

BIOASSAYS OF AMERICAN CYANAMID

FIBERS DIVISION

MILTON, SANTA ROSA COUNTY, FLORIDA

NPDES #FL0002593

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JANUARY 5, 1987?

Biology Section
Division of Environmental Programs

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MILTON, SANTA ROSA COUNTY, FLORIDA

NPDES #FL0002593

Biology Section
Bureau of Laboratories and
Special Programs
January 5, 1987

EXECUTIVE SUMMARY

American Cyanamid, NPDES #FL0002593, on 9 to 12 September, 1986.

Six separate 72 hour bioassay tests were performed on American Cyanamid's wastewater, including three definitive tests on final chlorinated effluent and three screening tests (100% concentration) on effluent that had been de-chlorinated. The final chlorinated effluent proved to be acutely toxic to all three test organisms, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi, producing 72 hour LC_{50}'s of 39.0%, 39.9%, and 90.8%, respectively. Conversely, the de-chlorinated effluent was not acutely toxic, since no waterfleas and only one fish had died by the end of the 72 hour tests. The toxicity, therefore, can mostly be attributed to the total residual chlorine (0.25 mg/l) measured in the sample. The presence of other toxic components, however, cannot be ruled out.

Ammonia was detected in Cyanamid's wastewater, but at concentrations below those causing toxicity. Chemical analyses indicated the presence of numerous unidentified organic compounds, and the metal silver (0.75 ug/l). These chemical constituents may have contributed to the toxicity exhibited by the American Cyanamid effluent.

Since toxicity was mostly attributed to the presence of residual chlorine, we recommend the installation of a de-chlorinated unit at this facility. Also, an attempt should be made to identify the organic compounds and to eliminate them from the discharge.

INTRODUCTION

American Cyanamid, Fibers Division, is located near Milton in Santa Rosa County, Florida (Fig. 1). According to the NPDES file, the facility produces acrylic fibers. Concentrated process water is disposed of in a deep well, while remaining wastewater is discharged through a long diffuser pipe to Escambia Bay.

The Biology Section has previously performed toxicity tests on effluent from this facility, in February, 1980, in October, 1980 and again, in May, 1984. The results of the February tests indicated that the effluent was extremely toxic to Daphnia pulex (48 hour LC_{50} = 7.2%) and also toxic to Mysidopsis bahia (48 hour LC_{50} = 74.8%) (FDER, 1980). Chlorine was present in the effluent during these tests, so a portion of the toxicity was attributed to that compound. The results of the October tests indicated that the effluent was not acutely toxic to Mysidopsis bahia, although significant mortality of shrimp was noted in one of the tests (FDER, 1981). Chlorine was not present in the effluent during these latter tests. Additionally, analyses for ammonia, a known ingredient of American Cyanamid's effluent (letter from Halladay to Kriegel), were not performed during these 1980 tests. The results of the May, 1984, test indicated acute toxicity to M. bahia (72 hour LC_{50} = 111.9%). Toxicity was mostly attributed to ammonia (FDER, 1984).

To again evaluate the toxicity of American Cyanamid's wastewater, the Northwest District requested additional bioassays be performed. Six separate 72 hour definitive and screening bioassays, using the freshwater test organisms, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi, were performed from 9 to 12 September, 1986. The results of these tests are presented in this report. Those involved in performing the tests and in preparation of this report were: M. L. Roll and P. G. Weiland.

