BIOASSAYS OF AIR PRODUCTS AND CHEMICALS, INC.

INCLUDING 96 HOUR FLOW THROUGH, 96 HOUR STATIC RENEWAL, 48 HOUR STATICS AND STANDARD REFERENCE TOXICANT ASSAY

PACE, SANTA ROSA COUNTY, FLORIDA

NPDES #FL0002313

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March 1, 1985

Biology Section
Division of Environmental Programs

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EXECUTIVE SUMMARY

Air Products, Santa Rosa County, NPDES #FL0002313, Flow Through and Static Bioassays, on January 13 to 17, 1985.

The Air Products facility produces fertilizer, amines, pvc plastic, and other chemical products. The principal component in the wastewater discharge ($\bar{x}$ = 1MGD) to Escambia Bay is ammonia, which reaches dangerously high concentrations during the cold months when the biological denitrification process in the treatment ponds is at its lowest level. Several previous bioassays, performed during the winter months by both DER and EPA, indicated acutely toxic conditions in Air Products' wastewater over a range of pH and/or temperatures. However, pH and temperature during these previous tests were usually somewhat higher than ambient pond conditions, which resulted in higher concentrations of toxic unionized ammonia than may have been found in the pond. Therefore, to more accurately define the toxic conditions in Air Products' wastewater, the Northwest District Industrial Waste staff requested that an on-site 96 hour flow-through bioassay be performed. In this test, the organisms can be continuously exposed to the wastewater and to as many of the wastewater's variable characteristics as possible, within the constraints of standard bioassay protocols.

The results of the on-site flow-through and static bioassays performed at the Air Products facility indicated that the wastewater was acutely toxic to the test organisms, Mysidopsis bahia and Pimephales promelas, but was not toxic to Daphnia pulex. The LC_{50}'s obtained from these tests are summarized below:

		LC_{50}			
Organism	Type of Test	24 Hours	48 Hours	72 Hours	96 Hours
M. bahia	96 Hour Flow-Through	a	177.9%	147.6%	68.3%
M. bahia	96 Hour Static Renewal	106.5%	74.8%	69.9%	64.1%
P. promelas	48 Hour Static	81.9%	70.3%	-	-
D. pulex	48 Hour Static	No Mortality	No Mortality	-	-

[a] = Insufficient Mortality

As can be seen, excellent agreement was obtained between the results of the M. bahia tests (96 hour LC_{50}'s = 68.3% and 64.7%) and the P. promelas test (48 hour LC_{50} = 70.3%). The lack of mortality in the D. pulex test may be due to the fact that, generally, daphnids are less sensitive to ammonia than are fish and shrimp.

The results of a Standard Reference Toxicant test, performed on-site using the principal test organism, M. bahia, indicated that the shrimp's sensitivity (48 hour LC_{50}) to the toxicant sodium lauryl sulfate was 8.9 ppm. This value is within both the EPA's recommended sensitivity range (1-10 ppm) and within DER's recommended range (8.0-19.2 ppm) (FDER 1984).

The toxicity exhibited by Air Products' wastewater may be attributed mostly, but not entirely, to the presence of total and unionized ammonia. The maximum ammonia concentrations measured during the flow-through test were 13.1 mg/l (total) and 0.466 mg/l (unionized). The latter value was measured at the end of the test when the temperature had increased due to a warming trend. These values are near, but still significantly below, the concentrations believed to be lethal to M. bahia (based on a previous Air Products bioassay). The concentrations of ammonia in the remaining static renewal and freshwater static tests ranged up to 21.0 mg/l (total) and 1.46 mg/l (unionized). This unionized fraction may have been mostly responsible for the toxicity observed in the P. promelas test. It should also be noted that an unionized ammonia concentration of 0.937 mg/l was measured in the D. pulex test, in which no mortality was observed.

Chemical analyses of Air Products' wastewater, collected during the flow-through test, failed to indicate the presence of any organic (acid, base neutral, or pesticide extractable) constituents. The metal analyses, however, indicated the presence of arsenic (8.6 ug/l), chromium (87 ug/l), and zinc (20 ug/l). These metals are routinely detected in Air Products' effluent and probably contributed only slightly to the observed toxicity.

INTRODUCTION

Air Products and Chemicals, Inc., is located on upper Escambia Bay near Pace, Santa Rosa County, Florida (Fig. 1). The facility produces a wide variety of industrial and agricultural chemicals, including pvc plastics, methanol, amines, nitric acid, ammonia, ammonium nitrate, urea and other chemical intermediates. Wastewater treatments include a percolation pond for the amines waste, and various equalization and neutralization processes for the remaining wastes (Fig. 2). The latter wastes are retained in a series of ponds for 60 to 90 days prior to being discharged through a ditch to Escambia Bay (Fig. 2).

The potential toxicity of this wastewater to aquatic organisms has been evaluated before by the FDER Biology Section (February 1980, October 1980, January 1984). The first series of tests indicated that the wastewater was acutely toxic to both the freshwater test organism, Daphnia pulex, and to the saltwater test organism, Mysidopsis bahia, producing 48 hour LC_{50}'s of 35% and 47%, respectively (FDER 1980). Little was known of the wastewater at that time, so a cause of the toxicity was not attributed. However, a review of certain data presented in the report (green algae present in the wastewater and high dissolved oxygen concentration) suggested that an algal growth stimulant, such as ammonia, may have been present. The second series of tests, conversely, indicated that the wastewater was not toxic to the same test organisms (FDER 1981). A review of notes recorded during these second tests indicated that ammonia (as measured with a pH/millivolt meter and a specific ion probe) was not present in the wastewater. The results of the January, 1984, tests indicated that the wastewater was again acutely toxic to Daphnia pulex, Simocephalus serrulatus, and to Mysidopsis bahia, producing 48 hour LC_{50}'s of 65.8%, 58.1%, and 71.3%, respectively (FDER 1984). This toxicity was attributed to the presence of total and unionized ammonia (32.0 mg/l and 2.59 mg/l, respectively) and to other chemical constituents detected in the wastewater. Air Products, however, responded that the toxicity was caused by an artificial increase in unionized ammonia, as a result of performing the tests at a higher pH and temperature than had been measured in the final treatment pond. While this may have been true, the DER Biology Section noted that the higher pH was caused by natural biological activity in the wastewater and was not a function of the bioassay test. The temperature difference was the result of following established EPA and DER bioassay protocols, which recommend performing bioassays at the temperature at which the test organisms had been previously acclimated. Nevertheless, during the winter months, the biological denitrification process in Air Products' treatment ponds is severely reduced, thereby resulting in an significant increase in ambient ammonia concentrations. This ammonia may very well be acutely toxic over a range of pH's and temperatures.

In addition to the DER tests, the U.S. EPA has evaluated the toxicity of Air Products' wastewater at least once, by performing on-site flow-through and static bioassays on January 7 to 11, 1979. Results of these tests indicated that the wastewater was acutely toxic to Mysidopsis bahia (flow-through), Cyprinodon variegatus (static), and to Pimephales promelas (static), producing

96 hour LC_{50}'s of 35%, 42.3%, and 47.6%, respectively (U.S. EPA 1979). It was noted, however, that the pH had increased during the C. variegatus static test. Test temperatures were maintained near those recommended for the test organisms.

