

BIOASSAYS OF MIAMI-DADE WATER AND SEWER AUTHORITY

CENTRAL REGIONAL WASTEWATER TREATMENT PLANT

MIAMI, DADE COUNTY, FLORIDA

NPDES #FL0024805

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 Central Regional WWTP (formerly Virginia Key STP), City of Miami, Dade County, NPDES #FL0020541, 25 to 27 August, 1987.

The MDWSA WWTP on Virginia Key near Miami treats approximately 130 MGD of combined municipal sewage, industrial and commercial wastes, and discharges to the Atlantic Ocean. Three static screening acute toxicity bioassays and one Microtox® Assay were performed on both chlorinated sample and a de-chlorinated portion of the sample. The chlorinated discharge was acutely toxic to the cladoceran arthropods (Water fleas), Daphnia pulex and Ceriodaphnia dubia, and the fish, Notropis leedsi (bannerfin shiner). All organisms were dead within 24 hours. All LC_{50}'s were less than the lowest screening concentration of 50% sample (24 hr $LC_{50} < 50\%$). Similar extreme toxicity was exhibited in the Microtox® Assay with an $EC_{50} = 22.2\%$ sample. Chlorine residual measured 0.7 mg/l by the Hach method and 0.8 mg/l by the Iodometric method.

The de-chlorinated sample killed all the Ceriodaphnia within 24 hours (24 hour $LC_{50} < 50\%$) in the lowest screening concntration. Daphnia pulex exhibited a less toxic response in the de-chlorinated portion but the sample still was acutely toxic with a 48 hour LC_{50} of 74%. Significant toxicity continued to be exhibited on the common marine bacterium, Photobacterium phosphoreum, used in the Microtox® Assay with an EC_{50} of 28.8%. There was no mortality of the fish Notropis leedsi in 48 hours in the de-chlorinated sample portion.

Chemical analyses performed on the reclaimed water from this facility indicated trace amounts of petroleum hydrocarbon residues, numerous unidentified pesticide extractable components sensitive to an Electron Capture Detector and a Nitrogen-Phosphorus Detector, and the metals silver .5 ug/l) and zinc (16 ug/l).

