


**REQUEST FOR CHANGE OF AUTHORIZATION
(CERTIFICATION AND SIGNATORY REQUIREMENTS)**

NPDES Permit Number: AR0033880 Facility Name: City of Hot springs

- Type of Change: New Cognizant Official (or duly authorized representative) (sections 1 and 2)
 (check one) New Responsible Official (complete section 2 only)
 Both (sections 1 and 2)

1. **NEW COGNIZANT OFFICIAL** (or duly authorized representative) (See 122.22(b); the individual, authorized by the ranking official in writing, as **having responsibility for the overall operation** of the regulated facility or activity responsibility, or having overall responsibility for environmental matters for the company.)

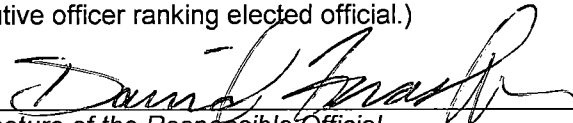
The ranking official hereby designates the following **individual** as the cognizant official, (duly authorized representative), for signing the permit required reports, etc., including Discharge Monitoring Reports (DMR) required by the permit, and other information requested by the Director:



Signature of the Cognizant Official (Duly Authorized Representative)
 James B Sorrells
 Name (First Name, MI, Last Name) Typed or Printed
 320 Davidson Dr Hot Springs AR 71901
 Mailing Address City, State, and Zip
 Facility Manager (501) 262-1125 262-0339
 Title A/C Phone Fax
 Email Address: jsorrells@cityhs.net

By signature below, the responsible official certifies that the above named **individual** is qualified to act as the duly authorized representative under the provisions of 40 CFR 122.22(b).

2. **RESPONSIBLE OFFICIAL** (Note: The responsible official is the person authorized to sign the permit application i/a/w 40 CFR 122.22(a). For a Corporation: it is the responsible corporate officer. Partnership or Sole Proprietorship: the general partner or proprietor. Municipality, State, Federal or other Public Agency: the principal executive officer ranking elected official.)



Signature of the Responsible Official 8/29/16
 Date
 David W. Frasher
 Name (First Name, MI, Last Name) Typed or Printed
 133 Convention Blvd Hot Springs, AR 71901
 Mailing Address City, State, and Zip
 City Manager (501) 321-6811 (501) 321-6814
 Title A/C Phone Fax
 Email Address: dfrasher@cityhs.net

Certification: I certify under penalty of law that this document and all attachments were prepared under my direct supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true, accurate, and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations.

Will the Responsible Official also be the person signing submittals? Yes No

September 2, 2016

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

Control No. 204941-1

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*
Outfall 001 - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

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

A handwritten signature in black ink, appearing to read 'John Overbey', is written over a horizontal line.

John Overbey
Chief Operating Officer

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	0.420	PASS
Control Growth CV < or = 40%	8.14	PASS
Growth Minimum Significant Difference 12 to 30%	10.2	BELOW
Critical Dilution CV < or = 40%	7.83	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	21.9	PASS
Control CV < or = 40% per Surviving Female	22.1	PASS
Reproduction Minimum Significant Difference 13 to 47%	21.7	PASS
Critical Dilution CV < or = 40%	22.7	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: Outfall 001
 - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.9	8.2	9.7
pH (standard units)	8.1	8.0	7.7
Alkalinity (mg/l as CaCO ₃)	58	87	41
Hardness (mg/l as CaCO ₃)	72	110	52
Conductivity (umhos/cm)	220	320	220
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	0.13	<0.1

2. Dilution Water Samples: Synthetic Soft Water #4356
 - a. Dates Prepared: August 19 through September 2, 2016
 - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.5	8.1	8.0
pH (standard units)	8.1	7.8	7.6
Alkalinity (mg/l as CaCO ₃)	32	33	33
Hardness (mg/l as CaCO ₃)	43	45	42
Conductivity (umhos/cm)	160	160	170
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05

C. Test Methods

1. Test methods used:

Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test 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: August 22, 2016 at 1405
Date & Time Test Terminated: August 30, 2016 at 1724
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: August 22, 2016 at 1430
Date & Time Test Terminated: August 29, 2016 at 1240
Type & Volume of Test Chamber: 30 ml disposable beaker
Volume of Sample: 15 ml
Number of Organisms per replicate: 1
Number of Replicates per dilution: 10

4. Acclimation of test organisms: Obtained from in-house cultures

5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms

1. Scientific Name

- a. Test 1000.0 *Pimephales promelas*
- b. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis

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

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

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

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

IV. Standard Reference Toxicants

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

Pimephales promelas (Fathead minnow)

Chronic reference tests are performed monthly.

A chronic reference test was performed on August 4, 2016 at 1350 to August 10, 2016 at 1537

The results were as follows: (Control No. 204418-1.)

Survival LC-50: 5123 mg/l

Growth IC-25: 2353 mg/l

Growth PMSD: 12.2

Ceriodaphnia dubia

Chronic reference tests are performed monthly.

A chronic reference test was performed on August 3, 2016 at 1405 to August 10, 2016 at 1440

The results were as follows: (Control No. 204418-2.)

Survival LC-50: 1871 mg/l

Growth IC-25: 1536 mg/l

Growth PMSD: 20.1

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	1.52
Hardness	EPA 200.7	100	1.05
pH	SM 4500-H+ B	101	0.00
Conductivity	EPA 120.1	104	2.83

VI. Organism History

Pimephales promelas (Fathead minnow)

Date: August 22, 2016

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: August 22, 2016

Age: <24 hours

Source: In-house culture

Water Chemistry Record:

Alkalinity: 57-64 mg/l

Hardness: 80-100 mg/l

Temperature: 25 deg.C

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

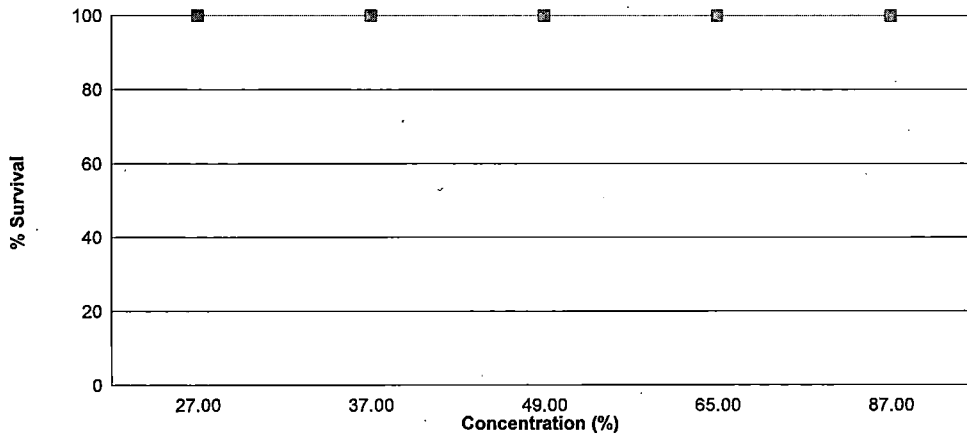
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

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

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

The test was initiated on August 22, 2016 at 1405 and continued through August 30, 2016 at 1724. 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 = 37 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.420
27 %	100	0.432
37 %	100	0.390
49 %	100	0.366 *
65 %	100	0.385
87 %	100	0.383

The significant difference noted at 49% effluent does not follow a dose-response pattern and is considered an anomaly.

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

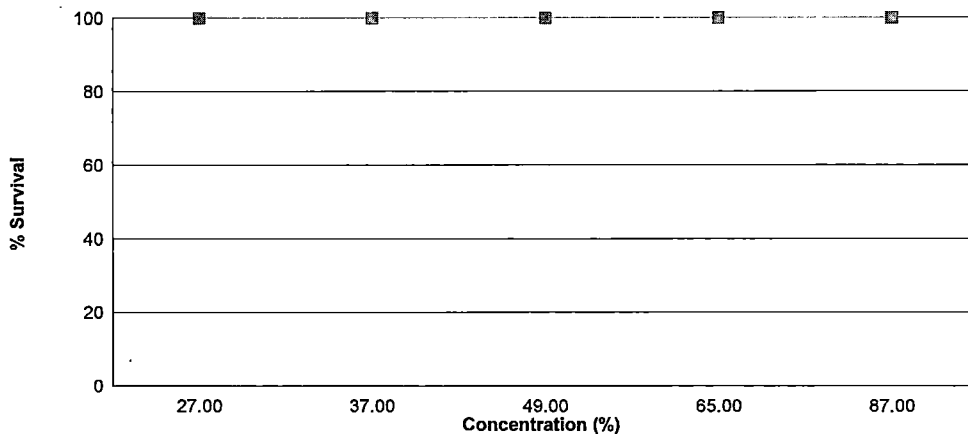
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

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

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

The test was initiated on August 22, 2016 at 1430 and continued through August 29, 2016 at 1240. 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	21.9
27 %	100	23.3
37 %	100	26.9
49 %	100	24.1
65 %	100	22.9
87 %	100	22.3

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: August 22, 2016 at 1405

Date and Time Test Terminated: August 30, 2016 at 1724

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

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: August 22, 2016 at 1405

Test Terminated: August 30, 2016 at 1724

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.92621	.92981	0.00360	8	0.450
	B	.92181	.92532	0.00351	8	0.439
	C	.92466	.92786	0.00320	8	0.400
	D	.92377	.92729	0.00352	8	0.440
	E	.92427	.92722	0.00295	8	0.369
27 %	A	.92312	.92641	0.00329	8	0.411
	B	.92364	.92756	0.00392	8	0.490
	C	.92111	.92475	0.00364	8	0.455
	D	.92433	.92769	0.00336	8	0.420
	E	.92665	.92972	0.00307	8	0.384
37 %	A	.92659	.92970	0.00311	8	0.389
	B	.92717	.93045	0.00328	8	0.410
	C	.92520	.92847	0.00327	8	0.409
	D	.92925	.93215	0.00290	8	0.362
	E	.92889	.93192	0.00303	8	0.379
49 %	A	.92408	.92682	0.00274	8	0.342
	B	.92448	.92757	0.00309	8	0.386
	C	.92438	.92741	0.00303	8	0.379
	D	.92443	.92734	0.00291	8	0.364
	E	.92367	.92656	0.00289	8	0.361
65 %	A	.92849	.93126	0.00277	8	0.346
	B	.92354	.92651	0.00297	8	0.371
	C	.93083	.93385	0.00302	8	0.378
	D	.92959	.93296	0.00337	8	0.421
	E	.92873	.93200	0.00327	8	0.409
87 %	A	.92915	.93199	0.00284	8	0.355
	B	.92504	.92821	0.00317	8	0.396
	C	.92680	.93009	0.00329	8	0.411
	D	.92508	.92810	0.00302	8	0.378
	E	.92501	.92800	0.00299	8	0.374

