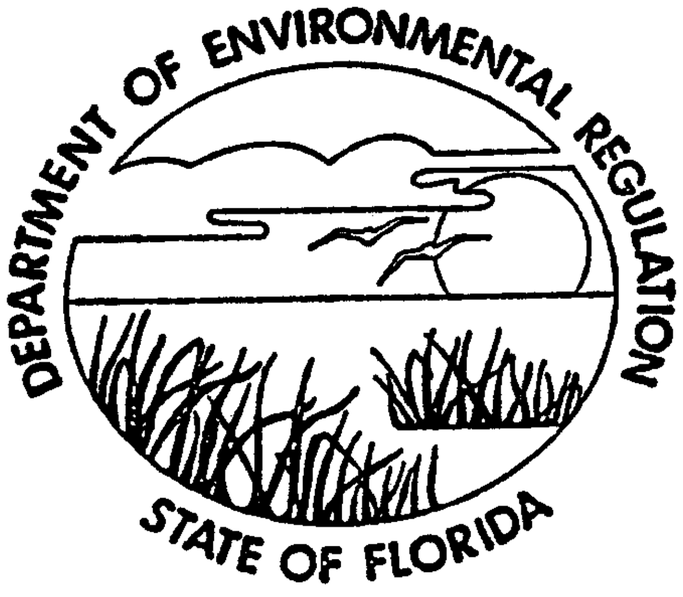


BIOASSAYS OF FT. MYERS SOUTH

SEWAGE TREATMENT PLANT

FT. MYERS, LEE COUNTY, FLORIDA

NPDES #FL0021270

SAMPLED 6/15/87

AUGUST 18, 1987

Biology Section
Division of Environmental Programs

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SEWAGE TREATMENT PLANT

FT. MYERS, LEE COUNTY, FLORIDA

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Biology Section
Bureau of Laboratories and
Special Programs
August 18, 1987

EXECUTIVE SUMMARY

Fort Myers South Sewage Treatment Plant, Ft. Myers, Lee County, Florida, NPDES #FL0021270, 16 to 19 June, 1987.

Four static acute toxicity bioassays were performed on samples of chlorinated and de-chlorinated recovered water collected from Ft. Myers South Sewage Treatment Plant. The chlorinated sample proved to be toxic to Ceriodaphnia dubia and Notropis leedsi, with 48 hour LC_{50}'s of 75.1% and 62.2%, respectively. The residual chlorine measured in this sample (0.45 mg/l HACH and 0.53 mg/l Iodometric) can probably account for part of the observed toxicity.

Although the de-chlorinated sample was not toxic to N. leedsi, 48 and 72 hour LC_{50}'s of 95.5% and 76.2%, respectively, were calculated for C. dubia. No ammonia was detected in this recovered water sample. This toxicity to C. dubia, therefore, suggests the presence of toxic components besides chlorine and ammonia in the Ft. Myers South discharge.

Chemical analyses indicated the presence of the pesticide lindane, several other unidentified organic compounds, and the metals silver and zinc in the Ft. Myers South sample. The toxicity noted above may very well have been partially due to these chemical constituents.

INTRODUCTION

The Ft. Myers South Sewage Treatment Plant is located at 1618 South Drive, in Ft. Myers, Lee County, Florida (Fig. 1). The plant primarily treats domestic wastes from a surrounding residential population of approximately 120,000. Advanced treatment at the Ft. Myers South facility includes nitrification. A schematic of the plant's wastewater treatment system is presented in Figure 2. The plant has a design capacity of 12 MGD, but treats an average wastewater volume of from 5 to 9 MGD. Final recovered water is chlorinated and discharged to the Callosahatchee River through a submerged outfall pipe.

