

BIOASSAYS OF MONSANTO TEXTILES

ESCAMBIA COUNTY, FLORIDA

NPDES #FL0002488

May 10, 1985

Biology Section
Division of Environmental Programs

BIOASSAYS OF MONSANTO TEXTILES

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NPDES #FL0002488

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Bureau of Laboratories
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EXECUTIVE SUMMARY

Monsanto Textiles Company, Outfalls #001 and #002, Escambia County, NPDES #FL0002488 on 27 to 29 March, 1985.

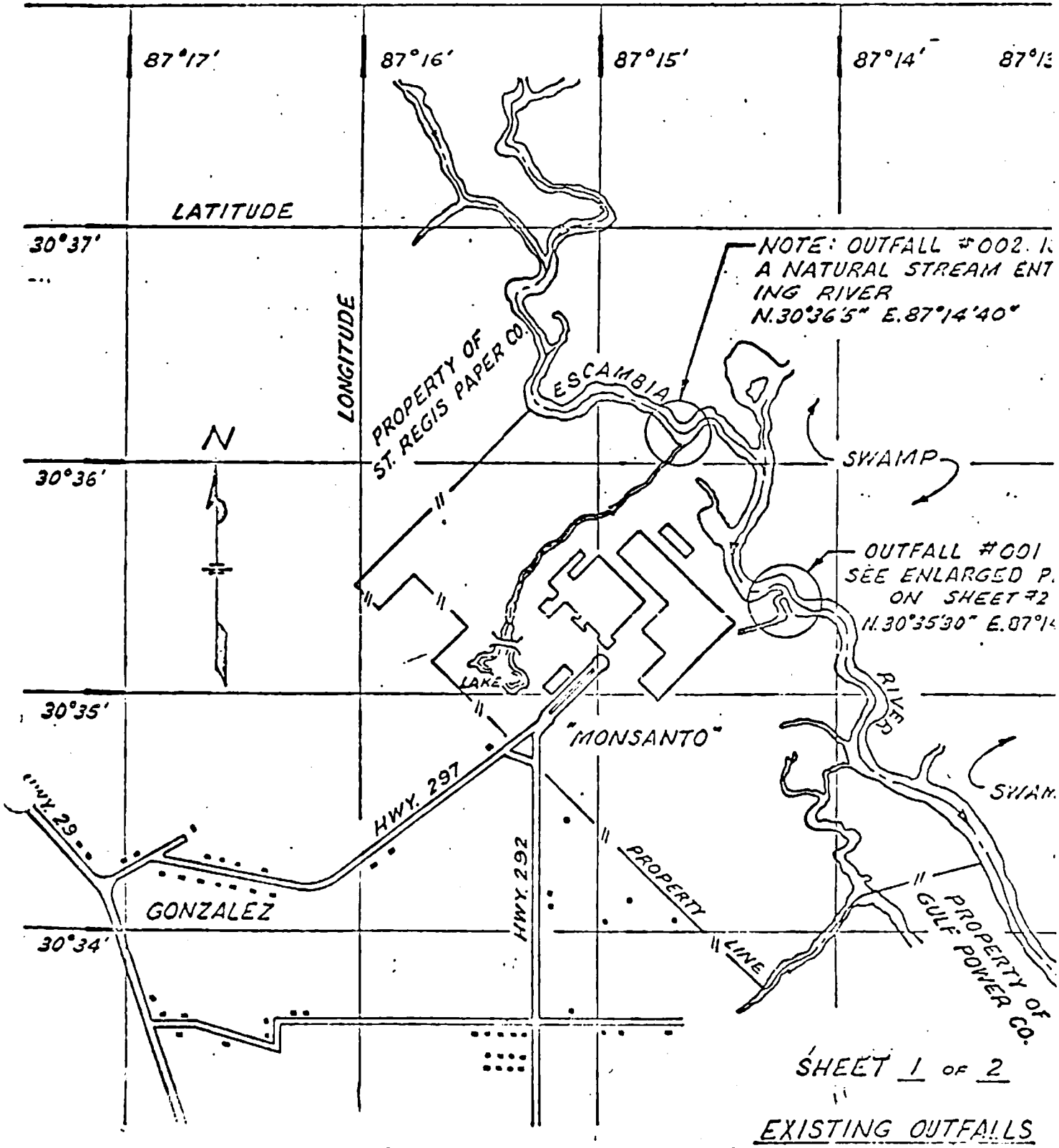
The sample of effluent collected from the Monsanto Textiles Company Outfall #001 (North Outfall) was not acutely toxic to the test organisms, Daphnia pulex, Notropis leedsi, and Mysidopsis bahia. In the D. pulex test, 10% of the organisms died in the 100% concentration, however no mortality occurred in the N. leedsi and M. bahia bioassays. A small amount of total ammonia (maximum measured = 0.149 mg/l) was detected in the sample. The sample of effluent collected from Outfall #002 (South Outfall) was acutely toxic to D. pulex and N. leedsi resulting in 48 hour LC_{50}'s of 77.8% and 59.1%, respectively. No mortality was noted in the M. bahia test. Ammonia was not detected in this sample. Chemical analyses performed on sample #001 indicated the presence of the metals arsenic (0.4 ug/l), chromium (102 ug/l), and zinc (76 ug/l) as well as N,N,4-trimethylbenzenesulfonamide (25 ug/l). The chemistry sample from Outfall #002 was not received due to a Monsanto lab accident.

