June 5, 2020

Biomonitoring Testing
for
East Effluent

Control No. 245515-1

Prepared for:

Ms. Whitney Young
City Water & Light of Jonesboro
5205 Ingels Road
Jonesboro, AR 72401

Prepared by:

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

City Water & Light of Jonesboro
ATTN: Ms. Whitney Young
5205 Ingels Road
Jonesboro, AR 72401

Re: *Ceriodaphnia dubia*
East Effluent
NPDES Permit No. AR0043401 AFIN16-00936

Dear Ms. Whitney Young:

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 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: **Due to a high coefficient of variance in the critical dilution, the test is invalid and will need to be repeated.** The data is attached for your review.

AMERICAN INTERPLEX CORPORATION

John Overbey
Chief Operating Officer

PDF cc: City Water & Light of Jonesboro
ATTN: Mr. Adam Saulsbury
asaulsbury@jonesborocwl.org

City Water & Light of Jonesboro
ATTN: Mr. Jody Gibson
jgibson@jonesborocwl.org

City Water & Light of Jonesboro
ATTN: Mr. Jay Earley
jearley@jonesborocwl.org

City Water & Light of Jonesboro
ATTN: Ms. Whitney Young
wyoung@jonesborocwl.org

City Water & Light of Jonesboro
ATTN: Ms. Tamara Blankenship
tblankenship@jonesborocwl.org

Table of Contents

I. Control Acceptance Criteria

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	17.7	PASS
Control CV < or = 40% per Surviving Female	23.4	PASS
Reproduction Minimum Significant Difference 13 to 47%	44.3	PASS
Critical Dilution CV < or = 40%	42.5	FAIL

II. Outlined Report

A. Introduction

1. Permit Number: AR0043401 AFIN16-00936
2. Test Requirements:

Test Method 1002.0

B. Source of Effluent/Dilution Water:

1. Effluent Samples:
   a. Sampling Point: East Effluent
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.9	8.2	7.7
pH (standard units)	7.7	7.7	7.7
Alkalinity (mg/l as CaCO3)	100	99	110
Hardness (mg/l as CaCO3)	130	130	110
Conductivity (umhos/cm)	620	620	640
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.24	0.30	0.18

2. Dilution Water Samples:
   Moderately Hard

Analysis	245464-1
Dissolved oxygen (mg/l)	7.1
pH (standard units)	8.0
Alkalinity (mg/l as CaCO3)	62
Hardness (mg/l as CaCO3)	85
Conductivity (umhos/cm)	300
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 Method 1002.0, *Ceriodaphnia dubia* Survival and Reproduction.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Ceriodaphnia dubia Survival and Reproduction Method 1002.0

Date & Time Test Initiated:	May 26, 2020 at 1350
Date & Time Test Terminated:	June 02, 2020 at 1322
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 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.

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.

Ceriodaphnia dubia

A chronic reference test was performed on April 01, 2020 at 1110 to April 07, 2020 at 1118
The results were as follows: (Control No. 243967-2.)

Survival LC-50: 1673.1 mg/l
Reproduction IC-25: 1072 mg/l
Reproduction PMSD: 14.2

V. Organism History

Ceriodaphnia dubia

Date: May 26, 2020
Age: <24 hours
Source: In-house culture
Water: Moderately hard synthetic
Temperature: 25 deg.C

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

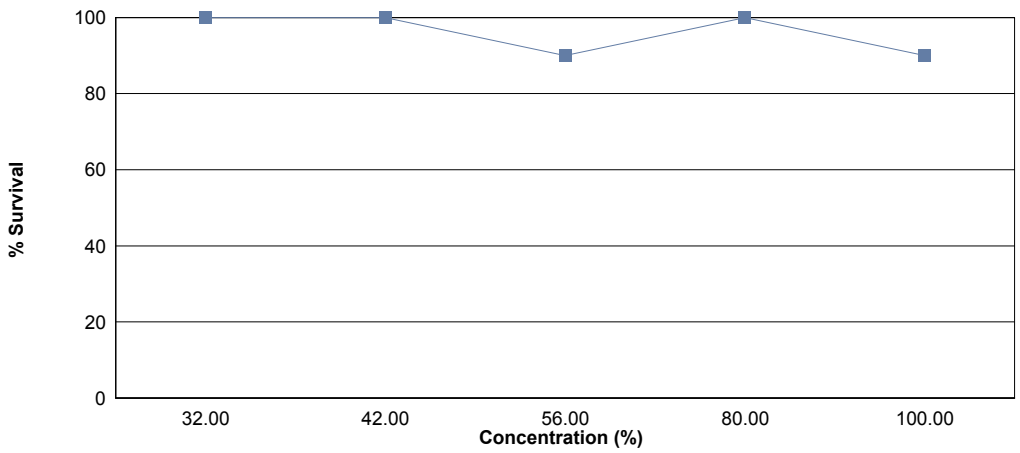
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

Effluent dilutions for this test were 32 %, 42 %, 56 %, 80 %, 100 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 100 % effluent.

