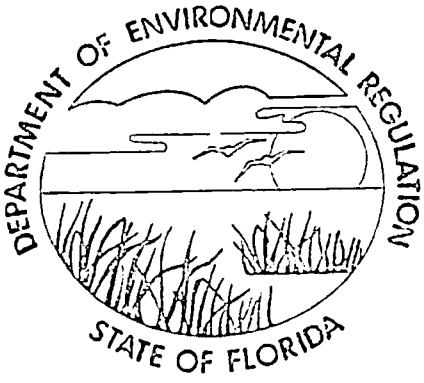


BIOASSAYS OF SEMINOLE KRAFT PAPER COMPANY

JACKSONVILLE, DUVAL COUNTY, FLORIDA

NPDES #FL0000400

APRIL 7, 1988

Biology Section
Division of Environmental Programs

BIOASSAYS OF SEMINOLE KRAFT PAPER COMPANY

JACKSONVILLE, DUVAL COUNTY, FLORIDA

NPDES #FL0000400

Biology Section
Bureau of Laboratories and
Special Programs
April 7, 1988

EXECUTIVE SUMMARY

Seminole Kraft Paper Company, Jacksonville, Duval County, Florida, NPDES #FL0000400, on 6 to 8 January, 1988.

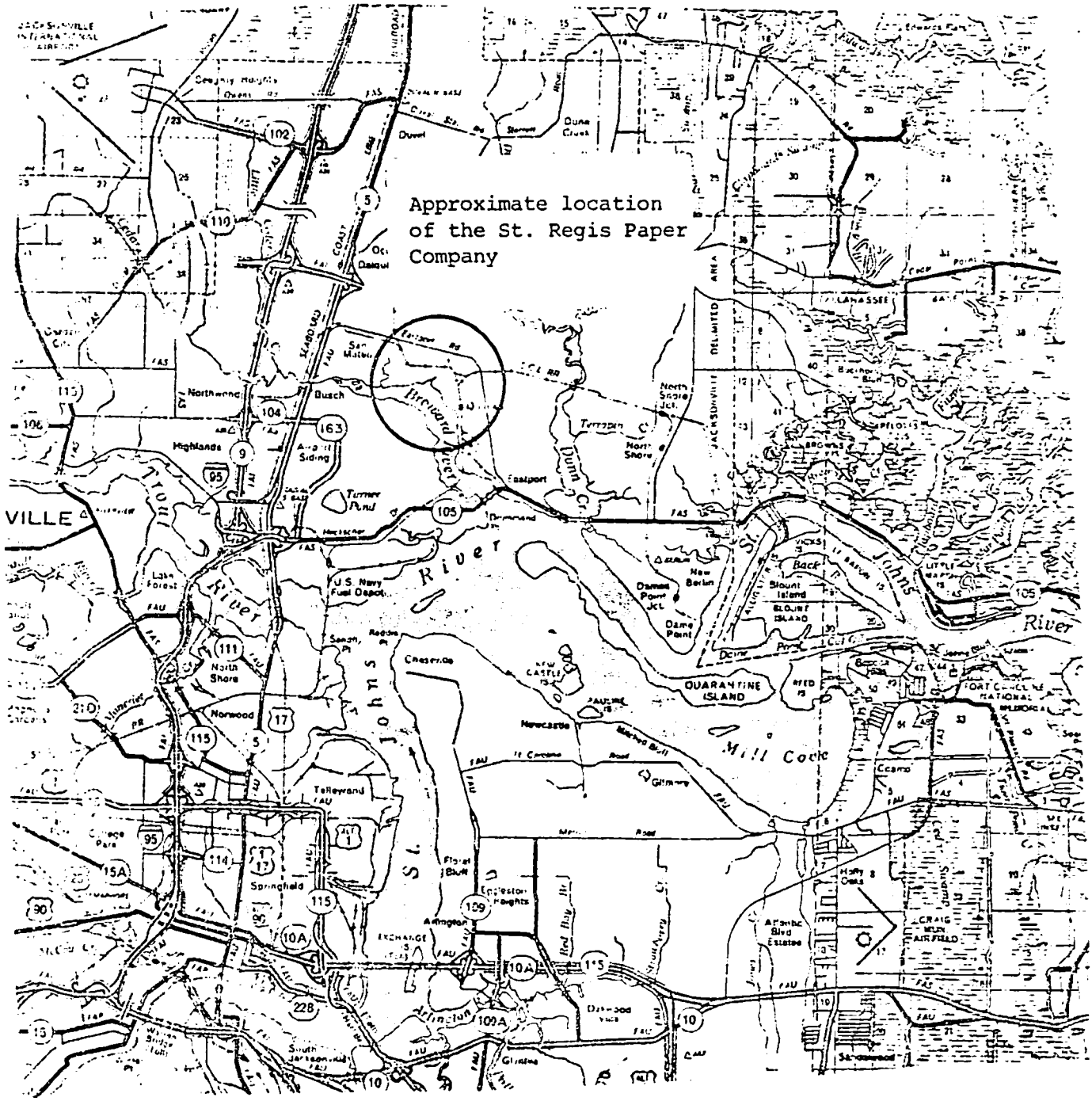
The sample of effluent from the Seminole Kraft Paper Company lagoon was not acutely toxic to the water fleas, Ceriodaphnia dubia or Daphnia pulex, or to the bannerfin shiner, Notropis leedsi. The Microtox® assay, using the bacterium, Photobacterium phosphoreum, was also non-toxic. Due to the non-toxic nature of the sample, chemical analyses were not performed.

