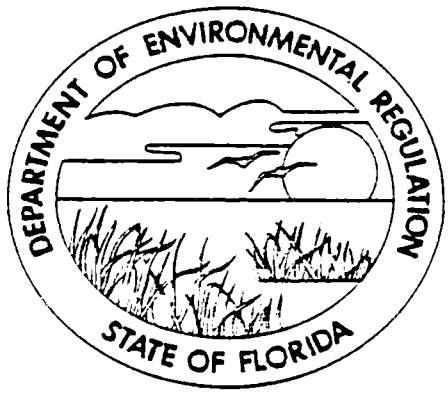


BIOASSAYS OF NITRAM CHEMICALS, INCORPORATED

TAMPA, HILLSBOROUGH COUNTY, FLORIDA

NPDES #FL0001643

Biology Section
Bureau of Water Analysis
January 11, 1984

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EXECUTIVE SUMMARY

Nitram Chemicals, Tampa, NPDES #FL0001643, on 13 to 15 December, 1983.

The sample of wastewater effluent from Nitram Chemicals, a phosphate fertilizer manufacturer, was not acutely toxic to the test organisms, Daphnia pulex and Mysidopsis bahia. No D. pulex and only two of 10 M. bahia in the 100% effluent concentration died during the 48 hour static tests. Ammonia concentrations of 2.8 mg/l (total) and 0.022 mg/l and 0.032 mg/l (unionized) were measured in the sample, which were insufficient to have caused the M. bahia mortality noted. Since the sample was not acutely toxic, further chemical analyses were not performed.

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 to a canal, and then 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 wastewater is temporarily retained on site in a holding pond. Treatment includes a diversion system for the retention of contaminants and an ammonium/ammonium nitrate recovery system prior to discharge to Delaney Creek.

Noncompliance with NPDES permit specifications was noted on 29 November 1974 when Nitram, Inc., received a Notice of Violation for exceeding the maximum pH limit in its effluent. Potentially toxic substances in the discharge water may include: chromium, zinc, oil and grease, ammonium, nitrate, nitrite, and chlorine.

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 to the biota of the receiving waters. 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 these latest tests are presented in this report. Those involved in performing the tests and in preparation of this report were: C. W. Dye, R. B. Frydenborg, and M. L. Roll.

