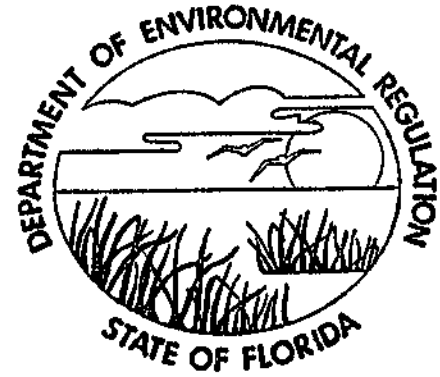


BIOASSAYS OF AIR PRODUCTS AND CHEMICALS, INC.

PACE, SANTA ROSA COUNTY, FLORIDA

NPDES #FL0002313

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APRIL 30, 1987

Biology Section
Division of Environmental Programs

BIOASSAYS OF AIR PRODUCTS AND CHEMICALS, INC.

PACE, SANTA ROSA COUNTY, FLORIDA

NPDES #FL0002313

Biology Section
Bureau of Laboratories and
Special Programs
April 30, 1987

EXECUTIVE SUMMARY

Air Products and Chemicals, Inc., NPDES #FL0002313, on 21 to 23 January, 1987.

The sample of wastewater collected from this chemical and fertilizer manufacturer proved to be acutely toxic to the test organisms, Ceriodaphnia dubia and Notropis leedsi, producing 48 hour LC_{50}'s of 96.2% and 44.8%, respectively. Mortality in the Daphnia pulex test was irregularly distributed and insufficient to calculate an LC_{50}. The observed toxicity was probably due to the concentrations of total ammonia (21.2 mg/l) and unionized ammonia (0.52 mg/l) measured in the effluent. However, the results of chemical analyses indicated the presence of several unidentified pesticide components and the metals chromium (115.0 ug/l) and zinc (49 ug/l). The presence of these organic and metal components, particularly the chromium, may well have contributed 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, January 1985, and September 1986). 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 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 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.

In January, 1985, the Biology Section performed on-site 96 hour flow-through and 48 hour static tests on Air Products' final effluent. Results indicated that the wastewater was acutely toxic to M. bahia (96 hour LC_{50} = 68.3%) and to P. promelas (48 hour LC_{50} = 70.3%), but was not toxic to D. pulex (FDER 1985a). 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.

To again evaluate the existence and extent of toxicity in Air Products' effluent, the Northwest District requested that acute toxicity bioassays be performed. Two 48 hour static bioassays were performed from 14 to 16 January, 1986, on a sample of final effluent. The results indicated that Air Products' effluent was acutely toxic to Notropis leedsi (48 hour LC_{50} = 39.9%), but was not toxic to Daphnia pulex (FDER 1986a). Toxicity was mostly attributed to ammonia.

During the late summer of 1986, fish kills were noted in Escambia Bay, which were thought to be related to the Air Products discharge since high levels of chlorine produced oxidants (CPO's) were detected in both the effluent and in the bay itself. The Northwest District requested that acute toxicity bioassays be performed to determine if indeed the CPO's detected in Air Products' discharge were responsible for the fish kills. Three separate 48 hour bioassays were performed using Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi. No toxicity was demonstrated in any of these tests, even though two separate methods of analysis indicated CPO concentrations as high as 31 mg/l. A third method of analysis did not detect any residual chlorine (a major CPO). Therefore, it is believed that the CPO's measured in Air Products' wastewater were either a result of interferences in the sample, or were bound in some way to make them unavailable to the test organisms (FDER 1986b).

In January, 1987, three definitive static acute toxicity bioassays were performed on a sample collected from Air Products' Outfall #001. Results are presented in this report. Those involved in performing the tests and in preparation of this report were: K. M. Lurding , M. L. Roll, and P. G. Weiland.

