed on April 2, 2013 at 1510 to April 9, 2013 at 1500

The results were as follows: (Control No. 166187-2.)

Survival LC-50: 1732 mg/l

Growth IC-25: 1250 mg/l

Growth PMSD: 8.05

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	99.8	0.151
pH	SM 4500-H+ B	100	0.134
Conductivity	EPA 120.1	103	0.647

VI. Organism History

Pimephales promelas (Fathead minnow)

Date: April 30, 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

Ceriodaphnia dubia

Date: April 30, 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

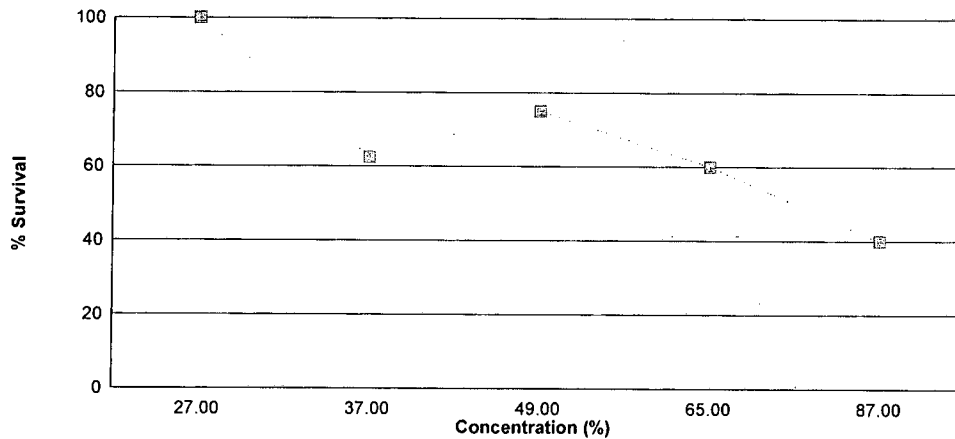
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

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 April 30, 2013 at 0930 and continued through May 7, 2013 at 0900. 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	0.307
27 %	100	0.253
37 %	62.5	0.178
49 %	75.0	0.252
65 %	60.0	0.183
87 %	40.0	0.121

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

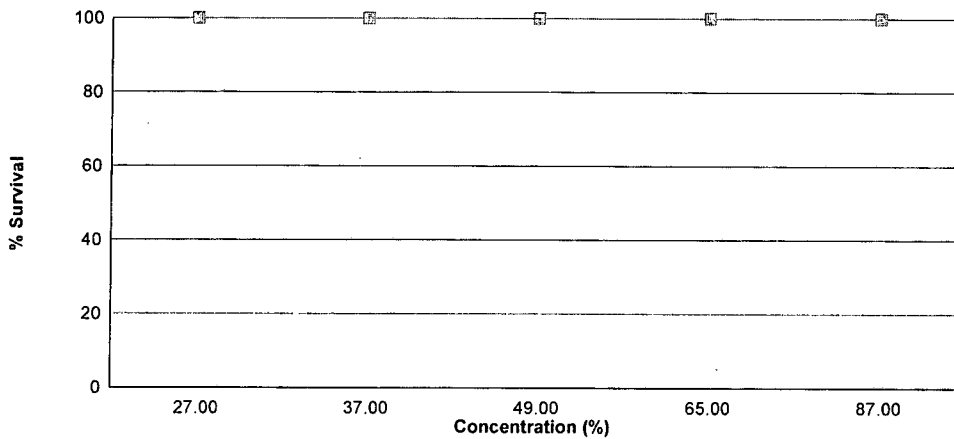
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

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 April 30, 2013 at 1205 and continued through May 7, 2013 at 1245. 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 <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	16.4
27 %	100	17.4
37 %	100	17.3
49 %	100	17.1
65 %	100	16.2
87 %	100	16.4

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: April 30, 2013 at 0930

Date and Time Test Terminated: May 7, 2013 at 0900

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	6	2	1	1
	E	8	8	8	8	3	0	0
49 %	A	8	8	8	8	7	7	7
	B	8	8	8	8	8	8	8
	C	8	8	8	5	3	0	0
	D	8	8	8	8	8	8	7
	E	8	8	8	8	8	8	8
65 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	6	5	2
	C	8	8	8	8	8	8	7
	D	8	8	8	8	8	6	6
	E	8	8	8	4	1	1	1
87 %	A	8	8	7	1	0	0	0
	B	8	8	8	8	8	8	8
	C	8	8	8	4	0	0	0
	D	8	8	8	1	0	0	0
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: April 30, 2013 at 0930
Test Terminated: May 7, 2013 at 0900

