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BIOASSAYS OF JEFFERSON SMURFIT

(ALTON PACKAGING COMPANY)

JACKSONVILLE, DUVAL COUNTY, FLORIDA

NPDES #FL0000892

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OCTOBER 9, 1986

Sampled July 7, 1986

Biology Section
Division of Environmental Programs

BIOASSAYS OF JEFFERSON SMURFIT

(ALTON PACKAGING COMPANY)

JACKSONVILLE, DUVAL COUNTY, FLORIDA

NPDES #FL0000892

Biology Section
Bureau of Laboratories and
Special Programs
October 9, 1986

EXECUTIVE SUMMARY

Jefferson Smurfit, Jacksonville, NPDES #FL0000892, on 8 to 11 July, 1986.

Three static acute toxicity bioassays were performed on a sample of this paper mill's (formerly Alton Packaging) wastewater, which is discharged to the St. Johns River. The wastewater proved not to be acutely toxic to the freshwater test organisms, Daphnia pulex and Notropis leedsi. Only a few random deaths of D. pulex were noted during the 72 hour test period. Conversely, the wastewater was immediately toxic to the saltwater shrimp, Mysidopsis bahia, producing a four hour LC_{50} of 54.6%. No further mortality was noted during the remainder of the 72 hour test period. No abnormalities (such as low dissolved oxygen) were observed during the tests, and neither ammonia nor chlorine was present in quantities sufficient to have caused any of the M. bahia toxicity. These latest results are very similar to previous DER bioassays of this facility, in which toxicity to Mysidopsis bahia has been frequently observed.

Results of chemical analyses indicated the presence of several organic constituents, including dehydroabietic acid and one large unidentified compound via the pesticide extractable analysis. Metal analyses indicated various concentrations of silver, zinc, nickel, and chromium. These chemical component probably contributed to the toxicity of the sample.

We recommend that the facility continue its biomonitoring program, perhaps on a more frequent schedule. Also, the pattern of this wastewater's toxicity (only to Mysidopsis bahia, and early in the test period) may suggest means to reduce the toxicity. Increasing the wastewater's retention time with constant aeration, and adjusting the pH of the discharge to > 7.0 units, may significantly improve the discharge.

INTRODUCTION

The Jefferson Smurfit (formerly Alton Packaging) Company is located at 1915 Wigmore Street, Jacksonville, Duval County, Florida (Figure 1). This paper industry facility uses pine wood to produce approximately 707 tons per day of Kraft pulp and linerboard. In the production process, 5 MGD of wastewater is generated which is discharged to the St. Johns River. Prior to discharge, the wastewater is treated by retention in a settling pond and by a Unox® treatment system. The wastewater is also fertilized with ammonia to promote the growth of microorganisms to aid in the degradation of wood fibers present in the wastewater. Sludges are separated from the wastewater prior to discharge.

This facility has, on occasion, been in violation for exceeding certain NPDES permit limitations. Additionally, the FDER Biology Section had previously performed a toxicity test on effluent from this facility, in September, 1981. The results of that test indicated that the effluent was extremely toxic to the test organism, Mysidopsis bahia, with a 48 hour LC_{50} of 15.7% (FDER 1981). Toxicity was attributed, in part, to the large concentrations of total and unionized ammonia determined in the sample at that time. However, Alton Company officials claimed that an ammonia spill occurred the same day that the sample was collected.

Effluent from this facility was retested, in March, 1982, again using Mysidopsis bahia as the test organism. Results of that test, however, were invalidated due to a large biochemical oxygen demand exerted by the effluent that depleted the dissolved oxygen in the test chambers and resulted in organism mortality. Therefore, that test was inconclusive in determining toxic aspects of the effluent. Ammonia was not detected in the effluent at that time (FDER 1982a).

