

BIOASSAYS OF THE ST. CLOUD WASTEWATER TREATMENT PLANT

OSCEOLA COUNTY, FLORIDA

NPDES #FL0028959

Biology Section
Division of Environmental Programs

OCTOBER 2, 1984

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EXECUTIVE SUMMARY

St. Cloud STP, NPDES #FL0028959, on 24 to 26 July, 1984.

The samples of pre-chlorinated, chlorinated, and de-chlorinated effluents from this domestic waste treatment facility were all acutely toxic to Daphnia pulex, producing 48 hour LC_{50} values of 40.2%, <5.6%, and 55.3%, respectively. Two additional bioassays on pre-chorinated and de-chlorinated effluents using Notropis leedsi as the test organism, showed little or no mortality. The toxicity of the chlorinated sample can mostly be attributed to the total residual chlorine (4.6 mg/l Iodometric Method) measured in that sample. The small concentrations of total ammonia (maximum measured = 1.36 mg/l) and of unionized ammonia (maximum = 0.03 mg/l) detected in these samples were insufficient to have contributed significantly to the observed mortality. Chemical analysis indicated the presence of the pesticides Lindane (0.2 ug/l) and Methoxychlor (0.2 and 0.3 ug/l), as well as several unidentified organic compounds in both the pre-chlorinated and final chlorinated effluents. Also present were the metals copper (157 ug/l), mercury (0.23 ug/l), silver (1.9 ug/l), and zinc (44 ug/l). The presence of these pesticides, organics, and metals most likely contributed to the toxicities noted above.

INTRODUCTION

The St. Cloud Sanitary Wastewater Treatment Plant is located on West Lakeshore Boulevard in St. Cloud, Osceola County, Florida (Fig. 1). This facility has been in operation since 1953 serving a population of approximately 9000. Secondary sewage treatment at this facility includes primary sedimentation, trickling filter, clarification and chlorination for a design capacity of 2.0 MGD. The effluent is discharged directly to the St. Cloud Flood Control Canal C-31 which flows into Lake Tohopekaliga (Fig. 2).

The Biology Section has previously performed toxicity bioassays on effluent from the St. Cloud STP, in January, 1980, September, 1981, and March, 1983. The results of these bioassays indicated that effluent from this plant is consistantly toxic (Table 1).

The St. John's River District requested that the Biology Section again evaluate the toxicity of the discharge. Five acute static toxicity bioassays were performed on 24 to 26 July, 1984, the results of which are presented in this report. Those involved in performing the tests and in preparation of this report are: Craig W. Dye, Marshall Faircloth, Russel B. Frydenborg, Manitia L. Moultrie and Martin L. Roll.

