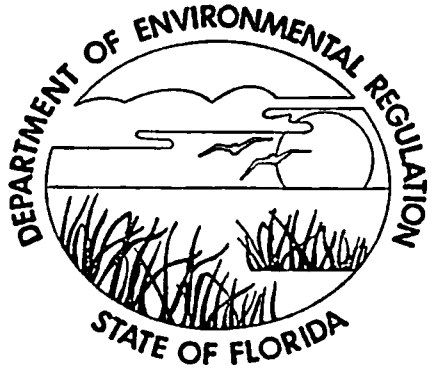


BIOASSAYS OF AIR PRODUCTS AND CHEMICALS, INC.

PACE, SANTA ROSA COUNTY, FLORIDA

NPDES #FL0002313

OCTOBER 8, 1986

Biology Section
Division of Environmental Programs

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Biology Section
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Special Programs
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EXECUTIVE SUMMARY

Air Products, Santa Rosa County, NPDES #FL0002313, on 3 to 5 September, 1986.

The Air Products facility is a large chemical manufacturing complex which discharges treated wastewater to Escambia Bay. During the late summer of 1986, fish kills were noted in the bay which were thought to be related to the Air Products' discharge, since high levels of chlorine produced oxidants (CPO) were detected in both the effluent and in the bay itself.

The Biology Section performed three 48 hour static acute toxicity bioassays on a sample of this effluent, using the freshwater test organisms, 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 anlaysis 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.

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, and January 1985). The first series of tests indicated that the wastewater was acutely toxic to both the freshwater test organisms, 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 resutls 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 bioassys 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, 1986). 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 is Air Products' discharge were responsible for the fish kills. Three separate 48 hour bioassays were performed from 3 to 5 September, 1986. The results of these latest tests are presented in this report. those involved in performing the tests and in preparation of this report were: R. B. Frydenborg, M. L. Roll, and P. G. Weiland.

