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DEPARTMENT OF ENVIRONMENTAL REGULATION
STATE OF FLORIDA

BIOASSAYS OF PANAMA CITY BEACH

WASTEWATER TREATMENT PLANT

PANAMA CITY BEACH, BAY COUNTY, FLORIDA

NPDES #FL0021512

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OCTOBER 16, 1987

Biology Section
Division of Environmental Programs

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PANAMA CITY BEACH, BAY COUNTY, FLORIDA

NPDES #FL0021512

Biology Section
Bureau of Laboratories and
Special Programs
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EXECUTIVE SUMMARY

Panama City Beach Wastewater Treatment Plant, Panama City Beach, Bay County, Florida, NPDES #FL0021512, 12 to 14 August, 1987.

Five static acute toxicity bioassays and two Microtox® assays were performed on samples of chlorinated and de-chlorinated recovered water collected from Panama City Beach WWTP. This facility discharges to an unnamed creek which flows to West Bay and eventually to St. Andrews Bay. Results indicated chlorine toxicity (residual chlorine = 1.3 mg/l HACH) to Ceriodaphnia dubia and Notropis leedsi, with 48 hour LC_{50}'s of 18.8% and < 50.0%, respectively. Additionally, the Microtox® assay indicated toxicity to Photobacterium phosphoreum, with an EC_{50} (15 minutes, 15°C) of 22.42%.

Bioassays with de-chlorinated sample resulted in acute toxicity to C. dubia (48 hour LC_{50} = 83.0%). Only one individual died in the Daphnia pulex test, and no mortality was observed in the N. leedsi bioassay. Likewise, the de-chlorinated sample was not toxic to P. phosphoreum EC_{50} (15 munites, 15°C) > 100%. The total and unionized ammonia (0.045 mg/l and 0.001 mg/l, respectively) were not sufficient to have caused the observed toxicity to C. dubia.

Chemical analyses indicated only a trace amount (< 3.0 ug/l) of lead, and the results for copper and zinc were invalidated. The cause of the toxicity in the de-chlorinated test was not apparent.

INTRODUCTION

The Panama City Beach Sewage Treatment Plant is located at 110 South Arnold Road, in Panama City Beach, Bay County, Florida (Fig. 1). According to the NPDES permit, this facility has a design flow capacity of 1.1 MGD, although peak flows of 2.0 MGD have occurred. The facility treats domestic waste from a population which fluctuates seasonally due to tourism from 6,000 to 10,000. Treatment consists of contact stabilization, activated sludge aeration and post-chlorination. Recovered water flows to a polishing pond and is discharged to an unnamed creek which flows to West Bay and eventually to St. Andrews Bay.

At the request of DER's Northwest District office, static acute toxicity bioassays were performed to evaluate the potential aquatic toxicity of this discharge to the biota of the receiving waters. Six bioassays were performed from 19 to 21 February, 1985. Results indicated chlorine toxicity to the freshwater test organisms, Daphnia pulex and Notropis leedsi, with 48 hour LC_{50}'s of 13.3% and 22.3%, respectively. In bioassays with a saltwater organism, Mysidopsis bahia, the chlorinated discharge produced a 48 hour LC_{50} of 17.8% and the de-chlorinated was also toxic at 66.1%. No cause for this toxicity was apparent from the organic chemical analysis, and the sample for metals analysis was invalidated (FDER 1985a).

To further evaluate the toxicity of this municipal discharge, the Biology Section performed five bioassays and a Microtox® assay on a sample collected in August, 1987. Results are presented in this report. Those involved in performing the tests and in preparation of this report were: J. Epler, J. M. Faircloth, R. B. Frydenborg, K. M. Lurding, M. L. Roll, and L. A. Williams.

