

**REPORT OF CHRONIC DEFINITIVE
TOXICITY TESTS ON POND 4 EFFLUENT
SPIKED WITH 6 MG/L AND 12 MG/L OF
ALUMINUM FROM
GEORGIA PACIFIC, PALATKA FLORIDA**

Prepared for:

**BROWN & CALDWELL
Nashville, Tennessee**

Prepared by:

**Empirical Laboratories, LLC
227 French Landing Drive
Nashville, Tennessee 37228
(615) 345-1115**

February 2010

This report contains 29 pages

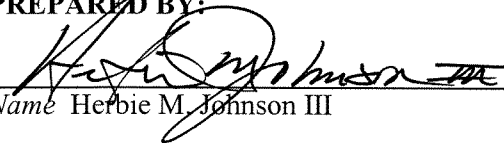


Empirical Laboratories, LLC

QUALITY ASSURANCE DOCUMENTATION

This document has been prepared, reviewed, and approved by the following
EMPIRICAL LABORATORIES of Tennessee personnel:

PREPARED BY:

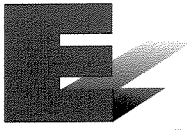

Name Herbie M. Johnson III

3/15/00

Date

Group Leader II

Title



Empirical Laboratories

March 15, 2010

Mr. Houston Flippin
Brown & Caldwell
501 Great Circle Rd., Suite 150,
Nashville, TN 37228

Dear Houston:

The results of the chronic definitive toxicity tests conducted on the two samples of Georgia Pacific Pond 4 effluent spiked with 6 and 12 mg/L of aluminum respectively, is presented in Appendix A. The test organisms were *Ceriodaphnia dubia*.

There were adverse effects on *C. dubia* reproduction in the effluent concentrations for both samples.

If you have any questions about the information in this report, please call me at (615) 345-1115.

Sincerely,

Empirical Laboratories, LLC

Herbie M. Johnson III
Group Leader II
Aquatic Toxicology Laboratory

Enclosures

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INTRODUCTION

Municipal and industrial dischargers are required, as part of the National Pollutant Discharge Elimination System (NPDES), to measure the toxicity of their wastewaters. The permit requirement may be for monitoring purposes only or may contain toxicity limits. The types of tests required vary from state to state and may depend on flow conditions or dilution by the receiving water. The most common tests required are acute tests, which measure the short-term toxicity of the wastewater. The test duration may vary from 24 hours to 96 hours, depending on the organism tested and the state requirements. Mini-chronic tests can be used to look at longer-term toxicity. These tests involve more subtle effects on the organism, e.g., growth and reproduction, and last seven days.

The organisms used in these tests have been limited to those that are easily cultured in the laboratory. This ensures that the quality and health of the organisms are consistent over time and do not affect the results of the test. The fish species most often used is the fathead minnow (*Pimephales promelas*). The invertebrates used are the water fleas (*Daphnia pulex*, *Daphnia magna*, and *Ceriodaphnia* sp.) which are freshwater crustaceans. The culturing methods for these organisms have been outlined by the U.S. Environmental Protection Agency (USEPA) and the American Society for Testing and Materials (ASTM).

METHODS

The test procedures adhered to the methods outlined in the U.S. Environmental Protection Agency Manual, "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms," Fourth Edition, EPA 821-R-02-013, October, 2002.

Samples were received on February 24, 2010, from the Brown & Caldwell. The samples were warmed to $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ in a warm water bath from their respective temperatures of 21.7°C . Water chemistry parameters were measured on each sample and the control water, and were recorded on the appropriate data sheets (Appendix B). The parameters measured were pH, dissolved oxygen, conductivity, temperature, hardness, alkalinity, and residual chlorine. In addition, dissolved oxygen, pH, and temperature measurements were made for each test and control solution before and after each renewal. Due to limited volume the alkalinity and hardness were not able to be run.

The tests with *C. dubia* were started with young who were less than 24 hours old. The organisms were placed individually in 30-mL plastic cups containing 15 mL of either serial dilutions of effluent or control water. The control and dilution water for the tests was laboratory reconstituted water made according to USEPA procedures and had an average hardness of 86 mg/L. The test concentrations used were 9, 18, 36, 72, and 100 percent plus a control. Ten cups were used in each exposure and the control. Each organism was fed a combination of chow (consisting of digested flake food, yeast, and cereal leaves) and algae (*Selenastrum capricornutum*) daily following solution renewal. The cups were checked daily for mortality and the number of young released over the test period. The total number of young released in each concentration was then compared to the controls to see if an effect had occurred. The tests were acceptable because at least 80.0 percent of the controls survived, 60.0 percent of the controls had three broods, and the mean number of young produced by surviving control adults was 15 or more.

QUALITY ASSURANCE

The *C. dubia* were obtained from cultures maintained by Empirical Laboratories, LLC according to USEPA recommended procedures (EPA 821-R-02-012 and EPA 821-R-02-013). The *C. dubia* stock was originally obtained from Aquatic BioSystems, Fort Collins, Colorado.

