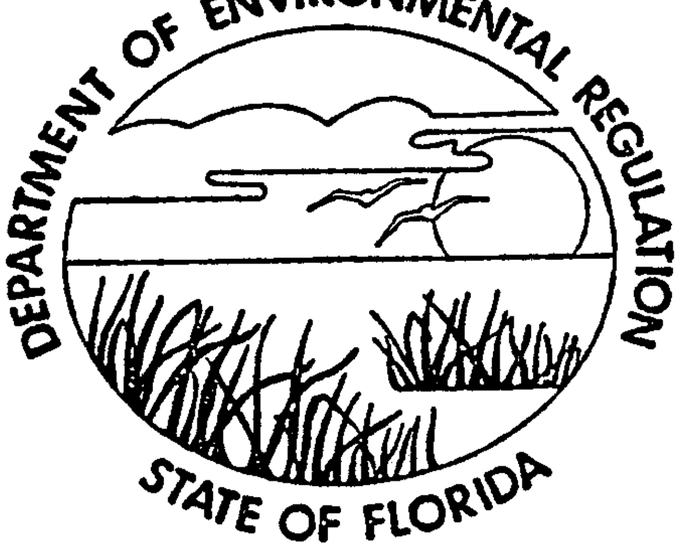


BIOASSAYS OF THE GEORGIA-PACIFIC CORPORATION

PALATKA, PUTNAM COUNTY, FLORIDA

NPDES #FL0002763

SAMPLED 4/27/87

MAY 22, 1987

Biology Section
Division of Environmental Programs

BIOASSAYS OF THE GEORGIA-PACIFIC CORPORATION

PALATKA, PUTNAM COUNTY, FLORIDA

NPDES #FL0002763

Biology Section
Bureau of Laboratories and
Special Programs
May 22, 1987

EXECUTIVE SUMMARY

Georgia-Pacific Corporation, Palatka, Putnam County, NPDES #FL0002763, 28 to 30 April, 1987.

The Biology Section performed three static acute toxicity bioassays and one Microtox® assay on a sample of effluent collected from Georgia-Pacific Pulp and Paper Mill. The sample was not toxic to the bioassay test organisms, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi. No mortality occurred during the 48 hour test. The total and unionized ammonia (2.9 mg/l and 0.08 mg/l, respectively) were insufficient to have caused any toxicity.

Likewise, the Microtox® bacterial toxicity assay indicated that the discharge was non-toxic, with an EC_{50} (15 minutes, 15°C) > 100% effluent. Samples for chemical analysis were not collected.

INTRODUCTION

The Georgia-Pacific Pulp and Paper Mill, formerly Hudson Pulp and Paper, is located on State Road 216, approximately 3 miles north of Palatka, Putnam County, Florida (Figure 1). The mill produces approximately 950 tons of paper products per day using an unbleached Kraft and bleached pulp process. Effluent of from 37 to 50 MGD is discharged, with approximately one half of this consisting of process wastewater. Treatment prior to discharge includes primary clarification and 40 days retention in four oxidation ponds. The effluent is aerated in the last pond using several mechanical aerators. Discharge is to Rice Creek, a tributary of the St. Johns River. A schematic of the mill and treatment system is presented in Figure 2.

In 1975 and 1976 the mill was in violation for exceeding its permit limitations for Biochemical Oxygen Demand (BOD) and Total Suspended Solids (TSS). It has also had chronic problems maintaining acceptable levels of dissolved oxygen in the receiving waters (Rice Creek). However, because Rice Creek has a low initial dissolved oxygen concentration, the mill has been granted a variance for this parameter. Additionally, from 5 to 30 MGD groundwater is pumped into Rice Creek to supplement its flow.

The Biological Section had previously performed toxicity bioassays on effluent from this facility, in June, 1981. The results of those tests indicated that the effluent was not toxic to either Daphnia pulex or to Pimephales promelas (FDER 1981).

However, to continue evaluating the effects of this effluent on the biota of the receiving waters, the Biological Section performed additional static acute toxicity bioassays on a grab sample from Outfall #001, on 20 to 22 July, 1982. These tests resulted in no mortality to Daphnia pulex or Heterandria formosa (FDER 1982).

