Bioassays of Gardinier, Incorporated

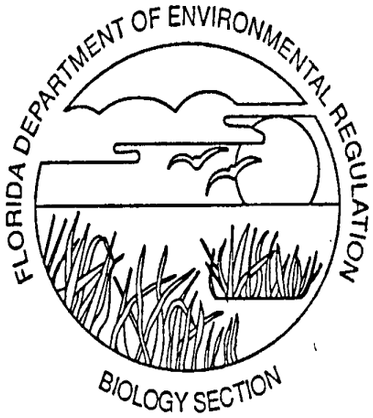
Tampa ,Hillsborough County, Florida

NPDES #FL0000761

Sampled Date 3/7/88

August 18, 1988

Biology Section
Division of Administrative and Technical Services



Bioassays of Gardinier, Incorporated

Tampa, Hillsborough County, Florida

NPDES #FL0000761

Sampled Date 3/7/88

Biology Section
Bureau of Laboratories
August 18, 1988

Executive Summary

Gardinier, Inc., Tampa, Hillsborough County, Florida, NPDES #FL0000761, 8 March to 10 March, 1988.

Three separate acute toxicity bioassays and one Microtox® assay were performed on a sample from the Gardinier facility in March, 1988. The sample was acutely toxic to the test organism, Ceriodaphnia dubia, producing an LC_{50} of 84.9%. The sample was not toxic to the bioassay organisms, *Daphnia pulex* and *Notropis leedsi*, nor to the Microtox® assay organism, *Photobacterium phosphoreum.*

Chemical analyses identified the presence of the metals arsenic, cadmium, chromium, copper, lead, and zinc. The zinc concentration of 439 µg/l probably contributed to the mortality observed in the *C. dubia* tests.