The laboratory control water used in the *C. dubia* test was reconstituted water made with Nanopure II ultrapure water to which were added appropriate amounts of KCl₂, MgCl₂, NaHCO₃, CaSO₄, and selenium. The pH, conductivity, dissolved oxygen, hardness, alkalinity, and residual chlorine of the laboratory water were checked prior to use. Adjustments to these parameters were made if they fell outside the acceptable ranges. The water quality data for the water were recorded in the Water Chemistry Log.

The statistical analyses of the toxicity tests are provided in Appendix C. Reference toxicant tests are conducted every month on all of the organisms cultured in our laboratory. The tests are acute and chronic, and the reference toxicant is sodium chloride. Data sheets with LC50, NOEC and IC25 values are kept in laboratory notebooks. Copies of the IC25 reference toxicant control charts for *C. dubia* are provided in Appendix D. A copy of the chain-of-custody form is provided in Appendix E.

This document has been prepared, reviewed, and approved by Empirical Laboratories, LLC.

RESULTS

There was a significant effect on *C. dubia* reproduction in the test concentrations for the 6 mg/L aluminum spiked sample. Survivals were 90 to 100 percent in the test concentrations and control. The total number of young produced in the control was 354, compared to 296, 294, 213, 100, and 60 in the 9.0, 18.0, 36.0, 72.0, and 100 percent effluent, respectively. The reproduction data was analyzed by Dunnett's Multiple Comparison Test using the TOXCALC® program. Significance was determined at the 0.05 confidence level. The NOEC was 18 percent, the LOEC was 36 percent, and the IC25 was 24.33 percent for reproduction, the most sensitive endpoint.

There was a significant effect on *C. dubia* reproduction in the test concentrations for the 12 mg/L aluminum spiked sample. Survivals were 90 to 100 percent in the test concentrations and control. The total number of young produced in the control was 369 compared to 351, 263, 182, 89, and 63 in the 9.0, 18.0, 36.0, 72.0, and 100 percent effluent, respectively. The reproduction data was analyzed by Dunnett's Multiple Comparison Test using the TOXCALC® program. Significance was determined at the 0.05 confidence level. The NOEC was 9 percent, the LOEC was 18 percent, and the IC25 was 16.59 percent for reproduction, the most sensitive endpoint.

Both tests are considered as passing.

APPENDIX A
Empirical Laboratories, LLC
TOXICITY TEST REPORT

REPORT OF AQUATIC TOXICITY TEST RESULTS

I. GENERAL INFORMATION

A. Plant Information

Industry: Georgia Pacific

Permit No.: Unknown

Address: Unknown

Contact: Unknown

Phone Number: Unknown

Type of Plant: Unknown

Plant Operating Schedule: Unknown

Receiving Stream: Unknown

B. Sample(s)

Source(s): Pond 4 Effluent

Collection Period: 2/24/10

Type of Collection: Grab

Collected by: Unknown

Empirical Laboratories, LLC Sample Log No.(s): 1003019, 01 & 02

REPORT OF AQUATIC TOXICITY TEST RESULTS (Cont'd)

II. DATA FOR WATER FLEA TEST ON POND 4 EFFLUENT SPIKED WITH 6 MG/L OF ALUMINUM

A. Test Type

Renewal ___ 24 hr Static ___ Acute
___ Nonrenewal ___ 48 hr ___ Flowthrough Chronic
___ 96 hr
 7 day

B. Test Organism

Common Name: Water Flea Scientific Name: *Ceriodaphnia dubia*
Age: <24 hr Source: Empirical Laboratories, LLC cultures
Observed Diseases: None
Treatment: None
Acclimation: Held in control water prior to testing

C. Test Method - Reference Material

Short-term Methods for Estimating Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, USEPA, Fourth Edition, October, 2002.

Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms, USEPA, Fifth Edition, October, 2002.

D. Dilution Water

Source: Reconstituted Water Collection Period: Daily
Type Collection: Grab Pretreatment: None
Alkalinity (mg/L as CaCO₃): 58* Hardness (mg/L as CaCO₃): 86*
Conductivity (µmhos/cm): 314* pH (standard units): 7.99*(7.85 – 8.06)
Test Temperature (°C): 25.4*(25.0-25.8)

* Mean values over seven days

REPORT OF AQUATIC TOXICITY TEST RESULTS (Cont'd)

E. Test Chambers

Composition: Plastic

Depth: 20 mm

Size: 30 mL

Test Volume per Chamber: 15 mL

F. Loading Technique

Organisms per concentration: 10

Replicates per concentration: 10

Organisms per replicate: 1

G. Testing Dates

Start: 2/25/10 (15:00 Hrs)

Complete: 3/3/10 (17:00 Hrs)

H. Summary Of Test Observations

1. Most sensitive index: Reproduction

LOEC: 36%

NOEC: 18%

ChV: 25.46

IC25: 24.33%

96 Hour LC50: >100%

95 percent confidence limits: 8.72 to 34.61%

Method: Dunnett's Multiple Comparison Test, ToxCalc v5.0.23

REPORT OF AQUATIC TOXICITY TEST RESULTS (Cont'd)

2. Data Summary

	Concentration (% Effluent)	Total Survival	Survival Rate (%)	Total Young Produced
1.	Control	10	100	354
3.	9.0	10	100	296
4.	18.0	10	100	294
5.	36.0	9	90.0	213*
6.	72.0	10	100	100*
7.	100	10	100	60*
8.				

