BIOASSAYS OF THE GRACEVILLE

WASTEWATER TREATMENT PLANT

GRACEVILLE, JACKSON COUNTY, FLORIDA

NPDES #FL0038555

SAMPLED 2/9/87

MAY 7, 1987

Biology Section
Division of Environmental Programs

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Bureau of Laboratories and
Special Programs
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EXECUTIVE SUMMARY

Graceville Wastewater Treatment Plant, NPDES #FL0038555, on 10 to 12 February, 1987.

The Biology Section performed six static acute toxicity bioassays on samples of chlorinated and de-chlorinated recovered water from the Graceville Wastewater Treatment Plant, a 1 MGD facility which discharges to Holmes Creek. The chlorinated sample proved to be acutely toxic to the test organisms, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi. Twenty-four hour LC_{50}'s of $< 50.0\%$ were noted for all three test organisms. The residual chlorine measured in this sample (0.7 mg/l, HACH Method) was apparently the main cause of the observed toxicity.

In the bioassays of the de-chlorinated sample, no mortality was noted in tests using Daphnia pulex and Notropis leedsi. In the C. dubia test, no mortality was observed at 24 hours. However, at 48 hours sufficient mortality occurred to calculate an LC_{50} of 206.7%. This LC_{50} would be in violation of state toxicity standards in the absence of a mixing zone. The concentrations of total ammonia (1.4 mg/l) and of unionized ammonia (0.006 mg/l) measured in this sample were insufficient to have contributed to the observed mortality. These data, therefore, indicate the presence of other toxic constituents. Samples for chemical analyses were not collected at the time of these bioassays.

INTRODUCTION

The City of Graceville operates the Graceville Wastewater Treatment Plant in Jackson County, Florida (Fig. 1). The facility serves a population of approximately 2700, as well as industrial waste from Goldkist Peanuts. According to the NPDES file, the facility was issued a new permit in April, 1984, which increased the facility's design flow capacity from 0.4 MGD to 1.1 MGD. The wastewater treatment system was expanded to include screening, grit removal, aeration in complete mix aerobic bio-reactors, clarification, and chlorination (Fig. 2). Sludge is digested aerobically, thickened, and dried on sludge drying beds prior to disposal in the Jackson East Sanitary Landfill. Recovered water is discharged to Holmes Creek.

At the request of Mr. Robert Reining of DER's Northwest District Branch Office, the Biology Section performed four static acute toxicity bioassays on 28 to 30 August, 1985, to evaluate the toxicity of this discharge. The pre-chlorinated sample proved to be acutely toxic to the test organisms, Daphnia pulex and Notropis leedsi, resulting in 48 hour LC_{50}'s of 76.2% and 50.0%, respectively. In the bioassay of the chlorinated sample, 40% of the D. pulex died in the 100% concentration. This toxicity, however, was insufficient to calculate an LC_{50}. This same chlorinated sample, conversely, was acutely toxic to N. leedsi, producing a 48 hour LC_{50} of 70.7%. A small amount of residual chlorine was measured in the sample (.06 mg/l, HACH Method), which probably did not contribute significantly to the observed toxicity. The small concentrations of total ammonia (maximum measured = .41 mg/l) and of unionized ammonia (maximum = .001 mg/l) measured in the samples were also insufficient to have contributed to the observed mortality (FDER 1985a).

In February, 1987, the Biology Section obtained another sample from this facility and six static acute toxicity bioassays were performed. Results of these tests are presented in this report. Those involved in performing the tests and in preparation of this report were: R. B. Frydenborg, K. M. Lurding, M. L. Roll, and P. G. Weiland.

