BIOASSAY OF [redacted], INC., U.S. PHOSPHORIC PRODUCTS

OUTFALL #012, TAMPA, HILLSBOROUGH COUNTY, FLORIDA

NPDES #FL0000761

SAMPLED 4/5/82

Biological Section
Bureau of Water Analysis
May 10, 1982

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EXECUTIVE SUMMARY

Gardinier, Inc., Outfall #012, NPDES# FL0000761, on 6 to 8 April, 1982.

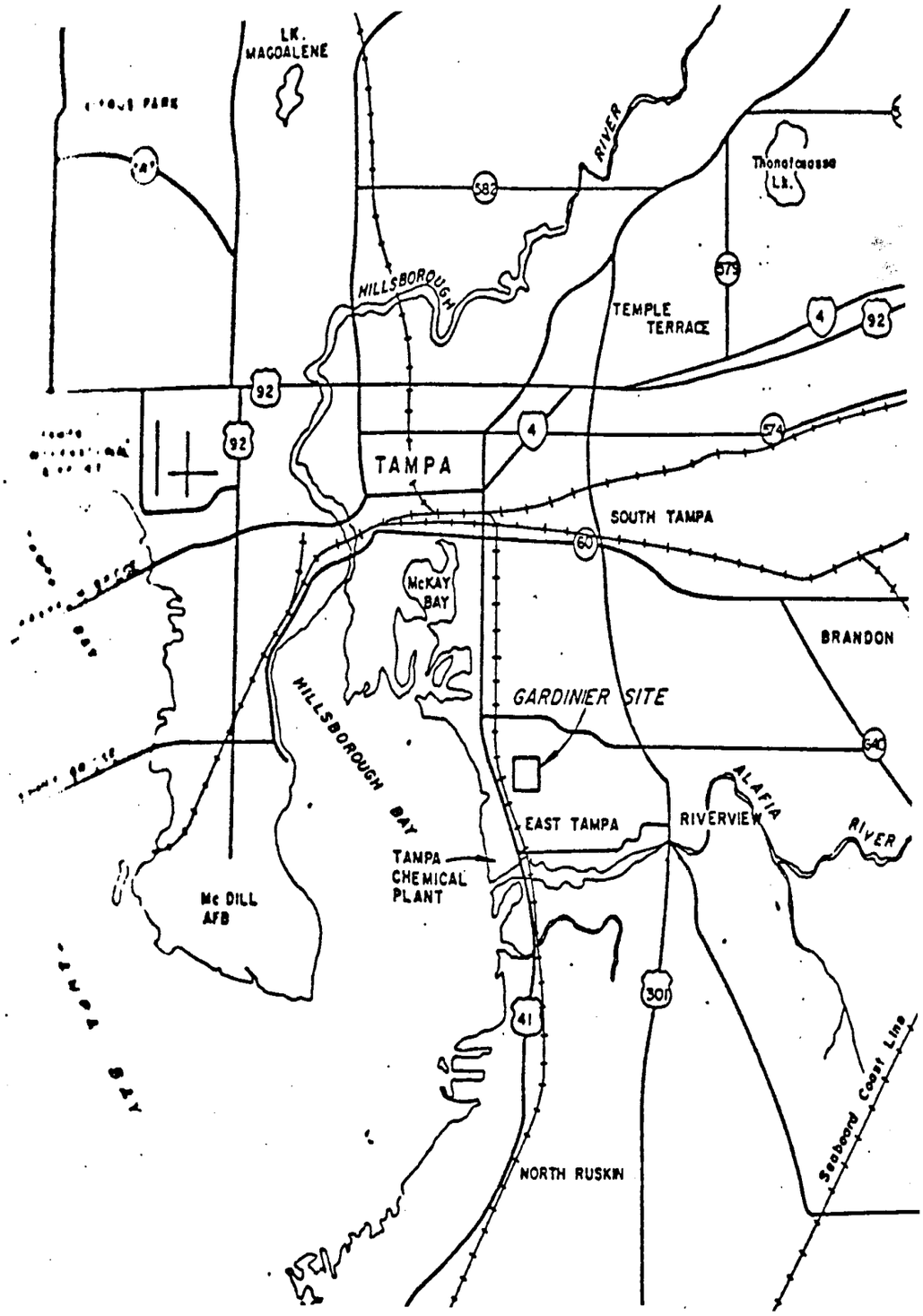
The sample of sludge pond overflow (Outfall #012) from this discharger, a manufacturer of fertilizer and inorganic chemicals located south of Tampa, was acutely toxic to the test organism, Mysidopsis bahia. A 48 hour LC_{50} of 75% was determined for this sample. The toxicity may be attributed, in part, to ammonia concentrations determined in the sample. Additionally, chemical analyses indicated the presence of significant concentrations of arsenic (240 ug/l) and of nickel (81 ug/l) in this effluent. These levels, however, are below those reported as being individually toxic to aquatic organisms, but may have adversely affected the test organisms.

