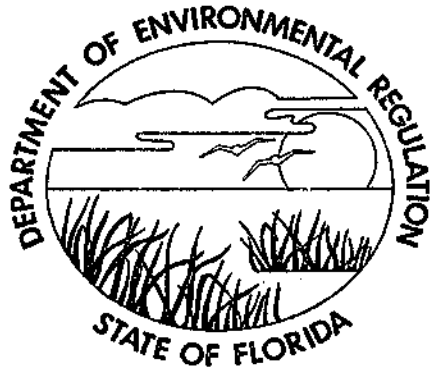


BIOASSAYS OF AMAX CHEMICALS PHOSPHATE FERTILIZER PRODUCTS, INCLUDING 96 HOUR FLOW THROUGH, 48 HOUR STATIC AND STANDARD REFERENCE TOXICANT ASSAYS PINEY POINT, MANATEE COUNTY, FLORIDA

NPDES #FL0000124

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June 24, 1985

Biology Section
Division of Environmental Programs

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Biology Section
Bureau of Laboratories and
Special Programs
June 24, 1985

EXECUTIVE SUMMARY

AMAX Chemicals, Piney Point, on 21-25 April, 1985.

The results of on-site 96 hour flow-through and 48 hour static bioassays of AMAX's wastewater discharge (Outfall #003) indicated significant improvement over the results of earlier (1983) bioassays. In the earlier tests, high levels of ammonia and pH rapidly resulted in acute toxicity to all the organisms tested. Since 1983, AMAX has installed a wastewater treatment system which removes most of the ammonia from the wastewater and controls the pH. In these latest tests, only a few random deaths of Notropis leedsi were noted by 96 hours. In the Daphnia pulex flow-through test, a sufficient number of organisms had died by 96 hours to produce an LC_{50} of 180.8%. This LC_{50}, however, is in compliance with the F.A.C. toxicity criteria regarding dischargers with mixing zones. Results of two additional D. pulex 48 hour static tests, performed concurrently with the flow-through, indicated no toxicity. Results of two concurrent Mysidopsis bahia 48 hour static tests, however, indicated that the wastewater was acutely toxic to these organisms (48 hour LC_{50}'s of 80.4% and 66.9%). The presence of ammonia may have contributed to this toxicity. Additionally, it should be noted that AMAX was not in operation during these tests. According to plant personnel, however, the wastewater provided for the tests was of worst case quality since it had been pumped directly from the plant holding ponds and through the treatment system.

The results of the on-site Standard Reference Toxicant (sodium lauryl sulfate) tests, using N. leedsi and D. pulex, indicated that the sensitivities of these organisms to the reference toxicant were within both the EPA and DER recommended ranges. However, the 48 hour LC_{50}'s obtained from these tests (8.4 ppm and 8.5 ppm, respectively) were slightly higher than recent LC_{50}'s obtained in the Tallahassee laboratory. This may suggest that the organisms used for the flow-through bioassays were more resistant to the standard toxicant than were organisms used recently in the Tallahassee lab. This increased resistance may have been due to the use of AMAX well water as diluent, which is of higher conductivity and hardness than Tallahassee well water.

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, 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 indicated that both samples were acutely toxic to all the test organisms, producing LC_{50}'s of <5.6% to 12.6%. Toxicity was attributed to significant concentrations of total and unionized ammonia (up to 109 mg/l and 78.9 mg/l, respectively), and to the elevated pH (9.4 s.u.) of the wastewater (FDER 1983). Since 1983, AMAX has installed a wastewater treatment system, consisting of double liming to remove ammonia and acid feed to control the pH (Mr. John Zibrida, AMAX, personal communication).

To again evaluate the toxicity of this wastewater, as part of a new AMAX discharge permit, the Southwest District requested that a flow-through bioassay be performed. AMAX, however, ceased operations in early 1985, due to a decreased demand for phosphate products. Under an agreement with AMAX, a wastewater discharge was provided for the test. The flow-through tests, and several concurrent statics, were performed from April 21 to 25, 1985. Wastewater, consisting of worst case quality (Mr. John Zibrida, AMAX) was pumped directly from AMAX's holding ponds, through the treatment system, and eventually to Outfall #003, the location of the DER mobile bioassay trailer. The results of these flow-through and static bioassays, using both fresh and saltwater test organisms, are presented in this report. Additionally, results of Standard Reference Toxicant (sodium lauryl sulfate) bioassays, performed on-site using the freshwater test organisms, are also presented here. Those involved in performing the tests and in preparation of this report were: C. W. Dye, R. B. Frydenborg, and M. L. Roll.