INTRODUCTION

Monsanto Textiles Company is located at the intersection of State Roads 292 and 297, near Gonzalez in Escambia County, Florida (Figure 1). This facility operates continuously producing nylon fibers and intermediate products. Process wastewater is generated by the production of adipic acid and nylon fibers. Additional sources of wastewater are cooling water and stormwater. A schematic of wastewater sources and flow is outlined in Figure 2. Wastewater from Outfall #001 may include chromium, copper, nickel, and zinc, while Outfall #002 may discharge chromium, copper, and nickel. Both outfalls discharge to the Escambia River, thence to Escambia Bay (data from NPDES file).

On 13 February, 1980, the Biology Section performed two 48 hour static bioassays on a 24 hour composite sample of effluent from Outfall #001. The results of those tests indicated that the sample was not acutely toxic to Pimephales promelas, however, it was toxic to Daphnia pulex, resulting in a 48 hour LC_{50} of 55%.

At the request of DER's Northwest District, additional bioassays were recently performed to further evaluate the toxicity of the effluent from this facility. Six 48 hour static acute toxicity bioassays were performed from 27 to 29 March, 1985. The results of these tests are presented in this report. Those involved in performing the tests and in preparation of this report were: R. B. Frydenborg, M. L. Moultrie, and M. L. Roll.



SHEET 1 OF 2

EXISTING OUTFALLS
In ESCAMBIA RIVER
At MONSANTO COMPANY
County of ESCAMBIA
State of FLORIDA
Date 5-28-71

FIGURE 1.

