BIOASSAYS OF ALTON PACKAGING COMPANY

JACKSONVILLE, DUVAL COUNTY, FLORIDA

NPDES #FL0000892

SAMPLED 3/26/84

FILE COPY
DO NOT REMOVE

Biology Section
Bureau of Water Analysis
June 22, 1984

BIOASSAYS OF ALTON PACKAGING COMPANY

JACKSONVILLE, DUVAL COUNTY, FLORIDA

NPDES #FL0000892

Biology Section
Bureau of Water Analysis
June 22, 1984

EXECUTIVE SUMMARY

Alton Packaging Company, Jacksonville, NPDES #FL0000892, on 27 to 29 March, 1984.

The wastewater effluent from Alton proved to be lethal to significant numbers of bioassay test organisms (Daphnia pulex, Notropis leedsi and Mysidopsis bahia), but some of the mortality noted had to be invalidated due to dissolved oxygen concentrations falling slightly below recommended levels. In the D. pulex bioassay, 25% of the organisms died in the 100% concentration while adequate dissolved oxygen was maintained, but this mortality was insufficient to calculate an LC_{50}. A 24 hour LC_{50} of 87.7% was calculated for the M. bahia bioassay, even though oxygen concentrations were adequate. At 48 hours, although no significant increase in mortality was noted, several test concentrations were abandoned due to dissolved oxygen concentrations falling to 24% - 28% of saturation. According to strict bioassay testing procedures, dissolved oxygen concentrations should not be permitted to fall below 40% of saturation. In the N. leedsi bioassay, only 10% of the organisms in the 100% concentration had died by 24 hours while maintaining adequate dissolved oxygen. At 48 hours, however, a significant increase in mortality was noted, but the dissolved oxygen in the affected chambers had fallen to 36% - 29% of saturation, thereby invalidating the mortality. The overall results of these bioassays indicated that Alton's effluent was acutely toxic to M. bahia, producing a valid 24 hour LC_{50} of 87.7%.

The total ammonia concentration (3.05 mg/1) and the unionized fraction (0.01 mg/1) measured in Alton's effluent were insufficient to have contributed significantly to the toxicity noted. Chemical analyses, however, indicated the presence of unidentified organic compounds, including a terpene (120 µg/1) and an oil (6 mg/1), and the metals arsenic (> 65 µg/1), copper (29 µg/1), cadmium (0.9 µg/1), mercury (0.2 µg/1) and selenium (16.8 µ/1). These components may have acted synergistically to have caused a portion of the toxicity noted above.

INTRODUCTION

The 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.