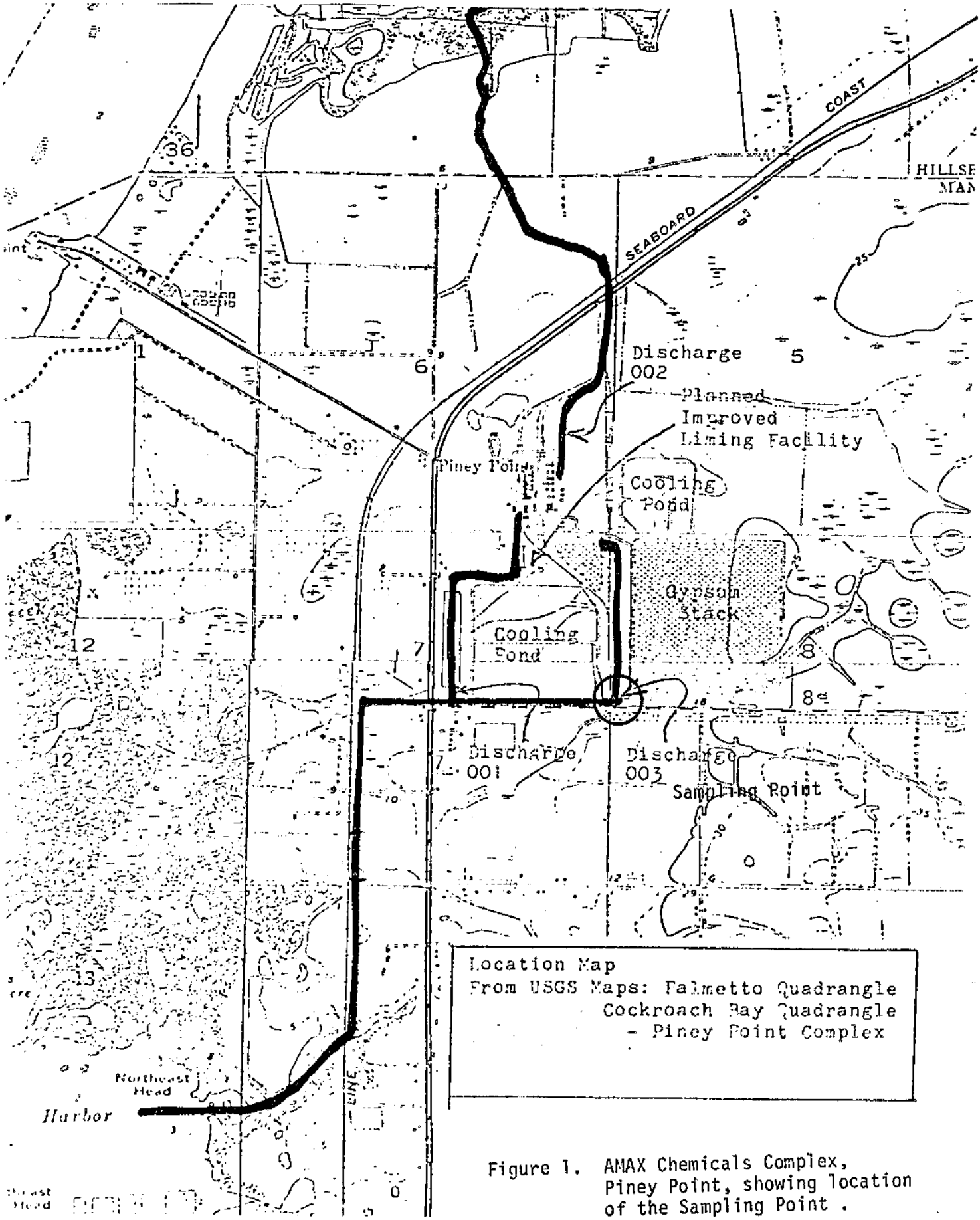


Figure 1. AMAX Chemicals Complex, Piney Point, showing location of the Sampling Point .

METHODS AND MATERIALS

Test methods used to conduct these flow-through and static bioassays are extensively described by Peltier and Weber (1985) and the FDER Biology Section (1985a). The organisms selected for this study include two species recommended by Peltier and Weber (1985), the U.S. EPA (1978), and the FDER Biology Section (1985a). 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.

The D. pulex used in the AMAX tests were obtained by siphoning individuals from each of our culturing aquaria into two 2 liter glass jars, each containing well water obtained from an AMAX process well. This AMAX well water was used as diluent for the on-site tests and, therefore, the organisms were pre-acclimated to this dilution water. The jars were transported to the site by state vehicle and immediately transferred to the mobile bioassay lab, and therefore, did not undergo any significant temperature change or disturbance. The D. pulex were fed prior to transport and there was no observed mortality of these organisms prior to the test.

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 N. leedsi used in the AMAX test began hatching in the Tallahassee lab on approximately April 5, 1985, in a 2-1/2 gallon all-glass aquarium. Water was replaced in this aquarium over a period of days with AMAX process well water (to be used as diluent in the on-site tests). Therefore, the fish were pre-acclimated to this dilution water. The fish were transported to the test site in this aquarium with constant aeration. The fish were fed daily with live, freshly hatched Artemia and were acclimated to 20°C in the mobile lab prior to the test. The fish did not undergo any significant temperature change or disturbance, nor was there any mortality observed prior to the test. The fish were 3 to 16 days old at the start 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 (15-30 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.

Approximately two weeks prior to the AMAX tests, three new 5 gallon aquaria with undergravel filtration were set up. Water used in these aquaria was seawater obtained from Piney Point, Tampa Bay, and adjusted with DER well water to obtain salinities of approximately 16 ppt, 19 ppt, and 22 ppt. Mysid shrimp were then introduced into these new aquaria from our stock aquaria of similar salinities. The aquaria were transported to the test site by state vehicle with constant aeration. The aquaria were transferred to the mobile bioassay lab upon arrival and, therefore, did not undergo any significant change in temperature or disturbance. The shrimp were acclimated to 20°C in the mobile lab prior to the test and were fed daily with live, freshly hatched, Artemia. No mortality of these organisms was noted prior to the test. Adult or large juvenile mysids were selected for these tests.