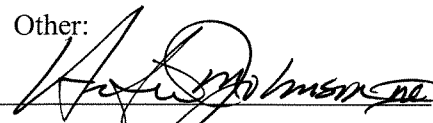
3. Comments:

I. Attachment

*Significantly different than the control ($\alpha=0.05$).

Data Summary Sheets

Other:

By:  Date: 3/15/10

Empirical Laboratories, LLC
621 Mainstream Drive
Nashville, TN 37228

(615) 345-1115

REPORT OF AQUATIC TOXICITY TEST RESULTS (Cont'd)

III. DATA FOR WATER FLEA TEST ON POND 4 EFFLUENT SPIKED WITH 12 MG/L OF ALUMINUM

A. Test Type

Renewal ___ 24 hr Static ___ Acute
___ Nonrenewal ___ 48 hr ___ Flowthrough Chronic
 ___ 96 hr
 7 day

B. Test Organism

Common Name: Water Flea Scientific Name: *Ceriodaphnia dubia*
Age: <24 hr Source: Empirical Laboratories, LLC cultures
Observed Diseases: None
Treatment: None
Acclimation: Held in control water prior to testing

C. Test Method - Reference Material

Short-term Methods for Estimating Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, USEPA, Fourth Edition, October, 2002.

Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms, USEPA, Fifth Edition, October, 2002.

D. Dilution Water

Source: Reconstituted Water Collection Period: Daily
Type Collection: Grab Pretreatment: None
Alkalinity (mg/L as CaCO₃): 58* Hardness (mg/L as CaCO₃): 86*
Conductivity (µmhos/cm): 314* pH (standard units): 7.99*(7.85 – 8.06)
Test Temperature (°C): 25.4*(25.0-25.8)

* Mean values over seven days

REPORT OF AQUATIC TOXICITY TEST RESULTS (Cont'd)

E. Test Chambers

Composition: Plastic

Depth: 20 mm

Size: 30 mL

Test Volume per Chamber: 15 mL

F. Loading Technique

Organisms per concentration: 10

Replicates per concentration: 10

Organisms per replicate: 1

G. Testing Dates

Start: 2/25/10 (15:15 Hrs)

Complete: 3/3/10 (16:10 Hrs)

H. Summary Of Test Observations

1. Most sensitive index: Reproduction

LOEC: 18%

NOEC: 9%

ChV: 12.73

IC25: 16.59%

96 Hour LC50: >100%

95 percent confidence limits: 8.24 – 26.21%

Method: Dunnett's Multiple Comparison Test, ToxCalc v5.0.23

REPORT OF AQUATIC TOXICITY TEST RESULTS (Cont'd)

2. Data Summary

	Concentration (% Effluent)	Total Survival	Survival Rate (%)	Total Young Produced
1.	Control	10	100	369
3.	9.0	10	100	351
4.	18.0	10	100	263*
5.	36.0	10	100	182*
6.	72.0	9	90.0	89*
7.	100	9	90.0	63*
8.				

3. Comments:

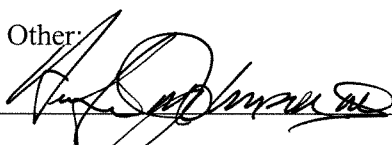
*Significantly different than the control ($\alpha=0.05$).

I. Attachment

Data Summary Sheets

Other:

By:



Date:

3/15/10

Empirical Laboratories, LLC
621 Mainstream Drive
Nashville, TN 37228

(615) 345-1115

APPENDIX B
RAW DATA SHEETS

WATER CHEMISTRY DATA SHEET
CHRONIC TOXICITY TESTS

Jug 7 7 7 7 4 4 4

DAY		0	1	2	3	4	5	6	7	8
INITIALS/FEED/CHEMISTRY			DK	DK	DK	DK	DK	DK	DK	DK
TIME OF RENEWAL			11:46	15:30	10:55	14:10	14:00			
pH (Standard Unit) Initial/Final	CK	8.06	7.95	8.03	7.88	8.12	7.84	7.85	7.89	7.98
	9/1	8.02	8.01	7.94	8.00	8.01	8.07	7.90	8.00	7.99
	18	7.99	8.05	8.06	7.96	8.07	8.00	7.98	7.91	8.08
	36	7.96	8.07	8.22	7.93	8.26	7.85	8.15	8.26	7.97
	72	7.92	7.97	8.41	7.90	8.45	7.91	8.28	7.94	8.34
	100	7.91	7.79	8.50	7.84	8.53	7.85	8.50	7.91	8.51
Dissolved Oxygen (mg/L) Initial/Final	CK	7.0	7.7	7.3	7.4	7.3	8.0	6.8	7.3	7.0
	9/1	7.0	7.5	7.3	7.5	7.2	8.0	6.8	7.2	7.0
	18	7.5	7.5	7.4	7.5	7.2	8.0	6.7	7.3	7.0
	36	7.5	7.3	7.4	7.5	7.2	8.0	6.6	7.5	6.9
	72	7.5	7.0	7.4	7.5	6.9	8.0	6.4	7.3	6.8
	100	7.5	6.9	7.4	7.2	6.9	8.0	6.5	7.9	6.7
Temperature (°C) Initial/Final	CK	25.8	25.7	25.2	25.3	25.4	25.2	25.6	25.3	25.7
	9/1	25.8	25.7	25.1	25.3	25.2	25.6	25.2	25.4	25.5
	18	25.9	25.7	25.2	25.4	25.1	25.7	25.0	25.4	25.8
	36	25.9	25.7	25.0	25.3	25.2	25.7	25.3	25.8	25.5
	72	25.9	25.7	24.9	24.0	25.3	25.4	25.3	25.6	25.3
	100	25.9	25.8	25.1	24.0	25.4	25.3	25.4	25.7	25.2
Conductivity (umho/cm) or Salinity (ppt)	CK	240	286	296	289	294	299			
	9/1	432	426	431	425	432	443			
	18	567	569	569	544	566	574			
	36	842	842	817	852	834	855			
	72	1339	1341	1006	1334	1333	1342			
	100	1707	1724	1599	1719	1721	1720			
Alkalinity (mg/L CaCO ₃)	CK	40	60	60	57.5	57.5	57.5	57.5		
	100	210								
Hardness (mg/L CaCO ₃)	CK	92	92	92	84	82	82	82		
	100	120								
Residual Chlorine	Amount Detected mg/L	100	0.02							
	Amount of Na ₂ S ₂ O ₃ Added (ml)									

Client Name : Georgia Pacific Pond 4 + 6 mg/L Al Set-up By AKS Terminated By AKS
 Date / Time Set-up 2/25/10 11:50:00 Test Species A. dubia Source: AKS Age: 22 hrs
 Date / Time Ended 3/3/10 11:17:00 Dilution Water: Jug No. C MOD. HARD
 Repetitions 10 Animals per Rep. 1 Organisms per Concentration 10 Stock Source/Temp.: 24.7°C

COMMENTS: All Temperatures are taken with Thermometer #5. Circled days indicate use of new samples. Randomization produced by Excel Macro.
 Empirical Laboratories Log No.(s): 1003019-01

Client Name: Car. Pond 4 + 6 mg/LA/
 Date: 2/25/10

DATA FOR CERIODAPHnia DUBIA 7 DAY TEST

REPLICATES

EFF. CONC	DAY OF TEST NO.	REPLICATES										TOTAL LIVE YOUNG	NO. LIVE ADULTS	
		1	2	3	4	5	6	7	8	9	10			
Ctr/ PCP 200 mg	1	L											0	10
	2	DK	L										0	10
	3	DK	3	2	2	L	L	3	2	L	4	L	16	10
	4	DK	8	10	9	8	6	8	11	7	10	7	84	10
	5	DK	L	L	L	8	11	L	L	9	L	11	39	10
	6	DK												
	Totals		36	38	42	32	32	42	38	34	24	34	235	10
9% (18ml)	1	L											0	10
	2	L											0	10
	3	4	L	L	3	L	2	L	3	3	2	17	10	
	4	L	3	3	6	5	5	4	5	6	L	37	10	
	5	8	11	11	10	13	L	10	11	6	L	80	10	
	6													
	Totals		38	29	30	19	36	26	32	19	43	24	296	10
18% (36ml)	1	L											0	10
	2	L											0	10
	3	5	4	3	3	L	3	4	L	2	L	24	10	
	4	9	2	L	7	6	8	8	9	6	6	46	10	
	5	11	L	L	L	12	L	12	L	L	L	35	10	
	6													
	Totals		31	33	34	37	27	32	31	29	24	14	294	10
36% (72ml)	1	L											0	10
	2	L											0	10
	3	L	3	2	3	L	L	L	L	2	L	10	10	
	4	4	5	3	9	5	0	4	7	7	7	51	10	
	5	8	9	L	L	10	1	L	L	L	L	22	9	
	6													
	Totals		27	32	17	26	22	0	18	24	25	12	203	9

Empirical Laboratories Log No. (s): 7003019-01 QA'd by: AM
100 % Survival 39.4 Mean Reproduction 10 # of Third Broods

