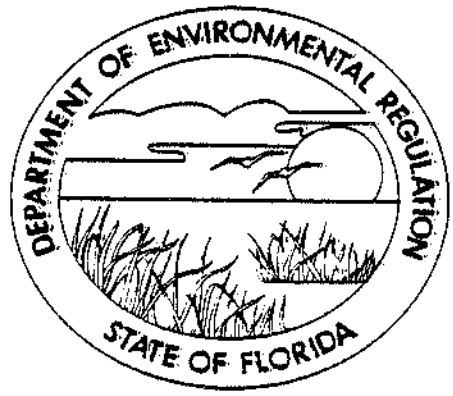


BIOASSAYS OF ALTON BOX PACKAGING COMPANY

JACKSONVILLE, DUVAL COUNTY, FLORIDA

NPDES #FL0000892

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Biology Section
Bureau of Water Analysis
June 21, 1983

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EXECUTIVE SUMMARY

Alton Box Packaging Company, Jacksonville, NPDES #FL0000892, on 24 to 26 May, 1983.

The sample of wastewater from this paper mill produced 30% mortality in the 100% effluent concentration in the Mysidopsis bahia bioassay, but no mortality was noted in the Heterandria formosa bioassay. The M. bahia mortality (3 dead of 10 organisms) was insufficient to calculate an LC_{50}. Therefore, Alton's effluent was in compliance with the F.A.C. Chapters 17-3 and 4 toxicity standards at the time of the sampling. Since the effluent was not in violation of standards, chemical analyses were not performed. The Biology Section recommends continued bioassay testing of this facility, perhaps including the use of a more definitive 96 hour flow-through test, to more adequately investigate the toxic properties of Alton's effluent.

INTRODUCTION

The Alton Box 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.

Therefore, to again evaluate the toxicity of this effluent, two 48 hour static acute toxicity bioassays were performed on 24 to 26 May, 1983. Organisms selected for these tests were again Mysidopsis bahia, to repeat the earlier tests, and Heterandria formosa, since the salinity of the St. Johns River had decreased to approximately 6 ppt at the time of sampling. 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: C. W. Dye, M. L. Roll, and K. A. Study.