INTRODUCTION

The Seminole Kraft Paper Company is a Kraft pulp and paper manufacturer located at 9469 Eastport Road, Jacksonville, Duval County, Florida (Figure 1). The mill produces approximately 1500 tons per day of unbleached Kraft packaging paper and paperboard. A 20 MGD design industrial wastewater treatment system, which includes a clarifier with settling and aeration lagoons, is used for treatment of approximately 17 MGD of post-process water. Discharge is to the St. Johns River.

It was noted in the NPDES permitting files that from February to May of 1987 the facility was in violation of various permitted parameters such as BOD, pH and TSS. These violations occurred after start-up of the facility which had been inoperative for 16 months. In August, 1981, the discharge from the facility (formerly (St. Regis Paper Company) was evaluated, by the FDER Biology Section, in a static acute bioassay. The results of the bioassay indicated that the discharge was not toxic in August of 1981.

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



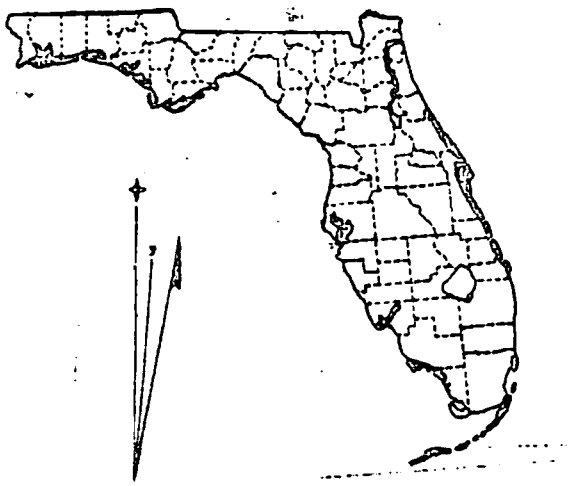


Figure 1. Portions of Jacksonville, Duval County, Florida, including study area.

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 32 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 toxicity bioassays and one Microtox® assay were performed on the sample of discharge from Outfall #001 of the Seminole Kraft Paper Company facility. The bioassays were performed from 1020 hours on 6 January to 1020 hours on 8 January, 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 Northwest District Branch Office at 0940 hours on January 5, 1988, using standard wastewater sampling procedures. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 25 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 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 water sample taken at Outfall #001 of the Seminole Kraft Paper Company industrial wastewater treatment facility was not acutely toxic to the test organisms Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi (Table 2 and 2a). The Microtox® assay was also non-toxic (EC_{50} > 100%), further supporting the conclusion that the recovered water from this facility was non-toxic on January 5, 1988.

Since the sample was not toxic, chemical analyses were not performed.

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 Seminole Kraft Paper Company discharge, Duval County, NPDES #FL0000400, on 6 to 8 January, 1988.

