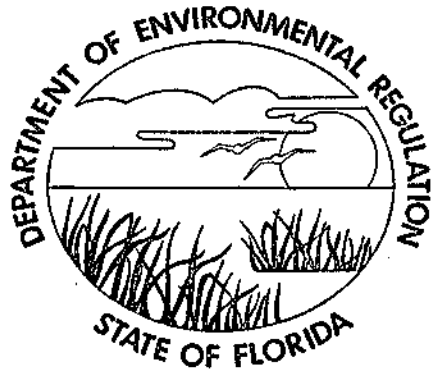


BIOASSAYS OF AMAX CHEMICALS PHOSPHATE FERTILIZER PRODUCTS, PINEY POINT, MANATEE COUNTY, FLORIDA

NPDES #FL0000124

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JULY 22, 1986

Biology Section
Division of Environmental Programs

BIOASSAYS OF AMAX CHEMICALS PHOSPHATE FETILIZER PRODUCTS, PINEY POINT, MANATEE COUNTY, FLORIDA

NPDES #FL0000124

Biology Section
Bureau of Laboratories and
Special Programs
July 22, 1986

EXECUTIVE SUMMARY

AMAX Chemicals, Piney Point, NPDES #FL0000124, on 6 to 8 May, 1986

The sample of this phosphate facility's process water discharge (Outfall #003) was not acutely toxic to the test organisms, *Daphnia pulex*, *Ceriodaphnia dubia*, and *Notropis leedsi*. No *D. pulex* died during the 48 hour static bioassays, however, a few *C. dubia* mortalities and 20% mortality of *N. leedsi* in the 100% concentration were noted. Moderate amounts of ammonia, 16.1 mg/l total and up to 0.05 mg/l unionized, were detected in AMAX's effluent, which may have contributed to the noted mortality. Since the sample was not acutely toxic, however, further chemical analyses were not performed.

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 eleveated 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 79.9 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 request 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 24, 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 those flow-through and static bioassays, using both fresh and saltwater test organisms, indicated that the effluent was not toxic to the freshwater fish used in the flow-through test. Some toxicity, however, was noted in the Daphnia pulex freshwater tests and in the Mysidopsis bahia saltwater tests (FDER 1985a).

Therefore, at the request of the Southwest District, additional bioassays were requested. A sample of effluent from Outfall #003 was collected on May 5, 1986, and three bioassays were performed using freshwater organisms. The results of these latest tests are presented in this report. Those involved in performing these latest tests and in preparation of this report were: M. L. Roll and P. G. Weiland.

