

FLOW-THROUGH BIOASSAYS OF NITRAM CHEMICALS

TAMPA, HILLSBOROUGH COUNTY, FLORIDA

NPDES #FL0001643

APRIL 16, 1986

Biology Section
Division of Environmental Programs

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Biology Section
Bureau of Laboratories and
Special Programs
April 16, 1986

EXECUTIVE SUMMARY

Nitram Chemcials, Flow-Through Bioassays, Tampa, on 16 to 20 February, 1986.

Nitram Chemicals is an ammonium nitrate fertilizer manufacturer with a 0.45 MGD discharge to Delaney Creek, which flows into Hillsborough Bay. Since this facility is suspected of discharging fluctuating amounts of ammonia, the Southwest District requested that an onsite 96 hour bioassay be performed. The results of these tests indicated that the wastewater being discharged during the bioassay was not actuely 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. Ammonia concentrations measured during the onsite bioassays reached a maximum of 22.1 mg/l (total) and 0.245 mg/l (unionized).

The results of the onsite Standard Reference Toxicant (sodium lauryl sulfate) tests, using N. leedsi and D. pulex, produced a 48 hour LC_{50} of 5.0 ppm and a 24 hour LC_{50} of 1.5 ppm for these organisms, respectively. These results, when compared to previous Biology Section values, suggest that the fish were healthy and viable at the time of the test, but that the D. pulex may have been stressed. Indeed, the D. pulex test had to be invalidated at 48 hours due to excessive control mortality, similar to the situation which occurred during the D. pulex flow-through bioassay.

Recommendation

Due to the fluctuations in ammonia discharges from the Nitram facility and to the possiblity of plant upsets or malfunctions, the Biology Section recommends closer scrutiny of the facility's wastewater discharge. This may include a more frequent inspection or self-monitoring sechedule, inspection of Nitram's wastewater treatment process and equipment, and the incorporation of a biomonitoring program in Nitram's permit.

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 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.

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 additinal sample was collectd 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 latest tests are presented in this report. Those involved in performing the tests and in preparation of this were: C. W. Dye, R. B. Frydenborg, and M. L. Roll.

