

BIOASSAYS OF SOUTHERN WOOD PIEDMONT

BALDWIN, DUVAL COUNTY, FLORIDA

NPDES #FL0000221

MAY 11, 1988

Biology Section
Division of Environmental Programs

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Biology Section
Bureau of Laboratories and
Special Programs
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EXECUTIVE SUMMARY

Southern Wood Piedmont, Baldwin, NPDES #FL0000221, on 2 to 4 March, 1988.

The sample of discharge from Southern Wood Piedmont was not acutely toxic to the water fleas, Ceriodaphnia dubia, Daphnia pulex, or to the bannerfin shiner, Notropis leedsi. The Microtox® assay, using the bacterium, Photobacterium phosphoreum, was also non-toxic. Although the bioassay results indicated that the sample was non-toxic, previously scheduled Compliance Sampling Toxics (CST) analyses were performed.

INTRODUCTION

The Southern Wood Piedmont Company was a wood treating operation located in Baldwin, Duval County, Florida, approximately 10 miles west of Jacksonville (Figure 1). The plant formerly pressure-treated a minimum of 50,000 cubic feet of wood per week with creosote, pentachlorophenol, and a solution of heavy metals to produce utility poles and crossties protected against weathering and decay. Although an elaborate treatment system with no discharge was used for process wastewater, freshly treeted wood was temporarily stored for drying near the non-contact water effluent ditch (Figure 2). Any significant rainfall may cause contaminated runoff, containing the aforementioned treatment chemicals, to enter the effluent ditch prior to its discharge to Baldwin Bay Swamp, which eventually drains into the St. Mary's River (FDER 1987).

As of 26 June, 1987, the facility was officially closed, and is now in the process of completing a Resource Conservation and Recovery Act closure. Groundwater wells are in place and will continue to be monitored for several years (personal communication, Mark Sittig, FDER NE District, Jacksonville).

The FDER Biology Section has previously performed toxicity tests on effluent from this facility with varing results. The 1979 flow-through assay indicated that the effluent was toxic to the organisms tested. The 1981 static bioassays indicated that the effluent was non-toxic to the test organisms; whereas, the 1982 static bioassays indicated that the effluent was acutely toxic to the test organism Heterandria formosa (least killifish) with some mortality to the other test organism (Daphnia pulex). The results of the 1981 and 1982 bioassays supported the suggestion that rainfall on the post treatment drying area may cause contaminated runoff to enter the facility discharge as there was no rainfall during the 1981 sampling period but significantly heavy rainfall prior to the 1982 sampling period (FDER 1987).

To again evaluate the existence and extent of the toxicity of the discharge from this now inoperative facility to the receiving waters, the Biology Section performed three acute toxicity bioassays and one Microtox® assay on a sample from this facility. The results of these tests are presented in this report. Those involved in performing the tests and in preparation of this report were D. A. Chasteen, J. M. Faircloth, and L. A. Williams.

