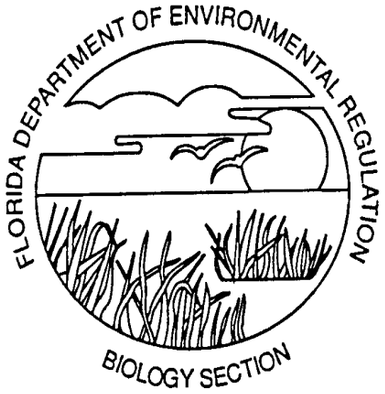


Bioassays of Clearwater East STP

Clearwater, Pinellas County, Florida

NPDES #FL0021865

Biology Section
Bureau of Laboratories
August 16, 1988

Executive Summary

Clearwater East Sewage Treatment Plant, Clearwater, Pinellas County, NPDES #FL0021865, on 2–4 February, 1988.

The sample of final chlorinated discharge from this 5.2 MGD domestic treatment facility proved to be acutely toxic to the test organisms, *Daphnia pulex*, and *Notropis leedsi*, producing 48 hour LC_{50}'s of less than 10% (i.e., all animals died in the lowest (10% sample) test concentration). This toxicity can be only partially attributed to the total residual chlorine (2.0 mg/l Iodometric) as the tests using dechlorinated sample were toxic to the test organisms, *Ceriodaphnia dubia*, *Daphnia pulex*, and *Notropis leedsi*, producing 48 hour LC_{50}'s of 40.5%, 79.2%, and 85.2%, respectively. The Microtox™ assay, using the bacterium *Photobacterium phosphorum*, was also toxic resulting in EC_{50}'s of 108.7% (chlorinated) and 123.7% (dechlorinated). The unionized ammonia concentration of 0.15 mg/l found in the sample is insufficient to account for the toxicity observed.

Chemical analyses indicated the presence of several pesticides including a probably toxic concentration of Diazinon (0.4 µg/l), and of the metals zinc (17 µg/l) and lead (3.5 µg/l), neither at levels likely to cause toxicity.

We recommend that the Clearwater East facility be required to meet the state toxicity standards or to undertake a Toxicity Reduction Evaluation with the goal of meeting those standards. Additionally, we recommend that the facility be required to reduce residual chlorine concentrations in their effluent.

Introduction

The Clearwater East Sewage Treatment Plant is located in Clearwater, Pinellas County, Florida (Figure 1). This facility is a 5.2 MGD activated sludge wastewater treatment plant with chlorinated discharge to Old Tampa Bay (Figure 2). The facility has been in violations of its permit on numerous previous occassions (e.g., 7/77, 2/81, 2/82, 12/84, 3/85) and had its permit amended based on a review of circumstances in 1985. Clearwater East has been scheduled to change to deep-well discharge since 1983 (the most recent attempt was scheduled for 6/88) but has not been successful in the conversion to date.

Bioassays were performed from February 2–4, 1988, on samples of the final treated and chlorinated effluent and on a dechlorinated subsample. The tests were performed using the organisms, *Ceriodaphnia dubia* and *Daphnia pulex* (water fleas) and *Notropis leedsi* (the bannerfin shiner). Those involved in performing the tests and in preparation of this report were D.A. Chasteen, J.M. Faircloth, and S.H. Wolfe.