INTRODUCTION

Gardinier, Inc., U.S. Phosphoric Products, is located at US 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 fluosilicic acids (11567 tons per day), triple super and super phosphate (2600 tons per day), ammonium phosphate (1728 tons per day), anhydrous ammonia (385 tons per day) and gypsum (14320 tons per day). Wastewater from the various operations is treated by liming and retention in three sludge ponds. As many as six NPDES permitted outfalls are located on the site, three of which (#001, #005 and #012) discharge to surface waters. Outfalls #001 and #005 contain cooling water, non-contact process water, treated process wastewater and wastewater from the sulfuric acid plant. They discharge to the Alafia River. Outfall #012 contains supernatant from the sludge ponds, and is discharged to Archie Creek, a tributary of Hillsborough Bay. A schematic of the facility and its wastewater outfalls is shown in Figure 2.

The Biological Section had previously performed toxicity bioassays on effluent from these three outfalls, in September, 1981. The results of those tests indicated that the effluents were not acutely toxic to the test organism, Mysidopsis bahia (FDER 1982). However, some lethality was noted in the highest test concentrations, particularily in effluent from Outfall #012. Additionally, significant amounts of total and unionized ammonia were determined in that sample, which may have partly caused the observed lethality. Chemical analyses were not performed on effluent from Outfall #012 at that time.

Based on those facts, the Biological Section recommended that a follow-up toxicity test be performed on effluent from Outfall #012. Therefore, to further evaluate the existence and extent of toxicity of this sludge pond overflow (Outfall #012) to the biota of the receiving waters, the Biological Section performed an additional static acute toxicity bioassay on a grab sample on 6 to 8 April, 1982. The results of this latest test are presented in this report.



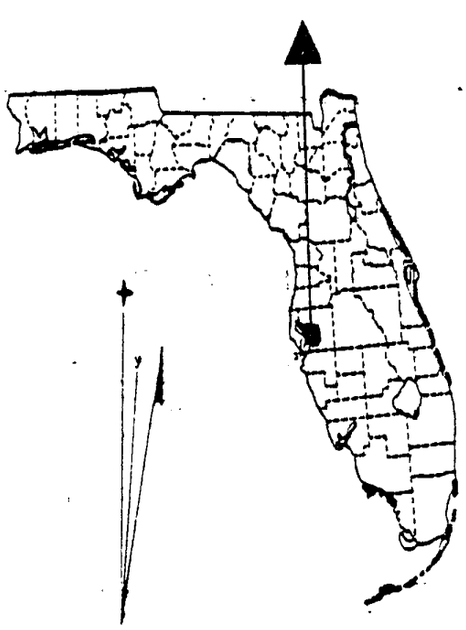


Figure 1. Portions of Hillsborough County, Florida, including the location of Gardinier, Inc.

METHODS AND MATERIALS

Test methods used to conduct this static bioassay are extensively described by Peltier (1978). The organism selected for this study is among those recommended by Peltier (1978) and the U.S.EPA (1978). The life history and maintenance procedures used to culture the organism are detailed below.

History and Maintenance of Bioassay Organisms

Mysidopsis bahia (mysid shrimp) - In September, 1978, approximately 300 adult mysid shrimp were obtained from the EPA laboratory in Gulf Breeze, Florida. The mysids are cultured under a 16:8 hour light-dark cycle in 10 and 15 gallon all glass aquaria equipped with undergravel filtration and aeration. The mysid shrimp are cultured over a wide range of salinities (10-30 ppt) to minimize acclimation periods to low or high salinity effluents. Seawater is obtained from the FSU Marine Laboratory located at Turkey Point, Florida (salinity range 17-31 ppt) and partial water changes are performed weekly. Freshwater, for dilution of the seawater as necessary, is obtained from a deep well near the DER laboratory. The mysids are fed twice daily with freshly hatched Artemia (brine shrimp) and are kept at a constant temperature of 22° plus or minus 1°C. Ten to fifteen day old (three-quarter adult size) mysids were used in this test.

