

NASHVILLE PUBLIC WORKS

426 North Main, Nashville, AR 71852
PH (870) 845-4015, FAX (870) 845-7409

January 7, 2013

STATE OF ARKANSAS
Arkansas Department of Environmental Quality
5301 Northshore Drive
North Little Rock, AR 72118-5317

Attn: Mr. Allen Anderson
Administrative Assistant, NPDES Enforcement


Re: NPDES Permit #AR0021776, AFIN # 31-00036
Bio-monitoring Results- Ceriodaphnia Dubia Retest

Dear Mr. Anderson:

Please find enclosed our results for the fourth quarter of 2012.
Results indicate a passing of all test except ceriodaphnia dubia reproduction.
A retest was completely with the same results. We are in the process of retesting
for the second time.

If you have any questions of concern, please contact me at 870-845-4015.

Sincerely,



Larry Dunaway
Public Works Director

cc: Pretreatment File, 2012

October 24, 2012

Test Results of
Fourth Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Outfall 001
Nashville, AR

Control No. 161739-1

**FOURTH QUARTER
BIOMONITORING
PASSED ALL BUT
CERIODAPHNIA DUBIA
REPRODUCTION**

Mr. Ed Carlyle
City of Nashville
426 North Main
Nashville, AR 71852

Prepared by:

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

City of Nashville
ATTN: Mr. Ed Carlyle
426 North Main
Nashville, AR 71852

Re: Chronic 7-Day Renewal utilizing *Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia*
Outfall 001 - Nashville, AR
NPDES Permit No. NPDES AR0021776 AFIN 31-00036

Dear Mr. Ed Carlyle:

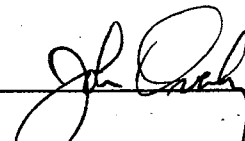
This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire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

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Method 1000.0 Chronic *Pimephales promelas* (Fathead minnow) Survival and Growth Test: The No Observable Effects Concentration (NOEC) for survival occurred at 97 % effluent, which is above the critical dilution of 73 %. The NOEC for growth occurred at 97 % effluent, which is above the critical dilution of 73 %. **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 97 % effluent, which is above the critical dilution of 73 %. The NOEC for reproduction occurred at 0 % effluent, which is below the critical dilution of 73 %. **The sample PASSED lethal effects, however, FAILED sub-lethal effects for the *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION



John Overbey
Laboratory Director

PDF cc: City of Nashville
ATTN: Mr. Ed Carlyle
mredcarlyle@yahoo.com

Table of Contents

- I. Control Acceptance Criteria
- II. Outlined Report
- III. Data Analysis
- IV. Standard Reference Toxicants
- V. Chemical Analysis/Quality Control
- VI. Organism History
- VII. Results Summary

Pimephales promelas (Fathead minnow)
Ceriodaphnia dubia

Appendix A: Raw Data

A1: Test 1000.0

Pimephales promelas (Fathead minnow) Survival and Growth

Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

A2: Statistics

A3: Water Chemistry

A4: Reference Toxicant

Appendix B: Chains of Custody

I. Control Acceptance Criteria

Pimephales promelas (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	95.0	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.376	PASS
Control Growth CV < or = 40%	7.56	PASS
Growth Minimum Significant Difference 12 to 30%	17.5	PASS
Critical Dilution CV < or = 40%	11.6	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	16.5	PASS
Control CV < or = 40% per Surviving Female	19.6	PASS
Reproduction Minimum Significant Difference 13 to 47%	18.3	PASS
Critical Dilution CV < or = 40%	87.9	FAIL

II. Outlined Report

A. Introduction

1. Permit Number: NPDES AR0021776 AFIN 31-00036
2. Test Requirements: Test Methods 1000.0 and 1002.0

3. Receiving Stream: Ouachita River Basin

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)	8.5	7.9	8.8
pH (standard units)	7.6	7.8	7.5
Alkalinity (mg/l as CaCO ₃)	110	110	120
Hardness (mg/l as CaCO ₃)	40	42	39
Conductivity (umhos/cm)	580	700	650
Residual Chlorine (mg/l)	<0.05	<0.05	0.050
Ammonia as N (mg/l)	<0.1	0.34	0.15

2. Dilution Water Samples: Synthetic Soft Water #3923
 - a. Dates Prepared: October 15 through October 29, 2012
 - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.4	8.2	8.4
pH (standard units)	7.8	7.7	7.7
Alkalinity (mg/l as CaCO ₃)	30	30	30
Hardness (mg/l as CaCO ₃)	47	44	44
Conductivity (umhos/cm)	150	190	180
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: October 16, 2012 at 1530
Date & Time Test Terminated: October 23, 2012 at 1445
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: October 16, 2012 at 1425
Date & Time Test Terminated: October 23, 2012 at 1350
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 September 5, 2012 at 1250 to September 12, 2012 at 1115

The results were as follows: (Control No. 160675-1.)