LOCATION MAP

SCALE IN MILES

1 3/4 1/2 1/4 0 1 2

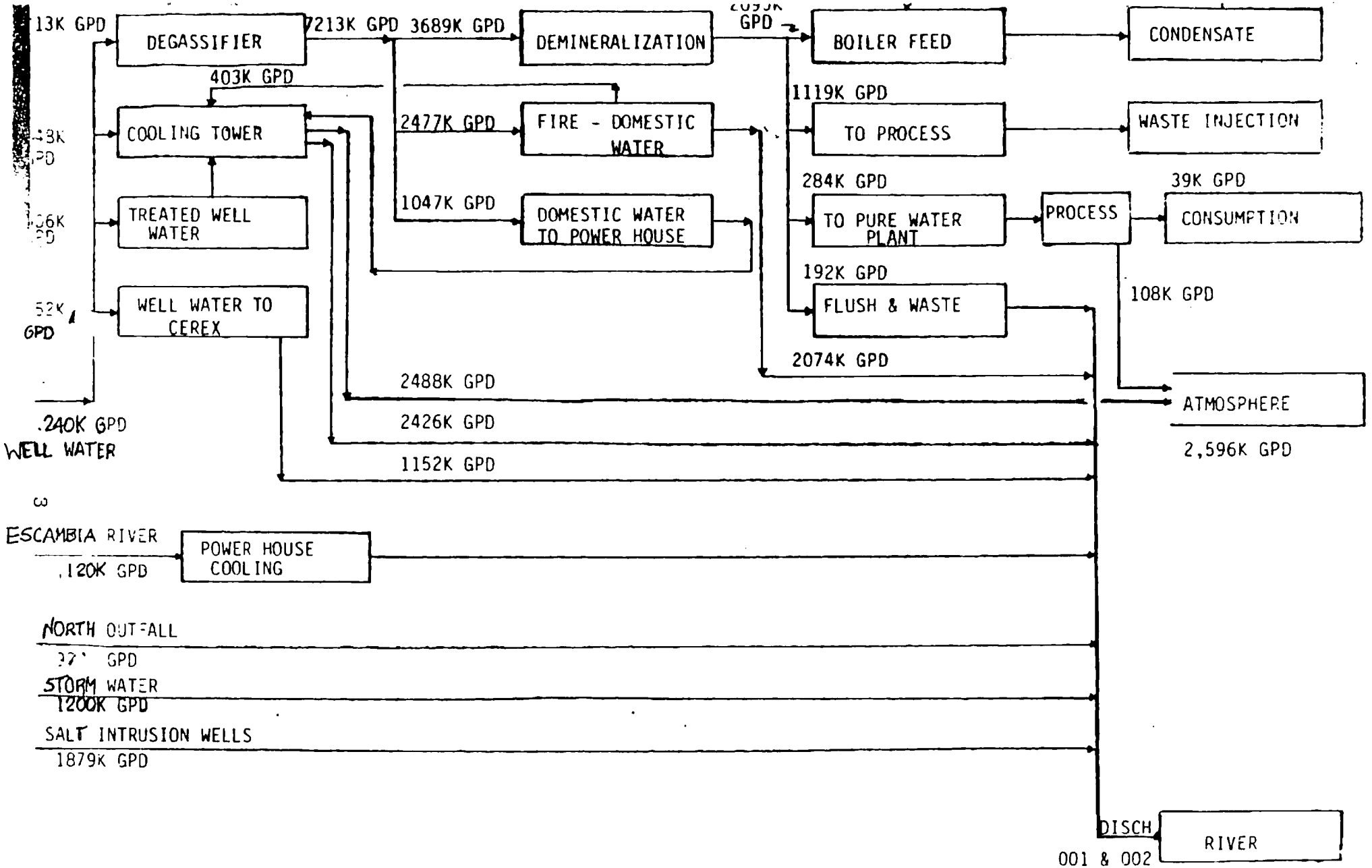


FIGURE 2

SCHEMATIC OF WATER FLOW
MONSANTO TEXTILES COMPANY
GONZALEZ, ESCAMBIA, FLORIDA
JULY 12, 1978

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 a species recommended by Peltier and Weber (1985), the U.S. EPA (1978), 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.

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.

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 fish used in this test were four 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, used for culturing the test organisms and as diluent in the bioassays, is prepared by mixing artificial sea salts (Forty Fathoms®) and well water to obtain a final salinity of 20-30 ppt. Freshwater, for dilution of the seawater as necessary, is obtained from a deep well near the DER laboratory. The mysids are fed twice daily and once daily on weekends with freshly hatched Artemia (brine shrimp) and are kept at a constant temperature of 23° ± 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

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

Six separate 48 hour static bioassays were performed on 24 hour composite samples of effluent collected from Outfalls #001 and #002 of the Monsanto Textiles Company. The tests were performed from 1115 hours on 27 March to 1100 hours on 29 March, 1985.

The samples were collected by Mr. Richard Singer of DER's Northwest District at 1025 hours on 26 March, 1985, 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.

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.

Salinity Adjustment

Since these effluents eventually enter an estuarine environment, the Biology Section also used a saltwater test organism (*Mysidopsis bahia*) to evaluate toxicity. Therefore, artificial sea salts (Forty Fathoms®) were added to the 100% concentration until a salinity of approximately 8 ppt was obtained. This salinity is well within the salinity tolerance of the test organism. Nevertheless, a Salinity Control was also employed, consisting of artificial seawater diluted with well water until a similar salinity of approximately 8 ppt was obtained. Additional information concerning salinity adjustment and saltwater tests is given below.

