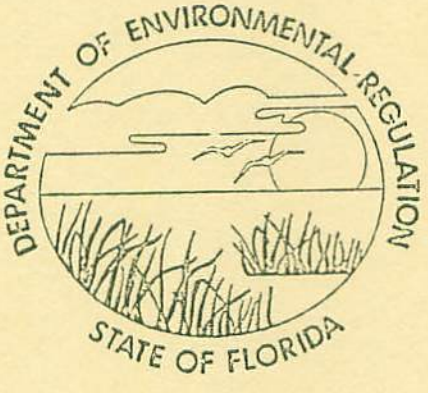


BIOASSAYS OF THE CITY OF NAPLES

WASTEWATER TREATMENT PLANT

NAPLES, COLLIER COUNTY, FLORIDA

NPDES #FL0026271

APRIL 6, 1988

Biology Section
Division of Environmental Programs

BIOASSAYS OF THE CITY OF NAPLES

WASTEWATER TREATMENT PLANT

NAPLES, COLLIER COUNTY, FLORIDA

NPDES #FL0026271

Biology Section
Bureau of Laboratories and
Special Programs
April 6, 1988

EXECUTIVE SUMMARY

City of Naples Wastewater Treatment Plant, Naples, Collier County, NPDES #FL0026271, on 9 to 11 February 1988.

The sample of chlorinated discharge from the City of Naples Wastewater Treatment Plant was acutely toxic to the two water fleas Ceriodaphnia dubia and Daphnia pulex, and to the bannerfin shiner, Notropis leedsi. The Microtox® assay, using the bacterium Photobacterium phosphoreum, also exhibited extreme toxicity producing and EC_{50} = 11.9% in the chlorinated sample.

The de-chlorinated sample of discharge from the City of Naples' WWTP was not acutely toxic to any of the test organisms in the bioassay, although there were three deaths of Ceriodaphnia dubia in the 100% test. The Microtox® assay indicated that he de-chlorinated sample was stimulatory to the bacterium, Photobacterium phosphoreum. Due to the non-toxic nature of the de-chlorinated sample, chemical analyses were not performed.

INTRODUCTION

The City of Naples Wastewater Treatment Plant is located at 1400 3rd Avenue North, Naples, Collier County, Florida (Figure 1). The WWTP has a design capacity of 6.0 MGD and treats an average of 4.6 MGD of waste while serving a population of 38,500. Treatment consists of activated sludge and chlorination (Figure 2). The effluent discharges first to a pond, then to the Gordon River.

To evaluate the existence and extent of the toxicity of the discharge from this facility to the receiving waters, the Biology Section performed three acute toxicity bioassays and one Microtox® assay on both chlorinated and de-chlorinated samples in February, 1988. The results are the subject of this report. Those involved in performing the tests and in preparation of this report were: D. A. Chasteen, J. M. Faircloth, and L. A. Williams.

