BIOASSAYS OF ST. REGIS PAPER COMPANY, KRAFT DIVISION,

PENSACOLA MILL

ESCAMBIA COUNTY, FLORIDA

NPDES #FL0002526

Biological Section
Bureau of Water Analysis
April 10, 1981

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INTRODUCTION

The St. Regis Paper Company's Pensacola Mill is located at the intersection of U.S. Highway 29 and State Road 184 in Cantonment, Escambia County (Fig. 1). According to the NPDES Permit (#FL0002526, issued in June 1979). This facility operates continuously, processing 1000 tons per day of pulpwood into paper products. Raw material required for this operation include: pulpwood (pine or hardwood), salt cake, lime, caustic soda, chlorine, salt, sodium chlorate, rosin size, alum, sulfuric acid, and cornstarch.

Figure 2 depicts the water flow through the St. Regis Plant in Cantonment. The following compounds are associated with the paper manufacturing process and may be found in the wastewater stream: pimaric acid, isopimaric acid, sandaracopimaric acid, abietic acid, dehydroabietic acid, 3, 4, 5, trichloroquaiacol, 3, 4, 5, 6, tetrachloroquaiacol, mono-chloro-dehydroabietic acid (2 isomers), 9, 10 epoxystearic acid, hydrogen sulfide, methyl mercaptan, and sodium sulfide. Prior to discharge to the headwaters of Eleven Mile Creek (Fig. 3), the wastewater is treated by a clarifier, a series of mechanically aerated lagoons and final settling in an additional lagoon. This system is designed for a 28 MGD wastewater flow. Eleven Mile Creek eventually empties into the northern portion of Perdido Bay approximately 12 miles downstream of the plant outfall.

On 17 to 19 February 1981, the Biological Section performed three separate static bioassays on effluent samples from the St. Regis Paper Company, Kraft Division, Pensacola Mill to determine the existence and extent of toxicity to the biota of the receiving waters. The results of these tests are reported here.

ST. REGIS PAPER CO.

