BIOASSAYS OF [redacted] PACKAGING COMPANY

JACKSONVILLE, DUVAL COUNTY, FLORIDA

NPDES #FL0000892

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Biology Section
Bureau of Water Analysis
August 27, 1982

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EXECUTIVE SUMMARY

Alton Packaging Company, Jacksonville, NPDES #FL0000892, on 14 to 16 July, 1982.

The sample of effluent from this discharger, a manufacturer of linerboard and paper pulp, was acutely toxic to Mysidopsis bahia and to Daphnia pulex. A 48 hour LC_{50} of 141.7% was determined for the Mysidopsis bioassay. Unfortunately, the Daphnia bioassay was invalidated due to missing organisms and to excessive control mortality (data not reported). Ammonia was present in the effluent, in sub-lethal concentrations. Chemical analyses for organic compounds indicated the presence of numerous compounds identified as belonging to the general class of terpenes, potentially large concentrations of numerous other unidentified compounds (none containing halogens), and butyl benzyl phthalate (0.4 µg/l). The analyses for metals indicated the presence of significant concentrations of arsenic (22 µg/l), cadmium (0.1 µg/l), chromium (77 µg/l), copper (32 µg/l), lead (14 µg/l) and zinc (124 µg/l), and, trace amounts of mercury and selenium. The presence of these organic compounds and metals may well have caused the observed toxicity.