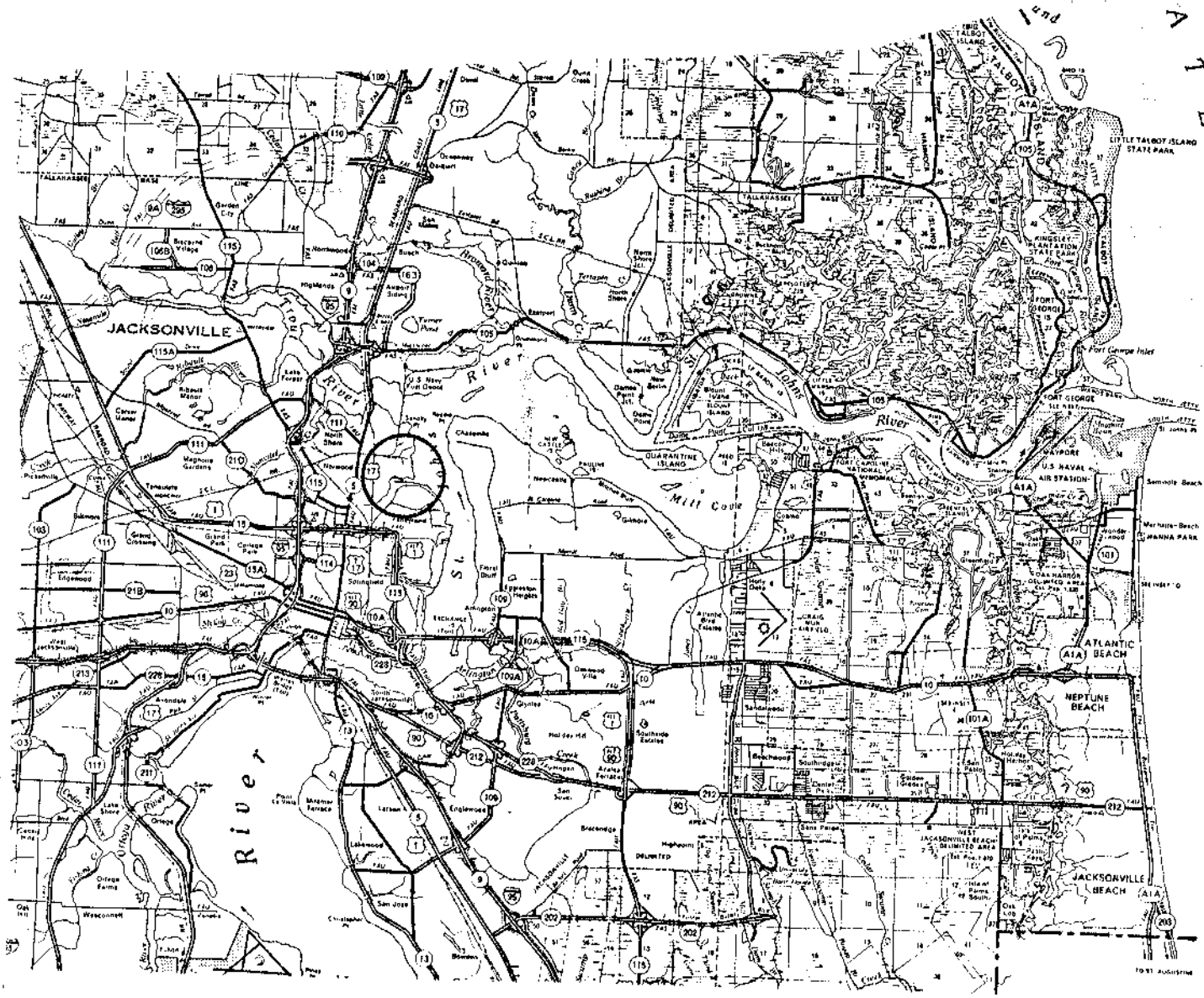
This facility has, on occasion, been in violation for exceeding certain NPDES permit limitations. Additionally, the FDER Biology Section had previously performed a toxicity test on effluent from this facility, in September, 1981. The results of that test indicated that the effluent was extremely toxic 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 ino 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. Due to this mortality, the Biology Section recommended continued bioassay testing of Alton's effluent.

Therefore, to again evaluate the toxicity of this effluent, three 48 hour static acute toxicity bioassays were performed on 27 to 29 March, 1984. Organisms selected for these tests were again Mysidopsis bahia, to repeat the earlier tests, Notropis leedsi (freshwater fish) and Daphnia pulex. The results of these latest tests are presented in this report. Those involved in performing these tests and in the preparation of this report were: P. D. Brooks, C. W. Dye, and M. L. Roll.



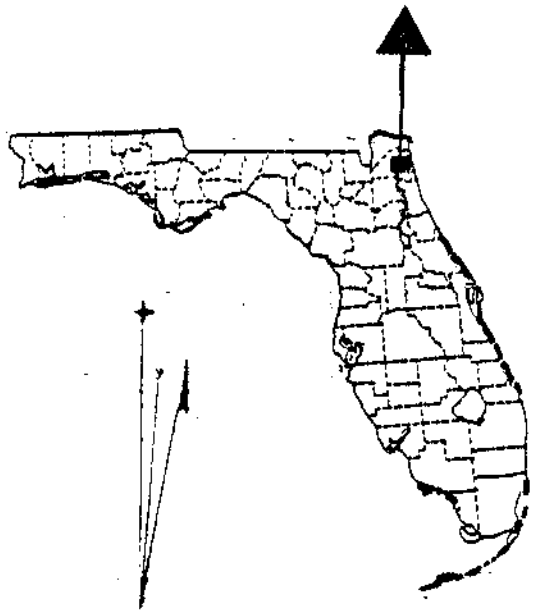


Figure 1. Portions of Jacksonville, Duval County, Florida, including approximate location of Alton Packaging Company.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978) and the FDER Biology Section (1983). The organisms selected for this study include two species recommended by Peltier (1978), the U.S. EPA (1978), and the FDER Biology Section (1983b). 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° plus or minus 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° plus or minus 2°C under a 16:8 hour light 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 9 to 21 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. 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 over a wide range of salinities (10-30 ppt) to minimize acclimation periods to low or high salinity effluents. Seawater is obtained from the FSU Marine Laboratory located at Turkey Point, Florida (salinity range 17-31 ppt) and partial water changes are performed weekly. 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° plus or minus 3°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

Three separate 48 hour static acute toxicity bioassays were performed on a grab sample of Alton's effluent. The tests were performed from 1015 hours on 27 March to 1045 hours on 29 March, 1984.