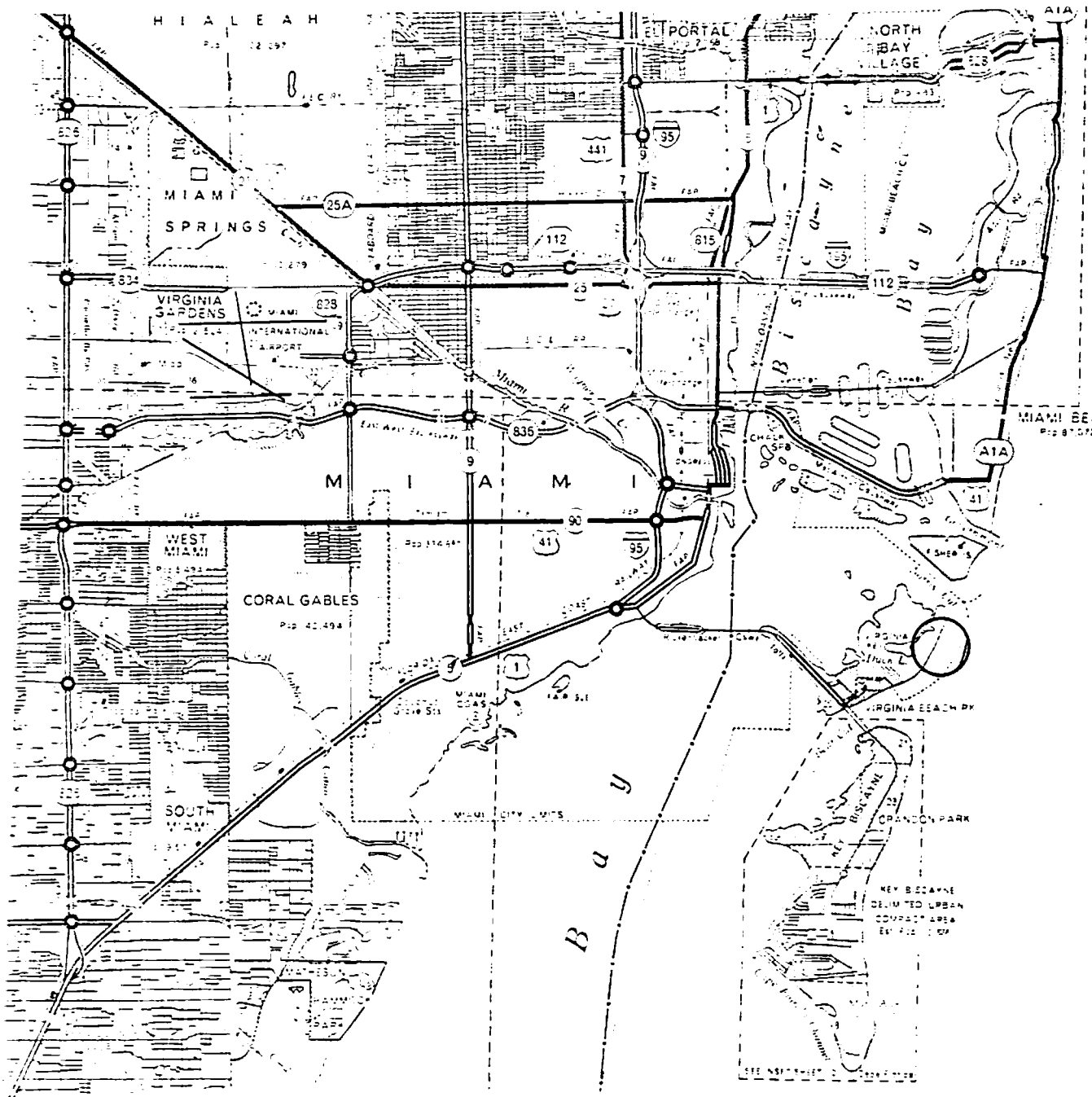
INTRODUCTION

The Miami-Dade Water and Sewer Authority Central District WWTP (formerly the Virginia Key STP) located in Miami, Dade County, Florida treats municipal wastes from a population of greater than 1.2 million. Included in this treatment are wastes from a variety of industrial and commercial sources, such as bakery, laundry, and dairy operations.

The plant consist of an older 48 MGD modified high rate activated sludge plant and a 73 MGD oxygen aeration module. The combined reclaimed water is discharged at a depth of 100 feet, approximately 3.5 miles offshore in the Atlantic Ocean.

On 21 to 23 April, 1982, a static acute bioassay was performed on the possum shrimp, Mysidopsis bahia, producing a LC_{50} value between 28.8% and 32.0% sample. The toxicity was attributed to the synergistic effects of ammonia, unidentified organic compound, and sublethal concentrations of arsenic and copper. Chlorine residual was negligible.

To further evaluate the extent of toxicity of this facility discharge to the receiving water biota, the Ecotoxicology Laboratory performed three static screening acute toxicity bioassays and one Microtox® Assay on both the chlorinated reclaimed water and on a portion of de-chlorinated sample on 25 to 27 August, 1987. Results are presented in this report. Those involved in performing the tests and in preparation of this report were: D. A. Chasteen, J. M. Faircloth, R. B. Frydenborg, K. M. Lurding, M. L. Roll, and L. A. Williams.