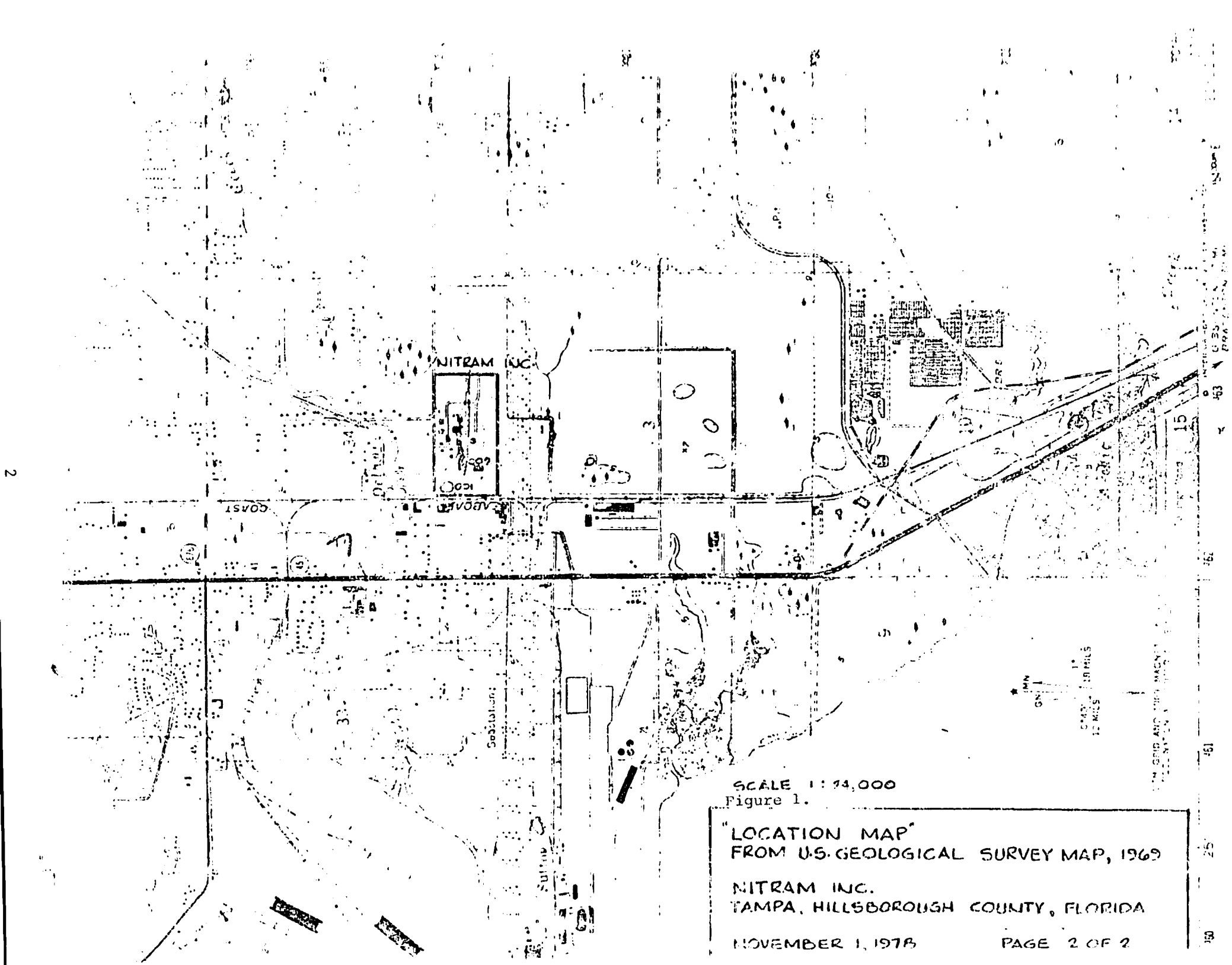


Figure 1.

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

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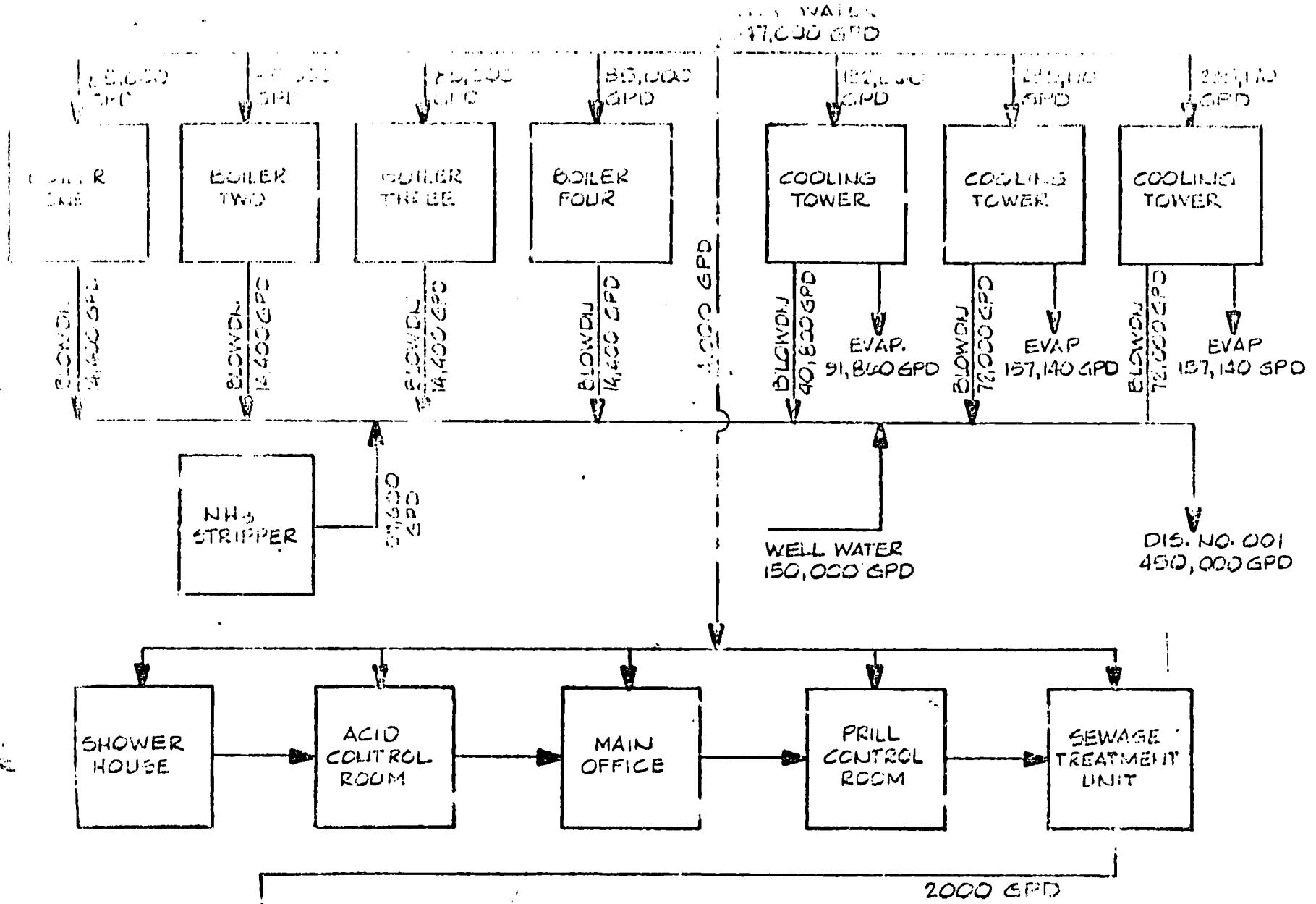


Figure .