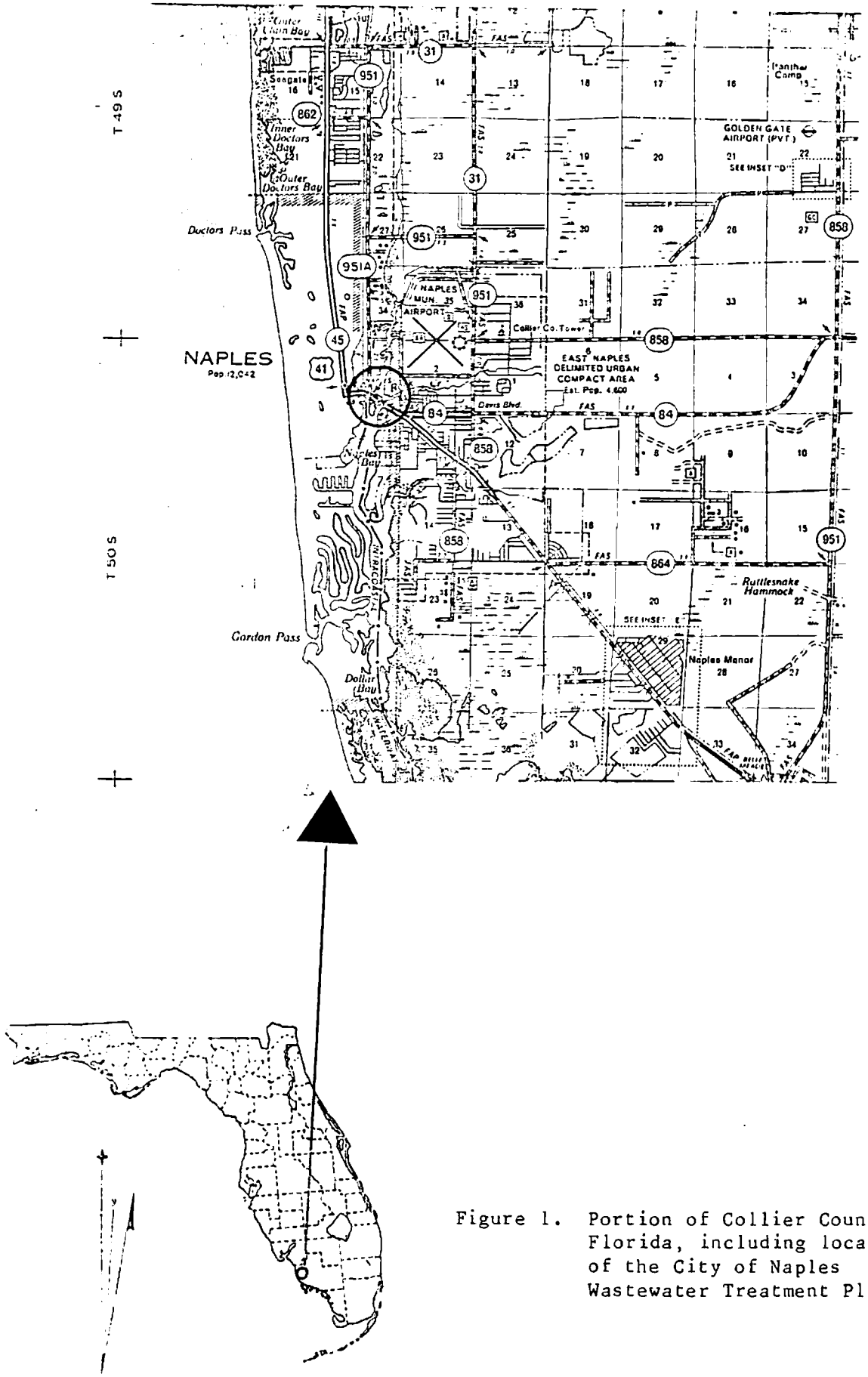


Figure 1. Portion of Collier County, Florida, including location of the City of Naples Wastewater Treatment Plant.

