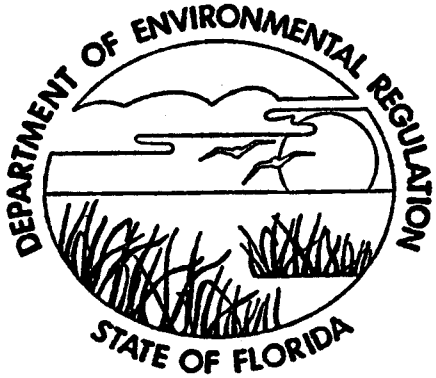


BIOASSAYS OF C-F INDUSTRIES

MULBERRY, POLK COUNTY, FLORIDA

NPDES #FL0000523

SAMPLED 10/8/85

NOVEMBER 8, 1985

Biology Section
Division of Environmental Programs

BIOASSAYS OF C-F INDUSTRIES

MULBERRY, POLK COUNTY, FLORIDA

NPDES #FL0000523

Biology Section
Bureau of Laboratories and
Special Programs

EXECUTIVE SUMMARY

C-F Industries, Mulberry, Polk County, Florida NPDES #FL0000523 on 9 to 11 October, 1985.

The sample of effluent collected from C-F Industries' outfall #003 was not acutely toxic to the freshwater test organisms, Daphnia pulex and Notropis leedsi. No mortality occurred in the D. pulex test and only one fish died in the N. leedsi 48 hour bioassay. A small amount of ammonia was measured in the sample, (maximum measured = 1.5 mg/l total and .002 mg/l unionized). Chemical analyses were not performed since the sample was not toxic.

INTRODUCTION

C-F Industries is located near Mulberry, Polk County, Florida (Fig. 1). The facility manufactures diammonium phosphate (DAP) and is permitted to discharge through outfalls #001 (recirculating cooling water) and #003 (non-process cooling water). The discharge averages 19 MGD and eventually enters the Alafia River (Fig. 2). Effluent from outfall #003 may contain ammonia, phosphorus and fluoride (from NPDES file).

To evaluate the toxicity of this effluent, the Biology Section performed two 48 hour static acute toxicity bioassays on a sample collected at outfall #003. The tests were performed from 9 to 11 October, 1985. Those involved in performing the tests and in preparation of this report were: R. B. Frydenborg, M. L. Moultrie and M. L. Roll.