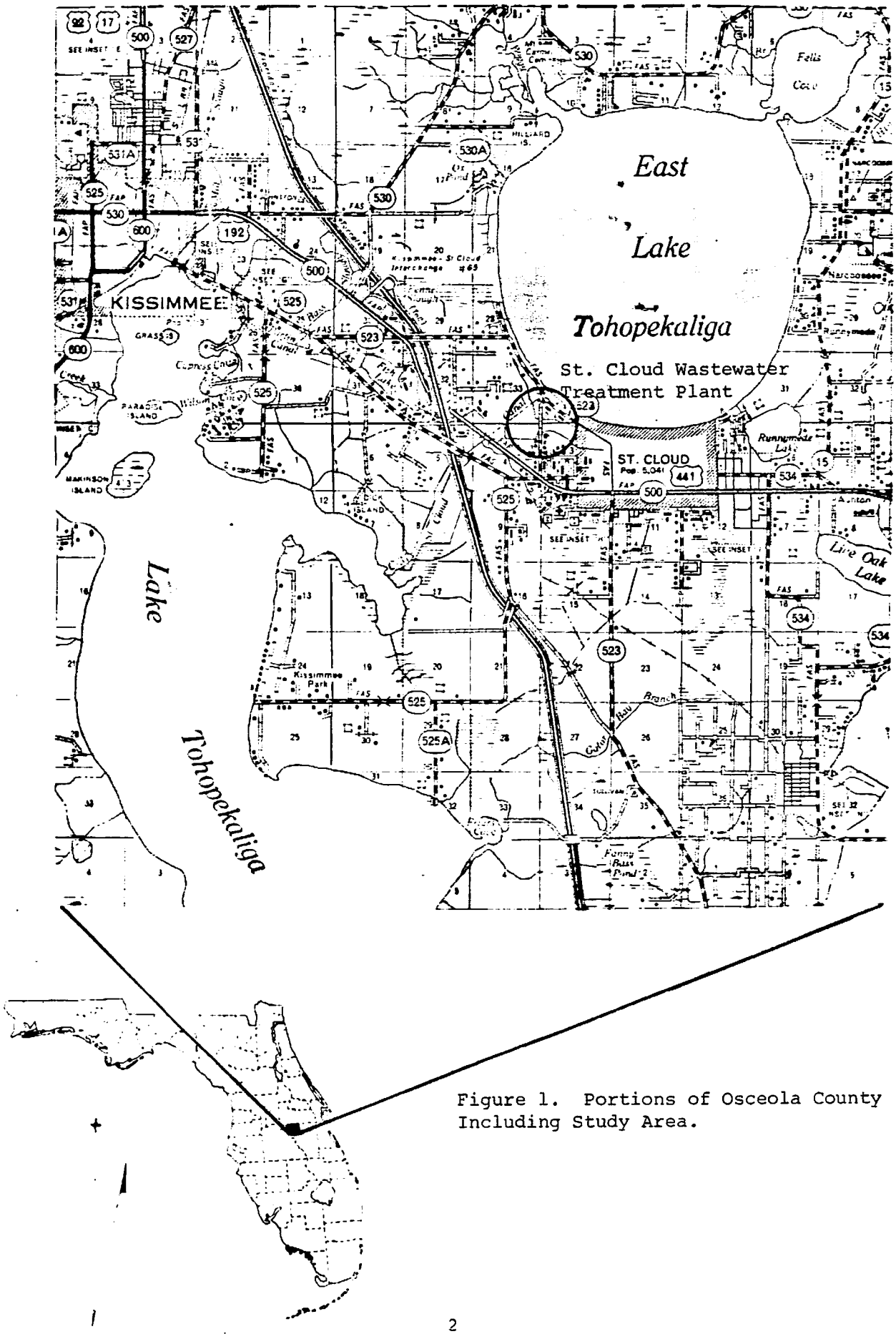


Figure 1. Portions of Osceola County Including Study Area.

