DEPARTMENT OF ENVIRONMENTAL REGULATION

STATE OF FLORIDA

BIOASSAYS OF THE FLORIDA STATE HOSPITAL

SEWAGE TREATMENT PLANT

CHATTAHOOCHEE, GADSDEN COUNTY, FLORIDA

NPDES #FL0031402

SAMPLED 10/5/87

DECEMBER 7, 1987

Biology Section
Division of Environmental Programs

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Biology Section
Bureau of Laboratories and
Special Programs
December 7, 1987

EXECUTIVE SUMMARY

Florida State Hospital STP, Chattahoochee, NPDES #FL0031402, on 6 to 8 October, 1987.

The sample of chlorinated de-chlorinated discharge from the Florida State Hospital's STP were not acutely toxic to the water fleas, Daphnia pulex or Ceriodaphnia dubia, or to the bannerfin shiner, Notropis leedsi. A chlorine residual of .2 mg/l Hach or .1 mg/l Cl_2 Iodometric was present which contributed to the minor amount of mortality in the 100% chlorinated sample. The Microtox® assay, using the bacterium, Photobacterium phosphoreum, was also non-toxic in both the chlorinated and de-chlorinated sample. Due to the non-toxic nature of the discharge, chemical analyses were not performed.

INTRODUCTION

The Florida State Hospital is located on US 90 in Chattahoochee, Gadsden County, Florida (Figure 1). The sewage treatment plant for this facility has a design capacity of 1.3 MGD and treats an average of .73 MGD of domestic waste from several thousand patients, staff, and workers. Treatment consists of activated sludge and chlorination (Figure 2). The effluent discharges through Outfall #001 (Figure 3) to a concrete cooling water ditch from the adjacent power plant. This water ultimately discharges to Mosquito Creek, which flows westward to the Apalachicola River.

The FDER Biology Section has performed toxicity tests of this facility in June, 1982 and November, 1983. In 1983 the 48 hour LC_{50} values were 28.9% for pre-chlorinated, 62.7% for chlorinated, and 81.0% for the de-chlorinated discharge to Daphnia pulex. The de-chlorinated sample was also toxic to Simocephalus serrulatus, producing a 24 hour LC_{50} of 71.3%. The fathead minnows had no mortality.

In June, 1982 all three effluents were considerably toxic to the water flea, Daphnia pulex, producing 48 hour LC_{50}'s of 23.3%, 8.6%, and 12.7%, respectively. The de-chlorinated effluent was toxic to Simocephalus serrulatus producing a 24 hour LC_{50} of 10.9%. A variety of metals and several unidentified organics were present and may have contributed to the toxicity, but a definite cause was not obvious.

To again evaluate the existence and extent of the toxicity of the discharge from this facility to the receiving waters, the Biology Section performed three acute toxicity bioassays and one Microtox® assay on both chlorinated and de-chlorinated samples. Those involved in performing the tests and in preparation of this report were: D. A. Chasteen, J. H. Epler, J. M. Faircloth, R. B. Frydenborg, and L. A. Williams.