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

Introduction

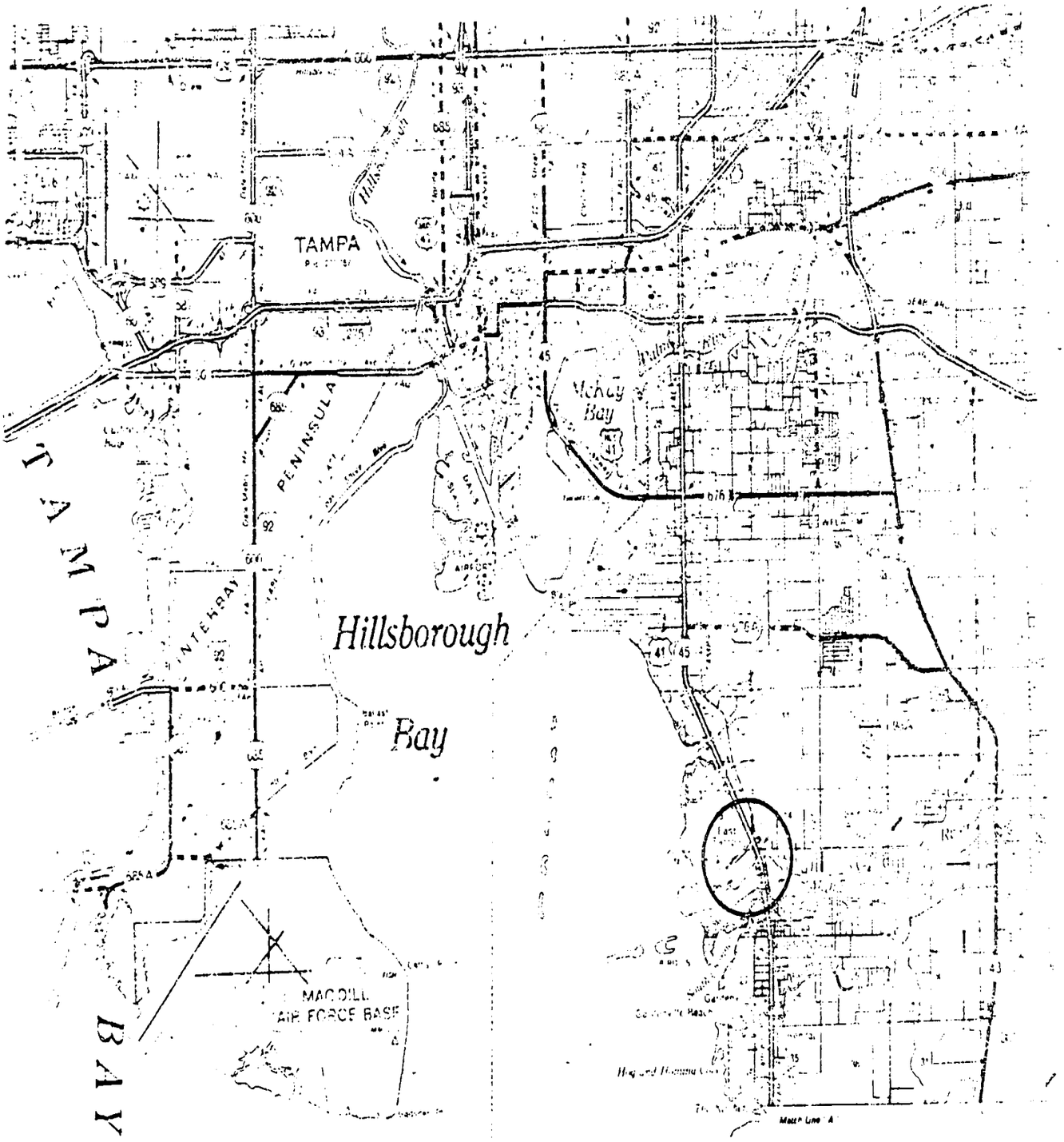
Gardinier, Inc., is located at U.S. 41 and Riverview Drive, just north of the mouth of the Alafia River on Hillsborough Bay, Tampa, Hillsborough County, Florida (Figure 1). This facility manufactures fertilizer and inorganic chemicals, including sulfuric, phosphoric, and fluorosilic acids, triple super and super phosphate, ammonium phosphate, anhydrous ammonia, and gypsum. Wastewater from the various operations is treated by liming and retention in sludge ponds. As many as six NPDES permitted outfalls are located on the site, three (#001, #005, and #012) that discharge to surface waters. Outfalls #001 and #005 contain water from the sulfuric acid plant. They discharge to the Alafia River. Outfall #012 contains supernatant from the sludge ponds, and is discharged to Archer Creek, a tributary of Hillsborough Bay. A schematic of the facility and its wastewater outfalls is shown Figure 2.