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 22, 2016 at 1430

Date and Time Test Terminated: August 29, 2016 at 1240

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	4	0	0	0	4	10	0.400	
4	3	3	4	4	4	4	0	0	3	4	29	10	2.90	
5	0	4	8	0	8	0	7	3	10	7	47	10	4.70	
6	6	0	10	8	10	11	16	9	10	0	80	10	8.00	
7	7	13	0	12	0	12	15E	0	14E	15	59	10	5.90	
8														
TOTAL	16	20	22	24	22	27	27	12	23	26	219	10	21.9	

E = Excluded fourth brood neonates

Concentration: 27 %														
Day	Replicate										No. of Young	No. of Adults	Young per Adult	
	1	2	3	4	5	6	7	8	9	10				
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
4	4	2	3	4	3	4	5	4	0	3	32	10	3.20	
5	8	1	9	0	7	10	9	8	7	8	67	10	6.70	
6	6	0	10	8	10	11	16	9	10	0	80	10	8.00	
7	7E	13	0	12	0	12E	15E	0	14	15	54	10	5.40	
8														
TOTAL	18	16	22	24	20	25	30	21	31	26	233	10	23.3	

E = Excluded fourth brood neonates

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	5	0	3	0	8	10	0.800	
4	4	2	1	0	4	4	0	4	0	4	23	10	2.30	
5	0	9	9	0	11	8	10	9	7	9	72	10	7.20	
6	11	12	12	12	12	13	14	15	16	13	130	10	13.0	
7	19	16E	0	17	0	0	15E	0	17E	0	36	10	3.60	
8														
TOTAL	34	23	22	29	27	25	29	28	26	26	269	10	26.9	

E = Excluded fourth brood neonates

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 22, 2016 at 1430

Date and Time Test Terminated: August 29, 2016 at 1240

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	4	0	2	0	6	10	0.600	
4	3	0	4	0	2	2	0	3	0	2	16	10	1.60	
5	8	5	8	0	7	11	8	8	8	4	67	10	6.70	
6	10	10	15	2	11	16	13	1	11	0	89	10	8.90	
7	0	14	0	16	0	0	1	15	16E	17	63	10	6.30	
8														
TOTAL	21	29	27	18	20	29	26	27	21	23	241	10	24.1	

E = Excluded fourth brood neonates

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	6	0	0	0	6	10	0.600
4	5	0	2	0	3	4	1	4	4	4	27	10	2.70
5	9	4	10	0	7	10	4	8	9	7	68	10	6.80
6	11	7	10	5	13	16	15	14	10	0	101	10	10.1
7	0	11	0	5	0	14E	20E	0	0	11	27	10	2.70
8													
TOTAL	25	22	22	10	23	30	26	26	23	22	229	10	22.9

E = Excluded fourth brood neonates

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	4	0	0	0	0	5	0	9	10	0.900
4	4	0	3	4	3	5	5	4	0	2	30	10	3.00
5	6	0	0	0	6	9	10	7	8	0	46	10	4.60
6	12	5	5	8	12	10	17	0	12	13	94	10	9.40
7	0	14	14	24E	0	0	0	16	18E	0	44	10	4.40
8													
TOTAL	22	19	22	16	21	24	32	27	25	15	223	10	22.3

E = Excluded fourth brood neonates

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

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

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

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

Steel's Many-One Rank Test			Transform: Arc Sin(Square Root(Y))		
Ho: Control < Treatment					
Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	27 %	27.50	16.00	5.00	
3	37 %	27.50	16.00	5.00	
4	49 %	27.50	16.00	5.00	
5	65 %	27.50	16.00	5.00	
6	87 %	27.50	16.00	5.00	
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality	No Transformation
D = 0.01976 W = 0.9729 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30) Data PASS normality test (alpha = 0.01).	

Bartlett's Test for Homogeneity of Variance	No Transformation
Calculated B1 statistic = 4.104 Critical B = 15.086 (alpha = 0.01, df = 5) Data PASS B1 homogeneity test at 0.01 level.	

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	0.01531	0.003062	3.717	
Within (Error)	24	0.01977	0.0008238		
Total	29	0.03508			
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	0.4196	0.4196			
2	27 %	0.432	0.432	-0.6831		
3	37 %	0.3898	0.3898	1.642		
4	49 %	0.3664	0.3664	2.931	*	
5	65 %	0.385	0.385	1.906		
6	87 %	0.3828	0.3828	2.027		
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	0.04284	10.2	-0.0124	
3	37 %	5	0.04284	10.2	0.0298	
4	49 %	5	0.04284	10.2	0.0532	
5	65 %	5	0.04284	10.2	0.0346	
6	87 %	5	0.04284	10.2	0.0368	

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 = 0.0859 D* = 0.6739 Critical D* = 1.035 (alpha = 0.01, N = 60)</p> <p>Data PASS normality test (alpha = 0.01).</p>	

Bartlett's Test for Homogeneity of Variance	No Transformation
<p>Calculated B1 statistic = 2.159 Critical B = 15.086 (alpha = 0.01, df = 5)</p> <p>Data PASS B1 homogeneity test at 0.01 level.</p>	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	162.9	32.58	1.532	
Within (Error)	54	1148	21.26		
Total	59	1311			
Critical F = 3.38 (alpha = 0.01, df = 5,54)					
2.38 (alpha = 0.05, df = 5,54)					
Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)					

Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	21.9	21.9			
2	27 %	23.3	23.3	-0.6789		
3	37 %	26.9	26.9	-2.425		
4	49 %	24.1	24.1	-1.067		
5	65 %	22.9	22.9	-0.485		
6	87 %	22.3	22.3	-0.194		
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	4.763	21.7	-1.4		
3	37 %	10	4.763	21.7	-5		
4	49 %	10	4.763	21.7	-2.2		
5	65 %	10	4.763	21.7	-1		
6	87 %	10	4.763	21.7	-0.4		

Appendix A3: Water Chemistry
Routine Chemical and Physical Data

Effluent Conc.: Control	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
DO, mg/l	Initial	7.5	8.1	8.1	8.0	8.0	8.1	8.2
	Final *1	7.9	8.2	8.3	7.5	7.0	6.9	7.9
	Final *2	8.1	8.4	8.5	7.5	7.4	6.6	7.6
pH, units	Initial	8.1	7.9	7.8	7.5	7.6	7.8	7.7
	Final *1	7.9	7.9	8.0	7.1	7.8	7.6	7.9
	Final *2	7.8	8.0	8.1	7.9	8.0	7.9	8.0
Alkalinity, mg CaCO ₃ /l	32	NA	33	NA	33	NA	NA	
Hardness, mg CaCO ₃ /l	43	NA	45	NA	42	NA	NA	
Conductivity, umhos/cm	160	170	160	150	170	170	150	
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	8.1	8.2	8.2	8.9	8.2
	Final *1	7.6	8.2	8.2	6.9	5.8	7.4	8.0
	Final *2	9.5	8.4	8.3	8.0	5.4	7.2	7.6
pH, units	Initial	8.1	8.0	7.8	7.7	7.7	7.8	7.7
	Final *1	7.9	8.0	8.1	7.1	8.0	7.8	7.8
	Final *2	7.9	8.1	7.9	8.2	8.4	8.2	8.3

Effluent Conc.: 37 %	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
DO, mg/l	Initial	8.1	7.3	8.2	8.4	8.2	8.6	8.2
	Final *1	7.7	8.2	8.3	3.7	5.8	7.6	8.0
	Final *2	8.4	8.4	7.8	6.6	5.7	7.6	7.7
pH, units	Initial	8.1	8.0	7.9	7.7	7.7	7.8	7.7
	Final *1	8.0	8.1	8.1	7.1	8.1	7.8	8.0
	Final *2	7.9	8.2	7.9	8.1	8.4	8.2	8.3

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.1	8.5	8.2	8.1	8.7	9.0	8.1
	Final *1	7.8	8.2	8.3	7.3	5.8	7.8	8.0
	Final *2	11	8.4	7.8	4.7	5.8	7.8	7.8
pH, units	Initial	8.1	8.0	7.9	7.9	7.8	7.8	7.8
	Final *1	8.0	8.1	8.1	7.3	8.2	7.9	8.0
	Final *2	8.0	8.2	7.9	8.1	8.5	8.3	8.2

Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.1	8.1	8.3	8.2	8.2	9.0	8.2
	Final *1	7.8	8.2	8.2	7.4	5.8	7.8	7.9
	Final *2	8.1	8.5	8.4	8.1	5.9	8.0	7.8
pH, units	Initial	8.1	8.1	8.0	7.9	7.8	7.9	7.8
	Final *1	8.0	8.2	8.2	7.4	8.2	7.9	8.0
	Final *2	8.0	8.2	8.2	8.4	8.6	8.2	8.4
Alkalinity, mg CaCO ₃ /l	48	NA	68	NA	38	NA	NA	
Hardness, mg CaCO ₃ /l	63	NA	92	NA	46	NA	NA	
Conductivity, umhos/cm	200	200	200	250	280	250	230	
Res. Chlorine, mg/l	<0.05	NA	<0.05	NA	<0.05	NA	NA	

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.2	8.0	8.1	8.3	8.2	6.1	8.2
	Final *1	7.8	8.4	8.3	7.2	5.8	7.8	7.9
	Final *2	9.9	8.5	8.5	8.2	6.0	8.1	7.8
pH, units	Initial	8.1	8.1	7.9	7.9	7.8	7.9	7.9
	Final *1	8.1	8.2	8.2	7.4	8.3	8.0	8.0
	Final *2	8.0	8.3	8.2	8.5	8.6	8.2	8.3

