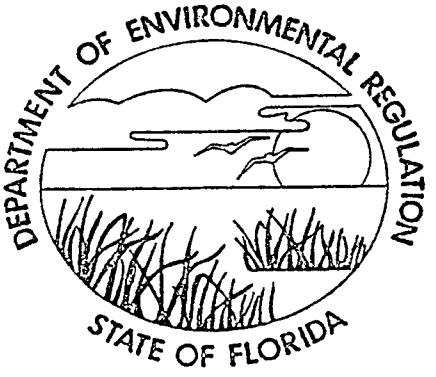


BIOASSAYS OF AMERICAN CYANAMID

FIBERS DIVISION

MILTON, SANTA ROSA COUNTY, FLORIDA

NPDES #FL0002593

SAMPLED 2/23/87

MAY 8, 1987

Biology Section
Division of Environmental Programs

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Biology Section
Bureau of Laboratories and
Special Programs
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EXECUTIVE SUMMARY

American Cyanamid, NPDES #FL0002593, on 24 to 26 February, 1987.

The sample of effluent collected from American Cyanamid's Outfall #001 was not acutely toxic to the test organisms, Ceriodaphnia dubia, Daphnia pulex, and Pimephales promelas. No mortality was noted at 48 hours in the P. promelas test, and only insignificant mortality was noted in the D. pulex test. In the C. dubia test, some individuals died at 24 and 48 hours, but the random distribution prevented the determination of an LC_{50}. No residual chlorine was detected in the effluent at this time. The total ammonia (10.8 mg/l) and unionized ammonia (0.06 mg/l) measured in this sample were insufficient to have caused any toxicity. Samples for chemical analyses were not collected.

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 definitive tests on the chlorinated effluent resulted in acute toxicity to all three test organisms, producing 72 hour LC_{50}'s of 39.0% (C. dubia), 39.9% (D. pulex), and 90.8% (N. leedsi). Conversely, the de-chlorinated effluent was not acutely toxic. The toxicity, therefore, was mostly attributed to the total residual chlorine (0.25 mg/l) measured in the sample, although the presence of other toxic components was not ruled out. Chemical analyses revealed the presence of numerous unidentified organic compounds, as well as the metal silver. These chemical constituents may have contributed to the observed toxicity. The installation of a de-chlorination system was recommended by the Biology Section, since toxicity was mostly attributed to the presence of residual chlorine. Additionally, it was recommended to attempt to identify the organic compounds and eliminate them from the discharge (FDER 1986).

In February, 1987, three additional definitive static bioassays were performed using Ceriodaphnia dubia, Daphnia pulex, and Pimephales promelas as test organisms. Results are presented in this report. Those involved in performing the tests and in preparation of this report were: M. J. Drawdy, K. M. Lurding, and M. L. Roll.

