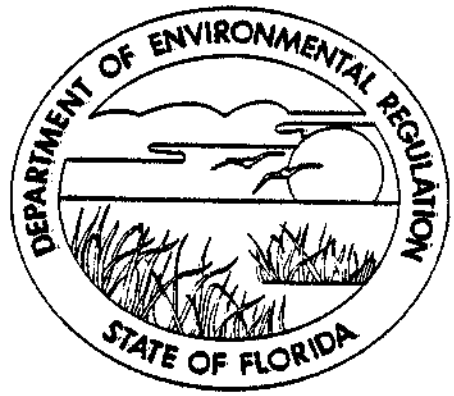


BIOASSAYS OF AIR PRODUCTS AND CHEMICALS

PACE, SANTA ROSA COUNTY, FLORIDA

NPDES #FL0002313

SAMPLED 1/30/84

FILE COPY
DO NOT REMOVE

Biology Section
Bureau of Water Analysis
April 30, 1984

BIOASSAYS OF AIR PRODUCTS AND CHEMICALS

PACE, SANTA ROSA COUNTY, FLORIDA

NPDES #FL0002313

Biology Section
Bureau of Water Analysis
April 30, 1984

EXECUTIVE SUMMARY

Air Products and Chemicals, NPDES #FL0002313 on 31 January to 2 February, 1984.

The sample of wastewater from this chemical and fertilizer manufacturer proved to be acutely toxic to the test organisms, Daphnia pulex, Simocephalus serrulatus and Mysidopsis bahia, producing 48 hour LC_{50}'s of 65.8%, 58.1% and 71.3%, respectively. This toxicity was most likely due to the significant concentrations of total ammonia (32.0 mg/l to 33.7 mg/l) and unionized ammonia (1.9 mg/l to 2.59 mg/l) measured in the wastewater. However, the results of chemical analyses indicated the presense of three unidentified base neutral organic compounds and various concentrations of the metals arsenic (6.8 µg/l), chromium (293 µg/l) and zinc (81 µg/l). The presence of these organic and metal components, particularly the chromium, may very well have contributed to the toxicity noted in the wastewater.

INTRODUCTION

Air Products and Chemicals 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 that may be found in both the discharge ditch and in Escambia Bay has been evaluated twice before by the FDER Biology Section (February 1980, October 1980). 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.

Since it has been a considerable time since the last series of bioassay tests, and since the NPDES permit for Air Products and Chemicals may be reissued, additional acute toxicity bioassays were requested by the Northwest District. A third series of three static acute toxicity bioassays were performed by the Biology Section, on January 31 to February 2, 1984. The results of these latest tests are presented in this report. Those involved in performing these tests and in preparation of this report were C. W. Dye and M. L. Roll.