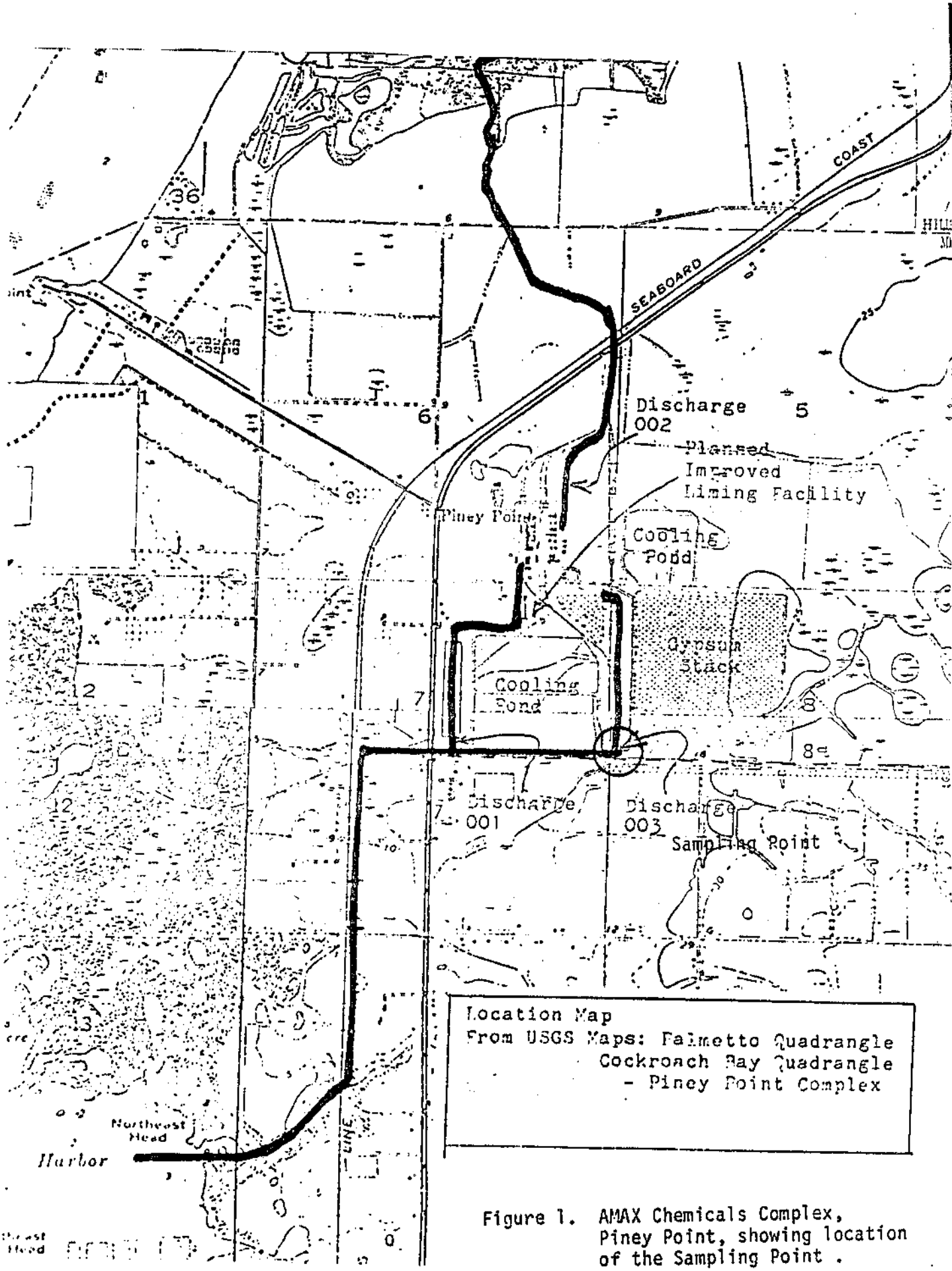


Figure 1. AMAX Chemicals Complex, Piney Point, showing location of the Sampling Point .

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier and Weber (1985) and the FDER Biology Section (1985b). The organisms selected for this study include 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.

Ceriodaphnia dubia (water flea) - A breeding culture of Ceriodaphnia dubia was obtained from the EPA laboratory in Athens, Georgia, in June, 1985. The culture is maintained 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®, Cerophyl® and Fleischmann's® yeast twice daily, once daily on weekends, and are kept at a constant temperature of 26° ± 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 -dark cycle and are fed Tetra-Min® and frozen Artemia (brin 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 approximately 18 days old at the time of the 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 (1985b). 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

Three separate 48 hour static acute toxicity bioassays were performed on a sample of AMAX's wastewater from Outfall #003. The tests were performed from 0930 hours on 6 May to 0930 hours on 8 May, 1986.

The sample was collected by Mr. David Demmi of DER's Southeast District at 1155 hours on 5 May, 1986, 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.

Freshwater Tests

Freshwater for dilution of the effluent sample 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 fleas, Ceriodaphnia dubia and 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. Ceriodaphnia were siphoned into a large culture dish and neonates were transferred by glass pipette, and loaded 10 per chamber, resulting in 20 per test concentration. Daphnia were siphoned into a large culture dish to isolate the proper size and age organisms (neonates). To accomplish

this, the Daphnia 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) bioassay. 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 this test.

Test Parameters

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 (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 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, 3, and 4.

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

Ceriodaphnia dubia (water flea)

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
50.0%[a]	100.0 ml	100.0 ml	200 ml
56.0%	88.0 ml	112.0 ml	200 ml
100.0%[a]	--------	200.0 ml	200 ml

Notropis leedsi (bannerfin shiner)

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

[a] Just these concentrations were used in the C. dubia test.

Table 2. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of AMAX Chemicals, Piney Point, NPDES #FL0000124, Outfall #003, on 6 to 8 May, 1986.

