

BIOASSAYS OF THE CITY OF MARIANNA

SEWAGE TREATMENT PLANT

MARIANNA, JACKSON COUNTY, FLORIDA

NPDES #FL0020117

Biology Section
Bureau of Water Analysis
January 19, 1983

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EXECUTIVE SUMMARY

Marianna STP, NPDES #FL0020117, on 30 November to 2 December, 1982

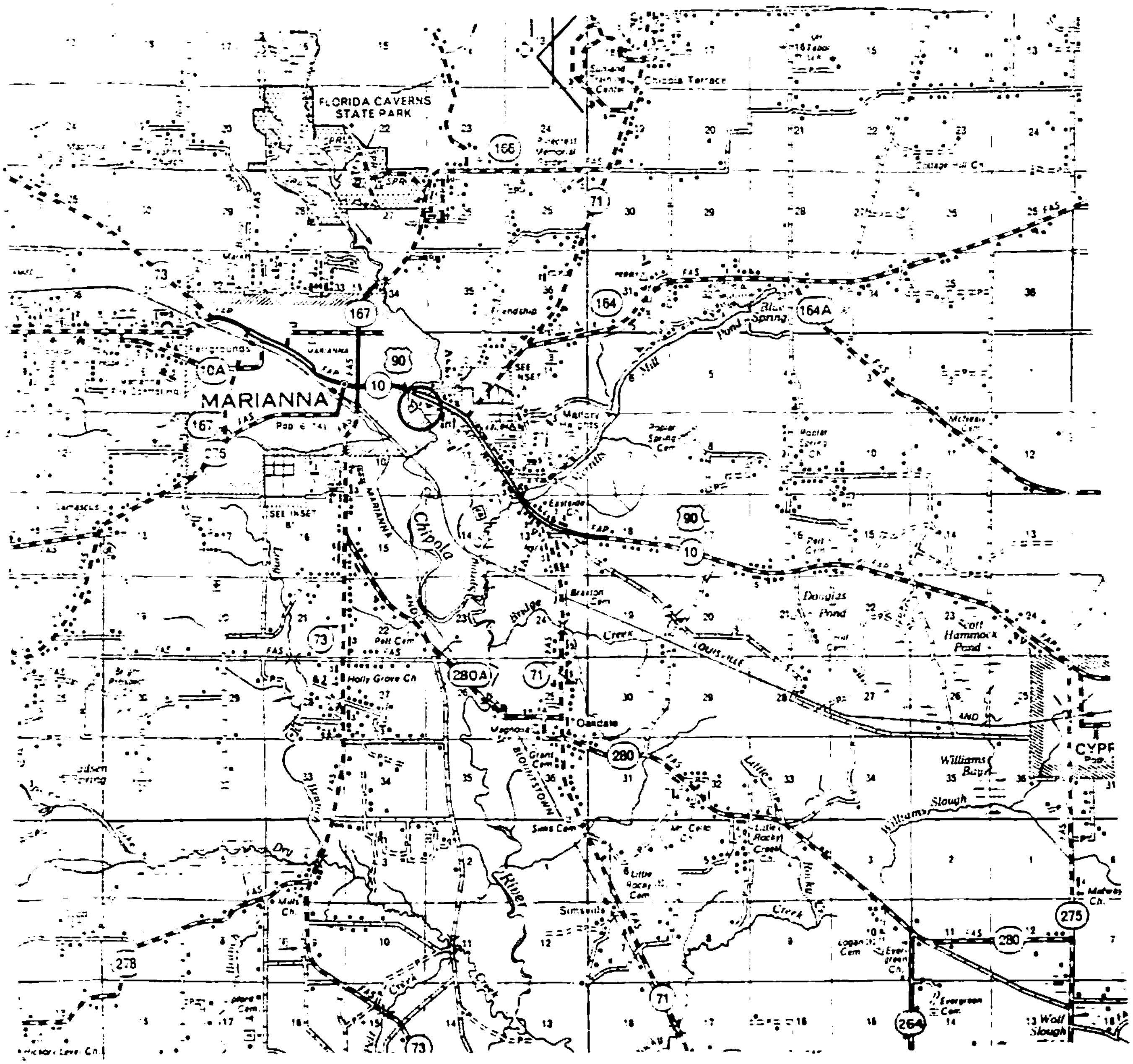
The sample of pre-chlorinated effluent from this municipal waste treatment facility was acutely toxic to Daphnia pulex, producing 24 hour LC_{50}'s of 54.2% to 56.0%. The 48 hour test was invalidated due to excessive control mortality. This same effluent was not toxic to Heterandria formosa. The chlorinated sample, in which the total chlorine residual had dissipated to a non-toxic level (0.04 mg/l), was acutely toxic to both Daphnia pulex and to Simocephalus serrulatus, producing 48 hour LC_{50}'s of 27.2% to 29.8% and 8.5% to 9.6%, respectively. Significant concentrations of total and unionized ammonia measured in these effluents (8.2 mg/l to 9.6 mg/l and .06 mg/l to .09 mg/l, respectively) may have contributed to the observed toxicity. Chemical analyses indicated the presence of the pesticide lindane (0.09 µg/l) and the metals arsenic (1.0 µg/l), lead (6.0 µg/l), silver (0.2 µg/l), and zinc (53 µg/l). These chemical constituents may have acted synergistically to have caused the observed toxicity, although, individually, the concentrations are subtoxic.

