

BIOASSAYS OF MILTON SEWAGE TREATMENT PLANT

MILTON, SANTA ROSA COUNTY, FLORIDA

NPDES #FL0021903

OCTOBER 1, 1987

Biology Section
Division of Environmental Programs

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Biology Section
Bureau of Laboratories and
Special Programs
October 1, 1987

EXECUTIVE SUMMARY

Milton Sewage Treatment Plant, Milton, Santa Rosa County, Florida, NPDES #FL0021903, 15 to 17 July, 1987.

Four static acute toxicity bioassays were performed on chlorinated and de-chlorinated samples of recovered water collected from Milton Sewage Treatment Plant, a facility which discharges into the Blackwater River. Ceriodaphnia dubia and Daphnia pulex were used as test organisms.

The chlorinated sample was toxic to C. dubia (48 hour LC_{50} = 83.0%), but mortality was not sufficient to calculate an LC_{50} in the D. pulex test. The residual chlorine measured in this sample (0.2 mg/l HACH) may have partially contributed to the observed toxicity.

In tests with the de-chlorinated sample, no mortality of D. pulex occurred. This sample was, however, toxic to C. dubia, with a 48 hour LC_{50} of 91.1%. The total and unionized ammonia measured in this sample (3.63 mg/l and 0.01 mg/l, respectively) was probably not sufficient to be responsible for this toxicity.

Chemical analyses indicated the presence of 0.1 ug/l of g-BHC, as well as numerous unidentified pesticide extractable components. Additionally, the metals cadmium (0.6 ug/l), silver (0.3 ug/l), and zinc (52.0 ug/l) were detected in this sample. These metals, as well as the pesticide components measured in this recovered water sample, may have contributed to the observed toxicity to C. dubia.

INTRODUCTION

The Milton Sewage Treatment Plant is located on West Walker Street in Milton, Santa Rosa County, Florida (Figure 1). This facility serves a population of approximately 10,000, and has a design flow capacity of 2.5 MGD (mean flow = 1.3 MGD). Treatment consists of screening, grit removal, aeration, secondary clarification, chlorination, and post aeration (Figure 2). Recovered water is discharged to the Blackwater River.

To evaluate the toxicity of this recycled water sample, the Biology Section performed four static acute toxicity bioassays on a sample collected in July, 1987. Results are presented in this report. Those involved in performing the tests and in preparation of this report were: J. M. Faircloth, R. B. Frydenborg, K. M. Lurding, and M. L. Roll.

