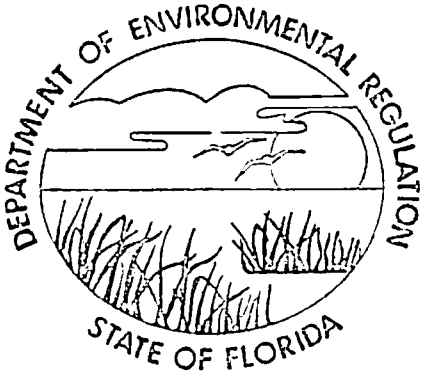



# BIOASSAYS OF NITRAM CHEMICALS INCORPORATED

TAMPA, HILLSBOROUGH COUNTY, FLORIDA

NPDES #FL0001643

MARCH 3, 1988

Biology Section
Division of Environmental Programs

# BIOASSAYS OF NITRAM CHEMICALS INCORPORATED

# TAMPA, HILLSBOROUGH COUNTY, FLORIDA

# NPDES #FL0001643

Biology Section
Bureau of Laboratories and
Special Programs
March 3, 1988

## EXECUTIVE SUMMARY

Nitram Chemicals Incorporated, Tampa, Hillsborough County, Florida, NPDES #FL0001643, on 12 to 14 January, 1988.

The sample from Nitram Chemicals Incorporated, a fertilizer manufacturer which discharges into Delaney Creek, was not acutely toxic to the test organisms, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi. Only one death was recorded in the 30% concentration of D. pulex. The total and unionized ammonia 0.981 mg/l and 0.01 mg/l, respectively were insufficient to have caused any mortality. The Microtox® assay, using the bacterium Photobacterium phosphoreum, indicated that the sample was stimulatory rather than toxic. Due to the non-toxic nature of the sample, chemical analyses were not performed.