At the request of DER's South Florida District, the Biology Section performed a series of acute toxicity bioassays on samples of recovered water from this Ft. Myers South facility to evaluate its toxicity. The tests were performed from 8 to 10 January, 1987. Results indicated that the sample was acutely toxic to the test organisms, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi, producing 48 hour LC_{50}'s of 55.4%, 83.5%, and 74.8%, respectively. A portion of this toxicity was attributed to the total residual chlorine (0.5 mg/l) measured in the sample. Three additional bioassays performed on de-chlorinated recovered water, in which the chlorine had been neutralized, indicated that the sample was acutely toxic to C. dubia and D. pulex (48 hour LC_{50} = 100% and 87.7%, respectively).

Chemical analyses indicated the presence of the pesticide lindane (0.08 ug/l), several unidentified compounds, and the metals arsenic and zinc. These components may have contributed to the the toxicity noted in the Ft. Myers STP sample (FDER 1987).

The South Florida District Office requested a follow-up bioassay to that performed in January, 1987. Therefore, the Biology Section tested a sample collected in June, 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.

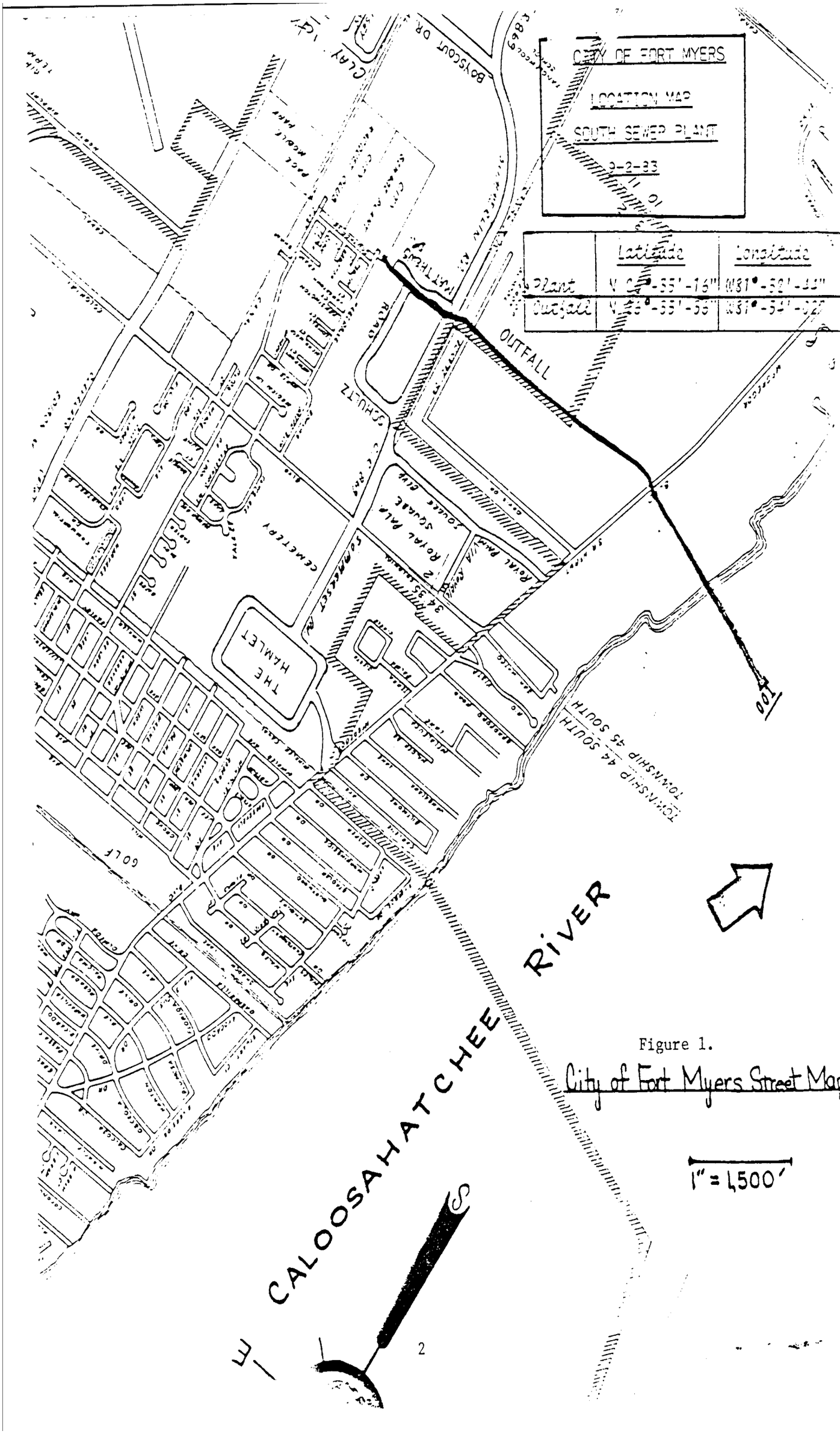


Figure 1.

City of Fort Myers Street Map

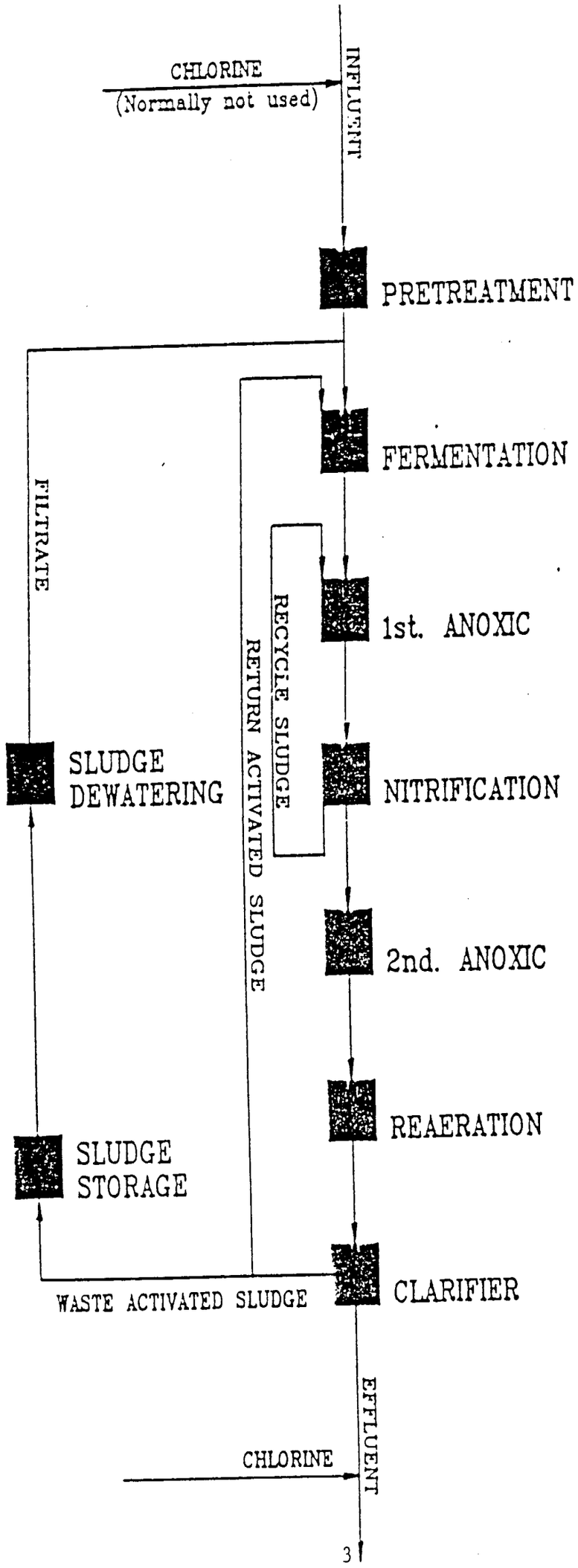


FIGURE 2

CENTRAL AND SOUTH WWTPs
FORT MYERS, FLORIDA

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 a species recommended by Peltier and Weber (1985) 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

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 (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

Four static acute toxicity bioassays were performed on a sample of recovered water collected from the Fort Myers South Sewage Treatment Plant. The tests were performed from 0930 hours on 16 June to 0930 hours on 19 June, 1987.

