

DOUGLASS FERTILIZER AND CHEMICAL, INC.

LAKE PLACID, HIGHLANDS COUNTY, FLORIDA

NO NPDES PERMIT

BIOASSAYS OF PERCOLATION POND WATER

ELUTRIATE BIOASSAYS OF SEDIMENT

Biology Section
Bureau of Water Analysis
June 4, 1984

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EXECUTIVE SUMMARY

Douglass Fertilizer and Chemical, Lake Placid, on 7 to 8 March, 1984.

The sample of percolation pond water from this fertilizer transfer facility proved to be extremely toxic to the test organisms, Daphnia pulex and Heterandria formosa, producing a 24 hour LC_{50} of 1.3% and a 4 hour LC_{50} of <5.6%, respectively. Additional screening tests of this pond water, using Notropis leedsi in a 2% concentration and Mysidopsis bahia in a 100% concentration, left all organsims dead after 4 hours and in less than 1 hour, respectively. This toxicity can be attributed to the substantial concentration of total ammonia (290 mg/l) and to the substantial ionic content (approximately 50,000 umhos) measured in the pond water.

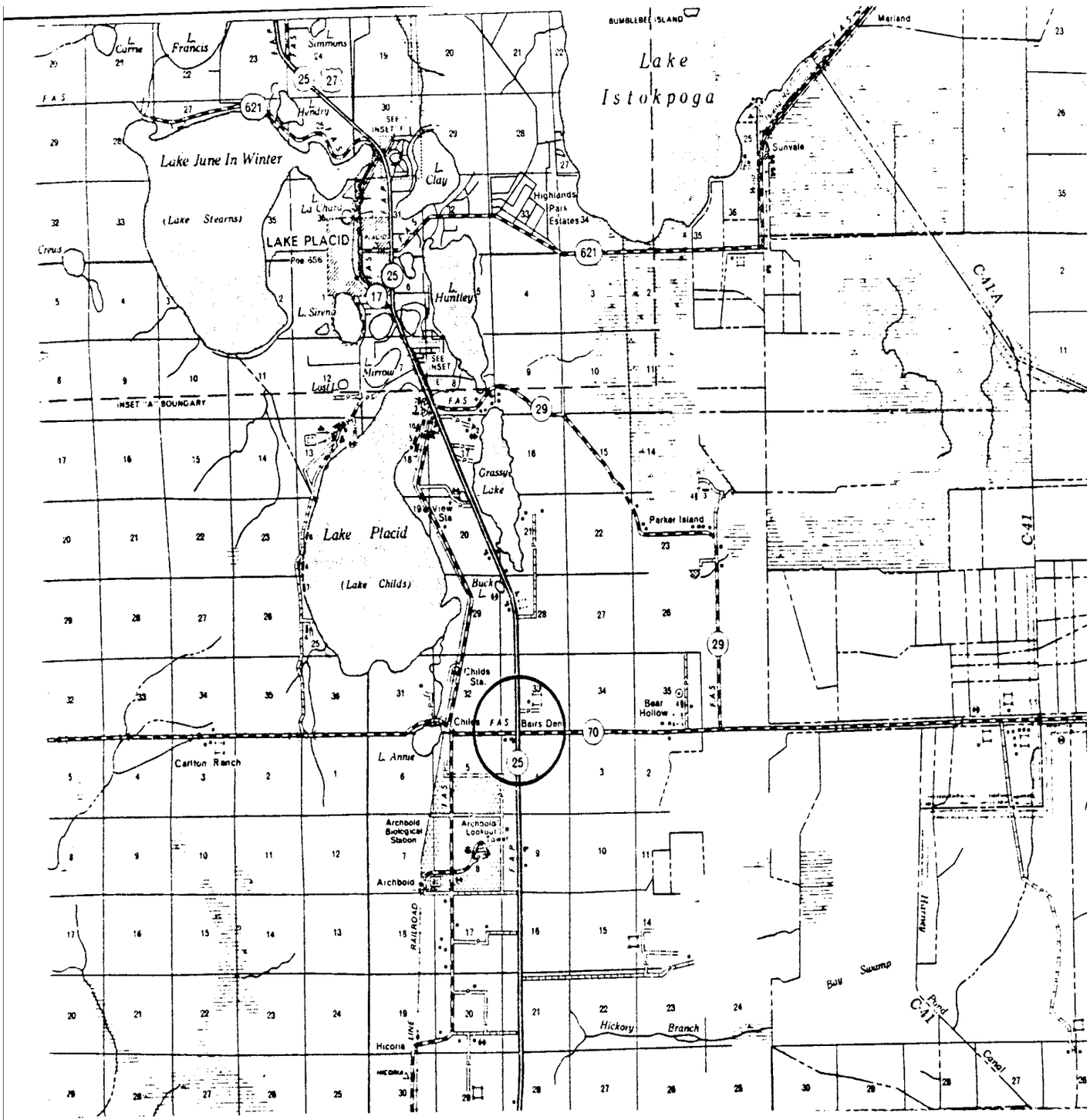
The elutriate bioassays performed on sediment collected from the percolation pond, using D. pulex and N. leedsi as test organisms, yielded similar results, with all organisms dead in the lowest test concentration (10.0%) by 24 hours. Again, this toxicity can be attributed, in part, to the ionic content of the elutriate sample.