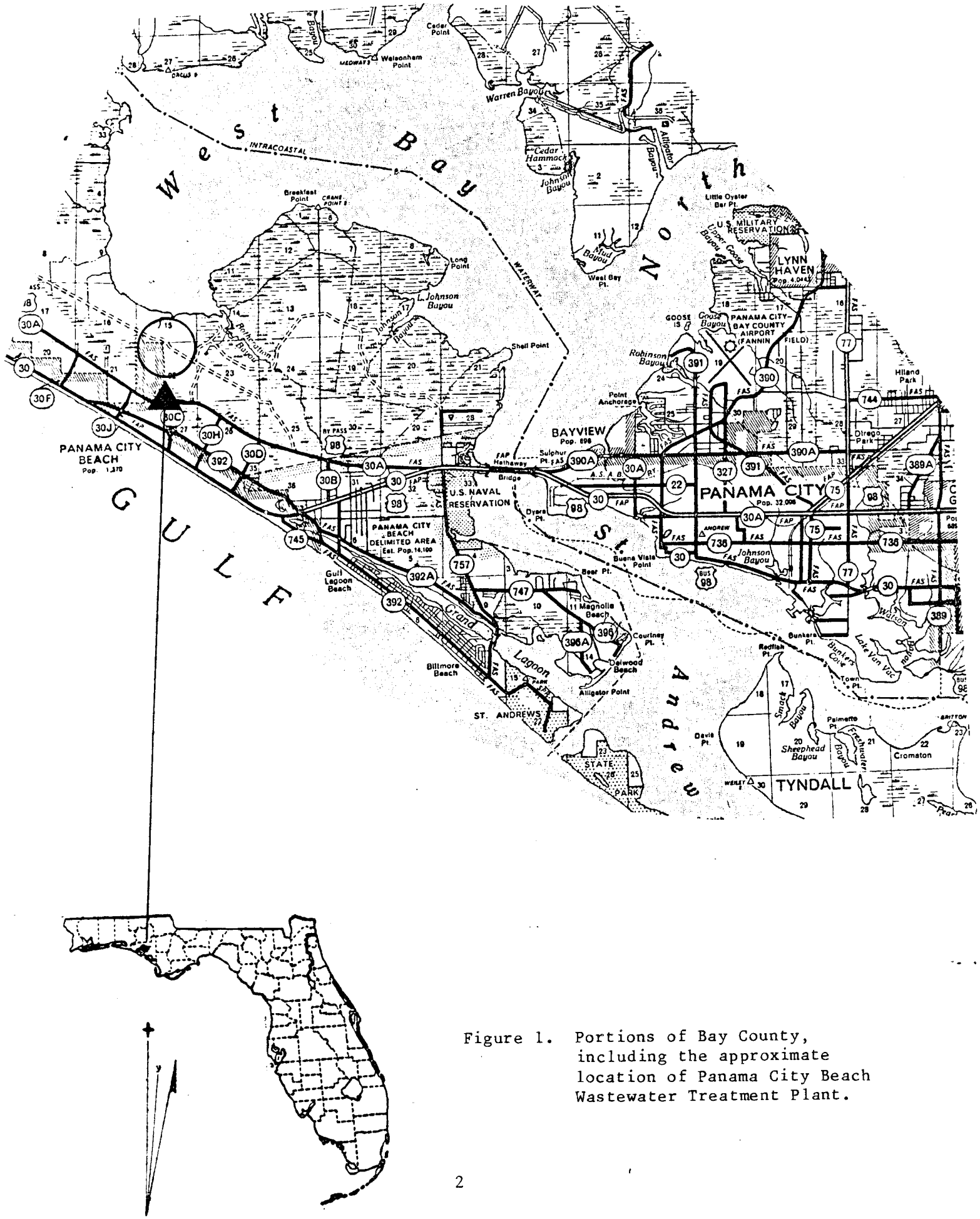


Figure 1. Portions of Bay County, including the approximate location of Panama City Beach Wastewater Treatment Plant.

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 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.

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 30 and 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 Tetra-Min® 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 Tetra Min®. The fish used in this test were 28 days old at the time of the test.

Photobacterium phosphoreum, a luminescent marine bacterium, is maintained as a freeze-dried culture, purchased from Microbics Corporation. Lot number M-603 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).

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.

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

Five separate static acute toxicity bioassays were performed on a sample of recovered water collected from Panama City Beach Wastewater Treatment Plant. The tests were performed from 1020 hours on 12 August to 1030 hours on 14 August, 1987.

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. Joe Villadsen of DER's Northwest District Branch office in Panama City at 1120 hours on 11 August, 1987, 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 sample in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the sample allowed to rise to 20° $\pm$ 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.

Total Residual Chlorine

Since many discharges are chlorinated, especially those originating from sewage treatment facilities, it is often desirable to differentiate between the toxicity resulting from chlorine and the toxicity associated with other components of the sample. To accomplish this, the Biology Section performed concurrent bioassays on a sample of chlorinated recovered water in which the chlorine had been neutralized. De-chlorination was accomplished by adding a calculated amount of 0.025N sodium thiosulfate. The total residual chlorine concentrations of the tested samples are given on the bioassay data forms. A comparison of the results of these tests will distinguish between the toxicity due solely to residual chlorine and the toxicity inherently contained in the recovered water.

