March 20, 2020

Biomonitoring Testing
for
Outfall 002A
Heber Springs, AR

Control No. 243380-1

Prepared for:

Mr. Paul Graham
Heber Springs Water & Sewer
1108 West Front Street
Heber Springs, AR 72543

Prepared by:

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

Heber Springs Water & Sewer
ATTN: Mr. Paul Graham
1108 West Front Street
Heber Springs, AR 72543

Re: Chronic*Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia*
Outfall 002A - Heber Springs, AR
NPDES Permit No. NPDES Permit AR0022381 AFIN 12-00029

Dear Mr. Paul Graham:

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 10 % effluent, which is above the critical dilution of 8 %. The NOEC for growth occurred at 10 % effluent, which is above the critical dilution of 8 %. **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 10 % effluent, which is above the critical dilution of 8 %. The NOEC for reproduction occurred at 10 % effluent, which is above the critical dilution of 8 %. **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: Heber Springs Water & Sewer
ATTN: Mr. Paul Graham
paul@heberspringswater.com

Heber Springs Water & Sewer
ATTN: Ms. Nora Mullabay
nora@heberspringswater.com

Table of Contents

I. Control Acceptance Criteria

Pimephales promelas (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	95	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.41	PASS
Control Growth CV < or = 40%	17	PASS
Growth Minimum Significant Difference 12 to 30%	18	PASS
Critical Dilution CV < or = 40%	14	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	19	PASS
Control CV < or = 40% per Surviving Female	26	PASS
Reproduction Minimum Significant Difference 13 to 47%	36	PASS
Critical Dilution CV < or = 40%	14	PASS

II. Outlined Report

A. Introduction

1. Permit Number: NPDES Permit AR0022381 AFIN 12-00029
2. Test Requirements: Chronic Biomonitoring, Quarterly
   Test Methods 1000.0 and 1002.0

B. Source of Effluent/Dilution Water:

1. Effluent Samples:
   a. Sampling Point: Outfall 002A
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.5	8.0	7.4
pH (standard units)	7.0	7.3	7.3
Alkalinity (mg/l as CaCO3)	41	44	42
Hardness (mg/l as CaCO3)	27	28	29
Conductivity (umhos/cm)	180	180	200
Residual Chlorine (mg/l)	0.050	0.060	<0.05
Ammonia as N (mg/l)	6.3	6.3	6.9

2. Dilution Water Samples:
   Soft

Analysis	242962-1
Dissolved oxygen (mg/l)	7.4
pH (standard units)	7.6
Alkalinity (mg/l as CaCO3)	31
Hardness (mg/l as CaCO3)	41
Conductivity (umhos/cm)	160
Residual Chlorine (mg/l)	<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:	March 10, 2020 at 1400
Date & Time Test Terminated:	March 17, 2020 at 1430
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 Reproduction Method 1002.0

Date & Time Test Initiated:	March 10, 2020 at 1335
Date & Time Test Terminated:	March 17, 2020 at 1532
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. Source 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 and following EPA method criteria.

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

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

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

IV. Standard Reference Toxicants

The sensitivity of the offspring is determined by performing a standard reference toxicant test monthly. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Pimephales promelas (Fathead minnow)

A chronic reference test was performed on February 18, 2020 at 1310 to February 25, 2020 at 1421
The results were as follows: (Control No. 242720-1.)

Survival LC-50: 4034 mg/l
Growth IC-25: 2477 mg/l
Growth PMSD: 6.56

Ceriodaphnia dubia

A chronic reference test was performed on February 25, 2020 at 1555 to March 03, 2020 at 1530
The results were as follows: (Control No. 242720-2.)

Survival LC-50: 1673.1 mg/l
Reproduction IC-25: 937.5 mg/l
Reproduction PMSD: 10.8

V. Organism History

Pimephales promelas (Fathead minnow)

Date: March 10, 2020
Age: <24 hours
Source: In-house culture
Water: Moderately hard synthetic
Temperature: 25 deg.C

Ceriodaphnia dubia

Date: March 10, 2020
Age: <24 hours
Source: In-house culture
Water: Moderately hard synthetic
Temperature: 25 deg.C

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

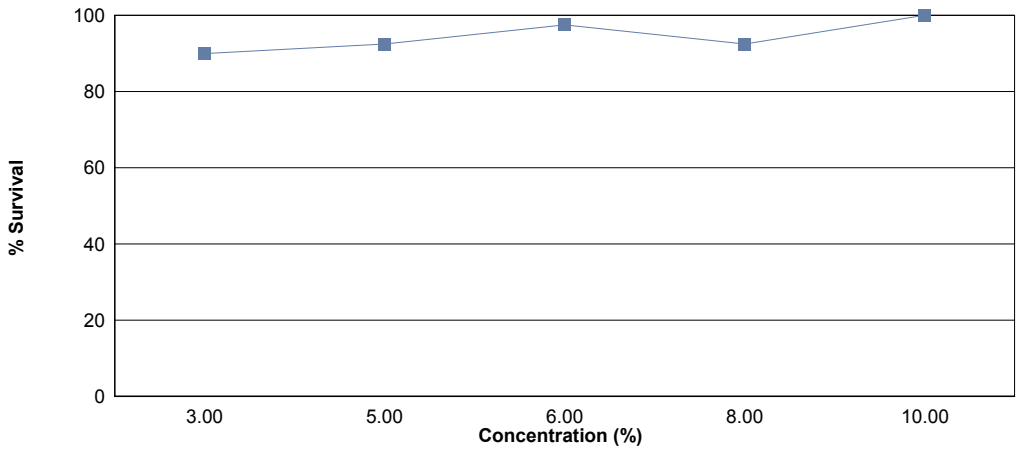
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

Effluent dilutions for this test were 3 %, 5 %, 6 %, 8 %, 10 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 8 % effluent.