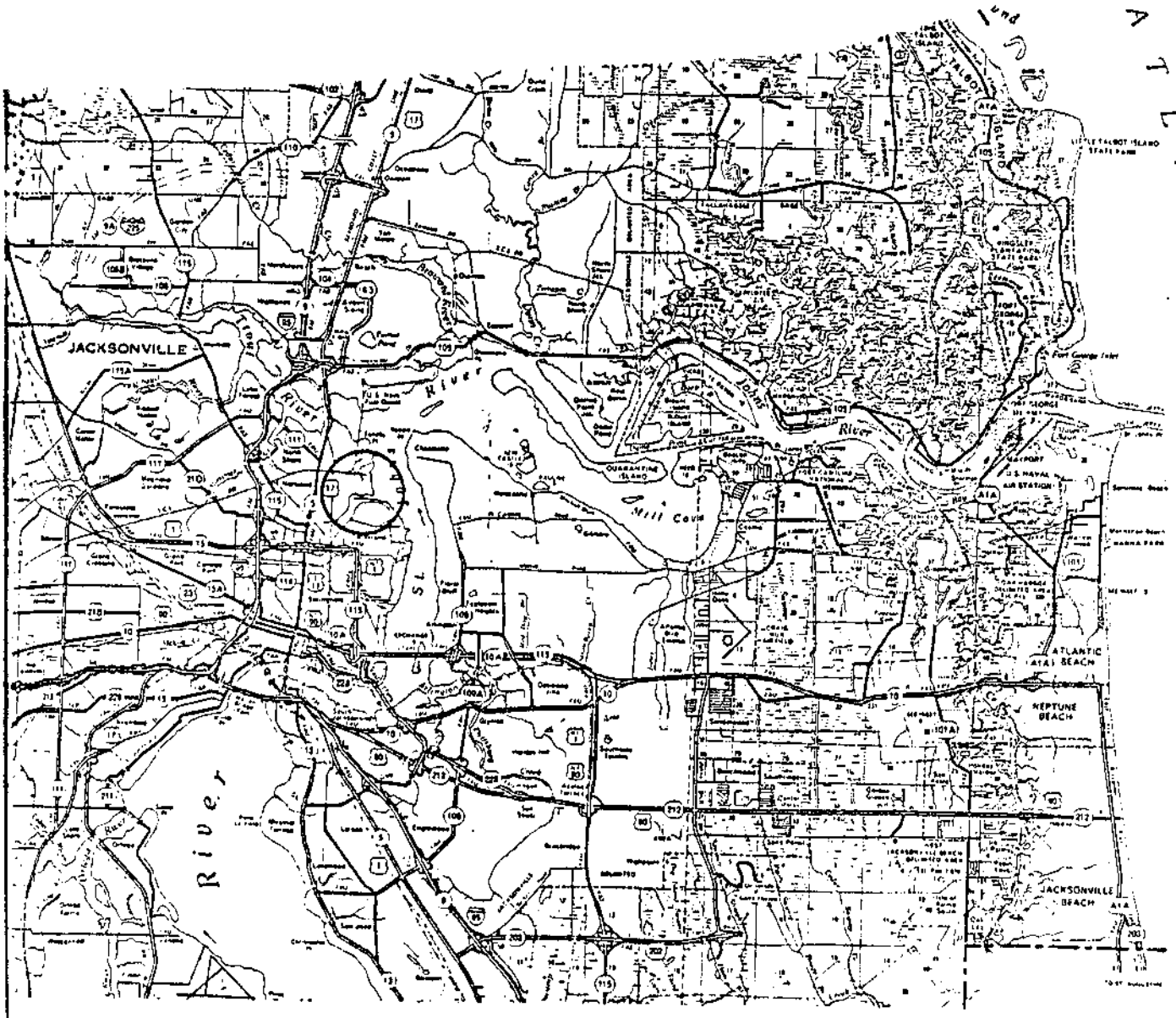
Since the March, 1982, test was invalidated due to depleted dissolved oxygen, two additional bioassays were performed in July, 1982, using Mysidopsis bahia and Daphnia pulex as test organisms. The higher test concentrations in these bioassays were continuously aerated to negate the effects of the effluent's high BOD. The M. bahia test produced an LC_{50} of 141.7%, a violation of the Chapter 17-3 acute toxicity standard. The D. pulex test was invalidated due to missing organisms in the extremely opaque effluent (FDER 1982b). However, after negotiating with DER's Jacksonville office, Alton Company was granted a mixing zone, thereby bringing the effluent into compliance with regard to acute toxicity as stated in the Chapter 17-4 mixing zone criteria.