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	9.3	7.8	7.6	8.0	8.0	7.9	22.3	22.7	19.5		
Control	10	10	10	9.3	7.8	7.6	8.0	8.0	7.9				232	239
10.0%	10	10	10	9.7	7.6	7.7	8.0	8.0	7.9	22.3	22.6	19.2		
10.0%	10	10	10	9.7	7.6	7.7	8.0	8.0	7.9				710	690
32.0%	10	10	10	10.2	7.4	7.8	7.8	7.9	7.9	22.3	22.5	19.0		
32.0%	10	10	10	10.2	7.4	7.8	7.8	7.9	7.9				1650	1530
56.0%	10	10	10	10.6	7.4	7.8	7.5	7.6	7.7	22.3	22.4	19.0		
56.0%	10	10	10	10.6	7.4	7.8	7.5	7.6	7.7				2650	2500
100.0%	10	10	10	11.6	7.6	7.8	6.5	6.8	6.9	22.4	22.4	19.2		
100.0%	10	10	10	11.6	7.6	7.8	6.5	6.8	6.9				4400	4150

Control Water

NH_3(as NH_3)(mg/l) 0

Total Alkalinity 107 (mg/l $CaCO_3$)

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

Total Hardness 134 (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) 0.03

Total Alkalinity 23 (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) 16.1

Total Hardness 120 (mg/l $CaCO_3$)

Table 3. Data recorded during 48 hour, Ceriodaphnia dubia, static acute toxicity bioassay of AMAX Chemicals, Piney Point, NPDES #FL0000124, Outfall #003, on 6 to 8 May, 1986.

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	11[a]	11	11	8.2	7.6	7.7	8.0	8.0	7.9	22.1	22.8	19.4		
Control	10	9	9	8.2	7.6	7.7	8.0	8.0	7.9				232	239
50.0%	11[a]	11	10	9.6	7.4	7.8	7.7	7.8	7.9	21.9	22.7	19.4		
50.0%	12[a]	11	11	9.6	7.4	7.8	7.7	7.8	7.9					
100.0%	9[a]	9	8	10.2	7.5	7.9	6.7	7.0	7.1	22.1	22.7	19.5		
100.0%	10	10	10	10.2	7.5	7.9	6.7	7.0	7.1				4400	4150

Control Water

NH_3(as NH_3)(mg/l): 0

Total Alkalinity (mg/l $CaCO_3$): 107

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

Total Hardness (mg/l $CaCO_3$): 134

100% Effluent

NH_3(as NH_3)(mg/l): 0.05

Total Alkalinity (mg/l $CaCO_3$): 23

Total ammonia (as N)(mg/l): 16.1

Total Hardness (mg/l $CaCO_3$): 120

[a] Due to small size of organism, more or less were loaded by mistake.

Table 4. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of AMAX Chemicals, Piney Point, NPDES #FL0000124, Outfall #003, on 6 to 8 May, 1986.

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	8.4	7.6	7.2	7.5	7.8	7.8	22.5	22.6	19.4		
Control	5	5	5	8.4	7.6	7.2	7.5	7.8	7.8				210	230
10.0%	5	5	5	8.6	7.4	7.6	7.6	7.8	7.9	22.4	22.5	19.3		
10.0%	5	5	5	8.6	7.4	7.6	7.6	7.8	7.9				700	650
32.0%	5	5	5	9.0	7.4	7.6	7.5	7.7	7.8	22.3	22.4	19.2		
32.0%	5	5	5	9.0	7.4	7.6	7.5	7.7	7.8				1610	1510
56.0%	5	5	5	9.5	7.0	7.2	7.3	7.5	7.6	22.3	22.4	19.3		
56.0%	6[a]	6	6	9.5	7.0	7.2	7.3	7.5	7.6				2650	2490
100.0%	5	4	4	10.4	6.8	6.9	6.4	6.7	6.8	22.3	22.4	19.3		
100.0%	5	4	4	10.4	6.8	6.9	6.4	6.7	6.8				4320	4180

Control Water

NH_3(as NH_3)(mg/l): 0

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

Total Alkalinity (mg/l $CaCO_3$): 107

Total Hardness (mg/l $CaCO_3$): 134

100% Effluent

NH_3(as NH_3)(mg/l): 0.02

Total ammonia (as N)(mg/l): 16.1

Total Alkalinity (mg/l $CaCO_3$): 23

Total Hardness (mg/l $CaCO_3$): 120

[a] Extra organism loaded by mistake, allowed to remain.

RESULTS AND DISCUSSION

The sample of AMAX Chemicals' process water discharge (Outfall #003) was not acutely toxic to the test organisms, Daphnia pulex, Ceriodaphnia dubia, and Notropis leedsi. No D. pulex died during the 48 hour static bioassays, however, a few C. dubia mortalities and 20% mortality of N. leedsi in the 100% concentration were noted. Moderate amounts of ammonia, 16.1 mg/l total and up to 0.05 mg/l unionized, were detected in AMAX's effluent, which may have contributed to the noted mortality. Since the sample was not acutely toxic, however, further chemical analyses were not performed.

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