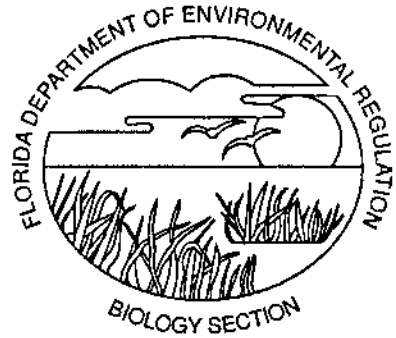


Bioassays of

Air Products and Chemicals, Inc.

Pace, Santa Rosa County, Florida

NPDES #FL0002313

Sampled 3/14/88

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September 19, 1988

Biology Section
Division of Administrative and Technical Services

Bioassays of

Air Products and Chemicals, Inc.

Pace, Santa Rosa County, Florida

NPDES #FL0002313

Biology Section
Bureau of Laboratories
September 19, 1988

Executive Summary

Air Products and Chemicals, Inc., Pace, Santa Rosa County, Florida, NPDES #FL0002313, on 15 to 17 March, 1980.

The sample from this facility was toxic to the bioassay test organism, *Notropis leedsi*, producing an LC_{50} of 74.8%. the sample was not toxic to the bioassay test organisms *Ceriodaphnia dubia* or *Daphnia pulex*. The sample was toxic to the Microtox® assay organism, *Photobacterium phosphoreum* producing an EC_{50} of 132.55%.

The total ammonia and unionized ammonia concentrations in the sample were 5.6 mg/l and 0.08 mg/l, respectively. Further chemical analyses detected three unidentified components sensitive to Electron Capture Detection (ECD) and the metals arsenic (4 µg/l), chromium (128 µg/l), and zinc (29 µg/l).

The unionized ammonia (0.08 mg/l) was of sufficient concentration to have caused the observed mortality in the *N. leedsi* test and is believed to have been the cause of mortality.

Introduction

Air Projects and Chemicals, Inc., is located on upper Escambia Bay near Pace, Santa Rosa County, Florida (Figure 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 (Figure 2). The latter wastes are retained in a series of ponds for 50 to 90 days prior to being discharged through a ditch to Escambia Bay (Figure 2). This discharger has been assigned a mixing zone.

The potential toxicity of this recovered water 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 test indicated that the sample 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 sample at that time, so a cause of the toxicity was not attributed. However, review of certain data presented in the report (green algae present in the sample 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 recovered water 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 recovered water 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 Product's final effluent. Results indicated that the recovered water 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' recovered water at least once, by performing on-site flow-through and static bioassays on January 7 to 11, 1979. Results of these tests indicted that the recovered water 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.

Two 48 hour static bioassays were performed from 14 to 16 January, 1986, on a sample of final recovered water. The results indicated that Air Products' recovered water 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' recovered water 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. The sample was 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 toxicity was attributed to the concentrations of total ammonia (21.2 mg/l) and unionized ammonia (0.52 mg/l) measured in the sample. Chemical analyses indicated the presence of several unidentified pesticide components and the metals, chromium (1150 µg/l) and zinc (49 µg/l) (FDER 1987).

To again evaluate the existence and extent of toxicity in the discharge from this facility, the Biology Section, at the request of the FDER Northwest District, performed three acute toxicity bioassays and one Microtox® assay on a sample from this facility. ?The results of these tests are presented in this report. Those involved in performing the tests and in preparation of this report were: D. A. Chasteen, J. M. Faircltoh, L. A. Williams, and S. H. Wolfe.

