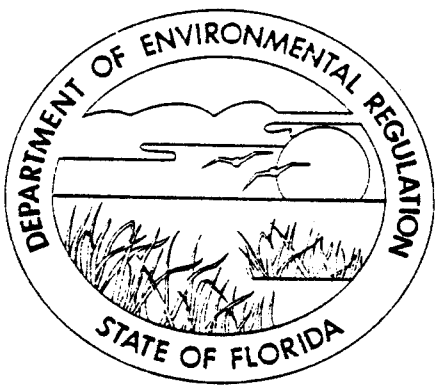


BIOASSAYS OF AMERICAN CYANAMID WASTE DISPOSAL SITE

MILTON, SANTA ROSA COUNTY, FLORIDA

NO NPDES PERMIT

SAMPLED 10/12/81

FILE COPY
DO NOT REMOVE

Biological Section
Bureau of Water Analysis
December 29, 1981

BIOASSAYS OF AMERICAN CYANAMID WASTE DISPOSAL SITE

MILTON, SANTA ROSA COUNTY, FLORIDA

NO NPDES PERMIT

Biological Section
Bureau of Water Analysis
December 29, 1981

EXECUTIVE SUMMARY

American Cyanamid Company, Waste Disposal Site

The American Cyanamid Company, Santa Rosa County, has requested the FDER Northwest District to allow the drainage of an unpermitted waste disposal lagoon to surface waters of the State. At the request of the Northwest District, the Biological Section performed static acute toxicity bioassays on a sample from this lagoon, on 13 to 15 October 1981, to evaluate the effects this discharge would have on the biota of the receiving waters. Results indicated acute toxicity to both fresh and saltwater test species (Daphnia pulex and Mysidopsis bahia, respectively) with 48-hour LC_{50}'s of 18.1% and 16.4%. Based on these results, we recommend that American Cyanamid not be allowed to discharge this waste to surface waters.

INTRODUCTION

The American Cyanamid Company is located near Milton, in Santa Rosa County, Florida (Figure 1). The facility manufactures acrylic fibers and industrial organic chemicals, during which a variety of wastewaters are produced. Non-contact wastewater is discharged to Escambia Bay (NPDES Outfall #001), while concentrated process wastewater is disposed of through a deep well injection system. Additionally, several non-discharging and non-permitted waste disposal lagoons and landfills are maintained on the site.

In February 1980, and again in October, 1980, the Biological Section performed series of static acute toxicity bioassays on effluent from American Cyanamid's Outfall #001 to Escambia Bay. The first series, using Daphnia pulex and Mysidopsis bahia, resulted in acute toxicity to both test species (48-hour LC_{50}'s = 7.2% and 74.8%, respectively, FDER 1980). The second series, using Mysidopsis bahia, was not acutely toxic although some mortality was noted (FDER 1981).

Recently, American Cyanamid requested the FDER Northwest District to allow the discharge of wastes from one of the non-permitted waste disposal lagoons into surface waters of the State. Therefore, the Northwest District requested the Biological Section to perform toxicity analysis (bioassays) on a sample of the waste material, to evaluate the potential effects the discharge would have on the biota of the receiving waters.

Two static acute toxicity bioassays, using Daphnia pulex and Mysidopsis bahia as test organisms, were performed on 13 to 15 October, 1981. Daphnia were used to test the effects the freshwater wastes would have on freshwater organisms, while Mysidopsis were used to test the effects the components of the wastes would have on estuarine and marine organisms. The results of these tests are presented in this report.

