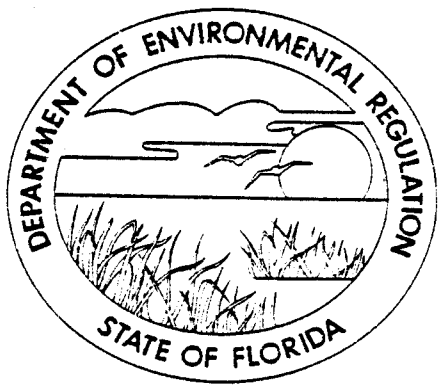


BIOASSAYS OF THE KANAPAHA ADVANCED WASTEWATER TREATMENT PLANT GAINESVILLE, ALACHUA COUNTY, FLORIDA NPDES #FL0032379

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Biological Section
Bureau of Water Analysis
August 2, 1982

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EXECUTIVE SUMMARY

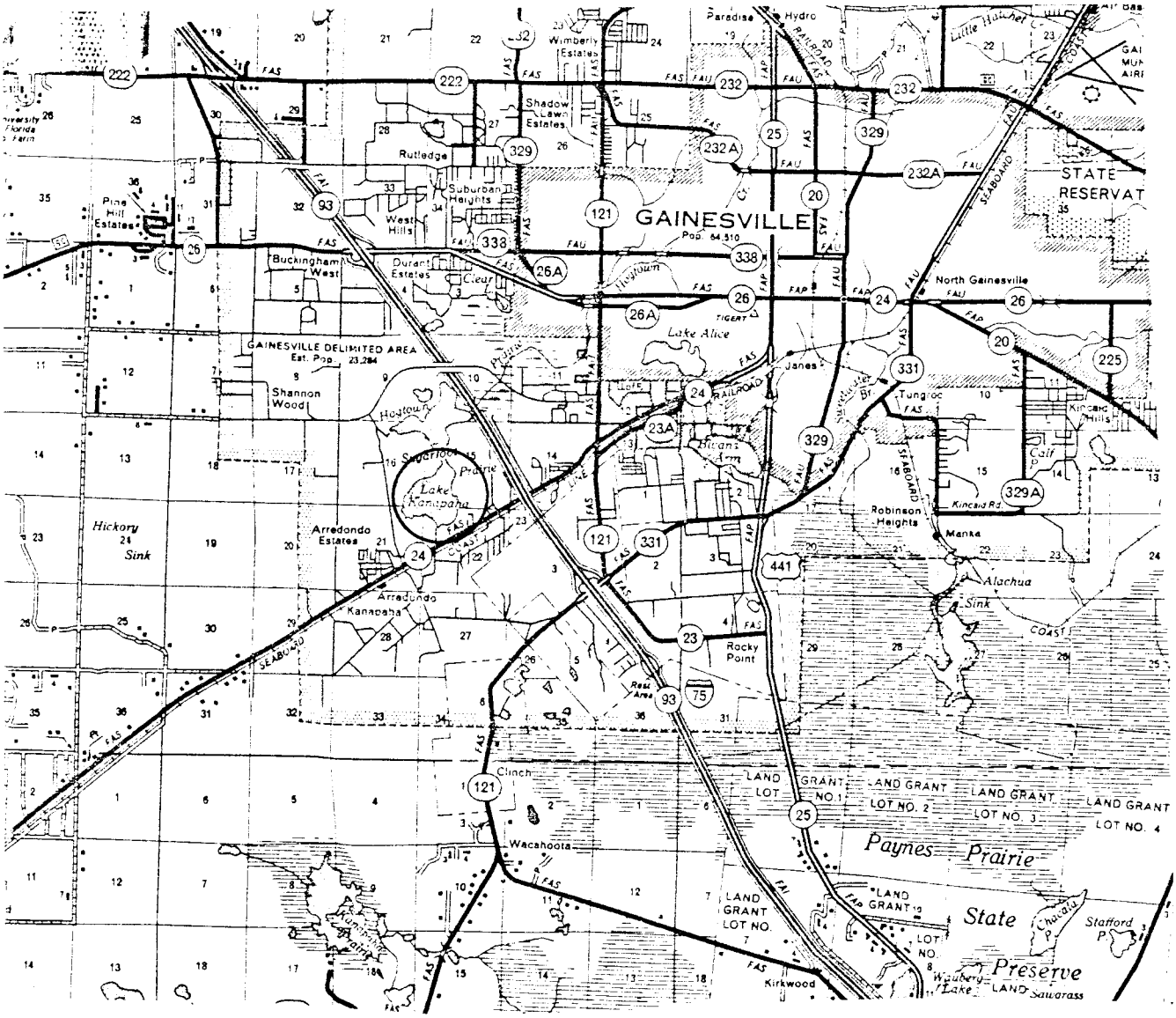
Kanapaha AWTP, Gainesville, NPDES #FL0032379, on 15 to 17 June, 1982