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. Additionally, on-site reference toxicant tests were performed in the mobile bioassay lab using Notropis leedsi and Daphnia pulex, to evaluate the sensitivity of these organisms after their transportation to the site and under the conditions in the mobile lab. The results of these tests would indicate whether the sensitivities of the organisms used in the flow-through tests had changed significantly from that experienced in the Tallahassee lab or had deviated from the recommended EPA and DER sensitivity ranges.

TEST METHODS

Dilution Water

Water used to acclimate the freshwater organisms prior to the test, and as dilution water in the flow-through and static renewal tests, was obtained from an AMAX process well. This water was characterized by an almost constant pH of 7.6 ± 0.1 standard units and a conductivity of 730 ± 20 umhos. Approximately 250 gallons of this dilution water was conveyed daily in a 300 gallon Nalgene® tank to the test site. We wish to thank Mr. John Zibrida of AMAX for providing us this source of dilution water and for his personal assistance throughout the test.

Saltwater used for dilution water in the M. bahia static tests was obtained from Piney Point, Tampa Bay (salinity approximately 19.5 ppt). This water was transported to the test site in a 5 gallon carboy and stored in the mobile lab until used.

Pre-test Conditions and Preparations

DER's mobile bioassay laboratory was constructed following EPA design specifications (Peltier and Weber, 1985) and is equipped with two solenoid valve operated, flow-through, proportional dilutor systems. The mobile lab also has suitable working area for performing static tests concurrently with the flow-through tests. The mobile laboratory was transported by commercial mover from Tallahassee to AMAX on April 19, 1985. The trailer was positioned adjacent to AMAX's NPDES Outfall #003. Electric power for the mobile lab was supplied by AMAX.

An effluent sampling pump was suspended into the wastewater sump and was positioned approximately 0.5 meters beneath the surface (at least 0.5 meters off the bottom of the sump). Effluent was conveyed directly and continuously to the mobile bioassay lab via a 50' length of braided reinforced Tygon® hose. Dilution water was transferred to a 500 gallon Nalgene® tank positioned adjacent to the mobile lab. A similar sampling pump was submerged in this tank and dilution water was conveyed directly and continuously to the mobile lab via a 15' length of identical Tygon® hose. Upon entering the mobile lab, both effluent and dilution water sampling lines passed through a cooled water bath adjusted to approximately 18°C. This water bath maintained the temperature of the relatively warm effluent and dilution water at 21°C ± 2°C.

Calibration of the proportional dilutor was performed as soon as the pumps were connected and were determined to be operating properly. Additionally, calibration was verified again just prior to the beginning of the test and at the end of the 96 hour test. The concentrations and volumes produced by the flow-through dilutor system are presented in Table 1. Flow rates were adjusted to operate the dilutor at approximately 12 cycles per hour, which provided for approximately 18 complete volume changes in the 8 liter flow-through aquaria every 24 hours. The dilutor system was operated for approximately 22 hours before the introduction of the test organisms at the beginning of the test at 1000 hours on Sunday,

April 21, 1985. The test was concluded at 1000 hours on Thursday, April 25, 1985.

Flow-Through Methods and Parameters

Two replicates of six concentrations and a control were used for the N. leedsi and D. pulex flow-through tests. To facilitate counting, the organisms were pipetted from culture dishes into separate retention chambers, which consisted of glass petri dishes to which cylinders of Nitex® screen (243 micron mesh size) were attached by silicone cement. These Nitex® retention chambers allowed much easier counting of test organisms when lifted from the test aquaria, while not retarding water flow in the aquaria. Ten organisms were loaded per retention chamber per aquarium, resulting in 20 test organisms per test concentration.

The number of live organisms, dissolved oxygen, pH, temperature and conductivity were recorded at 0, 24, 48, 72, and 96 hours for each aquarium. Ammonia concentrations, including total and unionized fractions (expressed in mg/l as N and NH_3, respectively), in each test concentration, were also recorded daily. Total alkalinity and total hardness were measured daily in the control and 100% concentrations, and at the beginning and end of the test in each intermediate concentration. These values appear in Tables 2 and 3.

Daphnia pulex and Mysidopsis bahia Static Tests

During the flow-through tests, four 48 hour static tests were performed in the mobile trailer using the test organisms, Daphnia pulex and Mysidopsis bahia. The tests were performed from 21 to 23, and again on 23 to 25 April, 1985. The D. pulex were tested to substantiate the results of the D. pulex flow-through test. The M. bahia were tested since AMAX's discharge eventually reaches an estuarine system (Tampa Bay).

Two replicates, each with six 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. Each test concentration (0 through 100%) was obtained directly from the flow-through aquaria, and therefore, the test concentrations were identical. Daphnia were transferred from their holding jars to a large culture dish to select the proper size and age organisms. Juvenile Daphnia were chosen for these tests. These organisms 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 six concentrations and a control, were used for the Mysidopsis bahia bioassays. The shrimp 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. The shrimp were fed Artemia nauplii during these tests (U.S. EPA 1978).

Dilution water used for these tests was ambient Tampa Bay surface water obtained approximately 50' offshore of Piney Point. Effluent was obtained directly from the overflow line of the flow-through dilutor system. This effluent was salinity adjusted to approximately 20 ppt and 14 ppt for the 21 April and 23 April tests, respectively. The salinity of the Tampa Bay diluent was approximately 19 ppt. The shrimp used in these tests were acclimated to $\pm$ 3 ppt of these salinities. Additionally, since the pH's of these salinity adjusted effluents and of the Tampa Bay diluent were significantly higher than the pH of AMAX's discharge, each test chamber in both tests, including the controls, was pH adjusted to approximately 6.7 standard pH units. This value was agreed to by AMAX's environmental representative. A dilute acid (0.1N HCl) was used for these pH adjustments. The concentration series was that recommended by APHA (1980) and is presented in Table 1.