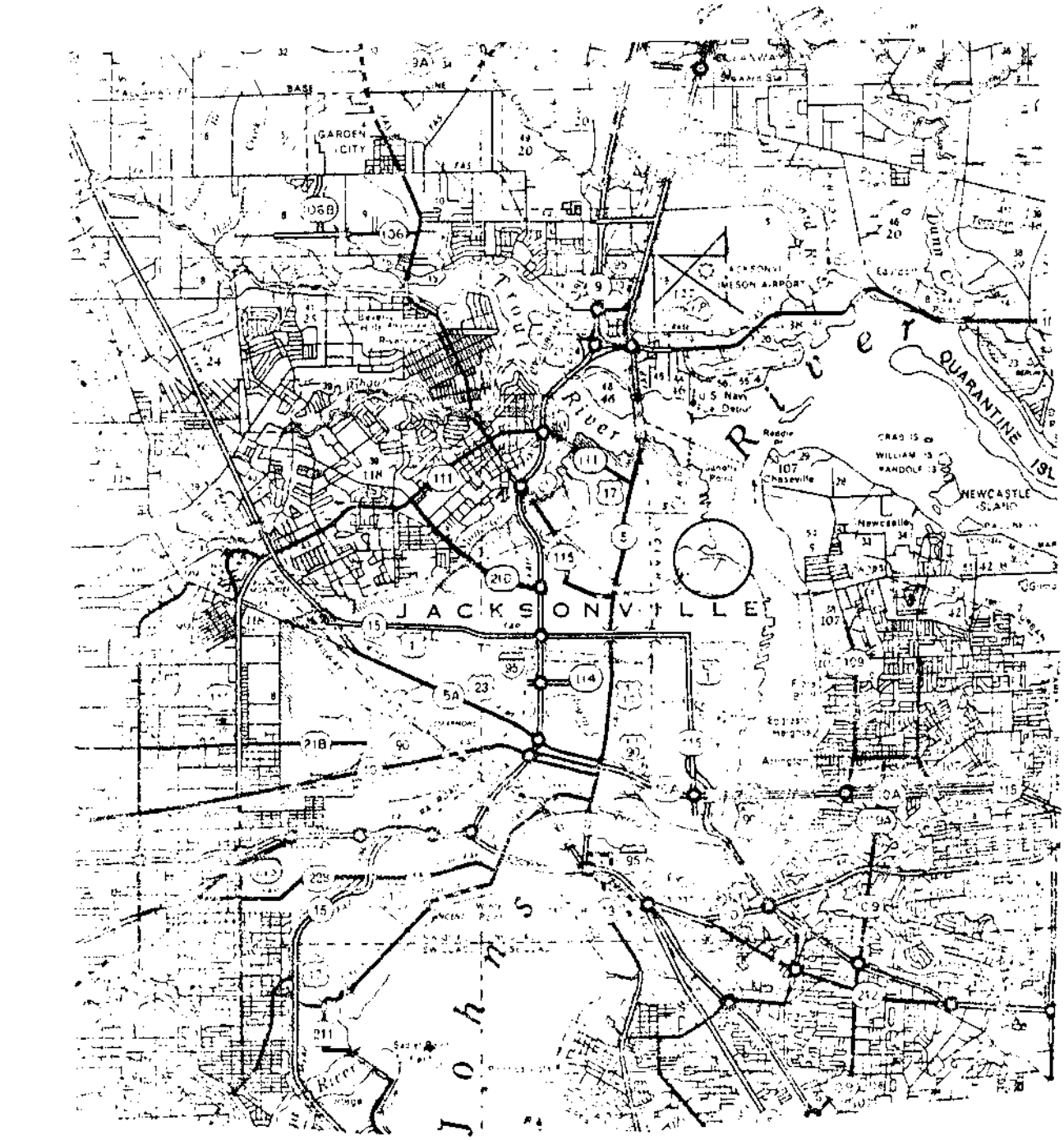
INTRODUCTION

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 Biological 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 1982).

Therefore, to again evaluate the effects of this effluent, two 48 hour static acute toxicity bioassays were performed on 14 to 16 July, 1982. Organisms selected for these tests were again Mysidopsis bahia, to repeat the earlier tests, and Daphnia pulex, since the salinity of the St. Johns River had decreased below 5 ppt due to recent rainfall. The results of these tests are presented in this report.



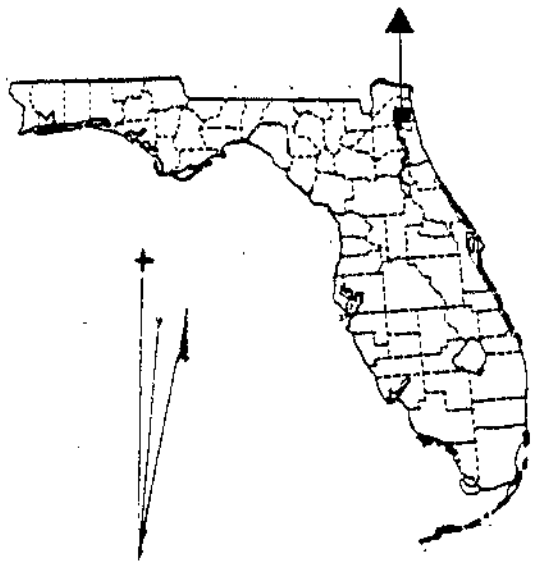


Figure 1. Portions of Jacksonville, Duval County, Florida, including study area.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978). The organisms selected for this study are among those recommended by Peltier (1978) and the U.S. EPA (1978). 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 liqufied blend of Purina Trout Chow® and Cerophyl® twice daily, and are kept at a constant temperature of 22° plus or minus 1°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms. They are then refilled 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.

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 16:8 hour lighting in 10 to 15 gallon all glass aquaria equipped with undergravelfiltration 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 1°C. Ten to fifteen day old (three-quarter adult size) mysids were used in this test.

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

Two separate 48 hour static acute toxicity bioassays were performed on a sample of effluent collected from Alton's NPDES sampling point. The tests were performed from 1415 hours on 14 July to 1500 hours on 16 July, 1982.

The sample was collected by Mr. Charles Love of DER's Jacksonville Office at 1515 hours on 13 July, 1982, using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples for metal analysis were preserved with HNO_3. Samples for organic chemical analysis and for the bioassays were unpreserved. The samples were iced and transported to the DER laboratory in Tallahassee for analysis within 24 hours of collection.

Since the St. Johns River was classified as freshwater (salinity= 2ppt) at the time of sampling, the effect of Alton's effluent on freshwater organisms was determined by testing with Daphnia pulex. Freshwater for dilution was obtained from the DER culture water well.

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 Daphnia were siphoned through a stainless steel screen. The trapped adults were then discarded. These neonates were then transferred by glass pipette, examined for injury, and were loaded 10 per chamber, resulting in 20 per test concentration. Water fleas were not fed during this test.