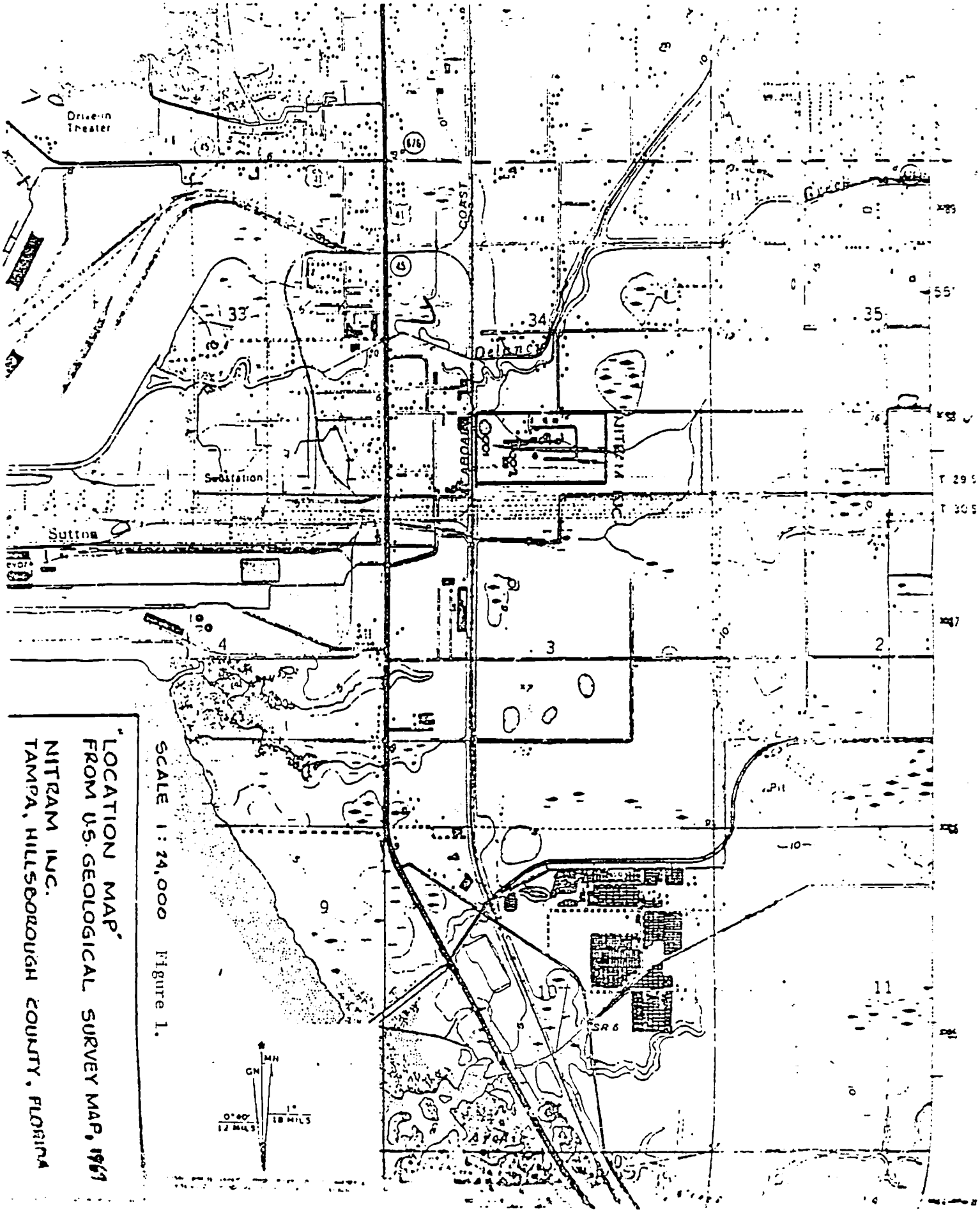
## INTRODUCTION

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 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.

Previous bioassays of samples from Nitram Chemicals, Incorporated, were performed in 1980, 1983, 1986, and 1987. The bioassays of 1980, 1983, and 1987 were static acute toxicity bioassays. The bioassay of 1986 was a flow-through bioassay. All of the bioassay results were non toxic, although during the flow-through bioassay an interaction between bacteria in the Nitram sample and the dilution water caused an excessive BOD problem and subsequent test organism mortality.

On 12 January, 1988 another sample was obtained by the Biology Section and three 48 hour static acute toxicity bioassays and one Microtox® assay were performed. Results of the bioassays are presented in this report. Those involved in performing the tests and in preparation of this report were: D. A. Chasteen, J. M. Faircloth, and L. A. Williams.




"LOCATION MAP"
FROM U.S. GEOLOGICAL SURVEY MAP, 1967
NITRAM INC.
TAMPA, HILLSBOROUGH COUNTY, FLORIDA

Figure 1.