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 collected by Mr. Bennie T. Shoemaker of DER's South Florida District office at 0830 hours on 15 June, 1987, using standard wastewater sampling procedures. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 25 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° $\pm$ 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 sample. 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 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 flea, Ceriodaphnia dubia. Flint glass jars of 60 ml capacity were used as test chambers, and 50 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. 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% 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% 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

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 72 hour, *Ceriodaphnia dubia* and *Notropis leedsi*, static acute toxicity bioassays of Ft. Myers South Sewage Treatment Plant, NPDES #FL0021270, 16 to 19 June, 1987.

Chlorinated Discharge[a]

Concentration or %	Number of Live Organisms				Dissolved Oxygen (mg/l)				pH				Temperature (°C)				Conductivity (umhos)		
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72	0	48	72
Ceriodaphnia dubia																			
Control	10	10	10	9	8.5	8.9	8.9	7.8	8.2	8.1	8.1	8.2	20.8	19.6	20.5	20.0			
Control	10	10	10	10	8.5	8.9	8.9	7.8	8.2	8.1	8.1	8.2							
50%	10	10	10	10	8.7	9.0	8.9	8.8	7.4	8.0	8.0	8.0	20.5	19.4	20.4	19.9			
50%	10	10	9	9	8.7	9.0	8.9	8.8	7.4	8.0	8.0	8.0							
100%	10	4	2	0	8.9	9.1	9.0	8.8	7.0	7.9	7.9	7.9	19.9	19.3	20.4	19.8			
100%	10	4	1	0	8.9	9.1	9.0	8.8	7.0	7.9	7.9	7.9							
Notropis leedsi																			
Control	5	5	5	5	8.7	8.4	8.4	8.3	7.6	7.8	7.9	8.0	21.4	19.7	20.3	19.9			
Control	5	5	5	5	8.7	8.4	8.4	8.3	7.6	7.8	7.9	8.0					203	218	210
50%	5	3	3	3	8.8	8.5	8.1	8.0	7.2	7.6	7.7	7.7	20.4	19.5	20.2	19.9			
50%	5	5	5	5	8.8	8.5	8.1	8.0	7.2	7.6	7.7	7.7					410	440	422
100%	5	0	-b	-b	8.9	8.8	-b	-b	6.9	7.4	-b	-b	19.0	19.4	-b	-b			
100%	5	0	-b	-b	8.9	8.8	-b	-b	6.9	7.4	-b	-b					700	-b	-b

Control Water

NH_3(as NH_3)(mg/l) 0

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

Total Alkalinity 114 (mg/l $CaOO_3$)

Total Hardness 113 (mg/l $CaOO_3$)

100% Sample

NH_3(as NH_3)(mg/l) 0

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

Total Alkalinity 72 (mg/l $CaOO_3$)

Total Hardness 137 (mg/l $CaOO_3$)

a Residual Chlorine = 0.45 mg/l HACH
0.53 mg/l Iodometric

b When all organisms dead at 24 hours, parameters not measured at 48 or 72 hours.

Table 3. Data recorded during 72 hour, *Ceriodaphnia dubia* and *Notropis leedsi*, static acute toxicity bioassays of Ft. Myers South Sewage Treatment Plant, NPDES #FL0021270, 16 to 19 June, 1987.

