

BIOASSAYS OF THE LAKE CITY SEWAGE TREATMENT PLANT COLUMBIA COUNTY, FLORIDA

NPDES #FL0025968

Biology Section
Bureau of Water Analysis
February 9, 1984

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EXECUTIVE SUMMARY

Lake City STP, NPDES #FL0025968, on 4 to 6 January, 1984

The pre-chlorinated sample of effluent from this municipal waste treatment facility proved to be acutely toxic to Daphnia pulex, producing a 48 hour LC_{50} of 93.0%. This same effluent, however, was not toxic to Notropis leedsi. The chlorinated effluent was slightly more toxic to D. pulex than was the pre-chlorinated effluent, producing a 48 hour LC_{50} of 78.7%. This was most likely due to the added effects of the total chlorine residual (0.55 mg/l) measured in that sample. In the bioassays of the de-chlorinated effluent, using D. pulex and Simocephalus serrulatus as test organisms, only random mortalities were noted, and therefore, no LC_{50}'s could be calculated. Ammonia was not detected in the effluents from this facility.

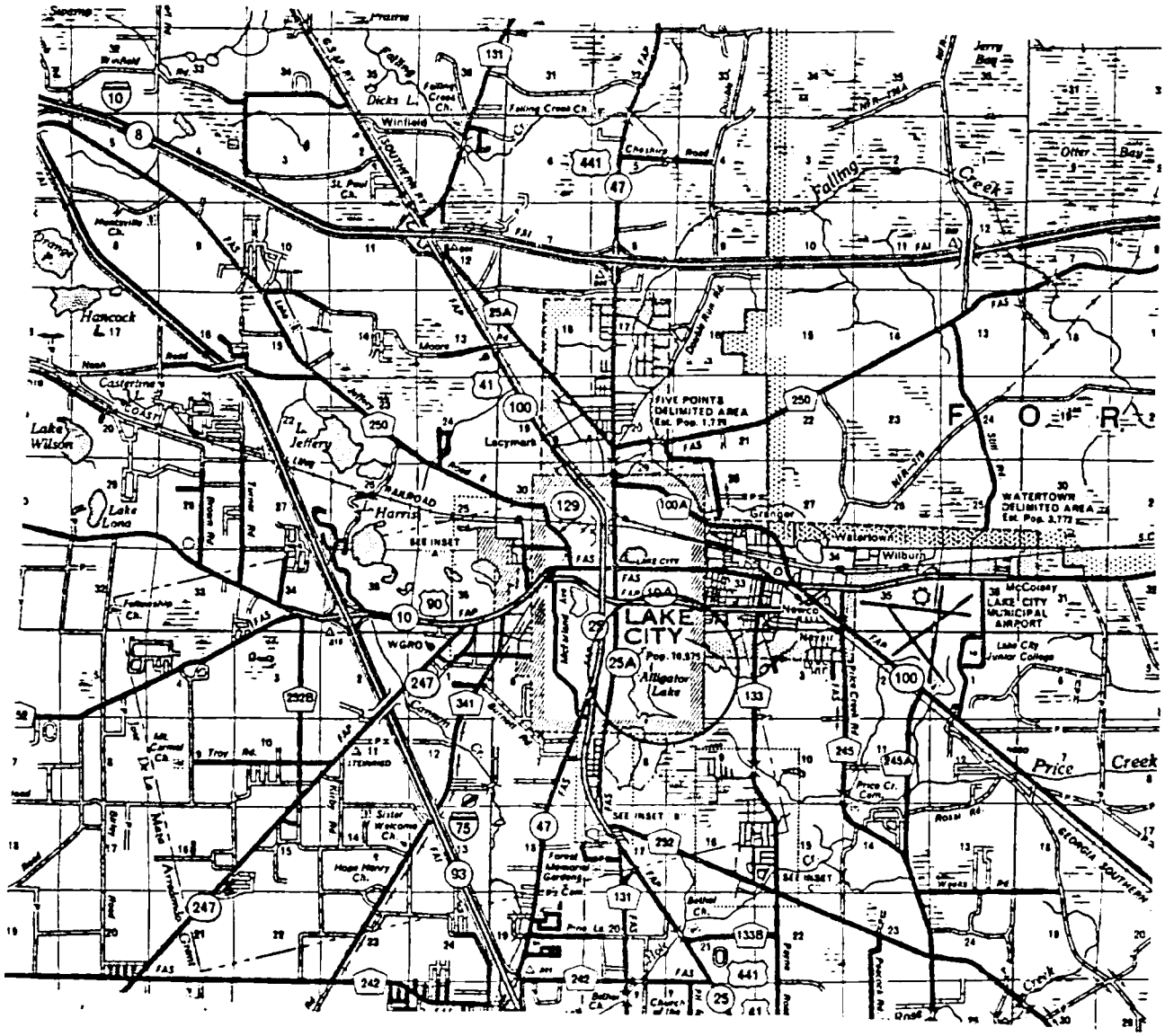
The DER Chemistry Section analyzed samples of effluents from the Lake City STP for certain organics and metals. These analyses failed to indicate the presence of any organic contaminant, but did indicate the presence of a measureable concentration of zinc (53.5 µg/l). This concentration of zinc probably did not contribute significantly to the observed mortality.