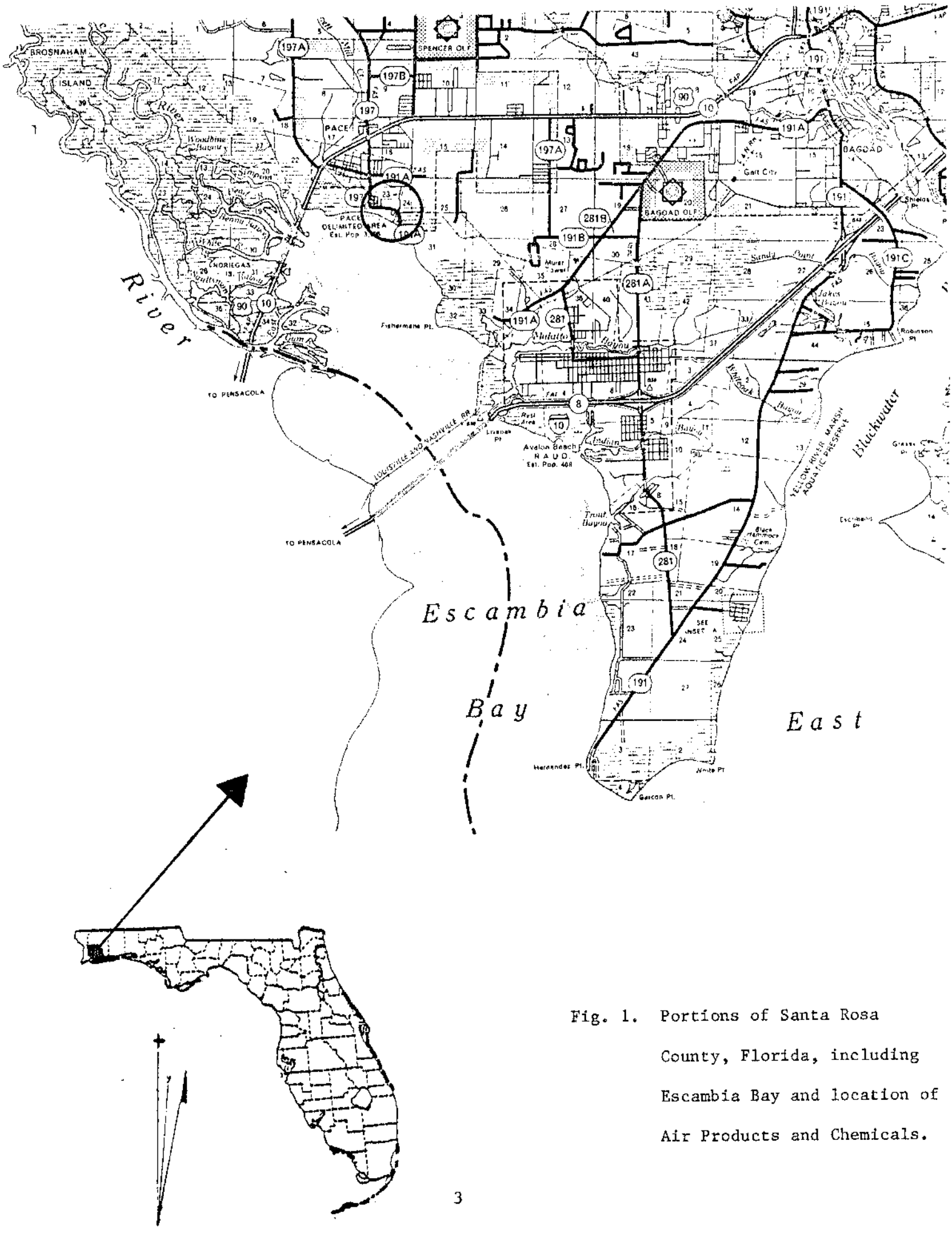
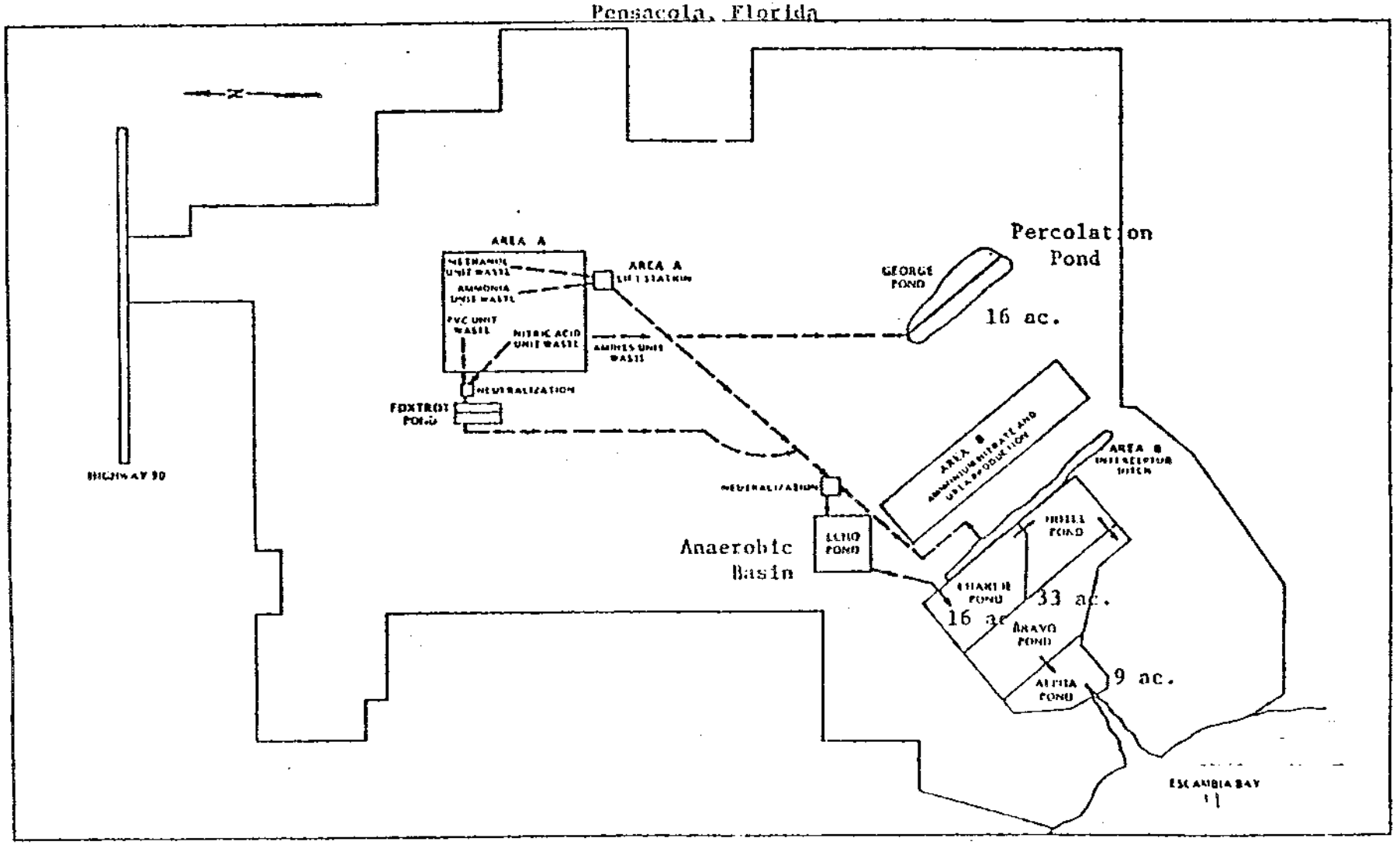