As a result of these tests, the DER Northwest District Industrial Waste Section included biological self-monitoring (bioassays) in Air Products' most recent NPDES permit. Specific requirements stipulate that a static test be performed in the summer and a 96 hour flow-through be performed in the winter. Additionally, the Northwest District requested the Biology Section perform their own on-site flow-through test during the winter of 1984-85, to validate the data generated by Air Products' consultants and to confirm the data of the previous static tests. These tests were scheduled for the week of January 13, 1985. On January 4, Mr. Russel Frydenborg, Ms. Manitia Moultrie, and Mr. Martin Roll visited the Air Products facility to meet with plant and DER representatives and to arrange for certain logistics such as obtaining a source of dilution water. Mr. Richard Sroufe, Air Products' Environmental Supervisor, was helpful in arranging for power requirments for DER's mobile bioassay lab and in explaining Air Products' operations and waste treatment processes. Mr. Charles Logue, Northwest District Industrial Waste engineer, was present and provided valuable input concerning Air Products's permit requirements and other District concerns.

DER's mobile bioassay lab and the personnel to perform the tests (R. Frydenborg, M. Moultrie, and M. Roll) arrived at the Air Products facility on January 11, 1985. The mobile lab was positioned near the overflow of Air Products' final treatment pond (NPDES Outfall #001, Fig. 2). Air Products' consultant (Environmental Science, Engineering, and their mobile bioassay lab) arrived the following day. Organisms selected for the DER tests included the estuarine shrimp, Mysidopsis bahia (used in the 96 hour flow-through and static renewal tests), and the freshwater organisms, Pimephales promelas and Daphnia pulex (used in the 48 hour static tests). A Standard Reference Toxicant test, using sodium lauryl sulfate, was also performed on M. bahia, for quality assurance purposes. All tests were performed in the DER mobile lab from January 13 to 17, 1985. The results of these tests, and of chemical analyses performed on Air Products' wastewater, are presented in this report. This report was prepared by Martin L. Roll.

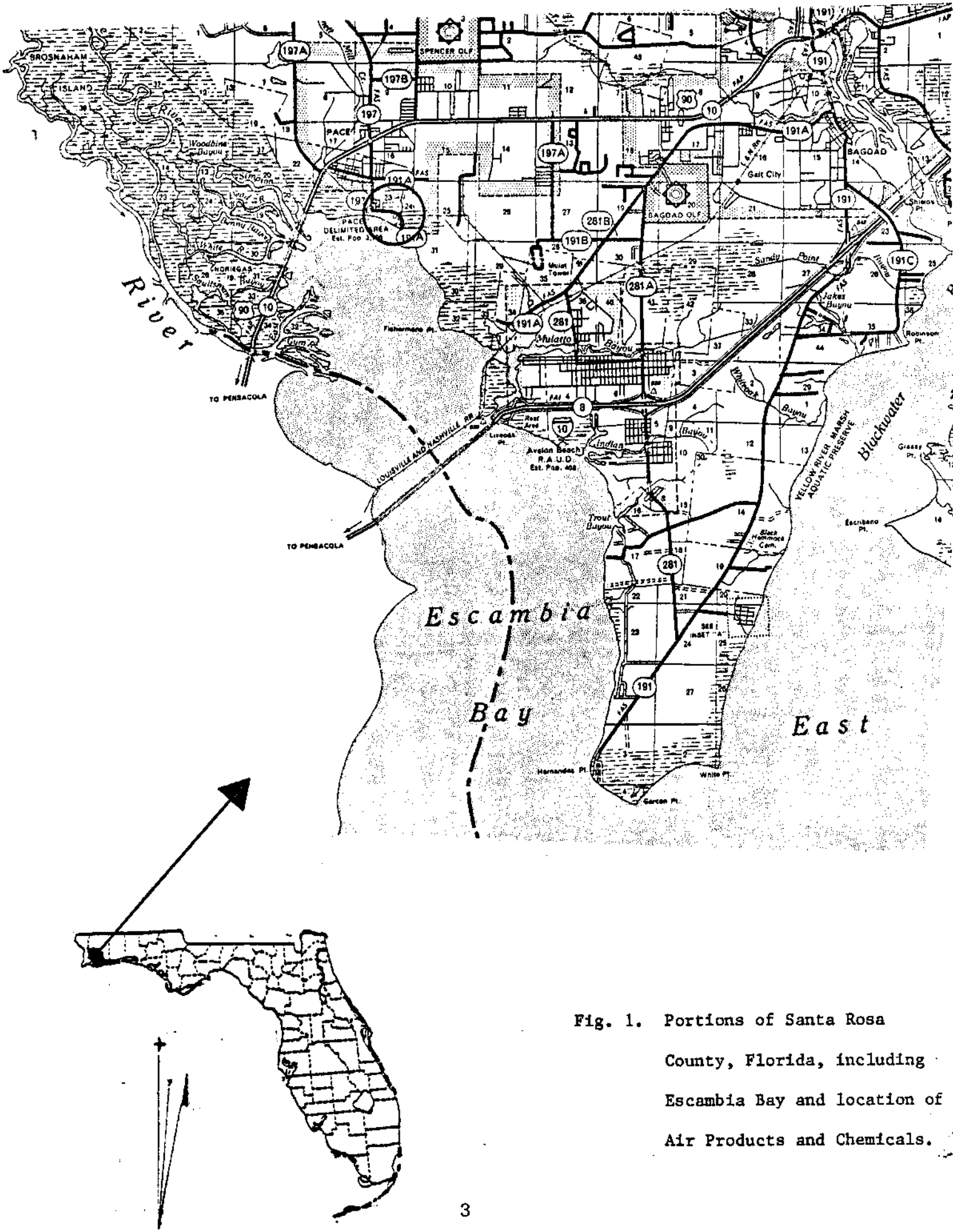
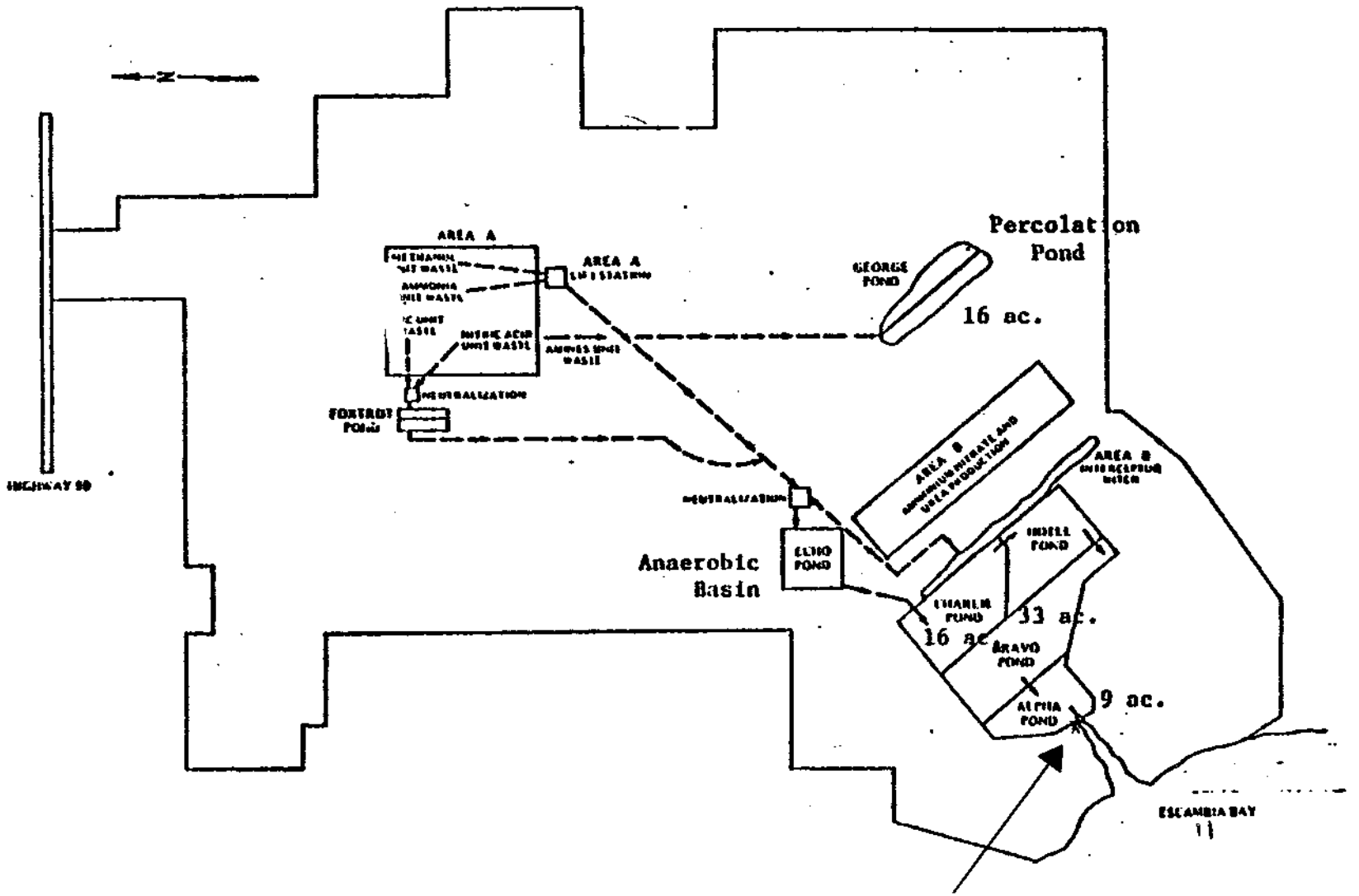


