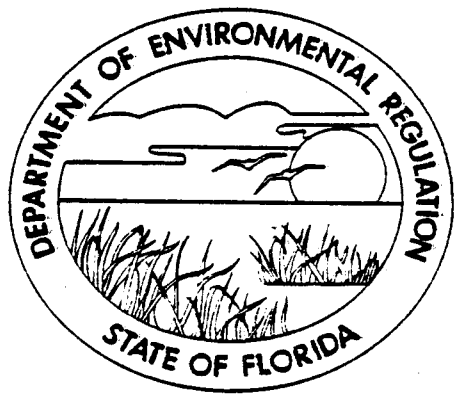


BIOASSAYS OF DIAMOND SHAMROCK

EXPERIMENTAL AGRICULTURAL STATION

NAPLES, COLLIER COUNTY, FLORIDA

NO NPDES PERMIT

SAMPLED 2/15/83

Biology Section
Bureau of Water Analysis
March 30, 1983

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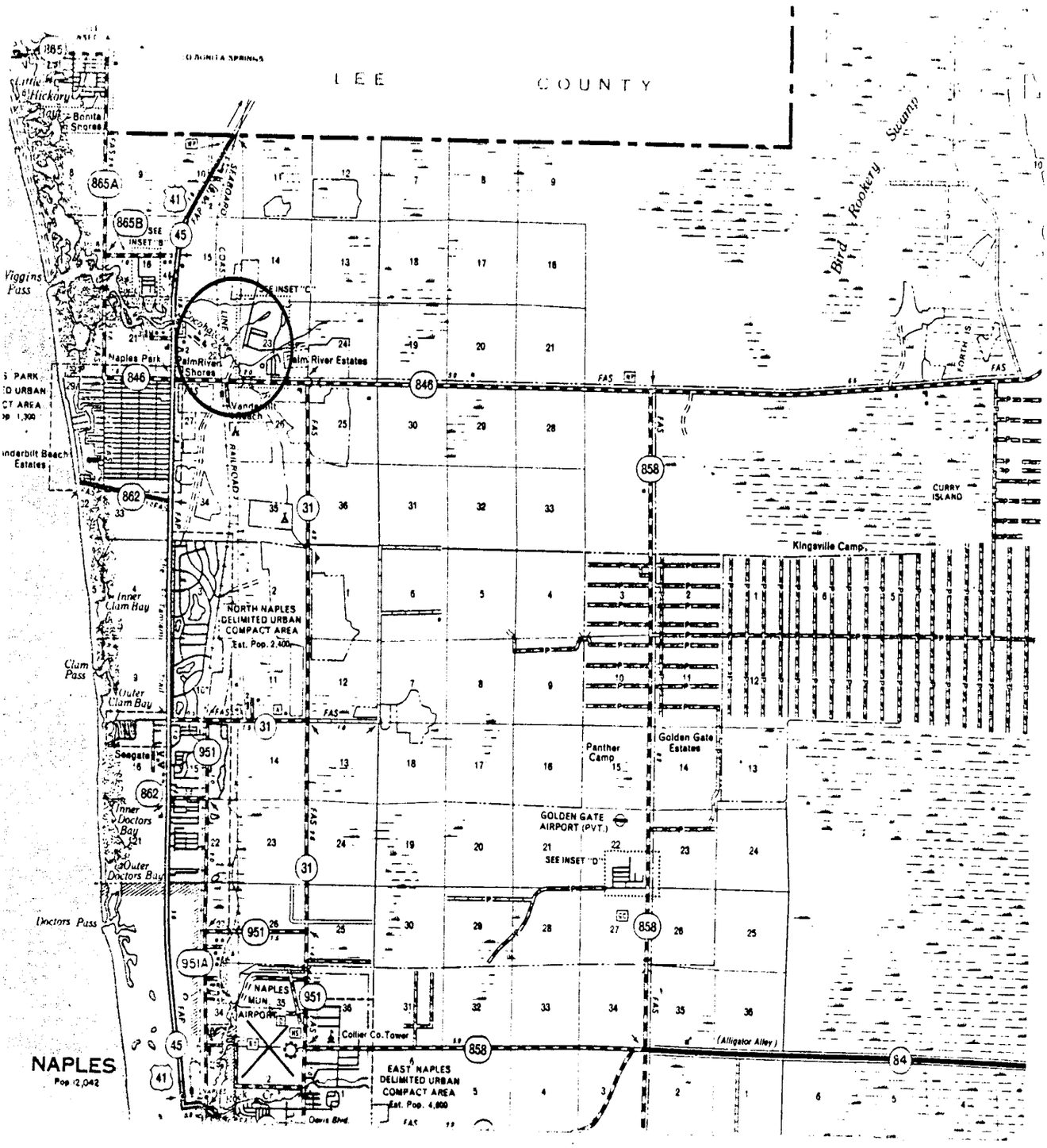
EXECUTIVE SUMMARY