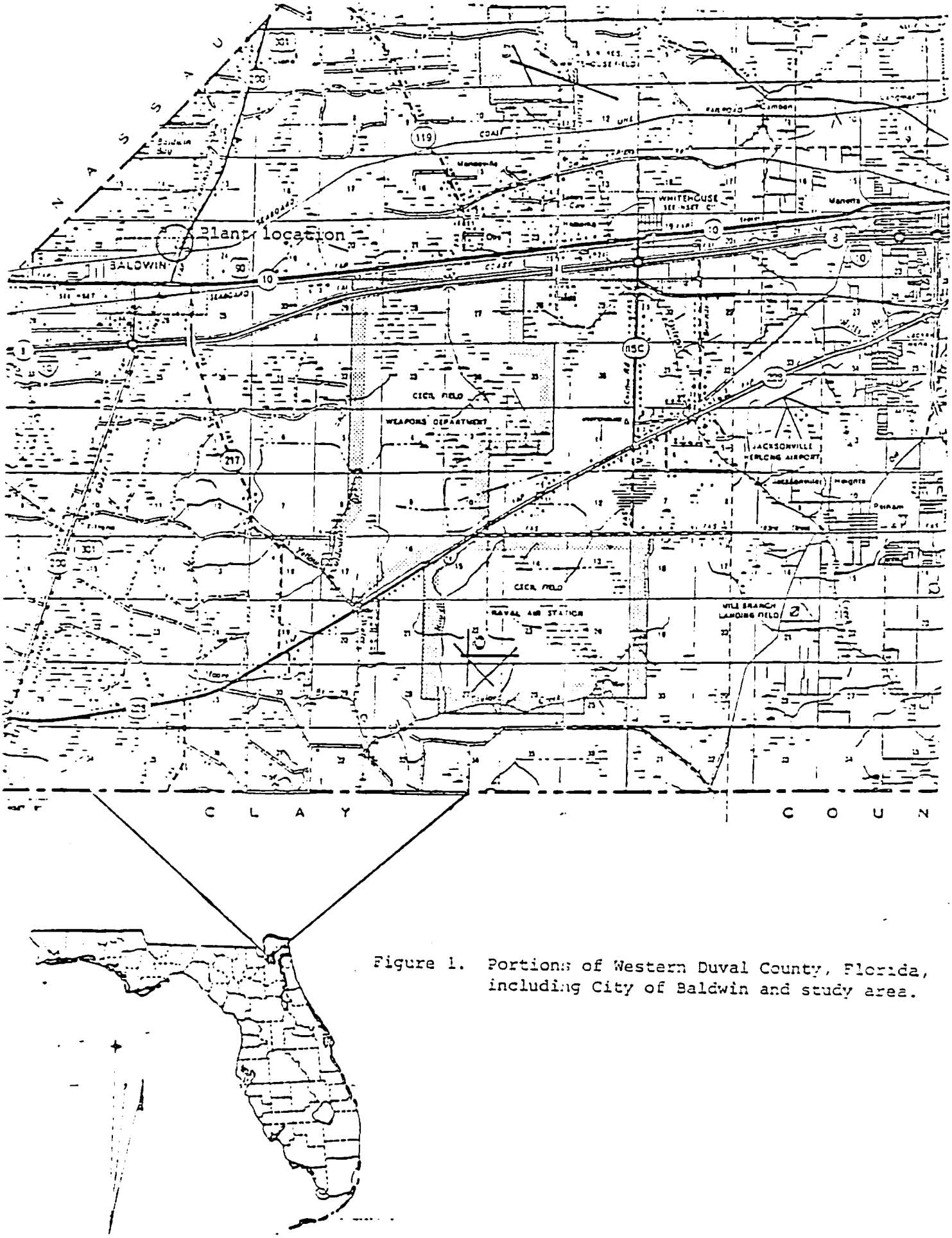
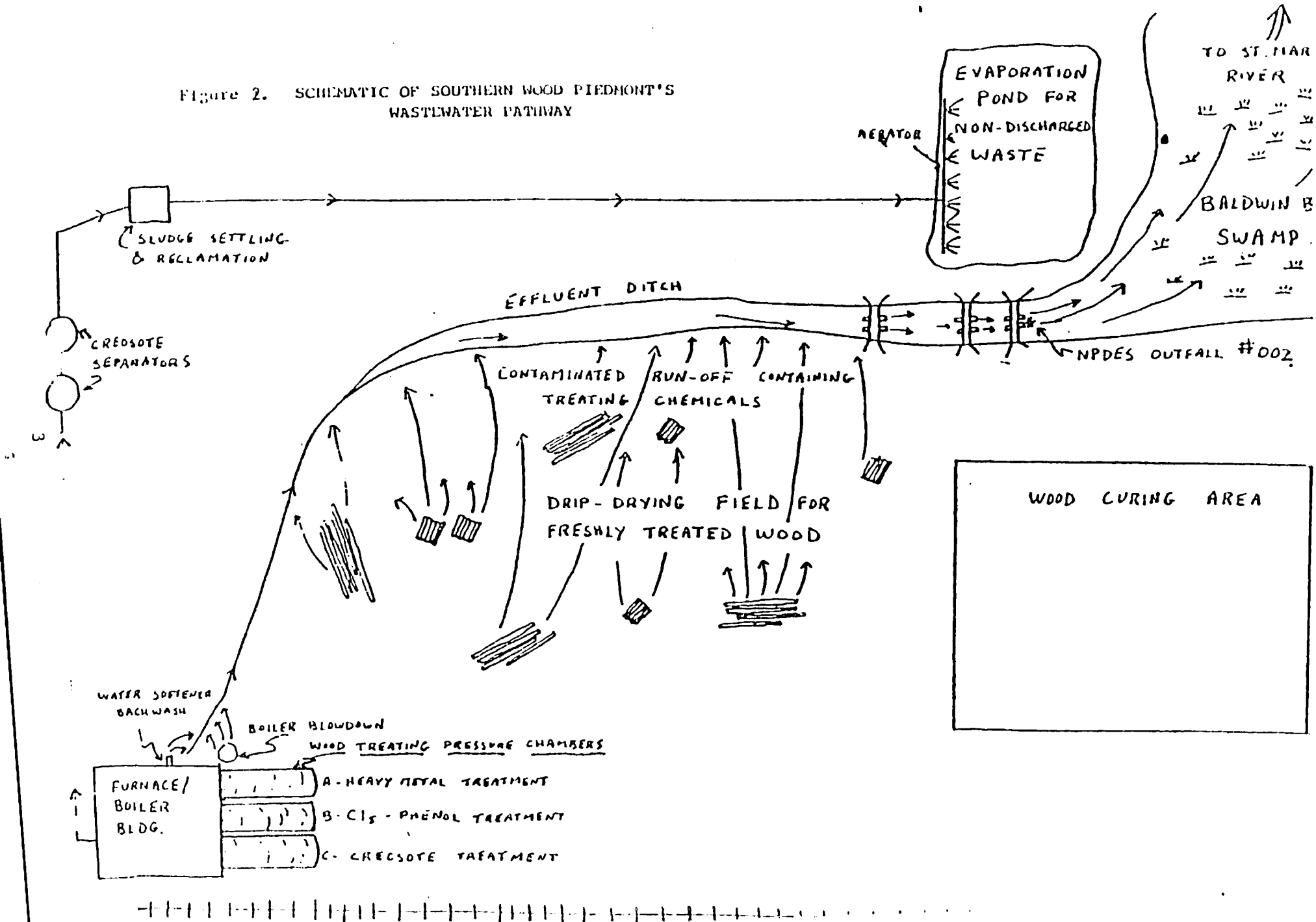


