

Sanitary Sewer Overflow (SSO) Monthly Report

Facility Name: Hot Springs Wastewater NPDES Permit No.: AR0033880 Monitoring Period (Month/Year) March / 2016

No Sanitary Sewer Overflows This Monitoring Period

Summary Report Code Descriptions				
Cause(s) of SSO	SSO Impact	Action(s) Taken	Ultimate Discharge Location	
CO -Construction	D -Debris	NEAH -No Evidence Adverse Health/Environmental Impact		CR -Creek/Stream/River (specify)
E -Equipment Failure	G -Grease	OEHC -Observed or Evidence of Human Contact	EC -Environmental Cleanup	DI -Ditch
HC -Hydro Clean	LF -Line Failure	EFK -Evidence of Fish Kill	HC -Hydro Cleaned	DR -Drop Inlet
R -Rainfall	RG -Roots/Grease		HR -Hand Rodded	GR -Ground Surface
RO -Roots	V -Vandalism		EN -Referred to Engineering	PA -Paved Area
			PN -Public Notification	CB -Contained in Building

Location	Manhole #	Start Date of SSO	End Date of SSO	Estimated Volume (in gallons)	Cause of SSO	Environmental Impact	Action(s) Taken to Address SSO	Discharge Location
615 McLeod St.	MH# 998	03/09/2016	03/11/2016	6000	R	NEAH	EC	CR--Stokes
519 McLeod St	MH# 995	03/09/2016	03/11/2016	5000	R	NEAH	EC	CR--Stokes
403 McLeod St.	MH# 991	03/09/2016	03/11/2016	4500	R	NEAH	EC	CR--Stokes
106 Forest View Ct.	MH# 12242	03/09/2016	03/11/2016	3500	R	NEAH	EC	CR--Hot Springs
106 Forest View Ct.	MH# 1864	03/09/2016	03/11/2016	4000	R	NEAH	EC	CR--Hot Springs
352 Fontana Rd.	MH# 1865	03/09/2016	03/11/2016	4000	R	NEAH	EC	CR--Hot Springs
362 Fontana Rd.	MH# 1866	03/09/2016	03/11/2016	4500	R	NEAH	EC	CR--Hot Springs
816 Carpenter Dam Rd.	MH# 5260	03/09/2016	03/11/2016	2500	R	NEAH	EC	CR--Unknown
857 Carpenter Dam Rd.	MH# 5259	03/09/2016	03/14/2016	12000	R	NEAH	EC	CR--Unknown
3047 Malvern Ave	MH# 1747	03/09/2016	03/14/2016	18000	R	NEAH	EC	CR--Gulpha
1539 Spring St	MH# 4118	03/09/2016	03/11/2016	3500	R	NEAH	EC	CR--Gulpha

Signature of Cognizant or Ranking Official

Date 4-12-2016

I certify under penalty of law that this document and all attachments were prepared under my direction or 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 of knowing violations.

11 / 60,300

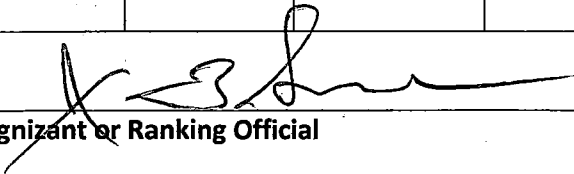
Sanitary Sewer Overflow (SSO) Monthly Report

Facility Name: Hot Springs Wastewater NPDES Permit No.: AR0033880 Monitoring Period (Month/Year) March / 2016

No Sanitary Sewer Overflows This Monitoring Period

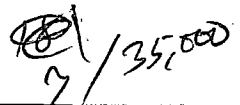
Summary Report Code Descriptions				
Cause(s) of SSO	SSO Impact	Action(s) Taken	Ultimate Discharge Location	
CO -Construction	D -Debris	NEAH -No Evidence Adverse Health/Environmental Impact		CR -Creek/Stream/River (specify)
E -Equipment Failure	G -Grease	OEHC -Observed or Evidence of Human Contact	EC -Environmental Cleanup	DI -Ditch
HC -Hydro Clean	LF -Line Failure	EFK -Evidence of Fish Kill	HC -Hydro Cleaned	DR -Drop Inlet
R -Rainfall	RG -Roots/Grease		HR -Hand Rodded	GR -Ground Surface
RO -Roots	V -Vandalism		EN -Referred to Engineering	PA -Paved Area
			PN -Public Notification	CB -Contained in Building

Location	Manhole #	Start Date of SSO	End Date of SSO	Estimated Volume (in gallons)	Cause of SSO	Environmental Impact	Action(s) Taken to Address SSO	Discharge Location
1539 Spring St.	MH# 4117	03/09/2016	03/11/2016	3000	R	NEAH	EC	CR--Gulpha
519 McLeod St.	MH# 995	03/31/2016	03/31/2016	3000	R	NEAH	EC	CR--Stokes
1539 Spring St.	MH# 4118	03/31/2016	03/31/2016	1000	R	NEAH	EC	CR--Gulpha
104 Forest View Ct.	MH# 1864	03/31/2016	03/31/2016	5000	R	NEAH	EC	CR--Hot Springs
615 McLeod St.	MH# 998	03/31/2016	03/31/2016	5000	R	NEAH	EC	CR--Stokes
857 Carpenter Dam Rd.	MH# 5259	03/31/2016	03/31/2016	8500	R	NEAH	EC	CR--Unknown
3047 Malvern Ave	MH# 1747	03/31/2016	03/31/2016	9500	R	NEAH	EC	CR--Gulpha


 Signature of Cognizant or Ranking Official

4-12-2016
 Date

I certify under penalty of law that this document and all attachments were prepared under my direction or 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 of knowing violations.


 3/35,000

March 3, 2016

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

Control No. 199512-1

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 %. 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 %. 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	0.279	PASS
Control Growth CV < or = 40%	4.40	PASS
Growth Minimum Significant Difference 12 to 30%	12.9	PASS
Critical Dilution CV < or = 40%	9.75	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	23.8	PASS
Control CV < or = 40% per Surviving Female	12.0	PASS
Reproduction Minimum Significant Difference 13 to 47%	14.0	PASS
Critical Dilution CV < or = 40%	12.9	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)	8.6	8.6	8.3
pH (standard units)	6.8	7.1	7.0
Alkalinity (mg/l as CaCO ₃)	49	55	28
Hardness (mg/l as CaCO ₃)	74	67	47
Conductivity (umhos/cm)	330	300	180
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	0.95	<0.1

2. Dilution Water Samples: Synthetic Soft Water #4302

- a. Dates Prepared: February 12 through February 26, 2016
- b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.0	8.6	8.1
pH (standard units)	7.3	7.2	7.1
Alkalinity (mg/l as CaCO ₃)	31	31	31
Hardness (mg/l as CaCO ₃)	48	48	48
Conductivity (umhos/cm)	160	150	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: February 23, 2016 at 1100
Date & Time Test Terminated: March 1, 2016 at 1005
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: February 23, 2016 at 1050
Date & Time Test Terminated: February 29, 2016 at 1115
Type & Volume of Test Chamber: 30 ml disposable beaker
Volume of Sample: 15 ml
Number of Organisms per replicate: 1
Number of Replicates per dilution: 10

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

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

D. Test Organisms

1. Scientific Name

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

III. Data Analysis

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

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

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

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

IV. Standard Reference Toxicants

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

Pimephales promelas (Fathead minnow)

Chronic reference tests are performed monthly.

A chronic reference test was performed on February 2, 2016 at 1425 to February 9, 2016 at 1340

The results were as follows: (Control No. 198842-1.)

Survival LC-50: 4893 mg/l

Growth IC-25: 3633 mg/l

Growth PMSD: 14.2

Ceriodaphnia dubia

Chronic reference tests are performed monthly.

A chronic reference test was performed on February 2, 2016 at 1610 to February 8, 2016 at 1450

The results were as follows: (Control No. 198842-2.)

Survival LC-50: 1777 mg/l

Growth IC-25: 720.1 mg/l

Growth PMSD: 21.4

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	98.6	1.97
pH	SM 4500-H+ B	101	0.145
Conductivity	EPA 120.1	101	3.97

VI. Organism History

Pimephales promelas (Fathead minnow)

Date: February 23, 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: February 23, 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

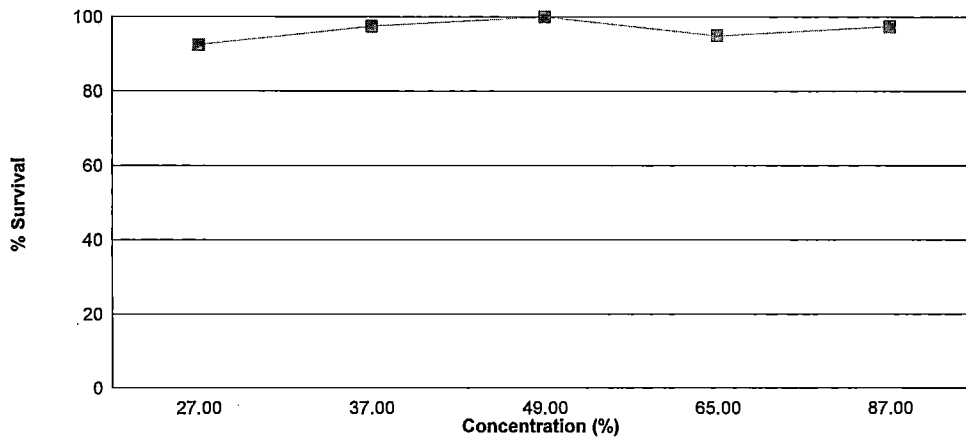
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

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 February 23, 2016 at 1100 and continued through March 1, 2016 at 1005. 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	0.279
27 %	92.5	0.268
37 %	97.5	0.276
49 %	100	0.276
65 %	95.0	0.257
87 %	97.5	0.269

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

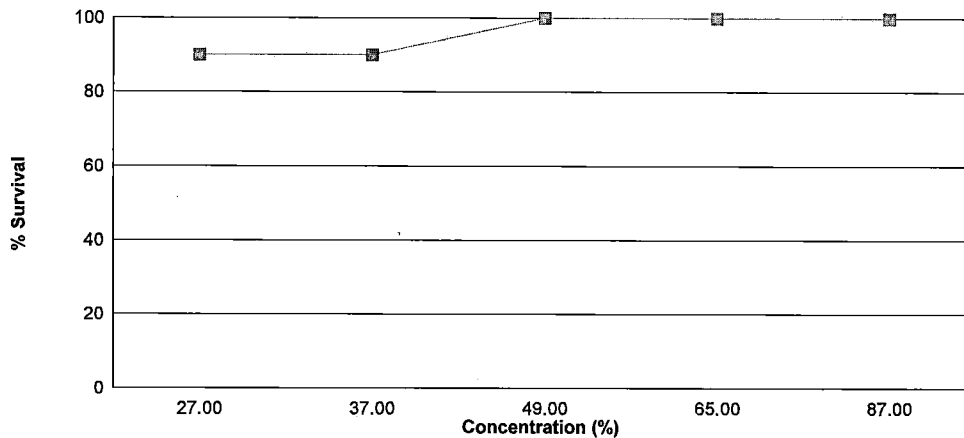
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

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 February 23, 2016 at 1050 and continued through February 29, 2016 at 1115. 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 6-day <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	23.8
27 %	90.0	24.6
37 %	90.0	24.0
49 %	100	25.6
65 %	100	23.9
87 %	100	22.6

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: February 23, 2016 at 1100

Date and Time Test Terminated: March 1, 2016 at 1005

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	7	6
	D	8	8	8	8	8	8	8
	E	8	7	7	7	7	7	7
37 %	A	8	8	8	8	8	8	7
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
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	7	7
	C	8	8	8	8	8	8	8
	D	8	8	7	7	7	7	7
	E	8	8	8	8	8	8	8
87 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	7	7
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: February 23, 2016 at 1100
Test Terminated: March 1, 2016 at 1005

Drying Started: February 29, 2016 at 1330
Drying Ended: March 3, 2016 at 0850

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.93923	.94132	0.00209	8	0.261
	B	.94517	.94749	0.00232	8	0.290
	C	.95345	.95568	0.00223	8	0.279
	D	.93860	.94080	0.00220	8	0.275
	E	.95194	.95427	0.00233	8	0.291
27 %	A	.95027	.95251	0.00224	8	0.280
	B	.94495	.94731	0.00236	8	0.295
	C	.94428	.94606	0.00178	8	0.222
	D	.93964	.94196	0.00232	8	0.290
	E	.94288	.94492	0.00204	8	0.255
37 %	A	.94262	.94455	0.00193	8	0.241
	B	.94097	.94307	0.00210	8	0.262
	C	.94198	.94437	0.00239	8	0.299
	D	.94195	.94416	0.00221	8	0.276
	E	.94903	.95145	0.00242	8	0.302
49 %	A	.94335	.94558	0.00223	8	0.279
	B	.94067	.94300	0.00233	8	0.291
	C	.94514	.94739	0.00225	8	0.281
	D	.94520	.94707	0.00187	8	0.234
	E	.94631	.94867	0.00236	8	0.295
65 %	A	.94193	.94402	0.00209	8	0.261
	B	.94697	.94899	0.00202	8	0.252
	C	.94319	.94529	0.00210	8	0.262
	D	.94475	.94652	0.00177	8	0.221
	E	.94512	.94745	0.00233	8	0.291
87 %	A	.94877	.95076	0.00199	8	0.249
	B	.94911	.95102	0.00191	8	0.239
	C	.94496	.94719	0.00223	8	0.279
	D	.95008	.95236	0.00228	8	0.285
	E	.94767	.95000	0.00233	8	0.291

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: February 23, 2016 at 1050

Date and Time Test Terminated: February 29, 2016 at 1115

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	5	0	0	3	3	3	4	2	3	2	25	10	2.50	
4	0	3	4	0	0	0	0	0	0	4	11	10	1.10	
5	10	10	10	10	8	8	5	11	8	9	89	10	8.90	
6	12	11	6	11	16	12	10	12	11	12	113	10	11.3	
7														
8														
TOTAL	27	24	20	24	27	23	19	25	22	27	238	10	23.8	

Concentration: 27 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	4	0	0	4	4	3	3	4	2	0	24	10	2.40
4	0	3	3	1	4	1	0	10	0	4	26	10	2.60
5	5	9	8	9	1	9	8	1	9	5	64	10	6.40
6	16	12X	15	10	15	12	13	15	10	14	132	9	14.7
7													
8													
TOTAL	25	24	26	24	24	25	24	30	21	23	246	10	24.6

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	X	0	0	0	0	0	9	0.00
3	1	0	0	3	5	X	3	3	0	0	15	9	1.67
4	0	5	4	0	0	X	0	0	3	0	12	9	1.33
5	9	8	9	10	9	X	7	9	7	10	78	9	8.67
6	18	11	10	19	16	X	13	20	14	14	135	9	15.0
7													
8													
TOTAL	28	24	23	32	30	0	23	32	24	24	240	10	24.0

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: February 23, 2016 at 1050

Date and Time Test Terminated: February 29, 2016 at 1115

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	3	0	0	0	4	4	3	3	2	0	19	10	1.90
4	0	2	4	4	0	0	0	0	0	3	13	10	1.30
5	9	6	10	6	10	8	7	10	11	11	88	10	8.80
6	11	12	13	12	14	12	12	19	18	13	136	10	13.6
7													
8													
TOTAL	23	20	27	22	28	24	22	32	31	27	256	10	25.6

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	4	0	0	0	4	4	3	3	2	0	20	10	2.00
4	0	6	4	4	0	0	0	1	0	5	20	10	2.00
5	8	8	10	10	9	1	6	10	9	8	79	10	7.90
6	11	12	12	13	12	13	10	12	14	11	120	10	12.0
7													
8													
TOTAL	23	26	26	27	25	18	19	26	25	24	239	10	23.9

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	3	4	3	4	0	21	10	2.10
4	0	3	4	4	3	0	0	2	0	2	18	10	1.80
5	8	9	8	9	1	6	7	7	8	5	68	10	6.80
6	10	12	13	12	13	12	11	12	13	11	119	10	11.9
7													
8													
TOTAL	21	24	25	25	21	21	22	24	25	18	226	10	22.6

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	0.75000	1.04720
2	27 %	4	1.00000	1.39310
2	27 %	5	0.87500	1.20940
3	37 %	1	0.87500	1.20940
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	0.87500	1.20940
5	65 %	3	1.00000	1.39310
5	65 %	4	0.87500	1.20940
5	65 %	5	1.00000	1.39310
6	87 %	1	1.00000	1.39310
6	87 %	2	0.87500	1.20940
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 = 0.1918 W = 0.861 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 %	22.50	16.00	5.00	
3	37 %	25.00	16.00	5.00	
4	49 %	27.50	16.00	5.00	
5	65 %	22.50	16.00	5.00	
6	87 %	25.00	16.00	5.00	
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality	No Transformation
<p>D = 0.01391 W = 0.9371 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30)</p> <p>Data PASS normality test (alpha = 0.01).</p>	

Bartlett's Test for Homogeneity of Variance	No Transformation
<p>Calculated B1 statistic = 2.694 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	0.001573	0.0003146	0.5424	
Within (Error)	24	0.01392	0.00058		
Total	29	0.01549			
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	0.2792	0.2792			
2	27 %	0.2684	0.2684	0.7091		
3	37 %	0.276	0.276	0.2101		
4	49 %	0.276	0.276	0.2101		
5	65 %	0.2574	0.2574	1.431		
6	87 %	0.2686	0.2686	0.6959		
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	0.03595	12.9	0.0108	
3	37 %	5	0.03595	12.9	0.0032	
4	49 %	5	0.03595	12.9	0.0032	
5	65 %	5	0.03595	12.9	0.0218	
6	87 %	5	0.03595	12.9	0.0106	

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 %	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
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	1	
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 = 0.1594 D* = 1.251 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 %	110.50	75.00	10.00	
3	37 %	117.00	75.00	10.00	
4	49 %	117.00	75.00	10.00	
5	65 %	106.50	75.00	10.00	
6	87 %	93.00	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	99.05	19.81	2.013	
Within (Error)	53	521.6	9.842		
Total	58	620.7			
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	23.8	23.8			
2	27 %	24.6	24.6	-0.5702		
3	37 %	26.667	26.667	-1.989		
4	49 %	25.6	25.6	-1.283		
5	65 %	23.9	23.9	-0.07128		
6	87 %	22.6	22.6	0.8553		
Dunnett's critical value = 2.31 (1 Tailed, alpha = 0.05, df [used] = 5,40) (Actual df = 5,53)						
WARNING - Unequal replicate sizes. Critical values assuming equal replicate sizes have been used.						

Dunnett's Test - Table 2 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control	
1	Control	10				
2	27 %	10	3.241	13.6	-0.8	
3	37 %	9	3.33	14	-2.867	
4	49 %	10	3.241	13.6	-1.8	
5	65 %	10	3.241	13.6	-0.1	
6	87 %	10	3.241	13.6	1.2	

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: February 23, 2016 at 0827

Date and Time Test Terminated: March 1, 2016 at 1005

Effluent Conc.: Control	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
DO, mg/l	Initial	8.0	7.2	8.6	8.8	8.1	8.4	8.8
	Final *1	7.5	5.8	8.4	7.9	8.2	8.2	7.7
	Final *2	7.6	8.0	8.6	7.4	7.8	8.6	
pH, units	Initial	7.3	6.2	7.2	7.0	7.1	7.2	7.2
	Final *1	7.2	7.0	7.3	6.9	7.2	7.3	7.2
	Final *2	7.5	7.2	7.4	7.4	7.4	7.5	
Alkalinity, mg CaCO ₃ /l	31	NA	31	NA	31	NA	NA	
Hardness, mg CaCO ₃ /l	48	NA	48	NA	48	NA	NA	
Conductivity, umhos/cm	160	150	150	160	160	160	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	8.0	8.3	8.5	9.0	7.6	8.1	8.8
	Final *1	7.6	5.5	8.2	8.1	8.2	8.4	7.5
	Final *2	7.6	8.1	8.6	7.7	7.9	8.8	
pH, units	Initial	7.1	7.1	7.2	7.1	7.0	7.2	7.1
	Final *1	7.3	6.9	7.3	7.0	7.3	7.3	7.1
	Final *2	7.5	7.3	7.5	7.4	7.4	7.5	

Effluent Conc.: 37 %	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
DO, mg/l	Initial	8.2	8.2	8.6	9.0	7.8	8.0	8.6
	Final *1	7.6	5.4	8.4	7.8	8.2	8.4	7.4
	Final *2	7.6	8.1	8.7	8.1	7.6	8.4	
pH, units	Initial	7.1	7.2	7.2	7.2	7.0	7.1	7.1
	Final *1	7.3	7.0	7.4	7.0	7.3	7.3	7.1
	Final *2	7.5	7.3	7.5	7.5	7.4	7.4	

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: February 23, 2016 at 0827

Date and Time Test Terminated: March 1, 2016 at 1005

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.3	8.2	8.4	8.7	8.0	8.0	8.7
	Final *1	7.4	5.4	8.5	7.6	8.1	8.4	7.7
	Final *2	7.6	8.1	8.8	7.8	7.7	8.3	
pH, units	Initial	7.0	7.2	7.1	7.1	7.0	7.1	7.0
	Final *1	7.4	7.0	7.3	7.0	7.3	7.3	7.1
	Final *2	7.5	7.4	7.5	7.5	7.4	7.4	

Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.3	8.1	8.5	8.8	8.0	8.3	9.0
	Final *1	7.2	5.4	8.4	7.8	8.1	8.4	7.8
	Final *2	7.5	8.1	8.7	7.7	7.8	8.6	
pH, units	Initial	7.0	6.8	7.1	7.1	7.0	7.1	7.0
	Final *1	7.3	7.1	7.3	7.0	7.4	7.2	7.1
	Final *2	7.6	7.4	7.6	7.5	7.5	7.4	
Alkalinity, mg CaCO ₃ /l	45	NA	46	NA	32	NA	NA	NA
Hardness, mg CaCO ₃ /l	69	NA	61	NA	49	NA	NA	NA
Conductivity, umhos/cm	270	260	250	250	170	170	170	170
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	8.4	8.3	8.4	9.0	8.2	8.3	8.7
	Final *1	7.4	5.2	8.6	7.9	8.2	8.5	7.6
	Final *2	7.5	8.2	8.6	8.0	7.9	8.8	
pH, units	Initial	7.0	7.3	7.1	7.1	7.0	7.1	6.9
	Final *1	7.3	7.1	7.4	7.1	7.4	7.3	7.1
	Final *2	7.6	7.5	7.6	7.6	7.3	7.4	

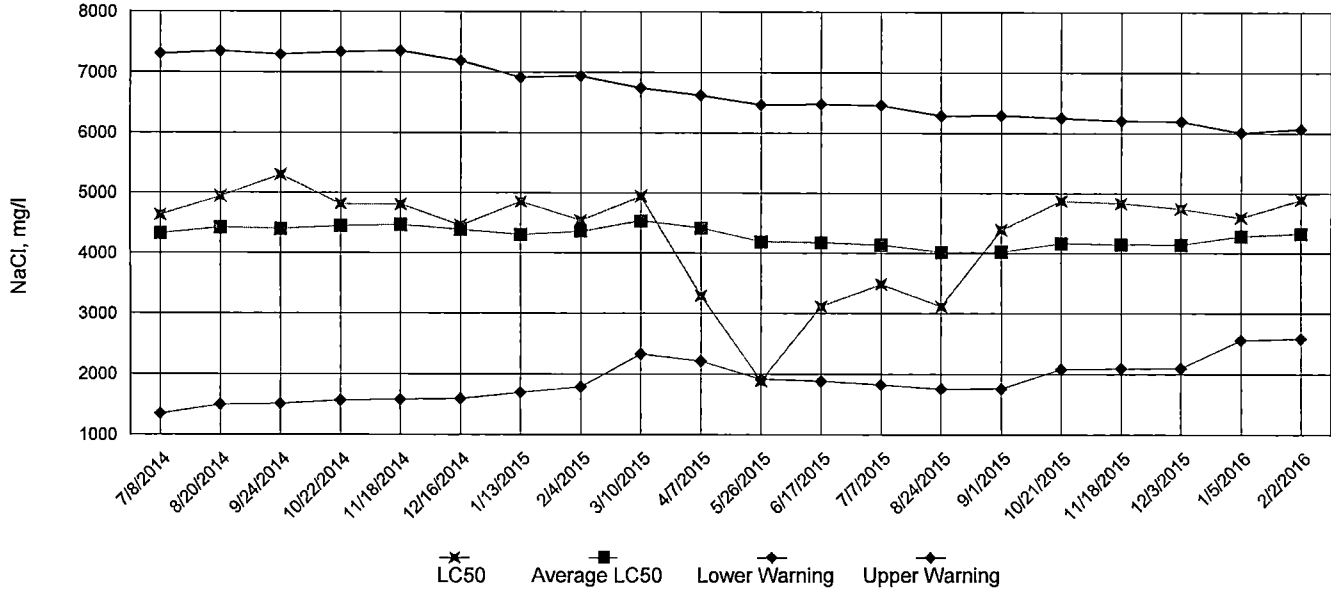
*1 = data from the *Pimephales promelas* (Fathead Minnow) test

*2 = data from the *Ceriodaphnia dubia* test

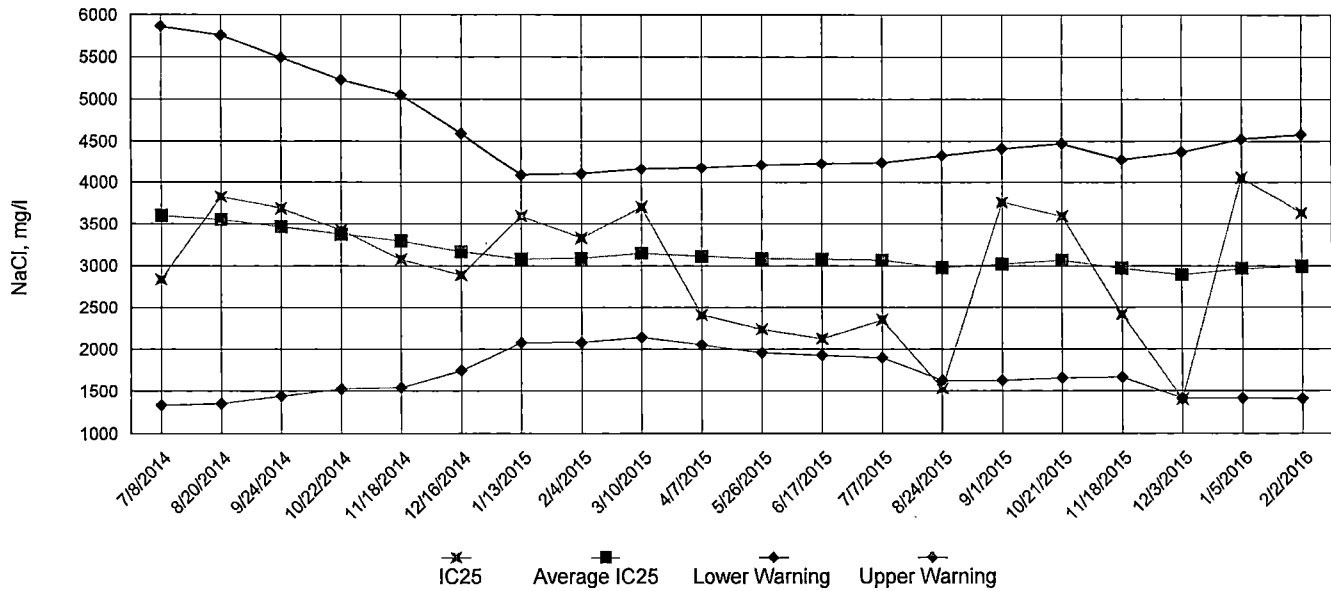
Appendix A4: Test 1000.0

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

LC50 Survival Data

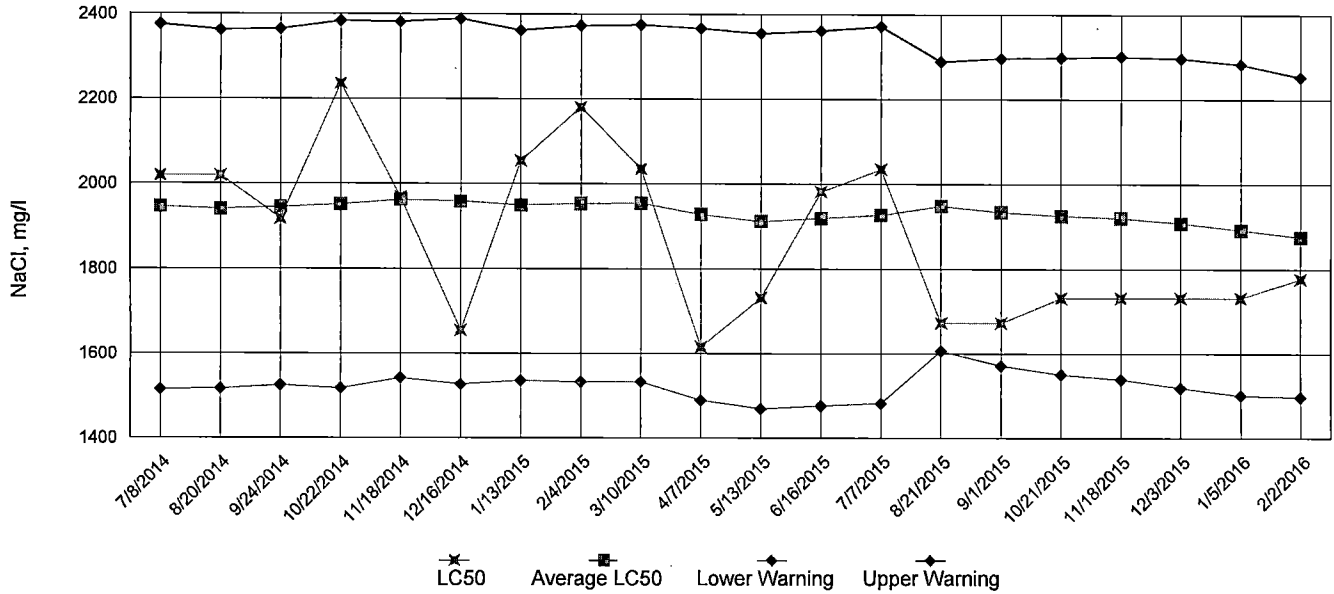


IC25 Growth Data

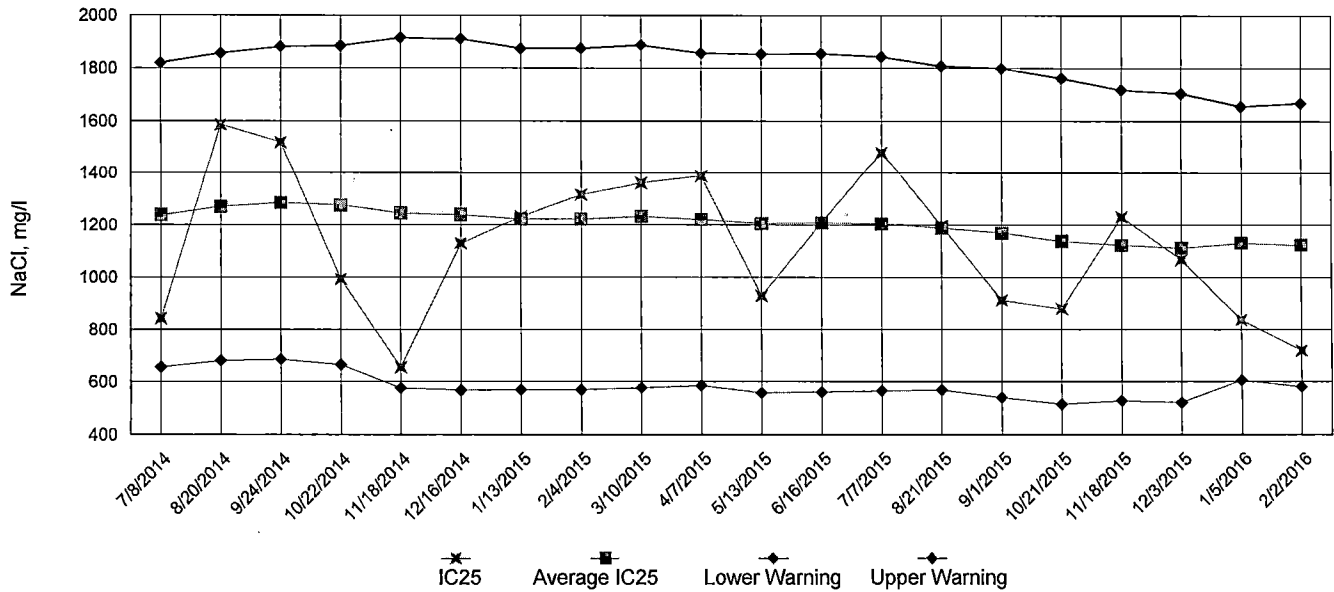


Appendix A4: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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: February 23, 2016 at 1100

Date and Time Test Terminated: March 1, 2016 at 1005

Dilution water used: Synthetic Soft Water #4302

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	75.0	100	87.5	100	97.5	92.5	12.1
37 %	87.5	100	100	100	100	100	100	97.5	5.73
49 %	100	100	100	100	100	100	100	100	0.00
65 %	100	87.5	100	87.5	100	100	100	95.0	7.21
87 %	100	87.5	100	100	100	100	100	97.5	5.73

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.261	0.290	0.279	0.275	0.291	0.279	4.40
27 %	0.280	0.295	0.222	0.290	0.255	0.268	11.2
37 %	0.241	0.262	0.299	0.276	0.302	0.276	9.28
49 %	0.279	0.291	0.281	0.234	0.295	0.276	8.85
65 %	0.261	0.252	0.262	0.221	0.291	0.257	9.75
87 %	0.249	0.239	0.279	0.285	0.291	0.269	8.61

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: 9.75 (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

2400
2400
2400

Test Initiated: DATE: February 23, 2016 TIME: 1100
Test Terminated: DATE: March 1, 2016 TIME: 1005

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.2	8.6	8.8	8.1	8.4	8.8
Final	7.5	5.8	8.4	7.9	8.2	8.2	7.7
pH Initial	7.3	6.2	7.2	7.0	7.1	7.2	7.2
Final	7.2	7.0	7.3	6.9	7.2	7.3	7.2
Alkalinity	31	NA	31	NA	31	NA	NA
Hardness	48	NA	48	NA	48	NA	NA
Conductivity	160	150	150	160	160	160	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	8.0	8.3	8.5	9.0	7.6	8.1	8.8
Final	7.6	5.5	8.2	8.1	8.2	8.4	7.5
pH Initial	7.1	7.1	7.2	7.1	7.0	7.2	7.1
Final	7.3	6.9	7.3	7.0	7.3	7.3	7.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	200	190	190	160	160	160
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 37 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.2	8.2	8.6	9.0	7.8	8.0	8.6
Final	7.6	5.4	8.4	7.8	8.2	8.4	7.4
pH Initial	7.1	7.2	7.2	7.2	7.0	7.1	7.1
Final	7.3	7.0	7.4	7.0	7.3	7.3	7.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	220	220	200	210	160	160	160
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 49 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.3	8.2	8.4	8.7	8.0	8.0	8.7
Final	7.4	5.4	8.5	7.6	8.1	8.4	7.7
pH Initial	7.0	7.2	7.1	7.1	7.0	7.1	7.0
Final	7.4	7.0	7.3	7.0	7.3	7.3	7.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	240	240	220	230	160	160	160
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 65 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.3	8.1	8.5	8.8	8.0	8.3	9.0
Final	7.2	5.4	8.4	7.8	8.1	8.4	7.8
pH Initial	7.0	6.8	7.1	7.1	7.0	7.1	7.0
Final	7.3	7.1	7.3	7.0	7.4	7.2	7.1
Alkalinity	45	NA	46	NA	32	NA	NA
Hardness	69	NA	61	NA	49	NA	NA
Conductivity	270	260	250	250	170	170	170
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 87 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.4	8.3	8.4	9.0	8.2	8.3	8.7
Final	7.4	5.2	8.6	7.9	8.2	8.5	7.6
pH Initial	7.0	7.3	7.1	7.1	7.0	7.1	6.9
Final	7.3	7.1	7.4	7.1	7.4	7.3	7.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	300	300	280	280	170	170	170
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: February 23, 2016 at 1050

Date and Time Test Terminated: February 29, 2016 at 1115

Dilution water used: Synthetic Soft Water #4302

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	90.0	100	100	100
6 day	100	90.0	90.0	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent				
		27 %	37 %	49 %	65 %	87 %
A	27	25	28	23	23	21
B	24	24	24	20	26	24
C	20	26	23	27	26	25
D	24	24	32	22	27	25
E	27	24	30	28	25	21
F	23	25	0	24	18	21
G	19	24	23	22	19	22
H	25	30	32	32	26	24
I	22	21	24	31	25	25
J	27	23	24	27	24	18
Mean per Adult	23.8	24.6	24.0	25.6	23.9	22.6
Mean per Surviving Adult	23.8	24.7	26.7	25.6	23.9	22.6
CV %	12.0	9.93	14.4	15.8	12.9	10.5

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

Appendix B: Test 1002.0
SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

1. Fisher's Exact Test:

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

a.) LOW FLOW OR CRITICAL DILUTION	(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: 12.9 (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

2400
2400
2400

Test Initiated: DATE: February 23, 2016 TIME: 1050
Test Terminated: DATE: February 29, 2016 TIME: 1115

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.2	8.6	8.8	8.1	8.4	8.8
Final	7.6	8.0	8.6	7.4	7.8	8.6	--
pH Initial	7.3	6.2	7.2	7.0	7.1	7.2	7.2
Final	7.5	7.2	7.4	7.4	7.4	7.5	--
Alkalinity	31	NA	31	NA	31	NA	NA
Hardness	48	NA	48	NA	48	NA	NA
Conductivity	160	150	150	160	160	160	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	8.0	8.3	8.5	9.0	7.6	8.1	8.8
Final	7.6	8.1	8.6	7.7	7.9	8.8	--
pH Initial	7.1	7.1	7.2	7.1	7.0	7.2	7.1
Final	7.5	7.3	7.5	7.4	7.4	7.5	--
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	200	190	190	160	160	160
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 37 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.2	8.2	8.6	9.0	7.8	8.0	8.6
Final	7.6	8.1	8.7	8.1	7.6	8.4	--
pH Initial	7.1	7.2	7.2	7.2	7.0	7.1	7.1
Final	7.5	7.3	7.5	7.5	7.4	7.4	--
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	220	220	200	210	160	160	160
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 49 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.3	8.2	8.4	8.7	8.0	8.0	8.7
Final	7.6	8.1	8.8	7.8	7.7	8.3	--
pH Initial	7.0	7.2	7.1	7.1	7.0	7.1	7.0
Final	7.5	7.4	7.5	7.5	7.4	7.4	--
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	240	240	220	230	160	160	160
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 65 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.3	8.1	8.5	8.8	8.0	8.3	9.0
Final	7.5	8.1	8.7	7.7	7.8	8.6	--
pH Initial	7.0	6.8	7.1	7.1	7.0	7.1	7.0
Final	7.6	7.4	7.6	7.5	7.5	7.4	--
Alkalinity	45	NA	46	NA	32	NA	NA
Hardness	69	NA	61	NA	49	NA	NA
Conductivity	270	260	250	250	170	170	170
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 87 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.4	8.3	8.4	9.0	8.2	8.3	8.7
Final	7.5	8.2	8.6	8.0	7.9	8.8	--
pH Initial	7.0	7.3	7.1	7.1	7.0	7.1	6.9
Final	7.6	7.5	7.6	7.6	7.3	7.4	--
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	300	300	280	280	170	170	170
Chlorine	NA	NA	NA	NA	NA	NA	NA



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>City of Hot Springs</u>			PO No. <u>16-88</u>		NO OF BOTTLES	ANALYSES REQUESTED										AIC CONTROL NO: <u>199512</u>		
Project Reference: <u>Bio-Monitoring</u>			MATRIX			<u>Bio Monitoring</u> <u>CO + FH</u>										AIC PROPOSAL NO:		
Project Manager: <u>Jim Sorrells</u>			W	S												Carrier:		
Sampled By: <u>H Mauldin</u>			G	C	W	S	Received Temperature C											
AIC No.	Sample Identification	Date/Time Collected	A	O	ATER	SOIL	6.1		<div style="border: 1px solid black; padding: 5px;"> Remarks </div>									
1	PLANT Effluent	2/21/16 @ 0000-2400	B	P	R	L	3											
Container Type			Preservative		P		NO		Field pH calibration 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 = (NH ₄) ₂ SO ₄ , NH ₄ OH			Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS															
Expedited results requested by: _____			Relinquished By: <u>[Signature]</u>			Date/Time <u>2/22/16 @ 10:00</u>			Received By: <u>m mann</u>			Date/Time <u>2-22-16 @ 10:00</u>						
Who should AIC contact with questions: _____			Relinquished By: <u>m. mann</u>			Date/Time <u>2-22-16 @ 10:55</u>			Received In Lab By: <u>[Signature]</u>			Date/Time <u>2/22/16 1055</u>						
Phone: _____ Fax: _____			Comments:															
Report Attention to: _____																		
Report Address to: _____																		
Email Address: _____																		



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <i>Hot Springs Wastewater</i>			PO No. <i>16-88</i>		NO OF BOTTLES	ANALYSES REQUESTED										AIC CONTROL NO: <i>199512</i>			
Project Reference: <i>Bio-Monitoring</i>			MATRIX			<i>Bio Monitoring</i>										AIC PROPOSAL NO:			
Project Manager: <i>Jim Sorrells</i>			WATER SOIL													Carrier: <i>HS DELIVERY</i>			
Sampled By: <i>H MAUCOIN</i>			GRA B	COMP	WATER	SOIL	NO OF BOTTLES	<i>Bio Monitoring</i>										Received Temperature C <i>0.2</i>	
AIC No.	Sample Identification	Date/Time Collected																Remarks	
<i>2</i>	<i>PLANT Effluent</i>	<i>2/23/16 @ 0000-2400</i>	<i>X</i>	<i>X</i>			<i>3</i>												
Container Type							<i>P</i>											Field pH calibration	
Preservative							<i>NO</i>											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: <i>A. Mauch</i>		Date/Time <i>2/24/16 @ 10:25</i>		Received By: <i>m. man</i>		Date/Time <i>2-24-16 @ 10:25</i>						
Expedited results requested by: _____							Relinquished By: <i>m. man</i>		Date/Time <i>2-24-16 @ 11:25</i>		Received in Lab By: <i>D. Brown</i>		Date/Time <i>2-24-16 11:25</i>						
Who should AIC contact with questions: Phone: _____ Fax: _____							Comments:												
Report Attention to: Report Address to: Email Address:																			



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Hot Springs Wastewater			PO No. 16-88		NO OF BOTTLES	ANALYSES REQUESTED Bro-Monitoring										AIC CONTROL NO: 199512	
Project Reference: Bo - Monitoring			MATRIX													AIC PROPOSAL NO:	
Project Manager: Jim Sorneus			W	S		Carrier: HS DELIVERY											
Sampled By: H. Maupin			G	C	Received Temperature C												
AIC No.	Sample Identification	Date/Time Collected	A	B	P	L	Remarks										
3	Plant Effluent	2/25/16 @ 0800-2400			X	X											
Container Type			Preservative			Field pH calibration on _____ @ _____											
G = Glass			P = Plastic			V = VOA vials		H = HCl to pH2		T = Sodium Thiosulfate		A = (NH ₄) ₂ SO ₄ , NH ₄ OH					
NO = none			S = Sulfuric acid pH2			N = Nitric acid pH2		B = NaOH to pH12		Z = Zinc acetate							
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS				Relinquished By: H. Maupin		Date/Time: 2/25/16 @ 10:00		Received By: M. Mann		Date/Time: 2-26-16 @ 10:00							
Expedited results requested by: _____				Relinquished By: M. Mann		Date/Time: 2-26-16 @ 10:52		Received in Lab By: D. Brown		Date/Time: 2-26-16 10:52							
Who should AIC contact with questions: _____				Comments:													
Phone: _____ Fax: _____																	
Report Attention to: _____ Report Address to: _____																	
Email Address: _____																	

March 3, 2016

Test Results of
First Quarter
Chronic 7-Day Renewal
Biomonitoring Testing

for

UV Treated Outfall #001

City of Hot Springs

Control No. 199511-1

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)
UV Treated 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.**

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)
- Appendix A: Raw Data
 - A1: Test 1000.0
 - Pimephales promelas* (Fathead minnow) Survival and Growth
 - 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	0.271	PASS
Control Growth CV < or = 40%	6.65	PASS
Growth Minimum Significant Difference 12 to 30%	11.1	BELOW
Critical Dilution CV < or = 40%	5.48	PASS

II. Outlined Report

A. Introduction

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

B. Source of Effluent/Dilution Water

1. Effluent Samples:

- a. Sampling Point: UV Treated Outfall 001
- b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.3	8.6	8.3
pH (standard units)	7.0	7.0	7.0
Alkalinity (mg/l as CaCO ₃)	50	47	28
Hardness (mg/l as CaCO ₃)	74	63	49
Conductivity (umhos/cm)	330	300	180
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	0.81	<0.1

2. Dilution Water Samples: Synthetic Soft Water #4302

- a. Dates Prepared: February 12 through February 26, 2015
- b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.2	8.5	7.7
pH (standard units)	7.2	7.3	6.9
Alkalinity (mg/l as CaCO ₃)	31	31	31
Hardness (mg/l as CaCO ₃)	48	48	48
Conductivity (umhos/cm)	160	150	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 Method 1000.0, Fathead Minnow Survival and Growth.

2. Endpoint: No Observable Effects Concentration (NOEC)

3. Test Conditions:

Pimephales promelas (Fathead minnow) Survival and Growth Method 1000.0

Date & Time Test Initiated: February 23, 2016 at 1050

Date & Time Test Terminated: March 1, 2016 at 0855

Type & Volume of Test Chamber: 500 ml disposable beaker

Volume of Sample: 250 ml

Number of Organisms per replicate: 8

Number of Replicates per dilution: 5

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

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

D. Test Organisms

1. Scientific Name

a. Test 1000.0 *Pimephales promelas*

III. Data Analysis

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

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

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

IV. Standard Reference Toxicants

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

Pimephales promelas (Fathead minnow)

Chronic reference tests are performed monthly.

A chronic reference test was performed on February 2, 2016 at 1425 to February 9, 2016 at 1340

The results were as follows: (Control No. 198842-1.)

Survival LC-50: 4893 mg/l

Growth IC-25: 3633 mg/l

Growth PMSD: 14.2

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	98.6	1.97
pH	SM 4500-H+ B	101	0.145
Conductivity	EPA 120.1	101	3.97

VI. Organism History

Pimephales promelas (Fathead minnow)

Date: February 23, 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

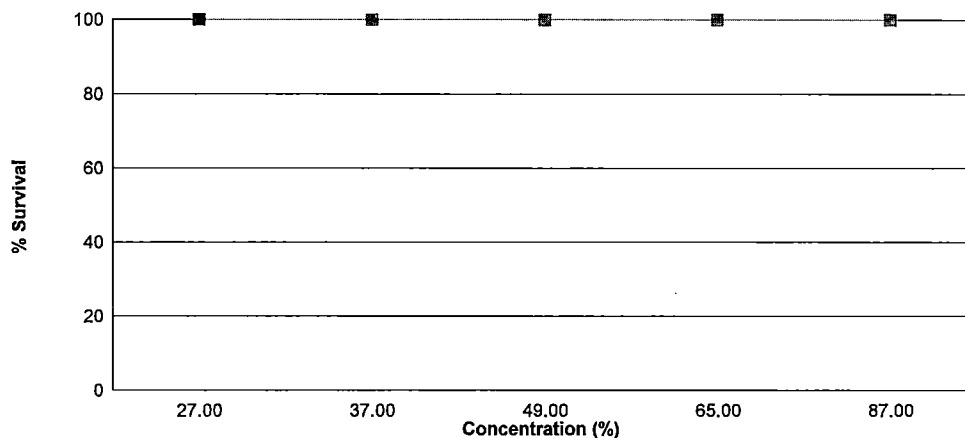
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

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 February 23, 2016 at 1050 and continued through March 1, 2016 at 0855. 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	0.271
27 %	100	0.271
37 %	100	0.292
49 %	100	0.311
65 %	100	0.316
87 %	100	0.310

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: February 23, 2016 at 1050

Date and Time Test Terminated: March 1, 2016 at 0855

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: February 23, 2016 at 1050
Test Terminated: March 1, 2016 at 0855

Drying Started: February 29, 2016 at 1415
Drying Ended: March 2, 2016 at 0945

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.94454	.94647	0.00193	8	0.241
	B	.93977	.94193	0.00216	8	0.270
	C	.93220	.93443	0.00223	8	0.279
	D	.94158	.94388	0.00230	8	0.288
	E	.94007	.94229	0.00222	8	0.278
27 %	A	.94403	.94622	0.00219	8	0.274
	B	.93941	.94136	0.00195	8	0.244
	C	.94392	.94615	0.00223	8	0.279
	D	.94126	.94367	0.00241	8	0.301
	E	.93632	.93839	0.00207	8	0.259
37 %	A	.94086	.94293	0.00207	8	0.259
	B	.93985	.94212	0.00227	8	0.284
	C	.94191	.94438	0.00247	8	0.309
	D	.93960	.94198	0.00238	8	0.298
	E	.93849	.94097	0.00248	8	0.310
49 %	A	.93918	.94170	0.00252	8	0.315
	B	.94129	.94368	0.00239	8	0.299
	C	.94137	.94398	0.00261	8	0.326
	D	.94091	.94367	0.00276	8	0.345
	E	.94171	.94388	0.00217	8	0.271
65 %	A	.94141	.94376	0.00235	8	0.294
	B	.94407	.94676	0.00269	8	0.336
	C	.94450	.94706	0.00256	8	0.320
	D	.94348	.94609	0.00261	8	0.326
	E	.94074	.94316	0.00242	8	0.302
87 %	A	.94299	.94539	0.00240	8	0.300
	B	.94036	.94296	0.00260	8	0.325
	C	.93947	.94202	0.00255	8	0.319
	D	.94123	.94367	0.00244	8	0.305
	E	.94671	.94913	0.00242	8	0.302

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 = 0.009781 W = 0.9686 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 = 3.087 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	0.01025	0.00205	5.031
Within (Error)	24	0.00978	0.0004075	
Total	29	0.02003		
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	0.2712	0.2712		
2	27 %	0.2714	0.2714	-0.01567	
3	37 %	0.292	0.292	-1.629	
4	49 %	0.3112	0.3112	-3.133	
5	65 %	0.3156	0.3156	-3.478	
6	87 %	0.3102	0.3102	-3.055	
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	0.03013	11.1	-0.0002
3	37 %	5	0.03013	11.1	-0.0208
4	49 %	5	0.03013	11.1	-0.04
5	65 %	5	0.03013	11.1	-0.0444
6	87 %	5	0.03013	11.1	-0.039

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: February 23, 2016 at 0827

Date and Time Test Terminated: March 1, 2016 at 0855

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.2	8.1	8.5	9.8	7.7	7.7	8.9
	Final	7.5	6.1	8.5	7.8	8.1	8.5	7.8
pH, units	Initial	7.2	6.9	7.3	7.0	6.9	7.0	7.2
	Final	7.3	6.8	7.2	6.9	7.3	7.3	7.2
Alkalinity, mg CaCO ₃ /l		31	NA	31	NA	31	NA	NA
Hardness, mg CaCO ₃ /l		48	NA	48	NA	48	NA	NA
Conductivity, umhos/cm		160	160	150	150	160	160	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	8.1	8.1	8.5	9.9	7.7	7.9	8.8
	Final	7.0	6.1	8.4	7.8	7.9	8.4	7.5
pH, units	Initial	7.1	7.3	7.2	6.9	6.9	7.0	7.1
	Final	7.2	6.9	7.3	6.9	7.3	7.3	7.1

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.3	8.2	8.6	9.9	9.6	8.0	8.8
	Final	7.0	6.0	8.4	8.0	8.1	7.9	7.6
pH, units	Initial	7.1	7.0	7.2	7.0	6.9	7.1	7.0
	Final	7.2	6.9	7.3	7.0	7.3	7.2	7.1

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: February 23, 2016 at 0827

Date and Time Test Terminated: March 1, 2016 at 0855

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.3	8.4	8.6	9.8	7.8	8.0	8.8
	Final	7.3	5.6	8.6	8.2	8.1	8.4	7.8
pH, units	Initial	7.0	7.0	7.1	7.0	6.9	7.1	7.0
	Final	7.3	6.8	7.4	7.1	7.4	7.3	7.2

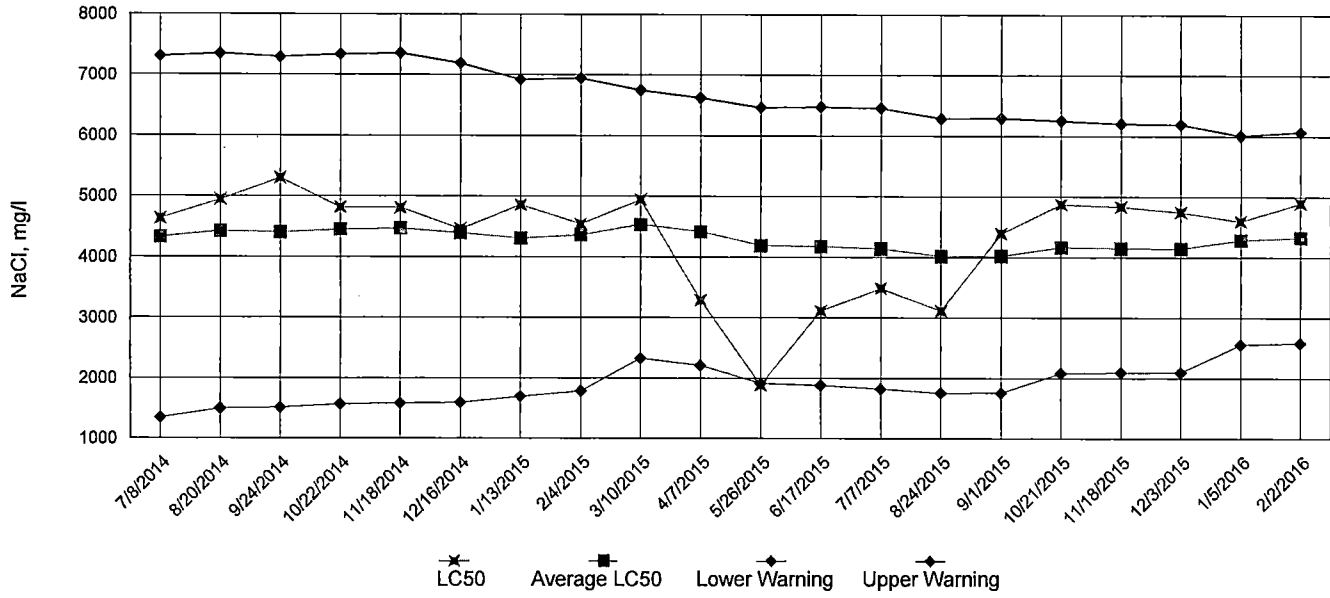
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.3	8.3	8.5	9.6	8.2	7.9	8.6
	Final	7.4	5.7	8.6	7.9	8.0	8.6	7.9
pH, units	Initial	7.0	6.9	7.1	7.0	7.0	7.1	6.9
	Final	7.3	7.0	7.4	7.1	7.4	7.3	7.2
Alkalinity, mg CaCO ₃ /l		50	NA	41	NA	30	NA	NA
Hardness, mg CaCO ₃ /l		66	NA	57	NA	50	NA	NA
Conductivity, umhos/cm		270	260	250	250	170	170	170
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	8.4	8.3	8.6	9.3	7.8	8.0	8.9
	Final	7.1	5.6	8.5	7.9	8.0	8.3	8.0
pH, units	Initial	7.0	6.8	7.1	7.0	7.0	7.1	6.8
	Final	7.4	7.0	7.4	7.1	7.4	7.3	7.2

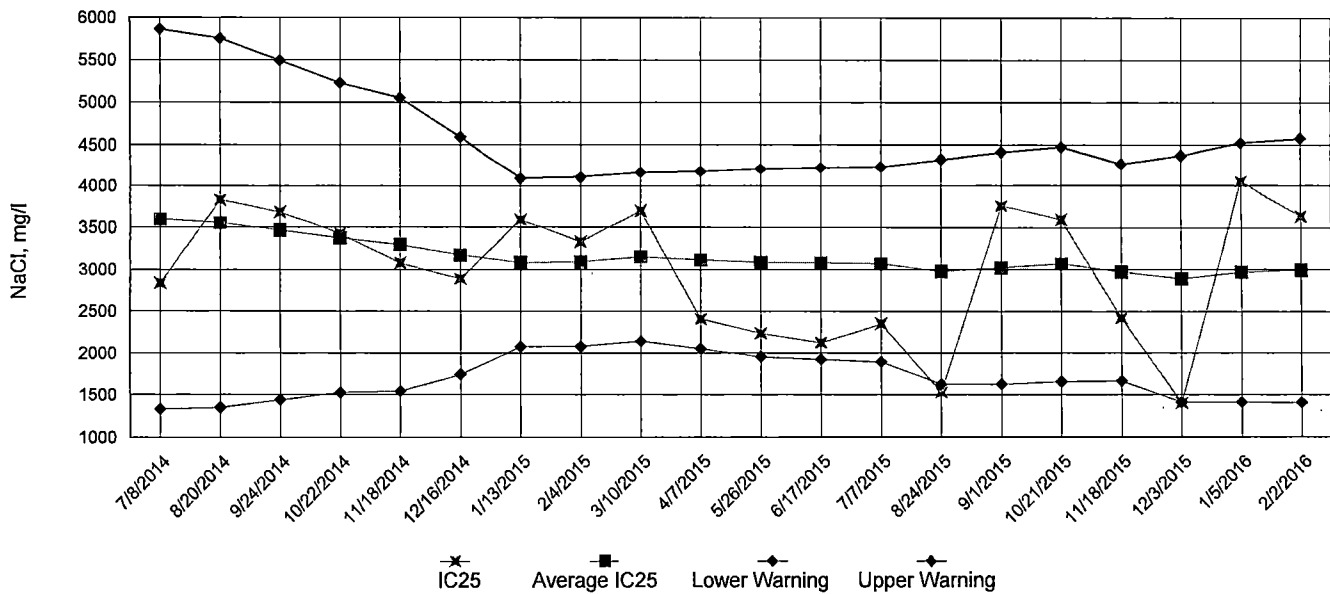
Appendix A4: Test 1000.0

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

LC50 Survival Data



IC25 Growth Data



Appendix B: Test 1000.0

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

Permittee: City of Hot Springs

NPDES No.: AR0033880 AFIN#26-00145

Date and Time Test Initiated: February 23, 2016 at 1050

Date and Time Test Terminated: March 1, 2016 at 0855

Dilution water used: Synthetic Soft Water #4302

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	0.241	0.270	0.279	0.288	0.278	0.271	6.65
27 %	0.274	0.244	0.279	0.301	0.259	0.271	7.91
37 %	0.259	0.284	0.309	0.298	0.310	0.292	7.27
49 %	0.315	0.299	0.326	0.345	0.271	0.311	9.00
65 %	0.294	0.336	0.320	0.326	0.302	0.316	5.48
87 %	0.300	0.325	0.319	0.305	0.302	0.31	3.59

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: 6.65 (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

2400
2400
2400

Test Initiated: DATE: February 23, 2016 TIME: 1050
Test Terminated: DATE: March 1, 2016 TIME: 0855

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	8.2	8.1	8.5	9.8	7.7	7.7	8.9
Final	7.5	6.1	8.5	7.8	8.1	8.5	7.8
pH Initial	7.2	6.9	7.3	7.0	6.9	7.0	7.2
Final	7.3	6.8	7.2	6.9	7.3	7.3	7.2
Alkalinity	31	NA	31	NA	31	NA	NA
Hardness	48	NA	48	NA	48	NA	NA
Conductivity	160	160	150	150	160	160	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	8.1	8.1	8.5	9.9	7.7	7.9	8.8
Final	7.0	6.1	8.4	7.8	7.9	8.4	7.5
pH Initial	7.1	7.3	7.2	6.9	6.9	7.0	7.1
Final	7.2	6.9	7.3	6.9	7.3	7.3	7.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	200	200	190	190	160	160	160
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
37 %							
D.O. Initial	8.3	8.2	8.6	9.9	9.6	8.0	8.8
Final	7.0	6.0	8.4	8.0	8.1	7.9	7.6
pH Initial	7.1	7.0	7.2	7.0	6.9	7.1	7.0
Final	7.2	6.9	7.3	7.0	7.3	7.2	7.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	220	210	200	200	160	160	170
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
49 %							
D.O. Initial	8.3	8.4	8.6	9.8	7.8	8.0	8.8
Final	7.3	5.6	8.6	8.2	8.1	8.4	7.8
pH Initial	7.0	7.0	7.1	7.0	6.9	7.1	7.0
Final	7.3	6.8	7.4	7.1	7.4	7.3	7.2
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	240	230	220	220	160	160	160
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
65 %							
D.O. Initial	8.3	8.3	8.5	9.6	8.2	7.9	8.6
Final	7.4	5.7	8.6	7.9	8.0	8.6	7.9
pH Initial	7.0	6.9	7.1	7.0	7.0	7.1	6.9
Final	7.3	7.0	7.4	7.1	7.4	7.3	7.2
Alkalinity	50	NA	41	NA	30	NA	NA
Hardness	66	NA	57	NA	50	NA	NA
Conductivity	270	260	250	250	170	170	170
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
87 %							
D.O. Initial	8.4	8.3	8.6	9.3	7.8	8.0	8.9
Final	7.1	5.6	8.5	7.9	8.0	8.3	8.0
pH Initial	7.0	6.8	7.1	7.0	7.0	7.1	6.8
Final	7.4	7.0	7.4	7.1	7.4	7.3	7.2
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	310	300	280	280	170	170	170
Chlorine	NA	NA	NA	NA	NA	NA	NA



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>City of Hot Springs</u>			PO No. <u>16-88</u>		NO OF BOTTLES	ANALYSES REQUESTED										AIC CONTROL NO: <u>199511</u>	
Project Reference: <u>Bio-Monitoring</u>			MATRIX			<u>Bio-Monitoring</u> <u>FMT</u>										AIC PROPOSAL NO:	
Project Manager: <u>Jim Sorrells</u>			WATER SOIL													Carrier:	
Sampled By: <u>H Maupin</u>			G	C		Received Temperature C: <u>0.1</u>											
AIC No.	Sample Identification	Date/Time Collected	A	B	P	L											Remarks
<u>1</u>	<u>PLANT Effluent</u>	<u>2/21/16 @ 0000-2400</u>	<u>X</u>	<u>X</u>			<u>3</u>										
Container Type			Preservative												Field pH calibration on _____ @ _____ Buffer:		
G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate			NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z = Zinc acetate A = (NH ₄) ₂ SO ₄ , NH ₄ OH														
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS					Relinquished By: <u>H Maupin</u>	Date/Time <u>2/21/16 @ 10:00</u>	Received By: <u>M. Mann</u>	Date/Time <u>2-22-16 @ 10:00</u>									
Expedited results requested by: _____					Relinquished By: <u>M. Mann</u>	Date/Time <u>2-22-16 @ 10:55</u>	Received in Lab By: <u>[Signature]</u>	Date/Time <u>2/22/16 1055</u>									
Who should AIC contact with questions: _____					Comments: <u>UV Testing</u>												
Phone: _____ Fax: _____																	
Report Attention to: _____																	
Report Address to: _____																	
Email Address: _____																	



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <i>Hot Springs Wastewater</i>			PO No. <i>16-88</i>		NO OF BOTTLES	ANALYSES REQUESTED										AIC CONTROL NO: <i>199511</i>			
Project Reference: <i>PLANT Bio-Monitoring</i>			MATRIX			<i>Bio-Monitoring FAT only</i>										AIC PROPOSAL NO:			
Project Manager: <i>JIM SORRELLS</i>			WATER SOIL													Carrier: <i>HS DELIVERY</i>			
Sampled By: <i>H MAULDIN</i>			GRA B	COMP	WATER	SOIL	NO OF BOTTLES											Received Temperature C: <i>0.1</i>	
AIC No.	Sample Identification	Date/Time Collected																Remarks	
<i>2</i>	<i>PLANT EFFLUENT</i>	<i>2-23-15 0000-2400</i>	<i>X</i>	<i>X</i>			<i>3</i>											Field pH calibration on _____ @ _____ Buffer:	
Container Type																			
Preservative																			
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: <i>H Mauldin</i>		Date/Time <i>2/24/16 09:25</i>		Received By: <i>M-mann</i>		Date/Time <i>2-24-16 @ 10:25</i>								
Expedited results requested by: _____					Relinquished By: <i>M. Mann</i>		Date/Time <i>2-24-16 @ 11:25</i>		Received in Lab By: <i>D. BROWN</i>		Date/Time <i>2-24-16 11:25</i>								
Who should AIC contact with questions: Phone: _____ Fax: _____ Report Attention to: Report Address to: Email Address: _____					Comments: <i>UV Testing</i>														



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>Hot Springs Wastewater</u>			PO No. <u>16-88</u>		NO OF BOTTLES	ANALYSES REQUESTED										AIC CONTROL NO: <u>199511</u>		
Project Reference: <u>Bio-Monitoring</u>			Project Manager: <u>Jim Sorrells</u>			MATRIX	Bio-Monitoring FHT ONLY										AIC PROPOSAL NO:	
Sampled By: <u>H MAULDIN</u>			G R A B														C O M P	
AIC No.	Sample Identification	Date/Time Collected													Received Temperature C	Remarks		
<u>3</u>	<u>PLANT Effluent</u>	<u>2/25/16 @ 2000-2400</u>		<u>X</u>	<u>X</u>										<u>HS DELIVERY 0</u>			
															Field pH calibration on _____ @ _____			
Container Type			Preservative												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: <u>H Mauldin</u>		Date/Time <u>2/26/16 @ 1000</u>		Received By: <u>M. Mann</u>		Date/Time <u>2-26-16 @ 10:00</u>							
Expedited results requested by: _____					Relinquished By: <u>M. Mann</u>		Date/Time <u>2-26-16 @ 10:52</u>		Received in Lab By: <u>D. Brown</u>		Date/Time <u>2-26-16 10:52</u>							
Who should AIC contact with questions: Phone: _____ Fax: _____ Report Attention to: Report Address to: Email Address: _____					Comments: <u>UV Testing</u>													

City of Hot Springs Wastewater
Treatment Plant
320 Davidson Dr.
Hot Springs, AR 71901



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

**RETURN RECEIPT
REQUESTED**