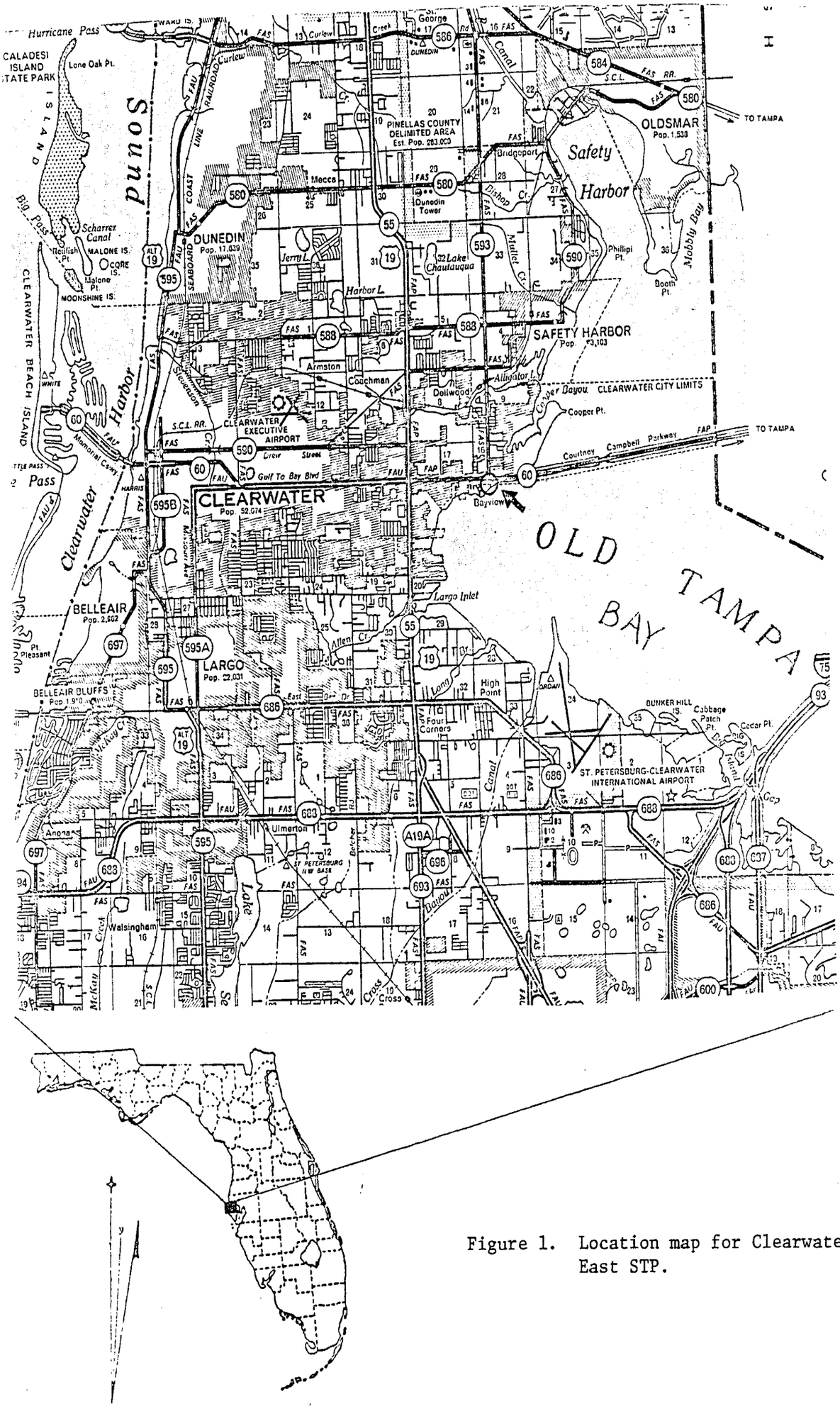


Figure 1. Location map for Clearwater East STP.

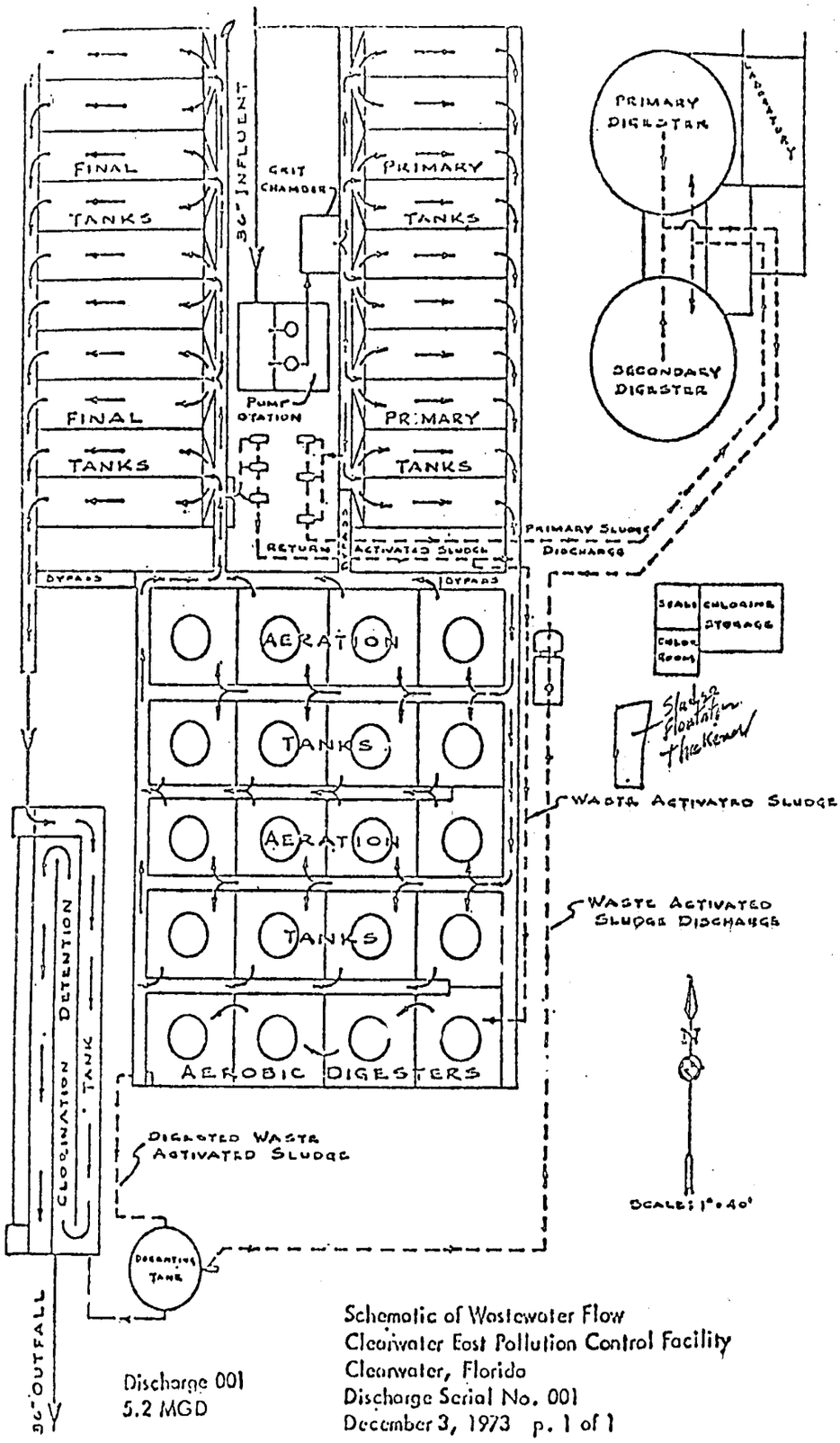


Figure 2. Plant layout

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), 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.

The Microtox® assay was run according to the manufacturer's protocol described in the Microtox® System Operating Manual (Beckman 1982).

1. 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 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 Aquarian® flake food 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 Aquarian® flake food. The fish used were 23 days old when the test commenced.

Photobacterium phosphoreum, a luminescent marine bacterium, is maintained as a freeze-dried culture, purchased from Microbics Corporation. Lot number M-606 was used in this assay. Immediately prior to the assay, the culture is rehydrated with 1.0 ml of specially purified water (Microtox Reconstitution Solution).

2. Standard Reference Toxicity Assays

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.

