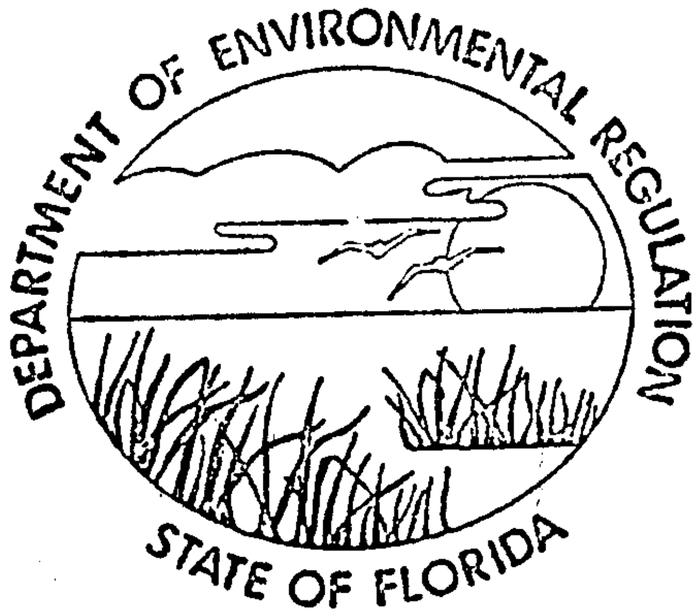


BIOASSAYS OF THE COOPER CITY EAST WASTEWATER TREATMENT PLANT DISCHARGE COOPER CITY, BROWARD COUNTY, FLORIDA

NPDES #FL0029211

SAMPLED 2/2/88

JUNE 30, 1988

Biology Section
Division of Environmental Programs

BIOASSAYS OF THE COOPER CITY EAST WASTEWATER TREATMENT PLANT DISCHARGE COOPER CITY, BROWARD COUNTY, FLORIDA NPDES #FL0029211

Biology Section
Bureau of Laboratories and
Special Programs
June 30, 1988

EXECUTIVE SUMMARY

Cooper City East Wastewater Treatment Plant, NPDES #FL0029211, on 3 to 5 February, 1988.

The sample of discharge from this facility contained high levels of residual chlorine and was acutely toxic to the water fleas Ceriodaphnia dubia and Daphnia pulex and to the fish Notropis leedsi. The Microtox® assay, using the bacterium Photobacterium phosphoreum, was also toxic.