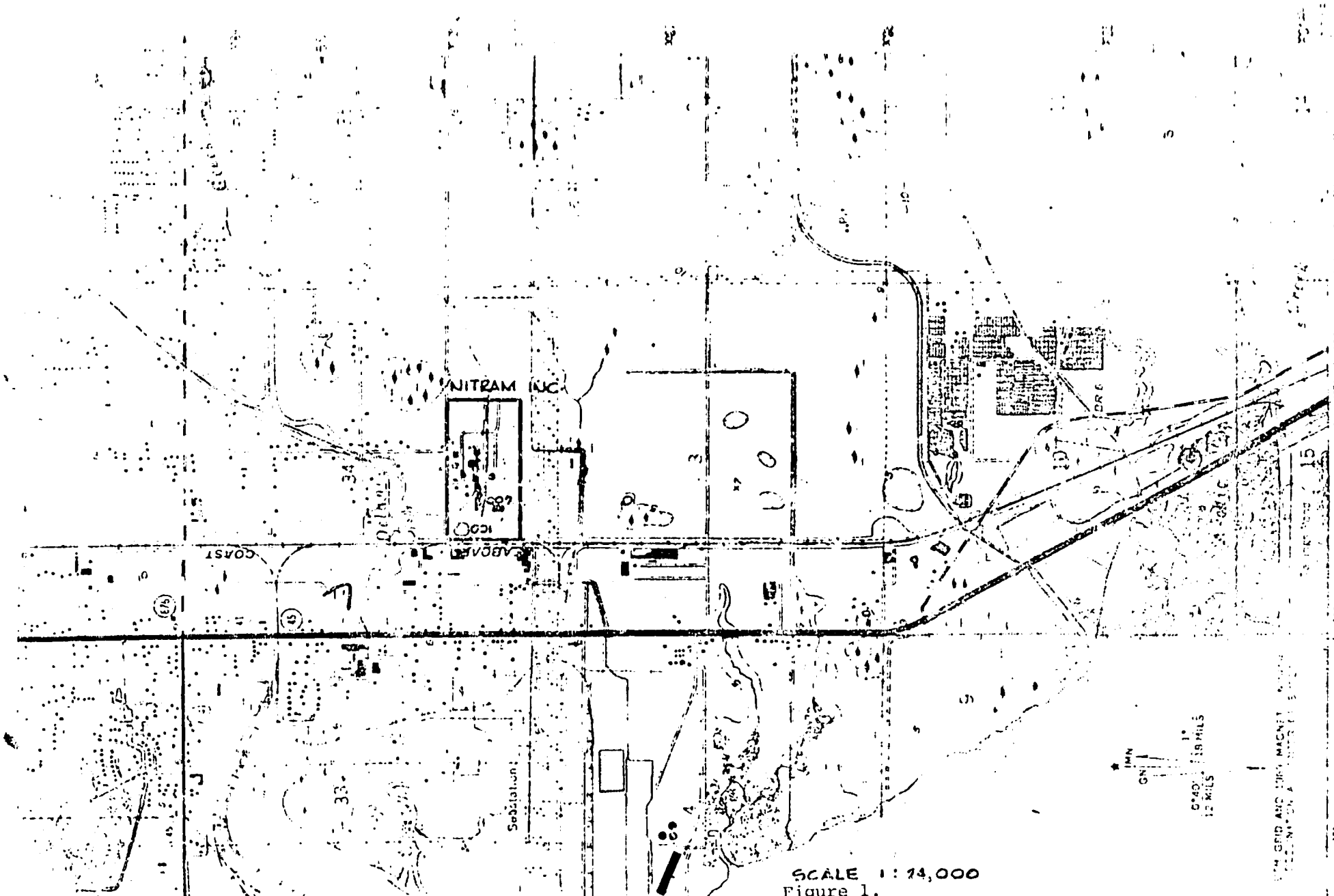


Figure 1.