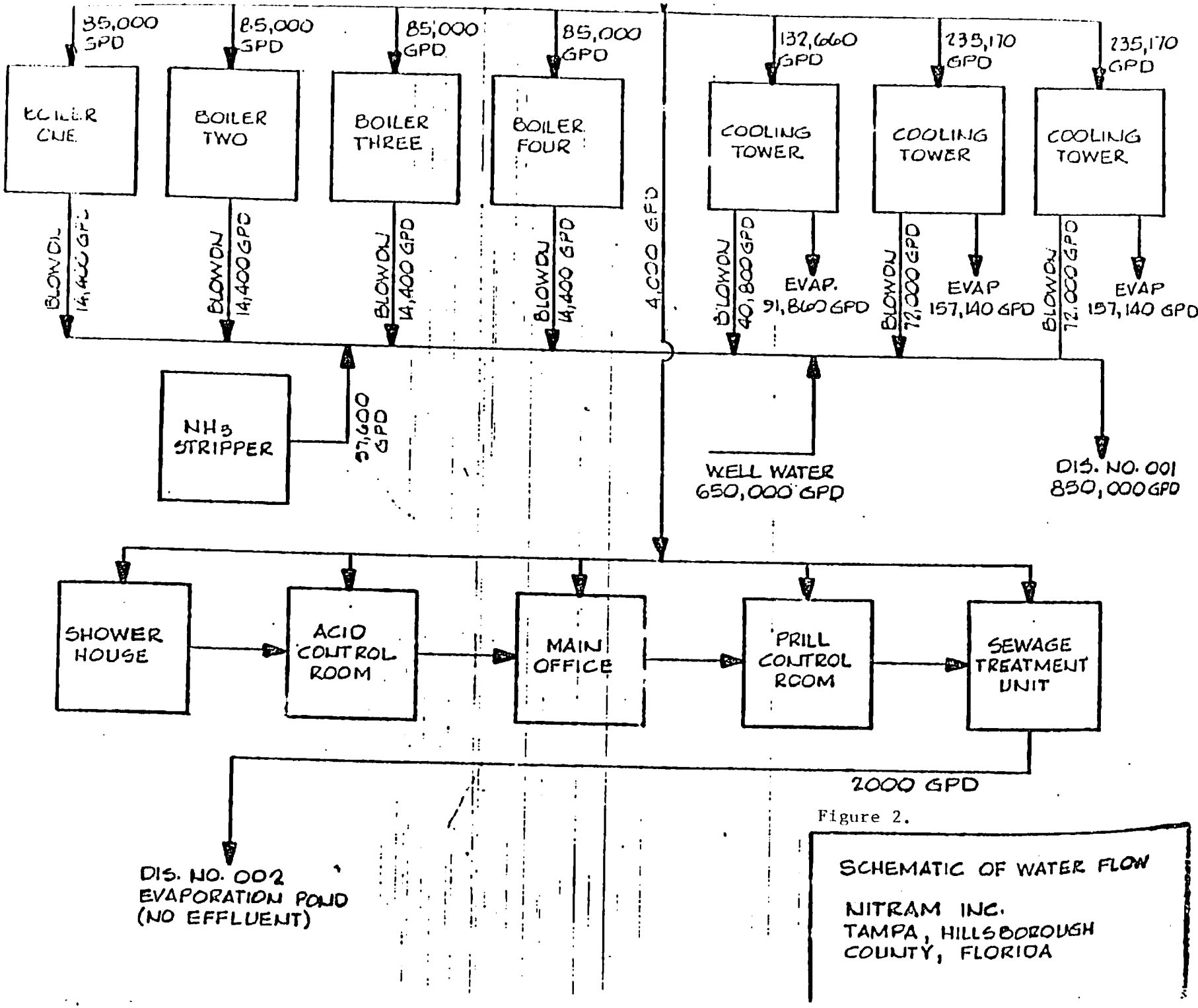



Figure 2.

SCHEMATIC OF WATER FLOW

NITRAM INC.
TAMPA, HILLSBOROUGH COUNTY, 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 (1985). The organisms selected for this study include species recommended by Peltier and Weber (1985) 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.

The Microtox® assay was run according to the manufacturer's protocol described in the Microtox® System Operating Manual (Beckman 1982).

### 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 June, 1987. 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 19 days old at the time of the test.

Photobacterium phosphoreum, a luminescent marine bacterium, is maintained as a freeze-dried culture, purchased from Microbics Corporation. Lot number M-606 was used in this assay. Immediately prior to the assay, the culture is rehydrated with 1.0 ml of specially purified water (Microtox® Reconstitution Solution).

## Standard Reference Toxicity Assays

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 and one Microtox® assay were performed on the sample collected from Nitram Chemicals, Incorporated. The tests were performed from 1100 hours on 12 January to 1100 hours on 14 January, 1988.

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 collected by Mr. David Demmi of DER's Southwest District Office at 1115 hours on 11 January, 1988, using standard wastewater sampling procedures. The samples were 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 samples in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the samples 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 recovered water 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 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 tests.

## 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% reclaimed water 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% reclaimed water 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 Table 2.

## Microtox® Test

Upon receipt at the DER laboratory, a 20 ml subsample was collected in a small beaker. The sample was adjusted to 2% salinity with reagent

grade dry sodium chloride to maintain the osmotic balance of the test bacterium during the assay. Four two fold dilutions were made of the test sample resulting in a test range of 97%, 49.5%, 24.75%, 12.38%, and 0%. An incubation temperature of 15°C was maintained throughout the assay. Ten microliters of bacterial suspension was added to each of the 1.0 ml test dilutions. Light output (luminescence) was read after 5 and 15 minutes incubation, using the Microtox® analyzer and recorded on a Perkin-Elmer strip chart recorder. In the presence of a toxicant, the luminescence of the test organisms will decrease. In the presence of a stimulant, the luminescence will increase. Toxicity is calculated as a function of the light lost in the test sample, normalized to the control, and is reported as the effective concentration at which a 50% reduction, of light output is determined in 15 minutes ($EC_{50}$). Results are given in Table 3.

## RESULTS AND DISCUSSION

The sample taken at Outfall #001 of Nitram Chemicals, Inc., was not acutely toxic to the test organisms, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi (Table 2). The Microtox® assay was also non toxic ($EC_{50}$ > 100%), further supporting the conclusion that the sample from this facility was non toxic on 11 January, 1988. Since the sample was not toxic, chemical analysis were not performed.

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

*Ceriodaphnia dubia* (water flea)

| Concentration | Dilution Water | Sample | Total Volume |
|---|---|---|---|
| Control | 50.0 ml | -------- | 50 ml |
| 30.0% | 35.0 ml | 15.0 ml | 50 ml |
| 60.0% | 20.0 ml | 30.0 ml | 50 ml |
| 100.0% | ------- | 50.0 ml | 50 ml |

*Daphnia pulex* (water flea)

*Simocephalus serrulatus* (water flea)

| Concentration | Dilution Water | Sample | Total Volume |
|---|---|---|---|
| Control | 200.0 ml | -------- | 200 ml |
| 30.0% | 140.0 ml | 60.0 ml | 200 ml |
| 60.0% | 80.0 ml | 120.0 ml | 200 ml |
| 100.0% | -------- | 200.0 ml | 200 ml |