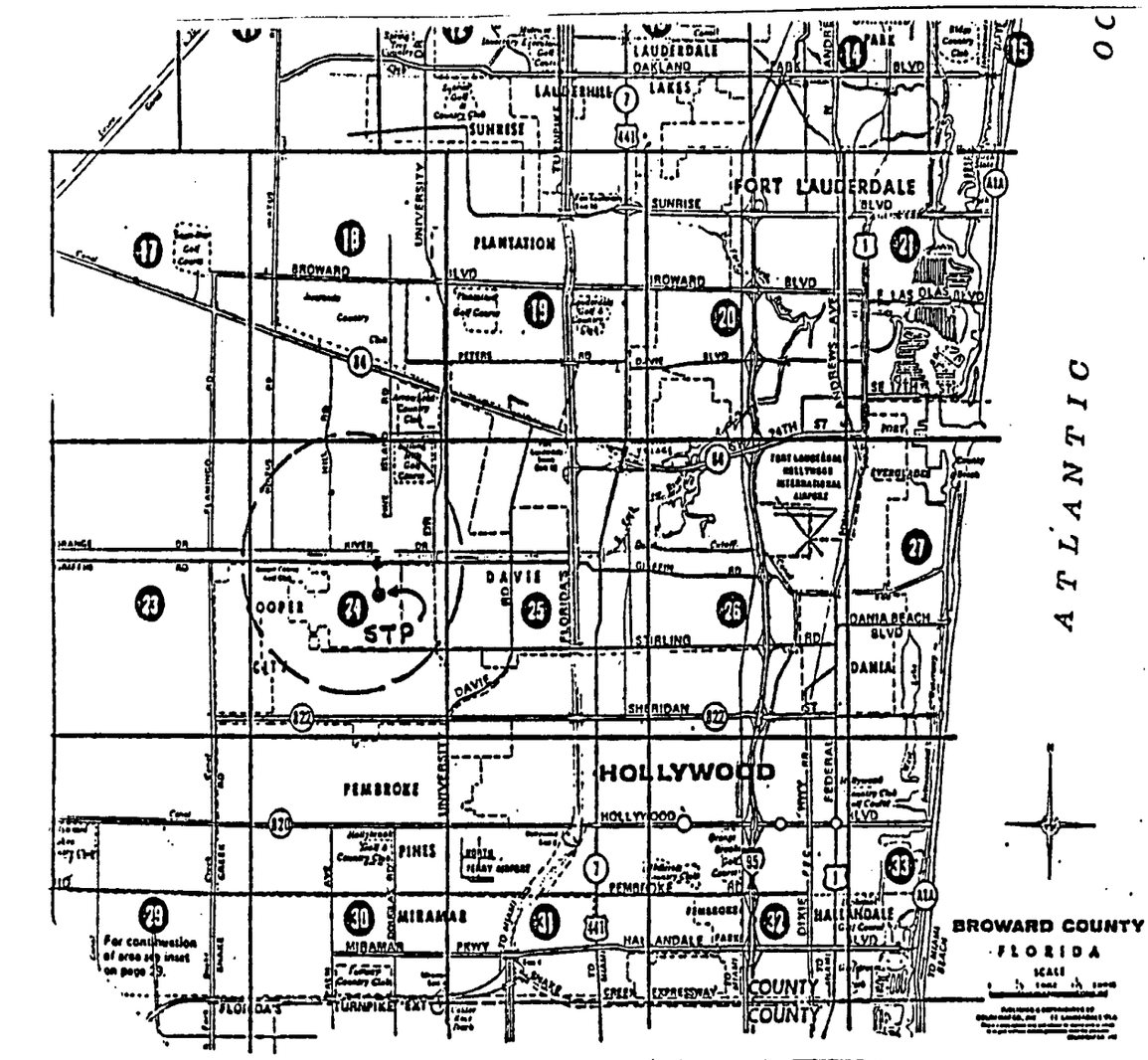
Bioassays performed using a dechlorinated sample were acutely toxic to the water flea Ceriodaphnia dubia (LC_{50} = 69%). The dechlorinated sample was also found to be toxic to Photobacterium phosphoreum (EC_{50} = 40.4%).

We recommend that the Cooper City East facility be required to reduce residual chlorine concentrations in their effluent to near the required 0.5 mg/l. Additionally, 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.

INTRODUCTION

The Cooper City East Wastewater Treatment Plant is located at 9070 SW 51st Street, Cooper City, Broward County, Florida (Figure 1). This facility is a 1.2 MGD contact stabilized waste treatment plant that is permitted to discharge 0.966 MGD, and that has a history of compliance problems. Originally privately owned, the plant was sold to the City of Sunrise in 1981, then to the City of Cooper City in 1983. Prior to early 1988 the plant discharged to the south fork of the North New River Canal. It now discharges to the Hollywood Wastewater Treatment Plant ocean outfall.

Bioassays were performed from February 3-5, 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. H. Epler, J. M. Faircloth, and S. H. Wolfe.



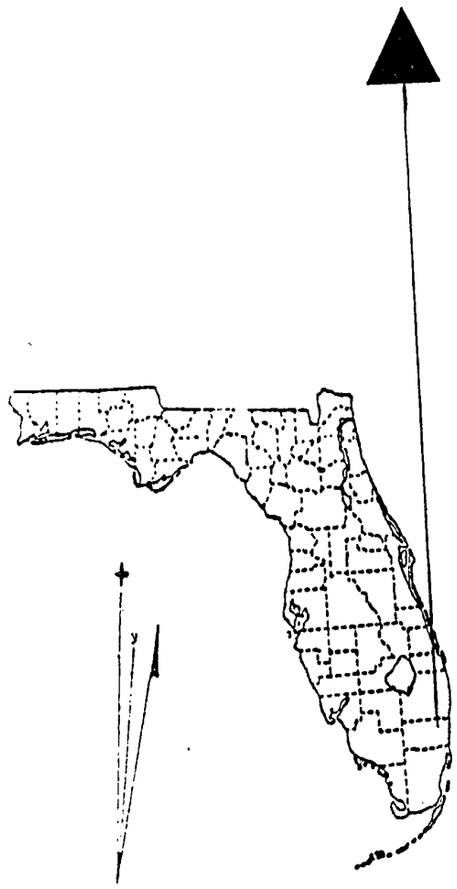


Figure 1. Portions of Cooper City and Broward County, Florida, including Cooper City East STP.