3. 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

Separate samples were obtained for chemical and biological testing. Samples for metal analyses were preserved with $HN0_3$. Samples for organic chemical analyses and for the bioassays were unpreserved. The samples were collected by using standard wastewater sampling procedures. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 24 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 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 reclaimed 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 reclaimed water. To accomplish this, the Biology Section performed concurrent bioassays on a sample of chlorinated reclaimed water in which the chlorine had been neutralized. Dechlorination was accomplished by adding a calculated amount of 0.025N sodium thiosulfate. The total residual chlorine concentrations of the tested sample of reclaimed water 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 reclaimed water.

Method

Freshwater for diluting the water samples being tested 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 the control and each test concentration were used in bioassays 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, respectively, 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. Ten were loaded per chamber, resulting in 20 per test concentration. *Daphnia* were siphoned into a stainless steel screen chamber placed into a large culture dish to isolate the proper size and age organisms (neonates). Then the screen chamber along with any retained adults were removed. The neonates remaining were then captured by glass pipette, examined for injury, and loaded 10 per chamber, resulting in 20 per test concentration. The 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 using a pipette into wide mouth quart jars containing 500 ml each of the appropriate test concentration. Five were loaded 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 Fisher Accuphast® 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% reclaimed water concentration. The specific conductance and 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 before use according to manufacturer's recommendations. Ammonia concentrations, including the total and unionized fraction, were recorded in the controls and the 100% reclaimed water 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.

Microtox® Test

Upon receipt at the DER laboratory, a 20 ml subsample was collected for Microtox® analysis. The sample was adjusted to 2% salinity with reagent grade dry sodium chloride to maintain the osmotic balance of the test bacterium during the assay. Four two-fold dilutions were made of the test sample resulting in a test range of 97%, 49.5%, 24.75%, 12.38%, and 0%. An incubation temperature of 15 °C was maintained throughout the assay. Ten µl of bacterial suspension was added to each of the 1.0 ml test dilutions. Light output (luminescence) was read after 5 and 15 minutes incubation, using the Microtox® analyzer and recorded on a Perkin-Elmer strip chart recorder. In the presence of a toxicant, the luminescence of the test organisms will decrease. In the presence of a stimulant, the luminescence will increase. Toxicity is calculated as a function of the light lost in the test sample, normalized to the control, and is reported as the effective concentration at which a 50% reduction, of light output is determined in 15 minutes (EC_{50}). Results are given in Table 4.

Results and Discussion

The sample of final chlorinated discharge water from the ~~Cooper City~~ CLEARWATER East Wastewater Treatment Plant proved to be acutely toxic to the test organisms, *Daphnia pulex* and *Notropis leedsi,* producing 48 hour LC_{50}'s of <10% in each bioassay (Table 5). The test of chlorinated discharge on *Ceriodaphnia dubia* was invalidated because of laboratory accident-induced mortality. However, this test indicated the same toxicity. This toxicity can be partially attributed to the total residual chlorine (2.0 mg/l Iodometric) measured in the sample.

The sample of discharge water that had been dechlorinated was acutely toxic to *Ceriodaphnia dubia, Daphnia pulex,* and *Notropis leedsi,* with respective LC_{50}'s of 40.5%, 79.2%, and 85.2%. Unionized ammonia was calculated to be 0.2 mg/l, which probably contributed to the toxicity.

Chemical analyses (Table 6) indicated the presence of three pesticides—δ-BHC (0.03 µg/l), γ-BHC (0.03 µg/l), and diazinon (0.4 µg/l), and four metals—lead (3.5 µg/l) and zinc (17 µg/l). The δ-BHC and γ-BHC are in trace concentrations unlikely to result in toxicity. The diazinon concentration is sufficient to cause toxicity, and probably accounts for most of that observed. The metals are in concentrations unlikely to cause toxicity.

Biology Section Recommendations

We recommend that the facility be required to meet the State toxicity standards or to undertake a Toxicity Reduction Evaluation with the goal of meeting those standards. Additionally, we recommend that the ~~Cooper City~~ CLEARWATER East facility be required to reduce residual chlorine concentrations in their effluent.

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

Ceriodaphnia dubia (water flea)

Concentration	Dilution Water	Sample	Total Volume
Chlorinated			
Control	50.0 ml	0.0 ml	50 ml
10.0%	45.0 ml	5.0 ml	50 ml
30.0%	35.0 ml	15.0 ml	50 ml
60.0%	20.0 ml	30.0 ml	50 ml
Dechlorinated			
Control	50.0 ml	0.0 ml	50 ml
30.0%	35.0 ml	15.0 ml	50 ml
60.0%	20.0 ml	30.0 ml	50 ml
100.0%	0.0 ml	50.0 ml	50 ml

Daphnia pulex (water flea)

Concentration	Dilution Water	Sample	Total Volume
Chlorinated			
Control	200.0 ml	0.0 ml	200 ml
10.0%	180.0 ml	20.0 ml	200 ml
30.0%	140.0 ml	60.0 ml	200 ml
60.0%	80.0 ml	120.0 ml	200 ml
Dechlorinated			
Control	200.0 ml	0.0 ml	200 ml
30.0%	140.0 ml	60.0 ml	200 ml
60.0%	80.0 ml	120.0 ml	200 ml
100.0%	0.0 ml	200.0 ml	200 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Sample	Total Volume
Chlorinated			
Control	500 ml	0 ml	500 ml
10.0%	450 ml	50 ml	500 ml
30.0%	350 ml	150 ml	500 ml
60.0%	200 ml	300 ml	500 ml
Dechlorinated			
Control	500 ml	0 ml	500 ml
30.0%	350 ml	150 ml	500 ml
60.0%	200 ml	300 ml	500 ml
100.0%	0 ml	500 ml	500 ml

Table 2. Data recorded during 48 hour static acute toxicity bioassays of Clearwater East Sewage Treatment Plant, chlorinated discharge, on 2 to 4 February, 1988.