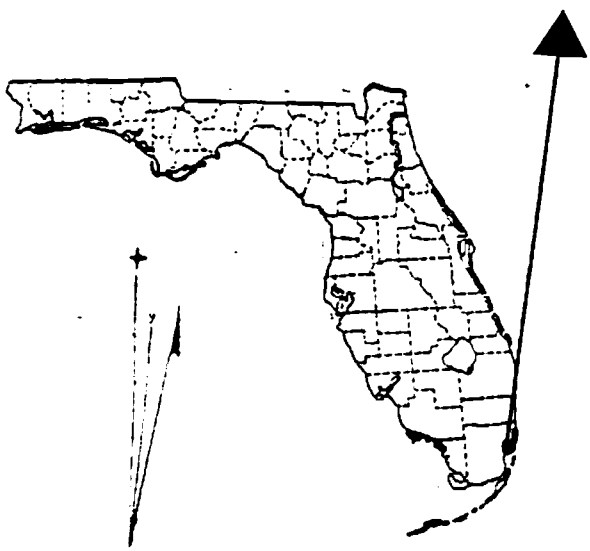


Figure 1. Portions of Dade County, Florida, including City of Miami and study area.

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 static screening acute toxicity bioassays and one Microtox® Assay was performed on both chlorinated reclaimed water and a portion of de-chlorinated sample from the discharge line sampling spigot of the MDWSA Central District WWTP. The bioassays were performed from 1050 hours on 25 August to 1050 hours on 27 August, 1987. The Microtox® Assay was performed at 0900 hours 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 placed on ice with no preservatives added. The samples were collected by Mr. Paul Strauss at 1200 hours on 24 August, 1987, 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° $\pm$ 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 recovered 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 recovered water. To accomplish this, the Biology Section performed concurrent bioassays on a sample of chlorinated recovered 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 recovered 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 recovered 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 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 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 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 from the MDWSA Central District WWTP (formerly Virginia Key STP) was acutely toxic to all test organisms with complete mortality in 24 hours (Table 2). This included two water fleas, Ceriodaphnia dubia and Daphnia pulex, and the fish, Notropis leedsi. Since the lowest concentration used in a static screening bioassay is 50% sample, we can say the 24 hour LC_{50} falls in the range of 0 to 50% sample concentration. The Microtox® Assay exhibited toxicity with an EC_{50} of 22.2% sample (Table 4) on the common marine bacterium, Photobacterium phosphoreum. The chlorine residual measured 0.7 mg/l by the Hach Method and 0.8 mg/l by the Iodometric Method. Total ammonia measured 7.1 mg/l and the unionized ammonia was calculated at 0.03 mg/l in the 100% sample concentration.

Table 3 contains the results of bioassays on the de-chlorinated sample portion. The cladoceran, Ceriodaphnia dubia, exhibited complete mortality by 24 hours. Daphnia pulex had only partial mortality in the 100% sample at 24 hours and only one out of 20 survived 48 hours. There was no mortality of D. pulex in the 50% sample concentration. The organism's threshold to the toxicants in the reclaimed water occurred when the LC_{50} was in the 50-100% range sample concentration. The Microtox® Assay produced an EC_{50} of 28.8% sample concentration (Table 4) supporting the determination of acute toxicity to the water fleas. No mortality occurred with the fish in the dechlorinated portion.

A fraction of the toxicity can be attributed to the chlorine residual, particularly to the fish and partially to the water flea, Daphnia pulex. Considerable toxicity remains after de-chlorination to both water fleas and the marine bacterium, Photobacterium phosphoreum.

Results of chemical analyses of the reclaimed water are presented in Table 5. Organics analyses found the presence of trace petroleum hydrocarbons and numerous unidentified pesticide extractable components sensitive to the electron capture detector and the nitrogen phosphorus detector. Metals found included silver at 0.5 ug/l and zinc at 16.0 ug/l. Silver seems particularly high compared to the Class III water quality standard of 0.07 ug/l.

The selective toxicity which affected the two water fleas, Ceriodaphnia dubia and Daphnia pulex is likely due to the effects of the unidentified pesticide extractable components sensitive to the electron capture detector and nitrogen phosphorus detector. The nitrogen-phosphorus detector indicates the presence of several unidentified nitrogen and phosphorus containing organic compounds. This analysis detects phosphorus based pesticides, nitrogen based pesicides, and other nitrogen containing compounds (i.e., nitrosamines) which may be toxic. Pesticides are very toxic compounds used to kill selected groups of organisms. Insecticides are a class of pesticides aimed at killing arthropods. Since the water fleas are arthropods in the Class Crustacean,

they are seriously affected by this discharge's pesticide extractable fraction. This causes concern for the arthropod (crustacean) community around the ocean outfall.