Pensacola Mill Site

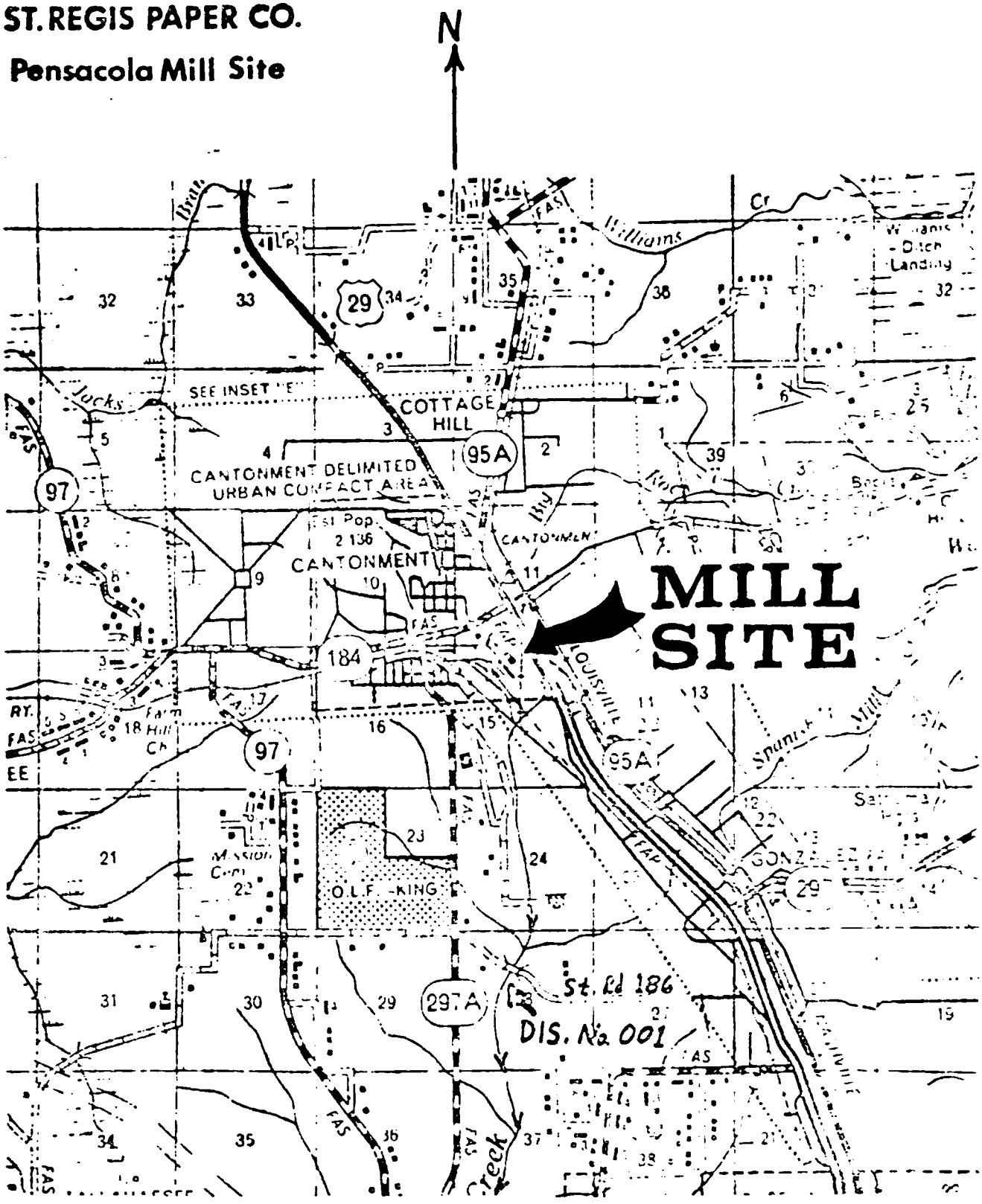