Diamond Shamrock Agricultural Station, Naples, on 16 to 18 February, 1983.

The sample of water from Diamond Shamrock's retention pond, collected at the discharge point to a nearby ditch, was not acutely toxic to the test organisms, Daphnia pulex, Pimephales promelas or Mysidopsis bahia. Only a few random D. pulex mortalities, no P. promelas mortality, and one M. bahia mortality were noted during the 48 hour static tests. Since the sample was not acutely toxic, chemical analyses were not performed.

INTRODUCTION

The Diamond Shamrock Experimental Agricultural Station is located on the Naples - Immokalee Road, near Naples, Collier County, Florida. Irrigation water used at the station is collected in a retention pond prior to being discharged to a series of nearby ditches. These ditches eventually connect to the Cocohatchee River, an estuary to the Gulf of Mexico (Fig. 1). According to Mr. Stuart Bradow, of DER's South Florida District, this irrigation water may contain certain pesticides used at the station which may impose a threat to the biota of the receiving waters. At the request of Mr. Bradow, the Biology Section performed three static bioassays to evaluate the existence and extent of toxicity of this irrigation water. Two bioassays were performed, on invertebrate and vertebrate freshwater test organisms, to evaluate the immediate effects of the discharge, while a third bioassay was performed on a saltwater test organism, to evaluate the effects the discharge may have upon reaching an esturine system. The results of these tests are presented in this report.



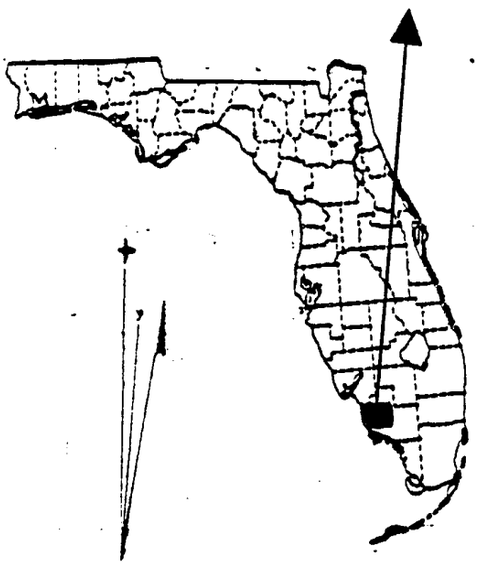


Figure 1. Portions of Collier County, Florida, including location of the Cocohatchee River.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978). The organisms selected for this study are among those recommended by Peltier (1978) and the U.S. EPA (1978). Life histories of these test organisms and maintenance procedures used to culture them are detailed below.

History and Maintenance of Bioassay Organisms

Daphnia pulex (water flea) - A breeding culture of Daphnia pulex was obtained from the EPA laboratory in Athens, Georgia, 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.

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 2 weeks old at the time of the test.

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 Biology Section conducts monthly standard reference toxicant bioassays using sodium lauryl sulfate as the reference toxicant.

Test Methods

Three separate 48 hour static acute toxicity bioassays were performed on a sample of water from Diamond Shamrock's retention pond. The tests were performed from 1115 hours on 16 February to 1130 hours on 18 February, 1983. The sample was collected by Mr. Stuart Bradow of DER's South Florida District at 1500 hours on 15 February, 1983, using standard wastewater sampling procedures. 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 iced and transported to the DER laboratory in Tallahassee for analyses within 24 hours of collection.