The samples of pre-chlorinated and chlorinated effluents from this discharger, a municipal waste treatment facility, were acutely toxic to the test organisms, Daphnia pulex and Simocephalus serrulatus. The pre-chlorinated effluent produced 48 hour LC_{50} values of 96% to 164% for Daphnia and 24 hour LC_{50} values of 80% to 96% for Simocephalus. The 48 hour Simocephalus bioassay was invalidated due to excessive control mortality. The chlorinated effluent produced a 24 hour LC_{50} value of 13% and a 48 hour value of 10% for Daphnia. The de-chlorinated effluent was lethal to a number of Daphnia in the higher test concentrations, but was not toxic to any Heterandria formosa. The cause of the toxicity in the chlorinated effluent samples can be attributed, in part, to the presence of total residual chlorine. Chemical analyses indicated the presence of the pesticide Lindane (0.1µg/l), numerous other organic compounds including five unidentified halogenated compounds, and, measurable concentrations of lead and zinc. These organic compounds and heavy metals may have acted synergistically to have caused the observed toxicity.

INTRODUCTION

The Kanapaha Advanced Wastewater Treatment Plant is located at 3901 S.W. 63rd Blvd. in Gainesville, Alachua County, Florida (Fig. 1). This facility has a design capacity of 7.0 MGD and treats an average of 5.9 MGD of domestic waste from a population of approximately 60,000. Included in this are approximately 10,000 GPD of industrial wastes. Treatment consists of grit removal, mechanical aeration, secondary clarification, denitrification, post aeration and chlorination. Discharge of the treated wastes is through deep well injection, but the facility does maintain an emergency outfall to Lake Kanapaha.

To further evaluate the existence and extent of toxicity of this effluent to standard bioassay test organisms, the Biological Section performed five static acute toxicity bioassays on grab samples, on 15 to 17 June, 1982. The results of these tests are presented in this report.



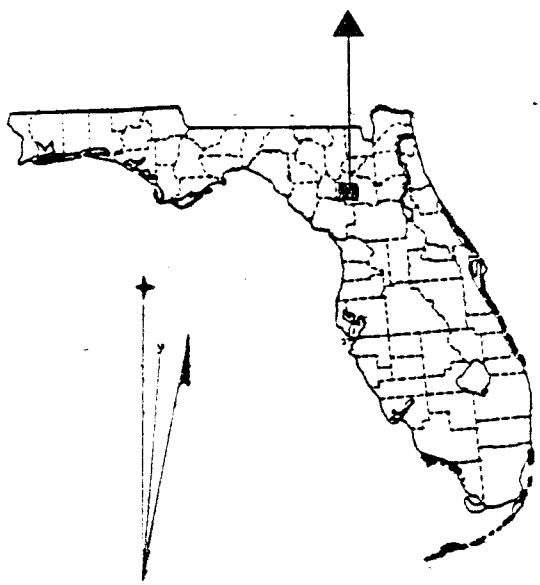


Figure 1. Portions of Alachua County, Florida, including Gainesville and study area.

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 3 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 Kanapaha AWTP. The tests were performed from 1130 hours on 15 June to 1150 hours on 17 June, 1982.