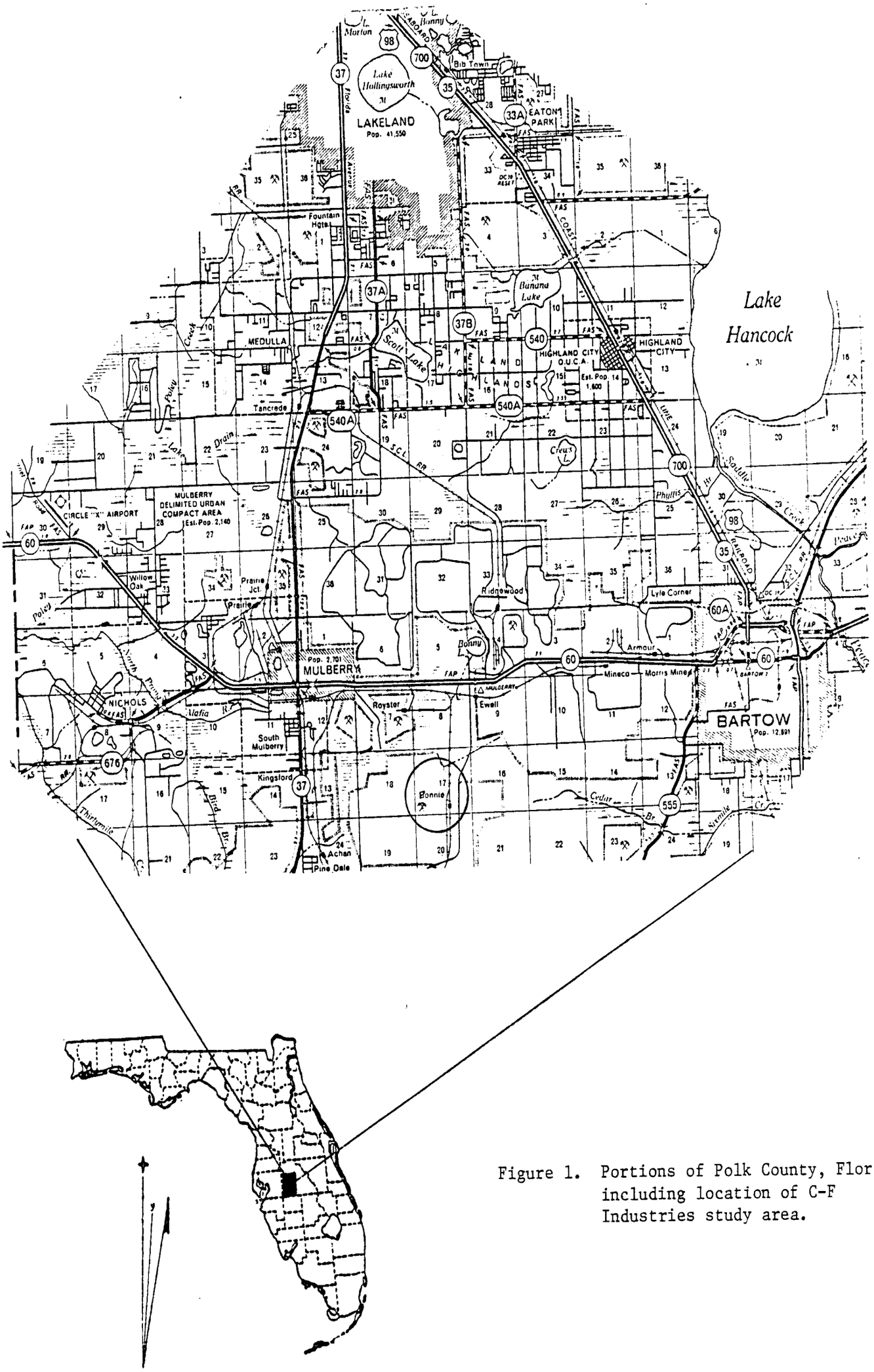


Figure 1. Portions of Polk County, Florida including location of C-F Industries study area.