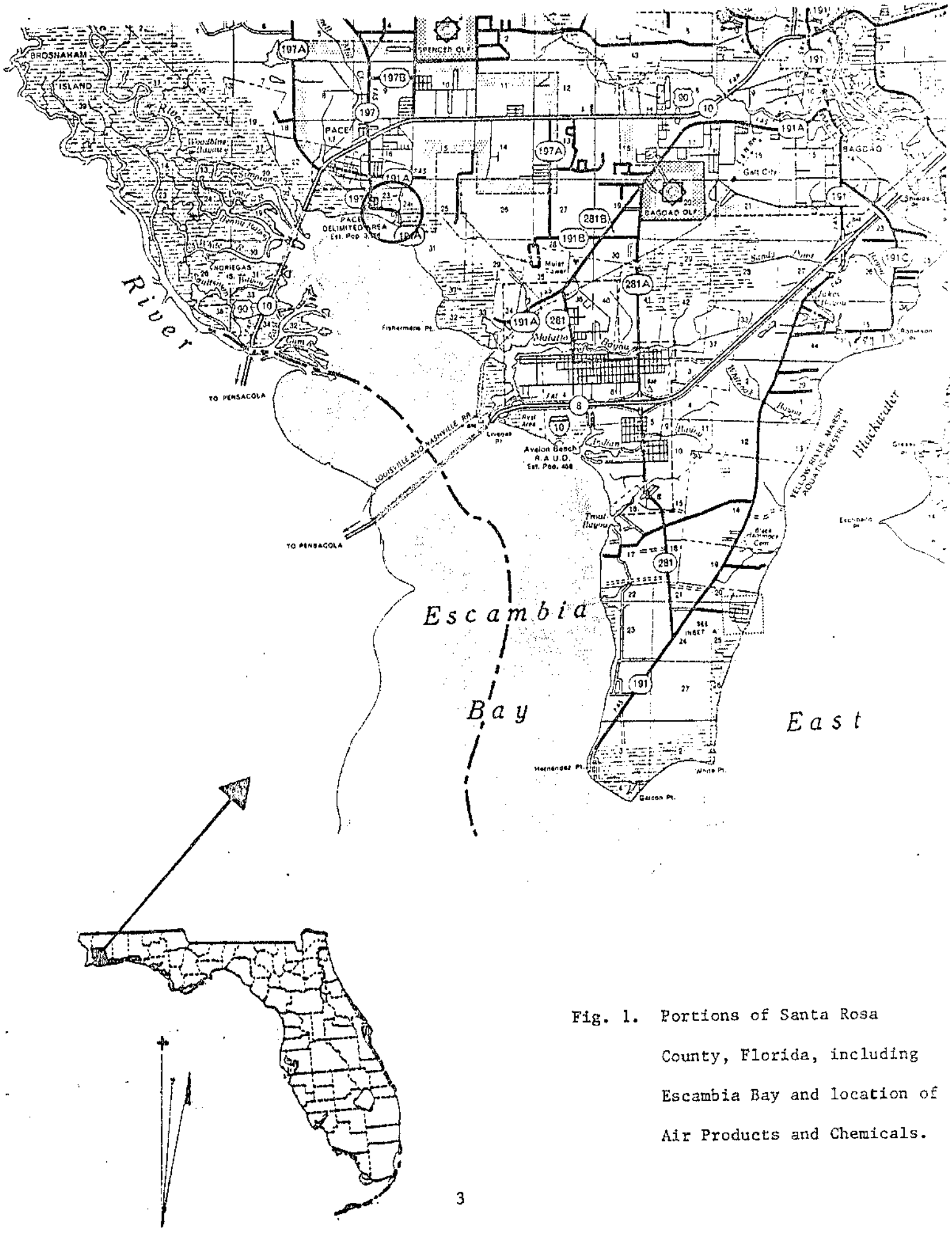
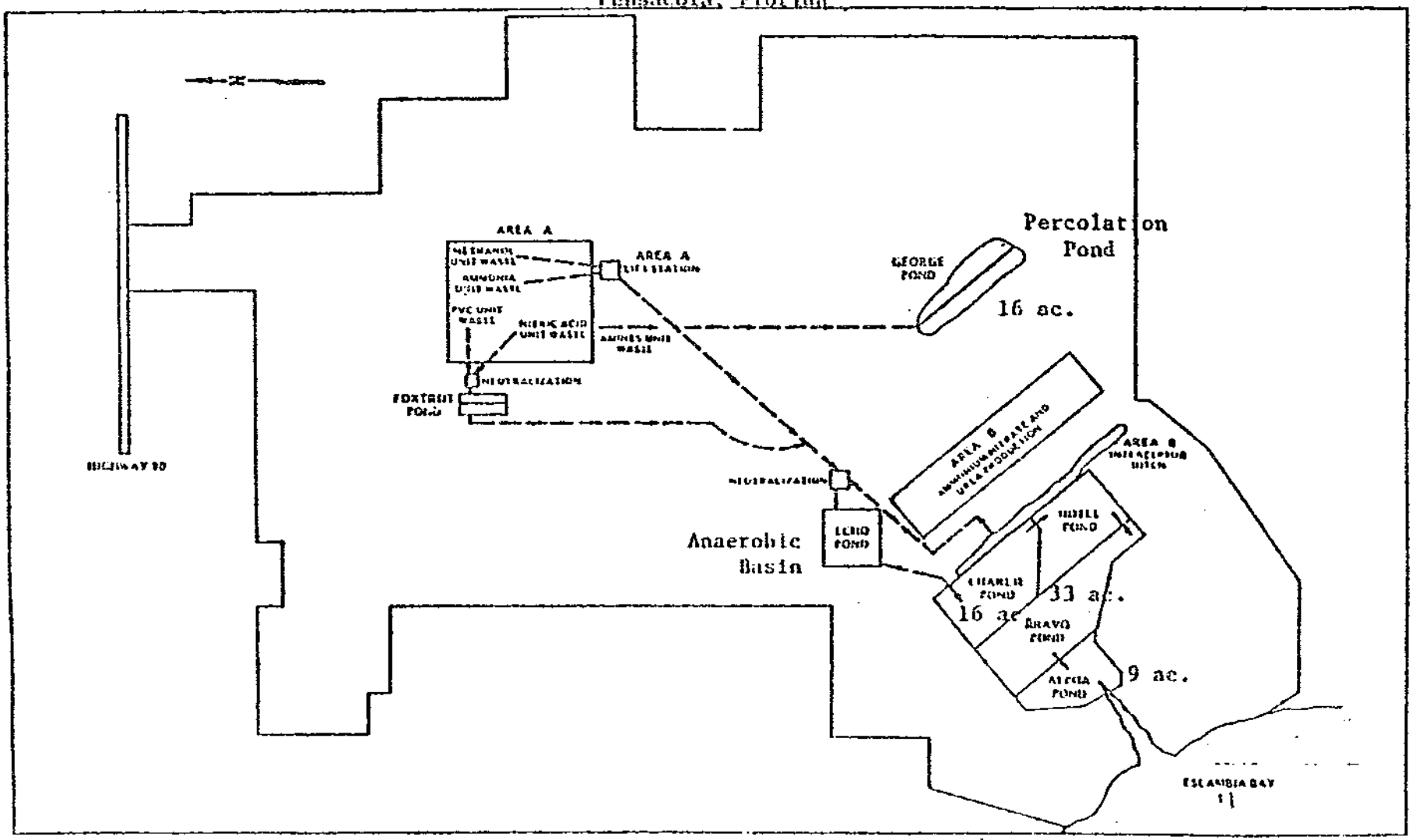


