

BIOASSAYS OF NITRAM CHEMICALS

TAMPA, HILLSBOROUGH COUNTY, FLORIDA

NPDES #FL0001643

APRIL 14, 1987

Biology Section
Division of Environmental Programs

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Biology Section
Bureau of Laboratories and
Special Programs
April 14, 1987

EXECUTIVE SUMMARY

Nitram Chemicals, Tampa, NPDES #FL0001643, on 3 to 5 February, 1987.

The sample of wastewater effluent from Nitram Chemicals, a phosphate fertilizer manufacturer which discharges to Delaney Creek, was not acutely toxic to the test organisms, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi. No significant mortality was observed during the 48 hour tests. The total and unionized ammonia (3.3 and 0.03 mg/l, respectively) was insufficient to have caused any toxicity.

INTRODUCTION

Nitram Chemicals, Incorporated, is located at 5321 Hartford Street in Tampa, Florida (Figure 1). This company discharges an average of 0.45 MGD wastewater from outfall #001 through a ditch to Delaney Creek, which flows into Hillsborough Bay.

Nitram Chemicals began operation in 1963, producing up to 1200 tons/day of ammonium nitrate fertilizer. The company operates two ammonium prilling plants and two nitric acid plants in the manufacturing process. The daily plant operation requires 1.10 MGD intake water. After its use, this water is split into domestic wastewater and process wastewater prior to treatment (Figure 2). Sanitary wastewater (0.002 MGD) is routed through a package sewage treatment plant and then to complete retention in an evaporation pond. Process water is temporarily retained on site in a holding pond. Treatment included a diversion system for the retention of contaminants and an ammonium/ammonium nitrate recovery system prior to discharge to Delaney Creek.

On 22 April, 1980, the Biology Section obtained an effluent sample from Nitram Chemicals, Inc., outfall #001 and performed a static bioassay to determine the existence and extent of toxicity. The results of that test indicated that the effluent was not toxic to the test organism, Daphnia pulex, at that time (FDER 1980).

To further evaluate the potential toxicity of effluent from Nitram Chemicals to aquatic organisms, an additional sample was collected on 12 December, 1983. Two 48 hour static acute toxicity bioassays were performed on 13 to 15 December, using Daphnia pulex and Mysidopsis bahia as test organisms. The results of those tests again indicated that Nitram's wastewater was not acutely toxic (FDER 1984). The Southwest District, however, has suspected Nitram of discharging fluctuating amounts of ammonia to the receiving waters. Therefore, an onsite 96 hour flow-through bioassay was requested. The flow-through tests were performed from 16 to 20 February, 1986. It should be noted that the Nitram facility was closed for maintenance during the week prior to the flow-through tests. The results of these tests indicated that the wastewater being discharged during the bioassay was not acutely toxic to Notropis leedsi. However, a serious abnormality occurred near hour 48 of the test which resulted in the death of significant numbers of N. leedsi. This abnormality can best be described as an unexpected interaction of bacteria in Nitram's wastewater with components in the dilution water which caused a BOD problem. This resulted in dissolved oxygen depletion and subsequent organism mortality. Mortality was observed only in diluted Nitram wastewater; no mortality occurred in either the control (dilution water) or in the 100% wastewater concentration. Since mortality was attributed to depleted oxygen, aeration was initiated in each test chamber and additional fish were exposed to each effluent concentration at hour 48. No mortality occurred during the remainder of the test. In the

Daphnia pulex flow-through bioassay, only one death was observed in the 100% concentration at hour 24. Unfortunately, this test had to be terminated at 48 hours due to excessive control mortality (FDER 1986).

On 2 February, 1987, another sample was obtained by the Biology Section and three 48 hour static acute toxicity bioassays were performed. Results are presented in this report. Those involved in performing the tests and in preparation of this report were: K. M. Lurding, M. L. Roll, and P. G. Weiland.

