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BIOASSAY OF ALTON BOX BOARD COMPANY

JACKSONVILLE, DUVAL COUNTY, FLORIDA

NPDES #FL0000892

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Biological Section
Bureau of Water Analysis
October 21, 1981

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EXECUTIVE SUMMARY

Bioassay of Alton Box Board Company (9 to 11 September 1981)

To evaluate the existence and extent of toxicity of this facility's wastewater discharge to the biota of the receiving water the Biological Section performed a static acute toxicity bioassay on a grab sample of effluent. Since Alton Box Board discharges to saline waters (St. Johns River, salinity = 17 ppt), Mysidopsis bahia (mysid shrimp) was selected for use in this test.

This particular grab sample of effluent was toxic to the test organism. The 48 hour LC_{50} was calculated to be 15.7%. The cause of this toxicity can be attributed, at least in part, to the unionized ammonia content. However, ammonia may not be the sole toxic agent.

INTRODUCTION

The Alton Box Board Company is located at 1915 Wigmore Street in Jacksonville, Duval County, Florida (Fig. 1). A diagram of this facility is presented in Figure 2 (from NPDES files). Using pine wood as the raw material, Alton Box Board produces 707 tons per day of Kraft pulp and liner board. In the manufacturing process, 5 MGD of wastewater is generated and subsequently discharged to the St. Johns River. In 1977, this facility was cited for exceeding the permit discharge limitations for biochemical oxygen demand and total suspended solids (NPDES permit files).

To evaluate the effect of Alton Box Board's wastewater discharge on the biota of the receiving water, the Biological Section conducted a static acute toxicity bioassay on 9 to 11 September, 1981. The results of this test are presented here.