Drying Started: May 2, 2013 at 1100
Drying Ended: May 8, 2013 at 1415

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.95640	.95851	0.00211	8	0.264
	B	.94833	.95082	0.00249	8	0.311
	C	.94811	.95090	0.00279	8	0.349
	D	.94889	.95170	0.00281	8	0.351
	E	.94926	.95133	0.00207	8	0.259
27 %	A	.95043	.95248	0.00205	8	0.256
	B	.95163	.95369	0.00206	8	0.258
	C	.95155	.95324	0.00169	8	0.211
	D	.95099	.95325	0.00226	8	0.282
	E	.95034	.95240	0.00206	8	0.258
37 %	A	.94903	.95126	0.00223	8	0.279
	B	.94850	.95105	0.00255	8	0.319
	C	.94767	.94996	0.00229	8	0.286
	D	.94611	.94617	0.00006	8	0.007
	E	.94537	.94537	0.00000	8	0.000
49 %	A	.94251	.94474	0.00223	8	0.279
	B	.94033	.94299	0.00266	8	0.332
	C	.93729	.93729	0.00000	8	0.000
	D	.93362	.93593	0.00231	8	0.289
	E	.93435	.93725	0.00290	8	0.362
65 %	A	.93431	.93657	0.00226	8	0.282
	B	.93524	.93586	0.00062	8	0.077
	C	.94679	.94900	0.00221	8	0.276
	D	.94774	.94997	0.00223	8	0.279
	E	.94952	.94953	0.00001	8	0.001
87 %	A	.94852	.94852	0.00000	8	0.000
	B	.94538	.94731	0.00193	8	0.241
	C	.94351	.94351	0.00000	8	0.000
	D	.94389	.94389	0.00000	8	0.000
	E	.94135	.94425	0.00290	8	0.362

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: April 30, 2013 at 1205

Date and Time Test Terminated: May 7, 2013 at 1245

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	2	2	0	4	3	0	2	0	0	4	17	10	1.70	
5	6	3	5	3	4	5	3	6	6	5	46	10	4.60	
6	0	0	6	0	0	5	0	4	4	0	19	10	1.90	
7	9	10	0	12	9	0	10	11	9	12	82	10	8.20	
8														
TOTAL	17	15	11	19	16	10	15	21	19	21	164	10	16.4	

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	2	2	2	0	2	0	4	3	0	4	19	10	1.90	
5	5	7	5	7	6	5	0	8	2	8	53	10	5.30	
6	0	0	0	6	0	4	5	0	4	0	19	10	1.90	
7	12	11	9	0	12	0	8	12	9	10	83	10	8.30	
8														
TOTAL	19	20	16	13	20	9	17	23	15	22	174	10	17.4	

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	2	2	4	0	0	0	2	2	0	4	16	10	1.60	
5	5	8	6	3	5	5	6	6	6	8	58	10	5.80	
6	0	0	0	5	4	5	0	0	4	0	18	10	1.80	
7	10	9	12	0	0	9	10	12	9	10	81	10	8.10	
8														
TOTAL	17	19	22	8	9	19	18	20	19	22	173	10	17.3	

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: April 30, 2013 at 1205

Date and Time Test Terminated: May 7, 2013 at 1245

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	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	0	2	0	2	3	0	2	0	0	9	10	0.900	
5	6	4	7	6	6	0	5	6	5	3	48	10	4.80	
6	4	4	0	5	0	4	6	0	5	4	32	10	3.20	
7	0	12	10	9	11	9	8	12	11	0	82	10	8.20	
8														
TOTAL	10	20	19	20	19	16	19	20	21	7	171	10	17.1	

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	0	2	2	0	0	2	4	2	2	2	16	10	1.60	
5	4	4	6	3	4	5	0	5	4	6	41	10	4.10	
6	4	0	0	6	5	0	4	0	0	0	19	10	1.90	
7	0	7	12	8	9	12	8	11	9	10	86	10	8.60	
8														
TOTAL	8	13	20	17	18	19	16	18	15	18	162	10	16.2	

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	2	0	0	2	3	0	2	0	2	2	13	10	1.30	
5	4	4	6	6	6	5	5	6	6	5	53	10	5.30	
6	0	4	5	0	0	6	0	5	0	0	20	10	2.00	
7	12	8	0	7	10	0	9	12	11	9	78	10	7.80	
8														
TOTAL	18	16	11	15	19	11	16	23	19	16	164	10	16.4	

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	0.12500	0.36137
3	37 %	5	0.00000	0.17771
4	49 %	1	0.87500	1.20940
4	49 %	2	1.00000	1.39310
4	49 %	3	0.00000	0.17771
4	49 %	4	0.87500	1.20940
4	49 %	5	1.00000	1.39310
5	65 %	1	1.00000	1.39310
5	65 %	2	0.25000	0.52360
5	65 %	3	0.87500	1.20940
5	65 %	4	0.75000	1.04720
5	65 %	5	0.12500	0.36137
6	87 %	1	0.00000	0.17771
6	87 %	2	1.00000	1.39310
6	87 %	3	0.00000	0.17771
6	87 %	4	0.00000	0.17771
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 = 5.14		
W = 0.9402		
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		Transform: Arc Sin(Square Root(Y))
Test can not be performed because at least one group has zero variance. Data FAIL to meet homogeneity of variance assumption.		

