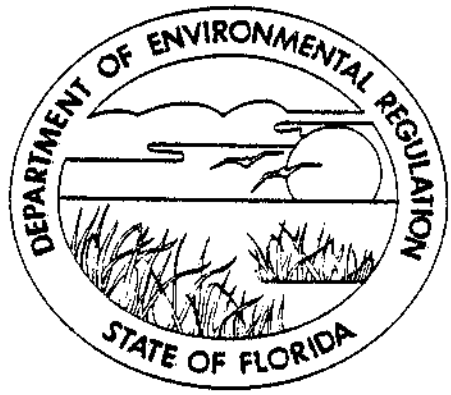


BIOASSAYS OF AMAX CHEMICALS

PHOSPHATE FERTILIZER PRODUCTS

PINEY POINT, MANATEE COUNTY, FLORIDA

NPDES #FL0000124

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Biology Section
Bureau of Water Analysis
September 22, 1983

BIOASSAYS OF AMAX CHEMICALS

PHOSPHATE FERTILIZER PRODUCTS

PINEY POINT, MANATEE COUNTY, FLORIDA

NPDES #FL0000124

Biology Section
Bureau of Water Analysis
September 22, 1983

EXECUTIVE SUMMARY

AMAX Chemicals, NPDES #FL0000124, on 31 August to 2 September, 1983.

A total of seven acute toxicity bioassays were performed on samples collected from this phosphate fertilizer manufacturer's discharge point and edge of mixing zone, using both freshwater and marine test organisms. The discharge point sample (Outfall #003) was extremely toxic to all the test organisms, producing 48 hour LC_{50}'s of 6.2% and 10.1% for Daphnia pulex and Mysidopsis bahia respectively, and, 4 hour LC_{50}'s of <5.6% for the fish, Pimephales promelas and Notropis leedsi. The edge of the mixing zone sample was almost equally as toxic, producing 48 hour LC_{50}'s of 11.7 %, 11.4%, and 12.6% for Daphnia pulex, Heterandria formosa, and Mysidopsis bahia, respectively.

This extreme toxicity was directly attributed to the large concentrations of total and unionized ammonia detected in the samples. Total ammonia concentrations of 102 mg/l and 70 mg/l, and unionized ammonia concentrations of 78.9 mg/l and 29.3 mg/l were measured in the discharge point and edge of mixing zone samples, respectively. These concentrations were sufficient to account for the toxicity noted in these bioassays.

INTRODUCTION

AMAX Chemicals is located on U.S. Highway 41 at Piney Point, Manatee County, Florida (Fig. 1). The facility manufactures various phosphate fertilizer products and related by-products, such as sulfuric and phosphoric acids, and ammonium phosphate. Non-process wastewater is discharged through Outfalls #001 and #002. Treated process wastewater is discharged sporadically through Outfall #003 (Fig. 2), a county drainage ditch to Piney Point Creek, which flows to Bishop Harbor on Tampa Bay.

In August, 1983, personnel from DER's Tampa Office detected elevated concentrations of ammonia in AMAX's process wastewater discharge #003. Acute toxicity bioassays were requested to evaluate the effects this discharge may have on the biota of the receiving waters. Samples were collected from the discharge point (Outfall #003) and from the edge of AMAX's designated mixing zone, approximately 800 meters downstream from the discharge point (Mr. Matt McCann, Tampa, personal communication). A total of seven acute toxicity bioassays were performed from August 31 to September 2, 1983, using both freshwater and marine test organisms on each sample. The results of these tests are reported here. Those involved in performing these tests and in preparation of this report were: C. W. Dye, R. B. Frydenborg, and M. L. Roll.