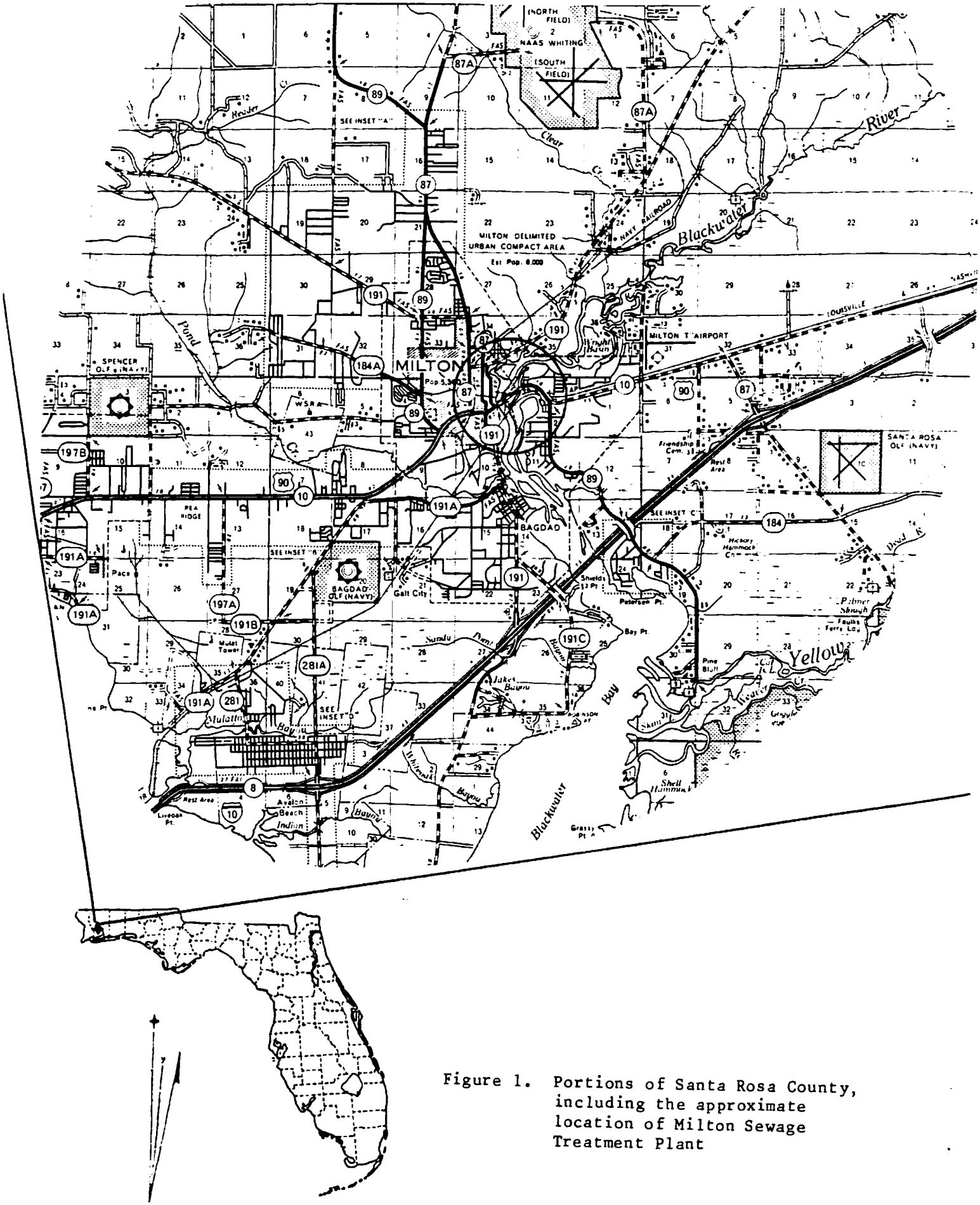
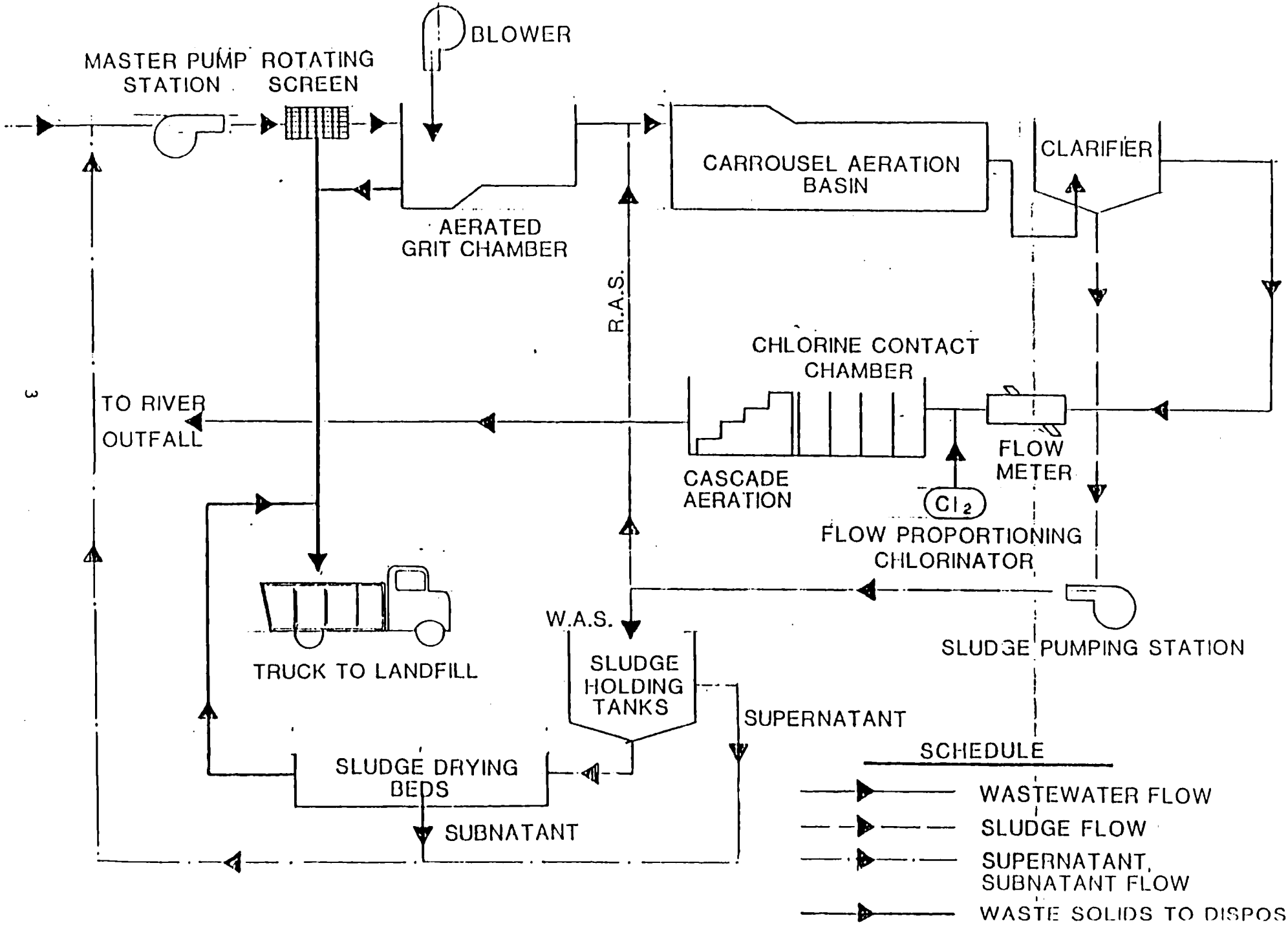