Water for dilution of the effluent sample for the freshwater tests 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 flea, Daphnia pulex. 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 were siphoned into a large culture dish 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 this test.

Two replicates, of six concentrations and a control, were used for the Pimephales promelas (fathead minnow) bioassay. 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 this test.

Saltwater for dilution of the effluent sample for the Mysidopsis bahia test was obtained from the FSU Marine Laboratory at Turkey Point (salinity = 28.0 ppt). For test concentrations of 56% and below, this diluent was mixed directly with the effluent, achieving a range of final salinities (13.0 ppt to 27.5 ppt). For the 100% effluent test concentration, freshly autoclaved artificial sea salts (Ocean 50®) were added until a salinity of approximately 7 ppt was achieved (the lower limit of mysid shrimp salinity tolerance). Two control groups (Table 4) 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:

1) Diluent Control - Turkey Point water (28.0 ppt). This represents the highest concentration of Turkey Point water used in the bioassays, and tests for toxicity in the diluent. This is also the highest salinity used in the bioassays, and therefore tests the tolerance of the organisms to this salinity.

2) Sea Salts Control - Well water, adjusted with artificial sea salts (Ocean 50®) to approximately 7 ppt salinity. This tests for toxicity of the highest concentration of artificial sea salts used in the bioassays, as well as for tolerance of the organisms to the lowest salinity used in the bioassays.

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, 20 ppt and 25 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. Total alkalinity and total hardness were recorded at the beginning of the test for the controls and the 100% effluent concentration. The specific conductance or salinity of each test concentration was recorded at the beginning and end of the test. Ammonia concentrations, including total and the unionized fraction (expressed in mg/l as $NH_3 + NH_4$ 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 2,3 and 4.

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

Daphnia pulex

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 minnow)

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, Daphnia pulex, static acute toxicity bioassay of Diamond Shamrock Agricultural Station, Naples, Collier County, Florida, on 16 to 18 February, 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	10	8.9	9.1	8.7	7.8	8.2	8.2	102		115		20.9	20.0	18.5	0/0	--		
Control	10	10	10	8.9	9.1	8.7	7.8	8.2	8.3										227	209
5.6%	10	10	10	8.9	9.2	8.7	7.8	8.2	8.3					20.8	19.8	18.6				
5.6%	10	10	10	8.9	9.2	8.7	7.8	8.2	8.3										282	259
10.0%	10	10	9	8.9	9.2	8.7	7.8	8.3	8.3					20.8	19.8	18.6				
10.0%	10	10	10	8.9	9.2	8.7	7.8	8.3	8.3										292	268
18.0%	10	10	10	8.9	9.2	8.7	7.8	8.3	8.3					20.7	19.7	18.6				
18.0%	10	10	9(10)[a]	9.0	9.1	8.6	7.8	8.3	8.3										332	307
32.0%	10	9	9	9.1	9.0	8.6	7.9	8.3	8.3					20.5	19.7	18.7				
32.0%	10	9	9	9.2	9.0	8.6	7.9	8.3	8.3										420	390
56.0%	10	10	10	9.3	8.9	8.6	7.8	8.3	8.4					20.4	19.7	18.7				
56.0%	10	10	10	9.5	8.9	8.6	7.8	8.3	8.4										590	488
100.0%	10	10	10	10.8	8.7	8.5	7.7	8.4	8.5	229		273		20.1	19.7	18.8	0/0	--		
100.0%	10	10	10	10.8	8.7	8.5	7.7	8.4	8.5										800	780

Remarks: [a] Number in parentheses used since one organism was stuck to side of glass.