Since the St. Johns River is usually saline (salinity >5 ppt), and the purpose of this test was to repeat the earlier invalidated bioassay, Mysidopsis bahia was used as the second test organism. 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 St. John's River at the time of the earlier bioassay (16 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 (11.2 ppt to 15.0 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). A series of 3 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: adjusted Turkey Point seawater (salinity=16 ppt), artificial sea salt (Ocean 50®) adjusted well water (salinity=6.8 ppt), and a salinity control consisting of Turkey Point seawater and well water (salinity= 7.4 ppt). The concentration series selected for this study (Table 1) is that recommended by the APHA (1980).

Two replicates of each 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 test. 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 10 per concentration. Based on the recommendations of EPA (1978), the mysids were fed bring shrimp (Artemia) nauplii at the rate of approximately 10 to 20 nauplii per mysid per day to minimize cannibalism.

Dissolved oxygen, pH, temperature, 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 of each test concentration was recorded at the beginning and end of the test. Ammonia concentrations, including total and unionized fractions (expressed in mg/l as N and as NH_3, respectively), were recorded in the controls and the 100% effluent test concentrations at the beginning and end of the test. Total ammonia concentrations were determined using an Orion® specific ion probe and an Orion Ionalyzer® meter. The unionized fraction was then determined 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

Daphnia pulex (water flea)

Concentration	Dilution Water	Effluent	Total Volume
Control	200 ml	---	200 ml
5.6%	188.8 ml	11.2 ml	200 ml
10.0%	180 ml	20 ml	200 ml
18.0%	164 ml	36 ml	200 ml
32.0%	136 ml	64 ml	200 ml
56.0%	88 ml	112 ml	200 ml
100.0%	--	200 ml	200 ml

Mysidopsis 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, Mysidopsis bahia, static acute toxicity bioassay of Alton Packaging Company, Jacksonville, Duval County, Florida, NPDES #FL0000892, on 14 to 16 July, 1982.

Concentration or %	Number of Live Organisms			Dissolved Oxygen[a] (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	7.9	8.0	7.7	8.0	8.0	8.0	21.3	21.0	21.4			15.0	15.5
5.6%	5	5	5	7.9	8.0	6.5	8.0	8.0	7.9							
10.0%	5	5	5	8.0	8.0	7.7	8.0	8.1	8.1	21.4	21.1	21.3			14.5	15.0
10.0%	5	5	5	7.7	8.0	7.7	7.9	8.1	8.0							
18.0%	5	5	5	7.9	8.0	8.0	7.9	8.1	8.1	21.4	21.2	21.2			13.5	13.0
18.0%	5	5	5	7.9	8.0	8.0	8.1	8.1	8.1							
32.0%	5	4	4	7.7	7.9	8.2	7.9	8.2	8.2	21.3	21.1	19.7			11.2	11.5
32.0%	5	5	5	7.6	8.1	8.2	7.8	8.2	8.2							
56.0%	5	5	5	7.4	7.6	8.3	8.1	8.2	8.3	21.1	21.2	21.0			7.7	7.8
56.0%	5	5	5	7.9	7.6	8.3	8.1	8.2	8.3							
100.0%	5	3	3	7.0	7.3	8.2	8.0	8.4	8.5	21.1	21.6	21.2	.08/1.89	.05/.42	7.5	7.5
100.0%	5	3	3	7.1	7.3	8.2	8.1	8.4	8.5							

Remarks: [a] All test chambers were aerated prior to loading the organisms and throughout the 48 hour test.

Table 3 . Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Alton Packaging Company, on 14 to 16 July, 1982.

Control Series[a]

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	8.0	7.5	7.4	8.0	7.9	8.1	20.4	21.4	21.5	.01/.20	.01/.20		
Seawater Control	5	5	5	7.9	7.5	7.4	8.0	7.9	8.1						16.0	16.0
Sea Salts Control	5	5	5	8.3	7.9	8.3	8.6	8.3	8.2	21.4	21.0	21.3				
Sea Salts Control	5	5	5	8.3	8.0	8.3	8.6	8.3	8.2						6.8	7.0
Salinity Control	5	5	5	8.2	8.4	6.9	8.0	8.1	7.9	21.4	20.2	21.4				
Salinity Control	5	5	5	8.2	8.5	6.9	8.0	8.1	7.9						7.4	7.3

Remarks:

[a] Each set of controls was aerated intermittently throughout the 48 hour test.