INTRODUCTION

The City of Marianna Sewage Treatment Plant is located at 1001 E. Franklin Street, in Marianna, Jackson County, Florida (Fig. 1). This municipal waste treatment facility has a design flow capacity of 1.14 MGD, but treats only an average of 0.557 MGD of waste. Included in this treatment are 0.019 MGD of industrial waste from a nearby meat rendering plant. Treatment consists of primary and secondary clarifiers, trickling filters and final chlorination (Fig. 2). The treated effluent is discharged through Outfall #001 to the Chipola River.

This facility has a record of numerous violations of treatment standards, stemming from infiltration problems in the sewer lines and from the poor quality of influent from the meat rendering plant (from NPDES file). Additionally, a direct discharge of sludge to the Chipola River was noted in July, 1981 (from NPDES file). Recent FDER Compliance Inspection Reports have noted the obsolescence of this facility and poor quality of treatment, resulting in unacceptable ratings.

To evaluate the effects this effluent may have on the biota of the receiving waters, the Biology Section performed four static acute toxicity bioassays on pre-chlorinated and chlorinated samples, from November 30 to December 2, 1982. The results of these tests are presented in this report.



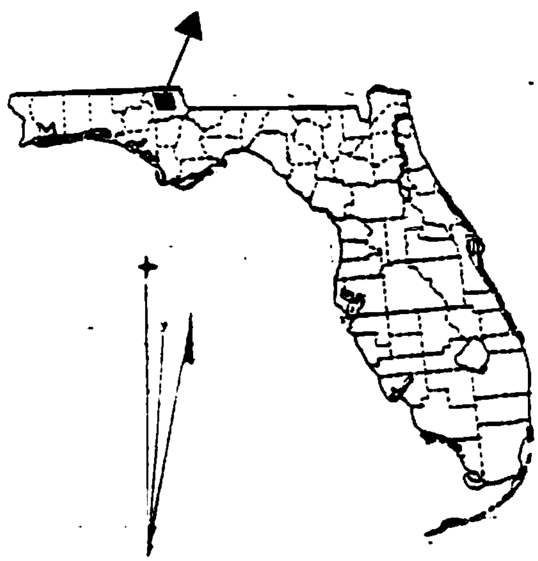
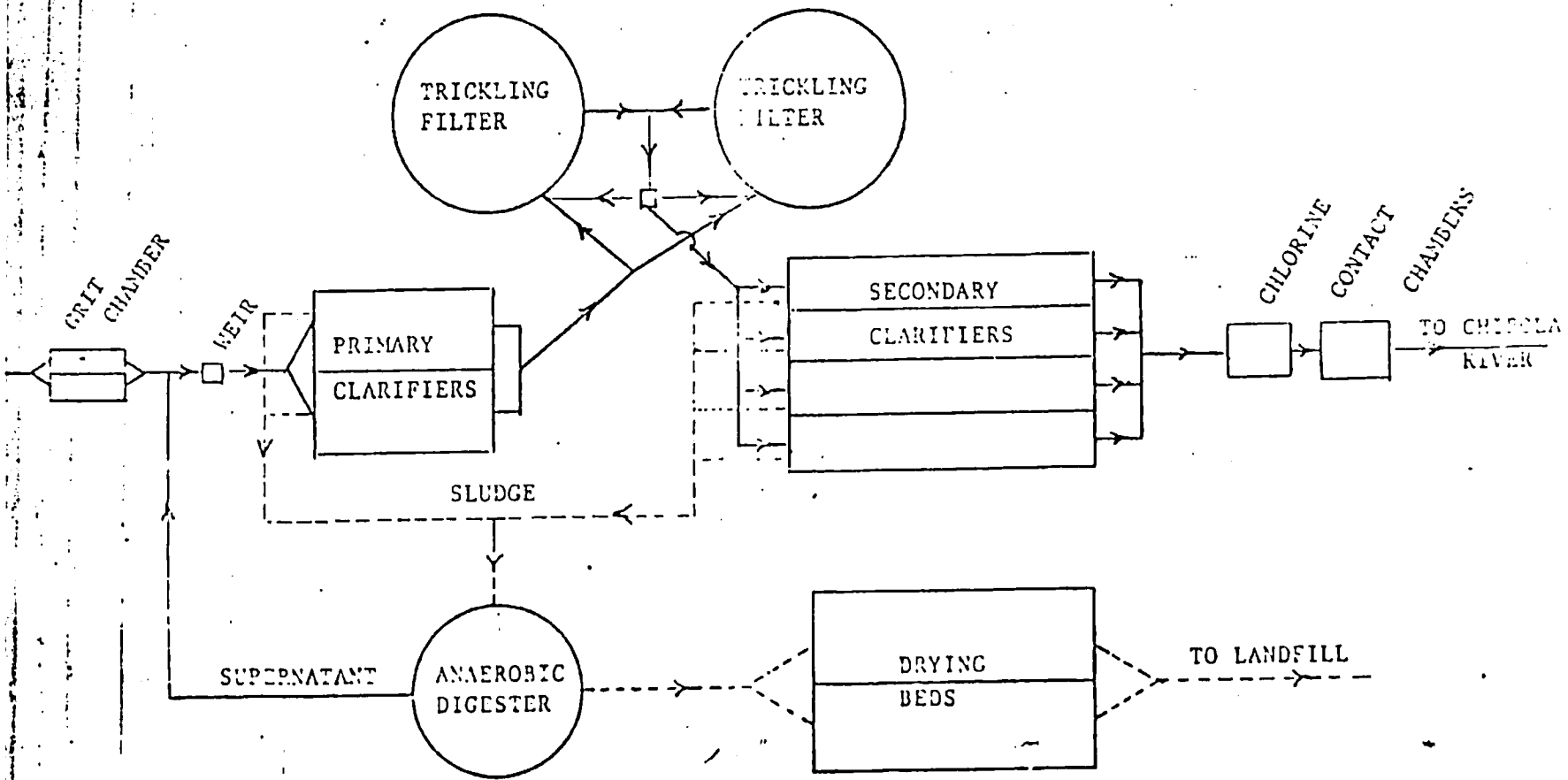


Figure 1. Portions of Jackson County, Florida, including City of Marianna and location of the Sewage Treatment Plant.