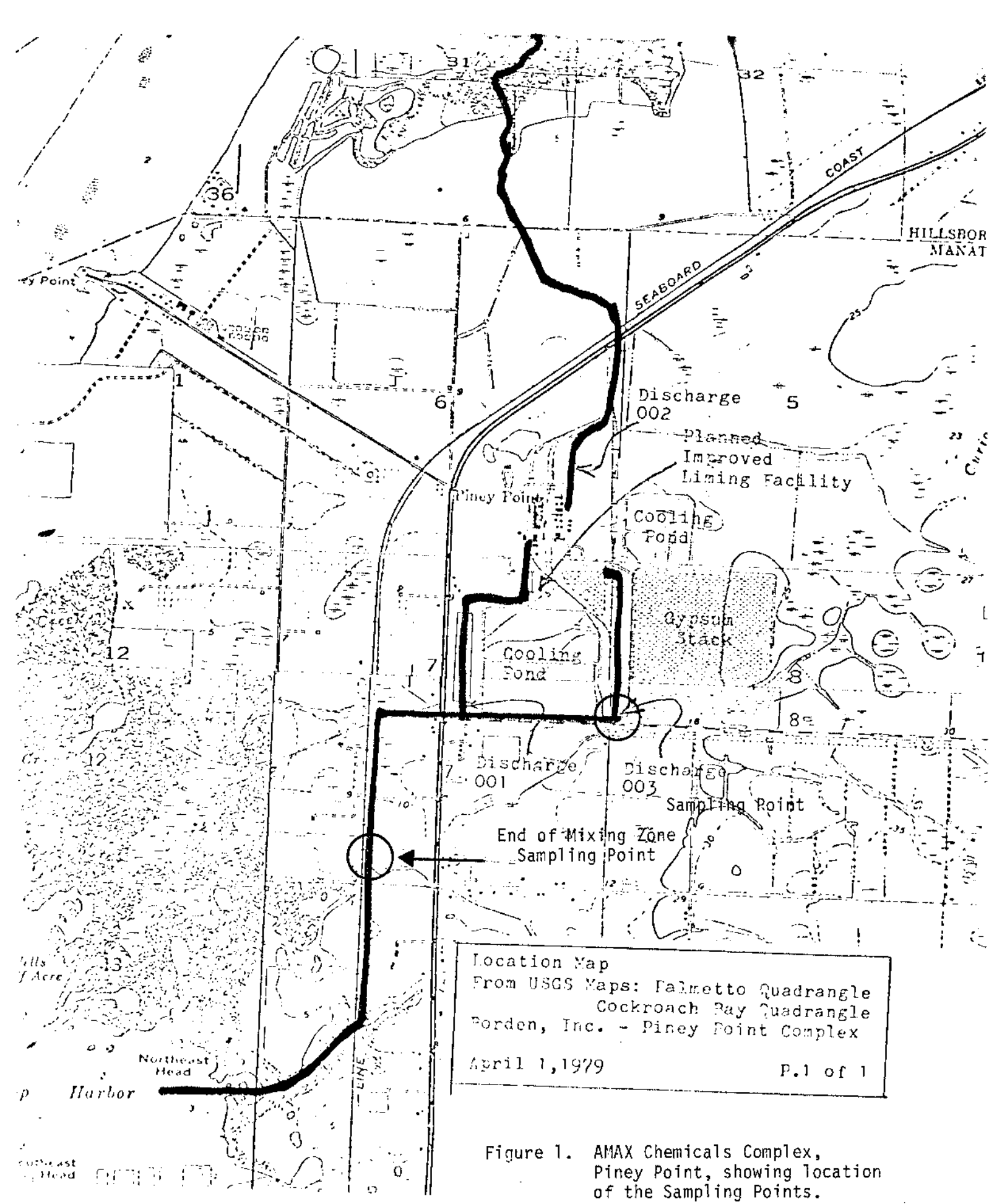
