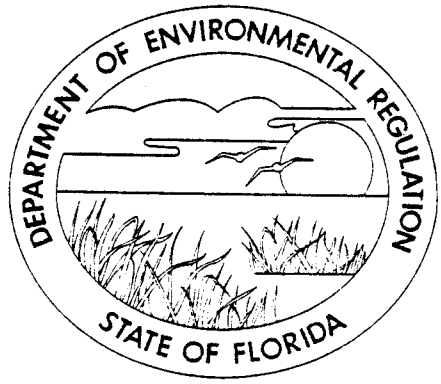


BIOASSAYS OF ARIZONA CHEMICAL COMPANY

STORMWATER RETENTION AREA OVERFLOW DITCH

AND SALTWATER DITCH OUTFALL TO ST. ANDREW BAY

PANAMA CITY, BAY COUNTY, FLORIDA

NO NPDES PERMIT

SAMPLED 11/3/81

Biological Section
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EXECUTIVE SUMMARY

Arizona Chemical Company/Saltwater Ditch Outfall - 4 to 6 November, 1981.

As a special request from the plant manager, and to follow up on the previous toxic bioassay reported at this site (FDER, 1981), the Biological Section performed two (2) 48 hour static acute toxicity bioassays using Mysidopsis bahia as the test organism. One (1) bioassay was performed on stormwater/wastewater collected from an overflow ditch as it left the Arizona Chemical Company's property. A second was performed on effluent entering St. Andrew Bay from the Saltwater Ditch Outfall, on Southwest Forest Industries' property. Results indicated that the stormwater/wastewater from the Arizona Chemical Company's overflow ditch was acutely toxic to the shrimp (48 hour LC_{50}'s = 25%). Effluent from the saltwater ditch was less toxic--some mortality was noted, however, a statistically sound LC_{50} could not be calculated. The toxicity may be due to one or more of the wide variety of organic compounds present in the samples.

INTRODUCTION

The Arizona Chemical Company is located next to the Southwest Forest Industries paper mill, in eastern Panama City, Bay County, Florida (Figure 1). It uses a tall oil byproducts stream from the paper mill to produce tall oil, resins, soap, esters, pinene, anethole, synthetic pine oil and various other related substances. Process wastewater is combined with wastewater from the paper mill and is pumped beneath St. Andrew Bay to the Bay County Wastewater Treatment Plant for secondary treatment. Stormwater falling on the site, including a substantial amount from the surrounding residential area, drains into a stormwater holding pond on the Arizona Chemical Company's property. This pond may also contain a certain amount of wastewater from runoff, spills, leakage, etc., from the site. Additionally, since this pond was once used to contain process wastewater before the present wastewater treatment system was installed, it may also contain residues from sludges, oils and other waste products. An overflow ditch drains this pond southward off the Company's property and towards St. Andrew Bay. Before it reaches the Bay, this overflow stream is joined by one or more streams of unknown origin from the paper mill. This combined discharge then broadens into the saltwater ditch, which has a continuous discharge to St. Andrew Bay (Figure 2).

The Biological Section had previously analyzed samples from this site in April, 1981 (FDER, 1981). Samples for chemical analyses were collected from the Arizona Chemical Company's overflow ditch at that time, and samples for both chemical and biological analyses were collected from the saltwater ditch outfall to St. Andrew Bay. Results indicated that the sample from the overflow ditch contained 13 organic compounds, including 2778 ug/l of 4-propyl phenol (FDER, 1981). Unfortunately, biological testing for toxicity was not performed on this sample. The saltwater ditch sample contained 5 organic compounds, including 589 ug/l of 4-propyl phenol, and was acutely toxic to Mysidopsis bahia with 24 and 48 hour LC_{50}'s of 53.3% to 60.3% (FDER, 1981). Toxicity was attributed to the synergistic effects of the organic compounds, low dissolved oxygen and to the presence of hydrogen sulfide. In that report it was incorrectly stated that the saltwater ditch was on Arizona Chemical Company property, when in fact, it is on Southwest Forest Industries' property. However, the results of the chemical analysis had suggested that some of the organic compounds, including the phenol, may have originated from the Arizona Chemical Company.

To identify the origin of the toxic discharge to St. Andrew Bay, Mr. Mike Warrell of Arizona Chemical Company requested additional chemical and biological analyses from the sites in question. On November 3, 1981, Mr. Charles Biedermann and Mr. Martin Roll of the DER Biological Section collected samples for both chemical and biological analyses from the overflow ditch as it left Arizona Chemical Company's property, and from the outfall to St. Andrew Bay from the saltwater ditch on Southwest Forest Industries' property. Mr. Warrell and Mr. Bert Lent (DER's Panama City Branch Office) were present during the sampling. Two (2) 48 hour static acute toxicity bioassays were performed on these samples, on 4 to 6 November, 1981, to evaluate the existence and extent of toxicity to the biota of the receiving waters. The results of both the chemical and biological analyses are presented in this report.