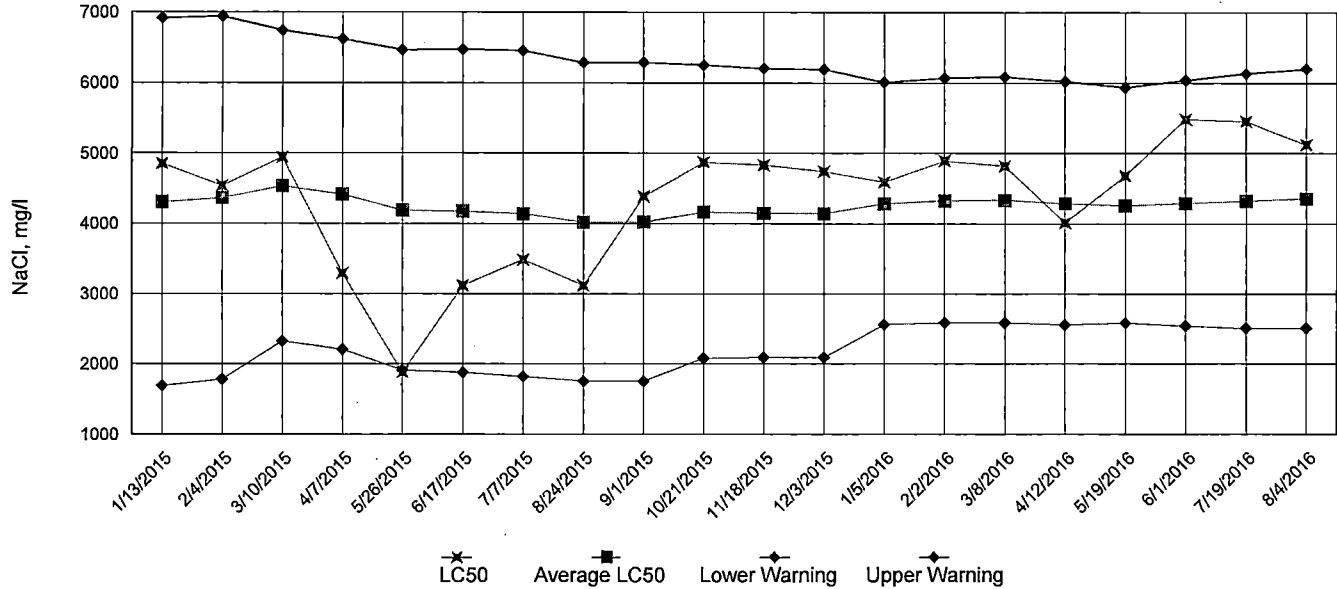
*1 = data from the *Pimephales promelas* (Fathead Minnow) test

*2 = data from the *Ceriodaphnia dubia* test

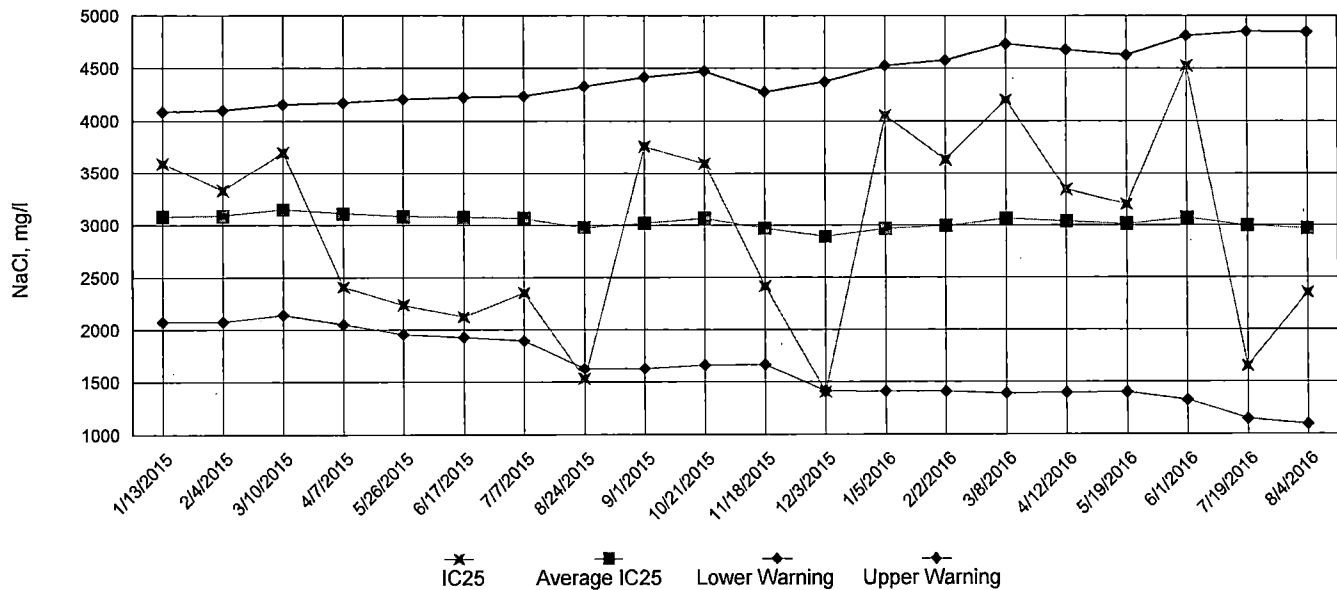
Appendix A4: Test 1000.0

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

LC50 Survival Data

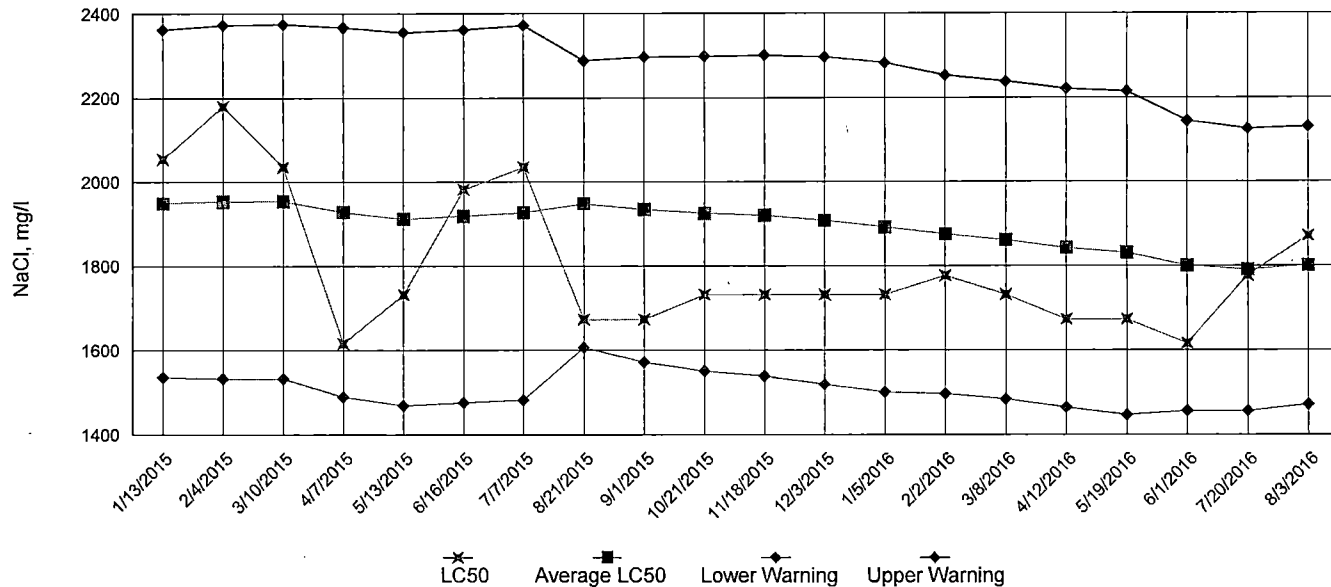


IC25 Growth Data

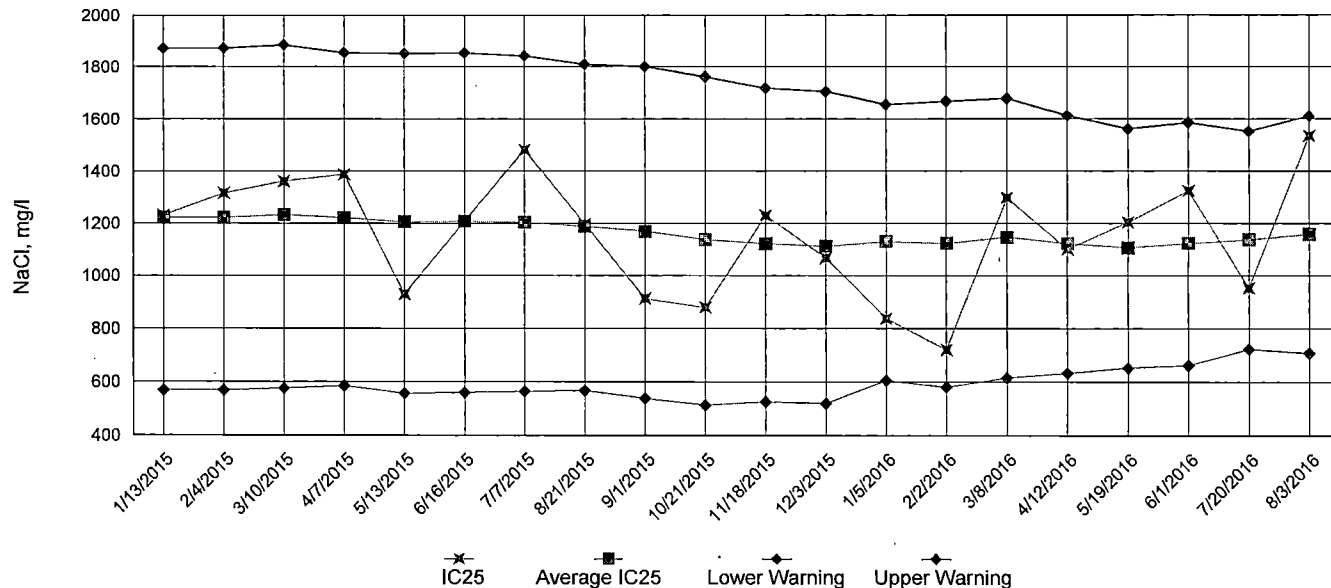


Appendix A4: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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: August 22, 2016 at 1405