Figure 1. Portions of Western Duval County, Florida, including City of Baldwin and study area.

Figure 2. SCHEMATIC OF SOUTHERN WOOD PIEDMONT'S WASTEWATER PATHWAY



METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier and Weber (1985) and the FDER Biology Section (1985). The organisms selected for this study include a species recommended by Peltier and Weber (1985) and the FDER Biology Section (1985). Additionally, the Biology Section maintains cultures of several species of organisms indigenous to the State of Florida. One of these indigenous species was also selected for testing. Life histories of these test organisms and maintenance procedures used to culture them are detailed below.

The Microtox® assay was run according to the manufacturer's protocol described in the Microtox® System Operating Manual (Beckman 1982).

History and Maintenance of Bioassay Organisms

Daphnia pulex (water flea) - A breeding culture of Daphnia pulex was obtained from the EPA laboratory in Athens, Georgia, in June, 1987. This culture has been maintained since its arrival in 2 1/2 gallon all-glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. Organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, once daily on weekends, and are kept at a constant temperature of 22° ± 2°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms, and then refilling with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances such as: declining populations, ephippia formation, or excessive fungal or bacterial growth.

Ceriodaphnia dubia (water flea) - A breeding culture of Ceriodaphnia dubia was obtained from the EPA laboratory in Athens, Georgia, in June, 1985. The culture is maintained in 2-1/2 gallon all-glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. Organisms are fed a liquified blend of Purina Trout Chow®, Cerophyl® and Fleischmann's® yeast twice daily, once daily on weekends, and are kept at a constant temperature of 26° ± 2°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms, and then refilling with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances such as: declining populations, ephippia formation, or excessive fungal or bacterial growth.

Notropis leedsi (bannerfin shiner) - Approximately 40 adult bannerfin shiners were obtained from an indigenous population in the upper Suwannee River during October, 1981. The breeding stock is maintained in 30 and 125 gallon all-glass aquaria equipped with a flow-through water exchange system. Water for this system is unchlorinated and is pumped from the DER deep well. The bannerfin shiners are reared at 22° ± 2°C under a 16:8 hour light-dark cycle and are fed Tetra-Min® and frozen Artemia (brine shrimp) twice daily, and once daily on weekends. Breeding and egg hatching are encouraged at 25°C. The eggs are laid between stacked red ceramic tiles separated by a 3mm bead of silicone. Most of the eggs

adhere to the tiles and are easily removed to heated, aerated hatching tanks. The narrow space between the tiles eliminates egg predation by adult fish. New hatching tanks are started regularly to segregate fry into even age groups. Newly hatched fry are fed a liquefied blend of Purina Trout Chow® and Cerophyl® twice daily. After one week, feeding is augmented with newly hatched Artemia nauplii and finely crushed Tetra Min®. The fish used in this test were 20 days old at the time of the test.