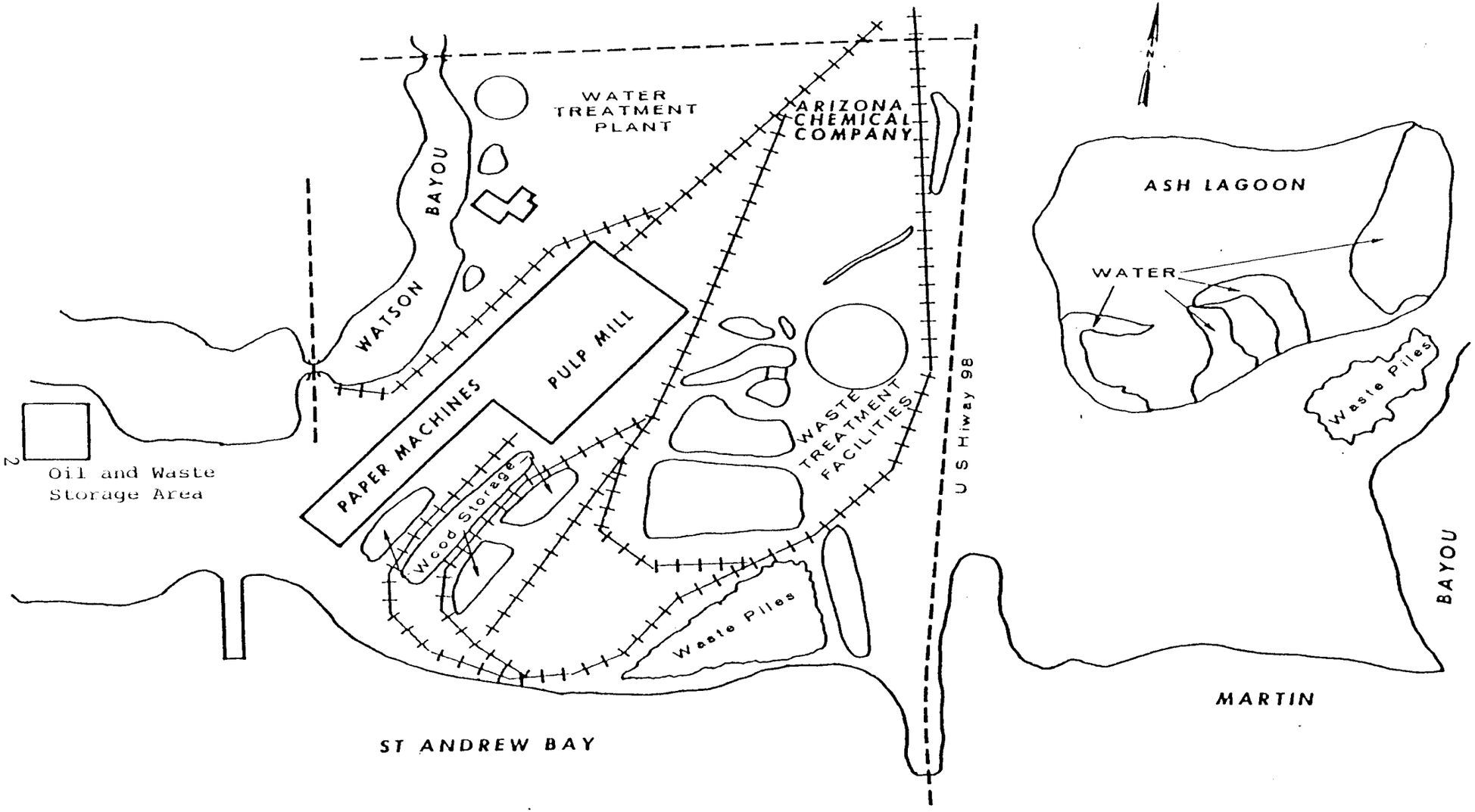


Figure 1. General layout of Southwest Forest Industries/Arizona Chemical Company Complex., Panama City, Bay County, Florida.