Date and Time Test Terminated: August 30, 2016 at 1724

Dilution water used: Synthetic Soft Water #4356

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	100	100	100	100	100	0.00
49 %	100	100	100	100	100	100	100	100	0.00
65 %	100	100	100	100	100	100	100	100	0.00
87 %	100	100	100	100	100	100	100	100	0.00

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.450	0.439	0.400	0.440	0.369	0.42	8.14
27 %	0.411	0.490	0.455	0.420	0.384	0.432	9.53
37 %	0.389	0.410	0.409	0.362	0.379	0.39	5.24
49 %	0.342	0.386	0.379	0.364	0.361	0.366	4.67
65 %	0.346	0.371	0.378	0.421	0.409	0.385	7.83
87 %	0.355	0.396	0.411	0.378	0.374	0.383	5.61

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

Appendix B: Test 1000.0

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

1. Steel's Many-One Rank Test:

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

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	<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: 8.14 (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, 304, 310, 314

Test Initiated: DATE: August 22, 2016 TIME: 1405
Test Terminated: DATE: August 30, 2016 TIME: 1724

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.5	8.1	8.1	8.0	8.0	8.1	8.2
Final	7.9	8.2	8.3	7.5	7.0	6.9	7.9
pH Initial	8.1	7.9	7.8	7.5	7.6	7.8	7.7
Final	7.9	7.9	8.0	7.1	7.8	7.6	7.9
Alkalinity	32	NA	33	NA	33	NA	NA
Hardness	43	NA	45	NA	42	NA	NA
Conductivity	160	170	160	150	170	170	150
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	8.1	8.2	8.2	8.9	8.2
Final	7.6	8.2	8.2	6.9	5.8	7.4	8.0
pH Initial	8.1	8.0	7.8	7.7	7.7	7.8	7.7
Final	7.9	8.0	8.1	7.1	8.0	7.8	7.8
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	180	180	180	190	220	200	180
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 37 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	7.3	8.2	8.4	8.2	8.6	8.2
Final	7.7	8.2	8.3	3.7	5.8	7.6	8.0
pH Initial	8.1	8.0	7.9	7.7	7.7	7.8	7.7
Final	8.0	8.1	8.1	7.1	8.1	7.8	8.0
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	180	190	190	210	230	220	200
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 49 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	8.5	8.2	8.1	8.7	9.0	8.1
Final	7.8	8.2	8.3	7.3	5.8	7.8	8.0
pH Initial	8.1	8.0	7.9	7.9	7.8	7.8	7.8
Final	8.0	8.1	8.1	7.3	8.2	7.9	8.0
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	190	190	190	230	250	230	200
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 65 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	8.1	8.3	8.2	8.2	9.0	8.2
Final	7.8	8.2	8.2	7.4	5.8	7.8	7.9
pH Initial	8.1	8.1	8.0	7.9	7.8	7.9	7.8
Final	8.0	8.2	8.2	7.4	8.2	7.9	8.0
Alkalinity	48	NA	68	NA	38	NA	NA
Hardness	63	NA	92	NA	46	NA	NA
Conductivity	200	200	200	250	280	250	230
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 87 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.2	8.0	8.1	8.3	8.2	6.1	8.2
Final	7.8	8.4	8.3	7.2	5.8	7.8	7.9
pH Initial	8.1	8.1	7.9	7.9	7.8	7.9	7.9
Final	8.1	8.2	8.2	7.4	8.3	8.0	8.0
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	210	210	290	320	280	250
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: August 22, 2016 at 1430

Date and Time Test Terminated: August 29, 2016 at 1240

Dilution water used: Synthetic Soft Water #4356

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	16	18	34	21	25	22
B	20	16	23	29	22	19
C	22	22	22	27	22	22
D	24	24	29	18	10	16
E	22	20	27	20	23	21
F	27	25	25	29	30	24
G	27	30	29	26	26	32
H	12	21	28	27	26	27
I	23	31	26	21	23	25
J	26	26	26	23	22	15
Mean per Adult	21.9	23.3	26.9	24.1	22.9	22.3
Mean per Surviving Adult	21.9	23.3	26.9	24.1	22.9	22.3
CV %	22.1	20.9	12.7	16.5	22.7	22.8

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. Dunnett's Test:

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

a.) LOW FLOW OR CRITICAL DILUTION	(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: 22.7 (TQP3B)

Appendix B: Test 1002.0
CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
CHEMICAL PARAMETERS CHART

PERMITTEE: City of Hot Springs
NPDES NO.: AR0033880 AFIN#26-00145
CONTACT: Mr. James Sorrells
ANALYST: 280, 304, 310, 314

Test Initiated: DATE: August 22, 2016 TIME: 1430
Test Terminated: DATE: August 29, 2016 TIME: 240

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.5	8.1	8.1	8.0	8.0	8.1	8.2
Final	8.1	8.4	8.5	7.5	7.4	6.6	7.6
pH Initial	8.1	7.9	7.8	7.5	7.6	7.8	7.7
Final	7.8	8.0	8.1	7.9	8.0	7.9	8.0
Alkalinity	32	NA	33	NA	33	NA	NA
Hardness	43	NA	45	NA	42	NA	NA
Conductivity	160	170	160	150	170	170	150
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	8.1	8.2	8.2	8.9	8.2
Final	9.5	8.4	8.3	8.0	5.4	7.2	7.6
pH Initial	8.1	8.0	7.8	7.7	7.7	7.8	7.7
Final	7.9	8.1	7.9	8.2	8.4	8.2	8.3
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	180	180	180	190	220	200	180
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
37 %							
D.O. Initial	8.1	7.3	8.2	8.4	8.2	8.6	8.2
Final	8.4	8.4	7.8	6.6	5.7	7.6	7.7
pH Initial	8.1	8.0	7.9	7.7	7.7	7.8	7.7
Final	7.9	8.2	7.9	8.1	8.4	8.2	8.3
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	180	190	190	210	230	220	200
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
49 %							
D.O. Initial	8.1	8.5	8.2	8.1	8.7	9.0	8.1
Final	11	8.4	7.8	4.7	5.8	7.8	7.8
pH Initial	8.1	8.0	7.9	7.9	7.8	7.8	7.8
Final	8.0	8.2	7.9	8.1	8.5	8.3	8.2
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	190	190	190	230	250	230	200
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
65 %							
D.O. Initial	8.1	8.1	8.3	8.2	8.2	9.0	8.2
Final	8.1	8.5	8.4	8.1	5.9	8.0	7.8
pH Initial	8.1	8.1	8.0	7.9	7.8	7.9	7.8
Final	8.0	8.2	8.2	8.4	8.6	8.2	8.4
Alkalinity	48	NA	68	NA	38	NA	NA
Hardness	63	NA	92	NA	46	NA	NA
Conductivity	200	200	200	250	280	250	230
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
87 %							
D.O. Initial	8.2	8.0	8.1	8.3	8.2	6.1	8.2
Final	9.9	8.5	8.5	8.2	6.0	8.1	7.8
pH Initial	8.1	8.1	7.9	7.9	7.8	7.9	7.9
Final	8.0	8.3	8.2	8.5	8.6	8.2	8.3
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	210	210	290	320	280	250
Chlorine	NA	NA	NA	NA	NA	NA	NA

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City of Hot Springs			PO No. 16-1336		NO OF BOTTLES	ANALYSES REQUESTED										AIC CONTROL NO: 204941				
Project Reference: Bio-Monitoring			MATRIX			Bio-Monitoring											AIC PROPOSAL NO:			
Project Manager: JIM SORRELLS			W	A			S											Carrier: IAS DELIVERY		
Sampled By: H MAULON			G	C	A	S											Received Temperature 0.1			
AIC No.	Sample Identification	Date/Time Collected	A	B	R	L											Remarks			
1	PLANT EFFLUENT	8/21/16 @ 0000-2400		X	X		3	X												
Container Type						P											Field pH calibration			
Preservative						NO											on _____ @ _____ Buffer:			
G = Glass NO = none			P = Plastic S = Sulfuric acid pH2			V = VOA vials N = Nitric acid pH2			H = HCl to pH2 B = NaOH to pH12			T = Sodium Thiosulfate Z = Zinc acetate			A = (NH ₄) ₂ SO ₄ , NH ₄ OH					
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS						Relinquished By: A. Cates		Date/Time: 8-22-16 @ 1000		Received By: M. Mann		Date/Time: 8-22-16 @ 10:00								
Expedited results requested by: _____						Relinquished By: M. Mann		Date/Time: 8-22-16 @ 11:05		Received in Lab By: D. BROWN		Date/Time: 8-22-16 WDS								
Who should AIC contact with questions: Phone: _____ Fax: _____ Report Attention to: SAME Report Address to: _____						Comments:														
Email Address: _____																				

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <i>City of Hot Springs</i>			PO No. <i>16-1336</i>		NO OF BOTTLES <i>Bio Monitoring</i>	ANALYSES REQUESTED										AIC CONTROL NO: <i>204941</i>				
Project Reference: <i>Bio-Monitoring</i>			MATRIX													AIC PROPOSAL NO:				
Project Manager: <i>JIM SORRELLS</i>			W	A		S											Carrier: <i>HS DELIVERY</i>			
Sampled By: <i>H. Mann</i>			G	C		T											Received Temperature C <i>0.1</i>			
AIC No.	Sample Identification	Date/Time Collected	R	O	E	I	L											Remarks		
<i>2</i>	<i>PLANT Effluent</i>	<i>8/23/16 @ 0000-2400</i>						<i>3</i>	<i>X</i>											
Container Type																Field pH calibration				
Preservative																on _____ @ _____				
																Buffer:				
G = Glass		P = Plastic		V = VOA vials		H = HCl to pH2		T = Sodium Thiosulfate		Z = Zinc acetate		A = (NH ₄) ₂ SO ₄ , NH ₄ OH								
NO = none		S = Sulfuric acid pH2		N = Nitric acid pH2		B = NaOH to pH12														
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS						Relinquished		Date/Time		Received		Date/Time								
Expedited results requested by: _____						By: <i>H. Mann</i>		<i>8/24/16 @ 10:50</i>		By: <i>M. Mann</i>		<i>8-24-16 @ 1050</i>								
Who should AIC contact with questions:						Relinquished		Date/Time		Received in Lab		Date/Time								
Phone: _____ Fax: _____						By: <i>M. Mann</i>		<i>8-24-16 @ 11:45</i>		By: <i>D. Brown</i>		<i>8-24-16 11:45</i>								
Report Attention to:						Comments:														
Report Address to:																				
Email Address:																				

