BIOASSAY OF AIR PRODUCTS AND CHEMICALS, INC.

SANTA ROSA, FLORIDA

NPDES PERMIT #FL0002313

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Biological Section
Bureau of Water Analysis
February 13, 1980

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INTRODUCTION

Air Products & Chemicals, Inc., is located adjacent to upper Escambia Bay near Pensacola, Florida (Fig. 1). This facility operates continuously, producing agricultural chemicals, PVC plastics, industrial organics and inorganics including chemical intermediates. Wastewater is passed through treatment facilities that include equalization, neutralization and a series of biological ponds (Fig. 2). The effluent has a calculated retention time, depending on flow, of 60 - 90 days (from EPA Bioassay report included in appendix). The plant discharges an average of 1.65 MGD of effluent into Escambia Bay.

On 13 February 1980 the Biological Section received effluent samples from outfall #001 of this facility and performed static bioassays to determine the existence and the extent of toxicity to the biota of the receiving water.

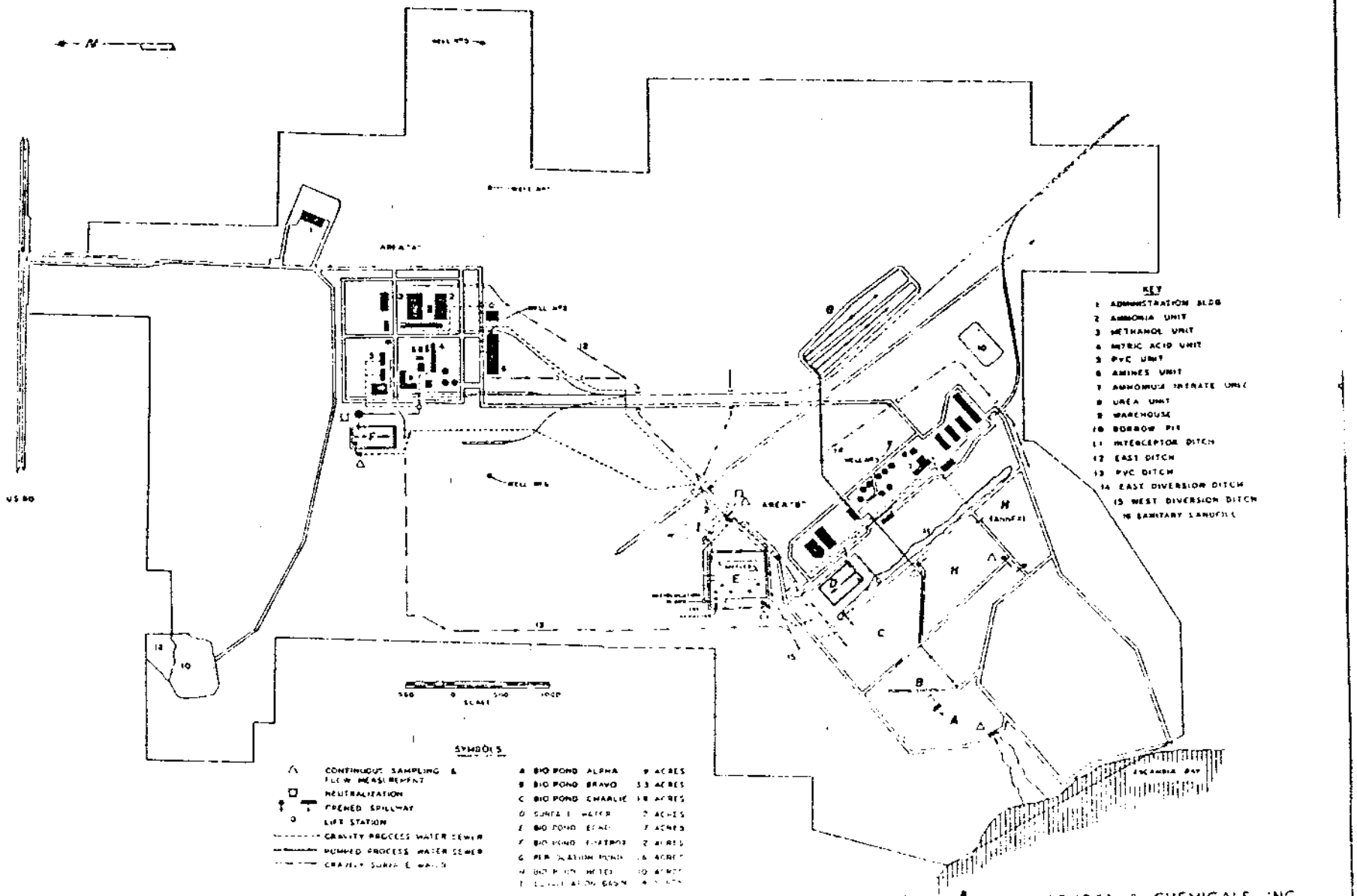


Figure 1. AIR PRODUCTS & CHEMICALS INC.
ESCAMBIA PLANT
WATER ABATEMENT SYSTEM

METHODS AND MATERIALS

Test methods used for conducting these static bioassays are extensively described by Peltier (1978). The organisms selected for this study are among those recommended by Peltier (1978) and the U.S. EPA (1978). 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 on March 9, 1979. This culture has been maintained continually since its arrival, in 2½ gallon all glass aquaria using a 16:8 hour light-dark cycle. Organisms have been fed a liquified blend of Purina Trout Chow^{R} and Cerophyl^{R} twice daily. Daphnia used in this bioassay were siphoned into a large culture dish through a series of nested stainless steel screens to isolate the proper size and age organisms (neonates). These neonates were then pipetted individually from the culture dish and placed randomly into the test chambers at the beginning of the test.

Mysidopsis bahia (Mysid shrimp)