Chemical analyses of this percolation pond water and sediment indicated the presence of extremely large concentrations of various metals, including arsenic, chromium, copper, nickel and zinc, in both the water and sediment. The presence of these metals, ammonia, and the ionic content were most likely the cause of the extreme toxicity of these samples.

INTRODUCTION

Douglass Fertilizer and Chemical, Inc., is located at the corner of U.S. 27 and S.R. 70 near Lake Placid, Highlands County, Florida (Fig. 1). The company apparently operates as a fertilizer and chemical transfer facility, loading and off-loading chemicals from trucks and other means of transport. The facility maintains at least one percolation pond on site which receives wash water and other runoff when the trucks are rinsed out. There is no discharge of this pond water, but there is direct percolation to the groundwater.

To determine if this percolation pond water and associated sediments are acutely toxic to aquatic organisms, Mr. Stuart Bradow of the South Florida District Hazardous Waste and Enforcement staff requested that toxicity bioassays be performed. Two static acute toxicity bioassays were performed on a sample of the pond water, and two static elutriate bioassays were performed on a sediment sample, on 7 to 8 March, 1984. Additional screening tests on the pond water were also performed. The results of these tests, and of certain chemical analyses performed on these samples, are presented in this report. Those involved in performing the tests and in preparation of this report were: P. D. Brooks, C. W. Dye and M. L. Roll.



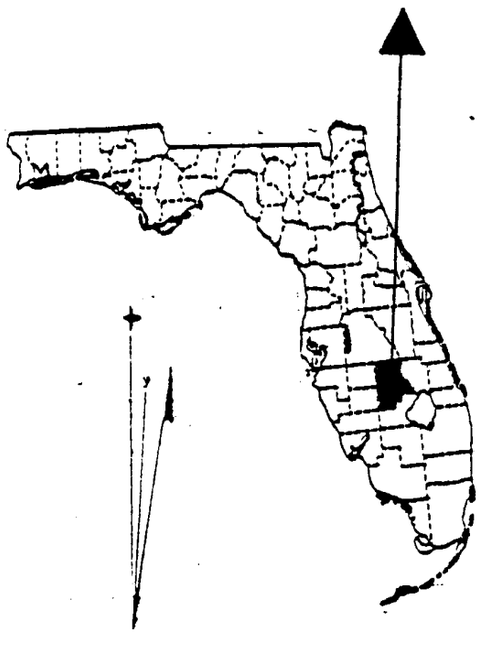


Figure 1. Map showing approximate location of Douglass Fertilizer and Chemical Inc. in Highlands County Florida.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978) and the FDER Biology Section (1983). The organisms selected for this study include species recommended by Peltier (1978), the U.S. EPA (1978), and the FDER Biology Section (1983). Additionally, the Biology Section maintains cultures of several species of organisms indigenous to the State of Florida. Two of these indigenous species were 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, 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.

Heterandria formosa (least killifish) - Approximately 100 adult least killifish were obtained from a wild population in the St. Marks Wildlife Refuge (Wakulla County, Florida) during August, 1980. The fish are maintained in two and one-half to 15 gallon all-glass aquaria equipped with a flow-through water exchange system. Water for this system is unchlorinated and is pumped from a deep well near the DER laboratory. The least killifish are reared at 22° plus or minus 2°C under a 16:8 hour light-dark cycle and are fed freshly hatched Artemia (brine shrimp) and Tetra-Min® twice daily. New born fish are initially maintained in the adult tanks, but are transferred to separate aquaria at weekly intervals, thereby segregating fish into even age groups. The fish used in this test were 10 to 17 days old.