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 in the flow-through test chambers. 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 tests. 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 concentration during the tests. 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 4 through 7.

48 Hour Standard Reference Toxicant Bioassays

During the first two days of the flow-through bioassays (21 to 23 April, 1985) two Standard Reference Toxicant bioassays were performed on-site, in the bioassay trailer. Sodium lauryl sulfate was used as the reference toxicant, in a stock concentration of 100 ppm. This stock concentration was diluted in AMAX dilution water to obtain a concentration series (Table 1) of from 1 to 10 ppm (the same series used in our monthly quality control test in the Tallahassee lab). Two replicates of each concentration was used, each containing either 10 Daphnia or 5 Notropis.

These tests ensured that the sensitivities of the Daphnia and Notropis used in the flow-through tests were not sifnificantly different from sensitivities obtained in previous tests in the Tallahassee laboratory. Dissolved oxygen, pH, temperature, and the number of live organisms were recorded for each test chamber at 0 Hours, 24 hours, and 48 hours. The conductivity of each test concentration was recorded at the beginning and end of the test. All data recorded during these tests are presented in Tables 8 and 9. The organisms were not fed during the tests.

Table 1. Diluent and Effluent Volumes, and Final Concentrations used in Flow-Through and Static Bioassays of AMAX Chemicals (21 to 25 April, 1985).

1. Flow-Through Bioassays (21 to 25 April, 1985), Daphnia pulex and Notropis leedsi

Diluent[a]		Effluent[a]		Total Volume[a]		Final Concentration[a]	
0 Hours	96 Hours	0 Hours	96 Hours	0 Hours	96 Hours	0 Hours	96 Hours
940 ml	907 ml	0 ml	0 ml	940 ml	907 ml	0%	0%
896 ml	888 ml	60 ml	65 ml	956 ml	953 ml	6.3%	6.8%
928 ml	924 ml	120 ml	111 ml	1048 ml	1035 ml	11.5%	10.7%
732 ml	724 ml	157 ml	159 ml	889 ml	883 ml	17.7%	18.0%
656 ml	649 ml	305 ml	300 ml	961 ml	949 ml	31.7%	31.6%
381 ml	383 ml	455 ml	457 ml	836 ml	840 ml	54.4%	55.4%
0 ml	0 ml	940 ml	952 ml	940 ml	952 ml	100.0%	100.0%

2. 48 Hour Static Bioassays (21 to 23 and 23 to 25 April, 1985), Daphnia pulex

Concentrations	Total Volume
0% through 100.0% Identical to Flow-Through Bioassay:	200 ml in each jar

[a] All values are the average of two or more replicates. Final concentrations are to nearest 0.1%.

3. 48 Hour Static Bioassays (21 to 23 and 23 to 25 April, 1985), Mysidopsis bahia

Concentration	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
100.0%	------	500 ml	500 ml

Table 1. (Cont.)

4. Notropis leedsi Standard Reference Toxicant Test (21 to 23 April, 1985)

Concentration	Dilution Water	Toxicant	Total Volume
Control	500 ml	-----	500 ml
1.0 ppm	495 ml	5 ml	500 ml
3.0 ppm	485 ml	15 ml	500 ml
7.0 ppm	465 ml	35 ml	500 ml
10.0 ppm	450 ml	50 ml	500 ml

5. Daphnia pulex Standard Reference Toxicant Test (21 to 23 April, 1985)

Concentration	Dilution Water	Toxicant	Total Volume
Control	200 ml	-----	200 ml
1.0 ppm	198 ml	2 ml	200 ml
3.0 ppm	194 ml	6 ml	200 ml
7.0 ppm	186 ml	14 ml	200 ml
10.0 ppm	180 ml	20 ml	200 ml

Table 2. Data recorded during 96 hour, Notropis leedsi, flow-through bioassay of AMAX Chemicals, Outfall #003, NPDES #FL0000124, Piney Point, Manatee County, Florida, on 21 to 25 April, 1985.