On 19 September 1978 approximately 300 adult mysid shrimp (Mysidopsis bahia) were obtained from the Gulf Breeze EPA Laboratory in Pensacola, Florida. The mysids were cultured under continuous lighting in four 15 gallon and two 10 gallon aquaria equipped with undergravel filtration. Sea water was obtained from the FSU Marine Laboratory located at Turkey Point, Florida (salinity range 17-31 o/oo). The mysids were fed twice daily with freshly hatched Artemia salina (brine shrimp) and kept at a constant temperature of $22^{\circ} \pm 1^{\circ}C$.

Immediately prior to the bioassay, a suitable number of mysids were transferred by net to a culture dish for observation and selection of individuals for the test. Sub-adult shrimp (10 days - 2 weeks old) were selected for this bioassay. These organisms selected for testing were transferred individually by glass tube and randomly placed in the test chambers.

Test Methods

Two separate 48 hour static bioassays were conducted from 13 February to 15 February 1980 on a 24 hr. composite sample of effluent from outfall #001. The sample was collected by Mr. Rick Bradburn of DER's Northwest District Office on 12 February 1980 using standard wastewater sampling procedures.

Separate samples were obtained for chemical and biological testing. Samples for metal analysis were preserved with HNO_3 (2ml/liter). Sample for organic chemical analysis and the bioassays were unpreserved. The samples were iced and transported to the DER laboratory in Tallahassee within 24 hours of collection.

Freshwater for dilution of the effluent sample, in the Daphnia pulex test, the same City of Tallahassee well that is used to culture the test organisms. The concentrations series selected for this study are those recommended by APHA (1976)(Table 1).

Two replicates, each with six concentrations and a control, were used to test with Daphnia pulex. Flint glass jars of 250 ml capacity were used as test chambers, 200 ml of the appropriate test concentration was placed in each chamber (Table 1). Daphnia were transferred individually by glass pipette and were loaded 10 per chamber, resulting in 20 per test concentration.

Dissolved oxygen, pH, and the number of live organisms were recorded for each test chamber at 0 hours, 24 hours, and 48 hours. Total alkalinity, total hardness and conductivity were recorded at the beginning of the test for the controls and the 100% concentration. These values were recorded daily on the bioassay data forms and are presented in Table 2. Organisms were not fed during this test.