Fig. 1. Portions of Santa Rosa County, Florida, including Escambia Bay and location of Air Products and Chemicals.

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



MAJOR SOURCE STREAMS AND WASTEWATER TREATMENT FACILITIES

ENGINEERING REPORT ON ATTAINMENT OF FINAL NPDES REQUIREMENT

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 (1985). The organisms selected for this study include a species recommended by Peltier and Weber (1985) 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.

The Microtox® assay was run according to the manufacturer's protocol described in the Microtox® System Operating Manual (Beckman 1982).

1. 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 June, 1987. 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 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 Aquarian® flake food 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 Aquarian® flake food. The fish used were 27 days old when the test commenced.

Photobacterium phosphoreum, a luminescent marine bacterium, is maintained as a freeze-dried culture, purchased from Microbics Corporation. Lot number M-606 was used in this assay. Immediately prior to the assay, the culture is rehydrated with 1.0 ml of specially purified water (Microtox Reconstitution Solution).

2. Standard Reference Toxicity Assays

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.

3. 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-hr acute definitive toxicity bioassays and one Microtox® assay were performed on a sample from the Air Products facility outfall #001. The tests were performed from 1040 hrs on 15 March, to 1040 hrs on 17 March, 1988.

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 collected by Mr. Barry Long of the FDER Northwest district office in Pensacola at 1110 hours on 14 March, 1988, using standard wastewater sampling procedures. 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 ± 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.

Aeration

Since the sample to be used for bioassay analysis contained a dissolved oxygen concentration of less than 40% of saturation, the entire sample was aerated to approximately 6.0 mg/l. This was achieved using a single airstone connected to the laboratory compressed air supply. Additionally, since the dissolved oxygen concentrations in certain individual test chambers had decreased to near 40% of saturation (due to BOD), then each of these affected test chambers and the controls were also aerated. The exact amounts and levels of aeration are noted on the bioassay data forms.

Method

Freshwater for diluting the water samples being tested was obtained from the same DER deep well that is used to culture the test organisms. The concentration series selected for this study is given in Table 1.

Two replicates of the control and each test concentration were used in bioassays 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, respectively, 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. Ten were loaded per chamber, resulting in 20 per test concentration. *Daphnia* were siphoned into a stainless steel screen chamber placed into a large culture dish to isolate the proper size and age organisms (neonates). Then the screen chamber along with any retained adults were removed. The neonates remaining were then captured by glass pipette, examined for injury, and loaded 10 per chamber, resulting in 20 per test concentration. The 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/bioassays]. The fish were transferred individually or by twos using a pipette into wide mouth quart jars containing 500 ml each of the appropriate test concentration. Five were loaded 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 Fisher Accuphast® 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% reclaimed water 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 before use 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% reclaimed water 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 Table 2.

Microtox® Test

Upon receipt at the DER laboratory, a 20 ml subsample was collected for Microtox® analysis. The sample was adjusted to 2% salinity with reagent grade dry sodium chloride to maintain the osmotic balance of the test bacterium during the assay. Four two-fold dilutions were made of the test sample resulting in a test range of 97%, 49.5%, 24.75%, 12.38%, and 0%. An incubation temperature of 15 °C was maintained throughout the assay. Ten µl of bacterial suspension was added to each of the 1.0 ml test dilutions. Light output (luminescence) was read after 5 and 15 minutes incubation, using the Microtox® analyzer and recorded on a Perkin-Elmer strip chart recorder. In the presence of a toxicant, the luminescence of the test organisms will decrease. In the presence of a stimulant, the luminescence will increase. Toxicity is calculated as a function of the light lost in the test sample, normalized to the control, and is reported as the effective concentration at which a 50% reduction, of light output is determined in 15 minutes (EC_{50}). Results are given in Table 3.