Photobacterium phosphoreum, a luminescent marine bacterium, is maintained as a freeze-dried culture, purchased from Microbics Corporation. Lot number m-606 was used in this assay. Immediately prior to the assay, the culture is rehydrated with 1.0 ml of specially purified water (Microtox® Reconstitution Solution).

Standard Reference Toxicity Assays

To ensure the uniform sensitivity of these test organisms to toxic substances, the Biology Section conducts monthly standard reference toxicant bioassays using sodium lauryl sulfate as the reference toxicant.

Test Methods

Methods used to perform acute toxicity bioassays are described by Peltier and Weber (1985) and the FDER Biology Section (1985). Some of the methods described below are more specific than those of Peltier and Weber. These will be noted in more detail.

Sampling Methods and Test Dates

Three static acute bioassays and one Microtox® assay were performed on a sample of effluent from Outfall #002 of the Southern Wood Piedmont facility. They were performed from 0930 hours on 2 March to 0930 hour on 4 March, 1988.

Separate samples were obtained for chemical and biological testing. Samples for metal analyses were preserved with HNO_3. Samples for organic chemical analyses and for the bioassays were unpreserved. The samples were collected by Mr. Mark Sittig of the FDER Northeast District Office at 0930 hours on 1 March, 1988, using standard wastewater sampling procedures. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 24 hours of collection.

Test Temperature

Upon arrival of the iced (4°C) bioassay samples in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the samples allowed to rise to 20° $\pm$ 2°C. Although this temperature may be different than the temperature of the sample during collection, it is the temperature range to which the test organisms are acclimatized and is the range recommended in the above referenced bioassay protocols. This avoids stressful effects caused by exposure to unusually cold or warm samples.

Freshwater Tests

Freshwater for dilution of the recovered water samples was obtained from the same DER deep well that is used to culture the test organisms. The concentration series selected for this study is given in Table 1.

Two replicates, of each concentration and a control, were used to test with the water fleas, Ceriodaphnia dubia and Daphnia pulex. Flint glass jars of either 60 ml (for C. dubia) or 250 ml (for D. pulex) capacity were used as test chambers, and either 50 ml or 200 ml of the appropriate test concentration was placed in each chamber. Ceriodaphnia were siphoned into a large culture dish and neonates were transferred by glass pipette, and loaded 10 per chamber, resulting in 20 per test concentration. Daphnia were siphoned into a large culture dish to isolate the proper size and age organisms (neonates). To accomplish this, the Daphnia were siphoned through a stainless steel screen. The trapped adults were then discarded. These neonates were then transferred by glass pipette, examined for injury, and were loaded 10 per chamber, resulting in 20 per test concentration. Water fleas were not fed during these tests.

Two replicates, of each concentration and a control, were used for the Notropis leedsi (bannerfin shiner) bioassay. The fish were transferred individually or by twos, into wide mouth quart jars containing 500 ml each of the appropriate test concentration, and were loaded 5 per chamber, resulting in 10 per concentration. Fish were not fed during this test.

Test Parameters

Dissolved oxygen (YSI Model 57 oxygen meter), pH (Corning Model 7 pH meter with Orion Ross® combination electrode), temperature (NBS calibrated glass thermometer), and the number of live organisms were recorded for each test concentration at 0 hours, 24 hours, and 48 hours. Total alkalinity and total hardness (measured as $CaCO_3$ using HACH Chemical Company reagents) were recorded at the beginning of the test for the controls and the 100% reclaimed water concentration. The specific conductance (YSI Model 33 S-C-T meter) of each test concentration was recorded at the beginning and end of the test. All instruments were calibrated daily according to manufacturer's recommendations. Ammonia concentrations, including the total and unionized fraction (expressed in mg/l as N and as NH_3, respectively), were recorded in the controls and the 100% reclaimed water test concentration at the beginning and end of the test. Total ammonia concentrations were determined using an Orion® specific ion probe and an Orion Ionalyzer®. The unionized fraction was then calculated based on the total ammonia concentration, and the pH, temperature, and salinity of the sample. These values were recorded on the bioassay data forms and are presented in Table 2.

