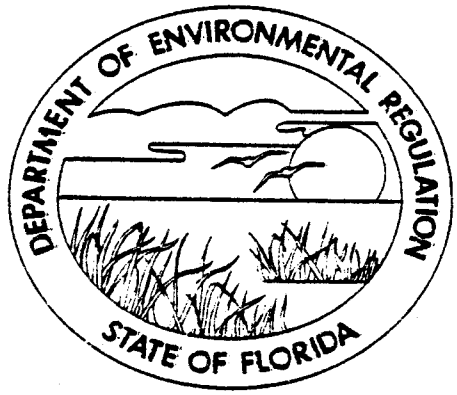


BIOASSAYS OF THE DUPONT TRAIL RIDGE MINE

STARKE, BRADFORD COUNTY, FLORIDA

NPDES #FL0000051

SAMPLED 1/4/83

Biology Section
Bureau of Water Analysis
February 11, 1983

BIOASSAYS OF THE DUPONT TRAIL RIDGE MINE

STARKE, BRADFORD COUNTY, FLORIDA

NPDES #FL0000051

Biology Section
Bureau of Water Analysis
February 11, 1983

EXECUTIVE SUMMARY

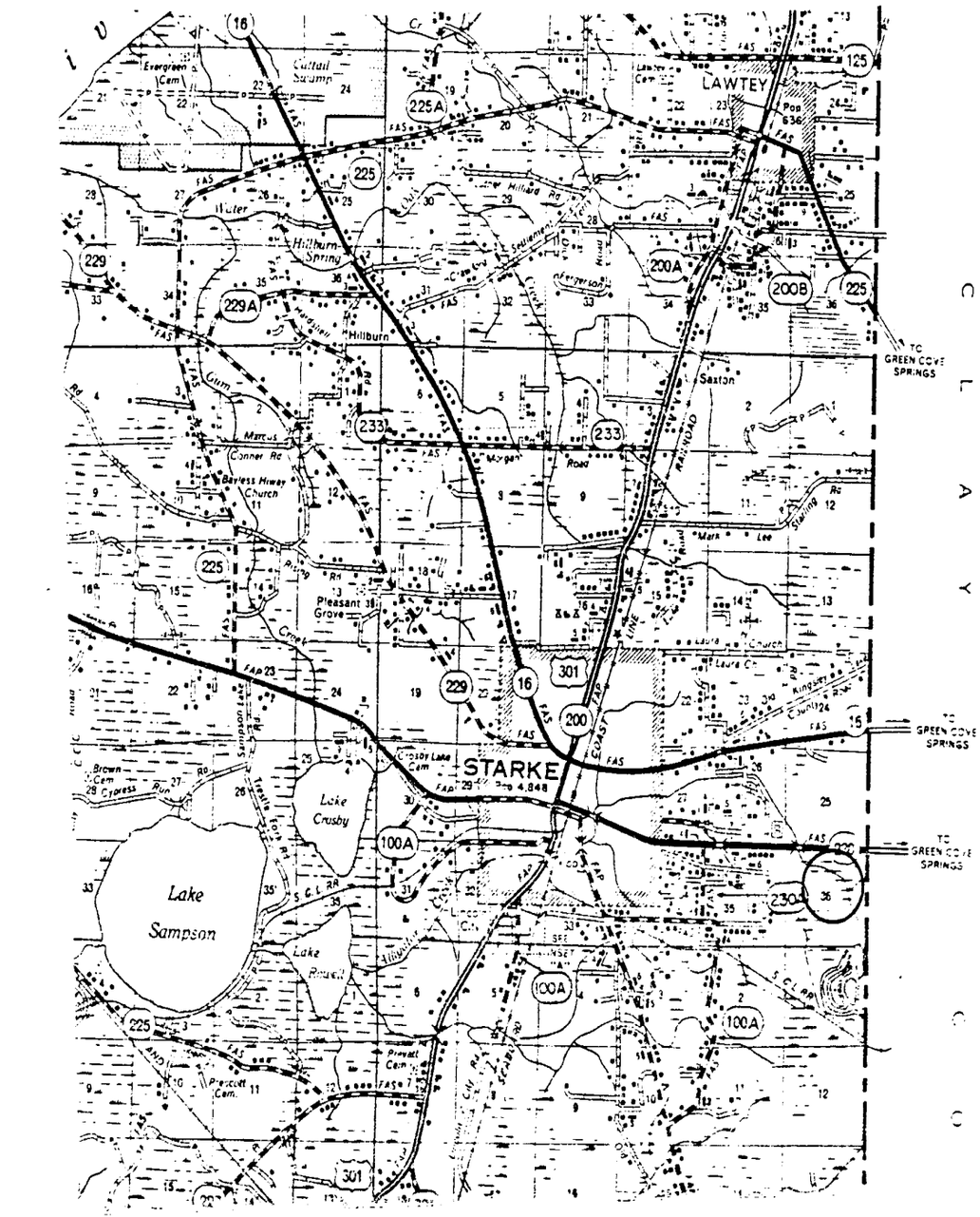
Dupont Trail Ridge Mine, NPDES #FL0000051, on 5 to 7 January, 1983.