The test was initiated on March 10, 2020 at 1400 and continued through March 17, 2020 at 1430. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 10 % effluent
b.) NOEC growth = 10 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	95.0	0.386
3 %	90.0	0.378
5 %	92.5	0.391
6 %	97.5	0.347
8 %	92.5	0.395
10 %	100	0.435

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

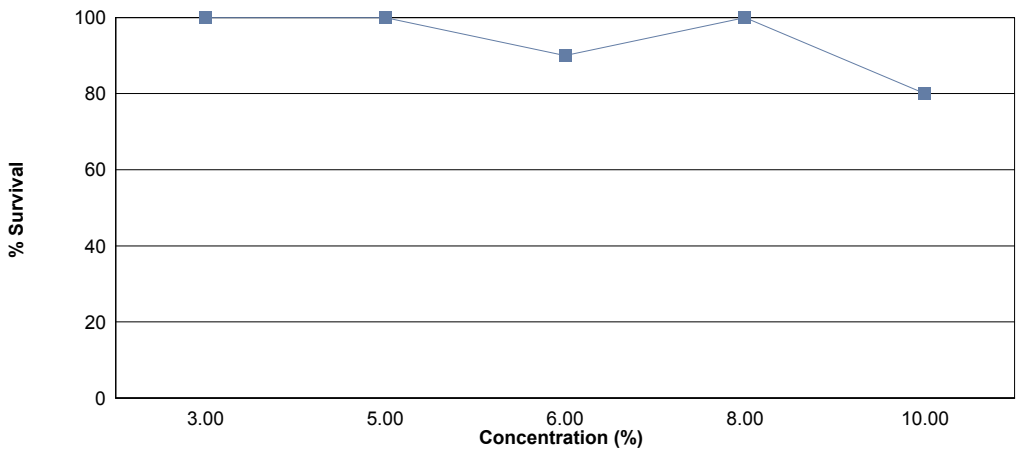
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

Effluent dilutions for this test were 3 %, 5 %, 6 %, 8 %, 10 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 8 % effluent.

The test was initiated on March 10, 2020 at 1335 and continued through March 17, 2020 at 1532. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 10 % effluent
b.) NOEC reproduction = 10 % effluent



Summary of the 7-day *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	19.2
3 %	100	22.7
5 %	100	25.9
6 %	90.0	22.4
8 %	100	27.8
10 %	80.0	20.7

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: March 10, 2020 at 1400
Date and Time Test Terminated: March 17, 2020 at 1430

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	7	7	7
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	7	7	7	7
3 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	7	7
	C	8	8	8	8	8	6	6
	D	8	8	8	8	8	8	8
	E	8	8	8	8	7	7	7
5 %	A	8	8	8	8	7	7	7
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	7	6	6
	E	8	8	8	8	8	8	8
6 %	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	7	7
	E	8	8	8	8	8	8	8
8 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	7	7
	C	8	8	8	8	8	8	7
	D	8	8	8	8	7	7	7
	E	8	8	8	8	8	8	8
10 %	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: March 10, 2020 at 1400
Test Terminated: March 17, 2020 at 1430

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	1.06836	1.07197	0.00361	8	0.451
	B	1.07241	1.07510	0.00269	8	0.336
	C	1.05818	1.06112	0.00294	8	0.368
	D	1.06854	1.07221	0.00367	8	0.459
	E	1.06678	1.06930	0.00252	8	0.315
3 %	A	1.08185	1.08500	0.00315	8	0.394
	B	1.06243	1.06518	0.00275	8	0.344
	C	1.07211	1.07466	0.00255	8	0.319
	D	1.07734	1.08083	0.00349	8	0.436
	E	1.05196	1.05512	0.00316	8	0.395
5 %	A	1.06467	1.06761	0.00294	8	0.368
	B	1.06313	1.06605	0.00292	8	0.365
	C	1.06466	1.06818	0.00352	8	0.440
	D	1.07043	1.07331	0.00288	8	0.360
	E	1.06130	1.06466	0.00336	8	0.420
6 %	A	1.07209	1.07514	0.00305	8	0.381
	B	1.06596	1.06859	0.00263	8	0.329
	C	1.06579	1.06856	0.00277	8	0.346
	D	1.05394	1.05635	0.00241	8	0.301
	E	1.06747	1.07048	0.00301	8	0.376
8 %	A	1.05952	1.06321	0.00369	8	0.461
	B	1.06837	1.07159	0.00322	8	0.402
	C	1.07035	1.07321	0.00286	8	0.358
	D	1.06484	1.06740	0.00256	8	0.320
	E	1.05829	1.06177	0.00348	8	0.435
10 %	A	1.08018	1.08361	0.00343	8	0.429
	B	1.07650	1.07999	0.00349	8	0.436
	C	1.06413	1.06729	0.00316	8	0.395
	D	1.07284	1.07643	0.00359	8	0.449
	E	1.05590	1.05963	0.00373	8	0.466

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: March 10, 2020 at 1335
Date and Time Test Terminated: March 17, 2020 at 1532