*Pimephales promelas* (fathead minnow)

*Heterandria formosa* (least killifish)

*Notropis leedsi* (bannerfin shiner)

*Mysidopsis bahia* (mysid shrimp)

| Concentration | Dilution Water | Sample | Total Volume |
|---|---|---|---|
| Control | 500 ml | -------- | 500 ml |
| 30.0% | 350 ml | 150 ml | 500 ml |
| 60.0% | 200 ml | 300 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 bioassays of Nitram Chemicals Incorporated, Tampa, Hillsborough County, NPDES #FL0001643, on 12 to 14 January, 1988.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Facility: | Nitram, Inc. | | | NPDES # FL0001643 | | Facility Type: | Industry |
| Location: | Hillsborough County | | | Contact/District: | D. Mooros | | |
| Sample Collection Date: | 1/11/88 | Time: | 1115 | Test type: Static Screening | | | |
| Test Beginning Date: | 1/12/88 | Time: | 1100 | # tests: | 3 | Receiving Water: | Delaney Creek |
| Test Ending Date: | 1/14/88 | Time: | 1100 | Chlorination Type: | Non-chlorinated | | |

| Parameter | |
|---|---|
| Total Residual Cl2: | 0 |
| Method: | Hach |
| By: | DAC |
| Salinity: | 2.5 |

| Ammonia: | Control Water | 100% Sample |
|---|---|---|
| Unionized NH3: | 0.00 | 0.01 |
| Total NH3: | 0 | 0.981 |

Organism: *Ceriodaphnia dubia* Age/Life stage: Neonates
LC50: Binomial: Moving Average: Probit:

| Concentrations | SURVIVAL # at 0 hour | Live at 24 hr | Live at 48 hr | Dissolved Oxygen 0 hour | 24 hour | 48 hour | pH 0 hour | 24 hour | 48 hour | Temperature 0 hour | 24 hour | 48 hour | Conductivity 0 hour | 48 hour |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 10 | 10 | 10 | 8.8 | 8.5 | 9 | 7.9 | 7.8 | 8 | 19 | 20.1 | 19.5 | | |
| Control | 10 | 10 | 10 | 8.8 | 8.5 | 9 | 8 | 7.9 | 8.1 | | | | | |
| 30% | 10 | 10 | 10 | 8.9 | 8.8 | 9.1 | 7.7 | 8 | 8.1 | 19.2 | 19.8 | 19.1 | | |
| 30% | 10 | 10 | 10 | 8.9 | 8.7 | 9.1 | 7.7 | 8 | 8.1 | | | | | |
| 60% | 10 | 10 | 10 | 9 | 8.8 | 9.3 | 7.5 | 8 | 8 | 19.2 | 19.6 | 19 | | |
| 60% | 10 | 10 | 10 | 9.1 | 8.8 | 9.3 | 7.5 | 8 | 8.1 | | | | | |
| 100% | 10 | 10 | 10 | 9.2 | 8.9 | 9.2 | 7.2 | 7.9 | 8 | 19.3 | 19.6 | 19.1 | | |
| 100% | 10 | 10 | 10 | 9.2 | 8.9 | 9.2 | 7.2 | 7.9 | 8 | | | | | |

| | Control water | 100% sample |
|---|---|---|
| Alkalinity: | 130 | 72 |
| Hardness: | 122 | 610 |

Organism: *Daphnia pulex* Age/Life stage: Neonates
LC50: Binomial: Moving Average: Probit:

| Concentrations | SURVIVAL # at 0 hour | Live at 24 hr | Live at 48 hr | Dissolved Oxygen 0 hour | 24 hour | 48 hour | pH 0 hour | 24 hour | 48 hour | Temperature 0 hour | 24 hour | 48 hour | Conductivity 0 hour | 48 hour |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 10 | 10 | 10 | 9 | 8.7 | 9 | 8.1 | 8 | 8.1 | 19.6 | 19.7 | 18.9 | | |
| Control | 10 | 10 | 10 | 9 | 8.7 | 9 | 8.1 | 8 | 8.1 | | | | 180 | 195 |
| 30% | 10 | 10 | 10 | 9.1 | 8.9 | 9.1 | 7.7 | 7.9 | 8.1 | 19.6 | 19.3 | 18.9 | | |
| 30% | 10 | 10 | 10 | 9.1 | 8.9 | 9 | 7.7 | 8 | 8.1 | | | | 1310 | 1300 |
| 60% | 10 | 10 | 10 | 9.3 | 9 | 9 | 7.3 | 7.9 | 8 | 19.8 | 19.5 | 18.8 | | |
| 60% | 10 | 10 | 10 | 9.3 | 8.9 | 9 | 7.3 | 7.9 | 8 | | | | 2330 | 2340 |
| 100% | 10 | 10 | 10 | 9.5 | 8.8 | 8.9 | 7 | 7.7 | 7.9 | 19.8 | 20.7 | 18.9 | | |
| 100% | 10 | 10 | 10 | 9.5 | 8.8 | 8.9 | 7 | 7.7 | 7.9 | | | | 3600 | 3650 |