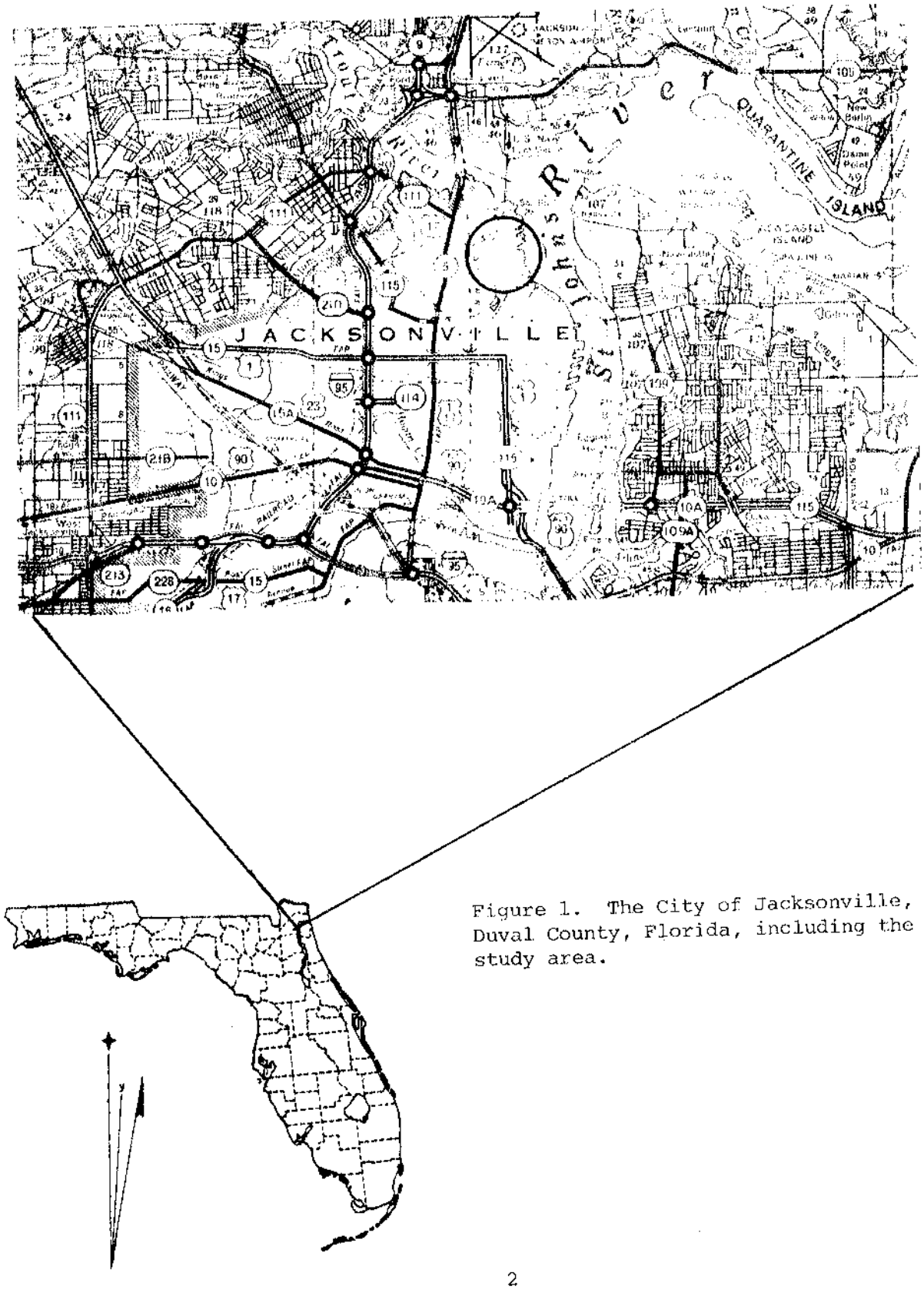


Figure 1. The City of Jacksonville, Duval County, Florida, including the study area.

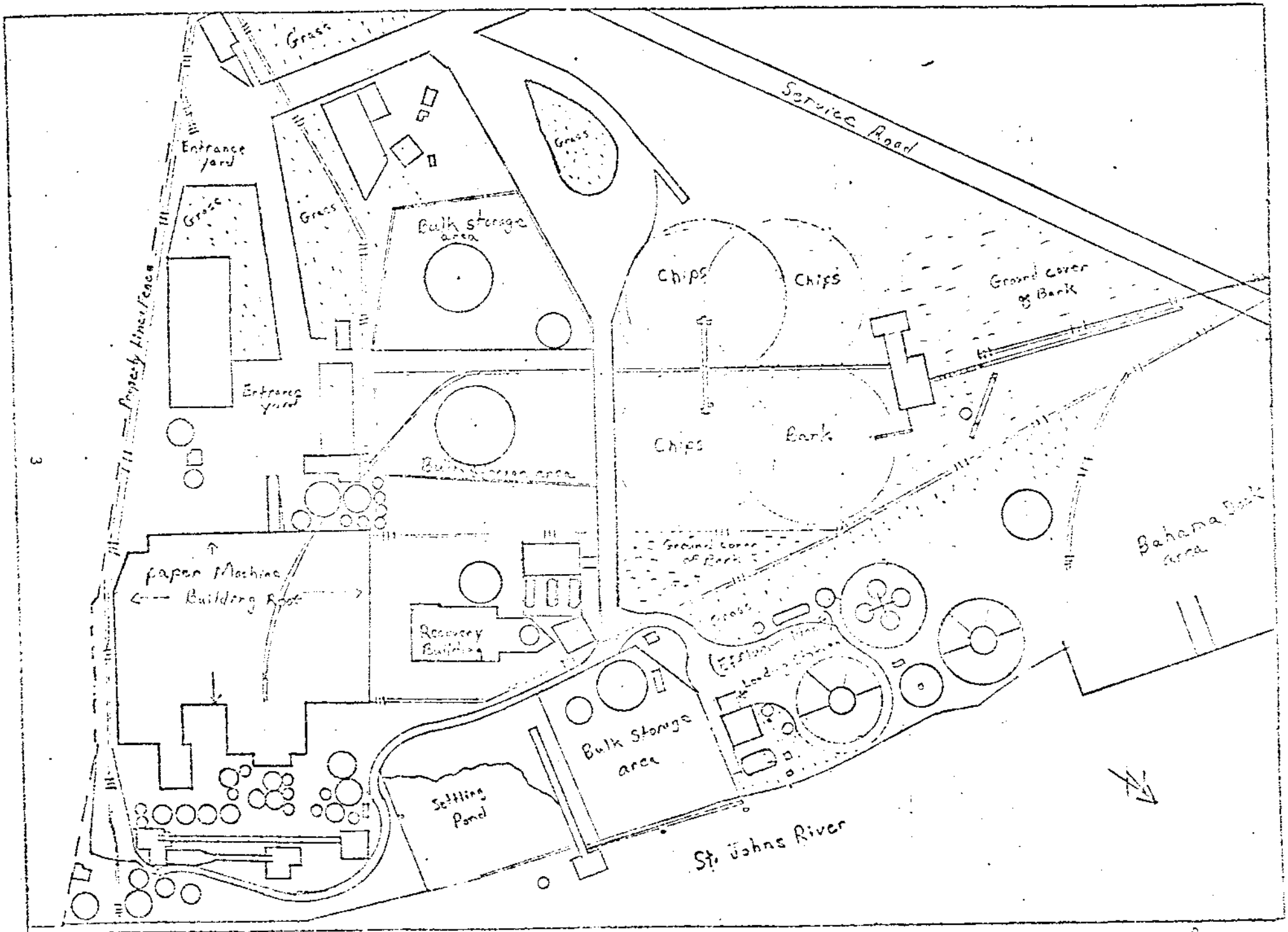


Figure 2. Alton Box Board Company Scale 1"=200'

METHODS AND MATERIALS

Test methods used to conduct this static bioassay are extensively described by Peltier (1978). The organism selected for this study is among those recommended by Peltier (1978) and the U.S.EPA (1978). The life history and maintenance procedures used to culture them are detailed below.