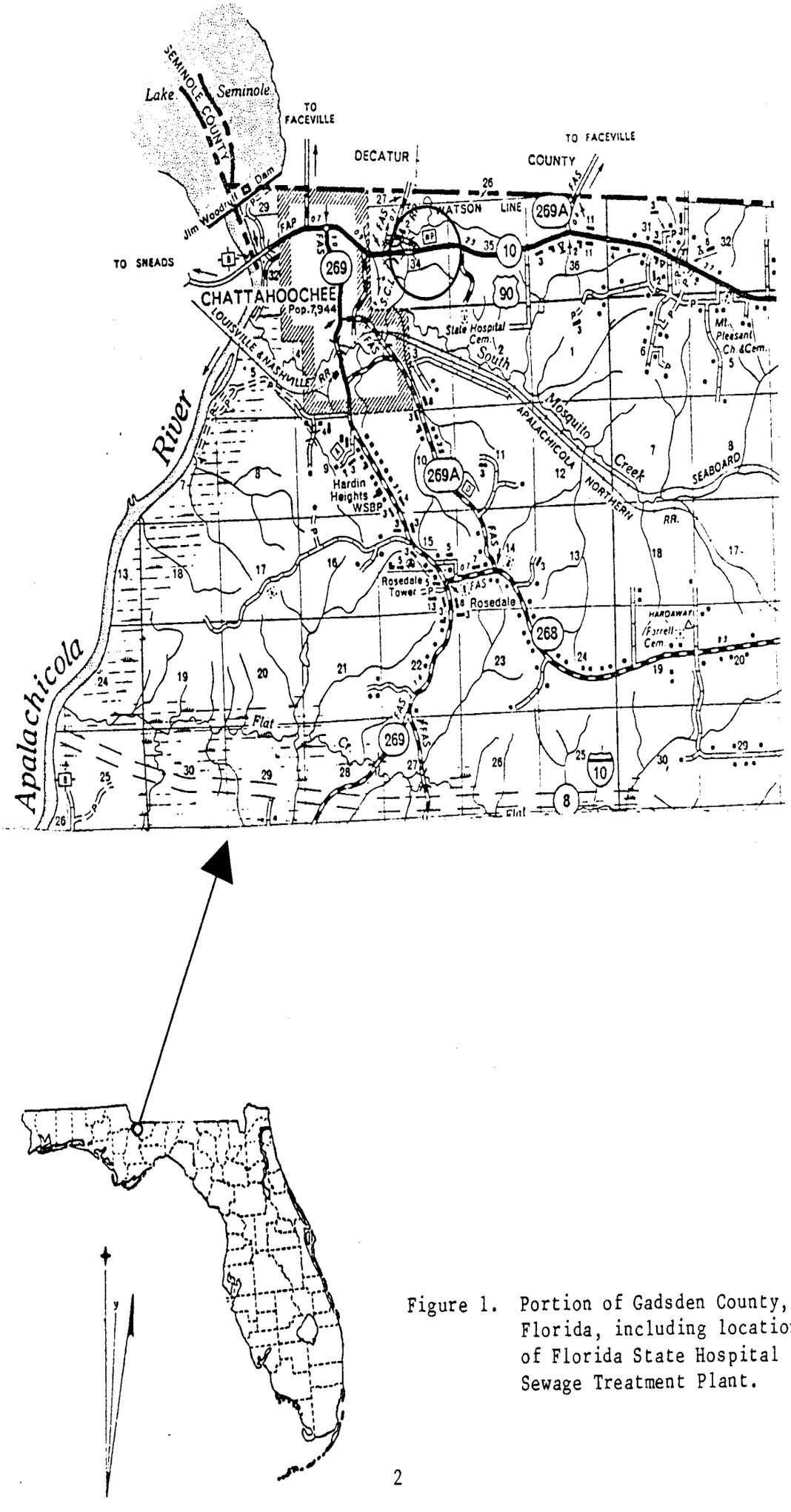


Figure 1. Portion of Gadsden County, Florida, including location of Florida State Hospital Sewage Treatment Plant.