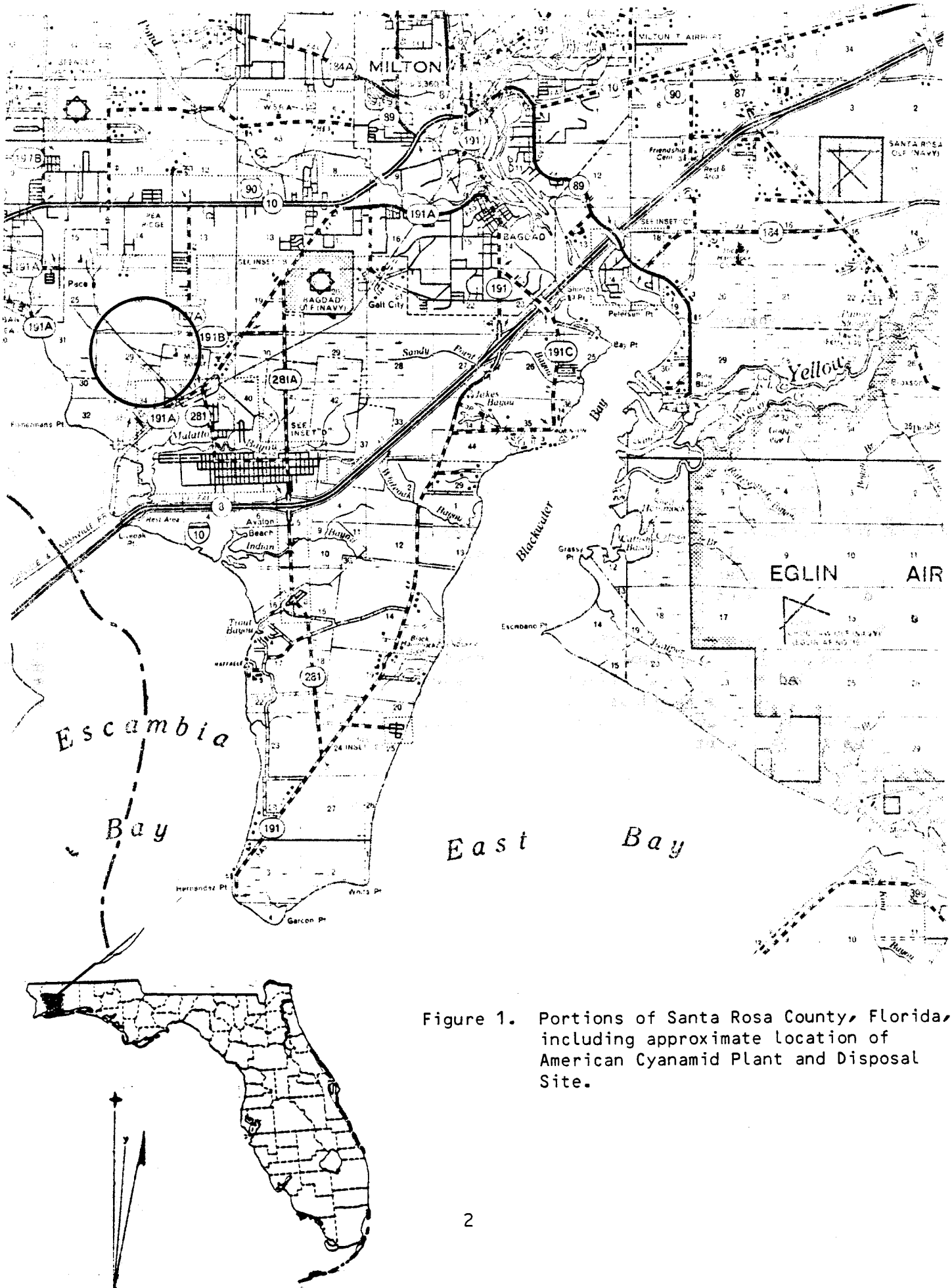


Figure 1. Portions of Santa Rosa County, Florida, including approximate location of American Cyanamid Plant and Disposal Site.

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 liquified 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 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 bioassays were conducted from 1300 hours on 13 October 1981 to 1300 hours, 15 October 1981 on grab samples of water from the waste disposal lagoon. The samples were collected by Mr. Laurence Dunelan of DER's Northwest District Office 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.

Freshwater Test

Freshwater for dilution of the samples 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[1], 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. The 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.

Saltwater Test

Saltwater for dilution of the sample was obtained from the FSU Marine Laboratory at Turkey Point (salinity= 31ppt). This saltwater diluent was then adjusted to the salinity of the receiving body of water (Judges Bayou = 16ppt) by adding an appropriate amount of DER well water. For test concentrations of 56% and below, this adjusted diluent was mixed directly with the sample, achieving a range of final salinities (8.3ppt to 15.5ppt). For the 100% effluent test concentration, freshly autoclaved artificial sea salts (Ocean 50®) were added until salinities of approximately 8ppt were achieved (the lower limit of mysid shrimp salinity tolerance). A series of three 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= 16ppt), artificial sea salt (Ocean 50®) adjusted well water (salinity= 8.3ppt), and a salinity control consisting of Turkey Point seawater and well water (salinity= 7.3ppt).

---

[1] Test concentrations ranged from 3.2% to 56%. A 100% test concentration was not used due to the highly colored nature of the sample, which would have made it impossible to count the Daphnia

Two replicates each of six test concentrations 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 10ppt and 15ppt salinity aquaria to culture dishes for observation and selection of individuals for the test. Ten to fifteen day old (three-quarter adult size) mysids were used in this 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 3ppt 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.