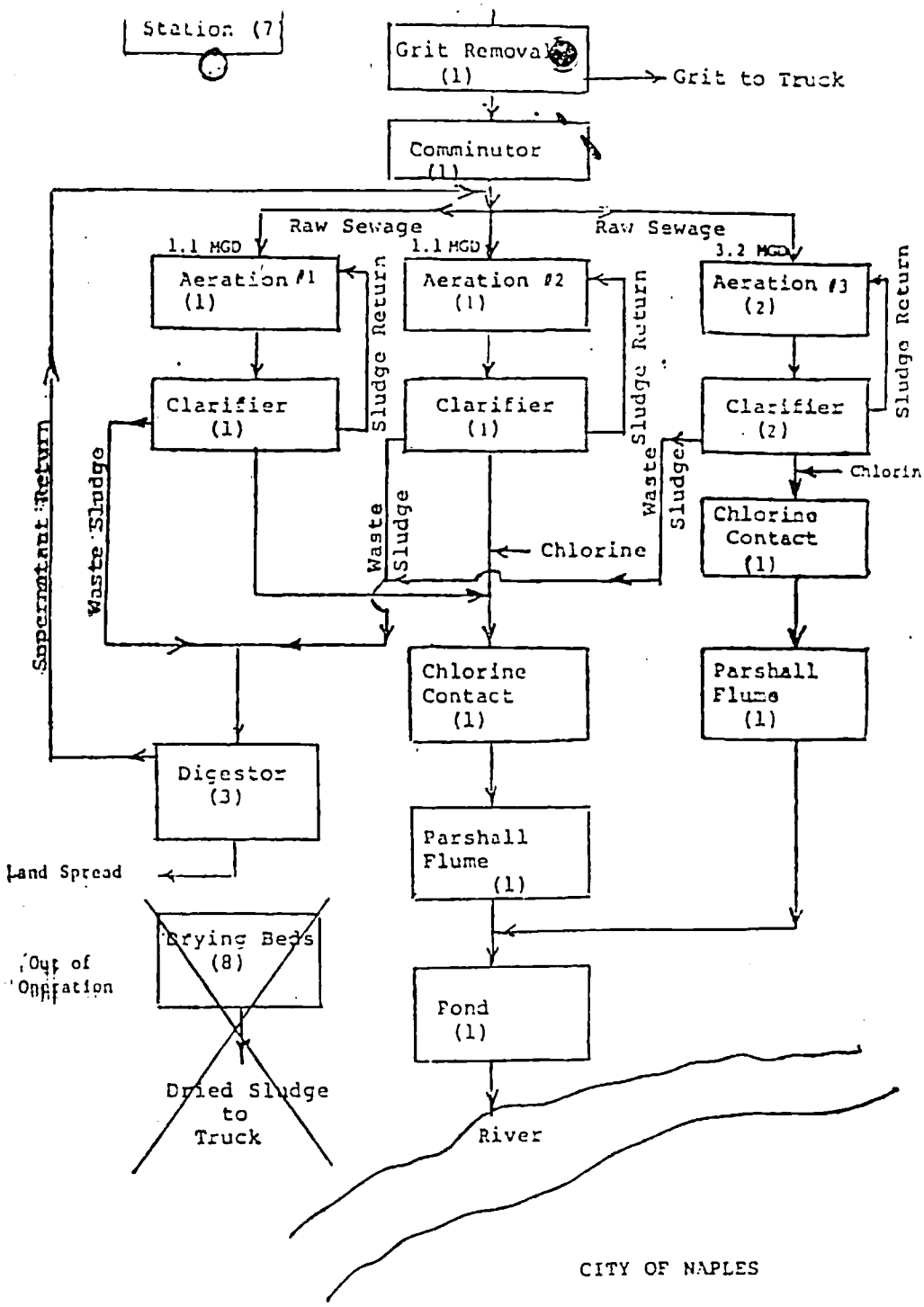


Figure 2. Flow schematic for the City of Naples Wastewater Treatment Plant.

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) 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 25 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

Three static acute toxicity bioassays and one Microtox® assay were performed on the chlorinated and de-chlorinated sample of discharge from the City of Naples WWTP (eight tests total). They were performed from 1045 hours on 9 February to 1100 hours on 11 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. Albert Walton, Jr. of the FDER's South Florida District, at 1140 hours on 8 February, 1988, 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 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. Due to loading mistakes there were 9 in the de-chlorinated control, 11 in the chlorinated 60% test, and 11 in the de-chlorinated 100% test. Fish were not fed during these 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. 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 water sample taken from the City of Naples Wastewater Treatment Plant was acutely toxic to all test organisms. This included the two water fleas, Ceriodaphnia dubia and Daphnia pulex, the fish, Notropis leedsi and the bacterium, Photbacterium phosphoreum. The water fleas and fish all died within 24 hours. The Microtox® assay using the bacterium produced an EC_{50} of 11.9%.

The de-chlorinated sample water was not acutely toxic to the two water fleas or the fish, although, four of the water fleas (Ceriodaphnia dubia) died in the 100% tests. The de-chlorinated sample was stimulatory to the test organism Photobacterium phosphoreum in the Microtox® assay (EC_{50} > 100%), further supporting the conclusion that the de-chlorinated sample was non-toxic.

The chlorine residual in the discharge was 1.2 mg/l Cl_2 by the Hach Method and 0.44 mg/l Cl_2 by the Iodometric Method. The toxicity in the water sample was attributed to the total residual chlorine.

Since the de-chlorinated sample was not toxic, chemical analysis were not performed.

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
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

Table 2. Data recorded during 48 hour, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi, static acute toxicity bioassay of the City of Naples Wastewater Treatment Plant, chlorinated discharge, Collier County, NPDES #FL0026271, on 9 to 11 February, 1988.

Facility: City of Naples STP — NPDES # FL0026271 — Facility Type: Municipal
Location: Collier county — Contact/District: B. Shoemaker
Sample Collection Date: 2/8/88 Time: 1140 — Test type: Static Screening
Test Beginning Date: 2/9/88 Time: 1045 — # tests: 3 — Receiving Water: Gordon River
Test Ending Date: 2/11/88 Time: 1045 — Chlorination Type: chlorinated

Parameter	
Total Residual Cl2:	1.2
Method:	Hach
By:	DAC
Salinity:	0.8

Ammonia:	Control Water	100% Sample
Unionized NH3:	0.00	0.04
Total NH3:	0	5.18

Organism: *Ceriodaphnia dubia* — Age/Life stage: Neonates
LC50: Binomial: Moving Average: Probit:
Toxic

Concentrations	SURVIVAL # at 0 hour	Live at 24 hr	Live at 48 hr	Dissolved Oxygen 0 hour	24 hour	48 hour	pH 0 hour	24 hour	48 hour	Temperature 0 hour	24 hour	48 hour	Conductivity 0 hour	48 hour
Control	10	10	10	8.8	8.7	9	7.9	8.1	7.6	20	19.9	19.7		
Control	10	10	10	8.8	8.8	9	7.9	7.9	7.7					
30%	10	0	0	8.8	9.2	-	7.7	8	-	20.2	19.8	-		
30%	10	0	0	8.8	9.2	-	7.7	8	-					
60%	10	0	0	8.7	9.2	-	7.5	8	-	20.5	19.6	-		
60%	10	0	0	8.6	9.2	-	7.4	7.9	-					
100%	10	0	0	8.4	9.4	-	7.2	7.9	-	20.4	19.6	-		
100%	10	0	0	8.3	9.3	-	7.2	7.9	-					