Survival LC-50: 7105 mg/l

Growth IC-25: 6473 mg/l

Growth PMSD: 32.2

Ceriodaphnia dubia

Chronic reference tests are performed monthly.

A chronic reference test was performed on September 5, 2012 at 1440 to September 11, 2012 at 1450

The results were as follows: (Control No. 160675-2.)

Survival LC-50: 2323 mg/l

Growth IC-25: 894.4 mg/l

Growth PMSD: 26.6

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	100	1.41
pH	SM 4500-H+ B	101	0.267
Conductivity	EPA 120.1	103	1.30

VI. Organism History

Pimephales promelas (Fathead minnow)

Date: October 16, 2012

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: October 16, 2012

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

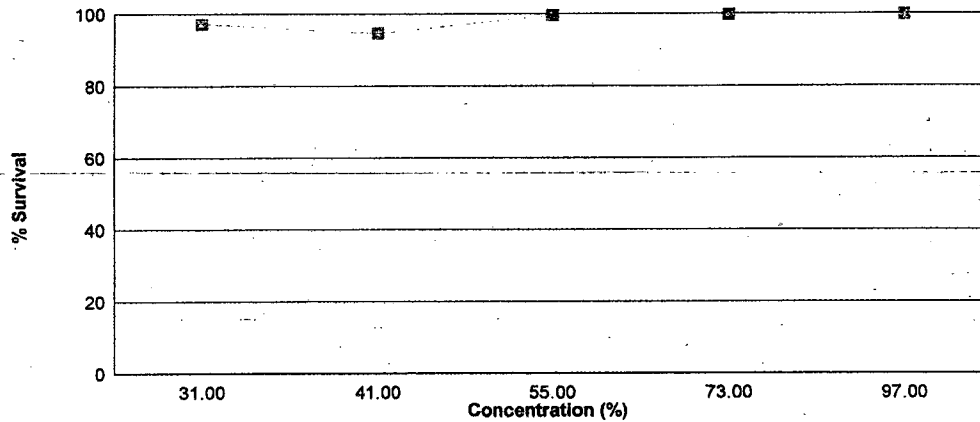
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

Effluent dilutions for this test were 31 %, 41 %, 55 %, 73 %, 97 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 73 % effluent.

The test was initiated on October 16, 2012 at 1530 and continued through October 23, 2012 at 1445. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 97 % effluent
- b.) NOEC growth = 97 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	95.0	0.357
31 %	97.5	0.359
41 %	95.0	0.378
55 %	100	0.362
73 %	100	0.404
97 %	100	0.381

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

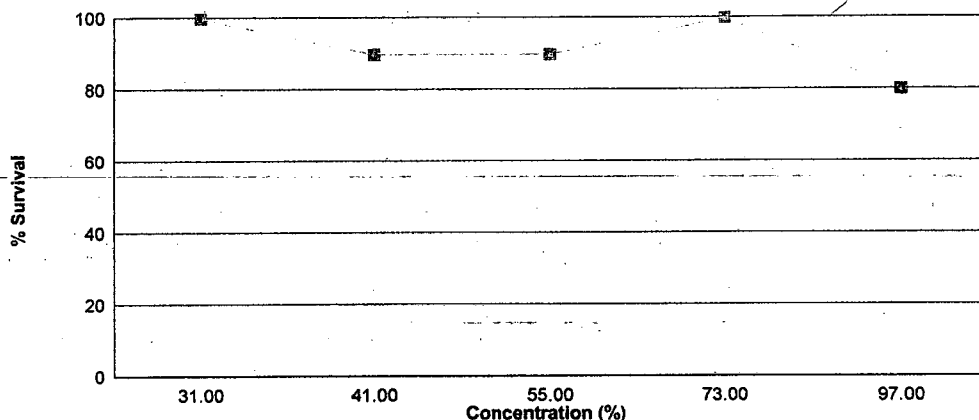
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 31 %, 41 %, 55 %, 73 %, 97 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 73 % effluent.