The pesticide extractable fraction of the reclaimed water discharge will likely 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 may be affected. Since these organisms play a dominant role in marine systems in terms of biomass, 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 combined effects of the pesticide extractable fraction and high silver concentration in the reclaimed water are the most likely cause of the acute toxicity in the de-chlorinated portion to the crustaceans and microbial test species. Chlorine increases the toxicity slightly to these organisms and causes acute toxicity to the fish species.

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

Ceriodaphnia dubia (water flea)

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

Daphnia pulex (water flea)

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

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 bioassays of Miami-Dade Water and Sewer Authority Central Regional, Miami, Dade County, NPDES #FL0024805, on 25 to 27 August, 1987.

Facility:	MDWSA Central Regional WWTP			NPDES # FL0024805		Facility Type:	Municipal WWTP
Location:	Dade County			Contact/District:	Paul Strauss/SE		
Sample Collection Date:	8/24/87	Time:	1200	Test type:	Static Screening		
Test Beginning Date:	8/25/87	Time:	1050	# tests:	3	Receiving Water:	Atlantic Ocean
Test Ending Date:	8/27/87	Time:	1050	Chlorination Type:	Chlorinated		

Parameter		
Total Residual Cl2:	0.7	0.8
Method:	Hach	Iodometric
By:	RBF	RBF

Ammonia:	Control Water	100% Sample
Unionized NH3:	0.03	0.03
Total NH3:	0.1	7.1

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	7	8.6	8.5	8.4	8	7.9	8	20.7	20.4	19.5		
Control	10	10	9	8.6	8.5	8.4	8	7.9	8					
50%	10	0	-	8.1	8.5	-	7.2	8.1	-	20	20.4	-		
50%	10	0	-	8.1	8.5	-	7.2	8.1	-					
100%	10	0	-	7.2	8.7	-	7	8.2	-	19.3	20.3	-		
100%	10	0	-	7.2	8.7	-	7	8.2	-					

	Control water	100% sample
Alkalinity:	105	164
Hardness:	117	424

Organism: *Daphnia pulex* 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	9	8	8.6	8.7	8.6	8.1	8	7.9	20.7	20.2	19.5		
Control	10	10	10	8.6	8.7	8.6	8.1	8	7.9				200	210
50%	10	0	-	8.1	8.7	-	7.1	7.9	-	20.1	20.1	-		
50%	10	0	-	8.1	8.7	-	7.1	7.9	-				1750	-
100%	10	0	-	7.1	8.8	-	6.9	7.8	-	19.4	20	-		
100%	10	0	-	7.1	8.8	-	6.9	7.8	-				3120	-

	Control water	100% sample
Alkalinity:	105	164
Hardness:	117	424

Organism: *Notropis leedsi* Age/Life stage: 27 days
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	5	5	5	8.2	8.5	8.4	7.6	7.7	7.7	21.6	19.9	19.4		
Control	5	5	5	8.2	8.5	8.4	7.6	7.7	7.7				210	210
50%	5	0	0	7.8	8.5	-	7	7.7	-	20.5	19.8	-		
50%	5	0	0	7.8	8.5	-	7	7.7	-				1800	-
100%	5	0	0	6.9	8.5	-	6.9	7.6	-	19.5	19.8	-		
100%	5	0	0	6.9	8.5	-	6.9	7.6	-				3200	-

	Control water	100% sample
Alkalinity:	105	164
Hardness:	117	424

Table 3. Data recorded during 48 hour, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi, static screening acute toxicity De-chlorinated bioassays of Miami-Dade Water and Sewer Authority Central Regional, Miami, Dade County, NPDES #FL0024805, on 25 to 27 August, 1987.