History and Maintenance of Bioassay Organisms

Mysidopsis bahia (mysid shrimp) - In September, 1978, approximately 300 adult mysid shrimp were obtained from the Gulf Breeze EPA Laboratory in Pensacola, Florida. The mysids have been cultured under continuous lighting 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 $22^{0} \pm 1^{0}$C. Immediately prior to the bioassay, a suitable number of mysids were transferred by net to a culture dish for observation and selection of individuals for the test. Fifteen day old (three-quarter adult size) mysids were used in this test. Organisms selected for testing were transferred individually by glass tube and randomly placed in the test chambers.

To ensure the uniform sensitivity of these test organisms to toxic substances, the Biological Section conducts monthly Standard Reference Toxicant Bioassays using sodium lauryl sulfate as the reference toxicant.

Test Methods

A 48 hour static bioassay was conducted from 1100 hours on 9 September to 1100 hours on 11 September, 1981. A grab sample of effluent (Outfall #001) was collected by Mr. Fred Birnie of DER's St. Johns River Subdistrict at 1330 hours on 8 September, 1981, using standard wastewater sampling procedures. The unpreserved sample was iced and transported to the DER laboratory in Tallahassee for testing within 24 hours of its collection.

Saltwater for dilution of the effluent sample was obtained from the FSU Marine Laboratory at Turkey Point (salinity = 30 ppt). This saltwater diluent was then adjusted to the salinity of the receiving body of water (St. Johns River = 17 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 (16.8 ppt to 8.5 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 toler-

ances). A series of three control groups (Table 2) 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: adjusted Turkey Point seawater (salinity = 17 ppt), artificial sea salt (Ocean 50®) adjusted DER well water (salinity = 7 ppt), and a salinity control consisting of adjusted Turkey Point seawater and DER well water (salinity = 7 ppt).

Two replicates of each test concentration and the controls were used in this static test. Immediately prior to the bioassay, 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 via 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 3 ppt salinity. The concentration series selected for this study (Table 1) are those recommended by the APHA (1976).

Five organisms were loaded per test chamber, giving ten per concentration. Temperature, pH, dissolved oxygen, and the number of live organisms were recorded at 0, 24, and 48 hours for each test chamber (Table 2). Additionally, ammonia concentrations[1] (including the total expressed in mg/l as N and the unionized fraction expressed in mg/l as NH_3) were determined for a control, a mid range, and a 100% test concentration at the beginning and end of the bioassay. According to EPA (1978), "Although it is generally undesirable to feed most fish and macroinvertebrates during static toxicity tests, an exception is necessary for M. bahia". Therefore, the mysids were fed brine shrimp (Artemia) nauplii at the rate of approximately 10 to 20 per mysid shrimp to minimize cannibalism.

[1] The total ammonia content was determined with an Orion specific ion probe and a Corning Model 10 pH/mv meter. The unionized fraction was then calculated based on pH, temperature and salinity.

Table 1. Concentration Series and Volumes Used for This Static Bioassay.

Concentration	Dilution Water	Effluent	Total Vol.
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%	---	500 ml	500 ml

Table 2. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of the Alton Box Board Company, Jacksonville, Duval County, Florida, NPDES #FL0000892, Outfall #001 on 9 to 11 September, 1981.

Test Concentrations

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
5.6%	5	5	5	7.1	6.4	4.9	7.8	7.8	7.6	20.9	20.6	21.8				
5.6%	5	5	5	7.2	6.4	5.3	7.8	7.8	7.6						16.8	16.0
10%	5	5	5	7.2	6.4	5.0	7.8	7.8	7.6	20.8	20.5	21.7				
10%	5	5	5	7.2	6.4	5.1	7.8	7.8	7.6						16.0	15.8
18%	5	3	1	6.5	6.9	4.1	7.8	7.9	7.7	20.7	20.4	21.6	---	.07/3.5		
18%	5	3	2	6.5	5.9	4.2	7.8	7.8	7.6						14.8	14.5
32%	5	0	0	6.5	6.3	5.0	7.8	7.9	7.7	20.6	20.2	21.5				
32%	5	1	0	6.4	6.2	5.5	7.8	7.9	7.8						12.0	12.0
56%	5	0	0	5.0	5.0	5.2	7.9	8.0	7.9	21.1	20.1	21.3				
56%	5	0	0	5.0	5.1	5.2	7.9	8.0	7.9						8.5	8.0
100%	5	0	0	3.5[a]	2.7[b]	4.1	8.2	8.1	8.0	21.5	19.9	21.1	1.17/18.2	0.88/21.7		
100%	5	0	0	3.6[a]	2.5[b]	4.2	8.2	8.2	8.1						7.0	7.0

Remarks:
a. This test concentration was aerated for 20 minutes (to saturation) prior to loading the test organisms. The values were recorded prior to aeration.
b. Since this dissolved oxygen level is below 60% saturation, this test concentration was not included in the lethality calculations.