De-chlorinated Discharge

Concentration or %	Number of Live Organisms				Dissolved Oxygen (mg/l)				pH				Temperature (°C)				Conductivity (umhos)		
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72	0	48	72
Ceriodaphnia dubia																			
Control	10	10	10	9	8.7	8.7	8.5	8.7	8.2	8.1	8.2	8.1	20.8	19.7	20.5	19.7			
Control	10	10	9	9	8.7	8.7	8.5	8.7	8.2	8.1	8.2	8.1							
50%	10	10	10	10	8.8	8.8	8.7	8.8	7.4	8.0	7.9	8.0	20.5	19.5	20.4	19.7			
50%	10	10	10	10	8.8	8.8	8.7	8.8	7.4	8.0	7.9	8.0							
100%	10	10	2	0	8.8	9.0	8.8	8.9	7.0	7.9	7.8	7.9	20.0	19.1	20.5	19.5			
100%	10	10	7	2	8.8	9.0	8.8	8.9	7.0	7.9	7.8	7.9							
Notropis leedsi																			
Control	5	5	5	5	8.7	8.4	8.4	8.3	7.6	7.8	7.9	8.0	21.4	19.7	20.3	19.9			
Control	5	5	5	5	8.7	8.4	8.4	8.3	7.6	7.8	7.9	8.0					203	218	210
50%	5	5	5	5	8.7	8.2	8.4	7.8	7.2	7.6	7.7	7.7	20.5	19.8	20.1	19.7			
50%	5	5	5	5	8.7	8.2	8.4	7.8	7.2	7.6	7.7	7.7					420	440	432
100%	5	5	5	5	8.6	8.2	8.4	7.9	6.9	7.4	7.5	7.6	19.7	19.6	20.0	19.6			
100%	5	5	5	5	8.6	8.2	8.4	7.9	6.9	7.4	7.5	7.6					720	750	730

Control Water

NH_3(as NH_3)(mg/l) 0

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

Total Alkalinity 114 (mg/l $CaOO_3$)

Total Hardness 113 (mg/l $CaOO_3$)

100% Sample

NH_3(as NH_3)(mg/l) 0

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

Total Alkalinity 74 (mg/l $CaOO_3$)

Total Hardness 142 (mg/l $CaOO_3$)

Table 4. LC_{50} Data for Bioassays of Ft. Myers South Sewage Treatment Plant, NPDES #FL0021270, 16 to 19 June, 1987.

Organism	Method of Data Analysis	48 Hour LC_{50} and 95% Confidence Intervals	72 Hour LC_{50} and 95% Confidence Intervals
Chlorinated Discharge			
Ceriodaphnia dubia	Binomial	75.1% (50 and 100)	67.6% (50 and 100)
	Moving Average	75.1% (66.5 and 86.2), a	b
	Probit	76.5% (65.0 and 88.8)	b
Notropis leedsi	Binomial	62.2% (50 and 100)	62.2% (50 and 100)
	Moving Average	b	b
	Probit	b	b
De-chlorinated Discharge			
C. dubia	Binomial	95.5% (50 and)	76.2% (50 and 100)
	Moving Average	b	b
	Probit	b	b
N. leedsi	Binomial		
	Moving Average	No Mortality	No Mortality
	Probit		

[a] This LC_{50} is reported in the text since it is the most statistically conservative value of the data set.

[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.

Table 5. Results of chemical analyses performed on a sample of recovered water from the Ft. Myers South STP, Lee County, NPDES #FL0021270, collected 15 June, 1987.

Organics

Acid Extractables:	N.D.
Base Neutral Extractables:	N.D.
Pesticide Extractables:	g-BHC (lindane), 0.02 ug/l
	Numerous other unidentified compounds

Metals

Arsenic	N.D.
Cadmium	N.D.
Chromium	N.D.
Copper	< 25 ug/l
Lead	-[a]
Mercury	N.D.
Nickel	N.D.
Selenium	N.D.
Silver	0.6 ug/l
Zinc	19 ug/l

N.D. = None Detected
[a] Lead analysis was invalidated

RESULTS AND DISCUSSION

The sample of final chlorinated recovered water collected from Fort Myers South Sewage Treatment Plant, a facility which discharges into the Caloosahatchee River, proved to be acutely toxic to the test organisms. Forty-eight and 72 hour LC_{50}'s of 75.1% and 67.6%, respectively, were calculated for Ceriodaphnia dubia. In the Notropis leedsi bioassay, a 48 hour LC_{50} of 62.2% was determined (Table 4). The residual chlorine measured in this sample (0.45 mg/l HACH and 0.53 mg/l Iodometric) probably contributed to the observed toxicity.

