BIOASSAYS OF [redacted]

BELLE GLADE, PALM BEACH COUNTY, FLORIDA

NPDES #FL0027740

SAMPLED 12/8/81

Biological Section
Bureau of Water Analysis
March 10, 1982

BIOASSAYS OF BELLE GLADE SEWAGE TREATMENT PLANTS #1 and #2

BELLE GLADE, PALM BEACH COUNTY, FLORIDA

NPDES #FL0027740

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Executive Summary

Belle Glade Sewage Treatment Plants #1 and #2, 9 to 11 December, 1981.

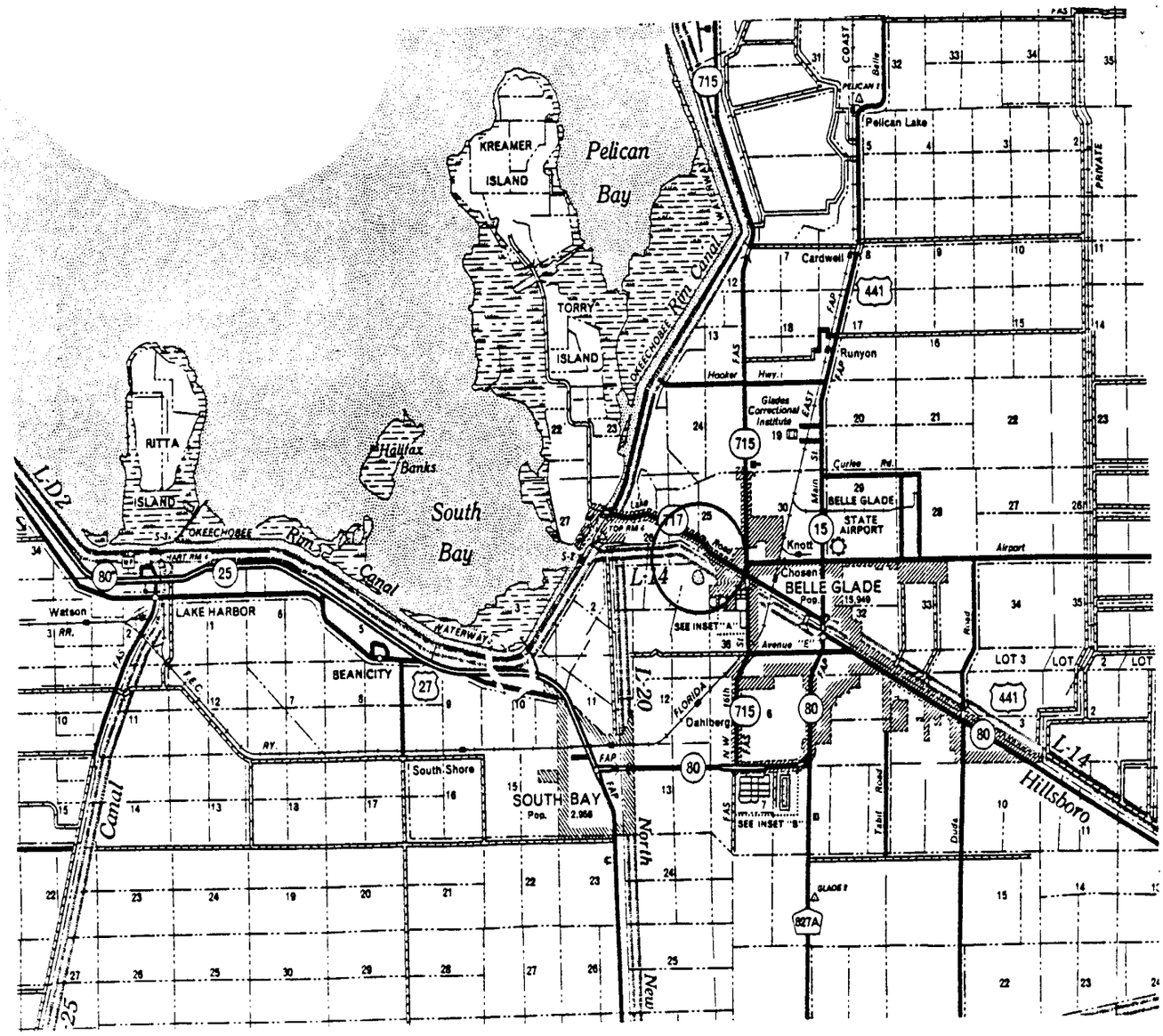
Five 48 hour static acute toxicity bioassays were performed on chlorinated and de-chlorinated effluents (Plant #1) and on pre-chlorinated, chlorinated and de-chlorinated effluents (Plant #2) from this facility. The water flea, Daphina pulex, was used as test organism for all tests. The chlorinated effluent from Plant #1 was extremely toxic to the test organism, with 24 and 48 hour LC_{50}'s of < 5.6%. In fact, every organism in every test concentration was dead by hour 24 of the test. The toxicity was attributed to high concentrations of residual chlorine. The de-chlorinated effluent from Plant #1 was not acutely toxic, and only random mortality was noted. The three samples tested from Plant #2 were not toxic, and only random mortality was noted.