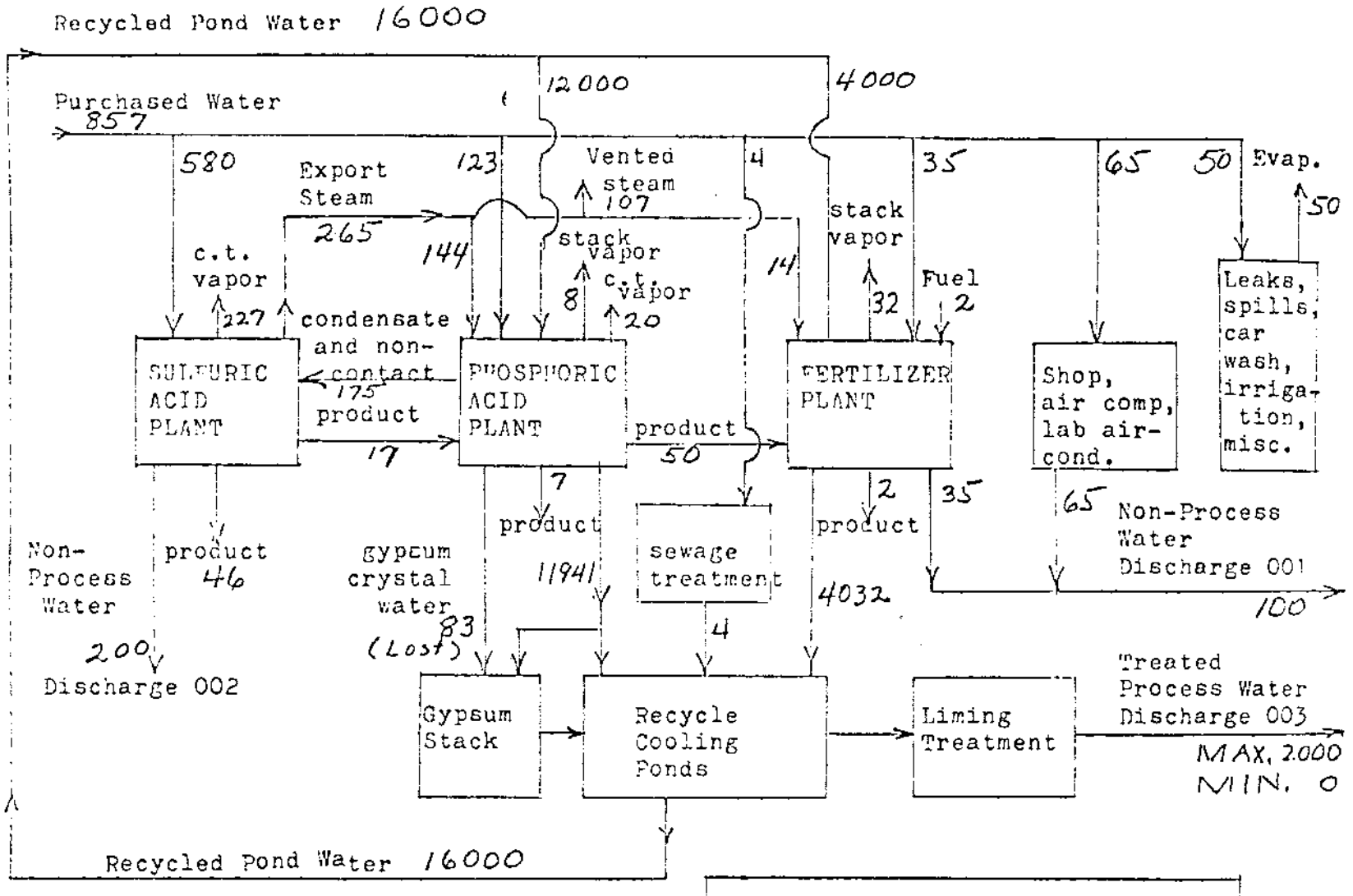