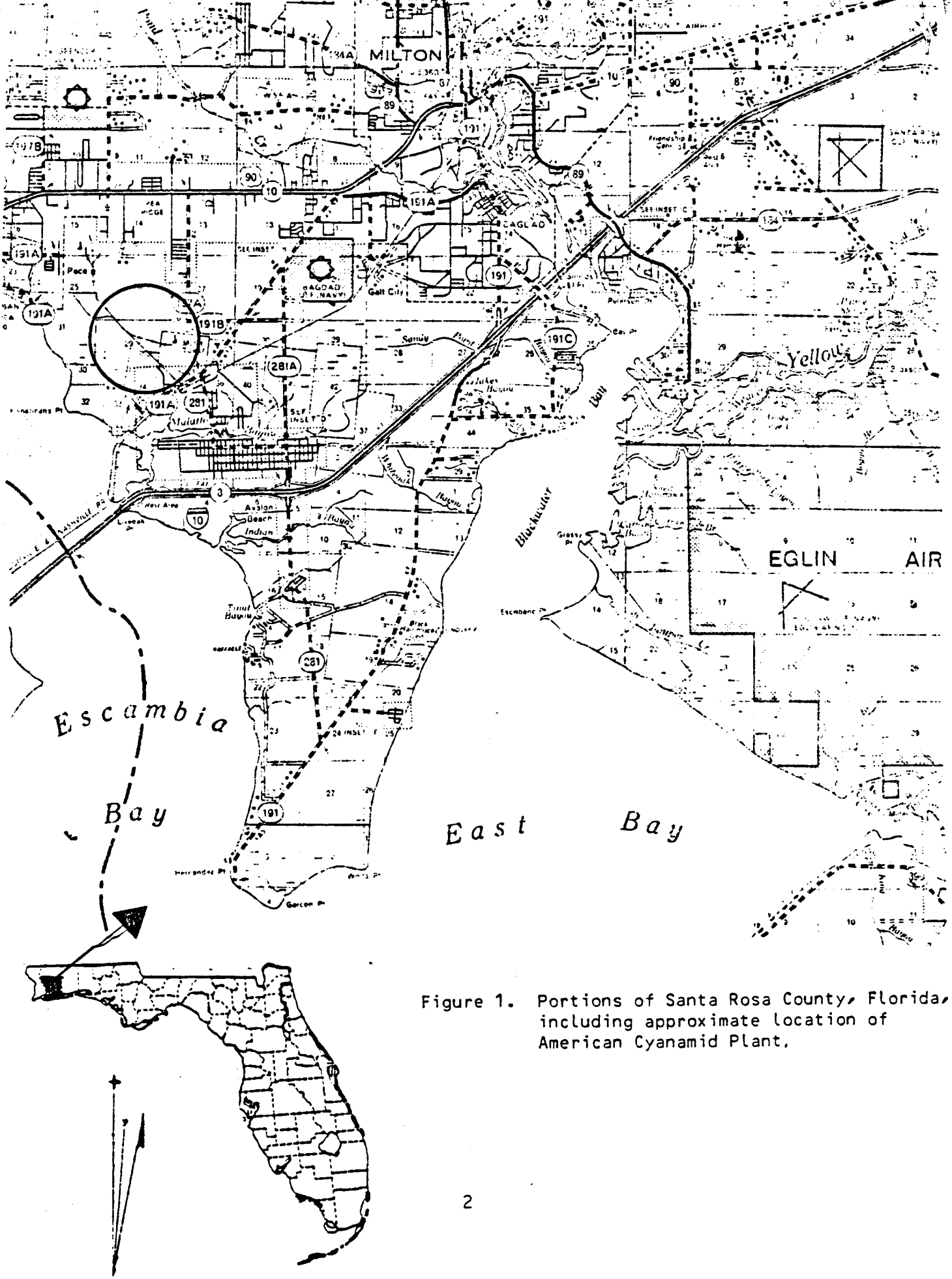


Figure 1. Portions of Santa Rosa County, Florida, including approximate location of American Cyanamid Plant.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier and Weber (1985) and the FDER Biology Section (1985). The organisms selected for this study include species recommended by Peltier and Weber (1985), the U.S. EPA (1978), and the FDER Biology Section (1985). 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

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, once daily on weekends, and are kept at a constant temperature of 22° ± 2°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms, and then refilling 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.

Ceriodaphnia dubia (water flea) - A breeding culture of Ceriodaphnia dubia was obtained from the EPA laboratory in Athens, Georgia, in June, 1985. The culture is maintained 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®, Cerophyl® and Fleischmann's® yeast twice daily, once daily on weekends, and are kept at a constant temperature of 26° ± 2°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms, and then refilling 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.