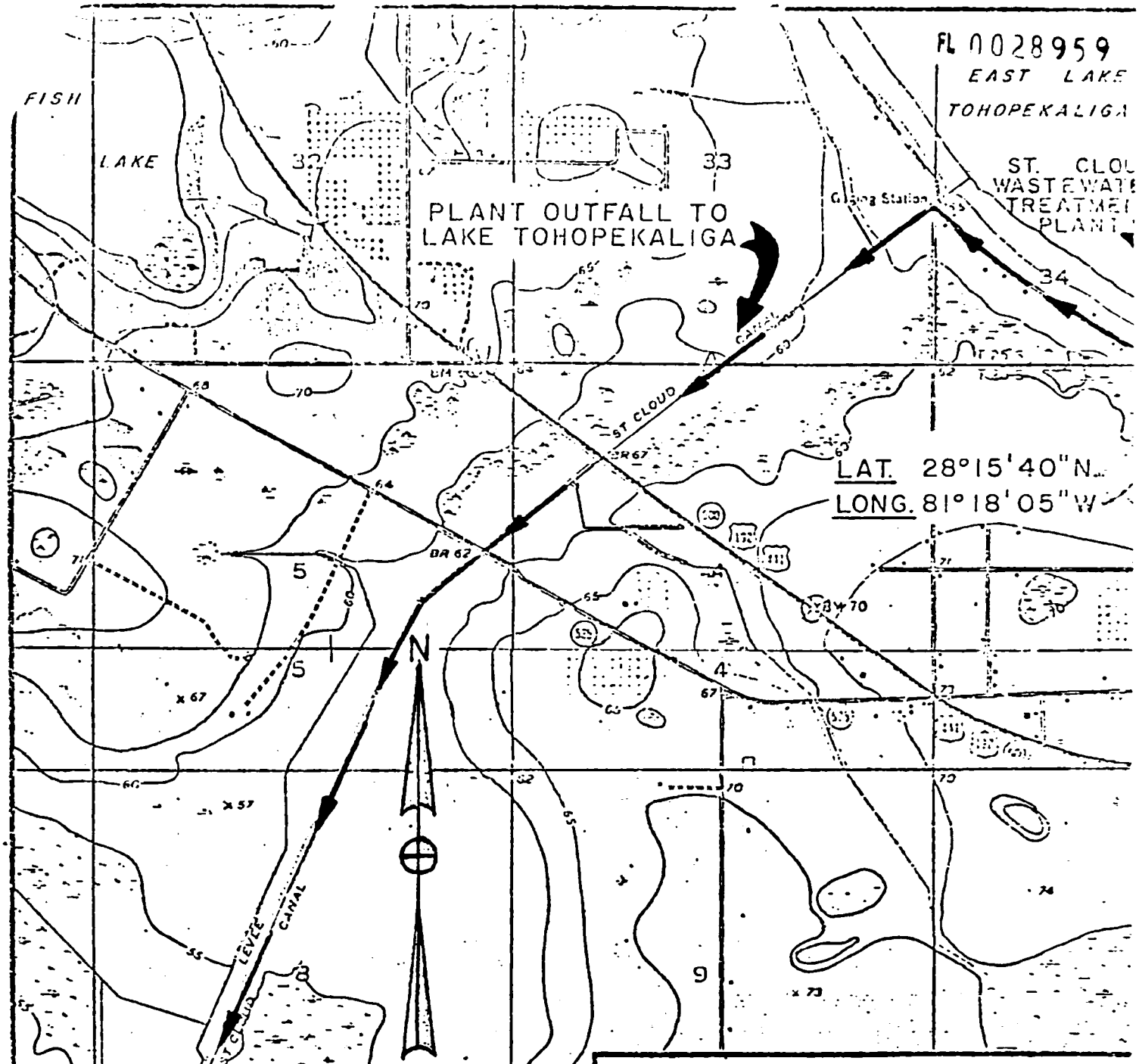


FIGURE 2

LOCATION MAP

ST. CLOUD WASTEWATE TREATMENT PLANT

ST. CLOUD, FLORIDA

MAY, 1975

Table 1. Summary of bioassays performed on St. Cloud STP by the FDER Biology Section.

Date	48 hour LC_{50}	Type Effluent	Organism
January 1980	81.6%	pre-Cl_2	Daphnia pulex
September, 1981	89.8%	pre-Cl_2	Daphnia pulex
September, 1981	<5.6%	Cl_2	Daphnia pulex
September, 1981	38.2%	Cl_2	Heterandria formosa
September, 1981	149.2%	de-Cl_2	D. pulex
March, 1983	78.0%	pre-Cl_2	D. pulex
March, 1983	a	pre-Cl_2	H. formosa
March, 1983	<5.6%	Cl_2	D. pulex
March, 1983	100 %	de-Cl_2	D. pulex
March, 1983	19.5%	de-Cl_2	Simocephalus Serrulatus
July 1984	40.2%	pre-Cl_2	D. pulex
July 1984	a	pre-Cl_2	Notropis leedsi
July 1984	<5.6%	Cl_2	D. pulex
July 1984	55.3%	de-Cl_2	D. pulex
July 1984	a	de-Cl_2	N. leedsi

[a] Due to an insufficient number of dead organisms or to the irregular distribution of mortalities, an LC_{50} value could not be calculated.

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. 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, once daily on weekends, 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, and then refilling with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances such as: declining populations, ephippia formation, or excessive fungal or bacterial growth.