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).

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 24 days old at the time of the test.

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).

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.

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

Six separate 48 hour static acute toxicity bioassays and two Microtox® assays were performed on chlorinated and dechlorinted samples from the Cooper City East facility. The tests were performed from 1030 hours on 3 February to 1230 hours on 5 February, 1988.

Separate samples were obtained for chemical and biological testing. Samples for metal analyses were preserved with HNO_3. Samples for organic chemical analyses and for the bioassays were unpreserved. The samples were collected by Mr. Paul Strauss of the FDER's Southeast District at 1030 hours on 2 February, 1988, using standard wastewater sampling procedures. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 25 hours of collection.

Test Temperature

Upon arrival of the iced (4°C) bioassay samples in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the samples 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 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. De-chlorination 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.

Freshwater Tests

Freshwater for dilution of the recovered water 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.

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

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% reclaimed water 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. The salinity of the sample

was recorded at the beginning 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% reclaimed water 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 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 in a small beaker. 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 microliters 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 East Wastewater Treatment Plant proved to be acutely toxic to the test organisms Ceriodaphnia dubia, Daphnia pulex and Notropis leedsi, producing 48 hour LC_{50}'s of 1.5%, <1%, and <1%, respectively (Table 5). The chlorinated discharge was also toxic to Photobacterium phosphoreum with an EC_{50} of 8.1%. This toxicity can be partially attributed to the total residual chlorine (>11 mg/l Iodometric) measured in the sample. The sample of discharge water that had been dechlorinated was acutely toxic to Ceriodaphnia dubia, with an LC_{50} of 68.8% and to Photobacterium phosphoreum with an EC_{50} of 40.4%. Unionized ammonia was calculated to be 0.01 mg/l; a non-toxic concentration.

The results of chemical analyses (Table 6) indicated the presence of two pesticides - d-BHC (0.7 ug/l) and chlorpyrifos (0.3 ug/l), and four metals - arsenic (3 ug/l), lead (4 ug/l), silver (0.1 ug/l), and zinc (46 ug/l). The pesticides are in trace concentrations unlikely to result in toxicity. The metals, with the possible exception of zinc, are in concentrations unlikely to cause toxicity. The zinc concentrations are near those toxic to Ceriodaphnia dubia, but it is likely that other, unidentified, components are responsible for part of the observed toxicity.

BIOLOGY SECTION RECOMMENDATION

We recommend that the Cooper City East facility be required to reduce residual chlorine concnetrations in their effluent to near the required 0.5 mg/l. Additionally, we recommend that the facility be required to meet the State toxicity standards or to undertake a Toxicity Reduction Evluation with the goal of meeting those standards.

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

DE-CHLORINATED

Ceriodaphnia dubia (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	50.0 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%	-------	50.0 ml	50 ml

Daphnia pulex (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	200.0 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%	--------	200.0 ml	200 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Sample	Total Volume
Control	500 ml	--------	500 ml
30.0%	350 ml	150 ml	500 ml
60.0%	200 ml	300 ml	500 ml
100.0%	------	500 ml	500 ml

CHLORINATED

Ceriodaphnia dubia (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	50.0 ml	--------	50.0 ml
1.0%	49.5 ml	0.5 ml	50.0 ml
10.0%	45.0 ml	5.0 ml	50.0 ml

Daphnia pulex (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	200.0 ml	0.0 ml	200.0 ml
1.0%	198.0 ml	2.0 ml	200.0 ml
10.0%	180.0 ml	20.0 ml	200.0 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Sample	Total Volume
Control	500.0 ml	0.0 ml	500.0 ml
1.0%	495.0 ml	5.0 ml	500.0 ml
10.0%	450.0 ml	50.0 ml	500.0 ml

Table 2. Data recorded during 48 hour, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi, static acute toxicity bioassays of Cooper City East Sewage Treatment Plant, Chlorinated discharge, NPDES #FL0029211, on 3 to 5 February, 1988.

