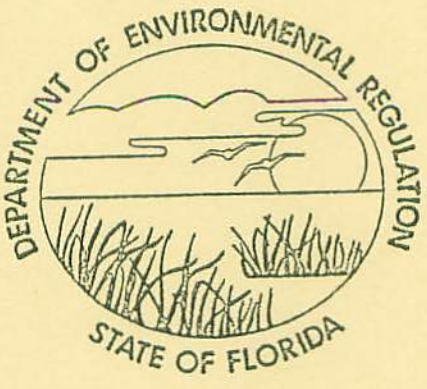


BIOASSAYS OF THE MIAMI-DADE WATER AND SEWER AUTHORITY

NORTH REGIONAL SEWAGE TREATMENT PLANT

NORTH MIAMI BEACH, DADE COUNTY, FLORIDA

NPDES #FL0032182

JANUARY 21, 1988

Biology Section
Division of Environmental Programs

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Biology Section
Bureau of Laboratories and
Special Programs
January 21, 1988

EXECUTIVE SUMMARY

Miami-Dade Water and Sewer Authority, North District WWTP, Dade County, NPDES #FL0032182, on 25 to 27 August, 1987.

The Miami-Dade Water and Sewer Authority North District WWTP treats nearly 80 MGD of combined municipal sewage and industrial wastes from the North Miami Beach area.

Both the reclaimed water and a de-chlorinated portion of the reclaimed water was acutely toxic to the water flea, Ceriodaphnia dubia with LC_{50}'s between 0 and 50% sample concentration. Partial mortalities occurred in the water flea, Daphnia pulex but were not acutely toxic. A chronic toxicity of approximately 134% could be calculated for the chlorinated sample portion. The de-chlorinated portion showed mortality. Neither the chlorinated or de-chlorinated sample exhibited any toxicity to the bannerfin shiner, Notropis leedsi. The Microtox® Assay showed toxicity to the bacterium in the chlorinated sample with an EC_{50} of 77.45%. The de-chlorinated portion was non-toxic. Chlorine residual was measured at .25 mg/l Hach method and 1.1 mg/l Iodometric.

Chemical analysis determined the presence of the organophosphorus pesticide, diazinon at .83 ug/l. Literature values of a 48 hour LC_{50} for Daphnia pulex fall between .6 - 1.1 ug/l. Ceriodaphnia dubia is generally more sensitive than Daphnia pulex. Numerous unidentified pesticide extractable compounds sensitive to the Electron Capture Detector and the Nitrogen Phosphorus Detector were present. The metals, silver (.8 ug/l) and zinc (49 ug/l), were found in concentration higher than the Class III receiving water standards of .07 ug/l and 30 ug/l, respectively. Toxicity to the water fleas (Class Crustacea) is most likely due to the presence of diazinon and the other unidentified pesticide extractable compounds. The high metal concentrations probably contributed to the toxicity.

INTRODUCTION

The Miami-Dade Water and Sewer Authority North District WWTP is located in North Miami Beach, Dade County, Florida (Figure 1). The facility treats the combined municipal and industrial sewage from North Miami Beach and discharges the reclaimed water to the Atlantic Ocean approximately two miles offshore. The plant is operating near its design capacity of 80 MGD with the highest average 30 day flow of 89.16 MGD. Treatment consists of prescreening, primary settling, degritting, followed by activated sludge, secondary clarification, disinfection, and ocean disposal.

To determine the existence and extent of toxicity from this very large WWTP, the Southeast District of DER requested the Ecotoxicology Laboratory to perform acute toxicity screening tests on the discharge. Previous bioassays have not been performed on this facility. In August, 1987, three static screening bioassays and one Microtox® Assay were performed on both the chlorinated discharge and a de-chlorinated portion of discharge. The results of these tests are presented in this report. Those involved in performing the tests and in preparation of this report were: D. A. Chasteen, R. B. Frydenborg, K. M. Lurding, M. L. Roll, and L. A. Williams.