Facility:	MDWSA Central Regional WWTP			NPDES # FL0024805		Facility Type:	Municipal WWTP
Location:	Dade County			Contact/District:	Paul Strauss/SE		
Sample Collection Date:	8/24/87	Time:	1200	Test type:	Static Screening		
Test Beginning Date:	8/25/87	Time:	1050	# tests:	3	Receiving Water:	Atlantic Ocean
Test Ending Date:	8/27/87	Time:	1050	Chlorination Type:	Dechlorinated		

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

Ammonia:	Control Water	100% Sample
Unionized NH3:	0.03	0.03
Total NH3:	0.1	7.1

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	8.5	8.5	8.5	8.1	8	7.9	20.3	19.4	19.4		
Control	10	10	10	8.5	8.5	8.5	8.1	8	7.9					
50%	10	0	-	8.1	8.6	-	7.2	8.1	-	20.3	19.3	-		
50%	10	0	-	8.1	8.6	-	7.2	8.1	-					
100%	10	0	-	7.3	8.4	-	7	8.2	-	20.1	19.3	-		
100%	10	0	-	7.3	8.4	-	7	8.2	-					

	Control water	100% sample
Alkalinity:	105	164
Hardness:	117	424

Organism: *Daphnia pulex* Age/Life stage: Neonates
LC50: Binomial: 74% Moving Average: Probit:
74% CI(50,100)

	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	8.6	8.5	8.6	8.1	8	7.9	20.2	20.2	19.3		
Control	10	10	10	8.6	8.5	8.6	8.1	8	7.9				200	205
50%	10	10	10	7.9	8.6	8.4	7.2	7.9	7.9	20.3	20.1	19.3		
50%	10	10	10	7.9	8.6	8.4	7.2	7.9	7.9				1730	1770
100%	10	5	0	6.8	8.5	8.1	7	7.9	8	20.3	20.1	19.5		
100%	10	7	1	6.8	8.5	8.1	7	7.9	8				3180	3200

	Control water	100% sample
Alkalinity:	105	164
Hardness:	117	424

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	5	5	8.3	8.2	8.2	7.6	7.8	7.8	21.4	20	19.4		
Control	5	5	5	8.3	8.2	8.2	7.6	7.8	7.8				210	210
50%	5	5	5	7.8	8.1	7.9	7.1	7.7	7.8	21.1	19.9	19.4		
50%	5	5	5	7.8	8.1	7.9	7.1	7.7	7.8				1800	1780
100%	5	5	5	7	7.8	7.4	6.9	7.7	7.9	20.7	19.9	19.5		
100%	5	5	5	7	7.8	7.4	6.9	7.7	7.9				3210	3200

	Control water	100% sample
Alkalinity:	105	164
Hardness:	117	424

Table 4. Microtox test results, MDWSA Central Regional WWTP discharge, Dade County, NPDES #FL0024805, on 25 to 27 August, 1987.

Microtox Test Culture Lot # M-603

Final Reclaimed Water

% Sample	Chlorinated		Dechlorinated	
	5-min	15-min	5-min	15-min
0	89	66	85	52
0	86	65	79	48
0	82	60	84	52
12.38	51	35	61	35
12.38	54	37	64.5	37
12.38	53	36	64	36
24.75	26.5	19	50	27
24.75	25.5	19	54	29
24.75	27	20	56	30.5
49.5	5	4.5	37	18
49.5	5	4	37.5	19
49.5	5	4.5	36	18
99	0.5	0.4	19	9
99	0.5	0.4	19.5	9
99	0.5	0.4	19	9

EC50 Chlorinated (15 minute, 15 degrees C) = 22.20% sample
EC50 Dechlorinated (15 minute, 15 degree C) > 28.79% sample

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. Chemical constituents present in the reclaimed water from the Miami Dade Water and Sewer Authority Central Regional Wastewater Treatment Plant (formerly Virginia Key STP), NPDES #FL0032182 on 24 August, 1987.