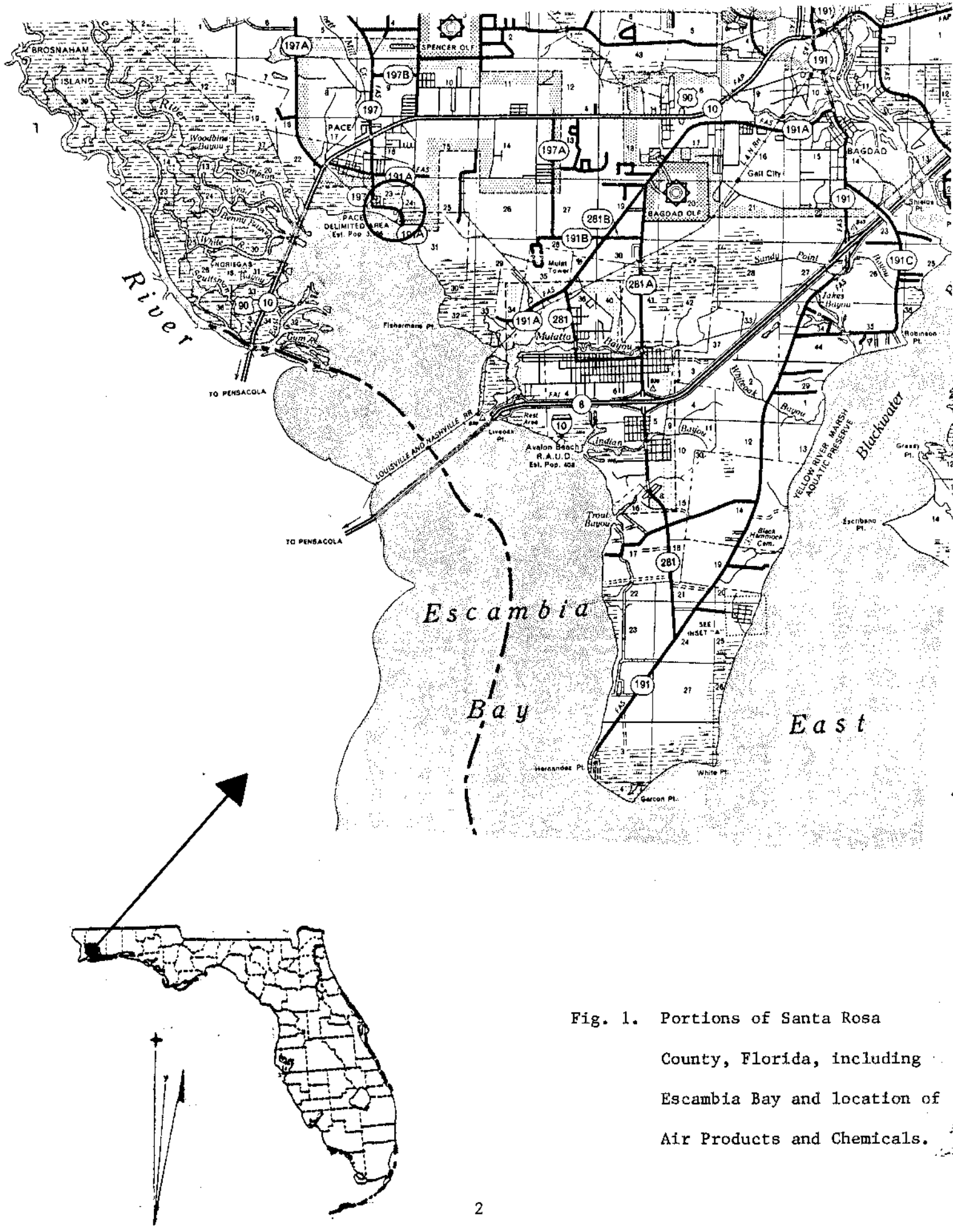
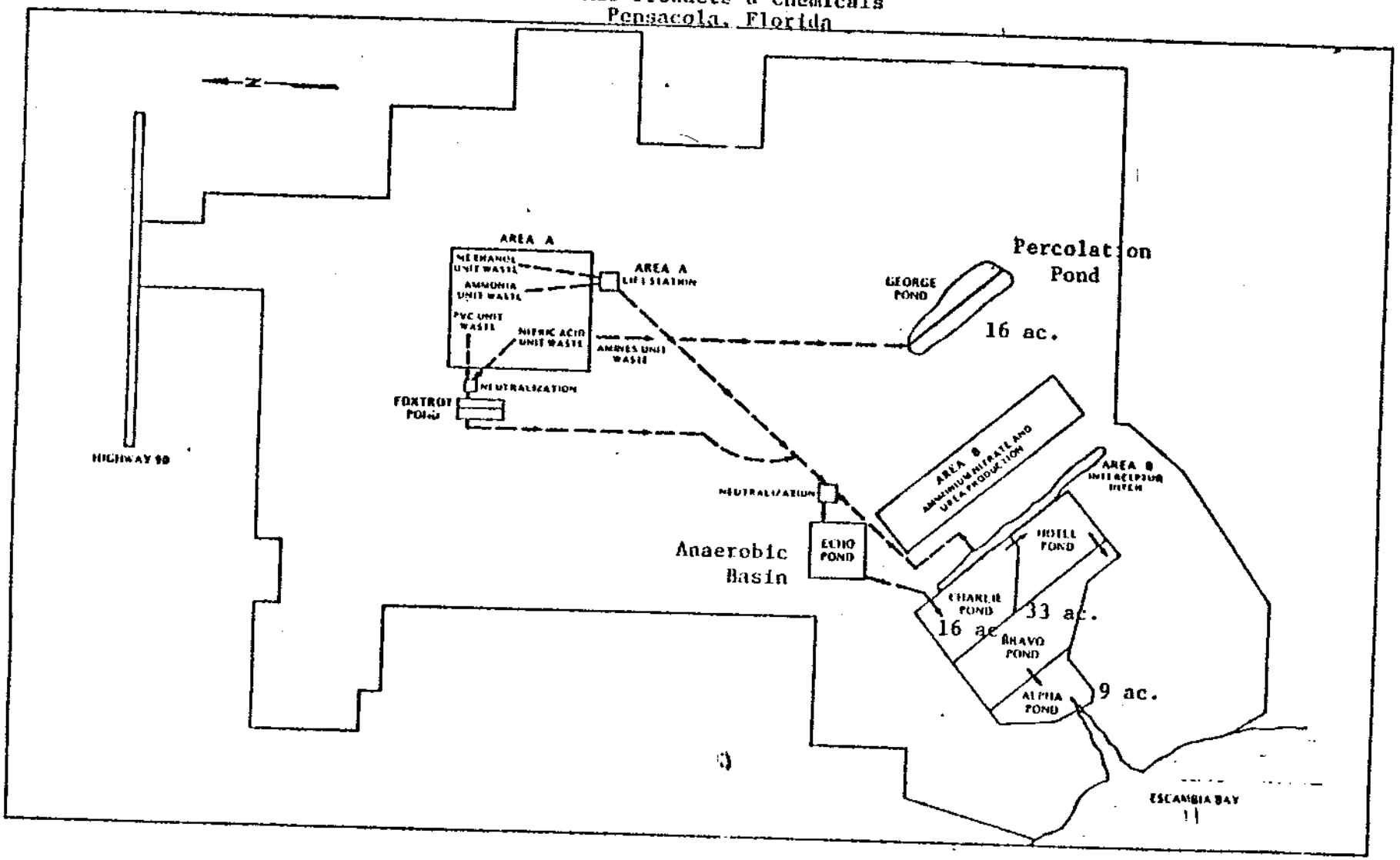