In 1973 Gardinier, Inc. was fined for damaging public lands (DER NPDES file). Since then, major modifications were introduced in the manufacturing of phosphate chemicals, including a new gypsum disposal field (Figure 3). These modifications reduced energy consumption and increased efficiency and production.

In September 1981, the Biology Section performed bioassays on samples from Outfalls #001, #005, and #012. Some mortality occurred in the test organism, *Mysidopsis bahia*, but an LC_{50} could not be calculated. Chemical analysis indicated that zinc and selenium were present in the sample from Outfall #005 (FDER 1981).

In June, 1987, a bioassay was performed on a sample from Outfall #005 of this facility. This sample produced an LC_{50} of 376.6% in the test organism, *Mysidopsis bahia.* In the absence of a mixing zone this sample met the criteria for chronic toxicity. Metals and unionized ammonia were thought to have contributed to the observed mortality in the test organisms (FDER 1987).

To again evaluate the existence and extent of toxicity in the discharge from Outfall #005 of this facility, the Biology Section performed three static acute toxicity bioassays and one Microtox® assay on a sample during March, 1988. 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. Faircloth, and L. A. Williams.



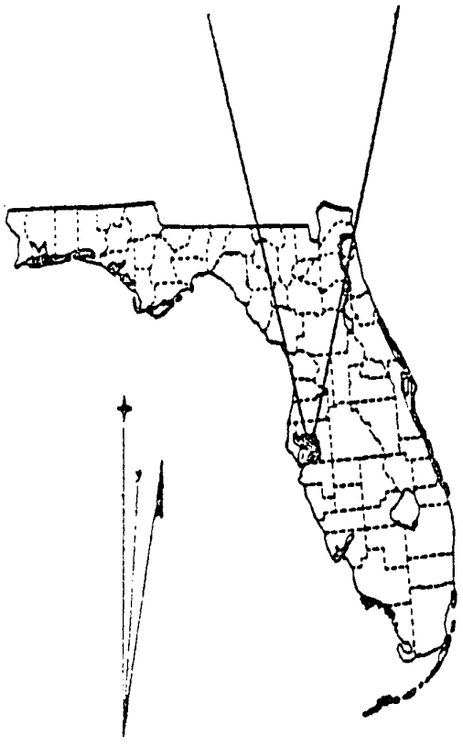
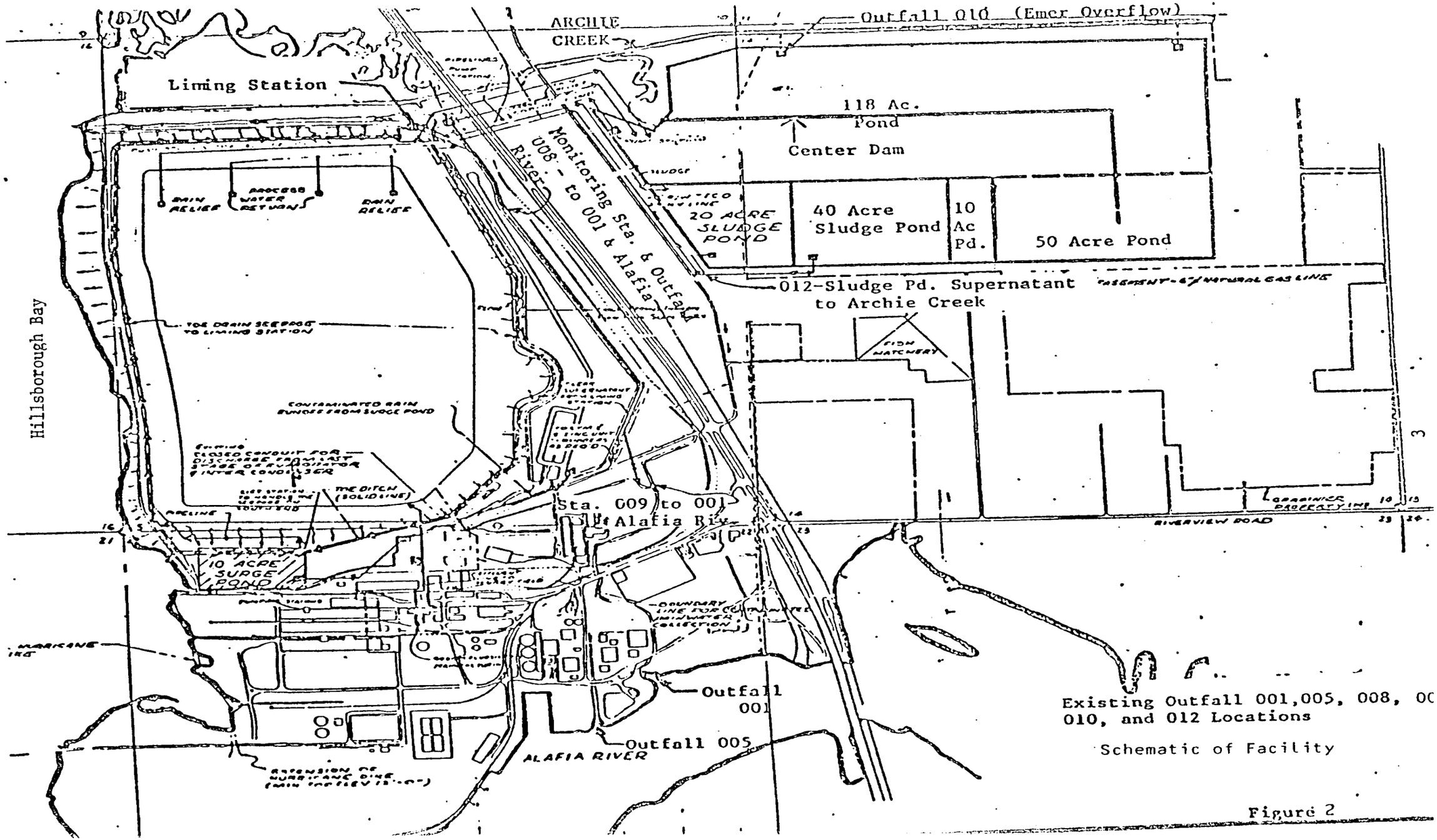


Figure 1. Portions of Tampa Hillsborough County, Florida, including study area.



