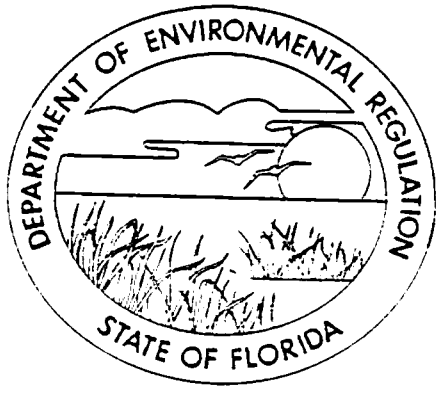


BIOASSAYS OF MONSANTO TEXTILES COMPANY

ESCAMBIA COUNTY, FLORIDA

NPDES PERMIT #FL0002488

Biological Section
Bureau of Water Analysis
April 8, 1981

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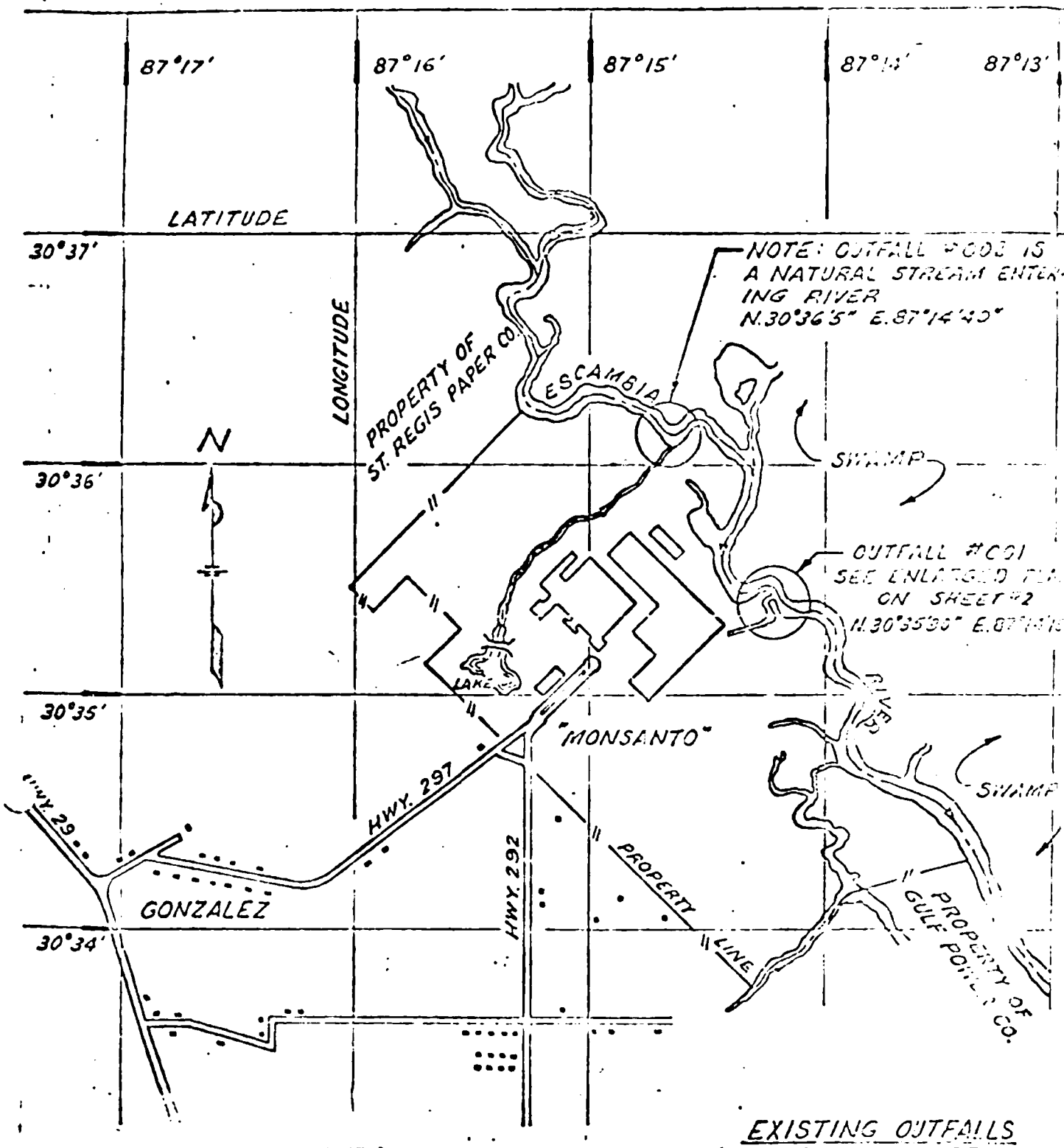
INTRODUCTION

The Monsanto Textiles Company is located approximately 15 miles north of Pensacola, near the town of Gonzalez, in Escambia County, Florida (Fig. 1). The facility maintains a continous operation, producing nylon fibers and intermediate products. The wastewater generated by the production of synthetic fibers is condensed, neutralized, treated with ion exchange material and collected in a holding pond prior to subsurface disposal through two injection wells. Approximately 85% of the cooling water is recycled. Several hundred maintenance personnel, with special materials and equipment, are available to minimize leakage. Additional waste and storm water, with possible added agricultural runoff, is discharged through outfall #001 (to a man made ditch), and outfall #002 (to a natural stream). Final discharge is to the Escambia River, a tributary of Escambia Bay.

In February 1980, the Biological Section of the Florida Department of Environmental Regulation received a composite effluent sample from Monsanto's #001 outfall to the Escambia River. Two acute, static, toxicity bioassays were conducted, using the water flea (Daphnia pulex) and the fathead minnow (Pimephales promelas) as test organisms. These tests were performed to determine the existence and extent of toxicity to the biota of the receiving waters. The results of these tests indicated that the effluent collected at outfall #001 was toxic to Daphnia pulex, 48 hour LC_{50}=55%). The same effluent was not toxic to Pimephales promelas.

To further evaluate the observed toxicity of Monsanto's #001 effluent to Daphnia pulex and possibly to other biota of the receiving waters additional bioassays were conducted by the Biological Section between 3 February and 5 February, 1981. These tests were performed at the requests of the Department's Northwest District and the Monsanto Textiles Company. Organisms used for these tests included: 1) the water flea Daphnia pulex; 2) the water flea Simocephalus serrulatus (an organism indigenous to Florida waters); 3) the fathead minnow Pimephales promelas; and 4) the mysid shrimp Mysidopsis bahia (an organism indigenous to Florida's estuarine waters). The mysid shrimp was selected for testing because water from the Escambia River affected by Monsanto's discharge eventually flows into Escambia Bay, an estuarine system.

The results of these latest toxicity bioassays are presented in this report.



PIGURE 1.

LOCATION MAP

SCALE IN MILES

1 ¾ ½ ¼ 0 1 2

EXISTING OUTFALLS

In ESCAMBIA RIVER
at MONSANTO COMPANY
County of ESCAMBIA
State of FLORIDA
Date 5-28-71

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978). The organisms selected for this study are among those recommended by Peltier (1978) and the U.S. EPA (1978). 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 2½ gallon all glass aquaria filled with unchlorinated DER well water, using 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 $22^{\circ} \pm 1^{\circ}C$. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess daphnids. 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.

Simocephalus serrulatus (water flea) - An indigenous breeding population of S. serrulatus was collected from Lake Jackson, Leon County, Florida, in March, 1980, by personnel from DER's Biological Section. Positive identification of the organism was performed by Mr. Jeff Foran of the Department of Environmental Engineering Sciences, University of Florida in Gainesville. The cultures are maintained in 2½ gallon all glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. The organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, and are kept at a constant temperature of $22^{\circ} \pm 1^{\circ}C$. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess population. They are then refilled with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances.

Pimephales promelas (fathead minnows) - A breeding culture of approximately 100 adult fathead minnows was obtained from the EPA laboratory in Cincinnati, Ohio in May, 1979. The fish have been maintained in four 125 gallon all glass aquaria equipped with a flow through water exchange system using unchlorinated well water obtained from a deep well near the DER laboratory. The fish have been reared at a temperature of $22^{\circ} \pm 2^{\circ}C$ under a 16:8 hour light-dark cycle. These fish are fed Glencoe Mills Trout Chow® and frozen Artemia (brine shrimp) at a rate of 0.75 g/30 fish, twice daily. Breeding has been encouraged by placing several halved clay pots in each tank to serve as substrates for eggs and to provide cover for the fish. Tanks are examined twice daily (at feeding time) for egg production. Clay pots containing egg clusters are immediately transferred to 15 gallon aerated aquaria for hatching. A new aquarium is prepared for each week's production of eggs, thereby segregating fish into even age groups to be used for testing. After hatching, fathead minnow fry are fed twice daily with freshly hatched Artemia. The fish used in this test were all <4 weeks old at the time of the test.

Mysidopsis bahia (mysid shrimp) - In September, 1978, approximately 300 adult mysid shrimp were obtained from the Gulf Breeze EPA Laboratory in Pensacola, Florida. The mysids have been cultured under continuous lighting 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 0/00) to minimize acclimation periods to low or high salinity effluents. Sea water is obtained from the FSU Marine Laboratory located at Turkey Point, Florida (salinity range 17-31 0/00) and partial water changes are performed weekly. Fresh water, for dilution of the sea water 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 $22^{\circ} \pm 1^{\circ}C$. Immediately prior to the bioassay, a suitable number of mysids were transferred by net to a culture dish for observation and selection of individuals for the test. Fifteen day old (three-quarter adult size) mysids were used in this test. Organisms selected for testing were transferred individually by glass tube and randomly placed in the test chambers.

To ensure the uniform sensitivity of these test organisms to toxic substances, the Biological Section conducts monthly Standard Reference Toxicant Bioassays using sodium lauryl sulfate as the reference toxicant.

Test Methods

Four separate 48 hour static bioassays were conducted from 1000 hours 3 February 1981 to 1045 hours 5 February 1981, on a 24 hour composite sample of effluent from outfall #001. The sample was collected by Mr. Rick Bradburn of DER's Northwest District Office at 1100 hours 2 February 1981 using standard wastewater sampling procedures. The samples were iced and transported to the DER laboratory in Tallahassee within 24 hours of collection.

Freshwater diluent for the Daphnia pulex, Simocephalus serrulatus and Pimephales promelas bioassays was obtained from the same DER well that is used to culture the test organisms. The concentration series selected for this study (Table 1) is that recommended by APHA (1976).

Two replicates, each with six concentrations and a control, were used in the Daphnia pulex and Simocephalus serrulatus bioassays. 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 and Simocephalus were siphoned into large culture dishes through a series of nested stainless steel screens to isolate the proper sized and aged organisms (neonates). These neonates were then transferred individually by glass pipette, examined for injury, and loaded 10 per chamber, resulting in 20 per test concentration. Two replicates, of six concentrations and a control, were also used for the fathead minnow bioassay. The fish were transferred individually or by twos, by net, into wide mouth quart jars containing 500 ml each of the appropriate test concentration. Fathead minnows were loaded 5 per chamber resulting in 10 per concentration.

Dissolved oxygen, temperature, pH, and the number of live organisms were recorded for each test chamber at 0 hours, 24 hours, and 48 hours. Total alkalinity, total hardness, conductivity of each test concentration were also recorded at the beginning of the test. Additionally, ammonia concentrations (unionized and total) were recorded at the beginning of the test for each test concentration in the fathead minnow bioassay (Table 4). These values were recorded on the bioassay data forms presented in Tables 2, 3 and 4. These organisms were not fed during this test.

Saltwater for dilution of the effluent in the Mysidopsis bahia test was obtained from the FSU marine laboratory at Turkey Point (salinity = 31.5 ppt). Effluent was adjusted to approximately 8.5 ppt salinity with autoclaved artificial sea salts (Ocean 50 ®). Turkey Point seawater was then used as the diluent in this bioassay. The salinity chosen for the effluent was the approximate lower salinity tolerance limit for Mysidopsis (Peltier, 1978). An additional control was performed at this lower salinity limit (7 ppt) to ensure that any observed mortality in the 100% effluent was not due to salinity shock.

Two replicates, each of six concentrations of effluent and two controls, were used in the mysid shrimp static test. Immediately prior to the bioassay, a suitable number of mysids were transferred by net to a culture dish for observation and selection of individuals for the test. Individual mysid shrimp were siphoned or pipetted via glass tube, carefully examined for injury and transferred to wide mouth quart jars, each containing 500 ml of the appropriate concentration. The concentration series selected for this study (Table 1) are those recommended by APHA (1976).

Five organisms were loaded per test chamber giving 10 per concentration. Salinity was measured in all chambers at the beginning of the saltwater test. The pH, temperature, dissolved oxygen, and the number of live organisms were recorded at 0, 24, and 48 hours (Table 5). According to U.S. EPA (1978), "Although it is generally undesirable to feed most fish and macroinvertebrates during static toxicity tests, an exception is necessary for M. bahia". Therefore, mysids were fed brine shrimp nauplii (Artemia) daily at a rate of approximately 10-20 per mysid to minimize cannibalism.

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

Water fleas (Daphnia pulex and Simocephalus serrulatus)

Concentration	Dilution Water	Effluent	Total Vol.
Control	200 ml	---	200 ml
5.6%	188.8 ml	11.2 ml	200 ml
10.0%	180 ml	20 ml	200 ml
18.0%	164 ml	36 ml	200 ml
32.0%	136 ml	64 ml	200 ml
56.0%	88 ml	112 ml	200 ml
100%	----	200 ml	200 ml

Fathead minnows and mysid shrimp (Pimephales promelas and Mysidopsis bahia)

Concentration	Dilution Water	Effluent	Total Vol.
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%	---	500 ml	500 ml

Table 2. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Monsanto Textiles Company, Escambia County, Florida, Outfall #001, FL0002488, on 3 to 5 February 1981.

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 (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Conductivity (µmhos)
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0
Control	10	10	9	7.8	8.5	8.8	7.9	8.2	8.3	116		118		20.5	20.1	19.8			205
Control	10	10	10	7.9	8.5	8.8	7.9	8.2	8.3										
5.6%	10	10	10	7.9	8.5	8.6	7.9	8.2	8.3	120		115		20.4	20.0	19.8			233
5.6%	10	10	9	7.9	8.5	8.7	7.9	8.2	8.3										
10%	10	10	9	8.0	8.5	8.7	7.9	8.2	8.2	120		118		20.3	20.0	19.8			255
10%	10	10	10	8.0	8.5	8.7	7.9	8.2	8.2										
18%	10	10	9	8.1	8.5	8.7	7.9	8.2	8.2	103		115		20.3	20.0	19.8			300
18%	10	10	8	8.1	8.5	8.7	7.9	8.2	8.2										
32%	10	9	9	8.2	8.2	8.6	7.9	8.2	8.2	92		106		20.3	20.0	19.8			372
32%	10	10	10	8.2	8.2	8.6	7.9	8.1	8.2										
56%	10	10	10	8.2	8.2	8.5	7.9	8.1	8.0	72		106		21.1	20.0	20.0			490
56%	10	10	8	8.2	8.2	8.5	7.9	8.0	8.0										
100%	10	10	7	8.3	8.2	8.4	7.8	7.7	7.9	30		106		21.6	20.0	21.3			770
100%	10	9	8	8.3	8.1	8.3	7.8	7.6	7.8										

Remarks:

Table 3. Data recorded during 48 hour, Simocephalus serrulatus, static acute toxicity bioassay of Monsanto Textiles Company, Escambia County, Florida, Outfall # 001, NPDES #FL0002488, on 3 to 5 February 1981.

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 (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Conductivity (µmhos)
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0
Control	10	10	10	7.9	8.5	8.7	7.9	8.2	8.2	116		118		20.7	20.0	19.7			205
Control	10	8	6	7.9	8.5	8.7	7.9	8.2	8.3										
5.6%	10	10	10	7.9	8.5	8.6	8.0	8.2	8.2	120		115		20.5	20.0	19.7			253
5.6%	10	10	9	8.0	8.5	8.6	7.9	8.2	8.2										
10%	10	10	10	8.0	8.4	8.6	7.9	8.2	8.3	120		118		20.4	20.0	20.1			255
10%	10	10	9	8.0	8.4	8.7	7.9	8.2	8.3										
18%	10	10	9	8.1	8.3	8.6	7.9	8.2	8.4	103		115		20.4	20.0	20.0			300
18%	10	10	9	8.1	8.3	8.7	7.9	8.2	8.4										
32%	10	9	9	8.2	8.3	8.6	7.9	8.1	8.3	92		106		20.0	20.0	19.9			372
32%	10	10	10	8.2	8.3	8.6	7.9	8.1	8.3										
56%	10	10	10	8.2	8.2	8.5	7.9	8.0	8.2	72		106		21.4	19.9	19.9			490
56%	10	10	10	8.2	8.2	8.5	7.9	8.0	8.2										
100%	10	10	9	8.3	8.0	8.3	7.8	7.8	7.8	30		106		21.8	19.9	20.1			770
100%	10	10	7	8.4	8.1	8.4	7.9	7.7	7.7										

Remarks:

Table 4. Data recorded during 48 hour, Pimephales promelas, static acute toxicity bioassay of Monsanto Textiles Company, Escambia County, Florida, Outfall #001, NPDES #FL0002488 on 3 to 5 February 1981.

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 (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Conductivity (µmhos)
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0
Control	5	5	5	8.6	8.1	8.2	7.9	7.9	8.1	116		118		20.3	19.9	19.7	.002/.05		205
Control	5	5	5	8.6	8.1	8.2	7.9	8.0	8.1										
5.6%	5	5	5	8.7	8.1	8.1	7.9	8.0	8.1	120		115		20.2	19.9	19.6	.002/.06		233
5.6%	5	5	5	8.7	8.1	8.2	7.9	8.0	8.1										
10%	5	5	5	8.7	8.1	8.0	7.9	8.0	8.0	120		118		20.1	19.9	19.6	.002/.06		255
10%	5	5	5	8.7	8.0	8.1	7.9	8.0	8.0										
18%	5	5	5	8.7	7.9	8.0	7.9	8.0	8.0	103		115		20.2	19.9	19.5	.003/.08		300
18%	5	5	5	8.7	7.9	8.1	7.9	8.0	8.0										
32%	5	5	5	8.8	8.0	8.1	7.9	8.0	8.0	92		106		20.0	20.0	19.6	.005/.14		372
32%	5	5	5	8.8	7.8	7.9	7.9	8.0	8.0										
56%	5	5	5	8.9	7.6	7.6	7.9	7.9	7.9	72		106		20.0	20.0	19.6	.008/.22		490
56%	5	5	5	8.9	7.7	7.7	7.9	7.8	7.9										
100%	5	5	5	9.1	7.5	7.7	7.8	7.8	7.7	30		106		20.6	20.0	19.6	.010/.10		770
100%	5	5	5	9.1	7.5	7.7	7.8	7.7	7.7										

Remarks: Fish weight: 0.013 g/each (average of 5)

Table 5. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Monsanto Textiles Company, Escambia County, Florida, Outfall #001, NPDES #FL0002488 on 3 to 5 February 1981.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Salinity (o/oo)
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0
Control	5	5	5	6.7	6.6	6.0	7.9	8.0	7.7	20.5	20.0	19.6			29.5
Control	5	5	5	6.7	6.7	6.1	7.9	8.0	7.8						
Control [a]	5	5	5	7.6	7.6	6.7	8.6	8.5	8.1	21.4	20.0	19.5			7.0
Control [a]	5	5	5	7.6	7.7	6.5	8.6	8.4	8.1						
5.6%	5	5	5	6.5	6.6	5.9	8.0	8.0	7.7	20.6	20.0	19.5			28.5
5.6%	5	5	5	6.6	6.5	5.8	8.0	7.9	7.7						
10%	5	4(5[b])	4(5[b])	6.5	6.4	5.9	8.0	7.9	7.7	20.5	20.0	19.6			27.5
10%	5	5	5	6.6	6.5	6.0	8.0	7.9	7.8						
18%	5	5	5	6.7	6.1	5.9	8.1	7.9	7.7	20.6	20.0	19.6			26.0
18%	5	5	5	6.7	6.3	6.0	8.1	7.9	7.7						
32%	5	5	5	6.9	6.3	5.9	8.2	8.0	7.7	20.9	20.0	19.7			23.0
32%	5	5	5	7.0	6.5	6.0	8.2	8.0	7.7						
56%	5	5	5	7.4	6.5	5.9	8.4	8.1	7.7	21.2	20.0	19.7			18.0
56%	5	5	5	7.4	6.5	6.0	8.5	8.1	7.7						
100%	5	5	5	8.0	6.7	6.4	9.0	8.5	7.7	21.9	20.0	19.7			8.5
100%	5	4(5[b])	4(5[b])	8.1	6.7	6.4	9.1	8.5	7.6						

Remarks: a. 7 0/00 salinity control consisting of DER well water adjusted for salinity with Ocean 50® sea salts.
b. Number in parentheses used in calculations since one organism jumped out of the test container.

RESULTS AND DISCUSSION

The sample of effluent collected at outfall #001 proved to be lethal to a number of Daphnia pulex (Table 2). Although the majority of mortality occurred in the 100% concentration, an insufficient number of organisms died for the calculation of statistically sound LC_{50} values (Table 6). The results of the Simocephalus test (Table 3) are somewhat suspect due to the amount of control mortality. In any case, as with Daphnia, a statistically sound LC_{50} value could not be calculated (Table 6). The same effluent was not toxic to the fathead minnow Pimephales promelas or to the mysid shrimp Mysidopsis bahia (Tables 4 and 5). Field parameters of the effluent, taken at the time of collection, are presented in Table 7.

Table 6. LC_{50} Data for Bioassay of Monsanto Textiles Company, Outfall #001, NPDES #FL0002488 on 3 to 5 February 1981.

Organism	Method of Data Analysis	24 hour LC_{50} and 95% confidence intervals	48 hour LC_{50} and 95% confidence intervals
Daphnia pulex	Binomial	A	A
	Moving average	A	A
	Probit	>100%,B	>100%,B
Simocephales serrulatus	Binomial	A	A
	Moving average	A	A
	Probit	A	> 100%, B
Pimephales promelas and Mysidopsis bahia	Binomial Moving average Probit	No mortality	No mortality

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. 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.

Table 7. Field Data of Monsanto Textiles Company's Outfall #001. NPDES #FL0002488.

Temperature:	25°C
Conductivity:	496 µmhos/cm
pH:	7.4
Dissolved Oxygen:	8.0 mg/l
Total Residual Chlorine:	not chlorinated
Salinity:	2 ppt
Receiving Body of Water:	Escambia River
Salinity:	2 ppt

Remarks: 24 hour composite from 1000 hours February 1, 1981 to 1000 hours February 2, 1981. Field measurements conducted by Mr. Rick Bradburn.

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