Fig. 1. Portions of Santa Rosa County, Florida, including Escambia Bay and location of Air Products and Chemicals.

Figure 2
Wastewater Treatment Facilities
Air Products & Chemicals
Pensacola, Florida



MAJOR SOURCE STREAMS AND WASTEWATER TREATMENT FACILITIES

ENGINEERING REPORT ON ATTAINMENT OF FINAL NPDES REQUIREMENTS
ESCAMBIA PLANT
Black, Crow & Eidsness, Inc., Engineers

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978) and the FDER Biology Section (1983). The organisms selected for this study include species recommended by Peltier (1978), the U.S. EPA (1978), and the FDER Biology Section (1983). Additionally, the Biology Section maintains cultures of several species of organisms indigenous to the State of Florida. 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, and are kept at a constant temperature of 22° plus or minus 2°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms. They are then refilled with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances such as: declining populations, ephippia formation, or excessive fungal or bacterial growth.

Simocephalus serrulatus (water flea) - An indigenous breeding population of Simocephalus serrulatus was collected from Lake Jackson, Leon County, Florida, in March, 1980, by personnel from DER's Biology Section. Positive identification of the organisms was made by Mr. Jeff Foran of the University of Florida Department of Environmental Engineering Sciences. The cultures are maintained in two and one-half gallon all-glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. The organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, and are kept at a constant temperature of 22° plus or minus 2°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms. They are then refilled with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances such as: declining populations, ephippia formation, or excessive fungal or bacterial growth.

Mysidopsis bahia (mysid shrimp) - In September, 1978, approximately 300 adult mysid shrimp were obtained from the EPA laboratory in Gulf Breeze, Florida. The mysids are

cultured under a 16:8 hour light-dark cycle in 10 and 15 gallon all-glass aquaria equipped with undergravel filtration and aeration. The mysid shrimp are cultured over a wide range of salinities (10-30 ppt) to minimize acclimation periods to low or high salinity effluents. Seawater is obtained from the FSU Marine Laboratory located at Turkey Point, Florida (salinity range 17-31 ppt) and partial water changes are performed weekly. Freshwater, for dilution of the seawater as necessary, is obtained from a deep well near the DER laboratory. The mysids are fed twice daily with freshly hatched Artemia (brine shrimp) and are kept at a constant temperature of 22° plus or minus 2°C. Adult or large juvenile mysids were used in this test.