Freshwater Tests

Freshwater for dilution of the recovered water samples 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 each concentration and a control, were used to test 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 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, and loaded 10 per chamber, resulting in 20 per test concentration. Daphnia were siphoned into a large culture dish to isolate the proper size and age organisms (neonates). To accomplish this, the Daphnia 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 each concentration and a control, were used for the Notropis leedsi (bannerfin shiner) 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 these tests.

Test Parameters

Dissolved oxygen (YSI Model 57 oxygen meter), pH (Corning Model 7 pH meter with Orion Ross® 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% 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 daily 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% 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 Tables 2 and 3.

Microtox® Test

Upon receipt at the DER laboratory, a 20 ml subsample was collected in a small beaker. 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 microliters 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 Tables 6 and 7.

RESULTS AND DISCUSSION

The chlorinated sample of recovered water proved to be acutely toxic to the test organisms, Ceriodaphnia dubia (48 hour LC_{50} = 18.8%) and Notropis leedsi (48 hour LC_{50} < 50.0%) (Table 4). This sample was also toxic to Photobacterium phosphoreum, with an EC_{50} (15 minutes, 15°C) of 22.42% (Table 6). The residual chlorine measured in this sample (1.3 mg/l HACH and 1.2 mg/l Iodometric) was largely responsible for the observed toxicity. The Daphnia pulex bioassay using chlorinated sample was invalidated due to excessive control mortality (see raw data).

The de-chlorinated sample was also toxic to C. dubia, with a 48 hour LC_{50} of 83.% (Table 4). One individual died in the D. pulex bioassay, and no mortality was observed in the N. leedsi test (Table 3). The Microtox® assay indicated no toxicity, with an EC_{50} (15 minutes, 15°C) of > 100% (Table 7). The total and unionized ammonia measured in this sample (0.045 mg/l and 0.001 mg/l, respectively) were not sufficient to have caused the toxicity to C. dubia.

Chemical analyses (Table 5) indicted only a trace amount of lead (< 3.0 ug/l). The results for copper and zinc were invalidated.

The toxicity measured here is in violation of water quality 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	--------	50 ml
10.0%	45.0 ml	5.0 ml	50 ml
50.0%	25.0 ml	25.0 ml	50 ml
100.0%	-------	50.0 ml	50 ml

Daphnia pulex (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	200.0 ml	--------	200 ml
10.0%	180.0 ml	20.0 ml	200 ml
50.0%	100.0 ml	100.0 ml	200 ml
100.0%	--------	200.0 ml	200 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Sample	Total Volume
Control	500 ml	--------	500 ml
10.0%	450 ml	50 ml	500 ml
50.0%	250 ml	250 ml	500 ml
100.0%	------	500 ml	500 ml

* The 10% and 50% concentrations were used for tests with chlorinated sample. The 50% and 100% concentrations were used for tests with de-chlorinated sample.

Table 2. Data recorded during 48 hour, Ceriodaphnia dubia and Notropis leedsi, static acute toxicity bioassays of Panama City Beach Wastewater Treatment Plant, NPDES #FL0021521, 12 to 14 August, 1987.

Chlorinated Discharge[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)	
	0	24	48	0	24	48[b]	0	24	48[b]	0	24	48[b]	0	48[b]
Ceriodaphnia dubia														
Control	10	10	10	8.5	8.2	8.2	8.0	7.9	7.9	20.9	20.9	22.0		
Control	10	10	10	8.6	8.2	8.2	8.0	7.9	7.9					
10%	10	10	9	8.5	8.3	8.3	7.9	8.0	7.9	20.8	20.8	20.7		
10%	10	10	9	8.6	8.3	8.3	7.9	8.0	7.9					
50%	10	0	-	8.6	8.4	-	7.8	8.0	-	20.7	20.9	-		
50%	10	0	-	8.6	8.4	-	7.8	8.0	-					
Notropis leedsi														
Control	5	5	5	8.1	8.0	7.7	7.6	7.7	7.7	21.7	20.8	20.5		
Control	5	5	5	8.1	8.0	7.7	7.6	7.7	7.7				210	220
10%	5	5	5	8.5	8.0	7.8	7.6	7.7	7.7	21.7	20.7	20.4		
10%	5	5	5	8.5	8.0	7.8	7.6	7.7	7.7				265	270
50%	5	0	-	8.7	8.1	-	7.7	7.8	-	21.1	20.6	-		
50%	5	0	-	8.7	8.1	-	7.7	7.8	-				460	-

Control Water	
NH_3(as NH_3)(mg/l) 0.001	Total Alkalinity 105 (mg/l $CaCO_3$)
Total ammonia (as N)(mg/l) 0.033	Total Hardness 115 (mg/l $CaCO_3$)

100% Sample	
NH_3(as NH_3)(mg/l) 0.001	Total Alkalinity 120 (mg/l $CaCO_3$)
Total ammonia (as N)(mg/l) 0.045	Total Hardness 175 (mg/l $CaCO_3$)

[a] Residual Chlorine: 1.3 mg/l HACH
1.2 mg/l Iodometric

[b] When all organisms dead at 24 hours, parameters not measured at 48 hours.