Due to the history of toxicity at this facility and the recent granting of a mixing zone to bring this toxicity into compliance, additional bioassay testing was requested by the Northeast District. Two 48 hour static bioassays were performed on a sample of Alton's effluent on 24 to 26 May, 1983, using Mysidopsis bahia and Heterandria formosa as test organisms. The results of these tests indicated 30% mortality of M. bahia in the 100% concentration, while no H. formosa mortality was noted (FDER 1983a). All test chambers were continuously aerated.

To continue to evaluate the toxicity of this effluent, three 48 hour static acute toxicity bioassays were performed on 27 to 29 March, 1984, using Mysidopsis bahia, Notropis leedsi and Daphnia pulex as test organisms. The M. bahia produced a valid 24 hour LC_{50} of 87.7%. However, at 48 hours, the dissolved oxygen in several concentrations had decreased to below recommended levels and additional organism mortality was not used in further LC_{50} calculations. In the D. pulex bioassay, 25% of the organisms died in the 100% concentration at 24 hours. At 48 hours, however, similar to the M. bahia test, insufficient dissolved oxygen was noted in the test chambers, and additional organism mortality was not used to calculate an LC_{50} (FDER 1984).

To again evaluate the toxicity of this discharge, three 48 hour static acute toxicity bioassays were performed on 8 to 10 January, 1985. Organisms selected for these tests were again Mysidopsis bahia, Heterandria formosa and Daphnia pulex. As in previous tests, results indicated some toxicity to M. bahia (30% mortality in the 100% effluent concentration), but no toxicity to the freshwater organisms (FDER 1985). All test chambers were aerated throughout the bioassays.

Due to the continual borderline toxicity problems at this facility, the Northeast District requested that further acute toxicity bioassay be performed. Three such tests, using D. pulex, N. leedsi, and M. bahia, were performed on 8 to 11 July, 1986. The results of these latest tests are presented in this report. Those involved in performing the tests and in preparation of this report were: M. L. Roll and P. G. Weiland.



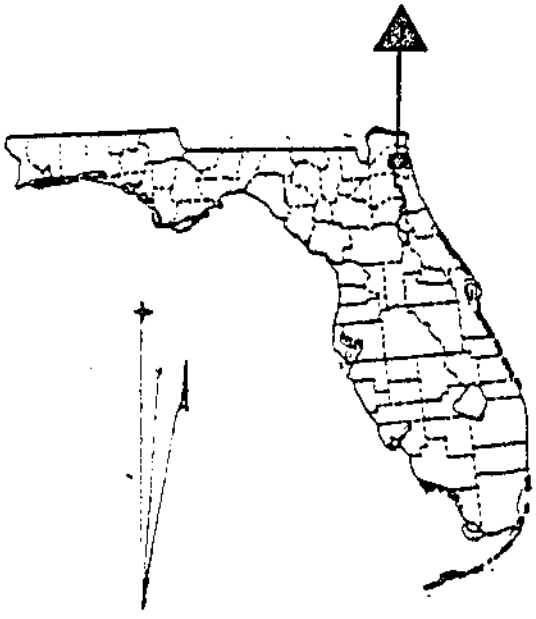


Figure 1. Portions of Jacksonville, Duval County, Florida, including approximate location of Alton Packaging Company.

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.

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.

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 approximately 25 days old at the time of the test.