Fig. 1. Portions of Santa Rosa County, Florida, including Escambia Bay and location of Air Products and Chemicals.

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 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 (1985b). 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 for this system is unchlorinated and is pumped from the DER deep well. The bannerfin shiners are reared at 22° ± 2°C under a 16:8 hour light-dark cycle and are fed Tetra-Min® and frozen Artemia (brine shrimp) twice daily, and once daily on weekends. Breeding and egg hatching are encouraged at 25°C. The eggs are laid between stacked red ceramic tiles separated by a 3mm bead of silicone. Most of the eggs adhere to the tiles and are easily removed to heated, aerated hatching tanks. The narrow space between the tiles eliminates egg predation by adult fish. New hatching tanks are started regularly to segregate fry

into even age groups. Newly hatched fry are fed a liquefied blend of Purina Trout Chow® and Cerophyl® twice daily. After one week, feeding is augmented with newly hatched Artemia nauplii and finely crushed Tetra Min®. The fish used in this test were 12 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 Air Products' wastewater effluent collected from outfall #001. The tests were performed from 1030 hours on 21 January to 1030 hours on 23 Janaury, 1987.

The samples were collected by Mr. Stephen Baisden of DER's Northwest District at 1348 hours on 20 January, 1987, 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 samples was obtained from the same DER deep well that is used to culture the test organisms. The concentration series selected for this study (Table 1) is 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 either 60 ml (for C. dubia) or 250 ml (for D. pulex) capacity were used as test chambers, and either 50 ml or 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 through 4.