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 %	22.50	16.00	5.00	
4	49 %	20.00	16.00	5.00	
5	65 %	17.50	16.00	5.00	
6	87 %	20.00	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 = 0.3857 W = 0.9626 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 = 14.00 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	0.1128	0.02256	1.404	
Within (Error)	24	0.3857	0.01607		
Total	29	0.4985			
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	0.3068	0.3068			
2	27 %	0.253	0.253	0.671		
3	37 %	0.1782	0.1782	1.604		
4	49 %	0.2524	0.2524	0.6785		
5	65 %	0.183	0.183	1.544		
6	87 %	0.1206	0.1206	2.322		
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	0.1892	61.7	0.0538	
3	37 %	5	0.1892	61.7	0.1286	
4	49 %	5	0.1892	61.7	0.0544	
5	65 %	5	0.1892	61.7	0.1238	
6	87 %	5	0.1892	61.7	0.1862	

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
<p>D = 0.1721 D* = 1.35 Critical D* = 1.035 (alpha = 0.01, N = 60)</p> <p>Data FAIL normality test (alpha = 0.01).</p>	

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 %	113.00	75.00	10.00	
3	37 %	115.50	75.00	10.00	
4	49 %	114.00	75.00	10.00	
5	65 %	103.00	75.00	10.00	
6	87 %	104.50	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	13.8	2.76	0.1569	
Within (Error)	54	949.8	17.59		
Total	59	963.6			
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	16.4	16.4			
2	27 %	17.4	17.4	-0.5332		
3	37 %	17.3	17.3	-0.4798		
4	49 %	17.1	17.1	-0.3732		
5	65 %	16.2	16.2	0.1066		
6	87 %	16.4	16.4	0		
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	4.333	26.4	-1	
3	37 %	10	4.333	26.4	-0.9	
4	49 %	10	4.333	26.4	-0.7	
5	65 %	10	4.333	26.4	0.2	
6	87 %	10	4.333	26.4	0	

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: April 30, 2013 at 0843

Date and Time Test Terminated: May 7, 2013 at 1245

Effluent Conc.: Control	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
DO, mg/l	Initial	8.0	7.8	7.6	8.2	8.1	7.6	8.0
	Final *1	7.4	7.1	7.7	7.3	7.6	7.8	6.5
	Final *2	7.6	8.0	8.2	7.8	7.7	7.9	7.5
pH, units	Initial	7.6	7.8	7.9	7.7	8.1	7.7	7.4
	Final *1	7.2	7.4	7.3	7.7	7.6	7.7	7.1
	Final *2	7.7	7.2	7.4	7.8	7.9	7.7	7.6
Alkalinity, mg CaCO ₃ /l	31	NA	31	NA	31	NA	NA	
Hardness, mg CaCO ₃ /l	41	NA	43	NA	44	NA	NA	
Conductivity, umhos/cm	160	160	170	190	160	210	160	
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	7.9	7.6	7.6	8.1	7.8	7.7	8.2
	Final *1	6.9	6.9	7.9	7.0	7.5	7.5	6.7
	Final *2	7.6	7.8	8.1	8.0	7.2	8.0	8.0
pH, units	Initial	7.4	7.6	7.8	7.6	7.8	7.7	7.4
	Final *1	7.1	7.4	7.3	7.4	7.6	7.6	7.1
	Final *2	7.7	7.2	7.4	7.7	7.8	7.6	7.7

Effluent Conc.: 37 %	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
DO, mg/l	Initial	7.6	7.5	7.8	8.1	7.8	7.8	8.2
	Final *1	7.2	6.7	7.7	7.2	7.3	7.2	6.6
	Final *2	7.6	7.8	7.8	7.8	7.9	7.9	8.0
pH, units	Initial	7.5	7.5	7.7	7.5	7.7	7.8	7.4
	Final *1	7.1	7.4	7.3	7.4	7.6	7.6	7.2
	Final *2	7.7	7.3	7.4	7.7	7.8	7.6	7.7