Microtox® Test

Upon receipt at the DER laboratory, a 20 ml subsample was collected in a small beaker. The sample was adjusted to 2% salinity with reagent

grade dry sodium chloride to maintain the osmotic balance of the test bacterium during the assay. Four two fold dilutions were made of the test sample resulting in a test range of 97%, 49.5%, 24.75%, 12.38%, and 0%. An incubation temperature of 15°C was maintained throughout the assay. Ten microliters of bacterial suspension was added to each of the 1.0 ml test dilutions. Light output (luminescence) was read after 5 and 15 minutes incubation, using the Microtox® analyzer and recorded on a Perkin-Elmer strip chart recorder. In the presence of a toxicant, the luminescence of the test organisms will decrease. In the presence of a stimulant, the luminescence will increase. Toxicity is calculated as a function of the light lost in the test sample, normalized to the control, and is reported as the effective concentration at which a 50% reduction, of light output is determined in 15 minutes (EC_{50}). Results are given in Table 3.

RESULTS AND DISCUSSION

The sample from the Southern Wood Piedmont facility was non-toxic (Table 2) to the water fleas, Ceriodaphnia dubia and Daphnia pulex, and the fish Notropis leedsi. The sample was also non-toxic to the Microtox® test organism, Photobacterium phosphoreum (Table 3).

Chemical analyses (Table 4) detected several unidentified components in the pesticide extractables analyses. The metals zinc (177 ug/l) and arsenic (5 ug/l) were detected in the metal analyses.

Zinc concentrations (USEPA 1976) of less than the concentration (177 ug/l) detected in the test sample have been shown to cause mortality in test organisms. Hardness, pH, temperature, and dissolved oxygen are among the factors which can mitigate the toxic effects of zinc to aquatic organisms and it is believed that one or more of these factors prevented the zinc concentration of the sample from exhibiting toxicity to the test organisms.

The three bioassays and the Microtox® assay showed that the sample from the Southern Wood Piedmont facility were not toxic on 1 March, 1988.

Table 1. Concentration Series and Volumes Used for These Static Bioassays.

Ceriodaphnia dubia (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	50.0 ml	--------	50 ml
30.0%	35.0 ml	15.0 ml	50 ml
60.0%	20.0 ml	30.0 ml	50 ml
100.0%	-------	50.0 ml	50 ml

Daphnia pulex (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	200.0 ml	--------	200 ml
30.0%	140.0 ml	60.0 ml	200 ml
60.0%	80.0 ml	120.0 ml	200 ml
100.0%	--------	200.0 ml	200 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Sample	Total Volume
Control	500 ml	--------	500 ml
30.0%	350 ml	150 ml	500 ml
60.0%	200 ml	300 ml	500 ml
100.0%	------	500 ml	500 ml

Table 2. Data recorded during 48 hour, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi, static acute toxicity bioassay of the Southern Wood Piedmont facility discharge, Duval County, NPDES #FL0000221 on 2 to 4 March, 1988.

Facility:	Southern Wood Piedmont			NPDES # FL0000221		Facility Type:	Industry
Location:	Duval County			Contact/District:	M. Sittig		
Sample Collection Date:	3/1/88	Time:	930	Test type: Static Screening			
Test Beginning Date:	3/2/88	Time:	930	# tests:	3	Receiving Water:	ST. Marys River
Test Ending Date:	3/4/88	Time:	930	Chlorination Type:	Non-chlorinated		

Parameter	
Total Residual Cl2:	0
Method:	Hach
By:	DAC
Salinity:	0

Ammonia:	Control Water	100% Sample
Unionized NH3:	0.00	0.00
Total NH3:	0	0

Organism: *Ceriodaphnia dubia* Age/Life stage: Neonates

LC50: Binomial: Moving Average: Probit:

non-toxic

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hour	Live at 24 hr	Live at 48 hr	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	48 hour
Control	10	10	10	8.8	8.9	9.2	8	8.1	7.8	20	19.8	19.9		
Control	10	10	10	8.8	9	9.2	8	8.1	7.8					
30%	10	10	10	8.9	9.2	9.3	7.8	8	7.8	19.9	19.7	19.8		
30%	10	10	10	8.9	9.2	9.3	7.8	8	7.8					
60%	10	10	10	8.9	9.2	9.3	7.6	8	7.8	20	19.6	19.8		
60%	10	10	10	8.9	9.2	9.3	7.6	8	7.8					
100%	10	10	10	8.7	9.1	9.2	7.4	7.9	7.7	20.3	19.7	19.8		
100%	10	10	10	8.7	9.1	9.2	7.4	7.9	7.7					