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

Ceriodaphnia dubia (water flea)

Concentration	Dilution Water	Effluent	Total Volume
Control	50.0 ml	--------	50 ml
10.0%	45.0 ml	5.0 ml	50 ml
32.0%	34.0 ml	16.0 ml	50 ml
56.0%	22.0 ml	28.0 ml	50 ml
100.0%	--------	50.0 ml	50 ml

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
56.0%	88.0 ml	112.0 ml	200 ml
100.0%	--------	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

Table 2. Data recorded during 48 hour, Ceriodaphnia dubia, static acute toxicity bioassay on Air Products, NPDES #FL0002313, on 21 to 23 Janaury, 1987.

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	7.8	8.4	8.8	8.1	7.7	8.0	20.9	20.2	19.8			Control Water
Control	10	9	9	7.8	8.4	8.8	8.1	7.7	8.0						NH_3(as NH_3)(mg/l) 0 / Total Alkalinity 117 (mg/l $CaOO_3$)
10.0%	10	9	9	7.9	8.6	8.8	8.1	7.8	8.0	20.6	20.0	19.7			
10.0%	10	9	9	7.9	8.6	8.8	8.1	7.8	8.0						Total ammonia (as N)(mg/l) 0 / Total Hardness 115 (mg/l $CaOO_3$)
32.0%	10	10	10	8.1	8.5	8.7	8.0	7.9	8.0	20.5	20.0	19.6			
32.0%	10	10	10	8.1	8.5	8.7	8.0	7.9	8.0						
56.0%	10	10	10	8.2	8.6	8.6	7.9	8.0	8.0	20.5	20.0	19.6			
56.0%	10	10	10	8.2	8.6	8.6	7.9	8.0	8.0						
100.0%	10	6	4	8.8	7.9	8.0	7.8	8.0	8.1	20.5	20.0	19.7			
100.05	10	6	5	8.8	7.9	8.0	7.8	8.0	8.1						100% Effluent
															NH_3(as NH_3)(mg/l) .65 / Total Alkalinity 355 (mg/l $CaOO_3$)
															Total ammonia (as N)(mg/l) 21.2 / Total Hardness 65 (mg/l $CaOO_3$)

Table 3. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay on Air Products, NPDES #FL0002313, on 21 to 23 January, 1987.

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	9	9	7.9	8.2	8.5	8.1	7.7	7.9	20.9	20.2	19.7			Control Water
Control	10	10	10	7.9	8.2	8.5	8.1	7.7	7.9				220	235	NH_3(as NH_3)(mg/l) 0 — Total Alkalinity 117 (mg/l $CaOO_3$)
10.0%	10	10	8	8.0	8.5	8.5	8.1	7.8	7.9	20.8	20.0	19.6			
10.0%	10	10	10	8.0	8.5	8.5	8.1	7.8	7.9				290	290	
32.0%	10	9	9	8.2	8.6	8.4	7.9	7.9	8.0	20.7	19.8	19.6			Total ammonia (as N)(mg/l) 0 — Total Hardness 115 (mg/l $CaOO_3$)
32.0%	10	10	10	8.2	8.6	8.4	7.9	7.9	8.0				440	430	
56.0%	10	10	10	8.4	8.1	7.4	7.8	7.9	7.9	20.6	19.9	19.5			
56.0%	10	10	10	8.4	8.1	7.4	7.8	7.9	7.9				690	680	
100.0%	10	10	10	9.2	7.5	6.2	7.7	7.9	7.9	20.6	20.1	19.6			
100.05	10	10	10	9.2	7.5	6.2	7.7	7.9	7.9				1000	990	100% Effluent
															NH_3(as NH_3)(mg/l) .52 — Total Alkalinity 355 (mg/l $CaOO_3$)
															Total ammonia (as N)(mg/l) 21.2 — Total Hardness 65 (mg/i $CaOO_3$)

Table 4. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of Air Products, NPDES #FL0002313, on 21 to 23 January, 1987.