Notropis leedsi (bannerfin shiner) - Approximately 40 adult bannerfin shiners were obtained from an indigenous population in the upper Suwannee River during October, 1981. The breeding stock is maintained in 30 and 125 gallon all-glass aquaria equipped with a flow-through water exchange system. Water for this system is unchlorinated and is pumped from the DER deep well. The bannerfin shiners are reared at 22° ± 2°C under a 16:8 hour light-dark cycle and are fed Tetra-Min® and frozen Artemia (brine shrimp) twice daily, and once daily on weekends. Breeding and egg hatching are encouraged at 25°C. The eggs are laid between stacked red ceramic tiles separated by a 3mm bead of silicone. Most of the eggs adhere to the tiles and are easily removed to heated, aerated hatching tanks. The narrow space between the tiles eliminates egg predation by

adult fish. New hatching tanks are started regularly to segregate fry into even age groups. Newly hatched fry are fed a liquefied blend of Purina Trout Chow® and Cerophyl® twice daily. After one week, feeding is augmented with newly hatched Artemia nauplii and finely crushed Tetra Min®. The fish used in this test were approximately 19 days old at the time of the 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

Methods used to perform acute toxicity bioassays are described by Peltier and Weber (1985) and the FDER Biology Section (1985). Some of the methods described below are more specific than those of Peltier and Weber. These will be noted in more detail.

Sampling Methods and Test Dates

Six separate 72 hour definitive and screening bioassays were performed on samples of final chlorinated and de-chlorinated effluents collected from American Cyanamid's Outfall #001. The tests were performed from 1000 hours on 9 September to 1000 hours on 12 September, 1986.

The sample were collected by Mr. Steve Baisden of DER's Northwest District at 1330 hours on 8 September, 1986, 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.

Test Temperature

Upon arrival of the iced (4°C) bioassay samples in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the samples allowed to rise to 20° $\pm$ 2°C. Although this temperature may be different than the temperature of the sample during collection, it is the temperature range to which the test organisms are acclimatized and is the range recommended in the above referenced bioassay protocols. This avoids stressful effects caused by exposure to unusually cold or warm samples.

Total Residual Chlorine

Since many wastewaters are chlorinated, it is often desirable to differentiate between the toxicity resulting from chlorine and the toxicity associated with other components of the wastewater. To accomplish this, the Biology Section performed concurrent screening bioassays (100% concentration only) on a sample of chlorinated wastewater

in which the chlorine had been neutralized. De-chlorination was accomplished by adding a calculated amount of 0.25N sodium thiosulfate. The total residual chlorine concentrations of the tested effluents are given on the bioassay data forms. A comparison of the results of these tests will distinguish between the toxicity due solely to residual chlorine and the toxicity inherently contained in the wastewater.

Freshwater Tests

Freshwater for dilution of the effluent sample in the definitive tests 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 an abbreviated version of that recommended by APHA (1980).

Two replicates, of each concentration and a control, were used to test with the water fleas, Ceriodaphnia dubia and Daphnia pulex. Flint glass jars of either 60 ml or 250 ml capacity were used as test chambers, and either 50 ml or 200 ml of the appropriate test concentration was placed in each chamber. Ceriodaphnia were siphoned into a large culture dish and neonates were transferred by glass pipette, and loaded 10 per chamber, resulting in 20 per test concentration. Daphnia were siphoned into a large culture dish to isolate the proper size and age organisms (neonates). To accomplish this, the water fleas 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 these tests.

Two replicates, of each concentration and a control, were used for the Notropis leedsi (bannerfin shiner) 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.

Test Parameters

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 concentration at 0 hours, 24 hours, 48 hours, and 72 hours. Total alkalinity and total hardness (measured as $CaCO_3$ using HACH Chemical Company reagents) were recorded at the beginning of the test for the controls and the 100% effluent concentration. The specific conductance (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, 3, and 4.