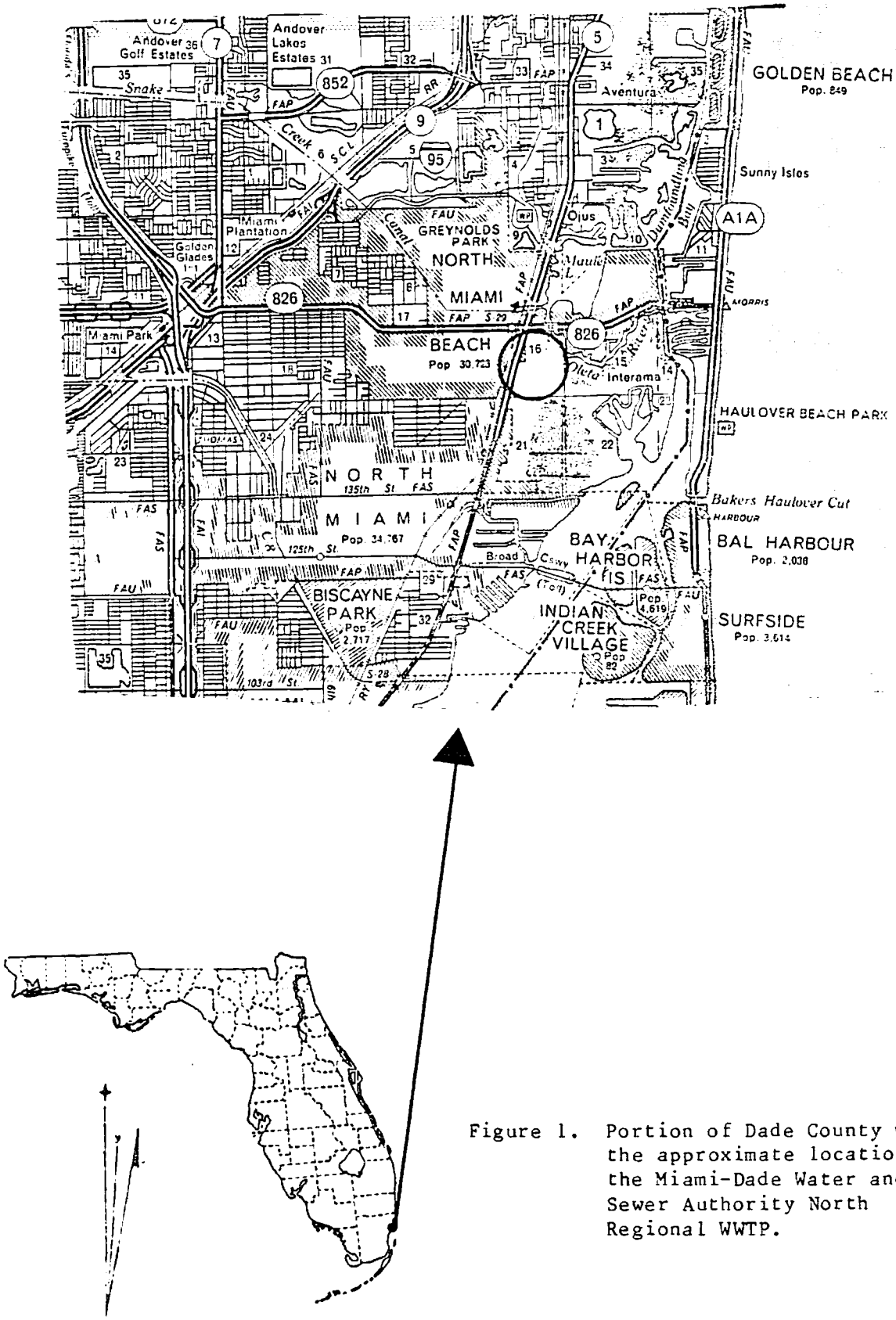


Figure 1. Portion of Dade County with the approximate location of the Miami-Dade Water and Sewer Authority North Regional WWTP.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier and Weber (1985) and the FDER Biology Section (1985). The organisms selected for this study include species recommended by Peltier and Weber (1985) and the FDER Biology Section (1985). Additionally, the Biology Section maintains cultures of several species of organisms indigenous to the State of Florida. One of these indigenous species was also selected for testing. 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 27 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-603 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

Three 48 hour static screening acute toxicity bioassays and one Microtox® Assay was performed on both the chlorinated discharge and a de-chlorinated portion of the discharge from the Miami-Dade Water and Sewer Authority North District WWTP. The bioassays were performed from 1030 hour on 25 August to 1040 hour on 27 August, 1987. The Microtox® Assay was performed at 1000 on 25 August, 1987.