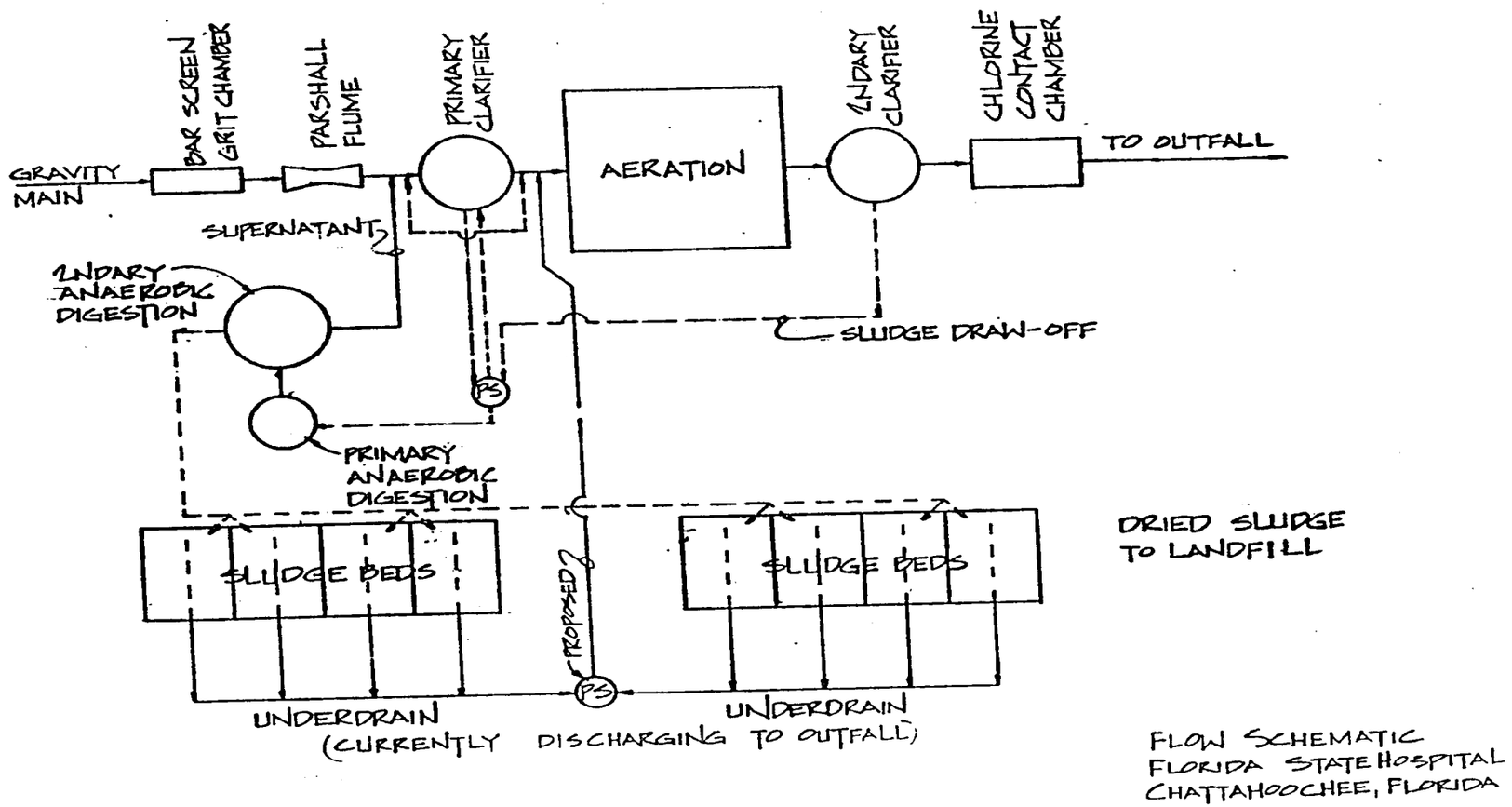


Figure 2. Flow schematic for the Florida State Hospital.