The samples were collected by Ms. Kathleen Hudek of DER's Jacksonville Office at 1300 hours on 14 June, 1982, 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 Biological Section adopted the following test procedure:

Test #1. Pre-Chlorinated Effluent using Daphnia pulex and Simocephalus serrulatus (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 Heterandria formosa (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 from 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. 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 flea)

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 Kanapaha AWTP, Gainesville, Alachua County, Florida, NPDES #FL0032379, on 15 to 17 June, 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	8.7	9.0	9.0	7.9	8.3	8.1					21.9	20.4	19.6	0/0	---		
Control	10	10	9	8.7	9.1	9.0	7.9	8.3	8.2										207	200
5.6%	10	10	10	8.6	8.9	9.1	7.8	8.3	8.2					21.9	20.3	19.5				
5.6%	10	9	9	8.5	8.9	9.2	7.9	8.3	8.2										222	215
10.0%	10	10	10	8.4	8.8	9.3	7.8	8.3	8.2					21.9	20.2	19.6				
10.0%	10	10	9	8.4	8.8	9.3	7.8	8.3	8.2										233	225
18.0%	10	10	10	8.4	8.8	9.4	7.8	8.2	8.2					21.8	20.1	19.5				
18.0%	10	10	9[b]	8.4	8.8	9.4	7.8	8.2	8.2										250	240
32.0%	10	10	10	8.3	8.7	9.4	7.7	8.2	8.2					21.7	20.1	19.5				
32.0%	10	10	10	8.3	8.7	9.4	7.7	8.2	8.2										280	270
56.0%	10	10	9	8.1	8.7	9.4	7.7	8.1	8.1					21.5	20.1	19.6				
56.0%	10	9	8	8.0	8.7	9.4	7.6	8.1	8.1										335	325
100.0%	10	9	4	6.5	8.6	9.3	7.5	8.0	8.0					21.0	20.2	19.8	0/0	---		
100.0%	11[a]	11	6	6.5	8.6	9.3	7.5	7.9	8.0										420	420

Remarks:

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

[b] Dead organism not found.

Table 3. Data recorded during 48 hour, Simocephalus serrulatus, static acute toxicity bioassay of the Kanapaha AWTP, Gainesville, Alachua County, Florida, NPDES #FL0032379, on 15 to 17 June, 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[a]	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	10	7	5	8.7	8.9	9.1	7.8	8.2	8.1					21.7	20.7	19.8	0/0	---		
Control	10	9	4	8.7	8.9	9.1	7.8	8.2	8.1										200	200
5.6%	10	6	5	8.6	8.7	9.1	7.8	8.2	8.1					21.6	20.5	19.6				
5.6%	10	9	7	8.6	8.7	9.2	7.9	8.2	8.2										212	210
10.0%	10	7	4	8.6	8.7	9.2	7.8	8.1	8.2					21.6	20.4	19.6				
10.0%	10	7	5	8.5	8.7	9.2	7.9	8.1	8.2										222	220
18.0%	10	8	6	8.4	8.6	9.2	7.9	8.1	8.2					21.6	20.3	19.6				
18.0%	10	7	7	8.4	8.6	9.2	7.9	8.1	8.2										238	235
32.0%	10	6	4	8.3	8.5	9.2	7.8	8.1	8.2					21.5	20.2	19.6				
32.0%	10	6	5	8.3	8.5	9.2	7.8	8.1	8.1										270	270
56.0%	10	8	6	8.1	8.4	9.1	7.7	8.0	8.1					21.2	20.2	19.7				
56.0%	10	8	3	7.9	8.4	9.1	7.7	8.0	8.1										320	320
100.0%	10	1	0	6.5	8.3	9.1	7.6	8.0	8.0					21.2	20.4	19.7	0/0	---		
100.0%	10	5	0	6.5	8.3	9.0	7.5	7.9	8.0										420	410

Remarks:

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

Table 4. Data recorded during 48 hour, *Daphnia pulex*, static acute toxicity bioassay of the Kanpaha AWTP, Gainesville, Alachua County, Florida, NPDES #FL0032379, on 15 to 17 June, 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	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	10	9	9	8.8	8.8	9.1	7.9	8.3	8.2					21.8	20.4	19.5	0/0	---		
Control	10	10	10	8.8	8.8	9.1	7.9	8.3	8.2										210	210
5.6%	10	10	10	8.8	9.0	9.1	7.8	8.3	8.2					21.9	20.1	19.3				
5.6%	10	9	9	8.8	9.0	9.0	7.8	8.3	8.2										230	220
10.0%	10	10	6	8.8	9.1	9.0	7.8	8.3	8.2					21.9	19.9	19.2				
10.0%	10	10	6	8.7	9.0	9.1	7.8	8.3	8.2										240	230
18.0%	10	0	0	8.7	9.1	--[b]	7.7	8.2	-[b]					21.9	19.8	-[b]				
18.0%	10	0	0	8.7	9.1	--	7.7	8.2	-										260	-[b]
32.0%	10	0	0	8.7	9.2	--	7.6	8.2	-					21.8	19.8	-				
32.0%	10	0	0	8.6	9.2	--	7.6	8.2	-										290	-
56.0%	10	0	0	8.4	9.1	--	7.5	8.1	-					21.8	19.8	-				
56.0%	10	0	0	8.4	9.1	--	7.4	8.1	-										345	-
100.0%	10	0	0	8.0	9.1	--	7.1	8.0	-					21.8	19.9	-	0/0	---		
100.0%	10	0	0	8.0	9.1	--	7.1	7.9	-										440	-

