

BIOASSAYS OF TRADEMARK NITROGEN

TAMPA, HILLSBOROUGH COUNTY, FLORIDA

NPDES #FL0000647

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APRIL 15, 1987

Biology Section
Division of Environmental Programs

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TAMPA, HILLSBOROUGH COUNTY, FLORIDA

NPDES #FL0000647

Biology Section
Bureau of Laboratories and
Special Programs
April 15, 1987

EXECUTIVE SUMMARY

Trademark Nitrogen, Tampa, NPDES #FL0000647, on 14 to 16 January, 1987.

The sample of wastewater collected from Trademark Nitrogen's Outfall #001 was not toxic to the test organisms, Daphnia pulex and Notropis leedsi. No mortality was noted in the D. pulex test at 24 hours, and 5% mortality was observed in the 50% concentration at 48 hours. Although 10% mortality was noted in the N. leedsi test in the 50% concentration at 24 hours, no mortality was observed in the 100% concentration. The total ammonia (2.0 mg/l) and unionized ammonia (0.05 mg/l) measured in this sample were insufficient to have caused any toxicity. Since the sample was not acutely toxic, chemical analyses were not performed.

INTRODUCTION

Trademark Nitrogen manufactures ammonium nitrate solutions and blended nitrogenous fertilizer solutions. The facility is located northeast of U.S. 301 and S.R. 60 in Tampa, Hillsborough County, Florida (Figure 1). This facility was formerly Kaiser Agricultural Chemicals, a division of Kaiser Aluminum.

The combined process wastewater generated at this facility consists of: cooling tower blowdown, rainwater runoff, backwash water, and boiler blowdown. The system is designed for a maximum flow of .08 MGD. Wastewater is discharged through NPDES Outfall #001 to an unnamed ditch to Six Mile Creek (Figure 2). According to the NPDES permit file, this wastewater may contain components such as: ammonia, chromium, zinc, and algicides.

To evaluate the toxicity of wastewater from Trademark Nitrogen, at the request of Mr. Don Moores of DER's Southwest District, the Biology Section performed two 48 hour bioassays on a sample of Outfall #001 effluent, on 16 to 18 October, 1984. The sample was not toxic to the test organisms, Daphnia pulex and Heterandria formosa, however, significant concentrations of total ammonia (maximum measured = 13.7 mg/l) and unionized ammonia (maximum measured = 0.32 mg/l) were detected in the wastewater (FDER 1985a).

Subsequent bioassays were performed on a sample of wastewater from the same outfall on 14 to 16 January, 1987. The results of these tests are presented in this report. Those involved in performing these tests and in preparation of this report were: K. M. Lurding, M. L. Roll, and P. G. Weiland.