Conc. or %	Test Container Number	Number of Live Organisms					Dissolved Oxygen (mg/l)					pH					Total Alkalinity (mg/l as $CaCO_3$)					Total Hardness (mg/l as $CaCO_3$)					Temperature				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control	1	10	10	10	10	10	7.0	6.6	6.9	7.4	6.6	7.7	7.5	7.5	7.7	7.6	138	132	130	140	135	338	350	350	345	332	21.5	21.2	21.2	20.7	21.4
Control	2	10	10	10	10	10	6.7	6.2	6.4	7.3	6.4	7.7	7.5	7.5	7.7	7.6	138										22.2	21.8	21.8	21.5	22.0
6.8%	3	10	10	10	9	9	6.4	6.1	6.2	7.0	6.3	7.6	7.5	7.4	7.6	7.5	135				125	325				318	21.5	21.4	21.4	21.0	21.7
6.8%	4	10	10	10	10	10	6.6	6.4	6.5	7.2	6.3	7.6	7.5	7.4	7.6	7.5	135										21.8	21.8	22.0	21.6	22.1
10.7%	5	10	10	10	10	10	6.4	6.1	6.0	6.5	5.8	7.6	7.5	7.4	7.6	7.4					120					325	21.6	21.5	21.5	21.2	21.8
10.7%	6	10	10	10	10	10	6.4	6.2	6.1	6.7	5.7	7.6	7.5	7.4	7.6	7.4											22.0	22.0	22.2	21.9	22.5
18.0%	7	10	10	10	10	9	6.3	5.8	5.9	6.6	5.5	7.5	7.4	7.3	7.6	7.4	122				115	320				315	21.7	21.6	21.6	21.2	21.9
18.0%	8	10	10	10	9	9	6.6	6.2	6.3	6.8	5.6	7.5	7.4	7.3	7.6	7.4	122										22.4	22.2	22.2	21.9	22.5
31.6%	9	10	10	10	9[a]	9	6.6	6.0	5.9	6.9	5.0	7.3	7.3	7.2	7.4	7.2					95					280	21.8	21.7	21.8	21.5	22.0
31.6%	10	10	10	10	10	10	6.7	6.4	6.2	6.5	5.0	7.3	7.3	7.2	7.4	7.2											22.5	22.3	22.3	22.1	22.7
54.4%	11	10	10	10	10	10	6.6	6.5	6.0	6.0	5.1	7.0	7.0	7.0	7.1	7.0	70				65	220				231	21.9	21.9	21.9	21.6	22.2
54.4%	12	10	10	10	10	9	7.0	6.8	6.3	6.3	5.6	7.0	7.0	7.0	7.1	7.0	70										22.3	22.2	22.3	22.0	22.6
100.0%	13	10	10	10	10	10	7.1	7.5	6.8	6.3	5.6	6.2	6.2	6.1	6.1	6.0	23	16	11	11	10	95	70	110	100	95	22.3	22.2	22.2	22.0	22.5
100.0%	14	10	10	10	10	10	7.1	7.4	6.7	6.1	5.5	6.2	6.2	6.1	6.1	6.0	23										22.6	22.5	22.5	22.5	22.9

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

[a] Organism trapped between screen and petri dish.

Table 2, continued

Conc. or %	Test Container Number	Conductivity (umhos)					Total Ammonia (mg/l as N)					Unionized Ammonia (mg/l as NH_3)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control	1	710	720	710	750	730	0.10	0	0	0.14	0.05	.003	0	0	.003	.001
Control	2	710	720	710	750	720										
6.8%	3	930	950	920	990	990	0.73	0.83	0.83	1.25	0.91	.015	.014	.001	.026	.016
6.8%	4	930	950	960	990	980										
10.7%	5	1080	1100	1080	1110	1100	1.10	1.60	1.61	2.15	1.51	.024	.027	.022	.045	.021
10.7%	6	1095	1100	1105	1150	1130										
18.0%	7	1295	1310	1290	1320	1310	1.70	2.90	3.01	3.55	5.26	.029	.040	.033	.060	.074
18.0%	8	1390	1360	1350	1410	1410										
31.6%	9	1810	1850	1810	1890	1890	2.80	5.30	5.81	6.55	7.48	.031	.058	.051	.089	.067
31.6%	10	1890	1900	1890	1910	1920										
54.4%	11	2650	2700	2650	2710	2740	4.80	9.10	11.8	10.9	13.4	.027	.050	.066	.075	.076
54.4%	12	2700	2700	2700	2730	2790										
100.0%	13	4240	4300	4260	4300	4390	8.60	16.5	17.8	19.3	27.2	.008	.015	.013	.014	.016
100.0%	14	4280	4300	4290	4350	4410										

Table 3, continued

Conc. or %	Test Container Number	Conductivity (umhos)					Total Ammonia (mg/l as N)					Unionized Ammonia (mg/l as NH_3)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control	1	710	720	710	750	730	0.10	0	0	0.14	0.05	.003	0	0	.003	.001
Control	2	710	720	710	750	720										
6.8%	3	930	950	920	990	990	0.73	0.83	0.83	1.25	0.91	.015	.014	.001	.026	.016
6.8%	4	930	950	960	990	980										
10.7%	5	1080	1100	1080	1110	1100	1.10	1.60	1.61	2.15	1.51	.024	.027	.022	.045	.021
10.7%	6	1095	1100	1105	1150	1130										
18.0%	7	1295	1310	1290	1320	1310	1.70	2.90	3.01	3.55	5.26	.029	.040	.033	.060	.074
18.0%	8	1390	1360	1350	1410	1410										
31.6%	9	1810	1850	1810	1890	1890	2.80	5.30	5.81	6.55	7.48	.031	.058	.051	.089	.067
31.6%	10	1890	1900	1890	1910	1920										
54.4%	11	2650	2700	2650	2710	2740	4.80	9.10	11.8	10.9	13.4	.027	.050	.066	.075	.076
54.4%	12	2700	2700	2700	2730	2790										
100.0%	13	4240	4300	4260	4300	4390	8.60	16.5	17.8	19.3	27.2	.008	.015	.013	.014	.016
100.0%	14	4280	4300	4290	4350	4410										

Table 4. Data recorded during 48 hour, Daphnia pulex, static bioassay of AMAX Chemicals, Outfall #003, NPDES #FL0000124, Piney Point, Manatee County, Florida, on 21 to 23 April, 1985.