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° plus or minus 2°C under a 16:8 hour light cycle and are fed Tetra-Min® and frozen Artemia (brine shrimp) twice daily. Breeding and egg hatching are encouraged at 25°C. The eggs are laid under a 1" thick slab of marble raised 3 mm above a removable horizontal pane of glass. Most of the eggs adhere to the glass and are easily removed to an aerated hatching tank. The narrow space between the marble slab and glass eliminates egg predation by adult fish. New hatching tanks are started weekly 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 17 to 21 days old at the time of the test.

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 static acute toxicity bioassays, two elutriate bioassays and two screening bioassays were performed on samples of percolation pond water and sediment collected from Douglass Fertilizer and Chemical. The tests were performed from 1030 and 1410 hours on 7 March to 1030 and 1410 hours on 8 March, 1984.

The samples were collected by Mr. Stuart Bradow of the DER's South Florida District on the 6th of March, 1984, at 1530 hours using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples for metal analyses were preserved with HNO_3. Samples for organic chemical analyses and for the bioassays were unpreserved. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 24 hours of collection.

Freshwater for dilution of the percolation pond and elutriate samples was obtained from the same DER deep well that is used to culture the test organisms. The concentration series selected for this study (Table 1) is that recommended by APHA (1980).

The elutriate was obtained by combining one part sediment to four parts well water in 1 liter flasks, shaking on a Wrist Action® shaker for 30 minutes, allowing the mixture to settle for 1 hour, and siphoning the supernatant into a separate flask. The elutriate was then filtered through pre-washed 0.45 m glass membrane filters to remove suspended particulates. All dilution and control water was also filtered through similar membranes.

Two replicates, each with seven (percolation pond bioassay) or four (elutriate bioassay) concentrations and controls, were used to test with the water flea, Daphnia pulex. Flint glass jars of 250 ml capacity were used as test chambers, and 200 ml of the appropriate test concentration was placed in each chamber. Daphnia were siphoned into a large culture dish to isolate the proper size and age organisms (neonates). To accomplish this, the water fleas were siphoned through a stainless steel screen. The trapped adults were then discarded. These neonates were then transferred by glass pipette, examined for injury, and were loaded 10 per chamber, resulting in 20 per test concentration. Water fleas were not fed during these tests.

For the pond sample, two replicates of six concentrations and a control were used for the Heterandria formosa (least killifish) bioassay, but only one chamber was used for the Notropis leedsi (bannerfin shiner) screening bioassay. For the elutriate bioassay, two replicates of three concentrations and a control were used with Notropis leedsi, except where noted. 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 these tests.

For the Mysidopsis bahia (mysid shrimp) screening bioassay, only one chamber was used containing 100% percolation pond water (salinity = 34.3 ppt). Shrimp acclimated to a salinity of 31.2 ppt were used for this test.

The N. leedsi screening test mentioined above, using a 2% concentration of percolation pond water, was initiated approximately one hour after the start of the H. formosa test, in which mortality was already being observed in the lowest test concentration (5.6%). This test attempted to determine the incipient level of toxicity. The M. bahia screening test, using 100% percolation pond water, also was initiated soon after the start of the other tests, in which mortality was already observed. Since the high ionic content (approximately 50,000 umhos) of this pond water corresponded to a salinity measurement of 34.3 ppt, it was necessary to establish that the water was toxic to saline test organisms as well as to freshwater organisms.

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 and 24 hours, unless complete mortality was observed prior to 4 hours. 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 percolation pond sample during 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 through 6.

Table 1. Concentration Series and Volumes Used for These Bioassays

1. Percolation Pond Bioassays

Daphnia pulex (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	200.0 ml	--------	200 ml
1.0%	198.0 ml	2.0 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

Heterandria formosa (least killifish)

Concentration	Dilution Water	Sample	Total Volume
Control	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

2. Elutriate Bioassays

Daphnia pulex (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	200.0 ml	-----	200.0 ml
10.0%	180.0 ml	20.0 ml	200.0 ml
25.0%	150.0 ml	50.0 ml	200.0 ml
50.0%	100.0 ml	100.0 ml	200.0 ml
100.0%	-----	168.0 ml	168.0 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Sample	Total Volume
Control	500 ml	----	500 ml
10.0%	450 ml	50 ml	500 ml
50.0%	250 ml	250 ml	500 ml
100.0%	-----	500 ml	500 ml

Table 1 Cont.