In April, 1987, the Biology Section performed three additional bioassays on an effluent sample from Georgia-Pacific as well as a Microtox® assay. Results are presented in this report. Those involved in performing the tests and in preparation of this report were: J. M. Faircloth, K. M. Lurding, M. L. Roll, and L. A. Williams.

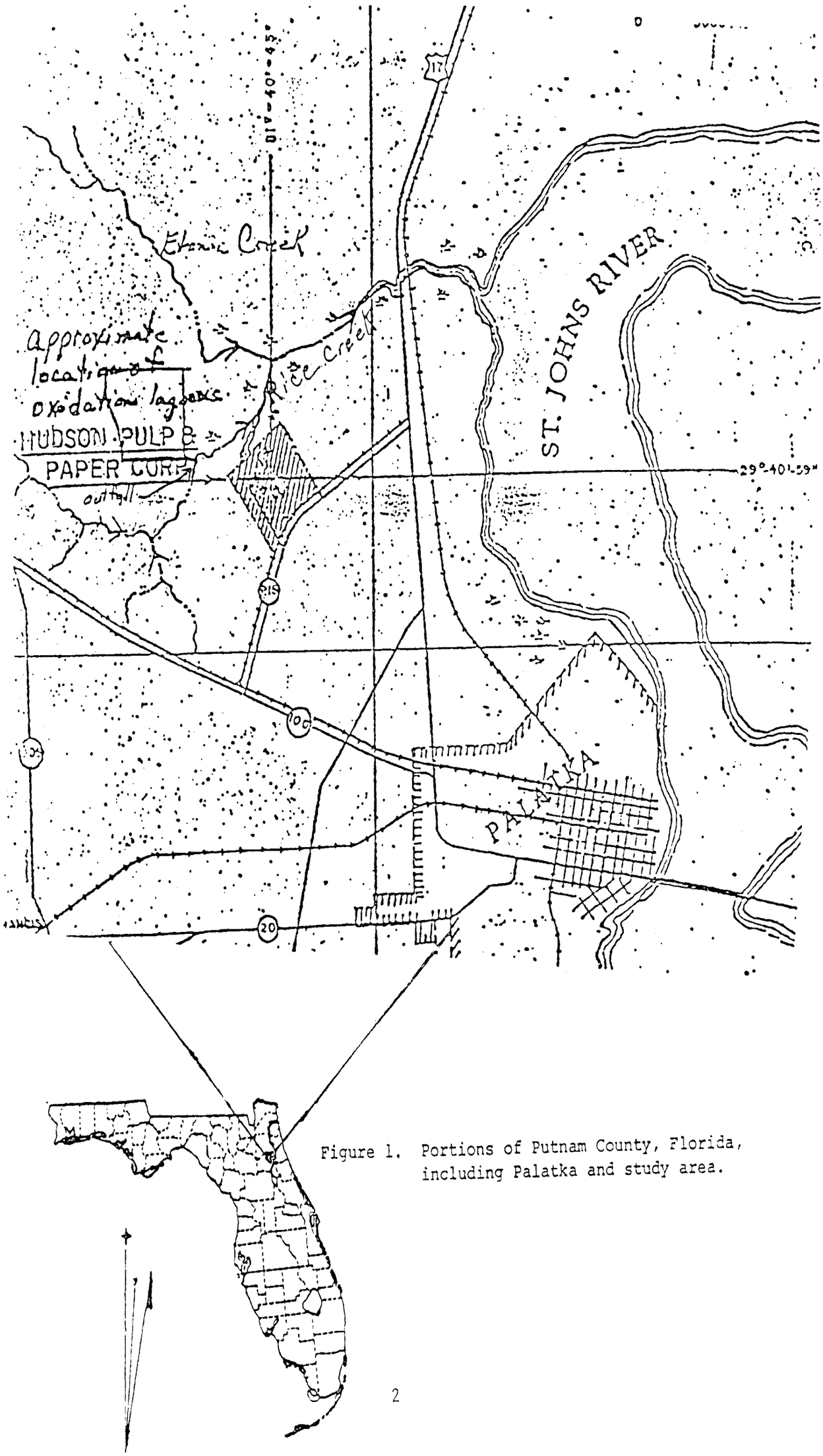
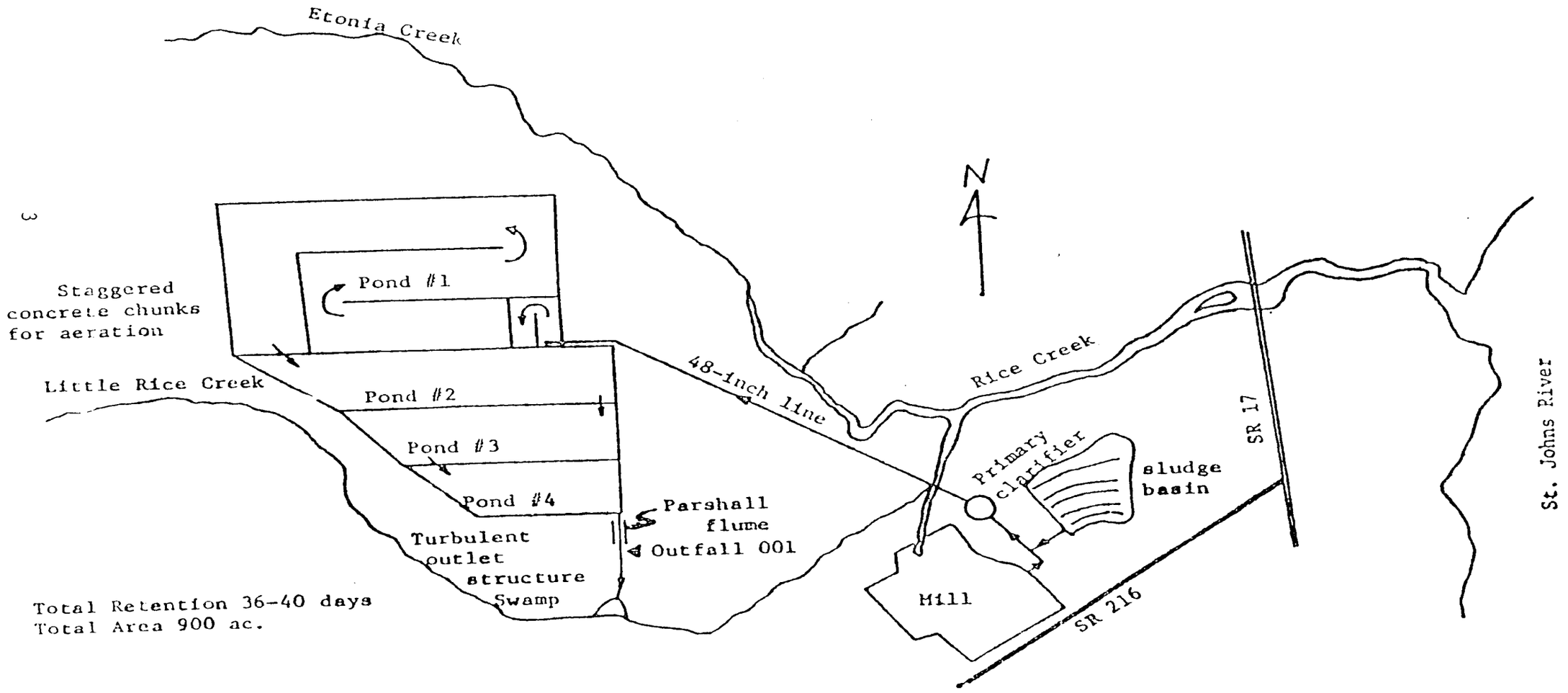