The sample of mining wastewater from this mineral dredging operation located near Starke, was not toxic to the organisms tested. Only a few random Daphnia pulex and Simocephalus serrulatus mortalities were noted, although excessive control mortality invalidated the Daphnia pulex test at 48 hours. No fish, Pimephales promelas or Heterandria formosa, died during the 48 hour static tests.

INTRODUCTION

The Dupont Trail Ridge Mine, encompassing portions of Bradford and Clay counties, is located east of Starke, Florida. The operation involves the mining of ancient beach sand to recover semi-precious minerals. Approximately 26,400 long tons of sand are dredged per day for the recovery of minerals such as ilmenite, zircon and staurolite. The wastewater produced, approximately 2.6 MGD, is flocculated with sulfuric acid and allowed to settle in ponds. Clean, acidified water overflowing from these ponds is neutralized with lime to a pH of from 6.0 to 8.5, allowed to settle again to remove sludge, and is discharged. The final effluent is discharged through Outfall #001 to Alligator Creek (Fig. 1), a tributary of Rowell Lake.

To evaluate the existence and extent of toxicity of this discharge to biota of the receiving waters, the Biology Section performed four, static acute toxicity bioassays on a sample of the effluent, from 5 to 7 January, 1983. The results of these tests are presented in this report.



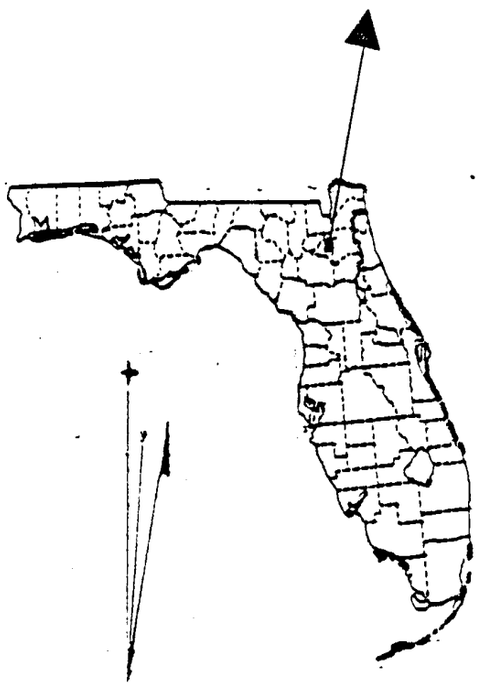


Figure 1. Portions of Bradford County, Florida, including Starke and approximate location of Outfall #001 to Alligator Creek.

METHODS AND MATERIALS

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

History and Maintenance of Bioassay Organisms

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

Simocephalus serrulatus (water flea) - An indigenous breeding population of Simocephalus serrulatus was collected from Lake Jackson, Leon County, Florida, in March, 1980, by personnel from DER's Biology Section. Positive identification of the organisms was made by Mr. Jeff Foran of the University of Florida Department of Environmental Engineering Sciences. The cultures are maintained in two and one-half gallon all glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. The organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, and are kept at a constant temperature of 22° plus or minus 1°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms. They are then refilled with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances such as: declining populations, ephippia formation, or excessive fungal or bacterial growth.