Remarks: [a] Total Residual Chlorine: field measurement 1.2 mg/l
lab measurement 0.4 mg/l (Iodometric Method I)

[b] Remaining parameters not recorded since all organisms were dead by hour 24.

Table 5. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of the Kanapaha AWTP, Gainesville, Alachua County, Florida, NPDES #FL0032379, on 15 to 17 June, 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[b]	8.8	9.1	9.2	7.8	8.3	8.2					21.8	20.6	19.3	0/0	---		
Control	10	10	10	8.8	9.1	9.2	7.9	8.3	8.2										212	205
5.6%	10	9	9	8.8	9.1	9.2	7.8	8.3	8.2					21.9	20.2	19.1				
5.6%	10	10	10	8.7	9.1	9.2	7.8	8.3	8.2										225	220
10.0%	10	10	10	8.8	9.0	9.2	7.8	8.2	8.2					22.0	20.0	19.1				
10.0%	10	10	10	8.8	9.0	9.1	7.8	8.2	8.2										245	230
18.0%	10	10	9	8.8	9.0	9.1	7.8	8.2	8.2					22.1	19.8	19.0				
18.0%	10	10	10	8.8	9.0	9.0	7.7	8.2	8.1										260	250
32.0%	10	10	10	8.7	9.0	9.0	7.7	8.1	8.1					22.2	19.8	19.2				
32.0%	10	10	10	8.7	9.0	9.0	7.7	8.1	8.1										295	280
56.0%	10	10	10	8.5	8.9	8.9	7.5	8.1	8.0					22.2	20.0	19.4				
56.0%	10	9	7	8.5	8.9	8.8	7.5	8.1	7.9										350	340
100.0%	10	10	9	8.3	8.9	8.7	7.2	7.9	7.8					22.5	20.4	19.7	0/0	---		
100.0%	10	8	8	8.2	8.9	8.6	7.2	7.9	7.7										455	440

Remarks: [a] Total Residual Chlorine: <0.05 mg/l (HACH DPD Method)

[b] Dead organism not found.

Table 6 . Data recorded during 48 hour, *Heterandria formosa*[a], static acute toxicity bioassay of the Kanapaha AWTP, Gainesville, Alachua County, Florida, NPDES #FL0032379, on 15 to 17 June, 1982.
Test #3 De-chlorinated Sample[b]

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Conductivity (µmhos)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	5	5	5	8.8	8.6	8.8	7.8	8.1	8.0					21.8	20.1	19.5	0/0	---		
Control	5	5	5	8.8	8.6	8.8	7.8	8.1	8.0										220	210
5.6%	5	5	5	8.8	8.5	8.9	7.8	8.1	8.1					21.8	19.9	19.3				
5.6%	5	5	5	8.7	8.5	8.9	7.8	8.1	8.1										235	225
10.0%	5	5	5	8.7	8.4	8.9	7.8	8.0	8.0					21.8	19.8	19.2				
10.0%	5	5	5	8.8	8.4	8.8	7.8	8.0	8.0										250	235
18.0%	5	5	5	8.8	8.4	8.8	7.7	8.0	8.0					21.8	19.8	19.3				
18.0%	5	5	5	8.8	8.4	8.8	7.7	8.0	8.0										260	255
32.0%	5	5	5	8.7	8.4	8.7	7.6	7.9	7.9					21.8	19.7	19.3				
32.0%	5	5	5	8.7	8.4	8.7	7.6	7.9	7.9										300	285
56.0%	5	5	5	8.7	8.3	8.5	7.5	7.8	7.8					22.3	19.8	19.3				
56.0%	5	5	5	8.8	8.3	8.5	7.5	7.8	7.8										360	340
100.0%	5	5	5	8.5	8.0	8.2	7.2	7.7	7.6					22.5	19.8	19.4	0/0	---		
100.0%	5	5	5	8.5	8.1	8.0	7.2	7.6	7.6										460	440

Remarks: [a] Weight of fish: 10.6 mg each, average of 10 fish.