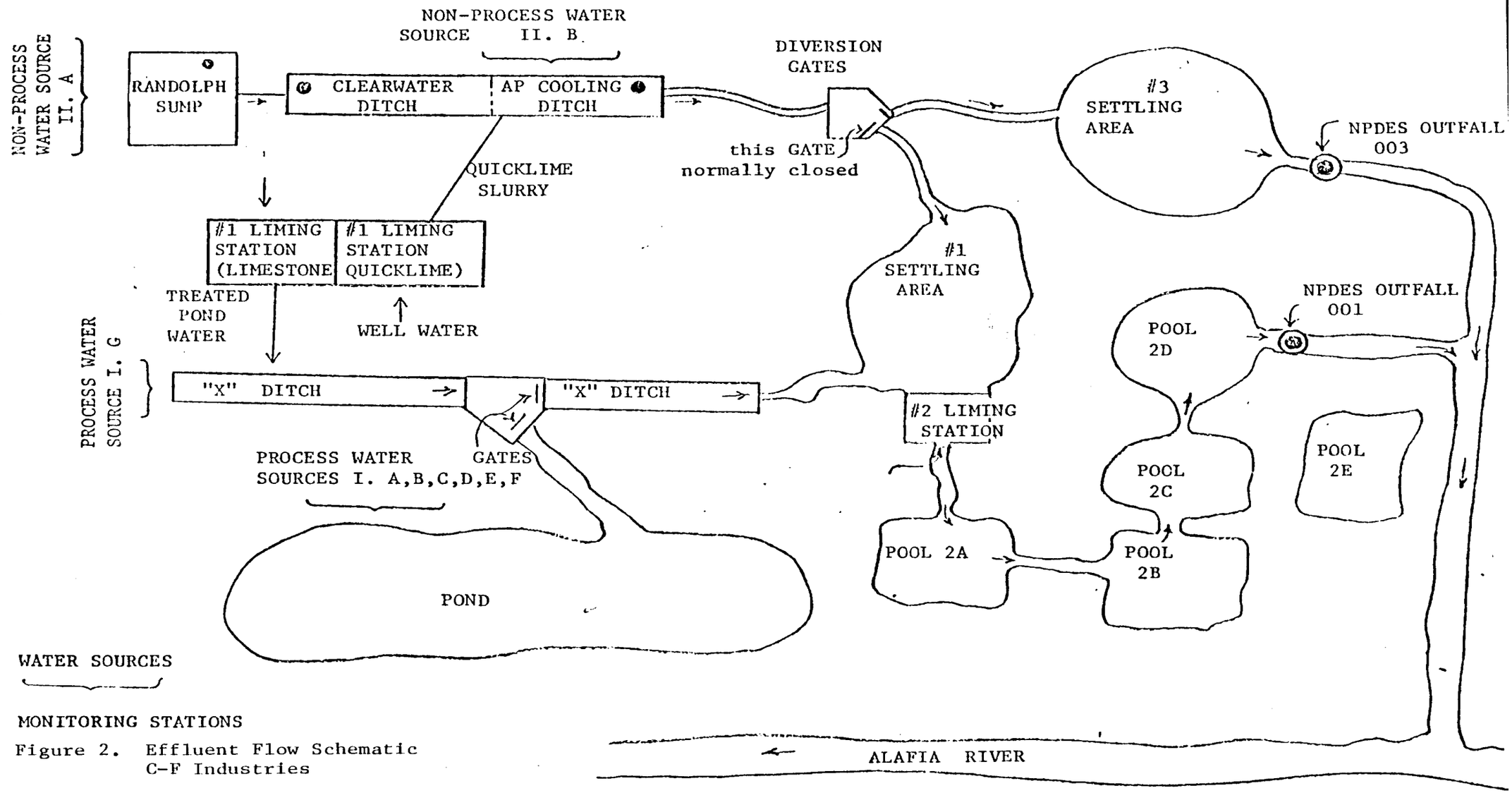


Figure 2. Effluent Flow Schematic
C-F Industries

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 a 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.

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 three weeks 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

Two 48 hour static acute toxicity bioassays were performed on a sample of effluent collected at C-F Industries' outfall #003 from 0930 hours on 9 October to 1030 hours on 11 October, 1985.

The sample was collected by Ms. Nan Baggett of DER's Southwest District office at 1245 hours on 7 October, 1985, 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.

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 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 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 fed during this test.

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 these tests.

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 control 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 and 3.

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

Daphnia pulex (water flea)

Concentration	Dilution Water	Effluent	Total Volume
Control	200.0 ml	--------	200 ml
10.0%	180.0 ml	20.0 ml	200 ml
32.0%	136.0 ml	64.0 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
100.0%	------	500 ml	500 ml

Table 2. Daphnia pulex

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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	8.0	7.8	8.1	7.6	8.2	8.0	22.3	21.7	21.4			
Control	10	10	10	7.9	7.8	8.1	7.6	8.2	8.0				201	210	
10.0%	10	10	10	8.1	7.8	8.3	7.4	8.1	8.0	22.3	21.7	21.4			
10.0%	10	10	10	8.1	7.8	8.3	7.4	8.0	8.0				250	250	
32.0%	10	10	10	8.1	7.5	8.3	7.1	7.8	7.9	22.3	21.6	21.4			
32.0%	10	10	10	8.0	7.5	8.3	7.1	7.8	7.9				340	340	
100.0%	10	10	10	7.3	7.5	8.3	6.5	7.1	7.2	22.4	21.7	21.5			
100.0%	10	10	10	7.4	7.4	8.3	6.5	7.1	7.2				690	650	

Control Water

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

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

Total Alkalinity (mg/l $CaCO_3$): 120

Total Hardness (mg/l $CaCO_3$): 122

100% Effluent

NH_3(as NH_3)(mg/l): .002

Total ammonia (as N)(mg/l): 1.5

Total Alkalinity (mg/l $CaCO_3$): 45

Total Hardness (mg/l $CaCO_3$): 252

Table 3. *Notropis leedsi*

C-F Industries, Bartow, Polk County, Florida, NPDES #FL0000523 on 9 to 11 October, 1985.

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	8.3	7.1	7.5	7.6	7.9	7.8	22.2	21.7	21.4		
Control	5	5	5	8.3	7.2	7.5	7.6	7.9	7.8				211	210
10.0%	5	5	5	8.2	7.3	7.5	7.4	7.8	7.8	22.2	21.7	21.4		
10.0%	5	5	5	8.2	7.3	7.6	7.4	7.8	7.8				255	258
32.0%	5	5	5	8.0	7.1	7.7	7.1	7.6	7.7	22.3	21.8	21.7		
32.0%	5	5	4	7.9	7.0	7.7	7.1	7.6	7.7				351	352
100.0%	5	5	5	7.1	6.7	7.5	6.5	6.8	7.0	22.3	21.7	21.8		
100.0%	5	5	5	7.1	6.7	7.5	6.5	6.8	7.0				600	690

Control Water

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

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

Total Alkalinity (mg/l $CaCO_3$): 120

Total Hardness (mg/l $CaCO_3$): 122

100% Effluent

NH_3(as NH_3)(mg/l): .002

Total ammonia (as N)(mg/l): 1.5

Total Alkalinity (mg/l $CaCO_3$): 45

Total Hardness (mg/l $CaCO_3$): 252

RESULTS AND DISCUSSIONS

The sample of effluent collected from C-F Industries' outfall #003 was not acutely toxic to the freshwater test organisms, Daphnia pulex and Notropis leedsi. No mortality occurred in the D. pulex test (Table 2) and only one fish died in the N. leedsi 48 hour bioassay (Table 3). A small amount of ammonia was measured in the sample (maximum measured = 1.5 mg/l total and .002 mg/l unionized). Chemical analyses were not performed since the sample was not toxic.

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