Pimephales promelas (fathead minnows) - A breeding culture of approximately 100 adult fathead minnows was obtained from the EPA laboratory in Cincinnati, Ohio, in May, 1979. This breeding population is augmented with fry hatched in our laboratory and with additional adult fish from EPA as required. The fish are maintained in four 125 gallon all glass aquaria equipped with a flow through water exchange system using unchlorinated well water obtained from a deep well near the DER laboratory. The fathead minnows are reared at a temperature of 22° plus or minus 2°C under a 16:8 hour light-dark cycle. These fish are fed Glencoe Mills "#3 size enriched trout fry granules", Tetra-Min®, and frozen Artemia (brine shrimp) at a rate of 0.75 g/30 fish, twice daily. Breeding has been encouraged by placing several halved clay pots in each tank to serve as substrates for eggs and to provide cover for the fish. Tanks are examined twice daily (at feeding time) for egg production. Clay pots containing egg clusters are immediately transferred to 15 gallon aerated

aquaria for hatching. A new aquarium is prepared for each week's production of eggs, thereby segregating fish into even age groups to be used for testing. After hatching, fathead minnow fry are fed twice daily with freshly hatched Artemia. The fish used in this test were approximately 4 weeks old at the time of the test.

Heterandria formosa (least killifish) - Approximately 100 adult least killifish were obtained from a wild population in the St. Marks Wildlife Refuge (Wakulla County, Florida) during August, 1980. The fish are maintained in two and one-half to 15 gallon all glass aquaria equipped with a flow through water exchange system. Water for this system is unchlorinated and is pumped from a deep well near the DER laboratory. The least killifish are reared at 22° plus or minus 2°C under a 16:8 hour light-dark cycle and are fed freshly hatched Artemia (brine shrimp) twice daily. New born fish are initially maintained in the adult tanks, but are removed at weekly intervals, thereby segregating fish into even age groups. The fish used in this test were 3 to 5 weeks old.

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

Four separate 48 hour static acute toxicity bioassays were performed on a sample of dishcarge from Outfall #001 of the Dupont Trail Ridge Mine. The tests were performed from 1130 hours on 5 January to 1200 hours on 7 January, 1983. The sample was collected by Mr. Chuck Love of DER's Jacksonville Office at 1217 hours on 4 January, 1983, using standard wastewater sampling procedures. The sample was iced and transported to the DER laboratory in Tallahassee for analyses within 24 hours of collection.

Freshwater for dilution of the effluent sample was obtained from the same DER deep well that is used to culture the test organisms. The concentration series selected for this study (Table 1) is that recommended by APHA (1980).

Two replicates, each with six concentrations and a control, were used to test with the water fleas, Daphnia pulex and Simocephalus serrulatus. Flint glass jars of 250 ml capacity were used as test chambers, and 200 ml of the appropriate test concentration was placed in each chamber. Daphnia and Simocephalus were siphoned into large culture dishes to isolate the proper size and age organisms (neonates). To accomplish this, the water fleas were siphoned through a stainless steel screen. The trapped adults were then discarded. These neonates were then transferred by glass pipette, examined for injury, and were loaded 10 per chamber, resulting in 20 per test concentration. Water fleas were not fed during these tests.

Two replicates, of six concentrations and a control, were used for the Pimephales promelas (fathead minnow) and the Heterandria formosa (least killifish) bioassays. 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 these tests.

Dissolved oxygen, pH, temperature, and the number of live organisms were recorded for each test chamber at 0 hours, 24 hours, and 48 hours. The specific conductance of each test concentration was recorded at the beginning and end of the test. Ammonia concentrations, including total and unionized fractions (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 determined 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 5.

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

Daphnia pulex and Simocephalus serrulatus (water fleas)

Concentration	Dilution Water	Effluent	Total Volume
Control	200 ml	---	200 ml
5.6%	188.8 ml	11.2 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%	--	200 ml	200 ml

Pimephales promelas (fathead minnows)

Heterandria formosa (least killifish)

Concentration	Dilution Water	Effluent	Total Volume
Control	500 ml	---	500 ml
5.6%	472 ml	28 ml	500 ml
10.0%	450 ml	50 ml	500 ml
18.0%	410 ml	90 ml	500 ml
32.0%	340 ml	160 ml	500 ml
56.0%	220 ml	280 ml	500 ml
100.0%	--	500 ml	500 ml

Table 2. Data recorded during 48 hour, *Daphnia pulex*, static acute toxicity bioassay of the Dupont Trail Ridge Mine, Outfall #001, Bradford County, Florida, NPDES #FL0000051, on 5 to 7 January, 1983.

