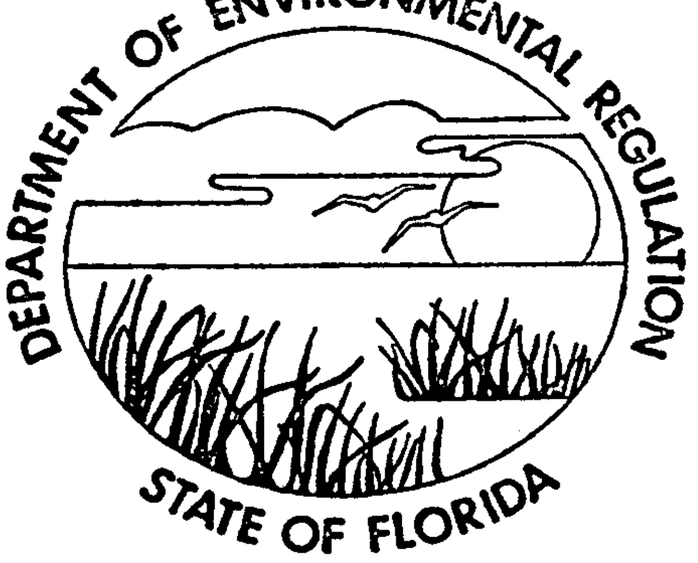


BIOASSAYS OF THE FT. MYERS CENTRAL SEWAGE TREATMENT PLANT FT. MYERS, LEE COUNTY, FLORIDA

NPDES #FL0021261

SAMPLED 12/17/85

MARCH 7, 1986

Biology Section
Division of Environmental Programs

BIOASSAYS OF THE FT. MYERS CENTRAL SEWAGE TREATMENT PLANT FT. MYERS, LEE COUNTY, FLORIDA NPDES #FL0021261

Biology Section
Bureau of Laboratories and
Special Programs
March 7, 1986

EXECUTIVE SUMMARY

Ft. Myers Central Sewage Treatment Plant, Lee County, NPDES #FL0021261, on 18 to 20 December, 1985.

Bioassays performed on samples of chlorinated and de-chlorinated effluent from this domestic waste treatment facility indicated that the samples were not acutely toxic to the test organisms, Daphnia pulex, Notropis leedsi, or Mysidopsis bahia. Only a few random M. bahia died during the 48 hour static tests. The residual chlorine detected in the final effluent (0.1 mg/l HACH) was insufficient to have caused any mortality. Ammonia was not detected in the effluent.

Result of chemical analyses indicated the presence of a few unidentified organic compounds and the metals beryllium (14 ug/l), lead (7 ug/l), and zinc (27 ug/l) in the final effluent.

INTRODUCTION

The Ft. Myers Central Sewage Treatment Plant is located at 1501 Raleigh Street, Ft. Myers, Lee County, Florida (Fig. 1). This 9.0 MGD design flow facility uses a 5.0 MGD pure oxygen activated sludge process and a 4.0 MGD trickling filter to treat primarily domestic waste (Fig. 2). Minor industrial facilities such as laundry and dry cleaning establishments and dairy products distributors may also contribute to this facility. Final effluent is chlorinated and then discharged to the Caloosahatchee River through a pipe 1300 feet from shore and five feet deep (data from NPDES file).

At the request of DER's South Florida District, the Biology Section performed static acute toxicity bioassays on samples of final chlorinated and de-chlorinated effluent from this facility, to determine the existence and extent of toxicity. The tests were peformed from 18 to 20 December, 1985. The results are presented in this report. Those involved in performing the tests and in preparation of this report were: R. B. Frydenborg, M. L. Roll and P. G. Weiland.

Figure 1.