Fig. 1. Portions of Santa Rosa County, Florida, including Escambia Bay and location of Air Products and Chemicals.

Figure 2. Air Products facility, including waste generating streams and treatment ponds.



DER's mobile bioassay lab adjacent to final treatment pond and the NPDES Outfall #001.

METHODS AND MATERIALS

Test methods used to conduct these flow-through and static bioassays are extensively described by Peltier (1978) and the FDER Biology Section (1983). The organisms selected for this study are among those recommended by Peltier (1978), the U.S. EPA (1978), and the FDER Biology Section (1983). 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° plus or minus 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 Air Products test were obtained by siphoning individuals from each of our culturing aquaria into two 2 liter glass jars, each containing fresh, aerated well 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 daily and there was no observed mortality of these organisms prior to the test.

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, and once daily on weekends. 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 2-1/2 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 P. promelas used in the Air Products test were hatched in the Tallahassee lab on approximately January 2, 1985, in a 2-1/2 gallon all-glass aquarium. 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 11 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 (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° plus or minus 3°C.

Approximately one week prior to the Air Products tests, four new 5 gallon aquaria with undergravel filtration were set up. Water used in these aquaria was seawater obtained from the EPA Gulf Breeze lab and adjusted with DER well water to obtain salinities of approximately 10 ppt, 16 ppt, 22 ppt, and 28 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, an on-site reference toxicant test was performed in the mobile bioassay lab using Mysidopsis bahia, to evaluate the sensitivity of the shrimp after their transportation to the site and under the conditions in the mobile lab. The results of this test would indicate whether the sensitivity of the shrimp used in the flow-through test 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 mysid shrimp prior to the test, and as dilution water in the flow-through and static renewal tests, was natural seawater obtained from the EPA Research Laboratory in Gulf Breeze, Florida. This water is pumped continuously from Santa Rosa Sound, filtered to remove debris, and undergoes rigorous quality assurance checks at the EPA lab. This water was characterized by an almost constant pH of 8.1 standard units and a salinity of 26 $\pm$ 1 ppt. Approximately 250 gallons of this dilution water was conveyed daily in a 300 gallon Nalgene® tank to the Air Products test site. We wish to thank Dr. James Clark of the Gulf Breeze lab for providing us this source of dilution water and for his personal assistance throughout the test.

Freshwater used for dilution water in the P. promelas and D. pulex static tests was the same DER well water used to culture the organisms. This water was transported to the test site in 13 gallon carboys and stored in the mobile lab until used.

Pre-test Conditions and Preparations

A few days prior to the test, a severe cold front passed through the Pensacola area, resulting in significant decreases in ambient air and water temperatures. This probably reduced the natural biological denitrification process in Air Products' waste treatment ponds. These conditions continued until the final day of the test (January 16-17) when rain and a warming trend significantly increased ambient pond and air temperatures.

DER's mobile bioassay laboratory was constructed following EPA design specifications (Peltier, 1978) and is equipped with two solenoid valve operated, flow-through, proportional dilutor systems (Fig. 3). 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 Air Products on January 11, 1985. The trailer was positioned adjacent to the overflow (NPDES Outfall #001) of Air Products' final treatment pond (Fig. 4). Electric power for the mobile lab was supplied by Air Products.

An effluent sampling pump was suspended from a dock leading into the final pond and was positioned approximately 0.5 meters beneath the surface (at least 0.75 meters off the bottom of the pond). Effluent was conveyed directly and continuously to the mobile bioassay lab via a 50' length of braided reinforced Tygon® hose. Gulf Breeze 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 heated water bath adjusted to

approximately 30°C. This water bath maintained the temperature of the relatively cold effluent and dilution water at 18°C ± 2°C, until the very end of the test when rain and a warming trend significantly increased outside air temperatures.

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. The highest concentration used in the flow-through test was 72.4%, since this was the highest concentration which provided an adequate salinity for the shrimp (8 ppt). Flow rates were adjusted to operate the dilutor at approximately 9 cycles per hour, which provided for approximately 12 complete volume changes in the 8 liter flow-through aquaria every 24 hours. The dilutor system was operated for approximately 40 hours before the introduction of the test organisms at the beginning of the test at 1045 hours on Sunday, January 13, 1985.

The only interruption of the flow-through test system was during the early evening hours of Monday, January 14, when a suspected power surge tripped several circuit breakers in the mobile lab. Power to the effluent and dilution water pumps was out for an estimated 5 hours, and, therefore, the system was static instead of functioning as flow-through during this period. An immediate check of the test organisms and chemical parameters indicated no significant changes.

Flow-Through Methods and Parameters

Two replicates of six concentrations and a control were used for the flow-through test. To facilitate counting, the mysids were pipetted from culture dishes into retention chambers, which consisted of 10 cm glass petri dishes to which 25 cm high 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 (see Figure 3). Ten mysids were loaded per retention chamber per aquarium, resulting in 20 test organisms per test concentration. For each test concentration, the mysids were pre-acclimated to within 3 ppt salinity.