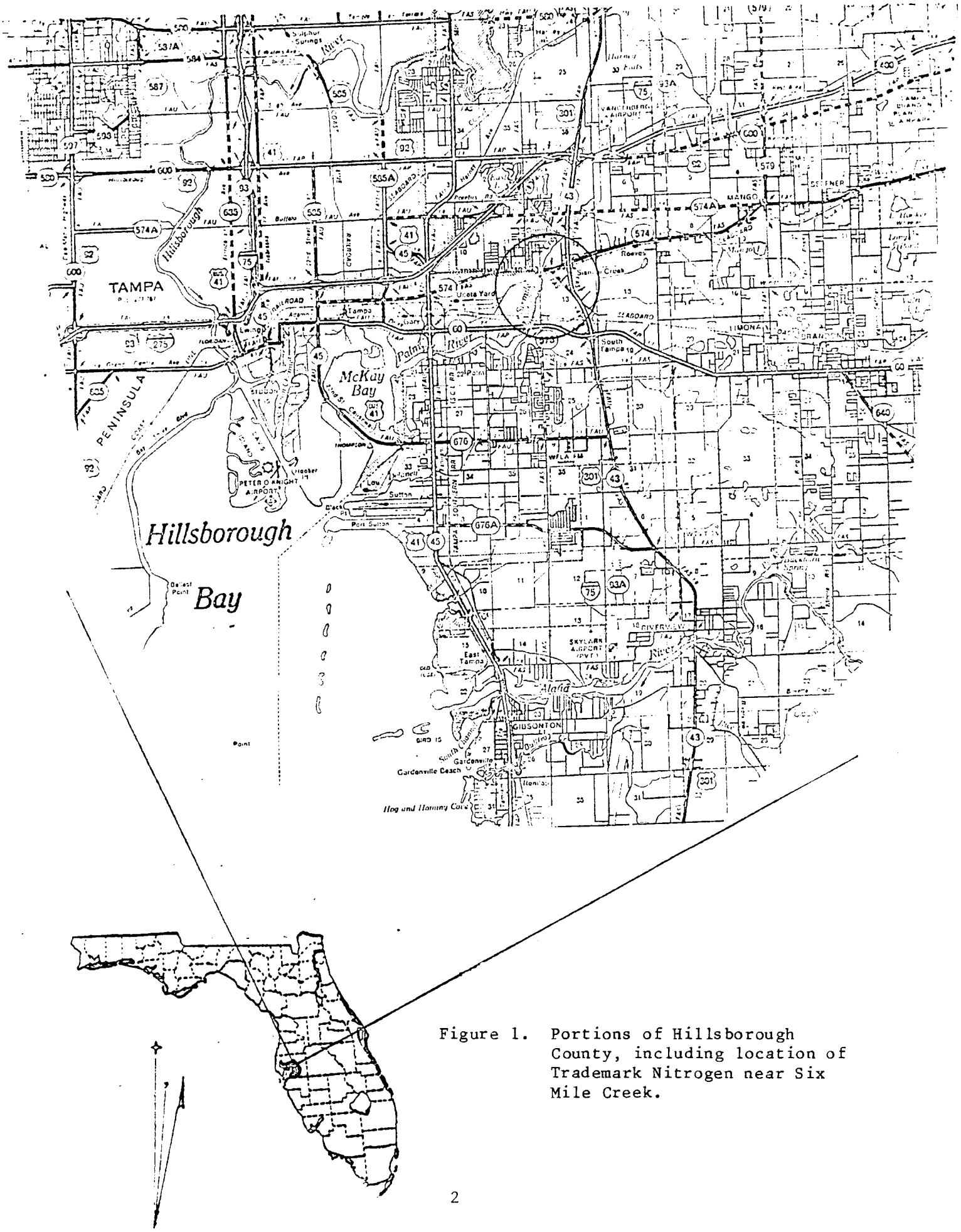


Figure 1. Portions of Hillsborough County, including location of Trademark Nitrogen near Six Mile Creek.