Introduction

The Belle Glade Sewage Treatment Plants # 1 and #2 are located at 2055 W. Canal St. South, Belle Glade, Palm Beach County, Florida (Fig. 1). Plant #1 has been in operation since 1957, while Plant #2 is relatively new, having been constructed in 1975. They provide waste treatment for the City of Belle Glade (population = 15,000), and for various industrial operations, including a nearby slaughterhouse. Treatment consists of primary sedimentation, aeration, secondary sedimentation, chlorination, and retention in a 5.4 acre evaporation pond. Discharge of the effluent (average 2 MGD) is to the Hillsboro Canal, which transects the City of Belle Glade (Fig. 2).

According to the NPDES file, this facility has constant problems in maintaining an efficient and adequate wastewater treatment system. Plant #1 has had persistent equipment failures including inoperable clarifers and aerators. The effluent is generally characterized as containing high amounts of solids and turbidity stemming from problems with influent from the slaughterhouse.

On 9 December, 1981, the Biological Section received samples of pre-chlorinated and chlorinated effluents from Plant #2, and chlorinated effluent from Plant #1, for toxicity analysis. Five static acute toxicity bioassays were performed to evaluate the existence and extent of toxicity to the biota of the receiving waters. The results of these tests are presented in this report.



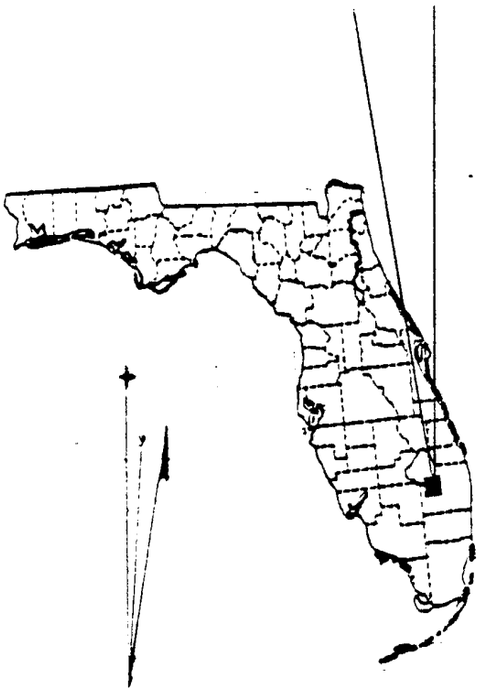


Figure 1. Portions of Palm Beach County Florida, including city of Belle Glade and study area.