Figure 1. AMAX Chemicals Complex, Piney Point, showing location of the Sampling Points.



Water Flows in GPM
Average for 343 Operating Days

Schematic of Water Flow
Borden, Inc. - Piney Point Complex
Palmetto, Manatee Co., Florida

April 1, 1979 P.1 of 1

Figure 2

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.

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. This breeding population is augmented with fry hatched in our laboratory and with additional adult fish from EPA as required. The fish are maintained in three 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 fathead minnows are reared at a temperature of 22° plus or minus 2°C under a 16:8 hour light-dark cycle. These fish are fed Glencoe Mills "#3 size enriched trout fry granules", Tetra-Min®, and frozen Artemia (brine shrimp) at a rate of 0.75g/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 6 to 8 days old at the time of the test.

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 16 to 19 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 approximately 15 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

A total of seven static acute toxicity bioassays were performed on samples collected from AMAX Chemical's discharge point (Outfall #003) and edge of mixing zone. The tests were performed from 1100 hours on 31 August to 1100 hours on 2 September, 1983. The samples were collected by Ms. Phyllis Giwer of DER's Tampa office at 1115 and 1145 hours on 30 August, 1983, using standard wastewater sampling procedures. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 24 hours of collection.

Freshwater for dilution of the 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).

Two replicates, each with five randomly arranged concentrations 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 five randomly arranged concentrations and a control, were used for the Pimephales promelas (fathead minnow), the Heterandria formosa (least killifish), and 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 for dilution of the samples for the M. bahia bioassays was obtained from the FSU Marine Laboratory at Turkey Point (salinity = 26.5 ppt). For test concentrations of 56% and below, this diluent was mixed directly with the samples, achieving a range of final salinities (13.0 ppt to 25.5 ppt).

The concentration series selected for this study (Table 1) is that recommended by APHA (1980). Two replicates of each randomly arranged 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 10 ppt, 15 ppt, 20 ppt, and 25 ppt 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

chamber at 0 hours, 24 hours, and at 48 hours, if live organisms were present. 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 samples. 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 in each 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 Tables 2 through 8.

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

Daphnia pulex (water flea)

Concentration[a]	Dilution Water	Effluent	Total Volume
Control	200.0 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

Pimephales promelas (fathead minnow)

Heterandria formosa (least killifish)

Notropis leedsi (bannerfin shiner)

Mydidopsis bahia (mysid shrimp)

Concentration[a]	Dilution Water	Effluent	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

[a] Due to the nature of the samples, and to conserve the samples for additional testing, 100% test concentrations were not used.

Table 2. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of AMAX Chemicals, NPDES #FL0000124, Piney Point, Florida.
Discharge Point (Outfall #003) Sample

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/N (mg/l)		Conductivity (µmhos)	
	0	4[a]	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	10	10	10	8	7.9	8.6	8.6	7.7	8.1	8.1	118		120		20.9	19.4	19.4	0/0	--		
Control	10	10	10	10	8.1	8.7	8.6	7.7	8.1	8.1										203	210
5.6%	10	10	9	5	8.1	8.8	8.6	8.8	8.4	8.1					20.9	19.2	19.5	1.38/5.4	--		
5.6%	10	10	9	8	8.1	8.7	8.6	8.8	8.4	8.1										444	450
10.0%	10	6	0	0	8.1	8.9	--[b]	9.0	8.5	--[b]					20.8	19.7	--[b]	3.8/10.6	--		
10.0%	10	2	0	0	8.1	8.8	--	9.0	8.5	--										720	--[b]
18.0%	10	0	0	0	8.0	8.8	--	9.1	8.7	--					20.8	19.1	--	6.93/16.5	--		
18.0%	10	0	0	0	8.0	8.7	--	9.1	8.7	--										1120	--
32.0%	10	0	0	0	7.6	8.8	--	9.2	8.8	--					20.7	19.0	--	14.5/30.1	--		
32.0%	10	0	0	0	7.6	8.7	--	9.2	8.8	--										1790	--
56.0%	10	0	0	0	7.2	8.8	--	9.3	8.9	--					20.5	19.2	--	26.3/48.1	--		
56.0%	10	0	0	0	7.1	8.7	--	9.3	8.9	--										2880	--
100.0%[c]								9.4			469		155					78.9/109		4850	

Remarks: [a] Since rapid mortality of organisms was observed, a 4 hour count of live organisms was performed.
[b] Remaining parameters were not recorded since all organisms were dead by hour 24.
[c] Parameters taken of the 100% sample are reported here.

Table 3. Data recorded during 48 hour, Pimephales promelas, static acute toxicity bioassay of AMAX Chemicals, NPDES #FL0000124, Piney Point, Florida.