Separate samples were obtained for chemical and biological testing. Samples for metal analyses were preserved with HNO_3. Samples for organic chemical analyses and for the bioassays were preserved on ice at 0°C. The samples were collected by Mr. Paul Strauss of the Southeast District of FDER at 1020 hour on 24 August, 1987, 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 sample in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the sample allowed to rise to 20° $\pm$ 2°C. Although this temperature may be different than the temperature of the sample during collection, it is the temperature range to which the test organisms are acclimatized and is

the range recommended in the above referenced bioassay protocols. This avoids stressful effects caused by exposure to unusually cold or warm samples.

Total Residual Chlorine

Since many 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) 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.

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. 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 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 reclaimed water exhibited acute toxicity (Table 2 and 3) to the water flea, Ceriodaphnia dubia in both the chlorinated and de-chlorinated discharge with LC_{50}'s between 0 and 50% sample concentration. In a screening test, the 50% sample concentration is the lowest dilution. Daphnia pulex, another water flea, had partial mortalities in both the 50% and 100% concentrations in the chlorinated and de-chlorinated sample portions. The bannerfin shiner (Notropis leedsi) exhibited no toxicity in either the chlorinated or de-chlorinated sample. The Microtox® Assay using the common marine bacterium, Photobacterium phosphoreum, exhibited toxicity to the chlorinated sample but not the de-chlorinated. The chlorine residual from disinfection should be removed by de-chlorination unless it dissipates during in pipe travel to the discharge point. A chlorine residual in the discharge may be harmful to the marine microbial communities.

Chemical analyses determined the presence of the metals (Table 5) silver at .8 ug/l and zinc at 49 ug/l. These are greater than the Class III water quality standards of .07 ug/l and 30 ug/l, respectively. Organics analysis did not find any acid extractable organics or herbicides. Trace petroleum hydrocarbons and di-n-butyl phthalate was detected by GC/MS in the base neutral extractable fraction. Analysis of the pesticide extractable fraction determined the presence of the organophosphorus pesticide diazinon at .83 ug/l and several unidentified components sensitive to the Electron Capture Detector and the Nitrogen-Phosphorus Detector.

The selective toxicity which affect the two water fleas, Ceriodaphnia dubia and Daphnia pulex, is likely due to the effects of diazinon and the unidentified pesticide extractable components sensitive to the electron capture detector and nitrogen-phosphorus detector. Pesticides are very toxic compounds used to kill selected groups of organisms. Insecticides are a class of pesticides aimed at killing arthropods. Diazinon, a common insecticide used primarily for terrestrial applications, is a problem when it contaminates aquatic systems through runoff or by passing relatively untreated through a WWTP. Laboratory aquatic toxicity tests show diazinon with 96 hour LC_{50}'s of 1.4 ug/l for the water flea Simocephalus, .8 ug/l for Daphnia pulex, and .20 ug/l for the amphipod, Gammarus fasciatus (Johnson and Finley, 1980). Unfortunately, diazinon, unlike the other common organophosphorus insecticides, appears to be inadequately removed by sewage treatment plants (McIntyre et al. 1981). The .83 ug/l of diazinon in the reclaimed water discharge will cause significant changes in the arthropod community around the ocean outfall. Florida lobster, commercial shrimps, and the predominant arthropod groups (i.e., amphipods, mysids, and other pericaridean orders) used as fish food organisms will be affected. Since these organisms play a dominant role in marine systems in terms of bioamass, diversity, food web structure, and economic activities of our society, the effects will cascade up and down the food chain. Efforts should be made to remove or treat the pesticide extractable fraction of MDWSA's discharge.

The N-P Detector detects nitrogen and phosphorus containing organic compounds. These compounds are phosphorus and nitrogen based pesticides and other potentially toxic nitrogen containing compounds (i.e., Nitrosamines...). The unidentified components of this fraction along with diazinon, most likely caused the toxicity observed to the two arthropods.

In summary, the reclaimed water contained high levels of the metals, silver, zinc, diazinon, and several unidentified pesticide extractable components, and exhibited significant acute toxicity to the standard representative crustaceans used in the bioassays. Recommended actions would be to attempt removal of the metals and pesticides by additional treatment, industrial pretreatment, and public education. The reclaimed water toxicity will continue to cause significant adverse effects to the Atlantic Ocean biota in the region of the outfall.

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

Ceriodaphnia dubia (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	50.0 ml	--------	50 ml
50.0%	25.0 ml	25.0 ml	50 ml
100.0%	-------	50.0 ml	50 ml

Daphnia pulex (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	200.0 ml	--------	200 ml
50.0%	100.0 ml	100.0 ml	200 ml
100.0%	--------	200.0 ml	200 ml

Notropis leedsi (bannerfin shiner)

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

Table 2. Data recorded during 48 hour, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi, static screening acute toxicity Chlorinated bioassays of the MDWSA North District WWTP, Dade County, NPDES #FL0032182, on 25 to 27 August, 1987.