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: PLANT Effluent			PO No. 16-1336		NO OF BOTTLES	ANALYSES REQUESTED										AIC CONTROL NO: 204941			
Project Reference: BIO-MONITORING			MATRIX			Bio-Monitoring											AIC PROPOSAL NO:		
Project Manager: JIM SORRELLS							3											Carrier: ITS DELIVERY	
Sampled By: HAROLD MAULDIN																		Received Temperature C	
AIC No.	Sample Identification	Date/Time Collected	G R A B	C O M P	W A T E R	S O I L	3											Remarks	
3	PLANT Effluent	8/25/16 @ 0900-2400		X	X			X											
			Container Type				P											Field pH calibration on _____ @ _____	
			Preservative				NO											Buffer:	
			G = Glass NO = none		P = Plastic S = Sulfuric acid pH2		V = VOA vials N = Nitric acid pH2		H = HCl to pH2 B = NaOH to pH12		T = Sodium Thiosulfate Z = Zinc acetate		A = (NH ₄) ₂ SO ₄ , NH ₄ OH						
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS							Relinquished By: A. Cates		Date/Time 8-26-16 @ 0940		Received By: M. Mann		Date/Time 8-26-16 @ 9:40						
Expedited results requested by: _____							Relinquished By: M. Mann		Date/Time 8-26-16 @ 10:40		Received in Lab By: D. BROWN		Date/Time 8-26-16 1040						
Who should AIC contact with questions:							Comments:												
Phone: _____ Fax: _____																			
Report Attention to:																			
Report Address to:																			
Email Address:																			



September 2, 2016

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

Control No. 204941-1

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*
Outfall 001 - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

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

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	0.420	PASS
Control Growth CV < or = 40%	8.14	PASS
Growth Minimum Significant Difference 12 to 30%	10.2	BELOW
Critical Dilution CV < or = 40%	7.83	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	21.9	PASS
Control CV < or = 40% per Surviving Female	22.1	PASS
Reproduction Minimum Significant Difference 13 to 47%	21.7	PASS
Critical Dilution CV < or = 40%	22.7	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: Outfall 001
 - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.9	8.2	9.7
pH (standard units)	8.1	8.0	7.7
Alkalinity (mg/l as CaCO ₃)	58	87	41
Hardness (mg/l as CaCO ₃)	72	110	52
Conductivity (umhos/cm)	220	320	220
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	0.13	<0.1

2. Dilution Water Samples: Synthetic Soft Water #4356

- a. Dates Prepared: August 19 through September 2, 2016
- b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.5	8.1	8.0
pH (standard units)	8.1	7.8	7.6
Alkalinity (mg/l as CaCO ₃)	32	33	33
Hardness (mg/l as CaCO ₃)	43	45	42
Conductivity (umhos/cm)	160	160	170
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05

C. Test Methods

1. Test methods used:

Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test 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: August 22, 2016 at 1405
Date & Time Test Terminated: August 30, 2016 at 1724
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: August 22, 2016 at 1430
Date & Time Test Terminated: August 29, 2016 at 1240
Type & Volume of Test Chamber: 30 ml disposable beaker
Volume of Sample: 15 ml
Number of Organisms per replicate: 1
Number of Replicates per dilution: 10

4. Acclimation of test organisms: Obtained from in-house cultures

5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms

1. Scientific Name

- a. Test 1000.0 *Pimephales promelas*
- b. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis

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

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

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

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

IV. Standard Reference Toxicants

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

Pimephales promelas (Fathead minnow)

Chronic reference tests are performed monthly.

A chronic reference test was performed on August 4, 2016 at 1350 to August 10, 2016 at 1537

The results were as follows: (Control No. 204418-1.)

Survival LC-50: 5123 mg/l

Growth IC-25: 2353 mg/l

Growth PMSD: 12.2

Ceriodaphnia dubia

Chronic reference tests are performed monthly.

A chronic reference test was performed on August 3, 2016 at 1405 to August 10, 2016 at 1440

The results were as follows: (Control No. 204418-2.)

Survival LC-50: 1871 mg/l

Growth IC-25: 1536 mg/l

Growth PMSD: 20.1

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	1.52
Hardness	EPA 200.7	100	1.05
pH	SM 4500-H+ B	101	0.00
Conductivity	EPA 120.1	104	2.83

VI. Organism History

Pimephales promelas (Fathead minnow)

Date: August 22, 2016

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: August 22, 2016

Age: <24 hours

Source: In-house culture

Water Chemistry Record:

Alkalinity: 57-64 mg/l

Hardness: 80-100 mg/l

Temperature: 25 deg.C

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

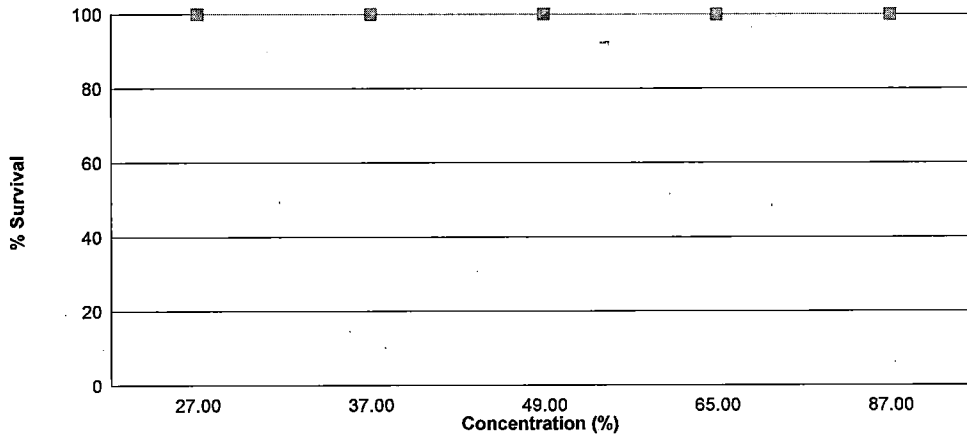
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

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

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

The test was initiated on August 22, 2016 at 1405 and continued through August 30, 2016 at 1724. 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 = 37 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.420
27 %	100	0.432
37 %	100	0.390
49 %	100	0.366 *
65 %	100	0.385
87 %	100	0.383

The significant difference noted at 49% effluent does not follow a dose-response pattern and is considered an anomaly.

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

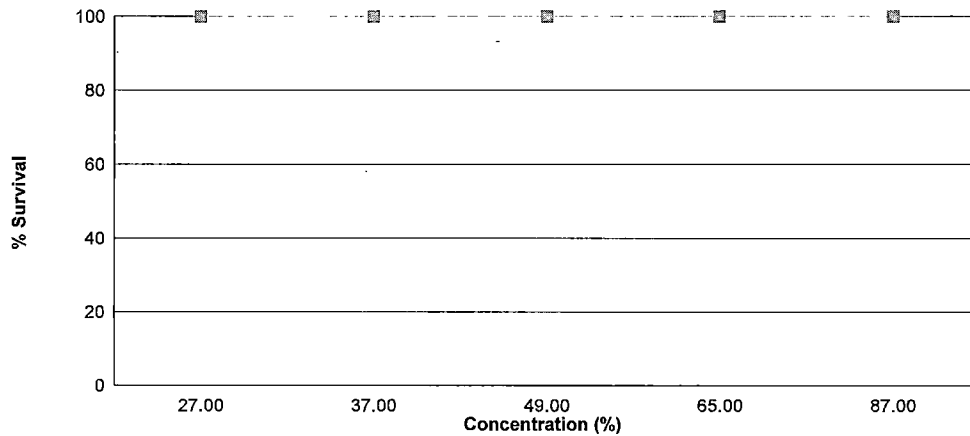
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

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

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

The test was initiated on August 22, 2016 at 1430 and continued through August 29, 2016 at 1240. 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	21.9
27 %	100	23.3
37 %	100	26.9
49 %	100	24.1
65 %	100	22.9
87 %	100	22.3

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: August 22, 2016 at 1405

Date and Time Test Terminated: August 30, 2016 at 1724

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

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: August 22, 2016 at 1405

Test Terminated: August 30, 2016 at 1724

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.92621	.92981	0.00360	8	0.450
	B	.92181	.92532	0.00351	8	0.439
	C	.92466	.92786	0.00320	8	0.400
	D	.92377	.92729	0.00352	8	0.440
	E	.92427	.92722	0.00295	8	0.369
27 %	A	.92312	.92641	0.00329	8	0.411
	B	.92364	.92756	0.00392	8	0.490
	C	.92111	.92475	0.00364	8	0.455
	D	.92433	.92769	0.00336	8	0.420
	E	.92665	.92972	0.00307	8	0.384
37 %	A	.92659	.92970	0.00311	8	0.389
	B	.92717	.93045	0.00328	8	0.410
	C	.92520	.92847	0.00327	8	0.409
	D	.92925	.93215	0.00290	8	0.362
	E	.92889	.93192	0.00303	8	0.379
49 %	A	.92408	.92682	0.00274	8	0.342
	B	.92448	.92757	0.00309	8	0.386
	C	.92438	.92741	0.00303	8	0.379
	D	.92443	.92734	0.00291	8	0.364
	E	.92367	.92656	0.00289	8	0.361
65 %	A	.92849	.93126	0.00277	8	0.346
	B	.92354	.92651	0.00297	8	0.371
	C	.93083	.93385	0.00302	8	0.378
	D	.92959	.93296	0.00337	8	0.421
	E	.92873	.93200	0.00327	8	0.409
87 %	A	.92915	.93199	0.00284	8	0.355
	B	.92504	.92821	0.00317	8	0.396
	C	.92680	.93009	0.00329	8	0.411
	D	.92508	.92810	0.00302	8	0.378
	E	.92501	.92800	0.00299	8	0.374

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 22, 2016 at 1430

Date and Time Test Terminated: August 29, 2016 at 1240

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	4	0	0	0	4	10	0.400	
4	3	3	4	4	4	4	0	0	3	4	29	10	2.90	
5	0	4	8	0	8	0	7	3	10	7	47	10	4.70	
6	6	0	10	8	10	11	16	9	10	0	80	10	8.00	
7	7	13	0	12	0	12	15E	0	14E	15	59	10	5.90	
8														
TOTAL	16	20	22	24	22	27	27	12	23	26	219	10	21.9	