Discharge Point (Outfall #003) Sample

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/N (mg/l)		Conductivity (µmhos)	
	0	4	24	48[a]	0	24	48[a]	0	24	48[a]	0	48	0	48	0	24	48[a]	0	48[a]	0	24
Control	5	5	5		7.7	8.7		7.7	8.0		118		120		21.5	19.3		0/0			
Control	5	5	5		7.4	8.8		7.7	8.0											208	198
5.6%	5	1	0		7.8	8.8		8.8	8.5						21.4	19.0		1.21/4.6			
5.6%	5	0	0		8.0	8.8		8.8	8.5											479	460
10.0%	5	0	0		8.0	8.8		9.0	8.7						21.3	18.9		2.87/7.8			
10.0%	5	0	0		8.0	8.8		9.0	8.7											780	750
18.0%	5	0	0		7.9	8.8		9.2	8.9						21.8	19.1		7.24/14.3			
18.0%	5	0	0		7.9	8.8		9.2	8.9											1170	1120
32.0%	5	0	0		7.7	8.8		9.3	9.0						21.1	19.1		15.4/27.5			
32.0%	5	0	0		7.5	8.8		9 3	9.0											1810	1740
56.0%	5	0	0		7.2	8.5		9.4	9.1						20.8	19.3		28.9/46.3			
56.0%	5	0	0		7.2	8.5		9.4	9.1											2900	2810
100.0%[b]								9.4			469		155					78.9/109		4850	

Remarks: [a] Test was terminated at 24 hours since all test organisms were dead.

[b] Parameters taken of the 100% sample are reported here.

Table 4. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of AMAX Chemical, #FL0000124. Piney Point, Florida, [illegible].

Discharge Point (Outfall #003) Sample

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/N (mg/l)		Conductivity (µmhos)	
	0	4	24	48[a]	0	24	48[a]	0	24	48[a]	0	48	0	48	0	24	48[a]	0	48[a]	0	24
Control	5	5	5		8.1	8.6		7.7	8.0		118		120		21.5	18.9		0/0			
Control	5	5	5		8.2	8.7		7.7	8.0											209	198
5.6%	5	0	0		8.2	8.8		8.8	8.6						21.4	18.6		1.21/4.6			
5.6%	5	0	0		8.2	8.8		8.8	8.6											480	460
10.0%	5	0	0		8.2	8.8		9.0	8.7						21.3	18.6		2.87/7.8			
10.0%	5	0	0		8.2	8.8		9.0	8.7											780	730
18.0%	5	0	0		7.9	8.8		9.2	8.9						21.3	18.6		7.1/14.3			
18.0%	5	0	0		8.0	8.8		9.2	8.9											1170	1120
32.0%	5	0	0		7.7	8.8		9.3	9.0						21.1	18.5		15.4/27.5			
32.0%	5	0	0		7.7	8.8		9.3	9.0											1820	1750
56.0%	5	0	0		7.3	8.6		9.4	9.1						20.8	18.8		28.8/46.3			
56.0%	5	0	0		7.2	8.6		9.4	9.1											2900	2810
100.0%[b]								9.4			469		155					78.9/109		4850	

Remarks: [a] Test was terminated at 24 hours since all test organisms were dead.

[b] Parameters taken of the 100% sample are reported here.

Table 6. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of AMAX Chemicals, NPDES #FL0000124, Piney Point, Florida.

Edge of Mixing Zone Sample

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/N (mg/l)		Conductivity (µmhos)	
	0	4	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	10	10	10	10	8.1	8.8	8.5	7.7	8.1	8.1	118		120		20.9	19.4	19.4	0/0	--		
Control	11[a]	11	11	11	8.1	8.7	8.4	7.7	8.1	8.1										200	210
5.6%	10	10	10	10	8.1	8.8	8.4	8.5	8.3	8.1					20.9	19.1	19.8	0.54/3.8	--		
5.6%	10	10	10	10	8.1	8.8	8.4	8.5	8.3	8.1										395	390
10.0%	10	7	7	7	8.1	8.9	8.5	8.7	8.4	8.1					20.8	19.5	19.8	1.24/5.9	--		
10.0%	10	9	9	8	8.0	9.1	8.3	8.7	8.3	8.1										610	600
18.0%	10	9	0	0	8.0	9.0	--[b]	8.8	8.5	--[b]					20.8	19.1	--[b]	2.57/10.1	--		
18.0%	10	2	0	0	7.9	8.9	--	8.8	8.5	--										970	--[b]
32.0%	10	0	0	0	7.4	8.9	--	9.0	8.6	--					20.7	18.9	--	6.46/18.1	--		
32.0%	10	0	0	0	7.4	8.7	--	9.0	8.6	--										1490	--
56.0%	10	0	0	0	7.0	8.7	--	9.1	8.7	--					20.9	19.0	--	15.1/35.8	--		
56.0%	10	0	0	0	6.9	8.6	--	9.1	8.7	--										2350	--
100.0%[c]								9.1			364		192					29.3/70	--	4190	