Facility:	MDWSA North Regional WWTP			NPDES # FL0032182		Facility Type:	Municipal WWTP
Location:	Dade County			Contact/District:	SE		
Sample Collection Date:	8/24/87	Time:	1020	Test type:	Static Screening Bioassay		
Test Beginning Date:	8/25/87	Time:	1030	# tests:	3	Receiving Water:	Atlantic Ocean
Test Ending Date:	8/27/87	Time:	1030	Chlorination Type:	Chlorinated		

Parameter		
Total Residual Cl2:	0.25	1.1
Method:	Hach	Iodometric
By:	RBF	RBF
Salinity:	0	

Ammonia:	Control Water	100% Sample
Unionized NH3:	0.00	0.01
Total NH3:	0.1	6

Organism: *Ceriodaphnia dubia* Age/Life stage: Neonates
LC50: Binomial: Moving Average: Probit:
<50%

	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	8.6	7.9	7.9	7.8	20.2	20.3	19.5		
Control	10	10	9	8.8	8.6	8.6	7.9	7.9	7.8					
50%	10	0	0	8.4	8.5	-	7	7.9	-	20	20.2	-		
50%	10	0	0	8.4	8.5	-	7	7.9	-					
100%	10	0	0	8.3	8.5	-	6.7	7.9	-	19.7	20.2	-		
100%	10	0	0	8.3	8.5	-	6.7	8	-					

	Control water	100% sample
Alkalinity:	105	148
Hardness:	117	274

Organism: *Daphnia pulex* Age/Life stage: Neonates
LC50: Binomial: Moving Average: Probit:
134.72%

	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	8.7	8.7	8	7.9	7.9	20.2	20.2	19.3		
Control	10	10	10	8.8	8.7	8.7	8	7.9	7.9				200	210
50%	10	10	9	8.8	8.8	8.7	6.9	7.7	7.9	20	20	19.2		
50%	10	10	9	8.8	8.8	8.7	6.9	7.7	7.9				1150	1180
100%	10	10	8	8.3	8.8	8.3	6.6	7.6	7.9	19.7	20	19.3		
100%	10	9	5	8.3	8.8	8.3	6.6	7.6	7.9				2000	2000

	Control water	100% sample
Alkalinity:	105	148
Hardness:	117	274

Organism: *Notropis leedsi* Age/Life stage: 27 days
LC50: Binomial: Moving Average: Probit:
non-toxic

	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	4	4	8.7	8.6	8.2	7.5	7.6	7.7	21.4	20.2	19.5		
Control	5	4	4	8.7	8.6	8.2	7.5	7.6	7.7				210	220
50%	5	4	4	8.6	8.5	8.1	6.8	7.3	7.7	20.8	20	19.4		
50%	5	5	5	8.6	8.5	8.1	6.8	7.3	7.7				1180	1200
100%	5	5	5	8.4	8.4	8.1	6.6	7.2	7.8	19.7	19.9	19.4		
100%	5	5	5	8.4	8.4	8.1	6.6	7.2	7.8				2010	2040

	Control water	100% sample
Alkalinity:	105	148
Hardness:	117	274

Table 3. Data recorded during 48 hour, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi static screening acute toxicity de-chlorinated bioassays of the MDWSA North District WWTP, Dade County, NPDES #FL0032182, on 25 to 27 August, 1987.

Facility:	MDWSA North Regional WWTP			NPDES # FL0032182		Facility Type:	Municipal WWTP
Location:	Dade County			Contact/District:	SE		
Sample Collection Date:	8/24/87	Time:	1020	Test type: Static Screening Bioassay			
Test Beginning Date:	8/25/87	Time:	1040	# tests:	3	Receiving Water:	Atlantic Ocean
Test Ending Date:	8/27/87	Time:	1040	Chlorination Type:	Dechlorinated		

Parameter	
Total Residual Cl2:	N.D.
Method:	Hach
By:	RBF
Salinity:	0

Ammonia:	Control Water	100% Sample
Unionized NH3:	0.00	0.02
Total NH3:	0.1	6.2

Organism: *Ceriodaphnia dubia* Age/Life stage: Neonates
LC50: Binomial: Moving Average: Probit:
<50%