Existing Outfall 001,005, 008, 0C
010, and 012 Locations

Schematic of Facility

Figure 2

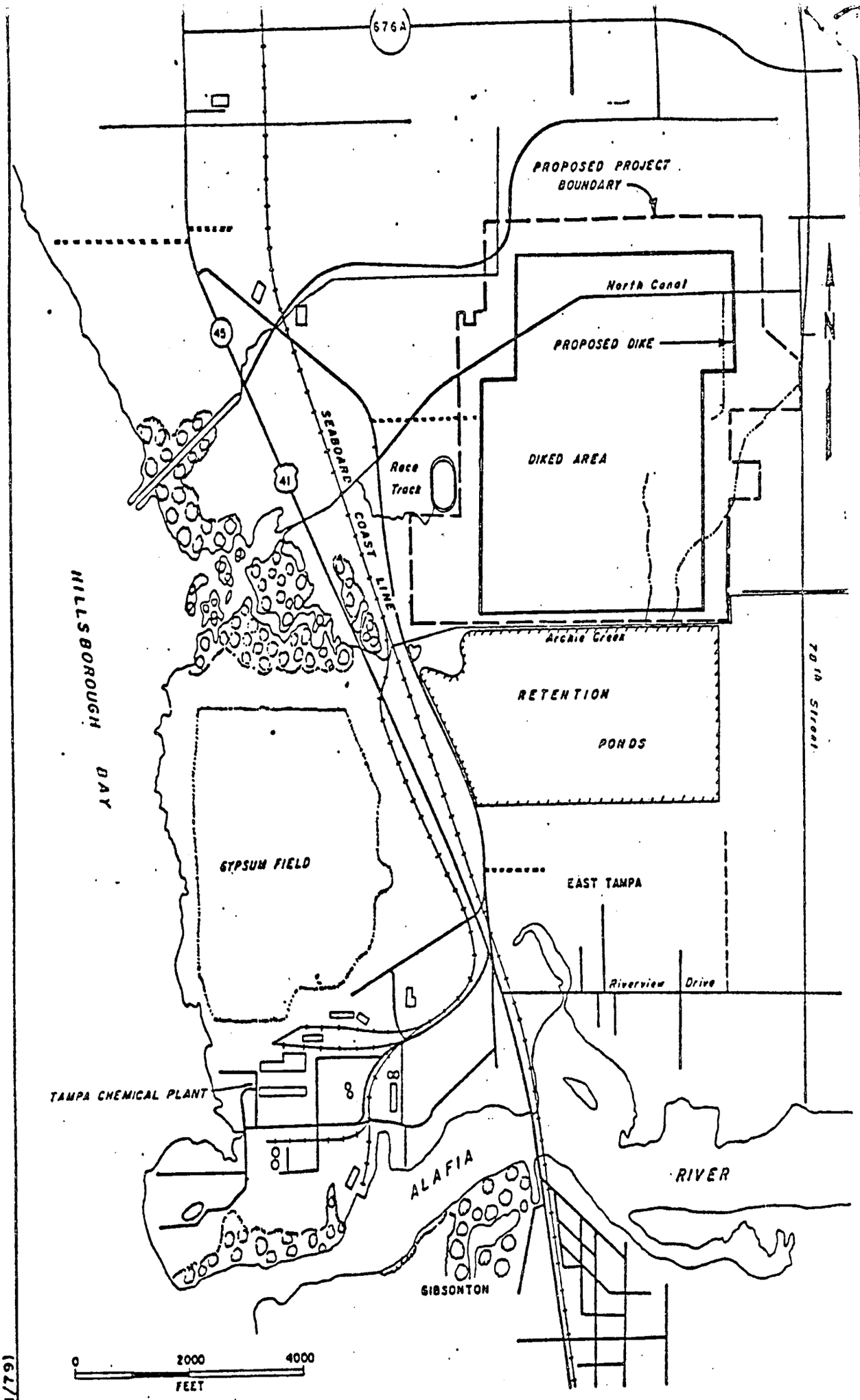


FIGURE 3 GYPSUM DISPOSAL FIELD LOCATION MAP

1147600326(11/79)

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), 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.

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 25 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 static acute toxicity screening bioassays and one Microtox® assay were performed on a sample of discharge from Outfall #005 of the Gardinier, Inc., facility in Tampa. The tests were performed from 1030 hours on 8 March to 1030 hours on 10 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. David Demmi of the FDER Southwest District Office at 0945 hours on 7 March, 1988, using standard wastewater sampling procedures. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 25 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.