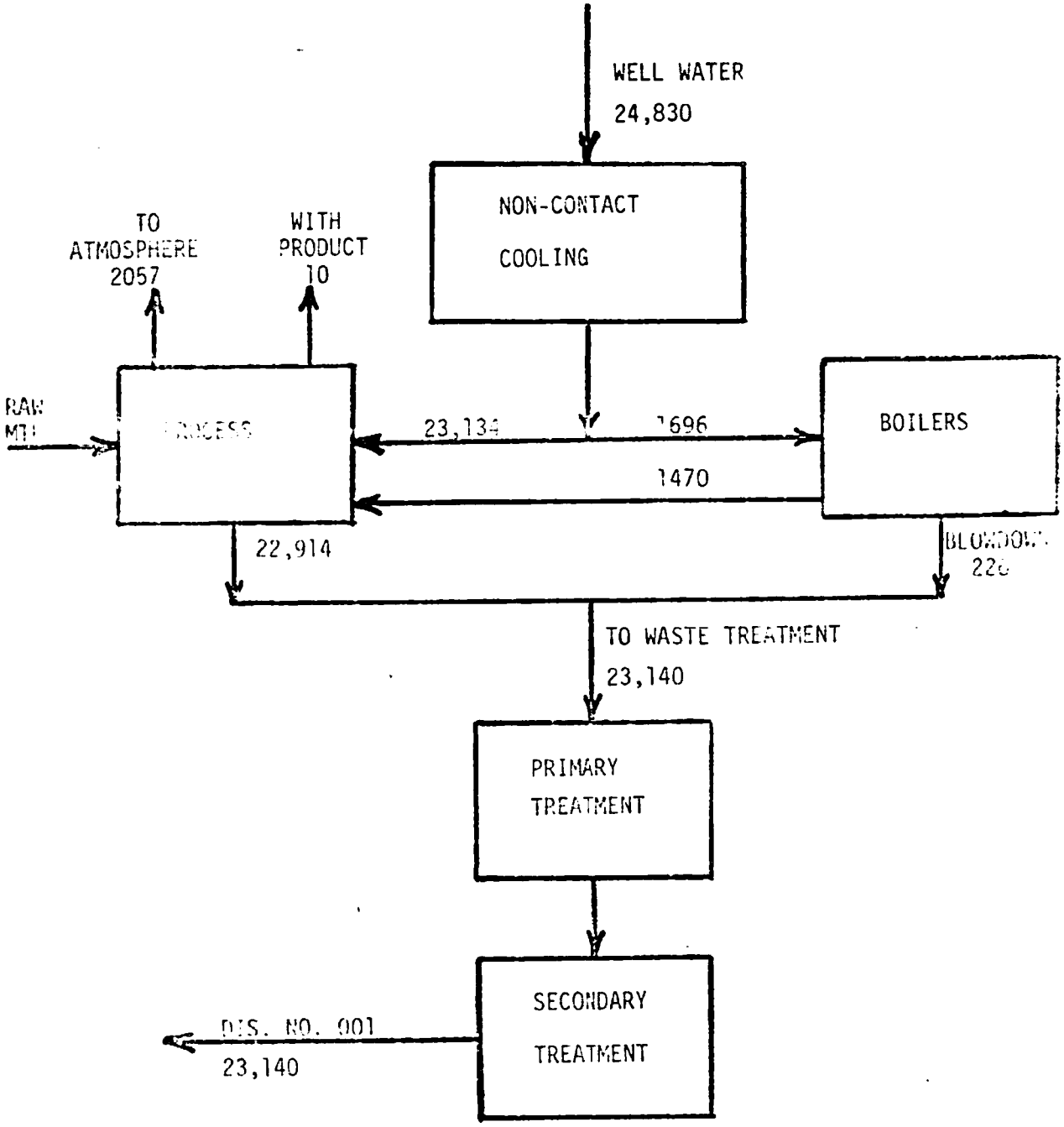