3. Screening Tests

Notropis leedsi (bannerfin shiner)
Mysidopsis bahia (mysid shrimp)[b]

Concentration	Dilution Water	Sample	Total Volume
2.0%	490 ml	10 ml	500 ml
100.0%[b]	----	500 ml	500 ml

[a] Due to a shortage of elutriate sample, only 168.0 ml was used for the 100% concentration.

Table 2. Data recorded during 24 hour, *Daphnia pulex*, static acute toxicity bioassay of Douglass Fertilizer and Chemical Inc. Lake Placid, Florida, on 7 to 8 March, 1984.

Percolation Pond Bioassay

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)		pH		Total Alakalinity (mg/l as $CaCO_3$)	Total Hardness (mg/l as $CaCO_3$)	Temperature (°c)		NH_3/N (mg/l)	Conductivity (umhos)	
	0	4	24	0	24	0	24	0	0	0	24		0	24
Control	10	10	10	8.6	8.8	7.7	8.2			19.1	18.9	0/0		
Control	10	9	9	8.6	8.8	7.7	8.2						188	209
1.0%	9[a]	9	4	8.2	8.8	7.2	7.8			21.4	18.8			
1.0%	10	10	7	8.2	8.8	7.2	7.8						480	780
5.6%	10	0	0	8.5	–[b]	7.1	–[b]			19.4	–[b]			
5.6%	10	0	0	8.5	–	7.1	–						3420	–[b]
10.0%	10	0	0	8.3	–	7.0	–			19.5	–			
10.0%	10	0	0	8.3	–	7.0	–						5020	–
18.0%	10	0	0	7.4	–	6.9	–			19.3	–			
18.0%	10	0	0	7.4	–	6.9	–						10,000	–
32.0%	10	0	0	6.3	–	6.8	–			19.3	–			
32.0%	10	0	0	6.3	–	6.8	–						17,500	–
56.0%	10	0	0	4.5	–	6.7	–			19.3	–			
56.0%	10	0	0	4.5	–	6.7	–						29,800	–
100.0%	10	0	0	1.4	–	6.6	–			19.3	–	0.14/290		
100.0%	10	0	0	1.4	–	6.6	–						48,900	–

Remarks: [a] Only 9 test organisms were loaded by mistake.

[b] Remaining parameters were not recorded since all organisms were dead by hour 4 of the test.

Table 3. Data recorded during 24 hour, Heterandria formosa, static acute toxicity bioassay of Douglass Fertilizer and Chemical Inc. Lake Placid, Florida, on 7 to 8 March, 1984.

Percolation Pond Bioassay

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)		pH		Total Alakalinity (mg/l as $CaCO_3$)	Total Hardness (mg/l as $CaCO_3$)	Temperature (°c)		NH_3/N (mg/l)	Conductivity (umhos)	
	0	4	24	0	24	0	24			0	24		0	24
Control	5	5	5	8.3	8.5	7.7	8.1			19.3	18.8	0 0		
Control	5	5	5	8.3	8.5	7.7	8.1						193	199
5.6%	5	0	0	7.8	–[a]	7.0	–[a]			21.8	–[a]			
5.6%	5	0	0	7.8	–	7.0	–						3720	–[a]
10.0%	5	0	0	7.6	–	7.0	–			19.5	–			
10.0%	5	0	0	7.6	–	7.0	–						5200	–
18.0%	5	0	0	7.1	–	6.9	–			19.5	–			
18.0%	5	0	0	7.1	–	6.9	–						10,300	–
32.0%	5	0	0	7.0	–	6.8	–			19.3	–			
32.0%	5	0	0	7.0	–	6.8	–						19,900	–
56.0%	5	0	0	4.0	–	6.7	–			19.3	–			
56.0%	5	0	0	4.0	–	6.7	–						30,000	–
100.0%	5	0	0	1.4	–	6.6	–			19.4	–	0.41 290		
100.0%	5	0	0	1.4	–	6.6	–						49,800	–

Remarks: [a] Remaining parameters were not recorded since all organisms were dead by hour 4 of the test.