Mysidopsis bahia (mysid shrimp) - In September, 1978, approximately 300 adult mysid shrimp were obtained from the EPA laboratory in Gulf Breeze, Florida. Additional mysid shrimp are obtained from EPA and private labortories as needed. The mysids are cultured under a 16:8 hour light-dark cycle in 10 and 15 gallon all-glass aquaria equipped with undergravel filtration and aeration. The mysid shrimp are cultured in

several salinities (20-30 ppt) to minimize acclimation effects to high salinity effluents. Low salinity effluents are adjusted to approximately 20 ppt. Seawater, used for culturing the test organisms and as diluent in the bioassays, is prepared by mixing artificial sea salts (Forty Fathoms®) and well water to obtain a final salinity of approximately 20 ppt. Freshwater, for dilution of the seawater as necessary, is obtained from a deep well near the DER laboratory. The mysids are fed twice daily and once daily on weekends with freshly hatched Artemia (brine shrimp) and are kept at a constant temperature of 23° ± 2°C. Adult or large juvenile mysids were used in this test.

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 72 hour static acute toxicity bioassays were performed on a sample of Jefferson Smurfit's wastewater discharge. The tests were performed from 1015 hours on 8 July to 1015 hours on 11 July, 1986.

The sample was collected by Mr. Mark Sittig of DER's Northeast District at 1030 hours on 7 July, 1986, using standard wastewater sampling procedures. 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 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.

Aeration

Since the initial dissolved oxygen concentration (5.8 mg/l) of the wastewater sample was significantly above 40% of saturation, pre-aeration of the sample was not necessary. By hour 30 of the test, however, the D.O. levels in several of the test chambers were observed to be slowly decreasing (lowest value measured = 5.3 mg/l). Therefore, aeration was begun in the controls and higher test concentrations at that time.

Salinity Adjustment

Since a saltwater test organism was also used to evaluate toxicity, artificial sea salts (Forty Fathoms®) were added to a portion of the sample until a salinity of 20 ppt was obtained. This salinity is well within the salinity tolerance of the test organism. A Salinity Control was also employed, consisting of well water with artificial sea salts added until a similar salinity of approximately 20 ppt was obtained. Additional information concerning salinity adjustment and saltwater tests is given below.

pH Adjustment

Since the toxicity, or lack of toxicity, in a bioassay sample may be dependent on the sample's pH, the Biology Section prefers, and recommends, that the pH of a sample at the start of the bioassay be similar to the pH measured during sampling. In this particular case, the sample and control used for the saltwater test were adjusted with an acid (0.1N HCl). This was necessary due to the pH having increased between sampling and initiating the test. This pH increase was likely a result of salinity adjusting the sample. The pH of the saltwater sample and control were adjusted to $\pm$ 0.3 s.u. of the value measured at sampling. This procedure provides the fairest test possible considering the varied chemical substances in complex wastewaters, the toxicities of which are dependent on pH. As stated, the control was adjusted with the same acid and to the same value as the sample. These adjustments are noted on the bioassay data forms.

Freshwater Tests

Freshwater for dilution of the effluent sample was obtained from the same DER deep well that is used to culture the test organisms. The concentration series selected for this study (Table 1) is that recommended by APHA (1980).

Two replicates, of each concentration and a control, were used to test with the water flea, Daphnia pulex. 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. Daphnia were siphoned into a large culture dish to isolate the proper size and age organisms (neonates). To accomplish this, the water fleas 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 this 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 test.

Saltwater Tests

Water used as the control and for dilution of the wastewater (50% concentration) was DER well water adjusted with artificial seasalts until a salinity of 20 ppt was obtained. As stated previously, the pH of this water and of the salinity adjusted wastewater was adjusted with an acid to 0.3 s.u. of the pH value measured at sampling. final salinities were all approximately 20 ppt.

Two replicates of each test concentration and of the control were used to test with Mysidopsis bahia. Immediately prior to the bioassay, a suitable number of mysids were transferred by net from the appropriate salinity aquarium to a culture dish for observation and selection of individuals for the tests. Individual mysid shrimp were siphoned using a glass tube, carefully examined for injury, and transferred to wide mouth quart jars, each containing 500 ml of the appropriate concentration. For each concentration, the mysid shrimp were pre-acclimated to within 4 ppt salinity. Five organisms were loaded per test chamber, giving ten per concentration. Based on the recommendations of EPA (1978), the mysids were fed brine shrimp (Artemia) nauplii at the rate of approximately 10 to 20 nauplii per mysid per day to minimize cannibalism.