	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	8.5	8	7.8	7.9	20.3	20.4	19.3		
Control	10	10	10	8.8	8.5	8.5	8	7.8	7.9					
50%	10	0	-	8.7	8.5	-	7	7.8	-	20.3	20.3	-		
50%	10	0	-	8.7	8.5	-	7	7.8	-					
100%	10	0	-	8.2	8.5	-	6.7	7.9	-	20.4	20.2	-		
100%	10	0	-	8.2	8.5	-	6.7	7.9	-					

	Control water	100% sample
Alkalinity:	105	148
Hardness:	117	274

Organism: *Daphnia pulex* Age/Life stage: Neonates
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	8.7	8.6	8.6	8.1	7.8	7.9	20.3	20.2	19.3		
Control	10	9	9	8.7	8.6	8.6	8.1	7.8	7.9				200	205
50%	10	10	10	8.7	8.7	8.5	6.9	7.5	7.9	20.3	20	19.3		
50%	10	10	9	8.7	8.7	8.5	6.9	7.5	7.9				1100	1150
100%	10	8	7	8.4	8.8	8.5	6.7	7.4	8	20.4	20	19.5		
100%	10	8	5	8.4	8.8	8.5	6.7	7.4	8				2000	2030

	Control water	100% sample
Alkalinity:	105	148
Hardness:	117	274

Organism: *Notropis leedsi* Age/Life stage:
LC50: Binomial: Moving Average: Probit:
non-toxic

	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	4	4	8.6	8.6	8.4	7.5	7.5	7.7	21.2	20	19.4		
Control	5	5	5	8.6	8.6	8.4	7.5	7.5	7.7				210	210
50%	5	5	5	8.6	8.3	8.3	6.9	7.2	7.7	21.2	19.9	19.4		
50%	5	5	5	8.6	8.3	8.3	6.9	7.2	7.7				1190	1190
100%	5	5	5	8.4	8.3	8	6.6	7	7.8	20.9	19.8	19.4		
100%	5	5	5	8.4	8.3	8	6.6	7	7.8				2050	2070

	Control water	100% sample
Alkalinity:	105	148
Hardness:	117	274

Table 4. Microtox test results, MDWSA North Regional WWTP discharge, Dade County, NPDES #FL0032182, on 25 August, 1987.

Microtox Test Culture Lot # M-603

Final Reclaimed Water

% Sample	Chlorinated		Dechlorinated	
	5-min	15-min	5-min	15-min
0	92	52	70.5	49
0	93	53	70	49
0	93	53	65	45
12.38	91	53	70	48
12.38	90	52	69	47
12.38	91	53	69.5	48
24.75	84	50	63	44
24.75	85	52	62	43
24.75	91	54.5	65	45
49.5	69	45	*	*
49.5	64.5	42	63.5	44
49.5	66	43	64	44
99	22	19	47	32
99	15	15	55	37
99	16	15	58	39

EC50 Chlorinated (15 minute, 15 degrees C) = 77.48% sample
EC50 Dechlorinated (15 minute, 15 degree C) > 100%

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.

* lab accident

Table 5. Chemical constituents present in the reclaimed water from the Miami Dade Water and Sewer Authority North District Wastewater Treatment Plant, NPDES #FL0032182 on 24 August, 1987.

Organics

Acid Extractables	None Detected
Base Neutral Extractables	Possible trace petroleum hydrocarbons trace di-n-butyl-phthalate
Herbicides-HPLC	None Detected
Pesticide Extractables	Diazinon (.83ug/L) Numerous unidentified components sensitive to ECD and NPD

Metals

Silver	0.8 ug/L
Zinc	49.0 ug/L

* Copper and Lead were invalidated due to contaminated field blank.

Discussion of the Toxicity Criteria

The toxicity standards of Chapters 17-3 and 17-4, F.A.C., are complex. Because of this complexity, they will be briefly reviewed here so that these test results may be more readily compared with the standards. For legal purposes, however, the language of the Florida Administrative Code should be consulted directly. 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. 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 can not exceed 30% sample concentration.

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

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

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.

Johnson, W. W. and M. T. Finley. 1980. Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. U.S. Department of Interior Fish and Wildlife Service.

McIntyre, A. E., J. N. Lester, and R. Perry. 1981. Persistence of organo-phosphorus insecticides in sewage sludges. Env. Technol-Letters 2 :111-118.

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.