| | Control water | 100% sample |
|---|---|---|
| Alkalinity: | 130 | 72 |
| Hardness: | 122 | 610 |

Organism: *Notropis leedsi* Age/Life stage: 19 days old
LC50: Binomial: Moving Average: Probit:

| Concentrations | SURVIVAL # at 0 hour | Live at 24 hr | Live at 48 hr | Dissolved Oxygen 0 hour | 24 hour | 48 hour | pH 0 hour | 24 hour | 48 hour | Temperature 0 hour | 24 hour | 48 hour | Conductivity 0 hour | 48 hour |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 5 | 5 | 5 | 8.5 | 8.5 | 8.4 | 7.5 | 7.9 | 8 | 19.8 | 19.8 | 19.1 | | |
| Control | 5 | 5 | 5 | 8.5 | 8.5 | 8.4 | 7.5 | 7.9 | 8 | | | | 205 | 208 |
| 30% | 5 | 4 | 4 | 8.8 | 8.5 | 8.4 | 7.3 | 7.7 | 7.8 | 19.9 | 19.4 | 18.7 | | |
| 30% | 5 | 5 | 5 | 8.8 | 8.5 | 8.4 | 7.3 | 7.8 | 7.8 | | | | 1375 | 1350 |
| 60% | 5 | 5 | 5 | 9.2 | 8.5 | 8.6 | 7.3 | 7.7 | 7.8 | 20.1 | 19 | 19.1 | | |
| 60% | 5 | 5 | 5 | 9.2 | 8.6 | 8.6 | 7.2 | 7.7 | 7.8 | | | | 2410 | 2350 |
| 100% | 5 | 5 | 5 | 9.5 | 8.6 | 8.7 | 6.9 | 7.5 | 7.7 | 20.3 | 19.2 | 18.8 | | |
| 100% | 5 | 5 | 5 | 9.5 | 8.6 | 8.7 | 6.9 | 7.5 | 7.7 | | | | 3710 | 3575 |

| | Control water | 100% sample |
|---|---|---|
| Alkalinity: | 115 | 72 |
| Hardness: | 120 | 610 |

Table 3. Microtox test results , Nitram Chemicals , Inc. , Tampa , Hillsborough County, NPDES # FL0001643 on 1/12/88.

Microtox Test Culture lot # M-606

Final Reclaimed Water

| % Sample | Non-chlorinated | |
|---|---|---|
| | 5-min | 15-min |
| 0 | 85 | 51 |
| 0 | 79 | 47 |
| 0 | 79 | 44 |
| 12.38 | 92 | 56 |
| 12.38 | 90 | 55 |
| 12.38 | 89 | 55 |
| 24.75 | 101 | 65 |
| 24.75 | 96 | 62 |
| 24.75 | 104 | 63 |
| 49.5 | 99 | 69 |
| 49.5 | 114 | 65 |
| 49.5 | 114 | 71 |
| 99 | 114 | 82 |
| 99 | 114 | 79 |
| 99 | 114 | 79 |

EC-50 Non-chlorinated (15 minute , 15 degree C ) > 100%

EC50 (15 minute, 15 degrees C) is calculated using the Data Reduction Computer Program developed by Microbics Corporation for the Sharp Model EL-5100 (Microbics) Systems Operating Manual, 1982.

## LITERATURE CITED

Beckman Instruments, Inc. 1982. Microtox System Operating Manual.

Florida Department of Environmental Regulation, Biology Section. 1985. Quality Assurance Manual for Performing Acute Toxicity Tests (Second Edition). FDER.

_____. 1987. Bioassays of Nitram Chemicals, Tampa, Hillsborough County, Florida. NPDES #FL0001643. FDER. 11 p.

Peltier, W. and C. I. Weber. 1985. Methods for measuring the acute toxicity of effluents to aquatic organisms (Third Edition). Environmental Research Laboratory. Athens, Georgia. EPA-600/4-85-012. 217 p.