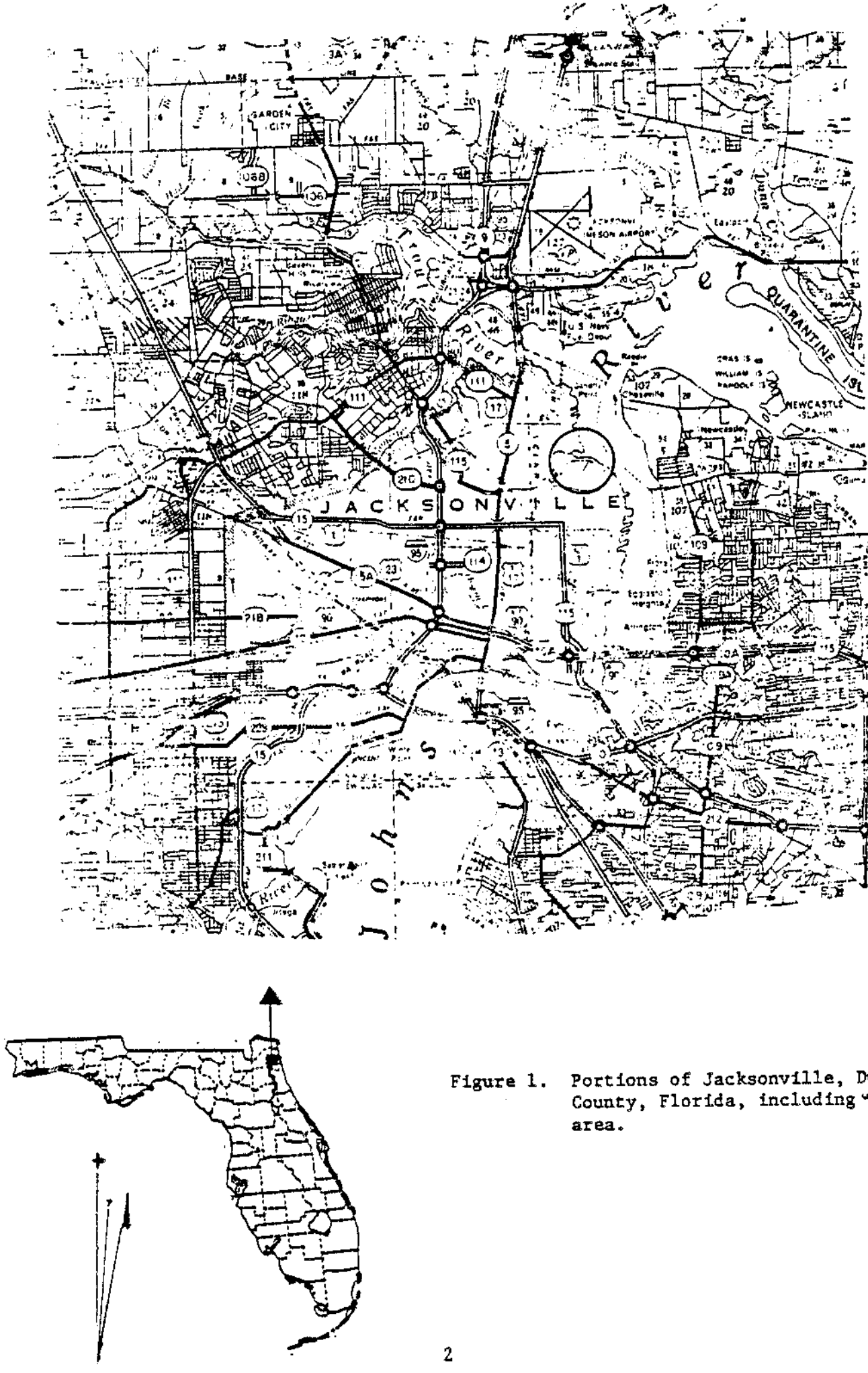


Figure 1. Portions of Jacksonville, Duval County, Florida, including study area.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978) and the FDER Biology Section (1983). The organisms selected for this study include a species recommended by Peltier (1978), the U.S. EPA (1978), and the FDER Biology Section (1983). 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

Heterandria formosa (least killifish) - Approximately 100 adult least killifish were obtained from a wild population in the St. Marks Wildlife Refuge (Wakulla County, Florida) during August, 1980. The fish are maintained in two and one-half to 15 gallon all-glass aquaria equipped with a flow-through water exchange system. Water for this system is unchlorinated and is pumped from a deep well near the DER laboratory. The least killifish are reared at 22° plus or minus 2°C under a 16:8 hour light-dark cycle and are fed freshly hatched Artemia (brine shrimp) and Tetra-Min® twice daily. New born fish are initially maintained in the adult tanks, but are transferred to separate aquaria at weekly intervals, thereby segregating fish into even age groups. The fish used in this test were 10 to 40 days old.

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 with freshly hatched Artemia (brine shrimp) and are kept at a constant temperature of 22° plus or minus 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

Two separate 48 hour static acute toxicity bioassays were performed on a grab sample of Alton's effluent. The tests were performed from 1430 hours on 24 May to 1445 hours on 26 May, 1983. The sample was collected by Ms. Teri Rhodes of DER's Jacksonville office at 0845 hours on 24 May, 1983, 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 Heterandria formosa bioassay 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 six randomly arranged concentrations and a control, were used for the Heterandria formosa (least killifish) 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 Mysidopsis bahia bioassay was obtained from the FSU Marine Laboratory at Turkey Point (salinity = 28 ppt). This saltwater diluent was then adjusted to a moderate salinity characteristic of the receiving body of water (St. Johns River = 16.2 ppt) by adding an appropriate amount of DER well water. For test concentrations of 56% and below, this adjusted diluent was mixed directly with the effluent, achieving a range of final salinities (8.1 ppt to 15.3 ppt). For the 100% effluent test concentration, freshly autoclaved artificial sea salts (Ocean 50®) were added until a salinity of approximately 7 ppt was achieved (the lower limit of mysid shrimp salinity tolerance). Two control groups (Table 3) 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 (28 ppt), adjusted with well water to a salinity of 16.2 ppt. This represents the highest concentrations of both Turkey Point water and well water used in the bioassays, and tests for toxicity in the diluent. This is also the highest salinity used in the bioassays, and therefore tests the tolerance of the organisms to this salinity.

