


# BIOASSAYS OF TRADEMARK NITROGEN

TAMPA, HILLSBOROUGH COUNTY, FLORIDA

NPDES PERMIT #FL0000647

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January 2, 1985

Biology Section
Division of Environmental Programs

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## EXECUTIVE SUMMARY

Trademark Nitrogen, Tampa, on 16 to 18 October, 1984.

The sample of wastewater collected from Trademark Nitrogen's Outfall #001 was not toxic to the test organisms, Daphnia pulex and Heterandria formosa. No mortality was noted in the D. pulex test at 24 hours. (The 48 hour test was invalidated.) No mortality was noted in the 48 hour H. formosa test. Although the sample was not toxic to the test organisms, significant concentrations of total ammonia (maximum measured = 13.7 mg/l) and of unionized ammonia (maximum measured = 0.32 mg/l) were detected in the wastewater. 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 results of these tests are presented in this report. Those involved in performing these tests and in preparation of this report were: R. B. Frydenborg, M. L. Moultrie, and M. L. Roll.

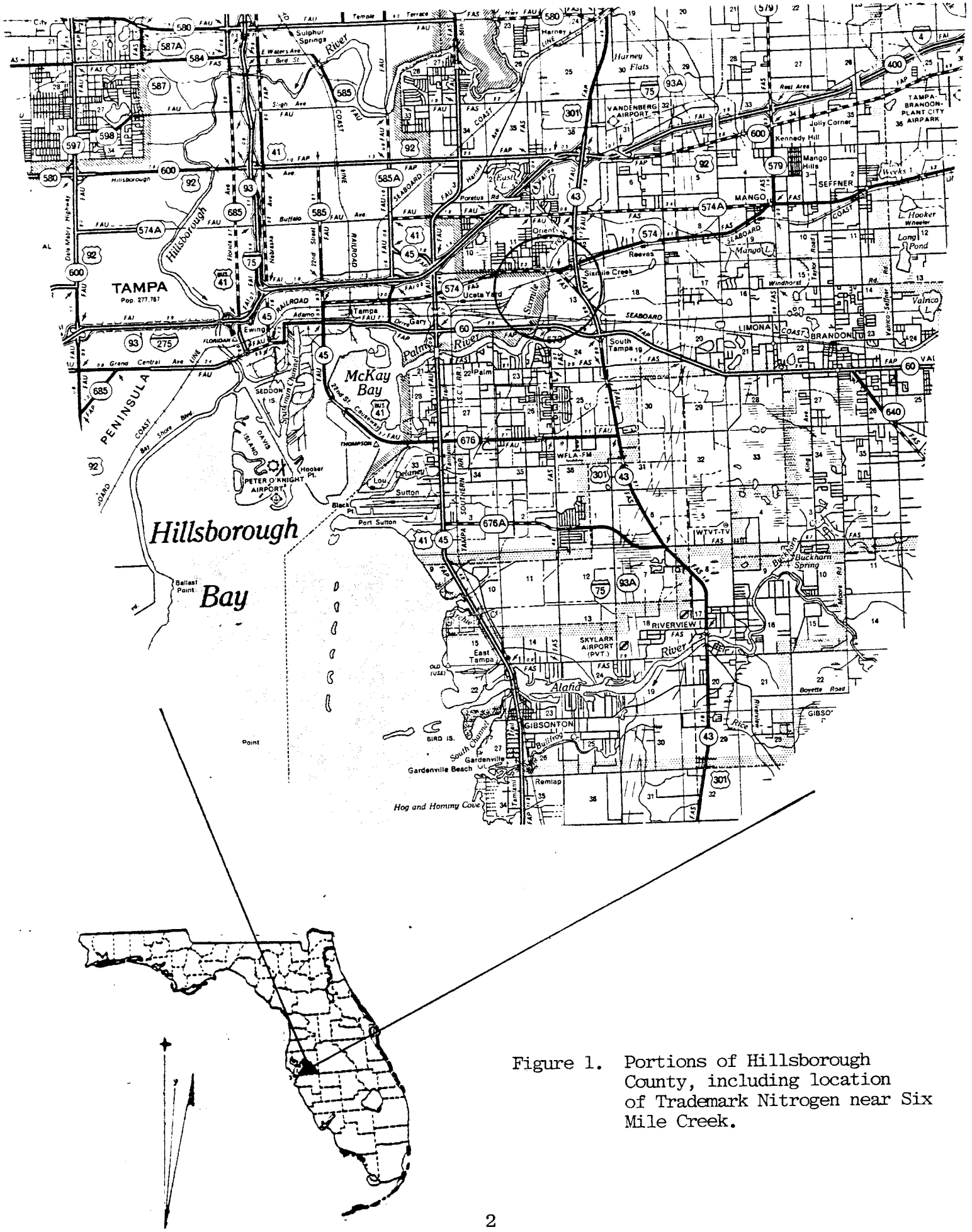



Figure 1. Portions of Hillsborough County, including location of Trademark Nitrogen near Six Mile Creek.

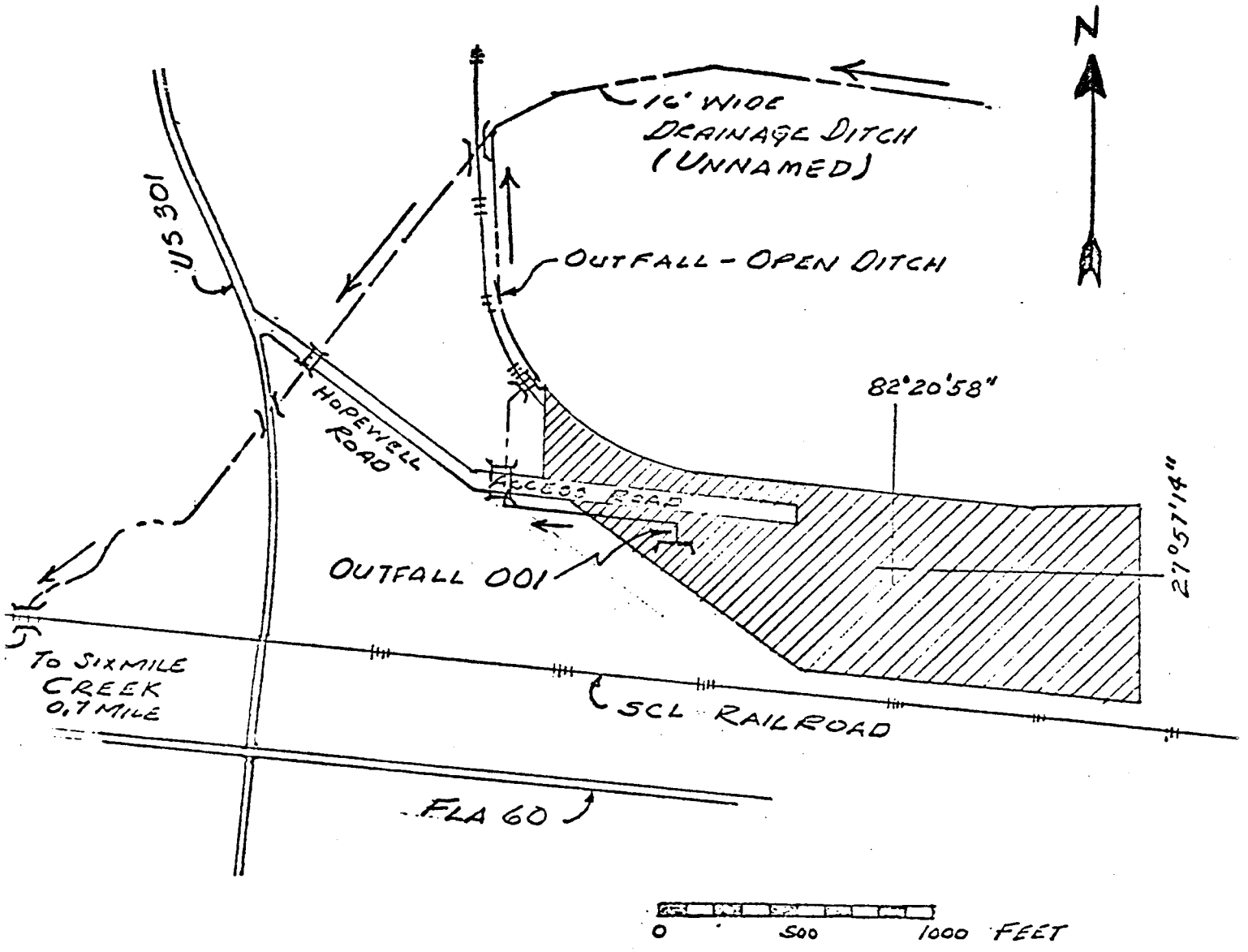



Figure 2. Location of Trademark Nitrogen's Outfall #001, discharged near Six Mile Creek, Tampa, Flordia.

## METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978) and the FDER Biology Section (1983). The organisms selected for this study include a species recommended by Peltier (1978), the U.S. EPA (1978), and the FDER Biology Section (1983). 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° plus or minus 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.

Heterandria formosa (least killifish) - Approximately 100 adult least killifish were obtained from a wild population in the St. Marks Wildlife Refuge (Wakulla County, Florida) during August, 1980. The fish are maintained in two and one-half to 15 gallon all-glass aquaria equipped with a flow-through water exchange system. Water for this system is unchlorinated and is pumped from a deep well near the DER laboratory. The least killifish are reared at 22° plus or minus 2°C under a 16:8 hour light-dark cycle and are fed freshly hatched Artemia (brine shrimp) and Tetra-Min® twice daily and once daily on weekends. New born fish are initially maintained in the adult tanks, but are transferred to separate aquaria at weekly intervals, thereby segregating fish into even age groups. The fish used in this test were 3 - 4 weeks old.

To ensure the uniform sensitivity of these test organisms to toxic substances, the Biology Section conducts monthly stndard reference toxicant bioassays using sodium lauryl sulfate as the reference toxicant.

## Test Methods

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 0945 hours on 16 October to 1010 hours on 18 October, 1984.

The sample was collected by Ms. Phyllis Giwer of DER's Southwest Florida District at 1425 hours on 15 October, 1984, 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.

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 (Table 1) is that recommended by APHA (1980).

Two replicates, each with five concentrations 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 not fed during this test.

Two replicates, of three concentrations and a control, were used for the Heterandria formosa (least killifish) 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.

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 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 |
| 18.0% | 164.0 ml | 36.0 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 |

*Heterandria formosa* (least killifish)

| Concentration | Dilution Water | Effluent | Total Volume |
|---|---|---|---|
| Control | 500 ml | ------ | 500 ml |
| 10.0% | 450 ml | 50 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, static acute toxicity bioassay of Trademark Nitrogen, FL0000647, on 16 to 18 October, 1984.

| Concentration or % | Number of Live Organisms | | | Dissolved Oxygen (mg/l) | | | pH | | | Temperature (°C) | | | Conductivity (umhos) | | $NH_3/N$ (mg/l) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 24 | 48 | 0 | 24 | 48 | 0 | 24 | 48 | 0 | 24 | 48 | 0 | 48 | 0 | 48 |
| Control | 10 | 10 | a | 8.7 | 8.0 | a | 7.7 | 8.0 | a | 21.4 | 20.7 | a | | | 0/0 | |
| Control | 10 | 10 | | 8.7 | 7.9 | | 7.7 | 8.0 | | | | | 212 | a | | |
| 10.0% | 10 | 10 | | 8.6 | 8.1 | | 7.7 | 8.1 | | 21.6 | 20.5 | | | | | |
| 10.0% | 10 | 10 | | 8.6 | 8.1 | | 7.7 | 8.1 | | | | | 333 | | | |
| 18.0% | 10 | 10 | | 8.5 | 8.2 | | 7.7 | 8.1 | | 21.6 | 20.4 | | | | | |
| 18.0% | 10 | 10 | | 8.5 | 8.1 | | 7.7 | 8.1 | | | | | 428 | | | |
| 32.0% | 10 | 10 | | 8.3 | 8.1 | | 7.7 | 8.0 | | 21.9 | 20.4 | | | | | |
| 32.0% | 10 | 10 | | 8.3 | 8.0 | | 7.7 | 8.0 | | | | | 570 | | | |
| 56.0% | 10 | 10 | | 7.8 | 7.7 | | 7.7 | 7.9 | | 22.3 | 20.4 | | | | | |
| 56.0% | 10 | 10 | | 7.8 | 7.7 | | 7.7 | 7.9 | | | | | 830 | | | |
| 100.0% | 10 | 10 | | 7.0 | 7.7 | | 7.6 | 7.9 | | 23.0 | 20.5 | | | | 0.32/13.7 | |
| 100.0% | 10 | 10 | | 7.0 | 7.7 | | 7.6 | 7.9 | | | | | 1320 | | | |
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Control Water