Organics

Acid Extractables	None Detected
Base Neutral Extractables	Trace petroleum hydrocarbons
Herbicides-HPLC	None Detected
Pesticide Extractables	One small unidentified component
	Numerous unidentified components sensitive to ECD and NPD

Metals

Silver	0.5 ug/L
Zinc	16.0 ug/L

* Results for lead were invalidated due to a 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.

Industry/Municipality: MIAMI Central STP
Address: VIRGINIA KEY
Contact/District:
Office of Serial Number: 001
NPDES Permit Number:

PRIMARY DATA SHEET

Test Number: 16
Test Type: (Static) Renewal, (Chronic) Conventional
Other: Cl2

Sample Collection: Date 8-21-87 Time 1200
Test Beginning: Date 8-25-87 Time 1050
Test Ending: Date 8-29-87 Time 1050
Test Organism: C. DUBIA
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	Cond. 0	24	48	72	96
CONTROL	51	10	10	7			8.6	8.5	8.4			8.0	7.9	8.0			20.7	20.4	19.5							
"	80	10	10	9			8.6	8.5	8.4			8.0	7.9	8.0												
50%	4	10	0	—			8.1	8.5	—			7.2	8.1	—			20.0	20.4	—							
"	99	10	0	—			8.1	8.5	—			7.2	8.1	—												
100%	65	10	0	—			7.2	8.7	—			7.0	8.2	—			19.3	20.3	—							
"	20	10	0	—			7.2	8.7	—			7.0	8.2	—												
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: 164/424
Other:
Measured by: KML

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: .1
100% Sample: 7.1
Other:

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

Signatures:
Kathleen Faichild
Martin S Bell
Russel Frydenborg

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7at7;9at9	9.0at21C	10Sat 10,26at30
24 Hours			
48 Hours			
72 Hours			
96 Hours			

Remarks:

Total Residual Chlorine:
Field data: 1.5 mg/l Method: HACH
Lab: 0.7 Method: HACH
Lab: 0.8 Method: DPD

De-chlorinated:
Measurements by: RBF

Reviewed by:
Date: 1/21/88

Industry/Toxicant MDWSA CENTRAL STP
Address VIRGINIA KEY
Contact/District
Effluent Serial Number 001
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 1200
Test Beginning: Date 8-25-87 Time 1100
Test Ending: Date 8-31-87 Time 1100
Test Organism D. PULEX
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	6	10	9	8			8.6	8.7	8.6			8.1	8.0	7.9			20.7	20.2	19.5							
"	15	10	10	10			8.6	8.7	8.6			8.1	8.0	7.9								200	210			
50%	2	10	0	—			8.1	8.7	—			7.1	7.9	—			20.1	20.1	—							
"	22	10	0	—			8.1	8.7	—			7.1	7.9	—								1750	—			
100%	7	10	0	—			7.1	8.8	—			6.9	7.8	—			19.4	20.0	—							
"	4	10	0	—			7.1	8.8	—			6.9	7.8	—								3120	—			
Initial of Investigator		MLR	MLR	MLR			MLR	MLR	MLR			MLR	MLR	MLR			RBF	MLR	MLR			MLR	MLR			

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 103/117
100% Sample: 164/424
Other:
Measured by: KMZ

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: .1
100% Sample: 7.1
Other:

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

Signatures:
Walter S. Nell
Joseph McCandlish
Russell 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: 1.5 Method: HACH
Lab: 0.7 Method: HACH
Lab: 0.8 Method: 1050
Reviewed by:
De-chlorinated:
Measurements by:
Date: 1/21/88

Facility: MADISON CENTRAL STP
Address: VIRGINIA RIV
Contact Person:
Effluent Special Number: 001
NPDES Permit Number:

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

Effluent Collection: Date 8-27-87 Time 1200
Test Beginning: Date 8-25-87 Time 1120
Test Ending: Date 8-27-87 Time 1120
Test Organism: N. COSSI
Age/Life Stage: 22 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	44	5	5	5			8.2	8.5	8.4			7.6	7.7	7.7			21.6	19.9	19.4							
"	30	5	5	5			8.2	8.5	8.4			7.6	7.7	7.7								210	210			
50%	38	5	0	—			7.8	8.5	—			7.0	7.7	—			20.5	19.8	—							
"	23	5	0	—			7.8	8.5	—			7.0	7.7	—								1800	—			
100%	17	5	0	—			6.9	8.5	—			6.9	7.6	—			19.5	19.8	—							
"	—	5	0	—			6.9	8.5	—			6.9	7.6	—								3200	—			
Initial of Investigator		MLR	MLR	MLR			MLR	MLR	MLR			MLR	MLR	MLR			RBF	MLR	MLR			MLR	MLR			

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 105/117
100% Sample: 164/42
Other:
Measured by: MLR

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: .1
100% Sample: 7.1
Other:

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

Signatures:
Martha S. Ball
Joseph M. Faircloth
Russell 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: 1.5 Method: HACH
Lab: 0.7 Method: HACH
Lab: 0.3 Method: 1000
De-chlorinated:
Measurements by: RBF

Reviewed by:
Date: 9/21/88

Industry/Toxicant MDWSA CENTRAL STP
Address VIRGINIA KEY
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-Cl_2

Sample Collection: Date 8-24-87 Time 1200
Test Beginning: Date 8-25-87 Time 1100
Test Ending: Date 8-27-87 Time 1100
Test Organism C. DUBIA
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	—	10	10	10			8.5	8.5	8.5			8.1	8.0	7.9			20.3	19.4	19.4							
"	88	10	10	10			8.5	8.5	8.5			8.1	8.0	7.9												
50%	90	10	0	—			8.1	8.6	—			7.2	8.1	—			20.3	19.3	—							
"	91	10	0	—			8.1	8.6	—			7.2	8.1	—												
100%	58	10	0	—			7.3	8.4	—			7.0	8.2	—			20.1	19.3	—							
"	62	10	0	—			7.3	8.4	—			7.0	8.2	—												
Initial of Investigator		KM	KM	KM			MLR	MLR	MLR			MLR	MLR	MLR			RBF	MLR	MLR							

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 105/117
100% Sample: 164/424
Other:
Measured by: KML

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: .1
100% Sample: 7.6
Other:

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

Signatures:
Jonathan Faucbott
KM Ludwig
Martin F Kell
Raoul 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: 1/21/88

Industry (Name): MIDWSA CENTRAL STP
Address: VIRGINIA KEY
Contact/District:
Effluent Serial Number: 001
NPDES Permit Number:

Test Number: 5 of 6
Test Type: (Static) Renewal, (Chronic) Conventional
Other: DE Cl2

Sample Collected: Date: 8-24-87 Time: 1200
Test Beginning: Date: 8-25-87 Time: 1120
Test Ending: Date: 8-27-87 Time: 1120
Test Organism: D. 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 0	24	48	72	96
CONTROL	~~27~~	10	10	10			8.6	8.5	8.6			8.1	8.0	7.9			20.2	20.2	19.3							
"	~~63~~	10	10	10			8.6	8.5	8.6			8.1	8.0	7.9								200	225			
50%	~~90~~ 40	10	10	10			7.9	8.6	8.4			7.2	7.9	7.9			20.3	20.1	19.3							
"	43	10	10	10			7.9	8.6	8.4			7.2	7.9	7.9								1730	1710			
100%	~~58~~ 65	10	5	0			6.8	8.5	8.1			7.0	7.9	8.0			20.3	20.1	19.5							
"	~~62~~ 50	10	7	1			6.8	8.5	8.1			7.0	7.9	8.0								3180	3200			
	RBF																									
Initial of Investigator		MLR	MLR	MLR			MLR	MLR	MLR			MLR	MLR	MLR			RBF	MLR	MLR			MLR	MLR			

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 105/117
100% Sample: 164/124
Other:
Measured by: KML