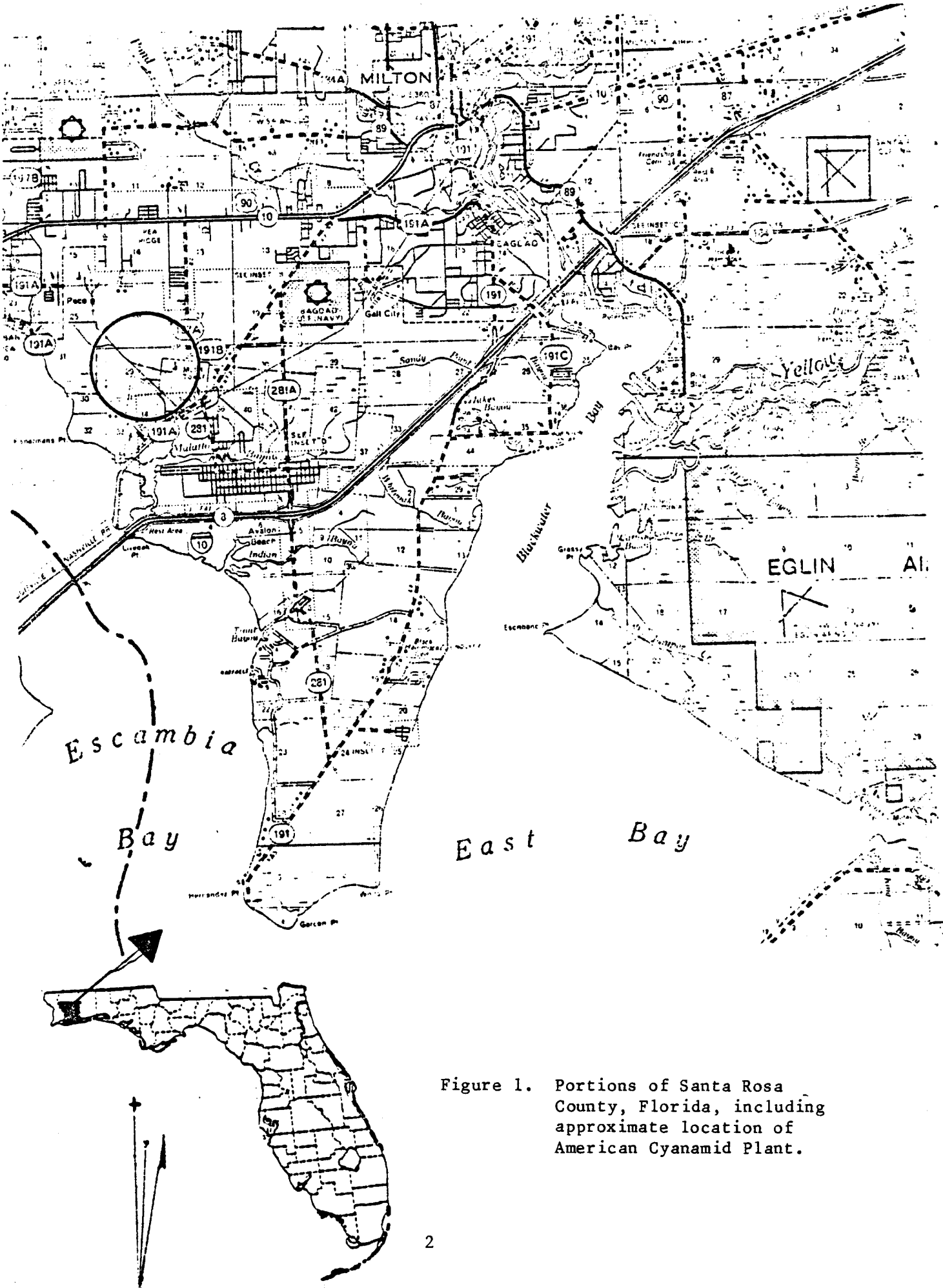


Figure 1. Portions of Santa Rosa County, Florida, including approximate location of American Cyanamid Plant.

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 are among those recommended by Peltier and Weber (1985), the U.S. EPA (1978), and the FDER Biology Section (1985). 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.

Pimephales promelas (fathead minnows) - A breeding culture of approximately 100 adult fathead minnows was obtained from the EPA laboratory in Cincinnati, Ohio, in May, 1979. This breeding population is augmented with fry hatched in our laboratory and with additional adult fish from EPA as required. The fish are maintained in all-glass aquaria (15 to 125 gallons), equipped with flow-through water exchange systems, using unchlorinated well water obtained from a deep well near the DER laboratory. The fathead minnows are reared at a temperature of 22° ± 2°C under a 16:8 hour light-dark cycle. These fish are fed Glencoe Mills "#3 size enriched trout fry granules" or Tetra-Min®, and frozen Artemia (brine shrimp) at a rate of 0.75g/30 fish, twice daily, and once daily on weekends. Breeding has been encouraged by placing several halved clay pots in each tank to serve as substrates for eggs and to provide cover for the fish. Tanks are examined twice daily (at feeding time) for egg production. Clay pots containing egg clusters are immediately transferred to 2-1/2 to 15 gallon aerated aquaria for hatching. A new aquarium is

prepared for each week's production of eggs, thereby segregating fish into even age groups to be used for testing. After hatching, fathead minnow fry are fed twice daily with freshly hatched Artemia. The fish used in this test were 21 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

Three separate 48 hour definitive static bioassays were performed on a sample of effluent collected from American Cyanamid's Outfall #001. The tests were performed from 0930 hours on 24 February to 0930 hours on 26 February, 1987.

The sample was collected by Mr. Stephen Baisden of DER's Northwest District at 1430 hours on 23 February, 1987, using standard wastewater sampling procedures. The sample was 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 sample in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the sample 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.

Freshwater Tests

Freshwater for dilution of the effluent sample 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 (for C. dubia) or 250 ml (for D. pulex) 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 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 these tests.

Two replicates, of each concentration and a control, were used for the Pimephales promelas (fathead minnow) 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, and 48 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

Pimephales promelas (fathead minnow)

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, NPDES #FL0002593, on 24 to 26, February, 1987.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Control	10	10	10	8.7	8.6	8.0	8.1	7.8	7.9	21.4	20.1	19.7		
Control	10	10	9	8.7	8.6	8.0	8.1	7.8	7.9					
10.0%	10	10	10	8.8	8.4	8.3	8.2	7.9	8.0	21.4	19.9	19.6		
10.0%	10	9	9	8.8	8.4	8.3	8.2	7.9	8.0					
32.0%	10	9	9	9.0	8.4	8.5	7.9	7.9	8.0	21.3	19.9	19.5		
32.0%	10	10	10	9.0	8.4	8.5	7.9	7.9	8.0					
56.0%	10	10	10	9.1	8.2	8.6	7.6	7.9	7.8	21.3	19.8	19.5		
56.0%	10	10	9	9.1	8.2	8.6	7.6	7.9	7.8					
100.0%	10	9	9	9.2	8.4	8.3	7.0	7.2	7.2	21.3	20.1	19.5		
100.0%	10	9	9	9.2	8.4	8.3	7.0	7.2	7.2					