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: April 30, 2013 at 0843

Date and Time Test Terminated: May 7, 2013 at 1245

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	7.8	7.8	8.2	8.0	7.6	8.1
	Final *1	7.3	6.9	7.6	7.1	7.5	7.3	6.6
	Final *2	7.7	7.6	8.1	8.0	8.0	8.1	7.4
pH, units	Initial	7.5	7.6	7.7	7.5	7.7	7.8	7.4
	Final *1	7.3	7.3	7.4	7.3	7.6	7.5	7.1
	Final *2	7.7	7.3	7.4	7.6	7.8	7.7	7.8

Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.1	7.4	7.6	8.0	7.7	7.6	8.1
	Final *1	7.2	6.6	7.6	7.4	7.4	7.5	6.3
	Final *2	7.9	7.6	8.0	7.8	7.7	8.2	7.9
pH, units	Initial	7.5	7.6	7.7	7.4	7.7	7.8	7.4
	Final *1	7.2	7.4	7.3	7.4	7.6	7.5	7.2
	Final *2	7.7	7.3	7.4	7.6	7.9	7.7	7.9
Alkalinity, mg CaCO ₃ /l	38	NA	46	NA	52	NA	NA	NA
Hardness, mg CaCO ₃ /l	54	NA	55	NA	59	NA	NA	NA
Conductivity, umhos/cm	230	240	240	260	250	290	260	260
Res. Chlorine, mg/l	<0.05	NA	<0.05	NA	<0.05	NA	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.2	7.8	8.1	7.8	NA	8.0
	Final *1	7.0	6.9	7.8	7.1	7.4	7.7	6.6
	Final *2	7.8	7.4	7.8	7.8	7.9	8.1	8.1
pH, units	Initial	7.3	7.5	7.6	7.8	7.7	NA	7.4
	Final *1	7.2	7.4	7.2	7.4	7.6	7.7	7.2
	Final *2	7.7	7.3	7.4	7.6	7.9	7.9	8.0

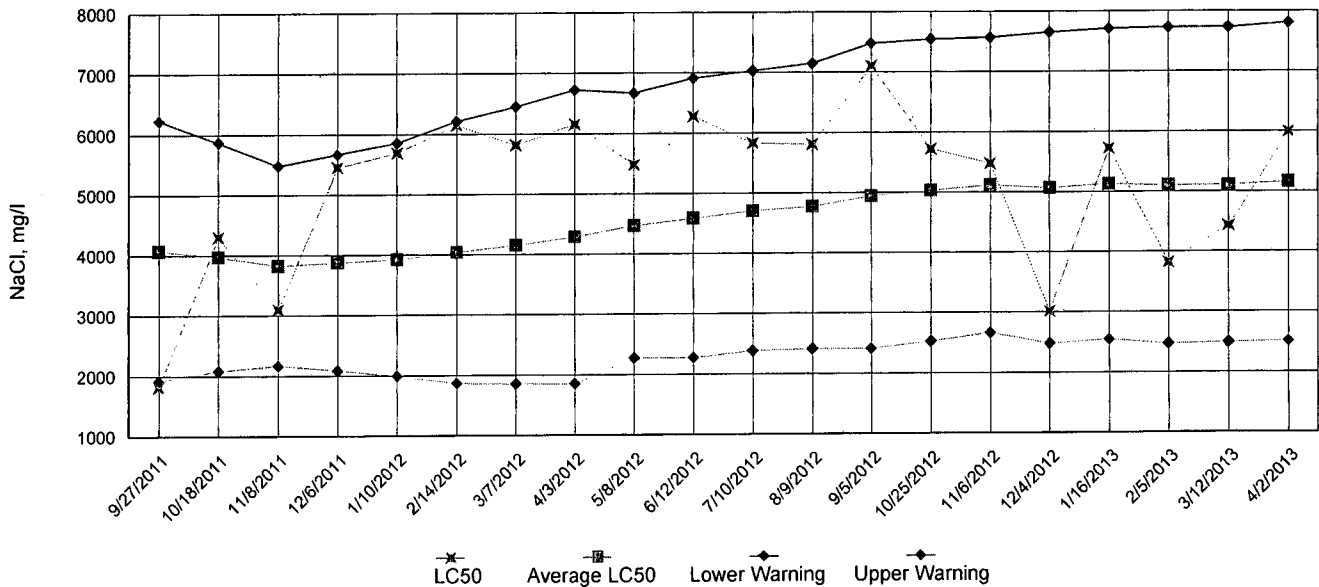
*1 = data from the *Pimephales promelas* (Fathead Minnow) test