Notropis leedsi (bannerfin shiner) - Approximately 40 adult bannerfin shiners were obtained from an indigenous population in the upper Suwannee River during October, 1981. The breeding stock is maintained in 30 and 125 gallon all-glass aquaria equipped with a flow-through water exchange system. Water for this system is unchlorinated and is pumped from the DER deep well. The bannerfin shiners are reared at 22° plus or minus 2°C under a 16:8 hour light cycle and are fed Tetra-Min® and frozen Artemia (brine shrimp) twice daily, and once daily on weekends. Breeding and egg hatching are encouraged at 25°C. The eggs are laid between stacked red ceramic tiles separated by a 3mm bead of silicone. Most of the eggs adhere to the tiles and are easily removed to heated, aerated hatching tanks. The narrow space between the tiles eliminates egg predation by adult fish. New hatching tanks are started regularly to segregate fry into even age groups. Newly hatched fry are fed a liquefied blend of Purina Trout Chow® and Cerophyl® twice daily. After one week, feeding is augmented with newly hatched Artemia nauplii and finely crushed Tetra Min®. The fish used in this test were 2 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 St. Cloud STP. The tests were performed from 1100 hours on 24 July to 1115 hours on 26 July, 1984. The samples were collected by Robert K. Laumer of DER's St. Johns River District at 1335 hours on 23 July, 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 3 and 4). 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 5). This test was performed on an effluent sample collected at the point of discharge.

Test #3. De-chlorinated Effluent (Tables 6 and 7). 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 2) is that recommended by APHA (1980).

Two replicates, each with six concentrations and a control, were used to test with the water flea, Daphnia pulex. 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 were siphoned into a large culture dish 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) bioassays. 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 these tests.

Dissolved oxygen (YSI Model 57 oxygen meter), pH (Corning Model 7 pH meter with Orion Ross® combination electrode), temperature (NBS calibrated glass thermometer), and the number of live organisms were recorded for each test concentration at 0 hours, 24 hours, and 48 hours. Total alkalinity and total hardness (measured as $CaCO_3$ using HACH Chemical Company reagents) were recorded at the beginning of the test for the controls and the 100% 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 end 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 3 through 7.

Table 2. Concentration Series and Volumes Used for These Static Bioassays

Daphnia pulex (water flea)

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 3. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of St. Cloud STP, #FL0028959 on 24 to 26 July, 1984.

Test 1 - Pre-chlorinated effluent[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/1)			pH			Temperature (°C)			Conductivity (umhos)		NH_3/N (mg/1)
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	48
Control	10	10	9	8.1	8.6	7.8	7.6	7.7	7.6	20.8	19.7	20.2			0/0
Control	10	10	10	8.2	8.6	7.8	7.6	7.7	7.6				188	185	
5.6%	10	10	10	8.3	8.7	7.9	7.6	7.8	7.7	21.0	19.8	20.4			
5.6%	10	10	10	8.3	8.7	7.9	7.6	7.8	7.7				202	190	
10%	10	10	10	8.3	8.8	8.1	7.6	7.8	7.8	21.2	19.0	20.2			
10%	10	10	10	8.3	8.7	8.0	7.6	7.8	7.8				210	195	
18%	10	10	10	8.2	8.7	8.0	7.6	7.8	7.8	21.2	19.0	19.9			
18%	10	10	10	8.2	8.5	8.0	7.6	7.8	7.8				225	205	
32%	10	10	8	7.7	8.6	7.8	7.5	7.8	7.8	21.2	19.0	19.9			
32%	10	10	5	7.7	8.5	7.8	7.5	7.8	7.8				250	230	
56%	10	9	3	6.1	7.1	7.4	7.4	7.8	7.7	21.3	19.5	20.1			
56%	10	9	2	6.1	7.2	7.4	7.4	7.8	7.7				290	270	
100%	10	1	0	5.8[b]	7.0	6.8	7.2	7.7	7.6	21.3	19.3	20.1			.017/.96
100%	10	2	0	5.8[b]	7.0	6.6	7.2	7.7	7.6				360	350	

Control Water

Total Alkalinity 100 (mg/l $CaCO_3$)

Total Hardness 130 (mg/l $CaCO_3$)

100% Sample

Total Alkalinity 65 (mg/l $CaCO_3$)

Total Hardness 95 (mg/l $CaCO_3$)

Remarks: [a] Total residual chlorine = none detected (HACH Method)

[b] Areated to value indicated prior to loading the organisms.