LOCATION MAP

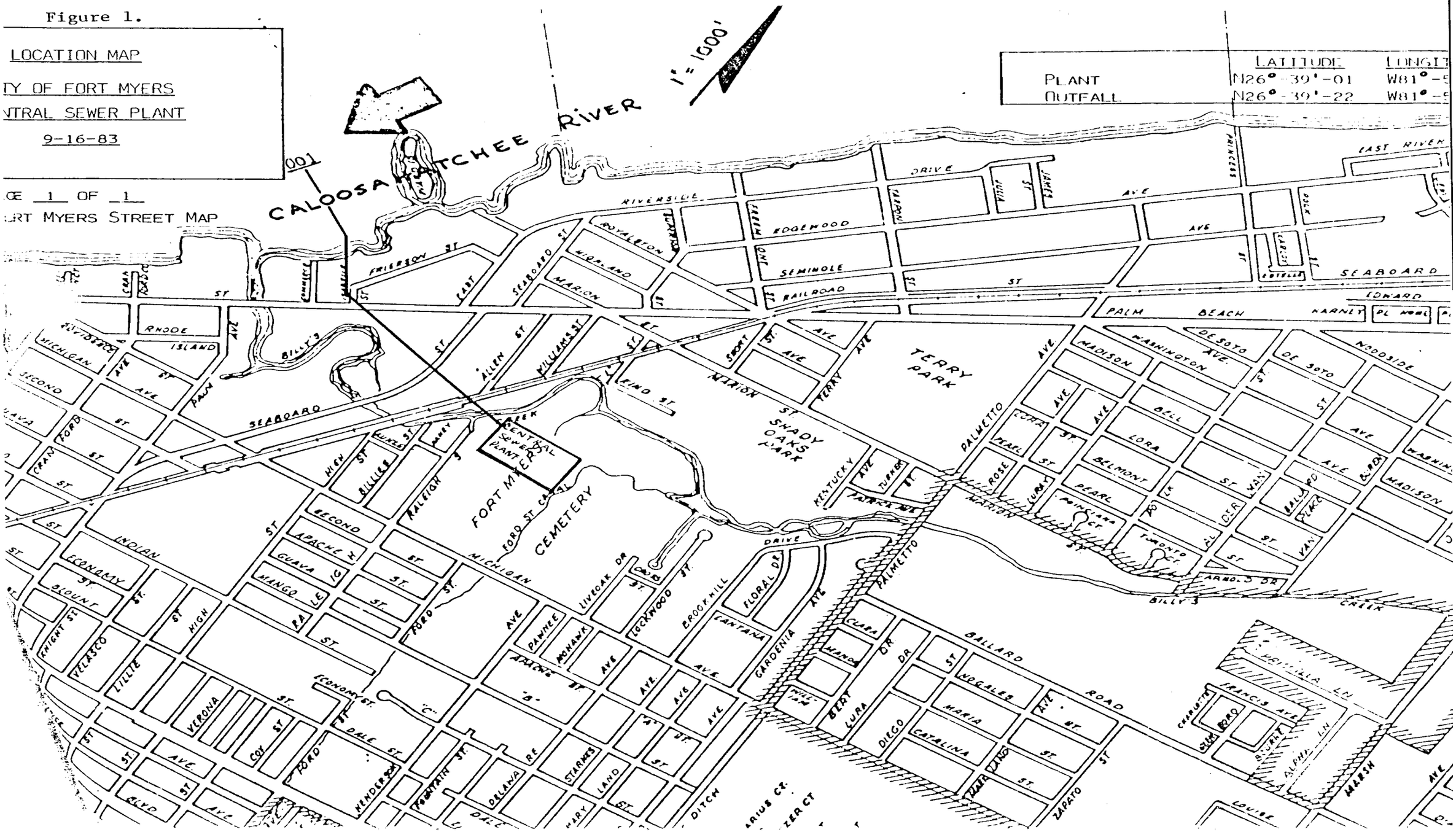
TY OF FORT MYERS

NTRAL SEWER PLANT

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RT MYERS STREET MAP



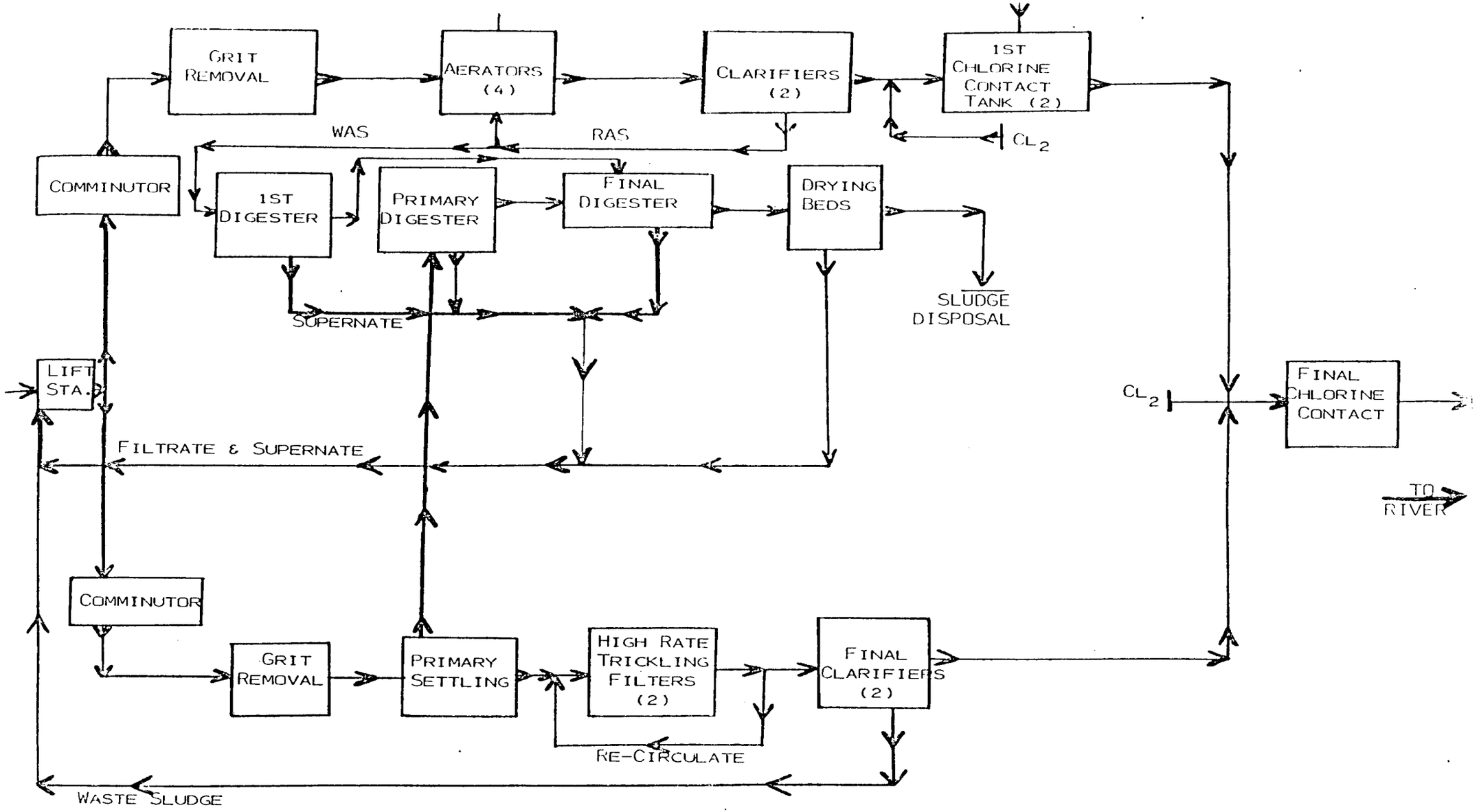


Figure 2. Waste Treatment and Flow.

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 labortory 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.

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 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 separted by 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 eliminated 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 crusted Tetra Min®. The fish used in this test were approximately three weeks old at the time of the test.

Mysidopsis bahia (mysid shrimp) - In September, 1978, approximately 300 adult mysid shrimp were obtained from the EPA laboratory in Gulf Breeze, Florida. The mysids are cultured under a 16:8 hour light-dark cycle in 10 and 15 gallon all-glass aquaria equipped with undergravel filtration and aeration. The mysid shrimp are cultured in several salinities (15-25 ppt) to minimize acclimation periods to low or high

salinity effluents. Seawater, used for culturing the test organisms and as diluent in the bioassays, is prepared by mixing artificial sea salts (Forty Fathoms®) and well water to obtain a final salinity of approximately 20 ppt. Freshwater, for dilution of the seawater as necessary, is obtained from a deep well near the DER laboratory. The mysids are fed twice daily and once daily on weekends with freshly hatched Artemia (brine shrimp) and are kept at a constant temperature of 23° ± 2°C. Adult or large juvenile mysids were used in this 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 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

A total of six separate 48 hour static acute toxicity bioassays were performed on samples of final chlorinated and de-chlorinated effluent from the Ft. Myers Central Sewage Treatment Plant. The tests were performed from 1010 hours on 18 December to 1010 hours on 20 December, 1985.