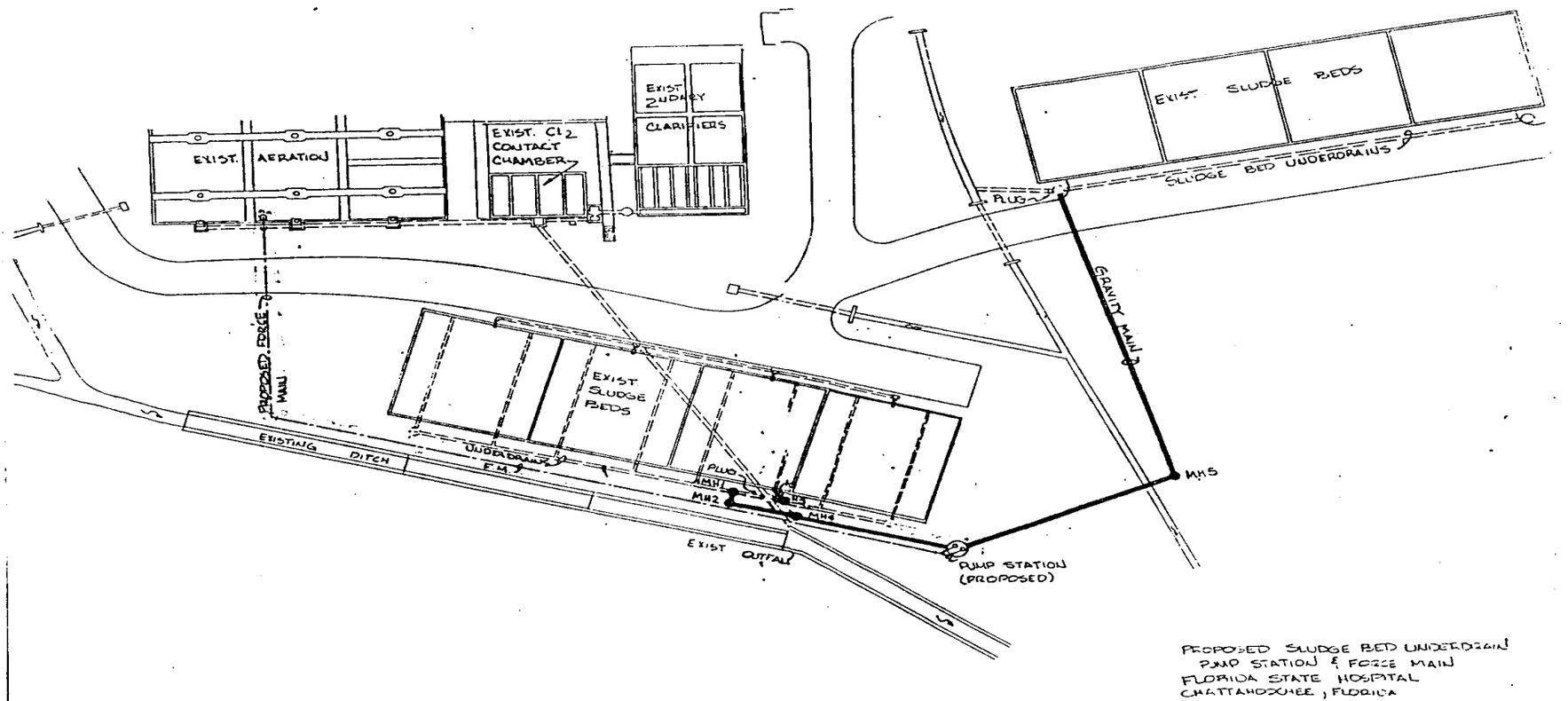


Figure 3. Florida State Hospital schematic showing location of outfall to cooling water ditch.

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 25 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

Three static acute toxicity bioassays and one Microtox® assay were performed on the chlorinated and de-chlorinated sample of discharge from the Florida State Hospital at Chattahoochee Outfall #001 (eight tests total). They were performed from 1110 hours on 6 October to 1110 hours on 8 October, 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. David Chasteen and Mr. Marshall Faircloth of the Tallahassee DER Biology Section at 1045 hours on October 5, 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 recovered waters 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 recovered water. 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 sample of recovered water 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 this 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% recovered 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 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% recovered 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 Tables 2 abd 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 4 and 5.

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
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
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
50.0%	250 ml	250 ml	500 ml
100.0%	------	500 ml	500 ml

Table 2. Data recorded during 48 hour, *Ceriodaphnia dubia*, *Daphnia pulex*, and *Notropis leedsi*, static acute toxicity bioassay of Florida State Hospital Sewage Treatment Plant, Chlorinated Discharge, Gadsden County, NPDES #FL0031402, on 6 to 8 October, 1987.

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	8.3	6.9	8.2	8.0	7.9	7.8	18.9	19.6	19.8		
Control	10	10	10	8.5	7.0	8.2	8.0	7.9	7.9					
50%	10	10	10	8.6	7.2	7.6	7.5	7.7	7.2	19.0	19.6	19.8		
50%	10	10	10	8.6	7.2	7.4	7.5	7.7	7.7					
100%	10	10	10	8.7	7.4	7.3	7.0	7.5	7.5	19.0	19.6	19.9		
100%	10	10	10	8.6	7.3	6.9	7.0	7.5	7.3					
Daphnia pulex														
Control	10	10	10	8.6	7.4	7.8	8.0	7.9	7.8	19.1	19.8	19.6		
Control	10	10	10	8.7	7.4	7.8	8.0	7.9	7.8				195	205
50%	10	10	10	8.7	7.5	7.0	7.5	7.7	7.5	19.2	19.5	19.9		
50%	10	10	10	8.8	7.5	6.8	7.5	7.7	7.5				240	260
100%	10	10	9	8.9	7.6	7.8	7.0	7.4	7.3	19.2	19.6	19.9		
100%	10	10	9	8.9	7.5	7.8	7.0	7.3	7.3				395	320
Notropis leedsi														
Control	5	5	5	8.5	7.3	6.8	7.6	7.7	7.6	20.0	19.7	19.5		
Control	5	5	5	8.9	7.3	7.0	7.6	7.7	7.6				195	210
50%	5	5	5	9.0	7.2	6.7	7.3	7.4	7.4	20.0	19.4	19.4		
50%	5	5	5	8.9	7.3	6.6	7.3	7.4	7.4				250	260
100%	5	5	5	8.9	7.5	6.7	6.9	7.2	7.2	19.7	19.5	19.6		
1005	5	5	5	9.1	7.5	6.6	6.9	7.2	7.2				300	320

Control Water

NH_3(as NH_3)(mg/1) 0

Total ammonia (as N)(mg/1) 0

Total Alkalinity 110 (mg/1 $CaOO_3$)

Total Hardness 118 (mg/1 $CaOO_3$)

100% Sample

NH_3(as NH_3)(mg/1) 0

Total ammonia (as N)(mg/1) 0

Total Alkalinity 48 (mg/1 $CaOO_3$)

Total Hardness 75 (mg/1 $CaOO_3$)

Table 3. Data recorded during 48 hour, *Ceriodaphnia dubia*, *Daphnia pulex*, and *Notropis leedsi*, static acute toxicity bioassay of the Florida State Hospital Sewage Treatment, De-chlorinated Discharge, Gadsden County, NPDES #FL0031402, on 6 to 8 October, 1987.