Results and Discussion

The sample of recovered water from the Air Products facility proved toxic to the bioassay test organism, *Notropis leedsi*, and to the Microtox® assay test organism, *Photobacterium phosphoreum*, producing an LC_{50} of 74.8% (Table 4) and an EC_{50} of 132.6% (Table 3). The sample was not toxic to the bioassay test organisms *Ceriodaphnia dubia* or *Daphnia pulex*.

Chemical analysis detected a total ammonia concentration of 5.6 mg/l and an unionized ammonia concentration of 0.08 mg/l. Further chemical analyses detected three unidentified components sensitive to Electron Capture Detection (ECD) and the metals arsenic (4 µg/l), chromium (128 µg/l), and zinc (29 µg/l) (Table 5).

The unionized ammonia (0.08 mg/l) was sufficient to have contributed to the observed mortality in the *N. leedsi* test. Unionized ammonia concentrations as low as 0.01 mg/l have been shown to have toxic effects on test organisms (U.S. EPA 1973).

As noted in the raw data sheet for the *N. leedsi* test, the dissolved oxygen content of the test water (100% sample) decreased at 24 hours. This decrease was not less than 40% saturation, however, the sample was aerated to prevent a potentially lethal decrease in the dissolved oxygen level.

In conclusion, the concentration of unionized ammonia in the sample may have contributed to the observed mortality in the N. leedsi bioassay test. The sample from this facility was toxic on 14 March, 1988.

Biology Section Recommendations

The Biology Section recommends that this facility be required to meet state toxicity standards or to undertake a Toxicity Reduction Evaluation with the goal of meeting those standards.

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

Ceriodaphnia dubia (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	50.0 ml	0.0 ml	50 ml
5.6%	47.2 ml	2.8 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%	0.0 ml	50.0 ml	50 ml

Daphnia pulex (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	200.0 ml	0.0 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%	0.0 ml	200.0 ml	200 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Sample	Total Volume
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

Table 2. Data recorded during 48 hour static definitive bioassay of the discharge of Air Products and Chemicals, Inc., Pace, Santa Rosa County, Florida NPDES #FL0002313 on 15 to 17 March, 1988.

Facility:	**Air Products and Chemicals**			NPDES #:	**FL0002313**	Facility Type:	**Industrial**
Location:	Milton, Florida	County:	Santa Rosa	Contact/District:	Barry Long		
				Test type:	Static definitive		
Sample Collection Date:	3/14/88	Time:	1110	# tests:	3	Receiving Water:	Escambia Bay
Test Beginning Date:	3/15/88	Time:	1030	Chlorination Type:	**Nonchlorinated**		
Test Ending Date:	3/17/88	Time:	1030		**Page 1 of 2**		

	Parameter	
Total Residual Cl2	0.0	-
Method:	Hach	-
Salinity (ppt):	0	

Ammonia:	Control Water	100% Sample
Unionized NH3:	0.00	0.08
Total NH3:	0.00	5.60

Organism: ***Ceriodaphnia dubia*** Life stage: neonate
LC50: Binomial: - Moving Average: - Probit: -
-

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentration:	# at 0 hr	Live at 24 hr	Live at 48 hr	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	48 hour
Control	10	10	10	9.0	9.0	8.8	8.2	7.9	8.0	19.4	18.8	19.2	-	-
Control	10	10	10	9.0	9.0	8.9	8.2	7.9	8.1	-	-	-	-	-
5.6%	10	10	10	9.0	8.9	8.9	8.0	8.0	8.1	19.4	18.6	19.0	-	-
5.6%	10	10	10	9.0	8.9	8.9	8.0	8.0	8.1	-	-	-	-	-
10%	10	10	10	9.0	8.9	9.0	7.8	8.0	8.1	19.5	18.4	18.9	-	-
10%	10	10	10	9.0	8.9	9.0	7.8	8.0	8.0	-	-	-	-	-
18%	10	10	10	9.0	9.0	8.8	7.5	8.0	8.1	19.5	18.4	19.0	-	-
18%	10	10	10	9.0	9.0	8.8	7.5	8.0	8.1	-	-	-	-	-
32%	10	10	10	9.0	8.8	8.6	7.4	8.0	8.1	19.4	18.4	18.9	-	-
32%	10	10	10	9.0	8.8	8.6	7.4	8.0	8.1	-	-	-	-	-
56%	10	10	10	9.0	8.4	8.1	7.3	8.0	8.1	19.5	18.4	19.1	-	-
56%	10	10	10	9.0	8.4	8.1	7.2	8.0	8.1	-	-	-	-	-
100%	10	10	10	9.0	8.0	7.4	7.0	8.1	8.1	19.5	18.5	19.3	-	-
100%	10	10	10	8.9	8.0	7.4	7.0	8.0	8.1	-	-	-	-	-