The number of live organisms, dissolved oxygen, pH, temperature and salinity 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 the control, mid and highest test concentrations, were recorded daily. These values appear in Table 2. Organisms used in this test were fed Artemia nauplii daily, as recommended by Peltier (1978) and DER (1983).

96 Hour Static Renewal Bioassay

Concurrently with the flow-through test, a 96 hour static renewal

bioassay was performed onsite, in the mobile bioassay lab. This test consisted of transferring, daily, approximately 500 ml of each test concentration, taken directly from the flow-through dilutor system, into quart size glass jars. Additionally, an 100% concentration was used in this test, by obtaining an effluent sample from the dilutor system and adjusting it with artificial sea salts (Forty Fathoms®) to approximately 7 ppt. The pH of this sample was then adjusted down to that of the original effluent. A control was used for this test, consisting of well water with similar salinity and pH adjustments. These procedures were performed daily at each renewal. Five mysid shrimp were pipetted into Nitex® retention chambers, (identical but smaller than the ones used in the flow-through aquaria) in each jar. Two replicates were used for each concentration, resulting in 10 test organisms per test concentration. At each 24 hour interval, the Nitex® chambers were removed and the contents of the jars discarded. Each jar was then immediately filled (500 ml) with the same test concentration as previously described. The mysid shrimp, still in their Nitex® chambers, were then re-introducted into their appropriate jars. Since these renewals were performed at the same time that the physical-chemical parameters were being recorded in the flow-through bioassay, the 0 hour through the 72 hour daily parameters were identical in the 96 hour renewal bioassay. The 96 hour parameters were recorded separately in the renewal bioassay. These values are reported in Table 3. The mysids used in this test were also fed Artemia nauplii daily.

Daphnia pulex and Pimephales promelas Freshwater Tests

Two replicates, each with five 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 transferred to a large culture dish to select the proper size and age organisms. Mid-size Daphnia were chosen for this test. 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 this test.

Two replicates, of five concentrations and a control, were used for the Pimephales promelas (fathead minnow) 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.

Dilution water used for these tests was DER well water. Effluent was obtained directly from the overflow line of the flow-through dilutor system. 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 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 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 concentrations each day 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 4 and 5.

48 Hour Standard Reference Toxicant Bioassay

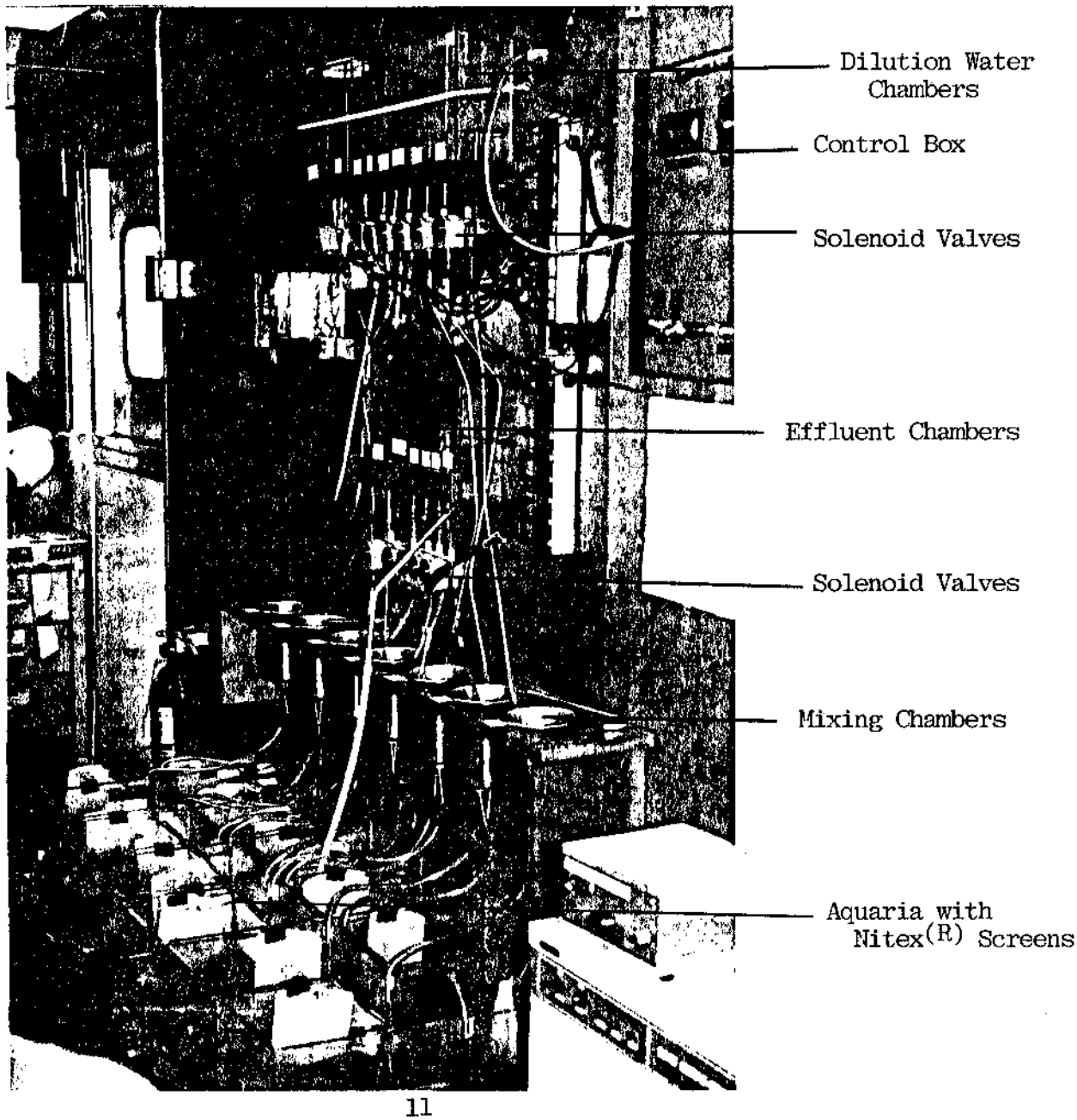
During the first two days of the flow-through bioassay (13 to 15 January, 1985) a Standard Reference Toxicant bioassay was performed onsite, in the bioassay trailer. Sodium lauryl sulfate was used as the reference toxicant, in a stock concentration of 100 ppm. This stock concentration was diluted in Gulf Breeze dilution water to obtain a concentration series (Table 1) of from 7 to 25 ppm (the same series used in our monthly quality control test in the Tallahassee lab). Two replicates of each concentration was used, each containing five mysids.

This test ensured that the sensitivities of the mysid shrimp used in these tests were not significantly different from sensitivities of shrimp used 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 salinity of each test concentration was recorded at the beginning and end of the test. All data recorded during this test are presented in Table 6. The mysids were fed *Artemia* nauplii daily.

Additional Investigations

While onsite during the flow-through test, Mr. Russel Frydenborg suspended a catch net in Air Products' NPDES Outfall overflow, to capture any macroinvertebrate drift organism that may have been inhabiting the treatment pond. The predominant organisms were identified and an evaluation was made as to their known pollution sensitivities.