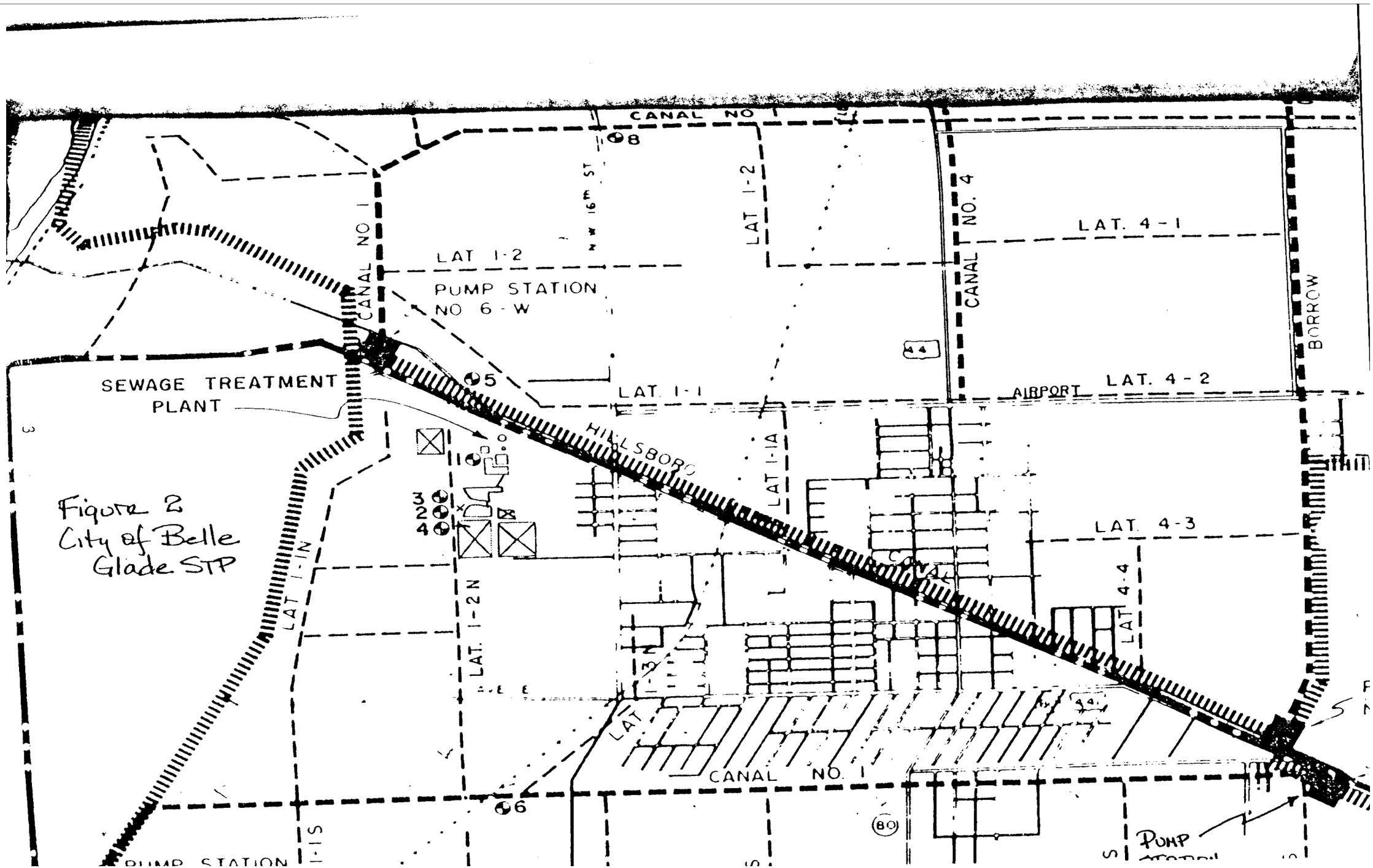


Figure 2
City of Belle Glade STP

Methods and Materials

Test methods used to conduct these static bioassays are extensively described by Peltier (1978). The organism selected for this study is among those recommended by Peltier (1978) and the U.S. EPA (1978). The life history and maintenance procedures used to culture the organism 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, ephippa formation, or excessive fungal or bacterial growth.

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

Test Methods

Five separate 48 hour static acute toxicity bioassays were performed on effluents collected from the Belle Glade Sewage Treatment Plants #1 and #2. The tests were performed from 1300 hours on 9 December, 1981, to 1330 hours on 11 December, 1981. A total of three samples were collected, consisting of: (1) a one gallon sample of chlorinated effluent from Plant #1, (2) a one gallon sample of pre-chlorinated effluent from Plant #2, and (3) a one gallon sample of chlorinated effluent from Plant #2. The samples were collected by Mr. Kevin Ruddick and Mr. Richard Stenburg of DER's Port St. Lucie Office at 1400 hours on 8 December, 1981, using standard wastewater sampling procedures. 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 procedures:

Test #1, Chlorinated Effluent from Plant #1 (Table2). This test was performed on an effluent sample collected at the point of discharge.

Test #2. De-chlorinated Effluent from Plant #1 (Table 3). 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.