INTRODUCTION

The Lake City Sewage Treatment Plant is located at 750 Margaret Street, in Lake City, Columbia County, Florida (Fig. 1). The facility has a design flow capacity of 3.0 MGD and began operation in 1975. During dry weather, an average of 2.31 MGD of wastes are treated, but during wet weather the volume of wastes may increase to 3.1 MGD. Treatment consists of extended aeration and final chlorination. This facility treats waste from a population of approximately 30,000 as well as sewage from at least three hospitals, one nursing home and one shopping center. A flow schematic of the Lake City STP is presented in Fig. 2. Final treated effluent is discharged to Lake Montgomery, which flows to Alligator Lake.

According to the NPDES file for this facility, the plant has major infiltration problems in its sewage collection system. Additionally, the facility has been in violation of its NPDES permit as recently as December, 1983, for not meeting final effluent limitations.

At the request of the Northeast District, and since the pesticide Lindane was previously detected in the effluent from this facility, acute toxicity bioassays were performed to evaluate the aquatic toxicity potential to the biota of the receiving waters. Five bioassay tests were performed from 4 to 6 January, 1984, and the results of these 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, M. L. Roll, and L. A. Williams.



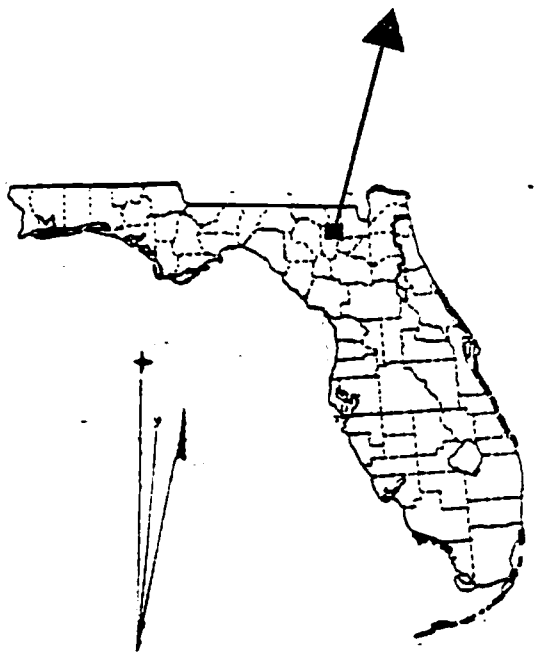
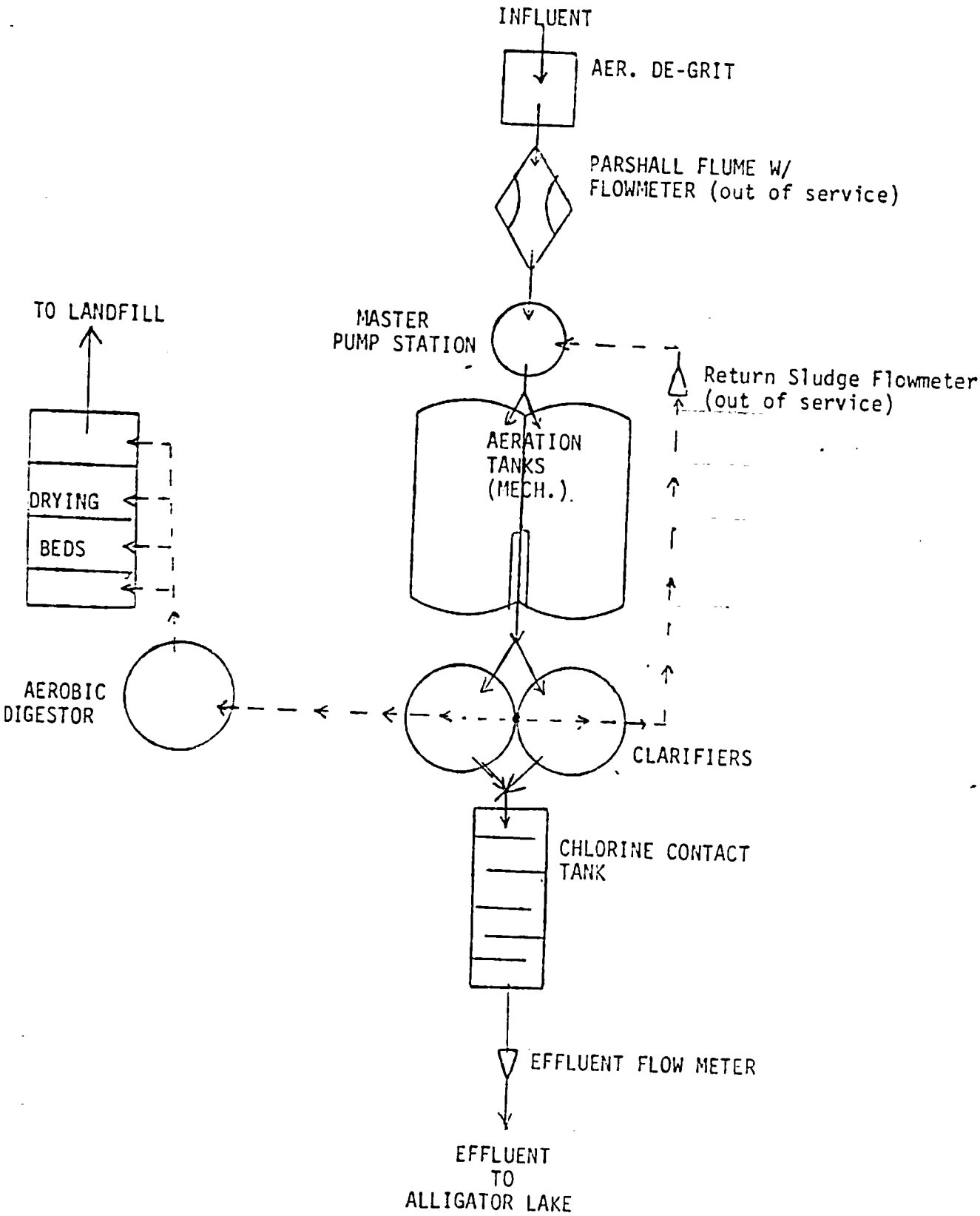


Fig. 1. Portions of Columbia County, Florida, including Lake City and Alligator Lake

LAKE CITY STP
FLOW SCHEMATIC
Figure 2

FL0025968



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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 a 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. 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 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.

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° plus or minus 2°C under a 16:8 hour light cycle and are fed Tetra-Min® and frozen Artemia (brine shrimp) twice daily. Breeding and egg hatching are encouraged at 25°C. The eggs are laid under a 1" thick slab of marble raised 3 mm above a removable horizontal pane of glass. Most of the eggs adhere to the glass and are easily removed to an aerated hatching tank. The narrow space between the marble slab and glass eliminates egg predation by adult fish. New hatching tanks are started weekly 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 approximately 27 days 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 Lake City STP. The tests were performed from 1045 hours on 4 January to 1110 hours on 6 January, 1984.

The samples were collected by Mr. David Woodbridge of DER's Northeast District at 1320 hours on 3 January, 1984, 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 associated with some other component of the effluent, the Biology Section adopted the following test procedure:

Test #1. Pre-chlorinated Effluent (Tables 2 and 3). This test was 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 (Table 4). This test was performed on an effluent sample collected at the point of discharge.