FIGURE 2

MARIANNA WASTEWATER TREATMENT PLANT

MARIANNA, FLORIDA



METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978). The organisms selected for this study include a species recommended by Peltier (1978) and the U.S. EPA (1978). Additionally, the Biological Section maintains cultures of several species of organisms indigenous to the State of Florida. Two of these indigenous species were 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, 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, ephippia formation, or excessive fungal or bacterial growth.

Simocephalus serrulatus (water flea) - An indigenous breeding population of Simocephalus serrulatus was collected from Lake Jackson, Leon County, Florida, in March, 1980, by personnel from DER's Biological Section. Positive identification of the organisms was made by Mr. Jeff Foran of the University of Florida Department of Environmental Engineering Sciences. The cultures are maintained in two and one-half gallon all glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. The 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, ephippia formation, or excessive fungal or bacterial growth.

Heterandria formosa (least killifish) - Approximately 100 adult least killifish were obtained from a wild population in the St. Marks Wildlife Refuge (Wakulla County, Florida) during August, 1980. The fish are maintained in two and one-half to 15 gallon all glass aquaria equipped with a flow through water exchange system. Water for this system is unchlorinated and is pumped from a deep well near the DER laboratory. The least killifish are reared at 22° plus or minus 2°C under a 16:8 hour light-dark cycle and are fed freshly hatched Artemia (brine shrimp) twice daily. New born fish are initially maintained in the adult tanks, but are removed at weekly intervals, thereby segregating fish into even age groups. The fish used in this test were 1-1/2 to 3 weeks old.

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

Test Methods

Four separate 48 hour static acute toxicity bioassays were performed on samples of pre-chlorinated and chlorinated effluents from the Marianna STP. The tests were performed from 1110 hours on November 30 to 1120 hours on December 2, 1982. The samples were collected by Mr. Thomas Andrews of DER's Tallahassee Branch Office at 1115 to 1130 hours on November 29, 1982, using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples for metal analyses were preserved with HNO_3. Samples for organic chemical analyses and for the bioassays were unpreserved. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 24 hours of collection.

To differentiate between the toxicity resulting from chlorine and the toxicity associated with some other component of the effluent, the Biological Section adopted the following test procedure:

Test #1. Pre-Chlorinated Effluent using Daphnia pulex and Heterandria formosa (Tables 2 and 3). This test was performed on an effluent sample collected at the end of the treatment process but prior to chlorination, and was intended to evaluate the toxicity of the effluent without the added toxic effects of chlorine.

Test #2. Chlorinated Effluent using Daphnia pulex and Simocephalus serrulatus (Tables 4 and 5). This test was performed on an effluent sample collected at the point of discharge.

Since the concentration of total residual chlorine in the chlorinated sample had dissipated to a less than toxic level (0.04 mg/1) by the time of the test, a test using de-chlorinated effluent was not performed.

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 fleas, Daphnia pulex and Simocephalus serrulatus. 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 and Simocephalus were siphoned into large culture dishes 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. Simocephalus neonates were separated from the adults visually in the culture dish prior to loading. These neonates were then transferred by glass pipette, examined for injury, and were loaded 10 per chamber, resulting in 20 per test concentration. Water fleas were not fed during these tests.

Two replicates, of six concentrations and a control, were used for the Heterandria formosa (least killifish) 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 this test.

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 concentration. 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 concentration at the beginning and end of the test. Total ammonia concentrations were determined using an Orion® specific ion probe and an Orion Ionalyzer®. 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 5.