Test #3. Pre-Chlorinated Effluent from Plant #2 (Table 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 #4. Chlorinated Effluent from Plant #2 (Table 5). This test was performed on an effluent sample collected at the point of discharge.

Test #5. De-Chlorinated Effluent from Plant #2 (Table 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 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 Daphnia 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.

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 concentrations. 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 a Corning® pH/millivolt meter. 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 (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

Table 2. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Belle Glade Sewage Treatment Plant #1, Belle Glade, Palm Beach County, Florida, NPDES #FL0027740, on 9 to 11 December, 1981.

Test #1, Chlorinated Effluent

Concentration 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/cm)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	10	10	9	8.2	8.6	8.2	7.8	8.0	8.0	112		114		21.3	21.9	20.8	.01/.23	--		
Control	10	10	10	8.2	8.6	8.2	7.8	8.0	8.0										210	220
5.6%	10	0	0	8.2	8.5	--[a]	7.7	8.1	-[a]					21.3	21.7	20.6				
5.6%	10	0	0	8.2	8.5	--	7.7	8.1	-										265	-[a]
10.0%	10	0	0	8.1	8.5	--	7.7	8.1	-					21.2	21.5	20.5				
10.0%	10	0	0	8.1	8.5	--	7.7	8.1	-										310	
18.0%	10	0	0	8.0	8.5	--	7.7	8.1	-					21.1	21.4	20.4				
18.0%	10	0	0	8.0	8.5	--	7.7	8.1	-										382	
32.0%	10	0	0	7.9	8.5	--	7.6	8.1	-					20.9	20.7	20.5				
32.0%	10	0	0	7.9	8.5	--	7.6	8.1	-										580	
56.0%	10	0	0	7.7	8.5	--	7.6	8.1	-					20.9	20.7	20.6				
56.0%	10	0	0	7.7	8.5	--	7.6	8.1	-										800	
100.0%	10	0	0	7.2	8.5	--	7.5	8.1	-	262		329		20.8	20.7	20.6	.14/8.68	--		
100.0%	10	0	0	7.0	8.5	--	7.5	8.1	-										1210	

a Data not reported since all organisms were dead by hour 24.

Total chlorine residuals: 5.6% — 0.35 mg/l
32.0% — 1.50 mg/l
100.0% — 2.90 mg/l (HACH DPM Method)
100.0% — 4.08 mg/l (Iodometric Method)

Table 3. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Belle Glade Sewage Treatment Plant #1, Belle Glade, Palm Beach County, Florida, NPDES #FL0027740, on 9 to 11 December, 1981.

Test #2. De-Chlorinated Effluent

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)		Total Hardness (mg/l as $CaCO_3$)		Temperature (°C)			NH_3/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	8.4	8.7	8.3	7.8	8.0	8.1	112		114		21.2	21.1	20.5	.01/.23	---		
Control	10	10	10	8.4	8.7	8.3	7.8	8.0	8.1										209	220
5.6%	10	10	10	8.3	8.6	7.9	7.8	8.0	8.1					21.2	20.9	20.6				
5.6%	10	10	10	8.3	8.6	7.9	7.8	8.0	8.1										267	275
10.0%	10	10	10	8.2	8.5	7.7	7.8	8.1	8.0					21.1	20.8	20.5				
10.0%	10	10	10	8.2	8.5	7.7	7.8	8.1	8.0										313	320
18.0%	10	9	9	8.2	8.5	7.6	7.7	8.1	8.0					21.1	20.7	20.5				
18.0%	10	10	10	8.2	8.5	7.6	7.7	8.1	8.0										389	394
32.0%	10	10	10	8.1	8.4	7.6	7.7	8.1	8.1					21.1	20.7	20.5				
32.0%	10	10	10	8.1	8.4	7.6	7.7	8.1	8.1										580	590
56.0%	10	10	9	7.9	8.2	7.5	7.7	8.1	8.1					21.0	20.7	20.6				
56.0%	10	10	10	7.9	8.2	7.5	7.7	8.1	8.1										810	840
100.0%	10	10	10	7.5	7.8	7.5	7.7	8.1	8.1	262		329		21.0	20.7	20.6	.32/12.6	---		
100.0%	10	10	10	7.5	7.8	7.5	7.7	8.1	8.1										1220	1220

Remarks:

Table 4. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Belle Glade Sewage Treatment Plant # 2, Belle Glade, Palm Beach County, Florida, NPDES, #FL0027740, on 9 to 11 December, 1981.