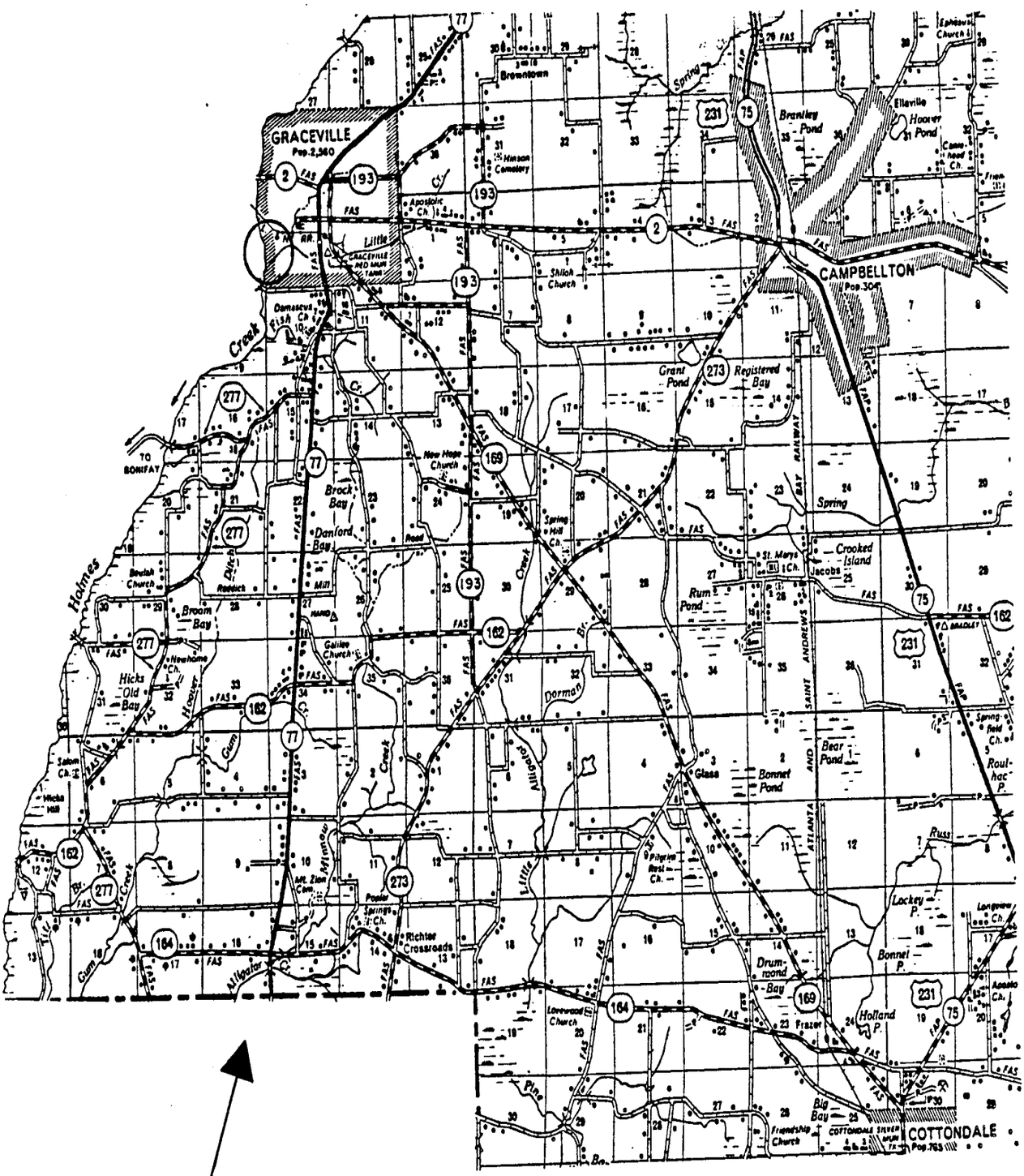
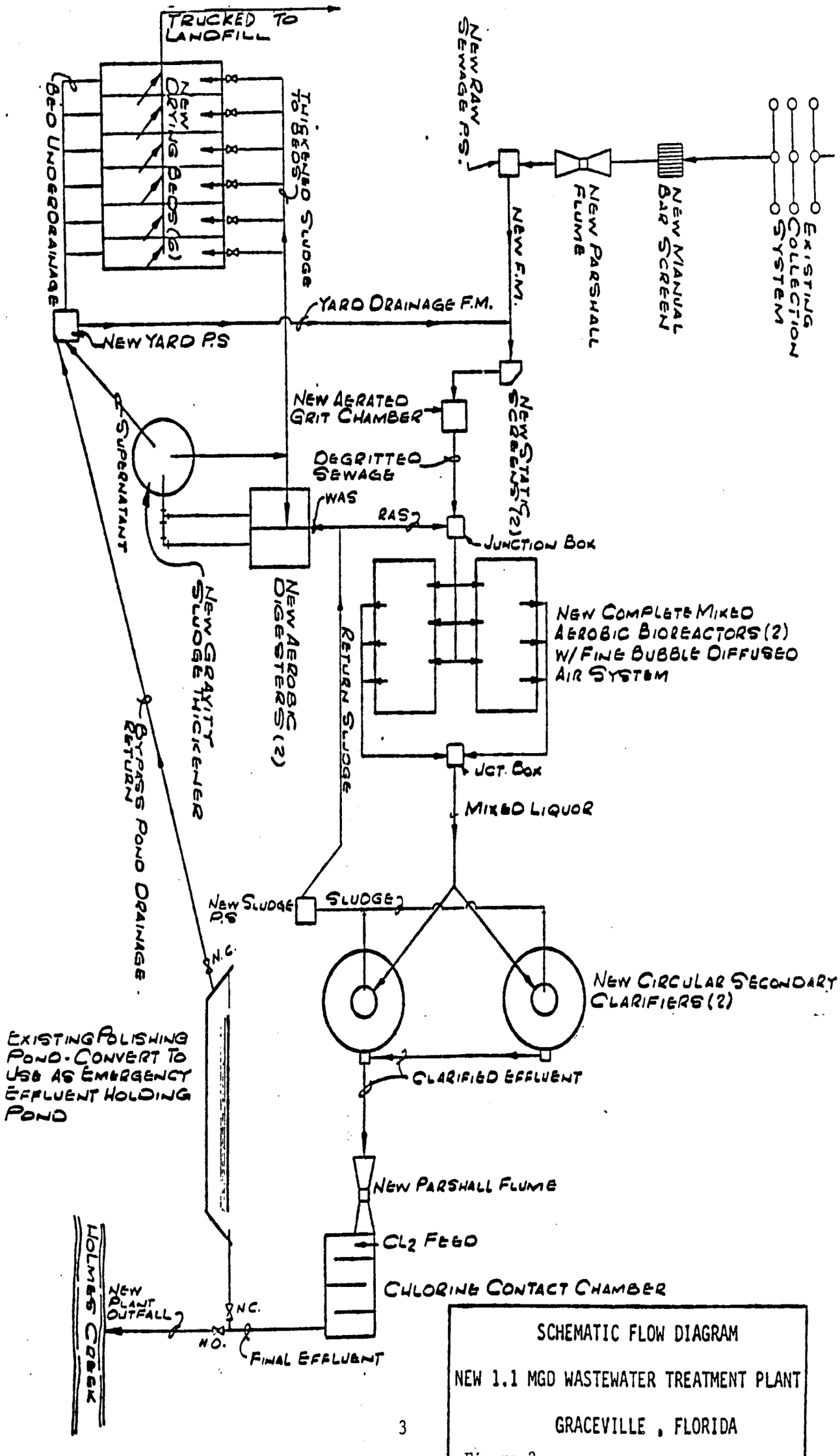