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, 48 hours, and 72 hours. Additionally, since M. bahia mortality was observed at 4 hours, D.O. measurements were also recorded then. 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 or salinity (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 Tables 2, 3, and 4.

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

Daphnia pulex (water flea)

Concentration	Dilution Water	Effluent	Total Volume
Control	200.0 ml	--------	200 ml
10.0%	180.0 ml	20.0 ml	200 ml
18.0%	164.0 ml	36.0 ml	200 ml
32.0%	136.0 ml	64.0 ml	200 ml
56.0%	88.0 ml	112.0 ml	200 ml
100.0%	--------	200.0 ml	200 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Effluent	Total Volume
Control	500 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
100.0%	------	500 ml	500 ml

Mysidopsis bahia (mysid shrimp)

Concentration	Dilution Water	Effluent	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 72 hour, Daphnia pulex, static acute toxicity bioassay of Jefferson Smurfit (Alton), Jacksonville, NPDES #FL0000892, on 8 to 11 July, 1986.

Concentration or %	Number of Live Organisms				Dissolved Oxygen (mg/l)				pH				Temperature (°C)				Conductivity (umhos)	
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72	0	72
Control	10	10	10	10	8.8	8.7	8.4	8.9	8.2	7.8	7.8	7.9	20.9	20.4	19.4	19.8		
Control	10	10	10	9	8.8	8.7	8.4	8.9	8.2	7.8	7.8	7.9					188	233
10.0%	10	10	10	10	8.8	8.6	8.4	8.9	7.8	7.9	7.9	8.0	20.7	20.3	19.4	19.7		
10.0%	10	10	10	10	8.8	8.6	8.4	8.9	7.9	7.9	7.9	8.0					370	405
18.0%	10	10	10	10	8.6	8.6	8.2	8.7	7.6	7.9	8.0	8.0	20.7	20.2	19.3	19.7		
18.0%	10	8	8	8	8.6	8.6	8.2	8.7	7.6	7.9	8.0	8.0					600	670
32.0%	10	10	10	10	8.3	8.3	7.8	8.3	7.3	7.9	8.0	8.0	20.6	20.2	19.3	19.8		
32.0%	10	10	10	10	8.3	8.3	7.8	8.3	7.3	7.9	8.0	8.0					850	920
56.0%	10	10	10	10	7.8	8.0	7.4	7.9	7.1	7.9	8.1	8.0	20.6	20.2	19.4	19.8		
56.0%	10	10	10	10	7.8	8.0	7.4	7.9	7.1	7.9	8.1	8.0					1320	1410
100.0%	10	10	10	10	6.9	7.5	7.1	7.4	6.9	7.9	8.1	8.1	20.6	20.3	19.4	19.8		
100.0%	10	10	10	10	6.9	7.5	7.1	7.4	6.9	7.9	8.1	8.1					2110	2200

Control Water

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

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

Total Alkalinity 118 (mg/l $CaCO_3$)

Total Hardness 120 (mg/l $CaCO_3$)

100% Effluent

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

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

Total Alkalinity 232 (mg/l $CaCO_3$)

Total Hardness 251 (mg/l $CaCO_3$)

Total Residual Chlorine: 0.06 mg/l (HACH DPD Method).

Table 3. Data recorded during 72 hour, Notropis leedsi, static acute toxicity bioassay of Jefferson Smurfit (Alton), Jacksonville, NPDES #FL0000892, on 8 to 11 July, 1986.