"LOCATION MAP"
FROM U.S. GEOLOGICAL SURVEY MAP, 1969

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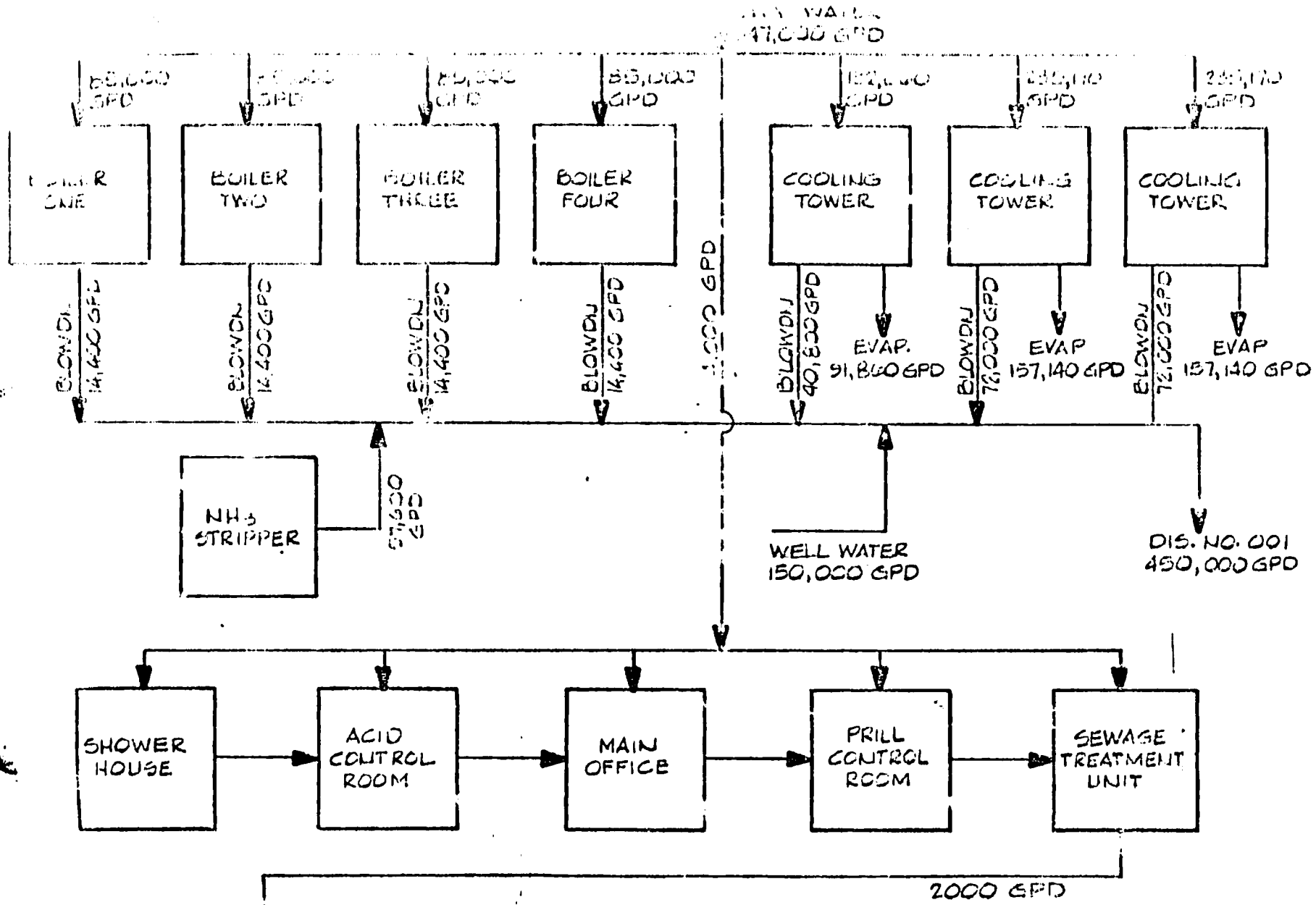


Figure .

SCHEMATIC OF WATER FLOW

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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 are among those recommended by Peltier (1978), the U.S. EPA (1978), and the FDER Biology Section (1983). 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, 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. They are then refilled 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.

Mysidopsis bahia (mysid shrimp) - In September, 1978, approximately 300 adult mysid shrimp were obtained from the EPA laboratory in Gulf Breeze, Florida. The mysids are cultured under a 16:8 hour light-dark cycle in 10 and 15 gallon all-glass aquaria equipped with undergravel filtration and aeration. The mysid shrimp are cultured over a wide range of salinities (10-30 ppt) to minimize acclimation periods to low or high salinity effluents. Seawater is obtained from the FSU Marine Laboratory located at Turkey Point, Florida (salinity range 17-31 ppt) and partial water changes are performed weekly. Freshwater, for dilution of the seawater as necessary, is obtained from a deep well near the DER laboratory. The mysids are fed twice daily with freshly hatched Artemia (brine shrimp) and are kept at a constant temperature of 22° plus or minus 2°C. Adult or large juvenile mysids were used in this 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

Two 48 hour static acute toxicity bioassays were performed on a sample of wastewater effluent from Nitram Chemicals. The tests were performed from 1020 hours on 13 December to 1030 hours on 15 December , 1983.

The sample was collected by Mr. Matt McCann of DER's Southwest District at 1100 hours on 12 December, 1983, using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples for metal analyses were preserved with HNO_3. The sample for the bioassays was 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 sample for the D. pulex bioassay 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 six concentrations and two controls, were used to test with the water flea, Daphnia pulex[1]. 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.