	Control water	100% sample
Alkalinity:	114	112
Hardness:	121	111

Organism: *Daphnia pulex* Age/Life stage: Neonates

LC50: Binomial: Moving Average: Probit:

non-toxic

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hour	Live at 24 hr	Live at 48 hr	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	48 hour
Control	10	10	10	9	9.3	9.1	8	8.1	7.9	20.1	19.7	19.6		
Control	10	10	10	9	9.3	9.2	8	8.1	7.9				198	215
30%	10	10	10	9	9.3	9.3	7.8	8	7.8	20.3	19.3	19.5		
30%	10	10	10	9	9.3	9.3	7.8	8	7.8				219	222
60%	10	10	10	9	9.4	9.2	7.5	7.9	7.7	20.6	19.2	19.4		
60%	10	10	10	9	9.3	9.2	7.5	7.9	7.7				230	240
100%	10	10	10	8.8	9.3	9.2	7.3	7.7	7.7	20.7	19.4	19.5		
100%	10	10	10	8.8	9.3	9.2	7.3	7.7	7.7				245	252

	Control water	100% sample
Alkalinity:	114	112
Hardness:	121	111

Organism: *Notropis leedsi* Age/Life stage: 20 days old

LC50: Binomial: Moving Average: Probit:

non-toxic

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hour	Live at 24 hr	Live at 48 hr	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	48 hour
Control	5	5	5	8.5	8.7	8.6	7.4	7.8	7.7	20.7	19.3	19.5		
Control	5	5	5	8.5	8.7	8.6	7.4	7.8	7.7				210	220
30%	5	5	5	8.7	8.8	8.6	7.4	7.7	7.6	20.9	19.2	19.3		
30%	5	5	5	8.7	8.7	8.7	7.4	7.7	7.6				222	230
60%	5	5	5	8.7	8.5	8.5	7.3	7.6	7.5	21	19	19.3		
60%	5	5	5	8.7	8.5	8.6	7.3	7.6	7.5				238	239
100%	5	5	5	8.9	8.4	8.3	7.2	7.5	7.4	21	19.3	19.4		
100%	5	5	5	8.9	8.4	8.3	7.2	7.5	7.4				250	255

	Control water	100% sample
Alkalinity:	124	112
Hardness:	125	111

Table 3. Microtox test results , Southern Wood Piedmont , Duval County , Florida NPDES# FL0000221 on 3/2/88.

Microtox Test Culture lot # M-606

Final Reclaimed Water

% Sample	Non-chlorinated	
	5-min	15-min
0	96	57
0	91	54
0	88	53
12.38	90	55
12.38	91	56
12.38	90	57
24.75	94	51
24.75	93	57
24.75	93	56
49.5	90	55
49.5	87	54
49.5	91	56
99	91	56
99	93	58
99	97	60

EC-50 Non-chlorinated (15 minute , 15 degree C) > 100%

EC50 (15 minute, 15 degrees C) is calculated using the Data Reduction Computer Program developed by Microbics Corporation for the Sharp Model EL-5100 (Microbics) Systems Operating Manual, 1982.

Table 4. Chemical constituents present in the water sample from the Southern Wood Piedmont facility, NPDES #FL0000221, on 1 March, 1988.

Organics

Acid Extractables	None detected
Base Neutral Extractables	None detected
Pesticide Extractables	Several unidentified components

Metals

		Class III Standards
Zinc	177 ug/l	30 ug/l
Arsenic	5 ug/l	50 ug/l

LITERATURE CITED

Beckman Instruments, Inc. 1982. Microtox System Operating Manual.

Florida Department of Environmental Regulation, Biology Section. 1985. Quality Assurance Manual for Performing Acute Toxicity Tests (Second Edition). FDER.

_____. 1987. Bioassays of Southern Wood Piedmont, Baldwin, Duval County, Florida, NPDES #FL0000221. FDER. 13 p.

Peltier, W. and C. I. Weber. 1985. Methods for measuring the acute toxicity of effluents to aquatic organisms (Third Edition). Environmental Research Laboratory. Athens, Georgia. EPA-600/4-85-012. 217 p.

U.S. Environmental Protection Agency. 1976. Quality Criteria for Water. U.S. EPA, Washington, D.C. 256 p.