Remarks:
[a] Extra organism loaded by mistake, allowed to remain.
[b] Remaining parameters were not recorded since all organisms were dead by hour 24.
[c] Parameters taken of the 100% sample are reported here.

Table 7. Data recorded during 48 hour, Heterandria formosa, static acute toxicity bioassay of AMAX Chemicals, NPDES #FL0000124, Piney Point, Florida.

Edge of Mixing Zone Sample

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/N (mg/l)		Conductivity (µmhos)	
	0			48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	5	5	5	5	8.2	8.4	8.2	7.7	8.0	7.9	118		120		21.4	18.8	19.0	0/0	--		
Control	5	5	5	5	8.3	8.4	8.2	7.7	8.0	7.9										208	199
5.6%	5	5	5	5	8.2	8.3	8.3	8.5	8.2	8.0					21.4	18.7	19.0	0.72/4.9	--		
5.6%	5	5	5	5	8.2	8.3	8.3	8.5	8.2	8.0										409	399
10.0%	5	5	3	3	8.2	8.6	8.3	8.7	8.4	8.1					21.3	18.8	19.0	1.35/6.2	--		
10.0%	5	5	4	4	8.1	8.6	8.2	8.7	8.4	8.0										670	600
18.0%	5	0	0	0	7.9	8.4	--[a]	8.8	8.6	--[a]					21.1	18.8	--[a]	2.87/11.1	--		
18.0%	5	0	0	0	7.8	8.5	--	8.8	8.6	--										990	--[a]
32.0%	5	0	0	0	7.6	8.3	--	9.0	8.8	--					21.0	18.7	--	6.89/19.0	--		
32.0%	5	0	0	0	7.6	8.3	--	9.0	8.8	--										1510	--
56.0%	5	0	0	0	6.7	7.6	--	9.1	8.9	--					20.3	18.6	--	14.2/34.6	--		
56.0%	5	0	0	0	6.6	7.6	--	9.1	8.9	--										2400	--
100.0%[b]								9.1			364		192					29.3/70	--	4190	

Remarks: [a] Remaining parameters were not recorded since all organisms were dead by hour 24.

[b] Parameters taken of the 100% sample are reported here.

Table 8. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of AMAX Chemicals, NPDES #FL0000124, Piney Point, Florida.

Edge of Mixing Zone Sample

Concentration or %	Number of Live Organisms				Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/N (mg/ℓ)		Salinity (ppt)	
	0	4	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
Control	5	5	5[a]	5[a]	7.1	6.9	6.4	8.2	8.0	7.9	21.8	18.8	18.8	0/0	--		
Control	5	5	5	5	7.2	6.9	6.4	8.2	8.0	7.9						26.0	25.0
5.6%	5	5	5	5	7.2	6.9	6.5	8.4	8.2	8.0	21.8	18.8	18.6	0.14/1.3	--		
5.6%	5	5	5	5	7.2	6.9	6.5	8.4	8.2	8.0						25.5	24.0
10.0%	5	5	5[a]	5[a]	7.2	7.0	6.6	8.6	8.4	8.1	21.9	18.8	18.6	0.6/3.7	--		
10.0%	5	5	4	4	7.2	7.0	6.6	8.6	8.4	8.1						24.5	23.0
18.0%	5	5	3	0	7.2	7.3	6.6	8.8	8.5	8.3	21.4	19.0	18.9	1.88/8.1	--		
18.0%	5	5	4	0	7.2	7.3	6.6	8.8	8.5	8.3						22.5	21.5
32.0%	5	0	0	0	7.2	7.3	--[b]	9.0	8.7	--[b]	21.0	18.9	--[b]	5.94/18	--		
32.0%	5	0	0	0	7.0	7.3	--	9.0	8.7	--						19.0	--[b]
56.0%	5	0	0	0	6.4	7.5	--	9.1	8.8	--	20.8	18.8	--	12/30.3	--		
56.0%	5	0	0	0	6.2	7.5	--	9.1	8.8	--						13.0	--

Remarks: [a] One organism was cannibalized, not counted as dead.

