BIOASSAYS OF THE FLORIDA STATE HOSPITAL
SEWAGE TREATMENT PLANT
CHATTAHOOCHEE, GADSDEN COUNTY, FLORIDA
NPDES #FL0031402

SAMPLED 11/6/83

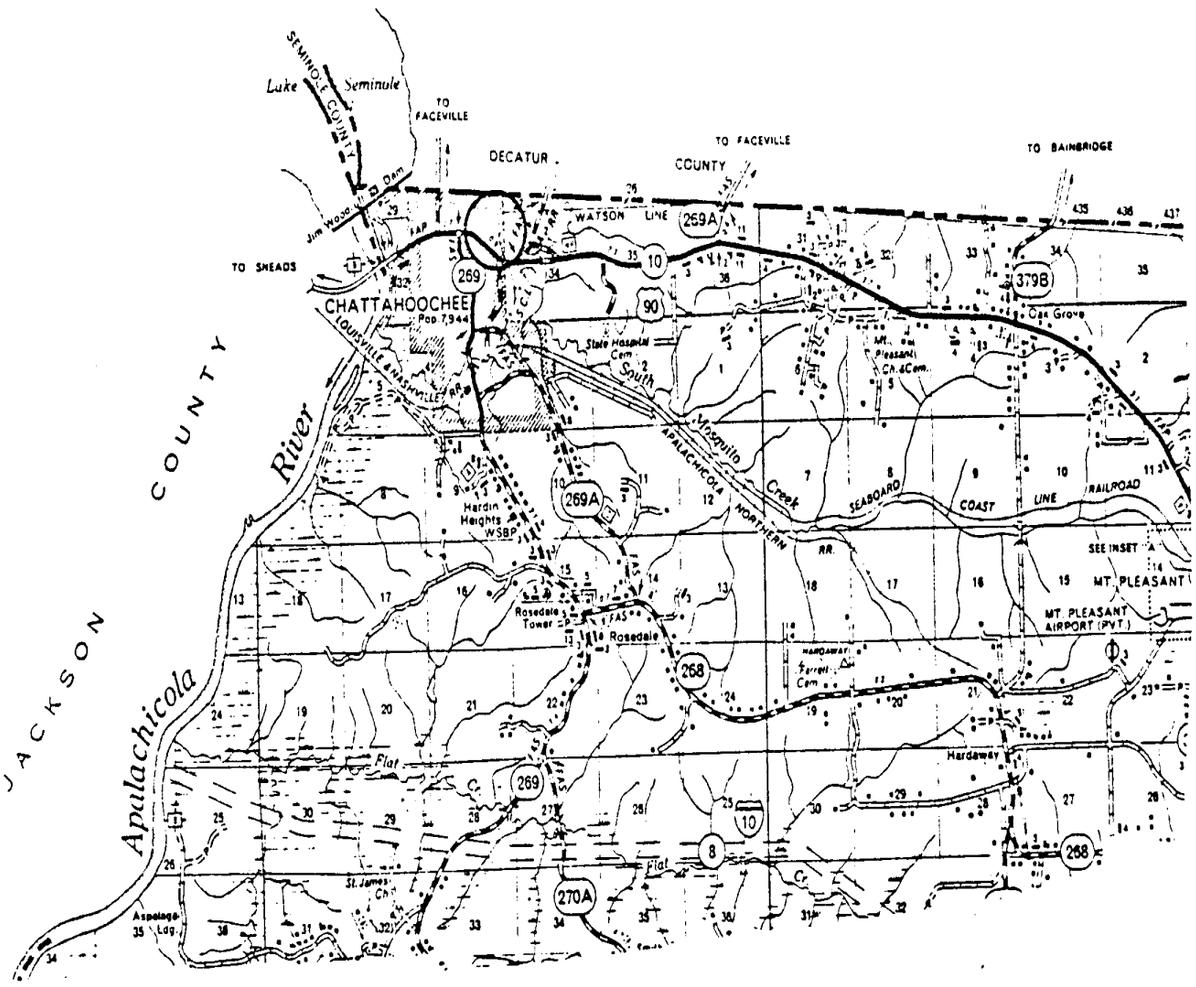
Biology Section
Bureau of Water Analysis
December 30, 1983