SCHEMATIC OF WATER FLOW

NITRAM INC.
TAMPA, HILLSBOROUGH
COUNTY, FLORIDA

NOVEMBER

METHODS AND MATERIALS

Test methods used to conduct these flow-through and static bioassays are extensively described by Peltier and Weber (1985) and the FDER Biology Section (1985a). 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 (1985a). 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.

The D. pulex used in the Nitram tests were obtained by siphoning individuals from each of our culturing aquaria into two 2 liter glass jars, each containing water obtained from Nitram during a preliminary site visit. The slight chlorine residual in this water had dissipated before introducing the D. pulex. This Nitram water was used as diluent for the on-site tests and, therefore, the organisms were pre-acclimated to this dilution water. The jars were transported to the site by state vehicle and immediately transferred to the mobil bioassay lab, and therefore, did not undergo any significant temperature change or disturbance. The D. pulex were fed prior to transport and there was no observed mortality of these organisms prior to the test.

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 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 N. leedsi used in the Nitram test began hatching in the Tallahassee lab on approximately February 5, 1986, in 2-1/2 gallon all-glass aquaria. Water was replaced in these aquaria over a period of days with Nitram water (to be used as diluent in the on-site tests). Therefore, the fish were pre-acclimated to this dilution water. The fish were transported to the test site in an aquarium with constant aeration. The fish were fed daily with live, freshly hatched Artemia and were acclimated to 20°C in the mobile lab prior to the test. The fish did not undergo any significant temperature change of disturbance, nor was there any mortality observed prior to the test. The fish were approximately 10 days old at the start 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. Additionally, on-site reference toxicant tests were performed in the mobile bioassay lab using Notropis leedsi and Daphnia pulex, to evaluate the sensitivity of these organisms after their transportaion to the site and under the conditions in the mobile lab. The results of these tests would indicate whether the sensitivities of the organisms used in the flow-through tests had changed significantly from that experienced in the Tallahassee lab or had deviated from the recommended EPA and DER sensitivity ranges.

Test Methods

Dilution Water

Water used to acclimate the organisms prior to the test, and as dilution water in the flow-through and Standard Reference Toxicant tests, was obtained from a Nitram (City of Tampa) water pipe. This water was characterized by a pH of 7.1 to 7.8 standard units and a conductivity of 375 to 500. Approximately 250 gallons of this dilution water was conveyed via hose daily to a 300 gallon Nalgene® tank at the test site. Any chlorine residual in this water was completely neutralized daily with additions of sodium thiosulfate. We wish to thank Mr. Dan Ross of Nitram for providing us this source of dilution water and for his personal assistance throughout the test.

Pre-test Conditions and Preparations

DER's mobile bioassay laboratory was constructed following EPA design specifications (Peltier and Weber, 1985) and is equipped with two solenoid valve operated, flow-through, proportional dilutor sustems. The mobile lab also has suitable working area for performing static tests concurrently with the flow-through tests. The mobile laboratory was transported by commerical mover from Tallahassee to Nitram on February 14, 1986. The trailer was positioned adjacent to Nitram's NPDES Outfall #001. Electric power for the mobile lab was supplied by Nitram.

An effluent sampling pump was suspended into the wastewater stream and was positioned approximately 0.3 meters beneath the surface (at least 0.3 meters off the bottom of the ditch). Effluent was conveyed directly and continuously to the mobile bioassay lab via a 100' length of braided reinforced Tygon® hose. Dilution water was transferred to a 350 gallon Nalgene® tank positioned near the mobile lab. A similar sampling pump was submerged in this tank and dilution water was conveyed directly and continuously to the mobile lab via a 50' length of identical Tygon® hose. Upon entering the mobile lab, both effluent and dilution water sampling lines passed through a cooled water bath adjusted to approximately 18°C. This water bath maintained the temperature of the effluent and dilution water at 22°C $\pm$ 2°C.

Calibration of the proportional dilutor was performed in Tallahassee prior to transport to Nitram. Additionally, calibration was verified again just prior to the beginning of the test and at the end of the 96 hour test. The concentrations and volumes produced by the flow-through dilutor system are presented in Table 1. Flow rates were adjusted to operate the dilutor at approximately 10.5 cycles per hour, which provided for approximately 15 complete volume changes in the 8 liter flow-through aquaria every 24 hours. The dilutor system was operated for approximately 18 hours before the introduction of the test organisms at the beginning of the test at 0715 hours on Sunday, February 16, 1986. The test was concluded at 0715 hours on Thursday, February 20, 1986.