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	8[a]	6.2	8.2	8.5	7.7	7.9	8.0	23.4	19.5	19.6		
Control	10	10	10	6.2	8.2	8.5	7.7	7.9	8.0				750	700
6.8%	10	10	10	6.8	7.9	8.4	7.6	7.9	8.0	20.7	19.5	19.6		
6.8%	10	10	10	6.8	7.9	8.4	7.6	7.9	8.0				910	900
10.7%	10	10	10	6.9	8.0	8.4	7.6	7.9	8.0	20.7	19.5	19.5		
10.7%	10	10	10	6.9	8.0	8.4	7.6	7.9	8.0				1060	1050
18.0%	10	10	10	6.8	7.6	8.4	7.5	7.8	7.9	20.7	19.5	19.5		
18.0%	10	10	10	6.8	7.6	8.4	7.5	7.8	7.9				1270	1260
31.6%	10	10	10	7.1	7.7	8.4	7.4	7.8	7.8	20.7	19.9	19.4		
31.6%	10	10	10	7.0	7.7	8.4	7.4	7.8	7.8				1750	1750
54.4%	10	10	10	7.2	7.6	8.2	7.1	7.6	7.6	20.9	19.5	19.4		
54.4%	10	10	10	7.2	7.6	8.2	7.1	7.6	7.6				2590	2510
100.0%	10	10	10	7.5	7.6	8.1	6.3	6.8	6.8	21.1	19.5	19.4		
100.0%	10	10	10	7.6	7.8	8.1	6.3	6.8	6.8				4020	4000

Control Water

NH_3(as NH_3)(mg/l) .003

Total ammonia (as N)(mg/l) 0.10

Total Alkalinity 138 (mg/l $CaCO_3$)

Total Hardness 338 (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) .008

Total ammonia (as N)(mg/l) 8.60

Total Alkalinity 23 (mg/l $CaCO_3$)

Total Hardness 95 (mg/l $CaCO_3$)

[a] Two organisms stuck to side of container.

Table 5. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of AMAX Chemicals, Outfall #003, NPDES #FL0000124, Piney Point, Manatee County, Florida, on 21 to 23 April, 1985.

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	7.0	6.6	6.8	6.7	7.2	7.1	21.3	20.0	19.5			Control Water
Control	5	5	5	7.1	6.6	6.8	6.7	7.2	7.1				19.4	20.4	NH_3(as NH_3)(mg/l) .0001 / Total Alkalinity (mg/l $CaCO_3$)
5.6%	5	5	5	7.0	6.4	6.7	6.7	7.2	7.2	21.3	19.5	19.4			
5.6%	5	5	5	7.0	6.5	6.7	6.7	7.2	7.2				19.5	20.4	
10.0%	5	5	5	7.0	6.4	6.3	6.7	7.2	7.1	21.4	19.5	19.4			Total ammonia (as N)(mg/l) .05 / Total Hardness (mg/l $CaCO_3$)
10.0%	5	5	5	7.0	6.7	6.5	6.7	7.2	7.1				18.0	18.8	
18.0%	5	5	5	7.1	6.8	6.7	6.7	7.2	7.2	21.5	19.8	19.3			
18.0%	5	5	5	7.1	6.8	6.7	6.7	7.2	7.2				19.5	20.4	
32.0%	5	5	5	7.2	6.8	6.4	6.7	7.2	7.1	21.5	19.5	19.3			
32.0%	5	5	5	7.2	6.9	6.6	6.7	7.2	7.1				19.5	20.7	100% Effluent
56.0%	5	5	4	7.5	6.6	6.7	6.7	7.2	7.2	21.6	19.8	19.3			NH_3(as NH_3)(mg/l) .015 / Total Alkalinity (mg/l $CaCO_3$)
56.0%	5	5	4	7.5	6.6	6.7	6.7	7.2	7.2				19.6	20.4	
100.0%	5	2	2	8.0	6.8	6.7	6.6	7.2	7.2	22.0	19.8	19.3			
100.0%	5	1	1	8.0	6.8	6.7	6.6	7.3	7.2				19.5	20.3	Total ammonia (as N)(mg/l) 7.8 / Total Hardness (mg/l $CaCO_3$)

Table 6. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of AMAX Chemicals, Outfall #003, NPDES #FL0000124, Piney Point, Manatee County, Florida, on 23 to 25 April, 1985.

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	9	6.9	8.5	8.3	7.7	7.9	7.9	21.5	19.0	19.8	710	
Control	10	10	9	6.4	8.5	8.3	7.7	7.9	7.9	22.2			710	705
6.8%	10	10	10	6.2	8.5	8.3	7.6	7.9	7.9	21.5	18.9	19.8	920	
6.8%	10	10	10	6.5	8.5	8.3	7.6	7.9	7.9	21.8			960	905
10.7%	10	10	10	6.0	8.4	8.3	7.6	7.8	7.9	21.6	18.9	19.8	1080	
10.7%	10	10	10	6.1	8.4	8.3	7.6	7.8	7.9	22.0			1105	1080
18.0%	10	10	10	5.9	8.4	8.2	7.5	7.8	7.8	21.7	18.9	19.8	1290	
18.0%	10	10	10	6.3	8.4	8.2	7.5	7.8	7.8	22.4			1350	1350
31.6%	10	10	9	5.9	8.4	8.1	7.3	7.7	7.7	21.8	18.9	19.8	1810	
31.6%	10	9	8	6.2	8.4	8.1	7.3	7.7	7.7	22.5			1890	1810
54.4%	10	9	7	6.0	8.3	7.9	7.0	7.5	7.5	21.9	18.9	19.9	2450	
54.4%	10	9	9	6.3	8.3	7.9	7.0	7.5	7.5	22.3			2700	2620
100.0%	10	10	10	6.8	8.4	8.2	6.2	6.8	6.8	22.3	19.0	20.0	4260	
100.0%	10	9	9	6.7	8.4	8.2	6.2	6.8	6.8	22.6			4290	4100