Figure 1. Portions of Santa Rosa County, including the approximate location of Milton Sewage Treatment Plant

Figure 2.

SCHEMATIC FLOW DIAGRAM - MILTON W.W.T.P.



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) 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. The bioassay performed on this organism, however, was invalidated due to excessive control mortality (see raw data sheets). 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 June, 1987. 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 27 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 screening bioassays were performed on samples of chlorinated and de-chlorinated recovered water collected from Outfall #001 of the Milton Sewage Treatment Plant. The tests were performed from 1020 hours on 15 July to 1020 hours on 17 July, 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 sample was collected by Mr. John Williams of DER's Northwest District Office at 1330 hours on 13 July, 1987, using standard wastewater sampling procedures. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 48 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 recycled waters 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 discharge. To accomplish this, the Biology Section performed concurrent bioassays on a sample of chlorinated 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 recovered 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.

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.

RESULTS AND DISCUSSION

The chlorinated sample of recovered water proved to be acutely toxic to Ceriodaphnia dubia, with a 48 hour LC_{50} of 83.0% (Table 4). Although a small amount of mortality occurred in the Daphnia pulex bioassay (Table 2), this was insufficient to calculate an LC_{50}. The residual chlorine measured in this sample (0.2 mg/l HACH) may have been a partial contributor to the observed toxicity.

In tests with the de-chlorinated sample, no mortality of D. pulex occurred (Table 3). A 48 hour LC_{50} of 91.1% was calculated, however, for C. dubia. The total and unionized ammonia measured in this sample (3.63 mg/l and 0.01 mg/l, respectively) was probably not sufficient to have accounted for this toxicity.

Chemical analyses (Table 5) indicated no acid or base neutral extractables. The pesticide extractable analysis revealed 0.1 ug/l of g-BHC, as well as numerous unidentified components. Analysis for metals indicated cadmium (0.6 ug/l), silver (0.3 ug/l), and zinc (52.0 ug/l). These metals, as well as the pesticide components detected in this recovered water sample, may have contributed to the observed toxicity to C. dubia.