Saltwater for dilution of the effluent sample in the Mysidopsis bahia test was obtained from the FSU marine laboratory at Turkey Point (Salinity = 23.5 o/oo). To minimize salinity differences between the various concentrations, freshly autoclaved artificial sea salt (Marinemix^R) was added to the effluent sample and mixed with a magnetic stirrer until the dry salt mix dissolved and a salinity of approximately 20.0 o/oo was achieved.

Two replicates, each of six concentrations of effluent and a control, were used to test with Mysidopsis (Table 1). Individual mysid shrimp were siphoned or pipetted via glass tube, carefully examined for injury and randomly transferred to wide mouth quart jars, each containing 500 ml of the appropriate concentration. The concentration series selected for this study (Table 1) are those recommended by APHA (1976).

Five organisms were loaded per test chamber giving 10 per concentration (Table 3). Salinity was measured in all chambers at the beginning of the saltwater test. Temperature, pH, dissolved oxygen and the number of live organisms were recorded at 0, 24 and 48 hours (Table 3). "Although it is generally undesirable to feed most fish and macroinvertebrates during static toxicity tests, an exception is necessary for M. bahia" (RPA 1978). Therefore, mysids were fed brine shrimp (Artemia) nauplii daily at a rate of approximately 10 - 20 per mysid to minimize cannibalism among the mysids.

Table 1

CONCENTRATION SERIES AND VOLUMES USED FOR THESE STATIC BIOASSAYS

Mysid shrimp (*Mysidopsis bahia*)

Concentration	Seawater diluent	Effluent	Total Vol.
Control	500 ml	0 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%	0 ml	500 ml	500 ml

Water flea (*Daphnia pulex*)

Concentration	Freshwater diluent	Effluent	Total Vol.
Control	200 ml	0 ml	200 ml
5.6%	189 ml	11 ml	200 ml
10.0%	180 ml	20 ml	200 ml
18.0%	164 ml	36 ml	200 ml
32.0%	136 ml	64 ml	200 ml
56.0%	88 ml	112 ml	200 ml
100.0%	0 ml	200 ml	200 ml

Table 2. Data recorded during 48-hour, *Daphnia pulex* static acute toxicity bioassay performed on Air Products and Chemicals, Inc., located in Escambia County, Florida. 13 February to 15 February 1980. NPDES Permit #FL0002313. Outfall #001.

Conc. or %	Test Container Number	Number of Live Organisms					Dissolved Oxygen (mg/l)					pH					Total Alkalinity (mg/l as $CaCO_3$)					Total Hardness (mg/l as $CaCO_3$)					Conductivity (umhos)				
		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	10	10	10			9.6	9.7	9.7			8.5	9.0	8.1			125					140					240				
Control	B	10	10	10			9.5	9.8	9.7			8.5	9.0	8.2													220				
5.6	A	10	10	10			9.4	10.2	10.4			8.4	8.9	8.2																	
5.6	B	10	10	10			9.4	10.1	10.4			8.4	8.8	8.2																	
10.0	A	10	9	9			9.4	10.6	10.7			8.3	8.8	8.3																	
10.0	B	10	10	10			9.4	10.5	10.7			8.3	8.8	8.3																	
18.0	A	10	10	9			9.5	11.2	11.1			8.2	8.3	8.4																	
18.0	B	10	9	8			9.5	10.9	11.2			8.3	8.8	8.4																	
32.0	A	10	9	7			9.5	11.9	12.1			8.2	8.8	8.5																	
32.0	B	10	9	9			9.7	11.8	12.0			8.2	8.8	8.5																	
56.0	A	10	8	0			9.8	12.6	13.0			8.1	8.7	8.5																	
56.0	B	10	7	2			9.9	12.3	12.9			8.1	8.7	8.5																	
100	A	10	0	-			10.0	12.8	-			8.0	8.6	-													800				
100	B	10	0	-			10.1	12.4	-			8.0	8.6	-			210					85					800				

Remarks:

Total residual chlorine = 0.0 at 100% concentration.