	Control water	100% sample
Alkalinity:	112	84
Hardness:	113	175

Organism: *Daphnia pulex* — Age/Life stage: Neonates
LC50: Binomial: Moving Average: Probit:
Toxic

Concentrations	SURVIVAL # at 0 hour	Live at 24 hr	Live at 48 hr	Dissolved Oxygen 0 hour	24 hour	48 hour	pH 0 hour	24 hour	48 hour	Temperature 0 hour	24 hour	48 hour	Conductivity 0 hour	48 hour
Control	10	10	10	8.8	8.9	8.9	8	8	7.7	20.4	19.7	19.6		
Control	10	10	10	8.8	8.9	8.9	8	8	7.7				196	195
30%	10	0	0	8.7	9.2	-	7.7	7.9	-	20.7	19.3	-		
30%	10	0	0	8.7	9.2	-	7.7	7.9	-				459	-
60%	10	0	0	8.6	9.2	-	7.4	7.9	-	21	19.2	-		
60%	10	0	0	8.6	9.3	-	7.4	7.9	-				790	-
100%	10	0	0	8.6	9	-	7.1	7.8	-	21.3	19.5	-		
100%	10	0	0	8.4	9	-	7.1	7.8	-				1110	-

	Control water	100% sample
Alkalinity:	112	84
Hardness:	113	175

Organism: *Notropis leedsi* — Age/Life stage: 25 days old
LC50: Binomial: Moving Average: Probit:
Toxic

Concentrations	SURVIVAL # at 0 hour	Live at 24 hr	Live at 48 hr	Dissolved Oxygen 0 hour	24 hour	48 hour	pH 0 hour	24 hour	48 hour	Temperature 0 hour	24 hour	48 hour	Conductivity 0 hour	48 hour
Control	5	5	5	8.3	8.1	8	7.5	7.7	7.5	21	19.6	19.6		
Control	5	5	5	8.3	8.1	8	7.5	7.7	7.5				218	209
30%	5	0	0	8.4	8.5	-	7.4	7.7	-	21.1	19.4	-		
30%	5	0	0	8.4	8	-	7.4	7.7	-				480	-
60%	5	0	0	8.4	8.7	-	7.3	7.7	-	21.4	19.3	-		
60%	5	0	0	8.4	9	-	7.3	7.7	-				800	-
100%	5	0	0	8.4	9	-	7.1	7.6	-	21.6	19.5	-		
100%	5	0	0	8.4	9.1	-	7.1	7.6	-				1140	-

	Control water	100% sample
Alkalinity:	119	84
Hardness:	125	175

Table 3. Data recorded during 48 hour, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi static acute toxicity bioassay of the City of Naples Wastewater Treatment Plant, de-chlorinated portion of sample, Collier County, on 9 to 11 February, 1988.

Facility:	City of Naples STP			NPDES # FL0026271		Facility Type:	Manucipal
Location:	Collier County			Contact/District:	B. Shoemaker		
Sample Collection Date:	2/8/88	Time:	1140	Test type:	Static Screening		
Test Beginning Date:	2/9/88	Time:	1105	# tests:	3	Receiving Water:	Gordon River
Test Ending Date:	2/11/88	Time:	1100	Chlorination Type:	de-chlorinated		

Parameter	
Total Residual Cl2:	1.2
Method:	Hach
By:	DAC
Salinity:	0.8

Ammonia:	Control Water	100% Sample
Unionized NH3:	0.00	0.05
Total NH3:	0	5.18

Organism: *Ceriodaphnia dubia* Age/Life stage: Neonates
LC50: Binomial: Moving Average: Probit:
non-toxic

Concentrations	SURVIVAL # at 0 hour	Live at 24 hr	Live at 48 hr	Dissolved Oxygen 0 hour	24 hour	48 hour	pH 0 hour	24 hour	48 hour	Temperature 0 hour	24 hour	48 hour	Conductivity 0 hour	48 hour
Control	10	10	10	8.5	9	8.5	8	8	7.7	20.5	20	19.9		
Control	10	10	10	8.6	9	8.8	8	8	7.7					
30%	10	10	10	8.7	9	8.8	7.8	8	7.6	20.6	19.8	19.9		
30%	10	10	10	8.6	9	8.8	7.7	8	7.6					
60%	10	10	10	8.6	9	8.8	7.5	8	7.6	21	19.8	19.8		
60%	10	10	10	8.6	9	8.8	7.5	8	7.6					
100%	10	10	7	8.6	8.9	8.7	7.3	7.9	7.5	21.2	19.9	20.1		
100%	10	10	9	8.3	8.9	8.6	7.3	7.9	7.5					