The test was initiated on October 16, 2012 at 1425 and continued through October 23, 2012 at 1350. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 97 % effluent
- b.) NOEC reproduction = 0 % effluent



Summary of the 7-day <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	16.5
31 %	100	7.90 *
41 %	90.0	4.80 *
55 %	90.0	4.40 *
73 %	100	1.70 *
97 %	80.0	0.800 *

*Significant difference when compared to the control (p=0.05)

Appendix B: Test 1000.0
SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

Permittee: City of Nashville

NPDES No.: NPDES AR0021776 AFIN 31-00036

Date and Time Test Initiated: October 16, 2012 at 1530

Date and Time Test Terminated: October 23, 2012 at 1445

Dilution water used: Synthetic Soft Water #3923

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	87.5	87.5	100	100	100	95.0	7.21
31 %	87.5	100	100	100	100	100	100	97.5	5.73
41 %	87.5	100	100	87.5	100	100	97.5	95.0	7.21
55 %	100	100	100	100	100	100	100	100	0.00
73 %	100	100	100	100	100	100	100	100	0.00
97 %	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	0.316	0.349	0.384	0.360	0.378	0.357	7.56
31 %	0.322	0.352	0.356	0.368	0.395	0.359	7.38
41 %	0.318	0.394	0.398	0.396	0.386	0.378	9.00
55 %	0.301	0.390	0.332	0.428	0.359	0.362	13.7
73 %	0.356	0.356	0.414	0.460	0.435	0.404	11.6
97 %	0.341	0.336	0.351	0.469	0.410	0.381	15.0

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	(73 %)	_____ 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	(73 %)	_____ 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: 97 % (TOP6C)

6. LOEC *Pimephales* Lethality: 97 % (TXP6C)

7. NOEC *Pimephales* Sublethality: 97 % (TPP6C)

8. LOEC *Pimephales* Sublethality: 97 % (TYP6C)

9. Coefficient of variation for *Pimephales* growth: 11.6 (TQP6C)

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

Permittee: City of Nashville

NPDES No.: NPDES AR0021776 AFIN 31-00036

Date and Time Test Initiated: October 16, 2012 at 1425

Date and Time Test Terminated: October 23, 2012 at 1350

Dilution water used: Synthetic Soft Water #3923

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent				
		31 %	41 %	55 %	73 %	97 %
24 hour	100	100	100	100	100	100
48 hour	100	100	100	100	100	90.0
7 day	100	100	90.0	90.0	100	80.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

Replicates	Control	Percent Effluent				
		31 %	41 %	55 %	73 %	97 %
A	14	8	7	6	2	0
B	15	7	9	2	1	1
C	16	8	2	5	0	1
D	15	7	15	5	0	0
E	17	4	1	3	4	4
F	21	7	6	5	4	0
G	12	7	2	0	0	0
H	22	10	0	8	2	2
I	14	8	3	3	2	0
J	19	13	3	7	2	0
Mean per Adult	16.5	7.90	4.80	4.40	1.70	0.800
Mean per Surviving Adult	16.5	7.90	5.22	4.89	1.70	1.00
CV %	19.6	29.5	88.7	40.2	87.9	141

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	(73 %)	_____ YES	<u> X </u> 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	(73 %)	<u> X </u> YES	_____ 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]: 1 (TGP3B)

5. NOEC Ceriodaphnia Lethality: 97 % (TOP3B)

6. LOEC Ceriodaphnia Lethality: 97 % (TXP3B)

7. NOEC Ceriodaphnia Sublethality: 0 % (TPP3B)

8. LOEC Ceriodaphnia Sublethality: 0 % (TYP3B)

9. Coefficient of variation for Ceriodaphnia Reproduction: 87.9 (TQP3B)

CITY OF NASHVILLE
426 NORTH MAIN STREET
NASHVILLE, AR 71852
870-845-4015

WASTE TREATMENT PLANT
LABORATORY ANALYSIS
FOR BIOMONITORING REPORTS

COLLECTION DATE: 10-16-2012

COLLECTION TIME: 0800-1600

COLLECTION PLACE: OUTFALL 001

CBOD	<u>3.54</u>	mg/L	#5210B
TSS	<u>10</u>	mg/L	#2540D
AMMN	<u>.14</u>	mg/L	#4500-NH3 A-B
FECAL COL.	<u>66</u>	mg/L	#9222D
CHLORINE	<u>.05</u>	mg/L	#4500-CI D
pH	<u>7.35</u>	mg/L	#4500 - H
DO	<u>8.44</u>	mg/L	#4500 - OG

ANALYST: EC COLLECTED BY: CC

Analysis include 10% replication
Test performed as required in Standards Methods
Samples are iced at time of collection