Client Name: G.P. Ponce 4 + 6mg/l A1
 Date: 2/25/10

DATA FOR CERIODAPHNIA DUBIA 7 DAY TEST

REPLICATES

EFF. CONC	DAY OF TEST NO.	REPLICATES										TOTAL LIVE YOUNG	NO. LIVE ADULTS
		1	2	3	4	5	6	7	8	9	10		
72% (144ml)	1 NG	L	—	—	—	—	—	—	—	—	—	0	10
	2 DK	L	—	—	—	—	—	—	—	—	—	0	10
	3 DK	L	3	3	4	2	L	5	2	L	3	22	10
	4 1/2	3	3	7	4	7	7	7	4	L	L	42	10
	5 1/2	L	—	—	—	—	—	—	—	—	—	0	10
	6	—	—	—	—	—	—	—	—	—	—	—	—
	6 1/2	8	6	6	L	8	2	3	3	L	L	36	10
	Totals	4	12	16	8	17	9	15	9	0	3	100	10
100%	1	L	—	—	—	—	—	—	—	—	—	0	10
	2	L	—	—	—	—	—	—	—	—	—	0	10
	3	L	L	L	L	L	L	3	2	3	L	8	10
	4	4	4	2	2	5	L	4	5	L	2	28	10
	5	4	L	4	L	L	L	—	—	—	—	8	10
	6	—	—	—	—	—	—	—	—	—	—	—	—
	Totals	11	4	10	2	8	2	7	7	5	4	60	10
1													
2													
3													
4													
5													
6													
7													
Totals													

Empirical Laboratories Log No. (s): 1003019-01 QA'd by: h/m
 _____ % Survival _____ Mean Reproduction _____ # of Third Broods

WATER CHEMISTRY DATA SHEET
CHRONIC TOXICITY TESTS

July 7 7 7 4 4 4

DAY		0	1	2	3	4	5	6	7	8			
INITIALS/FEED/CHEMISTRY			OK	OK	OK	OK	OK	OK	OK	OK			
TIME OF RENEWAL			11:30	15:40	10:13	14:35	14:30						
pH (Standard Unit) Initial/Final	Ctrl	8.06	7.95	7.78	8.00	7.97	8.12	7.91	7.85	7.94	7.98	7.58	7.91
	9.1	7.84	7.92	7.95	7.90	8.01	7.85	7.92	7.82	8.02	7.96	7.85	7.79
	18	7.78	7.90	8.01	7.82	8.07	7.79	7.97	7.80	8.08	7.90	7.86	7.90
	36	7.66	7.87	8.15	7.73	8.28	7.73	8.15	7.76	8.22	7.83	8.03	7.83
	72	7.63	7.59	8.33	7.62	8.38	7.64	8.35	7.74	8.39	7.79	8.28	7.87
	100	7.58	7.60	8.42	7.59	8.48	7.60	8.46	7.71	8.47	7.73	8.44	8.32
Dissolved Oxygen (mg/L) Initial/Final	Ctrl	7.7	7.7	7.4	7.4	7.1	7.3	6.9	7.4	7.2	7.7	7.1	6.2
	9.1	7.7	7.5	7.3	7.5	7.1	8.0	6.9	7.5	7.2	7.7	7.7	6.4
	18	7.6	7.3	7.1	7.6	7.2	8.0	6.9	7.5	7.0	7.6	7.2	6.7
	36	7.6	6.9	7.4	7.6	7.1	8.0	6.9	7.5	7.0	7.5	6.9	6.8
	72	7.6	7.0	7.5	7.6	7.0	8.0	6.8	7.6	6.9	7.5	6.9	6.8
	100	7.5	6.8	7.4	7.6	7.0	8.0	6.9	7.8	6.8	7.3	6.8	6.4
Temperature (°C) Initial/Final	Ctrl	25.8	25.7	24.4	25.7	24.8	25.0	25.1	25.2	25.3	25.3	25.4	25.5
	9.1	25.9	25.8	24.3	25.6	24.4	25.5	24.9	25.4	25.3	25.4	25.8	25.8
	18	25.9	25.8	24.6	25.6	24.5	25.6	24.8	25.5	25.4	25.4	25.6	24.6
	36	25.9	25.6	24.4	25.2	24.6	25.7	24.8	25.6	25.4	25.5	25.6	24.5
	72	25.9	25.6	24.3	24.4	24.9	25.4	24.8	25.6	25.1	25.6	25.5	24.3
	100	25.7	25.5	24.7	24.2	25.0	25.0	25.1	25.5	25.9	25.7	25.4	24.3
Conductivity (umho/cm) or Salinity (ppt)	Ctrl	290	286		296		289		290		299		
	9.1	434	436		439		429		447		439		
	18	571	574		559		579		572		577		
	36	849	854		842		844		846		859		
	72	1344	1360		1343		1355		1356		1364		
	100	1723	1751		1693		1712		1751		1744		
Alkalinity (mg/L CaCO ₃)	Ctrl	60	60		60		57.5		57.5		57.5		57.5
	100	180											
Hardness (mg/L CaCO ₃)	Ctrl	92	92		92		84		82		82		82
	100	136											
Residual Chlorine	Amount Detected mg/L	100	2.02										
	Amount of Na ₂ S ₂ O ₃ Added (ml)												

Client Name: Georgia Pacific Pond 4 + 12 mg/L Al Set-up By: AMO Terminated By: AK/10
 Date / Time Set-up: 2/25/10 1:15:15 Test Species: C. dubia Source: ABL Age: 24hrs
 Date / Time Ended: 3/3/10 1:16:10 Dilution Water: Jug No. C.MOD. HARD
 Repetitions: 10 Animals per Rep.: 1 Organisms per Concentration: 10 Stock Source/Temp.: 24.7°C