Concentration or %	Number of Live Organisms				Dissolved Oxygen (mg/l)				pH				Temperature (°C)				Conductivity (umhos)		Control Water	
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72	0	72		
Control	5	5	5	5	8.5	8.2[a]	8.5	8.7	7.6	7.8	8.0	8.1	21.1	20.2	19.2	19.7				
Control	5	5	5	5	8.5	8.2[a]	8.5	8.9	7.6	7.8	8.0	8.1					203	210	NH_3(as NH_3)(mg/l) 0	Total Alkalinity 118 (mg/l $CaCO_3$)
10.0%	5	5	5	5	8.3	8.2	7.8	8.4	7.4	7.8	7.9	8.0	21.1	20.0	19.2	19.5				
10.0%	5	5	5	5	8.3	8.2	7.8	8.4	7.4	7.8	7.9	8.0					393	392	Total ammonia (as N)(mg/l) 0	Total Hardness 120 (mg/l $CaCO_3$)
18.0%	5	5	5	5	8.2	8.0	7.4	8.4	7.3	7.7	7.9	8.0	21.1	19.9	19.2	19.5				
18.0%	5	5	5	5	8.2	8.0	7.4	8.2	7.3	7.7	7.9	8.0					600	620		
32.0%	5	5	5	5	8.0	7.3	6.7	7.3	7.2	7.7	8.0	7.9	21.0	19.9	19.2	19.4				
32.0%	5	5	5	5	8.0	7.3	6.7	7.3	7.2	7.7	8.0	7.9					900	910		
56.0%	5	5	5	5	7.8	6.7[a]	8.2	8.4	7.1	7.7	8.3	8.4	20.8	19.9	19.2	19.4				
56.0%	5	5	5	5	7.8	6.7[a]	8.2	8.6	7.1	7.7	8.3	8.4					1380	1400	100% Effluent	
100.0%	5	5	5	5	6.8	6.3[a]	8.3	8.6	6.9	7.7	8.5	8.6	20.5	20.1	19.2	19.4			NH_3(as NH_3)(mg/l) 0	Total Alkalinity 232 (mg/l $CaCO_3$)
100.0%	5	5	5	5	6.8	6.3[a]	8.3	8.6	6.9	7.7	8.5	8.6					2200	2220		
																			Total ammonia (as N)(mg/l) 0	Total Hardness 251 (mg/l $CaCO_3$)

Total Residual Chlorine: 0.06 mg/l (HACH DPD Method).

[a] Aeration begun in these test chambers at hour 30.

Table 4. Data recorded during 72 hour, Mysidopsis bahia, static acute toxicity bioassay of Jefferson Smurfit (Alton), Jacksonville, NPDES #FL0000892, on 8 to 11 July, 1986.

Concentration or %	Number of Live Organisms					Dissolved Oxygen (mg/l)					pH				Temperature (°C)				Salinity (ppt)	
	0	4	24	48	72	0	4	24	48	72	0	24	48	72	0	24	48	72	0	72
Control	5	5	5	5	5	7.6	8.2	5.3[a]	7.4	7.8	6.8	7.7	8.3	8.4	21.5	20.4	19.3	19.7		
Control	5	5	5	5	(5)[c]	7.6	8.2	5.3[a]	7.4	7.6	6.8	7.7	8.3	8.4					20.5	20.2
50.0%	5	2	2	2	2	7.4	7.7	5.5[a]	7.2	7.6	6.9	7.8	8.5	8.5	21.3	20.3	19.3	19.7		
50.0%	5	4	4	4	4	7.4	7.7	5.5[a]	7.2	7.6	6.9	7.8	8.5	8.5					20.5	20.2
100.0%	5	0	0	0	0	6.8	6.6	-b	-b	-b	6.8	7.8	-b	-b	21.0	20.4	-b	-b		
100.0%	5	0	0	0	0	6.8	6.6	-	-	-	6.8	7.8	-	-					20.2	20.0

Control Water

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

Total Alkalinity (mg/l $CaCO_3$)

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

Total Hardness (mg/l $CaCO_3$)

100% Effluent

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

Total Alkalinity (mg/l $CaCO_3$)

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

Total Hardness (mg/l $CaCO_3$)

Total Residual Chlorine: 0.06 mg/l (HACH DPD Method).

[a] Aeration begun in these test chambers at hour 30.

b Remaining parameters were not recorded since all organisms were dead by hour 4.

[c] Number in parentheses for calculations used, one organism was canabalized.

Table 5. LC_{50} Data for Bioassays of Jefferson Smurfit (Alton), NPDES #FL0000892, on 8 to 11 July, 1986.