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

Ceriodaphnia dubia (water flea)

Concentration	Dilution Water	Effluent	Total Volume
Control	50.0 ml	--------	50 ml
10.0%	45.0 ml	5.0 ml	50 ml
32.0%	34.0 ml	16.0 ml	50 ml
56.0%	22.0 ml	28.0 ml	50 ml
100.0%	-------	50.0 ml	50 ml

Daphnia pulex (water flea)

Concentration	Dilution Water	Effluent	Total Volume
Control	200.0 ml	--------	200 ml
10.0%	180.0 ml	20.0 ml	200 ml
32.0%	136.0 ml	64.0 ml	200 ml
56.0%	88.0 ml	112.0 ml	200 ml
100.0%	--------	200.0 ml	200 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Effluent	Total Volume
Control	500 ml	--------	500 ml
10.0%	450 ml	50 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, Ceriodaphnia dubia, static acute toxicity bioassay of American Cyanamid, Santa Rosa County, NPDES #FL0002593, on 9 to 12 September, 1986.

Concentration or %	Number of Live Organisms				Dissolved Oxygen (mg/l)				pH				Temperature (°C)				Conductivity (umhos)	
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72	0	72
Control	10	10	10	10	9.0	8.9	8.1	8.7	8.0	7.6	8.0	8.0	20.1	19.7	19.0	19.8		
Control	10	10	10	10	9.0	8.9	9.1	8.7	8.0	7.6	8.0	8.0					195	203
10.0%	10	10	10	10	8.9	8.8	9.2	8.6	8.0	7.7	8.0	8.0	20.0	19.7	18.9	19.6		
10.0%	10	10	10	10	8.9	8.8	9.2	8.6	8.0	7.7	7.9	8.0					205	208
32.0%	10	10	10	9	8.6	8.9	9.2	8.7	7.7	8.0	8.0	8.1	20.0	19.7	18.9	19.5		
32.0%	10	10	9	8	8.6	8.9	9.2	8.7	7.7	8.0	8.0	8.1					215	218
56.0%	10	6	0	0	8.4	9.0	9.1	-a	7.3	7.7	8.0	-a	19.9	19.7	18.9	-a		
56.0%	10	5	0	0	8.4	9.0	9.1	-	7.3	7.7	7.9	-					230	-a
100.0%	10	8	2	0	7.8	9.0	8.9	8.7	6.5	7.4	7.2	7.4	19.9	19.8	18.9	19.5		
100.0%	10	7	1	0	7.8	9.0	8.9	8.7	6.5	7.4	7.2	7.4					260	265
De-chlorinated																		
100.0%	10	10	10	10	7.8	9.0	8.9	8.7	6.5	7.2	7.0	7.2	20.0	19.9	19.0	19.5		
100.0%	10	10	10	10	7.8	9.0	8.9	8.7	6.5	7.2	7.0	7.2					270	275

Control Water

NH_3(as NH_3)(mg/l): 0

Total Alkalinity (mg/l $CaCO_3$): 125

Total ammonia (as N)(mg/l): 0

Total Hardness (mg/l $CaCO_3$): 123

100% Effluent

NH_3(as NH_3)(mg/l): 0.008

Total Alkalinity (mg/l $CaCO_3$): 32

Total ammonia (as N)(mg/l): 5.4

Total Hardness (mg/l $CaCO_3$): 12

Total Residual Chlorine: 0.25 mg/l (HACH DPD Method).

[a] Remaining parameters were not recorded since all organisms were dead by 48 hours.

Table 3. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of American Cyanamid, Santa Rosa County, NPDES #FL0002593, on 9 to 12 September, 1986.