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. 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 (as N) and unionized fraction (as NH_3) 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 discharge from Outfall #005 of Gardinier, Inc. was toxic to the bioassay test organism, Ceriodaphnia dubia, producing an LC_{50} of 84.9% (Table 4). The sample was not toxic to the bioassay organisms, Daphnia pulex and Notropis leedsi, nor was the sample toxic to the Microtox® assay organism, Photobacterium phosphoreum.

Chemical analyses indicated the presence of several metals including chromium (79 µg/l) and zinc (439 µg/l) (Table 5). Base neutral extractables indicated the presence of an estimated 0.005 µg/l petroleum hydrocarbons similar to SAE 30. Several small unidentified components sensitive to ECD were noted in the pesticide analysis. EPA (1976) gives zinc LC_{50} values for some Daphnia species. These values range from 33 µg/l at a hardness of 23 µg/l as $CaCO_3$ for Daphnia hyalina to 1524 µg/l at an unspecified hardness for Daphnia magna. Daphnia magna tests for zinc also resulted in LC_{50}'s of 60.9 µg/l to 308 µg/l in the hardness range from about 20mg/l as $CaCO_3$ to 45.3 mg/l as $CaCO_3$.

It is likely that the zinc concentration of 439 µg./l was responsible for part of the observed mortality in the Ceriodaphnia dubia tests even though the hardness was 438 mg/l as $CaCO_3$.

A concern of the Biology Section is that if the facility discharges to soft water, the toxicity of the diluted discharge may increase despite dilution because of the increasing zinc toxicity from the reduced hardness.

Biology Section Recommendations

The Biology Section recommends that the facility be required to meet state toxicity standards or to undergo 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
30.0%	35.0 ml	15.0 ml	50 ml
60.0%	20.0 ml	30.0 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
30.0%	140.0 ml	60.0 ml	200 ml
60.0%	80.0 ml	120.0 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
30.0%	350 ml	150 ml	500 ml
60.0%	200 ml	300 ml	500 ml
100.0%	0 ml	500 ml	500 ml

Table 2. Data recorded during 48 hour static acute toxicity bioassay of Gardinier, Inc., NPDES #FL0000761, 8 to 10 March, 1988.

Facility:	GARDINIER EAST			NPDES # FL0000761		Facility Type:	Industry
Location:	Hillsborough County			Contact/District:	D.Demmi		
Sample Collection Date:	3/7/88	Time:	945	Test type:	Static Screening		
Test Beginning Date:	3/8/88	Time:	1030	# tests:	3	Receiving Water:	Alafia Estuary
Test Ending Date:	3/10/88	Time:	1030	Chlorination Type:	Non-chlorinated		

Parameter	
Total Residual Cl2:	0 mg/l
Method:	Hach
By:	DAC
Salinity (ppt):	1

Ammonia:	Control Water	100% Sample
Unionized NH3 (mg/l	0.00	0.01
Total NH3 (mg/l):	0	8.54

Organism: *Ceriodaphnia dubia* — Age/Life stage: Neonates
LC50: Binomial: 84.5% Moving Average: - Probit: 84.9%
toxic

Concentrations	SURVIVAL # at 0 hour	Live at 24 hr	Live at 48 hr	Dissolved Oxygen 0 hour	24 hour	48 hour	pH 0 hour	24 hour	48 hour	Temperature 0 hour	24 hour	48 hour	Conductivity 0 hour	48 hour
Control	10	9	9	9.0	8.9	8.7	8.0	8.2	8.0	20.1	19.7	20.1	-	-
Control	10	10	10	9.0	8.9	8.7	8.0	8.2	8.0	-	-	-	-	-
30%	10	10	9	8.6	9.0	8.7	6.9	8.1	7.9	20.2	19.5	19.8	-	-
30%	10	10	9	8.6	9.0	8.6	6.9	8.1	7.9	-	-	-	-	-
60%	10	10	6	8.2	8.9	8.6	6.6	7.9	7.7	20.3	19.5	20.4	-	-
60%	10	10	8	8.2	8.9	8.7	6.6	7.9	7.7	-	-	-	-	-
100%	10	10	3	7.5	8.9	8.7	6.2	7.4	7.2	20.3	19.5	20.6	-	-
100%	10	10	5	7.5	8.9	8.7	6.2	7.7	7.2	-	-	-	-	-