Figure 1. LOCATION MAP
FROM AREA MAP ESCAMBIA COUNTY FLORIDA
ST. REGIS PAPER COMPANY
CANTONMENT
ESCAMBIA COUNTY
FLORIDA
JUNE 21, 1978 P. 1 of

SCALE 1" = 1 MILE



FLOW UNITS - 1000 GAL/DAY

Figure 2. SCHEMATIC OF WATER FLO
ST. REGIS PAPER COMPA
CANTONMENT
ESCAMBIA COUNTY
FLORIDA
JUNE 20, 1978 P. 1 OF 1

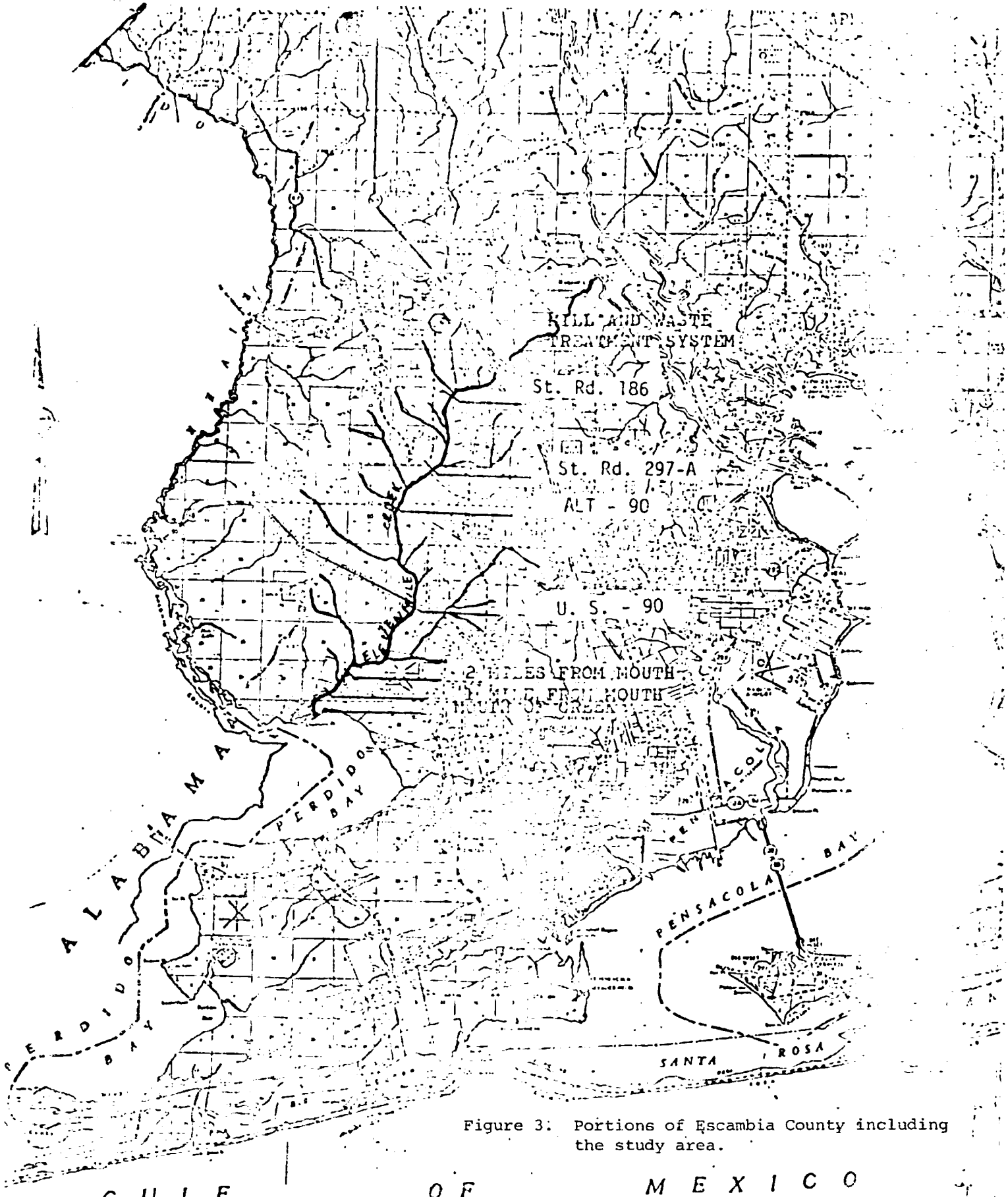


Figure 3: Portions of Escambia County including the study area.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978). The organisms selected for this study include species recommended by Peltier (1978) and the U.S. EPA (1978). Additionally, the Biological Section maintains cultures of several species of organisms indigenous to the State of Florida. Life histories of these test organisms and maintenance procedures used to culture them are detailed below.

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 March, 1979. This culture has been maintained since its arrival, in 2½ gallon all glass aquaria filled with unchlorinated DER well water, using a 16:8 hour light-dark cycle. Organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, and are kept at a constant temperature of $22^{\circ} \pm 1^{\circ}C$. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess daphnids. They are then refilled 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.

Simocephalus serrulatus (water flea) - An indigenous breeding population of S. serrulatus was collected from Lake Jackson, Leon County, Florida, in March, 1980, by personnel from DER's Biological Section. Positive identification of the organism was performed by Mr. Jeff Foran of the Department of Environmental Engineering Sciences, University of Florida in Gainesville. The cultures are maintained in 2½ gallon all glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. The organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, and are kept at a constant temperature of $22^{\circ} \pm 1^{\circ}C$. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess population. They are then refilled with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances.