Figure 1. Portions of Jackson County, including location of Graceville Wastewater Treatment Plant.



SCHEMATIC FLOW DIAGRAM

NEW 1.1 MGD WASTEWATER TREATMENT PLANT

GRACEVILLE , FLORIDA

Figure 2.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier and Weber (1985) and the FDER Biology Section (1985b). The organisms selected for this study include species recommended by Peltier and Weber (1985), the U.S. EPA (1978), and the FDER Biology Section (1985b). 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° ± 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.

Ceriodaphnia dubia (water flea) - A breeding culture of Ceriodaphnia dubia was obtained from the EPA laboratory in Athens, Georgia, in June, 1985. The culture is maintained 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®, Cerophyl® and Fleischmann's® yeast twice daily, once daily on weekends, and are kept at a constant temperature of 26° ± 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° ± 2°C under a 16:8 hour light-dark 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 28 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

Methods used to perform acute toxicity bioassays are described by Peltier and Weber (1985) and the FDER Biology Section (1985b). Some of the methods described below are more specific than those of Peltier and Weber. These will be noted in more detail.

Sampling Methods and Test Dates

Six 48 hour static acute toxicity bioassays were performed on samples of chlorinated and de-chlorinated recovered water collected downstream of the chlorine contact chamber of the Graceville Wastewater Treatment Plant. The tests were performed from 1000 hours on 10 February to 1000 on 12 February, 1987.

The sample was collected by Mr. Richard Sublette of DER's Northwest District Branch Office at 1124 hours on 9 February, 1987, using standard wastewater sampling procedures. The sample was iced and transported to the DER laboratory in Tallahassee for analyses within 24 hours of collection.

Test Temperature

Upon arrival of the iced (4°C) bioassay sample in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the sample allowed to rise to 20° ± 2°C. Although this temperature may be different than the temperature of the sample during collection, it is the temperature range to which the test organisms are acclimatized and is the range recommended in the above referenced bioassay protocols. This avoids stressful effects caused by exposure to unusually cold or warm samples.

Total Residual Chlorine

Since many discharges are chlorinated, especially those originating from sewage treatment facilities, it is often desirable to differentiate between the toxicity resulting from chlorine and the toxicity associated with other components of the water. To accomplish this, the Biology Section performed concurrent bioassays on a sample of chlorinated recovered water in which the chlorine had been neutralized.