pH Adjustment

Since the toxicity, or lack of toxicity, in a bioassay sample may be dependent on the sample's pH, the Biology Section prefers, and recommends, that the pH of a sample at the start of the bioassay be similar to the pH measured during sampling. In the *M. bahia* bioassays, the 100% samples

were adjusted with an acid (0.1N HCl). This was necessary due to the pH having increased between sampling and initiating the test. This pH increase was likely a result of salinity adjusting the samples. The pH of these 100% samples were adjusted to ± 0.3 s.u. of the value measured upon arrival in the laboratory and prior to any adjustments. This procedure provides the fairest test possible considering the varied chemical substances in complex wastewaters, the toxicities of which are dependent on pH. Two controls were also employed, consisting of dilution water and artificial seawater with its pH adjusted with the same acid to the value to which the sample was adjusted. These adjustments are noted on the bioassay data forms.

Freshwater Tests

Freshwater for dilution of the effluent 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 an abbreviated version of that recommended by APHA (1980).

Two replicates, of each concentration and a control, 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.

Two replicates, of each concentration and a control, were used for the Notropis leedsi (bannerfin shiner) bioassays. 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.

Saltwater Tests

Saltwater for dilution of the effluent samples was prepared from artificial sea salts (Forty Fathoms®), and adjusted with DER well water until a salinity of approximately 20 ppt was achieved. For test concentrations of 56% and below, this diluent was mixed directly with the effluent, achieving a range of final salinities (8.0 ppt to 20.0 ppt). For the 100% effluent test concentrations, freshly autoclaved artificial sea salts were added until salinities of approximately 8 ppt were achieved (well within the lower limit of mysid shrimp salinity tolerance). Two control groups were then used to ensure that any mortality in the test organisms was not the result of toxic properties associated with the diluent, the artificial sea salts, the pH adjustments, or the salinity range. The control groups included:

1) Diluent Control - Artificial seawater (20.0 ppt). This represents the highest concentration of artificial seawater used in the bioassays, and tests for toxicity in the diluent. This is also the highest salinity used in the bioassays, and therefore tests the tolerance of the organisms to this salinity.

2) Salinity - Acid Control - Artificial seawater (20.0 ppt) adjusted with well water to a salinity of approximately 8 ppt, with an amount of hydrochloric acid added needed to equal the pH achieved in the pH adjusted effluent.

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

Dissolved oxygen (YSI Model 57 oxygen meter), pH (Corning Model 7 pH meter with Orion Ross® combination electrode), temperature (NBS calibrated glass thermometer), and the number of live organisms were recorded for each test 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% effluent concentration. The specific conductance or salinity (YSI Model 33 S-C-T meter) of each test concentration was recorded at the beginning and end of the test. All instruments were calibrated daily according to manufacturer's recommendations. Ammonia concentrations, including the total and unionized fraction (expressed in mg/l as N and as NH_3, respectively), were recorded in the controls and the 100% effluent test concentrations at the beginning of the test. Total ammonia concentrations were determined using an Orion® specific ion probe and an Orion Ionalyzer®. The unionized fraction was then calculated based on the total ammonia concentration, and the pH, temperature, and salinity of the sample. These values were recorded on the bioassay data forms and are presented in Tables 2 through 7.

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

Daphnia pulex (water flea)

Concentration	Dilution Water	Effluent	Total Volume
Control	200.0 ml	--------	200 ml
10.0%	180.0 ml	20.0 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

Notropis leedsi (bannerfin shiner)

Mysidopsis bahia (mysid shrimp)

Concentration	Dilution Water	Effluent	Total Volume
Control	500 ml	--------	500 ml
10.0%	450 ml	50 ml	500 ml
32.0%	340 ml	160 ml	500 ml
56.0%	220 ml	280 ml	500 ml
100.0%	------	500 ml	500 ml

Table 2. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Monsanto Textiles Company, Escambia County, Florida, NPDES #FL0002488, on 27 to 29 March, 1985.