Concentration: Control													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	3	2	2	2	4	0	5	3	4	2	27	10	2.70
5	5	5	4	7	8	5	7	11	7	7	66	10	6.60
6	0	0	0	5	11	8	11	12	0	13	60	10	6.00
7	11	6	9	0	0	0	0	0	13	0	39	10	3.90
8													
TOTAL	19	13	15	14	23	13	23	26	24	22	192	10	19.2

Concentration: 3 %													
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	5	4	4	2	4	3	4	3	0	2	31	10	3.10
5	8	11	9	8	8	7	8	7	0	7	73	10	7.30
6	0	14	0	11	8	12	0	9	8	11	73	10	7.30
7	11	0	13	0	0	0	12	0	14	0	50	10	5.00
8													
TOTAL	24	29	26	21	20	22	24	19	22	20	227	10	22.7

Concentration: 5 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	2	5	4	3	4	4	4	3	4	2	35	10	3.50
5	7	9	8	0	9	7	9	11	11	6	77	10	7.70
6	0	14	0	13	14	15	12	14	0	11	93	10	9.30
7	13	0	14	14	0	0	0	0	13	0	54	10	5.40
8													
TOTAL	22	28	26	30	27	26	25	28	28	19	259	10	25.9

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: March 10, 2020 at 1335
Date and Time Test Terminated: March 17, 2020 at 1532

Concentration: 6 %													
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	X	0	9	0.00
4	5	2	4	4	2	3	4	4	4	X	32	9	3.56
5	10	6	10	11	10	11	10	9	9	X	86	9	9.56
6	0	0	0	11	13	14	0	15	0	X	53	9	5.89
7	13	13	12	0	0	0	14	0	1	X	53	9	5.89
8													
TOTAL	28	21	26	26	25	28	28	28	14	0	224	10	22.4

Concentration: 8 %													
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	5	4	4	4	5	6	5	4	5	2	44	10	4.40
5	10	10	8	10	11	10	8	10	13	6	96	10	9.60
6	0	15	0	14	14	14	12	14	0	11	94	10	9.40
7	16	0	14	0	0	0	0	0	14	0	44	10	4.40
8													
TOTAL	31	29	26	28	30	30	25	28	32	19	278	10	27.8

Concentration: 10 %													
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	X	0	0	9	0.00
3	0	0	0	0	0	0	0	0	X	0	0	9	0.00
4	5	4	5	4	4	4	X	4	X	4	34	8	4.25
5	9	11	9	9	10	12	X	8	X	7	75	8	9.38
6	0	14	0	11	12	11	X	12	X	12	72	8	9.00
7	14	0	12	0	0	0	X	0	X	0	26	8	3.25
8													
TOTAL	28	29	26	24	26	27	0	24	0	23	207	10	20.7

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

		Transformation of Data		Transform: Arc Sin(Square Root(Y))
Group	Identification	Rep	Value	Transformed
1	Control	1	1.00000	1.39310
1	Control	2	0.87500	1.20940
1	Control	3	1.00000	1.39310
1	Control	4	1.00000	1.39310
1	Control	5	0.87500	1.20940
2	3 %	1	1.00000	1.39310
2	3 %	2	0.87500	1.20940
2	3 %	3	0.75000	1.04720
2	3 %	4	1.00000	1.39310
2	3 %	5	0.87500	1.20940
3	5 %	1	0.87500	1.20940
3	5 %	2	1.00000	1.39310
3	5 %	3	1.00000	1.39310
3	5 %	4	0.75000	1.04720
3	5 %	5	1.00000	1.39310
4	6 %	1	1.00000	1.39310
4	6 %	2	1.00000	1.39310
4	6 %	3	1.00000	1.39310
4	6 %	4	0.87500	1.20940
4	6 %	5	1.00000	1.39310
5	8 %	1	1.00000	1.39310
5	8 %	2	0.87500	1.20940
5	8 %	3	0.87500	1.20940
5	8 %	4	0.87500	1.20940
5	8 %	5	1.00000	1.39310
6	10 %	1	1.00000	1.39310
6	10 %	2	1.00000	1.39310
6	10 %	3	1.00000	1.39310
6	10 %	4	1.00000	1.39310
6	10 %	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 = 0.2907	
W = 0.9458	
Critical W = 0.9	(alpha = 0.01, N = 30)
Critical W = 0.927	(alpha = 0.05, N = 30)
Data PASS normality test (alpha = 0.01).	

Bartlett's Test for Homogeneity of Variance	Transform: Arc Sin(Square Root(Y))
Test can not be performed because at least one group has zero variance. Data FAIL to meet homogeneity of variance assumption.	

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

Ho:Control<Treatment

Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	3 %	24.00	16.00	5.00	
3	5 %	26.50	16.00	5.00	
4	6 %	30.00	16.00	5.00	
5	8 %	25.00	16.00	5.00	
6	10 %	32.50	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 = 0.0516		
W = 0.9658		
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 = 4.148		
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	0.02047	0.004093	1.904
Within (Error)	24	0.0516	0.00215	
Total	29	0.07207		

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	0.3858	0.3858		
2	3 %	0.3776	0.3776	0.2796	
3	5 %	0.3906	0.3906	-0.1637	
4	6 %	0.3466	0.3466	1.337	
5	8 %	0.3952	0.3952	-0.3205	
6	10 %	0.435	0.435	-1.678	

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	3 %	5	0.06921	17.9	0.0082
3	5 %	5	0.06921	17.9	-0.0048
4	6 %	5	0.06921	17.9	0.0392
5	8 %	5	0.06921	17.9	-0.0094
6	10 %	5	0.06921	17.9	-0.0492

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	10	0	10
3 %	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
5 %	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
6 %	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
8 %	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
10 %	8	2	10
Total	18	2	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 8. 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	3 %	10	0	
2	5 %	10	0	
3	6 %	10	1	
4	8 %	10	0	
5	10 %	10	2	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality		No Transformation
D = 0.1443 D* = 1.132 Critical D* = 1.035	(alpha = 0.01, N = 60)	
Data FAIL normality test (alpha = 0.01).		