	Control water	100% sample
Alkalinity:	112	84
Hardness:	113	175

Organism: *Daphnia pulex* Age/Life stage: Neonates
LC50: Binomial: Moving Average: Probit:
non-toxic

Concentrations	SURVIVAL # at 0 hour	Live at 24 hr	Live at 48 hr	Dissolved Oxygen 0 hour	24 hour	48 hour	pH 0 hour	24 hour	48 hour	Temperature 0 hour	24 hour	48 hour	Conductivity 0 hour	48 hour
Control	10	10	10	8.8	9	8.7	8	8.1	7.7	20.6	19.8	19.6		
Control	10	10	10	8.8	9	8.8	8	8	7.7				202	195
30%	10	10	10	8.8	9	8.4	7.8	7.9	7.5	20.7	19.5	19.3		
30%	10	10	10	8.8	9	8.4	7.8	7.9	7.5				473	463
60%	10	10	10	8.6	8.9	8.4	7.5	7.9	7.4	20.8	19.5	19.3		
60%	10	10	10	8.6	8.9	8.3	7.5	7.9	7.5				799	790
100%	10	10	10	8.3	8.7	8.4	7.2	7.8	7.5	21	19.7	19.5		
100%	10	10	10	8.4	8.7	8.5	7.2	7.8	7.5				1120	1110

	Control water	100% sample
Alkalinity:	112	84
Hardness:	113	175

Organism: *Notropis leedsi* Age/Life stage: 25 days old
LC50: Binomial: Moving Average: Probit:
non-toxic

Concentrations	SURVIVAL # at 0 hour	Live at 24 hr	Live at 48 hr	Dissolved Oxygen 0 hour	24 hour	48 hour	pH 0 hour	24 hour	48 hour	Temperature 0 hour	24 hour	48 hour	Conductivity 0 hour	48 hour
Control	5	5	5	8.4	8.2	8	7.5	7.7	7.5	21	19.4	19.3		
Control	4	4	4	8.4	8.2	7.8	7.5	7.7	7.4				217	208
30%	5	5	5	8.4	8	7.8	7.4	7.7	7.4	21	19.2	19.1		
30%	5	5	5	8.4	8	7.8	7.4	7.6	7.3				479	451
60%	5	5	5	8.4	7.4	6.9	7.3	7.5	7.2	21.1	19.2	19.1		
60%	5	5	5	8.3	8	7.7	7.3	7.7	7.4				805	790
100%	6	6	6	8.3	7.9	7.8	7.2	7.6	7.4	21.5	19.5	19.4		
100%	5	5	5	8.4	7.9	7.8	7.2	7.6	7.4				1160	1110

	Control water	100% sample
Alkalinity:	119	84
Hardness:	125218	175

Table 4. Microtox test results , City of Naples STP discharge, Collier County, NPDES #FL0026271, on 9 February, 1988.

Microtox Test Culture Lot # M-606

Final Reclaimed Water

% Sample	Chlorinated		Dechlorinated	
	5-min	15-min	5-min	15-min
0	97	58	65	54
0	88	52	62	51
0	90	55	65	54
12.38	40	22	68	59
12.38	43	20	66	58
12.38	47	24	71	62
24.75	17	7	75	67
24.75	21	8	73	67
24.75	19	8	69	65
49.5	2	1	68	63
49.5	2	1	74	69
49.5	2	1	71	66
99	0.3	0.1	64	60
99	0.3	0.1	67	64
99	0.3	0.1	68	66

EC50 Chlorinated (15 minute, 15 degrees C) = 11.9% 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.

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.

Industry/Toxicant: City of Naples S.T.F.
Address: 1400 3rd Ave N Naples, Fl Collier Co.
Contact/District: B. Skogerboe SF
Effluent Serial Number: FL 0026271 AF
NPDES Permit Number: FL 0026271

BIOASSAY DATA FORM

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

Sample Collection: Date 2/8/88 Time 1140
Test Beginning: Date 2/9/88 Time 1045
Test Ending: Date 2/11/88 Time 1045
Test Organism: C. Dubia, Daphnia
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
Control	65	10	10	10			8.8	8.7	8.5			7.9	8.1	7.6			20.0	19.9	19.7		
Control	2	10	10	10			8.8	8.8	8.9			7.9	7.9	7.7							
30 %	36	10	0				8.8	9.2				7.7	8.0				20.2	19.6			
30 %	9	10	0				8.8	9.2				7.7	8.0								
60 %	83	10	0				8.7	9.2				7.5	8.0				20.5	19.6			
60 %	81	10	0				8.6	9.2				7.4	7.9								
100 %	62	10	0				8.4	9.4				7.2	7.9				20.4	19.6			
100 %	91	10	0				8.3	9.3				7.2	7.9								
Initial of Investigator		MB	DAC	MF			DAC	MF	DAC			DAC	DAC	DAC			DAC	DAC	DAC		