E = Excluded fourth brood neonates

Concentration: 27 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	2	3	4	3	4	5	4	0	3	32	10	3.20
5	8	1	9	0	7	10	9	8	7	8	67	10	6.70
6	6	0	10	8	10	11	16	9	10	0	80	10	8.00
7	7E	13	0	12	0	12E	15E	0	14	15	54	10	5.40
8													
TOTAL	18	16	22	24	20	25	30	21	31	26	233	10	23.3

E = Excluded fourth brood neonates

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	5	0	3	0	8	10	0.800
4	4	2	1	0	4	4	0	4	0	4	23	10	2.30
5	0	9	9	0	11	8	10	9	7	9	72	10	7.20
6	11	12	12	12	12	13	14	15	16	13	130	10	13.0
7	19	16E	0	17	0	0	15E	0	17E	0	36	10	3.60
8													
TOTAL	34	23	22	29	27	25	29	28	26	26	269	10	26.9

E = Excluded fourth brood neonates

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 22, 2016 at 1430
Date and Time Test Terminated: August 29, 2016 at 1240

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	4	0	2	0	6	10	0.600	
4	3	0	4	0	2	2	0	3	0	2	16	10	1.60	
5	8	5	8	0	7	11	8	8	8	4	67	10	6.70	
6	10	10	15	2	11	16	13	1	11	0	89	10	8.90	
7	0	14	0	16	0	0	1	15	16E	17	63	10	6.30	
8														
TOTAL	21	29	27	18	20	29	26	27	21	23	241	10	24.1	

E = Excluded fourth brood neonates

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	6	0	0	0	6	10	0.600	
4	5	0	2	0	3	4	1	4	4	4	27	10	2.70	
5	9	4	10	0	7	10	4	8	9	7	68	10	6.80	
6	11	7	10	5	13	16	15	14	10	0	101	10	10.1	
7	0	11	0	5	0	14E	20E	0	0	11	27	10	2.70	
8														
TOTAL	25	22	22	10	23	30	26	26	23	22	229	10	22.9	

E = Excluded fourth brood neonates

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	4	0	0	0	0	5	0	9	10	0.900	
4	4	0	3	4	3	5	5	4	0	2	30	10	3.00	
5	6	0	0	0	6	9	10	7	8	0	46	10	4.60	
6	12	5	5	8	12	10	17	0	12	13	94	10	9.40	
7	0	14	14	24E	0	0	0	16	18E	0	44	10	4.40	
8														
TOTAL	22	19	22	16	21	24	32	27	25	15	223	10	22.3	

E = Excluded fourth brood neonates

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

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

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Shapiro - Wilk's Test for Normality		Transform: Arc Sin(Square Root(Y))
<p>D = 0 W = 0 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30)</p> <p>Data FAIL normality test (alpha = 0.01).</p>		

Steel's Many-One Rank Test				Transform: Arc Sin(Square Root(Y))	
Ho:Control<Treatment					
Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	27 %	27.50	16.00	5.00	
3	37 %	27.50	16.00	5.00	
4	49 %	27.50	16.00	5.00	
5	65 %	27.50	16.00	5.00	
6	87 %	27.50	16.00	5.00	
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality	No Transformation
<p>D = 0.01976 W = 0.9729 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 = 4.104 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	0.01531	0.003062	3.717	
Within (Error)	24	0.01977	0.0008238		
Total	29	0.03508			
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	0.4196	0.4196			
2	27 %	0.432	0.432	-0.6831		
3	37 %	0.3898	0.3898	1.642		
4	49 %	0.3664	0.3664	2.931	*	
5	65 %	0.385	0.385	1.906		
6	87 %	0.3828	0.3828	2.027		
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	0.04284	10.2	-0.0124		
3	37 %	5	0.04284	10.2	0.0298		
4	49 %	5	0.04284	10.2	0.0532		
5	65 %	5	0.04284	10.2	0.0346		
6	87 %	5	0.04284	10.2	0.0368		

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 = 0.0859 D* = 0.6739 Critical D* = 1.035 (alpha = 0.01, N = 60)</p> <p>Data PASS normality test (alpha = 0.01).</p>	

Bartlett's Test for Homogeneity of Variance	No Transformation
<p>Calculated B1 statistic = 2.159 Critical B = 15.086 (alpha = 0.01, df = 5)</p> <p>Data PASS B1 homogeneity test at 0.01 level.</p>	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	162.9	32.58	1.532	
Within (Error)	54	1148	21.26		
Total	59	1311			
Critical F = 3.38 (alpha = 0.01, df = 5,54)					
2.38 (alpha = 0.05, df = 5,54)					
Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)					

Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	21.9	21.9			
2	27 %	23.3	23.3	-0.6789		
3	37 %	26.9	26.9	-2.425		
4	49 %	24.1	24.1	-1.067		
5	65 %	22.9	22.9	-0.485		
6	87 %	22.3	22.3	-0.194		
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	4.763	21.7	-1.4	
3	37 %	10	4.763	21.7	-5	
4	49 %	10	4.763	21.7	-2.2	
5	65 %	10	4.763	21.7	-1	
6	87 %	10	4.763	21.7	-0.4	

Appendix A3: Water Chemistry
Routine Chemical and Physical Data

Effluent Conc.: Control	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
DO, mg/l	Initial	7.5	8.1	8.1	8.0	8.0	8.1	8.2
	Final *1	7.9	8.2	8.3	7.5	7.0	6.9	7.9
	Final *2	8.1	8.4	8.5	7.5	7.4	6.6	7.6
pH, units	Initial	8.1	7.9	7.8	7.5	7.6	7.8	7.7
	Final *1	7.9	7.9	8.0	7.1	7.8	7.6	7.9
	Final *2	7.8	8.0	8.1	7.9	8.0	7.9	8.0
Alkalinity, mg CaCO ₃ /l	32	NA	33	NA	33	NA	NA	
Hardness, mg CaCO ₃ /l	43	NA	45	NA	42	NA	NA	
Conductivity, umhos/cm	160	170	160	150	170	170	150	
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	8.1	8.2	8.2	8.9	8.2
	Final *1	7.6	8.2	8.2	6.9	5.8	7.4	8.0
	Final *2	9.5	8.4	8.3	8.0	5.4	7.2	7.6
pH, units	Initial	8.1	8.0	7.8	7.7	7.7	7.8	7.7
	Final *1	7.9	8.0	8.1	7.1	8.0	7.8	7.8
	Final *2	7.9	8.1	7.9	8.2	8.4	8.2	8.3

Effluent Conc.: 37 %	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
DO, mg/l	Initial	8.1	7.3	8.2	8.4	8.2	8.6	8.2
	Final *1	7.7	8.2	8.3	3.7	5.8	7.6	8.0
	Final *2	8.4	8.4	7.8	6.6	5.7	7.6	7.7
pH, units	Initial	8.1	8.0	7.9	7.7	7.7	7.8	7.7
	Final *1	8.0	8.1	8.1	7.1	8.1	7.8	8.0
	Final *2	7.9	8.2	7.9	8.1	8.4	8.2	8.3

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.1	8.5	8.2	8.1	8.7	9.0	8.1
	Final *1	7.8	8.2	8.3	7.3	5.8	7.8	8.0
	Final *2	11	8.4	7.8	4.7	5.8	7.8	7.8
pH, units	Initial	8.1	8.0	7.9	7.9	7.8	7.8	7.8
	Final *1	8.0	8.1	8.1	7.3	8.2	7.9	8.0
	Final *2	8.0	8.2	7.9	8.1	8.5	8.3	8.2

Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.1	8.1	8.3	8.2	8.2	9.0	8.2
	Final *1	7.8	8.2	8.2	7.4	5.8	7.8	7.9
	Final *2	8.1	8.5	8.4	8.1	5.9	8.0	7.8
pH, units	Initial	8.1	8.1	8.0	7.9	7.8	7.9	7.8
	Final *1	8.0	8.2	8.2	7.4	8.2	7.9	8.0
	Final *2	8.0	8.2	8.2	8.4	8.6	8.2	8.4
Alkalinity, mg CaCO ₃ /l	48	NA	68	NA	38	NA	NA	NA
Hardness, mg CaCO ₃ /l	63	NA	92	NA	46	NA	NA	NA
Conductivity, umhos/cm	200	200	200	250	280	250	230	
Res. Chlorine, mg/l	<0.05	NA	<0.05	NA	<0.05	NA	NA	NA

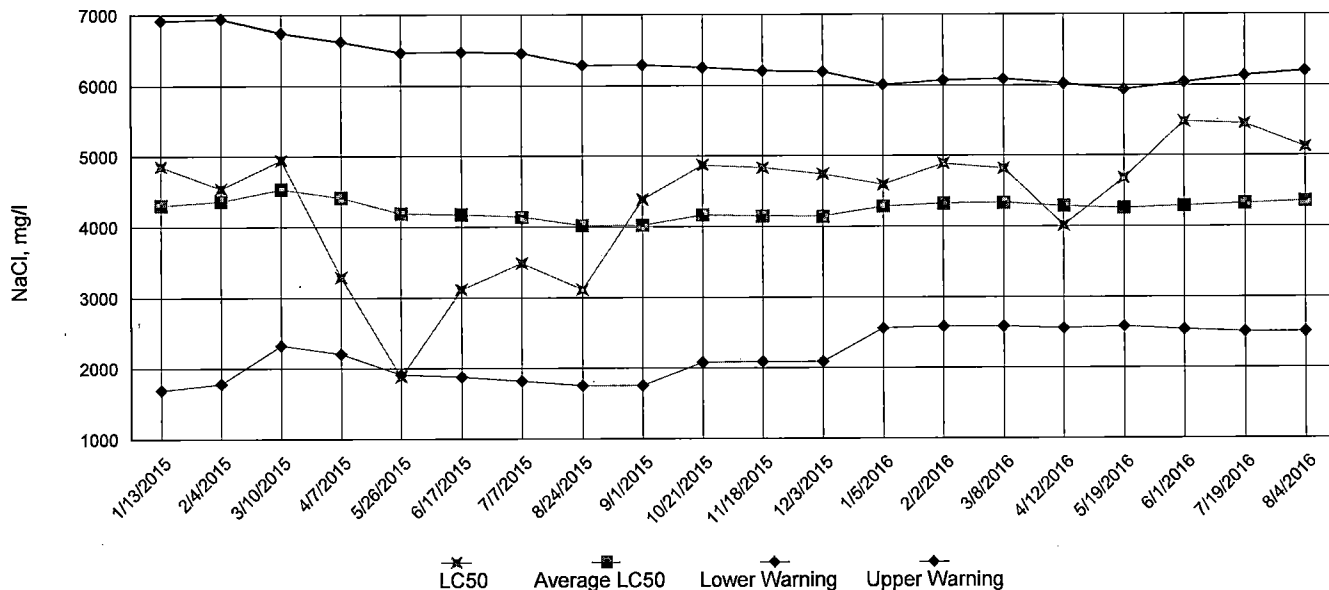
Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.2	8.0	8.1	8.3	8.2	6.1	8.2
	Final *1	7.8	8.4	8.3	7.2	5.8	7.8	7.9
	Final *2	9.9	8.5	8.5	8.2	6.0	8.1	7.8
pH, units	Initial	8.1	8.1	7.9	7.9	7.8	7.9	7.9
	Final *1	8.1	8.2	8.2	7.4	8.3	8.0	8.0
	Final *2	8.0	8.3	8.2	8.5	8.6	8.2	8.3