Note static screening concentration additions to reflect new laws

# BIOASSAY DATA FORM

Industry/Toxicant: Nitram, dNC.
Address: 5321 Hartford St, TAMPA, Fla
Contact/District: DON MOORES / SW
Effluent Serial Number: 001
NPDES Permit Number:

Test Number: 1 of 3
Test Type: (Static) Renewal, (Screening) Conventional
Other:

Sample Collection: Date 1-11-88 Time 1115
Test Beginning: Date 1-12-88 Time 1100
Test Ending: Date 1-15-88 Time 1100
Test Organism: Ceriodaphnia dubia
Age/Life Stage: neonate

recalibrate DC

| Conc. or % | Test Container Number | Number of Live Organisms 0 | 24 | 48 | 72 | 96 | Dissolved Oxygen (mg/l) 0 | 24 | 48 | 72 | 96 | pH 0 | 24 | 48 | 48 SRT | 48 | Temperature °C 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 36 | 10 | 10 | 10 | | | 8.8 | 8.5 | 9.0 | | | 7.9 | 7.8 | 8.2 | 7.9 | 8.0 | 19.0 | 20.1 | 17.5 | | | | | | | |
| Control | 1 | 10 | 10 | 10 | | | 8.8 | 8.5 | 9.0 | | | 8.0 | 7.9 | 8.3 | 7.9 | 8.1 | | | | | | | | | | |
| 30% | 71 | 10 | 10 | 10 | | | 8.9 | 8.8 | 9.1 | | | 7.7 | 8.0 | 8.4 | 8.0 | 8.1 | 19.2 | 19.8 | 17.1 | | | | | | | |
| 30% | 80 | 10 | 10 | 10 | | | 8.9 | 8.7 | 9.1 | | | 7.7 | 8.0 | 8.4 | 7.9 | 8.1 | | | | | | | | | | |
| 60% | 20 | 10 | 10 | 10 | | | 9.0 | 8.8 | 9.3 | | | 7.5 | 8.0 | 8.3 | 8.0 | 8.0 | 19.2 | 17.6 | 19.0 | | | | | | | |
| 60% | 85 | 10 | 10 | 10 | | | 9.1 | 8.8 | 9.3 | | | 7.5 | 8.0 | 8.3 | 8.0 | 8.1 | | | | | | | | | | |
| 100% | 51 | 10 | 10 | 10 | | | 9.2 | 8.9 | 9.2 | | | 7.2 | 7.9 | 8.2 | 7.9 | 8.0 | 17.3 | 19.6 | 19.1 | | | | | | | |
| 100% | 9 | 10 | 10 | 10 | | | 9.2 | 8.9 | 9.2 | | | 7.2 | 7.9 | 8.2 | 7.9 | 8.0 | | | | | | | | | | |
| Initial of Investigator | | DAC | DAC | DAC | | | JAF | JAF | JAF | | | JAF | JAF | JAF | JAF | JAF | JAF | JAF | DAC | | | | | | | |

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 130 / 12
100% Sample: 72 / 610
Other:
Measured by: mf

Ammonia unionized/total (mg/l as $NH_3/N$)
Hour 0
Control Water: 0.0
100% Sample: 0.981
Other:

Hour ____
Control Water:
100% Sample:
Other:
Measured by: MF

Signatures:
Marshall Faircloth
David Chandler

Instrument Calibrations:

| | pH Meter: | DO Meter: | S-C-T Meter: |
|---|---|---|---|
| 0 Hours | 7.0; 9.9 | 9.2 @ 20°C | 10.5 o 10.27.5 @ 30 |
| 24 Hours | 7.01 9.89 | 8.9 @ 21.5°C | — DC |
| 48 Hours | 7.07 9.09 | 9.2 @ 19°C DC | 10.2 o 10.28.0 @ 30 |
| 72 Hours | | | |
| 96 Hours | | | |

Total Residual Chlorine:
Field data: 0 Method:
Lab: 0 Method: Hach
Lab: Method:

De-chlorinated:
Measurements by: DAC

Ammonia meter slope - 58.9 ??

Reviewed by:
Date: 3/3/88

Remarks:

Industry/Toxicant: Nitram, dNC.
Address: 532 Hartford St. Tampa Fl,
Contact/District: DON MOORE
Effluent Serial Number:
NPDES Permit Number:

BIOASSAY DATA FORM

Test Number: 2 of 3
Test Type: Static, Renewal, Screening, Conventional
Other: recirculate