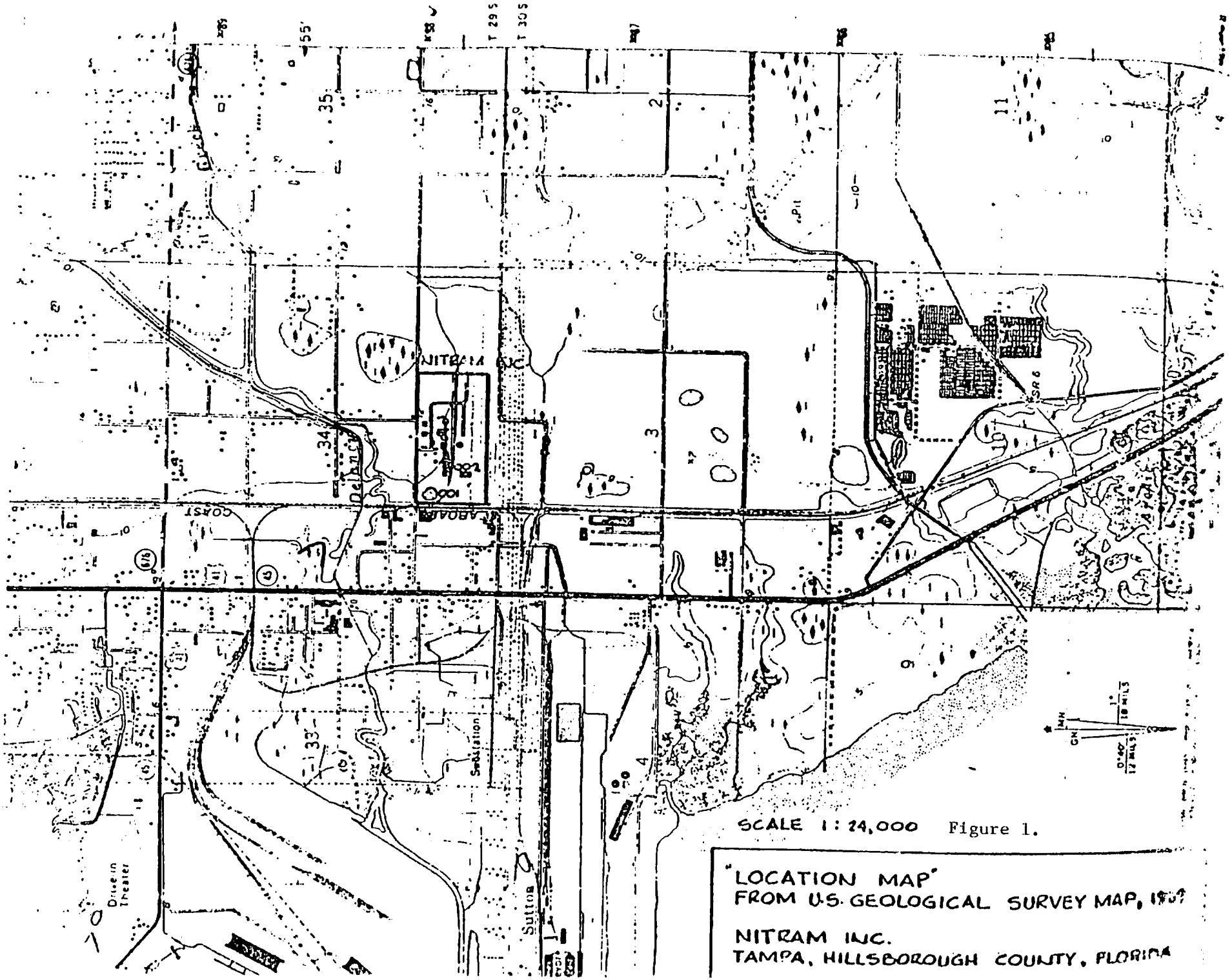


Figure 1.

"LOCATION MAP"
FROM U.S. GEOLOGICAL SURVEY MAP, 1957

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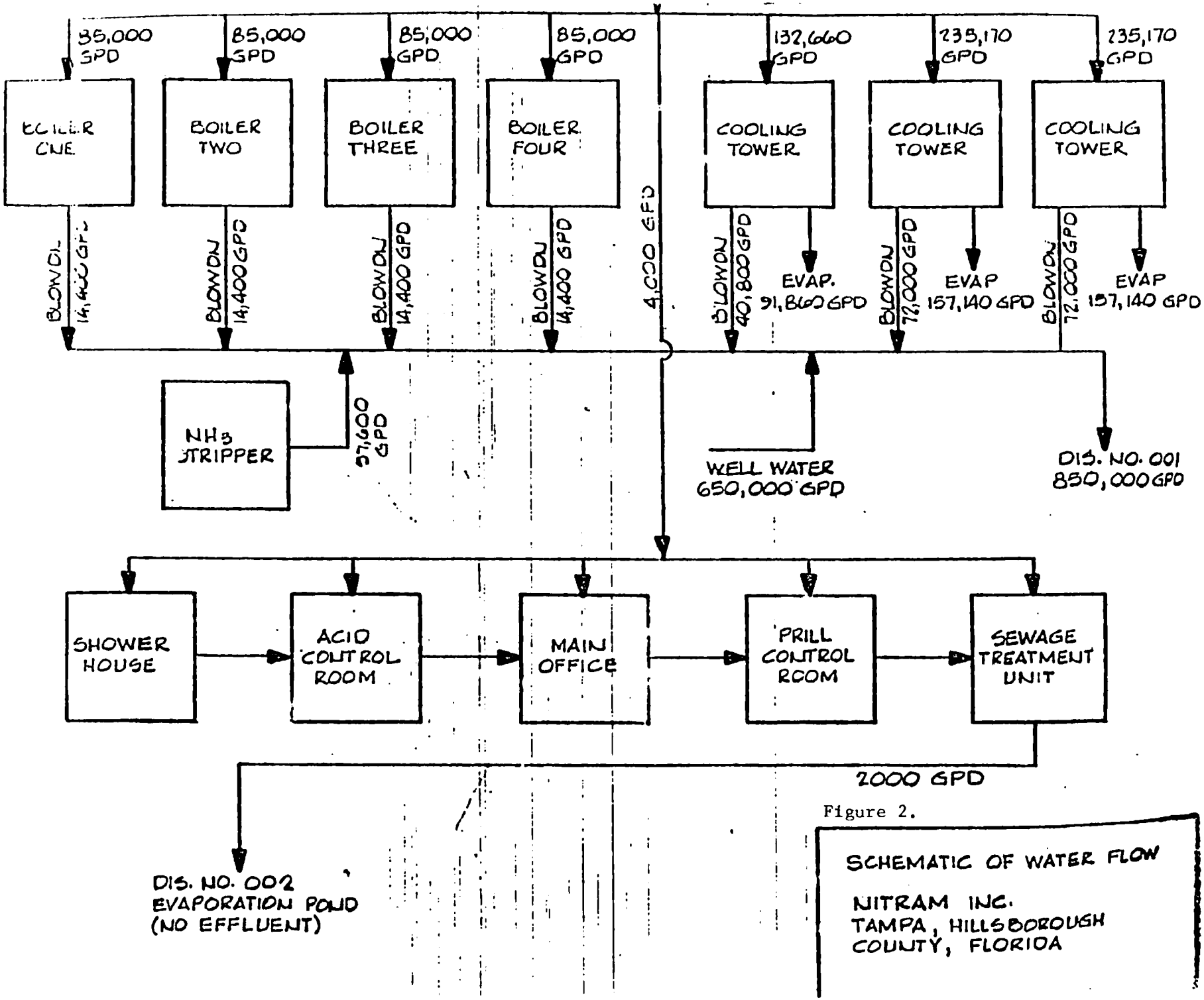