To ensure the uniform sensitivity of these test organisms to toxic substances, the Biology Section conducts monthly standard reference toxicant bioassays using sodium lauryl sulfate as the reference toxicant.

Test Methods

Three separate 48 hour static acute toxicity bioassays were performed on a sample of wastewater from Air Products and Chemicals Outfall #001. The tests were performed from 1030 hours on 31 January to 1045 hours on 2 February, 1984.

The sample was collected by Mr. Charles Logue of DER's Northwest District at 1155 hours on 30 January 1984, 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.

1. 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 that recommended by APHA (1980).

Two replicates, each with six concentrations and a control, were used to test with the water fleas, Daphnia pulex and Simocephalus serrulatus. 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 and Simocephalus were siphoned into large culture dishes to isolate the proper size and age organisms (neonates). To accomplish this, the water fleas were siphoned through a stainless steel screen. The trapped adults were then discarded. These neonates were then transferred by glass pipette, examined for injury, and were loaded 10 per chamber, resulting in 20 per test concentration. Water fleas were not fed during these tests.

2. Saltwater Test

Saltwater for dilution of the effluent sample was obtained from the FSU Marine Laboratory at Turkey Point (salinity = 27.0 ppt). For test concentrations of 56% and below, this diluent was mixed directly with the effluent, achieving a range of final salinities (12.5 ppt to 25.0 ppt). For the 100% effluent test concentration, freshly autoclaved artificial sea salts were added until a salinity of approximately 7 ppt was achieved (the lower limit of mysid shrimp salinity tolerance). Two control groups (Table 4) were then used to ensure that any mortality in the test

organisms was not the result of toxic properties associated with the diluent, the artificial sea salts, or the salinity range. The control groups included:

1) Diluent Control - Turkey Point water (27.0 ppt). This represents the highest concentration of Turkey Point water used in the bioassay, and tests for toxicity in the diluent. This is also the highest salinity used in the bioassay, and therefore tests the tolerance of the organisms to this salinity.

2) Sea Salts Control - Well water, adjusted with artificial sea salts to approximately 7 ppt salinity. This tests for toxicity of the highest concentration of artificial sea salts used in the bioassay, as well as for tolerance of the organisms to the lowest salinity used in the bioassay.

The concentration series selected for this study (Table 1) is that recommended by APHA (1980). Two replicates of each test concentration and of the controls were used to test with Mysidopsis bahia. Immediately prior to the bioassay, a suitable number of mysids were transferred by net from the 10 ppt, 15 ppt, 20 ppt and 25 ppt salinity aquaria to culture dishes for observation and selection of individuals for the tests. Individual mysid shrimp were siphoned using a glass tube, carefully examined for injury, and transferred to wide mouth quart jars, each containing 500 ml of the appropriate concentration. For each concentration, the mysid shrimp were pre-acclimated to within 4 ppt salinity. Five organisms were loaded per test chamber, giving ten per concentration. Based on the recommendations of EPA (1978), the mysids were fed brine shrimp (Artemia) nauplii at the rate of approximately 10 to 20 nauplii per mysid per day to minimize cannibalism.

Dissolved oxygen (YSI Model 57 oxygen meter), pH (Corning Model 7 pH meter with Orion Ross® combination electrode), temperature (NBS calibrated glass thermometer), and the number of live organisms were recorded for each test 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 freshwater tests for the controls and the 100% effluent concentration. The specific conductance or salinity (YSI Model 33 S-C-T meter) of each test concentration was recorded at the beginning and end of the test. All instruments were calibrated daily according to manufacturer's recommendations. Ammonia concentrations, including the total and unionized fraction (expressed in mg/l as N and as NH_3, respectively), were recorded in the controls and the 100% effluent test concentration at the beginning and end 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

Daphnia pulex and *Simocephalus serrulatus* (water fleas)

Concentration	Dilution Water	Effluent	Total Volume
Control	200.0 ml	--------	200 ml
5.6%	188.8 ml	11.2 ml	200 ml
10.0%	180.0 ml	20.0 ml	200 ml
18.0%	164.0 ml	36.0 ml	200 ml
32.0%	136.0 ml	64.0 ml	200 ml
56.0%	88.0 ml	112.0 ml	200 ml
100.0%	--------	200.0 ml	200 ml