The sample was collected by Mr. Jay Carver of DER's Northeast District at 1045 hours on 26 March, 1984, 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.

Freshwater for dilution of the effluent sample for the D. pulex and N. leedsi tests 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, each with six concentrations 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 six concentrations 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 for dilution of the effluent sample for the M. bahia test was obtained from the FSU Marine Laboratory at Turkey Point (salinity = 22.0 ppt). For test concentrations of 56% and below, this diluent was mixed directly with the effluent, achieving a range of final salinities (10.8 ppt to 22.0 ppt). For the 100% effluent test concentration, freshly autoclaved artificial sea salts (Instant Ocean®) were added until a salinity of approximately 7 ppt was achieved (the lower limit of mysid shrimp salinity tolerance). Two control groups (Table 4) were then used to ensure that any mortality in the test organisms was not the result of toxic properties associated with the diluent, the artificial sea salts, or the salinity range. The control groups included:

1) Diluent Control - Turkey Point water (22.0 ppt). This represents the highest concentration of Turkey Point water used in the bioassay, and tests for toxicity in the diluent. This is also the highest salinity used in the bioassay, and therefore tests the tolerance of the organisms to this salinity.

2) Sea Salts Control - Well water, adjusted with artificial sea salts (Instant Ocean®) to approximately 7 ppt salinity. This tests for

toxicity of the artificial sea salts used in the bioassay, as well as for tolerance of the organisms to the lowest salinity used in the bioassay.

The concentration series selected for this study (Table 1) is that recommended by APHA (1980). Two replicates of each test concentration and of the controls were used to test with Mysidopsis bahia. Immediately prior to the bioassay, a suitable number of mysids were transferred by net from the 10 ppt, 15 ppt and 20 ppt salinity aquaria to culture dishes 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.

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 freshwater tests 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
5.6%	188.8 ml	11.2 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)

Mysidopsis bahia (mysid shrimp)

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

Table 2. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Alton Packaging Company, Jacksonville, Duval County, Florida, NPDES #FL0000892, on 27 to 29 March, 1984.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alakalinity (mg/l as $CaCO_3$)		Total Hardness (mg/l as $CaCO_3$)		Temperature (°c)			NH_3/N (mg/l)		Conductivity (umhos)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	10	10	10	9.0	9.4	9.3	7.7	8.2	8.2	91		138		20.4	19.5	19.1	0/0	—		
Control	10	10	10	9.0	9.4	9.3	7.7	8.2	8.2										177	177
5.6%	10	10	10	8.7	9.1	9.1	7.5	8.1	8.1					20.4	19.4	18.8				
5.6%	10	10	10	8.7	9.1	9.1	7.5	8.1	8.1										265	261
10.0%	10	10	10	8.5	8.8	8.9	7.4	8.0	8.1					20.3	19.4	18.7				
10.0%	10	10	10	8.5	8.8	8.9	7.4	8.0	8.1										335	330
18.0%	10	10	10	8.2	8.3	8.5	7.3	7.9	8.0					20.2	19.4	18.7				
18.0%	10	10	10	8.2	8.2	8.5	7.3	7.9	8.0										455	449
32.0%	10	10	10	7.4	7.4	7.7	7.2	7.8	7.9					20.0	19.4	18.8				
32.0%	10	10	10	7.4	7.4	7.7	7.2	7.8	7.9										695	680
56.0%	10	10	10	6.0	5.7	6.3	7.0	7.7	7.8					19.9	19.5	18.8				
56.0%	10	10	10	6.0	5.7	6.3	7.0	7.7	7.8										1100	1100
100.0%	10	10	9	[a] 3.6	[b] 4.1	4.5	6.8	7.5	7.7	158		319		19.9	19.5	19.1	.01/3.05	—		
100.0%	10	10	6	[a] 3.2	[b] 3.9	4.3	6.8	7.5	7.7										1740	1780

Remarks: [a] Aerated to 4.5 mg/l and 4.4 mg/l, respectively, prior to loading the organisms.
[b] Aerated to 5.2 mg/l and 5.7 mg/l, respectively, at 24 hours, and again, briefly, at 46 hours.