Facility:	Cooper City East			NPDES # FL0029211		Facility Type:	Municipal STP
Location:	9070 SW 51st St			Contact/District:	Paul Strauss/SE		
Sample Collection Date:	2/2/88	Time:	1000	Test type: Static Screening			
Test Beginning Date:	2/3/88	Time:	1030	# tests:	6	Receiving Water:	Old Tampa Bay
Test Ending Date:	2/5/88	Time:	1230	Chlorination Type:	Chlorinated		

Parameter		
Total Residual Cl2:	out of range	11.1+
Method:	Hach	Iodometric
By:	DAC	
Salinity (ppt):	0	

Ammonia:	Control Water	100% Sample
Unionized NH3:	#VALUE!	#VALUE!
Total NH3:	0	3.88

Organism: *Ceriodaphnia dubia* Age/Life stage: neonate
LC50: Binomial: 1.5% Moving Average: n/a Probit: n/a

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hour	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	838	8	7.9	8	20.7	20.5	19.9	-	-
Control	10	10	10	8.7	8.6	8.8	8	8	8	-	-	-	-	-
1%	10	9	4	8.8	8.6	8.8	8	8	8	20.8	20.6	19.8	-	-
1%	10	9	9	8.8	8.7	8.8	8	8	8	-	-	-	-	-
10%	10	0	0	8.6	8.8	-	7.6	8	-	20.8	20.3	-	-	-
10%	10	0	0	8.7	8.8	-	7.6	8	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	Control water	100% sample
Alkalinity:	118	102
Hardness:	119	157

Organism: *Daphnia pulex* Age/Life stage: neonate
LC50: <1% Binomial: n/a Moving Average: n/a Probit: n/a

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hour	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	8.9	8.8	8.9	8	8	8	21.1	20.1	19.7	-	-
Control	10	10	10	8.9	8.9	8.9	8	8	8	-	-	-	190	201
1%	10	4	4	8.9	9	8.8	8	8	8	20.9	20	19.6	-	-
1%	10	5	2	8.9	9	8.8	8	8	8	-	-	-	200	209
10%	10	0	-	8.9	9	-	7.7	7.9	-	21	19.9	-	-	-
10%	10	0	-	8.9	9	-	7.6	7.9	-	-	-	-	238	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	Control water	100% sample
Alkalinity:	118	102
Hardness:	119	157

Organism: *Notropis leedsi* Age/Life stage: 24 days
LC50: <1% Binomial: n/a Moving Average: n/a Probit: n/a

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hour	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 w/dechlor	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Control w/dechlor	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1%	10	1	1	8.5	8.6	7	7.4	7.8	7.7	21.1	19.8	19.6	-	-
1%	10	0	0	8.5	8.6	7	7.4	7.8	-	-	-	-	212	221
10%	10	0	0	8.5	8.7	-	7.2	7.7	-	21.3	19.7	-	-	-
10%	10	0	0	8.5	8.7	-	7.2	7.7	-	-	-	-	255	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	Control water	100% sample
Alkalinity:	118	102
Hardness:	119	157

Table 3. Data recorded during 48 hour, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi, static acute toxicity bioassay of Cooper City East Sewage Treatment Plant, dechlorinated discharge, NPDES #FL0029211, on 3 to 5 February, 1988.

Facility:	Cooper City East			NPDES # FL0029211		Facility Type:	Municipal STP
Location:	9070 SW 51st St			Contact/District:	Paul Strauss/SE		
Sample Collection Date:	2/2/88	Time:	1000	Test type: Static Screening			
Test Beginning Date:	2/3/88	Time:	1030	# tests: 6		Receiving Water: Old Tampa Bay	
Test Ending Date:	2/5/88	Time:	1230	Chlorination Type:	Dechlorinated		

Parameter		
Total Residual Cl2:	out of range	11.1+
Method:	Hach	Iodometric
By:	DAC	
Salinity (ppt):	0	

Ammonia:	Control Water	100% Sample
Unionized NH3:	-	0.01
Total NH3:	-	3.88

Organism: ***Ceriodaphnia dubia*** Age/Life stage: neonate
LC50: Binomial: 68.8% Moving Average: n/a Probit: n/a

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hour	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	8.6	8.8	8	8	8	20.8	21.2	19.8	-	-
Control	10	10	10	8.8	8.6	8.8	8	8	8	-	-	-	-	-
30%	10	10	10	8.8	8.6	8.6	7.3	7.9	8	21	20.6	19.9	-	-
30%	10	10	10	8.8	8.5	8.6	7.3	7.69	8	-	-	-	-	-
60%	10	9	6	8.2	8.5	8.7	7.1	7.9	7.9	21.2	20.3	20	-	-
60%	10	10	9	8.3	8.5	8.7	7.1	7.9	7.9	-	-	-	-	-
100%	10	4	0	7.6	8.3	8.7	6.8	7.9	7.9	21.3	20.3	20	-	-
100%	10	4	0	7.6	8.3	8.5	6.8	7.9	7.9	-	-	-	-	-