Test #3. De-chlorinated Effluent (Tables 5 and 6). This test was 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 six concentrations 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 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 controls and the 100% effluent 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% 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

Notropis leedsi (bannerfin shiner)

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 Lake City STP, NPDES #FL0025968, on 4 to 6 January, 1984

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 (°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	8.7	9.0	8.8	7.8	8.2	8.4	79		135		20.4	20.0	19.9	0.0/0.0			
Control	10	9	8	8.7	9.0	8.8	7.8	8.2	8.4										202	205
5.6%	10	10	10	8.5	8.9	8.8	7.8	8.3	8.4					20.3	19.9	19.9				
5.6%	10	10	9	8.5	8.9	8.8	7.8	8.3	8.4										203	208
10.0%	10	10	10	8.4	8.9	8.8	7.8	8.3	8.3					20.2	19.9	19.8				
10.0%	10	10	10	8.4	8.9	8.8	7.8	8.3	8.3										205	209
18.0%	10	10	10	8.2	8.9	8.8	7.7	8.3	8.3					20.2	19.8	19.8				
18.0%	10	10	10	8.2	8.9	8.8	7.7	8.3	8.3										210	211
32.0%	10	10	10	8.2	8.9	8.8	7.7	8.2	8.3					20.2	19.8	19.8				
32.0%	10	10	10	8.2	8.9	8.8	7.7	8.2	8.3										212	218
56.0%	10	10	10	8.0	8.8	8.8	7.6	8.1	8.3					20.0	19.8	19.8				
56.0%	10	10	10	8.0	8.8	8.8	7.6	8.1	8.3										222	230
100.0%	10	10	2	7.6	8.7	8.8	7.1	8.0	8.0	0		78		20.0	19.9	20.3	0.0/0.0			
100.0%	10	10	6	7.6	8.7	8.7	7.1	8.0	8.0										243	257

Remarks: [a] Total Residual Chlorine: None Detected.

Table 3. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of the Lake City STP, NPDES #FL0025968, on 4 to 6 January, 1984.

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 (°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	5	5	5	8.3	8.3	8.3	7.7	8.0	8.3	79		135		20.5	19.9	19.9	0.0/0.0			
Control	5	5	5	8.3	8.3	8.3	7.7	8.0	8.3										201	203
5.6%	5	5	5	8.3	8.3	8.3	7.7	8.1	8.3					20.4	19.7	19.7				
5.6%	5	5	5	8.3	8.3	8.4	7.7	8.1	8.3										203	205
10.0%	5	5	5	8.3	8.3	8.4	7.7	8.1	8.2					20.4	19.7	19.7				
10.0%	5	5	5	8.3	8.3	8.4	7.7	8.1	8.2										207	209
18.0%	5	5	5	8.3	8.3	8.4	7.6	8.1	8.2					20.3	19.6	19.6				
18.0%	5	5	5	8.3	8.3	8.4	7.6	8.1	8.2										210	211
32.0%	5	5	5	8.2	8.3	8.4	7.6	8.1	8.2					20.2	19.6	19.6				
32.0%	5	4	4	8.2	8.3	8.4	7.6	8.1	8.2										212	219
56.0%	5	5	5	7.9	8.2	8.4	7.5	8.0	8.1					20.0	19.6	19.6				
56.0%	5	5	5	7.9	8.2	8.4	7.5	8.0	8.1										225	229
100.0%	5	5	5	7.6	8.2	8.4	7.0	7.6	7.8	0		78		19.9	19.6	19.6	0.0/0.0			
100.0%	5	5	5	7.6	8.2	8.4	7.0	7.6	7.8										245	250

Remarks: [a] Total Residual Chlorine : None Detected
Weight of fish: 9.15 mg each, average of 10 fish.