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

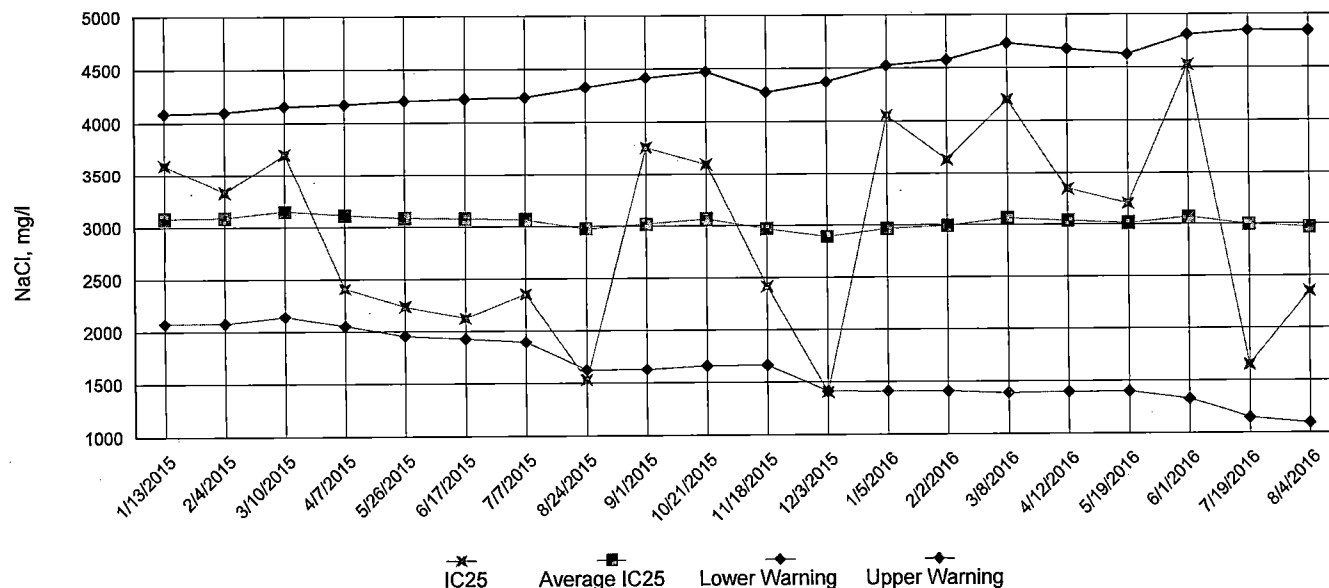
Appendix A4: Test 1000.0

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

LC50 Survival Data

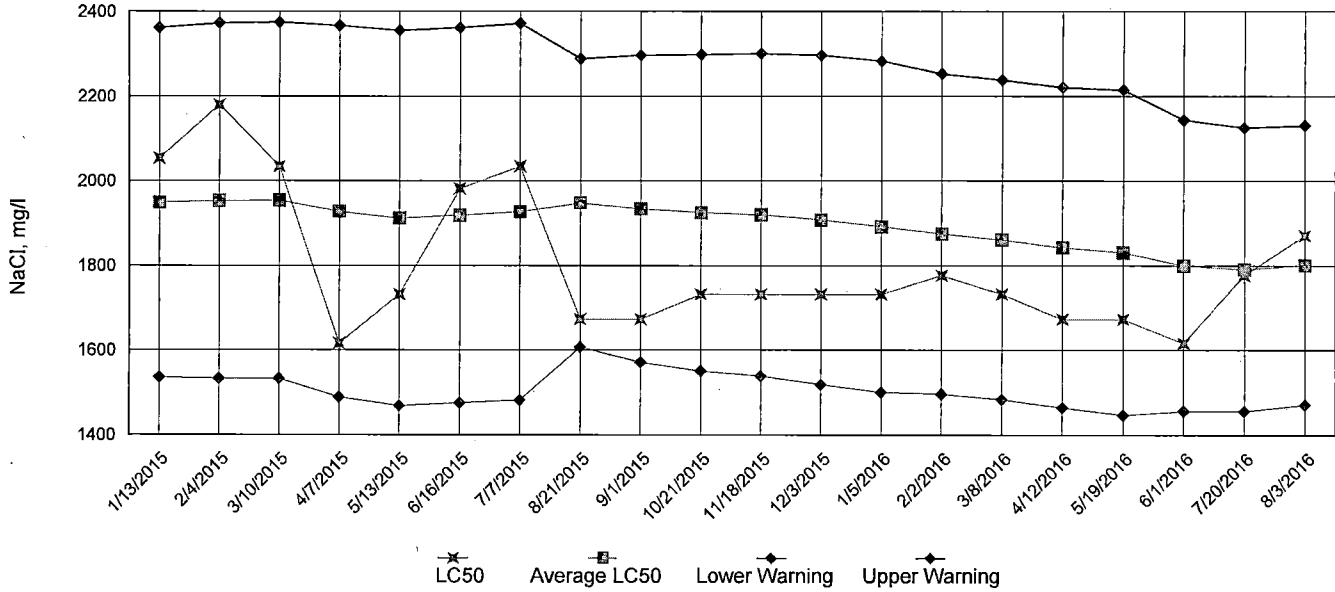


IC25 Growth Data

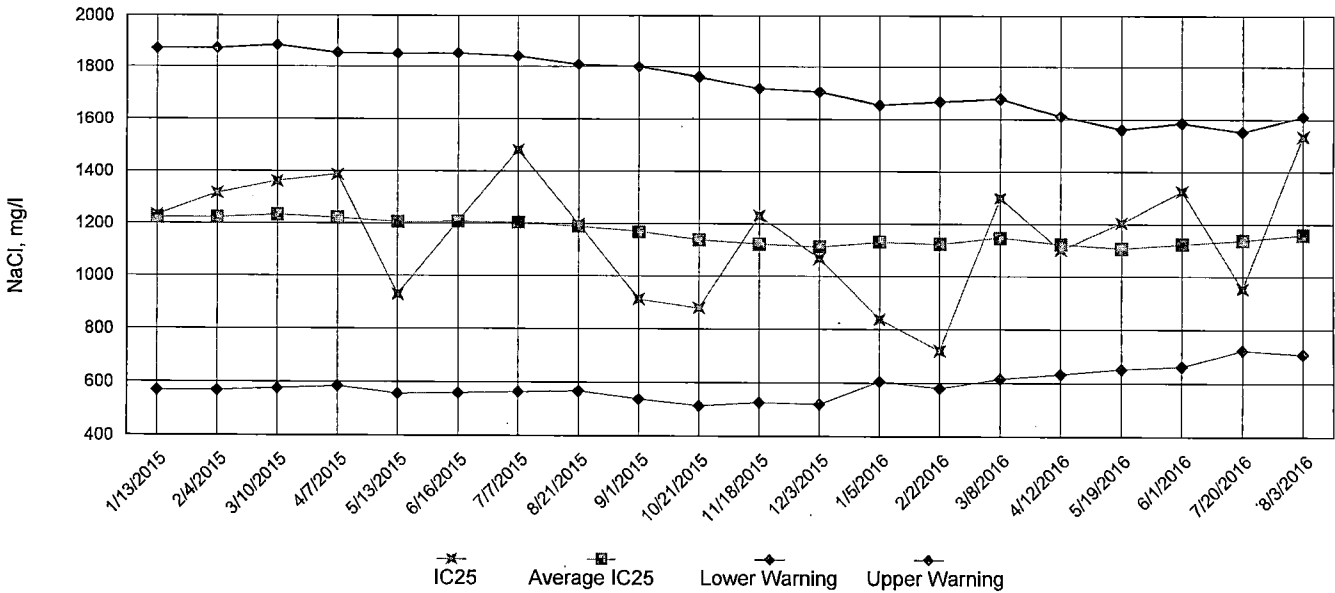


Appendix A4: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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: August 22, 2016 at 1405

Date and Time Test Terminated: August 30, 2016 at 1724

Dilution water used: Synthetic Soft Water #4356

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	100	100	100	100	100	0.00
49 %	100	100	100	100	100	100	100	100	0.00
65 %	100	100	100	100	100	100	100	100	0.00
87 %	100	100	100	100	100	100	100	100	0.00

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.450	0.439	0.400	0.440	0.369	0.42	8.14
27 %	0.411	0.490	0.455	0.420	0.384	0.432	9.53
37 %	0.389	0.410	0.409	0.362	0.379	0.39	5.24
49 %	0.342	0.386	0.379	0.364	0.361	0.366	4.67
65 %	0.346	0.371	0.378	0.421	0.409	0.385	7.83
87 %	0.355	0.396	0.411	0.378	0.374	0.383	5.61

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

Appendix B: Test 1000.0

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

1. Steel's Many-One Rank Test:

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

a.) LOW FLOW OR CRITICAL DILUTION	(65 %)	<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: 8.14 (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, 304, 310, 314

Test Initiated: DATE: August 22, 2016 TIME: 1405
Test Terminated: DATE: August 30, 2016 TIME: 1724