Table 3. Data recorded during 48 hour, Notropis leedsi[a], static acute toxicity bioassay of Alton Packaging Company, Jacksonville, Duval County, Florida, NPDES #FL0000892, on 27 to 29 March, 1984.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alakalinity (mg/l as $CaCO_3$)		Total Hardness (mg/l as $CaCO_3$)		Temperature (°c)			NH_3/N (mg/l)		Conductivity (umhos)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	5	5	5	8.7	8.1	8.1	7.7	7.9	8.0	91		138		20.7	19.5	18.9	0/0	—		
Control	[b]7	7	7	8.7	8.2	8.3	7.7	7.9	8.0										183	183
5.6%	5	5	5	8.5	8.1	8.2	7.5	7.9	7.9					20.7	19.3	18.7				
5.6%	5	4	4	8.5	7.8	7.9	7.5	7.9	7.9										273	270
10.0%	5	5	5	8.4	7.7	7.7	7.4	7.8	7.8					20.5	19.2	18.6				
10.0%	5	5	5	8.4	7.7	7.8	7.4	7.8	7.8										345	333
18.0%	[b]6	6	6	8.1	7.1	6.8	7.3	7.7	7.7					20.4	19.1	18.6				
18.0%	5	5	5	8.1	7.1	6.8	7.3	7.7	7.7										465	452
32.0%	5	5	5	7.2	6.0	5.4	7.2	7.5	7.6					20.1	19.1	18.6				
32.0%	5	5	5	7.2	5.8	5.4	7.2	7.5	7.6										710	700
56.0%	[b]4	4	4	6.3	4.7	4.4	7.0	7.5	7.8					20.0	19.1	18.7				
56.0%	[b]4	4	[d]0	6.3	4.8	[d]3.4	7.0	7.5	7.8										1110	1480
100.0%	5	5	[d]4	[c]3.6	4.3	[d]3.1	6.8	7.6	7.8	158		319		19.5	19.1	18.8	.01/3.05	—		
100.0%	5	4	[d]1	[c]3.4	4.3	[d]2.7	6.8	7.6	7.7										1780	1790

Remarks:
[a] Weight of fish: 12.55 mg each, average of 10 fish
[b] More or less organisms loaded by mistake.
[c] Aerated to 5.1 mg/l and 5.1 mg/l, respectively, prior to loading the organisms
[d] These data are invalid due to DO concentrations falling to 36%, 33% and 29% of saturation at 46 hours. Mortality not used for LC_{50} determination.

Table 4. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Alton Packaging Company, Jacksonville, Duval County, Florida, NPDES #FL0000892, on 27 to 29 March, 1984.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			NH_3/N (mg/l)		Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
Diluent Control	5	5	5	7.5	6.4	5.8	7.9	7.7	7.6	20.5	19.5	19.0	0/0	—		
Diluent Control	5	5	5	7.5	6.3	5.8	7.9	7.7	7.6						22.0	22.2
Sea Salts Control	5	5	5	8.3	6.9	6.4	8.1	8.0	7.9	20.5	19.6	19.2				
Sea Salts Control	5	5	5	8.3	7.1	6.6	8.1	8.1	7.9						7.3	7.7
5.6%	5	5	5	7.8	6.3	5.8	7.9	7.7	7.6	20.1	19.2	18.8				
5.6%	5	5	5	7.8	6.6	6.0	7.9	7.8	7.6						22.0	22.2
10.0%	5	5	5	7.4	6.1	4.4	7.4	7.6	7.5	19.9	19.3	18.8				
10.0%	5	5	5	7.4	6.1	4.7	7.4	7.6	7.5						19.5	19.8
18.0%	5	5	5	7.3	6.1	4.3	7.3	7.6	7.5	19.8	19.3	18.8				
18.0%	5	5	5	7.3	6.1	4.2	7.3	7.6	7.5						18.8	18.9
32.0%	5	5	5	6.9	5.5	3.5	7.1	7.5	7.5	19.7	19.4	19.0				
32.0%	5	5	5	6.9	5.5	3.7	7.1	7.5	7.5						16.0	16.0
56.0%	6[a]	6	5[c]	6.0	4.8	2.1[c]	6.9	7.4	7.6	19.5	19.7	19.1				
56.0%	5	5	5[c]	6.0	4.8	2.2[c]	6.9	7.4	7.6						10.8	10.8
100.0%	5	3	2[c]	4.9[b]	6.1	2.4[c]	6.9	7.6	7.8	18.5	19.7	19.2	.01/3.05	—		
100.0%	5	0	0	4.8[b]	6.5	—[d]	6.9	7.6	7.8						7.5	7.9

Remarks:

[a] Extra organism loaded by mistake, allowed to remain.