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 112/113 0.0F
100% Sample: 84/185
Other:
Measured by: DAC

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: 0.0
100% Sample: 5.18
Other:

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

slope - 56.6

Signatures:
Marshall Faircloth
Donna Chastain

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7@7.9@9	9.0@21°C	10.1@10, 28.9@30
24 Hours	7@7.9@9	9.0@21°C	
48 Hours	7@7.9@9	9.0@21°C	1294 10.12@ 27.6@30
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: 1.3 Method: Hach
Lab: 1.2 Method: Hach
Lab: 0.44 Method: Colorimeter

De-chlorinated:
Measurements by: DAC

Reviewed by:
Date: 4/6/88

Remarks: Ohm. 21°C 1305 and calibrate 1294.7
48 hr.

TOXIC

Industry/Effluent: City of Wpt. STP
Address: 1400 3rd Ave/Wpt. Ft Collins Co.
Contact/District: G Sherwin SF
Effluent Serial Number:
NPDES Permit Number: FL0026271

BIOASSAY DATA SHEET

Test Number: 2 of 6
Test Type: Static Renewal, Screening Conventional
Other: Cl

Sample Collection: Date 2/8/88 Time 1140
Test Beginning: Date 2/9/88 Time 10:45
Test Ending: Date 2/14/88 Time 10:45
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	42	10	10	10			8.8	8.9	8.9			8.0	8.0	7.7			20.4	19.7	19.6							
C.T.C	25	10	10	10			8.8	8.9	8.9			8.0	8.0	7.7								196		195		
30%	66	10	0				8.7	9.2				7.7	7.9				20.7	19.3								
30%	39	10	0				8.7	9.2				7.7	7.9									459				
60%	61	10	0				8.6	9.2				7.4	7.9				21.0	19.2								
60%	67	10	0				8.6	9.3				7.4	7.9									790				
100%	2	10	0				8.6	9.0				7.1	7.8				21.3	19.5								
100%	11A	10	0				8.4	9.0				7.1	7.8									1110				
Initial of Investigator		DAC	DAC	DAC			DAC	MF	DAC			DAC	DAC	DAC			DAC	DAC	DAC			DAC		DAC		

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 112/103
100% Sample: 84/175
Other:
Measured by: DAC

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: 0.0
100% Sample: 5.18
Other:
Hour ____
Control Water:
100% Sample:
Other:
Measured by: ML

slope - 56.6

Signatures:

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7.9@21°C	7.0@21°C	10,000 @ 25°C
24 Hours	7.9@21°C	9.0@21°C	
48 Hours			
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: 1.3 Method: Hach
Lab: 1.2 Method: Hach
Lab: 0.44 Method: dechlorinator

De-chlorinated:
Measurements by: DAC

0.8 salinity by DAC

Reviewed by:
Date: 4/6/88

Remarks:

130 Survive at 1299.7

UNC

Industry/Toxicant: City of Naples STP
Address: 1400 3rd Ave N Naples FL Collier Co
Contact/District: B Shannon / SF
Effluent Serial Number:
NPDES Permit Number: FL0026271

BIOASSAY DATA FORM

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

Sample Collection: Date 2/5/98 Time 1140
Test Beginning: Date 2/6/98 Time 1100
Test Ending: Date 2/11/98 Time 1045
Test Organism: Mysidopsis bahia
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	4	5	5	5			8.3	8.1	8.0			7.5	7.7	7.5			21.0	19.6	19.6							
Control	32	5	5	5			8.3	8.1	8.0			7.5	7.7	7.5								218		209		
30%	31	5	0				8.4	8.5				7.4	7.7				21.1	19.4								
30%	234	5	0				8.4	8.0				7.4	7.7									480				
60%	131	5	0				8.4	8.7				7.3	7.7				21.4	19.3								
60%	25	6	0				8.4	9.0				7.3	7.7									800				
100%	6	5	0				8.4	9.0				7.1	7.6				21.6	19.5								
100%	228	5	0				8.4	9.1				7.1	7.6									1140				
		1																								
Initial of Investigator		DAC	DAC	MF			DAC	MF	DAC			DAC	DAC	DAC			DAC	DAC	DAC			MF		DAC		

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 119/125
100% Sample: 84/175
Other:
Measured by: DAC

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0 — Tot.
Control Water: 0.0
100% Sample: 5.18
Other:
Hour ____
Control Water:
100% Sample:
Other:
Measured by: MF

Aqua - 56.6

Signatures:
Marshall Faircloth
Carl Chandler

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7.00 7.9 9	9.0 @ 21°C	10.1 @ 25.7 0 3 0
24 Hours	7.01 7.9 9	9.0 @ 21°C	
48 Hours			
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: 1.3 Method: Hach
Lab: 1.2 Method: Hach
Lab: 0.44 Method: Hach

0.8 ml by DAC

De-chlorinated:
Measurements by: DAC

Reviewed by:
Date: 4/6/98

Remarks:
ch. 1305 @ 12997 Cond. cal.