Table 4. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of St. Cloud STP, #FL0028959 on 24 to 26 July, 1984.

Test 1 - Pre-chlorinated[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)		NH_3/N (mg/l)
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	48
Control	5	5	5	8.1	8.2	7.8	7.6	7.8	7.9	21.2	19.1	20.2			0/0
Control	5	4	4	8.1	8.1	7.8	7.6	7.8	7.9				192	195	
5.6%	5	5	5	8.2	8.2	7.8	7.6	7.8	7.9	21.3	18.9	19.6			
5.6%	5	5	5	8.2	8.2	7.9	7.6	7.8	7.9				214	200	
10%	5	5	5	8.2	8.2	7.9	7.6	7.9	7.9	21.8	18.7	19.5			
10%	5	5	5	8.2	8.2	7.9	7.6	7.9	7.9				212	205	
18%	5	5	5	7.5	8.0	7.8	7.6	7.9	7.9	21.9	18.5	19.5			
18%	5	5	5	7.5	8.0	7.8	7.6	7.9	7.9				230	218	
32%	5	5	5	6.8	8.0	7.4	7.5	7.9	7.9	21.9	18.5	19.4			
32%	5	5	5	6.8	8.0	7.5	7.5	7.9	7.9				252	240	
56%	5	5	5	5.1	7.9	6.8	7.4	7.8	7.7	21.9	18.5	19.3			
56%	5	5	5	5.1	7.8	6.8	7.4	7.8	7.7				295	230	
100%	5	5	5	4.8[b]	6.1	5.3	7.2	7.7	7.6	21.5	18.5	19.3			.017/.96
100%	5	5	5	4.7[b]	6.2	5.3	7.2	7.7	7.6				370	350	

Control Water

Total Alkalinity 100 (mg/l $CaCO_3$)

Total Hardness 130 (mg/l $CaCO_3$)

100% Sample

Total Alkalinity 65 (mg/l $CaCO_3$)

Total Hardness 95 (mg/l $CaCO_3$)

Remarks:

[a] Total residual chlorine = none detected (HACH Method)

[b] Areated to value indicated prior to loading the organisms.

Table 5. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of St. Cloud STP, #FL0028959 on 24 to 26 July, 1984.

Test 2 - Chorinated[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)		NH_3/N (mg/l)
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	48
			[b]												
Control	10	10	*	8.0	8.1		7.6	7.8		21.0	18.8				0/0
Control	10	10		8.0	8.2		7.6	7.8					190		
5.6%	10	0		8.1	8.2		7.6	7.9		21.1	18.8				
5.6%	10	0		8.1	8.1		7.6	7.9					205		
10%	10	0		8.2	8.1		7.6	7.9		21.0	18.8				
10%	10	0		8.2	8.2		7.6	7.9					212		
18%	10	0		8.1	8.0		7.5	8.0		21.0	18.8				
18%	10	0		8.1	8.0		7.5	8.0					229		
32%	10	0		7.8	8.0		7.4	8.0		20.9	18.8				
32%	10	0		7.8	8.0		7.4	8.0					251		
56%	10	0		7.1	7.9		7.2	7.9		20.9	18.9				
56%	10	0		7.1	7.8		7.2	7.9					290		
100%	10	0		6.1	6.1		7.0	7.8		20.8	19.1				
100%	10	0		6.1	6.2		7.0	7.8					360		

Control Water

Total Alkalinity 100 (mg/l $CaCO_3$)

Total Hardness 130 (mg/l $CaCO_3$)

100% Sample

Total Alkalinity 85 (mg/l $CaCO_3$)

Total Hardness 100 (mg/l $CaCO_3$)

Remarks: [a] Total residual chlorine = 2.5 mg/l (HACH) and 4.6 mg/l (Iodometric Method).

[b] Due to 100% mortality at 24 hours, parameters not performed at 48 hours.