Pimephales promelas (fathead minnows) - A breeding culture of approximately 100 adult fathead minnows was obtained from the EPA laboratory in Cincinnati, Ohio in May, 1979. The fish have been maintained in four 125 gallon all glass aquaria equipped with a flow through water exchange system using unchlorinated well water obtained from a deep well near the DER laboratory. The fish have been reared at a temperature of $22^{\circ} \pm 2^{\circ}C$ under a 16:8 hour light-dark cycle. These fish are fed Glencoe Mills Trout Chow® and frozen Artemia (brine shrimp) at a rate of 0.75 g/30 fish, twice daily. Breeding has been encouraged by placing several halved clay pots in each tank to serve as substrates for eggs and to provide cover for the fish. Tanks are examined twice daily (at feeding time) for egg production. Clay pots containing egg clusters are immediately transferred to 15 gallon aerated aquaria for hatching. A new aquarium is prepared for each week's production of eggs, thereby segregating fish into even age groups to be used for testing. After hatching, fathead minnow fry are fed twice daily with freshly hatched Artemia. The fish used in this test were all two weeks old at the time of the test.

To ensure the uniform sensitivity of these test organisms to toxic substances, the Biological Section conducts monthly Standard Reference Toxicant Bioassays using sodium lauryl sulfate as the reference toxicant.

Test Methods

Three separate 48 hour static bioassays were conducted from 1000 hours on 17 February to 1000 hours on 19 February, 1981, on a composite effluent sample from outfall #001. The sample was composited between 1100 hours on 15 February and 1100 hours on 16 February 1981. The samples were collected at 1100 hours on 16 February by Mr. Rick Bradburn of DER's Northwest District Office using standard wastewater sampling procedures. Separate samples were collected for chemical and biological testing. Samples for metal analysis were preserved with HNO_3. Samples for organic chemical analysis and for the bioassays were unpreserved. The samples were iced and transported to the DER laboratory in Tallahassee within 24 hours of collection.

Freshwater for dilution of the effluent sample was obtained from the same DER well that is used to culture the test organisms. The concentration series selected for this study (Table 1) is that recommended by APHA (1976).

Two replicates, each with 6 concentrations and a control, were used to test with Daphnia pulex and Simocephalus serrulatus. Flint glass jars of 250 ml capacity were used as test chambers, and 200 ml of the appropriate test concentration was placed in each chamber. Since numerous indigenous cladocerans were observed in the effluent sample, each test concentration was strained through a #20 mesh nylon plankton net to avoid confusing these organisms with the bioassay test species. The Daphnia and Simocephalus were carefully siphoned into respective culture dishes for observation. Individuals were then transferred by glass pipette, examined for injury and were loaded 10 per chamber, resulting in 20 per test concentration. Because of the dark color of this effluent sample, large mature water fleas were selected to making counting easier.

Two replicates, of six concentrations and a control, were used for the fathead minnow bioassay. The fish were transferred individually or by twos, by net, into wide mouth quart jars containing 500 ml each of the appropriate test concentration. Fathead minnows were loaded 5 per chamber resulting in 10 per concentration.

Dissolved oxygen, pH, temperature, and the number of live organisms were recorded for each test chamber at 0 hours, 24 hours, and 48 hours. Total alkalinity, total hardness, and conductivity were recorded at the beginning of the test for the controls and the 100% effluent concentration. These values were recorded on the bioassay data forms and are presented in Tables 2, 3, and 4. Organisms were not fed during these tests. Total and unionized ammonia levels were also monitored using an Orion specific ion electrode. Since these concentrations remained negligible throughout the test they are not reported here.

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

Water fleas (Daphnia pulex and Simocephalus serrulatus)

Concentration	Dilution Water	Effluent	Total Vol.
Control	200 ml	---	200 ml
5.6%	188.8 ml	11.2 ml	200 ml
10.0%	180 ml	20 ml	200 ml
18.0%	164 ml	36 ml	200 ml
32.0%	136 ml	64 ml	200 ml
56.0%	88 ml	112 ml	200 ml
100%	----	200 ml	200 ml

Fathead minnows (Pimephales promelas)

Concentration	Dilution Water	Effluent	Total Vol.
Control	500 ml	---	500 ml
5.6%	472 ml	28 ml	500 ml
10.0%	450 ml	50 ml	500 ml
18.0%	410 ml	90 ml	500 ml
32.0%	340 ml	160 ml	500 ml
56.0%	220 ml	280 ml	500 ml
100%	---	500 ml	500 ml

Table 2. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of St. Regis Paper Company, Kraft Division, Pensacola Mill, Escambia County, Florida, NPDES Permit # FL0002526, Outfall #001, on 17 February to 19 February 1981.