2) Sea Salts Control - Well water, adjusted with artificial sea salts (Ocean 50®) to approximately 7 ppt salinity. This tests for toxicity of the highest concentration of artificial sea salts used in the bioassays, as well as for tolerance of the organisms to the lowest salinity used in the bioassays.

The concentration series selected for this study (Table 1) is that recommended by APHA (1980). Two replicates of each randomly arranged test concentration and of the controls were used to test with Mysidopsis bahia.

Immediately prior to the bioassays, a suitable number of mysids were transferred by net from the 10 ppt and 15 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 chamber at 0 hours, 24 hours, and 48 hours. 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 and 3.

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

Heterandria formosa (least killifish)

Mydidopsis bahia (mysid shrimp)

Concentration	Dilution Water	Effluent	Total Volume
Control	500 ml	------	500 ml
5.6%	472 ml	28 ml	500 ml
10.0%	450 ml	50 ml	500 ml
18.0%	410 ml	90 ml	500 ml
32.0%	340 ml	160 ml	500 ml
56.0%	220 ml	280 ml	500 ml
100.0%	------	500 ml	500 ml

Table 2. Data recorded during 48 hour, Heterandria formosa,[a] static acute toxicity bioassay of Alton Box Company, Jacksonville, Duval County, Florida, NPDES #FL0000892, on 24 to 26 May, 1983.

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Conductivity (µmhos)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	5	5	5	8.8	8.5	8.2	7.8	8.0	8.0					21.5	19.7	19.9	0/.02	--		
Control	5	5	5	8.8	8.5	8.2	7.8	8.0	8.0										233	230
5.6%	5	5	5	8.5	7.3	7.9	7.7	8.0	8.0					21.5	19.8	19.9				
5.6%	5	5	5	8.5	7.4	7.9	7.7	8.0	8.0										311	302
10.0%	5	5	5	7.9	7.0	7.7	7.6	8.0	8.0					21.4	19.7	19.9				
10.0%	5	5	5	7.9	7.0	7.7	7.6	8.0	8.0										385	380
18.0%	5	5	5	7.4	6.3	7.0	7.4	7.9	8.0					21.2	19.8	19.9				
18.0%	5	5	5	7.4	6.1	7.0	7.4	7.9	8.0										600	590
32.0%	5	5	5	7.0[b]	8.6[b]	8.3[b]	7.4	8.4	8.3					20.9	20.2	19.9				
32.0%	5	5	5	7.3[b]	8.8[b]	8.3[b]	7.4	8.4	8.3										900	895
56.0%	5	5	5	5.2[b]	8.8[b]	8.5[b]	7.3	8.4	8.4					20.5	19.9	19.9				
56.0%	5	5	5	6.3[b]	8.8[b]	8.5[b]	7.3	8.5	8.4										1250	1210
100.0%	5	5	5	4.1[b]	8.8[b]	8.5[b]	7.2	8.6	8.5					19.9	20.0	20.0	.001/.13	--		
100.0%	5	5	5	4.9[b]	8.8[b]	8.5[b]	7.2	8.6	8.5										1900	1890

Remarks:

[a] Weight of fish: 10.5 mg each, average of 9 fish.

[b] These test chambers were aerated for 10 minutes prior to loading the organisms, and continuously throughout the test. Dissolved oxygen concentration of effluent at sampling time: 0.0 mg/l (Mr. Dennis Murrin personal communication).