	Control water	100% sample
Alkalinity (mg/l):	120	60
Hardness (mg/l):	124	438

Organism: *Daphnia pulex* — Age/Life stage: Neonates
LC50: Binomial: Moving Average: Probit:
non-toxic

Concentrations	SURVIVAL # at 0 hour	Live at 24 hr	Live at 48 hr	Dissolved Oxygen 0 hour	24 hour	48 hour	pH 0 hour	24 hour	48 hour	Temperature 0 hour	24 hour	48 hour	Conductivity 0 hour	48 hour
Control	10	10	10	9.0	9.0	8.7	8.0	8.3	8.0	20.3	19.7	19.7	-	-
Control	10	10	10	9.0	9.0	8.7	8.0	8.3	8.0	-	-	-	210	215
30%	10	10	10	8.6	9.0	8.7	6.8	8.9	7.7	20.4	19.3	19.5	-	-
30%	10	10	8	8.9	9.0	8.7	6.8	7.8	7.8	-	-	-	610	640
60%	10	10	10	8.0	8.9	8.7	6.5	7.5	7.5	20.6	19.2	19.5	-	-
60%	10	10	10	8.0	8.9	8.7	6.5	7.5	7.5	-	-	-	990	1010
100%	10	10	10	7.4	8.7	8.5	6.2	7.0	7.0	20.7	19.3	19.5	-	-
100%	10	10	10	7.4	8.7	8.5	6.2	7.0	7.0	-	-	-	1490	1495

	Control water	100% sample
Alkalinity (mg/l):	120	60
Hardness (mg/l):	124	438

Organism: *Notropis leedsi* — Age/Life stage: 25 days old
LC50: Binomial: Moving Average: Probit:
non-toxic

Concentrations	SURVIVAL # at 0 hour	Live at 24 hr	Live at 48 hr	Dissolved Oxygen 0 hour	24 hour	48 hour	pH 0 hour	24 hour	48 hour	Temperature 0 hour	24 hour	48 hour	Conductivity 0 hour	48 hour
Control	5	4	4	8.2	8.8	7.4	7.5	7.8	7.6	21.5	19.5	19.3	-	-
Control	5	5	5	8.2	8.8	7.4	7.5	7.8	7.6	-	-	-	210	212
30%	5	5	5	8.3	8.8	7.5	6.8	7.5	7.5	21.3	19.3	19	-	-
30%	5	5	5	8.3	8.8	7.6	6.8	7.5	7.5	-	-	-	650	620
60%	5	5	5	8.0	8.8	7.7	6.5	7.3	7.3	21.1	19.1	19	-	-
60%	5	5	5	8.0	8.8	7.5	6.5	7.2	7.3	-	-	-	1040	1000
100%	5	5	5	7.4	8.5	7.5	6.2	6.8	6.9	21.0	19.3	19	-	-
100%	5	5	5	7.4	8.5	7.5	6.2	6.8	6.9	-	-	-	1520	1490

	Control water	100% sample
Alkalinity (mg/l):	121	60
Hardness (mg/l):	119	438

Table 3. Microtox test results , Gardinier East , Tampa , Hillsborough County , NPDES # FL0000761 on 3/08/88.

Microtox Test Culture lot # M-606

Final Reclaimed Water

% Sample	Non-chlorinated	
	5-min	15-min
0	98	58
0	92	57
0	103	65
12.38	90	60
12.38	92	62
12.38	91	62
24.75	77	54
24.75	91	65
24.75	87	60
49.5	86	64
49.5	85	64
49.5	87	65
99	82	63
99	77	57
99	86	66

EC-50 Non-chlorinated (15 minute , 15 degree C) = Stimulatory.

EC50 (15 minute, 15 degrees 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 of Gardinier Inc., Tampa, Hillsborough County, Florida, NPDES #FL0000761, on 8 to 10 March, 1988.

Organism	Method of Data Analysis	48 Hour LC_{50} (95% Confidence Intervals)
Ceriodaphnia dubia	Binomial	84.5% (45.45%, + ∞)
	Moving Average	a
	Probit	84.9% (64.5%, 156.0%),b

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 This LC_{50} is reported in the text since it is the most statistically conservative value of the data set.

Table 5. Chemical constituents present in the reclaimed water from the Gardinier, Incorporated facility Outfall #005.

Organics

Acid Extractables	None Detected
Base Neutral Extactables	Petroleum Hydrocarbons similar to SAE 30. Estimated amount = 0.005 ug/l
Pesticide Extractables	Several small unidentified components sensitive to ECD.