Mysidopsis bahia (mysid shrimp)

Concentration	Dilution Water	Effluent	Total Volume
Controls	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 2 . Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Air Products and Chemicals, Pace, Santa Rosa County, Florida, NPDES #FL0002313, on 31 January to 2 February 1984

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)		Total Hardness (mg/l as $CaCO_3$)		Temperature (°c)			NH_3/N (mg/l)		Conductivity (µmhos)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	10	10	10	8.1	8.9	8.7	7.7	8.2	8.3	98		140					0/0	.01/.15		
Control	10	10	10	8.1	8.9	8.7	7.7	8.2	8.3					20.8	20.4	18.6			195	195
5.6%	10	10	10	8.3	8.9	8.7	7.7	8.2	8.2											
5.6%	10	10	10	8.3	8.9	8.7	7.7	8.2	8.2					20.8	20.0	18.4			220	220
10.0%	10	10	10	8.4	8.9	8.7	7.8	8.2	8.2											
10.0%	10	10	10	8.4	8.9	8.7	7.8	8.2	8.2					20.7	19.9	18.3			245	240
18.0%	10	10	10	8.6	9.0	8.8	7.8	8.2	8.2											
18.0%	10	10	10	8.6	9.0	8.8	7.8	8.2	8.2					20.5	19.9	18.3			280	270
32.0%	10	10	10	9.1	9.0	8.9	7.9	8.2	8.2											
32.0%	10	10	10	9.1	9.0	8.9	7.9	8.2	8.2					20.4	19.9	18.3			340	340
56.0%	10	9	5	10.4	9.1	9.0	8.1	8.2	8.2											
56.0%	10	7	5	10.4	9.1	9.0	8.1	8.2	8.2					20.0	20.0	18.5			470	450
100%	10	7	4	12.0	9.0	8.9	8.1	8.1	8.2	196		51								
100%	10	3	2	12.0	9.0	8.9	8.1	8.1	8.2					19.7	20.1	18.7	1.9 33.7	2.0 30.7	780	780

Remarks: Total Residual Chlorine: None Detected.

Table 3. Data recorded during 48 hour, Simocephalus serrulatus, static acute toxicity bioassay of Air Products and Chemicals, Pace, Santa Rosa County Florida, NPDES #F10002313, on 31 January to 2 February 1984

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)		Total Hardness (mg/l as $CaCO_3$)		Temperature (°c)			NH_3/N (mg/l)		Conductivity (µmhos)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	10	10	9	8.5	8.9	8.8	7.7	8.2	8.2	98		140		20.8	20.0	18.6	0/0	.01/.15	190	220
Control	10	9	7	8.5	8.9	8.8	7.7	8.2	8.2											
5.6%	10	10	10	8.5	8.9	8.6	7.7	8.2	8.2					20.7	19.9	18.5			215	210
5.6%	10	10	10	8.5	8.9	8.7	7.7	8.2	8.2											
10%	10	10	10	8.5	8.9	8.7	7.8	8.2	8.2					20.6	19.8	18.5			230	230
10%	10	10	10	8.5	8.9	8.7	7.8	8.2	8.2											
18%	10	10	10	8.8	9.0	8.6	7.9	8.2	8.2					20.5	19.8	18.5			285	270
18%	10	9	9	8.8	9.0	8.6	7.9	8.2	8.2											
32%	10	10	10	9.3	9.0	8.6	8.0	8.2	8.2					20.3	19.9	18.5			340	330
32%	10	10	8	9.3	9.0	8.6	8.0	8.2	8.2											
56%	10	9	5	10.4	9.0	8.7	8.1	8.2	8.2					19.9	19.9	18.5			400	420
56%	10	8	6	10.4	9.0	8.7	8.1	8.2	8.2											
100%	10	4	2	12.0	8.7	8.6	8.2	8.1	8.2	196		51		19.5	20.2	18.7	2.33/33.7	1.98/33.7	850	800
100%	10	5	0	12.0	8.7	8.5	8.2	8.1	8.1											

Remarks:

Total Residual Chlorine: None Detected

Table 4 . Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Air Products and Chemicals, Pace, Santa Rosa County, Florida, NPDES #FL0002313, on 3 January to 2 February 1984

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			NH_3/N (mg/l)		Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
Sea Water Control	5	4	4	7.3	7.1	7.6	8.2	8.1	7.9	20.4	19.9	18.0	0/0	.02/0.4		
Sea Water Control	5	5	5	7.6	7.1	7.6	8.2	8.1	7.9						27.0	28.5
Sea Salt Control	5	4	4	8.0	8.0	7.7	8.3	8.2	8.2	20.7	19.8	18.2				
Sea Salt Control	5	5	5	8.0	8.0	7.7	8.3	8.2	8.2						8.0	8.0
5.6%	5	5	5	7.5	7.0	7.0	8.2	8.1	7.9	20.4	19.8	18.5				
5.6%	5	5	5	7.5	7.0	7.0	8.2	8.1	7.9						25.0	25.5
10%	5	5	5	7.6	7.0	7.1	8.2	8.1	8.0	20.3	19.7	18.4				
10%	5	5	5	7.6	7.0	7.1	8.2	8.1	8.0						24.5	25.5
18%	5	5	5	8.1	7.1	7.2	8.2	8.1	8.0	20.0	19.6	18.3				
18%	5	5	5	8.1	7.1	7.3	8.2	8.1	8.0						22.5	23.0
32%	5	5	5	8.7	7.3	7.5	8.2	8.1	8.1	19.8	19.6	18.2				
32%	5	5	5	8.7	7.3	7.5	8.2	8.1	8.1						19.0	20.0
56%	5	3	3	10.1	7.8	8.0	8.2	8.2	8.2	19.5	19.7	18.0				
56%	5	4	4	10.1	7.8	8.0	8.2	8.2	8.2						12.5	13.0
100%	5	3	1	10.6	7.8	8.2	8.3	8.2	8.2	19.3	19.9	18.0	2.59/32.0	2.38/38.5		
100%	5	2	1	10.6	7.8	8.2	8.3	8.2	8.2						7.0	8.0

Remarks:

Total Residual Chlorine: None Detected

Table 5. LC_{50} Data for Bioassays of Air Products and Chemicals, NPDES #FL0002313, on 31 January to 2 February, 1984.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
D. pulex	Binomial	100.0% (56 and ∞),a	56.0% (32 and ∞)
	Moving Average	100.0% (75 and ∞),a	65.8% (55 and 82),b
	Probit	96.3% (78 and 150),a,b	68.8% (57 and 86)
S. serrulatus	Binomial	93.5% (56 and ∞)a	59.3% (32 and 100)
	Moving Average	93.5% (75 and 199), a	58.1% (48 and 70),b
	Probit	102.4% (77 and 196), a,b	56.3% (47 and 70)
M. bahia	Binomial	100.0% (32 and ∞),a	70.3% (32 and ∞)
	Moving Average	100.0% (0 and ∞),a	70.2% (56 and 100)
	Probit	91.9% (67 and 236), a,b	71.3% (56 and 96),b

[a] 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.

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

Table 6. Results of chemical analyses performed on Wastewater from Air Products and Chemicals, NPDES #FL0003213, collected on 30 January, 1984.

Organics

Acid Extractables	N.D.
Base Neutral Extractables	3 Unidentified Compounds
Pesticide Extractables	N.D.

Metals

Antimony	N.D.
Arsenic	6.8 µg/l
Beryllium	N.D.
Cadmium	N.D.
Chromium	293 µg/l
Copper	N.D.
Lead	–[a]
Mercury	<0.2 µg/l[b]
Nickel	N.D.
Selenium	<6.8 µg/l[b]
Silver	N.D.
Thallium	N.D.
Zinc	81 µg/l

N.D. = None Detected
[a] The lead analysis was invalidated.
[b] Actual valve is known to be less than value given.