Total Alkalinity 88 (mg/l $CaCO_3$)

Total Hardness 113 (mg/l $CaCO_3$)

100% Sample

Total Alkalinity 76 (mg/l $CaCO_3$)

Total Hardness 364 (mg/l $CaCO_3$)

[a] 48 hour test invalidated due to excessive control mortality, parameters not recorded.

Table 3. Data recorded during 48 hour, Heterandria formosa, static acute toxicity bioassay of Trademark Nitrogen, FL0000647, on 16 to 18 October, 1984.

| Concentration or % | Number of Live Organisms | | | Dissolved Oxygen (mg/l) | | | pH | | | Temperature (°C) | | | Conductivity (umhos) | | $NH_3/N$ (mg/l) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 24 | 48 | 0 | 24 | 48 | 0 | 24 | 48 | 0 | 24 | 48 | 0 | 48 | 0 | 48 |
| Control | 5 | 5 | 5 | 8.4 | 7.0 | 7.9 | 7.7 | 7.7 | 7.8 | 21.8 | 20.5 | 19.7 | | | 0/0 | |
| Control | 5 | 5 | 5 | 8.4 | 7.1 | 7.9 | 7.7 | 7.7 | 7.8 | | | | 218 | 217 | | |
| 10.0% | 5 | 5 | 5 | 8.4 | 7.2 | 8.0 | 7.7 | 7.7 | 7.8 | 21.8 | 20.3 | 19.4 | | | | |
| 10.0% | 5 | 5 | 5 | 8.4 | 7.2 | 8.0 | 7.7 | 7.7 | 7.8 | | | | 340 | 328 | | |
| 50.0% | 5 | 5 | 5 | 7.8 | 7.2 | 7.9 | 7.7 | 7.8 | 7.8 | 22.2 | 20.2 | 19.3 | | | | |
| 50.0% | 5 | 5 | 5 | 7.8 | 7.2 | 7.9 | 7.7 | 7.8 | 7.8 | | | | 790 | 770 | | |
| 100.0% | 5 | 5 | 5 | 6.9 | 7.0 | 7.8 | 7.6 | 7.8 | 7.7 | 23.0 | 20.2 | 19.3 | | | 0.32/13.7 | |
| 100.0% | 5 | 5 | 5 | 6.9 | 7.0 | 7.8 | 7.6 | 7.8 | 7.7 | | | | 1360 | 1290 | | |
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Control Water

Total Alkalinity 88 (mg/l $CaCO_3$)

Total Hardness 113 (mg/l $CaCO_3$)

100% Sample

Total Alkalinity 76 (mg/l $CaCO_3$)

Total Hardness 364 (mg/l $CaCO_3$)

Table 4. $LC_{50}$ Data for Bioassays of Trademark Nitrogen, FL0000647, on 16 to 18 October, 1984.

| Organism | Method of Data Analysis | 24 Hour $LC_{50}$ and 95% Confidence Intervals | 48 Hour $LC_{50}$ and 95% Confidence Intervals |
|---|---|---|---|
| D. pulex | Binomial<br>Moving Average<br>Probit | No mortality | a |
| H. formosa | Binomial<br>Moving Average<br>Probit | No mortality | No mortality |



[a] Test was invalidated due to excessive control mortality.

## RESULTS AND DISCUSSION

The sample of wastewater collected from Trademark Nitrogen's Outfall #001 was not toxic to the test organisms, Daphnia pulex and Heterandria formosa. No mortality was noted in the D. pulex test at 24 hours. The 48 hour test was invalidated due to excessive control mortality (Table 2). Likewise, no mortality was noted during the 48 hour H. formosa test (Table 3). Although the sample was not toxic to the test organisms, significant concentrations of total ammonia (maximum measured = 13.7 mg/l) and of unionized ammonia (maximum measured = .32 mg/l) were detected in this wastewater. Since the sample was not acutely toxic, chemical analyses were not performed.

## LITERATURE CITED

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