Not Acute / Toxic

BIOASSAY DATA FORM

Industry/Toxicant: Cotton Nash STP
Address: 1400 3rd St NW Naples Ft Collins, Co.
Contact/District: B Shanks / SF
Effluent Serial Number:
NPDES Permit Number: FL 0026271

Test Number: 4 of 6
Test Type: Static Renewal, Screening Conventional
Other: de-cl

Sample collection: Date 2/8/88 Time 1140
Test Beginning: Date 2/9/88 Time 1105
Test Ending: Date 2/11/88 Time 1150
Test Organism: C. Dubia Daphnia
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					
Control	93	10	10	10			8.5	9.0	8.5			8.0	8.0	7.7			20.5	20.0	19.9							
Control	1	10	10	10			8.6	9.0	8.8			8.0	8.0	7.7												
30%	63	10	10	10			8.7	9.0	8.8			7.8	8.0	7.6			20.6	19.8	19.9							
30%	92	10	10	10			8.6	9.0	8.8			7.7	8.0	7.6												
60%	67	10	10	10			8.6	9.0	8.8			7.5	8.0	7.6			21.0	19.8	19.8							
60%	5	10	10	10			8.6	9.0	8.8			7.5	8.0	7.6												
100%	95	10	10	7			8.6	8.9	8.7			7.3	7.9	7.5			21.2	19.9	20.1							
100%	82	10	10	9			8.3	8.9	8.6			7.3	7.9	7.5												
Initial of Investigator		MF	MF	DAC			DAC	DAC	DAC			DAC	DAC	DAC			DAC	DAC	DAC							

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 112/113
100% Sample: 84/175
Other:
Measured by: DAC

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0 tbl
Control Water: 0.0
100% Sample: 5.18
Other:
Hour
Control Water:
100% Sample:
Other:
Measured by: MF

slope - 56.6

Signatures:
Mary Ann Fondell
Dave Chaplin

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7.0, 7.00	9.02 [illegible]	10.0, 10, 28.9 c 30
24 Hours	7.0, 7.00	9.0 a 21°C	
48 Hours			
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: 1.3 Method: Hach
Lab: 1.2 Method: Hach
Lab: 0.44 Method: Hach
De-chlorinated: 0.0
Measurements by: DAC

Reviewed by:
Date: 4/6/88

Remarks:
Olw, 1305c 1299.7 Cond

Industry/Toxicant: City of Naples STF
Address: 1400 3rd Ave N, Naples FL Collier Co.
Contact/District: B Shoemaker / SE
Effluent Serial Number:
NPDES Permit Number: FL0026271

BIOASSAY DATA FORM

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

Sample Collection: Date 2/8/88 Time 1140
Test Beginning: Date 2/9/88 Time 1050
Test Ending: Date 2/11/88 Time 1100
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	70	10	10	10			9.9	9.0	8.7			8.0	8.1	7.7			20.6	19.8	19.6							
Control	68	10	10	10			8.8	9.0	8.8			8.0	8.0	7.7								202		195		
30 %	7	10	10	10			8.8	9.0	8.4			7.8	7.9	7.5			20.7	19.5	19.3							
30 %	50	10	10	10			8.8	9.0	8.4			7.8	7.9	7.5								473		463		
60 %	41	10	10	10			8.6	8.4	8.4			7.5	7.9	7.4			20.8	19.5	19.3							
60 %	132	10	10	10			8.6	8.9	8.3			7.5	7.9	7.5								799		790		
100 %	46	10	10	10			8.3	8.7	8.4			7.2	7.8	7.5			21.0	19.7	19.5							
100 %	48	10	10	10			8.4	8.7	8.5			7.2	7.8	7.5								1120		1110		
Initial of Investigator		DAC	DAC	DAC			DAC	DAC	DAC			DAC	DAC	DAC			DAC	DAC	DAC			DAC		DAC		

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 112/113
100% Sample: 84/175
Other:
Measured by: DAC