Flow-Through Methods and Parameters

Two replicates of six concentrations and a control were used for the N. leedsi and D. pulex flow-through tests. To facilitate counting, the organisms were pipetted from culture dishes into separate retention chambers, which consisted of glass petri dishes to which cylinders of Nitex® screen (243 micron mesh size) were attached by silicone cement. These Nitex® retention chambers allowed much easier counting of test organisms when lifted from the test aquaria, while not retarding water flow in the aquaria. Ten organisms were loaded per retention chamber per aquarium, resulting in 20 test organisms per test concentration.

The number of live organisms, dissolved oxygen, pH, temperature and conductivity were recorded at 0, 24, 48, 72, and 96 hours for each aquarium. Ammonia concentrations, including total and unionized fractions (expressed in mg/l as N and NH_3, respectively), in each test concentration, were also recorded daily. Total alkalinity total hardness were also measured daily in each concentration. These values appear in Tables 2, 3, and 4.

An abnormality was observed in the Notropis leedsi flow-through test at 48 hours. At this time, significant mortality of fish was noted in test concentrations between 0% and 100%, with corresponding low dissolved oxygen measurements. Apparently, an oxygen demand had developed in these intermediate test concentrations, resulting in oxygen depletion and organism mortality. Aeration was immediately initiated in all the

aquaria, and 10 new fish were introduced in each aquarium as soon as sufficient oxygen was available. For the purpose of this bioassay, these intermediate concentrations were terminated, but the control and 100% concentrations remained through to their 96 hour conclusion (Table 2 - Test #1, Original Test). The new fish introduced at hour 48 were considered as a separate 48 hour flow-through bioassay (Table 3 - Test #2, Second Test). The Daphnia pulex flow-through bioassay was terminated at 48 hours due to excessive control mortality (Table 4).

48 Hour Standard Reference Toxicant Bioassays

During the first two days of the flow-through bioassays (16 to 18 February, 1986) two Standard Reference Toxicant bioassays were performed on-site, in the bioassay trailer. Sodium lauryl sulfate was used as the reference toxicant, in a stock concentration of 100 ppm. This stock concentration was diluted in Nitram dilution water to obtain a concentration series (Table 1) of from 1 to 15 ppm. Two replicates of each concentration was used, each containing either 10 Daphnia or 5 Notropis.

These tests would indicate if the sensitivities of the Daphnia and Notropis used in the flow-through tests were significantly different from sensitivities obtained in previous tests in the Tallahassee laboratory. Dissolved oxygen, pH, temperature, and the number of live organisms were recorded for each test chamber at 0 hours, 24 hours, and 48 hours. The conductivity of each test concentration was recorded at the beginning and end of the test. All data recorded during these tests are presented in Tables 5 and 6. The organisms were not fed during the tests.

Table 1. Diluent and Effluent Volumes, and Final Concentrations used in Flow-Through Bioassays of Nitram Chemicals (15 to 20 February, 1986.

Flow-Through Bioassays (16 to 20 February, 1986), Daphnia pulex and Notropis leedsi

Diluent[a]		Effluent[a]		Total Volume[a]		Final Concentration[a]	
0 Hours	96 Hours	0 Hours	96 Hours	0 Hours	96 Hours	0 Hours	96 Hours
915 ml	885 ml	0 ml	0 ml	915 ml	885 ml	0%	0%
885 ml	829 ml	50 ml	57 ml	935 ml	886 ml	5.3%	6.4%
968 ml	960 ml	107 ml	110 ml	1075 ml	1070 ml	10.0%	10.3%
751 ml	743 ml	156 ml	161 ml	907 ml	904 ml	17.1%	17.8%
679 ml	693 ml	321 ml	218 ml	1000 ml	1011 ml	32.1%	31.5%
363 ml	355 ml	481 ml	483 ml	844 ml	838 ml	57.0%	57.6%
0 ml	0 ml	969 ml	958 ml	969 ml	958 ml	100.0%	100.0%

Notropis leedsi Standard Reference Toxicant Test (16 to 18 February, 1986)

Concentration	Dilution Water	Toxicant	Total Volume
Control	500 ml	-----	500 ml
1.0 ppm	495 ml	5 ml	500 ml
3.0 ppm	485 ml	15 ml	500 ml
5.0 ppm	475 ml	25 ml	500 ml
7.0 ppm	465 ml	35 ml	500 ml
10.0 ppm	450 ml	50 ml	500 ml
15.0 ppm	425 ml	75 ml	500 ml

Daphnia pulex Standard Reference Toxicant Test (16 to 18 February, 1986)

Concentration	Dilution Water	Toxicant	Total Volume
Control	200 ml	-----	200 ml
1.0 ppm	198 ml	2 ml	200 ml
3.0 ppm	194 ml	6 ml	200 ml
5.0 ppm	190 ml	10 ml	200 ml
7.0 ppm	186 ml	14 ml	200 ml
10.0 ppm	180 ml	20 ml	200 ml
15.0 ppm	170 ml	30 ml	200 ml

[a] All values are the average of two or more replicates. Final concentrations are to nearest 0.1%.