Concentration or %	Number of Live Organisms				Dissolved Oxygen (mg/l)				pH				Temperature (°C)				Conductivity (umhos)	
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72	0	72
Control	10	10	10	10	8.9	8.7	8.7	8.7	8.0	7.8	7.8	7.9	20.6	20.1	19.0	19.7		
Control	10	10	10	10	8.9	8.7	8.7	8.7	8.0	7.8	7.8	7.9					195	203
10.0%	10	10	10	10	8.9	8.7	8.7	8.8	8.0	7.8	7.8	7.9	20.4	20.0	19.0	19.7		
10.0%	10	10	10	10	8.9	8.7	8.7	8.8	8.0	7.8	7.8	7.9					205	208
32.0%	10	10	9	9	8.6	8.8	8.6	8.8	7.7	7.8	7.8	7.9	20.3	19.9	19.0	19.6		
32.0%	10	10	8	7	8.6	8.8	8.6	8.8	7.7	7.8	7.8	7.9					215	218
56.0%	10	10	3	2	8.4	8.8	8.5	8.8	7.2	7.6	7.6	7.9	20.2	19.9	19.0	19.6		
56.0%	10	6	0	0	8.4	8.8	8.5	-a	7.2	7.6	7.6	-a					230	229
100.0%	10	1	0	0	7.7	8.9	7.8	-	6.4	7.1	6.9	-	20.0	19.9	19.0	-a		
100.0%	10	2	0	0	7.7	8.9	7.8	-	6.4	7.1	6.9	-					260	-a
De-chlorinated																		
100.0%	10	10	10	10	7.8	8.9	7.8	8.5	6.3	7.0	6.8	7.0	20.0	20.0	19.0	19.6		
100.0%	10	10	10	10	7.8	8.9	7.8	8.5	6.3	7.0	6.8	7.0					270	275

Control Water

NH_3(as NH_3)(mg/l): 0

Total ammonia (as N)(mg/l): 0

Total Alkalinity (mg/l $CaCO_3$): 125

Total Hardness (mg/l $CaCO_3$): 123

100% Effluent

NH_3(as NH_3)(mg/l): 0.007

Total ammonia (as N)(mg/l): 5.4

Total Alkalinity (mg/l $CaCO_3$): 33

Total Hardness (mg/l $CaCO_3$): 12

Total Residual Chlorine: 0.25 mg/l (HACH DPD Method).

[a] Remaining parameters were not recorded since all organisms were dead by 48 hours.

Table 4. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of American Cyanamid, Santa Rosa County, NPDES #FL0002593, on 9 to 12 September, 1986.

Concentration or %	Number of Live Organisms				Dissolved Oxygen (mg/l)				pH				Temperature (°C)				Conductivity (umhos)	
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72	0	72
Control	5	5	5	5	8.6	8.1	7.8	8.2	7.6	7.5	7.5	7.6	21.5	20.2	19.2	19.7		
Control	5	5	5	5	8.6	8.1	7.8	8.2	7.6	7.5	7.5	7.6					200	240
10.0%	5	5	5	5	8.5	8.1	7.8	8.2	7.6	7.6	7.6	7.7	21.3	20.0	19.2	19.5		
10.0%	5	5	5	5	8.5	8.1	8.1	8.2	7.6	7.6	7.6	7.7					210	240
32.0%	5	5	5	5	8.5	8.3	8.3	8.3	7.3	7.6	7.6	7.7	21.1	19.7	18.9	19.4		
32.0%	5	5	5	5	8.5	8.3	8.3	8.3	7.3	7.6	7.6	7.7					215	240
56.0%	5	4	4	4	8.2	8.4	8.3	8.3	7.1	7.4	7.4	7.6	20.5	19.7	18.8	19.3		
56.0%	5	5	5	5	8.2	8.4	8.3	8.3	7.1	7.4	7.4	7.6					230	265
100.0%	5	4	4	4	7.8	8.0	7.7	7.7	6.3	6.9	6.8	7.0	20.0	19.6	18.8	19.4		
100.0%	5	0	0	0	7.8	8.0	-a	-	6.3	6.9	-a	-					265	307
De-chlorinated																		
100.0%	5	5	5	5	7.8	7.8	7.6	7.5	6.3	6.9	6.8	6.9	20.1	19.8	19.0	19.5		
100.0%	5	4	4	4	7.8	7.8	7.6	7.5	6.3	6.9	6.8	6.9					275	311

Control Water

Total Alkalinity 125 (mg/l $CaCO_3$)

NH_3(as NH_3)(mg/l) 0

Total Hardness 123 (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) 0

100% Effluent

Total Alkalinity 33 (mg/l $CaCO_3$)

NH_3(as NH_3)(mg/l) 0.005

Total Hardness 12 (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) 5.4

Total Residual Chlorine: 0.25 mg/l (HACH DPD Method)

[a] Remaining parameters were not recorded since all organisms were dead by 24 hours.