Facility:	SEMINOLE KRAFT PAPER CO.	Facility Type:	Industrial	Sample Collection Date:	1/5/88 Time: 940
NPDES#:	FL0000400	Test Type:	Conventional	Test Beginning Date:	1/6/88 Time: 1020
Location:	DUVAL COUNTY	No. of Tests:	3	Test Ending Date:	1/8/88 Time: 1020
District/Contact	NE / D. Murrin	Chlorination Type:	Non-chlorinated		
Receiving Water	ST. Johns River				

					Alkalinity	Hardness	Total NH3	Unionized NH3
Total Residual Chlorine:	0							
Method:	Hach		100% Sample:	All taxa	115	120	0.496	0.01
By:	DAC		Conditioned Water:	Cladocerans	290	160	0	0
100% Sample salinity:	0		Well Water:	Fish	120	118	0	0

Organism:	*Ceriodaphnia dubia*	Age/Life stage:	Neonates	Diluent:	Conditioned Water
Calculation Technique:	Binomial	Moving Average		Probit	
24 Hour LC50:	non-toxic				
48 Hour LC50:					
Lower 95% C.L.:					
Upper 95% C.L.:					

	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	8.7	8.9	8	7.8	7.9	19	19.8	19.1		
Control	10	10	10	9	8.7	8.9	8	7.9	8					
5.60%	10	10	10	9.2	8.7	8.9	8	7.9	8.1	18.8	19.6	18.7		
5.60%	10	10	10	9.2	8.7	8.9	8	7.9	8.1					
10%	10	10	10	839	8.5	9.1	7.9	7.9	8.1	18.7	19.7	18.7		
10%	10	10	10	8.9	8.5	9.1	7.9	7.9	8.1					
18%	10	10	10	8.5	8.3	9.2	7.9	7.9	8.2	18.8	19.6	18.6		
18%	10	10	10	8.5	8.4	9.1	7.9	7.9	8.2					
32%	10	10	10	8.3	8.5	8.9	7.8	8	8.3	18.9	19.6	18.6		
32%	10	10	10	8.3	8.5	8.9	7.8	8	8.3					
56%	10	10	10	7.4	8.5	8.8	7.8	8.2	8.4	19.2	19.8	18.6		
56%	10	9	9	7.4	8.5	8.9	7.8	8.2	8.4					
100%	10	10	10	5.9	8.2	8.7	7.7	8.3	8.5	19.9	19.8	18.7		
100%	10	10	10	5.7	8.3	8.7	7.7	8.3	8.5					

Continued.

Table 2. Continued.

Organism: *Daphnia pulex* Age/Life stage: Neonates Diluent: Conditioned Water
Calculation Technique: Binomial Moving Average Probit
24 Hour LC50: non-toxic
48 Hour LC50:
Lower 95% C.L.:
Upper 95% C.L.:

Concentrations	SURVIVAL # at 0 hour	Live at 24 hr	Live at 48 hr	Dissolved Oxygen 0 hour	24 hour	48 hour	pH 0 hour	24 hour	48 hour	Temperature 0 hour	24 hour	48 hour	Conductivity 0 hour	48 hour
Control	10	10	10	9.2	9	9	7.9	7.9	8	18.7	19.6	18.5		
Control	10	10	10	9.2	9	9	8	7.9	8				198	208
5.60%	10	10	10	9.3	8.7	9.1	8	7.9	8	18.7	19.2	18.4		
5.60%	10	10	10	9.3	8.7	9.1	8	7.9	8				285	285
10%	10	9	9	9.2	8.4	9	7.9	7	8	18.4	19.2	18.2		
10%	10	10	10	9.1	8.4	9	7.9	7.9	8				365	365
18%	10	9	8	8.7	8.3	8.9	7.9	7.9	8.1	18.3	19.3	18.5		
18%	10	10	10	8.7	8.3	8.8	7.9	7.9	8.1				490	490
32%	10	10	10	8.2	8.4	8.8	7.8	7.9	8.2	18.6	19.1	18.3		
32%	10	10	10	8.2	8.3	8.8	7.8	7.9	8.2				750	750
56%	10	10	10	7.1	7.9	8.7	7.7	8	8.3	19.1	19.2	18.3		
56%	10	10	10	7.1	7.9	8.6	7.7	8	8.3				1120	1190
100%	10	10	10	5.5	7.4	8.2	7.6	8.1	8.4	20	19.5	18.6		
100%	10	10	10	5.1	7.4	8.2	7.6	8.1	8.4				1810	1810