Table 4 . LC_{50} Data for Bioassays of Alton Packaging Company, NPDES #FL0000892, on 14 to 16 July, 1982.

Organism	Method of Data Analysis	24 LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
D. pulex	Binomial Moving Average Probit	Invalidated	Invalidated
M. bahia	Binomial	a	a
	Moving Average	a	a
	Probit	141.7% (85 and 266471),b	141.7% (85 and 266471), b

[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.

[b] Due to the distribution of mortality, the LC_{50} value calculated by this method of statistical analysis does not meet the criteria established by the Committee on Methods for Toxicity Tests with Aquatic Organisms.

RESULTS AND DISCUSSION

The sample of effluent collected from Alton's NPDES Outfall was acutely toxic to Mysidopsis bahia, producing 24 and 48 hour LC_{50} values of 141.7% (Table 4). All the mortality in this test occurred within 24 hours, suggesting that the constant aeration of the test chambers rendered the effluent non-toxic after 24 hours. Although aeration was necessary, since the initial dissolved oxygen concentration of the effluent was <2.0 mg/1, aeration "can reduce the apparent toxicity of an effluent by stripping it of highly volatile toxic substances" (Peltier 1978). The toxicity of this effluent cannot be attributed to ammonia, since only sub-lethal amounts of total and unionized ammonia were present. The 96 hour LC_{50} for this effluent would be ≤141.7%. Unfortunately, the Daphnia pulex bioassay was invalidated due to missing organisms and to excessive control mortality. The extreme opacity and large amount of sediment in the sample precluded accurate counting of test organisms.

The DER Chemistry Section has analyzed samples of this effluent for certain organic compounds and metals (Table 5). Chemical analyses for organic compounds indicated the presence of numerous compounds identified as belonging to the general class of terpenes, potentially large concentrations of numerous other unidentified compounds (none containing halogens), and butyl benzyl phthalate (0.4 µg/1), The analyses for metals indicated the presence of significant concentrations of arsenic (22 µg/1), cadmium (0.1 µg/1), chromium (77 µg/1), copper (32 µg/1), lead (14 µg/1), and zinc (124 µg/1), and trace amounts of mercury and selenium. Although individually these concentrations of metals are below levels reported to be acutely toxic to aquatic organisms (U.S. EPA 1976), they may have acted synergistically to have contributed to the observed toxicity. Therefore, the toxicity of this effluent may be attributed to the additive effects of the numerous organic compounds and metals present.

Since this facility has no provision for a mixing zone (Mr. Fred Birnie, personal communication), the LC_{50} value obtained for this effluent (141.7%, Table 4) is in violation of Chapter 17-3.021(1), which prohibits discharges that are acutely toxic. Acute toxicity is defined in LC_{50} terms as being ≤300%. Please see below for further discussion of the toxicity criteria in the Florida Administrative Code.

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 (3), 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 sustance 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, whether as 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)(o)); 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, but rather to the average of a number of points within 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 the mixing zone (i.e., at any point within the mixing zone), from exceeding the 96 hour LC_{50}. Therefore, at any point within a mixing zone, including the point of discharge, one-half of the test organisms cannot be killed in 96 hours.

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} standard. 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, 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.

Table 5. Results of Chemical Analyses Performed on Effluent from Alton Packaging Company, NPDES #FL0000892, collected 13 July, 1982.

Organics

Parameter	Compound
Acid Extractables	1. Numerous compounds detected by GC[a]
Base Neutral Extractables	1. Numerous compounds detected by GC[a]
Pesticide Extractables	1. Numerous compounds in large concentrations detected by GC[b]
	2. Butyl benzyl phthalate (0.4 µg/l)

Metals

Antimony	N.D.
Arsenic	22 µg/l
Beryllium	N.D.
Cadmium	0.1 µg/l
Chromium	77 µg/l
Copper	32 µg/l
Lead	14 µg/l
Mercury	<0.2 µg/l[c]
Nickel	N.D.
Selenium	<10 µg/l[c]
Silver	N.D.
Thallium	N.D.
Zinc	124 g/l

N.D. = None Detected

[a]Identified by GC/MS as belonging to the general class of terpenes.

[b]None of these compounds contained halogens.

[c]Actual value is known to be less than value given.

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