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

Table 2. Data recorded during 48 hour, Ceriodaphnia dubia and Daphnia pulex, static acute toxicity bioassays of Milton Sewage Treatment Plant, NPDES #FL0021903, 15 to 17 July, 1987.

Chlorinated Discharge[a]

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	8.7	8.3	8.5	8.0	8.1	7.9	18.9	20.6	20.9		
Control	10	10	10	8.7	8.3	8.5	8.0	8.1	8.0					
50%	10	10	10	8.4	8.0	8.3	7.4	7.8	7.7	19.1	20.6	20.6		
50%	10	10	10	8.4	8.0	8.3	7.4	7.8	7.7					
100%	10	9	3	7.8	6.4	8.3	6.9	7.4	7.5	19.4	20.6	20.7		
100%	10	10	2	7.8	6.4	8.3	6.9	7.4	7.5					
Daphnia pulex														
Control	10	10	10	8.7	8.3	8.5	8.0	8.1	7.9	19.4	20.5	20.5		
Control	10	10	10	8.7	8.3	8.5	8.0	8.1	7.9				200	210
50%	10	10	10	8.2	8.2	7.2	7.3	7.7	7.5	19.4	20.5	20.3		
50%	10	10	10	8.2	8.2	7.2	7.3	7.7	7.5				220	222
100%	10	10	10	7.5	8.2	6.8	6.8	7.6	7.3	19.7	20.5	20.4		
100%	10	10	9	7.5	8.2	6.8	6.8	7.6	7.3				240	242

Control Water

NH_3(as NH_3)(mg/l) 0

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

Total Alkalinity 115 (mg/l $CaCO_3$)

Total Hardness 109 (mg/l $CaCO_3$)

100% Sample

NH_3(as NH_3)(mg/l) 0.01

Total ammonia (as N)(mg/l) 3.63

Total Alkalinity 75 (mg/l $CaCO_3$)

Total Hardness 23 (mg/l $CaCO_3$)

[a] Residual chlorine = 0.2 mg/l (HACH)
0.0 mg/l (Iodometric)

Table 3. Data recorded during 48 hour, Ceriodaphnia dubia and Daphnia pulex, static acute toxicity bioassays of Milton Sewage Treatment Plant, NPDES #FL0021903, 15 to 17 July, 1987.

De-chlorinated Discharge[a]

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	8.6	8.3	8.7	8.0	8.1	8.0	19.8	20.7	20.4		
Control	10	10	10	8.6	8.3	8.7	8.0	8.1	8.0					
50%	10	10	10	8.4	6.0	8.1	7.4	7.6	7.7	19.7	20.7	20.8		
50%	10	10	9	8.4	6.0	8.1	7.4	7.6	7.7					
100%	10	10	4	7.8	5.4	8.1	6.9	7.3	7.5	19.6	20.7	20.8		
100%	10	10	4	7.8	5.7	8.1	6.9	7.3	7.4					
Daphnia pulex														
Control	10	10	10	8.7	8.1	8.4	8.0	8.0	7.9	19.8	20.6	20.4		
Control	10	10	10	8.7	8.1	8.4	8.0	8.0	7.9				200	228
50%	10	10	10	8.4	6.1	7.4	7.3	7.5	7.5	19.7	20.5	20.3		
50%	10	10	10	8.4	6.1	7.4	7.3	7.5	7.5				225	230
100%	10	10	10	7.6	8.0	6.9	6.9	7.5	7.3	19.9	20.5	20.3		
100%	10	10	10	7.6	8.0	6.8	6.9	7.5	7.3				245	248

Control Water

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

Total Alkalinity 115 (mg/l $CaOO_3$)

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

Total Hardness 109 (mg/l $CaOO_3$)

100% Sample

NH_3(as NH_3)(mg/l): 0.01

Total Alkalinity 75 (mg/l $CaOO_3$)