CITY OF NASHVILLE
426 NORTH MAIN STREET
NASHVILLE, AR 71852
870-845-4015

WASTE TREATMENT PLANT
LABORATORY ANALYSIS
FOR BIOMONITORING REPORTS

COLLECTION DATE: 10-18-2012
COLLECTION TIME: 0800-1600
COLLECTION PLACE: OUTFALL 001

CBOD	<u>3.60</u>	mg/L	#5210B
TSS	<u>10</u>	mg/L	#2540D
AMMN	<u>.17</u>	mg/L	#4500-NH3 A-B
FECAL COL.	<u>80</u>	mg/L	#9222D
CHLORINE	<u>.03</u>	mg/L	#4500-CI D
pH	<u>6.92</u>	mg/L	#4500 - H
DO	<u>8.38</u>	mg/L	#4500 - OG

ANALYST: EC COLLECTED BY: CC

Analysis include 10% replication
Test performed as required in Standards Methods
Samples are iced at time of collection

CITY OF NASHVILLE
426 NORTH MAIN STREET
NASHVILLE, AR 71852
870-845-4015

WASTE TREATMENT PLANT
LABORATORY ANALYSIS
FOR BIOMONITORING REPORTS

COLLECTION DATE: 10-20-2012

COLLECTION TIME: 0800-1600

COLLECTION PLACE: OUTFALL 001

CBOD	<u>3.49</u>	mg/L	#5210B
TSS	<u>9</u>	mg/L	#2540D
AMMN	<u>.18</u>	mg/L	#4500-NH3 A-B
FECAL COL.	<u>28</u>	mg/L	#9222D
CHLORINE	<u>.05</u>	mg/L	#4500-Cl D
pH	<u>7.18</u>	mg/L	#4500 - H
DO	<u>7.01</u>	mg/L	#4500 - OG

ANALYST: EC COLLECTED BY: CC

Analysis include 10% replication
Test performed as required in Standards Methods
Samples are iced at time of collection

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: CITY OF NASHVILLE			PO No.		No of	Analyser Requested								AIC Control No: 161739	
Project Reference: BIOMONITORING 4th Q			Sample Matrix			BOTTLES	FATHERD	MINNOW	CERIODARIUM	DUBIA	AIC Proposal No:				
Project Manager: ED CARLYLE JR			W	S	L						Carrier: ED				
Sampled By: Ed Carlyle Jr			A	O		S	Received Temperature °C 2°C								
AIC No.	Sample Identification	Date/Time Collected	B	P	E		Remarks								
1	NASHUB104Q	10/15/2012	X	X		3	X	X							
		0800-1600	8												
			HR		1										
					G										
					A										
					L										
					P										
					NO										
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						
NORMAL TURNAROUND CONTACT: ED CARLYLE, JR. 870-557-3143 FAX: 870-845-4522 REPORT TO: ED CARLYLE, JR. 426 NORTH MAIN NASHVILLE, AR 71852					Relinquished By: Ed Carlyle Jr	Date/Time: 10:15	Received By:	Date/Time:							
					Relinquished By:	Date/Time:	Received in Lab By: [Signature]	Date/Time: 10-16-12 10:15A							
					Comments: 1st of 3 samples - hand delivered on ice										

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: CITY OF NASHVILLE			PO No.		No of BOTTLES	Analyses Requested										AIC Control No: 161939								
Project Reference: BIDMONITORING 4th Q.			Sample Matrix			WATER	SOIL	SLUDGE	FATHEAD	MINNOW	CERIODAPHNA	DUBIA											AIC Proposal No:	
Project Manager: ED CARLYLE, JR.			G R A B C O M P																				Carrier: Ed	
Sampled By: Ed Carlyle Jr.															Received Temperature °C 2									
AIC No.	Sample Identification	Date/Time Collected	G R A B	C O M P	WATER	SOIL	SLUDGE	FATHEAD	MINNOW	CERIODAPHNA	DUBIA											Remarks		
2	NASHUBIO4A	10/17/2012	8			X		X		X														
2		0800-1600	HR																					
															Field pH calibration									
			Container Type												on _____ @ _____									
			Preservative												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																					
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS					Relinquished By: Ed Carlyle Jr.					Date/Time: 10/18/2012 10:40					Received By:									
Expedited results requested by: _____					Relinquished By:					Date/Time:					Received in Lab By: Laura K...									
Who should AIC contact with questions: _____					Comments:										Date/Time: 10-18-12 1040									
Phone: _____ Fax: _____					SECOND SAMPLE HAND DELIVERED ON ICE																			
Report Attention to: _____																								
Report Address to: _____																								