[b] Total Residual Chlorine: <0.05 mg/l (HACH DPD Method)

Table 7. LC_{50} Data for Bioassays of Kanapaha AWTP, Gainesville, on 15 to 17 June, 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	Binomial	a	96.7% (56 and ∞),b
Pre-chlorinated	Moving Average	a	96.7% (77 and 261),b
Sample	Probit	a	164% (89 and 981),b
Test #2:S.serrulatus	Binomial	79.7% (56 and∞)	
Pre-chlorinated	Moving Average	79.7% (66 and 104)	c
Sample	Probit	96.4% (39 and ∞)	
Test #2: D. pulex	Binomial	13.4% (10 and 18)	10.8% (5.6 and 18)
Chlorinated	Moving Average	a	10.1% (8.8 and 12)
Sample	Probit	a	10.1% (8.7 and 12)
Test #3:D. pulex	Binomial	a	a
De-chlorinated	Moving Average	a	a
Sample	Probit	a	>1000%
Test #3:P.formosa	Binomial		
De-chlorinated	Moving Average	No Mortality	No Mortality
Sample	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]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]The 48 hour Simocephalus bioassay was invalidated due to excessive control mortality.

RESULTS AND DISCUSSION

The pre-chlorinated effluent from the Kanapaha AWTP proved to be acutely toxic to Daphnia pulex, with 48 hour LC_{50} values of 96.7% to 164% (Table 7). The same effluent also was acutely toxic to Simocephalus serrulatus, producing 24 hour LC_{50} values of 79.7% to 96.4% (Table 7). The 48 hour Simocephalus bioassay was invalidated due to excessive control mortality (>20%). Although this test was invalidated, the results did suggest that the pre-chlorinated effluent was toxic to Simocephalus, since a distinct trend of increasing mortality was noted with increasing effluent concentration (Table 3). The chlorinated effluent was extremely toxic to Daphnia pulex, producing a 24 hour LC_{50} value of 13.4% and 48 hour values of 10.1% to 10.8% (Table 7). The toxicity of this chlorinated effluent can be attributed, in part, to the total residual chlorine measured in the sample (1.2 mg/l field, 0.4 mg/l lab, Table 4). The sample of de-chlorinated effluent was not acutely toxic to either Daphnia pulex or to Heterandria formosa. Only a few Daphnia died in the higher effluent concentrations while no Heterandria died during the 48 hour static test. The 96 hour LC_{50} values for the pre-chlorinated and chlorinated effluents would be less than or equal to their 24 and 48 hour values.

The DER Chemistry Section has analyzed samples of effluent from the Kanapaha AWTP for certain organic compounds and metals (Table 8). A hexane extraction method for the identification of pesticide-like compounds determined the presence of g-BHC (Lindane) at a concentration of 0.1µg/l. Additionally, numerous other compounds sensitive to the electron capture detector and five unidentified halogenated compounds were present in the effluent. The analyses for metals determined the presence of small concentrations of lead (8µg/l) and zinc (28µg/l) in the effluent. These organic compounds and heavy metals may have acted synergistically to have caused the observed toxicity.

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 limits allowable for discharge to surface waters.

Table 8. Results of Chemical Analyses Performed on Effluent from the Kanapaha AWTP, Gainesville, collected 14 June, 1982.

Organics

Parameter	Compound
Acid Extractables	N.D.
Base Neutral Extractables	N.D.
Pesticide Extractables	g-BHC (Lindane) 0.1µg/l Numerous compounds sensitive to the electron capture detector. Five unidentified halogenated compounds.

Metals

Antimony	N.D.
Arsenic	N.D.
Beryllium	N.D.
Cadmium	N.D.
Chromium	N.D.
Copper	<30µg/l[a]
Lead	8µg/l
Mercury	<0.2µg/l[a]
Nickel	N.D.
Selenium	N.D.
Silver	N.D.
Thallium	N.D.
Zinc	28µg/l

N.D. = None Detected

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

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