Total ammonia (as N)(mg/l): 3.63

Total Hardness 23 (mg/l $CaOO_3$)

Table 4. LC_{50} Data for Bioassays of Milton Sewage Treatment Plant, NPDES #FL0021903, 15 to 17 July, 1987.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Chlorinated Discharge			
Ceriodaphnia dubia	Binomial	a	83.0% (50 and 100)
	Moving Average	a	a
	Probit	a	a
Daphnia pulex	Binomial		a
	Moving Average	No Mortality	a
	Probit		a
De-chlorinated Discharge			
C. dubia	Binomial		89.7% (50 and ∞)
	Moving Average	No Mortality	89.7% (75.3 and 125.2)
	Probit		91.1% (76.0 and 119.3), b
D. pulex	Binomial		
	Moving Average	No Mortality	No Mortality
	Probit		

[a] 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.

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

Table 5. Results of chemical analyses performed on recovered water from the Milton STP, NPDES #FL0021903, collected 13 July, 1987.

Organics

Acid Extractables	N.D.
Base Neutral Extractables	N.D.
Pesticide Extractables	0.1 ug/l g-BHC
	Numerous unidentified components

Metals

Arsenic	N.D.
Cadmium	0.6 ug/l
Chromium	N.D.
Copper	___[a]
Lead	___[a]
Mercury	N.D.
Nickel	N.D.
Selenium	N.D.
Silver	0.3 ug/l
Zinc	52.0 ug/l

N.D. = None Detected

[a] Results for copper and lead invalidated due to contaminated field blank.

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

Florida Department of Environmental Regulation, Biology Section. 1985. Quality Assurance Manual for Performing Acute Toxicity Tests (Second Edition). FDER.

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 Cl2

Facility/Toxicant MILTON STP
Address W25T WALKER STR. SANTA ROSA Co
NPDES Number MILTON Fla. FL0021903
Outfall Number 001

Test # 1,2,3
Type Screening
Organism C. Dubia
D. pulex N. leedsi

Sample Collected: Date 7-13-87 Time 1330
Test Beginning: Date 7-15-87 Time 1020
Test Ending: Date 7-17-87 Time 1020

Test Concentration	Chamber Number	Live Organisms 0	24	48	Dissolved Oxygen (mg/L) 0	24	48	pH 0	24	48	Temperature °C 0	24	48	Conductivity 0	48	Ammonia Total 0	48	Unionized 0	48
control	21	10	10	10	8.7	8.3	8.5	8.0	8.1	7.9	18.4	20.6	20.9			0		0	
control	47	10	10	10	8.7	8.3	8.5	8.0	8.1	8.0									
C Dubia 50 %	6	10	10	10	8.4	8.0	8.3	7.4	7.8	7.7	19.1	20.6	20.6						
50 %	96	10	10	10	8.4	8.0	8.3	7.4	7.8	7.7									
100 %	93	10	9	3	7.8	6.4	6.3	6.9	7.4	7.5	19.4	20.1	20.7			36.3		.01	
100 %	97	10	10	2	7.8	6.4	6.3	6.9	7.4	7.5									
	44																		
control	6 44	10	10	10	8.7	8.3	8.5	8.0	8.1	7.9*	19.4	20.8	20.5	200		0		0	
control	— 6	10	10	10	8.7	8.3	8.5	8.0	8.1	7.9				200	210				
Dpulex 50 %	#45 1 -	10	10	10	8.2	8.2	7.2	7.3	7.7	7.5	19.4	20.5	20.3						
50 %	#45 59	10	10	10	8.2	8.2	7.2	7.3	7.7	7.5				220	222				
100 %	#45 13	10	10	10	7.5	8.2	6.4	6.8	7.6	7.3	19.3	20.5	20.4						
100 %	#45 138	10	10	9	7.5	8.2	6.9	6.8	7.6	7.3				240	242	36.3		.01	
	#45 1	2																	
control	#45 10	5	3	3	8.3			7.5			20.8					0.0		0	
control	#20 231	5	4	4	8.3			7.5						210					
N Leedsi 50 %	#45 55	5	5	5	8.1			7.2			20.0								
50 %	#36 70	5	5	5	8.1			7.2						228					
100 %	113	5	4	4	7.5			6.3			20.4								
100 %	36	5	5	5	7.5			6.4						248		36.3		.01	
		MLR	MLR	MLR	MLR			MLR			MLR			MLR		RBF		MLR	