Table 6. Data recorded during 48 hour, *Daphnia pulex*, static acute toxicity bioassay of St. Cloud STP, #FL0028959 on 24 to 26 July, 1984.

Test 3 - De-chlorinated

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)		NH_3/N (mg/l)
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	48
Control	10	10	10	8.0	8.7	8.0	7.6	7.9	7.8	21.0	18.8	19.6			0/0
Control	10	10	9	8.0	8.7	8.1	7.6	7.9	7.9				195	190	
5.6%	10	10	8	8.1	8.7	7.8	7.6	8.0	7.9	21.0	18.6	19.6			
5.6%	10	10	8	8.1	8.7	7.8	7.6	8.0	7.9				210	200	
10%	10	10	9	8.2	8.8	8.0	7.6	8.0	8.0	21.0	18.5	19.6			
10%	10	10	10	8.2	8.9	8.0	7.6	8.0	8.0				218	210	
18%	10	10	10	8.2	8.8	8.0	7.6	8.1	7.9	21.0	18.7	19.6			
18%	10	10	8	8.2	8.9	8.0	7.6	8.1	7.9				235	225	
32%	10	10	9	8.1	8.9	7.7	7.6	8.1	7.9	20.9	18.8	19.6			
32%	10	10	9	8.1	8.8	7.7	7.6	8.1	7.9				262	250	
56%	10	10	9	7.8	8.7	7.6	7.5	8.0	7.9	20.8	18.8	19.8			
56%	10	10	5	7.8	8.7	7.7	7.5	8.0	7.9				310	300	
100%	10	3	0	6.2	8.7	6.2	7.3	7.9	7.7	20.7	19.2	19.9			.032/1.36
100%	10	3	0	6.3	8.7	6.3	7.3	7.9	7.7				395	380	

Control Water

Total Alkalinity 100 (mg/l $CaCO_3$)

Total Hardness 130 (mg/l $CaCO_3$)

100% Sample

Total Alkalinity 85 (mg/l $CaCO_3$)

Total Hardness 100 (mg/l $CaCO_3$)

Remarks:

Table 7. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of St. Cloud STP, #FL0028959 on 24 to 26 July, 1984.

Test 3 - De-chlorinated

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)		NH_3/N (mg/l)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	48 hr	
Control	5	5	5	8.2	8.5	7.8	7.6	7.9	7.8	20.9	18.6	19.5			0/0	Control Water
Control	5	5	5	8.2	8.5	7.9	7.6	7.9	7.9				195	195		Total Alkalinity 100 (mg/l $CaCO_3$)
5.6%	5	5	5	8.2	8.5	7.8	7.6	7.9	7.9	21.2	18.5	19.2				
5.6%	5	4	4	8.2	8.6	7.9	7.6	7.9	7.9				210	200		Total Hardness 130 (mg/l $CaCO_3$)
10%	5	5	5	8.2	8.4	7.7	7.6	8.0	7.9	21.2	18.3	19.1				
10%	5	5	5	8.2	8.4	7.8	7.6	8.0	7.9				220	208		
18%	5	5	5	8.2	8.4	7.8	7.6	8.0	7.9	21.2	18.3	19.0				
18%	5	5	5	8.2	8.4	7.8	7.6	8.0	7.8				235	210		
32%	5	5	5	8.1	8.4	7.4	7.5	7.9	7.8	21.2	18.3	19.0				
32%	5	5	5	8.1	8.4	7.4	7.5	7.9	7.8				262	245		100% Sample
56%	5	5	5	7.7	8.0	6.7	7.4	7.9	7.8	21.0	18.4	19.0				Total Alkalinity 85 (mg/l $CaCO_3$)
56%	5	5	5	7.7	8.0	6.7	7.4	7.9	7.7				312	290		
100%	5	4	3	7.3	7.9	5.3	7.2	7.8	7.6	20.8	18.7	19.1			.032/1.36	Total Hardness 100 (mg/l $CaCO_3$)
100%	5	5	5	7.3	7.8	5.4	7.2	7.8	7.6				398	390		

Remarks:

Table 8. LC_{50} Data for Bioassay of St. Cloud STP, #FL0028959, on 24 to 26 July, 1984.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Test #1:	Binomial	76.7% (56 and 100)	39.3% (18 and 56)
D. pulex	Moving Average	76.7% (68.3 and 86.9)	40.7% (34.2 and 49.9)
Pre-chlorinated Sample	Probit	77.2% (67.3 and 88.6)	40.2% (34.1 and 47.3),b
Test #1:	Binomial	No Mortality	No Mortality
N. leedsi	Moving Average		
Pre-chlorinated Sample	Probit		
Test #2:			
D. pulex		<5.6%,d	<5.6%,d
Chlorinated Sample			
Test #3:	Binomial	87.7% (56 and ∞)	63.8% (32 and 100)
D. pulex	Moving Average	a	55.3% (46.2 and 69.1),b
De-chlorinated Sample	Probit	a	57.5% (0 and ∞),c
Test #3:	Binomial	a	a
N. leedsi	Moving Average	a	a
De-chlorinated 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] This LC_{50} is reported in the text since it is the most statistically conservative of the data set.

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

[d] Since every organism was dead in the lowest test concentration (5.6%), the LC_{50} is <5.6%.

Table 9. Results of chemical analyses performed on effluents from the St. Cloud STP, NPDES #FL0028959, collected on 23 July, 1984.

	Organics	
	Pre-chlorinated	Chlorinated
Acid Extractables:	N.D.	N.D.
Base Neutral Extractables:	N.D.	N.D.
Pesticide Extractables:	Lindane 0.2 ug/l Methoxychlor 0.2 ug/l Several unidentified compounds	Lindane 0.2 ug/l Methoxychlor 0.3 ug/l Several unidentified compounds

Metals	
Antimony	N.D.
Arsenic	N.D.
Beryllium	N.D.
Cadmium	N.D.
Chromium	N.D.
Copper	157 ug/l
Lead	N.D.
Mercury	0.23 ug/l
Nickel	N.D.
Selenium	N.D.
Silver	1.9 ug/l
Thallium	N.D.
Zinc	44 ug/l

N.D. = NONE DETECTED

RESULTS AND DISCUSSION

The samples of pre-chlorinated, chlorinated, and de-chlorinated effluents from this domestic waste treatment facility were all acutely toxic to Daphnia pulex, producing 48 hour LC_{50} values of 40.2%, <5.6%, and 55.3%, respectively. Two additional bioassays on pre-chlorinated and de-chlorinated effluents using Notropis leedsi as the test organism, showed little or no mortality.

The toxicity of the chlorinated sample can be attributed to the total residual chlorine (4.6 mg/l Iodometric Method) measured in that sample. The small concentrations of total ammonia (maximum measured = 1.36 mg/l) and of unionized ammonia (maximum = 0.03 mg/l) detected in these samples were insufficient to have contributed significantly to the observed mortality.

The toxicity noted in the earlier DER bioassays (FDER 1983) was attributed to significant concentrations of various chemical constituents in the St. Cloud Effluent. As many as 15 unidentified organic components were present, including: 3 acid extractables, 7 base neutral extractables and 5 halogenated pesticide extractables, and, the pesticide Lindane (0.04 ug/l). In addition, the final effluent from the St. Cloud facility contained a very significant concentration of copper (440 ug/l), a significant amount of silver (1.9 ug/l) and zinc (39 ug/l). Results of the latest chemical analyses (Table 9) again indicated the presence of the pesticide Lindane, at a significantly higher concentration of 0.2 ug/l in both the pre-chlorinated and final chlorinated effluents. The pesticide Methoxychlor was also present in both effluents at concentrations of 0.2 ug/l and 0.3 ug/l, respectively. These analyses also indicated the presence of several unidentified organic compounds in both effluents. The metal analyses indicated the presence of significant concentrations of copper (157 ug/l), mercury (0.23 ug/l) and silver (1.9 ug/l), and, a moderate amount of zinc (44 ug/l) in the final effluent. The presence of these constituents, particularly the pesticides, copper, mercury and silver, most likely contributed to the toxicities noted in the St. Cloud STP effluents.

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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