Table 4. Data recorded during 24 hour, Daphnia pulex, static acute elutriate toxicity bioassay of Douglass Fertilizer and Chemical Inc. Lake Placid, Florida, on 7 to 8 March, 1984.

Elutriate Sediment Bioassay

Concentration or %	Number of Live Organisms		Dissolved Oxygen (mg/l)		pH		Total Alakalinity (mg/l as $CaCO_3$)	Total Hardness (mg/l as $CaCO_3$)	Temperature (°c)		NH_3/N (mg/l)	Conductivity (umhos)	
	0	24	0	24	0	24			0	24		0	24
Control	10	10	8.1	8.8	7.6	8.2			19.2	18.7			
Control	10	10	8.1	8.8	7.6	8.2						166	189
10.0%	10	0	7.5	– [b]	7.2	– [b]			19.3	– [b]			
10.0%	10	0	7.5	–	7.2	–						2920	– [b]
25.0%	10	0	7.1	–	7.0	–			19.5	–			
25.0%	10	0	7.1	–	7.0	–						5900	–
50.0%	10	0	6.4	–	6.9	–			19.7	–			
50.0%	10	0	6.4	–	6.9	–						13,700	–
100.0% [a]	10	0	4.6	–	6.8	–			20.4	–			
100.0% [a]	10	0	4.6	–	6.8							26,200	–

Remarks: [a] Total volume of this test concentration was 168 ml.

[b] Remaining parameters were not recorded since all organisms were dead early in the test.

Table 5. Data recorded during 24 hour, Notropis leedsi, static acute elutriate toxicity bioassay of Douglass Fertilizer and Chemical Inc. Lake Placid, Florida, on 7 to 8 March, 1984.

Elutriate Sediment Bioassay

Concentration or %	Number of Live Organisms		Dissolved Oxygen (mg/l)		pH		Total Alakalinity (mg/l as $CaCO_3$)	Total Hardness (mg/l as $CaCO_3$)	Temperature (°c)		NH_3/N (mg/l)	Conductivity (umhos)	
	0	24	0	24	0	24			0	24		0	
Control	5	5	7.6	8.7	7.6	8.1			19.3	18.5			
Control	5	5	7.6	8.7	7.6	8.1						182	188
10.0%	5	0	7.5	–[b]	7.2	–[b]			19.3	–[b]			
10.0%	5	0	7.5	–	7.2	–						3220	–[b]
50.0%	5	0	6.1	–	6.9	–			19.9	–			
50.0%	5	0	6.1	–	6.9	–						14,000	–
100%[a]	5	0	3.8	–	6.8	–			20.8	–		26,100	–

Remarks: [a] Due to shortage of elutriate, only one 100% test concentration was used.
[b] Remaining parameters were not recorded since all organisms were dead early in the test.

Table 6. Data recorded during Notropis leedsi and Mysidopsis bahia screening tests of Douglass Fertilizer and Chemical, Inc., Lake Placid, Florida on 7 to 8 March, 1984

Percolation Pond Sample

Concentration or %	Number of Live Organisms 0	4	24	Dissolved Oxygen (mg/l) 0	24	pH 0	24	Total Alakalinity (mg/l as $CaCO_3$)	Total Hardness (mg/l as $CaCO_3$)	Temperature (°c) 0	24	NH_3/N (mg/l)	Conductivity (umhos) 0	24
N. leedsi														
2.0%	5	0	0	8.7	[a] –	7.2	[a] –			20.3	[a] –		1320	[a] –
M. bahia														
100.0%	5	[b] 0	0	4.0	[a] –	6.6	[a] –			18.8	[a] –		[c] 49800	[a] –

Remarks:
[a] Remaining parameters were not recorded since all organisms were dead by hour 4 of the test.
[b] Complete mortality was actually observed 30 to 60 minutes after the start of this test.
[c] This conductivity corresponded to a salinity measurement of 34.3 ppt.

Table 7. Results of chemical analyses performed on Percolation Pond Water and sediment samples, Douglass Fertilizer and Chemical, Lake Placid, collected 6 March, 1984.