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: CITY OF NASHVILLE			PO No.		No of BOTTLES	Analyses Requested											AIC Control No: 161739							
Project Reference: BIOMONITORING 4Q			Sample Matrix			FATHERD MINNOW CERIODAPHNIA DUBIA												AIC Proposal No:						
Project Manager: ED CARLYLE JR			WATER SOIL WASTE	COMPOST														Carrier: Ed						
Sampled By: Ed Carlyle Jr.							GRAB	P												Received Temperature °C: 2				
AIC No.	Sample Identification	Date/Time Collected																		Remarks				
3	NASHUB104Q	10-19-2012			8				3	X	X													
-3		0800-1600			HR																			
				1																				
				G																				
				A																				
				L																				
				P																				
				NO																				
Container Type														Field pH calibration										
Preservative														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												
TV EW PI Ri Ri	NORMAL TURNAROUND CONTACT: ED CARLYLE, JR. 870-557-3143 FAX: 870-845-4522 REPORT TO: ED CARLYLE, JR. 426 NORTH MAIN NASHVILLE, AR 71852				Relinquished By: Ed Carlyle Jr.		Date/Time: 10-19-2012 5:00		Received By: Amanda Mangus		Date/Time: 10-20-12 8:00													
					Relinquished By:		Date/Time:		Received in Lab By: Amanda Mangus		Date/Time: 10-20-12 8:00													
					Comments: hand delivered on ice - third sample																			

December 21, 2012

Test Results of
Fourth Quarter
Chronic
Biomonitoring Testing
for
Outfall 001

Control No. 163252-1

**FIRST CERIODAPHNIA
DUBIA REPRODUCTION
RETEST FAILED**

Prepared for:

Mr. Ed Carlyle
City of Nashville
426 North Main
Nashville, AR 71852

Prepared by:

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



City of Nashville
ATTN: Mr. Ed Carlyle
426 North Main
Nashville, AR 71852

Re: Chronic utilizing *Ceriodaphnia dubia*
Outfall 001
NPDES Permit No. NPDES AR0021776 AFIN 31-00036

Dear Mr. Ed Carlyle:

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

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Other daphnids were observed in the test solutions which may have interfered with the test results.

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: The No Observable Effects Concentration (NOEC) for survival occurred at 97 % effluent, which is above the critical dilution of 73 %. The NOEC for reproduction occurred at 41 % effluent, which is below the critical dilution of 73 %. The sample **PASSED** lethal effects, however, **FAILED** sub-lethal effects for the *Ceriodaphnia dubia* test.

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Nashville
ATTN: Mr. Ed Carlyle
mredcarlyle@yahoo.com

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
Ceriodaphnia dubia

Appendix A: Raw Data

- A1: 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

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	16.6	PASS
Control CV < or = 40% per Surviving Female	9.07	PASS
Reproduction Minimum Significant Difference 13 to 47%	18.4	PASS
Critical Dilution CV < or = 40%	41.2	FAIL

II. Outlined Report

A. Introduction

1. Permit Number: NPDES AR0021776 AFIN 31-00036

2. Test Requirements: Test Method 1002.0

3. Receiving Stream: Ouachita River Basin

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)	8.2	8.7	8.6
pH (standard units)	7.6	7.9	7.7
Alkalinity (mg/l as CaCO ₃)	120	99	100
Hardness (mg/l as CaCO ₃)	21	35	34
Conductivity (umhos/cm)	650	650	650
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.14	0.19	0.54

2. Dilution Water Samples: Synthetic Soft Water #3939

a. Dates Prepared: November 29 through December 12, 2012

b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.2	7.7	7.5
pH (standard units)	7.7	7.5	7.6
Alkalinity (mg/l as CaCO ₃)	31	31	31
Hardness (mg/l as CaCO ₃)	44	41	41
Conductivity (umhos/cm)	160	160	180
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 1002.0, *Ceriodaphnia dubia* Survival and Reproduction.

2. Endpoint: No Observable Effects Concentration (NOEC)

3. Test Conditions:

Ceriodaphnia dubia Survival and Growth Method 1002.0

Date & Time Test Initiated: December 12, 2012 at 1600
Date & Time Test Terminated: December 19, 2012 at 1430
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 1002.0 *Ceriodaphnia dubia*

III. Data Analysis

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

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was analyzed using Kolmogorov's Test for Normality and Bartlett's test and analyzed with 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.

Ceriodaphnia dubia

Chronic reference tests are performed monthly.

A chronic reference test was performed on December 4, 2012 at 1640 to December 11, 2012 at 1700

The results were as follows: (Control No. 162913-2.)