North Outfall

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	10	10	10	8.3	8.5	7.9	7.7	8.1	8.0	22.1	21.9	21.4		
Control	10	9	9	8.3	8.5	7.9	7.7	8.1	8.0				212	220
10.0%	10	10	10	8.4	8.5	8.1	7.8	8.1	8.0	22.1	21.9	21.2		
10.0%	10	10	10	8.4	8.5	8.1	7.8	8.1	8.0				210	210
32.0%	10	10	10	8.5	8.4	8.2	7.8	8.0	7.9	22.1	21.8	21.1		
32.0%	10	10	10	8.5	8.4	8.2	7.8	8.0	7.9				175	175
56.0%	10	10	10	8.8	8.3	8.2	7.6	7.9	7.8	22.1	21.6	21.1		
56.0%	10	10	10	8.8	8.3	8.2	7.6	7.8	7.8				331	332
100.0%	10	10	9	9.1	8.1	7.7	7.3	7.3	7.5	22.1	21.6	21.2		
100.0%	10	10	9	9.1	8.1	7.7	7.3	7.3	7.5				268	271

Control Water

NH_3(as NH_3)(mg/l) 0

Total ammonia (as N)(mg/l) 0

Total Alkalinity 98 (mg/l $CaCO_3$)

Total Hardness 106 (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) .001

Total ammonia (as N)(mg/l) .149

Total Alkalinity 5 (mg/l $CaCO_3$)

Total Hardness 10 (mg/l $CaCO_3$)

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

Table 3. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of Monsanto Textiles Company, Escambia County, Florida, NPDES #FL0002488, on 27 to 29 March, 1985.

North Outfall

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	8.4	7.6	7.7	7.9	7.8	21.9	21.7	21.0			Control Water
Control	5	5	5	8.5	8.4	7.5	7.7	8.0	7.8				225	260	NH_3(as NH_3)(mg/l) 0 — Total Alkalinity (mg/l $CaCO_3$) 98
10.0%	5	5	5	8.5	8.4	7.6	7.7	7.9	7.8	21.8	21.4	20.8			
10.0%	5	5	5	8.5	8.4	7.6	7.7	7.9	7.8				210	241	
32.0%	5	5	5	8.6	8.3	7.7	7.7	7.8	7.8	21.8	21.1	20.8			Total ammonia (as N)(mg/l) 0 — Total Hardness (mg/l $CaCO_3$) 106
32.0%	5	5	5	8.6	8.3	7.7	7.7	7.8	7.7				179	210	
56.0%	5	5	5	9.0	8.2	7.0	7.6	7.7	7.5	21.9	21.1	20.7			
56.0%	5	5	5	9.0	8.2	7.1	7.6	7.7	7.5				250	284	
100.0%	5	5	5	9.3	7.9	6.7	7.2	7.0	7.0	21.9	21.0	20.9			
100.0%	5	5	5	9.3	7.9	6.8	7.2	7.0	7.0				205	242	100% Effluent
															NH_3(as NH_3)(mg/l) .001 — Total Alkalinity (mg/l $CaCO_3$) 5
															Total ammonia (as N)(mg/l) .149 — Total Hardness (mg/l $CaCO_3$) 10

Table 4. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Monsanto Textiles Company, Escambia County, Florida, NPDES #FL0002488, on 27 to 29 March, 1985.

North Outfall

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Control	5	5	5	8.0	6.8	6.2	8.8	8.6	8.5	22.6	21.8	21.3		
Control	5	5	5	8.0	6.8	6.3	8.8	8.6	8.5				20.7	19.5
Acid/Salinity Control	5	5	5	8.3	6.7	6.5	6.9	7.7	7.9	22.2	21.5	21.0		
Acid/Salinity Control	5	5	5	8.3	6.8	6.6	6.9	7.7	7.9				8.4	8.2
10.0%	5	5	5	8.0	6.6	6.5	8.8	8.6	8.5	22.5	21.4	20.8		
10.0%	5	5	5	8.0	6.6	6.5	8.8	8.6	8.5				18.5	17.5
56.0%	5	5	5	8.6	6.7	6.6	8.9	8.4	8.1	22.2	21.1	20.8		
56.0%	5	5	5	8.6	6.7	6.6	8.9	8.4	8.1				9.9	9.2
100.0%	5	5	5	8.5	6.6	6.6	6.9	7.4	7.3	22.7	21.1	20.9		
100.0%	5	5	5	8.5	6.6	6.5	6.9	7.3	7.3				7.9	7.5

Control Water

NH_3(as NH_3)(mg/l) 0

Total Alkalinity ____ (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) 0

Total Hardness ____ (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) .0006

Total Alkalinity ____ (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) .149

Total Hardness ____ (mg/l $CaCO_3$)

Table 5. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Monsanto Textiles Company, Escambia County, Florida, NPDES #FL0002488, on 27 to 29 March, 1985.