In tests using de-chlorinated recovered water, 48 and 72 hour LC_{50}'s of 95.5% and 76.2%, respectively, were calculated for C. dubia. No mortality was noted in the N. leedsi bioassay (Table 3). No ammonia was detected in this sample, and, therefore, other toxic components may have been present.

Chemical analyses (Table 5) indicated the presence of the pesticide lindane, several other unidentified organic compounds, and the metals silver and zinc in the Ft. Myers South discharge. These chemical constituents may very well have contributed to the toxicities noted above.

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 recovered water 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.

LITERATURE CITED

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_____. 1987. Bioassays of Ft. Myers South Sewage Treatment Plant, Ft. Myers, Lee County, Florida, NPDES #FL0021270. FDER. 20 p.

Peltier, W. and C. I. Weber. 1985. Methods for measuring the acute toxicity of effluents to aquatic organisms (Third Edition). Environmental Research Laboratory. Athens, Georgia. EPA-600/4-85-012. 217 p.

BIOASSAY DATA FORM

Facility/Toxicant Ft. Myers South AWT Test # 1,2
Address Lee County
NPDES Number FL0021270
Outfall Number

Type Screen
Organism C. dubia, N. leedsi

Sample Collected: Date 6/15/87 Time 0830
Test Beginning: Date 6/16/87 Time 0930
Test Ending: Date 6/19/87 Time 0930

Test Concentration	Chamber Number	Live Organisms 0	24	48	72	Dissolved Oxygen (mg/L) 0	24	48	72	pH 0	24	48	72	Temperature °C 0	24	48	72	Cond 0	48		Ammonia Total 0	48	Union-ized 0	48
C. dubia																								
Control	50	10	10	10	9	8.5	8.9	8.9	7.8	8.2	8.1	8.1	8.2	20.8	19.6	20.5	20.0				0		0	
Control	81	10	10	10	10	8.5	8.9	8.9	7.8	8.2	8.1	8.1	8.2											
50%	93	10	10	10	10	8.7	9.0	8.9	8.8	7.4	8.0	8.0	8.0	20.5	19.4	20.4	19.9							
50%	30	10	10	9	9	8.7	9.0	8.9	8.8	7.4	8.0	8.0	8.0											
100%	20	10	4	2	0	8.9	9.1	9.0	8.3	7.0	7.9	7.9	7.9	19.9	19.3	20.0	19.8				0		0	
100%	62	10	4	1	0	8.9	9.1	9.0	8.8	7.0	7.9	7.9	7.9											
N. leedsi																								
Control	—	5	5	5	5	8.7	8.4	8.4	8.3	7.6	7.8	7.9	8.0	21.4	19.7	20.3	19.9				0		0	
Control	25	5	5	5	5	8.7	8.4	8.4	8.3	7.6	7.8	7.9	8.0				[illegible]	203	218	210				
50%	100	5	3	3	3	8.8	8.5	8.1	8.0	7.2	7.6	7.7	7.7	20.4	19.5	20.1	19.9							
50%	—	5	3	5	5	8.8	8.5	8.1	8.0	7.2	7.6	7.7	7.7				[illegible]	410	410	422				
100%	33	5	0	—	—	8.9	8.8	—		6.9	7.4	—		19.0	19.4	—					0		0	
100%	30	5	0	—	—	8.9	8.8	—		6.9	7.4	—					[illegible]	700	—					