NON TOXIC

BIOASSAY DATA FORM

Industry/Toxicant Weatherwood Piedmont
Address Duval County (Baldwin)
Contact/District NE Florida Siting
Effluent Serial Number EA 002
NPDES Permit Number FL0002771

Test Number: 1 of 3
Test Type: (Static) Renewal, (Screening) Conventional
Other: CST

Sample Collection: Date 3/1/88 Time 0930
Test Beginning: Date 3/2/88 Time 0930
Test Ending: Date 3/9/88 Time 0930
Test Organism Ceriodaphnia dubia
Age/Life Stage neonates

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96					
Control	20	10	10	10			8.8	8.9	9.2			8.0	8.1	7.8			20.0	19.8	19.9							
Control	63	10	10	10			8.8	9.0	9.2			8.0	8.1	7.8												
30%	21	10	10	10			8.9	9.2	9.3			7.8	8.0	7.8			19.9	19.7	19.8							
30%	92	10	10	10			8.9	9.2	9.3			7.8	8.0	7.8												
60%	1	10	10	10			8.9	9.2	9.3			7.6	8.0	7.8			20.0	19.6	19.8							
60%	36	10	10	10			8.9	9.2	9.3			7.6	8.0	7.8												
100%	6	10	10	10			8.7	9.1	9.2			7.4	7.9	7.7			20.3	19.7	19.8							
100%	93	10	10	10			8.7	9.1	9.2			7.4	7.9	7.7												
Initial of Investigator	DAC	DAC	DAC	DAC			UNF	UNF	UNF			UNF	UNF	UNF			UNF	UNF	UNF							

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 114 | 121
100% Sample: 112 | 111
Other:
Measured by: DAC

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: Not recorded
100% Sample: run by DAC
Other:
Hour previous Test 2/81
Control Water: was 0.0
100% Sample: NF
Other:
Measured by: ____

Operator: Carol Clark, Marshall Fairchild

Instrument Calibrations:

	pH Meter:	DO Meter:	Cond. Meter:
0 Hours	7@7 9@4	9.2 @ 20°C	10@10 25@30
24 Hours	7@7 9@4	9.0 @ 21°C	10@10 25@30
48 Hours	7@7 9@4	9.0 @ 21°C	9.5@10 27.5@30
72 Hours			
96 Hours			

Remarks: *1050 = 19°C
1140 = 20.5°C
1230 = 21°C

Total Residual Chlorine:
Field Data: ____ Method: ____
Lab: N.D. Method: Hach
Lab: ____ Method: ____
Dechlorinated:
Dechlorinated by: DAC

Reviewed by: [signature]
Date: 5/11/88

NON-TOXIC

BIOASSAY DATA FORM

Industry/Toxicant: Southern Wood Piedmont
Address: Durant County (Belhaven)
Contact/District: NC Mark Sitlig
Effluent Serial Number: 002
NPDES Permit Number: FP0000221

Test Number: 2 of 3
Test Type: (Static) Renewal, (Screening) Conventional
Other:

Sample Collection: Date 3/1/88 Time 0930
Test Beginning: Date 3/2/88 Time 0930
Test Ending: Date 3/4/88 Time 0930
Test Organism: Daphnia pulex
Age/Life Stage: Neonates

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96	Conductivity 0	24	48	72	96
Control	1	10	10	10			9.0	9.3	9.1			8.0	8.1	7.9			20.1	19.7	19.6							
Control	39	10	10	10			9.0	9.3	9.2			8.0	8.1	7.9								198	–	215		
30%	60	10	10	9			9.0	9.3	9.3			7.8	8.0	7.8			20.3	19.3	19.5							
30%	11A	10	10	10			9.0	9.3	9.3			7.8	8.0	7.8								219	–	222		
60%	27	10	10	10			9.0	9.4	9.2			7.5	7.9	7.7			20.6	19.2	19.4							
60%	50	10	10	10			9.0	9.3	9.2			7.5	7.9	7.7								230	–	240		
100%	119	10	10	10			8.8	9.3	9.2			7.3	7.7	7.7			20.7	19.7	19.5							
100%	61	10	10	10			8.8	9.3	9.2			7.3	7.7	7.7								245	–	252		
Initial of Investigator		DAC	DAC	DAC			JMF	JMF	JMF			JMF	JMF	JMF			JMF	JMF	JMF			JMF		JMF		