Survival LC-50: 2125 mg/l
Growth IC-25: 972.8 mg/l
Growth PMSD: 17.9

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	102	2.63
pH	SM 4500-H+ B	101	0.267
Conductivity	EPA 120.1	102	0.660

VI. Organism History

Ceriodaphnia dubia

Date: December 12, 2012

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 *Ceriodaphnia dubia*, Cladoceran Survival and Reproduction Test -- Method 1002.0

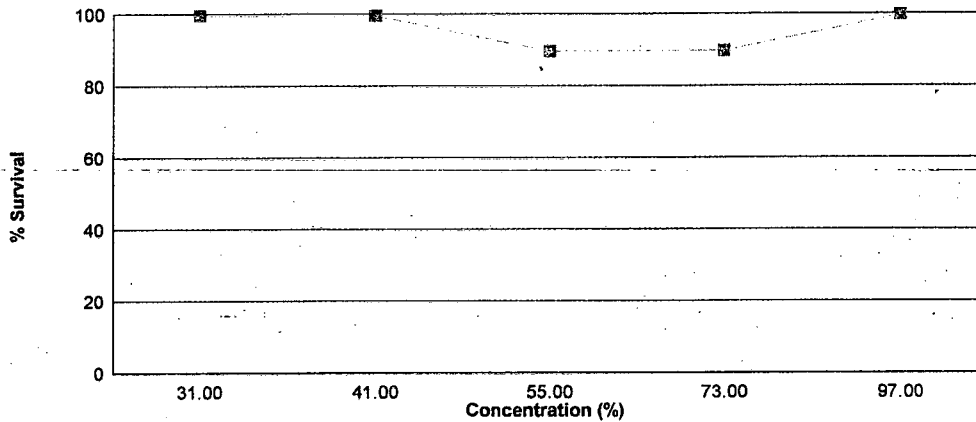
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 31 %, 41 %, 55 %, 73 %, 97 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 73 % effluent.

The test was initiated on December 12, 2012 at 1600 and continued through December 19, 2012 at 1430. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 97 % effluent
- b.) NOEC reproduction = 41 % effluent



Concentration	Percent Survival	Mean Reproduction
Control	100	16.6
31 %	100	15.9
41 %	100	13.4
55 %	90.0	12.7 *
73 %	90.0	9.00 *
97 %	100	10.0 *

*Significant difference when compared to the control (p=0.05)

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

Permittee: City of Nashville

NPDES No.: NPDES AR0021776 AFIN 31-00036

Date and Time Test Initiated: December 12, 2012 at 1600

Date and Time Test Terminated: December 19, 2012 at 1430

Dilution water used: Synthetic Soft Water #3939

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent				
		31 %	41 %	55 %	73 %	97 %
24 hour	100	100	100	100	100	100
48 hour	100	100	100	90.0	100	100
7 day	100	100	100	90.0	90.0	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

Replicates	Control	Percent Effluent				
		31 %	41 %	55 %	73 %	97 %
A	18	15	16	15	15	16
B	20	12	13	14	14	8
C	16	15	17	14	12	10
D	16	16	18	11	5	8
E	17	16	12	0	7	9
F	15	22	15	9	4	9
G	15	15	14	16	14	11
H	16	19	9	14	11	10
I	17	13	9	19	8	9
J	16	16	11	15	0	10
Mean per Adult	16.6	15.9	13.4	12.7	9.00	10.0
Mean per Surviving Adult	16.6	15.9	13.4	14.1	10.0	10.0
CV %	9.07	17.9	23.7	20.2	41.2	23.1

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	(73 %)	<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	(73 %)	<u> X </u> YES	<u> </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]: 1 (TGP3B)
5. NOEC *Ceriodaphnia* Lethality: 97 % (TOP3B)
6. LOEC *Ceriodaphnia* Lethality: 97 % (TXP3B)
7. NOEC *Ceriodaphnia* Sublethality: 41 % (TPP3B)
8. LOEC *Ceriodaphnia* Sublethality: 55 % (TYP3B)
9. Coefficient of variation for *Ceriodaphnia* Reproduction: 41.2 (TQP3B)

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

PERMITTEE: City of Nashville SAMPLE No. 1 COLLECTED ending: DATE: December 11, 2012 TIME: 0800
NPDES NO.: NPDES AR0021776 AFIN 31-000 1600
CONTACT: Mr. Ed Carlyle 1600
ANALYST: 280, 298, 304, 307

Test Initiated: DATE: December 12, 2012 TIME: 1600
Test Terminated: DATE: December 19, 2012 TIME: 1430