The test was initiated on May 26, 2020 at 1350 and continued through June 02, 2020 at 1322. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 100 % effluent
b.) NOEC reproduction = 100 % effluent



Summary of the 7-day *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	17.7
32 %	100	21.9
42 %	100	26.4
56 %	90.0	23.3
80 %	100	28.5
100 %	90.0	20.9

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: May 26, 2020 at 1350
Date and Time Test Terminated: June 02, 2020 at 1322

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	0	0	0	2	2	2	3	4	0	2	15	10	1.50
5	4	6	4	6	8	0	8	11	5	6	58	10	5.80
6	7	0	8	0	0	6	0	10	9	0	40	10	4.00
7	7	8	0	9	8	9	13	0	0	10	64	10	6.40
8													
TOTAL	18	14	12	17	18	17	24	25	14	18	177	10	17.7

Concentration: 32 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	2	2	4	0	2	3	4	3	4	28	10	2.80
5	10	7	7	10	2	8	8	11	6	8	77	10	7.70
6	0	14	0	12	0	0	0	15	10	14	65	10	6.50
7	16	0	0	0	7	13	13	0	0	0	49	10	4.90
8													
TOTAL	30	23	9	26	9	23	24	30	19	26	219	10	21.9

Concentration: 42 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	2	3	3	4	3	4	4	4	34	10	3.40
5	9	8	9	8	9	9	9	10	9	10	90	10	9.00
6	0	0	0	12	0	0	11	13	11	0	47	10	4.70
7	15	13	15	0	13	12	0	0	10	15	93	10	9.30
8													
TOTAL	28	24	26	23	25	25	23	27	34	29	264	10	26.4

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: May 26, 2020 at 1350
Date and Time Test Terminated: June 02, 2020 at 1322

Concentration: 56 %													
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	4	0	4	10	0.400
4	4	0	X	4	3	0	6	4	0	4	25	9	2.78
5	11	8	X	10	8	3	12	11	9	8	80	9	8.89
6	0	0	X	15	0	0	0	18	17	0	50	9	5.56
7	16	11	X	0	3	12	19	0	0	13	74	9	8.22
8													
TOTAL	31	19	0	29	14	15	37	33	30	25	233	10	23.3

Concentration: 80 %													
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	4	0	4	10	0.400
4	3	3	2	3	4	4	6	4	0	4	33	10	3.30
5	9	10	9	9	9	10	10	12	10	10	98	10	9.80
6	0	12	0	17	13	0	0	13	16	15	86	10	8.60
7	16	0	16	0	0	16	16	0	0	0	64	10	6.40
8													
TOTAL	28	25	27	29	26	30	32	29	30	29	285	10	28.5

Concentration: 100 %													
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	3	0	3	10	0.300
4	3	4	0	3	2X	5	6	0	0	4	27	9	3.00
5	9	9	6	10	X	11	12	2	8	11	78	9	8.67
6	0	8	10	11	X	0	0	0	14	0	43	9	4.78
7	13	0	0	0	X	14	19	0	0	12	58	9	6.44
8													
TOTAL	25	21	16	24	2	30	37	2	25	27	209	10	20.9

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	10	0	10
32 %	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
42 %	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
56 %	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
80 %	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
100 %	9	1	10
Total	19	1	20

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

Summary of Fisher's Exact Test

Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	32 %	10	0	
2	42 %	10	0	
3	56 %	10	1	
4	80 %	10	0	
5	100 %	10	1	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality		No Transformation
D = 0.1383 D* = 1.085 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	32 %	127.50	75.00	10.00	
3	42 %	148.50	75.00	10.00	
4	56 %	127.50	75.00	10.00	
5	80 %	154.50	75.00	10.00	
6	100 %	123.50	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	755.3	151.1	2.628
Within (Error)	54	3105	57.5	
Total	59	3860		

Critical F = 3.38 (alpha = 0.01, df = 5,54)
2.38 (alpha = 0.05, df = 5,54)

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	17.7	17.7		
2	32 %	21.9	21.9	-1.239	
3	42 %	26.4	26.4	-2.565	
4	56 %	23.3	23.3	-1.651	
5	80 %	28.5	28.5	-3.185	
6	100 %	20.9	20.9	-0.9436	