Table 3. Data recorded during 48 hour, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi, static acute toxicity bioassays of Panama City Beach Wastewater Treatment Plant, NPDES #FL0021512, 12 to 14 August, 1987.

De-chlorinated Discharge

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Ceriodaphnia dubia														
Control	10	10	10	8.1	8.1	8.7	8.0	7.9	7.9	21.1	20.9	20.7		
Control	10	10	9	8.2	8.1	8.7	8.0	8.0	7.9					
50%	10	10	10	8.5	8.1	8.7	7.8	7.9	7.9	20.9	20.8	20.6		
50%	10	10	10	8.9	8.1	8.7	7.8	7.9	7.9					
100%	10	7	2	9.4	7.8	8.8	7.8	8.0	7.9	20.6	20.8	20.6		
100%	10	8	3	9.6	7.8	8.8	7.8	8.0	7.9					
Daphnia pulex														
Control	10	10	10	8.5	8.2	8.7	8.0	7.9	7.9	21.5	20.7	20.5		
Control	10	10	10	8.5	8.2	8.7	8.0	7.9	7.9				195	210
50%	10	10	10	8.8	8.3	8.8	7.8	7.9	7.8	21.1	20.6	20.5		
50%	10	10	10	8.8	8.3	8.8	7.8	7.9	7.8				460	470
100%	10	10	10	10.2	8.4	8.4	7.8	8.0	7.8	20.7	20.6	20.4		
100%	10	10	9	10.3	8.4	8.4	7.8	8.0	7.8				790	820
Notropis leedsi														
Control	5	5	5	8.4	7.8	8.3	7.6	7.7	7.7	21.7	20.5	20.3		
Control	5	5	5	8.5	7.8	8.3	7.6	7.7	7.7				212	210
50%	5	5	5	9.3	8.0	8.2	7.7	7.8	7.7	21.3	20.4	20.2		
50%	5	5	5	9.3	8.0	8.2	7.7	7.8	7.7				468	460
100%	5	5	5	10.2	8.0	8.1	7.7	7.8	7.8	20.7	20.7	20.2		
100%	5	5	5	10.2	8.0	8.1	7.7	7.8	7.8				810	810

Control Water

NH_3(as NH_3)(mg/l) 0.001

Total ammonia (as N)(mg/l) 0.033

Total Alkalinity 105 (mg/l $CaOO_3$)

Total Hardness 115 (mg/l $CaOO_3$)

100% Sample

NH_3(as NH_3)(mg/l) 0.001

Total ammonia (as N)(mg/l) 0.045

Total Alkalinity 120 (mg/l $CaOO_3$)

Total Hardness 175 (mg/l $CaOO_3$)

Table 4. LC_{50} Data for Bioassays of Panama City Beach Wastewater Treatment Plant, NPDES #FL0021512, 12 to 14 August, 1987.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Chlorinated Discharge			
Ceriodaphnia dubia	Binomial	22.4% (10 and 50)	18.8% (10 and 50)
	Moving Average	a	a
	Probit	a	a
Notropis leedsi	Binomial		
	Moving Average	< 50.0%, b	< 50.0%, b
	Probit		
De-chlorinated Discharge			
C. dubia	Binomial	a	83.0% (50 and 100)
	Moving Average	a	a
	Probit	a	a
D. pulex	Binomial		a
	Moving Average	No Mortality	a
	Probit		a
N. leedsi	Binomial		
	Moving Average	No Mortality	No Mortality
	Probit		

[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] The LC_{50} is < 50.0% since all the organisms died in this test concentration (50.0%).

Table 5. Results of chemical analyses performed on recovered water from Panama City Beach Wastewater Treatment Plant, NPDES #FL0021512, collected 11 August, 1987.

Organics	
Acid Extractables	N.D.
Base Neutral Extractables	N.D.
Pesticide Extractables	N.D.

Metals	
Arsenic	N.D.
Cadmium	N.D.
Chromium	N.D.
Copper	___[a]
Lead	< 3.0 ug/l[b]
Mercury	N.D.
Nickel	N.D.
Selenium	N.D.
Silver	N.D.
Zinc	___[a]

N.D. = None Detected

[a] Results for copper and zinc were invalidated due to contamination of field blank.