COMMENTS: All Temperatures are taken with Thermometer #5. Circled days indicate use of new samples. Randomization produced by Excel Macro.
 Empirical Laboratores Log No.(s): 100 3019-02

Client Name: G.P. Pond 4 + 12mg/LA1
 Date: 2/25/10

DATA FOR CERIODAPHНИЯ DUBIA 7 DAY TEST

REPLICATES

EFF. CONC	DAY OF TEST NO.											TOTAL LIVE YOUNG	NO. LIVE ADULTS	
		1	2	3	4	5	6	7	8	9	10			
Ctrl per 200ml	1	L											0	10
	2 DK	L											0	10
	3 DK	L			2	4	L	2	6	4	L	18	10	
	4 18	4	3	2	6	L	6	4	6	6	5	42	10	
	5 18	9	L	L			11	11	L	L	L	31	10	
	6													
	6 1/2 J10	20	14	30	36	40	17	31	32	38	20	278	10	
Totals	33	17	32	44	55	34	37	44	48	25	369	10		
9% (18ml)	1	L										0	10	
	2	L										0	10	
	3	L					2	L			2	10		
	4	6	6	7	4	5	8	4	8	4	21	56	10	
	5	18	10	4	10	L	12	9	L	9	8	62	10	
	6													
	7	10	37	17	29	30	29	27	14	34	4	231	10	
Totals	28	53	28	43	35	39	40	22	47	16	351	10		
18% (36ml)	1	L										0	10	
	2	L										0	10	
	3	L					3				3	10		
	4	5	4	4	2	L	5	4	3	6	4	37	10	
	5	8	9	8	L	5	L	L	L	L	L	30	10	
	6													
	7	19	17	28	9	L	33	4	34	22	27	193	10	
Totals	32	30	40	11	5	41	8	37	28	31	263	10		
36% (72ml)	1	L										0	10	
	2	L										0	10	
	3	3	L	L			2	4	L	L	9	10		
	4	4	3	5	7	6	8	8	L	4	6	51	10	
	5	L	L	11	8	L	L	L	L	6	L	25	10	
	6													
	7	8	26	12	11	6	7	9	2	12	4	97	10	
Totals	15	29	28	24	12	15	19	4	22	10	182	10		

Empirical Laboratories Log No. (s): 100 3019-02

QA'd by: Amst

100

% Survival

36.9

Mean Reproduction

7

of Third Broods

Client Name: 6 P. Pond 4 + 12 mg/L
 Date: 2/25/10

DATA FOR CERIODAPHNIA DUBIA 7 DAY TEST

REPLICATES

EFF. CONC	DAY OF TEST NO.	REPLICATES										TOTAL LIVE YOUNG	NO. LIVE ADULTS	
		1	2	3	4	5	6	7	8	9	10			
72% (144ml)	1/10	L										0	10	
	2/10	L										0	10	
	3/10	L	L	L	2	L						2	10	
	4/10	L	5	3	L	L	4	3	D	3	6	24	9	
	5/10	L	L							1	4	4	8	9
	6											55		
	7/10	5	3	2	7	9	6	4	L	12	7	55	9	
	Totals	5	8	5	9	9	10	7	0	19	17	89	9	
100%	1	L										0	10	
	2	L										0	10	
	3	L	L	2	L	L	2	L	L	5	L	9	10	
	4	3	6	5	5	L	2	3	L	L	11	24	9	
	5	L	8	L	3	L						11	9	
	6													
	7	L	3	L	4	5	2	5	L	L		19	9	
	Totals	3	17	7	12	5	6	8	0	5	0	63	9	
	1													
	2													
	3													
	4													
	5													
	6													
	7													
	Totals													
	1													
	2													
	3													
	4													
	5													
	6													
	7													
	Totals													

Empirical Laboratories Log No. (s): 100 3019 -02

QA'd by: [Signature]

_____ % Survival _____ Mean Reproduction _____ # of Third Broods

APPENDIX C
STATISTICAL ANALYSES

Georgia Pacific Pond 4 + 6mg/L Al-Reproduction

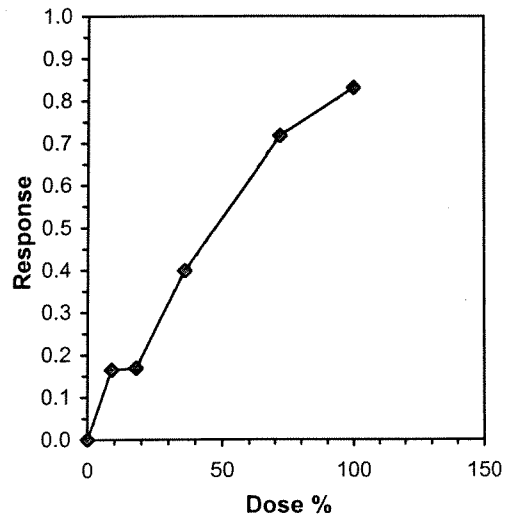
Start Date: 2/25/2010 15:00 Test ID: GP6al Sample ID: XX9999999-NPDES Permit #
 End Date: 3/3/2010 17:00 Lab ID: -EMPIRICAL Sample Type: EFF2-Industrial
 Sample Date: Protocol: EPAF 94-EPA Freshwater Test Species: CD-Ceriodaphnia dubia
 Comments: Pond 4 with 6 mg/L of Aluminum added