Table 4. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of the Lake City STP, NPDES #FL0025968, on 4 to 6 January, 1984

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	9	8.3	8.7	8.8	7.7	8.3	8.4	79		135		20.3	20.0	19.8	0.0/0.0			
Control	10	10	10	8.3	8.7	8.8	7.7	8.3	8.4										198	203
5.6%	10	10	10	8.4	8.8	8.8	7.7	8.3	8.3					20.2	19.8	19.8				
5.6%	10	10	8	8.4	8.8	8.8	7.7	8.3	8.3										200	205
10.0%	10	10	10	8.4	8.8	8.8	7.7	8.3	8.4					20.1	19.8	19.8				
10.0%	10	10	9	8.4	8.8	8.8	7.7	8.3	8.4										202	209
18.0%	10	10	9	8.5	8.8	8.9	7.7	8.3	8.4					20.0	19.8	19.8				
18.0%	10	10	10	8.5	8.8	8.9	7.7	8.3	8.4										205	212
32.0%	10	10	9	8.5	8.8	8.9	7.6	8.3	8.4					19.9	19.8	20.1				
32.0%	10	10	9	8.5	8.8	8.9	7.6	8.3	8.4										212	220
56.0%	10	10	10	8.5	8.8	8.9	7.5	8.2	8.3					19.8	19.8	19.9				
56.0%	10	10	9	8.5	8.8	8.9	7.5	8.2	8.3										220	232
100.0%	10	4	3	8.7	8.8	8.9	6.9	7.8	8.0	10		68		19.8	19.8	20.4	0.0/0.0			
100.0%	10	1	0	8.7	8.8	8.9	6.9	7.8	8.0										243	258

Remarks: [a] Total Chlorine Residual: 0.55 mg/l HACH DPD Method, 0.35 mg/l Iodometric Method I

Table 5. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of the Lake City STP, NPDES #FL0025968, on 4 to 6 January, 1984

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	8	8.2	8.8	8.8	7.7	8.0	8.2	79		135		20.3	19.7	20.0	0.0/0.0			
Control	10	10	9	8.2	8.8	8.8	7.7	8.0	8.2										198	205
5.6%	10	10	10	8.3	8.9	8.8	7.7	8.1	8.3					20.2	19.7	19.8				
5.6%	10	10	10	8.3	8.9	8.8	7.7	8.1	8.3										200	205
10.0%	10	9	8	8.4	8.9	8.9	7.7	8.2	8.3					20.1	19.7	19.8				
10.0%	10	9	8	8.4	8.9	8.9	7.7	8.2	8.3										202	209
18.0%	11[b]	11	9	8.4	8.9	8.9	7.6	8.2	8.3					20.0	19.7	19.8				
18.0%	11[b]	11	9	8.4	8.9	8.9	7.6	8.2	8.3										205	212
32.0%	10	10	9	8.5	8.9	8.9	7.6	8.2	8.2					19.8	19.7	20.1				
32.0%	10	10	9	8.5	8.9	8.9	7.6	8.2	8.2										212	221
56.0%	10	10	10	8.5	8.9	8.9	7.6	8.2	8.2					19.6	19.7	20.2				
56.0%	10	10	9	8.5	8.9	8.9	7.6	8.2	8.2										222	237
100.0%	10	10	9	8.7	8.9	8.8	7.0	7.9	8.0	10		78		19.5	19.7	20.2	0.0/0.0			
100.0%	10	9	9	8.7	8.9	8.8	7.0	7.9	8.0										248	265

Remarks: [a] Total Chlorine Residual: approximately 0.09 mg/l (HACH DPD Method). Could not de-chlorinate further even with excess sodium thiosulfate.
[b] Extra organism loaded by mistake, allowed to remain.

Table 6. Data recorded during 48 hour, Simocephalus serrulatus, static acute toxicity bioassay of the Lake City STP, NPDES #FL0025968, on 4 to 6 January, 1984