	Control Water	100% Sample
Alkalinity:	123	250
Hardness:	125	60

Organism: ***Daphnia pulex*** Life stage: neonate
LC50: Binomial: - Moving Average: - Probit: -
-

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentration:	# at 0 hr	Live at 24 hr	Live at 48 hr	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	48 hour
Control	10	10	10	8.2	8.0	8.1	8.2	8.0	8.1	19.4	18.6	18.9	-	-
Control	10	10	10	8.3	8.0	8.1	8.3	8.0	8.1	-	-	-	218	225
5.6%	10	10	10	8.1	8.0	8.0	8.1	8.0	8.0	19.6	18.3	18.7	-	-
5.6%	10	10	10	8.0	8.0	8.0	8.0	8.0	8.0	-	-	-	252	240
10%	10	10	10	7.9	7.9	8.0	7.9	7.9	8.0	19.6	18.2	18.6	-	-
10%	10	10	10	7.9	7.9	8.0	7.9	7.9	8.0	-	-	-	280	270
18%	10	10	10	7.7	7.9	8.0	7.7	7.9	8.0	19.6	18.6	18.6	-	-
18%	10	10	10	7.7	7.9	8.0	7.7	7.9	8.0	-	-	-	330	310
32%	10	10	10	7.3	7.8	7.9	7.3	7.8	7.9	19.6	18.1	18.6	-	-
32%	10	10	10	7.3	7.8	7.9	7.3	7.8	7.9	-	-	-	410	390
56%	10	10	10	7.1	7.7	7.9	7.1	7.7	7.9	19.7	18.2	18.8	-	-
56%	10	10	10	7.1	7.7	7.9	7.1	7.7	7.9	-	-	-	610	550
100%	10	10	10	7.0	7.6	7.9	7.0	7.6	7.9	19.7	18.5	19.1	-	-
100%	10	10	10	7.0	7.6	7.9	7.0	7.6	7.9	-	-	-	860	870

	Control water	100% sample
Alkalinity:	123	250
Hardness:	125	60

Table 2. Continued.

Facility:	**Air Products and Chemicals**		NPDES #:	**FL0002313**	Facility Type: **Industrial**
Location:	Milton, Florida	County: Santa Rosa	Contact/District:	Barry Long	
			Test type:	Static definitive	
Sample Collection Date:	3/14/88	Time: 1110	# tests:	3	Receiving Water: Escambia Bay
Test Beginning Date:	3/15/88	Time: 1030	Chlorination Type:	**Nonchlorinated**	
Test Ending Date:	3/17/88	Time: 1030			**Page 2 of 2**

	Parameter	
Total Residual Cl2	0.0	-
Method:	Hach	-
Salinity (ppt):	0	

Ammonia:	Control Water	100% Sample
Unionized NH3:	0.00	0.08
Total NH3:	0.00	5.60

Organism: ***Notropis leedsi*** Age: 27 days
LC50: Binomial: 74.8% Moving Average: - Probit: -

	Control water	100% sample
Alkalinity:	120	250
Hardness:	122	60

-

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentration	# at 0 hr	Live at 24 hr	Live at 48 hr	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	48 hour
Control	5	5	5	8.5	7.9	7.6	7.6	7.6	7.9	20.6	18.7	18.9	-	-
Control	5	5	5	8.5	7.9	7.6	7.6	7.6	7.8	-	-	-	216	210
5.6%	5	5	5	8.5	7.8	7.7	7.5	7.6	7.8	20.6	18.3	18.8	-	-
5.6%	5	5	5	8.6	7.8	7.7	7.6	7.6	7.8	-	-	-	250	240
10%	5	5	5	8.6	7.6	7.6	7.5	7.6	7.8	20.3	18.2	18.7	-	-
10%	5	5	5	8.6	7.5	7.6	7.5	7.6	7.8	-	-	-	279	268
18%	5	5	5	8.6	7.7	7.5	7.4	7.5	7.8	20.4	18.0	18.6	-	-
18%	5	5	5	8.6	7.7	7.5	7.4	7.5	7.8	-	-	-	325	310
32%	5	5	5	8.6	6.9	6.6	7.3	7.5	7.7	20.3	18.0	18.7	-	-
32%	5	5	5	8.7	6.9	6.6	7.3	7.5	7.7	-	-	-	409	382
56%	5	5	5	8.7	6.2	5.9	7.1	7.4	7.7	20.1	18.1	18.8	-	-
56%	5	5	5	8.8	6.3	5.5	7.1	7.4	7.7	-	-	-	600	590
100%	5	5	0	9.0	5.5	7.9	7.0	7.6	8.2	19.8	18.5	19.3	-	-
100%	5	3	0	8.9	5.6	7.7	7.0	7.5	8.1	-	-	-	880	850

Table 3. Microtox test results, Air Products, Pace, Santa Rosa County, NPDES #FL0002313, on 3/15/88.