South Outfall

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	10	10	10	8.4	8.0	8.2	7.8	8.2	8.2	22.3	21.7	21.2		
Control	10	10	10	8.4	8.0	8.2	7.8	8.2	8.2				230	230
10.0%	10	10	8	8.5	8.1	8.2	7.8	8.2	8.2	22.2	21.5	21.2		
10.0%	10	10	10	8.5	8.1	8.2	7.8	8.2	8.2				225	222
32.0%	10	9	5	8.6	8.1	8.3	7.8	8.1	8.1	22.2	21.5	21.3		
32.0%	10	10	10	8.6	8.1	8.3	7.8	8.1	8.1				227	225
56.0%	10	10	7	8.8	8.2	8.3	7.7	7.9	8.0	22.1	21.5	21.4		
56.0%	10	10	9	8.8	8.2	8.3	7.7	7.9	8.0				227	225
100.0%	10	6	1	9.5	8.3	8.1	7.3	7.4	7.7	22.2	21.9	21.5		
100.0%	10	9	2	9.5	8.3	8.1	7.3	7.4	7.7				230	232

Control Water

NH_3(as NH_3)(mg/l) 0

Total ammonia (as N)(mg/l) 0

Total Alkalinity (mg/l $CaCO_3$) 98

Total Hardness (mg/l $CaCO_3$) 106

100% Effluent

NH_3(as NH_3)(mg/l) 0

Total ammonia (as N)(mg/l) 0

Total Alkalinity (mg/l $CaCO_3$) 22

Total Hardness (mg/l $CaCO_3$) 35

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

Table 6. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of Monsanto Textiles Company, Escambia County, Florida, NPDES #FL0002488, on 27 to 29 March, 1985.

South Outfall

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	8.4	7.6	7.7	7.9	7.8	21.9	21.7	20.9		
Control	5	5	5	8.5	8.4	7.5	7.7	8.0	7.8				227	260
10.0%	5	5	5	8.6	8.0	7.8	7.7	7.9	8.0	21.8	21.3	20.9		
10.0%	5	5	5	8.6	8.0	7.8	7.7	7.9	8.0				228	250
32.0%	5	5	3	8.8	8.0	7.5	7.7	7.8	7.8	21.9	21.2	20.9		
32.0%	5	5	2	8.8	8.0	7.5	7.7	7.8	7.8				229	251
56.0%	5	3	0	9.0	8.1	7.4	7.6	7.7	7.6	22.1	21.2	21.0		
56.0%	5	1	0	9.0	8.1	7.4	7.6	7.7	7.6				229	251
100.0%	5	1	0	9.4	8.2	7.5	7.3	7.3	7.3	22.1	21.5	21.0		
100.0%	5	1	0	9.4	8.0	7.5	7.3	7.3	7.3				231	251

Control Water

NH_3(as NH_3)(mg/l) 0

Total ammonia (as N)(mg/l) 0

Total Alkalinity (mg/l $CaCO_3$) 98

Total Hardness (mg/l $CaCO_3$) 106

100% Effluent

NH_3(as NH_3)(mg/l) 0

Total ammonia (as N)(mg/l) 0

Total Alkalinity (mg/l $CaCO_3$) 22

Total Hardness (mg/l $CaCO_3$) 35

Table 7. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Monsanto Textiles Company, Escambia County, Florida, NPDES #FL0002488, on 27 to 29 March, 1985.