Control Water

NH_3(as NH_3)(mg/l) .009

Total ammonia (as N)(mg/l) 0.25 at 24 hours

Total Alkalinity 130 (mg/l $CaCO_3$)

Total Hardness 350 (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) .048

Total ammonia (as N)(mg/l) 17.2 at 24 hours

Total Alkalinity 11 (mg/l $CaCO_3$)

Total Hardness 110 (mg/l $CaCO_3$)

Table 7. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of AMAX Chemicals, Outfall #003, NPDES #FL0000124, Piney Point, Manatee County, Florida, on 23 to 25 April, 1985.

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	7.4	6.0	4.7	6.6	6.9	7.0	19.8	18.9	19.7		
Control	5	5	5	7.4	6.3	5.0	6.6	7.0	7.0				19.3	19.1
5.6%	5	5	5	7.4	5.5	4.5	6.6	6.9	7.0	20.0	18.9	19.7		
5.6%	5	5	5	7.4	4.2	4.0	6.6	6.8	7.0				19.0	18.9
10.0%	5	5	5	7.4	5.8	4.4	6.7	7.0	7.0	20.0	18.9	19.7		
10.0%	5	5	5	7.4	5.8	4.8	6.7	7.0	7.0				19.0	18.5
18.0%	5	5	5	7.5	5.8	4.9	6.7	7.0	7.0	20.2	18.9	19.7		
18.0%	5	5	5	7.5	5.4	4.0	6.7	6.9	7.0				18.0	18.2
32.0%	5	5	5	7.5	5.6	3.9	6.7	6.9	6.9	20.5	19.0	19.7		
32.0%	5	5	5	7.5	5.9	4.4	6.7	6.9	6.9				17.5	17.2
56.0%	5	4	3	7.6	5.4	4.7	6.7	6.8	7.1	21.3	19.0	19.7		
56.0%	5	5	4	7.5	5.8	5.2	6.6	6.9	7.0				16.0	16.4
100.0%	5	0	0	7.6	6.5	5.8	6.7	6.9	6.9	22.0	19.0	19.8		
100.0%	5	1	1	7.6	6.5	5.2	6.7	6.8	6.8				14.0	14.0

Control Water

NH_3(as NH_3)(mg/l) 0 .002

Total ammonia (as N)(mg/l) 0 0.65

0 24 HOURS

Total Alkalinity (mg/l $CaCO_3$)

Total Hardness (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) .024 .060

Total ammonia (as N)(mg/l) 9.5 18.4

0 24 HOURS

Total Alkalinity (mg/l $CaCO_3$)

Total Hardness (mg/l $CaCO_3$)

Table 8. Data recorded during 48 hour, Notropis leedsi, static acute bioassay of sodium lauryl sulfate (Standard Reference Toxicant), performed during AMAX Flow-Through bioassay, on 21 to 23 April, 1985.

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	6.1	7.4	8.2	7.6	7.8	8.0	23.3	20.0	19.4			Control Water
Control	5	5	5	6.1	7.4	8.2	7.6	7.8	8.0				795	705	NH_3(as NH_3)(mg/l) / Total Alkalinity (mg/l CaO_3)
1.0 ppm	5	5	5	5.9	7.4	8.2	7.6	7.8	8.0	23.4	19.8	19.3			
1.0 ppm	5	5	5	5.9	7.4	8.2	7.6	7.8	8.0				780	695	
3.0 ppm	5	5	5	6.0	7.2	8.0	7.6	7.7	7.9	23.4	19.5	19.2			Total ammonia (as N)(mg/l) / Total Hardness (mg/l CaO_3)
3.0 ppm	5	5	5	6.0	7.0	8.0	7.6	7.7	7.9				760	690	
7.0 ppm	5	5	5	6.0	6.3	7.5	7.6	7.7	7.8	23.4	19.5	19.1			
7.0 ppm	5	5	5	6.0	6.5	7.5	7.6	7.7	7.8				750	695	
10.0 ppm	5	0	0	6.0	6.2	–[a]	7.6	7.6	–[a]	23.2	19.5	19.1			
10.0 ppm	5	0	0	6.0	6.0	–	7.6	7.6	–				740	695	100% Effluent
															NH_3(as NH_3)(mg/l) / Total Alkalinity (mg/l CaO_3)
															Total ammonia (as N)(mg/l) / Total Hardness (mg/l CaO_3)

[a] Remaining parameters were not recorded since all organisms were dead at 24 hours.

Table 9. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of sodium lauryl sulfate (Standard Reference Toxicant), performed during AMAX Flow-Through bioassay on 21 to 23 April, 1985.