Table 2. Data recorded during 96 hour Notropis leedsi flow-through bioassay of Nitram Chemicals, Tampa, NPDES #FL0001643, on 16 to 20 February, 1986.

Test #1. Original Test

Conc. or %	Test Container Number	Number of Live Organisms					Dissolved Oxygen (mg/l)					pH					Total Alkalinity (mg/l as $CaCO_3$)					Total Hardness (mg/l as $CaCO_3$)					Temperature				
		0	24	48	72	96	0	24	48[b]	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control	A	10	10	10	10	10	8.1	9.8	7.2	7.1	7.0	7.7	7.1	7.4	7.4	7.8	92	100	95	95	95	155	145	145	150	150	20.5	22.6	22.5	21.5	22.5
Control	B	10	10	10	10	10	8.2	9.8	7.2	7.2	6.7	7.7	7.2	7.5	7.5	7.7	92					163					20.5	22.8	22.7	21.5	22.5
5.9%	A	10	10	-[a]			8.1	9.7	-			7.7	7.2	-[a]			94	105	-[a]			165	175	-[a]			20.5	22.7	-[a]		
5.9%	B	10	10	-			8.2	9.7	-			7.7	7.2	-			94					174					20.7	22.8	-		
10.2%	A	10	10	-			8.0	9.6	-			7.6	7.2	-			98	100	-			210	190	-			20.7	22.5	-		
10.2%	B	10	9	-			7.9	9.5	-			7.6	7.2	-			100					210					20.7	22.9	-		
17.5%	A	10	10	-			7.9	9.3	-			7.5	7.1	-			110	100	-			240	240	-			20.6	22.7	-		
17.5%	B	10	10	-			7.9	9.2	-			7.5	7.1	-			110					240					20.6	22.8	-		
31.8%	A	10	10	-			7.5	8.7	-			7.4	7.1	-			112	105	-			300	290	-			20.5	22.7	-		
31.8%	B	10	10	-			7.6	8.8	-			7.4	7.1	-			113					300					20.5	22.8	-		
57.3%	A	10	10	-			7.2	7.6	-			7.2	7.0	-			120	115	-			415	490	-			20.5	22.5	-		
57.3%	B	10	10	-			7.3	7.6	-			7.2	7.0	-			119					415					20.5	22.7	-		
100.0%	A	10	10	10	10	10	6.1	6.9	4.4	5.9	5.4	7.0	6.8	7.2	7.2	7.1	135	140	135	140	107	580	685	670	655	620	20.3	22.5	22.4	22.0	23.1
100.0%	B	10	10	10	10	10	6.6	7.4	4.7	6.6	6.6	7.0	6.9	7.1	7.4	7.2	145					560					20.3	22.5	22.5	22.2	23.4

[a] Test concentrations terminated at 48 hours due to low dissolved oxygen concentrations, data not reported.

[b] Aeration initiated in all test chambers at 48 hours and continued for the duration of the test.

Table 2. Continued

Conc. or %	Test Container Number	Conductivity (umhos)					Total Ammonia (mg/l as N)					Unionized Ammonia (mg/l as NH_3)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control	A	380	490	480	480	418	0.58	0.50	0.83	0.77	0.74	.014	.004	.013	.011	.024
Control	B	375	500	478	490	412										
5.9%	A	490	700	-a			0.61	0.75	-a			.015	.007	-a		
5.9%	B	490	700	-												
10.2%	A	650	890	-			0.78	0.99	-			.015	.009	-		
10.2%	B	660	890	-												
17.5%	A	900	1110	-			1.95	1.25	-			.031	.009	-		
17.5%	B	900	1150	-												
31.8%	A	1310	1600	-			2.00	1.80	-			.025	.013	-		
31.8%	B	1310	1600	-												
57.3%	A	2000	2450	-			3.10	3.11	-			.024	.018	-		
57.3%	B	2000	2400	-												
100.0%	A	3250	3800	3720	3600	3700	4.25	5.35	17.1	22.1	7.01	.021	.022	.137	.245	.060
100.0%	B	3250	3800	3720	3610	3710										

[a] Test concentrations terminated at 48 hours due to low dissolved oxygen concentrations, data not reported.

[b] Aeartion initiated in all test chambers at 48 hours and continued for the duration of the test.

Table 3. Data recorded during 48 hour Notropis leedsi flow-through bioassay of Nitram Chemicals, Tampa, NPDES #FL0001643, on 18 to 20 February, 1986.