Microtox Test Culture Lot # M-603

% Sample	Final Reclaimed Water Nonchlorinated 5-min	15-min
0	71	36
0	60	41
0	55	48
12.38	59	37
12.38	52	37
12.38	61	33
24.75	57	39
24.75	58	36
24.75	35	21
49.5	47	24
49.5	49	24
49.5	-	29
99	34	28
99	44	20
99	-	31

EC_{50} Chlorinated (15 minute, 15 °C) = 132.55% sample

EC_{50} (15 minute, 15 °C) is calculated using the Data Reduction Computer Program developed by Microbics Corporation for the Sharp Model EL-5100 (Microbics) Systems Operating Manual, 1982.

Table 4. LC_{50} Data for Bioassay

Organism	Method of Data Analysis	48 Hour LC_{50} (95% Confidence Intervals)
Nonchlorinated discharge		
Notropis leedsi	Binomial	74.8% (56%–100%)
	Moving Average	a
	Probit	a

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.

Table 5. Chemical constituents detected in the recovered water from Air Products and Chemicals, Inc., Pace, Santa Rosa County, Florida, NPDES #FL0002313.

Organics	
Purgeables	None Detected
Acid Extractables	None Detected
Base Neutral Extractables	None Detected
Pesticide Extractables	Three unidentified components sensitive to ECD.

Metals	
Arsenic	4 µg/l
Chronium	128 µg/l
Zinc	29 µg/l

Table 4. LC_{50} Data for Bioassay

Organism	Method of Data Analysis	48 Hour LC_{50} (95% Confidence Intervals)
Nonchlorinated discharge		
Notropis leedsi	Binomial	74.8% (56%–100%)
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	Probit	a

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Table 5. Chemical constituents detected in the recovered water from Air Products and Chemicals, Inc., Pace, Santa Rosa County, Florida, NPDES #FL0002313.

Organics	
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Pesticide Extractables	Three unidentified components sensitive to ECD.

Metals	
Arsenic	4 µg/l
Chronium	128 µg/l
Zinc	29 µg/l

Literature Cited

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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. Applicable portions of the F.A.C. are: 17-3.021(1), 17-3.021(4), 17-3.051(1)(d), 17-3.061(3)(p), 17-3.402(1), 17-4.244(4), and 17-4.244(6).

The particular restrictions applied to a given discharger depend upon the type of discharge, the type of water body which is impacted, and the type of mixing zone, if any, which has been permitted. The most stringent toxicity limitations which apply to the various combinations of these factors are listed below, specified in terms of the most dilute concentration of effluent which is allowed to kill 50% of the test organisms in 96 hours. Note that any listed concentration greater than 100% is extrapolated from toxicity curves determined from testing various effluent concentrations of 100% or less. Violations of the toxicity standards occur in the following conditions.

I. Discharges to Surface Water

   A. With no mixing zone, or with only a dredge and fill mixing zone the discharge may not exhibit an $LC_{50} < 2000\%$ at the point of discharge.

   B. With a non-dredge and fill mixing zone the discharge may not exhibit an $LC_{50} < 100\%$ at the point of discharge and may not exhibit an $LC_{50} < 2000\%$ at the edge of the mixing zone.

   C. With a non-dredge and fill mixing zone, and either discharging to the open ocean through multiport diffusers or meeting the provisions of 17-4.244(4)(b) the LC_{50} at the point of discharge cannot exceed 30% sample concentration.

II. Discharges to ground water may not exceed an LC_{50} of 300% at the point of contact with surface waters

A violation of these provisions occurs if toxicity is found at a more dilute concentrations of the sampled discharge than allowed above.

Industry/Toxicant: Air Products
Address: Milton, FL / Santa Rosa Co.
Contact/District: NW / Barry Long
Effluent Serial Number: 001 Alpha Pond
NPDES Permit Number: FL0002313

BIOASSAY DATA FORM

Test Number: 1 of 3
Test Type: Static Renewal, Screening (Conventional)
Other: CST

Sample Collection: Date 3/14/88 Time 1110
Test Beginning: Date 3/15/88 Time 1030
Test Ending: Date 3/17/88 Time 1030
Test Organism: Ceriodaphnia dubia
Age/Life Stage: < 8 hours