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

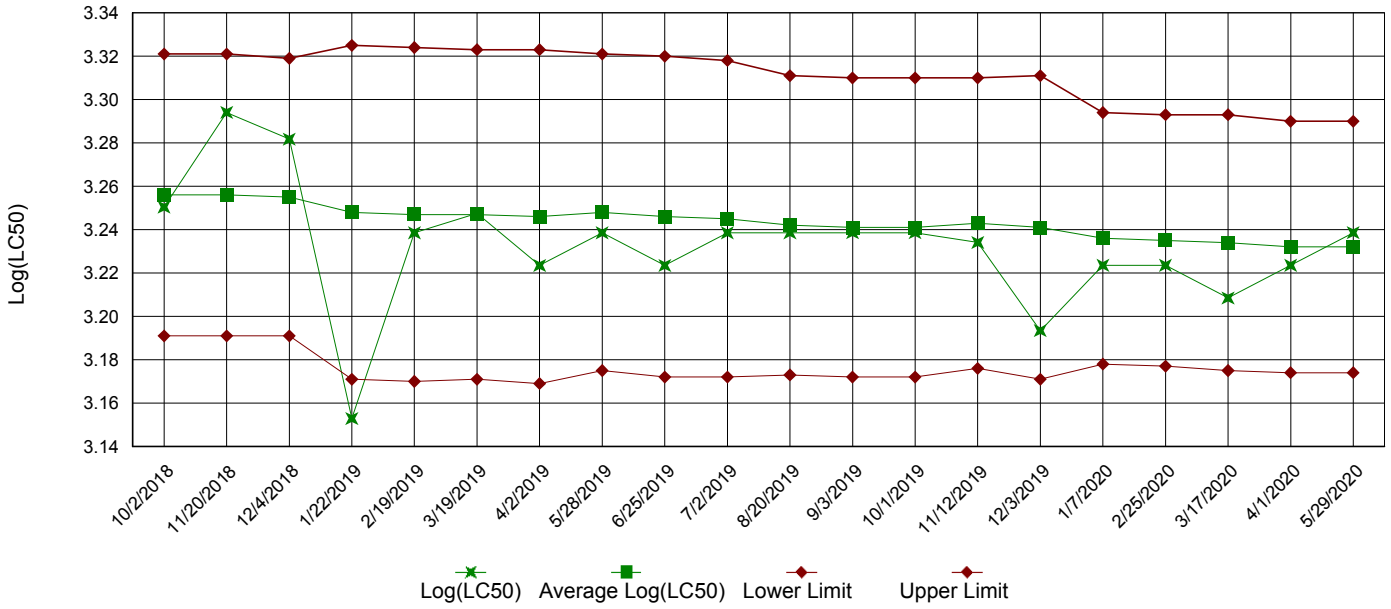
Ho:Control<Treatment

Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	10			
2	32 %	10	7.834	44.3	-4.2
3	42 %	10	7.834	44.3	-8.7
4	56 %	10	7.834	44.3	-5.6
5	80 %	10	7.834	44.3	-10.8
6	100 %	10	7.834	44.3	-3.2