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Conductivity (µmhos)	
	0	24	48	0	24	48[a]	0	24	48[a]	0	48	0	48	0	24	48[a]	0	48	0	48[a]
Control	10	10	0	8.6	9.1	--	7.7	8.3	--					20.7	19.6	--	0/0	--		
Control	10	10	10	8.6	9.1	--	7.7	8.3	--										210	--
5.6%	10	10	10	8.6	9.2	--	7.7	8.3	--					20.7	19.5	--				
5.6%	10	10	9	8.6	9.1	--	7.7	8.3	--										210	--
10.0%	10	9	9	8.6	9.2	--	7.8	8.3	--					20.7	19.4	--				
10.0%	10	10	10	8.6	9.1	--	7.8	8.3	--										212	--
18.0%	10	10	10	8.7	9.2	--	7.8	8.3	--					20.6	19.4	--				
18.0%	10	10	10	8.7	9.1	--	7.8	8.3	--										215	--
32.0%	10	10	10	8.8	9.2	--	7.8	8.2	--					20.5	19.4	--				
32.0%	10	10	10	8.8	9.1	--	7.8	8.2	--										220	--
56.0%	10	10	9	8.9	9.2	--	7.8	8.2	--					20.3	19.5	--				
56.0%	10	10	10	8.9	9.1	--	7.8	8.2	--										230	--
100.0%	10	10	10	9.0	9.2	--	7.9	8.1	--					20.0	19.6	--	0/0	--		
100.0%	10	10	10	9.0	9.2	--	7.9	8.1	--										248	--

Remarks: [a] The 48 hour test is invalidated due to excessive control mortality, therefore, the remaining parameters are not recorded.

Table 3 . Data recorded during 48 hour, Simocephalus serrulatus, static acute toxicity bioassay of the Dupont Trail Ridge Mine, Outfall #001, Bradford County, Florida, NPDES #FL0000051, on [illegible] January, 1983.

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Conductivity (μmhos)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	10	10	8(9)[a]	8.5	9.4	8.9	7.7	8.2	8.3					20.8	19.3	19.7	0/0	--		
Control	10	10	8	8.5	9.4	8.9	7.7	8.3	8.3										210	220
5.6%	10	10	9	8.5	9.3	8.6	7.8	8.3	8.3					20.8	19.2	19.6				
5.6%	10	10	10	8.5	9.3	8.6	7.8	8.3	8.3										212	220
10.0%	10	10	10	8.5	9.3	8.6	7.8	8.3	8.3					20.6	19.2	19.5				
10.0%	10	10	10	8.5	9.3	8.6	7.8	8.3	8.3										212	220
18.0%	10	10	10	8.6	9.3	8.5	7.8	8.3	8.3					20.5	19.2	19.3				
18.0%	10	10	9	8.6	9.3	8.6	7.8	8.3	8.2										215	222
32.0%	10	10	10	8.8	9.3	8.6	7.8	8.2	8.2					20.4	19.1	19.3				
32.0%	10	10	9	8.8	8.3	8.6	7.8	8.2	8.2										218	230
56.0%	10	10	10	8.8	9.3	8.5	7.9	8.2	8.2					20.4	19.0	19.3				
56.0%	10	10	10	8.8	9.3	8.5	7.9	8.2	8.2										230	238
100.0%	10	9	9	8.9	9.4	8.7	7.9	8.1	8.2					20.0	19.2	19.3	0/0	--		
100.0%	10	10	9	8.9	9.3	8.7	7.9	8.1	8.2										250	258

Remarks: [a] Number in parentheses used since one organism was not found in the jar.