Test #2. Second Test

Conc. or %	Test Container Number	Number of Live Organisms					Dissolved Oxygen (mg/l)					pH					Total Alkalinity (mg/l as $CaCO_3$)					Total Hardness (mg/l as $CaCO_3$)					Temperature				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control	A	10	10	10			7.9	7.1	7.0			7.5	7.4	7.8			95	95	95			145	150	150			22.5	21.5	22.5		
Control	B	11[a]	11	11			7.9	7.2	6.7			7.5	7.5	7.7													22.7	21.5	22.5		
5.9%	A	10	9	9			6.9	6.3	7.3			7.3	7.3	7.6			100	95	95			170	170	180			22.4	21.3	22.5		
5.9%	B	10	10	10			6.4	6.7	6.6			7.3	7.4	7.5													22.6	21.7	22.5		
10.2%	A	10	10	10			5.6	6.5	5.1			7.3	7.4	7.3			100	95	100			195	180	200			22.5	21.4	22.3		
10.2%	B	10	10	10			6.3	6.7	6.0			7.3	7.4	7.3													22.8	21.8	22.7		
17.5%	A	10	10	10			5.7	5.1	5.2			7.2	7.3	7.2			100	100	100			240	210	230			22.7	21.5	22.3		
17.5%	B	10	10	10			6.7	5.9	5.0			7.2	7.3	7.2													22.8	21.9	22.9		
31.8%	A	10	10	10			6.0	6.5	6.3			7.2	7.4	7.3			100	110	100			280	285	280			22.6	21.5	22.4		
31.8%	B	10	10	10			6.9	5.8	6.2			7.2	7.3	7.3													22.8	21.8	22.9		
57.3%	A	10	10	10			5.2	6.0	6.8			7.2	7.3	7.4			110	120	105			470	480	450			22.5	21.7	22.6		
57.3%	B	10	10	10			6.2	5.3	6.1			7.1	7.2	7.3													22.6	21.8	22.9		
100.0%	A	10	10	10			6.9	5.9	5.4			7.2	7.2	7.1			135	140	107			670	655	620			22.4	22.0	23.1		
100.0%	B	10	10	10			7.4	6.6	6.6			7.1	7.4	7.2													22.5	22.2	23.4		

[a] Extra organism loaded by mistake, allowed to remain.

[b] All test chambers were continuously aerated during the test.

Table 3. Continued

Test #2. Second Test

Conc. or %	Test Container Number	Conductivity (umhos)					Total Ammonia (mg/l as N)					Unionized Ammonia (mg/l as NH_3)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control	A	480	490	418			0.83	0.77	0.74			.013	.011	.024		
Control	B	478	490	412												
5.9%	A	680	710	605			3.40	1.80	1.20			.040	.021	.024		
5.9%	B	660	710	595												
10.2%	A	820	870	730			2.10	3.16	1.46			.024	.042	.017		
10.2%	B	805	870	730												
17.5%	A	1080	1130	1040			3.00	4.84	2.12			.028	.052	.019		
17.5%	B	1090	1100	990												
31.8%	A	1640	1510	1440			5.30	7.95	2.69			.049	.096	.031		
31.8%	B	1640	1540	1520												
57.3%	A	2380	2310	2320			8.00	14.0	4.04			.063	.134	.053		
57.3%	B	2340	2300	2300												
100.0%	A	3720	3600	3700			17.1	22.1	7.01			.137	.245	.060		
100.0%	B	3720	3610	3710												

Table 4. Data recorded during 96 hours Daphnia pulex flow-through bioassay of Nitram Chemicals, Tampa, NPDES #FL0001643, on 16 to 20 February, 1986.

Conc. or %	Test Container Number	Number of Live Organisms					Dissolved Oxygen (mg/l)					pH					Total Alkalinity (mg/l as $CaCO_3$)					Total Hardness (mg/l as $CaCO_3$)					Temperature				
		0	24	48[a]	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control	A	10	10				8.1	9.8				7.7	7.1				92	100				155	145				20.5	22.6			
Control	B	10	10				8.2	9.8				7.7	7.2				92					163					20.5	22.8			
5.9%	A	10	10				8.1	9.7				7.7	7.2				94	105				165	175				20.5	22.7			
5.9%	B	10	10				8.2	9.7				7.7	7.2				94					174					20.7	22.8			
10.2%	A	10	10				8.0	9.6				7.6	7.2				98	100				210	190				20.7	22.5			
10.2%	B	10	10				7.9	9.5				7.6	7.2				100					210					20.7	22.9			
17.5%	A	10	10				7.9	9.3				7.5	7.1				110	100				240	240				20.6	22.7			
17.5%	B	10	10				7.9	9.2				7.5	7.1				110					240					20.6	22.8			
31.8%	A	10	10				7.5	8.7				7.4	7.1				112	105				300	290				20.5	22.7			
31.8%	B	10	10				7.6	8.8				7.4	7.1				113					300					20.5	22.8			
57.3%	A	10	10				7.2	7.6				7.2	7.0				120	115				415	490				20.5	22.5			
57.3%	B	10	10				7.3	7.6				7.2	7.0				119					415					20.5	22.7			
100.0%	A	10	9				6.1	6.9				7.0	6.8				135	140				580	684				20.3	22.5			
100.0%	B	10	10				6.6	7.4				7.0	6.9				145					560					20.3	22.5			