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	24	48
Control	10	10	8[a]	6.2	8.2	8.5	7.7	7.9	8.0	23.4	19.5	19.6			
Control	10	10	10	6.2	8.2	8.5	7.7	7.9	8.0				750	700	700
1.0 ppm	10	10	10	6.1	8.2	8.4	7.7	7.9	8.0	23.8	19.5	19.5			
1.0 ppm	10	10	10	6.1	8.2	8.4	7.7	7.9	8.0				750	700	700
3.0 ppm	10	10	9	5.9	7.7	8.4	7.7	7.9	8.0	23.8	19.5	19.5			
3.0 ppm	10	10	9	5.9	7.7	8.4	7.7	7.9	8.0				750	700	695
7.0 ppm	10	10	7	6.0	7.4	8.3	7.7	7.8	7.9	23.7	19.5	19.5			
7.0 ppm	10	10	8	6.0	7.4	8.3	7.7	7.8	7.9				740	700	700
10.0 ppm	10	6	3	5.8	7.4	8.2	7.7	7.8	7.9	23.5	19.5	19.6			
10.0 ppm	10	7	3	5.8	7.4	8.2	7.7	7.8	7.9				740	700	705

Control Water

NH_3(as NH_3)(mg/l) _____ Total Alkalinity _____ (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) _____ Total Hardness _____ (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) _____ Total Alkalinity _____ (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) _____ Total Hardness _____ (mg/l $CaCO_3$)

[a] Two organisms stuck to side of container.

Table 10. LC_{50} Data for Flow-Through Bioassays of AMAX Chemicals, Piney Point, on 21-25 April, 1985.

Organism	Method of Data Analysis	48 Hour LC_{50} and 95% Confidence Intervals	72 Hour LC_{50} and 95% Confidence Intervals	96 Hour LC_{50} and 95% Confidence Intervals
Notropis leedsi	Binomial Moving Average Probit	No Mortality	Random Mortality	Random Mortality
Daphnia pulex	Binomial Moving Average Probit	Random Mortality	a a 953.1% (182 and ∞)	a a 180.8% (95.9 and 1397.9)

[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 11. LC_{50} Data for Static Bioassays of AMAX Chemicals, 21-23 and 23-25 April, 1985.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
21-23 April			
Daphnia pulex	Binomial Moving Average Probit	No Mortality	No Mortality
Mysidopsis bahia	Binomial	87.6% (56 and ∞)	79.7% (32 and ∞)
	Moving Average	a	79.7% (54 and 197)
	Probit	a	80.4% (62 and 119),b
23-25 April			
Daphnia pulex	Binomial Moving Average Probit	Random Mortality	Random Mortality
Mysidopsis bahia	Binomial	74.8% (56 and 100)	67.1% (32 and 100)
	Moving Average	74.8% (61 and 103)	66.2% (54 and 87)
	Probit	74.8% (61 and 92)	66.9% (53 and 85),b

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

Table 12. LC_{50} Data for Standard Reference Toxicant Bioassays Performed During AMAX Flow-Through, on 21-23 April, 1985.

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	8.5 ppm (7 and ∞)
	Moving Average	a	8.5 ppm (7.3 and 10.3),b
	Probit	a	8.4 ppm (6.6 and 12.6)
Notropis leedsi	Binomial	8.4 ppm (7 and 10)	8.4 ppm (7 and 10)
	Moving Average	a	a
	Probit	a	a

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

RESULTS AND DISCUSSION

The results of the on-site 96 hour flow-through bioassays of AMAX's wastewater discharge (Outfall #003) indicated that the wastewater was relatively innocuous to the test organism, Notropis leedsi. Only a few random N. leedsi deaths were noted during the 96 hour test. A sufficient number of Daphnia pulex died, however, to calculate a 96 hour LC_{50} of 180.8% (Table 10). AMAX has an approved mixing zone, and this LC_{50} is in compliance with the Chapter 17-4 (F.A.C.) water quality criteria regarding allowable toxicity within mixing zones.

These results indicate a significant improvement over the results of earlier bioassays performed in 1983. At that time, high levels of ammonia and pH in the AMAX discharge resulted in rapid and extreme toxicity to all the organisms tested (FDER 1983). Since 1983, AMAX has installed a treatment system which reduces ammonia and pH levels in the wastewater. The results of these measures have significantly reduced the toxicity of AMAX's wastewater. Although AMAX was not operating at the time of these flow-through tests, the wastewater provided was of worst case quality since it was pumped directly from AMAX's holding ponds and through the treatment system (Mr. John Zibrida, AMAX, personal communication).

The results of 48 hour static tests, performed during the flow-through tests in the mobile trailer, were conflicting. Little or no mortality was noted during the D. pulex tests (21 to 23 and 23 to 25, April), which substantiates the results of the D. pulex flow-through test. The two Mysidopsis bahia tests, however, produced 48 hour LC_{50}'s of 80.4% and 66.9% (Table 11). The cause of this toxicity to the shrimp is not apparent, but was probably due to a combination of low pH, ammonia, and to other unknown components of the wastewater.

The results of the on-site Standard Reference Toxicant (sodium lauryl sulfate) tests, using N. leedsi and D. pulex, indicated that the sensitivities of these organisms to the reference toxicant were within both the EPA and DER recommended ranges. However, the 48 hour LC_{50}'s obtained from these tests (8.4 ppm and 8.5 ppm, respectively, Table 12) were slightly higher than recent LC_{50}'s obtained in the Tallahassee laboratory. Results of recent Tallahassee SRT tests indicated average LC_{50}'s of approximately 3.5 ppm and 4.5 ppm for D. pulex and N. leedsi, respectively (FDER 1985b). This may suggest that the organisms used for the flow-through bioassays were more resistant to the standard toxicant than were organisms used recently in the Tallahassee lab. This increased resistance may have been due to the use of AMAX well water as diluent, which is of higher conductivity and hardness than Tallahassee well water.

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