Sample Collection: Date 1-11-88 Time 1115
Test Beginning: Date 1-12-88 Time 1100
Test Ending: Date 1-14-88 Time 1100
Test Organism: Daphnia pulex
Age/Life Stage: Neonate

| Conc. or % | Test Container Number | Number of Live Organisms 0 | 24 | 48 | 72 | 96 | Dissolved Oxygen (mg/l) 0 | 24 | 48 | 72 | 96 | pH 0 | 24 | 48 DC | 72 DC | 96 DC | Temperature °C 0 | 24 | 48 | 72 | 96 | Conductivity 0 | 24 | 48 | 72 | 96 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 65 | 10 | 10 | 10 | 10 | | 9.0 | 8.7 | 9.0 | | | 8.1 | 8.0 | 8.3 | 8.0 | 8.1 | 19.6 | 19.7 | 18.7 | | | | | | | |
| Control | 36 | 10 | 10 | 10 | 10 | | 9.0 | 8.7 | 9.0 | | | 8.1 | 8.0 | 8.3 | 8.0 | 8.1 | | | | | | 180 | – | 195 | | |
| 30% | — | 10 | 10 | 10 | 9 | | 9.1 | 8.9 | 9.1 | | | 7.7 | 7.9 | 8.2 | 7.9 | 8.1 | 19.6 | 19.3 | 18.9 | | | | | | | |
| 30% | 66 | 10 | 10 | 10 | 10 | | 9.1 | 8.9 | 9.0 | | | 7.7 | 8.0 | 8.3 | 8.0 | 8.1 | | | | | | 1310 | – | 1300 | | |
| 60% | 71 | 10 | 10 | 10 | 10 | | 9.3 | 9.0 | 9.0 | | | 7.3 | 7.9 | 8.2 | 8.0 | 8.0 | 19.8 | 19.5 | 18.8 | | | | | | | |
| 60% | 26 | 10 | 10 | 10 | 10 | | 9.3 | 8.9 | 9.0 | | | 7.3 | 7.9 | 8.2 | 8.0 | 8.0 | | | | | | 2330 | – | 2340 | | |
| 100% | 8 | 10 | 10 | 8 dead | 10 | | 9.5 | 8.8 | 8.9 | | | 7.0 | 7.7 | 8.1 | 7.9 | 7.9 | 19.8 | 20.7 | 18.9 | | | | | | | |
| 100% | 38 | 10 | 10 | 10 | 10 | | 9.5 | 8.8 | 8.9 | | | 7.0 | 7.7 | 8.1 | 7.9 | 7.9 | | | | | | 3600 | – | 3650 | | |
| Initial of Investigator | | JE | DAC | DAC JMF | JMF JMP | | JMF | JMF | JMF | | | JMF | JMF | JMF | JMF | JMF | JMF | JMF | JMF | | | DAC | | JMF | | |

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 130/122
100% Sample: 72/610
Other:
Measured by: MF

Ammonia unionized/total (mg/l as $NH_3/N$)
Hour 0
Control Water: 0.0
100% Sample: 0.981
Other:

Hour ____
Control Water:
100% Sample:
Other:
Measured by: MF

Signatures:
Marshall Faircloth
David Acosta

Instrument Calibrations:

| | pH Meter: | DO Meter: | S-C-T Meter: |
|---|---|---|---|
| 0 Hours | | | |
| 24 Hours | | | |
| 48 Hours | | | |
| 72 Hours | | | |
| 96 Hours | | | |

Remarks:

Total Residual Chlorine:
Field data: _____ Method: _____
Lab: _____ Method: _____
Lab: _____ Method: _____

De-chlorinated:
Measurements by: _____

Reviewed by:
Date: 3/3/88

Industry/Toxicant: Ni, Trans, dNC.
Address: 5321 Hanford ST. Tampa Fl.
Contact/District: Don Moores
Effluent Serial Number:
NPDES Permit Number:

# BIOASSAY DATA FORM

Test Number: 3 of 3
Test Type: Static, Renewal, Screening, Conventional
Other:

Sample Collection: Date 1-11-88 Time 1115
Test Beginning: Date 1-12-88 Time 1100
Test Ending: Date 1-14-88 Time 1100
Test Organism: Mysidopsis bahia
Age/Life Stage: 19 days old