[b] Actual value is known to be less than value given.

Table 6. Microtox test results, Panama City Beach WWTP, NPDES #FL0021512, 12 August 1987. Chlorinated discharge.

Microtox Test Culture Lot #M-603

	Final Readings	
% Sample	5-min (I_5)	15-min (I_{15})
0	56	41
0	52	38
0	55	40
12.38	40.5	25
12.38	40	25
12.38	42	26
24.75	27	15
24.75	37	20
24.75	38	21.5
49.5	26.5	12.5
49.5	31	15.5
49.5	25	12
99	6.5	4
99	5	3
99	5	3

EC_{50} (15 min., 15°C) = 22.42% sample
EC_{50} (15 min, 15°C) calculated using the Data Reduction Computer Program developed by Microbics Corporation for the Sharp Model EL-5100 (Microtox®) Systems Operating Manual, 1982.

Table 7. Microtox test results, Panama City Beach WWTP, NPDES #FL0021512, 12 August, 1987. De-chlorinated discharge

Microtox Test Culture Lot #M-603

% Sample	Final Readings 5-min (I_5)	15-min (I_{15})
0	58	36
0	56	36
0	59	38
12.38	57	36
12.38	55	35.5
12.38	57	36
24.75	60	38
24.75	62	40
24.75	59	39
49.5	54	34
49.5	57.5	37
49.5	53	34
99	70	43
99	49.5	30
99	50	32

EC_{50} (15 min., 15°C) > 100% sample
EC_{50} (15 min, 15°C) calculated using the Data Reduction Computer Program developed by Microbics Corporation for the Sharp Model EL-5100 (Microtox®) Systems Operating Manual, 1982.

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 (4), 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 onetwentieth 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 substance 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, whetheras 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)(p)]; 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 not designated for dredge and fill permitting, but rather to the average of a number of points within such 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 such a mixing zone (i.e., at any point within the mixing zone), from exceeding the 96 hour LC_{50}. Therefore, at any point within such a mixing zone, including the point of discharge, one-half of the test organisms cannot be killed in 96 hours. Within a mixing zone designated for dredge and fill permitting, the acute toxicity standard (one-third of the 96 hour LC_{50}) applies at all points, including the point of discharge.

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}. 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 not designated for dredge and fill permitting, 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 recovered water samples, representative of the 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, 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.

LITERATURE CITED

Florida Department of Environmental Regulation, Biology Section. 1985a. Bioassays of Panama City Beach Sewage Treatment Plant, Panama City Beach, Bay County, Florida, NPDES #FL0021512. FDER. 18 p.

_____. 1985b. Quality Assurance Manual for Performing Acute Toxicity Tests (Second Edition). FDER.

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.

Industry/Toxicant PANAMA CITY BEACH W.W.T.P. BIOASSAY DATA FORM

Address 206 N. GULF BLVD. PANAMA CITY BEACH, FL. 32407

Contact/District Richard Sublette

Effluent Serial Number 001

NPDES Permit Number FL0021512

Test Number: 1 of 6

Test Type: (Static) Renewal, (Screening) Conventional

Other: Cl_2

Sample Collection: Date 8-11-87 Time 1120

Test Beginning: Date 8-12-87 Time 1025

Test Ending: Date 8-14-87 Time 1030

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	Conductivity 0	24	48	72	96
CONT	39	10	10	10			8.5	8.2	8.2			8.0	7.9	7.9			20.9	20.9	22.0							
"	95	10	10	10			8.6	8.2	8.2			8.0	7.9	7.9			20.9	[illegible]								
10%	87	10	10	9			8.5	8.3	8.3			7.9	8.0	7.9			20.8		20.7							
"	1	10	10	9			8.6	8.3	8.3			7.9	8.0	7.9			20.8	20.8								
50%	20	10	0	0			8.6	8.4	-			7.8	8.0	-			20.7		—							
"	68	10	0	0			8.6	8.4	-			7.8	8.0	-			20.7	20.9	-							
Initial of Investigator		JE	MLR	MLR			RBF	MLR	MLR			MLR	MLR	MLR			MLR	MLR	MT							

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

Control Water: 105/115

100% Sample: 120/175

Other:

Measured by: JMF

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

Hour 0

Control Water: .033

100% Sample: .045

Other:

Hour 3

Control Water:

100% Sample:

Other:

Measured by: RBF

Signatures:

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7.07; 9.09	8.9@21.5°C	10.4@10.0; 26.2@30
24 Hours	7.07; 9.09	8.9@21.5°C	
48 Hours	7.07; 9.09	9.0@21.0°C	Meter 26030
72 Hours			
96 Hours			