Organism	Method of Data Analysis	4-72 Hour LC_{50}'s and 95% Confidence Intervals
Daphnia pulex	Binomial	Random Mortality
	Moving Average	
	Probit	
Notropis leedsi	Binomial	No Mortality
	Moving Average	
	Probit	
Mysidopsis bahia	Binomial	54.6% (0 and 100)
	Moving Average	a
	Probit	a

[a] Due to an insufficient number of dead organisms, or to the irregular distribution of mortality, an LC_{50} value cannot be calculated using this method of statistical analysis.

Table 6. Results of Chemical Analyses Performed on Wastewater from Jefferson Smurfit (Alton) Company, Jacksonville, NPDES #FL0000892, collected July 7, 1986.

Organics

Acid Extractables:	Dehydroabietic acid (10 ug/l)[a] Numerous other unidentified organic of a natural origin
Base Neutral Extractables:	Numerous unidentified compounds
Pesticide Extractables:	One large unidentified compound

Metals

Antimony	N.D.
Arsenic	< 4 ug/l
Beryllium	N.D.
Cadmium	< 0.5 ug/l
Chromium	< 25 ug/l
Copper	N.D.
Lead	N.D.
Mercury	N.D.
Nickel	< 50 ug/l
Selenium	N.D.
Silver	0.4 ug/l
Thallium	N.D.
Zinc	41 ug/l

[a] Estimated concentration

RESULTS AND DISCUSSION

The results of this latest series of bioassays on wastewater from the Jefferson Smurfit (Alton) facility were very similar to previous DER tests. No toxicity was evident in the freshwater tests using Daphnia pulex and Notropis leedsi. Only a few random D. pulex deaths were noted during the 72 hour test period (Table 2). However, immediate acute toxicity (4 hour LC_{50} = 54.6%) was noted in the Mysidopsis bahia saltwater test (Table 4). The shrimp appeared stressed as soon as they were introduced to the wastewater. No additional mortlity was noted, however, after that initially observed at 4 hours. No abnormalities (such as low D.O.) were noted, and neither chlorine nor ammonia was present to have caused this toxicity. A reason for this toxicity, therefore, is not readily apparent.

The pattern of this toxicity, in this and in previous DER tests - frequent toxicity to M. bahia early during the test period using unaerated wastewater - may suggest an explanation. The wastewater may contain components specifically toxic to M. bahia that are easily rendered non-toxic by aeration (volatilization) or by an increase in pH (neutralization). Test data have indicated that toxicity ceases after aeration or after an increase in pH (caused by aeration among other things). Of course, further bioassay testing is needed to substantiate this.

The results of chemical analyses (Table 6) indicated the presence of numerous organic constituents, including dehydroabietic acid and one large unidentified compound via the pesticide extractable analysis. Metal analyses indicated various concentrations of zinc (41 ug/l), silver (0.4 ug/l), nickel (< 50 ug/l), chromium (< 25 ug/l), cadmium (< 0.5 ug/l), and arsenic (< 4 ug/l) in Smurfit's wastewater. These chemical components probably contributed to the toxicity of the sample.

Recommendation

Due to the frequent toxicity exhibited by the Jefferson Smurfit wastewater, we recommend the continuation of their biomonitoring program, perhaps on a more frequent schedule. Also, we would like to review all bioassay test data generated at this facility, and to closely review test methodology (i.e., pre-aeration of sample).

Also, we would recommend that the discharger undertake bench-scale experiments to reduce the toxicity of their wastewater. We suggest such measures as increasing the retention time of the effluent while under aeration, and increasing the pH of the discharge to > 7.0 units.

Discussion of the Toxicity Criteria

The toxicity standards of Chapters 17-3 and 17-4, F.A.C., are complex. Because of this complexity, they will be briefly reviewed here so that these test results may be more readily compared with the standards. For legal purposes, however, the language of the Florida Administrative Code should be consulted directly.