[b] These chambers were not aerated initially since sufficient DO was present. Aeration began at hour 22 since DO's had fallen to 3.6 mg/l and 3.8 mg/l (approximately 43% of saturation). DO was increased to values shown at hour 24.

[c] These data are invalid due to DO concentrations falling to 24%, 25% and 28% of saturation of 46 hours. Mortality not used for 48 hour LC_{50} determination.

[d] This DO was not reported since all organisms in this chamber were dead by 24 hours.

Table 5. LC_{50} Data for Bioassays of Alton Packaging Company, NPDES #FL0000892, on 27 to 29 March, 1984.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
D. pulex	Binomial	No Mortality	[a]
	Moving Average		[a]
	Probit		[a]
N. leedsi	Binomial	[a]	Invalid
	Moving Average	[a]	
	Probit	[a]	
M. bahia	Binomial	87.7% (56 and ∞)	Invalid
	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 Analysis Performed on Samples of Alton's Effluent, collected 26 March, 1984.

Organics

Pesticide Extractables:	One unidentified compound
Base Neutral Extractables:	A terpene compound (approximately 120 ug/l)
	An oil (approximately 6 mg/l)

Metals

Arsenic	>65	ug/l[a]
Copper	29	ug/l[b]
Chromium	<25	ug/l
Cadmium	0.9	ug/l[b]
Lead	<30	ug/l
Mercury	0.2	ug/l
Nickel	N.D.	
Selenium	16.8	ug/l
Silver	<0.5	ug/l[b]
Zinc	----	

N.D. = None Detected

[a]Actual value is known to be greater than value given

[b]Actual value is known to be less than value given

The zinc analysis was invalidated

RESULTS

The sample of wastewater effluent from Alton Packaging Company proved to be lethal to significant numbers of bioassay test organisms (Daphnia pulex, Mysidopsis bahia and Notropis leedsi) even while maintaining sufficient dissolved oxygen concentrations in the test chambers. However, additional mortality (particularly of N. leedsi) observed in the later stages of the tests had to be invalidated due to dissolved oxygen concentrations falling to slightly below recommended levels. In the D. pulex bioassay (Table 2), 25% of the organisms had died by 48 hours in the 100% concentration while maintaining adequate dissolved oxygen with intermittant aeration. This mortality, however, was insufficient to calculate an LC_{50} (Table 5). In the N. leedsi bioassay (Table 3), 10% of the organisms had died by 24 hours while maintaining adequate dissolved oxygen. Near the end of the test, however, dissolved oxygen concentrations were measured at 36% to 29% of saturation. Therefore, the additional mortality observed in these affected test chambers at that time had to be invalidated. According to strict bioassay testing procedures (Peltier 1978, FDER 1983), dissolved oxygen concentrations should not be permitted to fall below 40% of saturation. In the M. bahia bioassay (Table 4), aeration was not started until hour 22 of the test when dissolved oxygen concentrations in the 100% chambers were measured at approximately 43% of saturation. Nevertheless, the mortality noted in these chambers was sufficient to calculate a 24 hour LC_{50} of 87.7% (Table 5). Near the end of the test, however, dissolved oxygen concentrations had fallen to 24% to 28% of saturation, thereby invalidating the small increase in mortality noted at that time. The final results of these bioassays indicated that Alton's effluent was acutely toxic to M. bahia, producing a 24 hour LC_{50} of 87.7%. This toxicity was not due to the small concentrations of total ammonia (3.05 mg/l) or of unionized ammonia (0.01 mg/l) measured in the effluent.

From the above results and the data presented in Tables 2, 3 and 4, it is apparent that the toxicity of Alton's effluent is not dependent so much on low dissolved oxygen concentrations, but more on the effects of aeration on the effluent prior to and during the bioassays. This will be explained more in detail below. First of all, it must be pointed out that the lowest dissolved oxygen concentrations measured in the test chambers where mortality occured (2.4 mg/l for M. bahia and 2.7 mg/l to 3.4 mg/l for N. leedsi) were not likely the cause of the toxicity. The recommended level of 40% of saturation is merely to ensure that the organisms are not unduly stressed during the tests and to guard against total depletion of oxygen in a sample with a significant BOD.