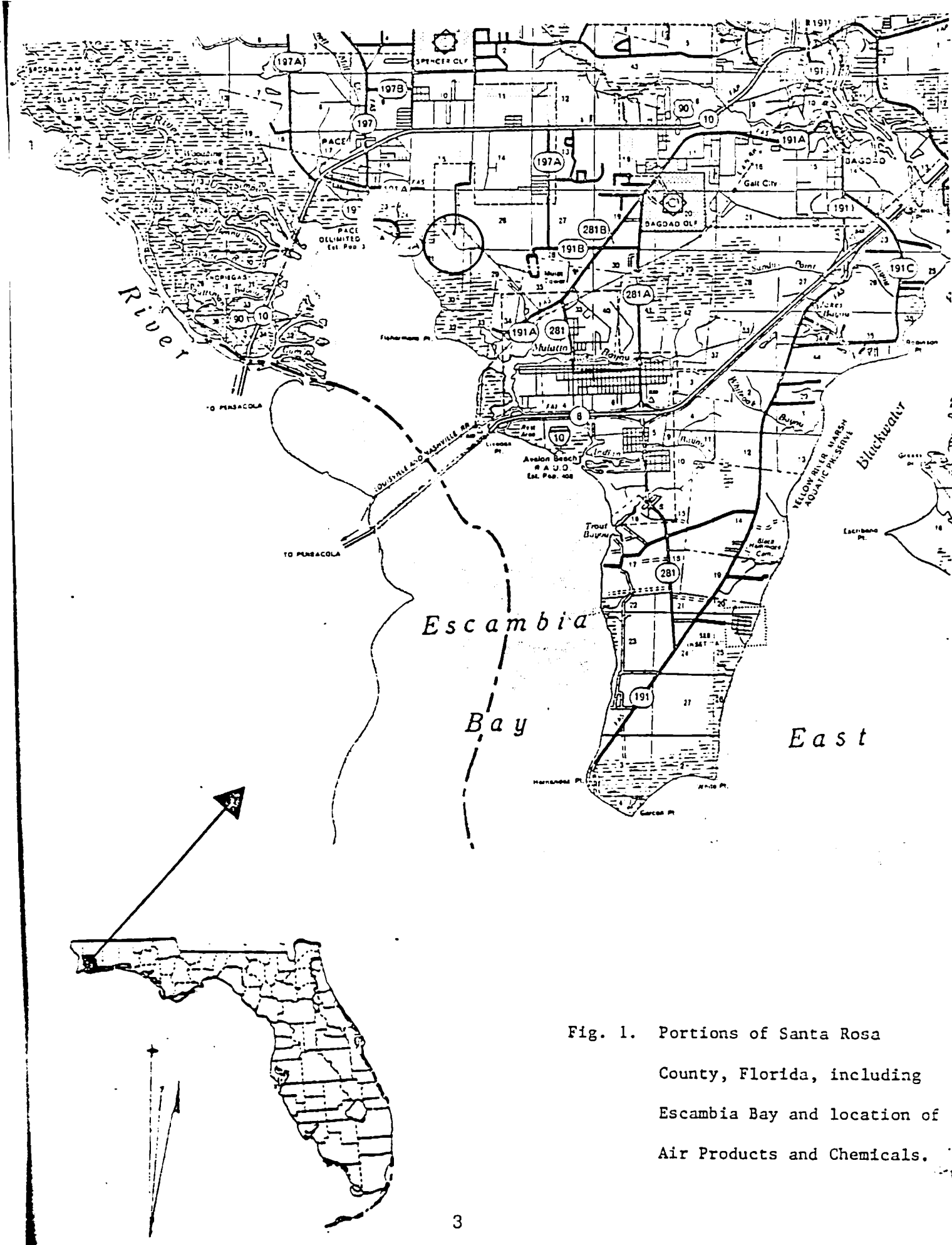
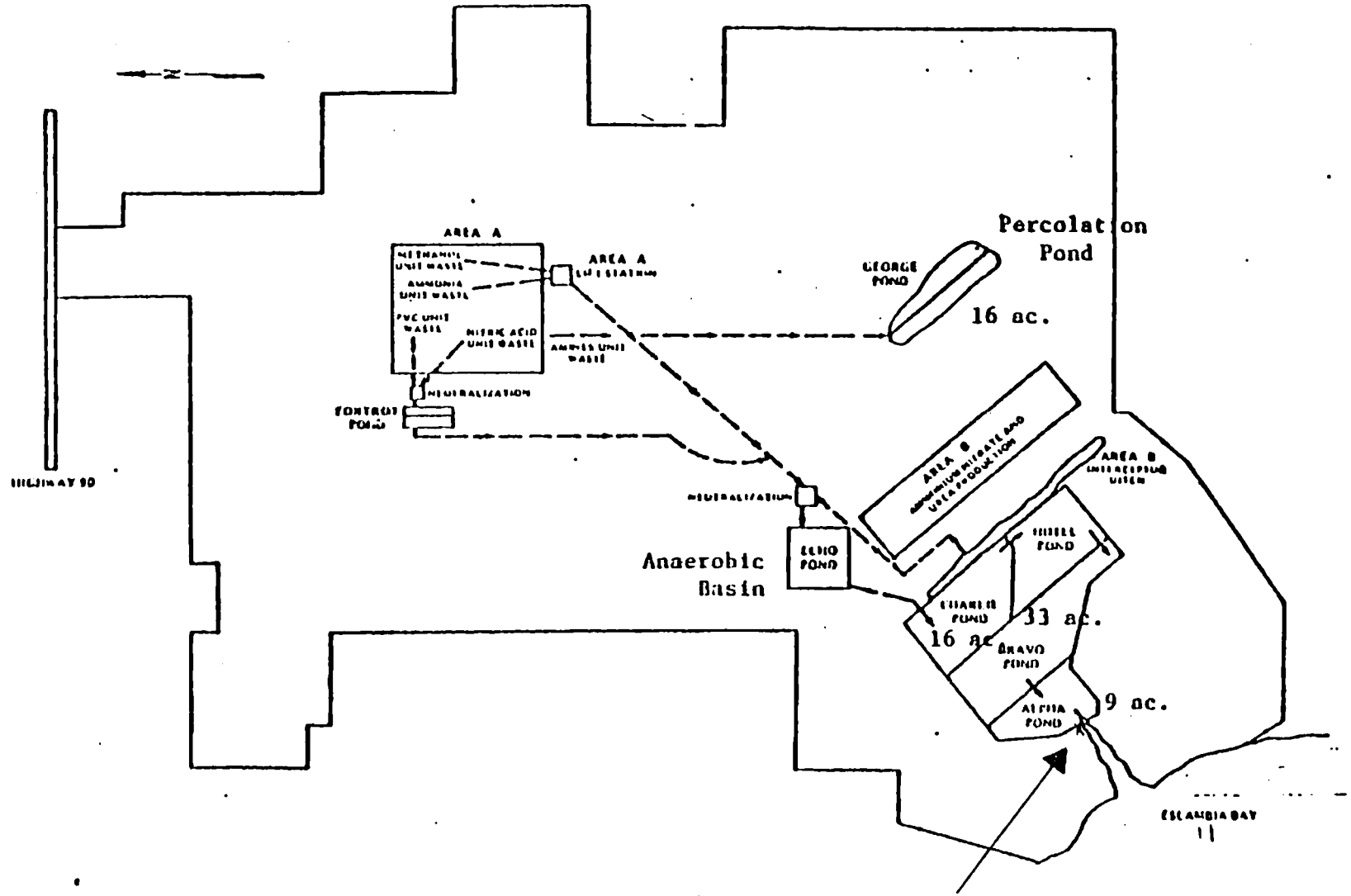


