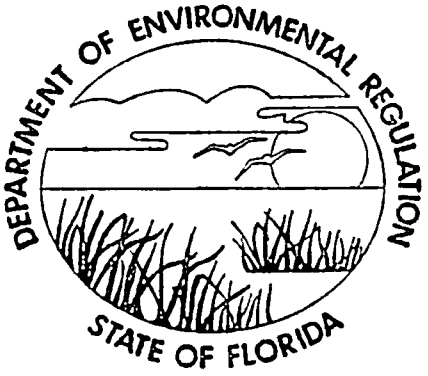


BIOASSAYS OF LAKE MANOR PROPERTIES, INC.

JENSEN BEACH, MARTIN COUNTY, FLORIDA

NPDES #FL0023060

MAY 13, 1987

Biology Section
Division of Environmental Programs

BIOASSAYS OF LAKE MANOR PROPERTIES, INC.

JENSEN BEACH, MARTIN COUNTY, FLORIDA

NPDES #FL0023060

Biology Section
Bureau of Laboratories and
Special Programs
May 13, 1987

EXECUTIVE SUMMARY

Lake Manor Properties, Inc. Sewage Treatment Plant, NPDES #FL0023060, on 24 to 26 March, 1987.

Five separate static acute toxicity bioassays were performed on samples of chlorinated and de-chlorinated recycled waters collected from Lake Manor Porperties, Inc., a mobile home park in Jensen Beach. The chlorinated sample was found to be toxic to Daphnia pulex and to Notropis leedsi (24 hour LC_{50} < 10.0%). This can be attributed to the residual chlorine (2.5 mg/l) measured in this sample.

The de-chlorinated sample proved to be acutely toxic to Ceriodaphnia dubia, with a 48 hour LC_{50} of 75.1%. Significant mortality was also noted in the Notropis leedsi bioassay of this sample. However, due to oxygen depletion in the affected test chambers, the mortality will not be used for an LC_{50} determination. Because the concentrations of total and unionized ammonia (7.45 mg/l and 0.12 mg/l, respectively) were probably not sufficient to have caused the observed toxicity, it is likely that other toxic compounds were present in the recovered water sample. Chemical analyses were not performed at this time.

INTRODUCTION

Lake Manor Properties, Inc. is a mobile home park located in Jensen Beach, Florida (Figure 1). The sewage treatment plant which serves this residential community has a design flow of 0.01 MGD, and utilizes an extended aeration type treatment. Recovered water is discharged to a freshwater drainage ditch which flows into the Indian River.

To evaluate the toxicity of this discharge, the Biology Section performed five static acute toxicity bioassays in March, 1987. Results are presented in this report. Those involved in performing the tests and in preparation of this report were: R. B. Frydenborg, K. M. Lurding, and M. L. Roll.