Facility: **Clearwater East STP** — NPDES #: **FL0021865** — Facility Type: **Municipal STP**
Location: Clearwater — County: Pinellas — Contact/District: Dave Demmi/SW
Test type: static screen
Sample Collection Date: 2/1/88 — Time: 1015 — # tests: 6 — Receiving Water: Old Tampa Bay
Test Beginning Date: 2/2/88 — Time: 1000 — Chlorination Type: chlorinated
Test Ending Date: 2/4/88 — Time: 1000

Parameter		
Total Residual Cl2:	2.1	2.0
Method:	Hach	Iodometric
Salinity (ppt):	0	

Ammonia:	Control Water	100% Sample
Unionized NH3:	0.00	0.15
Total NH3:	0.00	12.10

Organism: ***Ceriodaphnia dubia*** — Life stage: neonate
LC50: — — Binomial: — — Moving Average: — — Probit: —

SURVIVAL				Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hr	Live at 24 hr	Live at 48 hr	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	48 hour
Control	10	0	0	8.6	8.6	-	7.9	7.8	-	20.0	20.7	-	-	-
Control	10	10	10	8.7	8.6	-	7.9	7.8	-	-	-	-	-	-
10%	10	0	-	8.8	8.8	-	7.8	7.7	-	20.8	20.3	-	-	-
10%	10	0	-	8.8	8.8	-	7.7	7.9	-	-	-	-	-	-
30%	10	0	-	8.4	8.9	-	7.5	8.0	-	20.8	20.1	-	-	-
30%	10	0	-	8.4	8.9	-	7.5	8.0	-	-	-	-	-	-
60%	10	0	-	8.2	8.8	-	7.4	8.1	-	20.9	19.4	-	-	-
60%	10	0	-	8.2	8.8	-	7.4	8.1	-	-	-	-	-	-

	Control Water	100% Sample
Alkalinity:	113	251
Hardness:	116	200

Organism: ***Daphnia pulex*** — Life stage: neonate
LC50: <10% Binomial: — — Moving Average: — — Probit: —

SURVIVAL				Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hr	Live at 24 hr	Live at 48 hr	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	48 hour
Control	10	10	10	8.8	8.7	-	8.0	7.9	-	20.7	20.5	-	-	-
Control	10	10	10	8.8	8.7	-	8.0	7.9	-	-	-	-	175	-
10%	10	0	-	8.8	8.9	-	7.8	8.0	-	20.9	20.1	-	-	-
10%	10	0	-	8.8	8.9	-	7.8	8.0	-	-	-	-	238	-
30%	10	0	-	8.5	9.0	-	7.6	8.0	-	21.0	19.8	-	-	-
30%	10	0	-	8.5	9.0	-	7.6	8.0	-	-	-	-	350	-
60%	10	0	-	8.2	9.0	-	7.4	8.0	-	21.0	19.9	-	-	-
60%	10	0	-	8.2	9.0	-	7.4	8.0	-	-	-	-	590	-

	Control water	100% sample
Alkalinity:	113	251
Hardness:	116	200

Organism: ***Notropis leedsi*** — Age: 23 days
LC50: <10% Binomial: — — Moving Average: — — Probit: —

SURVIVAL				Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hr	Live at 24 hr	Live at 48 hr	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	48 hour
Control	5	5	5	8.5	8.4	-	7.4	7.8	-	21.0	20.4	-	-	-
Control	5	5	5	8.5	8.4	-	7.4	8.8	-	-	-	-	208	-
10%	5	0	-	8.3	8.8	-	7.4	7.8	-	21.1	19.9	-	-	-
10%	5	0	-	8.4	8.8	-	7.4	7.8	-	-	-	-	270	-
30%	5	0	-	8.2	8.9	-	7.4	7.9	-	21.1	19.7	-	-	-
30%	5	0	-	8.2	8.9	-	7.4	7.9	-	-	-	-	380	-
60%	5	0	-	8.1	8.9	-	7.4	7.9	-	21.1	19.7	-	-	-
60%	5	0	-	8.2	9.0	-	7.4	7.9	-	-	-	-	600	-

	Control water	100% sample
Alkalinity:	118	251
Hardness:	119	200

Table 3. Data recorded during 48 hour static acute toxicity bioassays of Clearwater East Sewage Treatment Plant, dechlorinated discharge, on 2 to 4 February, 1988.

Facility: **Clearwater East STP** | NPDES #: **FL0021865** | Facility Type: **Municipal STP**
Location: Clearwater | County: Pinellas | Contact/District: Dave Demmi/SW
Test type: static screen
Sample Collection Date: 2/1/88 | Time: 1015 | # tests: 6 | Receiving Water: Old Tampa Bay
Test Beginning Date: 2/2/88 | Time: 1000 | Chlorination Type: **dechlorinated**
Test Ending Date: 2/4/88 | Time: 1000

Parameter		
Total Residual Cl2:	2.1	2.0
Method:	Hach	Iodometric
Salinity (ppt):	0	

Ammonia:	Control Water	100% Sample
Unionized NH3:	0.00	0.20
Total NH3:	0.00	12.10

	Control Water	100% Sample
Alkalinity:	113	251
Hardness:	116	200

Organism: ***Ceriodaphnia dubia*** | Life stage: neonate
LC50: Binomial: 40.5% | Moving Average: — | Probit: —

SURVIVAL				Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hr	Live at 24 hr	Live at 48 hr	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	48 hour
Control	10	10	10	8.7	8.6	8.5	8.0	7.9	7.9	20.7	20.2	20.1	-	-
Control	10	10	10	8.8	8.6	8.5	8.0	7.9	7.9	-	-	-	-	-
30%	10	10	10	8.5	8.7	8.7	7.6	8.0	8.0	20.7	20.2	20.0	-	-
30%	10	10	9	8.5	8.7	8.7	7.6	8.0	8.0	-	-	-	-	-
60%	10	0	0	8.3	8.7	8.7	7.6	8.2	8.1	20.8	20.3	20.0	-	-
60%	10	0	0	8.3	8.6	8.7	7.5	8.2	8.1	-	-	-	-	-
100%	10	8	0	7.8	8.7	8.6	7.5	8.3	8.2	21.3	20.1	20.1	-	-
100%	10	8	0	7.8	8.7	8.6	7.5	8.3	8.2	-	-	-	-	-