De-chlorination was accomplished by adding a calculated amount of 0.025N sodium thiosulfate. The total residual chlorine concentrations of the tested samples are given on the bioassay data forms. A comparison of the results of these tests will distinguish between the toxicity due solely to residual chlorine and the toxicity inherently contained in the water.

Freshwater Tests

Freshwater for dilution of the samples was obtained from the same DER deep well that is used to culture the test organisms. The concentration series selected for this study is given in Table 1.

Two replicates, of each concentration and a control, were used to test with the water fleas, Ceriodaphnia dubia and Daphnia pulex. Flint glass jars of either 60 ml (for C. dubia) or 250 ml (for D. pulex) capacity were used as test chambers, and either 50 ml or 200 ml of the appropriate test concentration was placed in each chamber. Ceriodaphnia were siphoned into a large culture dish and neonates were transferred by glass pipette, and loaded 10 per chamber, resulting in 20 per test concentration. 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.

Two replicates, of each concentration 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.

Test Parameters

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% sample 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% sample test concentration 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 and 3.

Table 1. Concentration Series and Volumes Used for These Static Bioassays.

Ceriodaphnia dubia (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	50.0 ml	--------	50 ml
50.0%	25.0 ml	25.0 ml	50 ml
100.0%	-------	50.0 ml	50 ml

Daphnia pulex (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	200.0 ml	--------	200 ml
50.0%	100.0 ml	100.0 ml	200 ml
100.0%	--------	200.0 ml	200 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Sample	Total Volume
Control	500 ml	--------	500 ml
50.0%	250 ml	250 ml	500 ml
100.0%	------	500 ml	500 ml

Table 2. Data recorded during 48 hour, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi, static acute toxicity bioassays of Graceville Sewage Treatement Plant, NPDES #FL0038555, on 10 to 12 February, 1987.

Chlorinated Sample[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)	
	0	24	48[b]	0	24	48	0	24	48	0	24	48	0	48
Ceriodaphnia dubia														
Control	10	10		8.9	8.7		8.0	8.0		20.0	19.6			
Control	10	10		8.9	8.7		8.0	8.0						
50.0%	10	0		8.9	8.7		7.6	7.9		20.1	19.5			
50.0%	10	0		8.9	8.7		7.6	7.9						
100.0%	10	0		9.0	9.0		6.9	7.7		20.4	19.5			
100.0%	10	0		9.0	9.0		6.9	7.7						
Daphnia pulex														
Control	10	10		8.0	7.9		8.0	7.9		20.2	19.7			
Control	12	12		8.0	7.9		8.0	7.9					210	
50.0%	10	0		7.5	7.9		7.5	7.9		20.3	19.6			
50.0%	10	0		7.5	7.9		7.5	7.9					185	
100.0%	10	0		6.9	7.5		6.9	7.5		20.9	19.6			
100.0%	10	0		6.9	7.5		6.9	7.5					160	
Notropis leedsi														
Control	5	5		7.8	7.8		7.8	7.8		20.5	19.8			
Control	5	5		7.8	7.8		7.8	7.8					210	
50.0%	5	0		8.8	8.9		7.4	7.5		20.9	19.7			
50.0%	5	0		8.8	8.9		7.4	7.5					190	
100.0%	5	0		8.8	8.9		6.9	7.3		21.3	19.6			
100.0%	5	0		8.8	8.9		6.9	7.3					160	

Control Water

NH_3(as NH_3)(mg/l): 0

Total ammonia (as N)(mg/l): 0

Total Alkalinity (mg/l $CaCO_3$): 120

Total Hardness (mg/l $CaCO_3$): 115

100% Sample

NH_3(as NH_3)(mg/l): 0.006

Total ammonia (as N)(mg/l): 1.4

Total Alkalinity (mg/l $CaCO_3$): 55

Total Hardness (mg/l $CaCO_3$): 40

[a] Total Chlorine Residual: 0.7 mg/l (HACH DPD Method).

[b] Test terminated at 24 hours due to complete mortality of test organisms exposed to sample concentrations.

Table 3. Data reocrded during 48 hour, *Ceriodaphnia dubia*, *Daphnia pulex*, and *Notropis leedsi*, static acute toxicity bioassay of Graceville Sewage Treatment Plant, NPDES #FL0038555, on 10 to 12 February, 1987.