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 (^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	9[b]	9	9	8.4	8.8	8.8	7.7	8.2	8.2	79		135		20.4	19.7	20.0	0.0/0.0			
Control	10	9	8	8.4	8.8	8.8	7.7	8.2	8.2										198	202
5.6%	11[b]	11	10	8.4	8.9	8.8	7.7	8.2	8.3					20.2	19.7	19.8				
5.6%	9[b]	8	7	8.4	8.9	8.9	7.7	8.2	8.3										200	207
10.0%	10	10	9	8.4	8.9	8.8	7.7	8.2	8.3					20.1	19.7	19.8				
10.0%	10	10	10	8.4	8.9	8.9	7.7	8.2	8.3										202	209
18.0%	10	10	10	8.5	9.0	8.9	7.6	8.2	8.3					20.0	19.7	19.7				
18.0%	10	10	10	8.5	9.0	8.9	7.6	8.2	8.3										205	211
32.0%	10	10	9	8.5	9.0	8.9	7.6	8.2	8.2					19.8	19.7	19.7				
32.0%	10	10	9	8.5	9.0	8.9	7.6	8.2	8.2										212	220
56.0%	10	10	8	8.6	9.0	8.9	7.5	8.2	8.2					19.6	19.7	19.8				
56.0%	10	10	10	8.6	9.0	8.9	7.5	8.2	8.2										222	235
100.0%	10	10	10	8.7	9.0	8.9	7.0	7.9	8.0	10		78		19.5	19.7	19.9	0.0/0.0			
100.0%	10	9	9	8.7	9.0	8.8	7.0	7.9	8.0										247	262

Remarks: [a] Total Chlorine Residual: approximately 0.09 mg/l (HACH DPD Method). Could not de-chlorinate further even with excess sodium thiosulfate.
[b] By mistake, either 9 or 11 organisms, instead of 10, were loaded into these chambers at the start of the test.

Table 7. LC_{50} Data for Bioassays of the Lake City STP, NPDES #FL0025968, on 4 to 6 January, 1984.

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*	Binomial	No Mortality	93.0% (56 and ∞)
Pre-chlorinated	Moving Average	No Mortality	a
Sample	Probit	No Mortality	141.2% (0 and ∞), b
Test #1: *N. leedsi*	Binomial	a	a
Pre-chlorinated	Moving Average	a	a
Sample	Probit	a	a
Test #2: *D. pulex*	Binomial	85.6% (56 and ∞)	78.7% (56 and 100)
Chlorinated	Moving Average	a	78.7% (71 and 88)
Sample	Probit	a	94.5% (0 and ∞), b
Test #3: *D. pulex*	Binomial	a	a
De-chlorinated	Moving Average	a	a
Sample	Probit	a	a
Test #3: *S. serrulatus*	Binomial	a	a
De-chlorinated	Moving Average	a	a
Sample	Probit	a	a

[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, results calculated using the probit method are not statistically sound.

Table 8. Results of Chemical Analyses Performed on Effluents from the Lake City STP, NPDES #FL0025968, collected 3 January, 1984.

Organics	
Base Neutral Extractables:	N.D.
Pesticide Extractables:	N.D.
Metals	
Antimony	N.D.
Arsenic	N.D.
Beryllium	N.D.
Cadmium	N.D.
Chromium	N.D.
Copper	<25 µg/l[a]
Lead	N.D.
Mercury	<0.2 µg/l[a]
Nickel	N.D.
Selenium	N.D.
Silver	N.D.
Thallium	N.D.
Zinc	53.5 µg/l

N.D. = None Detected
[a] Actual value is known to be less than value given.

RESULTS AND DISCUSSION

The pre-chlorinated effluent from the Lake City STP was not toxic to Daphnia pulex at 24 hours, but was at 48 hours (48 hour LC_{50} = 93.0%, Table 7). This same effluent, however, was not toxic to Notropis leedsi (only one of 10 fish died in the 32% test concentration, Table 3). The final chlorinated effluent, conversly, was acutely toxic to D. pulex at both 24 and 48 hours, producing LC_{50}'s of 85.6% and 78.7%, respectively (Table 7). This slight increase in toxicity was most likely due to the added effects of the total chlorine residual (0.55 mg/l) measured in that sample. In the bioassays of the de-chlorinated effluent, using D. pulex and Simocephalus serrulatus as test organisms, only random mortalities were noted, and therefore, LC_{50}'s could not be calculated. Ammonia was not detected in the effluents from this facility. The 96 hour LC_{50}'s for the pre-chlorinated and chlorinated effluents discussed above would be less than or equal to their 48 hour values.

The DER Chemistry Section has analyzed effluents from the Lake City STP for certain organics and metals (Table 8). These analyses failed to indicate the presence of any organic contaminants, but did indicate the presence of a measureable concentration of zinc (53.5 µg/l). This concentration, however, probably did not contribute significantly to the observed mortality.

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.

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