Submitter: MDWSA North Regional STP
Address: NE 151 St @ Biscayne Blvd.
Contact/District:
Federal Social Number: 001
NPDES Permit Number:

Test Number: 1 of 6
Test Type: Definitive / Renewal / Screening / Conventional
Other: Cl_2

Sample Collection: Date 8/24/87 Time 1020
Test Beginning: Date 8/25/87 Time 1030
Test Ending: Date 8/27/87 Time 1030
Test Organism: Ceriodaphnia Dubia
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 0	24	48	72	96
Control	75	10	10	10			8.8	8.6	8.6			7.9	7.9	7.8			20.2	20.3	19.5							
Control	92	10	10	9			8.8	8.6	8.6			7.9	7.9	7.8			3									
50%	95	10	0	—			8.4	8.5	—			7.0	7.9	—			20.0	20.2	—							
50%	36	10	0	—			8.4	8.5	—			7.0	7.9	—												
100%	83	10	0	—			8.3	8.5	—			6.7	7.9	—			19.7	20.2	—							
100%	87	10	0	—			8.3	8.5	—			6.7	8.0	—												
Initial of Investigator		KML	KML	KML			MLR	MLR	MLR			MLR	MLR	MLR			RBF	MLR	MLR							

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 105/117
100% Sample: 148/27
Other:
Measured by: KML

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: .01
100% Sample: 6.0
Other:
Hour ____
Control Water:
100% Sample:
Other:
Measured by: MLR

Signatures:
Kathy Lurding
Martin J. Rell
Russel Frydenborg

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7 at 7; 9 at 9	9.0 at 21°C	10.5 at 10, 26 at 30
24 Hours	7 at 7; 9 at 9	9.0 at 21°C	
48 Hours	7 at 7; 9 at 9	9.2 at 20°C	10.5 at 10, 25 at 30
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: 1.0 ppm Method:
Lab: 0.25 mg/l Method: HACH
Lab: 1.1 Method: IODO NITRATE?

Reviewed by:
Date: 1/19/88

De-chlorinated:
Measurements by: RBF

Remarks:

Industry/Toxicant: MOWSA North Regional STP
Address: BISCAYNE BLVD.
Contact/District:
Effluent Serial Number: 601
NPDES Permit Number:

BIOASSAY DATA FORM

Test Number: 2 of 6
Test Type: Static Renewal (Screening) Conventional
Other: Cl_2

Sample Collection: Date 8/24/87 Time 1020
Test Beginning: Date 8/25/87 Time 1030
Test Ending: Date 8/27/87 Time 1030
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 0	24	48	72	96
Control	39	10	10	10			8.8	8.7	8.7			8.0	7.9	7.9			20.2	20.2	19.3							
Control	- x -	10	10	10			8.8	8.7	8.7			8.0	7.9	7.9								200	210			
50%	35	10	10	9			8.8	8.8	8.7			6.9	7.7	7.9			20.0	20.0	19.2							
50%	67	10	10	9			8.8	8.8	8.7			6.9	7.7	7.9								1150	1130			
100%	10	10	10	8			8.3	8.8	8.3			6.6	7.6	7.9			19.1	20.0	19.3							
100%	55	10	9	5			8.3	8.8	8.3			6.6	7.6	7.9								2000	2000			
Initial of Investigator		MLR	MLR	MLR			[illegible]	MLR	MLR			MLR	MLR	MLR			RBF	MLR	MLR			MLR	MLR			

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 105/117
100% Sample: 148/274
Other:
Measured by: KMH

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: .1
100% Sample: 6.0
Other:

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

Signatures:
K/Lurding
Master's Rel
Rural Engineering

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7at7; 9at9	9.0at31°C	10.5at10; 24at30
24 Hours			
48 Hours			
72 Hours			
96 Hours			

Remarks: Probe leaks

Total Residual Chlorine:
Field data: 1.0 ppm Method:
Lab: 0.2 mg/l Method: HACH
Lab: 1.1 Method: IODO

De-chlorinated:
Measurements by:

Reviewed by:
Date: 1/19/88

Laboratory/Facility: MIAMI NORTH Regional STP

Address: BISCAYNE BLVD

Contact/District:

Effluent Serial Number: 001

NPDES Permit Number:

TEST DATA SHEET

Test Number: 3 of 6

Test Type: Static Renewal (Screening) Conventional

Other: Cl_2

Sample Collection: Date 8/24/87 Time 1020

Test Beginning: Date 8/25/87 Time 1110

Test Ending: Date 8/27/87 Time 1110

Test Organism: Nitrocra leedsi

Age/Life Stage:

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
Control	70	5	4	4			8.7	8.6	8.2			7.5	7.6	7.7			~~23.5~~	20.2	19.5			~				
Control	22	5	4	4			8.7	8.6	8.2			7.5	7.6	7.7			21.4					210	220			
50%	3	5	4	4			8.6	8.5	8.1			6.8	7.3	7.7			20.8	20.0	19.4							
50%	55	5	5	5			8.6	8.5	8.1			6.8	7.3	7.7								1180	1200			
100%	—	5	5	5			8.4	8.4	8.1			6.6	7.2	7.8			19.7	19.9	19.4							
100%	52	5	5	5			8.4	8.4	8.1			6.6	7.2	7.8								2010	2040			
Initial of Investigator		KMH	RBF	MZ			MLR	MLR	MLR			MLR	MLR	MLR			RBF	MLR	MLR			MLR	MLR			

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

Control Water:

100% Sample: 105/117

Other: 148/92

Measured by: KMH

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

Hour 0

Control Water: .1

100% Sample: 6.0

Other:

Hour ____

Control Water:

100% Sample:

Other:

Measured by: MLR

Signatures:

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7 at 7; 9 at 9	9.0 at 21°C	10.5 at 15, 26 at 30
24 Hours			
48 Hours			
72 Hours			
96 Hours			

Total Residual Chlorine:

Field data: 1.0 Method: HACH

Lab: 0.25 Method: HACH

Lab: 1.1 Method: 1050

De-chlorinated:

Measurements by: RBF

Reviewed by:

Date: 1/19/88

Remarks:

Industry/Toxicant MDWSA North ~~Central~~ Regional STP
Address BISCAYNE BLVD
Contact/District
Effluent Serial Number 001
NPDES Permit Number

BIOASSAY DATA FORM

Test Number: 4 of 6
Test Type: Static Renewal (Screening) Conventional
Other: De-Cl2

Sample Collection: Date 8/24/87 Time 1020
Test Beginning: Date 8/25/87 Time 1040
Test Ending: Date 8/29/87 Time 1040
Test Organism C. dubia
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 0	24	48	72	96	Alkalinity/Hardness (mg/l as $CaCO_3$) 0	24	48	72	96
Control	50	10	10	10			8.8	8.5	8.5			8.0	7.8	7.9			20.3	20.4	19.3												
Control	5	10	10	10			8.8	8.5	8.5			8.0	7.8	7.9																	
50%	39	10	0	–			8.7	8.5	–			7.0	7.8	–			20.3	20.3	–												
50%	-x-	10	0	–			8.7	8.5	–			7.0	7.8	–																	
100%	85	10	0	–			8.2	8.5	–			6.7	7.9	–			20.4	20.2	–												
100%	45	10	0	–			8.2	8.5	–			6.7	7.9	–																	
Initial of Investigator		KML	KML	KML			MLR	MLR	MLR			MLR	MLR	MLR			RBF	MLR	MLR												

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 105/117
100% Sample: 148/174
Other:
Measured by: KML

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: .1
100% Sample: 6.2
Other:

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

Signatures:
K M Lurding
Martin J. Kell
Ronald Frydenborg

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: ND Method: HACH
Lab: ____ Method: ____
De-chlorinated:
Measurements by: RBF

Reviewed by:
Date: 1/19/88

Regional

Facility/Location: MDWSA North Central STP
Address: BISCAYNE BLVD
Contact/District:
Effluent Serial Number: 001
NPDES Permit Number:

TEST DATA FORM

Test Number: 5 of 6
Test Type: Static Renewal, Screening, Conventional
Other: DE-Cl2

Sample Collection: Date 8/24/87 Time 1020
Test Beginning: Date 8/25/87 Time 1040
Test Ending: Date 8/27/87 Time 1040
Test Organism: D. 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 0	24	48	72	96
Control	78	10	10	10			8.7	8.6	8.6			8.1	7.8	7.9			20.3	20.2	19.3							
Control	41	10	9	9			8.7	8.6	8.6			8.1	7.6	7.9								200	205			
50%	36	10	10	10			8.7	8.7	8.5			6.9	7.5	7.9			20.3	20.0	19.3							
50%	49	10	10	9			8.7	8.7	8.5			6.9	7.5	7.9								1100	1150			
100%	69	10	8	7			8.4	8.8	8.5			6.7	7.4	8.0			20.4	20.0	19.5							
100%	64	10	8	5			8.4	8.8	8.5			6.7	7.4	8.0								2000	2030			
Initial of Investigator		MLR	MLR				MLR	MLR	MLR			MLR	MLR	MLR			RBE	MLR	MLR			MLR	MLR			