Remarks:

Total Residual Chlorine:

Field data: — Method: —

Lab: 1.3 Method: HACH

Lab: 1.2 Method: IODO

De-chlorinated: [illegible]

Measurements by: RBF

Reviewed by: [signature]

Date: 10/14/87

Industry/Toxicant PANAMA CITY BEACH WWTP
Address
Contact/District
Effluent Serial Number 001
NPDES Permit Number FL0023512

BIOASSAY DATA FORM

Test Number: 2 of 6
Test Type: (Static) Renewal, (Screening) Conventional
Other: Cl_2

Sample Collection: Date 8-11-87 Time 1120
Test Beginning: Date 8-12-87 Time 1100
Test Ending: Date 8-14-87 Time 1200
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	48	72	96
CONT	18	10	5[a]	0			8.4	8.3	8.2			8.0	7.9	7.9			21.4	20.8	20.6				48			
"	26	10	10	10			8.6	8.3	8.2			8.0	7.9	7.9								190	200			
10%	38	10	10	10			8.6	8.4	8.0			7.9	7.9	7.8			21.2	20.7	20.4							
"	23	10	10	10			8.7	8.4	8.0			7.9	7.9	7.8								240	250			
50%	7	10	0	0			8.9	8.5	-			7.8	7.9	-			20.8	20.6	-							
"	54	10	0	0			8.9	8.5	-			7.8	7.9	-								445	-			
Initial of Investigator		MLA	MLR	MLR			JE	MLR	MLR			MLR	MLR	MLR			MLR	MLR	MLR			MLR	MLR			

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 105/115
100% Sample: 120/175
Other:
Measured by: JMF

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: .033
100% Sample: .045
Other:
Hour D
Control Water:
100% Sample:
Other:
Measured by: RBF

Signatures:
Marten S Roll
Joseph Fairbrother
Russell Frydenborg
J H Eplee

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours			
24 Hours		SEE TEST 1	
48 Hours			
72 Hours			
96 Hours			

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

De-chlorinated:
Measurements by: RBF

Reviewed by:
Date: 10/14/87

Remarks: [a] Test invalidated. Jar # 18 believed contaminated by pH probe MLR

Industry/Toxicant: PANAMA CITY BEACH WWTP
Address:
Contact/District:
Effluent Serial Number: 001
NPDES Permit Number: FL0021512

BIOASSAY DATA FORM

Test Number: 3 of 6
Test Type: (Static) Renewal, (Screening) Conventional
Other: Cl_2

Sample Collection: Date 8-11-87 Time 1120
Test Beginning: Date 8-12-87 Time 1020
Test Ending: Date 8-14-87 Time 1090
Test Organism: NOTROPIS LEEDSI
Age/Life Stage: 28 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	48	72	96
CONT	37	5	5	5			8.1	8.0	7.7			7.6	7.7	7.7			21.7	20.8	20.5							
"	52	5	5	5			8.1	8.0	7.7			7.6	7.7	7.7								210	220			
10%	51	5	5	5			8.5	8.0	7.8			7.6	7.7	7.7			21.7	20.7	20.4							
"	42	5	5	5			8.5	8.0	7.8			7.6	7.7	7.7								265	270			
50%	47	5	0	0			8.7	8.1	—			7.7	7.8	—			21.1	20.6	—							
"	72	5	0	0			8.7	8.1	—			7.7	7.8	—								460	—			
Initial of Investigator		MLR	MLR	MLR			SE	MLR	MLR			MLR	MLR	MLR			MLR	MLR	MLR			MLR	MLR			

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

Control Water: 115/120
100% Sample: 120/175
Other:
Measured by: JMF

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

Control Water: .035
100% Sample: .045
Other:

Hour 0

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

Signatures:

Martin S Roll
Joseph Faudoth
Russel Frydenborg
JH Capt

Instrument Calibrations:

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

Remarks:

Total Residual Chlorine:

Field data: _____ Method: _____
Lab: _____ Method: _____
Lab: _____ Method: _____

De-chlorinated:
Measurements by: RBF

Reviewed by:

Date: 10/14/87

Industry/Toxicant PANAMA CITY BEACH WWTP

Address

Contact/District

Effluent Serial Number 001

NPDES Permit Number FL0021512

BIOASSAY DATA FORM

Test Number: 4 of 6

Test Type: (Static) Renewal, (Screening) Conventional

Other: DE-Cl_2

Sample Collection: Date 8-11-87 Time 1120

Test Beginning: Date 8-12-87 Time 1050

Test Ending: Date 8-14-87 Time 1030

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
CONT	5	10	10	10			8.1	8.1	8.7			8.0	7.9	7.9			21.1	20.9	20.7		
"	—	10	10	9			8.2	8.1	8.7			8.0	8.0	7.9							
50%	85	10	10	10			8.5	8.1	8.7			7.8	7.9	7.9			20.9	20.8	20.6		
"	47	10	10	10			8.9	8.1	8.7			7.8	7.9	7.9							
100%	45	10	7	2			9.4	7.8	8.8			7.8	8.0	7.9			20.6	20.8	20.6		
"	92	10	8	3			9.6	7.8	8.8			7.8	8.0	7.9							
Initial of Investigator		JE	MLR	MLR			JE	MLR	MLR			MLR	MLR	MLR			MLR	MLR	MLR		

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

Control Water: 105/115

100% Sample: 120/175

Other:

Measured by: JMF

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

Hour 0

Control Water: .033

100% Sample: .045

Other:

Hour 0

Control Water:

100% Sample:

Other:

Measured by: RBF

Signatures:

Martin S Rall

Joseph Fairchild

Russell Frydenborg

Instrument Calibrations:

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

Remarks:

Total Residual Chlorine:

Field data: _____ Method: _____

Lab: _____ Method: _____

Lab: _____ Method: _____

(De-chlorinated) YES

Measurements by: RBF

Reviewed by:

Date: 10/14/87

Industry/Toxicant PANAMA CITY BEACH WWTP

BIOASSAY DATA FORM

Address

Contact/District

Effluent Serial Number

NPDES Permit Number

Test Number: 5 of 6

Test Type: (Static) Renewal, (Screening) Conventional

Other: DE-Cl2

Sample Collection: Date 8-11-87 Time 1120

Test Beginning: Date 8-12-87 Time 1050

Test Ending: Date 8-14-87 Time 1030

Test Organism ~~CERIO~~ DAPHNIA ~~SHEEPSHEAD MINNOW~~ MYSID

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	~~48~~	72	96
CONT	66	10	10	10			8.5	8.2	8.7			8.0	7.7	7.9			21.5	20.7	20.5							
"	50	10	10	10			8.5	8.2	8.7			8.0	7.9	7.9								195	210			
50%	2	10	10	10			8.8	8.3	8.8			7.8	7.9	7.8			21.1	20.6	20.5							
"	78	10	10	10			8.8	8.3	8.8			7.8	7.9	7.8								460	470			
100%	4	10	10	10			10.2	8.4	8.4			7.8	8.0	7.8			20.7	20.6	20.4							
"	3	10	10	9			10.3	8.4	8.4			7.8	8.0	7.8								790	820			
Initial of Investigator		MLR	MLR	MLR			JE	MLR	MLR			MLR	MLR	MLR			MLR	MLR	MLR			MLR	MLR			

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

Control Water: 105/115

100% Sample: 120/175

Other:

Measured by: JMF

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

Hour 0

Control Water: .033

100% Sample: .045

Other:

Hour 0

Control Water:

100% Sample:

Other:

Measured by: RBF

Signatures:

Martin L. Hill

Joseph Faircloth

Russell Lindenberg

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours			
24 Hours			
48 Hours	YES	TEST 1	
72 Hours			
96 Hours			

Remarks:

Total Residual Chlorine:

Field data: _____ Method: _____

Lab: _____ Method: _____

Lab: _____ Method: _____

(De-chlorinated): YES

Measurements by: RBF

Reviewed by:

Date: 10/14/87

Industry/Toxicant PANAMA CITY BEACH WWTP
Address
Contact/District
Effluent Serial Number 001
NPDES Permit Number FL0021512

BIOASSAY DATA FORM

Test Number: 6 of 6
Test Type: Static Renewal, Screening, Conventional
Other: DE-Cl_2

Sample Collection: Date 8-11-87 Time 1120
Test Beginning: Date 8-12-87 Time 1020
Test Ending: Date 8-14-87 Time 1030
Test Organism NOTROPIS LEEDSI
Age/Life Stage 28 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
CONT	68	5	5	5			8.4	7.8	8.3			7.6	7.7	7.7			21.7	20.5	20.3				48			
"	13	5	5	5			8.5	7.8	8.3			7.6	7.7	7.7								212	210			
50%	—	5	5	5			9.3	8.0	8.2			7.7	7.8	7.7			21.3	20.4	20.2							
"	70	5	5	5			9.3	8.0	8.2			7.7	7.8	7.7								468	460			
100%	6	5	5	5			10.2	8.0	8.1			7.7	7.8	7.8			20.7	20.3	20.2							
"	33	5	5	5			10.2	8.0	8.1			7.7	7.8	7.8								810	810			
Initial of Investigator		MLR	MLR	MLR			JE	MLR	MLR			MLR	MLR	MLR			MLR	HC	MLR			MLR	MLR			