Test #3. Pre-Chlorinated Effluent

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°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	8.2	8.6	8.1	7.8	8.1	8.1	112		114		21.4	20.8	20.7	.01/.23	---		
Control	10	10	10	8.2	8.6	8.1	7.8	8.1	8.1										205	213
5.6%	10	10	10	7.9	7.9	8.1	7.8	8.0	8.1					21.2	20.7	20.7				
5.6%	10	10	10	7.9	7.9	8.1	7.8	8.0	8.1										260	268
10.0%	10	10	10	7.5	7.7	7.9	7.7	8.0	8.1					21.1	20.7	20.6				
10.0%	10	10	10	7.5	7.7	8.0	7.7	8.0	8.1										303	312
18.0%	10	10	10	7.1	7.6	7.8	7.7	8.0	8.1					21.0	20.6	20.5				
18.0%	10	10	10	7.2	7.6	7.8	7.7	8.0	8.1										383	390
32.0%	10	10	10	6.4	7.3	7.3	7.6	8.0	8.0					20.9	20.6	20.5				
32.0%	10	10	10	6.4	7.3	7.3	7.6	8.0	8.0										580	580
56.0%	10	10	10	5.1[a]	6.7	6.8	7.5	8.0	8.0					20.9	20.6	20.5				
56.0%	10	10	9	5.1[a]	6.7	6.8	7.5	8.0	8.0										800	829
100.0%	10	10	10	2.4[a]	6.1	5.8	7.4	8.0	8.0	268		344		20.8	21.1	20.5	.17/13.16	---		
100.0%	10	10	10	2.4[a]	6.1	5.8	7.4	8.0	8.0										1210	1210

Remarks: [a] Aerated to 7.4, 7.3, 7.4, 7.6, respectively, before loading.

Table 5 . Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Belle Glade Sewage Treatment Plant #2, Belle Glade, Palm Beach County, Florida, NPDES #FL0027740, on 9 to 11 December, 1980.

Test #4 Chlorinated Effluent

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)		Total Hardness (mg/l as $CaCO_3$)		Temperature (°C)			NH_3/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	7.9	8.6	7.8	7.8	8.1	8.1	112		114		21.3	21.0	20.8	.01/.23	--		
Control	10	10	10	7.9	8.6	7.8	7.8	8.1	8.1										204	213
5.6%	10	10	10	7.9	7.9	7.6	7.7	8.0	8.1					21.2	20.9	20.6				
5.6%	10	10	10	7.9	7.9	7.6	7.7	8.0	8.1										262	270
10.0%	10	10	10	7.9	7.4	7.6	7.7	8.0	8.0					21.1	20.7	20.6				
10.0%	10	9	9	7.8	7.4	7.5	7.7	8.0	8.0										308	313
18.0%	10	10	10	7.6	6.6	7.0	7.6	7.9	8.0					21.1	20.6	20.6				
18.0%	10	10	9	7.6	6.6	7.1	7.6	7.9	8.0										383	395
32.0%	10	10	10	7.1	5.3[b]	6.5	7.5	7.8	7.9					20.9	20.6	20.6				
32.0%	10	10	10	7.1	5.3[b]	6.6	7.5	7.8	8.0										570	580
56.0%	10	10	10	6.6	3.5[b]	5.3	7.4	7.7	8.0					20.8	20.5	20.5				
56.0%	10	9	9	6.5	3.5[b]	5.3	7.4	7.7	8.0										800	810
100.0%	10	10	10	4.4[a]	1.4[b]	4.6	7.3	7.7	8.0	270		342		20.8	20.6	20.5	.16/15.68	--		
100.0%	10	10	10	4.5[a]	1.4[b]	4.6	7.3	7.7	8.0										1200	1220

Remarks:
a. Aerated to 8.4 and 8.2, respectively, before loading.
b. Aerated to 7.2, 7.2, 6.6, 7.5, 6.1, 6.1, respectively.