To ensure the uniform sensitivity of these test organisms to toxic substances, the Biological Section conducts monthly standard reference toxicant bioassays using sodium lauryl sulfate as the reference toxicant.

Test Methods

A 48 hour static acute toxicity bioassay was performed on sludge pond overflow from Gardinier's Outfall #012. The test was performed from 1045 hours hours on 6 April to 1050 hours on 8 April, 1982.

The sample was collected by Ms. Phyllis Jones of DER's Southwest District at 1100 hours on 5 April, 1982, using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples for metal analysis were preserved with HNO_3. Samples for organic chemical analysis and for the bioassays were unpreserved. The samples were iced and transported to the DER laboratory in Tallahassee for analysis within 24 hours of collection.

Saltwater for dilution of the effluent sample was obtained from the FSU Marine Laboratory at Turkey Point (salinity = 26 ppt). This saltwater diluent was then adjusted between the salinities of the receiving bodies of water (Archie Creek = 9.5 ppt and Hillsborough Bay = 23.2 ppt) by adding an appropriate amount of DER well water. The final salinity of the diluent was adjusted to 19 ppt. For test concentrations of 56% and below, this adjusted diluent was mixed directly with the effluent, achieving a range of final salinities (11 ppt to 18 ppt). For the 100% effluent test concentration, freshly autoclaved artificial sea salts (Ocean 50®) were added until salinities of approximately 7 ppt were achieved (the lower limit of mysid shrimp salinity tolerance). A series of 3 control groups (Table 3) were then used to ensure that any mortality in the test organisms was not the result of toxic properties associated with the diluent, the artificial sea salts, or the salinity range. The control groups included: adjusted Turkey Point seawater (salinity = 19 ppt), artificial sea salt (Ocean 50®) adjusted well water (salinity = 7 ppt), and a salinity control consisting of Turkey Point seawater and well water (salinity = 9.5 ppt). The concentration series selected for this study (Table 1) is that recommended by the APHA (1980).

Two replicates of each test concentration and of the controls were used to test with Mysidopsis bahia. Immediately prior to the bioassay, a suitable number of mysids were transferred by net from the 10 ppt, 15 ppt and 20 ppt salinity aquaria to culture dishes for observation and selection of individuals for the test. Ten to fifteen day old (three-quarter adult size) mysids were used in this test. Individual mysid shrimp were siphoned using a glass tube, carefully examined for injury, and transferred to wide mouth quart jars, each containing 500 ml of the appropriate concentration. For each concentration, the mysid shrimp were pre-acclimated to within 4 ppt salinity. Five organisms were loaded per test chamber, giving ten per concentration. Based on the recommendations of EPA (1978), the mysids were fed brine shrimp (Artemia) nauplii at the rate of approximately 10 to 20 nauplii per mysid per day to minimize cannibalism.

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 salinity 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 seawater control and the 100% effluent test concentration at the beginning and end 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 and 3.

Table 1. Concentration Series and Volumes Used for This Static Bioassay

Mysidopsis bahia (mysid shrimp)