Fig. 1. Portions of Santa Rosa County, Florida, including Escambia Bay and location of Air Products and Chemicals.

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



final treatment pond and the NPDES Outfall #001.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier and Weber (1985) and the FDER Biology Section (1985). The organisms selected for this study include species recommended by Peltier and Weber (1985), the U.S. EPA (1978), and the FDER Biology Section (1985). Additionally, the Biology Section maintains cultures of several species of organisms indigenous to the State of Florida. One of these indigenous species was also selected for testing. Life histories of these test organisms and maintenance procedures used to culture them are detailed below.

History and Maintenance of Bioassay Organisms

Daphnia pulex (water flea) - A breeding culture of Daphnia pulex was obtained from the EPA laboratory in Athens, Georgia, in March, 1979. This culture has been maintained since its arrival in 2 1/2 gallon all-glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. Organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, once daily on weekends, and are kept at a constant temperature of 22° ± 2°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms, and then refilling with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances such as: declining populations, ephippia formation, or excessive fungal or bacterial growth.

Ceriodaphnia dubia (water flea) - A breeding culture of Ceriodaphnia dubia was obtained from the EPA laboratory in Athens, Georgia, in June, 1985. The culture is maintained in 2-1/2 gallon all-glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. Organisms are fed a liquified blend of Purina Trout Chow®, Cerophyl® and Fleischmann's® yeast twice daily, once daily on weekends, and are kept at a constant temperature of 26° ± 2°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms, and then refilling with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances such as: declining populations, ephippia formation, or excessive fungal or bacterial growth.

Notropis leedsi (bannerfin shiner) - Approximately 40 adult bannerfin shiners were obtained from an indigenous population in the upper Suwannee River during October, 1981. The breeding stock is maintained in 30 and 125 gallon all-glass aquaria equipped with a flow-through water exchange system. Water 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 approximately 13 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 (1985). 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 1015 hours on 3 September to 1015 hours on 5 September, 1986.

The sample was collected by Mr. Steve Baisden of DER's Northwest District at 1440 hours on 2 September, 1986, using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples for metal analyses were preserved with HNO_3. Samples for organic chemical analyses and for the bioassays were unpreserved. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 24 hours of collection.

Test Temperature

Upon arrival of the iced (4°C) bioassay samples in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the samples allowed to rise to 20° $\pm$ 2°C. Although this temperature may be different than the temperature of the sample during collection, it is the temperature range to which the test organisms are acclimatized and is the range recommended in the above referenced bioassay protocols. This avoids stressful effects caused by exposure to unusually cold or warm samples.