	Control water	100% sample
Alkalinity:	118	102
Hardness:	119	157

Organism: ***Daphnia pulex*** Age/Life stage: neonate
LC50: Binomial: - Moving Average: - Probit: -

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hour	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	8.5	8.6	8	8	8	21.1	20	19.5	-	-
Control	10	10	10	8.9	8.5	8.7	8	8	8.1	-	-	-	195	199
30%	10	10	10	8.5	8.7	8.7	7.4	7.9	7.9	21.2	19.8	19.4	-	-
30%	10	10	10	8.5	8.7	8.6	7.4	7.9	7.9	-	-	-	340	345
60%	10	10	10	8.2	8.5	8.4	7.1	7.8	7.9	21.5	19.8	19.5	-	-
60%	10	10	10	8.2	8.5	8.4	7.1	7.8	7.9	-	-	-	500	488
100%	10	10	10	7.7	8.2	8.4	6.8	7.7	7.9	21.9	19.9	19.6	-	-
100%	10	10	10	7.7	8.2	8.3	6.8	7.7	7.9	-	-	-	710	720

	Control water	100% sample
Alkalinity:	118	102
Hardness:	119	157

Organism: ***Notropis leedsi*** Age/Life stage: 24 days
LC50: Binomial: - Moving Average: - Probit: -

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hour	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	8.6	8.3	7.9	7.5	7.8	7.9	21.2	19.8	19.4	-	-
Control	5	5	5	8.6	8.3	8.2	7.5	7.8	7.9	-	-	-	215	217
30%	5	5	5	8.3	7.8	7.8	7.3	7.6	7.8	21.5	19.7	19.4	-	-
30%	5	5	5	8.4	7.9	7.8	7.2	7.6	7.8	-	-	-	358	353
60%	5	5	5	8	7.7	7.8	7.1	7.5	7.7	21.8	19.8	19.5	-	-
60%	5	5	5	8	7.7	7.7	7.1	7.5	7.7	-	-	-	540	491
100%	5	5	5	7.7	7.3	7.2	6.9	7.3	7.7	22	20	19.7	-	-
100%	5	5	5	7.7	7.3	7.2	6.9	7.4	7.7	-	-	-	720	723

	Control water	100% sample
Alkalinity:	118	102
Hardness:	119	157

Table 4. Microtox test results, Cooper City East WWTP discharge, Broward County, NPDES #FL0029211, on 3 February, 1988.

Microtox Test Culture Lot # M-603

	Final Reclaimed Water			Final Reclaimed Water	
% Sample	Chlorinated		% Sample	Dechlorinated	
	5-min	15-min		5-min	15-min
0	74	48	0	99	55
0	72	48	0	93	54
0	72	48	0	84	47
0.0013	72	47	12.38	77	61
0.0013	70	45	12.38	70	37
0.0013	71	46	12.38	70	38
0.025	70	46	24.75	71	36
0.025	67	45	24.75	57	29
0.025	71	48	24.75	66	34
0.5	60	42	49.5	57	25
0.5	55	40	49.5	52	23
0.5	60	48	49.5	52	23
10	0	0	99	35	13
10	0	0	99	37	15
10	0	0	99	38	16

EC50 Chlorinated (15 minute, 15 degree C) = 8.1%
EC50 Dechlorinated (15 minute, 15 degree C) = 40.4%

EC50 (15 minute, 15 degrees 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 Cooper City East Wastewater Treatment Plant, Cooper City, Broward County, Florida, NPDES #FL0029211.

Organism	Method of Data Analysis	48 Hour LC_{50} and 95% Confidence Intervals
Chlorinated Discharge		
Ceriodaphnia dubia	Binomial	1.5% (0 and 10)
	Moving Average	a
	Probit	a
Daphnia pulex	Binomial	<1%, a
	Moving Average	a
	Probit	a
Notropis leedsi	Binomial	<1%, a
	Moving Average	a
	Probit	a
De-chlorinated Discharge		
Ceriodaphnia dubia	Binomial	68.84% (60 and 100)
	Moving Average	a
	Probit	a
Daphnia pulex	Binomial	No Mortality
	Moving Average	
	Probit	
Notropis leedsi	Binomial	No Mortality
	Moving Average	
	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.