De-chlorinated Sample

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Ceriodaphnia dubia														
Control	10	10	10	8.9	8.6	7.9	8.0	8.0	7.9	20.0	19.7	20.4		
Control	10	10	10	8.9	8.6	7.9	8.0	8.0	7.9					
50.0%	10	10	10	8.7	8.5	8.0	7.6	7.8	7.8	20.4	19.5	20.3		
50.0%	10	10	9	8.7	8.5	8.0	7.6	7.8	7.8					
100.0%	10	10	8	8.5	8.2	8.2	6.9	7.5	7.6	21.4	19.5	20.4		
100.0%	10	10	8	8.5	8.2	8.2	6.9	7.5	7.6					
Daphnia pulex														
Control	10	10	8	8.9	8.8	7.9	8.0	8.0	7.8	20.2	19.5	20.5		
Control	10	10	10	8.9	8.8	7.9	8.0	8.0	7.8				210	
50.0%	10	10	10	8.7	8.3	7.9	7.5	7.7	7.7	20.8	19.4	20.1		
50.0%	10	10	10	8.7	8.3	7.9	7.5	7.7	7.7				185	
100.0%	10	10	10	8.6	8.4	8.1	6.9	7.4	7.4	22.0	19.5	20.1		
100.0%	10	10	10	8.6	8.4	8.1	6.9	7.4	7.4				160	
Notropis leedsi														
Control	5	5	5	8.9	7.9	7.5	7.8	7.8	7.6	20.5	19.8	20.4		
Control	5	5	5	8.9	7.9	7.5	7.8	7.8	7.6				210	
50.0%	5	5	5	8.6	7.7	7.6	7.4	7.5	7.5	21.4	19.5	20.2		
50.0%	5	5	5	8.6	7.7	7.6	7.4	7.5	7.5				190	
100.0%	6[a]	6	6	8.7	7.4	7.7	6.9	7.3	7.3	22.5	19.5	20.2		
100.0%	5	5	5	8.7	7.4	7.7	6.9	7.3	7.3				160	

Control Water

NH_3(as NH_3)(mg/l) _____

Total ammonia (as N)(mg/l) 0

Total Alkalinity (mg/l $CaOO_3$) 120

Total Hardness (mg/l $CaOO_3$) 115

100% Effluent

NH_3(as NH_3)(mg/l) 0.006

Total ammonia (as N)(mg/l) 1.4

Total Alkalinity (mg/l $CaOO_3$) 55

Total Hardness (mg/l $CaOO_3$) 40

[a] Extra organism, allowed to remain.

Table 4. LC_{50} Data for Bioassays of Graceville Wastewater Treatment Plant, Graceville, Jackson County, Florida, NPDES #FL0038555, on 10 to 12 February, 1987.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Chlorinated Sample			
Ceriodaphnia dubia	Binomial	< 50.0%, a	< 50.0%, a
	Moving Average		
	Probit		
Daphnia pulex	Binomial	< 50.0%, a	< 50.0%, a
	Moving Average		
	Probit		
Notropis leedsi	Binomial	< 50.0%, a	< 50.0%, a
	Moving Average		
	Probit		
De-chlorinated Sample			
C. dubia	Binomial	No Mortality	b
	Moving Average		b
	Probit		206.7% (110.4 and ∞)
D. pulex	Binomial	No Mortality	No Mortality
	Moving Average		
	Probit		
N. leedsi	Binomial	No Mortality	No Mortality
	Moving Average		
	Probit		

[a] The LC_{50} is < 50.0% since all the organisms died in the smallest test concentration (50.0%).

[b] 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.

RESULTS AND DISCUSSION

The sample of final chlorinated recovered water from the Graceville Wastewater Treatment Plant proved to be acutely toxic to the test organisms, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi. Twenty-four hour LC_{50}'s of < 50.0% were observed in bioassays of all three test organisms (Table 4). The residual chlorine measured in this sample (0.7 mg/l, HACH Method) was apparently the main cause of the observed toxicity.

In the bioassays of the de-chlorinated sample, no mortality was noted in tests using Daphnia pulex and Notropis leedsi. In the C. dubia test, no mortality was observed at 24 hours. However, at 48 hours sufficient mortality was noted to calculate an LC_{50} of 206.7%. This LC_{50} would be in violation of state toxicity standards in the absence of a mixing zone. The concentrations of total ammonia (1.4 mg/l) and of unionized ammonia (0.006 mg/l) measured in this sample were insufficient to have contributed to the observed mortality. These data, therefore, indicate the presence of other toxic constituents. Samples for chemical analyses were not collected at the time of these bioassays.

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 onetwentieth 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, whetheras 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)(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 discharge samples, representative of the 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, 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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