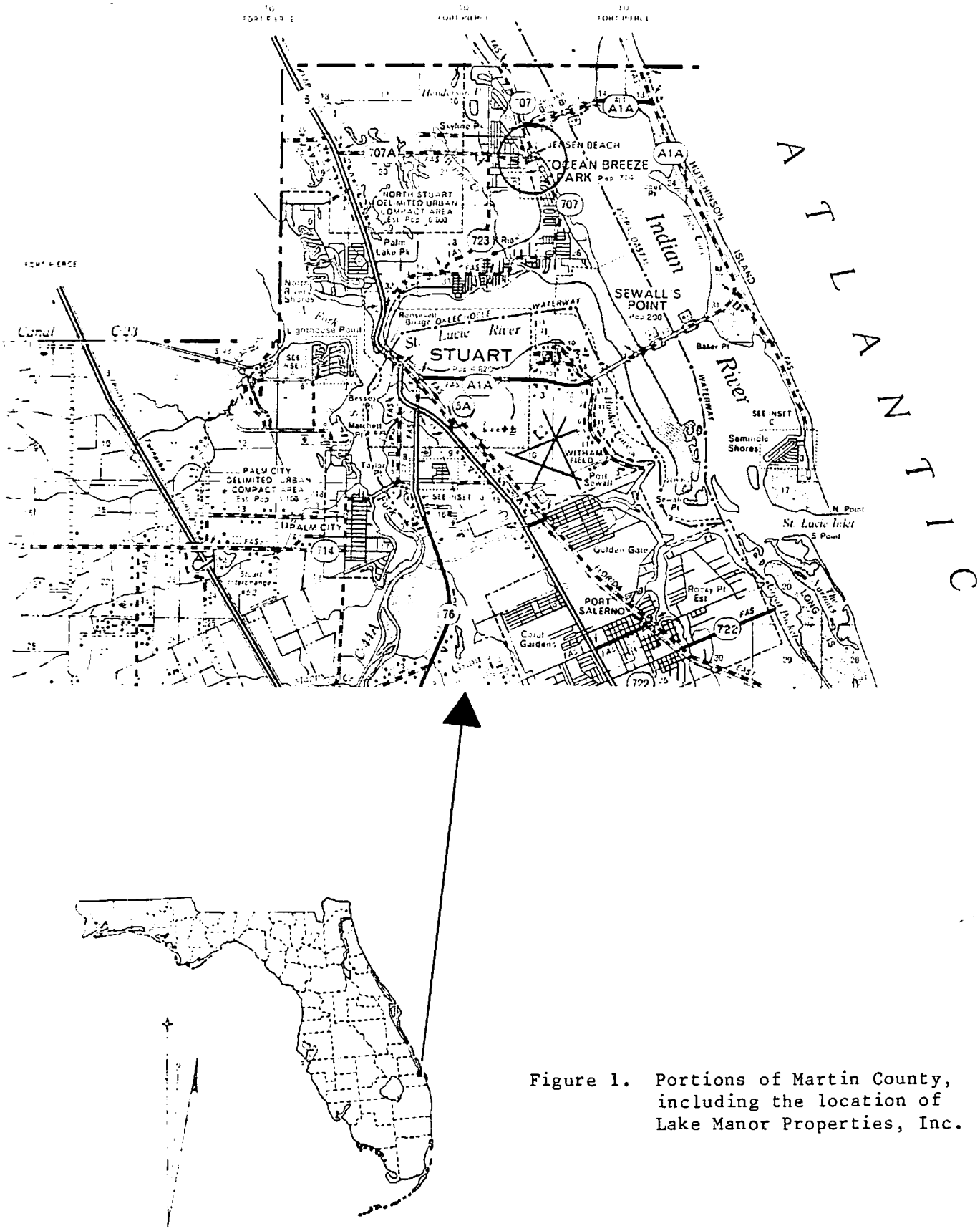


Figure 1. Portions of Martin County, including the location of Lake Manor Properties, Inc.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier and Weber (1985) and the FDER Biology Section (1985). The organisms selected for this study include species recommended by Peltier and Weber (1985), the U.S. EPA (1978), and the FDER Biology Section (1985). 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 23 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 (1985). 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

Five 48 hour static acute toxicity bioassays were performed on a sample of recovered water collected from the chlorine contact chamber of Lake Manor's sewage treatment facility. The tests were performed from 1125 hours on 24 March to 1125 hours on 26 March, 1987.

The sample was collected by Mr. Paul Strauss of DER's Southeast District Branch Office at 0930 hours on 23 March, 1987, using standard wastewater sampling procedures. The sample was iced and transported to the DER laboratory in Tallahassee for analyses within 26 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.

Aeration

Since the sample to be used for bioassay analysis contained a dissolved oxygen concentration of less than 40% of saturation, the entire sample was aerated to approximately 6.0 mg/l. This was achieved using a single airstone connected to the laboratory compressed air supply. Additionally, since the dissolved oxygen concentrations in certain individual test chambers had decreased to near 40% of saturation (due to BOD), then each of these affected test chambers and the controls were also aerated. The exact amounts and levels of aeration are noted on the bioassay data forms.

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 discharge 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 of 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 sample.

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) 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 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 24 hours. 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.

Chlorinated Sample

Daphnia pulex (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	200.0 ml	--------	200 ml
10%	180.0 ml	20.0 ml	200 ml
50%	100.0 ml	100.0 ml	200 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Sample	Total Volume
Control	500 ml	-------	500 ml
10%	450 ml	50 ml	500 ml
50%	250 ml	250 ml	500 ml

De-chlorinated Sample

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, Daphnia pulex and Notropis leedsi, static acute toxicity bioassay of Lake Manor Properties, Inc., NPDES #FL0023060, on 24 to 26 March, 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[b]	0	24	48[b]	0	24	48[b]	0	48[b]
Daphnia pulex														
Control	10	10		8.7	8.9		8.2	8.3		19.9	20.7			
Control	10	10		8.7	8.9		8.2	8.3					220	
10%	10	0		8.8	9.2		8.0	8.2		20.0	20.5			
10%	10	0		8.8	9.2		8.0	8.2					245	
50%	10	0		8.1	9.2		7.3	8.1		20.1	20.4			
50%	10	0		8.1	9.2		7.3	8.1					335	
Notropis leedsi														
Control	5	5	5	8.9	9.0	8.4	7.9	7.8	7.9	20.3	20.9	20.4		
Control	6[c]	6	6	8.9	9.0	8.4	7.9	8.1	7.9				215	210
10%	5	1	1	8.9	9.2	8.0	7.7	8.0	7.9	20.4	20.5	20.3		
10%	5	2	2	8.9	9.2	8.0	7.7	8.0	7.9				240	235
50%	5	0	0	8.3	9.3		7.3	7.9		20.5	20.4			
50%	5	0	0	8.3	9.3		7.3	7.9					340	

Control Water

NH_3(as NH_3)(mg/l) 0.02

Total Alkalinity 123 (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) 0.48

Total Hardness 118 (mg/l $CaCO_3$)

100% Sample

NH_3(as NH_3)(mg/l) 0.12

Total Alkalinity 121 (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) 7.45[d]

Total Hardness 60 (mg/l $CaCO_3$)

[a] Residual Cl_2 = 2.5 mg/l (HACH).