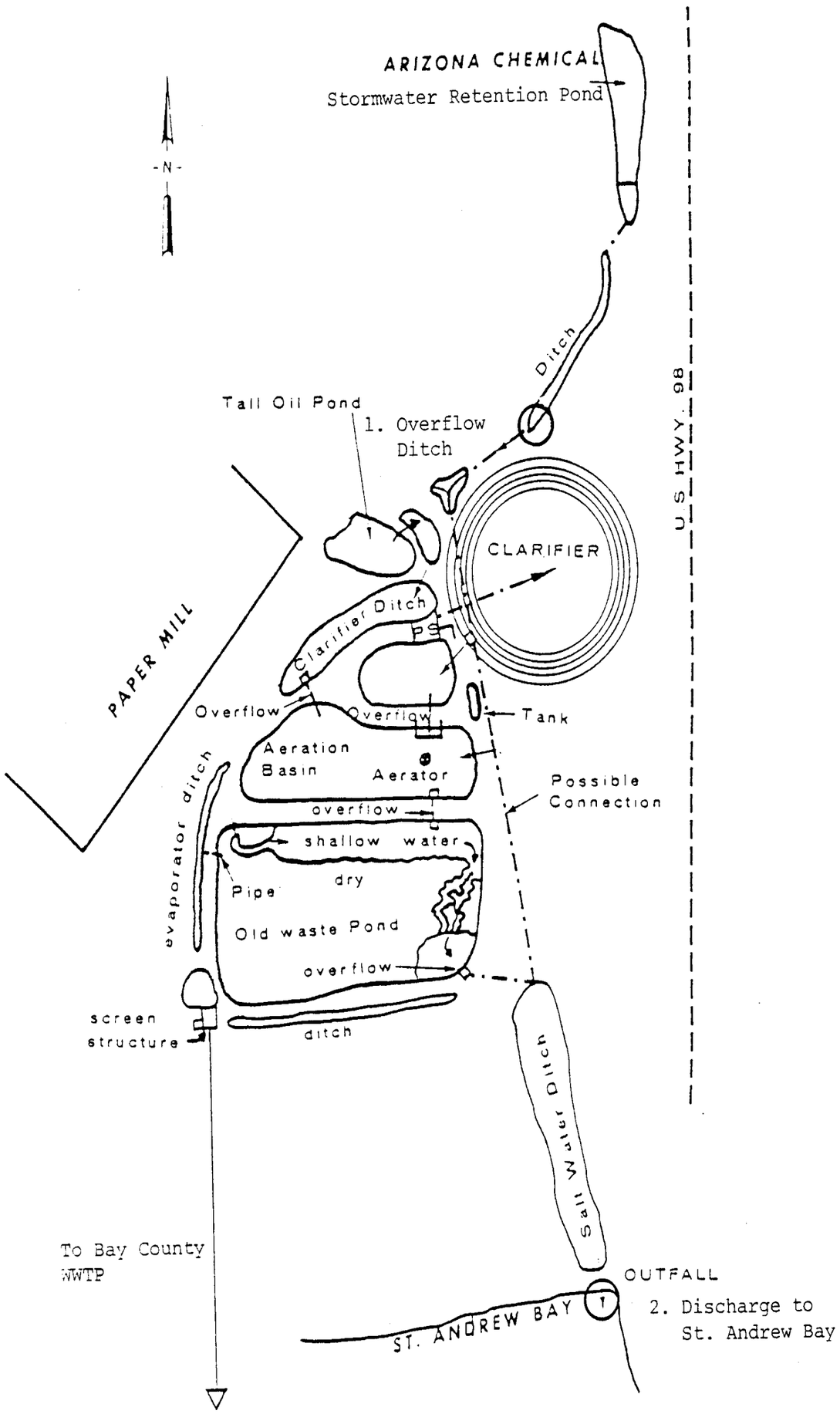


Figure 2. Southwest Forest Industries/Arizona Chemical Company Complex, including sampling locations.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays 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 the organism are detailed below.

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 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° 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 samples collected from the overflow ditch on Arizona Chemical Company property, and from the outfall to St. Andrew Bay from the Saltwater Ditch on Southwest Forest Industries' property. The tests were performed from 0930 hours on 4 November, 1981, to 0950 hours on 6 November, 1981. The samples were collected by Mr. Charles Biedermann and Mr. Martin Roll of DER's Biological Section at 1120 hours on 3 November, 1981, 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.