Table 6. Chemical constituents present in the discharge water from the Cooper City East Wastewater Treatment Plant, NPDES #FL0029211, on 2 February 1988.

	Organics	
Pesticide extractables		
	d-BHC	0.7 µg/l
	chlorpyrifos	0.3 µg/l
	Metals	
	Arsenic	3 µg/l
	Lead	4 µg/l
	Silver	0.1 µg/l
	Zinc	46 µg/l

LITERATURE CITED

Beckman Instruments, Inc. 1982. Microtox System Operating Manual.

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.

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(1), 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 $LC_{50} \leq 2000\%$ at the point of discharge.

B. With a non-dredge and fill mixing zone the discharge may not exhibit an $LC_{50} \leq 100\%$ at the point of discharge and may not exhibit an $LC_{50} \leq 2000\%$ 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 concentration of the sampled discharge than allowed above.

Industry/Toxicant Cooper City WWTP
Address
Contact/District SE/Paul Strauss
Effluent Serial Number
NPDES Permit Number

BIOASSAY DATA FORM

Test Number: 1 of 6
Test Type: (Static) Renewal, (Screening) Conventional
Other: Cl_2

Sample Collection: Date 2/2/88 Time 1000
Test Beginning: Date 2/3/88 Time 1030
Test Ending: Date 2/5/88 Time 1230
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	0	24	48	72	96
Control	5	10	10	10			8.7	8.6	8.8			8.0	7.9	8.0			20.7	20.5	19.9							
Control	68	10	10	10			8.7	8.6	8.8			8.0	8.0	8.0												
1%	65	10	9	4			8.8	8.6	8.8			8.0	8.0	8.0			20.8	20.6	19.8							
1%	82	10	9	9			8.8	8.7	8.8			8.0	8.2	8.0												
10%	87	10	0	0			8.6	8.8	-			7.6	8.0	-			20.8	20.3	-							
10%	47	10	0	0			8.7	8.8	-			7.6	8.0	-												
Initial of Investigator		JE	DAC/DAC				JMF	JMF				JMF	JMF	DAC ~~JMF~~			JMF	JMF	DAC							

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 118/119
100% Sample: 102/152
Other:
Measured by: DAC
13.88

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

Signatures:
David Chandler
Jeff Steele
Joseph Faircloth

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	707 909	89.0 mm/Hg 9.0 mg/l 8.8 mg/l	
24 Hours	707 909	9.0 mg/l	
48 Hours	707 909	9.0 mg/l	
72 Hours			
96 Hours			

Remarks:

Total Residual Chlorine:
Field data: Method: >8.0 PS
Lab: Method: out of range Hach
Lab: 11.1 Method: Test-N-Tube Reviewed by:
6/30/88
Dechlorinated:
Measurements by: DAC

Industry/Toxicant Cooper City WWTP
Address
Contact/District SE / Paul Stvanss
Effluent Serial Number
NPDES Permit Number

BIOASSAY DATA FORM

Test Number: 2 of 6
Test Type: (Static) Renewal, (Screening) Conventional
Other: C/D

Sample Collection: Date 2/2/89 Time 1000
Test Beginning: Date 2/3/85 Time 1030
Test Ending: Date 2/5/85 Time 1230
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 0	24	48	72	96
Control	60	10	10	11			8.9	8.8	8.9			8.0	8.0	8.0			21.1	20.1	19.7							
Control	49	10	10	10			8.9	8.9	8.9			8.0	8.0	8.0								190		201		
1%	67	10	4	4			8.9	9.0	8.8			8.0	8.0	8.0			20.9	20.0	19.6							
1%	27	10	5	2			8.9	9.0	8.8			8.0	8.0	8.0								200		209		
10%	11	10	0	—			8.9	9.0	-			7.7	7.9	-			21.0	19.9	—							
10%	64	10	0	—			8.9	9.0	-			7.6	7.9	-								238		—		
Initial of Investigator		JMF	DAC	DAC			JMF	JMF	CAC			JMF	SAF				JMF	JMF	DAC			JMF				

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

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

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0

Control Water:
100% Sample:
Other:

Hour

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

Signatures: David Taylor
Joseph Candelaria

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:

Reviewed by:
Date: 6/30/89

Industry/Toxicant Cooper City WWTP
Address
Contact/District SE
Effluent Serial Number
NPDES Permit Number

BIOASSAY DATA FORM

Test Number: 3 of 6
Test Type: Static Renewal, Screening Conventional
Other: Cl_2

Sample Collection: Date 2/2/88 Time 1000
Test Beginning: Date 2/3/88 Time 1030
Test Ending: Date 2/5/88 Time 1230
Test Organism Nitrocris
Age/Life Stage 24 hours

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 0	24	48	72	96
1%	69	5	1	1			8.5	8.6	[illegible]	[illegible]	7.0	7.4	7.8	7.7			21.1	19.8	19.6							
1%	281	5	0	0			8.5	8.6	[illegible]		7.0	7.4	7.8	-								212		221		
10%	131	5	0	0			8.5	8.7				7.2	7.7	-			21.3	19.7								
10%	31	5	0	0			8.5	8.7				7.2	7.7	-								255		—		
* Controls w/ dechlorinated samples - test 1%																										
Initial of Investigator		JAF	JAF	DAC			JAF	JAF				VM	VM				VM	VM				JAF		DAC		

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water:
100% Sample:
Other:
Measured by:

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

Salinity: 0

Signatures:
Richard Chanton
Joseph Fanbell

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:

Reviewed by:
Date: 6/30/88

Industry/Toxicant: Cooper City WWTP
Address:
Contact/District: SE
Effluent Serial Number:
NPDES Permit Number:

BIOASSAY DATA FORM

Test Number: 4 of 6
Test Type: Static Renewal, Screening Conventional
Other: dc. Cl_2

Sample Collection: Date 2/2/88 Time 1000
Test Beginning: Date 2/3/88 Time 1045
Test Ending: Date 2/5/88 Time 1230
Test Organism: Ceriodaphnia dubia
Age/Life Stage: neonates

Conc. or %	Test Container Number	Number of Live Organisms					Dissolved Oxygen (mg/l)					pH					Temperature °C					Conductivity				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control	62	10	10	10			8.8	8.6	8.8			8.0	8.0	8.0			20.8	21.2	19.8							
Control	36	10	10	10			8.8	8.6	8.8			8.0	8.0	8.0												
30%	93	10	10	10			8.8	8.6	8.6			7.3	7.9	8.0			21.0	20.6	19.9							
30%	81	10	10	10			8.8	8.5	8.6			7.3	7.9	8.0												
60%	85	10	9	6*			8.2	8.5	8.7			7.1	7.9	7.9			21.2	20.3	20.0							
60%	71	10	10	9			8.3	8.5	8.7			7.1	7.9	7.9												
100%	100	10	4	0			7.6	8.3	8.7			6.8	7.9	7.9			21.3	20.3	20.0							
100%	6	10	4	0			7.6	8.3	8.5			6.8	7.9	7.9												
Initial of Investigator		JE	DAC JNF	DAC			JNF	JNF	DAC			JNF	JNF	DAC			JNF	JNF	DAC							

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water:
100% Sample:
Other:
Measured by:

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

Signatures:
Jane F. Fendall

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: N.D. Method: Hach
Lab: Method:
De-chlorinated:
Measurements by:

Reviewed by:
Date: 6/30/88

Remarks: * 3 near death - slight movement

Industry/Toxicant Cooper City
Address
Contact/District SE
Effluent Serial Number
NPDES Permit Number

BIOASSAY DATA FORM

Test Number: 5 of 6
Test Type: Static Renewal, Screening Conventional
Other: de Cl_2

Sample Collection: Date 2/2/85 Time 1000
Test Beginning: Date 2/6/85 Time 1045
Test Ending: Date 2/15/85 Time 1230
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 micromhos cm⁻¹ 0	24	48	72	96
Control	37	10	10	10			8.8	8.5	8.6			8.0	8.0	8.0			21.1	20.0	19.5							
Control	4	10	10	10			8.9	8.5	8.7			8.0	8.0	8.1								195		199		
3%	24	10	10	10			8.5	8.7	8.7			7.4	7.9	7.9			21.2	19.8	19.4							
30%	23	10	10	10			8.5	8.7	8.6			7.4	7.9	7.9								340		345		
60%	65	10	10	10			8.2	8.5	8.4			7.1	7.8	7.9			21.5	19.8	19.5							
60%	119	10	10	10			8.2	8.5	8.4			7.1	7.8	7.9								500		488		
100%	17	10	10	10			7.7	8.2	8.4			6.8	7.7	7.9			21.9	19.9	19.6							
100%	31	10	10	10			7.7	8.2	8.3			6.8	7.7	7.9								710		720		
Initial of Investigator		MF	MF	DAC			MF	MF	DAC			MF	MF	DAC			MF	MF				MF		DAC		