Metals

Arsenic	6.0 µg/l
Cadmium	1.2 µg/l
Chromium	79.0 µg/l
Copper	26.0 µg/l
Lead	26.0 µg/l
Zinc	439.0 µg/l

Literature Cited

Beckman Instruments, Inc. 1982. Microtox system operating manual.

Florida Department of Environmental Regulation. 1985. Quality assurance manual for performing acute toxicity tests (Second Edition). Biology Section, Tallahassee.

_____. 1987. Bioassay of Gardinier, Inc., U. S. Phosphoric Products, Tampa, Hillsborough County, Florida, NPDES #FL0000761. FDER. 15 p.

Peltier, W. and C. I. Weber. 1985. Methods for measuring the acute toxicity of effluents to aquatic organisms (Third Edition). Environmental Research Laboratory. Athens, Georgia. EPA-600/4-85-012. 217 p.

U.S. Environmental Protection Agency. 1976. Quality criteria for water. U.S. EPA, Washington, D.C. 256 p.

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.

TOXIC

BIOASSAY DATA FORM

Industry/Toxicant Gardinier East
Address 6571 Hillsborough Co.
Contact/District SW / Dade Dennis
Effluent Serial Number 005
NPDES Permit Number FL0020761

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

Sample Collection: Date 3-7-88 Time 0945
Test Beginning: Date 3-8-88 Time 1030
Test Ending: Date 3-10-88 Time 1130
Test Organism Ceriodaphnia dubia
Age/Life Stage neonates

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	0	24	48	72	96
Control	83	10	9 DAC	9			9.0	8.9	8.7			8.0	8.2	8.0			20.1	19.7	20.1							
Control	5	10	10	10			9.0	8.9	8.7			8.0	8.2	8.0												
30%	92	10	10	9			8.6	9.0	8.7			6.9	8.1	7.9			20.2	19.5	19.8							
30%	99	10	10	9			8.6	9.0	8.6			6.9	8.1	7.9												
60%	6	10	10	6			8.2	8.9	8.6			6.6	7.9	7.7			20.3	19.5	20.4							
60%	19	10	10	8			8.2	8.9	8.7			6.6	7.9	7.7												
100%	63	10	10	3			7.5	8.9	8.7			6.2	7.4	7.2			20.3	19.5	20.6							
100%	82	10	10	5			7.5	8.9	8.7			6.2	7.4	7.2												
Initial of Investigator		DAC	MF	MF			MF	MF	MF			MF	MF	MF			MF	MF	MF							

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 120 | 124
100% Sample: 60 | 438
Other:
Measured by: DAC

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: 0 | 0
100% Sample: | 8.5
Other:
Hour

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

Conductivity, 401
Salinity: 57.7 MA

Signature: Mansell Fairbeth

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7.07 9.09	9.0 @ 21°C	9.3 @ 20 11.1 @ 30
24 Hours	7.01 9.09	9.2 @ 20°C	
48 Hours	7.07 9.01	9.2 @ 20°C	9.5 @ 20 11.2 @ 30
72 Hours			
96 Hours			

Remarks: Cond. 1295 @ 21°C
Microbx ©
45 in 12 KO @ 20°C

Total Residual Chlorine:
Field data: — Method: —
Lab: 0.0 Method: Hach
Lab: Method:

De-chlorinated:
Measurements by: DAC

Reviewed by:
Date: 8/15/88

NonToxic

BIOASSAY DATA FORM

Industry/Toxicant Gardinier East
Address Hwy 41 Hillsborough Co.
Contact/District SW / Dave Bennet
Effluent Serial Number 005
NPDES Permit Number FL0000761

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

Sample Collection: Date 3-7-88 Time 0945
Test Beginning: Date 3-8-88 Time 1030
Test Ending: Date 3-10-88 Time 1030
Test Organism Daphnia pulex
Age/Life Stage Neonates