Organism: *Notropis leedsi* Age/Life stage: 32 days Diluent: Well Water
Calculation Technique: Binomial Moving Average Probit
24 Hour LC50: non-toxic
48 Hour LC50:
Lower 95% C.L.:
Upper 95% C.L.:

Concentrations	SURVIVAL # at 0 hour	Live at 24 hr	Live at 48 hr	Dissolved Oxygen 0 hour	24 hour	48 hour	pH 0 hour	24 hour	48 hour	Temperature 0 hour	24 hour	48 hour	Conductivity 0 hour	48 hour
Control	5	5	5	9.1	8	7.8	7.8	7.7	7.7	18.7	19.3	18.4		
Control	5	5	5	9.2	8.1	7.8	7.8	7.7	7.7				205	225
5.60%	5	5	5	9.2	6.9	7.5	7.8	7.5	7.8	18.4	19	18.2		
5.60%	5	5	5	9.2	7	7.5	7.8	7.5	7.8				290	300
10%	5	5	5	9	7	7.5	7.8	7.6	7.8	18.3	18.8	18		
10%	5	5	5	9	7	7.5	7.8	7.6	7.8				362	370
18%	5	5	5	8.7	6.8	7.4	7.8	7.6	7.8	18.5	18.8	18		
18%	5	5	5	8.7	6.5	7.1	7.8	7.6	7.8				498	500
32%	5	5	5	8	6.7	7.2	7.7	7.7	7.9	18.8	18.8	18		
32%	5	5	5	8	6.7	7.2	7.7	7.7	7.9				790	790
56%	5	5	5	7	6	6.7	7.7	7.7	8	19.4	18.9	18.1		
56%	5	5	5	7.1	6.1	6.8	7.7	7.7	8				1200	1170
100%	5	5	5	5.4	5.4	6.1	7.6	7.9	8.1	20.5	19.4	18.4		
100%	5	5	5	5.3	5.1	6	7.6	7.9	8.1				1900	1820

Table 3. Microtox test results, Seminole Kraft Paper Company, Jacksonville,Duval County, NPDES # FL0000400 on 1/6/88.

Microtox Test Culture lot # M-606

Final Reclaimed Water

% Sample	Non-chlorinated	
	5-min	15-min
0	98	64
0	97	60
0	98	63
12.38	99	63
12.38	96	60
12.38	99	65
24.75	93	60
24.75	93	59
24.75	92	59
49.5	90	59
49.5	92	59
49.5	84	54
99	73	52
99	74	51
99	73	50

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.

LITERATURE CITED

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

Florida Department of Environmental Regulation, Biology Section. 1981. Bioassays of St. Regis Paper Company, Jacksonville, Duval County, Florida, NPDES #FL0000400. 9 p.

_____. 1985. Quality Assurance Manual for Performing Acute Toxicity Tests (Second Edition). FDER.

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.

Nontoxic

Industry/Toxicant Seminole Kraft Paper Co.
Address Duval Co. /
Contact/District NE
Effluent Serial Number 201
NPDES Permit Number FL0000400

BIOASSAY DATA FORM

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

Sample Collection: Date 1/5/88 Time 2340
Test Beginning: Date 1/6/88 Time 1020
Test Ending: Date 1/10/88 Time 1020
Test Organism Ceriodaphnia dubia
Age/Life Stage neonates