Organism: ***Daphnia pulex*** | Life stage: neonate
LC50: Binomial: 79.2% | Moving Average: 79.2% | Probit: 80.0%

SURVIVAL				Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hr	Live at 24 hr	Live at 48 hr	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	48 hour
Control	10	10	10	8.7	8.7	8.7	8.0	8.0	7.9	20.6	20.2	19.8	-	-
Control	10	10	10	8.8	8.8	8.7	8.0	8.0	7.9	-	-	-	200	195
30%	10	10	10	8.6	8.5	8.7	7.7	8.0	8.0	20.9	20.1	19.8	-	-
30%	10	10	10	8.6	8.5	8.6	7.7	8.0	8.0	-	-	-	380	360
60%	10	10	10	8.2	8.5	8.6	7.5	8.1	8.0	21.2	19.9	19.8	-	-
60%	10	10	9	8.2	8.5	8.3	7.5	8.0	8.0	-	-	-	600	550
100%	10	10	1	7.7	8.7	8.2	7.4	8.1	8.1	21.5	20.0	19.8	-	-
100%	10	10	1	7.8	8.7	8.2	7.4	8.1	8.1	-	-	-	850	780

	Control water	100% sample
Alkalinity:	113	251
Hardness:	116	200

Organism: ***Notropis leedsi*** | Age: 23 days
LC50: Binomial: 85.2% | Moving Average: — | Probit: —

SURVIVAL				Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hr	Live at 24 hr	Live at 48 hr	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	48 hour
Control	5	5	5	8.3	8.5	8.1	7.4	7.8	7.8	21.0	19.8	19.7	-	-
Control	5	5	5	8.6	8.5	8.1	7.5	7.8	7.8	-	-	-	208	210
30%	5	5	5	8.6	8.4	7.7	7.4	7.8	7.9	21.1	19.7	19.6	-	-
30%	5	5	5	8.3	8.3	7.7	7.4	7.9	7.9	-	-	-	380	382
60%	5	5	5	8.0	8.3	7.7	7.4	7.9	8.0	21.2	19.7	19.6	-	-
60%	5	5	5	8.1	8.3	7.4	7.4	7.9	8.0	-	-	-	600	590
100%	5	5	2	7.7	8.2	6.8	7.4	8.0	8.0	21.5	19.8	19.6	-	-
100%	5	5	0	7.7	8.2	6.5	7.4	8.0	8.0	-	-	-	850	800

	Control water	100% sample
Alkalinity:	118	251
Hardness:	119	200

Table 4. Microtox test results, Clearwater East STP discharge, Pinellas County, NPDES #FL0021865, on 2 to 4 February, 1988.

Microtox Test — Culture Lot # M-606

% Sample	Final Reclaimed Water			
	Chlorinated		Dechlorinated	
	5-min	15-min	5-min	15-min
0	86	90	91	61
0	83	87	87	60
0	85	93	95	66
12.38	31	30	87	56
12.38	13	9	85	55
12.38	15	10	88	57
24.75	5	2	74	42
24.75	6	3	86	56
24.75	2	1	79	51
49.5	2	0.8	71	43
49.5	2	0.3	69	43
49.5	0.5	0.3	72	45
99	0.2	0.2	52	34
99	0.2	0.1	54	35
99	0.2	0.1	53	36

EC_{50} Chlorinated (15 minute, 15 °C) = 6.04% sample
EC_{50} Dechlorinated (15 minute, 15 °C) = 123.7% sample

EC_{50} (15 minute, 15 °C) is calculated using the Data Reduction Computer Program developed by Microbics Corporation for the Sharp Model EL-5100 (Microbics) Systems Operating Manual, 1982.

Table 5. LC_{50} Data for Bioassay of Clearwater East STP.

Organism	Method of Data Analysis	48 Hour LC_{50} (95% Confidence Intervals)
Chlorinated discharge		
Ceriodaphnia dubia	Binomial	a,c
	Moving Average	c
	Probit	c
Daphnia pulex	Binomial	c
	Moving Average	c
	Probit	c
Notropis leedsi	Binomial	c
	Moving Average	c
	Probit	c
Dechlorinated discharge		
Ceriodaphnia dubia	Binomial	40.54% (30% and 60%)
	Moving Average	b
	Probit	b
Daphnia pulex	Binomial	79.22% (60% and 100%)
	Moving Average	79.22 % (72.81% and 86.64%),d
	Probit	79.96% (71.19% and 89.03%)
Notropis leedsi	Binomial	85.15% (60% and 9,999%)
	Moving Average	b
	Probit	b

a As the result of a laboratory accident resulting in control mortality, this test is invalid

b Due to an insufficient number of dead organisms, or to the irregular distribution of mortality, an LC_{50} value cannot be calculated using this method of statistical analysis.

c The LC_{50} is < 10% since all the organisms died in the lowest test concentration (10%).

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

Table 6. Chemical constituents present in the discharge water from the Clearwater East Sewage Treatment Plant, NPDES #FL0021865, on 1 February 1988.

	Organics	
Pesticide extractables		
	δ-BHC	0.3 µg/l
	γ-BHC	0.3 µg/l
	diazinon	0.4 µg/l
	Metals	
	lead	3.5 µg/l
	zinc	17 µg/l

Literature Cited

American Public Health Association. 1980. Standard methods for the examination of water and wastewater. 15th ed. 1,134 p.

Beckman Instruments, Inc. 1982. Microtox system operating manual.

Florida Department of Environmental Regulation. 1985. Quality assurance manual for performing acute toxicity tests (Second Edition). Biology Section, Tallahassee.