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Conductivity (μmhos)
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0
Control #111	10	10	9	8.9	8.7	8.8	7.8	8.3	8.1	111		111		21.6	21.1	21.0			209
Control #89	10	10	10	8.9	8.7	8.9	7.8	8.3	8.1										
5.6% #41	10	10[a]	10[a]	8.8	8.7	9.0	7.9	8.2	8.2					21.4	20.9	21.0			249
5.6% #86	10	10[a]	10[a]	8.9	8.7	9.0	7.9	8.2	8.2										
10.0% #95	10	10[a]	10[a]	9.0	8.5	8.9	7.9	8.2	8.2					21.2	20.8	21.0			280
10.0% #55	10	10[a]	10[a]	9.0	8.3	8.8	7.9	8.2	8.2										
18.0% #19	10	10[a]	10[a]	8.9	9.3	8.5	7.9	8.1	8.1					21.1	21.2	21.0			333
18.0% #52	10	10	10[a]	8.8	8.2	8.5	7.9	8.1	8.1										
32.0% #2	10	10[a]	10[a]	8.9	7.7	8.3	7.9	8.1	8.0					21.0	21.1	20.9			437
32.0% #143	10	10[a]	10[a]	8.9	7.7	8.3	7.9	8.1	8.0										
56.0% #79	10	10[a]	10[a]	8.7	7.3	7.7	7.9	8.0	8.0					20.7	21.1	21.2			640
56.0% #53	10	10[a]	10[a]	8.7	7.2	7.8	7.9	8.0	8.0										
100% #116	10	10[a]	10[a]	8.5	6.6	7.1	7.8	7.9	7.9					20.4	21.9	21.0			970
100% #118	10	10[a]	10[a]	8.5	6.4	7.1	7.8	7.9	7.9	156		114							

Remarks: [a] More than 10 organisms observed in the test concentration
(Since an indigenous population of cladocerans was observed in the effluent sample only the larger organisms were counted).

Table 3. Data recorded during 48 hour, Simocephalus serrulatus, static acute toxicity bioassay of St. Regis Paper Company, Kraft Division, Pensacola Mill, Escambia County, Florida, NPDES Permit #FL0002526, Outfall #001, on 17 February to 19 February 1981.

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Conductivity (μmhos)
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0
Control #96	10	10	9	8.5	9.2	9.1	7.8	8.3	8.2	111		111		21.5	21.5	20.7			
Control #97	10	10	10	8.5	9.2	9.0	7.9	8.3	8.2										210
5.6% #59	10	10[a]	10[a]	8.4	9.1	9.0	7.9	8.2	8.2					21.3	21.3	20.8			
5.6% #48	10	10[a]	10[a]	8.4	9.1	8.9	7.9	8.2	8.2										250
10.0% #82	10	10	10	8.4	9.0	8.8	7.9	8.2	8.2					21.3	21.0	20.9			
10.0% #35	10	10	10	8.4	9.0	8.7	7.9	8.2	8.2										285
18.0% #29	10	10[a]	10[a]	8.4	8.7	8.7	7.9	8.1	8.1					21.2	21.0	20.9			
18.0% #91	10	10	10	8.3	8.8	8.5	7.9	8.1	8.1										340
32.0% #125	10	10	10	8.2	8.5	8.4	7.9	8.1	8.1					21.0	20.9	20.9			
32.0% #132	10	10[a]	10[a]	8.2	8.5	8.4	7.9	8.1	8.0										430
56.0% #5	10	10[a]	10[a]	8.2	7.9	7.9	7.9	8.0	8.0					20.6	21.7	21.0			
56.0% #112	10	10[a]	10[a]	8.2	7.9	7.8	7.9	8.0	8.0										660
100% #137	10	10	10	8.0	7.1	7.1	7.8	7.9	7.9					20.4	21.5	21.6			
100% #133	10	10[a]	10[a]	8.0	7.1	7.0	7.8	7.9	7.9	156		114							980

Remarks: [a] More than 10 organisms observed in the test concentration (since an indigneous population of cladocerans was observed in the effluent sample, only the larger organisms were counted).