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

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

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0

Control Water:
100% Sample:
Other:

Hour

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

Signatures:
Richard Charlton
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: 6/30/85

Industry/Toxicant: Cooper City WWTP
Address:
Contact/District: SC
Effluent Serial Number:
NPDES Permit Number:

BIOASSAY DATA FORM

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

Sample Collection: Date 2/2/88 Time 1000
Test Beginning: Date 2/3/88 Time 1045
Test Ending: Date 2/5/88 Time 1230
Test Organism: Notropis Imperialis
Age/Life Stage: 24 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 µmhos cm-1 0	24	48	72	96
Control	13	5	5	5			8.6	8.3	7.9			7.5	7.8	7.9			21.2	19.8	19.4							
Control	99	5	5	5			8.6	8.3	8.2			7.5	7.8	7.9								215		217		
30%	66	5	5	5			8.3	7.8	7.8			7.3	7.6	7.8			21.5	19.7	19.4							
30%	55	5	5	5			8.4	7.9	7.8			7.2	7.6	7.8								358		353		
60%	76	5	5	5			8.0	7.7	7.8			7.1	7.5	7.7			21.8	19.8	19.5							
60%	49	5	5	5			8.0	7.7	7.7			7.1	7.5	7.7								540		491		
100%	113	5	5	5			7.7	7.3	7.2			6.9	7.3	7.7			22.0	20.0	19.7							
100%	30	5	5	5			7.7	7.3	7.2			6.9	7.4	7.7								720		723		
Initial of Investigator		MF	DAC				MF	MF	DAC			MF	MF	DAC			MF	MF				MF		DAC		

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

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

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0

Control Water:
100% Sample:
Other:

Hour

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

Signatures:
David Charlton
Marshall Faulkner

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:

Reviewed by:
Date: 6/30/88

066

MICROTOX™ Data Reduction Sheet

SOURCE: Copper City WWTP

GRAPH CALC EC 50 (15 MIN, 15 °C) = 40.4 (%SAMPLE) mg/L

SAMPLE DATA:

SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent · deCl2

DATE ASSAYED: ~~9:15Am~~ 2-03-88 TIME: 9:15Am

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	Γ5	Γ15	NOTES
A1	0	99	55	X	X	
B1		93	~~57~~			
C1		84	47			
A2	12.38	77	41	.27	.34	
B2		70	37			
C2		70	38			
A3	24.75	71	36	.42	.58	
B3		57	29			
C3		66	34			
A4	49.5	~~57~~	25	.71	1.14	
B4		52	23			
C4		52	23			
A5	99	35	13	1.51	2.54	
B5		37	15			
C5		38	16			
EC_{50} (MIN, °C)				~~65.84~~	40.4	

REVIEWED BY:

DATE: 6/30/88

067

MICROTOX™ Data Reduction Sheet

SOURCE: Cooper City WWTP

GRAPH CALC EC 50 (15 MIN, 15 °C) = 8.1 (%SAMPLE) mg/L

SAMPLE DATA:

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

DATE ASSAYED: 02-03-88 TIME: 9:45AM

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: 1:10

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

REMARKS:

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	Γ5	Γ15	NOTES
A1		74	48	X	X	—
B1	0	72	48			
C1		72	48			
A2		72	~~47~~			
B2	~~12.38~~	70	45	.024	.044	.5:10 = .05 X .00025 = .000013
C2	.0013%	71	46			
A3		70	46			
B3	~~2.475~~	67	45	.048	.036	.5:10 = .05 X .005 = .00025
C3	.025%	71	48			
A4		60	42			
B4	~~.495~~	55	40	.255	.161	.5:10 = .05 X .10 = .005
C4	.5%	60	42			
A5		0	0	—	—	
B5	~~.99~~	0	0			1:10 = .10
C5	10%	0	0			
EC_{50} (MIN, °C)				1.63	8.15	

REVIEWED BY:

DATE: 6/30/88