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 115/120
100% Sample: 105/125
Other:
Measured by: JMF

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: .035
100% Sample: .045
Other:

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

Signatures:
Martin S. Hill
Joseph Fairbrother
Russel Frydenborg

Instrument Calibrations:

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

Remarks:

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

Reviewed by:
Date: 10/14/87

MICROBICS — MICROTOX DATA REDUCTION SHEET

SAMPLE ID NO. _____ COLLECTED BY: _____

SOURCE: Panama City Beach DATE: _____ TIME: _____

REPORTED RESULTS: TOXIC ☐ NON-TOXIC ☐ MAX Γ = _____ FOR CONCENTRATION = _____

GRAPH ☐
CALC ☒ EC 50 (15 MIN, 15 °C) = 22 ☒ % SAMPLE ☐ mg/L

CONFIDENCE FACTOR = _____ ☐ OTHER _____

95% CONFIDENCE INTERVAL _____ TO _____

REMARKS: _____

SAMPLE DATA: SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent - Cl2

DATE ASSAYED: 8 - 12 - 87; TIME: 1:00 PM

VISUAL COLOR: none; CORRECTION REQUIRED: YES ☐ NO ☐

VISUAL TURBIDITY: none; SEPARATION REQUIRED: YES ☐ NO ☐

INITIAL pH: 7.8; pH ADJUSTED TO: _____ WITH _____

OSMOTICALLY ADJUSTED WITH: MOAS ☐ DRY NaCl ☒ OTHER ☐ _____

PRIMARY DILUTION OF SAMPLE: none

OPERATOR: Leslie; REAGENT VIAL LOT NO. M – 603

REMARKS: _____

MICROTOX 99% TEST

Sample Cuvette	% Sample	Final Readings 5 min	Final Readings 15 min	Γ5	Γ15	Notes
A1	0	56	41			
B1		52	38			
C1		53	40			
A2	12.28	40.5	25	.33	.57	
B2		40	25			
C2		42	26			
A3	24.75	27	15	.60	1.11	
B3		37	20			
C3		38	21.5			
A4	49.5	26.5	12.5	.98	1.98	
B4		31	15.5			
C4		25	12			
A5	99	6.5	4	8.33	10.90	
B5		5	3			
C5		5	3			

EC50 (15 min, 15 °C) By Graph

By Calculator 32.46 22.42

Reviewed by _____

Date 10/14/87

MICROBICS MICROTOX™ DATA REDUCTION SHEET

SAMPLE ID NO. _____ COLLECTED BY: _____

SOURCE: Panama City Beach DATE: _____

TIME: _____

REPORTED RESULTS: TOXIC ☐ NON-TOXIC ☐ MAX Γ = _____ FOR CONCENTRATION = _____

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

CONFIDENCE FACTOR = _____ ☐ OTHER _____

95% CONFIDENCE INTERVAL _____ TO _____

REMARKS: _____

SAMPLE DATA: SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent/deCl2

DATE ASSAYED: 8 - 12 - 87; TIME: 11:30AM

VISUAL COLOR: none; CORRECTION REQUIRED: YES ☐ NO ☐

VISUAL TURBIDITY: none; SEPARATION REQUIRED: YES ☐ NO ☐

INITIAL pH: 7.8; pH ADJUSTED TO: _____ WITH _____

OSMOTICALLY ADJUSTED WITH: MOAS ☐ DRY NaCl ☒ OTHER ☐ _____

PRIMARY DILUTION OF SAMPLE: none

OPERATOR: Lesee; REAGENT VIAL LOT NO. M - 603

REMARKS: _____

MICROTOX 99% TEST

Sample Cuvette	% Sample	Final Readings 5 min	Final Readings 15 min	Γ5	Γ15	Notes
A1	0	53	36			
B1		56	36			
C1		59	38			
A2	12.28	57	36	.02	.02	
B2		55	35.5			
C2		57	36			
A3	24.75	60	38	-.04	-.06	
B3		62	40			
C3		[illegible]	39			
A4	49.5	54	34	.05	.05	
B4		57.5	37			
C4		60	34			
A5	99	50	43	.02	.05	
B5		47.5	30			
C5		50	32			
EC50 (15 min, 15 °C)		By Graph				
		By Calculator		152.9	>100	

Reviewed by _____

Date 10/14/87