Instrument Calibrations:

	pH Meter:	D.O. Meter:	S-C-T Meter:
0 Hours	7.0 at 7, 9.0 at 9	9.0 at 21°C	10.5 at 10; 27.2 at 30
24 Hours	7.0 at 7, 9.0 at 9	9.2 at 20°C	
48 Hours	[illegible] at 7, [illegible] 9	9.0 at 21°C	10.9 at 10; 27.7 at 30
	7.0 at 7, 9.0 at 9	9.2 at 20°C	10.2 at 10, 26.5 at 30

NOTES

Control Water
Total Alkalinity (mg/l $CaCO_3$) 114
Total Hardness (mg/l $CaCO_3$) 113

100% Sample
Total Alkalinity (mg/l $CaCO_3$) 72
Total Hardness (mg/l $CaCO_3$) 137

Age of Test Organisms 28 days
Weight

Cl_2 0.45 HACH
0.53 IODOMETRIC

Reviewed by: [signature]
Date: 8/17/87

BIOASSAY DATA FORM

Facility/Toxicant Ft. Myers Seattle August — Test # 3, 4 — Sample Collected: Date 6/15/87 Time 0830

Address Lee County — Type Screen — Test Beginning: Date 6/16/87 Time 0930

NPDES Number FL0021270 — Organism C. dubia N. reads — Test Ending: Date 19 June 87 Time 0930

Outfall Number

Test Concentration	Chamber Number	Live Organisms 0	24	48		Dissolved Oxygen (mg/l) 0	24	48		pH 0	24	48	72	Temperature °C 0	24	48	72	Cond. 0	48		Ammonia Total 0	48	Unionized 0	48
Control	95	10	10	10	9	8.7	8.7	8.5	8.7	8.2	8.1	8.2	8.1	20.6	19.7	20.5	19.9				0		0	
Control	36	10	10	9	9	8.7	8.7	8.5	8.7	8.2	8.1	8.2	8.1											
50%	15	10	10	10	10	8.8	8.8	8.7	8.8	7.4	8.0	7.9	8.0	20.5	19.5	20.4	19.7							
50%	4	10	10	10	10	8.8	8.8	8.7	8.8	7.4	8.0	7.9	8.0											
100%	88	10	10	8	0	8.8	9.0	8.8	8.9	7.0	7.9	7.8	7.9	20.0	19.1	20.5	19.3				0		0	
100%	18	10	10	7	2	8.8	9.0	8.8	8.9	7.0	7.9	7.8	7.9											
50%	70	5	5	5	5	8.7	8.2	8.4	7.8	7.2	7.6	7.7	7.7	20.5	19.5	20.1	19.7							
50%	3	5	5	5	5	8.7	8.2	8.4	7.8	7.2	7.6	7.7	7.7					420	440	432				
100%	66	5	5	5	5	8.6	8.2	8.4	7.9	6.9	7.4	7.5	7.6	19.7	19.6	20.0	19.6							
100%	44	5	5	5	5	8.6	8.2	8.4	7.9	6.9	7.4	7.5	7.6					720	750	730	0		0	
		RBF MLR	MLR	MLR RBF	MLR	MLR	MLR	MLR	MLR	MLR	MLR	MLR	RBF	MLR	MLR	MLR	RBF	MLR	MLR	MLR	MLR	MLR	MLR	

NOTES

Control Water

Total Alkalinity (mg/l $CaCO_3$) 114

Total Hardness (mg/l $CaCO_3$) 163

100% Sample

Total Alkalinity (mg/l $CaCO_3$) 74

Total Hardness (mg/l $CaCO_3$) 142

Age of Test Organisms 28 days

Weight

Instrument Calibrations: pH Meter: D.O. Meter: S-C-T Meter:

0 Hours

24 Hours

48 Hours

DE-Cl2

Reviewed by:

Date: 6/17/87