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.2	8.1	7.7	7.6	7.5	7.3	7.7
Final	8.1	7.7	7.8	7.8	7.6	7.9	7.6
pH Initial	7.7	7.7	7.5	7.8	7.6	7.9	7.6
Final	8.0	7.8	8.0	8.0	7.9	7.9	7.9
Alkalinity	31	NA	31	NA	31	NA	NA
Hardness	44	NA	41	NA	41	NA	NA
Conductivity	160	160	160	170	180	170	170
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 31 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.3	8.1	8.1	7.7	7.7	7.6	7.8
Final	8.3	7.8	8.1	7.8	8.0	7.4	7.6
pH Initial	7.6	7.8	7.7	7.8	7.8	7.9	7.7
Final	8.2	8.2	8.3	8.3	8.2	8.1	8.0
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	310	310	310	320	340	320	320
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 41 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.2	8.2	8.1	7.8	7.4	7.6	7.8
Final	8.2	8.1	7.7	7.4	8.0	7.5	7.9
pH Initial	7.7	7.8	7.7	7.8	7.8	7.9	7.7
Final	8.3	8.3	8.4	8.4	8.3	8.1	8.2
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	360	360	360	360	380	360	370
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 55 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.3	8.3	8.0	7.9	7.6	7.4	7.6
Final	8.3	8.0	7.8	7.6	7.9	7.7	7.7
pH Initial	7.6	7.7	7.8	7.7	7.9	7.9	7.7
Final	8.4	8.4	8.4	8.4	8.4	8.1	8.2
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	430	430	430	430	450	430	440
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 73 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.6	8.3	8.0	8.0	7.5	7.4	7.6
Final	8.4	8.0	7.7	7.9	8.2	7.6	7.6
pH Initial	7.6	7.8	7.8	7.8	8.0	8.0	7.8
Final	8.5	8.4	8.5	8.5	8.5	8.2	8.3
Alkalinity	97	NA	82	NA	74	NA	NA
Hardness	35	NA	37	NA	36	NA	NA
Conductivity	520	510	520	520	540	520	530
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 97 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	8.1	8.0	7.8	7.6	7.6	7.7
Final	8.5	8.1	7.9	7.9	8.4	7.4	7.9
pH Initial	7.6	7.8	7.8	7.8	8.1	8.0	7.8
Final	8.6	8.6	8.6	8.6	8.7	8.3	8.3
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	630	620	630	630	660	640	650
Chlorine	NA	NA	NA	NA	NA	NA	NA

CITY OF NASHVILLE
426 NORTH MAIN STREET
NASHVILLE, AR 71852
870-845-4015

WASTE TREATMENT PLANT
LABORATORY ANALYSIS
FOR BIOMONITORING REPORTS

COLLECTION DATE: 12-11-2012
COLLECTION TIME: 0800 - 0800
COLLECTION PLACE: OUTFALL 001

CBOD	<u>4.08</u>	mg/L	#5210B
TSS	<u>8</u>	mg/L	#2540D
AMMN	<u>no results from Lab.</u>	mg/L	#4500-NH3 A-B
FECAL COL.	<u>29</u>	mg/L	#9222D
CHLORINE	<u>10.49</u>	mg/L	#4500-CI D
pH	<u>7.05</u>	mg/L	#4500 - H
DO	<u>11.43</u>	mg/L	#4500 - OG

ANALYST: EC COLLECTED BY: CC

Analysis include 10% replication
Test performed as required in Standards Methods
Samples are iced at time of collection

CITY OF NASHVILLE
426 NORTH MAIN STREET
NASHVILLE, AR 71852
870-845-4015

WASTE TREATMENT PLANT
LABORATORY ANALYSIS
FOR BIOMONITORING REPORTS

COLLECTION DATE: 12-13-2012
COLLECTION TIME: 0800-1600
COLLECTION PLACE: OUTFALL 001

CBOD	<u>2.61</u>	mg/L	#5210B
TSS	<u>8</u>	mg/L	#2540D
AMMN	<u>-</u>	mg/L	#4500-NH3 A-B
FECAL COL.	<u>57</u>	mg/L	#9222D
CHLORINE	<u>.05</u>	mg/L	#4500-CI D
pH	<u>7.05</u>	mg/L	#4500 - H
DO	<u>11.13</u>	mg/L	#4500 - OG

ANALYST: EC COLLECTED BY: CC

Analysis include 10% replication
Test performed as required in Standards Methods
Samples are iced at time of collection