[a] Test terminated at 48 hours due to excessive control mortality, data not reported.

Table 4. Continued

Conc. or %	Test Container Number	Conductivity (umhos)					Total Ammonia (mg/l as N)					Unionized Ammonia (mg/l as NH_3)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control	A	380	490				0.58	0.50				.014	.004			
Control	B	375	500													
5.9%	A	490	700				0.61	0.75				.015	.007			
5.9%	B	490	700													
10.2%	A	650	890				0.78	0.99				.015	.009			
10.2%	B	660	890													
17.5%	A	900	1110				1.95	1.23				.031	.009			
17.5%	B	900	1150													
31.8%	A	1310	1600				2.00	1.80				.025	.013			
31.8%	B	1310	1600													
57.3%	A	2000	2450				3.10	3.11				.024	.018			
57.3%	B	2000	2400													
100.0%	A	3250	3800				4.25	5.35				.021	.022			
100.0%	B	3250	3800													

Table 5. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of Sodium Lauryl Sulfate (Standard Reference Toxicant), performed during Nitram flow-through bioassay on 16 to 18 February, 1986.

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	8.5	7.0	7.4	7.8	7.6	7.4	17.0	21.3	21.4			Control Water
Control	5	5	5	8.5	7.0	7.5	7.8	7.6	7.4				345	395	NH_3(as NH_3)(mg/l) _____ Total Alkalinity _____ (mg/l $CaCO_3$)
1.0 ppm	5	5	5	8.5	7.0	7.6	7.8	7.7	7.4	16.8	21.3	21.2			
1.0 ppm	5	5	5	8.5	7.0	7.6	7.8	7.7	7.4				349	395	
3.0 ppm	5	5	5	8.5	6.9	7.0	7.7	7.7	7.5	16.8	21.2	21.1			Total ammonia (as N)(mg/l) _____ Total Hardness _____ (mg/l $CaCO_3$)
3.0 ppm	5	5	5	8.5	6.9	7.1	7.8	7.7	7.5				350	390	
5.0 ppm	5	2	2	8.5	5.4	6.5	7.8	7.6	7.4	16.8	21.2	21.0			
5.0 ppm	5	3	3	8.5	5.7	6.5	7.9	7.6	7.4				350	390	
7.0 ppm	5	0	0	8.4	4.3	-[a]	7.8	7.5	-[a]	16.9	21.2	-[a]			
7.0 ppm	5	0	0	8.5	4.3	-	7.8	7.5	-				351	-[a]	100% Effluent
10.0 ppm	5	0	0	8.5	4.5	-	7.8	7.4	-	17.0	21.1	-			NH_3(as NH_3)(mg/l) _____ Total Alkalinity _____ (mg/l $CaCO_3$)
10.0 ppm	5	0	0	8.5	4.3	-	7.8	7.4	-				350	-	
15.0 ppm	5	0	0	8.6	3.1	-	7.8	7.4	-	17.0	20.9	-			
15.0 ppm	5	0	0	8.6	3.2	-	7.8	7.4	-				350	-	Total ammonia (as N)(mg/l) _____ Total Hardness _____ (mg/l $CaCO_3$)

[a] Remaining parameters were not recorded since all organisms were dead by 24 hours.