*2 = data from the *Ceriodaphnia dubia* test

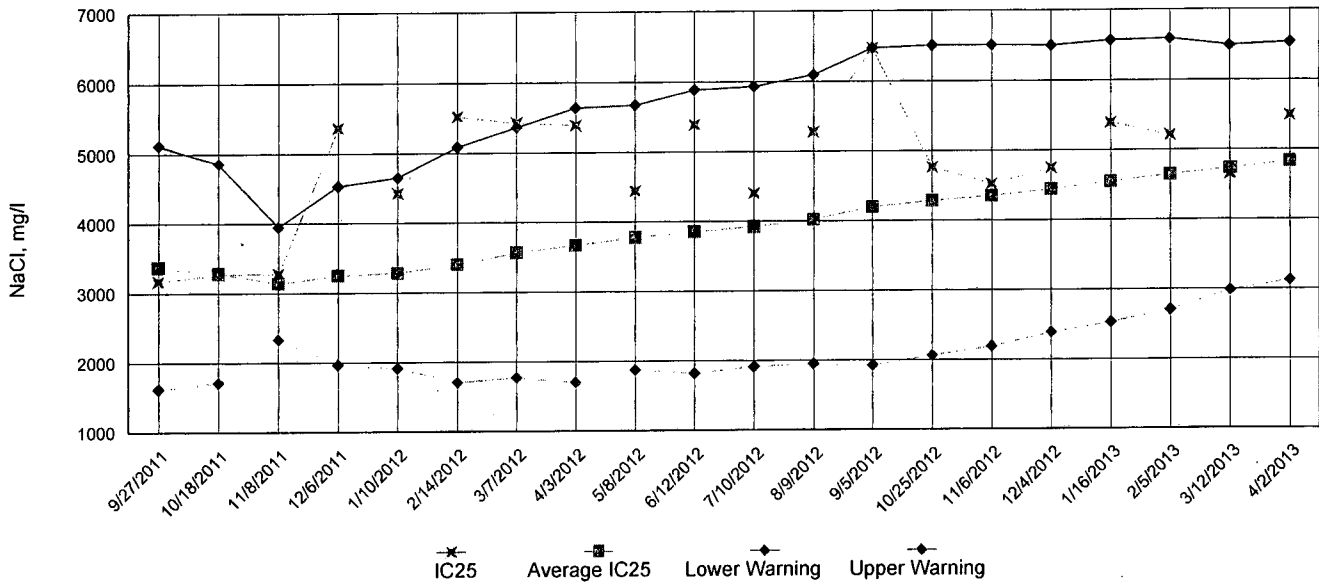
Appendix A4: Test 1000.0

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

LC50 Survival Data

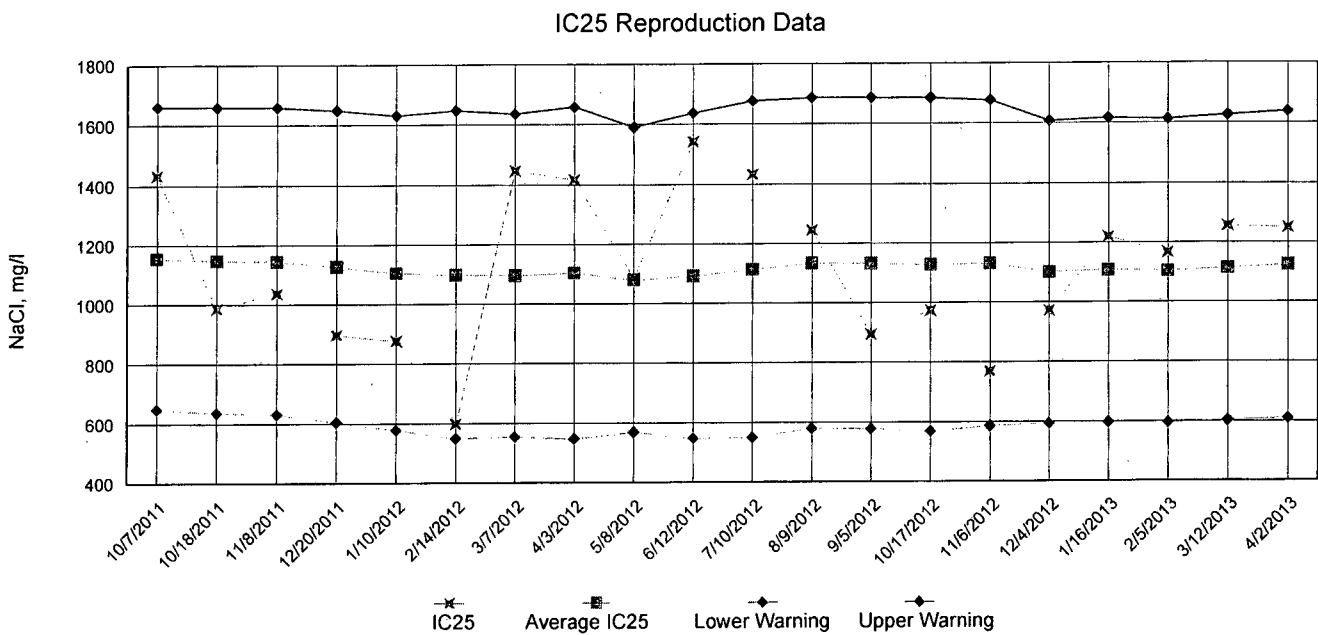
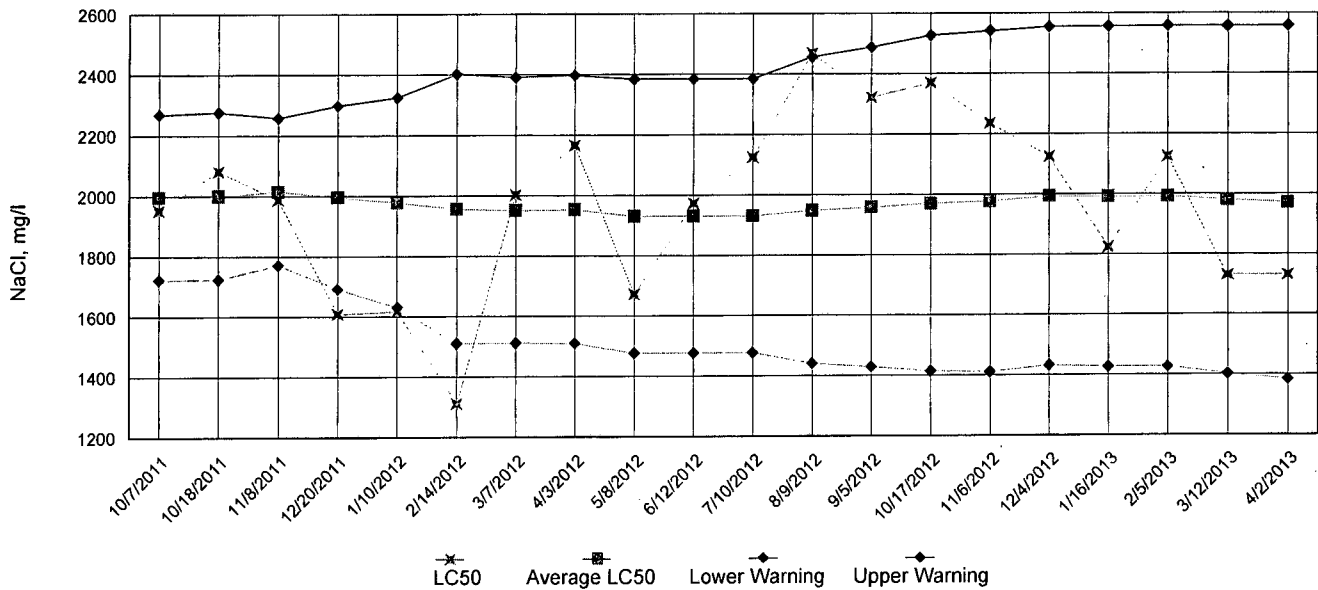


IC25 Growth Data



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

LC50 Survival Data



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: April 30, 2013 at 0930

Date and Time Test Terminated: May 7, 2013 at 0900

Dilution water used: Synthetic Soft Water #3984

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
37 %	100	100	100	12.5	0.00	100	100	62.5	82.5
49 %	87.5	100	0.00	87.5	100	100	100	75.0	56.5
65 %	100	25.0	87.5	75.0	12.5	100	100	60.0	64.9
87 %	0.00	100	0.00	0.00	100	100	100	40.0	137

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.264	0.311	0.349	0.351	0.259	0.307	14.5
27 %	0.256	0.258	0.211	0.282	0.258	0.253	10.2
37 %	0.279	0.319	0.286	0.007	0.000	0.178	89.9
49 %	0.279	0.332	0.000	0.289	0.362	0.252	57.4
65 %	0.282	0.077	0.276	0.279	0.001	0.183	73.3
87 %	0.000	0.241	0.000	0.000	0.362	0.121	141

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

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: 280, 298, 304, 307

2400
2400
2400

Test Initiated: DATE: April 30, 2013 TIME: 0930
Test Terminated: DATE: May 7, 2013 TIME: 0900