Data Recorded for the Fresh and Saltwater Tests

Dissolved oxygen, pH, temperature, and the number of live organisms were recorded for each test chamber at 0 hours, 24 hours, and 48 hours. Total alkalinity and total hardness were recorded at the beginning of the test for the controls and the 56% sample concentration in the freshwater test. 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 highest test concentrations at the beginning and end 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

Freshwater Test with *Daphnia pulex* (water fleas)

Concentration	Dilution Water	Sample	Total Volume
Control	200 ml	---	200 ml
3.2%	193.6 ml	6.4 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

Saltwater Test with *Mysidopsis bahia* (mysid shrimp)

Concentration	Dilution Water	Sample	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, Daphnia pulex, static acute toxicity bioassay of American Cyanamid Waste Disposal Site, Santa Rosa County, Florida, on 13 to 15 October, 1981.

Freshwater Test

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)			$NH_4 NH_3+NH_4$ (mg/l)		Conductivity (umhos/cm)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
ontrol	10	10	9	8.7	8.7	8.4	7.8	8.3	8.2	110	120	119	122	21.9	21.8	21.6	.01/.28	.01/.09		
ontrol	10	10	10	8.7	8.7	8.4	7.8	8.3	8.2										220	215
.2%	10	10	10	8.7	8.9	8.4	7.9	8.3	8.2					21.7	21.8	21.5				
.2%	10	10	10	8.7	8.9	8.5	7.9	8.3	8.2										325	310
.6%	10	10	10	8.7	8.9	8.6	7.9	8.2	8.3					21.7	21.8	21.5				
.6%	10	10	10	8.7	9.0	8.7	7.9	8.2	8.3										390	405
0%	10	10	6	8.7	9.2	8.8	7.9	8.2	8.3					21.6	21.8	21.8				
0%	10	9	6	8.7	9.2	8.8	7.8	8.2	8.3										600	600
8%	10	8	6	8.7	9.3	8.9	7.8	8.2	8.3					21.5	21.9	21.8				
8%	10	8	4	8.7	9.3	9.0	7.8	8.2	8.2										880	900
2%	10	6	2	8.6	9.3	9.2	7.8	8.1	8.2					21.4	22.1	21.9				
2%	10	4	1	8.6	9.3	9.2	7.8	8.1	8.1										1350	1375
6%	11[a]	5	1	8.4	9.2	9.2	7.7	7.9	8.0	98	95	73	81	21.3	22.7	21.9	———	1.04/19.6		
6%	10	6	2	8.4	9.2	9.2	7.7	7.9	8.0										2110	2190
00% [b]	———			—	9.0	9.2	7.6	7.8	7.8	80[c]		44[c]		20.6	22.4	22.0	.58/33.6	1.2/3 5		

Remarks: a. Extra organism loaded by mistake, allowed to remain
b. Certain parameters were recorded in the 100% waste sample, which are presented here.
c. Estimated values due to color interference.

Table 3. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of American Cyanamid Waste Disposal Site, Santa Rosa County, Florida, on 13 to 15 October, 1981.

Saltwater Test

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	2(4)[a]	2(4)[a]	8.1	7.7	6.7	7.9	8.0	8.0	21.4	21.5	21.6				
5.6%	5	4(5)[a]	4(5)[a]	8.1	7.7	6.5	7.9	8.0	7.9						15.0	15.5
10.0%	5	2	2	8.0	7.6	6.6	7.9	8.0	7.9	21.4	21.6	21.6				
10.0%	5	5	5	8.0	7.6	6.6	7.9	8.0	7.9						14.8	15.0
18.0%	5	3	1	7.9	7.9	6.4	7.9	8.0	7.9	21.3	21.7	21.8				
18.0%	5	4	3	8.0	7.9	6.5	7.9	8.0	7.9						13.3	13.9
32.0%	5	5	4	8.0	8.1	6.7	7.9	8.0	8.0	21.2	21.8	21.8				
32.0%	5	1	0	8.1	8.1	6.8	7.9	8.0	7.9						11.3	12.0
56.0%	5	1	0	8.0	7.9	7.0	7.8	7.9	7.9	21.1	22.0	21.9				
56.0%	5	0	0	7.9	7.8	7.0	7.8	7.9	7.9						8.3	8.8
100%	5	0	0	7.6	6.7	6.2	8.2	7.8	7.8	21.1	22.1	22.0	2.11[b]/33.6	1.27/46.4		
100%	5	0	0	7.7	6.7	6.3	8.2	7.8	7.9						8.0	9.0

Remarks: a. Numbers in parentheses used in calculations since organisms jumped out between 0 and 24 hours.
b. The concentration of unionized ammonia reflects the rise in pH caused by the addition of artificial sea salts. If the pH of the unadjusted sample (7.6 units) is used inthe calculations, then the concentration of unionized ammonia would be 0.56 mg/l as NH_3.