[b] Remaining parameters were not recorded since all organisms were dead by hour 24.

Table 9. LC_{50} Data for Bioassays of AMAX Chemical, NPDES #FL0000124, Discharge Point Sample, on 31 August to 2 September, 1983.

Organism	Method of Data Analysis	4 Hour LC_{50} and 95% Confidence Intervals	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
D. pulex	Binomial	9.3% (5.6 and 18)	7.0% (5.6 and 10)	6.2% (0 and 10)
	Moving Average	a	a	a
	Probit	a	a	a
P. promelas		<5.6%, b	<5.6%, b	<5.6%, b
N. leedsi		<5.6%, b	<5.6%, b	<5.6%, b
M. bahia	Binomial	20.5% (10 and 32)	12.6% (10 and 18)	11.4% (0 and 18)
	Moving Average	a	11.2% (8 and 15)	10.1% (8 and 13), c
	Probit	a	11.5% (9 and 15) ,c	9.7% (7 and 13)

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

[b] Since almost every test organism was dead in even the lowest effluent concentration (5.6%), the LC_{50}'s for these time periods are all <5.6%.

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

Table 10. LC_{50} Data for Bioassays of AMAX Chemical, NPDES #FL0000124, Edge of Mixing Zone Sample, on 30 August to 2 September, 1983.

Organism	Method of Data Analysis	4 Hour LC_{50} and 95% Confidence Intervals	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
	Binomial	18.7% (10 and 32)	12.0% (10 and 18)	11.7% (10 and 18)
D. pulex	Moving Average	15.5% (13 and 18)	a	a
	Probit	16.2% (14 and 19), b	a	a
	Binomial	13.4% (10 and 18)	11.4% (5.6 and 18)	11.4% (5.6 and 18)
H. formosa	Moving Average	a	a	a
	Probit	a	a	a
	Binomial	24.0% (18 and 32)	20.5% (10 and 32)	12.6% (10 and 18)
M. bahia	Moving Average	a	17.6% (13 and 23)	a
	Probit	a	18.6% (14 and 24), b	a

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

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

RESULTS AND DISCUSSION

Both samples of wastewater collected from AMAX Chemical's discharge point (Outfall #003) and from the edge of their mixing zone (800 meters downstream) were extremely toxic to the freshwater and marine organisms used in these bioassays. The discharge point sample produced 4 hour LC_{50}'s of <5.6% for the freshwater fish, Pimephales promelas and Notropis leedsi, and 48 hour LC_{50}'s of 6.2% and 10.1% for the freshwater crustacean Daphnia pulex, and the marine shrimp, Mysidopsis bahia, respectively (Table 9). The edge of mixing zone sample was almost equally as toxic, producing 48 hour LC_{50}'s of 11.7%, 11.4%, and 12.6% for Daphnia pulex, Heterandria formosa (freshwater fish), and Mysidopsis bahia, respectively (Table 10). It was noted that every test organism was dead within minutes after being introduced into the higher test concentrations. This rapid and extreme toxicity can directly be attributed to the large concentrations of total and unionized ammonia detected in the samples. Total ammonia concentrations of 109 mg/l and 70 mg/l, and unionized ammonia concentrations of 78.9 mg/l and 29.3 mg/l were measured in the discharge point and edge of mixing zone samples, respectively. The U.S. EPA (1976) and Thurston, et al. (1981) have determined that the toxicity of unionized ammonia to certain freshwater fish begins at 0.2 mg/l. The concentration of unionized ammonia measured in the discharge point sample (Outfall #003) was approximately 400 times the reported incipient toxicity value.

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 existance of a mixing zone, whether as a specific part of the permit or based on the existance 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 maximum 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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