Conc-%	1	2	3	4	5	6	7	8	9	10
D-Control	36.000	38.000	42.000	32.000	32.000	42.000	38.000	34.000	26.000	34.000
9	38.000	29.000	30.000	19.000	36.000	26.000	32.000	19.000	43.000	24.000
18	31.000	33.000	34.000	37.000	27.000	32.000	31.000	29.000	26.000	14.000
36	37.000	32.000	17.000	26.000	22.000	0.000	18.000	24.000	25.000	12.000
72	11.000	12.000	16.000	8.000	17.000	9.000	15.000	9.000	0.000	3.000
100	11.000	4.000	10.000	2.000	8.000	2.000	7.000	7.000	5.000	4.000

Conc-%	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%					Mean	N-Mean
D-Control	35.400	1.0000	35.400	26.000	42.000	13.852	10				35.400	1.0000
9	29.600	0.8362	29.600	19.000	43.000	26.801	10	1.916	2.287	6.921	29.600	0.8362
18	29.400	0.8305	29.400	14.000	37.000	21.464	10	1.982	2.287	6.921	29.400	0.8305
*36	21.300	0.6017	21.300	0.000	37.000	48.843	10	4.659	2.287	6.921	21.300	0.6017
*72	10.000	0.2825	10.000	0.000	17.000	54.772	10	8.392	2.287	6.921	10.000	0.2825
*100	6.000	0.1695	6.000	2.000	11.000	52.116	10	9.714	2.287	6.921	6.000	0.1695

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.81048	1.035	-0.5588	1.58524						
Bartlett's Test indicates equal variances (p = 0.02)	13.5788	15.0863								
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	18	36	25.4558	5.55556	6.92085	0.1955	1385.11	45.8019	1.6E-14	5, 54

Linear Interpolation (200 Resamples)				
Point	%	SD	95% CL	Skew
IC25	24.333	5.550	8.729 34.610	-0.3388



Georgia Pacific Pond 4 + 12mg/L Al-Reproduction

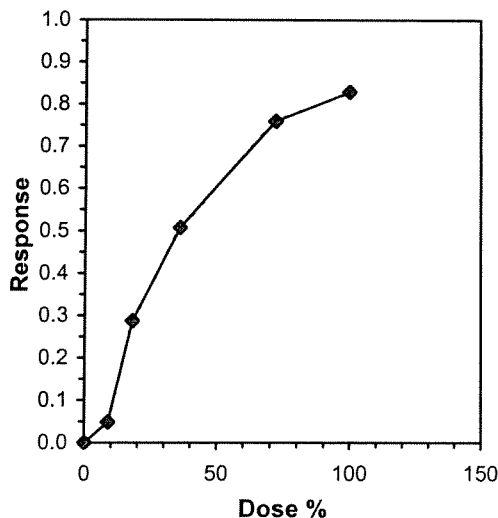
Start Date: 2/25/2010 15:15 Test ID: GP12a1 Sample ID: XX9999999-NPDES Permit #
 End Date: 3/3/2010 16:10 Lab ID: -EMPIRICAL Sample Type: EFF2-Industrial
 Sample Date: Protocol: EPAF 94-EPA Freshwater Test Species: CD-Ceriodaphnia dubia
 Comments: Pond 4 with 12 mg/L of Aluminum added

Conc-%	1	2	3	4	5	6	7	8	9	10
D-Control	33.000	17.000	32.000	44.000	55.000	34.000	37.000	44.000	48.000	25.000
9	28.000	53.000	28.000	43.000	35.000	39.000	40.000	22.000	47.000	16.000
18	32.000	30.000	40.000	11.000	5.000	41.000	8.000	37.000	28.000	31.000
36	15.000	29.000	28.000	26.000	12.000	15.000	19.000	6.000	22.000	10.000
72	5.000	8.000	5.000	9.000	9.000	10.000	7.000	0.000	19.000	17.000
100	3.000	17.000	7.000	12.000	5.000	6.000	8.000	0.000	5.000	0.000

Conc-%	Mean	N-Mean	Transform: Untransformed					N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%	Mean					N-Mean	
D-Control	36.900	1.0000	36.900	17.000	55.000	30.459	10				36.900	1.0000	
9	35.100	0.9512	35.100	16.000	53.000	32.910	10	0.417	2.287	9.880	35.100	0.9512	
*18	26.300	0.7127	26.300	5.000	41.000	50.888	10	2.453	2.287	9.880	26.300	0.7127	
*36	18.200	0.4932	18.200	6.000	29.000	43.480	10	4.328	2.287	9.880	18.200	0.4932	
*72	8.900	0.2412	8.900	0.000	19.000	62.995	10	6.480	2.287	9.880	8.900	0.2412	
*100	6.300	0.1707	6.300	0.000	17.000	82.665	10	7.082	2.287	9.880	6.300	0.1707	