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0 — Tod
Control Water: 0.0
100% Sample: 5.1
Other:
Hour ____
Control Water:
100% Sample:
Other:
Measured by: mf

slope - 56.6

Signatures:
Laurel A Lyndall
David Chambers

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7.0 4.0 9	9.0 @ 21°C	12.880, 25.0 @ 30
24 Hours	7.0 9.0 9	9.0 @ 21°C	
48 Hours			
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: 1.3 Method: Hach
Lab: 1.2 Method: Hach
Lab: 0.44 Method: Colorimetric
De-chlorinated: 0.0 Hach
Measurements by: DAC

Reviewed by:
Date: 4/6/88

Remarks:
Ch 1305 @ 1299.7 (21°C) and 5%

NON - TOXIC

BIOASSAY DATA FORM

Industry/Facility: City of Naples STP
Address: 1400 3rd Ave N. Naples FL Collier Co.
Contact/District: B. Shumake / SE
Effluent Serial Number:
NPDES Permit Number: FL0026271

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

Sample Collection: Date 2/5/88 Time 1140
Test Beginning: Date 2/9/88 Time 1105
Test Ending: Date 2/11/88 Time 1100
Test Organism: Mysidopsis bahia
Age/Life Stage: 25 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	16	5	5	5			8.4	8.2	8.0			7.5	7.7	7.5			21.0	19.4	19.3							
control	1	4	4	4			8.4	8.2	7.8			7.5	7.7	7.4								217		218		
30%	86	5	5	5			8.4	8.0	7.8			7.4	7.7	7.4			21.0	19.2	19.1							
30%	43	5	5	5			8.4	8.0	7.8			7.4	7.6	7.3								479		451		
60%	34	5	5	5			8.4	7.4	6.9			7.3	7.5	7.2			21.1	19.2	19.1							
60%	2	5	5	5			8.3	8.0	7.7			7.3	7.7	7.4								805		796		
100%	62	6	6	6			8.3	7.9	7.8			7.2	7.6	7.4			21.5	19.5	19.4							
100%	25	5	5	5			8.4	7.9	7.8			7.2	7.6	7.4								1160		1110		
Initial of Investigator		DAC	DAC	DAC			DAC	DAC	DAC			DAC	DAC	DAC			DAC	DAC	DAC			DAC		DAC		

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

Control Water: 119/125
100% Sample: 84/175
Other:
Measured by: DAC

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

Hour 0 — tot

Control Water: 0.0
100% Sample: 5.18
Other:

Hour ____

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

slope = 56.6

Signatures:

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7@7.9@9	9@8.2°C	10,410 µS@25
24 Hours	7@7.9@9	9@8.2°C	
48 Hours			
72 Hours			
96 Hours			

Total Residual Chlorine:

Field data: 4.3 Method: Hach
Lab: 4.2 Method: Hach
Lab: 0.44 Method: colorimetric

De-chlorinated: 0.0 Hach
Measurements by: DAC

Reviewed by:

Date: 4/6/88

Remarks:

Ohm 1305 @ 1299.7 (25°C cond. STD)

068

MICROTOX™ Data Reduction Sheet

SOURCE: Naples STP

GRAPH CALC EC 50 (15 MIN, 15 °C) = 11.9 (%SAMPLE) mg/l.

SAMPLE DATA:

SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent Cl 2

DATE ASSAYED: 02-09-88 TIME:

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-

REMARKS:

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	Γ5	Γ15	NOTES
A1	0	88	58			
B1		96	52			
C1		97	55			
A2	12.38	40	22	1.12	1.50	
B2		43	20			
C2		47	24			
A3	24.75	17	7	3.83	6.17	
B3		21	8			
C3		19				
A4	49.5	2	1	14.83	54.0	
B4		2	1			
C4		2	1			
A5	99	3	1	304.56	549.0	
B5		3	1			
C5		3	1			
EC_{50} (MIN, °C)				13.24	11.89	

REVIEWED BY:

DATE: 4/6/88

069

MICROTOX™ Data Reduction Sheet

SOURCE: Naperville STP (stimulatory)

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

SAMPLE DATA:

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

DATE ASSAYED: 02-09-88 TIME:

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: Pete Williams REAGENT VIAL LOT NO. M-

REMARKS:

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	Γ5	Γ15	NOTES
A1	0	65	54	X	X	
B1		62	51			
C1		65	54			
A2	12.38	68	59	-.063	-.112	
B2		66	58			
C2		71	62			
A3	24.75	75	67	-.115	-.201	
B3		73	67			
C3		69	65			
A4	49.5	68	63	-.099	-.197	
B4		74	69			
C4		71	66			
A5	99	64	60	-.035	-.163	
B5		67	64			
C5		68	66			
EC_{50} (MIN, ° C)						stimulatory

REVIEWED BY:

DATE: 4/6/88