Figure 2.

SCHEMATIC OF WATER FLOW

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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 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 48 hour static acute toxicity bioassays were performed on an effluent sample collected from Nitram Chemicals, Inc. The tests were initiated at 0910 hours on 3 February, 1987 and lasted until 0910 hours on 5 February, 1987.

The sample was collected by Mr. David Demmi of DER's Southwest district office at 0929 hours on 2 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° ± 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 recovered water sample 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 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 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 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 form and are presented in Table 2.

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

Ceriodaphnia dubia (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	50.0 ml	--------	50 ml
50.0%	25.0 ml	25.0 ml	50 ml
100.0%	-------	50.0 ml	50 ml

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, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi, static acute toxicity bioassay of Nitram Chemicals, NPDES #FL0001643, on 3 to 5 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
Ceriodaphnia dubia														
Control	10	10	10	8.4	8.1	6.7	8.0	8.0	8.0	21.4	20.5	20.8		
Control	10	10	10	8.4	8.1	6.7	8.0	8.0	8.0					
50.0%	10	10	10	8.1	7.8	6.9	7.6	8.1	8.0	21.5	20.3	20.7		
50.0%	10	10	10	8.1	7.8	6.9	7.6	8.1	8.0					
100.0%	10	10	10	7.3	7.5	7.3	7.2	8.1	8.0	21.6	20.3	20.7		
100.0%	10	10	10	7.3	7.5	7.3	7.2	8.1	8.0					
Daphnia pulex														
Control	10	10	10	8.9	8.2	5.7	8.0	8.1	8.0	21.5	20.4	20.7		
Control	10	10	9	8.9	8.2	5.7	8.0	8.1	8.0				205	220
50.0%	10	10	10	8.2	7.9	6.8	7.5	8.0	7.9	21.5	20.2	20.6		
50.0%	10	10	10	8.2	7.9	6.8	7.5	8.0	7.9				1920	1920
100.0%	10	10	10	7.4	6.5	7.3	7.2	7.9	7.9	21.6	20.2	20.6		
100.0%	10	9	9	7.4	6.5	7.3	7.2	7.9	7.9				3480	3490
Notropis leedsi														
Control	5	5	5	8.0	7.6	5.6	7.6	7.9	7.7	21.9	20.4	20.7		
Control	5	4	4	8.0	7.6	5.6	7.6	7.9	7.7				215	235
50.0%	5	5	5	7.8	6.7	6.9	7.3	7.7	7.8	21.8	20.1	20.5		
50.0%	5	5	5	7.8	6.7	7.2	7.3	7.7	7.8				1970	1930
100.0%	5	5	5	7.3	5.5	7.0	7.1	7.6	7.8	21.7	20.1	20.4		
100.0%	6[a]	6	6	7.3	5.3	7.2	7.1	7.6	7.8				3500	3500

Control Water

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

Total Alkalinity (mg/l $CaCO_3$): 115

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

Total Hardness (mg/l $CaCO_3$): 116

100% Effluent

NH_3(as NH_3)(mg/l): 0.03

Total Alkalinity (mg/l $CaCO_3$): 150

Total ammonia (as N)(mg/l): 3.3

Total Hardness (mg/l $CaCO_3$): >500

[a] Extra organism loaded by mistake - allowed to remain

RESULTS AND DISCUSSION

The effluent sample collected from Nitram Chemicals, Inc., was not acutely toxic to the test organisms, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi. No significant mortality was noted during the 48 hour static tests (Table 2). The total ammonia (3.3 mg/l) and unionized ammonia (0.03 mg/l) were insufficient to have caused any toxicity.

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