RESULTS AND DISCUSSION

The results of the third series of acute toxicity bioassays performed on wastewater form Air Products and Chemicals indicated that the wastewater was acutely toxic to all three test organisms, Daphnia pulex, Simocephalus serrulatus and Mysidopsis bahia. A 24 hour LC_{50} of 96.3% and a 48 hour value of 65.8% were calculated for the freshwater test organism, D. pulex (Table 5). Statistically similar 24 and 84 hour LC_{50}'s of 102.4% and 58.1%, respectively, were calculated for our indigenous freshwater test organism, S. serrulatus (Table 5). Likewise, the 24 and 48 hour LC_{50}'s calculated for the saltwater organism, Mysidopsis bahia, 91.9% and 71.3%, respectively (Table 5), were similar to the values calculated for the freshwater organisms. The 96 hour LC_{50}'s for these test organisms would be less than or equal to their 48 hour values.

These latest results were very similar to the results of the first series of bioassay tests performed on this wastewater, in February, 1980, in which LC_{50} values of 35% and 47% were obtained for D. pulex and M. bahia, respectively (FDER 1980). The toxicity at that time could not be attributed to specific causes, but it was suspected that an algal growth stimulant, such as ammonia, may have been present. The toxicity observed in these latest tests can be attributed mostly to the presence of ammonia, since significant concentrations of total ammonia (32.0 mg/l to 33.7 mg/l) and unionized ammonia (1.9 mg/l to 2.59 mg/l) were measured in the wastewater (Tables 2, 3 and 4). According to Thurston, et al. (1981), the toxicity of unionized ammonia to certain sensitive freshwater fish begins at approximately 0.2 mg/l. The concentrations of unionized ammonia measured in this wastwater were significantly above this value. It was also noted that certain biological and chemical parameters (green algae present and high dissolved oxygen concentrations) were observed in both the first series of tests (February 1980) and in these latest tests (February 1984).

To further chemically characterize this wastewater, the DER Chemistry Section has performed analyses for certain metals and organics (Table 6). These analyses indicated the presence of three unidentified base neutral organic compounds and various concentrations of the metals arsenic (6.8 µg/l), chromium (293 µg/l) and zinc (81 µg/l). These chemical components may very well have contributed to the toxicity noted in the wastewater. Particular emphasis should be placed on the chromium, since concentrations of hexavalent chromium as low as 19 µg/l to 81 µg/l were reported as being acutely toxic to Daphnia magna (U.S. EPA 1980).

Discussion of the Toxicity Criteria

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

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

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

From the above discussion, it should be clear that if the least stringent toxicity standard, the 96 hour LC_{50}, is exceeded at any point, from the point of discharge outwards, by any discharger, then a violation of the standards has occurred. This is an important consideration since the test

procedure here reported involves the measurement of the 50% lethal concentration, and not some fraction of that concentration. Any discharger required to meet the one-twentieth of the 96 hour LC_{50} standard is therefore being tested very leniently. Any reduction in the test period from 96 hours provides an added degree of leniency to a discharger.

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

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

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

The toxicity reported here is in excess of allowable limits.

LITERATURE CITED

American Public Health Association. 1980. Standard Methods for the Examination of Water and Wastewater. 15th ed. 1134 p.

Florida Department of Environmental Regulation, Biology Section. 1980. Bioassay of Air Products and Chemicals, Inc., Santa Rosa, Florida. NPDES #FL0002313. FDER. 10 p.

Florida Department of Environmental Regulation, Biology Section. 1981. Bioassays of Air Products and Chemicals, Inc., Santa Rosa County, Florida. NPDES #FL0002313. FDER. 14 p.

Florida Department of Environmental Regulation, Biology Section. 1983. Quality Assurance Manual for Performing Acute Toxicity Tests. FDER. 22 p.

Peltier, W. 1978. Methods for measuring the acute toxicity of effluents to aquatic organisms. Environmental Research Laboratory. Athens, Georgia. EPA-600/4-78-012 (Revised July 1978). 51 p.

Thurston, R.V., Russo, R.C. and G.A. Vinogradov. 1981. Ammonia toxicity to fishes: Effect of pH on the toxicity of the unionized species. Env. Sci. Tech. 15(7): 837-840.

U.S. Environmental Protection Agency. 1978. Bioassay procedures for the Ocean Disposal Permit Program. Environmental Research Laboratory. Gulf Breeze, Florida. EPA-600/9-78-010. 121 p.

U.S. Environmental Protection Agency. 1980. Ambient Water Quality Criteria for Chromium. U.S. EPA, Washington, D.C. EPA 440/5-80-035. 105 p.