Figure 3. Inside of DER's Mobile Bioassay Lab Showing Solenoid Valve Operated Flow-Through Dilutor System and Aquaria.



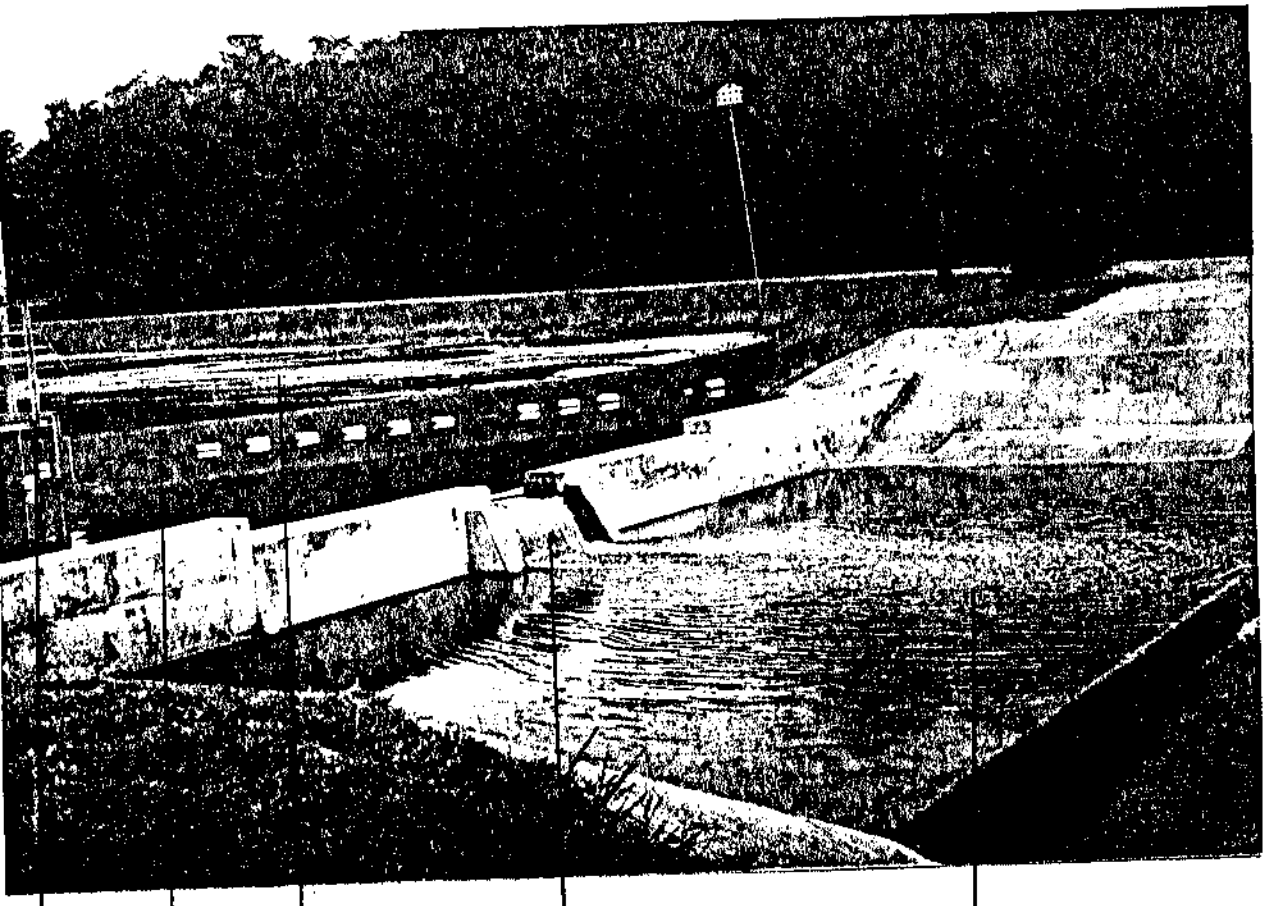


Figure 4. Air Products Final Treatment Pond and NPDES Outfall #001.

Table 1. Diluent and Effluent Volumes, and Final Concentrations used in Flow-Through, Static Renewal and Static Bioassays of Air Products (13 to 17 January, 1985.

1. Flow-Through Bioassay (13 to 17 January, 1985), Mysidopsis bahia

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
909 ml	904 ml	0 ml	0 ml	909 ml	904 ml	0%	0%
890 ml	890 ml	60 ml	58 ml	950 ml	948 ml	6.3%	6.1%
919 ml	917 ml	107 ml	104 ml	1026 ml	1021 ml	10.4%	10.2%
722 ml	721 ml	152 ml	147 ml	874 ml	868 ml	17.4%	16.9%
641 ml	640 ml	296 ml	292 ml	937 ml	932 ml	31.6%	31.3%
349 ml	360 ml	448 ml	447 ml	797 ml	807 ml	56.2%	55.4%
262 ml	258 ml	679 ml	682 ml	941 ml	940 ml	72.2%	72.6%

2. 96 Hour Static Renewal Bioassay (13 to 17 January, 1985), Mysidopsis bahia

Concentrations	Total Volume
0% through 72.4% Identical to Flow-Through Bioassay:	500 ml in each jar
100% and Acid Control:	500 ml in each jar

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

3. Daphnia pulex Freshwater Static Test (13 to 15 January, 1985)

Concentration	Dilution Water	Effluent	Total Volume
Control	200.0 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
100.0%	--------	200.0 ml	200 ml

Table 1. (Cont.)

4. Pimephales promelas Freshwater Static Test (13 to 15 January, 1985)

Concentration	Dilution Water	Effluent	Total Volume
Control	500 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

5. Mysidopsis bahia Standard Reference Toxicant Test (13 to 15 January, 1985)

Concentration	Dilution Water	Toxicant	Total Volume
Control	500 ml	--------	500 ml
7.0 ppm	465 ml	35 ml	500 ml
13.0 ppm	435 ml	65 ml	500 ml
19.0 ppm	405 ml	95 ml	500 ml
25.0 ppm	375 ml	125 ml	500 ml

Table 2. Data recorded during 96 hour, Mysidopsis bahia, flow-through bioassay of Air Products and Chemicals, NPDES #FL0002313, Santa Rosa County, Florida, on 13 to 17 January, 1985.