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 116/121
100% Sample: 112/111
Other:
Measured by: DAC

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: Not
100% Sample: recorded
Other:
Measured by DAC
Hour
Control Water: 2/87 Previous Test
100% Sample: NF
Other:
Measured by:

Signature: Randall Fairbrother

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours			
24 Hours			
48 Hours			
72 Hours			
96 Hours			

Remarks:

Total Residual Chlorine:
Field data: _____ Method: _____
Lab: ND Method: Hach
Lab: _____ Method: _____ Reviewed by:
De-chlorinated:
Measurements by: DAC
Date: 5/11/88

NON-TOXIC

BIOASSAY DATA FORM

Industry/Toxicant: Southern Wood Piedmont
Address: Duval County (Baldwin)
Contact/District: NE / Mark Sutley
Effluent Serial Number: 002
NPDES Permit Number: FL0000221

Test Number: 3 of 3
Test Type: (Static) Renewal, (Screening) Conventional
Other:

Sample Collection: Date 3-1-88 Time 0820
Test Beginning: Date 3-2-88 Time 0930
Test Ending: Date 3-4-88 Time 0930
Test Organism: Notropis kedsi
Age/Life Stage: 20 days

Conc. or -%	Test Container Number	Number of Live Organisms					Dissolved Oxygen (mg/l)					pH					Temperature °C					Conductivity				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control	51	5	5	5			8.5	8.7	8.6			7.4	7.8	7.7			20.7	18.3	19.5							
Control	52	5	5	5			8.5	8.7	8.6			7.4	7.8	7.7								210	–	220		
30%	44	5	5	5			8.7	8.8	8.6			7.4	7.7	7.6			21.9	19.2	19.3							
30%	68	5	5	5			8.7	8.7	8.7			7.4	7.7	7.6								222	–	230		
60%	239	5	5	5			8.7	8.5	8.5			7.3	7.6	7.5			21.0	19.0	19.3							
60%	47	5	5	5			8.7	8.5	8.6			7.3	7.6	7.5								238	–	239		
100%	54	5	5	5			8.9	8.4	8.3			7.2	7.5	7.4			21.0	19.3	19.4							
100%	15	5	5	5			8.9	8.4	8.3			7.2	7.5	7.4								250	–	255		
Initial of Investigator	DAC	INF	INF	INF			INF	INF	INF			INF	INF	INF			INF	INF	INF			INF	–	INF		

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 124 / 125
100% Sample: 112 / 111
Other:
Measured by: DAC

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: NOT recorded
100% Sample: now by DAC
Other:
Hour: previous test
Control Water: was 0.0 mg
100% Sample:
Other:
Measured by:

Signature: David Hostetler
Standard Standard

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours			
24 Hours			
48 Hours			
72 Hours			
96 Hours			

Remarks:

Total Residual Chlorine:
Field data: _____ Method: _____
Lab: _____ Method: _____
Lab: _____ Method: _____
Dechlorinated:
Measurements by:

Reviewed by: [signature]
Date: 5/11/88

074

MICROTOX™ Data Reduction Sheet

SOURCE: Southern Wood Piedmont

GRAPH CALC EC 50 (15 MIN, 15 °C) = >100 (%SAMPLE) mg/L stimulatory

SAMPLE DATA:

SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent

DATE ASSAYED: 3-2-88 TIME: 0900

VISUAL COLOR: NONE CORRECTION REQUIRED: YES NO

VISUAL TURBIDITY: NONE SEPARATION REQUIRED: YES NO

INITIAL pH: pH ADJUSTED TO: WITH:

OSMOTICALLY ADJUSTED WITH: MOAS (DRY NaCl) OTHER

PRIMARY DILUTION OF SAMPLE:

OPERATOR: Jezlee A. Willson by fmf and Joseph M. Faircloth REAGENT VIAL LOT NO. M-606

REMARKS:

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	Γ5	Γ15	NOTES
A1	0	96	57	X	X	
B1		91	54			
C1		88	53			
A2	12.38	90	55	.015	-.024	
B2		91	56			
C2		90	57			
A3	24.75	84	31	.019	-.035	
B3		93	57			
C3		93	56			
A4	49.5	90	53	.026	-.006	
B4		87	54			
C4		91	56			
A5	99	91	56	-.021	-.057	
B5		93	58			
C5		97	60			
EC_{50} (MIN, °C)				>100	—	

REVIEWED BY:

DATE: 5/11/88