Steel's Many-One Rank Test					No Transformation
Ho:Control<Treatment					
Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	3 %	122.00	75.00	10.00	
3	5 %	143.00	75.00	10.00	
4	6 %	130.50	75.00	10.00	
5	8 %	148.00	75.00	10.00	
6	10 %	128.00	75.00	10.00	
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Dunnett's Test for PMSD Calculation

ANOVA Table — No Transformation

SOURCE	DF	SS	MS	F
Between	5	515.5	103.1	2.348
Within (Error)	54	2371	43.91	
Total	59	2886		

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	19.2	19.2		
2	3 %	22.7	22.7	-1.181	
3	5 %	25.9	25.9	-2.261	
4	6 %	22.4	22.4	-1.08	
5	8 %	27.8	27.8	-2.902	
6	10 %	20.7	20.7	-0.5062	

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

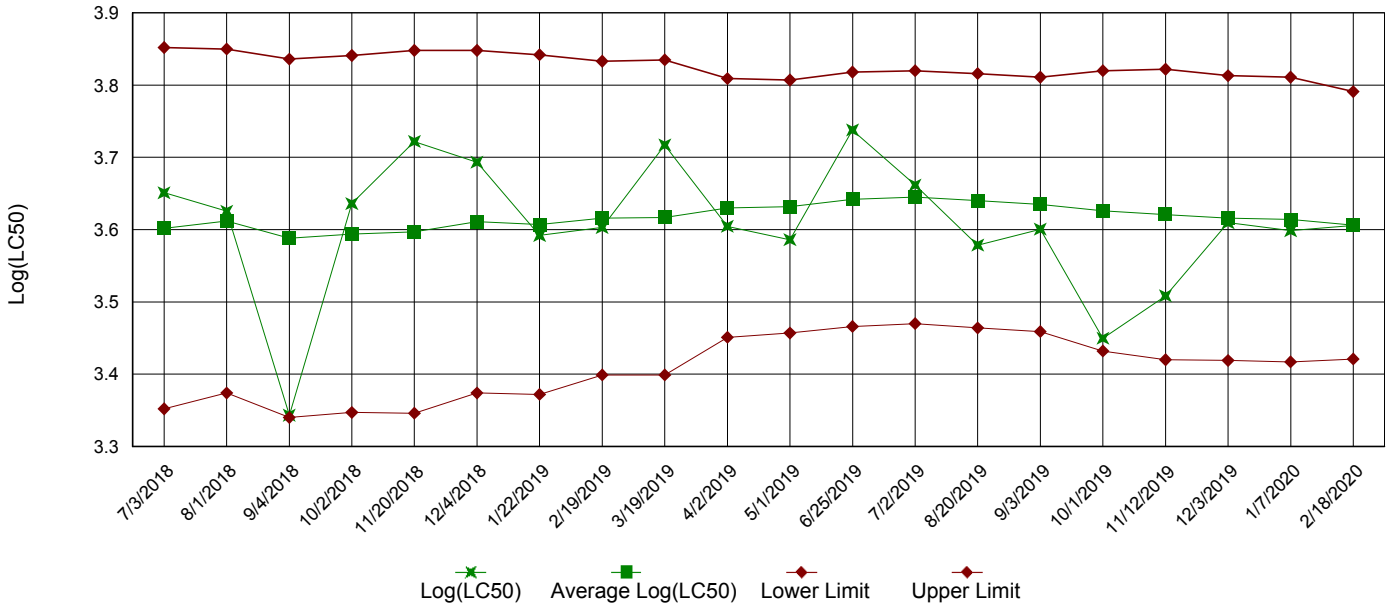
Ho:Control<Treatment

Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	10			
2	3 %	10	6.846	35.7	-3.5
3	5 %	10	6.846	35.7	-6.7
4	6 %	10	6.846	35.7	-3.2
5	8 %	10	6.846	35.7	-8.6
6	10 %	10	6.846	35.7	-1.5