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96
Control	62	10	10	10			9.0	9.0	8.8			8.2	7.9	8.0			19.4	18.8	19.2		
Control	85	10	10	10			9.0	9.0	8.9			8.2	7.9	8.1							
5.6%	83	10	10	10			9.0	8.9	8.9			8.0	8.0	8.1			19.4	18.6	19.0		
5.6%	5	10	10	10			9.0	8.9	8.9			8.0	8.0	8.1							
10%	19	10	10	10			9.0	8.9	9.0			7.8	8.0	8.1			19.5	18.4	18.7		
10%	72	10	10	10			9.0	8.9	9.0			7.8	8.0	8.0							
18%	92	10	10	10			9.0	9.0	8.8			7.5	8.0	8.1			19.5	18.4	19.0		
18%	67	10	10	10			9.0	9.0	8.8			7.5	8.0	8.1							
32%	99	10	10	10			9.0	8.8	8.6			7.4	8.0	8.1			19.4	18.4	18.9		
32%	63	10	10	10			9.0	8.8	8.6			7.4	8.0	8.1							
56%	87	10	10	10			9.0	8.4	8.1			7.3	8.0	8.1			19.5	18.4	19.1		
56%	93	10	10	10			9.0	8.4	8.1			7.2	8.0	8.1							
100%	82	10	10	10			9.0	8.0	7.7			7.0	8.1	8.1			19.5	18.5	19.3		
100%	6	10	10	10			8.9	8.0	7.4			7.0	8.0	8.1							
Initial of Investigator	DAF	MF	MF	MF			DAF	MF	MF			DAF	MF	MF			DAF	DAF	MF		

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 123 | 125
100% Sample: 252 | 60
Other:
Measured by: DAF

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: 0.0 | 0.0
100% Sample: | 5.60
Other:

Hour 0
Control Water:
100% Sample:
Other:
Measured by: MF

Slope = -56.4

Signatures:
David Porter
Marshall Faircloth

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7.07 9.09	9.1 @ 20.5	1.8 uhos 27.1 @ 25° 1299 @ 25°
24 Hours	7.07 9.09	9.2 @ 20.5°C	
48 Hours	7.07 9.09	9.0 @ 21°C	1.8 uhos 27.6 @ 25° 1305 @ 21°
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: — Method: —
Lab: N.D. Method: Hach
Lab: Method:

De-chlorinated:
Measurements by: DAF

Reviewed by:
Date: 9/16/88

Industry/Toxicant A.C Products
Address Milton, FL / Santa Rosa Co.
Contact/District NW / Barry Long
Effluent Serial Number 001 Alpha Pond
NPDES Permit Number FL0002313

BIOASSAY DATA FORM

Test Number: 2 of 3
Test Type: Static Renewal, Screening (Conventional)
Other: CST

Sample Collection: Date 3/14/88 Time 1110
Test Beginning: Date 3/15/88 Time 1030
Test Ending: Date 3/17/88 Time 1030
Test Organism Daphnia pulex
Age/Life Stage neonates - mid 1/2 brood

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96	Conductivity 0	24	48	72	96
Control	4	10	10	10			9.2	8.8	8.6			8.2	8.0	8.1			19.4	18.6	18.9							
Control	132	10	10	10			9.1	8.8	8.7			8.3	8.0	8.1								218		225		
5.6%	50	10	10	10			9.1	9.0	8.8			8.1	8.0	8.0			19.6	18.3	18.7							
5.6%	23	10	10	10			9.1	9.0	8.8			8.0	<D	8.0								252		240		
10%	58	10	10	10			9.1	8.8	8.8			7.9	7.9	8.0			19.6	18.2	18.6							
10%	65	10	10	10			9.1	8.8	8.8			7.9	7.9	8.0								280		270		
18%	46	10	10	10			9.1	8.7	8.5			7.7	7.9	8.0			19.6	18.0	18.6							
18%	138	10	10	10			9.1	8.7	8.5			7.7	7.9	8.0								330		310		
32%	55	10	10	10			9.1	8.3	8.0			7.3	7.8	7.9			19.6	18.5	18.6							
32%	49	10	10	10			9.1	8.2	7.8			7.3	7.8	7.9								410		390		
56%	2	10	10	10			9.1	7.6	6.9			7.1	7.7	7.9			19.7	18.2	18.8							
56%	119	10	10	10			9.1	7.6	6.9			7.1	7.7	7.9								410		550		
100%	7	10	10	10			9.1	6.5	5.8			7.0	7.6	7.9			19.7	18.5	19.1							
100%	26	10	10	10			9.0	6.5	5.8			7.0	7.6	7.9								360		310		
Initial of Investigator		DAC	DAC	DAC			DAC	MF	MF			DAC	DAC MF	MF			DAC	DAC	MF			DAC		MF		

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 127 | 12
100% Sample: 250 | 60
Other:
Measured by: DAC

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: 0.0 | 0.
100% Sample: | 5.0
Other:
Hour ____
Control Water:
100% Sample:
Other:
Measured by: MF