INTRODUCTION

The Florida State Hospital is located on U.S. 90 in Chattahoochee, Gadsden County, Florida (Fig. 1). The sewage treatment plant for this facility has a design capacity of 1.3 MGD and treats an average of 0.73 MGD of domestic waste from several thousand patients, staff and workers. Treatment consists of activated sludge and chlorination. Discharge of effluent is through Outfall #001 to Mosquito Creek, which flows westward to the Apalachicola River.

The FDER Biology Section has previously performed toxicity bioassays on samples of pre-chlorinated, chlorinated, and de-chlorinated effluents from this facility, in June, 1982. The results of those tests indicated that all three effluents were considerably toxic to Daphnia pulex, producing 48 hour LC_{50}'s of 23.3%, 8.6% and 12.7%, respectively. The de-chlorinated effluent was also acutely toxic to Simocephalus serrulatus at that time (24 hour LC_{50}=10.9%). The toxicities of the effluents at that time could not be attributed to specific chemical constituents, although at least one organic and several metal components were present (FDER 1982).

To again evaluate the existence and extent of toxicity of these effluents to the biota of the receiving waters, the Biology Section performed five additional static acute toxicity bioassays on pre-chlorinated, chlorinated and de-chlorinated samples, on 7 to 9 November, 1983. The results of these latest tests are presented in this report. Those involved in performing the tests and in preparation of this report were: C. W. Dye, R. B. Frydenborg, and M. L. Roll.



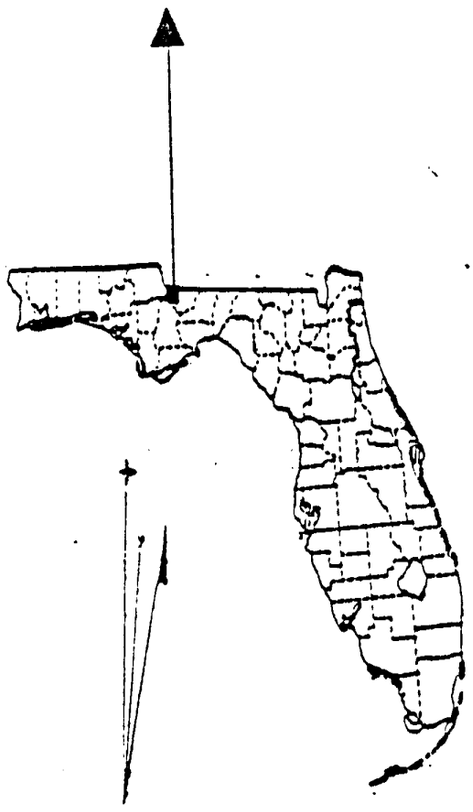


Figure 1. Portions of Gadsden County, Florida, including Chattahoochee and study area.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978) and the FDER Biology Section (1983). The organisms selected for this study include species recommended by Peltier (1978), the U.S. EPA (1978), and the FDER Biology Section (1983). 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.

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 2°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.

Simocephalus serrulatus (water flea) - An indigenous breeding population of Simocephalus serrulatus was collected from Lake Jackson, Leon County, Florida, in March, 1980, by personnel from DER's Biology Section. Positive identification of the organisms was made by Mr. Jeff Foran of the University of Florida Department of Environmental Engineering Sciences. The cultures are maintained in two and one-half gallon all-glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. The 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 2°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 three 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 0.75g/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 17 to 19 days weeks old at the time of the 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

Five separate 48 hour static acute toxicity bioassays were performed on samples of pre-chlorinated, chlorinated and de-chlorinated effluents from the Florida State Hospital STP. The tests were performed from 1530 hours on 7 November to 1530 hours on 9 November, 1983.