Table 2. Continued

Control Series

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
Seawater Control	5	5	5	7.0	7.0	6.5	7.8	7.9	7.6	20.9	19.5	20.9	.02/.84[b]	0/.08		
Seawater Control	5	5	5	7.0	6.6	6.5	7.8	7.8	7.6						17.0	17.0
Sea Salt Control	5	5	5	7.8	7.2	6.6	8.6	8.1	7.8	21.0	19.5	20.9				
Sea Salt Control	5(4)[a]	4	4	8.0	7.2	6.4	8.6	8.2	7.9						7.0	7.5
Salinity Control	5(4)[a]	4	4	8.0	7.2	5.4	8.0	8.0	7.8	21.0	19.6	21.0				
Salinity Control	5	5	5	8.1	7.2	6.1	8.0	7.9	7.8						7.0	7.3

Remarks: a. Number in parentheses used in calculations since one death was due to cannibalism.
b. These ammonia values are suspect due to membrane clogging. Since these ammonia concentrations are still well below the toxic threshold the sample was not retested with a new membrane.

RESULTS AND DISCUSSION

This particular grab sample of effluent from the Alton Box Board Company was toxic to the test organism (Mysidopsis bahia). The 24 hour LC_{50} ranged from 19.9% to 20.3% and the 48 hour LC_{50} was calculated to be 15.7% (Table 3). The 96 hour LC_{50} for this effluent sample would therefore be less than or equal to 15.7%. Note that since the dissolved oxygen content in the 100% test containers dropped to below 60% saturation within 24 hours (Table 2), this concentration was not used in the LC_{50} calculations.

The cause of toxicity in this effluent sample may be partly attributed to the substantial levels of ammonia. Since unionized ammonia concentrations ranging between .2 and 2.0 mg/l as NH_3 have demonstrated toxicity to freshwater fish (U.S. EPA 1976, Thurston and Russo 1981) we expect the concentrations found in this sample (1.17 to 0.88 mg/l as NH_3, Table 2) to be toxic to saltwater organisms. However, ammonia may not be the sole toxic substance involved since the 18% concentration (unionized ammonia = 0.07 mg/l as NH_3, Table 2) also showed partial mortality. Without further chemical characterization of Alton Box Board's discharge, it is impossible to attribute all of the mortality observed in this test to specific toxic substances.

Chapter 17-3.051, Florida Administrative Code, indicates that "all waters of the State shall at all places and at all times be free from...components of discharges which...are acutely toxic." "Acute toxicity" is defined in Chapter 17-3.021(1) to mean the "the presence of one or more substances...in amounts which are greater than one-third (1/3) of the amount lethal to 50% of the test organisms in 96 hours (96 hour LC_{50})...". In the absence of a mixing zone, this 1/3 of the 96 hour LC_{50} applies at the point of discharge. Chapter 17-4.244(4) states that "in no event shall the maximum concentration of wastes in the mixing zone exceed the amount lethal to 50% of the test organisms in 96 hours (96 hour LC_{50}) for a species significant to the indigenous aquatic community...". This criterion applies at the point of discharge if there is a mixing zone. The distinction between presence or absence of a mixing zone is not relevant here, however, since the testing procedure used does not involve effluent concentration. The 1/3 of the 96 hour LC_{50} criterion is three times as stringent as the 96 hour LC_{50} criterion, and therefore, in the absence of a mixing zone, the procedure used is very lenient. The procedure followed is also lenient in that testing is only done for 48 hours. The 96 hour LC_{50} derived from this testing is therefore reported as "less than or equal to" the 48 hour measurement. The value found after 48 hours can never be more restrictive than the value found after 96 hours. It must be noted that all organisms 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.

Table 3. LC_{50} Data for Bioassay of Alton Box Board Company on 9 to 11 September 1981.

Organism	Method of Data Analysis	24 hour LC_{50} and 95% confidence intervals	48 hour LC_{50} and 95% confidence intervals
Mysidopsis bahia	Binomial	19.9% (10 and 32)	15.7% (10 and 32)
	Moving Average	20.5% (16 and 27)	A
	Probit	20.3% (16 and 26)	A

A. Due to an irregular distribution of mortality, an LC_{50} value cannot be calculated using this method of statistical analysis.

LITERATURE CITED

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