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

Daphnia pulex and *Simocephalus serrulatus* (water fleas)

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

Heterandria formosa (least killifish)

Concentration	Dilution Water	Effluent	Total Volume
Control	500 ml	---	500 ml
5.6%	472 ml	28 ml	500 ml
10.0%	450 ml	50 ml	500 ml
18.0%	410 ml	90 ml	500 ml
32.0%	340 ml	160 ml	500 ml
56.0%	220 ml	280 ml	500 ml
100.0%	--	500 ml	500 ml

Table 2 . Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of the Marianna STP, Jackson Co., Florida, NPDES #FL0020117, from November 30 to December 2, 1982

Test #1 Pre-chlorinated Sample

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[a]	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	10	9	0	8.9	9.2	8.9	7.5	7.9	8.1	113		116		20.6	20.0	20.1	0/0	0/.04		
Control	10	10	0	8.9	9.2	8.9	7.5	7.9	8.1										200	200
5.6%	10	9	9	8.9	9.1	8.9	7.6	7.9	8.1					20.5	19.9	20.0				
5.6%	10	10	10	8.9	9.0	8.9	7.6	7.9	8.1										220	220
10.0%	10	10	10	8.8	8.9	8.7	7.6	7.9	8.1					20.4	19.8	20.0				
10.0%	10	9	9	8.7	8.9	8.7	7.6	7.9	8.1										230	230
18.0%	10	9	4	8.6	8.7	8.6	7.6	7.9	8.1					20.4	19.8	20.0				
18.0%	10	10	3	8.5	8.7	8.6	7.6	7.9	8.1										260	260
32.0%	10	9	3	8.1	8.3	8.4	7.5	7.8	8.1					20.2	19.8	20.0				
32.0%	10	6	0	8.1	8.3	8.4	7.5	7.8	8.1										270	310
56.0%	10	5	1	7.5	7.1	7.7	7.3	7.7	8.0					20.0	19.8	20.2				
56.0%	10	5	0	7.5	7.1	7.7	7.3	7.7	8.0										360	385
100.0%	10	1(4)[b]	0	6.0	6.6	6.6	7.1	7.7	7.9	167		147		19.8	20.1	20.2	.06/9.6	.08/2.2		
100.0%	10	0	0	5.8	6.6	6.6	7.1	7.7	7.9										600	600

Remarks: [a] The 48 hour test is invalidated due to control mortality.

[b] Number in parentheses used in calculations since three organisms were not found.

Table 3. Data recorded during 48 hour, Heterandria formosa[a], static acute toxicity bioassay of the Marianna STP, Jackson Co., Florida, NPDES #FL0020117, from November 30 to December 2, 1982

Test #1 Pre-chlorinated Sample

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
Contol	5	5	5	8.8	8.6	8.4	7.6	7.8	8.1	113		116		20.9	19.7	20.0	0/0	0/.04		
Control	5	5	5	8.8	8.6	8.4	7.6	7.8	8.1										210	210
5.6%	5	5	5	8.7	8.5	8.4	7.6	7.8	8.1					20.8	19.5	19.7				
5.6%	5	5	5	8.7	8.5	8.4	7.6	7.8	8.1										230	225
10.0%	5	5	5	8.8	8.4	8.2	7.6	7.8	8.0					20.7	19.0	19.7				
10.0%	5	5	5	8.8	8.4	8.2	7.6	7.8	8.0										245	240
18.0%	5	5	5	8.6	8.2	8.0	7.5	7.8	8.0					20.6	19.0	19.5				
18.0%	5	5	5	8.6	8.2	7.9	7.5	7.7	8.0										275	255
32.0%	5	5	5	8.3	7.6	7.5	7.4	7.7	7.9					20.4	19.0	19.5				
32.0%	5	5	5	8.3	7.6	7.5	7.4	7.7	7.9										320	280
56.0%	5	5	5	7.5	7.0	6.7	7.2	7.6	7.8					20.2	19.0	19.5				
56.0%	5	5	5	7.5	7.0	6.7	7.2	7.6	7.8										405	340
100.0%	5	5	5	5.9	6.3	5.9	7.1	7.6	7.8	167		147		19.8	19.5	19.5	.06/9.6	.06/2.2		
100.0%	5	5	5	5.9	6.3	5.9	7.1	7.6	7.8										600	550

Remarks: [a]Weight of fish: 11.66 mg each, average of 9 fish.