Table 4. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of American Cyanamid Waste Disposal Site, Santa Rosa County, Florida on 13 to 15 October, 1981.

Saltwater Test 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	7.9	7.4	6.7	7.7	7.7	7.8	21.6	21.9	21.8	.01/.61	0/.18		
Seawater Control	5	5	5	7.9	7.4	6.7	7.8	7.8	7.8						16	16
Salinity Control	5	5	5	8.3	7.5	7.1	7.9	8.0	8.0	21.7	21.9	21.8				
Salinity Control	5	5	5	8.4	7.6	7.0	7.9	8.0	8.0						7.3	7.8
Sea Salts Control	5	5	5	8.3	7.6	7.4	8.6	8.4	8.3	21.8	22.0	22.0	0/.01	——		
Sea Salts Control	5	5	5	8.5	7.7	7.4	8.7	8.4	8.3						8.3	8.6

RESULTS AND DISCUSSION

The sample from the American Cyanamid Waste Disposal Site proved to be acutely toxic to both Daphnia pulex and to Mysidopsis bahia. The 48-hour LC_{50}'s for these organisms were 17.3% to 18.1% and 14.8% to 16.4%, respectively (Table 5). The 96-hour LC_{50}'s for these organisms would therefore be $\leq$18.1% and $\leq$16.4%, respectively. The FDER Chemistry Section has chemically characterized a sample of this waste, with results presented in Table 6. Copper was the only chemical quantified in this priority pollutant scan. Copper, in the quantity detected (26.8 ug/l) would not result in the mortality observed in these bioassays. Possible causes of toxicity include the high levels of unionized and total ammonia (U.S. EPA 1976, Thurston and Russo 1981) quantified in Tables 2 and 3. Additionally, personal communication with Mr. Neil Sharitz of American Cyanamid indicates the sample tested may also contain sodium thiocyanate (not analyzed for by DER's Chemistry Section in Tallahassee), a known toxicant.

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. Based on these biological test results, which indicate that the waste disposal lagoon discharge would adversely affect the aquatic biota of the receiving water, we recommend that American Cyanamid not discharge these untreated wastes.

Table 5. LC_{50} Data for Bioassays of American Cyanamid's Waste Disposal Site, Santa Rosa County, Florida, on 13 to 15 October 1981.

Organism	Method of Data Analysis	24 LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Freshwater Test	Binomial	a	18.0% (5.6 to 32)
Daphnia pulex	Moving Average	a	18.1% (14 to 24)
	Probit	45.1% (33 to 77),b	17.3% (14 to 22)
Saltwater Test	Binomial	35.4% (0 to 56)	14.8% (0 to 56)
Mysidopsis bahia	Moving Average	22.8% (15 to 34)	16.4% (11 to 23)
	Probit	22.8% (15 to 34)	16.0% (10 to 23)

[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.

Table 6. Results of Chemical Analyses Performed on Samples Collected from the American Cyanamid Waste Disposal Site, Santa Rosa County, Florida.

Organics

Volatiles:	Not Detected
Acid Extractables:	Not Detected
Base Neutral Extractables:	Not Detected
Pesticides Extractables:	Not Detected

Metals

Antimony	trace[a]
Arsenic	trace[a]
Beryllium	Not Detected (10 ug/l)[b]
Cadmium	Not Detected (10 ug/l)[b]
Chromium	Not Detected (50 ug/l)[b]
Copper	26.8 ug/l
Lead	trace[a]
Mercury	Not Detected (0.2 ug/l)[b]
Nickel	Not Detected (50 ug/l)[b]
Selenium	Not Detected (10 ug/l)[b]
Silver	trace[a]
Thallium	trace[a]
Zinc	trace[a]

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

[b] The number given is the minimum detection limit.

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, Biological Section. 1980. Bioassays of American Cyanamid Company, Fibers Division, Santa Rosa County, Florida. NPDES #FL0002593. FDER. 12 p.

Florida Department of Environmental Regulation, Biological Section. 1981. Bioassays of American Cyanamid Company, Fibers Division, Santa Rosa County, Florida. NPDES #FL0002593. FDER. 11 p.

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.

Thurston, R. V. and R. C. Russo. 1981. Ammonia toxicity to fishes: Effect of pH on the toxicity of the unionized species. Env. Sci. Tech. 15(7): 837-840.

U.S. Environmental Protection Agency. 1976. Quality Criteria for Water. U.S. EPA, Washington, D.C. 256 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.