Table 4. Data recorded during 48 hour, Pimephales promelas, static acute toxicity bioassay of St. Regis Paper Company, Kraft Division, Pensacola Mill, Escambia County, Florida, NPDES Permit #FL0002526, Outfall #001, on 17 February to 19 February 1981.

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Conductivity (μmhos)
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0
Control #34	6[a]	6	6	8.6	8.0	7.6	7.8	8.1	8.1	111		111		21.4	21.2	20.9			
Control #35	5	5	5	8.4	8.1	7.9	7.9	8.1	8.1										210
5.6% #29	5	5	5	8.4	7.9	7.9	7.9	8.1	8.1					21.2	21.2	20.9			
5.6% #22	5	5	5	8.3	7.9	7.8	7.9	8.1	8.1										250
10.0% #59	5	5	5	8.3	7.7	7.5	7.9	8.1	8.1					21.1	21.1	20.9			
10.0% #16	5	5	5	8.3	7.7	7.4	7.9	8.1	8.1										285
18.0% #52	5	5	5	8.3	7.6	7.1	7.9	8.0	8.0					21.0	21.1	20.9			
18.0% #45	5	5	5	8.3	7.5	7.1	7.9	8.0	8.0										340
32.0% #17	5	5	5	8.2	7.3	7.1	7.9	8.0	8.0					20.9	21.2	20.9			
32.0% #39	6[a]	6	6	8.2	7.3	7.1	7.9	8.0	8.0										430
56.0% #58	5	5	5	8.1	6.8	6.4	7.9	8.0	7.9					20.8	21.2	21.0			
56.0% #10	5	5	5	8.1	6.8	6.4	7.9	7.9	7.9										660
100% #30	5	5	5	8.0	5.4	5.6	7.8	7.9	7.8					20.6	21.8	21.3			
100% #3	5	5	5	8.0	5.8	5.5	7.8	7.9	7.8	156		114							980

Remarks: Fathead minnows used in this test were approximately two weeks old and weighed an average of 0.0051 g each.

a. Since it is difficult to remove extra organisms without disturbing the test, extra healthy fish were allowed to remain in the containers for the duration of this test.

RESULTS AND DISCUSSION

This particular composite sample from St. Regis Paper Company, Pensacola Mill, was not toxic to any of the organisms tested (Daphnia pulex, Simocephalus serrulatus and Pimephales promelas). Extensive chemical characterization of this composite effluent sample is presented in Table 5. Chromium (57 ug/l), mercury (0.3 ug/l), and zinc (69 ug/l) were detected in the sample. These metals were not toxic to the organisms tested under the chemical and physical conditions of the bioassays.

Table 5. Results of Chemical Analyses Performed on Composite Effluent Samples Collected on 16 February 1981, from the St. Regis Paper Company's Pensacola Mill.

Parameter	Result (u/gl)
Organics	
Pesticides	Not Detected
Base/Neutral Extractables	Not Detected
Acid Extractables	Not Detected
Metals	
Antimony	200 k
Arsenic	50 k
Beryllium	10 k
Cadmium	10 k
Chromium	57
Copper	50 k
Lead	50 k
Mercury	0.3
Nickel	50 k
Selenium	50 k
Silver	10 k
Tellurium	100 k
Zinc	69

k - actual value is known to be less than value given.

LITERATURE CITED

American Public Health Association. 1976. Standard Methods for the Examination of Water and Wastewater. 14th ed. pp. 1193.

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