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.

Thurston, R.V., Russo, R.C. and G.A. Vinogradov. 1981. Ammonia toxicity to fishes: Effect of pH on the toxicity of the unionized species. Env. Sci. Tech. 15(7): 837-840.

U.S. Environmental Protection Agency. 1976. Quality criteria for water. U.S. EPA, Washington, D.C. 256 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.

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. Applicable portions of the F.A.C. are: 17-3.021(l), 17-3.021(4), 17-3.051(1)(d), 17-3.061(3)(p), 17-3.402(1), 17-4.244(4), and 17-4.244(6).

The particular restrictions applied to a given discharger depend upon the type of discharge, the type of water body which is impacted, and the type of mixing zone, if any, which has been permitted. The most stringent toxicity limitations which apply to the various combinations of these factors are listed below, specified in terms of the most dilute concentration of effluent which is allowed to kill 50% of the test organisms in 96 hours. Note that any listed concentration greater than 100% is extrapolated from toxicity curves determined from testing various effluent concentrations of 100% or less. Violations of the toxicity standards occur in the following conditions.

I. Discharges to Surface Water

   A. With no mixing zone, or with only a dredge and fill mixing zone the discharge may not exhibit an $LC_{50} < 2000\%$ at the point of discharge.

   B. With a non-dredge and fill mixing zone the discharge may not exhibit an $LC_{50} < 100\%$ at the point of discharge and may not exhibit an $LC_{50} < 2000\%$ at the edge of the mixing zone.

   C. With a non-dredge and fill mixing zone, and either discharging to the open ocean through multiport diffusers or meeting the provisions of 17-4.244(4)(b) the LC_{50} at the point of discharge cannot exceed 30% sample concentration.

II. Discharges to ground water may not exceed an LC_{50} of 300% at the point of contact with surface waters

A violation of these provisions occurs if toxicity is found at a more dilute concentrations of the sampled discharge than allowed above.

TOXIC Cl_2

BIOASSAY DATA FORM

Industry/Toxicant Clearwater East STP
Address Clearwater
Contact/District Sid/Dave Demmi
Effluent Serial Number EB 001
NPDES Permit Number FL0021865

Test Number: 1 of 6
Test Type: ~~Static~~ Renewal, (Screening) Conventional
Other:

Sample Collection: Date 2/1/88 Time 1015
Test Beginning: Date 2/2/88 Time 1000
Test Ending: Date 2/4/88 Time 1000
Test Organism Ceriodaphnia dubia
Age/Life Stage neonates

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96
Control	75	10	0*	0			8.6	8.6				7.9	7.8				20.0	20.7			
Control	67	10	10	10			8.7	8.6				7.9	7.8				~~21.7~~				
10%	85	10	0	0			8.8	8.8				7.8	7.9				20.8	20.3			
10%	88	10	0	0			8.8	8.8				7.7	7.9				~~21.~~				
30%	63	10	0	0			8.4	8.9				7.5	8.0				20.8	20.1			
30%	44	10	0	0			8.4	8.9				7.5	8.0								
60%	21	10	0	0			8.2	8.8				7.4	8.1				20.9	19.4			
60%	49	10	0	0			8.2	8.8				7.4	8.1								
Initial of Investigater	DAC	MF	MF	DAC			MF	MF				MF	MF				MF	MF			

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 113/116
100% Sample: 251/260
Other:
Measured by: DAC

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: 0.0 | 0.0
100% Sample: | 12.1
Other:

Hour
Control Water:
100% Sample:
Other:
Measured by: MF

slope -55.6

Signature: Randall Parker
Marshall Faircloth

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7.01 9.09	9.1 @ 20.5	
24 Hours	7.01 9.09	9.0 @ 21°C	
48 Hours	7.02 9.09	9.0 @ 21°C	
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: 2.5 Method: Hach
Lab: 2.1 Method: Hach
Lab: 2.5 (1.95) Method: Jenco
Dechlorinated:
Measurements by: DAC

Reviewed by:
Date: 5/4/88

Remarks: * inadvertent addition of Sample to control - shown as on other samples (DAC)

TOXIC Cl2

BIOASSAY DATA FORM

Industry/Toxicant: Clearwater East STP
Address:
Contact/District: SW
Effluent Serial Number: 001
NPDES Permit Number: FL0021565

Test Number: 2 of 6
Test Type: (Static) Renewal (Screening) Conventional
Other:

Sample Collection: Date 2/1/88 Time 1015
Test Beginning: Date 2/2/88 Time 1015
Test Ending: Date 2/4/88 Time 1000
Test Organism: Daphnia pulex
Age/Life Stage: neonate

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96	Conductivity umhos 0	24	48	72	96
Control	47	10	10	10			8.8	8.7				8.0	7.9				20.7	20.5								
Control	132	10	10	10			8.8	8.7				8.0	7.9									175				
10%	18	10	0	0			8.8	8.9				7.8	8.0				20.9	20.1								
10%	138	10	0	-			8.8	8.9				7.8	8.0									235				
30%	13	10	0	-			8.5	9.0				7.6	8.0				21.0	19.8 20.1								
30%	50	10	0	-			8.5	9.0				7.6	8.0									350				
60%	48	10	0	-			8.2	9.0				7.4	8.0				21.0	19.7								
60%	12	10	0	-			8.2	9.0				7.4	8.0									590				
Initial of Investigator	DAC	DAC	DAC	DAC			MF	MF				MF	MF				MF	MF				MF				

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 113/116
100% Sample: 251/201
Other:
Measured by: DAC

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: 0.0 | 0.0
100% Sample: | 12.1
Other:

Hour ____
Control Water:
100% Sample:
Other:
Measured by: mf

Slope = -55.6

Signatures: Carol Chandler
Marshall Faircloth

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours			
24 Hours			
48 Hours			
72 Hours			
96 Hours			

Remarks:

Total Residual Chlorine:
Field data: ____ Method: ____
Lab: ____ Method: ____
Lab: ____ Method: ____

De-chlorinated:
Measurements by:

100% salinity = 0

Reviewed by:
Date: 5/4/88

TOXIC

BIOASSAY DATA FORM

Industry/Toxicant: Clearwater East STP
Address:
Contact/District: SW / Dave Dennis
Effluent Serial Number: 001
NPDES Permit Number: FL0021865

Test Number: 3 of 6
Test Type: Static Renewal, Screening, Conventional
Other: Cl2

Sample Collection: Date 2/1/88 Time 1015
Test Beginning: Date 2/2/88 Time 0940
Test Ending: Date 2/4/88 Time 1000
Test Organism: Mysidopsis bahia
Age/Life Stage: 23 days

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96	Conductivity (umhos cm⁻¹) 0	24	48	72	96
Control	2	5	5	5			8.5	8.4				7.4	7.8				21.0	20.4 DAC								
Control	285	5	5	5			8.5	8.4				7.4	7.8									228				
10%	234	5	0	-			8.3	8.8				7.4	7.8				21.1	19.9								
10%	228	5	0	-			8.4	8.8				7.4	7.8									270				
30%	32	5	0	-			8.2	8.9				7.4	7.9				21.1	19.7								
30%	18	5	0	-			8.2	8.9				7.4	7.9									380				
60%	70	5	0	-			8.1	8.9				7.4	7.9				21.1	19.7								
60%	27	5	0	-			8.2	9.0				7.4	7.9									600				
Initial of Investigator	DAC	MF	MF				JMF	JMF				JMF	DAC				JMF	MF				JMF				

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 118 / 119
100% Sample: 251 / 200
Other:
Measured by: DAC

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: 0.0 / 0.0
100% Sample: / 12.1
Other:

Hour
Control Water:
100% Sample:
Other:
Measured by: MF

Signature: David Dardeen
Marshall Faircloth

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours			
24 Hours			
48 Hours			
72 Hours			
96 Hours			

Remarks:

Total Residual Chlorine:
Field data: Method:
Lab: Method:
Lab: Method:

Dechlorinated:
Measurements by:

60% salinity = 0.1

Reviewed by:
Date: 5/4/88

Industry/Toxicant Clearwater East STP TOXIC de-Clz

BIOASSAY DATA FORM

Address

Contact/District SW / Dave Dennis

Effluent Serial Number 001

NPDES Permit Number FL0021865

Test Number: 4 of 6

Test Type: Static Renewal, Screening Conventional

Other:

Sample Collection: Date 2/1/88 Time 1015

Test Beginning: Date 2/1/88 Time 1000

Test Ending: Date 2/4/88 Time 1000

Test Organism Ceriodaphnia dubia

Age/Life Stage neonates

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96					
Control	92	10	10	10	10		8.7	8.6	8.5			8.0	7.9	7.9			20.7	20.2	20.1							
Control	2	10	10	10	10		8.8	8.6	8.5			8.0	7.9	7.9												
30%	99	10	10*	10	10		8.5	8.7	8.7			7.6	8.0	8.0			20.7	20.2	20.0							
30%	91	10	10*	9	9		8.5	8.7	8.7			7.6	8.0	8.0												
60%	20	10	0	0			8.3	8.7	8.7			7.6	8.2	8.1			20.8	20.3	20.0							
60%	83	10	0	0			8.3	8.6	8.7			7.5	8.2	8.1												
100%	95	10	8*	0			7.8	8.7	8.6			7.5	8.3	8.2			21.3	20.1	20.1							
100%	9	10	8*	0			7.8	8.7	8.6			7.5	8.3	8.2												
Initial of Investigator DAC		JMF	MF	DAC			JMF	JMF	JMF			JMF	JMF	JMF			JMF	DAC	JMF							

Alkalinity/Hardness (mg/l as $CaCO_3$)

Control Water: 113/116

100% Sample: 257/200

Other:

Measured by: DAC

Ammonia unionized/total (mg/l as NH_3/N)

Hour 0

Control Water: 0.0 | 0.0

100% Sample: | 12.1

Other:

Hour

Control Water:

100% Sample:

Other:

Measured by: MF

Signatures:

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours			
24 Hours			
48 Hours			
72 Hours			
96 Hours			

Total Residual Chlorine:

Field data: Method:

Lab: 0.0 Method: Hach

Lab: Method:

De-chlorinated:

Measurements by: DAC

Reviewed by:

Date: 8/14/88

Remarks: * only slight movement

TOXIC de-Cl2

Industry/Toxicant Clearwater East STP
Address
Contact/District SW / Dave Demmi
Effluent Serial Number 001
NPDES Permit Number FL0020346

BIOASSAY DATA FORM

Test Number: 5 of 6
Test Type: (Static) Renewal (Screening) Conventional
Other:

Sample Collection: Date 2/1/88 Time 1015
Test Beginning: Date 2/2/88 Time 1045
Test Ending: Date 2/4/88 Time 1045
Test Organism Daphnia pulex
Age/Life Stage neonates

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96	Conductivity umhos cm-1 0	24	48	72	96
Control	42	10	10	10			8.7	8.7	8.7			8.0	8.0	7.9			20.6	20.2	19.8							
Control	26	10	10	10			8.8	8.8	8.7			8.0	8.0	7.9								200		195		
30%	72	10	10	10			8.6	8.5	8.7			7.7	8.0	8.0			20.9	20.1	19.8							
30%	10	10	10	10 DAC			8.6	8.5	8.6			7.7	8.0	8.0								380		360		
60%	25	10	10	10			8.2	8.5	8.6			7.5	8.1	8.0			21.2	19.9	19.8							
60%	7	10	10	9			8.2	8.5	8.6			7.5	8.0	8.0								600		550		
100%	61	10	10	1			7.7	8.7	8.2			7.4	8.1	8.1			21.5	20.0	19.9							
100%	8	10	10	1			7.8	8.7	8.2			7.4	8.1	8.1								850		780		
Initial of Investigator		DAC	DAC/MF	DAC			JAF	JAF	JAF			JAF	JAF	JAF			JAF	JAF	JAF			JAF		JAF		