The samples were collected by Mr. Albert S. Walton of DER's South Florida District at 1107 hours on 16 December, 1985, 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 labortory in Tallahassee for analyses within 48 hours of collection.

Test Temperature

Upon arrival of the iced (4°C) bioassay samples in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the samples allowed to rise to 20° ± 2°C. Although this temperture 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.

Salinity Adjustment

Since this particular bioassay sample eventually enters an estuarine environment, the Biology Section used a saltwater test organism to help evaluate toxicity. Therefore, artificial sea salts (Forty Fathoms®) were added to portions of the samples until salinities of approximately 21-22 ppt were obtained. This salinity is well within the salinity tolerance of the test organism. The control used for this test and for use as the diluent consisted of artificial seawater at a similar salinity of approximately 20 ppt.

pH Adjustment

Since the toxicity, or lack of toxicity, in a bioassay sample may be dependent on the sample's pH, the Biology Section prefers, and recommends, that the pH of a sample at the start of the bioassay be similar to the pH measured during sampling. In this particular case, the salinity adjusted samples used in the saltwater tests were adjusted with an acid (0.1N HCl). This was necessary due to the pH having increased between sampling and initiating the test. This pH increase was likely a result of salinity adjusting the samples. The pH of the saltwater samples was adjusted to $\pm$ 0.3 s.u. of the value measured at sampling. This procedure provides the fairest test possible considering the varied chemical substances in complex wastewaters, the toxicities of which are dependent on pH.

Total Residual Chlorine

Since many wastewaters 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 wastewater. To accomplish this, the Biology Section performed concurrent bioassays on a sample chlorinated wastewater 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 effluents 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 wastewater.

Freshwater Tests

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 is presented in Table 1.

Two replicates, of each concentration and a control, were used to test with the water flea, Daphnia pulex. 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 were siphoned into a large culture dish to isolate the proper size and age organisms (neonates). To accomplish this, the water fleas were siphoned through a stainless steel screen. The trapped adults were then discarded. These neonates were then transferred by glass pipette, examined for injury, and were loaded 10 per chamber, resulting in 20 per test concentration. Water fleas were not fed during these tests.

Two replicates, of each concentration and a control, were used for the Notropis leedsi (bannerfin shiner) bioassays. The fish were transferred individually or by twos, into wide mouth quart jars containing 500 ml each of the appropriate test concentration, and were loaded 5 per chamber, resulting in 10 per concentration. Fish were not fed during these tests.

Saltwater Tests

The concentration series selected for the saltwater tests is presented in Table 1. Two replicates of each test concentration and of the control were used to test with Mysidopsis bahia. Immediately prior to the bioassays, a suitable number of mysids were transferred by net from the appropriate salinity aquarium to a culture dish for observation and selection of individuals for the tests. Individual mysid shrimp were siphoned using a glass tube, carefully examined for injury, and transferred to wide mouth quart jars, each containing 500 ml of the appropriate concentration. For each concentration, the mysid shrimp were pre-acclimated to within 4 ppt salinity. Five organisms were loaded per test chamber, giving ten per concentration. Based on the recommendations of EPA (1978), the mysids were fed brine shrimp (Artemia) nauplii at the rate of approximately 10 to 20 nauplii per mysid per day to minimize cannibalism.

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 control and 100% effluent concentration. The specific conductance or salinity (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 control and the 100% effluent 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 through 5.

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

Daphnia pulex (water flea)

Concentration	Dilution Water	Effluent	Total Volume
Control	200.0 ml	---------	200 ml
10.0%	180.0 ml	20.0 ml	200 ml
50.0%	100.0 ml	100.0 ml	200 ml
100.0%	--------	200.0 ml	200 ml

Notropis leedsi (bannerfin shiner)

Mysidopsis bahia (mysid shrimp)

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

Table 2. Bioassay of Ft. Myers Central Sewage Treatment Plant, NPDES #FL0021261, on 18 to 20 December, 1985.
Daphnia pulex: Chlorinated [a] Effluent.