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.54815	1.035	-0.3105	-0.1664						
Bartlett's Test indicates equal variances (p = 0.03)	12.18	15.0863								
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	9	18	12.7279	11.1111	9.88002	0.26775	1689.27	93.3426	1.7E-10	5, 54

Linear Interpolation (200 Resamples)					
Point	%	SD	95% CL	Skew	
IC25	16.594	4.702	8.236	26.210	0.2810

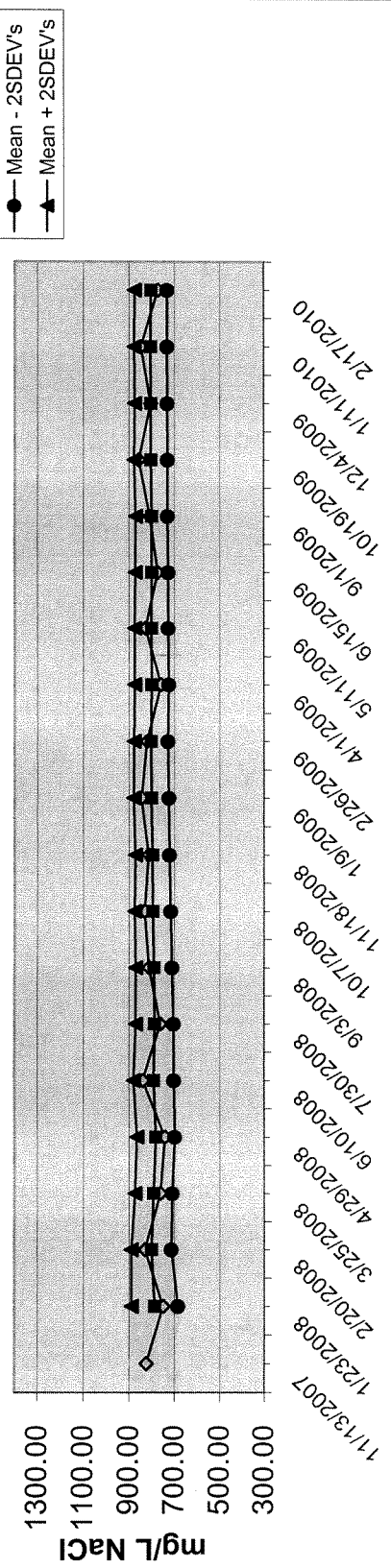


APPENDIX D
REFERENCE TOXICANT DATA

Updated February 17, 2010
 IC25 NaCl Reference Toxicant Tests
 Control Chart for *C. dubia*

Source	Date	IC25	Mean	Standard Deviation	2STDEV's	Mean - 2SDEV's	Mean + 2SDEV's	CV%
Empirical Labs	11/13/2007	822.82						
Empirical Labs	1/23/2008	750.00	786.41	51.49	102.98	683.43	889.39	6.55
Empirical Labs	2/20/2008	829.17	800.66	43.99	87.98	712.68	888.64	5.49
Empirical Labs	3/25/2008	760.71	790.68	41.10	82.20	708.48	872.87	5.20
Empirical Labs	4/29/2008	741.23	780.79	41.90	83.81	696.98	864.59	5.37
Empirical Labs	6/10/2008	838.91	790.47	44.36	88.72	701.75	879.19	5.61
Empirical Labs	7/30/2008	761.81	786.38	41.92	83.84	702.54	870.22	5.33
Empirical Labs	9/3/2008	811.48	789.52	39.81	79.62	709.89	869.14	5.04
Empirical Labs	10/7/2008	830.25	794.04	39.64	79.28	714.77	873.32	4.99
Empirical Labs	11/18/2008	812.93	795.93	37.84	75.69	720.24	871.62	4.75
Empirical Labs	1/9/2009	843.24	800.23	38.63	77.27	722.97	877.50	4.83
Empirical Labs	2/26/2009	819.68	801.85	37.26	74.52	727.33	876.37	4.65
Empirical Labs	4/1/2009	757.43	798.44	37.74	75.48	722.95	873.92	4.73
Empirical Labs	5/11/2009	826.58	800.45	37.03	74.07	726.38	874.51	4.63
Empirical Labs	6/15/2009	772.32	798.57	36.42	72.83	725.74	871.41	4.56
Empirical Labs	9/1/2009	806.82	799.09	35.24	70.49	728.60	869.57	4.41
Empirical Labs	10/19/2009	856.32	802.45	36.84	73.68	728.77	876.13	4.59
Empirical Labs	12/4/2009	802.66	802.46	35.74	71.48	730.99	873.94	4.45
Empirical Labs	1/11/2010	846.57	804.79	36.18	72.35	732.43	877.14	4.50
Empirical Labs	2/17/2010	772.60	803.18	35.94	71.88	731.30	875.05	4.47

C. dubia Chronic Control Chart



APPENDIX E
CHAIN-OF-CUSTODY FORM