The two basic concepts utilized in the standards are acute and chronic toxicity (defined in 17-3.021(1) and (4), respectively). Simplifying the actual F.A.C. language somewhat, acute toxicity is defined as the presence of a substance in a concentration one-third as great as that concentration which kills one-half of the test organisms in 96 hours, i.e., one-third of the 96 hour LC_{50}. Chronic toxicity is similarly defined as onetwentieth of the 96 hour LC_{50}. Therefore, if a test solution kills one- half or more of a group of test organisms in 96 hours, it must contain at least three times the defined "acutely toxic" concentration and at least twenty times the "chronically toxic" concentration of some substance or substances. If a test solution causes that same mortality in less than 96 hours, (for example, in 24 or 48 hours), it is at least as toxic as the example given above, and actually is probably considerably more toxic.

The particular restrictions applied to a given discharger depend upon the existence of a mixing zone, whetheras a specific part of the permit or based on the existence of a currently valid permit predating the language in Chapter 17-4 requiring mixing zone designation. In the absence of a mixing zone, the general criteria for surface waters [17-3.061] are applicable at all points, beginning at the point of discharge. This section prohibits the discharge of substances in concentrations which are chronically toxic [17-3.061(2)(p)]; that is, the concentrations cannot exceed one-twentieth of the 96 hour LC_{50} as discussed above. If a mixing zone does exist, then this chronic toxicity standard is only applicable from the edge of the mixing zone and beyond. Within the mixing zone, the less stringent acute toxicity standard of the minimum criteria for all waters [17-3.051(1)(d)] applies: one-third of the 96 hour LC_{50}. The acute toxicity standard does not apply, however, to each point within a mixing zone not designated for dredge and fill permitting, but rather to the average of a number of points within such a mixing zone, based on the provisions of the surface water mixing zones section of Chapter 17-4 [17-4.244(4)]. At the same time, though, this same rule prohibits waste concentrations within such a mixing zone (i.e., at any point within the mixing zone), from exceeding the 96 hour LC_{50}. Therefore, at any point within such a mixing zone, including the point of discharge, one-half of the test organisms cannot be killed in 96 hours. Within a mixing zone designated for dredge and fill permitting, the acute toxicity standard (one-third of the 96 hour LC_{50}) applies at all points, including the point of discharge.

From the above discussion, it should be clear that if the least stringent toxicity standard, the 96 hour LC_{50}, is exceeded at any point, from the point of discharge outwards, by any discharger, then a violation of the standards has occurred. This is an important consideration since the test procedure here reported involves the measurement of the 50% lethal concentration, and not some fraction of that concentration. Any discharger required to meet the one-twentieth of the 96 hour LC_{50} standard is therefore being tested very leniently. Any reduction in the test period from 96 hours provides an added degree of leniency to a discharger.

A number of different dilutions were examined in order to determine by how much, if any, the discharge exceeds the 96 hour LC_{50}. For example, if the sample, after two-fold dilution with diluent (a 33% concentration), kills one-half of the test organisms after 96 hours, then the sample is three times as toxic as is allowed by that standard. Similarly, if a 5% solution of the sample kills 50% of the organisms, the sample is twenty times as toxic as is allowed. Toxicities are reported here in that manner, i.e., in terms of the percentage dilution of the test sample. The smaller the percentage which is reported means a correspondingly greater toxicity.

Note that even if a discharger receives a very large mixing zone not designated for dredge and fill permitting, the regulations require that the maximum toxicity at any point within the mixing zone be restricted to the 96 hour LC_{50}. The results here reported are based on actual effluent samples, representative of the effluent concentration which enters waters of the state at the point of discharge. If it is demonstrated that, under the conditions existing at the point of discharge, effluent concentrations will be less than at the actual sampling point (if the two points are different), the reported bioassay results will reflect the reduced discharge concentration.

With respect to the species of organisms used in these tests, the language of the F.A.C. indicates that they should be "significant to the indigenous aquatic community". All species used in these tests are significant to the indigenous aquatic community since they are sensitive to toxic substances which may be expected to impact the indigenous community.

The toxicity reported here is in excess of allowable limits.

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