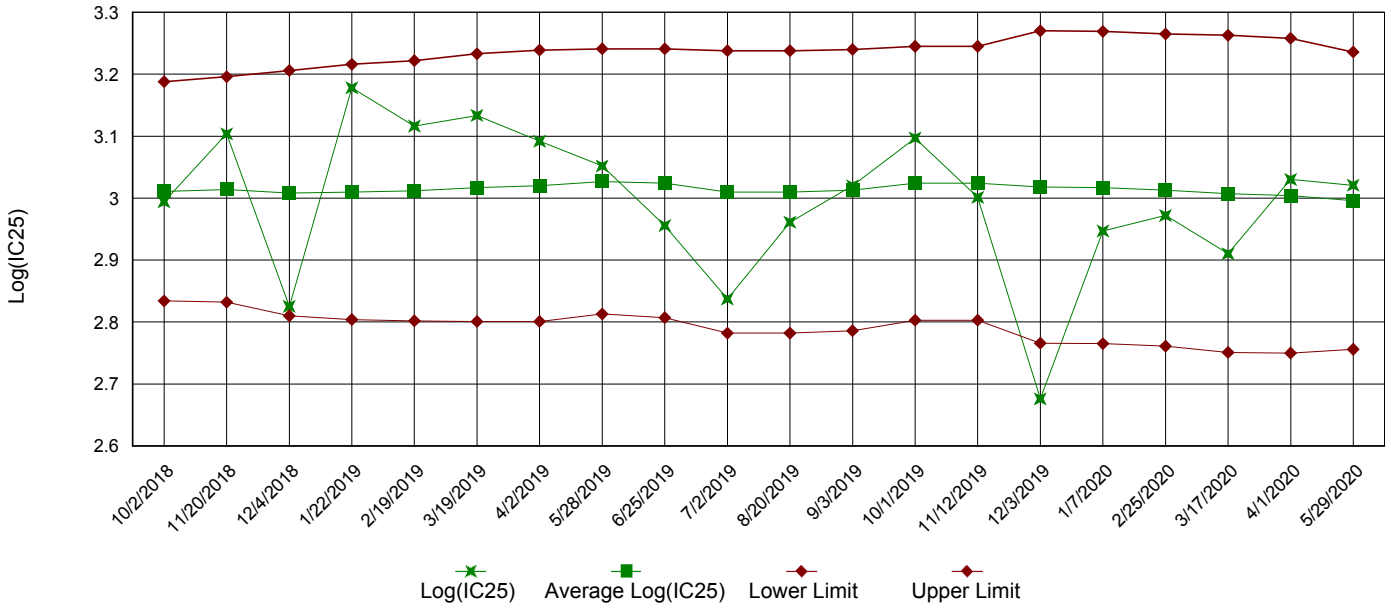
Appendix A3: Test 1002.0

Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Jonesboro CWL
Project Reference: Biomonitoring
Project Manager: Whitney Young
Sampled By: MH/JH

PO No.

AIC CONTROL NO: 245515
AIC PROPOSAL NO:
Carrier:
Received Temperature C: 1.3

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	SAMPLE MATRIX: WATER	SOIL	NO OF BOTTLES	ANALYSES REQUESTED: C. dubia	P. promelas	Remarks
1	East Effluent	5/25-26/20 8:00-7:00am		X	X		3	X		No Chlorine
	West Effluent	5/25-26/20 8:00-7:00am		X	X		3	X	X (AIC 245516)	No Chlorine
		Container Type								Field pH calibration
		Preservative								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 ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions:
Phone:_____Fax:
Report Attention to:_____
Report Address to:

Relinquished By:	Date/Time	Received By:	Date/Time
	5/26/20 10:57am		
Relinquished By:	Date/Time	Received in Lab By: DAndy Brow	Date/Time 5-26-20 10:57AM

Comments: West: parallel testing w/ UV (AIC 245517)

FORM 0060

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: City Water + Light
Project Reference: East & West Bio-Monitoring
Project Manager: Whitney Young
Sampled By: JH
PO No.

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	SAMPLE MATRIX: WATER	SOIL	NO OF BOTTLES	C. Dubia	P. Promelas	ANALYSES REQUESTED	AIC CONTROL NO: 245515 / Remarks
2	EAST Effluent	5/26-27/20 10:00am-9:00am		X	X		3	X			East
	WEST Effluent	5/26-27/20 10:00Am 9:00Am		X	X		3	X	X	(AIC 245516/245517)	Cl = 0.00
		Container Type									Field pH calibration
		Preservative									on _____ @ _____
											Buffer:

AIC PROPOSAL NO:
Carrier: CW&L
Received Temperature C: 1.8 / 1.3

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 ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions:
Phone:_____Fax:
Report Attention to:_____
Report Address to:

Relinquished By:	Date/Time	Received By:	Date/Time
	5/27/20 12:43pm		
Relinquished By:	Date/Time	Received in Lab By: D.BROWN	Date/Time 5.27.20 1243

Comments:

FORM 0060

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Jonesboro CWL
Project Reference: WET Testing
Project Manager: Whitney Young
Sampled By: RS/JE

PO No.

SAMPLE MATRIX: WATER, SOIL

NO OF BOTTLES

ANALYSES REQUESTED: C. dubia; P. promelas

AIC CONTROL NO: 245515
AIC PROPOSAL NO:
Carrier:
Received Temperature C: 0.9°C/0.8°C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL	NO OF BOTTLES	C. dubia	P. promelas	Remarks
3	East Effluent	5/28-29/20 10:00-9:00AM		X	X		3	X		No Chlorine
	West Effluent	5/28-29/20 10:00-9:00 PM		X	X		3	X	X	(AIC 245516/245517)
		Container Type								Field pH calibration
		Preservative								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 ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions:
Phone:_____Fax:
Report Attention to:_____
Report Address to:

Relinquished By:	Date/Time	Received By:	Date/Time
	5/29/20 1:20pm		
Relinquished By:	Date/Time	Received in Lab By:	Date/Time
			5-29-20 1320

Comments:

FORM 0060