Conc. or %	Test Container Number	Number of Live Organisms					Dissolved Oxygen (mg/l)					pH					Salinity (ppt)					NH_3/N-Total (mg/l)					Temperature (°C)				
		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	A	11[a]	11	11	11	10	8.1	8.4	8.4	8.9	8.1	8.1	8.0	8.1	8.1	8.1	26.4	25.5			25.5	.006/.15		0/0		0/0	17.9		17.7		22.0
																	26.4		23.8								17.7		17.1		
Control	B	10	10	10	10	10	8.1	8.5	8.4	8.9	8.1	8.1	8.0	8.1	8.1	8.1	26.5	25.5			25.4		0/0		0/0		18.1		17.5		22.0
																	26.4		23.8								17.7		17.0		
6.2%	A	10	9	9	9	9	8.0	8.5	8.4	8.9	8.1	8.1	8.1	8.1	8.1	8.0	25.2	24.0			24.0						18.2		17.5		21.9
																	25.0		22.5								17.7		17.0		
6.2%	B	10	10	10	10	10	8.0	8.5	8.4	8.9	8.1	8.1	8.1	8.1	8.1	8.0	25.1	24.0			24.0						18.2		17.4		21.8
																	24.9		22.3								17.7		16.8		
10.3%	A	10	10	10	10	10	8.1	8.5	8.5	9.0	8.1	8.1	8.1	8.0	8.0	8.0	24.7	23.0			23.0						18.1		17.4		21.7
																	24.0		21.5								17.5		16.6		
10.3%	B	10	10	10	10	10	8.2	8.6	8.5	9.1	8.1	8.1	8.1	8.0	8.0	8.0	24.2	23.0			23.0						17.8		17.2		21.8
																	24.0		21.3								17.5		16.6		
17.2%	A	10	10	10	10	10	8.2	8.5	8.5	9.0	8.1	8.0	8.0	8.0	8.0	8.0	22.9	21.2			21.5						17.9		17.3		21.8
																	22.0		20.0								17.5		16.6		
17.2%	B	11[a]	11	11	11	11	8.3	8.6	8.5	9.0	8.1	8.1	8.0	8.0	8.0	8.0	22.9	21.6			21.8						17.7		17.3		21.9
																	22.5		20.1								17.5		16.7		
31.5%	A	10	10	9	9	9	8.2	8.7	8.5	9.1	8.2	8.0	8.0	7.9	7.9	7.9	19.3	18.4			18.4	.186/5.25		.157/4.14			18.2		17.4		22.0
																	19.0		17.0			.095/3.5					17.5		16.7		
31.5%	B	10	10	10	10	10	8.4	8.7	8.5	9.1	8.2	8.0	8.0	7.9	7.9	7.9	19.3	18.6			18.4			.120/4.6			18.0		17.3		22.1
																	19.0		17.0			.190/5.6					17.5		16.6		
55.8%	A	10	10	7	7	7	8.2	8.7	8.5	9.1	8.0	7.9	7.9	7.9	7.9	7.9	13.5	13.0			12.8						18.8		17.4		22.2
																	13.2		11.5								17.6		16.7		
55.8%	B	10	10	10	10	10	8.2	8.7	8.6	9.1	8.0	7.9	7.9	7.9	7.9	7.9	13.2	12.1			12.5						18.6		17.4		22.4
																	12.5		11.3								17.6		16.6		
72.4%	A	10	8	6	6	4	8.0	8.8	9.0	8.9	8.0	7.8	7.9	7.9	7.9	7.9	8.7	8.8			8.1	.331/13.1		.466/11.3			18.7		17.3		22.3
																	8.8		7.8			.255/8.85					17.5		16.5		
72.4%	B	10	9	7	6	4	8.0	8.9	9.0	8.9	8.0	7.8	7.9	7.9	7.9	7.9	8.2	8.0			8.0	.383/13.1					18.5		17.3		22.4
																	8.1		7.5					.339/12.4			17.5		16.5		

Remarks: Parameters of 100% effluent from diluter system: 9.3 7.9 995 μmhos conductivity .794/23.7 .765/17.6

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

Table 3. Data recorded during 96 hour, Mysidopsis bahia, static renewal bioassay of Air Products and Chemicals, NPDES #FL0002313, Santa Rosa County, Florida, on 13 to 17 January, 1985.

Conc. or %	Test Container Number	Number of Live Organisms					Dissolved Oxygen (mg/l)					pH					Salinity (ppt)					NH_3/NH_3+NH_4 (mg/l)					Temperature (°C)				
		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	A	5	5	5	5	5	8.1	8.4	8.4	8.9	-[b]	8.1	8.0	8.1	8.1	-[b]	26.4	26.4	25.5	23.8	-[b]					-[b]	17.9	17.7	17.7	17.1	-[b]
Control	B	5	5	5	5	5	8.1	8.5	8.4	8.9	-[b]	8.1	8.0	8.1	8.1	-[b]	26.5	26.4	25.3	23.8	-[b]	.006/.15				-[b]	18.1	17.7	17.5	17.0	-[b]
6.2%	A	5	5	5	5	5	8.0	8.5	8.4	8.9	5.2	8.1	8.1	8.1	8.1	7.7	25.2	25.0	24.0	22.5	25.0						18.2	17.7	17.5	17.0	23.9
6.2%	B	5	5	5	5	5	8.0	8.5	8.4	8.9	5.2	8.1	8.1	8.1	8.1	7.7	25.1	24.9	24.0	22.3	25.1						18.2	17.7	17.4	16.8	23.8
10.3%	A	5	5	5	5	5	8.1	8.5	8.5	9.0	5.2	8.1	8.1	8.0	8.0	7.8	24.7	24.0	23.0	21.5	22.9						18.1	17.5	17.4	16.6	24.3
10.3%	B	5	5	5	5	5	8.2	8.6	8.5	9.1	5.1	8.1	8.1	8.0	8.0	7.8	24.2	24.0	23.0	21.3	23.1						17.8	17.5	17.2	16.6	24.4
17.2%	A	5	5	5	5	5	8.2	8.5	8.5	9.0	5.2	8.0	8.0	8.0	8.0	7.9	22.9	22.0	21.2	20.0	22.9						17.9	17.5	17.3	16.6	24.4
17.2%	B	5	5	5	5	5	8.3	8.6	8.5	9.0	5.3	8.1	8.0	8.0	8.0	7.9	22.9	22.5	21.6	20.1	22.0						17.7	17.5	17.3	16.7	24.4
31.5%	A	5	5	5	5	5	8.2	8.7	8.5	9.1	5.1	8.0	8.0	7.9	7.9	7.9	19.3	19.0	18.4	17.0	18.1						18.2	17.5	17.4	16.7	24.4
31.5%	B	5	5	5	5	5	8.4	8.7	8.5	9.1	5.0	8.0	8.0	7.9	7.9	7.9	19.3	19.0	18.6	17.0	18.1						18.0	17.5	17.3	16.6	24.4
55.8%	A	5	5	5	5	5	8.2	8.7	8.5	9.1	5.2	7.9	7.9	7.9	7.9	7.9	13.5	13.2	13.0	11.5	13.0						18.8	17.6	17.4	16.7	24.4
55.8%	B	5	5	4	4	4	8.2	8.7	8.6	9.1	4.6	7.9	7.9	7.9	7.9	7.9	13.2	12.5	12.1	11.3	12.2						18.6	17.6	17.4	16.6	24.4
72.4%	A	5	4	4	3	1	8.0	8.8	9.0	8.9	4.2	7.8	7.9	7.9	7.9	8.1	8.7	8.8	8.8	7.8	7.5	.331/13.1			1.17/15.6		18.7	17.5	17.3	16.5	24.6
72.4%	B	5	5	3	2	1	8.0	8.9	9.0	8.9	4.7	7.8	7.9	7.9	7.9	8.1	8.2	8.1	8.0	7.5	8.1	.272/9.45					18.5	17.5	17.3	16.5	24.7
100%	A	5	3	0	-[a]	-[a]	9.1	8.3	7.3	-[a]	-[a]	7.8	7.9	8.2	-[a]	-[a]	7.1	7.5	-[a]	-[a]	-[a]	.753/21.0		-[a]	1.1/12.4	-[a]	23.4	22.5	-[a]	-[a]	-[a]
100%	B	5	3	0	-	-	9.0	8.3	7.4	-	-	7.8	7.9	8.2	-	-	7.1	7.5	-	-	-	.843/19.8			-	-	23.4	22.9	-	-	-
Acid Control	A	5	5	4	-	-	8.4	8.1	5.7	-	-	7.8	7.9	7.8	-	-	7.0	7.5	-	-	-	.01/.29		.005/.135	-	-	23.4	21.0	24.0	-	-
Acid Control	B	5	5	4	-	-	8.4	8.1	6.0	-	-	7.8	7.9	7.8	-	-	7.1	7.5	-	-	-	.01/.33			-	-	23.3	20.9	26.0	-	-