Figure 1. Portions of Putnam County, Florida, including Palatka and study area.

FIGURE 2
Hudson Pulp and Paper Company
Palatka, Florida



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 species recommended by Peltier and Weber (1985), the U.S. EPA (1978), 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.

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 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 18 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 #M603 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).

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 separate 48 hour static acute toxicity bioassays were performed on a sample of effluent collected from the Georgia-Pacific Outfall #001. The tests were performed from 0915 hours on 28 April to 0915 hours on 30 April, 1987.

The sample was collected by Mr. Mark Sittig of DER's Northeast Florida District Office at 1000 hours on 27 April, 1987, using standard wastewater sampling procedures. The sample was 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 sample in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the sample allowed to rise to 20° ± 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 effluent 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 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 test.

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 tests.

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 Microtox® osmotic adjusting solution to maintain the osmotic balance of the test bacterium during the assay. Four two-fold dilutions were made of the test sample resulting in test concentrations of 45%, 22.5%, 11.25%, 5.625 and 0%. An incubation temperature of 15°C was maintained throughout the assay. Ten microliters of bacterial suspension were added to each of the 1.5 ml test dilutions. Light output (luminescence) was read after 0, 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.

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% effluent 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% effluent test concentration at the beginning 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.

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
50.0%	25.0 ml	25.0 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
50.0%	100.0 ml	100.0 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
50.0%	250 ml	250 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 bioassays of Georgia-Pacific, NPDES #FL0002763, 28 to 30 April, 1987.

Concentration %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Ceriodaphnia dubia														
Control	10	10	10	8.5	8.4	8.6	8.5	8.4	8.4	22.0	20.4	21.1		
Control	10	10	10	8.5	8.4	8.6	8.5	8.4	8.5					
50%	10	10	10	8.0	8.3	8.4	8.1	8.4	8.4	21.8	20.3	20.9		
50%	10	10	10	8.0	8.3	8.4	8.1	8.4	8.4					
100%	10	10	10	7.3	8.1	8.3	7.8	8.5	8.5	21.5	20.2	21.0		
100%	10	10	10	7.3	8.1	8.3	7.8	8.5	8.5					
Daphnia pulex														
Control	10	10	10	8.4	8.3	8.4	8.5	8.4	8.4	22.3	20.5	20.7		
Control	10	10	10	8.4	8.3	8.4	8.5	8.4	8.4				217	230
50%	10	10	10	7.9	8.2	8.3	8.0	8.2	8.3	22.2	20.1	20.6		
50%	10	10	10	7.9	8.2	8.3	8.0	8.3	8.3				920	910
100%	10	10	10	7.1	8.0	8.0	7.8	8.3	8.3	21.9	20.1	20.6		
100%	10	10	10	7.1	8.0	8.0	7.8	8.3	8.3				1530	1530
Notropis leedsi														
Control	5	5	5	8.4	8.1	8.1	7.9	8.2	8.2	22.3	20.4	20.7		
Control	5	5	5	8.4	8.1	8.1	7.9	8.2	8.2				215	220
50%	5	5	5	7.8	7.7	8.0	7.8	8.1	8.2	22.1	20.1	20.7		
50%	5	5	5	7.8	7.7	8.0	7.8	8.1	8.2				920	900
100%	5	5	5	7.3	7.3	7.3	7.7	8.1	8.2	22.0	20.1	20.5		
100%	5	5	5	7.3	7.3	7.3	7.7	8.1	8.2				1550	1530

Control Water

NH_3(as NH_3)(mg/l): 0

Total ammonia (as N)(mg/l): 0

Total Alkalinity (mg/l $CaCO_3$): 110

Total Hardness (mg/l $CaCO_3$): 108

100% Effluent

NH_3(as NH_3)(mg/l): 0.08

Total ammonia (as N)(mg/l): 2.9

Total Alkalinity (mg/l $CaCO_3$): 230

Total Hardness (mg/l $CaCO_3$): 160

Table 3. Microtox Test Results.

Microtox Test	Culture Lot #M603		
Final Assay Conc. (%)	Initial Photometer Reading (I_0)	5-min (I_5)	15-min (I_{15})
0	37.5	37.5	28
0	38	38	29
5.625	40.5	31	26.5
5.625	36.5	37	23
11.25	40	33	29
11.25	36	30	25.5
22.5	38	32	28
22.5	39	33	28.5
45	39	33.5	29.5
45	39.5	33	29

$EC_{50} > 100\%$
EC_{50} (15 min, 15°C) calculated using the Data Reduction Computer Program developed by Microbics Corporation for the Sharp Model EL-5100 (Microtox®) Systems Operating Manual, 1982.

RESULTS AND DISCUSSION

The sample of wastewater effluent collected from Georgia-Pacific's Outfall #001 was not toxic to any of the three bioassay test organisms, Ceriodaphnia dubia, Daphnia pulex, or Notropis leedsi. No mortality was noted for the duration of the 48 hour test (Table 2). The total and unionized ammonia (2.9 mg/l and 0.08 mg/l, respectively) were not sufficient to have caused any toxicity.

The Microtox® assay indicated that the pulp and paper mill discharge was non-toxic, with an EC_{50} (15 minutes, 15°C) > 100% (Table 3). Samples for chemical analysis were not collected.

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