South Outfall

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Control	5	5	5	8.0	6.8	6.2	8.8	8.6	8.5	22.1	21.8	21.3		
Control	5	5	5	8.0	6.8	6.3	8.8	8.6	8.5				20.7	19.5
Acid/Salinity Control	5	5	5	8.3	6.7	6.5	6.9	7.7	7.9	22.2	21.5	21.0		
Acid/Salinity Control	5	5	4	8.3	6.8	6.6	6.9	7.7	7.9				8.4	8.2
10.0%	5	5	5	8.2	6.6	6.5	8.7	8.6	8.4	22.4	21.4	20.8		
10.0%	5	5	5	8.2	6.6	6.5	8.7	8.6	8.4				18.7	18.5
56.0%	5	5	5	8.7	6.8	6.8	8.9	8.4	8.2	22.5	21.2	20.9		
56.0%	5	5	5	8.7	6.8	6.8	8.9	8.4	8.2				9.9	9.5
100.0%	5	5	5	8.4	7.0	6.7	7.1	7.4	7.5	20.4	21.4	21.0		
100.0%	5	5	5	8.4	7.0	6.7	7.1	7.4	7.5				8.4	8.1

Control Water

NH_3(as NH_3)(mg/l) 0

Total Alkalinity _____ (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) 0

Total Hardness _____ (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) 0

Total Alkalinity _____ (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) 0

Total Hardness _____ (mg/l $CaCO_3$)

Table 8. LC_{50} Data for Bioassay of Monsanto Textiles Company (North Outfall), Escambia County, Florida, NPDES #FL0002488, on 27 to 29 March, 1985.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
D. pulex	Binomial	a	a
	Moving Average	a	a
	Probit	a	a
N. leedsi	Binomial	No Mortality	No Mortality
	Moving Average		
	Probit		
M. bahia	Binomial	No Mortality	No Mortality
	Moving Average		
	Probit		

[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.

Table 9. LC_{50} Data for Bioassay of Monsanto Textiles Company (South Outfall), Escambia County, Florida, NPDES #FL0002488, on 27 to 29 March, 1985.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
D. pulex	Binomial	b	77.8% (56 and 100),[c]
	Moving Average	b	70.7% (55.2 and 105.1)
	Probit	206.6% (112.7 and ∞)	63.9% (0 and ∞)
N. leedsi	Binomial	52.1% (32 and ∞)	32.0% (10 and 56)
	Moving Average	59.1% (46.1 and 77.6),[c]	a
	Probit	60.8% (46.1 and 82.6)	a
M. bahia	Binomial	No Mortality	No Mortality
	Moving Average		
	Probit		

[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.

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

Table 10. Results of Chemical Analyses Performed on Monsanto's Wastewater from Outfall #001 (North)[a], Escambia County, NPDES #FL0002488, Collected on 26 March, 1985.

Organics	
Base Neutral Extractables:	
N,N,4-trimethylbenzenesulfonamide	25 ug/l
Acid Neutral Extractables:	N.D.
Pesticide Extractables:	N.D.
Metals	
Antimony	N.D.
Arsenic	0.4 ug/l
Beryllium	N.D.
Cadmium	N.D.
Chromium	102 ug/l
Copper	N.D.
Lead	N.D.
Mercury	<0.2 ug/l
Nickel	N.D.
Selenium	N.D.
Silver	N.D.
Thallium	N.D.
Zinc	76 ug/l

N.D. = None Detected

[a] Chemistry samples from Outfall #002 (South) were not collected due to a Monsanto lab accident.

RESULTS AND DISCUSSION

The sample of effluent collected from the Monsanto Textiles Company Outfall #001 (North) was not acutely toxic to the test organisms, Daphnia pulex, Notropis leedsi, and Mysidopsis bahia. In the D. pulex test, 10% of the organisms died in the 100% concentration (Table 2). No mortality was noted in the N. leedsi and M. bahia bioassays (Tables 3 and 4). A small amount of total ammonia (maximum measured = 0.149 mg/l) was detected in the sample. The sample of effluent collected from Outfall #002 was acutely toxic to D. pulex and N. leedsi, resulting in 48 hour LC_{50}'s of 77.8% and 59.1%, respectively (Table 9). No mortality was observed in the M. bahia test (Table 7). Ammonia was not detected in this sample. Chemical analyses performed on the sample from Outfall #001 indicated the presence of the metals arsenic (0.4 ug/l), chromium (102 ug/l), and zinc (76 ug/l) as well as N,N,4-trimethylbenzenesulfonamide (25 ug/l). The chemistry sample from Outfall #002 was not received due to a Monsanto lab accident.

Discussion of the Toxicity Criteria

The toxicity standards of Chapters 17-3 and 17-4, F.A.C., are complex. Because of this complexity, they will be briefly reviewed here so that these test results may be more readily compared with the standards. For legal purposes, however, the language of the Florida Administrative Code should be consulted directly.