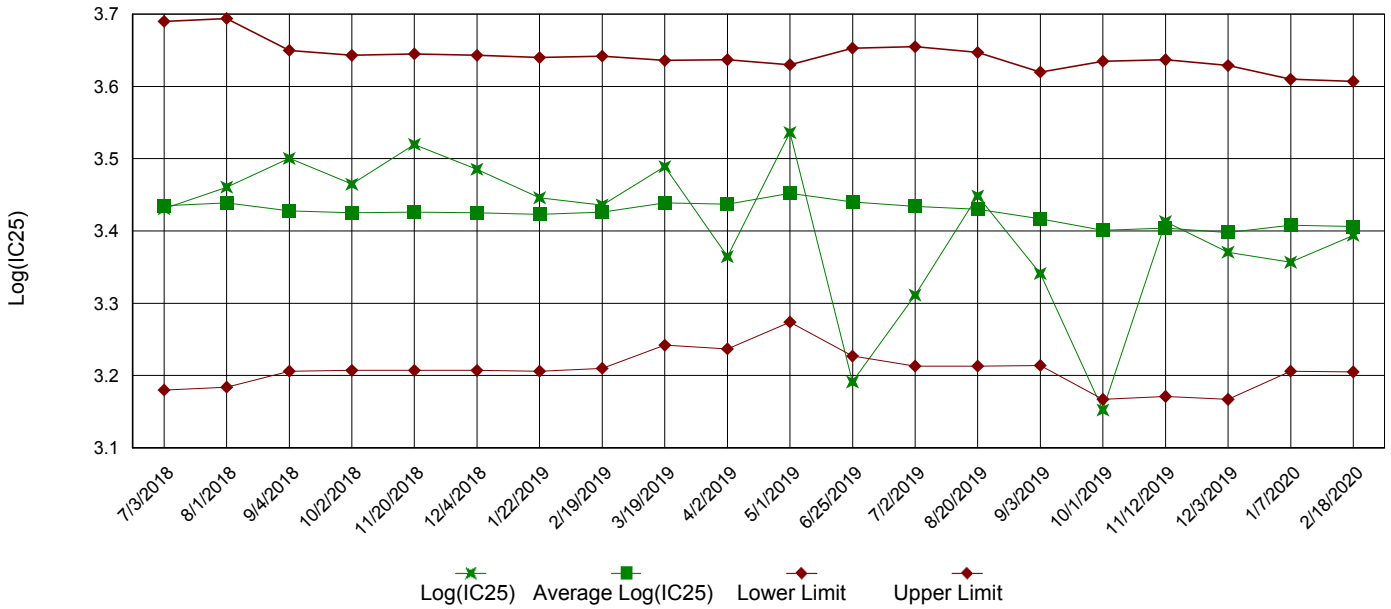
Appendix A3: Test 1000.0

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

LC50 Survival Data



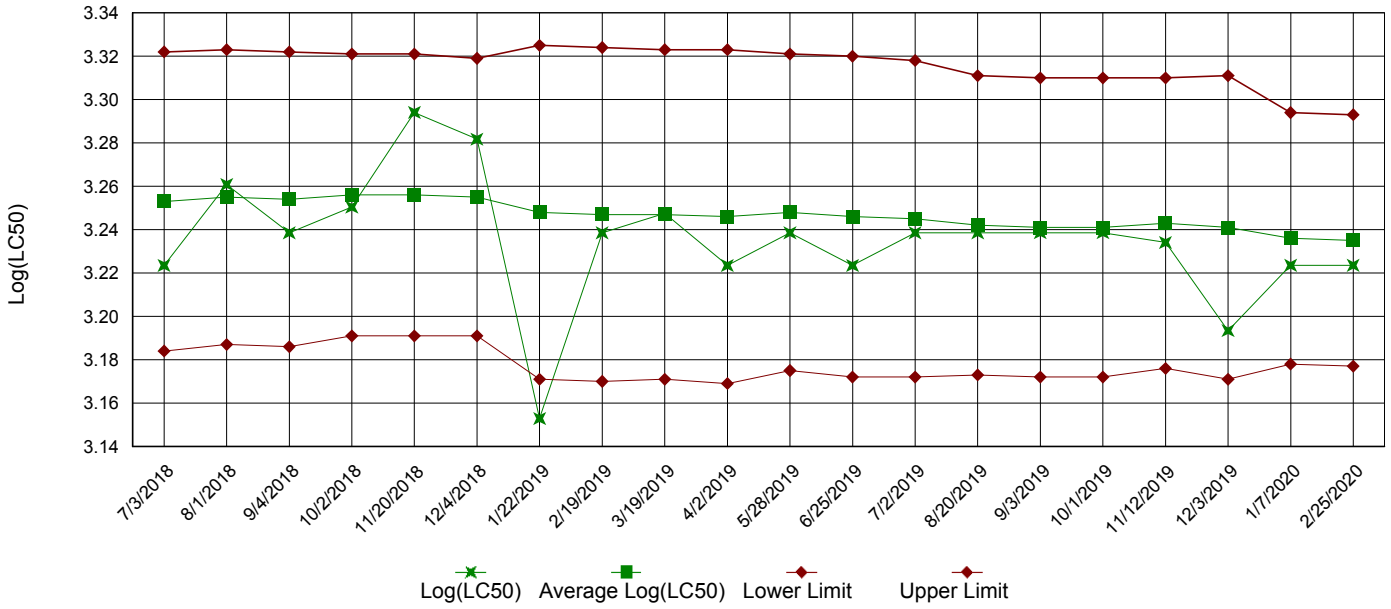
IC25 Growth Data



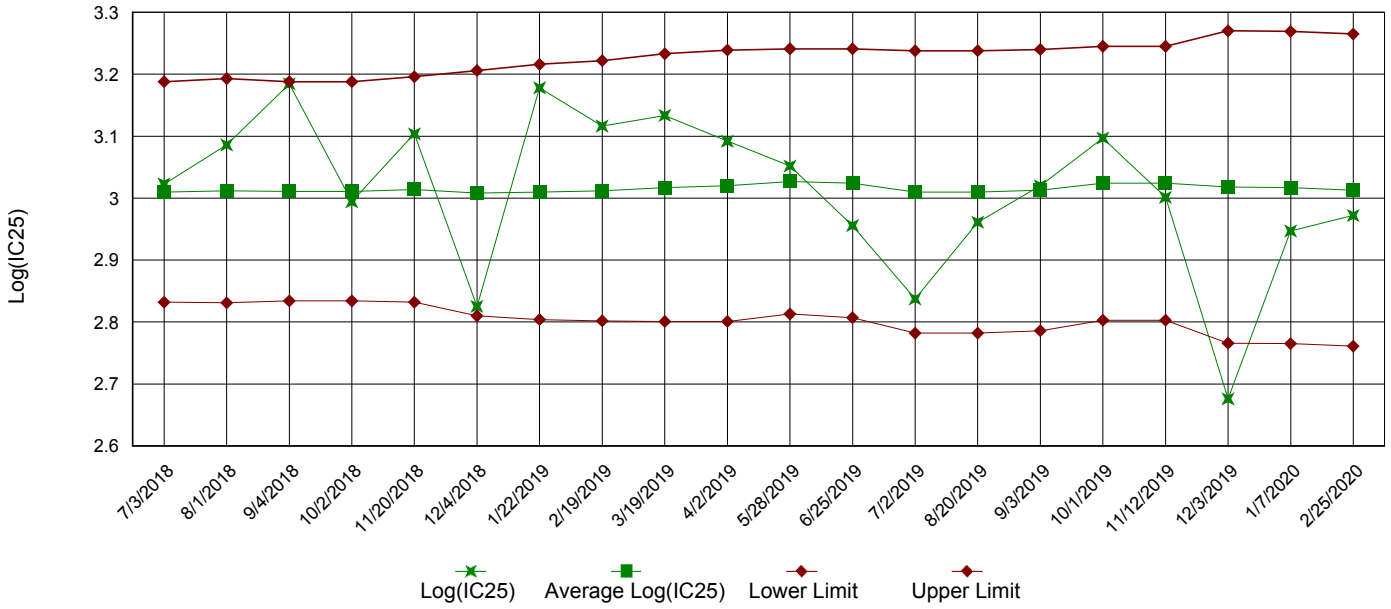
Appendix A3: Test 1002.0

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: Heber Springs Water & Sewer

NPDES No.: NPDES Permit AR0022381 AFIN 12-00029

Date and Time Test Initiated: March 10, 2020 at 1400
Date and Time Test Terminated: March 17, 2020 at 1430
Dilution water used: Soft

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in replicate chambers A	B	C	D	E	Mean percent survival 24 hr	48 hr	7 days	CV%
Control	100	87.5	100	100	87.5	100	100	95.0	7.21
3 %	100	87.5	75.0	100	87.5	100	100	90.0	11.6
5 %	87.5	100	100	75.0	100	100	100	92.5	12.1
6 %	100	100	100	87.5	100	100	100	97.5	5.73
8 %	100	87.5	87.5	87.5	100	100	100	92.5	7.40
10 %	100	100	100	100	100	100	100	100	0.00

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers A	B	C	D	E	Mean dry weight, mg	CV%
Control	0.451	0.336	0.368	0.459	0.315	0.386	17.1
3 %	0.394	0.344	0.319	0.436	0.395	0.378	12.2
5 %	0.368	0.365	0.440	0.360	0.420	0.391	9.41
6 %	0.381	0.329	0.346	0.301	0.376	0.347	9.61
8 %	0.461	0.402	0.358	0.320	0.435	0.395	14.4
10 %	0.429	0.436	0.395	0.449	0.466	0.435	6.08

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 (8 %) _____YES X NO
   b.) 1/2 LOW FLOW DILUTION (NA) _____YES _____NO

2. Dunnett's Test:

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

   a.) LOW FLOW OR CRITICAL DILUTION (8 %) _____YES X NO
   b.) 1/2 LOW FLOW DILUTION (NA) _____YES _____NO

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

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

5. NOEC Pimephales Lethality: 10 % (TOP6C)

6. LOEC Pimephales Lethality: 10 % (TXP6C)

7. NOEC Pimephales Sublethality: 10 % (TPP6C)

8. LOEC Pimephales Sublethality: 10 % (TYP6C)

9. Coefficient of variation for Pimephales growth: 17.1 (TQP6C)

AMERICAN INTERPLEX CORPORATION LABORATORIES

Appendix B: Test 1000.0

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

PERMITTEE: Heber Springs Water & Sewer
NPDES NO.: NPDES Permit AR0022381 AFIN
CONTACT: Mr. Paul Graham
ANALYST: 280, 310, 343, 345

Test Initiated: DATE: March 10, 2020 TIME: 1400
Test Terminated: DATE: March 17, 2020 TIME: 1430

DILUTION Control		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.4	7.5	7.5	7.2	7.4	8.0	7.3
	Final	6.6	5.7	6.1	5.7	7.7	6.0	6.7
pH	Initial	7.6	8.0	7.6	8.0	8.0	8.1	7.8
	Final	7.7	7.4	7.6	7.5	8.0	7.5	7.6

DILUTION 3 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.8	7.5	7.5	7.1	7.1	8.1	8.1
	Final	6.5	4.6	5.8	5.8	7.7	6.2	5.7
pH	Initial	7.5	8.1	7.7	7.8	7.8	7.9	7.8
	Final	7.6	7.2	7.4	7.4	7.9	7.4	7.4

DILUTION 5 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	8.0	7.7	7.7	7.0	7.2	8.1	8.1
	Final	6.7	4.7	5.9	6.2	7.8	6.4	5.9
pH	Initial	7.6	8.1	7.6	7.8	7.8	7.8	7.7
	Final	7.6	7.2	7.4	7.4	7.8	7.4	7.4