Freshwater Tests

Freshwater for dilution of the effluent 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 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 or 250 ml 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 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.

Two replicates, of each concentration and a control, were used for the Notropis leedsi (bannerfin shiner) bioassay. The fish were transferred individually or by twos, into wide mouth quart jars containing 500 ml each of the appropriate test concentration, and were loaded 5 per chamber, resulting in 10 per concentration. Fish were not fed during this test.

Test Parameters

Dissolved oxygen (YSI Model 57 oxygen meter), pH (Corning Model 7 pH meter with Orion Ross® combination electrode), temperature (NBS calibrated glass thermometer), and the number of live organisms were recorded for each test concentration at 0 hours, 24 hours, and 48 hours. Total alkalinity and total hardness (measured as $CaCO_3$ using HACH Chemical Company reagents) were recorded at the beginning of the test for the controls and the 100% effluent concentration. The specific conductance (YSI Model 33 S-C-T meter) of each test concentration was recorded at the beginning and end of the test. All instruments were calibrated daily according to manufacturer's recommendations. Ammonia concentrations, including the total and unionized fraction (expressed in mg/l as N and as NH_3, respectively), were recorded in the controls and the 100% effluent test concentration at the beginning of the test. Total ammonia concentrations were determined using an Orion® specific ion probe and an Orion Ionalyzer®. The unionized fraction was then calculated based on the total ammonia concentration, and the pH, temperature, and salinity of the sample. These values were recorded on the bioassay data forms and are presented in Tables 2, 3, and 4.

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

Ceriodaphnia dubia (water flea)

Concentration	Dilution Water	Effluent	Total Volume
Control	50.0 ml	--------	50 ml
10.0%	45.0 ml	5.0 ml	50 ml
18.0%	41.0 ml	9.0 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
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

Notropis leedsi (bannerfin shiner)

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

TABLE 2. Data recorded during 48 hour, Ceriodaphnia dubia, static acute toxicity bioassay of Air Products, Santa Rosa County, NPDES #FL0002313, on 3 to 5 September, 1986.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Control	10	10	10	9.9	8.4	9.1	7.7	7.9	7.9	20.6	19.9	19.3		
Control	10	10	10	9.9	8.4	9.1	7.7	7.9	7.9					
10.0%	10	10	10	9.9	8.5	9.2	7.8	8.0	8.0	20.6	19.8	19.3		
10.0%	10	10	10	9.9	8.5	9.2	7.8	8.0	8.0					
32.0%	10	10	10	9.7	8.5	9.3	7.9	8.0	8.0	20.6	19.8	19.4		
32.0%	10	10	10	9.7	8.5	9.3	7.9	8.0	8.0					
56.0%	10	10	10	9.5	8.6	9.3	7.9	8.1	8.2	21.0	19.8	19.4		
56.0%	10	10	10	9.5	8.6	9.3	7.9	8.1	8.2					
100.0%	10	10	10	9.2	8.7	9.4	7.9	8.3	8.3	21.5	19.9	19.4		
100.0%	10	10	10	9.2	8.7	9.4	7.9	8.3	8.3					

Control Water

NH_3(as NH_3)(mg/l) 0.01

Total Alkalinity 114 (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) 0.5

Total Hardness 109 (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) 0.24

Total Alkalinity 207 (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) 5.8

Total Hardness 35 (mg/l $CaCO_3$)

Chlorine Produced Oxidants: 31 mg/l (PAO Method)
29 mg/l (Iodometric Method)
0 mg/l (HACH DPD Method)