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
Control	10	10	10	8.2	8.5	8.0	7.8	8.1	8.3	21.2	22.4	20.5		
Control	10	10	10	8.2	8.5	8.0	7.8	8.1	8.3				238	272
10.0%	10	10	10	8.2	8.4	8.0	7.7	8.0	8.3	21.2	22.1	20.3		
10.0%	10	10	10	8.2	8.4	8.0	7.7	8.0	8.3				330	358
50.0%	10	10	10	8.2	8.0	8.0	7.5	7.9	8.2	20.8	22.0	20.2		
50.0%	10	10	10	8.2	8.0	8.0	7.5	7.9	8.2				720	805
100.0%	10	10	10	8.4	8.0	7.8	7.3	7.8	8.2	20.3	22.1	20.2		
100.0%	10	10	10	8.4	8.0	7.8	7.3	7.8	8.2				1220	1320

Control Water

NH_3(as NH_3)(mg/l) 0

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

Total Alkalinity (mg/l $CaOO_3$) 110

Total Hardness (mg/l $CaOO_3$) 115

100% Effluent

NH_3(as NH_3)(mg/l) 0

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

Total Alkalinity (mg/l $CaOO_3$) 115

Total Hardness (mg/l $CaOO_3$) 223

[a] Total Residual Chlorine: 0.1 mg/l (HACH DPD Method).

Table 3. Bioassays of Ft. Myers Sewage Treatment Plant, NPDES #FL0021261, on 18 to 20 December, 1985.
Daphnia pulex; De-Chlorinated [a] Effluent.

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
Control	10	10	10	8.3	8.4	7.9	7.8	8.1	8.4	21.3	22.1	20.2		
Control	10	10	8	8.3	8.4	7.9	7.8	8.1	8.4				229	257
10.0%	10	10	10	8.3	8.3	7.9	7.7	8.0	8.3	21.3	22.0	20.2		
10.0%	10	10	10	8.3	8.3	7.9	7.7	8.0	8.3				330	349
50.0%	10	10	10	8.3	8.2	7.8	7.5	7.9	8.3	21.0	22.0	20.3		
50.0%	10	10	10	8.3	8.2	7.9	7.5	7.9	8.3				720	805
100.0%	10	10	10	8.4	7.5	7.8	7.3	7.7	8.2	20.7	22.0	20.4		
100.0%	10	10	10	8.4	7.5	7.8	7.3	7.7	8.2				1220	1330

Control Water

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

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

Total Alkalinity (mg/l $CaCO_3$): 110

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

100% Effluent

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

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

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

Total Hardness (mg/l $CaCO_3$): 223

[a] Total Residual Chlorine: None Detected (HACH DPD Method).

Table 4. Bioassays of Ft. Myers Sewage Treatment Plant, NPDES #FL0021261, on 18 to 20 December, 1985.
Notropis leedsi; Chlorinated and De-Chlorinated Effluent.

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
Chlorinated Effluent														
Control	5	5	5	8.3	8.2	7.7	7.7	8.0	8.3	21.2	21.7	20.1		
Control	5	5	5	8.3	8.2	7.7	7.7	8.0	8.3				232	249
50.0%	5	5	5	8.3	8.2	7.7	7.5	7.8	8.2	20.6	21.6	19.9		
50.0%	5	5	5	8.3	8.2	7.7	7.5	7.8	8.2				730	795
100.0%	5	5	5	8.4	8.1	7.6	7.3	7.7	8.1	20.0	21.6	19.9		
100.0%	6[a]	6	6	8.4	8.1	7.6	7.3	7.7	8.1				1220	1310
De-chlorinated Effluent														
Control	5	5	5	8.3	8.2	7.7	7.7	8.0	8.3	21.2	21.7	20.1		
Control	5	5	5	8.3	8.2	7.7	7.7	8.0	8.3				232	249
50.0%	5	5	5	8.4	8.2	7.6	7.5	7.8	8.2	20.8	21.8	19.9		
50.0%	5	5	5	8.4	8.2	7.6	7.5	7.8	8.2				730	820
100.0%	5	5	5	8.5	8.0	7.4	7.3	7.7	8.1	20.5	21.8	20.1		
100.0%	5	5	5	8.5	8.0	7.4	7.3	7.8	8.1				1230	1310