Table 3. Data recorded during 48 hour, Pimephales promelas, static acute toxicity bioassay of Diamond Shamrock Agricultural Station, Naples, Collier County, Florida, on 16 to 18 February, 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	8.7	8.3	7.7	8.1	8.1	102		115		21.0	19.4	18.4	0/0	--		
Control	5	5	5	8.5	8.7	8.4	7.7	8.1	8.1										220	205
5.6%	5	5	5	8.5	8.7	8.6	7.8	8.1	8.2					20.9	19.3	18.3				
5.6%	5	5	5	8.5	8.7	8.6	7.8	8.1	8.2										253	233
10.0%	5	5	5	8.5	8.8	8.6	7.8	8.2	8.2					20.8	19.2	18.3				
10.0%	5	5	5	8.5	8.8	8.6	7.8	8.2	8.2										279	258
18.0%	5	5	5	8.6	8.7	8.6	7.8	8.1	8.2					20.7	19.2	18.3				
18.0%	5	5	5	8.7	8.6	8.5	7.8	8.1	8.2										320	299
32.0%	5	5	5	8.8	8.5	8.6	7.8	8.2	8.3					20.6	19.3	18.4				
32.0%	5	5	5	9.0	8.5	8.6	7.8	8.2	8.3										393	318
56.0%	5	5	5	9.4	8.5	8.6	7.8	8.2	8.3					20.3	19.4	18.5				
56.0%	5	5	5	9.4	8.5	8.6	7.8	8.2	8.3										590	485
100.0%	5	5	5	10.6	8.3	8.4	7.7	8.3	8.4	229		273		20.0	19.6	18.7	0/0	--		
100.0%	5	5	5	10.6	8.3	8.4	7.7	8.3	8.4										800	790

Remarks:

Table 4. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Diamond Shamrock Agricultural Station, Naples, Collier County, Florida, on 16 to 18 February, 1983.

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	6.9	6.7	7.0	8.1	8.0	7.9	20.9	19.6	18.5	0/0	--		
Seawater Control	5	5	5	6.9	6.7	7.0	8.1	8.0	7.9						27.5	27.0
Sea Salts Control	5	5	5	8.1	7.8	7.6	8.8	8.5	8.3	21.2	19.5	18.4				
Sea Salts Control	5	5	5	8.1	7.8	7.6	8.8	8.5	8.3						7.2	7.2
5.6%	5	5	5	7.0	7.0	7.1	8.1	8.0	7.9	20.7	19.3	18.3				
5.6%	5	5	5	7.0	7.0	7.1	8.1	8.0	7.9						26.0	25.3
10.0%	5	5	5	7.0	6.9	7.2	8.1	8.0	8.0	20.3	19.1	18.2				
10.0%	5	5	5	7.0	6.9	7.2	8.1	8.0	8.0						25.0	24.3
18.0%	6[a]	6	6	7.2	6.8	7.2	8.0	8.0	8.0	20.3	19.1	18.1				
18.0%	5	5	5	7.3	6.8	7.2	8.0	8.0	8.0						22.9	22.3
32.0%	5	5	5	7.9	7.0	7.3	8.0	8.1	8.1	20.1	19.1	18.2				
32.0%	5	5	5	7.9	7.0	7.3	8.0	8.1	8.1						19.2	18.8
56.0%	5	5	5	8.8	7.3	7.5	7.9	8.1	8.2	20.0	19.2	18.2				
56.0%	5	5	4(5)[b]	8.8	7.3	7.5	7.9	8.1	8.2						13.0	12.5
100.0%	5	5	4	9.3	7.9	7.7	8.5	8.5	8.5	19.7	19.3	18.4	0/0	--		
100.0%	5	5	5	9.3	7.9	7.7	8.5	8.5	8.5						7.2	7.2

Remarks: [a] Extra organism loaded by mistake, allowed to remain.

[b] Number in parentheses used since one organism was cannibalized between 24 and 48 hours.

RESULTS AND DISCUSSION

The sample of water collected from Diamond Shamrock's retention pond was not acutely toxic to the test organisms, *Daphnia pulex*, *Pimephales promelas* and *Mysidopsis bahia*. Only a few random *D. pulex* mortalities, no *P. promelas* mortality, and one *M. bahia* mortality were noted during the 48 hour static tests. Since the sample was not acutely toxic, chemical analyses were not performed.

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