The samples were collected by Mr. Thomas Andrews of DER's Northwest Branch Office at 1120 hours on 7 November, 1983, 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.

To differentiate between the toxicity resulting from chlorine and the toxicity associated with some other component of the effluent, the Biology Section adopted the following test procedure:

Test #1. Pre-chlorinated Effluent using Daphnia pulex and Pimephales promelas (Tables 2 and 3). These tests were performed on an effluent sample collected at the end of the treatment process but prior to chlorination, and was intended to evaluate the toxicity of the effluent without the added toxic effects of chlorine.

Test #2. Chlorinated Effluent using Daphnia pulex (Table 4). This test was performed on an effluent sample collected at the point of discharge.

Test #3. De-chlorinated Effluent using Daphnia pulex and Simocephalus serrulatus (Tables 5 and 6). These tests were performed on a subsample of the chlorinated effluent sample in which the chlorine had been neutralized. De-chlorination was accomplished by adding a calculated amount of 0.025 N sodium thiosulfate. This test was intended to evaluate the combined toxicity of the effluent and the chlorine reaction products.

Freshwater for dilution of the effluent samples 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 fleas, Daphnia pulex and Simocephalus serrulatus. 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 and Simocephalus were siphoned into large culture dishes 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 these tests.

Two replicates, of five 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.

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 chamber 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 100% effluent concentra tion. 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% effluent test concentrations 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 through 6).

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

Daphnia pulex and *Simocephalus serrulatus* (water fleas)

Concentration	Dilution Water	Effluent	Total Volume
Control	200.0 ml	--------	200 ml
5.6%	188.8 ml	11.2 ml	200 ml
10.0%	180.0 ml	20.0 ml	200 ml
18.0%	164.0 ml	36.0 ml	200 ml
32.0%	136.0 ml	64.0 ml	200 ml
56.0%	88.0 ml	112.0 ml	200 ml
100.0%	--------	200.0 ml	200 ml

Pimephales promelas (fathead minnow)

Concentration	Dilution Water	Effluent	Total Volume
Control	500 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 the Florida State Hospital STP, Chattahoochee, Gadsden County, Florida, NPDES #FL0031402, on 7 to 9 November, 1983.

Test #1 Pre-chlorinated Effluent[a]

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 (^oc)			NH_3/N (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	8.5	8.8	8.5	7.7	8.1	8.2					20.7	21.2	20.8	0/0	---		
Control	10	10	10	8.5	8.8	8.5	7.7	8.1	8.2										200	210
5.6%	10	10	10	8.5	8.8	8.6	7.7	8.1	8.2					20.8	20.7	20.8				
5.6%	10	10	10	8.5	8.8	8.6	7.7	8.1	8.2										207	210
10.0%	10	10	10	8.5	9.0	8.7	7.8	8.1	8.2					20.8	21.6	20.7				
10.0%	10	10	10	8.5	8.9	8.7	7.8	8.1	8.2										212	220
18.0%	10	10	10	8.4	8.9	8.7	7.8	8.1	8.2					20.8	20.7	20.7				
18.0%	10	10	10	8.4	8.9	8.7	7.8	8.1	8.2										220	225
32.0%	10	6	3	8.3	9.0	8.7	7.7	8.0	8.2					21.0	20.8	20.7				
32.0%	10	10	4	8.3	8.9	8.7	7.7	8.0	8.1										233	240
56.0%	10	2	0	8.2	8.9	8.7	7.5	7.8	8.1					21.1	20.8	20.7				
56.0%	10	4	0	8.2	8.9	8.7	7.5	7.8	8.0										258	257
100.0%	10	0	0	7.9	8.9	8.7	7.0	7.2	7.7	10		70		21.5	20.7	20.7	0/0	---		
100.0%	10	0	0	7.9	8.7	8.7	7.0	7.2	7.5										303	310

Remarks: [a] Total Chlorine Residual: None Detected (HACH DPD Method).

Table 3. Data recorded during 48 hour, Pimephales promelas, static acute toxicity bioassay of the Florida State Hospital STP, Chattahoochee, Gadsden County, Florida, NPDES #FL0031402, on 7 to 9 November, 1983.

Test #1 Pre-chlorinated Effluent

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 (^oc)			NH_3/N (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	8.4	8.8	8.7	7.7	8.1	8.0					21.2	20.7	20.9	0/0	---		
Control	5	5	5	8.4	8.8	8.7	7.7	8.0	8.1										202	215
10.0%	5	5	5	8.4	8.8	8.7	7.8	8.1	8.1					21.5	20.4	20.8				
10.0%	4[a]	4	4	8.4	8.8	8.7	7.8	8.1	8.1										220	220
18.0%	5	5	5	8.3	8.7	8.6	7.7	8.0	8.1					21.5	20.4	20.6				
18.0%	5	5	5	8.3	8.8	8.6	7.7	8.0	8.1										229	230
32.0%	5	5	5	8.3	8.8	8.6	7.6	7.9	8.1					21.6	20.3	20.6				
32.0%	5	5	5	8.3	8.8	8.7	7.6	7.9	8.1										242	240
56.0%	5	5	5	8.1	8.8	8.6	7.4	7.7	8.0					21.5	20.3	20.5				
56.0%	5	5	5	8.1	8.5	8.6	7.4	7.7	8.0										269	265
100.0%	5	4	4	7.7	8.1	8.6	6.9	7.1	7.6	10		70		21.8	20.3	20.5	0/0	---		
100.0%	5	5	5	7.7	8.4	8.5	6.9	7.1	7.4										315	310

Remarks: [a] Due to a shortage of test organisms, only four fish were loaded into this test chamber.

Table 4. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of the Florida State Hospital STP, Chattahoochee, Gadsden County, Florida, NPDES #FL0031402, on 7 to 9 November, 1983.

Test #2 Chlorinated Effluent[a]

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/N (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	8.5	9.1	8.7	7.7	8.3	8.2					20.8	20.7	20.8	0/0	---		
Control	10	10	10	8.5	9.1	8.7	7.7	8.3	8.2										202	220
5.6%	10	10	10	8.4	9.1	8.7	7.7	8.3	8.2					20.9	20.6	20.8				
5.6%	10	9	9	8.4	9.1	8.7	7.7	8.3	8.2										212	219
10.0%	10	10	10	8.5	9.2	8.7	7.7	8.3	8.2					21.0	20.5	20.8				
10.0%	10	10	10	8.5	9.1	8.7	7.7	8.3	8.2										219	205
18.0%	10	10	10	8.5	9.2	8.7	7.7	8.2	8.2					21.0	20.5	20.8				
18.0%	10	10	8	8.5	9.2	8.7	7.7	8.2	8.2										227	225
32.0%	10	10	10	8.5	9.2	8.7	7.6	8.2	8.2					20.8	20.5	20.8				
32.0%	10	10	9	8.5	9.2	8.7	7.6	8.2	8.2										238	230
56.0%	10	10	8	8.6	9.2	8.7	7.4	7.8	8.1					20.4	20.5	20.8				
56.0%	10	10	8	8.6	9.1	8.7	7.4	7.8	8.1										262	260
100.0%	10	0	0	8.7	9.2	8.8	6.9	7.4	7.5	0		70		20.0	20.7	21.1	0/.07	---		
100.0%	10	1	0	8.7	9.1	8.7	6.9	7.4	7.5										307	315

Remarks: [a] Total Chlorine Residual: 0.70 mg/l HACH DPD Method
0.75 mg/l Iodometric Method I

Table 5. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of the Florida State Hospital STP, Chattahoochee, Gadsden County, Florida, NPDES #FL0031402, on 7 to 9 November, 1983.

Test #3 De-chlorinated Effluent[a]

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/N (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	8.4	9.1	8.7	7.7	8.3	8.1					20.9	20.5	20.6	0/0	---		
Control	10	10	10	8.4	9.1	8.7	7.7	8.3	8.1										210	220
5.6%	10	10	10	8.5	9.1	8.7	7.7	8.3	8.2					21.1	20.3	20.6				
5.6%	10	10	10	8.5	9.1	8.7	7.7	8.3	8.2										215	215
10.0%	10	10	10	8.5	9.2	8.6	7.8	8.3	8.2					21.1	20.3	20.5				
10.0%	10	10	10	8.5	9.2	8.6	7.8	8.3	8.2										220	215
18.0%	10	10	10	8.5	9.2	8.6	7.7	8.2	8.2					21.1	20.4	20.8				
18.0%	10	10	10	8.5	9.2	8.6	7.7	8.2	8.2										230	225
32.0%	10	10	8	8.6	9.2	8.6	7.7	8.1	8.2					21.0	20.4	20.8				
32.0%	10	10	8	8.6	9.2	8.6	7.7	8.1	8.2										247	250
56.0%	10	10	10	8.6	9.1	8.7	7.5	8.0	8.2					20.8	20.3	20.8				
56.0%	10	10	8	8.6	9.0	8.6	7.5	8.0	8.1										272	270
100.0%	10	6	1	8.7	9.0	8.7	7.1	7.6	7.9	0		70		20.7	20.7	21.0	0/0	---		
100.0%	10	10	4	8.7	9.0	8.5	7.0	7.6	7.7										322	330

Remarks: [a] Total Chlorine Residual: 0.08 mg/l HACH DPD Method. Sample could not be de-chlorinated further, even with excess sodium thiosulfate.

Table 6. Data recorded during 48 hour, Simocephalus serrulatus, static acute toxicity bioassay of the Florida State Hospital STP, Chattahoochee, Gadsden County, Florida, NPDES #FL0031402, on 7 to 9 November, 1983.

Test #3 De-chlorinated Effluent

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/N (mg/l)		Conductivity (µmhos)	
	0	24	48[a]	0	24	48[a]	0	24	48[a]	0	48	0	48	0	24	48[a]	0	48	0	48[a]
Control	10	10		8.4	8.9		7.7	8.2						20.9	20.6		0/0	---		
Control	10	10		8.4	8.9		7.7	8.2											203	
5.6%	10	10		8.5	8.9		7.8	8.2						21.0	20.3					
5.6%	10	9		8.5	9.0		7.8	8.2											212	
10.0%	10	10		8.5	9.0		7.8	8.1						21.0	20.3					
10.0%	10	9		8.5	9.1		7.8	8.1											220	
18.0%	10	8		8.5	9.0		7.7	8.1						21.0	20.3					
18.0%	10	8		8.5	9.0		7.7	8.0											229	
32.0%	10	10		8.6	9.1		7.6	7.9						20.9	20.3					
32.0%	10	9		8.6	9.1		7.7	7.9											245	
56.0%	10	5		8.6	9.1		7.6	7.7						20.6	20.2					
56.0%	10	5		8.6	9.1		7.6	7.6											272	
100.0%	10	5		8.7	9.0		7.0	7.5		0		70		20.6	20.6		0/0	---		
100.0%	10	4		8.7	9.0		7.0	7.4											322	

Remarks: [a] The 48 hour test was invalidated due to excessive control mortality (>20%), data not reported.

Table 7. LC_{50} Data for Bioassays of the Florida State Hospital, Chattahoochee, NPDES #FL0031402, on 7 to 9 November, 1983.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Test #1: *D. pulex* Pre-chlorinated Sample	Binomial	45.0% (32 and 100)	28.9% (18 and 56)
	Moving Average	44.4% (38 and 51.7),d	a
	Probit	44.7% (38 and 52.3)	a
Test #1: *P. promelas* Pre-chlorinated Sample	Binomial	a	a
	Moving Average	a	a
	Probit	a	a
Test #2: *D. pulex* Chlorinated Sample	Binomial	77.7% (56 and 100)	67.1% (56 and 100)
	Moving Average	a	62.7% (55 and 72),d
	Probit	80.4% (0 and ∞),c	61.7% (0 and ∞),c
Test #3: *D. pulex* De-chlorinated Sample	Binomial	a	81.0% (56 and 100)
	Moving Average	a	81.0% (71 and 96),d
	Probit	a	77.1% (62 and 109)
Test #3: *S. serrulatus* De-chlorinated Sample	Binomial	56.0% (32 and ∞),b	e
	Moving Average	71.3% (55 and 113),b,d	
	Probit	85.5% (55 and 206),b	

[a] Due to an insufficient number of dead organisms, or to the irregular distribution of mortality, an LC_{50} value cannot be calculated using this method of statistical analysis.

[b] Due to the distribution of mortality, the LC_{50} value calculated by this method of statistical analysis does not meet the criteria established by the Committee on Methods for Toxicity Tests with Aquatic Organisms.

[c] Due to the distribution of mortality, results calculated using the probit method are not statistically sound.

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

[e] The 48 hour test was invalidated due to excessive control mortality (>20%).

Table 8. Results of Chemical Analyses Performed on Effluent Samples from the Florida State Hospital STP, Chattahoochee, Collected November 7, 1983.

ORGANICS

	Pre-chlorinated Effluent	Chlorinated Effluent
Base Neutral Extractables:	N.D.	N.D.
Pesticide Extractables:	Several Unidentified Compounds	Several Unidentified Compounds

METALS

Cadmium	N.D.
Chromium	N.D.
Copper	<25 µg/l
Mercury	<0.2 µg/l
Silver	N.D.
Zinc	30.7 µg/l

N.D.=None Detected

RESULTS AND DISCUSSION

The samples of pre-chlorinated, chlorinated, and de-chlorinated effluents from the Florida State Hospital STP at Chattahoochee proved to be acutely toxic to the test organism, Daphnia pulex, producing 48 hour LC_{50} values of 28.9%, 62.7%, and 81.0%, respectivelty (Table 7). An additional bioassay of the pre-chlorinated effluent using Pimephales promelas was not acutely toxic, only one of 10 fish died in the 100% test concentration (Table 3). An additional bioassay of the de-chlorinated effluent using Simocephalus serrulatus was acutely toxic, producing a 24 hour LC_{50} of 71.3% (Table 7). The 48 hour S. serrulatus test was invalidated due to excessive control mortality. The 96 hour LC_{50}'s for these effluents would be less than or equal to their 24 or 48 hour values.

The results of these latest bioassays were very similar to the results of earlier bioassays performed in June, 1982, although these latest results did show a significant decrease in toxicity from the 1982 tests, suggesting that some improvement has been made at the facility. However, these latest tests still indicated the presence of acute toxicity in the effluents from the Florida State Hospital STP. Additionaly, it was noted that the pre-chlorinated effluent was significantly more toxic to D. pulex than was the chlorinated effluent, which contained a substantial total chlorine residual. This may suggest that the chlorination process lessened the toxicity of the effluent.

Samples of pre-chlorinated and chlorinated effluents, and a sample of chlorinated effluent, have been analyzed by the FDER Chemistry Section for certain organics and metals, respectively (Table 8). These analyses indicated the presence of several unidentified organic compounds in both the pre-chlorinated and final chlorinated effluents, and, the presence of zinc (30.7 µg/l) in the final effluent. These chemical constituents may have contributed to the toxicities noted, but without a more complete analysis, a definite cause cannot be attributed to any specific constituent at this time.

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 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 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 existance of a mixing zone, whether as a specific part of the permit or based on the existance 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 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.

LITERATURE CITED

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