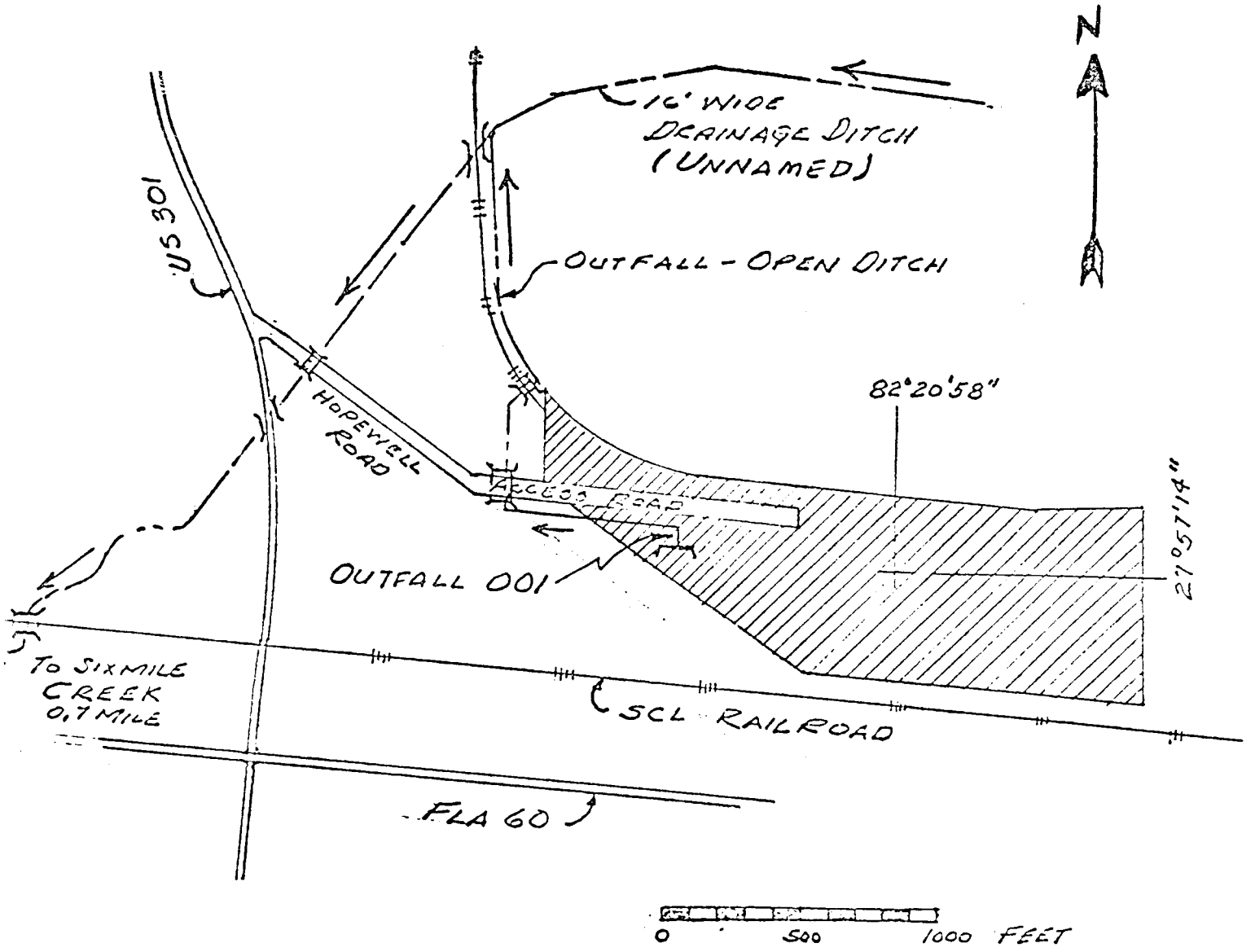


Figure 2. Location of Trademark Nitrogen's Outfall #001, discharged near Six Mile Creek, Tampa, Florida.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier and Weber (1985) and the FDER Biology Section (1985b). The organisms selected for this study include species recommended by Peltier and Weber (1985), the U.S. EPA (1978), and the FDER Biology Section (1985b). 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 32 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 (1985b). 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 separate 48 hour static acute toxicity bioassays were performed on the sample of wastewater from Trademark Nitrogen's Outfall #001. The tests were performed from 0915 hours on 14 January, 1987 to 0930 hours on 16 January, 1987.

The sample was collected by Mr. David Demmi of DER's Southwest Florida District at 0930 hours on 13 January, 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 samples was obtained from the same DER deep well that is used to culture the test organisms. The concentration series selected for this study is given in Table 1.

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 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 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 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 the HACH colormetric test. 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 Table 2.

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

Daphnia pulex (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	200.0 ml	--------	200 ml
50.0%	100.0 ml	100.0 ml	200 ml
100.0%	--------	200.0 ml	200 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Sample	Total Volume
Control	500 ml	--------	500 ml
50.0%	250 ml	250 ml	500 ml
100.0%	------	500 ml	500 ml

Table 2. Data recorded during 48 hour, *Daphnia pulex* and *Notropis leedsi*, static acute toxicity bioassay of Trademark Nitrogen, NPDES #FL0000647, on 14 to 16 January, 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
Daphnia pulex														
Control	10	10	10	8.5	7.8	8.1	8.0	7.9	7.8	21.1	20.7	20.7		
Control	10	10	10	8.5	7.8	8.1	8.0	7.9	7.8				202	225
50.0%	10	10	9	8.4	7.8	8.0	7.8	7.9	7.8	21.7	20.4	20.4		
50.0%	10	10	10	8.4	7.8	8.0	7.8	7.8	7.8				320	340
100.0%	10	10	10	8.0	8.3	7.9	7.6	8.0	7.9	22.8	20.5	20.4		
100.0%	10	10	10	8.0	8.3	7.9	7.6	8.0	7.9				440	440
Notropis leedsi														
Control	5	5	5	8.3	7.4	7.0	7.7	7.7	7.8	21.5	20.5	20.4		
Control	5	5	5	8.3	7.4	7.0	7.7	7.7	7.8				200	220
50.0%	5	5	5	8.0	7.3	7.0	7.6	7.6	7.7	22.0	20.4	20.3		
50.0%	5	4	4	8.0	7.3	7.0	7.6	7.6	7.7				330	340
100.0%	5	5	5	7.9	7.3	7.2	7.5	7.6	7.7	23.1	20.3	20.3		
100.0%	5	5	5	7.9	7.3	7.2	7.5	7.6	7.9				455	450

Control Water	
NH_3(as NH_3)(mg/l) 0	Total Alkalinity (mg/l $CaOO_3$) 117
Total ammonia (as N)(mg/l) 0	Total Hardness (mg/l $CaOO_3$) 115

100% Effluent	
NH_3(as NH_3)(mg/l) .05	Total Alkalinity (mg/l $CaOO_3$) 175
Total ammonia (as N)(mg/l) 2	Total Hardness (mg/l $CaOO_3$) 245

RESULTS AND DISCUSSION

The sample of wastewater collected from Trademark Nitrogen's Outfall #001 was not acutely toxic to the test organisms, Daphnia pulex and Notropis leedsi. No mortality was noted in the D. pulex test at 24 hours, and 5% mortality was observed in the 50% concentration at 48 hours. Although 10% mortality was noted in the N. leedsi test in the 50% concentration at 24 hours, no mortality was observed in the 100% concentration. The total ammonia (2.0 mg/l) and unionized ammonia (0.05 mg/l) measured in this sample were insufficient to have caused any toxicity. Since the sample was not acutely toxic, chemical analyses were not performed.

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