Table 4 . Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of the Marianna STP, Jackson Co., Florida, NPDES #FL0020117, from November 30 to December 2, 1982

Test #2 Chlorinated Sample[a]

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	9	7(8)[b]	8.9	8.7	8.4	7.6	8.0	8.2	113		116		20.5	19.9	19.9	0/0	0/.04		
Control	10	10	9	8.9	8.7	8.4	7.7	8.0	8.2										210	212
5.6%	10	10	10	8.9	8.7	8.5	7.7	8.0	8.2					20.5	19.8	19.8				
5.6%	10	10	10	8.9	8.7	8.5	7.7	8.9	8.2										230	225
10.0%	10	10	10	8.8	8.7	8.5	7.7	8.0	8.2					20.3	19.7	19.8				
10.0%	10	10	10	8.8	8.7	8.5	7.7	8.0	8.2										240	235
18.0%	10	10	8	8.7	8.6	8.3	7.7	8.0	8.2					20.1	19.7	19.8				
18.0%	10	10	9	8.7	8.6	8.3	7.7	8.0	8.2										255	258
32.0%	10	7	3	8.4	8.5	7.9	7.6	8.0	8.2					19.8	19.8	19.9				
32.0%	10	7	4	8.4	8.5	7.9	7.6	8.0	8.2										270	292
56.0%	10	2	0	7.8	8.0	7.5	7.5	7.9	8.1					19.5	20.0	19.9				
56.0%	10	1	1	7.8	8.0	7.5	7.5	7.9	8.1										350	352
100.0%	10	0	0	6.4	7.2	6.5	7.4	7.9	8.1	189		149		19.4	20.0	19.9	.09/8.2	.09/1.5		
100.0%	10	2	1	6.4	7.2	6.5	7.4	7.9	8.1										450	452

Remarks: [a]Total Residual Chlorine: 0:04 mg/l (HACH DPD Method)

[b]Number in parentheses used since one organism was not found.

Table 5 . Data recorded during 48 hour, Simocephalus serrulatus, static acute toxicity bioassay of the Marianna STP, Jackson Co., Florida NPDES #FL0020117, from November 30 to December 2, 1982

Test #2 Chlorinated Sample[a]

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	9.0	8.8	8.5	7.6	7.9	8.2	113		116		20.7	20.2	20.2	0/0	0/.04		
Control	10	10	7	9.0	8.8	8.5	7.6	8.0	8.2										200	208
5.6%	10	10	9	9.0	8.7	8.4	7.7	8.0	8.2					20.5	20.2	20.1				
5.6%	10	10	10	9.0	8.7	8.4	7.7	8.0	8.3										220	218
10.0%	10	8	1	8.9	8.7	8.4	7.7	8.0	8.2					20.4	20.3	20.1				
10.0%	10	10	5	8.9	8.7	8.4	7.7	8.0	8.2										235	230
18.0%	10	7	0	8.7	8.5	8.1	7.7	8.0	8.2					20.2	20.5	20.4				
18.0%	10	9	3	8.7	8.5	8.1	7.7	8.0	8.2										250	252
32.0%	10	5	0	8.4	8.4	8.0	7.6	7.9	8.2					19.9	20.6	20.5				
32.0%	10	6	0	8.4	8.4	8.0	7.6	7.9	8.2										290	287
56.0%	10	1	0	7.9	7.9	7.5	7.5	7.9	8.1					19.5	20.6	20.4				
56.0%	10	0	0	7.9	7.9	7.5	7.5	7.9	8.1										350	350
100.0%	10	0	0	6.3	7.0	6.7	7.4	7.9	8.1	189		149		19.2	20.5	20.2	.09/8.2	.09/1.5		
100.0%	10	0	0	6.3	7.0	6.7	7.4	7.9	8.1										450	450

Remarks: [a]Total Residual Chlorine: 0.04 mg/l (HACH DPD Method).