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	D.O. 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	36	10	10	10		9.0	8.7	8.9			8.0	7.8	7.9			19.0	19.8	19.1							
Control	51	10	10	10		9.0	8.7	8.9			8.0	7.9	8.0												
5.6%	95	10	10	10		9.2	8.7	8.9			8.0	7.9	8.1			18.8	19.6	18.7							
5.6%	9	10	10	10		9.2	8.7	8.9			8.0	7.9	8.1												
10%	20	10	10	10		8.9	8.5	9.1			7.9	7.9	8.1			18.7	19.7	18.7							
10%	75	10	10	10		8.9	8.5	9.1			7.9	7.9	8.1												
18%	92	10	10	10		8.5	8.3	9.2			7.9	7.9	8.2			18.8	19.6	18.6							
18%	21	10	10	10		8.5	8.4	9.1			7.9	7.9	8.2												
32%	65	10	10	10		8.3	8.5	8.9			7.8	8.0	8.3			18.9	19.6	18.6							
32%	1	10	10	10		8.3	8.5	8.9			7.8	8.0	8.3												
56%	80	10	10	10		7.4	8.5	8.8			7.8	8.2	8.4			19.2	19.8	18.6							
56%	85	10	9	9		7.4	8.5	8.9			7.8	8.2	8.4												
100%	13	10	10	10		5.4	8.2	8.7			7.7	8.3	8.5			19.9	19.8	18.7							
100%	71	10	10	10		5.7	8.3	8.7			7.7	8.3	8.5												
Initial of Investigator		DAC	DAC JMF			JMF	JMF	JMF			JMF	JMF	JMF			JMF	JMF	JMF							

Alkalinity/Hardness (mg/l as $CaCO_3$)

Control Water: 115/120
100% Sample: 290/160
Other:
Measured by: JMF

Ammonia unionized/total (mg/l as NH_3/N) 0.24
Hour 0

Control Water: 0.0
100% Sample: 0.496
Other:

Hour ____

Control Water:
100% Sample:
Other:
Measured by: JMF

Signature:
David Chanter
Marshall Faircloth

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7@7.9@9	9.0@21°C	10@10,27.5@30
24 Hours	7@7.9@9	9.0@19°C	—
48 Hours	7@7.9@9	7.3@19°C	10@10 27.5@30
72 Hours			
96 Hours			

Remarks:

Total Residual Chlorine:

Field data: ____ Method: ____
Lab: 0.0 Method: Hach DAC
Lab: ____ Method: ____ Reviewed by:

Dechlorinated:
Measurements by: DAC

Date: 4/6/88

Non Toxic

Industry/Toxicant Seminole Kraft Paper
Address Duval Co.
Contact/District NE
Effluent Serial Number 001
NPDES Permit Number FL0000400

BIOASSAY DATA FORM

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

Sample Collection: Date 1/5/88 Time 0940
Test Beginning: Date 1/6/88 Time 1020
Test Ending: Date 1/8/88 Time 1020
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	70	10	10	10			9.2	9.0	9.0			7.9	7.9	8.0			18.7	19.6	18.5							
Control	8	10	10	10			9.2	9.0	9.0			8.0	7.7	8.0								198	208			
5.6%	1	10	10	10			9.3	8.7	9.1			8.0	7.9	8.0			18.7	19.2	18.4							
5.6%	65	10	10	10			9.3	8.7	9.1			8.0	7.9	8.0								285	285			
10.0%	55	10	9	9			9.2	8.4	9.0			7.9	7.9	8.0			18.4	19.2	18.2							
10.0%	43	10	10	10			9.1	8.4	9.0			7.9	7.9	8.0								365	365			
18%	58	10	9	8			8.7	8.3	8.9			7.9	7.9	8.1			18.3	19.3	18.5							
18%	17	10	10	10			8.7	8.3	8.8			7.9	7.9	8.1								490	490			
32%	23	10	10	10			8.2	8.4	8.8			7.8	7.9	8.2			18.6	19.1	18.3							
32%	36	10	10	10			8.2	8.3	8.8			7.8	7.9	8.2								750	750			
56%	66	10	10	10			7.1	7.9	8.7			7.7	8.0	8.3			19.1	19.2	18.3							
56%	16	10	10	10			7.1	7.9	8.6			7.7	8.0	8.3								1120	1190			
100%	7	10	10	10			5.5	7.4	8.2			7.6	8.1	8.4			20.0	19.5	18.6							
100%	71	10	10	10			5.1	7.4	8.2			7.6	8.1	8.4								1810	1810			
Initial of Investigator		DAC	DAC	MB			JMF	JMF	JMF			JMF	JMF	JMF			JMF	JMF	JMF			JMF	JMF			