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	8.0	7.8	7.6	8.2	8.1	7.6	8.0
Final	7.4	7.1	7.7	7.3	7.6	7.8	6.5
pH Initial	7.6	7.8	7.9	7.7	8.1	7.7	7.4
Final	7.2	7.4	7.3	7.7	7.6	7.7	7.1
Alkalinity	31	NA	31	NA	31	NA	NA
Hardness	41	NA	43	NA	44	NA	NA
Conductivity	160	160	170	190	160	210	160
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
27 %							
D.O. Initial	7.9	7.6	7.6	8.1	7.8	7.7	8.2
Final	6.9	6.9	7.9	7.0	7.5	7.5	6.7
pH Initial	7.4	7.6	7.8	7.6	7.8	7.7	7.4
Final	7.1	7.4	7.3	7.4	7.6	7.6	7.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	180	190	190	200	200	220	200
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
37 %							
D.O. Initial	7.6	7.5	7.8	8.1	7.8	7.8	8.2
Final	7.2	6.7	7.7	7.2	7.3	7.2	6.6
pH Initial	7.5	7.5	7.7	7.5	7.7	7.8	7.4
Final	7.1	7.4	7.3	7.4	7.6	7.6	7.2
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	200	200	200	210	210	240	220
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
49 %							
D.O. Initial	8.0	7.8	7.8	8.2	8.0	7.6	8.1
Final	7.3	6.9	7.6	7.1	7.5	7.3	6.6
pH Initial	7.5	7.6	7.7	7.5	7.7	7.8	7.4
Final	7.3	7.3	7.4	7.3	7.6	7.5	7.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	220	210	230	220	260	240
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
65 %							
D.O. Initial	8.1	7.4	7.6	8.0	7.7	7.6	8.1
Final	7.2	6.6	7.6	7.4	7.4	7.5	6.3
pH Initial	7.5	7.6	7.7	7.4	7.7	7.8	7.4
Final	7.2	7.4	7.3	7.4	7.6	7.5	7.2
Alkalinity	38	NA	46	NA	52	NA	NA
Hardness	54	NA	55	NA	59	NA	NA
Conductivity	230	240	240	260	250	290	260
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
87 %							
D.O. Initial	7.7	7.2	7.8	8.1	7.8	NA	8.0
Final	7.0	6.9	7.8	7.1	7.4	7.7	6.6
pH Initial	7.3	7.5	7.6	7.8	7.7	NA	7.4
Final	7.2	7.4	7.2	7.4	7.6	7.7	7.2
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	250	260	260	290	280	NA	300
Chlorine	NA	NA	NA	NA	NA	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: April 30, 2013 at 1205

Date and Time Test Terminated: May 7, 2013 at 1245

Dilution water used: Synthetic Soft Water #3984

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent				
		27 %	37 %	49 %	65 %	87 %
24 hour	100	100	100	100	100	100
48 hour	100	100	100	100	100	100
7 day	100	100	100	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

Replicates	Control	Percent Effluent				
		27 %	37 %	49 %	65 %	87 %
A	17	19	17	10	8	18
B	15	20	19	20	13	16
C	11	16	22	19	20	11
D	19	13	8	20	17	15
E	16	20	9	19	18	19
F	10	9	19	16	19	11
G	15	17	18	19	16	16
H	21	23	20	20	18	23
I	19	15	19	21	15	19
J	21	22	22	7	18	16
Mean per Adult	16.4	17.4	17.3	17.1	16.2	16.4
Mean per Surviving Adult	16.4	17.4	17.3	17.1	16.2	16.4
CV %	23.2	24.7	28.3	27.9	21.7	22.3

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 %)	<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	(65 %)	<u> </u> YES	<u> X </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]: 0 (TGP3B)
5. NOEC *Ceriodaphnia* Lethality: 87 % (TOP3B)
6. LOEC *Ceriodaphnia* Lethality: 87 % (TXP3B)
7. NOEC *Ceriodaphnia* Sublethality: 87 % (TPP3B)
8. LOEC *Ceriodaphnia* Sublethality: 87 % (TYP3B)
9. Coefficient of variation for *Ceriodaphnia* Reproduction: 23.2 (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: April 30, 2013 TIME: 1205
Test Terminated: DATE: May 7, 2013 TIME: 1245