Control Water

NH_3(as NH_3)(mg/l) 0

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

Total Alkalinity 109 (mg/l $CaOO_3$)

Total Hardness 118 (mg/l $CaOO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) 0.06

Total ammonia (as N)(mg/l) 10.8

Total Alkalinity 29 (mg/l $CaOO_3$)

Total Hardness 11 (mg/l $CaOO_3$)

Table 3. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of American Cyanamid, NPDES #FL0002593, on 24 to 26 February, 1987.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)			
	0	24	48	0	24	48	0	24	48	0	24	48	0	48		
Control	10	10	10	8.8	8.5	8.1	8.0	7.9	7.9	21.2	19.9	19.7			Control Water	
Control	10	10	9	8.8	8.5	8.1	8.0	7.9	7.9				235	240	NH_3(as NH_3)(mg/l)	Total Alkalinity 109 (mg/l $CaOO_3$)
10.0%	10	10	9	9.0	8.5	8.3	8.0	7.9	7.9	21.5	19.8	19.5			0	
10.0%	10	10	10	9.0	8.5	8.3	8.0	7.9	7.9				240	240		
32.0%	10	10	10	9.1	8.5	8.5	7.9	8.0	7.9	21.4	19.7	19.5			Total ammonia (as N)(mg/l)	Total Hardness 118 (mg/l $CaOO_3$)
32.0%	10	10	10	9.1	8.5	8.5	7.9	8.0	7.9				250	242	0	
56.0%	10	10	10	9.2	8.4	8.5	7.6	7.7	7.7	21.3	19.8	19.4				
56.0%	10	10	10	9.2	8.4	8.5	7.6	7.7	7.7				265	252		
100.0%	10	10	10	9.2	8.4	8.0	7.0	7.2	7.3	21.2	20.0	19.5				
100.0%	10	10	10	9.2	8.4	8.0	7.0	7.2	7.3				295	290	100% Effluent	
															NH_3(as NH_3)(mg/l)	Total Alkalinity 29 (mg/l $CaOO_3$)
															0.06	
															Total ammonia (as N)(mg/l)	Total Hardness 11 (mg/l $CaOO_3$)
															10.8	

Table 4. Data recorded during 48 hour, Pimephales promelas, static acute toxicity bioassay of American Cyanamid, NPDES #FL0002593, on 24 to 26 February, 1987.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)		
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	
Control	5	5	5	8.7	8.3	7.7	7.5	7.6	7.6	21.8	20.2	19.5			Control Water
Control	5	5	5	8.7	8.3	7.7	7.5	7.6	7.6				220		NH_3(as NH_3)(mg/l) 0 — Total Alkalinity 109 (mg/l $CaOO_3$)
10.0%	5	5	5	8.8	8.7	8.0	7.5	7.7	7.6	21.5	19.6	19.5			
10.0%	5	5	5	8.8	8.7	8.0	7.5	7.7	7.6				222		Total ammonia (as N)(mg/l) 0 — Total Hardness 118 (mg/l $CaOO_3$)
32.0%	5	5	5	8.9	8.3	8.3	7.5	7.7	7.7	21.7	19.7	19.5			
32.0%	5	5	5	8.9	8.3	8.3	7.5	7.7	7.7				242		
56.0%	6[a]	6	6	9.0	8.2	8.4	7.4	7.5	7.5	21.4	19.8	19.5			
56.0%	5	5	5	9.0	8.2	8.4	7.4	7.5	7.5				260		
100.0%	5	5	5	9.2	7.9	7.7	7.0	7.0	7.2	21.2	20.2	19.6			
100.0%	5	5	5	9.2	7.9	7.7	7.0	7.0	7.2				300		100% Effluent
															NH_3(as NH_3)(mg/l) 0.06 — Total Alkalinity 29 (mg/l $CaOO_3$)
															Total ammonia (as N)(mg/l) 10.8 — Total Hardness 11 (mg/l $CaOO_3$)

RESULTS AND DISCUSSION

The sample of effluent collected from American Cyanamid's Outfall #001 was not acutely toxic to the test organisms, Ceriodaphnia dubia, Daphnia pulex, and Pimephales promelas (Tables 2-4). No mortality was noted at 48 hours in the P. promelas test, and only insignificant mortality was noted in the D. pulex test. In the C. dubia test, some individuals died at 24 and 48 hours, but the random distribution prevented the determination of an LC_{50}. No residual chlorine was detected in the effluent at this time. The total ammonia (10.8 mg/l) and unionized ammonia (0.06 mg/l) measured in this sample were insufficient to have caused any toxixcity. Samples for chemical analyses were not collected.

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