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 115/120
100% Sample: 270/160
Other:
Measured by: MF

Ammonia unionized/total (mg/l as NH_3/N) 0.49%
Hour 0
Control Water: 0.0
100% Sample: 0.49%
Other:

Hour ____
Control Water:
100% Sample:
Other:
Measured by: JMF

Signature: David Chambers
Marshall Faubett

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7.0; 9.99	9.2 mg/L 0°C	1000 27450
24 Hours			
48 Hours			
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: ____ Method: ____
Lab: 0 Method: Hach DAC
Lab: ____ Method: ____
Reviewed by:

De-chlorinated:
Measurements by: DAC
Date: 4/6/88

Remarks:

Non toxic

Industry/Toxicant Seminole Kraft Paper
Address Duval Co.
Contact/District NE
Effluent Serial Number 001
NPDES Permit Number FL0000400

BIOASSAY DATA FORM

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

Sample Collection: Date 1/5/88 Time 0940
Test Beginning: Date 1/6/88 Time 1020
Test Ending: Date 1/8/88 Time 1020
Test Organism Notropis lutrensis
Age/Life Stage 32 days (older than 7 day sensitive age)

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	13	5	5	5			9.1	8.0	7.8			7.8	7.7	7.7			18.7	19.3	18.4							
Control	71	5	5	5			9.2	8.1	7.8			7.8	7.7	7.7								205	225			
5.6%	54	5	5	5			9.2	6.9	7.5			7.8	7.5	7.8			18.4	19.0	18.2							
5.6%	69	5	5	5			9.2	7.0	7.5			7.8	7.5	7.8								290	300			
10%	234	5	5	5			9.0	7.0	7.5			7.8	7.6	7.8			18.3	18.8	18.0							
10%	71	5	5	5			9.0	7.0	7.5			7.8	7.6	7.8								362	370			
18%	4	5	5	5			8.7	6.8	7.4			7.8	7.6	7.8			18.5	18.8	18.0							
18%	70	5	5	5			8.7	6.5	7.1			7.8	7.6	7.8								498	500 DAC			
32%	239	5	5	5			8.0	6.7	7.2			7.7	7.7	8.0	7.9		18.8	18.8	18.0							
32%	131	5	5	5			8.0	6.7	7.2			7.7	7.7	8.0	7.9							790	790 DAC			
56%	44	5	5	5			7.0	6.0	6.7			7.7	7.6	8.0			19.4	18.9	18.1							
56%	31	5	5	5			7.1	6.1	6.8			7.7	7.7	8.0								1200	1170			
100%	281	5	5	5			5.4	5.4	6.1			7.6	7.9	8.1			20.5	19.4	18.4							
100%	15	5	5	5			5.3	5.1	6.0			7.6	7.9	8.1								1900	1820			
Initial of Investigator		MF	MF	MF DAC			JMF	JMF	JMF			JMF	JMF	JMF			JMF	JMF	JMF			JMF	JMF			

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: ~~DAC~~ 120/118
100% Sample: 290/160
Other:
Measured by: MF

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: 0.0
100% Sample: 0.496
Other:

Hour ____
Control Water:
100% Sample:
Other:
Measured by: JMF

Salinity: 1.2 ppt

Signature: David Charles
Marshall Faircloth

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: 4/16/88

046

MICROTOX™ Data Reduction Sheet

SOURCE: Seminole Kraft Paper Co

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

SAMPLE DATA:

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

DATE ASSAYED: 01-06-88 TIME: 8:15 AM

VISUAL COLOR: pale yellow 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: none

OPERATOR: Steve Williams REAGENT VIAL LOT NO. M-606

REMARKS: no res'd

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	Γ5	Γ15	NOTES
A1	0	98	64	X	X	
B1		97	60			
C1		98	63			
A2	12.38	91	63	-.003	-.005	
B2		96	60			
C2		99	65			
A3	24.75	93	60	.054	.051	
B3		93	59			
C3		92	59			
A4	49.5	90	59	0.102	.087	
B4		92	59			
C4		84	54			
A5	99	73	52	0.332	0.322	
B5		74	51			
C5		73	50			
EC_{50} (MIN, °C)				234.9	413.2	

REVIEWED BY:

DATE: 4/6/88