NOTES

Control Water

Total Alkalinity (mg/l $CaCO_3$) 115

Total Hardness (mg/l $CaCO_3$) 109

100% Sample

Total Alkalinity (mg/l $CaCO_3$) 75

Total Hardness (mg/l $CaCO_3$) 23

MLR

Age of Test Organisms Each ± 27 days old

Weight

Instrument Calibrations:

	pH Meter:	D.O. Meter:	S-C-T Meter:
0 Hours	7.07 7.09	9.2 @ 20°C	1000 25.3 @ 30
24 Hours	7.07 7.09	8.8 @ 21.5°C	
48 Hours	7.0 7.0	9.0 9.2	

Reviewed by

Date 9/29/87

detected Cl_2 0.2 by Hach method
0.0 by iodometric method

De Cl_2

BIOASSAY DATA FORM

Facility/Toxicant Milton STP Test # RA 4,5,6 Sample Collected: Date 7-13-87 Time 1330

Address West Mulloy St Santa Rosa Co. Milton Fl Type Screening static Test Beginning: Date 7-15-87 Time 1020

NPDES Number FL0021903 Organism C Dubia Test Ending: Date 7-17-87 Time 1020

Outfall Number 001 D. pulex N. loedsi

Test Concentration	Chamber Number	Live Organisms 0	24	48	Dissolved Oxygen (mg/L) 0	24	48	pH 0	24	48	Temperature °C 0	24	48	Conductivity 0	48	Ammonia Total 0	48	Ammonia Unionized 0	48
control	72	10	10	10	N/R	8.3	8.7	8.0	8.1	8.0	19.8	20.1	20.4			0		0	
control	20	10	10	10	8.6	8.3	6.7	8.0	8.1	8.0									
50%	67	10	10	10	8.4	6.0	6.1	7.4	7.6	7.7	19.7	20.1	20.8						
50%	98	10	10	9	8.4	6.0	6.1	7.4	7.6	7.7				MLR					
100%	2	10	10	4	2.8	5.4	6.1	6.9	7.3	7.5	19.6	20.1	20.8	383		353		.01	
100%	50	10	10	4	7.8	5.7	6.1	6.9	7.3	7.4									
control	57	10	10	10	8.7	8.1	8.4	8.0	8.0	7.9	19.8	20.4	20.4			0		0	
control	11	10	10	10	8.7	8.1	8.4	8.0	8.0	7.9				200	225				
50%	2	10	10	10	8.4	6.1	7.4	7.3	7.5	7.5	19.7	20.5	20.3						
50%	14	10	10	10	8.4	6.1	7.4	7.3	7.5	7.5				225	230				
100%	11A	10	10	10	7.6	8.0	6.4	6.9	7.5	7.3	19.9	20.5	20.3						
100%	22	10	10	10	7.6	8.0	6.6	6.9	7.5	7.3				245	246	353		.01	
50%	35	5	4	4	8.0			7.1	8.1		20.1					0			
50%	62	5	5	5	8.0			7.1	8					225					
100%	30	5	5	5	7.6			6.8			20.6								
100%	46	5	5	5	7.6			6.8						250		353		.01	
		MLR	MLR	MLR	MLR			MLR			MLR	MLR		MLR		RDS		MLR	

NOTES

Control Water

Total Alkalinity (mg/l $CaCO_3$) 115

Total Hardness (mg/l $CaCO_3$) 109

100% Sample

Total Alkalinity (mg/l $CaCO_3$) 75

Total Hardness (mg/l $CaCO_3$) 24 MLR

Age of Test Organisms Fish 2 days

Weight ____

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

0 Hours — 20 Test #1

24 Hours

48 Hours

Reviewed by

Date 9/29/87