Saltwater for dilution of the effluent samples was obtained from the FSU Marine Laboratory at Turkey Point (salinity= 30ppt). This saltwater diluent was then adjusted to the salinity of the receiving body of water (St. Andrew Bay = 27ppt) 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 (12ppt to 25ppt). For the 100% effluent test concentrations, freshly autoclaved artificial sea salts (Ocean 50®) were added until salinities of approximately 7ppt were 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= 27ppt), artificial sea salt (Ocean 50®) adjusted well water (salinity= 9ppt), and a salinity control consisting of Turkey Point seawater and well water (salinity= 12.5ppt). 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, 15, 20 and 25ppt salinity aquaria to culture dishes for observation and selection of individuals for the tests. Ten to fifteen day old (three-quarter adult size) mysids were used in these 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, pH, temperature, and the number of live organisms were recorded for each test chamber at 0 hours, 24 hours, and 48 hours. Additionally, the number of live organisms was counted 5.5 hours into the test since early mortality was noted. The 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 seawater control and the 100% test concentrations at the beginning of the test. Total ammonia concentrations were determined using an Orion® specific ion probe and a Corning® pH/millivolt 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, 3 and 4.

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

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 Arizona Chemical Company and overflow ditch, Panama City, Bay County, Florida, on 4 to 6 November, 1981.

Concentration or %	Number of Live Organisms				Dissolved Oxygen (mg/l)			pH			Temperature (°C)			NI_3/NI_3+NI_4 (mg/l)		Salinity (ppt)	
	0	5.5	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
5.6%	5	5	5	5	7.6	7.7	6.5	8.0	7.9	7.9	21.5	21.3	21.3				
5.6%	5	5	5	5	7.6	7.6	6.5	8.0	7.9	7.9						25.0	26.0
10.0%	5	5	5	5	7.5	7.4	6.4	7.9	7.9	7.9	21.3	21.3	21.2				
10.0%	5	5	5	5	7.5	7.4	6.3	7.9	7.9	7.9						24.0	25.0
18.0%	5	5	5	4	7.2	7.5	6.0	7.9	7.9	7.9	21.0	21.3	21.1				
18.0%	5	5	5	4	7.2	7.4	6.2	7.9	7.9	7.9						22.0	23.0
32.0%	5	5	2	1(2) [a]	6.5	7.4	5.5	7.8	7.9	7.8	20.4	21.3	21.1				
32.0%	5	5	3	1	6.5	7.4	5.6	7.8	7.9	7.8						18.3	19.5
56.0%	5	2	0	0	4.8[b]	5.7	5.0	7.7	7.8	7.8	22.3	21.3	21.1				
56.0%	5	5	0	0	4.8[b]	5.7	5.0	7.7	7.8	7.8						12.0	13.0
100.0%	5	1	0	0	2.3[b]	2.6	3.9	8.4	7.9	8.0	21.6	21.4	21.3	.01/.07	—		
100.0%	5	1	0	0	2.2[b]	2.5	3.9	8.4	7.9	8.0						8.5	9.5

Remarks: a. Number in parentheses used in calculations since one organism jumped out between 24 and 48 hours.
b. These test chambers aerated to >60% saturation. Values recorded prior to aeration and to loading the organisms.

Table 3. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Arizona Chemical Company and saltwater ditch on Southwest Forest Industries' property, Panama City, Bay County, Florida, on 4 to 6 November, 1981.

Concentration or %	Number of Live Organisms				Dissolved Oxygen (mg/l)			pH			Temperature (°C)			NI_3/NI_3+NI_4 (mg/l)		Salinity (ppt)	
	0	5.5	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
5.6%	5	5	5	5	7.6	7.4	6.4	8.0	7.9	8.0	21.7	21.6	21.5				
5.6%	5	5	5	5	7.6	7.4	6.4	8.0	7.9	8.0						25.0	26.0
10.0%	5	5	5	5	7.4	7.4	6.4	8.0	8.0	8.1	21.5	21.6	21.5				
10.0%	5	5	5	5	7.4	7.4	6.5	8.0	8.0	8.1						24.5	25.5
18.0%	5	5	5	5	7.2	7.4	6.6	7.9	8.0	8.1	21.3	21.6	21.5				
18.0%	5	5	5	5	7.2	7.4	6.6	7.9	8.0	8.1						23.0	24.0
32.0%	5	5	5	5	6.7	7.4	6.8	7.9	8.0	8.2	21.1	21.7	21.6				
32.0%	5	5	5	5	6.7	7.4	6.8	7.9	8.0	8.2						20.5	21.5
56.0%	5	5	5	5	5.6	7.3	7.0	7.8	8.1	8.3	20.6	21.7	21.6				
56.0%	5	5	5	5	5.6	7.3	7.1	7.8	8.1	8.3						16.0	17.0
100.0%	6[a]	6	6	6	3.3[b]	7.3	8.0	7.8	8.3	8.5	20.1	22.1	22.0	0/.12	—		
100.0%	5	2	1	1	3.3[b]	7.3	8.1	7.8	8.3	8.5						7.0	7.5

Remarks: a. Extra organism loaded by mistake, allowed to remain.
b. These test chambers aerated to >60% saturation. Values recorded prior to loading the organisms.