Table 4 . Data recorded during 48 hour, *Pimephales promelas*,[a] static acute toxicity bioassay of the Dupont Trail Ridge Mine, Outfall #001, Bradford County, Florida, NPDES #FL0000051, on 5 to 7 January, 1983.

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Conductivity (μmhos)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	5	5	5	8.5	8.4	8.1	7.7	7.9	8.0					20.8	19.1	19.5	0/0	--		
Control	5	5	5	8.5	8.4	8.1	7.7	7.9	8.0										202	202
5.6%	5	5	5	8.6	8.1	7.9	7.7	7.9	8.0					20.7	19.0	19.3				
5.6%	5	5	5	8.6	7.7	7.5	7.7	7.9	8.0										210	215
10.0%	5	5	5	8.6	7.7	7.7	7.8	7.9	8.0					20.6	18.9	19.3				
10.0%	5	5	5	8.6	7.8	7.6	7.8	7.9	8.0										215	205
18.0%	5	5	5	8.6	7.8	7.5	7.8	7.9	8.0					20.6	18.9	19.3				
18.0%	5	5	5	8.6	7.8	7.5	7.8	7.9	8.0										218	215
32.0%	5	5	5	8.7	8.0	7.7	7.8	7.9	8.0					20.8	18.9	19.3				
32.0%	5	5	5	8.7	8.0	7.5	7.8	7.9	8.0										222	225
56.0%	5	5	5	8.7	7.8	7.6	7.9	7.8	8.0					20.3	19.0	19.4				
56.0%	5	5	5	8.7	7.7	7.4	7.9	7.8	8.0										232	230
100.0%	5	5	5	8.8	7.9	7.5	7.9	7.8	7.9					20.1	19.3	19.5	0/0	--		
100.0%	5	5	5	8.8	7.9	7.5	7.9	7.8	7.9										250	258

Remarks: [a] Weight of fish = 39.0 mg each, average of 10 fish.

Table 5 . Data recorded during 48 hour, Heterandria formosa,[a] static acute toxicity bioassay of the Dupont Trail Ridge Mine, Outfall #001, Bradford County, Florida, NPDES #FL0000051, on 5 to 7 January, 1983.

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Conductivity (µmhos)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	5	5	5	8.6	9.1	8.1	7.7	8.0	8.1					20.4	19.0	19.3	0/0	--		
Control	5	5	5	8.6	9.1	8.1	7.7	8.0	8.1										208	215
5.6%	5	5	5	8.5	9.0	8.0	7.7	8.1	8.1					20.3	18.7	19.0				
5.6%	5	5	5	8.5	9.0	8.1	7.7	8.1	8.1										208	215
10.0%	5	5	5	8.5	8.9	8.0	7.8	8.1	8.1					20.6	18.6	18.9				
10.0%	5	5	5	8.5	8.9	8.1	7.8	8.1	8.1										218	215
18.0%	5	5	5	8.6	8.9	8.1	7.8	8.0	8.1					20.6	18.6	18.8				
18.0%	5	5	5	8.6	8.9	8.1	7.8	8.0	8.1										218	218
32.0%	5	5	5	8.6	8.9	7.9	7.8	8.0	8.0					20.5	18.6	18.8				
32.0%	5	5	5	8.6	8.9	7.9	7.8	8.0	8.0										222	222
56.0%	5	5	5	8.7	8.9	8.0	7.9	8.0	8.0					20.2	18.7	18.9				
56.0%	5	5	5	8.7	8.9	8.0	7.9	8.0	8.0										228	232
100.0%	5	5	5	8.8	9.0	8.1	7.9	7.9	8.0					20.1	19.0	19.1	0/0	--		
100.0%	5	5	5	8.9	9.0	8.1	7.9	7.9	8.0										251	258

Remarks: [a] Weight of fish = 12.86 mg each, average of 10 fish.

RESULTS AND DISCUSSION

The sample of wastewater discharge from Outfall #001 of the Dupont Trail Ridge Mine was not acutely toxic to the organisms tested. Only a few random Daphnia pulex and Simocephalus serrulatus mortalities were noted, although excessive control mortality invalidated the Daphnia pulex test at 48 hours. No fish, Pimephales promelas or Heterandria formosa, died during the 48 hour static tests.

LITERATURE CITED

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

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

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