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: .1
100% Sample: 7.6
Other:
Hour
Control Water:
100% Sample:
Other:
Measured by: MLR

Signatures:
Virginia Fairchild
Kathleen Lynch
Martin L. Hall
Russell 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:)
Measured by: RBF

Reviewed by:
Date: 1/21/88

Industry/Toxicant: ATHENS CENTRAL STP
Address: VIRGINIA HWY
Contact/District:
Effluent Serial Number: 001
NPDES Permit Number:

BIOASSAY DATA FORM

Test Number: 6 of 6
Test Type: Static Renewal, Continuing Conventional
Other: DE Cl_2

Sample Collection: Date 9-24-87 Time 1200
Test Beginning: Date 9-25-87 Time 1125
Test Ending: Date 10-2-87 Time 1125
Test Organism: N. LEUDSI
Age/Life Stage: 2 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	26	5	5	5			8.3	8.2	8.2			7.6	7.8	7.8			21.4	20.0	19.4							
"	67	5	5	5			8.3	8.2	8.2			7.6	7.8	7.8								210	210			
50%	65	5	5	5			7.8	8.1	7.9			7.1	7.7	7.8			21.1	19.9	19.4							
"	281	5	5	5			7.8	8.1	7.9			7.1	7.7	7.8								1800	1780			
100%	46	5	5	5			7.0	7.8	7.4			6.9	7.7	7.9			20.7	19.9	19.5							
"	10	5	5	5			7.0	7.8	7.4			6.9	7.7	7.9								3210	3200			
Initial of Investigator		KML	JF	KML			MLR	MLR	MLR			MLR	MLR	MLR			RBF	MLR	MLR			MLR	MLR			

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 105/117
100% Sample: 164/424
Other:
Measured by: KML

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: .1
100% Sample: 7.6
Other:
Hour ____
Control Water:
100% Sample:
Other:
Measured by: MLR

Signatures:
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: ____
Reviewed by:
(De-chlorination):
Measurements by: RBF
Date: 1/21/88

206

MICROTOX™ Data Reduction Sheet

SOURCE: Central Miami Ocean Outfall

GRAPH (CALC) EC 50 (15 MIN, 15 °C) = 22.2 (%SAMPLE) mg/L

SAMPLE DATA:

SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent - Cl$_2$

DATE ASSAYED: 8-25-87 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: [illegible] REAGENT VIAL LOT NO. M-1-05

REMARKS:

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	Γ5	Γ15	NOTES
A1	0	89	66			
B1		86	65			
C1		82	60			
A2	12.38	51	35	.63	.21	
B2		51	37			
C2		53	36			
A3	24.75	26.5	17	2.21	1.22	
B3		25 5	19			
C3		27	20			
A4	49.5	35.5	4.5	16.31	9.08	
B4			4			
C4			4.5			
A5	99	.5	.4	170 13	168 17	
B5		.5	.4			
C5		.5	.4			
EC_{50} (MIN, ° C)				16.61	22.24	

REVIEWED BY: [signature]

DATE: 11/21/88

010

MICROTOX™ Data Reduction Sheet

SOURCE: Central Miami Creek Outfall

GRAPH CALC EC 50 (15 MIN, 15 °C) = 29 (%SAMPLE) mg/L

SAMPLE DATA:

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

DATE ASSAYED: 8-25-87 TIME: 9:45 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: [illegible] REAGENT VIAL LOT NO. M-603

REMARKS:

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	Γ5	Γ15	NOTES
A1	0	85	52			
B1		77	48			
C1		84	52			
A2	12.38	61	33	.31	.41	
B2		64.5	27			
C2		64	36			
A3	24.75	50	27	.55	.76	
B3		54	29			
C3		56	30.5			
A4	49.5	37	18	1.24	1.76	
B4		37.5	19			
C4		36	18			
A5	99	19	9	3.31	4.63	
B5		19.5	9			
C5		19	9			
EC_{50} (MIN, °C)				37.71	28.71	

REVIEWED BY: [signature]

DATE: 1/21/88