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	7.7	8.0	8.2	7.5	7.5	7.7	21.2	20.2	19.9		
Control	5	5	5	7.7	8.0	8.2	7.5	7.5	7.7				210	215
10.0%	5	5	5	7.8	8.2	8.2	7.6	7.6	7.7	21.0	20.0	19.5		
10.0%	5	5	5	7.8	8.2	8.2	7.6	7.6	7.7				280	275
32.0%	5	5	4	8.0	8.2	7.6	7.6	7.7	7.7	20.8	19.9	19.5		
32.0%	5	5	5	8.0	8.2	7.6	7.6	7.7	7.7				435	425
56.0%	5	0	0	8.3	7.4	6.5	7.7	7.8	7.7	20.7	19.9	19.5		
56.0%	5	2	2	8.3	7.4	6.5	7.7	7.8	7.7				690	680
100.0%	5	0	0	8.9	6.6	-a	7.7	7.8	-a	20.1	20.2	-a		
100.05	5	0	0	8.9	6.6	-	7.7	7.8	-				1000	-a

Control Water

NH_3(as NH_3)(mg/l) 0

Total Alkalinity 117 (mg/l $CaCO_3$)

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

Total Hardness 115 (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) .52

Total Alkalinity 355 (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) 21.2

Total Hardness 65 (mg/l $CaCO_3$)

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

Table 5. LC_{50} Data for Bioassay of Air Products and Chemicals, Inc., NPDES #FL0002313, 21 to 23 January, 1987.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Ceriodaphnia dubia	Binomial	a	96.2% (56 and ∞)
	Moving Average	a	a
	Probit	472.1% (0 and ∞), b	193.3% (0 and ∞), b
Daphnia pulex	Binomial	a	a
	Moving Average	a	a
	Probit	a	a
Notropis leedsi	Binomial	50.0% (32 and 100)	44.4% (32 and 100)
	Moving Average	a	42.0% (30.2 and 57.2)
	Probit	b	44.8% (35.8 and 55.9), c

[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, results calculated using the probit method are not statistically sound.

[c] This LC_{50} is reported in the text 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 #FL0002313, collected on 20 Janaury, 1987.

Organics	
Acid Extractables	N.D.
Base Neutral Extractables	N.D.
Pesticide Extractables	Several unidentified components

Metals	
Antimony	N.D.
Arsenic	< 3.0 ug/l[a]
Beryllium	N.D.
Cadmium	N.D.
Chromium	115.0 ug/l
Copper	N.D.
Lead	N.D.
Mercury	N.D.
Nickel	N.D.
Selenium	N.D.
Silver	N.D.
Thallium	N.D.
Zinc	49 ug/l

N.D. = None Detected
[a] Actual value is known to be less than value given.

RESULTS AND DISCUSSION

Results of bioassays performed on the sample of effluent collected from Air Products and Chemicals, Inc., which produces a wide variety of industrial and agricultural chemicals, are presented in Tables 2-4. In tests using Ceriodaphnia dubia, a 48 hour LC_{50} of 96.2% was calculated (Table 5). In the Daphnia pulex test, the distribution of mortality was irregular and was not sufficient to determine an LC_{50} (Table 3). The bioassay using Notropis leedsi as the test organism, however, indicated 24 and 48 hour LC_{50}'s of 50.0% and 44.8%, respectively. This toxicity can mostly be attributed to the presence of ammonia. The total ammonia measured in this sample was 21.2 mg/l, and unionized ammonia was 0.52 mg/l (Table 4). According to Thurston et al. (1981), the toxicity of unionized ammonia to certain sensitive freshwater fish begins at approximately 0.2 mg/l. Unionized ammonia levels detected in the Air Products' wastewater were significantly higher.

To further chemically characterize this effluent, the DER Chemistry Section performed analyses for certain metals and organics (Table 6). These analyses indicated the presence of several unidentified pesticide components, as well as the metals chromium (115.0 ug/l) and zinc (49 ug/l). These chemical components probably contributed to the observed toxicity, particularly the chromium. Concentrations of hexavalent chromium as low as 19 ug/l to 81 ug/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 onetwentieth 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, whetheras 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.

From the above discussion, it should be clear that if the least stringent toxicity standard, the 96 hour LC_{50}, is exceeded at any point, from the point of discharge outwards, by any discharger, then a violation of the standards has occurred. This is an important consideration since the test procedure here reported involves the measurement of the 50% lethal concentration, and not some fraction of that concentration. Any discharger required to meet the one-twentieth of the 96 hour LC_{50} standard is therefore being tested very leniently. Any reduction in the test period from 96 hours provides an added degree of leniency to a discharger.

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

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

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

The toxicity reported here is in excess of allowable limits.

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