Table 5. LC_{50} Data for Bioassays of American Cyanamid, Santa Rosa County, NPDES #FL0002593, on 9 to 12 September, 1986.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals	72 Hour LC_{50} and 95% Confidence Intervals
Chlorinated Sample				
Ceriodaphnia dubia	Binomial	a	40.8% (32 and 56)	39.0% (32 and 56)
	Moving Average	a	35.2% (22.9 and 49.0)	a
	Probit	133.5% (0 and ∞)	45.8% (0 and ∞)	a
Daphnia pulex	Binomial	72.9% (56 and 100)	42.3% (32 and 56)	40.3% (32 and 56)
	Moving Average	72.4% (62.4 and 88.0)	38.8% (31.1 and 47.4)	35.6% (28.0 and 43.8)
	Probit	72.9% (62.9 and 85.1)	42.3% (36.7 and 48.6)	39.9% (34.6 and 45.9), b
Notropis leedsi	Binomial	90.0% (56 and ∞)	90.0% (56 and ∞)	90.0% (56 and ∞)
	Moving Average	90.0% (70.0 and 299.8)	90.0% (70.0 and 299.8)	90.0% (70.0 and 299.8)
	Probit	90.8% (70.9 and 161.2)	90.8% (70.9 and 161.2)	90.8% (70.9 and 161.2), b
De-chlorinated Sample				
C. dubia and D. pulex	Binomial	No Mortality	No Mortality	No Mortality
	Moving Average			
	Probit			
N. leedsi	Binomial	a	a	a
	Moving Average			
	Probit			

[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] This LC_{50} is reported in the text since it is the most statistically conservative value of the data set.

Table 6. Results of Chemical Analyses Performed on Wastewater from American Cyanamid, NPDES #FL0002593, Collected on 8 September, 1986.

Organics

Acid Extractables:	N.D.
Base Neutral Extractables:	Numerous unidentified compounds
Pesticide Extractables:	Several unidentified compounds

Metals

Arsenic	N.D.
Cadmium	N.D.
Chromium	N.D.
Copper	N.D.
Lead	N.D.
Mercury	N.D.
Nickel	N.D.
Selenium	N.D.
Silver	0.75 ug/l
Zinc	N.D.

N.D. = None Detected

RESULTS AND DISCUSSION

The sample of final chlorinated effluent collected from American Cyanamid's Outfall #001 proved to be acutely toxic to all three test organisms, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi, producing 72 hour LC_{50}'s of 39.0%, 39.9%, and 90.8%, respectively. Conversely, acute toxicity was not evident in the de-chlorinated sample, since no waterfleas and only one fish died during the 72 hour tests. The above toxicity, therefore, can mostly be attributed to the total residual chlorine (0.25 mg/l) measured in the sample. The presence of other toxic components in American Cyanamid's wastewater, however, is a distinct possibility, since the toxicity noted in the waterflea bioassays was more severe than would have been expected from the chlorine alone.

The ammonia measured in this wastewater, 5.4 mg/l total and up to 0.008 mg/l unionized, was insufficient to have contributed significantly to the toxicity. The results of chemical analyses (Table 6) indicated the presence of numerous organic compounds, and the metal silver (0.75 ug/l). These chemical components may have contributed to the toxicity noted in American Cyanamid's effluent.

Recommendation

Because of the obvious toxicity due to residual chlorine, we recommend the installation of a de-chlorination system at the American Cyanamid facility. Additionally, the identity of the organic compounds should be investigated, and these, along with the silver, should eventually be eliminated from the discharge.

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 (4), 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 onetwentieth 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, whetheras 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)(p)]; 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 not designated for dredge and fill permitting, but rather to the average of a number of points within such 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 such a mixing zone (i.e., at any point within the mixing zone), from exceeding the 96 hour LC_{50}. Therefore, at any point within such a mixing zone, including the point of discharge, one-half of the test organisms cannot be killed in 96 hours. Within a mixing zone designated for dredge and fill permitting, the acute toxicity standard (one-third of the 96 hour LC_{50}) applies at all points, including the point of discharge.

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}. 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 not designated for dredge and fill permitting, 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.

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