Saltwater for dilution of the effluent sample for the M. bahia bioassay was obtained from the FSU Marine Laboratory at Turkey Point (salinity = 27.0 ppt). For test concentrations of 56% and below, this diluent was mixed directly with the effluent, achieving a range of final salinities (14.0 ppt to 25.8 ppt). For the 100% effluent test concentration, freshly autoclaved artificial sea salts (Ocean 50®) were added until a salinity of approximately 7 ppt was achieved (the lower limit of mysid shrimp salinity tolerance). Two control groups (Table 3) were then used to ensure that any mortality in the test organisms was not the result of toxic properties associated with the diluent, the artificial sea salts, or the salinity range. The control groups included:

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[1] Since the sample had a salinity of approximately 2 ppt, and it was not known if D. pulex could tolerate this salinity, an additional control consisting of well water adjusted to 2 ppt salinity using artificial sea salts was added. This ensures that any toxicity obsrved in the bioassay is not due to the salinity.

1) Diluent Control - Turkey Point water (27.0 ppt). This represents the highest concentration of Turkey Point water used in the bioassay, and tests for toxicity in the diluent. This is also the highest salinity used in the bioassay, and therefore tests the tolerance of the organisms to this salinity.

2) Sea Salts Control - Well water, adjusted with artificial sea salts (Ocean 50®) to approximately 7 ppt salinity. This tests for toxicity of the highest concentration of artificial sea salts used in the bioassay, as well as for tolerance of the organisms to the lowest salinity used in the bioassay.

The concentration series selected for this study (Table 1) is that recommended by APHA (1980). Two replicates of each test concentration and of the controls were used to test with Mysidopsis bahia. Immediately prior to the bioassay, a suitable number of mysids were transferred by net from the 10 ppt, 15 ppt, 20 ppt, and 25 ppt salinity aquaria to culture dishes for observation and selection of individuals for the test. Individual mysid shrimp were siphoned using a glass tube, carefully examined for injury, and transferred to wide mouth quart jars, each containing 500 ml of the appropriate concentration. For each concentration, the mysid shrimp were pre-acclimated to within 4 ppt salinity. Five organisms were loaded per test chamber, giving ten per concentration. Based on the recommendations of EPA (1978), the mysids were fed brine shrimp (Artemia) nauplii at the rate of approximately 10 to 20 nauplii per mysid per day to minimize cannibalism.

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 chamber 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 D. pulex test for the control and the 100% effluent concentration. The specific conductance or salinity (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
Controls	200.0 ml	--------	200 ml
5.6%	188.8 ml	11.2 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

Mydidopsis bahia (mysid shrimp)

Concentration	Dilution Water	Effluent	Total Volume
Controls	500 ml	------	500 ml
5.6%	472 ml	28 ml	500 ml
10.0%	450 ml	50 ml	500 ml
18.0%	410 ml	90 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, Daphnia pulex, static acute toxicity bioassay of Nitram Chemicals, Tampa, Hillsborough County, Florida on 13 to 15 December, 1983.