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, Mysidopsis bahia, static acute toxicity bioassay of Gardinier, Inc., Tampa, Hillsborough County, Florida, NPDES #FL0000761, Outfall #012, on 6 to 8 April, 1982.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
5.6%	5(4)[a]	4	4	8.0	7.3	6.8	7.9	7.9	7.8	22.0	19.3	19.7				
5.6%	5	5	5	8.0	7.3	6.8	7.9	7.9	7.8						18.3	18.0
10.0%	5	5	5	8.2	7.4	6.9	7.6	7.9	7.8	21.9	19.1	19.7				
10.0%	5	5	5	8.2	7.5	6.9	7.6	7.8	7.8						17.8	17.3
18.0%	5	5	5	8.2	7.5	6.9	7.3	7.7	7.7	21.9	19.1	19.7				
18.0%	5	5	5	8.2	7.5	7.0	7.3	7.7	7.7						16.5	16.0
32.0%	5	5	5	8.3	7.6	7.1	7.0	7.5	7.6	21.8	19.3	19.7				
32.0%	5	5	5	8.3	7.5	7.1	7.0	7.5	7.6						14.5	14.5
56.0%	5	5	5	8.5	7.5	7.1	6.7	7.2	7.3	21.8	19.5	19.7				
56.0%	5	5	5	8.5	7.5	7.1	6.7	7.1	7.3						11.0	11.0
100.0%	5	0	0	8.5	7.4	7.3	6.7	6.9	7.0	21.7	19.7	19.8	.06/25.3	.09/23.2		
100.0%	5	1	0	8.5	7.4	7.4	6.7	6.9	7.0						7.0	7.0

Remarks: [a] Number in parentheses used in calculations since one organism was cannibalized between 0 and 24 hours.

Table 3. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Gardinier, Inc., on 6 to 8 April, 1982.

Control Series

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
Seawater Control	5	5	5	8.0	7.5	7.5	7.9	8.0	7.9	22.2	19.5	19.9	.01/.44	.02/.80		
Seawater Control	5	5	5	8.0	7.5	7.4	7.9	8.0	7.9						19.0	19.0
Sea Salt Control	5	5	5	8.5	8.1	7.7	8.5	8.4	8.2	22.2	19.3	19.8				
Sea Salt Control	5	5	5	8.5	8.1	7.6	8.5	8.4	8.2						7.0	7.5
Salinity Control	5	5	5	8.2	8.0	7.5	8.0	8.1	8.0	22.1	19.3	19.8				
Salinity Control	5	5	5	8.2	8.0	7.5	8.0	8.1	8.0						9.5	9.5

Remarks:

Table 4. LC_{50} Data for Bioassay of Gardinier, Inc., Outfall #012, on 6 to 8 April, 1982.

Organism	Method of Data Analysis	24 LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Mysidopsis bahia	Binomial	79.6% (56 and 100)	74.8% (56 and 100)
	Moving Average	a	a
	Probit	a	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.

RESULTS AND DISCUSSION

The sample of sludge pond overflow from Gardinier's Outfall #012 proved to be acutely toxic to the test organism, Mysidopsis bahia. A 24 hour LC_{50} of 79.6% and a 48 hour LC_{50} of 74.8% were determined for this effluent (Table 4). The 96 hour LC_{50} for this sample would, therefore, be $\leq$ 74.8%. This toxicity may be attributed, in part, to the ammonia concentrations determined in the sample. Although the concentrations determined here (25.3 to 23.2 mg/l total, and .06 to .09 mg/l unionized, Table 2) are below the levels reported to be acutely toxic at the specific pH of our test to certain freshwater fish (Thurston et al. 1981), some adverse effects may occur to mysid shrimp. This is especially true when this low pH effluent (pH=6.7, Table 2) containing ammonia reaches the higher pH marine waters of Hillsborough Bay. Since the percent unionized ammonia increases dramatically with increasing pH, a substantial increase in toxic unionized ammonia may occur when this effluent enters Hillsborough Bay.

The DER Chemistry Section has analyzed a sample of this effluent for certain organics and metals. The results of these analyses are presented in Table 5. No organic compounds, as determined by the acid, base or pesticide extractable methods of analyses, were identified. Metal analyses did indicate the presence of significant concentrations of arsenic (240 ug/l) and of nickel (81 ug/l) in the effluent. These concentrations, however, are below those reported as being individually toxic to aquatic organisms (U.S. EPA 1976). The cause of the toxicity in this effluent, therefore, cannot be attributed to a single factor, but may be related to the synergistic effects of ammonia, arsenic and nickel.

TABLE 5. RESULTS OF CHEMICAL ANALYSES PERFORMED ON EFFLUENT FROM GARDINIER'S OUTFALL #012, collected 5 APRIL, 1982.

Organics

Parameter	Compound
Acid Extractables	N.D.
Base Neutral Extractables	N.D.
Pesticide Extractables	N.D.