The two basic concepts utilized in the standards are acute and chronic toxicity (defined in 17-3.021(1) and (4), respectively). Simplifying the actual F.A.C. language somewhat, acute toxicity is defined as the presence of a substance in a concentration one-third as great as that concentration which kills one-half of the test organisms in 96 hours, i.e., one-third of the 96 hour LC_{50}. Chronic toxicity is similarly defined as one-twentieth of the 96 hour LC_{50}. Therefore, if a test solution kills one-half or more of a group of test organisms in 96 hours, it must contain at least three times the defined "acutely toxic" concentration and at least twenty times the "chronically toxic" concentration of some substance or substances. If a test solution causes that same mortality in less than 96 hours, (for example, in 24 or 48 hours), it is at least as toxic as the example given above, and actually is probably considerably more toxic.

The particular restrictions applied to a given discharger depend upon the existence of a mixing zone, whether as a specific part of the permit or based on the existence of a currently valid permit predating the language in Chapter 17-4 requiring mixing zone designation. In the absence of a mixing zone, the general criteria for surface waters [17-3.061] are applicable at all points, beginning at the point of discharge. This section prohibits the discharge of substances in concentrations which are chronically toxic [17-3.061(2)(p)]; that is, the concentrations cannot exceed one-twentieth of the 96 hour LC_{50} as discussed above. If a mixing zone does exist, then this chronic toxicity standard is only applicable from the edge of the mixing zone and beyond. Within the mixing zone, the less stringent acute toxicity standard of the minimum criteria for all waters [17-3.051(1)(d)] applies: one-third of the 96 hour LC_{50}. The acute toxicity standard does not apply, however, to each point within a mixing zone not designated for dredge and fill permitting, but rather to the average of a number of points within such a mixing zone, based on the provisions of the surface water mixing zones section of Chapter 17-4 [17-4.244(4)]. At the same time, though, this same rule prohibits waste concentrations within such a mixing zone (i.e., at any point within the mixing zone), from exceeding the 96 hour LC_{50}. Therefore, at any point within such a mixing zone, including the point of discharge, one-half of the test organisms cannot be killed in 96 hours. Within a mixing zone designated for dredge and fill permitting, the acute toxicity standard (one-third of the 96 hour LC_{50}) applies at all points, including the point of discharge.

From the above discussion, it should be clear that if the least stringent toxicity standard, the 96 hour LC_{50}, is exceeded at any point, from the point of discharge outwards, by any discharger, then a violation of the standards has occurred. This is an important consideration since the test procedure here reported involves the measurement of the 50% lethal concentration, and not some fraction of that concentration. Any discharger required to meet the one-twentieth of the 96 hour LC_{50} standard is therefore being tested very leniently. Any reduction in the test period from 96 hours provides an added degree of leniency to a discharger.

A number of different dilutions were examined in order to determine by how much, if any, the discharge exceeds the 96 hour LC_{50}. For example, if the sample, after two-fold dilution with diluent (a 33% concentration), kills one-half of the test organisms after 96 hours, then the sample is three times as toxic as is allowed by that standard. Similarly, if a 5% solution of the sample kills 50% of the organisms, the sample is twenty times as toxic as is allowed. Toxicities are reported here in that manner, i.e., in terms of the percentage dilution of the test sample. The smaller the percentage which is reported means a correspondingly greater toxicity.

Note that even if a discharger receives a very large mixing zone not designated for dredge and fill permitting, the regulations require that the <u>maximum</u> toxicity at any point within the mixing zone be restricted to the 96 hour LC_{50}. The results here reported are based on actual effluent samples, representative of the effluent concentration which enters waters of the state at the point of discharge. If it is demonstrated that, under the conditions existing at the point of discharge, effluent concentrations will be less than at the actual sampling point (if the two points are different), the reported bioassay results will reflect the reduced discharge concentration.

With respect to the species of organisms used in these tests, the language of the F.A.C. indicates that they should be "significant to the indigenous aquatic community". All species used in these tests are significant to the indigenous aquatic community since they are sensitive to toxic substances which may be expected to impact the indigenous community.

The toxicity reported here is in excess of allowable limits.

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