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)		Total Hardness (mg/l as $CaCO_3$)		Temperature (^oc)			NH_3/N (mg/l)		Conductivity (µmhos)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	10	10	10	8.4	8.8	8.5	7.7	8.2	8.3	94		123		20.9	20.3	20.5	0/0	---		
Control	10	10	10	8.4	8.8	8.5	7.7	8.2	8.3										197	222
Salinity Control	10	10	10	8.2	8.6	8.7	7.9	8.4	8.5					21.7	20.1	20.6				
Salinity Control	10	10	10	8.2	8.6	8.7	7.9	8.4	8.5										3990	4090
5.6%	10	10	10	8.4	8.8	8.6	7.7	8.2	8.3					20.9	20.2	20.3				
5.6%	10	10	10	8.4	8.8	8.6	7.7	8.2	8.3										370	409
10.0%	10	10	10	8.3	8.7	8.7	7.7	8.2	8.3					20.9	20.1	20.2				
10.0%	10	10	10	8.3	8.7	8.7	7.7	8.2	8.3										580	600
18.0%	10	10	10	8.2	8.7	8.7	7.6	8.2	8.3					20.8	20.1	20.2				
18.0%	10	10	10	8.2	8.7	8.7	7.6	8.2	8.3										820	880
32.0%	10	10	10	8.0	8.6	8.8	7.5	8.1	8.3					20.8	20.1	20.3				
32.0%	10	10	10	8.0	8.6	8.8	7.5	8.1	8.3										1300	1330
56.0%	10	10	10	7.7	8.4	8.9	7.4	8.1	8.3					20.8	20.1	20.4				
56.0%	10	10	10	7.7	8.4	8.9	7.4	8.1	8.3										2050	2120
100.0%	10	10	10	6.7	8.3	8.9	7.2	8.0	8.3	108		752		21.0	20.0	20.5	.022/2.8	---		
100.0%	10	10	10	6.7	8.3	8.9	7.2	8.0	8.3										3390	3470

Remarks: Total Residual Chlorine: None detected (HACH DPD Method).

Table 3. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Nitram Chemicals, Tampa, Hillsborough County, Florida, on 13 to 15 December, 1983.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			NH_3/N (mg/l)		Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
Diluent Control	5	5	5	7.3	6.9	7.5	8.3	8.1	8.2	21.0	20.2	20.3	0/0	---		
Diluent Control	5	5	5	7.3	6.9	7.5	8.3	8.1	8.2						27.0	27.8
Sea Salts Control	5	5	5	8.2	8.0	8.4	8.2	8.3	8.4	21.3	20.0	20.2				
Sea Salts Control	5	5	5	8.2	8.0	8.4	8.2	8.3	8.4						7.0	7.5
5.6%	5	5	5	7.4	7.0	7.5	8.2	8.1	8.2	20.9	19.9	20.1				
5.6%	5	5	5	7.4	7.0	7.5	8.2	8.1	8.2						25.8	25.7
10.0%	5	5	5	7.4	6.9	7.6	8.1	8.1	8.2	20.8	19.9	20.2				
10.0%	5	5	5	7.4	6.9	7.6	8.1	8.1	8.2						25.0	25.0
18.0%	5	5	5	7.3	7.0	7.7	8.0	8.1	8.2	20.7	19.9	20.3				
18.0%	5	5	5	7.3	7.0	7.7	8.0	8.1	8.2						23.0	23.1
32.0%	5	5	5	7.3	7.1	7.9	7.8	8.0	8.2	20.7	19.9	20.4				
32.0%	5	5	5	7.3	7.1	7.9	7.8	8.0	8.2						19.5	19.8
56.0%	5	5	5	7.2	7.3	8.1	7.6	8.0	8.2	20.7	19.9	20.4				
56.0%	5	5	5	7.2	7.3	8.1	7.6	8.0	8.2						14.0	14.1
100.0%	5	5	3	7.3	7.4	8.2	7.4	7.9	8.2	20.2	20.0	20.6	.032/2.8	---		
100.0%	5	5	5	7.2	7.4	8.2	7.4	7.9	8.2						7.2	7.7

Remarks: Total Residual Chlorine: None Detected (HACH DPD Method).

RESULTS AND DISCUSSION

The sample of wastewater effluent from Nitram Chemicals, a phosphate fertilizer manufacturer, was not acutely toxic to the test organisms, *Daphnia pulex* and *Mysidopsis bahia*. No *D. pulex* and only two of 10 *M. bahia* in the 100% effluent concentration died during the 48 hour static tests. Ammonia concentrations of 2.8 mg/l (total) and 0.022 mg/l and 0.032 mg/l (unionized) were measured in the sample, which were insufficient to have caused the *M. bahia* mortality noted. Since the sample was not acutely toxic, further chemical analyses were not performed.

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