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.5	8.1	8.1	8.0	8.0	8.1	8.2
Final	7.9	8.2	8.3	7.5	7.0	6.9	7.9
pH Initial	8.1	7.9	7.8	7.5	7.6	7.8	7.7
Final	7.9	7.9	8.0	7.1	7.8	7.6	7.9
Alkalinity	32	NA	33	NA	33	NA	NA
Hardness	43	NA	45	NA	42	NA	NA
Conductivity	160	170	160	150	170	170	150
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	8.1	8.2	8.2	8.9	8.2
Final	7.6	8.2	8.2	6.9	5.8	7.4	8.0
pH Initial	8.1	8.0	7.8	7.7	7.7	7.8	7.7
Final	7.9	8.0	8.1	7.1	8.0	7.8	7.8
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	180	180	180	190	220	200	180
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 37 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	7.3	8.2	8.4	8.2	8.6	8.2
Final	7.7	8.2	8.3	3.7	5.8	7.6	8.0
pH Initial	8.1	8.0	7.9	7.7	7.7	7.8	7.7
Final	8.0	8.1	8.1	7.1	8.1	7.8	8.0
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	180	190	190	210	230	220	200
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 49 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	8.5	8.2	8.1	8.7	9.0	8.1
Final	7.8	8.2	8.3	7.3	5.8	7.8	8.0
pH Initial	8.1	8.0	7.9	7.9	7.8	7.8	7.8
Final	8.0	8.1	8.1	7.3	8.2	7.9	8.0
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	190	190	190	230	250	230	200
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 65 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	8.1	8.3	8.2	8.2	9.0	8.2
Final	7.8	8.2	8.2	7.4	5.8	7.8	7.9
pH Initial	8.1	8.1	8.0	7.9	7.8	7.9	7.8
Final	8.0	8.2	8.2	7.4	8.2	7.9	8.0
Alkalinity	48	NA	68	NA	38	NA	NA
Hardness	63	NA	92	NA	46	NA	NA
Conductivity	200	200	200	250	280	250	230
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 87 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.2	8.0	8.1	8.3	8.2	6.1	8.2
Final	7.8	8.4	8.3	7.2	5.8	7.8	7.9
pH Initial	8.1	8.1	7.9	7.9	7.8	7.9	7.9
Final	8.1	8.2	8.2	7.4	8.3	8.0	8.0
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	210	210	290	320	280	250
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: August 22, 2016 at 1430

Date and Time Test Terminated: August 29, 2016 at 1240

Dilution water used: Synthetic Soft Water #4356

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	16	18	34	21	25	22
B	20	16	23	29	22	19
C	22	22	22	27	22	22
D	24	24	29	18	10	16
E	22	20	27	20	23	21
F	27	25	25	29	30	24
G	27	30	29	26	26	32
H	12	21	28	27	26	27
I	23	31	26	21	23	25
J	26	26	26	23	22	15
Mean per Adult	21.9	23.3	26.9	24.1	22.9	22.3
Mean per Surviving Adult	21.9	23.3	26.9	24.1	22.9	22.3
CV %	22.1	20.9	12.7	16.5	22.7	22.8

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. Dunnett's Test:

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

a.) LOW FLOW OR CRITICAL DILUTION	(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: 22.7 (TQP3B)

Appendix B: Test 1002.0
CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
CHEMICAL PARAMETERS CHART

PERMITTEE: City of Hot Springs
NPDES NO.: AR0033880 AFIN#26-00145
CONTACT: Mr. James Sorrells
ANALYST: 280, 304, 310, 314

Test Initiated: DATE: August 22, 2016 TIME: 1430
Test Terminated: DATE: August 29, 2016 TIME: 240

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.5	8.1	8.1	8.0	8.0	8.1	8.2
Final	8.1	8.4	8.5	7.5	7.4	6.6	7.6
pH Initial	8.1	7.9	7.8	7.5	7.6	7.8	7.7
Final	7.8	8.0	8.1	7.9	8.0	7.9	8.0
Alkalinity	32	NA	33	NA	33	NA	NA
Hardness	43	NA	45	NA	42	NA	NA
Conductivity	160	170	160	150	170	170	150
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	8.1	8.2	8.2	8.9	8.2
Final	9.5	8.4	8.3	8.0	5.4	7.2	7.6
pH Initial	8.1	8.0	7.8	7.7	7.7	7.8	7.7
Final	7.9	8.1	7.9	8.2	8.4	8.2	8.3
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	180	180	180	190	220	200	180
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
37 %							
D.O. Initial	8.1	7.3	8.2	8.4	8.2	8.6	8.2
Final	8.4	8.4	7.8	6.6	5.7	7.6	7.7
pH Initial	8.1	8.0	7.9	7.7	7.7	7.8	7.7
Final	7.9	8.2	7.9	8.1	8.4	8.2	8.3
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	180	190	190	210	230	220	200
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
49 %							
D.O. Initial	8.1	8.5	8.2	8.1	8.7	9.0	8.1
Final	11	8.4	7.8	4.7	5.8	7.8	7.8
pH Initial	8.1	8.0	7.9	7.9	7.8	7.8	7.8
Final	8.0	8.2	7.9	8.1	8.5	8.3	8.2
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	190	190	190	230	250	230	200
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
65 %							
D.O. Initial	8.1	8.1	8.3	8.2	8.2	9.0	8.2
Final	8.1	8.5	8.4	8.1	5.9	8.0	7.8
pH Initial	8.1	8.1	8.0	7.9	7.8	7.9	7.8
Final	8.0	8.2	8.2	8.4	8.6	8.2	8.4
Alkalinity	48	NA	68	NA	38	NA	NA
Hardness	63	NA	92	NA	46	NA	NA
Conductivity	200	200	200	250	280	250	230
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
87 %							
D.O. Initial	8.2	8.0	8.1	8.3	8.2	6.1	8.2
Final	9.9	8.5	8.5	8.2	6.0	8.1	7.8
pH Initial	8.1	8.1	7.9	7.9	7.8	7.9	7.9
Final	8.0	8.3	8.2	8.5	8.6	8.2	8.3
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	210	210	290	320	280	250
Chlorine	NA	NA	NA	NA	NA	NA	NA

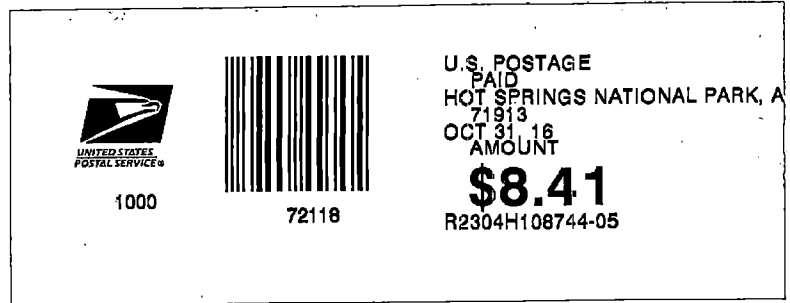
CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <i>City of Hot Springs</i>		PO No. <i>16-1336</i>		NO OF BOTTLES <i>Bio Monitoring</i>		ANALYSES REQUESTED										AIC CONTROL NO: <i>204941</i>	
Project Reference: <i>Bio-Monitoring</i>		MATRIX				AIC PROPOSAL NO:											
Project Manager: <i>JIM SCROLLS</i>		G R A B C O M P		W A T E R S O I L												Received Temperature C <i>0.1</i>	
Sampled By: <i>H. MAURIN</i>		Date/Time Collected <i>8/23/16 @ 0000-2400</i>														Remarks	
AIC No.	Sample Identification																
<i>2</i>	<i>PLANT Effluent</i>			<i>X X</i>		<i>3</i>		<i>X</i>									
Container Type						<i>P</i>										Field pH calibration on _____ @ _____	
Preservative						<i>NO</i>										Buffer:	
G = Glass . P = Plastic		NO = none S = Sulfuric acid pH2		V = VOA vials N = Nitric acid pH2		H = HCl to pH2 B = NaOH to pH12		T = Sodium Thiosulfate Z = Zinc acetate		A = (NH ₄) ₂ SO ₄ , NH ₄ OH							
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS						Relinquished By: <i>H. Maurin</i>		Date/Time <i>8/24/16 @ 10:50</i>		Received By: <i>M. Mann</i>		Date/Time <i>8-24-16 @ 1050</i>					
Expedited results requested by: _____						Relinquished By: <i>M. Mann</i>		Date/Time <i>8-24-16 @ 11:45</i>		Received in Lab By: <i>D. Brown</i>		Date/Time <i>8-24-16 11:45</i>					
Who should AIC contact with questions: _____						Comments:											
Phone: _____ Fax: _____																	
Report Attention to: _____																	
Report Address to: _____																	
Email Address: _____																	

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: PLANT Effluent			PO No. 16-1336		NO OF BOTTLES	ANALYSES REQUESTED										AIC CONTROL NO: 204941					
Project Reference: BIO-MONITORING			MATRIX			Bio-Monitoring											AIC PROPOSAL NO:				
Project Manager: JIM SORRELLS							3											Carrier: ITS DELIVERY			
Sampled By: HAROLD MAULDIN																		Received Temperature C			
AIC No.	Sample Identification	Date/Time Collected	G R A B	C O M P	W A T E R	S O I L	3											Remarks			
3	PLANT Effluent	8/25/16 @ 2000-2400		X	X			X													
Container Type							P											Field pH calibration on _____ @ _____			
Preservative							NO											Buffer:			
G = Glass NO = none			P = Plastic S = Sulfuric acid pH2		V = VOA vials N = Nitric acid pH2		H = HCl to pH2 B = NaOH to pH12			T = Sodium Thiosulfate Z = Zinc acetate			A = (NH ₄) ₂ SO ₄ , NH ₄ OH								
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS							Relinquished By: A. Cates			Date/Time 8-26-16 @ 0940			Received By: M. Mann			Date/Time 8-26-16 @ 9:40					
Expedited results requested by: _____							Relinquished By: M. Mann			Date/Time 8-26-16 @ 10:40			Received in Lab By: D. BROWN			Date/Time 8-26-16 1040					
Who should AIC contact with questions: Phone: _____ Fax: _____							Comments:														
Report Attention to: Report Address to: Email Address:																					

City of Hot Springs
Wastewater Treatment Plant
320 Davidson Drive
Hot Springs, Ar 71901



RETURN RECEIPT
REQUESTED

Arkansas Department of Environmental
Quality
5301 North Shore Drive
North Little Rock, AR 72118-5317