Green algae present in effluent.

Table 3. Data recorded during 48-hour, *Mysidopsis bahia* static, acute toxicity bioassay performed on Air Products and Chemicals, Inc., located in Escambia County, Florida. 13 February to 15 February 1980. NPDES Permit #FL0002313. Outfall #001.

Conc. or %	Test Container Number	Number of Live Organisms					Dissolved Oxygen (mg/l)					pH					Total Alkalinity (mg/l as $CaCO_3$)					Total Hardness (mg/l as $CaCO_3$)					Salinity (o/oo)				
		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			10.2	8.7	8.5			7.6	7.3	7.6													24				
Control	B	5	5	5			9.9	8.9	8.2			7.7	7.4	7.6													24				
5.6	A	5	5	5			9.9	8.5	8.3			7.8	7.5	7.7													23				
5.6	B	5	5	5			9.9	8.8	8.4			7.8	7.5	7.7													23				
10.0	A	5	5	5			10.0	8.5	8.6			8.0	7.6	7.8													23				
10.0	B	5	5	5			10.0	8.6	8.7			8.0	7.6	7.8													23				
18.0	A	5	5	5			10.1	8.3	7.5			8.1	7.8	7.8													23				
18.0	B	5	5	5			10.0	8.3	7.6			8.2	7.8	7.9													23				
32.0	A	5	5	5			10.0	7.8	6.6			8.3	8.0	7.9													22.5				
32.0	B	5	4	4			10.0	7.9	7.1			8.4	8.0	8.0													22.5				
56.0	A	5	4	0			10.0	7.2	6.2			8.5	8.2	8.1													21.5				
56.0	B	5	5	3			10.0	7.5	6.4			8.6	8.2	8.1													21				
100	A	5	0	-			10.1	5.8	-			8.7	8.3	-													19.5				
100	B	5	0	-			10.0	6.3	-			8.8	8.4	-													19.5				

Remarks:

Autoclaved artificial sea salts added to effluent to adjust salinity to 20.0 o/oo.
Total residual chlorine = 0.0 at 100% concentration.
Green algae present in effluent.
Shrimp size & age - 10 days - 2 weeks old.

RESULTS & DISCUSSION

Effluent from outfall #001 at Air Products and Chemicals, Inc. was toxic. The 48 hour LC_{50} for Daphnia pulex was calculated to be 35%, and for the marine species, Mysidopsis bahia, the 48 hour LC_{50} was 47% (Table 4). Little information was available regarding the chemical composition of this effluent, which precluded any speculation as to a specific cause for the toxicity.

Chapter 17-3.051, Florida Administrative Code, indicates that "all waters of the State shall at all places and at all times be free from...components of discharges which...are acutely toxic". "Acute toxicity" is defined in Chapter 17-3.021(1) to mean "the presence of one or more substances... in amounts which are greater than one-third (1/3) of the amount lethal to 50% of the test organisms in 96 hours (96 hr LC_{50})...". Mixing zones are not relevant to this criterion, and the restriction on acute toxicity, therefore, applies at the point of discharge. Essentially this provision means that an effluent at the point of discharge can not cause 50% mortality in the test organisms even if the effluent is concentrated three-fold. Since the testing procedure used here does not involve any concentrating of the effluent, and since mortality is only measured for 48 hours, any toxicity reported here may be considered to be substantially in excess of allowable limits.

Table 4. LC_{50} Data

Date of Sample	Organism	Type of Test	Method of Data Analysis	24 hr LC_{50}	48 hr LC_{50}
13 February 80	*Daphnia pulex*	Static (Freshwater)	Binomial test	65.5%	40.3%
			Moving average method	60.1%	34.4%
			Probit method	57.5%	34.6%
13 February 80	*Mysidopsis bahia*	Static (Saltwater)	Binomial test	70.4%	47.0%
			Moving average method	66.4%	47.0%
			Probit method	64.5%	47.3%

LITERATURE CITED

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