Table 6. LC_{50} Data for Bioassays of the Marianna STP, NPDES #FL0020117, on November 30 to December 2, 1982

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Test #1: D. pulex	Binomial	56.0% (32 and 100)	
Pre-chlorinated	Moving Average	54.2% (43 and 75)	a
Sample	Probit	54.5% (41 and 80)	
Test #1: H. formosa	Binomial		
Pre-chlorinated	Moving Average	No Mortality	No Mortality
Sample	Probit		
Test #2: D. pulex	Binomial	38.9% (18 and 56)	27.2% (18 and 56)
Chlorinated	Moving Average	41.2% (34 nad 49)	29.8% (24 and 39)
Sample	Probit	42.1% (35 and 51)	29.0% (24 and 35)
Test #2: S. serrul.	Binomial	33.5% (18 and 56)	8.5% (5.6 and 18)
Chlorinated	Moving Average	26.7% (22 and 33)	9.3% (7.6 and 11)
Sample	Probit	27.8% (23 and 34)	9.6% (7.9 and 11)

[a]This test was invalidated due to control mortality.

TABLE 7. Results of Chemical Anaylses Performed on Effluent from the Marianna STP, NPDES #FL0020117, collected November 29, 1982.

Organics

Acid Extractables	N.D.
Base Neutral Extractables	N.D.
Pesticide Extractables	g-BHC (Lindane) 0.09 µg/l

Metals

Arsenic	1.0 µg/l
Cadmium	N.D.
Chromium	N.D.
Copper	< 25 µg/l[a]
Lead	6 µg/l
Mercury	N.D.
Nickel	< 50 µg/l[a]
Selenium	N.D.
Silver	0.2 µg/l
Zinc	53 µg/l

N.D. = None Detected

[a] Actual value is known to be less than value given.

RESULTS AND DISCUSSION

The sample of pre-chlorinated effluent from the Marianna STP proved to be acutely toxic to Daphnia pulex, producing 24 hour LC_{50} values of 54.2% to 56.0% (Table 6). The 48 hour test was invalidated due to excessive control mortality. This same effluent, however, was not toxic to any Heterandria formosa. The chlorinated effluent from this facility, in which the total residual chlorine had apparently dissipated to a less than toxic concentration (0.04% mg/1), was, nevertheless, acutely toxic to both Daphnia pulex and to Simocephalus serrulatus. This effluent produced 24 hour LC_{50} values of 38.9% to 42.1% and 48 hour values of 27.2% to 29.8% for Daphnia, and, 24 hour LC_{50} values of 26.7% to 33.5% and 48 hour values of 8.5% to 9.6% for Simocephalus (Table 6). These data suggest that Simocephalus may have been more sensitive to the toxicity of this effluent than were Daphnia. The 96 hour LC_{50}'s for these effluents would be less than or equal to their 24 or 48 hour values.

Significant concentrations of total and unionized ammonia (8.2 mg/1 to 9.6 mg/1 and .06 mg/1 to .09 mg/1, respectively) measured in these effluents may have contributed to the observed toxicity. However, these concentrations are substantially below those reported as being acutely toxic to certain freshwater fish (Thurston et al. 1981).

The DER Chemistry Section has analyzed samples of the final effluent from the Marianna STP for certain metals and organics (Table 7). These analyses indicated the presence of the pesticide Lindane (0.09 µg/1) and the metals arsenic (1.0 µg/1), lead (6.0 µg/1), silver (0.2 µg/1) and zinc (53 µg/1). Although these chemical constituents are in sub-lethal concentrations individually, they may have acted synergistically to have caused the observed toxicity.

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 (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 sustance 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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