Organics

	Water	Sediment
Base Neutral Extractables	N.D.	----
Pesticide Extractables	One Unidentified Compound	Several Unidentified Compounds

Metals

	Water		Sediment	
Arsenic	132	µg/l	3.3	mg/kg
Cadmium	72	µg/l	69	mg/kg
Chromium	1206	µg/l	120	mg/kg
Copper	24300	µg/l	3491	mg/kg
Lead	91	µg/l	83	mg/kg
Mercury	0.26	µg/l	<0.04	mg/kg
Nickel	493	µg/l	90	mg/kg
Selenium	---[a]		---[a]	
Silver	0.7	µg/l	0.14	mg/kg
Zinc	31370	µg/l	3163	mg/kg

[a] Selenium results not available due to strong interferences.

Table 8. LC_{50} data for Bioassays of Douglass Fertilizer and Chemical Lake Placid, Highlands Co., Florida, on 7 to 8 March, 1984.

Organism	Method of Data Analysis	4 Hour LC_{50} and 95% Confidence Intervals	24 Hour LC_{50} and 95% Confidence Intervals
Pond Water *Daphnia Pulex*	Binomial	2.4% (1 and 5.6),b	1.3% (0 and 5.6),b
	Moving Average	a	a
	Probit	a	a
Heterandria formosa		<5.6,c	<5.6%,c
Sediment Elutriate: *Daphnia pulex*		----	<10.0%,d
Notropis leedsi		----	<10.0%,d

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

[b] Due to the distribution of mortality, the LC_{50} value calculated by this method of statistical analysis does not meet the criteria established by the Committee on Methods for Toxicity Tests with Aquatic Organisms.

[c] Since all organisms were dead in the lowest test concentration (5.6%) of this test, the LC_{50} is <5.6%.

[d] Since all organisms were dead in the lowest test concentrations of these tests (10.0%), the LC_{50} is <10.0%.

RESULTS AND DISCUSSION

The sample of percolation pond water collected from Douglass Fertilizer and Chemical proved to be extremely toxic to the test organisms, Daphnia pulex and Heterandria formosa, in a very short time. A 4 hour LC_{50} of 2.4% and a 24 hour LC_{50} of 1.3% were calculated for D. pulex (Table 8). For the H. formosa test, 4 and 24 hour LC_{50}'s of <5.6% were reported (Table 8), since this lowest test concentration had killed all the fish by 4 hours. In an attempt to establish the incipient level of toxicity to fish, a screening test using Notropis leedsi in a 2% pond water concentration was also performed. However, all fish in this test were also dead by 4 hours (Table 6). This toxicity can be attributed to the substantial concentration of total ammonia (290 mg/l) and to the substantial ionic content (approximately 50,000 µmhos) measured in the pond water. Since the high conductivity of the pond water corresponded to a salinity measurement of 34.3 ppt. it was necessary to establish that the pond water was also toxic to saline test organisms as well as to freshwater organisms. Therefore, an additional screening test was performed using Mysidopsis bahia in a 100% pond water sample. It was noted that all shrimp were dead within minutes of being introduced to this sample (Table 6).

The elutriate bioassays performed on sediments collected from this percolation pond, using Notropis leedsi and Daphnia pulex as test organisms, yielded similar results. Twenty four hour LC_{50}'s of <10% were reported for these organisms, since all organisms were dead in this lowest test concentration well before 24 hours (Table 8). This toxicity can be attributed, in part, to the high ionic content of the elutriate sample. Measurements of ammonia were not made in the elutriate tests.

Results of chemical analyses of these percolation pond water and sediment samples are presented in Table 7. The organic analyses indicated the presence of only unidentified compounds in the water and sediment. Herbicides, suspected components of this pond water (Mr. Stuart Bradow, personal communication), were not tested for. The metal analyses, however, indicated the presence of extremely large concentrations of metals in both the water and sediment. Included in the pond water were arsenic (132 µg/l), cadmium (72 µg/l), chromium (1206 µg/l), copper (24300 µg/l), lead (91 µg/l), mercury (0.26 µg/l), nickel (493 µg/l), silver (0.7 µg/l) and zinc (31370 µg/l). The sediment contained relatively similar values in the mg/kg (ppm) range. These metals may very well account for some of the ionic content measured in these tests, and, are near or significantly above concentrations known to be acutely toxic to aquatic organisms. The extreme toxicity of these samples, therefore, was likely due to the combined presence of these metals, ionic content, and ammonia.

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