DILUTION 6 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.5	7.5	7.9	7.3	7.5	8.2	7.8
	Final	6.6	4.5	5.8	6.4	7.6	5.7	5.6
pH	Initial	7.5	8.2	7.7	7.8	7.8	7.8	7.7
	Final	7.6	7.1	7.3	7.4	7.8	7.4	7.4

DILUTION 8 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.5	7.4	7.6	7.2	7.3	7.6	7.9
	Final	6.5	4.9	5.8	6.1	7.8	6.6	6.0
pH	Initial	7.5	8.2	7.6	7.7	7.7	7.8	7.7
	Final	7.6	7.2	7.3	7.4	7.8	7.5	7.4

DILUTION 10 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.9	7.6	7.5	7.2	7.7	8.1	8.0
	Final	6.7	4.6	5.8	6.0	7.8	6.6	5.8
pH	Initial	7.5	8.1	7.6	7.7	7.8	7.9	7.7
	Final	7.6	7.1	7.4	7.5	7.8	7.4	7.4

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
41	27	180	0.050	Outfall 002A Effl H.S. WWTP 10-MAR-20
44	28	180	0.060	Outfall 002A Effl H.S. WWTP 11-MAR-20
42	29	200	<0.05	Outfall 002A Effl H.S. WWTP 13-MAR-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
31	41	160	<0.05	242962-1

Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

Permittee: Heber Springs Water & Sewer

NPDES No.: NPDES Permit AR0022381 AFIN 12-00029

Date and Time Test Initiated: March 10, 2020 at 1335
Date and Time Test Terminated: March 17, 2020 at 1532
Dilution water used: Soft

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent 3 %	5 %	6 %	8 %	10 %
24 hour	100	100	100	100	100	100
48 hour	100	100	100	100	100	90.0
7 day	100	100	100	90.0	100	80.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

Replicates	Control	Percent Effluent 3 %	5 %	6 %	8 %	10 %
A	19	24	22	28	31	28
B	13	29	28	21	29	29
C	15	26	26	26	26	26
D	14	21	30	26	28	24
E	23	20	27	25	30	26
F	13	22	26	28	30	27
G	23	24	25	28	25	0
H	26	19	28	28	28	24
I	24	22	28	14	32	0
J	22	20	19	0	19	23
Mean per Adult	19.2	22.7	25.9	22.4	27.8	20.7
Mean per Surviving Adult	19.2	22.7	25.9	24.9	27.8	25.9
CV %	26.2	13.6	12.5	18.8	13.5	8.12

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	(8 %)	_____YES	__X__ NO
b.) 1/2 LOW FLOW DILUTION	(NA)	_____YES	_____ 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	(8 %)	_____YES	__X__ NO
b.) 1/2 LOW FLOW DILUTION	(NA)	_____YES	_____ NO

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 0 (TLP3B)

4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 0 (TGP3B)

5. NOEC Ceriodaphnia Lethality: 10 % (TOP3B)

6. LOEC Ceriodaphnia Lethality: 10 % (TXP3B)

7. NOEC Ceriodaphnia Sublethality: 10 % (TPP3B)

8. LOEC Ceriodaphnia Sublethality: 10 % (TYP3B)

9. Coefficient of variation for Ceriodaphnia Reproduction: 26.2 (TQP3B)

AMERICAN INTERPLEX CORPORATION LABORATORIES

Appendix B: Test 1002.0

CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
CHEMICAL PARAMETERS CHART

PERMITTEE: Heber Springs Water & Sewer
NPDES NO.: NPDES Permit AR0022381 AFIN
CONTACT: Mr. Paul Graham
ANALYST: 280, 310, 343, 345

Test Initiated: DATE: March 10, 2020 TIME: 1335
Test Terminated: DATE: March 17, 2020 TIME: 1532

DILUTION Control		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.4	7.5	7.5	7.2	7.4	8.0	7.3
	Final	7.0	7.4	7.6	7.7	7.7	7.5	7.2
pH	Initial	7.6	8.0	7.6	8.0	8.0	8.1	7.8
	Final	8.0	8.0	8.0	8.2	8.0	7.9	7.8

DILUTION 3 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.8	7.5	7.5	7.1	7.1	8.1	8.1
	Final	7.0	7.2	7.2	7.5	7.3	7.5	7.2
pH	Initial	7.5	8.1	7.7	7.8	7.8	7.9	7.8
	Final	8.0	8.0	8.0	8.2	8.0	7.9	7.9

DILUTION 5 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	8.0	7.7	7.7	7.0	7.2	8.1	8.1
	Final	7.0	7.2	7.2	7.9	7.4	8.0	7.4
pH	Initial	7.6	8.1	7.6	7.8	7.8	7.8	7.7
	Final	8.0	8.0	7.9	8.1	7.9	7.9	7.9

DILUTION 6 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.5	7.5	7.9	7.3	7.5	8.2	7.8
	Final	7.0	7.2	7.2	8.0	7.2	8.0	7.0
pH	Initial	7.5	8.2	7.7	7.8	7.8	7.8	7.7
	Final	8.0	7.9	7.9	8.1	7.9	7.9	7.9

DILUTION 8 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.5	7.4	7.6	7.2	7.3	7.6	7.9
	Final	7.0	7.2	7.5	7.7	7.5	7.8	7.1
pH	Initial	7.5	8.2	7.6	7.7	7.7	7.8	7.7
	Final	8.0	7.9	7.9	8.1	7.9	7.9	7.8