Remarks: Parameters reported for the Control through 72.4% concentrations and from 0 through 72 hours are those measured in the flow-through test. All 96 hour parameters and those of the 100% and Acid Control samples were recorded separately.

Footnotes: [a] Since all the shrimp had died in the 100% sample by hour 48, there were no further renewals of this or of the Acid Control sample, and therefore, no further parameters (72 and 96 hours) were recorded. Additionally, certain parameters, such as salinity and temperature, were not recorded at 48 hours.

[b] By mistake, the control jars were emptied before final parameters were recorded.

Table 4. Data recorded during 48 hour, Pimephales promelas, static acute toxicity bioassay of Air Products and Chemicals, NPDES #FL0002313, Santa Rosa County, on 13 to 15 January, 1985.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)			NH_3/N (mg/l)		
	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48
Control	5	5	5	9.0	8.6	7.2	7.8	8.0	7.9	19.2	23.3	23.9				0 0	.01 .2	0 .05
Control	5	5	5	9.0	8.5	7.0	7.8	8.0	7.9				210	240	279			
10.0%	5	3	3	9.1	8.4	7.0	7.8	8.0	8.0	18.9	22.8	23.4						
10.0%	5	5	5	9.1	8.4	7.0	7.8	8.0	8.0				293	325	370			
18.0%	5	5	5	9.0	8.4	7.2	7.8	8.0	8.0	18.9	22.5	23.0						
18.0%	5	5	5	9.0	8.4	7.1	7.8	8.0	8.0				362	400	425			
32.0%	5	5	5	9.2	8.3	7.2	7.9	8.1	8.0	19.1	22.1	22.9						
32.0%	5	5	5	9.2	8.3	7.2	7.9	8.1	8.0				480	500	520			
56.0%	5	4	4	9.4	7.7	6.5	8.0	8.1	8.0	19.3	22.2	22.7						
56.0%	5	5	5	9.4	7.7	6.1	8.0	8.1	8.0				670	710	790			
100.0%	5	0	0	9.8	6.6	[b]	8.0	8.1	[b]	19.9	23.1	23.5				.87 18.9	1.46 20.5	.84 9.23
100.0%	6[a]	3	0	9.8	6.6	8.4[c]	8.0	8.1	8.3				1070	1150	1410			

Control Water

Total Alkalinity 105 (mg/l $CaCO_3$)

Total Hardness 123 (mg/l $CaCO_3$)

100% Sample

Total Alkalinity 365 (mg/l $CaCO_3$)

Total Hardness 57 (mg/l $CaCO_3$)

Footnotes:
[a] Extra organism loaded by mistake, allowed to remain.
[b] Remaining parameters were not recorded since all fish in this jar had died by hour 24.
[c] This jar was aerated starting at hour 44 of the test since the DO had decreased to 4.0 mg/l.

Table 5. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Air Products and Chemicals, NPDES #FL0002313, Santa Rosa County, on 13 to 15 January, 1985.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)			NH_3/N (mg/l)		
	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48
Control	10	10	10	9.0	8.2	7.1	7.9	7.9	7.9	19.8	23.1	24.0				0 0	.01 .165	.004 .09
Control	10	10	10	9.0	8.2	7.1	7.9	7.9	8.0				211	238	288			
10.0%	10	10	10	9.1	8.1	7.1	7.8	7.9	8.0	19.5	22.6	23.3						
10.0%	11[a]	11	11	9.1	8.1	7.1	7.8	7.9	8.0				293	320	279			
18.0%	10	10	10	9.0	8.3	7.6	7.9	8.0	8.0	19.9	22.6	22.5						
18.0%	11[a]	11	11	9.0	8.3	7.6	7.9	8.0	8.0				358	390	442			
32.0%	11[a]	11	11	9.1	8.2	7.7	7.9	8.1	8.1	20.2	23.2	22.5						
32.0%	11[a]	11	11	9.1	8.2	7.7	7.9	8.1	8.1				483	520	580			
56.0%	10	10	10	9.4	7.8	7.9	8.0	8.1	8.2	20.4	23.8	22.9						
56.0%	10	10	10	9.4	7.8	7.9	8.0	8.1	8.2				650	750	790			
100.0%	10	10	10	9.6	7.3	7.5	8.0	8.2	8.2	21.0	23.5	23.8				.937 18.9		.678 7.32
100.0%	10	10	10	9.6	7.3	7.5	8.0	8.2	8.2				1100	1200	1300			

Control Water

Total Alkalinity 105 (mg/l $CaCO_3$)

Total Hardness 123 (mg/l $CaCO_3$)

100% Sample

Total Alkalinity 365 (mg/l $CaCO_3$)

Total Hardness 57 (mg/l $CaCO_3$)

Footnotes: [a] Extra organism added by mistake, allowed to remain.

Table 6. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of sodium lauryl sulfate (Standard Reference Toxicant), performed during Air Products flow-through, on 12 to 15 January, 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	9.5	5.7	5.9	8.0	7.8	7.7	19.0	23.5	25.2		
Control	5	5	5	9.5	5.4	5.9	8.0	7.8	7.7				25.5	26.5
7.0 ppm	5	5	5	9.6	4.5	5.1	8.1	7.8	7.6	19.1	23.4	25.1		
7.0 ppm	5	3	3	9.6	4.6	5.4	8.1	7.8	7.6				25.1	25.5
13.0 ppm	5	3	1	9.6	4.6	4.4	8.1	7.7	7.6	19.3	23.4	25.2		
13.0 ppm	5	1	0	9.6	4.5	4.4	8.1	7.7	7.6				25.0	24.2
19.0 ppm	5	0	0	9.5	4.4	–[a]	8.1	7.7	–[a]	19.1	23.4	–[a]		
19.0 ppm	5	0	0	9.5	4.5	–	8.1	7.7	–				25.1	–[a]
25.0 ppm	5	0	0	9.5	4.0	–	8.1	7.7	–	19.1	23.5	–		
25.0 ppm	5	0	0	9.4	4.3	–	8.1	7.7	–				24.8	–

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 $CaCO_3$)

Footnotes: [a]Remaining parameters were not recorded since all the shrimp had died by hour 24.