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

Control Water: 105 / 11
100% Sample: 148 / 2
Other:
Measured by: KML

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

Control Water: .1
100% Sample: 6.2
Other:

Hour

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

Signatures:

Martin L Rell

Russel Frydenborg

Instrument Calibrations:

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

Remarks: No emergence in dissolved pH was observed

Total Residual Chlorine:

Field data: Method:
Lab: ND Method: HACH
Lab: Method:

De-chlorinated:
Measurements by: RBE

Reviewed by:

Date: 9/19/88

Industry/Toxicant: MOWSA North (East)
Address: BISCAYNE BLVD
Contact/District:
Effluent Serial Number: 001
NPDES Permit Number:

BIOASSAY DATA FORM

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

Sample Collection: Date 8/24/87 Time 1000
Test Beginning: Date 8/25/87 Time 1115
Test Ending: Date 8/29/87 Time 1115
Test Organism: N. tenuis
Age/Life Stage: 2-7 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 0	24	48	72	96
Control	58	5	4	4			8.6	8.6	8.4			7.5	7.5	7.7			21.2	20.0	19.4							
Control	34	5	5	5			8.6	8.6	8.4			7.5	7.5	7.7								210	210			
50%	49	5	5	5			8.6	8.3	8.3			6.9	7.2	7.7			21.2	19.9	19.4							
50%	6	5	5	5			8.6	8.3	8.3			6.9	7.2	7.7								1190	1190			
100%	50	5	5	5			8.4	8.3	8.0			6.6	7.0	7.8			20.9	19.8	19.4							
100%	25	5	5	5			8.4	8.3	8.0			6.6	7.0	7.8								2050	2070			
Initial of Investigator							MLR	MLR	MLR			MLR	MLR	MLR			RBF	MLR	MLR			MLR	MLR			

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 105/117
100% Sample: 148/274
Other:
Measured by:

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: .1
100% Sample: 6.2
Other:

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

Signatures:
Kathy Harding
Martin P. Roll
Russel Frydenborg

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: ND Method: HACH
Lab: Method:
(De-chlorinated)
Measurements by: RBF

Reviewed by:
Date: 1/19/88

014

MICROTOX™ Data Reduction Sheet

SOURCE: North Miami Ocean Outfall

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

SAMPLE DATA:

SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent - [illegible]

DATE ASSAYED: 8-25-87 TIME: 10:00 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: none

OPERATOR: Leslie Williams REAGENT VIAL LOT NO. M-603

REMARKS:

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	Γ5	Γ15	NOTES
A1	0	70.5	49	X	X	
B1		70	49			
C1		65	45			
A2	12.38	70	48	-.01	.00	
B2		69	47			
C2		69.5	48			
A3	24.75	63	44	.08	.08	
B3		62	43			
C3		65	45			
A4	49.5	63.5	44	.08	.08	
B4		64	44			
C4						
A5	99	47	32	.28	.32	
B5		50	34			
C5		50	34			
EC_{50} (MIN, °C)				28+3	32+4	

REVIEWED BY:
DATE: 1/19/88

8/25-27/87
2FL 003102

009

MICROTOX™ Data Reduction Sheet

SOURCE: North Miami Ocean Outfall

GRAPH / CALC EC 15 (15 MIN, 15° C) = 77.5 (%SAMPLE) mg/L

SAMPLE DATA:

SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent 012

DATE ASSAYED: 8-25-87 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: Susie Quintana REAGENT VIAL LOT NO. M- 6[illegible]E

REMARKS:

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	Γ5	Γ15	NOTES
A1	0	92	52	X	X	
B1		93	53			
C1		93	53			
A2	12.38	91	53	.02	.00	
B2		90	52			
C2		91	53			
A3	24.75	84	50	.07	.01	
B3		85	52			
C3		91	54.5			
A4	49.5	61	45	.39	.22	
B4		64.5	42			
C4		66	43			
A5	99	22	14	4.25	2.22	
B5		15	15			
C5		16	15			
EC_{50} (MIN, ° C)				62.18	77.48	

REVIEWED BY:

DATE: 1/19/88