According to a review of pertinent literature, paper mill effluents are considerably less toxic at higher pH's (a function in our test). For example, McLeay et al. (1979a) reported that "bioassays with six samples of Kraft whole mill effluents show clearly that these effluents are considerably less toxic at alkaline rather than neutral pH values." In a follow-up paper, McLeay et al. (1979b) explained the mechanism of this pH - toxicity relationship. Briefly, the toxicity of the toxic components of paper mill effluents (resin, abietic and dehydroabietic acids) decreases substantially with increasing ionization (corresponding to an increase in pH). Bioassays performed by Zanella (1983) strongly support this finding. Apparently, an increase in pH (as a result of

aeration) of a paper mill effluent may very well render the effluent non-toxic. This increase in pH as a result of aeration is most likely due to the increased concentration of dissolved carbon dioxide in the aerated sample. In the current DER bioassays, the 100% concentrations in the M. bahia test which were not aerated initially, were by far the most toxic. In these chambers, the pH rose almost a full unit after 48 hours after periods of aeration at 22 and 46 hours. Correspondingly, there was almost no increase in mortality after 24 hours.

The DER Chemistry Section has analyzed Alton's effluent for certain metals and organics (Table 6). These analyses indicated the presence of an unidentified pesticide - like compound, a "terpene - class" compound at an approximate concentration of 120 µg/l, and, a lightweight oil at an approximate concentration of 6 mg/l. Also present in Alton's effluent were the metals arsenic (>65 µg/l), cooper (29 µg/l), cadmium (0.9 µg/l), mercury (0.2 µg/l) and selenium (16.8 µg/l). Although individually these metals are in sublethal concentrations, they may very well have acted synergistically with the organic compounds to have caused a portion of the observed toxicity.

From the data presented above, it is suggested that Alton Packaging Company does indeed have a toxicity problem with its effluent, and, that this toxicity is dependent upon the aeration (dissolved oxygen concentration) and the pH of the sample. Therefore, we recommend a careful review of previous bioassay reports (DER's, EPA's, and ESE's) to determine the possible detoxifying effects that "over-aeration" may have had on Alton's effluent during previous bioassays. Additionally, when performing future bioassays of Alton, we recommend that the dissolved oxygen concentration be maintained near the minimum recommended level of 40% of saturation, and that the pH of the tests be maintained at a value somewhere between that of the original effluent at sampling and that of the receiving body of water.

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 one-twentieth 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 existance of a mixing zone, whether as a specific part of the permit or based on the existance 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.

LITERATURE CITED

American Public Health Association. 1980. Standard Methods for the Examination of Water and Wastewater. 15th ed. 1134 p.

Florida Department of Environmental Regulation, Biology Section. 1981. Bioassay of Alton Box Board Company, Duval County, Florida NPDES #FL0000892. FDER. 11 p.

Florida Department of Environmental Regulation, Biology Section. 1982a. Bioassay of Alton Box Board Packaging Company, Jacksonville, Duval County, Florida. NPDES #FL0000892. FDER. 11 p.

Florida Department of Environmental Regulation, Biology Section. 1982b. Bioassays of Alton Box Board Packaging Company, Jacksonville, Duval County, Florida. NPDES #FL0000892. FDER. 14 p.

Florida Department of Environmental Regulation, Biology Section. 1983a. Bioassays of Alton Box Packaging Company, Jacksonville, Duval County, Florida. NPDES #FL0000892. FDER. 10 p.

Florida Department of Environmental Regulation, Biology Section. 1983b. Quality Assurance Manual for Performing Acute Toxicity Tests. FDER. 22 p.

McLeay, D. J., C. C. Walden and J. R. Munro. 1979a. Effect of pH on toxicity of Kraft pulp and paper mill effluent to salmonid fish in freshwater and seawater. Water Res. 13, 249-254.

_____. 1979b. Influence of dilution water on the toxicity of Kraft pulp and paper mill effluent, including mechanisms of effect. Water Res. 13, 151-158.

Peltier, W. 1978. Methods for measuring the acute toxicity of effluents to aquatic organisms. Environmental Research Laboratory. Athens, Georgia. EPA-600/4-78-012 (Revised July 1978). 51 p.

U.S. Environmental Protection Agency. 1978. Bioassay procedures for the Ocean Disposal Permit Program. Environmental Research Laboratory. Gulf Breeze, Florida. EPA-600/9-78-010. 121 p.

Zanella, E. 1983. Effect of pH on acute toxicity of dehydroabietic acid and chlorinated dehydroabietic to fish and Daphnia. Bull. Environm. Coutam. Toxicol. 30, 133-140.