CITY OF NASHVILLE
426 NORTH MAIN STREET
NASHVILLE, AR 71852
870-845-4015

WASTE TREATMENT PLANT
LABORATORY ANALYSIS
FOR BIOMONITORING REPORTS

COLLECTION DATE: 12-15-2012
COLLECTION TIME: 0800-1600
COLLECTION PLACE: OUTFALL 001

CBOD	<u>3.41</u>	mg/L	#5210B
TSS	<u>11</u>	mg/L	#2540D
AMMN	<u>~</u>	mg/L	#4500-NH3 A-B
FECAL COL.	<u>55</u>	mg/L	#9222D
CHLORINE	<u>.07</u>	mg/L	#4500-CI D
pH	<u>7.42</u>	mg/L	#4500 - H
DO	<u>11.43</u>	mg/L	#4500 - OG

ANALYST: EC COLLECTED BY: CC

Analysis include 10% replication
Test performed as required in Standards Methods
Samples are iced at time of collection



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: CITY OF NASHVILLE			PO No.		No of BOTTLES	Analyses Requested										AIC Control No: 163252						
Project Reference: BIDMONITORING-CERIOD-RES			Sample Matrix			CERIODAPHA DUBIA																AIC Proposal No:
Project Manager: ED CARLYLE, JR.			W	S			W															
Sampled By: Ed Carlyle Jr.			G	C																		Received Temperature °C 2°C
AIC No.	Sample Identification	Date/Time Collected	GRA	COMP	WATER	SOIL	WASTE															Remarks
	NASHVIDEER	12/10-11/12		24			X	3														
	RES	0800-0800		HR																		
								1														
								G														
								A														
								L														
								P														Field pH calibration
								NO														on _____ @ _____
																						Buffer:

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

NORMAL TURNAROUND
CONTACT: ED CARLYLE, JR.
870-557-3143 FAX: 870-845-4522
REPORT TO: ED CARLYLE, JR.
426 NORTH MAIN
NASHVILLE, AR 71852

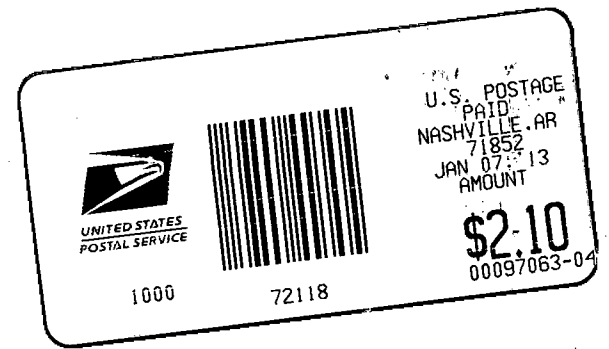
Relinquished By: Ed Carlyle Jr.	Date/Time: 12/11/12 11:38	Received By:	Date/Time:
Relinquished By:	Date/Time:	Received in Lab By: Sam P...	Date/Time: 12-11-12 11:38AM
Comments: FIRST SAMPLE OF 3 HAND DELIVERED ON ICE			



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: CITY OF NASHVILLE			PO No.			No of BOTTLES	Analyses Requested										AIC Control No: 103252	
Project Reference: BIOMONITORING CER-RES			Sample Matrix														CERIODAPHNIA	
Project Manager: ED CARLYLE JR			WATER SOIL WASTE								Carrier:							
Sampled By: Ed Carlyle Jr			GRA B			COM P			Received Temperature °C			Remarks						
AIC No.	Sample Identification	Date/Time Collected																
2	NASHUBIO CER-RES	12/12/12 0800-1600	8			X	3											
							1											
							G											
							A											
							L											
							P											
		Container Type					NO							Field pH calibration on _____ @ _____				
		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							
Turnaround Time Requested: (Please circle) _NORMAL or EXPEDITED IN ___ DAYS							Relinquished By: Ed Carlyle Jr		Date/Time: 12/13/12 10:40		Received By:		Date/Time					
Expedited results requested by:							Relinquished By:		Date/Time:		Received in Lab By: [Signature]		Date/Time: 12-13-12 1040					
Who should AIC contact with questions:							Comments: hand delivered on ice - second sample of three											
Phone: _____ Fax: _____																		
Report Attention to: _____																		
Report Address to: _____																		

**CITY OF NASHVILLE
LARRY DUNAWAY
PUBLIC WORKS
DIRECTOR
426 NORTH MAIN STREET
NASHVILLE, AR 71852**



**Arkansas Department of Environmental
Quality
Attn: Mr. Allen Anderson
Enforcement Assistant, NPDES Section
5301 Northshore Drive
Little Rock, AR 72118-5317**