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	8.0	7.8	7.6	8.2	8.1	7.6	8.0
Final	7.6	8.0	8.2	7.8	7.7	7.9	7.5
pH Initial	7.6	7.8	7.9	7.7	8.1	7.7	7.4
Final	7.7	7.2	7.4	7.8	7.9	7.7	7.6
Alkalinity	31	NA	31	NA	31	NA	NA
Hardness	41	NA	43	NA	44	NA	NA
Conductivity	160	160	170	190	160	210	160
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
27 %							
D.O. Initial	7.9	7.6	7.6	8.1	7.8	7.7	8.2
Final	7.6	7.8	8.1	8.0	7.2	8.0	8.0
pH Initial	7.4	7.6	7.8	7.6	7.8	7.7	7.4
Final	7.7	7.2	7.4	7.7	7.8	7.6	7.7
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	180	190	190	200	200	220	200
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
37 %							
D.O. Initial	7.6	7.5	7.8	8.1	7.8	7.8	8.2
Final	7.6	7.8	7.8	7.8	7.9	7.9	8.0
pH Initial	7.5	7.5	7.7	7.5	7.7	7.8	7.4
Final	7.7	7.3	7.4	7.7	7.8	7.6	7.7
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	200	200	200	210	210	240	220
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
49 %							
D.O. Initial	8.0	7.8	7.8	8.2	8.0	7.6	8.1
Final	7.7	7.6	8.1	8.0	8.0	8.1	7.4
pH Initial	7.5	7.6	7.7	7.5	7.7	7.8	7.4
Final	7.7	7.3	7.4	7.6	7.8	7.7	7.8
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	220	210	230	220	260	240
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
65 %							
D.O. Initial	8.1	7.4	7.6	8.0	7.7	7.6	8.1
Final	7.9	7.6	8.0	7.8	7.7	8.2	7.9
pH Initial	7.5	7.6	7.7	7.4	7.7	7.8	7.4
Final	7.7	7.3	7.4	7.6	7.9	7.7	7.9
Alkalinity	38	NA	46	NA	52	NA	NA
Hardness	54	NA	55	NA	59	NA	NA
Conductivity	230	240	240	260	250	290	260
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
87 %							
D.O. Initial	7.7	7.2	7.8	8.1	7.8	NA	8.0
Final	7.8	7.4	7.8	7.8	7.9	8.1	8.1
pH Initial	7.3	7.5	7.6	7.8	7.7	NA	7.4
Final	7.7	7.3	7.4	7.6	7.9	7.9	8.0
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	250	260	260	290	280	NA	300
Chlorine	NA	NA	NA	NA	NA	NA	NA



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs			PO No. 13-2030		No of BOTTLES	Analyses Requested										AIC Control No: 106946										
Project Reference: Plant Effluent			Sample Matrix			Chronic.CD. Chronic.FH														AIC Proposal No:						
Project Manager: James Sorrells			GRA B	COMP	WATER SOIL	3	X											Carmer:								
Sampled By: A. ROSE																		Received Temperature °C: 2.3								
AIC No.	Sample Identification	Date/Time Collected																	Remarks							
1	Plant Effluent	4-28-13 2020-2400	X	X																						
Container Type						P											Field pH calibration									
Preservative						NO											on _____ @ _____									
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						Relinquished By: J. Thomason		Date/Time: 4-29-13 1025		Received By: M. Mann		Date/Time: 4-29-13 10:25am														
Expedited results requested by: _____						Relinquished By: M. Mann		Date/Time: 4-29-13 11:40am		Received in Lab By: [Signature]		Date/Time: 4-29-13 11:42am														
Who should AIC contact with questions: _____						Comments:																				
Phone: _____ Fax: _____																										
Report Attention to: Mr. James Sorrells																										
Report Address to: 320 Davidson Road Hot Springs, AR 71901																										



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs		PO No. 13-2030		No of Analyses Requested										AIC Control No: 1166946			
Project Reference: Plant Effluent		Sample Matrix		BOTTLES Chronic CD, Chronic FH										AIC Proposal No:			
Project Manager: James Sorrells		WATER SOIL												AIC Control No:			
Sampled By: H. Mauldin		GRA B		COMP		Remarks										Carrier: Hot Springs Shuttle	
AIC No. 3		PRESERVATIVE		RECEIVED TEMPERATURE °C: 2													
Sample Identification: PLANT EFFLUENT		Date/Time Collected: 5/2/13 @ 0000-2100		CONTAINER TYPE: X X		Field pH calibration on _____ @ _____ Buffer: _____											
Container Type		Preservative		NO													
G = Glass		P = Plastic		V = VOA vials		H = HCl to pH2		T = Sodium Thiosulfate		A = (NH4)2SO4							
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>H. Mauldin</u>		Date/Time: 5/3/13 @ 11:15		Received By: <u>Mr Mann</u>		Date/Time: 5/3/13 @ 11:15							
Expedited results requested by: <u>SAMR</u>				Relinquished By: <u>M. Mann</u>		Date/Time: 5/3/13 @ 12:20		Received in Lab By: <u>Steve Pulane</u>		Date/Time: 5-3-13 12:20							
Who should AIC contact with questions: <u>SORRELLS</u>				Comments:													
Phone: <u>501-262-1125</u> Fax: <u>501-262-0339</u>																	
Report Attention to: <u>Mr. James Sorrells</u>																	
Report Address to: 320 Davidson Road Hot Springs, AR 71901																	

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