Table 6. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Sodium Lauryl Sulfate (Standard Reference Toxicant), performed during Nitram flow-through bioassay on 16 to 18 February, 1986.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)		
	0	24	48 [a]	0	24	48	0	24	48	0	24	48	0	48	
Control	10	8		8.1	7.3		7.8	7.5		16.9	21.6				Control Water
Control	10	10		8.2	7.3		7.8	7.5					345		NH_3(as NH_3)(mg/l) Total Alkalinity (mg/l $CaCO_3$)
1.0 ppm	10	5		8.4	7.4		7.8	7.5		16.8	21.2				
1.0 ppm	10	8		8.4	7.4		7.9	7.5					350		
3.0 ppm	10	1		8.4	7.2		7.8	7.5		16.8	21.0				Total ammonia (as N)(mg/l) Total Hardness (mg/l $CaCO_3$)
3.0 ppm	10	3		8.4	7.2		7.9	7.5					350		
5.0 ppm	10	0		8.4	6.9		7.8	7.6		17.0	20.9				
5.0 ppm	10	4		8.4	7.1		7.8	7.6					351		
7.0 ppm	10	0		8.4	7.0		7.8	7.6		17.0	20.8				
7.0 ppm	10	0		8.4	7.2		7.8	7.6					350		100% Effluent
10.0 ppm	10	0		8.5	7.1		7.8	7.7		17.0	20.8				NH_3(as NH_3)(mg/l) Total Alkalinity (mg/l $CaCO_3$)
10.0 ppm	10	0		8.5	7.2		7.8	7.7					350		
15.0 ppm	10	0		8.5	7.4		7.8	7.7		17.0	20.7				
15.0 ppm	10	0		8.5	7.4		7.8	7.7					349		Total ammonia (as N)(mg/l) Total Hardness (mg/l $CaCO_3$)

[a] Test was invalidated at 48 hours due to excessive control mortality, data not reported.

Table 7. LC_{50} Data for Bioassays of Nitram Chemicals and Standard Reference Toxicant, 16 to 20 February, 1986.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Flow-Through Tests			
Notropis leedsi	Binomial	No Mortality	No Mortality
	Moving Average		
	Probit		
Daphnia pulex	Binomial	One Mortality	[b]
	Moving Average		
	Probit		
Standard Reference Toxicant			
Notropis leedsi	Binomial	5.0 ppm (3 and 7)	5.0 ppm (3 and 7)
	Moving Average	[a]	[a]
	Probit	[a]	[a]
Daphnia pulex	Binomial	1.4 ppm (0 and 3)	
	Moving Average	1.4 ppm (0.7 and 2.1)	[b]
	Probit	1.5 ppm (0.9 and 2.0), [c]	

[a] Due to an insufficient number of dead organisms, or to the irregular distribution of mortality, an LC_{50} value cannot be calculated using this method of statistical analysis.

[b] Test invalidated due to excessive control mortality.

[c] This LC_{50} is reported in the text since it is the most statistically conservative value of the data set.

RESULTS AND DISCUSSION

The results of the Biology Section's on-site testing of the Nitram facility indicated that the wastewater discharge was not acutely toxic. There was no mortality noted in the Notropis leedsi exposed to 100% wastewater for a full 96 hours (Table 2). There was significant mortality noted, however, in all intermediate (between 0% and 100%) wastewater concentrations at 48 hours (Table 2). This mortality was attributed to insufficient dissolved oxygen in these test chambers, apparently caused by an increase in the BOD. This BOD may have been a result of an unexpected interaction between bacteria in Nitram's wastewater and components in the dilution water. As stated before, there was not mortality in either the control (dilution water) or in full strength 100% Nitram wastewater. Aeration was immediately initiated in all test chambers and additional fish were introduced to each chamber as soon as sufficient oxygen was available. Only one random N. leedsi mortality ocurred during this 48 hour flow-through bioassay (Table 3). Since the mortality of N. leedsi noted above was attributed to low dissolved oxygen, the affected test concentrations were considered invalid and no LC_{50} is given.

In the Daphnia pulex flow-through bioassay, only one mortality was noted in the 100% concentration at hour 24 (Table 4). Unfortunately, this test had to be terminated at 48 hours due to excessive control mortality.

Ammonia concentrations measured during the on-site bioassays reached a maximum of 22.1 mg/l (total) and 0.245 mg/l (unionized).

The results of the on-site Standard Reference Toxicant (sodium lauryl sulfate) tests, using N. leedsi and D. pulex, produced a 48 hour LC_{50} of 5.0 ppm and a 24 hour LC_{50} of 1.5 ppm for these organisms, respectively. These results, when compared to previous Biology Section values, suggest that the fish were healthy and viable at the time of the test, but that the D. pulex may have been stressed. Indeed, the D. pulex test had to be invalidated at 48 hours due to excessive control mortality, similar to the situation which occurred during the D. pulex flow-through bioassay.

Additional factors should be considered when evaluating this bioassay and the overall operation of Nitram Chemicals. First, the facility was closed for approximately one week prior to our arrival for maintenance. What took place during this period is unknown. Also, upon arrival, an abundance of dead crawfish was noted in Nitram's wastewater ditch. The plant representative admitted to a malfunction within the facility and the subsequent release of an undertermined amount of ammonia.

Recommendation

Due to the fluctuations in ammonia discharges from the Nitram facility and to the possiblility of plant upsets or malfunctions, the Biology Section recommends closer scrutiny of the facility's wastewater discharge. This may include a more frequent inspection or self-monitoring schedule, inspection of Nitram's wastewater treatment process and equipment, and the incorpor ation of a biomonitoring program in Nitram's permit.

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