Metals

Antimony	N.D.
Arsenic	240 µg/l
Beryllium	N.D.
Cadmium	<0.5 µg/l
Chromium	N.D.
Copper	<25 µg/l
Lead	N.D.
Mercury	N.D.
Nickel	81 µg/l
Selenium	<5 µg/l
Silver	N.D.
Thallium	N.D.
Zinc	<25 µg/l

N.D. = None Detected

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.

The two basic concepts utilized in the standards are acute and chronic toxicity, (defined in 17-3.021(1) and (3), respectively). Simplifying the actual F.A.C. language somewhat, acute toxicity is defined as the presence of a substance in a concentration one-third as great as that concentration which kills one-half of the test organisms in 96 hours, i.e., one-third of the 96 hour LC_{50}. Chronic toxicity is similarly defined as one-twentieth of the 96 hour LC_{50}. Therefore, if a test solution kills one-half or more of a group of test organisms in 96 hours, it must contain at least three times the defined "acutely toxic" concentration and at least twenty times the "chronically toxic" concentration of some sustance or substances. If a test solution causes that same mortality in less than 96 hours, (for example, in 24 or 48 hours), it is at least as toxic as the example given above, and actually is probably considerably more toxic.

The particular restrictions applied to a given discharger depend upon the existence of a mixing zone, whether as a specific part of the permit or based on the existence of a currently valid permit predating the language in Chapter 17-4 requiring mixing zone designation. In the absence of a mixing zone, the general criteria for surface waters (17-3.061) are applicable at all points, beginning at the point of discharge. This section prohibits the discharge of substances in concentrations which are chronically toxic (17-3.061(2)(o)); that is the concentrations cannot exceed one-twentieth of the 96 hour LC_{50} as discussed above. If a mixing zone does exist, then this chronic toxicity standard is only applicable from the edge of the mixing zone and beyond. Within the mixing zone, the less stringent acute toxicity standard of the minimum criteria for all waters (17-3.051(1)(d)) applies: one-third of the 96 hour LC_{50}. The acute toxicity standard does not apply, however, to each point within a mixing zone, but rather to the average of a number of points within a mixing zone, based on the provisions of the surface water mixing zones section of Chapter 17-4 (17-4.244(4)). At the same time, though, this same rule prohibits waste concentrations within the mixing zone (i.e., at any point within the mixing zone), from exceeding the 96 hour LC_{50}. Therefore, at any point within a mixing zone, including the point of discharge, one-half of the test organisms cannot be killed in 96 hours.

From the above discussion, it should be clear that if the least stringent toxicity standard, the 96 hour LC_{50}, is exceeded at any point, from the point of discharge outwards, by any discharger, then a violation of the standards has occurred. This is an important consideration since the test procedure here reported involves the measurement of the 50% lethal concentration, and not some fraction of that concentration. Any discharger required to meet the one-twentieth of the 96 hour LC_{50} standard is therefore being tested very leniently. Any reduction in the test period from 96 hours provides an added degree of leniency to a discharger.

A number of different dilutions were examined in order to determine by how much, if any, the discharge exceeds the 96 hour LC_{50} standard. For example, if the sample, after two-fold dilution with diluent (a 33% concentration), kills one-half of the test organisms after 96 hours, then the sample is three times as toxic as is allowed by that standard. Similarly, if a 5% solution of the sample kills 50% of the organisms, the sample is twenty times as toxic as is allowed. Toxicities are reported here in that manner, i.e., in terms of the percentage dilution of the test sample. The smaller the percentage which is reported means a correspondingly greater toxicity.

Note that even if a discharger receives a very large mixing zone, the regulations require that the maximum toxicity at any point within the mixing zone be restricted to the 96 hour LC_{50}. The results here reported are based on actual effluent samples, representative of the effluent concentration which enters waters of the state at the point of discharge. If it is demonstrated that, under the conditions existing at the point of discharge, effluent concentrations will be less than at the actual sampling point (if the two points are different), the reported bioassay results will reflect the reduced discharge concentration.

With respect to the species of organisms used in these tests, the language of the F.A.C. indicates that they should be "significant to the indigenous aquatic community". All species used in these tests are significant to the indigenous aquatic community since they are sensitive to toxic substances which may be expected to impact the indigenous community.

The toxicity reported here is in excess of allowable limits.

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