Table 7. LC_{50} Data for Bioassays Performed at the Air Products Facility, NPDES #FL0002313, Santa Rosa County, on 13 to 17 January, 1985.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals	72 Hour LC_{50} and 95% Confidence Intervals	96 Hour LC_{50} and 95% Confidence Intervals
Mysidopsis bahia	Binomial	a	a	a	68.3% (54 and ∞), b
96 Hour Flow-Through	Moving Average	a	a	a	68.3% (62 and 86), b, d
	Probit	a	177.9% (57 and ∞), b, c	147.6% (51 and), b, c	94.5% (0 and ∞), b, c
Mysidopsis bahia	Binomial	a	77.9% (53 and 100)	72.4% (53 and 100)	63.8% (53 and 100)
96 Hour Static Renewal	Moving Average	a	72.4% (63 and 86)	68.6% (59 and 81)	60.2% (51 and 74)
	Probit	106.5% (89 and 1209), b	74.0% (65 and 85), d	70.0% (61 and 80), d	64.1% (57 and 72), d
Pimephales promelas	Binomial	81.9% (0 and ∞)	70.3% (0 and 100), d		
48 Hour Static	Moving Average	81.9% (66 and 115), d	a	——	——
	Probit	111.6% (0 and ∞), c	67.9% (0 and ∞), c		
Daphnia pulex	Binomial				
48 Hour Static	Moving Average	No Mortality	No Mortality	——	——
	Probit				
Mysidopsis bahia	Binomial	11.2 ppm (0 and 19)	9.0 ppm (0 and 13)		
Standard Reference Toxicant	Moving Average	10.6 ppm (8.0 and 13), d	9.0 ppm (6.87 and 11.2)	——	——
	Probit	10.3 ppm (7.7 and 13)	8.9 ppm (6.93 and 11.1), d		

[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] Due to the distribution of mortality, results calculated using the probit method are not statistically sound.

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

Table 8. Results of Chemical Analyses Performed on Wastewater from Air Products and Chemicals, NPDES #FL0002313, Collected during Flow-Through Bioassay, on January 16, 1985

Organics

Acid Extractables:	N.D.
Base Neutral Extractables:	N.D.
Pesticide Extractables:	N.D.

Metals

Arsenic	8.6 µg/l
Cadmium	N.D.
Chromium	87 µg/l
Copper	N.D.
Lead	--[a]
Mercury	N.D.
Nickel	N.D.
Selenium	N.D.
Silver	N.D.
Zinc	20 µg/l

N.D. = None Detected
[a] Lead analysis was invalidated

RESULTS AND DISCUSSION

The results of the bioassays performed at the Air Products facility indicated that the wastewater was acutely toxic to the test organisms, Mysidopsis bahia and Pimephales promelas. The M. bahia 96 hour flow-through test produced a 96 hour LC_{50} of 68.3% (Table 7). The M. bahia 96 hour static renewal test produced similar 48, 72, and 96 hour LC_{50}'s of 74.0%, 70.0%, and 64.1%, respectively (Table 7). The P. promelas 48 hour static test produced 24 and 48 hour LC_{50}'s of 81.9% and 70.3%, respectively (Table 7). These results are very similar, indicating excellent agreement between the flow-through and static renewal tests, and between the saltwater shrimp (M. bahia) and the freshwater fish (P. promelas). A portion of this toxicity can be attributed to the significant concentrations of total ammonia (up to 23.7 mg/l) and unionized ammonia (up to 1.46 mg/l in the P. promelas test) measured during the various tests. However, ammonia was not the sole toxic agent since the maximum unionized ammonia concentration measured during the M. bahia flow-through test was 0.466 mg/l (Table 2), which is believed to be below the concentration necessary to be lethal to these organisms.

Air Products' wastewater was not toxic to any Daphnia pulex during the 48 hour static test performed on-site concurrently with the flow-through. This may have been due to the fact that daphnids are less sensitive to ammonia than are fish and shrimp (William Peltier, personal communication). It should be noted, however, that a significantly high unionized ammonia concentration (0.937 mg/l) was measured at the beginning of the D. pulex test (Table 5).

The results of the Standard Reference Toxicant Bioassay, performed on-site during the flow-through bioassay, indicated that the mysid shrimp used in these tests exhibited comparable sensitivities to sodium lauryl sulfate as shrimp used in previous toxicity testing. The 48 hour LC_{50} value of 8.9 ppm (Table 7) obtained in this test is within the 8.0 ppm to 19.2 ppm range suggested for mysid shrimp in the most recent Standard Reference Toxicant Bioassays (FDER, 1984b). This value is also within the EPA recommended sensitivity range (1-10 ppm) for bioassay test organisms, using sodium lauryl sulfate as the toxicant. This indicates that the shrimp used in the Air Products flow-through and static renewal bioassays were not significantly different from shrimp used in previous bioassays in the Tallahassee laboratory, with respect to sensitivity to sodium lauryl sulfate.

The results of the organic chemical analyses of Air Products' wastewater failed to detect the presence of any contaminants (Priority Pollutants) in measurable concentrations (Table 8). The metal analyses, however, indicated the presence of arsenic (8.6 µg/l), chromium (87 µg/l), and zinc (20 µg/l). These metals are routinely detected in Air Products' effluent and probably contributed only slightly to the observed toxicity.

The primary factor responsible for the toxicity noted in our tests of effluent from Air Products' wastewater is, undoubtedly, the presence of

ammonia. Potentially lethal concentrations of this substance were detected, or believed to have existed, in previous bioassays (FDER 1980 and 1984, U.S. EPA 1979). The results of these latest DER tests are consistent with these earlier results. However, it is not possible to attribute this latest toxicity, especially that found in the flow-through test, solely to the presence of ammonia. Another possible source of toxicity in Air Products' wastewater is cyanide. As much as 150 ug/l of this substance has been detected in the wastewater (Mr. Charles Logue, personal communication). According to the U.S. EPA (1976), from 50 ug/l to 200 ug/l of cyanide is toxic to most fish.

Macroinvertebrates

A net was placed at the #001 Alpha outfall between 1000 and 1300 hours on 14 January, 1985, to capture organisms drifting from the final treatment pond. The organisms were placed in 70% ethanol, sorted from detritus and stored in bottles. In the lab, the macroinvertebrates were identified to the lowest practical taxonomic level under an Olympus dissecting microscope, using appropriate taxonomic keys. A list of species found is presented in the following table.

Air Products Drift Macroinvertebrate Taxonomic List

Hemiptera

Buenoa cf. *margaritacea* (abundant)
Immature nymph of Corixidae (abundant)

Diptera

Chaoborus sp. (rare)
cf. *Euparyphus* sp. (rare)
Chironomus stigmaterus (common)
Glyptotendipes lobiferus (rare)

Odonata

Enallagma civile (rare, only 1 individual found)
Anax junius (rare, only 1 individual found)

The two most numerous organisms, making up approximately 95% of the drift net sample, were *Buenoa* cf. *margaritacea* and an immature hemipteran of the family Corixidae. *Buenoa* sp. and most corixids are ubiquitous air-breathing species often found living in sewage lagoons, temporary rain puddles and unused swimming pools. The remainder of the organisms found appear to be indifferent (i.e. tolerant) to water quality conditions. The fact that pollution tolerant organisms dominated the macroinvertebrate drift from the treatment ponds indicates that conditions in the ponds may be stressful to aquatic life.

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.

rom 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.