[b] When all organisms dead at 24 hours parameters not measured.

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

[d] Total ammonia measured at 24 hours.

Table 3. Data recorded during 48 hour Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi, static acute toxicity bioassay of Lake Manor Properties, Inc., NPDES #FL0023060, on 24 to 26 March, 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	9.0	9.0	8.0	8.0	8.3	8.1	20.0	20.7	20.3		
Control	10	10	10	9.0	8.0	8.0	8.0	8.3	8.1					
50%	10	10	10	8.7	7.5	8.0	7.7	7.9	8.0	20.2	20.5	20.2		
50%	10	10	9	8.7	7.5	8.0	7.7	7.9	8.0					
100%	10	7	1	8.0	7.2	7.9	7.5	7.9	8.1	20.2	20.6	20.2		
100%	10	7	2	8.0	7.2	7.9	7.5	7.9	8.1					
Daphnia pulex														
Control	10	10	10	8.9	8.8	8.0	8.0	8.2	8.1	20.4	20.5	20.2		
Control	10	10	10	8.9	8.8	8.0	8.0	8.2	8.1				220	225
50%	10	10	10	8.2	4.7	7.9	7.7	7.8	8.0	20.4	20.3	20.1		
50%	10	10	10	8.2	4.7	7.9	7.7	7.8	8.0				390	390
100%	10	10	10	7.4	3.7	7.7	7.5	7.7	8.1	20.5	20.4	20.1		
100%	10	10	10	7.4	3.7	7.7	7.5	7.7	8.1				650	640
Notropis leedsi														
Control	5	5	5	8.9	9.0	8.4	7.9	7.8	7.9	20.3	20.9	20.4		
Control	6[a]	6	6	8.9	9.0	8.4	7.9	8.1	7.9				215	210
50%	5	4	2	8.0	4.5	8.2	7.6	7.7	8.1	20.9	20.5	20.2		
50%	5	5	5	8.0	5.2	8.2	7.6	7.7	8.1				395	395
100%	5	0[c]	0	7.5	1.5[c]	-	7.5	7.5	-	20.9	20.4	-		
100%	5	4[c]	0	7.5	2.2[c]	-	7.5	7.5	-				650	-

Control Water

NH_3(as NH_3)(mg/l) 0.02

Total Alkalinity 123 (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) 0.48

Total Hardness 118 (mg/l $CaCO_3$)

100% Sample

NH_3(as NH_3)(mg/l) 0.12

Total Alkalinity 121 (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) 7.45[b]

Total Hardness 60 (mg/l $CaCO_3$)

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

[b] Total ammonia measured at 24 hours.

[c] Because the D.O. dropped below 40% saturation, this mortality will not be used for LC_{50} determination.

Table 4. LC_{50} Data for Bioassays of Lake Manor Properties, Inc., Martin County, Florida, NPDES #FL0023060, on 24 to 26 March, 1987.

Chlorinated Discharge

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Daphnia pulex	Binomial Moving Average Probit	< 10.0%, a	< 10.0%, a
Notropis leedsi	Binomial Moving Average Probit	< 10.0%, a	< 10.0%, a

De-chlorinated Discharge

Ceriodaphnia dubia	Binomial	b	75.1% (50 and 100)
	Moving Average	b	75.1% (66.5 and 86.2)
	Probit	b	76.5% (65.0 and 88.8)
Daphnia pulex	Binomial Moving Average Probit	No Mortality	No Mortality
Notropis leedsi	Binomial Moving Average Probit	c	c

[a] The LC_{50} is < 10.0% since more than half the organisms died in the smallest test concentration (10.0%).

[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.

[c] Test invalidated due to dissolved oxygen deletion (< 40% saturation).

RESULTS AND DISCUSSION

The chlorinated discharge of Lake Manor Properties was found to be acutely toxic to Daphnia pulex and to Notropis leedsi, with 24 hour LC_{50}'s of < 10.0% (Table 4). The residual chlorine measured in this sample (2.5 mg/l, HACH) was probably mostly responsible for the observed toxicity.

In the bioassay with de-chlorinated sample water, no mortality of D. pulex was noted in 48 hours. In the N. leedsi test, dissolved oxygen dropped below 40% saturation in the 100% concentration, therefore, this mortality could not be used to calcualte an LC_{50} (Table 3). In the test using Ceriodaphnia dubia as the test organism, this sewage treatment plant discharge proved to be acutely toxic, with a 48 hour LC_{50} of 75.1% (Table 4). The total and unionized ammonia (7.45 mg/l and 0.12 mg/l, respectively) were probably not sufficient to have caused the observed mortality. Therefore, other toxic compounds may have been present. Chemical analyses were not performed on this sample.

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 samples, representative of the discharge 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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