TABLE 3. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Air Products, Santa Rosa County, NPDES #FL0002313, on 3 to 5 September, 1986.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Control	10	10	10	10.2	8.8	9.0	8.0	7.8	7.8	19.8	19.7	19.2		
Control	10	10	10	10.2	8.8	9.0	8.0	7.8	7.8				190	212
10.0%	10	10	10	10.2	8.6	9.1	8.0	7.9	7.8	19.9	19.6	19.0		
10.0%	10	10	10	10.2	8.6	9.1	8.0	7.9	7.8				230	248
32.0%	10	10	10	10.0	8.6	9.2	8.0	8.0	7.9	20.4	19.5	19.1		
32.0%	10	9	9	10.0	8.6	9.2	8.0	8.0	7.9				330	332
56.0%	10	10	10	9.8	8.7	9.3	8.0	8.1	8.1	21.2	19.5	19.0		
56.0%	11	11	11	9.8	8.7	9.3	8.0	8.1	8.1				435	440
100.0%	10	10	10	9.2	8.8	9.4	7.9	8.2	8.3	21.5	19.6	19.2		
100.0%	10	10	10	9.2	8.8	9.4	7.9	8.2	8.3				700	700

Control Water

NH_3(as NH_3)(mg/l) 0.02

Total Alkalinity 114 (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) 0.5

Total Hardness 109 (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) 0.21

Total Alkalinity 207 (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) 5.8

Total Hardness 35 (mg/l $CaCO_3$)

Chlorine Produced Oxidants: 31 mg/l (PAO Method)
29 mg/l (Iodometric Method)
0 mg/l (HACH DPD Method)

TABLE 4. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of Air Products, Santa Rosa County, NPDES #FL0002313, on 3 to 5 September, 1986.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)		
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	
Control	5	5	5	10.2	8.7	9.4	8.1	8.0	8.0	20.0	19.5	19.2			Control Water
Control	5	5	5	10.2	8.8	9.4	8.1	8.0	8.0				200	217	NH_3(as NH_3)(mg/l) 0 — Total Alkalinity 114 (mg/l $CaCO_3$)
10.0%	5	5	5	10.2	8.7	9.3	8.0	8.0	8.0	20.3	19.4	19.0			
10.0%	5	5	5	10.2	8.7	9.3	8.0	8.0	8.0				250	252	
32.0%	5	5	5	10.0	8.6	9.2	8.0	8.0	8.0	20.6	19.2	18.9			Total ammonia (as N)(mg/l) 0 — Total Hardness 109 (mg/l $CaCO_3$)
32.0%	5	5	5	10.0	8.6	9.2	8.0	8.0	8.0				342	340	
56.0%	5	5	5	9.8	8.6	9.2	7.9	8.0	8.1	21.7	19.4	18.9			
56.0%	5	5	5	9.8	8.6	8.2	7.9	8.0	8.1				455	440	
100.0%	5	5	5	8.8	8.5	9.4	7.9	8.3	8.3	22.9	19.5	19.1			
100.0%	5	5	5	8.8	8.5	9.4	7.9	8.3	8.3				750	700	100% Effluent
															NH_3(as NH_3)(mg/l) 0.26 — Total Alkalinity 207 (mg/l $CaCO_3$)
															Total ammonia (as N)(mg/l) 5.8 — Total Hardness 35 (mg/l $CaCO_3$)

Chlorine Produced Oxidants: 31 mg/l (PAO Method)
29 mg/l (Iodometric Method)
0 mg/l (HACH DPD Method)

RESULTS AND DISCUSSION

The sample of wastewater collected from Air Products' outfall #001 was not acutely toxic to the test organisms, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi. No noteable mortality was observed during the 48 hour static bioassays. These results were somewhat of a surprise since two methods of analysis for detecting chlorine produced oxidants measured up to 31 mg/l of this substance(s) in the sample. However, a method for measuring residual chlorine detected none of this substance. Therefore, it is believed that either the CPO readings were caused by interferences in the sample, or the substances were somehow bound and biologically unavailable to the test organism.

The ammonia measured in the sample, 5.8 mg/l total and 0.26 mg/l unionized, was apparently insufficient to have caused any mortality. Since the sample was not acutely toxic, further chemical anlayses were not performed.

Recommendation

We recommend that the source and cause of the chlorine produce oxidant readings in Air Products' wastewater be investigated and explained. Further investigation should be made to determine the exact effects of these substances on the biota of the receiving waters. Otherwise, routine inspection and biomonitoring of the Air Products facility and wastewater should continue as scheduled.

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