Salinity 1.1 ppt

Signatures: Darl Hooster, Marshall Faircloth

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter: 4990 µC
0 Hours	7.07 9.09	9.1 @ 20.5°C	9.8 @ 10 22.1 @ X
24 Hours			
48 Hours			
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: ____ Method: ____
Lab: ____ Method: ____
Lab: ____ Method: ____

De-chlorinated:
Measurements by: ____

Reviewed by: [signature]
Date: 9/16/88

Remarks: ~~100% aerated after 24 hours~~

Industry/Toxicant: Air Products
Address: Milton FL / Santa Rosa Co.
Contact/District: NW / Barry Lee
Effluent Serial Number: 001 Alpha Pond
NPDES Permit Number: FL0002313

BIOASSAY DATA FORM

Test Number: 3 of 3
Test Type: Static Renewal, Screening, (Conventional)
Other: CST

Sample Collection: Date 3/14/88 Time 1110
Test Beginning: Date 3/15/88 Time 1040
Test Ending: Date 3/17/88 Time 1040
Test Organism: Mysidopsis bahia
Age/Life Stage: 27 days

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96	Conductivity 0	24	48	72	96
Control	62	5	5	5			8.5	7.9	7.6			7.6	7.6	7.9			20.6	18.7	18.9							
Control	6	5	5	5			8.5	7.9	7.6			7.6	7.6	7.8								216		210		
5.6%	31	5	5	5			8.5	7.8	7.7			7.5	7.6	7.8			20.6	18.3	18.8							
5.6%	43	5	5	5			8.6	7.8	7.7			7.6	7.6	7.8								250		240		
10%	33	5	5	5			8.6	7.6	7.6			7.5	7.6	7.8			20.3	18.2	18.7							
10%	38	5	5	5			8.6	7.5	7.6			7.5	7.6	7.8								279		268		
18%	239	5	5	5			8.6	7.7	7.5			7.4	7.5	7.8			20.4	18.0	18.6							
18%	58	5	5	5			8.6	7.7	7.5			7.4	7.5	7.8								325		310		
32%	15	5	5	5			8.6	6.9	6.6			7.3	7.5	7.7			20.3	18.0	18.7							
32%	70	5	5	5			8.7	6.9	6.6			7.3	7.5	7.7								409		382		
56%	17	5	5	5			8.7	6.2	5.9			7.1	7.4	7.7			20.1	18.1	18.8							
56%	3	5	5	5			8.8	6.3	5.5			7.1	7.4	7.7								600		590		
100%	67	5	5	0*			9.0	5.5	7.9			7.0	7.6	8.2			19.8	18.5	19.3							
100%	50	5	3	0*			8.9	5.6	7.7			7.0	7.5	8.1								880		850		
Initial of Investigator		mf	mf	mf			DAC	JMF	JMF			DAC	JMF	JMF			DAC	DAC	JMF			DAC		JMF		

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 120 | 122
100% Sample: 250 | 60
Other:
Measured by: DAC

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: 0.0 | 0.0
100% Sample: | 5.6
Other:
Hour ____
Control Water:
100% Sample:
Other:
Measured by: MF

Salinity
15 ppt

Signature: David Chester
Marshall Faudeth

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours			
24 Hours			
48 Hours			
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: ____ Method: ____
Lab: ____ Method: ____
Lab: ____ Method: ____
De-chlorinated:
Measurements by:

Reviewed by: [signature]
Date: 9/16/88

Remarks: * 100% sample - lots of suspended algae
Lab, Lab + Room.
- aerated after 24 hours

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MICROTOX™ Data Reduction Sheet

SOURCE: Air Products

GRAPH CALC EC 50 (15 MIN, 15 °C) = 7100 (%SAMPLE) mg/L

SAMPLE DATA:

SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent

DATE ASSAYED: 3-15-88 TIME:

VISUAL COLOR: none CORRECTION REQUIRED: YES (NO)

VISUAL TURBIDITY: none SEPARATION REQUIRED: YES (NO)

INITIAL pH: pH ADJUSTED TO: WITH:

OSMOTICALLY ADJUSTED WITH: MOAS (DRY NaCl) OTHER

PRIMARY DILUTION OF SAMPLE:

OPERATOR: Marshall Faircloth REAGENT VIAL LOT NO. M-606

REMARKS:

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	Γ5	Γ15	NOTES
A1 B1 C1	0	71 60 55	36 41 48	X	X	
A2 B2 C2	12.38	59 52 61	37 37 33	.081	.168	
A3 B3 C3	24.75	67 58 35	31 36 37-21	.240	.302	
A4 B4 C4	49.5	47 49 —	24 24 29	.292	.623	
A5 B5 C5	99	34 44 —	28 28 31	.525	.582	
EC_{50} (MIN, °C)					137.55	

REVIEWED BY:

DATE: 9/16/88