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	8.5	7.3	8.0	8.0	8.0	7.9	19.0	19.7	20.1		
Control	10	10	9	8.5	7.3	8.0	8.0	8.0	7.9					
50%	10	10	10	8.5	7.5	7.2	7.5	7.8	7.6	19.1	19.7	19.7		
50%	10	10	10	8.5	7.5	7.2	7.5	7.8	7.6					
100%	10	10	9	8.6	7.7	7.0	7.0	7.5	7.3	19.1	19.9	19.9		
100%	10	10	10	8.7	7.6	7.0	7.0	7.5	7.3					
Daphnia pulex														
Control	10	10	10	8.8	7.3	7.6	8.0	8.0	7.9	19.2	20.0	19.8		
Control	10	10	10	8.4	7.3	7.8	8.0	8.0	7.9				195	210
50%	10	10	10	8.6	7.5	6.7	7.5	7.7	7.5	19.4	19.5	19.4		
50%	10	10	10	8.7	7.4	6.8	7.5	7.7	7.5				250	260
100%	10	10	10	8.8	7.7	6.8	6.9	7.4	7.3	19.3	19.6	19.6		
100%	10	10	10	8.8	7.7	6.7	6.9	7.4	7.3				300	325
Notropis leedsi														
Control	5	5	5	8.4	7.2	6.4	7.6	7.7	7.7	19.9	19.5	19.5		
Control	5	5	5	8.2	7.3	6.9	7.6	7.7	7.7				195	210
50%	5	5	5	8.6	7.4	7.2	7.2	7.5	7.5	20.0	19.4	19.7		
50%	5	5	5	8.7	7.4	7.2	7.2	7.5	7.5				250	260
100%	5	5	5	9.0	7.6	6.8	6.9	7.2	7.2	19.8	19.5	19.6		
100%	5	5	5	9.0	7.5	7.0	6.9	7.2	7.2				310	325

Control Water

NH_3(as NH_3)(mg/l) 0

Total ammonia (as N)(mg/l) 0

Total Alkalinity 110 (mg/l $CaOO_3$)

Total Hardness 118 (mg/l $CaOO_3$)

100% Sample

NH_3(as NH_3)(mg/l) 0

Total ammonia (as N)(mg/l) 0

Total Alkalinity 48 (mg/l $CaOO_3$)

Total Hardness 70 (mg/l $CaOO_3$)

Table 4. Microtox test results, Florida State Hospital chlorinated discharge, Gadsden County, NPDES #FL0031402, on 6 to 8 October, 1987.

Microtox Test Culture Lot #M-603

	Final Readings	
% Sample	5-min (I_5)	15-min (I_{15})
0	89	44
0	98	50
0	90	45
12.38	80	41
12.38	84	42
12.38	88	45
24.75	79	40
24.75	79	40
24.75	85	43
49.5	78	38
49.5	78	38
49.5	84	40
99	70	31
99	68	30
99	70	31

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.

Table 5. Microtox test results, Florida State Hospital chlorinated discharge, Gadsden County, NPDES #FL0031402, on 6 to 8 October, 1987.

Microtox Test Culture Lot #M-603

	Final Readings	
% Sample	5-min (I_5)	15-min (I_{15})
0	62	36
0	65	37
0	68	40
12.38	61	36
12.38	67.5	40
12.38	66	38
24.75	63	36
24.75	68	40
24.75	63	38
49.5	59	34
49.5	63	36
49.5	56	32
99	58	31
99	56	30
99	54	29

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.

RESULTS AND DISCUSSION

The water sample taken at Outfall #001 of the Florida State Hospital Sewage Treatment Plant was not acutely toxic to the test organisms Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi in either the chlorinated or de-chlorinated tests (Table 2 and 3). The Microtox® assay was also non-toxic (EC_{50} > 100%) in both de-chlorinated and chlorinated tests (Table 4 and 5) further supporting the conclusion that the recovered water from this facility was non-toxic on October 5, 1987.

Discharging to the cooling water (concrete lined) ditch, well before reaching Mosquito Creek, provides additional dilution and aeration to the recovered water. This may allow the excess chlorine to dissipate.

Since the sample was not toxic, chemical analyses were not performed.

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