Table 4. Control Series for Arizona Chemical Company, bioassays of 4 to 6 November, 1981

Concentration or %	Number of Live Organisms				Dissolved Oxygen (mg/l)			pH			Temperature (°C)			NI_3/NI_3+NI_4 (mg/l)		Salinity (ppt)	
	0	5.5	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
Seawater Control	5	5	5	5	7.4	7.5	6.8	7.9	8.1	7.9	21.9	21.8	21.8	0/.10	—		
Seawater Control	5	5	5	5	7.4	7.5	6.8	7.9	8.1	7.9						27.0	27.8
Sea Salts Control	5	5	5	5	8.5	8.2	7.3	8.7	8.5	8.3	22.0	21.9	21.8				
Sea Salts Control	5	5	5	5	8.5	8.2	7.3	8.7	8.5	8.3						9.0	8.8
Salinity Control	5	5	5	5	8.3	8.1	7.0	8.0	8.0	8.0	21.9	22.1	22.1				
Salinity Control	5	5	5	5	8.3	8.1	7.0	8.0	8.0	8.0						12.5	13.3

RESULTS AND DISCUSSION

The sample collected from the stormwater/wastewater overflow ditch on Arizona Chemical Company's property proved to be acutely toxic to Mysidopsis bahia, with 5.5 to 48 hour LC_{50}'s of 71.3% to 24.9% (Table 5). Therefore, the 96 hour LC_{50} would be less than or equal to the 48 hour value ($\leq$25.3%). The sample collected at the saltwater ditch outfall to St. Andrew Bay, on Southwest Forest Industries' property, was less toxic to the shrimp. Some mortality was noted in the 100% test concentration (Table 3), however, this mortality was not sufficient for the calculation of a statistically sound LC_{50}.

The DER Chemistry Section in Tallahassee analyzed these samples for selenium and organic phenols. Results are presented in Table 6. Trace amounts of selenium was found in the saltwater ditch sample, but it was below the limit for accurate quantification. The organic analysis of the sample from the Arizona Chemical's stormwater ditch indicated that as many as 18 major and minor compounds were present, including a number of substituted phenols, biphenyls, naphthalene, sulfur, furan and ethanone. The analysis of the sample from the saltwater ditch outfall, on Southwest Forest Industries' property, indicated the presence of 7 major and minor compounds, including terpene, biphenyl, ether, ethane and naphthalene. The toxicity noted in the samples may be due to the presence of one or more of these compounds.

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 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, 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 <u>maximum</u> 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 f 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. LC_{50} Data for Bioassays of Arizona Chemical Company and Saltwater Ditch Outfall, 4 to 6 November, 1981.

Organism	Method of Data Analysis	5.5 Hour LC_{50} and 95% Confidence Intervals	24 LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Mysidopsis bahia Arizona Chemical Company overflow ditch.	Binomial	70.3% (32 and ∞)	32.0%(18 and 56)	25.5% (10 and 56)
	Moving Average	70.2% (56 and 100)	a	24.9% (18 and 35)
	Probit	71.3% (56 and 96)	a	25.3% (20 and 32)
Mysidopsis bahia Saltwater Ditch Outfall to St. Andrew Bay	Binomial	a	a	a
	Moving Average	a	a	a
	Probit	a	a	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 Samples from Arizona Chemical Company/Saltwater Ditch Outfall, Panama City, Florida, collected 3 November, 1981.

	Selenium
Arizona Chemical Company Overflow Ditch	Not Detected (10ug/l)[a]
Saltwater Ditch Outfall to St. Andrew Bay	trace[b]

	Organics Compound
Arizona Chemical Company Overflow Ditch	1. 1,1'-biphenyl 2. Biphenyl ether 3. 4-propyl-phenol (46 ug/l)[c] 4. 1,2,3,4-tetrahydro-1,6,8-trimethyl-naphthalene 5. Sulfur 6. 2-butyl tetrahydrofuran 7. 1-(2-furanyl)-ethanone 8. 3-(2-phenylethyl) phenol (43 ug/l)[c] 9. 10 other unidentified compounds
Saltwater Ditch Outfall to St. Andrew Bay	1. Highly condensed terpene 2. Biphenyl ether 3. 1,1-bis(p-ethylphenyl)-ethane 4. 1,2,3,4-tetrahydro-1,6,8-trimethyl-naphthalene 5. 3 other unidentified compounds

[a] Number given is the minimum detection limit.

[b] Trace amount detected, however, the actual concentration is insufficient for accurate quantification.

[c] Estimated concentration.

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