| % Conc. or | Test Container Number | Number of Live Organisms 0 | 24 | 48 | 72 | 96 | Dissolved Oxygen (mg/l) 0 | 24 | 48 | 72 | 96 | pH 0 | 24 | 48 | 72 | 96 | Temperature °C 0 | 24 | 48 | 72 | 96 | conductivity 0 | 24 | 48 | 72 | 96 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 71 | 5 | 5 | 5 | | | 8.5 | 8.5 | 8.4 | | | 7.5 | 7.9 | 8.1 | 7.8 | 8.0 | 19.8 | 19.8 | 21.1 | | | | | | | |
| Control | 239 | 5 | 5 | 5 | | | 8.5 | 8.5 | 8.4 | | | 7.5 | 7.9 | 8.1 | 7.8 | 8.0 | | | | | | 205 | - | 208 | | |
| 30% | 44 | 5 | 4 | 4 | | | 8.8 | 8.5 | 8.4 | | | 7.3 | 7.7 | 8.0 | 7.8 | 7.8 | 19.9 | 19.4 | 18.7 | | | | | | | |
| 30% | 70 | 5 | 5 | 5 | | | 3.8 | 8.5 | 8.4 | | | 7.3 | 7.8 | 8.1 | 7.8 | 7.8 | | | | | | 13 75 | - | 1350 | | |
| 60% | 15 | 5 | 5 | 5 | | | 9.2 | 8.5 | 8.6 | | | 7.2 | 7.7 | 8.1 | | 7.8 | 20.1 | 19.0 | 21.1 | | | | | | | |
| 60% | 281 | 5 | 5 | 5 | | | 9.2 | 8.6 | 8.6 | | | 7.2 | 7.7 | 8.1 | | 7.8 | | | | | | 24 10 | - | 2350 | | |
| 100% | 54 | 5 | 5 | 5 | | | 9.5 | 8.6 | 8.7 | | | 6.9 | 7.5 | 8.0 | | 7.7 | 20.3 | 19.2 | 18.8 | | | | | | | |
| 100% | 234 | 5 | 5 | 5 | | | 9.5 | 8.6 | 8.7 | | | 6.9 | 7.5 | 8.0 | | 7.7 | | | | | | 3 10 | - | 3575 | | |
| Initial of Investigator | | MF | MF | MF | | | MF | MF | MF | | | MF | MF | | | MF | MF | MF | MF | | | DAC | | MF | | |

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 115/120
100% Sample: 72/610
Other:
Measured by: MF

Ammonia unionized/total (mg/l as $NH_3/N$)
Hour 0
Control Water: 0.0
100% Sample: 0.981
Other:

Hour ____
Control Water:
100% Sample:
Other:
Measured by: MB

Signatures:
Marshall Faircloth
David Chesteen

Instrument Calibrations:

| | pH Meter: | DO Meter: | S-C-T Meter: |
|---|---|---|---|
| 0 Hours | | | |
| 24 Hours | | | |
| 48 Hours | | | |
| 72 Hours | | | |
| 96 Hours | | | |

Remarks:

Total Residual Chlorine:
Field data: _____ Method: _____
Lab: _____ Method: _____
Lab: _____ Method: _____

De-chlorinated:
Measurements by: _____

Sal. control = 0.0
Salinity = 2.5 ppt

Reviewed by:
Date: 3/3/88

# MICROTOX™ Data Reduction Sheet

SOURCE: Nitram

Stimulatory

GRAPH (CALC) EC 50 ( 15 MIN, 15 °C) = >100 (%SAMPLE) mg/L

## SAMPLE DATA:

SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent

DATE ASSAYED: 01-12-88 (MM) TIME: 10:00

VISUAL COLOR: none CORRECTION REQUIRED: YES (NO)

VISUAL TURBIDITY: none SEPARATION REQUIRED: YES (NO)

INITIAL pH: pH ADJUSTED TO: WITH:

OSMOTICALLY ADJUSTED WITH: MOAS (DRY NaCl) OTHER

PRIMARY DILUTION OF SAMPLE: none

OPERATOR: Lisa Williams REAGENT VIAL LOT NO. M-606

REMARKS: no res'd.

### MICROTOX™ 99% TEST

| SAMPLE CUVETTE | % SAMPLE | READINGS 5 MIN | READINGS 15 MIN | Γ5 | Γ15 | NOTES |
|---|---|---|---|---|---|---|
| A1 | 0 | 85 | 51 | X | X | |
| B1 | | 79 | 47 | | | |
| C1 | | 79 | 44 | | | |
| A2 | 12.38 | 92 | 56 | -.103 | -.145 | |
| B2 | | 90 | 55 | | | |
| C2 | | 89 | 55 | | | |
| A3 | 24.75 | 101 | 65 | -.185 | -.253 | |
| B3 | | 96 | 62 | | | |
| C3 | | 104 | 63 | | | |
| A4 | 49.5 | 99 | 69 | -.257 | -.307 | |
| B4 | | 114 | 65 | | | |
| C4 | | 114 | 71 | | | |
| A5 | 99 | 114 | 82 | -.289 | -.408 | Stimulatory |
| B5 | | 114 | 79 | | | |
| C5 | | 114 | 79 | | | |
| $EC_{50}$ ( MIN, °C) | | | | — | — | |

REVIEWED BY:
DATE: