BIOASSAYS OF THE GAINESVILLE MAIN STREET

SEWAGE TREATMENT PLANT

GAINESVILLE, ALACHUA COUNTY, FLORIDA

NPDES #FL0027251

SAMPLED 7/26/82

Biology Section
Bureau of Water Analysis
October 4, 1982

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EXECUTIVE SUMMARY

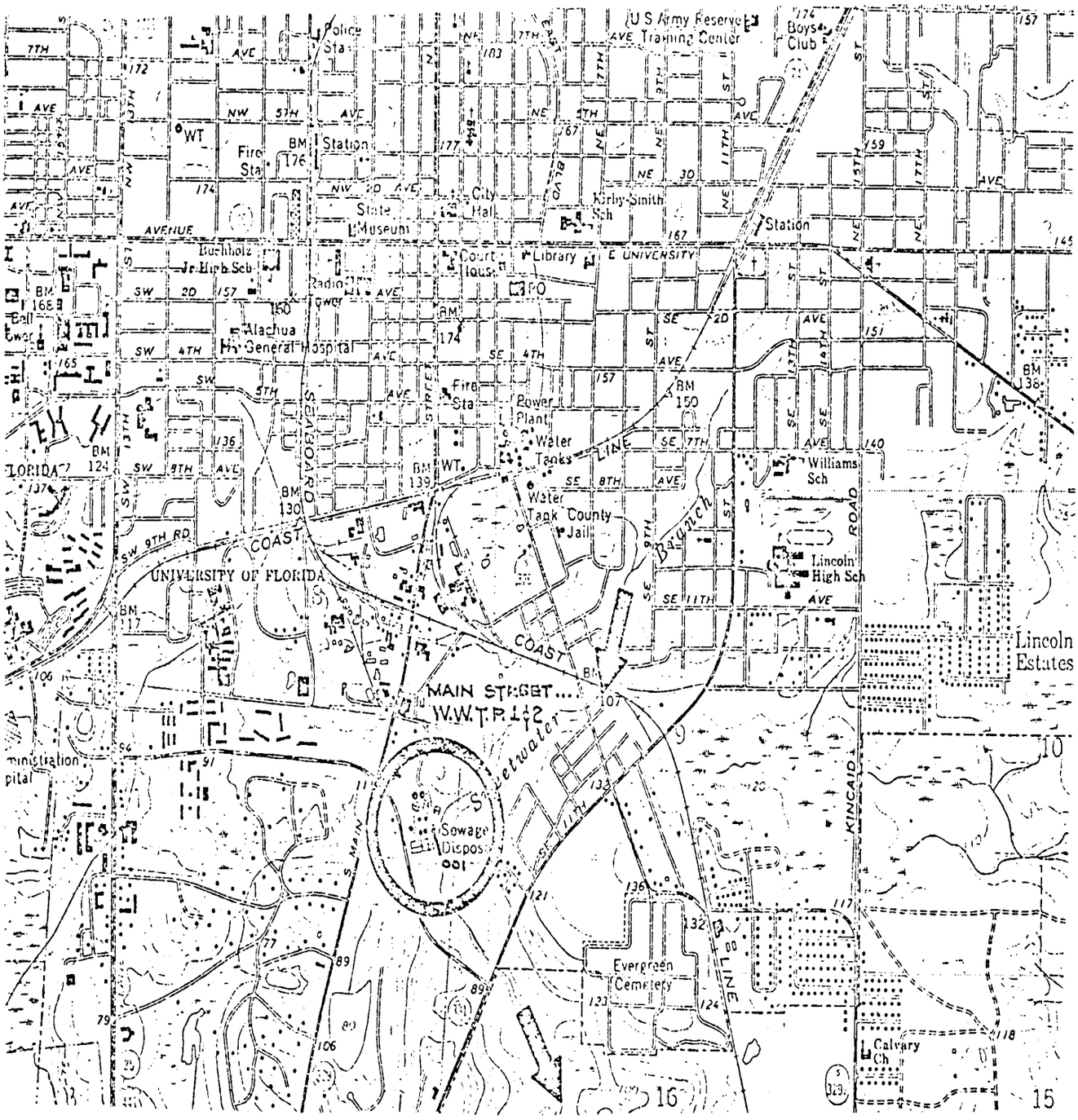
Gainesville Main St. STP, NPDES #FL0027251, on 27 to 29 July, 1982

The samples of pre-chlorinated, chlorinated and de-chlorinated effluents from this discharger, a domestic waste treatment facility, were all acutely toxic to Daphnia pulex, with 48 hour LC_{50} values of 188%, <5.6% and 133%, respectively. A second bioassay of the pre-chlorinated effluent, using Heterandria formosa, was not toxic. A second bioassay of the de-chlorinated effluent, using Simocephalus serrulatus, resulted in LC_{50} values of 27% to 37%. The toxicity of the chlorinated sample can be attributed to the high concentration of total residual chlorine measured in that sample. Chemical analyses of the chlorinated effluent determined the presence of the pesticide Lindane (0.2 µg/l) and the metals arsenic (22 µg/l), mercury (0.4 µg/l), zinc (59 µg/l), beryllium (<25 µg/l) and silver (<10 µg/l). Although individually sub-lethal, these chemical constituents of the effluent may have acted synergistically to have caused the observed toxicity.

INTRODUCTION

The Gainesville Main Street Sewage Treatment Plant is located in the southern portion of Gainesville, Alachua County, Florida (Fig. 1). This waste treatment facility has a design capacity of 9.5 MGD and treats an average of 5.3 MGD of waste. Included in this total are 30,000 GPD of industrial wastes. Treatment at this facility, consisting of two separate systems, employs trickling filters, contact stabilization, activated sludge and final chlorination. A diagram of this operation is presented in Figure 2. Discharge of effluent is to Sweetwater Branch, which flows to Paynes Prairie.

To further evaluate the existence and extent of toxicity of this effluent to the biota of the receiving waters, the Biological Section performed static acute toxicity bioassays on pre-chlorinated, chlorinated and de-chlorinated grab samples, on 27 to 29 July, 1982. The results of these tests are presented in this report.



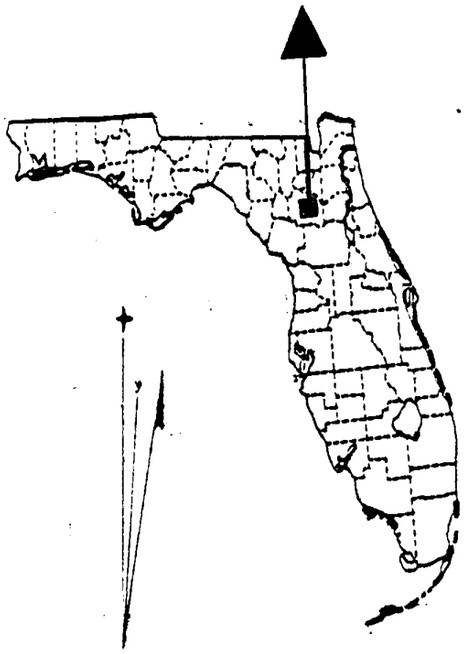


Figure 1. Portions of Gainesville, Alachua County, Florida, including study area.

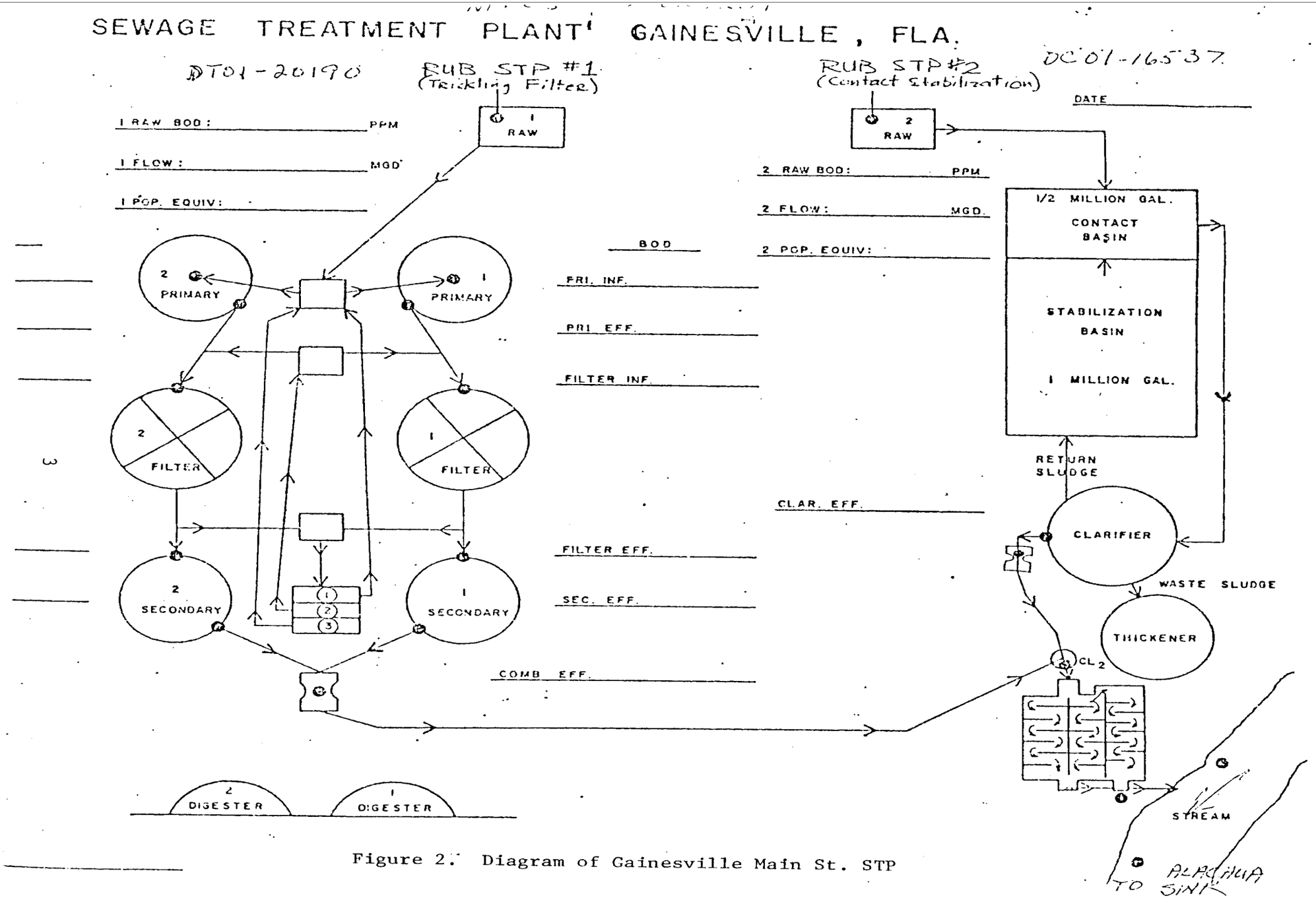


Figure 2. Diagram of Gainesville Main St. STP

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978). The organisms selected for this study include a species recommended by Peltier (1978) and the U.S. EPA (1978). Additionally, the Biological Section maintains cultures of several species of organisms indigenous to the State of Florida. Two of these indigenous species were 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 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.

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 Biological 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 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.

Heterandria formosa (least killifish) - Approximately 100 adult least killifish were obtained from a wild population in the St. Marks Wildlife Refuge (Wakulla County, Florida) during August, 1980. The fish are maintained in two and one-half to 15 gallon all glass aquaria equipped with a flow through water exchange system. Water for this system is unchlorinated and is pumped from a deep well near the DER laboratory. The least killifish are reared at 22° plus or minus 2°C under a 16:8 hour light-dark cycle and are fed freshly hatched Artemia (brine shrimp) twice daily. New born fish are initially maintained in the adult tanks, but are removed at weekly intervals, thereby segregating fish into even age groups. The fish used in this test were 2 to 4 weeks old.

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

Test Methods

Five separate 48 hour static acute toxicity bioassays were performed on samples of pre-chlorinated, chlorinated and de-chlorinated effluents from the Gainesville Main St. STP. The tests were performed from 1420 hours on 27 July to 1440 hours on 29 July, 1982. The samples were collected by Mr. Charles Love of DER's Jacksonville Office at 1440 hours on 26 July, 1982, 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.

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

Test #1. Pre-Chlorinated Effluent using Daphnia pulex and Heterandria formosa (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. These tests were 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 Daphnia were siphoned through a stainless steel screen. The trapped adults were then discarded. Simocephalus neonates were separated ffrom the adults visually in the culture dish prior to loading. 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 six concentrations and a control, were used for the Heterandria formosa (least killifish) 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, 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 of each test concentration was recorded at the beginning and end of the test. Ammonia concentrations, including total and unionized fractions (expressed in mg/l as N and as NH_3, respectively), were recorded in the 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 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 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 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

Heterandria formosa (least killifish)

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 the Gainesville Main St. STP, Gainesville, Alachua County, Florida, NPDES #FL0027251, on 27 to 29 July, 1982.

Test #1 Pre-chlorinated Sample

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	9.2	9.1	8.9	7.7	8.2	8.0	119		117		20.8	19.8	19.9	0/0	---		
Control	10	10	10	9.2	9.2	8.9	7.7	8.2	8.1										200	210
5.6%	10	10	10	9.2	9.2	8.9	7.8	8.2	8.1					20.8	19.7	19.8				
5.6%	10	10	10	9.2	9.2	8.9	7.8	8.2	8.1										210	220
10.0%	10	10	10	9.1	9.3	8.9	7.8	8.2	8.1					21.1	19.7	19.7				
10.0%	10	10	10	9.1	9.2	8.9	7.8	8.2	8.1										220	230
18.0%	10	10	10	9.1	9.2	8.8	7.8	8.2	8.1					21.0	19.7	19.7				
18.0%	10	10	9	9.1	9.2	8.8	7.8	8.2	8.1										240	245
32.0%	10	10	10	9.1	9.2	8.8	7.8	8.1	8.1					20.8	19.7	19.7				
32.0%	10	9	9	9.1	9.1	8.8	7.8	8.1	8.1										265	270
56.0%	10	10	8	8.9	8.8	8.8	7.5	8.1	8.0					20.7	19.7	19.7				
56.0%	10	9	7	8.9	8.8	8.8	7.5	8.0	8.0										310	320
100.0%	10	8	8	8.9	8.3	8.6	7.2	7.8	7.9	60		115		20.6	19.8	19.9	.10/.63	--		
100.0%	10	9	8	8.9	8.3	8.7	7.2	7.8	7.9										390	395

Remarks:

Table 3 . Data recorded during 48 hour, Heterandria formosa[a], static acute toxicity bioassay of the Gainesville Main St. STP, Gainesville, Alachua County, Florida, NPDES #FL0027251, on 27 to 29 July, 1982.

Test #1 Pre-chlorinated Sample

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	9.1	8.6	8.6	7.7	8.1	8.0	119		117		21.5	21.1	19.8	0/0	--		
Control	5	5	5	9.1	8.6	8.6	7.7	8.1	8.0										210	210
5.6%	5	5	5	9.0	8.5	8.5	7.8	8.0	8.0					21.4	19.9	19.7				
5.6%	5	5	5	9.0	8.5	8.5	7.8	8.0	8.0										220	220
10.0%	5	5	5	9.1	8.5	8.4	7.8	8.0	8.0					21.2	19.7	19.6				
10.0%	5	5	5	9.1	8.5	8.4	7.8	8.0	8.1										230	230
18.0%	5	5	5	9.1	8.0	8.1	7.8	7.9	8.0					21.1	19.7	19.5				
18.0%	5	5	5	9.1	8.1	8.1	7.8	7.9	8.0										250	245
32.0%	5	5	5	8.9	8.1	8.1	7.7	7.9	8.0					21.0	19.6	19.5				
32.0%	5	5	5	8.9	8.2	8.1	7.7	7.9	8.0										275	270
56.0%	5	5	5	8.9	8.0	8.0	7.5	7.8	7.9					20.9	19.7	19.6				
56.0%	5	5	5	8.9	8.0	8.0	7.5	7.8	7.9										320	315
100.0%	5	5	5	8.5	7.8	7.9	7.2	7.7	7.8	60		115		20.7	19.8	19.6	.01/.63	--		
100.0%	5	5	5	8.5	7.8	7.9	7.2	7.6	7.7										400	395

Remarks: [a] Weight of fish: 12.14 mg each, average of 10 fish.

Table 4 . Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of the Gainesville Main St. STP, Gainesville, Alachua County, Florida, NPDES #FL0027251, on 27 to 29 July, 1982.

Test #2 Chlorinated Sample[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/NH_3+NH_4 (mg/l)		Conductivity (µmhos)
	0	24	48[b]	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0
Control	10	10		9.0	9.1		7.7	8.2		119		117		20.8	20.0		0/0	---	
Control	10	10		9.0	9.1		7.7	8.2											195
5.6%	10	0		9.1	9.3		7.8	8.2						20.8	19.9				
5.6%	10	0		9.1	9.3		7.8	8.2											205
10.0%	10	0		9.0	9.3		7.8	8.2						20.8	19.9				
10.0%	10	0		9.0	9.3		7.8	8.2											215
18.0%	10	0		8.9	9.3		7.7	8.2						20.8	19.9				
18.0%	10	0		8.9	9.3		7.7	8.2											225
32.0%	10	0		8.9	9.3		7.7	8.2						20.7	20.0				
32.0%	10	0		8.9	9.3		7.7	8.2											245
56.0%	10	0		8.9	9.3		7.5	8.1						20.7	20.0				
56.0%	10	0		8.9	9.3		7.4	8.1											290
100.0%	10	0		8.8	9.3		7.0	7.8		37		104		20.7	20.1		0/.10	---	
100.0%	10	0		8.8	9.3		7.0	7.8											350

Remarks: [a] Total Residual Chlorine: 4.1 mg/l (Iodometric Method I).

[b] Since all organisms in the effluent concentrations were dead by hour 24, the test was terminated at that time.

Table 5. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of the Gainesville Main St. STP, Gainesville, Alachua County, Florida, NPDES #FL0027251, on 27 to 29 July, 1982.

Test #3 De-chlorinated Sample[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/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	9.2	9.0	8.8	7.8	8.2	8.1	119		117		20.7	19.9	19.8	0/0	---		
Control	10	10	10	9.2	9.1	8.8	7.8	8.2	8.1										205	215
5.6%	10	10	10	9.2	9.1	8.7	7.9	8.2	8.1					20.7	19.9	19.8				
5.6%	10	10	10	9.2	9.1	8.7	7.9	8.2	8.1										220	220
10.0%	10	10	10	9.2	9.2	8.7	7.9	8.2	8.1					20.9	19.9	19.9				
10.0%	10	10	10	9.2	9.2	8.6	7.9	8.3	8.1										230	230
18.0%	10	10	10	9.2	9.2	8.6	7.8	8.2	8.1					20.9	20.0	19.9				
18.0%	10	10	10	9.2	9.2	8.5	7.8	8.2	8.1										245	240
32.0%	10	10	10	9.2	9.2	8.5	7.8	8.2	8.0					21.0	20.4	20.0				
32.0%	10	10	10	9.2	9.2	8.5	7.7	8.2	7.9										275	275
56.0%	10	9	8	9.1	9.1	8.5	7.6	8.0	7.9					21.1	20.4	20.1				
56.0%	10	10	10	9.1	9.0	8.4	7.6	8.0	7.9										320	320
100.0%	10	7	6	9.1	8.8	8.1	7.2	7.8	7.7					21.2	20.5	20.1	0/.10	---		
100.0%	10	9	8	9.1	8.8	8.0	7.2	7.8	7.6	37		104							400	400

Remarks: [a] Total Residual Chlorine: <0.02 mg/1 (HACH DPC Method).

Table 6 . Data recorded during 48 hour, Simocephalus serrulatus, static acute toxicity bioassay of the Gainesville Main St. STP, Gainesville, Alachua County, Florida, NPDES #FL0027251, on 27 to 29 July, 1982.

Test #3 De-chlorinated Sample[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/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	9	9.1	9.0	8.7	7.8	8.2	8.1	119		117		20.8	19.8	19.8	0/0	---		
Control	10	10	9	9.1	9.0	8.8	7.8	8.2	8.2										205	215
5.6%	10	10	8	9.2	9.1	8.7	7.9	8.2	8.2					20.7	19.8	19.7				
5.6%	10	9	9	9.2	9.1	8.7	7.9	8.2	8.1										220	220
10.0%	10	10	9	9.2	9.1	8.7	7.9	8.3	8.2					20.7	19.7	19.7				
10.0%	10	10	10	9.2	9.1	8.7	7.9	8.3	8.2										230	230
18.0%	10	10	7	9.2	9.2	8.7	7.8	8.2	8.1					20.9	19.8	19.7				
18.0%	10	10	9	9.2	9.2	8.6	7.8	8.2	8.1										245	245
32.0%	10	10	8	9.1	9.1	8.6	7.7	8.2	8.0					21.0	19.8	19.8				
32.0%	10	10	7	9.1	9.1	8.5	7.7	8.1	8.0										275	270
56.0%	10	10	0	9.1	9.0	8.4	7.6	8.1	7.9					21.1	20.0	19.9				
56.0%	10	7	0	9.1	9.0	8.4	7.6	8.0	7.9										320	320
100.0%	10	3	0	9.1	8.6	8.2	7.2	7.8	7.8	37		104		21.1	20.3	20.0	0/.10	---		
100.0%	10	2	0	9.1	8.6	8.2	7.2	7.8	7.7										400	400

Remarks: [a] Total Residual Chlorine: <0.02 mg/1 (HACH DPD Method)

Table 7. LC_{50} Data for Bioassays of the Gainesville Main St. STP, NPDES #FL0027251, on 27 to 29 July, 1982.

Organism	Method of Data Analysis	24 LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Test #1: D. pulex Pre-chlorinated Sample	Binomial	a	a
	Moving Average	a	a
	Probit	350.8% (137 and ∞), b	187.9% (105 and 2031), b
Test #1: H. formosa Pre-chlorinated Sample	Binomial		
	Moving Average	No Mortality	No Mortality
	Probit		
Test #2: D. pulex Chlorinated Sample	Binomial		
	Moving Average	<5.6%, c	<5.6%, c
	Probit		
Test #3: D. pulex De-chlorinated Sample	Binomial	a	
	Moving Average	a	
	Probit	165.3% (109 and ∞), b	133.4% (97 and 629), b
Test #3: S. serrulatus De-chlorinated Sample	Binomial	79.1% (56 and 100)	37.2% (32 and 56)
	Moving Average	79.1% (68 and 96)	27.3% (21 and 37)
	Probit	91.3% (0 and ∞), d	27.9% (0 and ∞), d

[a] Due to the pattern 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] Since all organisms were dead in the lowest test concentration (5.6%) by hour 24, the LC_{50}'s are <5.6%.
[d] Due to the distribution of mortality, results calculated using the probit method are not statistically sound.

RESULTS AND DISCUSSION

The sample of pre-chlorinated effluent from the Gainesville Main St. STP was acutely toxic to Daphnia pulex, producing a 48 hour LC_{50} value of 187.9% (Table 7). This same effluent was not toxic to Heterandria formosa. The chlorinated effluent from this facility was extremely toxic to Daphnia pulex, since all organisms were dead in every effluent concentration by hour 24 of the test. Based on this mortality, the 24 and 48 hour LC_{50}'s are <5.6% (Table 7). The toxicity of this chlorinated effluent can be attributed to the high total residual chlorine concentration (4.1 mg/1, Table 4) measured in that sample. The de-chlorinated effluent was acutely toxic to Daphnia pulex, producing a 48 hour LC_{50} value of 133.4% (Table 7). This same effluent was also acutely toxic to Simocephalus serrulatus, producing 24 hour LC_{50} values of 79.1% to 91.3% and 48 hour values of 27.3% to 37.2% (Table 7). The 96 hour LC_{50}'s for these effluents would be less than or equal to their 48 hour values. Small concentrations of ammonia were determined in these effluents, but they were significantly below reported toxic levels (Thurston et. al 1981).

The DER Chemistry Section has analyzed samples of effluent from the Gainesville Main St. STP for certain organic compounds and metals (Table 8). These analyses determined the presence of the pesticide Lindane (0.2 µg/1) and the metals arsenic (22 µg/1), mercury (0.4 µg/1) and zinc (59 µg/1), and, trace amounts of beryllium (<25 µg/1) and silver (<10 µg/1). Although the concentrations of these constituents may individually be sub-lethal (U.S. EPA 1976), they may have acted synergistically to have caused the observed toxicity. Additionally, the organic species of mercury at concentrations of 0.4 µg/1 and below have been shown to cause distinct chronic effects to Daphnia and to cause long term (3 months exposure) toxicity to Pimephales promelas (U.S. EPA 1976). And, concentrations of silver significantly less than 10 µg/1 have been shown to be toxic to Daphnia and to stickleback fish (U.S. EPA 1976). But, without a more definitive quantification of silver, it is not known exactly what effects this metal may have had on the test organisms.

DISCUSSION OF THE TOXICITY CRITERIA

The toxicity standards of Chapters 17-3 and 17-4, F.A.C., are complex. Because of this complexity, they will be briefly reviewed here so that these test results may be more readily compared with the standards. For legal purposes, however, the language of the Florida Administrative Code should be consulted directly.

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

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

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

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

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

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

The toxicity reported here is in excess of allowable limits.

Table 8. Results of Chemical Analyses Performed on Effluent from the Gainesville Main St. STP, NPDES #FL0027251, collected 26 July, 1982

Organics

Parameter	Compound
Acid Extractables	N.D.
Base Neutral Extractables	N.D.
Pesticide Extractables	g-BHC (Lindane), 0.2 µg/l

Metals

Antimony	N.D.
Arsenic	22 µg/l
Beryllium	<25 µg/l[a]
Cadmium	N.D.
Chromium	N.D.
Copper	N.D.
Lead	N.D.
Mercury	0.4 µg/l
Nickel	N.D.
Selenium	N.D.
Silver	<10 µg/l[a]
Thallium	N.D.
Zinc	59 µg/l

N.D. = None Detected

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

LITERATURE CITED

American Public Health Association. 1980. Standard Methods for the Examination of Water and Wastewater. 15th ed. 1134 p.

Peltier, W. 1978. Methods for measuring the acute toxicity of effluents to aquatic organisms. Environmental Research Laboratory. Athens, Georgia. EPA-600/4-78-012 (Revised July 1978). 51 p.

Thurston, R.V., Russo, R.C. and G.A. Vinogradov. 1981. Ammonia toxicity to fishes: Effect of pH on the toxicity of the unionized species. Env. Sci. Tech. 15(7): 837-840.

U.S. Environmental Protection Agency. 1976. Quality Criteria for Water. U.S. EPA, Washington, D.C. 256 p.

U.S. Environmental Protection Agency. 1978. Bioassay procedures for the Ocean Disposal Permit Program. Environmental Research Laboratory. Gulf Breeze, Florida. EPA-600/9-78-010. 121 p.