DILUTION 10 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.9	7.6	7.5	7.2	7.7	8.1	8.0
	Final	6.9	7.2	7.3	7.4	7.2	7.5	7.2
pH	Initial	7.5	8.1	7.6	7.7	7.8	7.9	7.7
	Final	8.0	7.9	7.9	8.2	7.9	7.9	7.8

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
41	27	180	0.050	Outfall 002A Effl H.S. WWTP 10-MAR-20
44	28	180	0.060	Outfall 002A Effl H.S. WWTP 11-MAR-20
42	29	200	<0.05	Outfall 002A Effl H.S. WWTP 13-MAR-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
31	41	160	<0.05	242962-1

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Heber Springs Water & Wastewater Dept.
Project Reference: 1st Quarter Bio Monitoring
Project Manager: Paul Graham
Sampled By: Joey Massey

PO No. 16118

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	SAMPLE MATRIX: WATER	SOIL	NO OF BOTTLES	ANALYSES REQUESTED: 1st Quarter Bio Monitoring Chronic
1	Outfall 002A Effl. H.S. W.W.T.P.	3-9-20 8 AM TO 3-10-20 8 AM		24HR Comp	✓		3	
		Container Type						
		Preservative						

AIC CONTROL NO: 243380
AIC PROPOSAL NO:
Carrier: H.S
Received Temperature C: 0.4°
Remarks
Field pH calibration on _____ @ _____
Buffer:

G = Glass, P = Plastic, V = VOA vials, H = HCl to pH2, T = Sodium Thiosulfate
NO = none, S = Sulfuric acid pH2, N = Nitric acid pH2, B = NaOH to pH12, Z = Zinc acetate, A=(NH4)2, NH4OH

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN 7 DAYS
Expedited results requested by: Paul Graham
Who should AIC contact with questions: Joey Massey
Phone 362-3422 Fax: 501-362-3538
Report Attention to: Paul Graham
Report Address to: Heber Springs Water, 1108 West Front St., Heber Springs Water AR 72543

Relinquished By:	Date/Time	Received By:	Date/Time
Relinquished By: Joey Massey	3-10-20 11:10	Received in Lab By: JW348	10-mar-20 11:10

Comments:

FORM 0060

Every 2 HRS, Samples = 12
10:00 AM, 12pm, 2pm, 4pm, 6pm, 8pm
10pm, 12 AM, 2 AM, 4 AM, 6AM, 8AM

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Heber Springs Water & Waste Water Dept.
Project Reference: 1st Quarter WW Monitoring
Project Manager: Paul Graham
Sampled By: Joey Massey
PO No. 16118
AIC CONTROL NO: 243380
AIC PROPOSAL NO:
Carrier: —
Received Temperature C: 0.5
Remarks

ANALYSES REQUESTED: Chronic BD Monitoring 1st Quarter

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	SAMPLE MATRIX: WATER	SOIL	NO OF BOTTLES
2	outfall 002 24HR Comp KS WWTP	3-10-20 8AM 3-11-20 8AM		24 HR	✓		3
		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=(NH4)2, NH4OH

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN 7 DAYS
Expedited results requested by: Paul Graham
Who should AIC contact with questions: Joey Massey
Phone: 362-3422 Fax: 501-362-3358
Report Attention to: Paul Graham
Report Address to: Heber Springs Water
1108 west Front St.
Heber Springs AR 72543

Relinquished By:	Date/Time	Received By:	Date/Time
Relinquished By: Joey Massey	Date/Time 3-11-20 1055AM	Received in Lab By: DANNY BROWN	Date/Time 3-11-20 1055

Comments:

FORM 0060

Every 2 HRS = Samples 10:00Am, 12pm, 2pm, 4pm, 6pm, 8pm, 10pm, 12Am, 2Am, 4AM, 6Am, 8AM

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Heber Springs Water & Wastewater Dept.
PO No.: 16118
Project Reference: 1st Quarter Bio Monoriting
Project Manager: Paul Graham
Sampled By: C.J. & Joey Massey

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL	NO OF BOTTLES
3	Out Fall 002 A Eff. H.S. W.W.TP	3-12-20 8 Am TO 3-13-20 8 Am		24 HR	✓		3
		Container Type					
		Preservative					

ANALYSES REQUESTED: Chronic Bio Monoriting 1st Quarter

AIC CONTROL NO: 243380
AIC PROPOSAL NO:
Carrier:
Received Temperature C: 0.1°C
Remarks
Field pH calibration on _____ @ _____
Buffer:

G = Glass, P = Plastic, V = VOA vials, H = HCl to pH2, T = Sodium Thiosulfate
NO = none, S = Sulfuric acid pH2, N = Nitric acid pH2, B = NaOH to pH12, Z = Zinc acetate, A=(NH4)2, NH4OH

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN 2 DAYS
Expedited results requested by: Paul Graham
Who should AIC contact with questions: Joey Massey
Phone: 362-3422 Fax: 501-362-3338
Report Attention to: Paul Graham
Report Address to: Heber Springs Water
1108 West Front St.
Heber Springs AR 72543

Relinquished By:	Date/Time	Received By:	Date/Time
Relinquished By: Joey Massey	Date/Time 3-13-20 11:00Am	Received in Lab By:	Date/Time 3-13-20 - 1100

Comments:

FORM 0060

Every 2 HRS = Samples = 12
10:00 Am 12pm, 2pm, 4pm, 6pm, 8pm, 10pm
12AM, 2AM, 4AM, 6AM, 8AM