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			9.0	9.0	8.7			8.0	8.3	8.0			20.3	19.7	19.7							
Control	26	10	10	10			9.0	9.0	8.7			8.0	8.3	8.0								210		215		
30%	17	10	10	10			8.6	9.0	8.7			6.8	7.9	7.7			20.4	19.3	19.5							
30%	8	10	10	8			8.6	9.0	8.7			6.8	7.8	7.8								610		640		
60%	38	10	10	10			9.0	8.9	8.7			6.5	7.5	7.5			20.6	19.2	19.5							
60%	25	10	10	10			9.0	8.9	8.7			6.5	7.5	7.5								990		1010		
100%	7	10	10	10			7.4	8.7	8.5			6.2	7.0	7.0			20.7	19.3	19.5							
100%	132	10	10	10			7.4	8.7	8.5			6.2	7.0	7.0								1490		1495		
Initial of Investigator		DAC	DAC	MF	MF			MF	MF	MF		MF	MF	MF			MF	MF	MF			MF		MF		

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 120 | 124
100% Sample: 60 | 438
Other:
Measured by:

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0

	U	T
Control Water:	0	0
100% Sample:		8.54
Other:		

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

Signature: David Chanton
Marshall Fairbull

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: 0.0 Method: Hach
Lab: Method:

Dechlorinated:
Measurements by: DAC

Reviewed by:
Date: 3/15/88

Remarks: Nearly all D.p. at 24 hr were caught on the surface film. D.O. at 48 hr.

Nontox16

BIOASSAY DATA FORM

Industry/Toxicant Gardinier East
Address Hwy 41, Hillsborough Co.
Contact/District EW/Dave/Dennis
Effluent Serial Number 005
NPDES Permit Number FL0006761

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

Sample Collection: Date 3-7-88 Time 0945
Test Beginning: Date 3-8-88 Time 1030
Test Ending: Date 3-10-88 Time 1030
Test Organism Notropis leedsi
Age/Life Stage 25 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	5	5	4	4			8.2	8.8	7.4			7.5	7.8	7.6			20.5	19.5	19.3							
Control	49	5	5	5			8.2	8.8	7.4			7.5	7.8	7.6								210		212		
30%	66	5	5	5			8.3	8.8	7.5			6.8	7.5	7.5			21.3	19.3	19.0							
30%	69	5	5	5			8.3	8.8	7.6			6.8	7.5	7.5								650		620		
60%	234	5	5	5			8.0	8.8	7.7			6.5	7.3	7.3			21.1	19.1	19.0							
60%	16	5	5	5			8.0	8.8	7.5			6.5	7.2	7.3								1040		1000		
100%	52	5	5	5			7.4	8.5	7.5			6.2	6.8	6.9			21.0	19.3	19.0							
100%	34	5	5	5			7.4	8.5	7.5			6.2	6.8	6.9								1520		1490		
Initial of Investigator		DAC	MF	MF	MF		JNF	JNF	MF			MF	JNF	MF			JNF	JNF	MF			JNF		MF		

Alkalinity/Hardness (mg/l as $CaCO_3$)

Control Water:	121	119
100% Sample:	60	438
Other:		

Measured by: DAC

Ammonia unionized/total (mg/l as NH_3/N)

Hour 0	U	T
Control Water:	0	0
100% Sample:		8.54
Other:		
Hour ____		
Control Water:		
100% Sample:		
Other:		

Measured by: MF

100% salinity: 1.0

Signatures: David Chambers
Marshall Faircloth

Instrument Calibrations:

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

Remarks:

Total Residual Chlorine:

Field data: — Method: —
Lab: 0.0 Method: Hach
Lab: ____ Method: ____

De-chlorinated:
Measurements by: DAC

Reviewed by:

Date: 5/15/88

075

MICROTOX™ Data Reduction Sheet

SOURCE: Hardwicke

Stimulatory

GRAPH / CALC EC 50 (15 MIN, 15 °C) = >100 (%SAMPLE) mg/L

SAMPLE DATA:

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

DATE ASSAYED: 3-08-88 TIME: 9:00

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: Lesley Sullivan REAGENT VIAL LOT NO. M-606

REMARKS:

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15-MIN	Γ5	Γ15	NOTES
A1	0	98	58	X	X	
B1		92	57			
C1		103	65			
A2	12.38	90	60	.073	-.022	
B2		92	62			
C2		91	62			
A3	24.75	77	54	.149	.006	
B3		91	65			
C3		87	60			
A4	49.5	86	64	.136	-.067	
B4		85	64			
C4		87	65			
A5	99	82	63	.196	-.032	
B5		77	57			
C5		86	66			
EC_{50} (MIN, °C)				>100	Stimulatory	

REVIEWED BY:

DATE: 8/15/88