Control Water

NH_3(as NH_3)(mg/l) 0

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

Total Alkalinity (mg/l $CaCO_3$) 110

Total Hardness (mg/l $CaCO_3$) 115

100% Effluent

NH_3(as NH_3)(mg/l) 0

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

Total Alkalinity (mg/l $CaCO_3$) 115

Total Hardness (mg/l $CaCO_3$) 223

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

Tabel 5. Bioassay of Ft. Myers Sewage Treatment Plant, NPDES #FL0021261, on 18 to 20 December 1985.
Mysidopsis bahia; Chlorinated and De-chlorinated Effluent.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Chlorianted Effluent														
Control	5	5	5	7.2	6.9	6.5	8.3	8.1	8.3	21.0	21.7	20.0		
Control	5	5	5	7.2	6.9	6.5	8.3	8.1	8.3				20.0	18.0
50.0%	5	5	4	7.5	6.9	6.3	7.8	7.9	8.1	20.5	21.5	19.8		
50.0%	5	5	5	7.5	6.9	6.3	7.8	7.9	8.1				20.3	18.8
100.0%	5	5	5	7.8	6.7	6.3	7.2	7.7	8.0	20.1	21.5	19.8		
100.0%	5(4)[a]	4	4	7.8	6.7	6.3	7.2	7.7	8.0				21.0	19.3
De-chlorinated Effluent														
Control	5	5	5	7.2	6.9	6.5	8.3	8.1	8.2	21.0	21.7	20.0		
Control	5	5	5	7.2	6.9	6.5	8.3	8.1	8.3				20.0	18.0
50.0%	5	5	5	7.5	6.5	6.3	7.9	7.9	8.1	20.7	21.7	19.9		
50.0%	5	4	3	7.5	6.5	6.3	7.9	7.9	8.1				20.8	19.3
100.0%	5	5	5	7.8	6.5	6.2	7.2	7.7	8.0	20.5	21.7	20.0		
100.0%	5	5	5	7.8	6.5	6.2	7.2	7.7	8.0				22.0	20.3

Control Water	
NH_3(as NH_3)(mg/l) 0	Total Alkalinity (mg/l $CaOO_3$)
Total ammonia (as N)(mg/l) 0	Total Hardness (mg/l $CaOO_3$)

100% Effluent	
NH_3(as NH_3)(mg/l) 0	Total Alkalinity (mg/l $CaOO_3$)
Total ammonia (as N)(mg/l) 0	Total Hardness (mg/l $CaOO_3$)

[a] Number in parentheses used since one organism jumped from container.

Table 6. Results of Chemical Analyses Performed on Final Effluent from the Ft. Myers Central Sewage Treatment Plant, NPDES #FL0021261, Collected 16 December, 1985.

Organics

Pesticide Extractables:	Several unidentified compounds

Metals

Antimony	N.D.
Arsenic	N.D.
Beryllium	14 ug/l
Cadmium	N.D.
Chormium	N.D.
Copper	N.D.
Lead	7 ug/l
Mercury	N.D.
Nickel	N.D.
Selenium	N.D.
Silver	< 0.2 ug/l
Thallium	N.D.
Zinc	27 ug/l

N.D. = None Detected

RESULTS AND DISCUSSION

Bioassays performed on samples of chlorinated and de-chlorinated effluent from the Ft. Myers Central waste treatment facility indicated that the samples were not acutely toxic to the test organisms, Daphnia pulex, Notropis leedsi, or Mysidopsis bahia. Only a few random M. bahia died during the 48 hour static tests (Table 5). The residual chlorine detected in the final effluent (0.1 mg/l HACH) was insufficient to have cause any mortality. Ammonia was not detected in the effluent.

Results of chemical analyses (Table 6) indicated the presence of a few unidentified organic compounds and the metals beryllium (14 ug/l), lead (7 ug/l), and zinc (27 ug/l) in the final effluent.

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.

U.S. Environmental Protection Agency. 1978. Bioassay procedures for the Ocean Disposal Permit Program. Environmental Research Laboratory. Gulf Breeze, Florida. EPA-600/9-78-010. 121 p.