Table 3. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Alton Box Company, Jacksonville, Duval County, Florida, NPDES #FL0000892, on 24 to 26 May, 1983.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			NH_3/NH_3+NH_4 (mg/l)		Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
Diluent Control	5(4)[a]	4	4	8.5	7.1	7.0	8.3	8.1	8.0	21.5	19.7	19.5	0/0	--		
Diluent Control	5	5	5	8.5	7.2	7.0	8.3	8.2	8.0						16.2	16.2
Sea Salts Control	5	5	5	8.6	8.0	6.8	8.4	8.3	8.0	21.1	19.4	19.5				
Sea Salts Control	5	5	5	8.6	8.0	6.8	8.4	8.3	8.0						7.2	7.3
5.6%	5	5	5	8.2	6.9	6.5	8.1	8.0	7.9	21.4	19.3	19.5				
5.6%	5	5	5	8.2	6.9	6.6	8.1	8.0	7.9						15.3	15.2
10.0%	5	5	5	8.1	6.2	6.6	7.9	7.9	7.9	21.4	19.4	19.4				
10.0%	5	5	5	8.1	6.2	6.6	7.9	7.9	7.9						14.9	14.8
18.0%	5	5	5	7.5	5.6	6.2	7.6	7.8	7.8	21.4	19.3	19.3				
18.0%	5	5	5	7.5	5.3	6.2	7.6	7.8	7.8						13.9	13.8
32.0%	5	5	5	6.9[b]	7.9[b]	8.0[b]	7.4	8.3	8.2	21.3	19.5	19.5				
32.0%	5	5	5	7.0[b]	8.1[b]	8.0[b]	7.4	8.3	8.2						12.0	11.9
56.0%	5	5	5	6.2[b]	8.3[b]	8.2[b]	7.3	8.4	8.3	21.2	19.8	19.9				
56.0%	5	5	5	6.4[b]	8.3[b]	8.3[b]	7.3	8.4	8.3						8.1	8.5
100.0%	5	3	3	6.5[b]	8.1[b]	8.3[b]	7.5	8.5	8.5	20.9	20.2	19.7	.001/.13	--		
100.0%	5	5	4	4.4[b]	8.0[b]	8.2[b]	7.3	8.4	8.4						7.0	7.1

Remarks: [a] Number in parentheses used since one organism was cannibalized between 0 and 24 hours.

[b] These test chambers were aerated for 10 minutes prior to loading the organisms, and continuously throughout the test. Dissolved oxygen concentration of effluent at sampling time: 0.0 mg/l (Mr. Dennis Murrin personal communication).

RESULTS AND DISCUSSION

The sample of wastewater effluent from Alton's Outfall #001 was not toxic to Heterandria formosa, but produced 30% mortality (3 dead of 10 shrimp) in the 100% test concentration of the Mysidopsis bahia bioassay (Table 3). However, the mortality was insufficient to calculate an LC_{50}. Therefore, these bioassays indicated that Alton's effluent was apparently in compliance with the end-of-pipe toxicity standards in Chapter 17-4 regarding mixing zones. Since the effluent was in compliance, chemical analyses were not performed. The small amount of ammonia detected in the effluent could not have had an effect on the toxicity.

Since the initial dissolved oxygen concentration of the effluent at the time of sampling was 0.0 mg/l (Mr. Dennis Murrin, personal communication), the higher test concentrations were aerated for 10 minutes prior to loading the organisms, and continuously throughout the tests. This constant aeration increased the dissolved oxygen levels in these chambers to near saturation (Tables 2 and 3). The aeration may have reduced the toxicity of this effluent by stripping it of highly volatile toxic substances (Peltier 1978, FDER 1983). Additionally, the aeration apparently increased the pH of the affected test concentrations at least a full unit or more (Tables 2 and 3), further adding an unknown factor in evaluating the toxicity of Alton's effluent. Therefore, the Biology Section recommends continued bioassay testing of this facility, including the use of more definitive 96 hour static renewal and flow-through bioassays, to more properly investigate the toxic characteristics of this effluent.

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