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 113/116
100% Sample: 251/200
Other:
Measured by: DAC

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: 0.0 | 0.0
100% Sample: | 12.1
Other:

Hour
Control Water:
100% Sample:
Other:
Measured by: MF

Signature: David Chandler
Marshall Faircloth

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours			
24 Hours			
48 Hours			
72 Hours			
96 Hours			

Remarks:

Total Residual Chlorine:
Field data: Method:
Lab: Method:
Lab: Method:

Dechlorinated:
Measurements by:

Reviewed by:
Date: 5/4/88

Industry/Toxicant: Clearwater East STP — TOXIC — BIOASSAY DATA FORM

Address:
Contact/District: SW / Dave Dennis
Effluent Serial Number: 001
NPDES Permit Number: FL0021865

Test Number: 6 of 6
Test Type: (Static) Renewal, (Screening) Conventional
Other: de-Cl2

Sample Collection: Date 2/1/88 Time 1015
Test Beginning: Date 2/2/88 Time 1040
Test Ending: Date 2/4/88 Time 1000
Test Organism: Mysidopsis bahia
Age/Life Stage: 23 days

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96	Conductivity, umhos/cm 0	24	48	72	96
Control	25	5	5	5			8.3	8.5	8.1			7.4	7.8	7.8			21.0	19.8	19.7							
Control	46	5	5	5			8.3	8.5	8.1			7.5	7.8	7.8								208		210		
30%	34	5	5	5			8.3	8.4	7.7			7.4	7.8	7.9			21.1	19.7	19.6							
30%	62	5	5	5			8.3	8.3	7.7			7.4	7.9	7.9								380		382		
60%	86	5	5	5			8.0	8.3	7.7			7.4	7.9	8.0			21.2	19.7	19.6							
60%	6	5	5	5			8.1	8.3	7.4			7.4	7.9	8.0								400		590		
100%	25	5	5	2			7.7	8.2	6.8			7.4	8.0	8.0			21.5	19.8	19.6							
100%	43	5	5	0			7.7	8.2	6.5			7.4	8.0	8.0								850		800		
Initial of Investigator	DAC	MF	MF	DAC			MF	MF	MF			MF	MF	MF			MF	MF	MF			MF				

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 118/119
100% Sample: 257/200
Other:
Measured by: DAC

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: 0.0 / 0.0
100% Sample: 12.1
Other:
Hour ____
Control Water:
100% Sample:
Other:
Measured by: mc

Signatures: Darrell Carter
Marshall Sanchez

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours			
24 Hours			
48 Hours			
72 Hours			
96 Hours			

Remarks:

Total Residual Chlorine:
Field data: ____ Method: ____
Lab: ____ Method: ____
Lab: ____ Method: ____

De-chlorinated:
Measurements by:

100% salinity = .3
Reviewed by:
Date: 8/4/88

063

MICROTOX™ Data Reduction Sheet

SOURCE: Seawater East STP

GRAPH / CALC EC 50 (15 MIN, 15 °C) = 6.04 (%SAMPLE) mg/L

SAMPLE DATA:

SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent - Cl2

DATE ASSAYED: 02-02-88 TIME: 9:00 AM

VISUAL COLOR: none CORRECTION REQUIRED: YES (NO)

VISUAL TURBIDITY: none SEPARATION REQUIRED: YES (NO)

INITIAL pH: pH ADJUSTED TO: WITH:

OSMOTICALLY ADJUSTED WITH: MOAS (DRY NaCl) OTHER

PRIMARY DILUTION OF SAMPLE:

OPERATOR: Leslie Williams REAGENT VIAL LOT NO. M- 606

REMARKS:

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	I5	I15	NOTES
A1	0	86	90	X	X	span readj → 100% 15 min reading
B1		83	87			
C1		85	93			
A2	12.38	31	30	3.31	4.51	
B2		13	9			
C2		15	10			
A3	24.75	5	2	18.54	44.0	
B3		6	3			
C3		2	1			
A4	49.5	2	.8	55.44	191.9	
B4		2	.3			
C4		.5	.3			
A5	99	.2	.2	422.33	674.0	
B5		.2	.1			
C5		.2	.1			
EC_{50} (MIN, °C)				7.42	6.04	

REVIEWED BY:

DATE: 8/4/88

064

MICROTOX™ Data Reduction Sheet

SOURCE: Clearwater East STP

GRAPH CALC EC 50 (15 MIN, 15 °C) = ≥100 (%SAMPLE) mg/L

SAMPLE DATA:

SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent - de Cl_2

DATE ASSAYED: 02-02-88 TIME: 9:30 AM

VISUAL COLOR: none CORRECTION REQUIRED: YES (NO)

VISUAL TURBIDITY: none SEPARATION REQUIRED: YES (NO)

INITIAL pH: pH ADJUSTED TO: WITH:

OSMOTICALLY ADJUSTED WITH: MOAS (DRY NaCl) OTHER

PRIMARY DILUTION OF SAMPLE:

OPERATOR: Lee Williams REAGENT VIAL LOT NO. M-606

REMARKS:

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	Γ5	Γ15	NOTES
A1	0	91	61	X	X	
B1		87	60			
C1		95	66			
A2	12.38	87	56	.05	.113	
B2		85	55			
C2		88	57			
A3	24.75	74	42	.142	.255	
B3		86	56			
C3		79	51			
A4	49.5	71	43	.288	.427	
B4		69	43			
C4		72	45			
A5	99	52	34	.717	.781	
B5		54	35			
C5		53	36			
EC_{50} (MIN, ° C)				108.7	123.7	

REVIEWED BY:

DATE: 8/4/88