Total chlorine residuals:
5.6% — 0.1 mg/1
32.0% — 0.32 mg/1
100.0% — 0.9 mg/1 (HACH DPD Method)
100.0% — Not Detected (Iodometric Method)

Table 6. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Belle Glade Sewage Treatment Plant # 2, Belle Glade, Palm Beach County, Florida, NPDES #FL0027740, on 9 to 11 December, 1981.

Test #5. De-Chlorinated Effluent

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)		Total Hardness (mg/l as $CaCO_3$)		Temperature (°C)			NH_3/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	8.0	8.8	7.9	7.8	8.1	8.1	112		114		21.1	20.7	20.5	.01/.23	--		
Control	10	9	9	8.0	8.8	8.1	7.8	8.1	8.1										208	210
5.6%	10	10	10	8.0	8.1	7.5	7.7	8.0	8.1					21.0	20.7	20.4				
5.6%	10	10	10	8.0	8.1	7.5	7.7	8.0	8.1										263	268
10.0%	10	10	10	7.9	7.5	7.5	7.7	7.9	8.1					21.0	20.6	20.3				
10.0%	10	10	10	7.9	7.5	7.5	7.7	7.9	8.0										310	313
18.0%	10	10	10	7.8	6.6	7.5	7.6	7.8	7.9					21.0	20.6	20.3				
18.0%	10	10	10	7.8	6.6	7.5	7.6	7.8	7.9										385	392
32.0%	10	10	10	7.5	5.5[a]	6.6	7.5	7.8	7.8					21.1	20.5	20.3				
32.0%	10	9	7	7.5	5.5[a]	6.7	7.5	7.8	7.9										570	590
56.0%	10	10	10	7.1	3.7[a]	5.9	7.5	7.8	7.8					21.1	20.6	20.2				
56.0%	10	9	9	7.1	3.7[a]	5.9	7.5	7.8	7.8										800	810
100.0%	10	9	8	6.2	1.6[a]	4.3	7.4	7.7	7.7	270		342		21.2	21.1	20.2	.21/16.1	--		
100.0%	10	8	8	6.0	1.4[a]	4.3	7.4	7.7	7.8										1200	1230

Remarks: [a] Aerated to 6.4, 7.0, 6.5, 6.6, 6.3, 6.9, respectively.

Table 7. LC_{50} Data for Bioassays of Belle Glade Sewage Treatment Plants #1 and #2, on 9 to 11 December, 1981.

Organism	Method of Data Analysis	24 LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Test #1: D. pulex Chlorinated Sample, Plant #1	a	<5.6%, a	<5.6%, a
Test #2: D. pulex De-chlorinated Sample, Plant #1	Binomial	b	b
	Moving Average	b	b
	Probit	b	b
Test #3: D. pulex Pre-chlorinated Sample, Plant #2	Binomial	No mortality	b
	Moving Average		b
	Probit		b
Test #4: D. pulex Chlorinated Sample, Plant #2	Binomial	b	b
	Moving Average	b	b
	Probit	b	b
Test #5: D. pulex De-chlorinated Sample, Plant #2	Binomial	b	b
	Moving Average	b	b
	Probit	>100%, c	>100%, c

[a] Since every organism in every test concentration (5.6% - 100%) were dead by hour 24, the LC_{50} value is <5.6%.

[b] Due to an insufficient number of dead organisms, an LC_{50} value cannot be calculated using this method of statistical analysis.

[c] 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.

Results and Discussion

The chlorinated effluent from Plant #1 (Test #1, Table 2) was extremely toxic to the test organism, Daphnia pulex (24 and 48 hour LC_{50}'s of <5.6%) (Table 7). In fact, every organism in every test concentration (5.6% - 100%) was dead by hour 24 of the test. The 96 hour LC_{50} for this sample would, therefore, be <5.6%. This toxicity can be attributed to the concentrations of residual chlorine determined in this sample (Table 2) particularly since the de-chlorinated sample (Table 3) was not toxic. Even the residual chlorine concentration determined in the lowest test concentration (0.35 mg/l-5.6%; Table 2) is higher than the reported chlorine toxicity level (U.S. EPA 1976). No other sample tested from either Plants #1 or #2 exhibited significant lethality, although some random mortality was noted. Although significant concentrations of total ammonia were determined in these sample (Tables 2 through 6) the test organism, Daphnia pulex, was apparently unaffected.

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 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 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.

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