DEPARTMENT OF ENVIRONMENTAL REGULATION

STATE OF FLORIDA

BIOASSAYS OF THE [redacted] PIERCE UTILITIES AUTHORITY

WASTEWATER TREATMENT PLANT

ST. LUCIE COUNTY, FLORIDA

NPDES #FL0027278

SAMPLED 8/3/87

JANUARY 19, 1988

Biology Section
Division of Environmental Programs

BIOASSAYS OF THE FT. PIERCE UTILITIES AUTHORITY

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ST. LUCIE COUNTY, FLORIDA

NPDES #FL0027278

Biology Section
Bureau of Laboratories and
Special Programs
January 19, 1988

EXECUTIVE SUMMARY

Fort Pierce Utilities Authority Wastewater Treatment Plant, NPDES #FL0027278, St. Lucie County, on 4 to 6 August, 1987.

The Ft. Pierce Utilities Authority WWTP located near the Ft. Pierce inlet to the Indian River treats approximately 4.6 MGD of combined municipal and industrial sewage from the Ft. Pierce area. Three static screening bioassays and one Microtox® Assay were performed on both the chlorinated sample and a de-chlorinated portion of sample of reclaimed water from the Ft. Pierce Utilities Authority WWTP.

Extreme toxicity with complete mortality was observed at 24 hours for both water fleas (Ceriodaphnia dubia and Daphnia pulex) and the bannerfin shiner (Notropis leedsi) in the chlorinated discharge. The chlorinated discharge was also toxic to the common marine bacterium, Photobacterium phosphoreum, producing an EC_{50} of 15.03% in the Microtox® Assay. The total chlorine residual measured .6 mg/l by the Hach DPD method and .71 mg/l by the Iodometric method.

The de-chlorinated sample exhibited acute toxicity with LC_{50}'s between 0 - 50% at 24 hours to the water flea Ceriodaphnia dubia and a 48 hour LC_{50} between 50% and 100% to the water flea, Daphnia pulex. Microtox® Assay results for the de-chlorinated portion also exhibited toxicity to Photobacterium phosphoreum producing an EC_{50} of 81.5% sample. No mortality occurred with the bannerfin shiner, Notropis leedsi. Total ammonia measured .8 mg/l, below toxic concentrations.

Chemical analyses for the pesticide extractable organic fraction indicated the presence of g-BHC (lindane) at .12 ug/l. Metals samples were not collected. Numerous other unidentified components sensitive to electron capture detectors (ECD) and nitrogen-phosphorus detectors (NPD) were present contributing to the toxicity. NPD's are specific to nitrogen and phosphorus containing compounds which include a broad range of pesticides and other toxic compounds (i.e., nitrosamines etc.). Toxicity may be attributed to lindane and unidentified pesticide extractables.

INTRODUCTION

The Ft. Pierce Utilities Authority Wastewater Treatment Plant is located on Causeway Island, Ft. Pierce, St. Lucie County, Florida (Figure 1). The facility provides secondary treatment to 4.6 MGD (average combined flow) of domestic and industrial wastes from a population of 38,000. Alhthough the design capacity is 6.5 MGD, peak flows of 8.51 MGD have occurred. After comminution, grit removal, aeration (including both activated sludge and contact stabilization), clarification, digestion, sludge removal, and chlorination, the reclaimed water is discharged to the Indian River (data from NPDES file).

A previous bioassay, performed in September, 1980 on a sample of pre-chlorinated effluent from this facility, using Daphnia pulex as the test organism, resulted in a 48 hour LC_{50} of 73.2% (FDER 1980). Additional bioassays were performed on samples of chlorinated and de-chlorinated reclaimed waters in October, 1984 and October 1985. In 1984, both samples were acutely toxic to the test organism, Mysidopsis bahia (48 hour LC_{50}'s = 31.5% and 44.8%, respectively) (FDER 1984). Toxicity was attributed to several unidentified organic compounds and the metals copper, mercury, and zinc.

In 1985, the pre-chlorinated sample was acutely toxic to the water flea, Daphnia pulex and the opossum shrimp, Mysidopsis bahia with LC_{50}'s of 80.9% and 44.8%, respectively. The chlorinated discharge was only slightly more toxic with LC_{50}'s of < 32% and 50.1%, respectively. It was also toxic to the bannerfin shiner (Notropis leedsi) with an LC_{50} of 56.5%. Toxicity was attributed to the pesticide lindane (.02 ug/l), several other unidentified organic compounds, and the volatile compounds (biomodichloromethane (20 ug/l), chloroform (70 ug/l), dilromochloromethane (4.3 ug/l), and other unidentified volatiles (10 ug/l).

The Ecotoxicology Lab recently performed additional bioassays and Microtox® Assays on samples of chlorinated and de-chlorinated portions of sample from this facility on 4 to 6 August, 1987. 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, J. M. Faircloth, K. M. Lurding, M. L. Roll, and L. A. Williams.

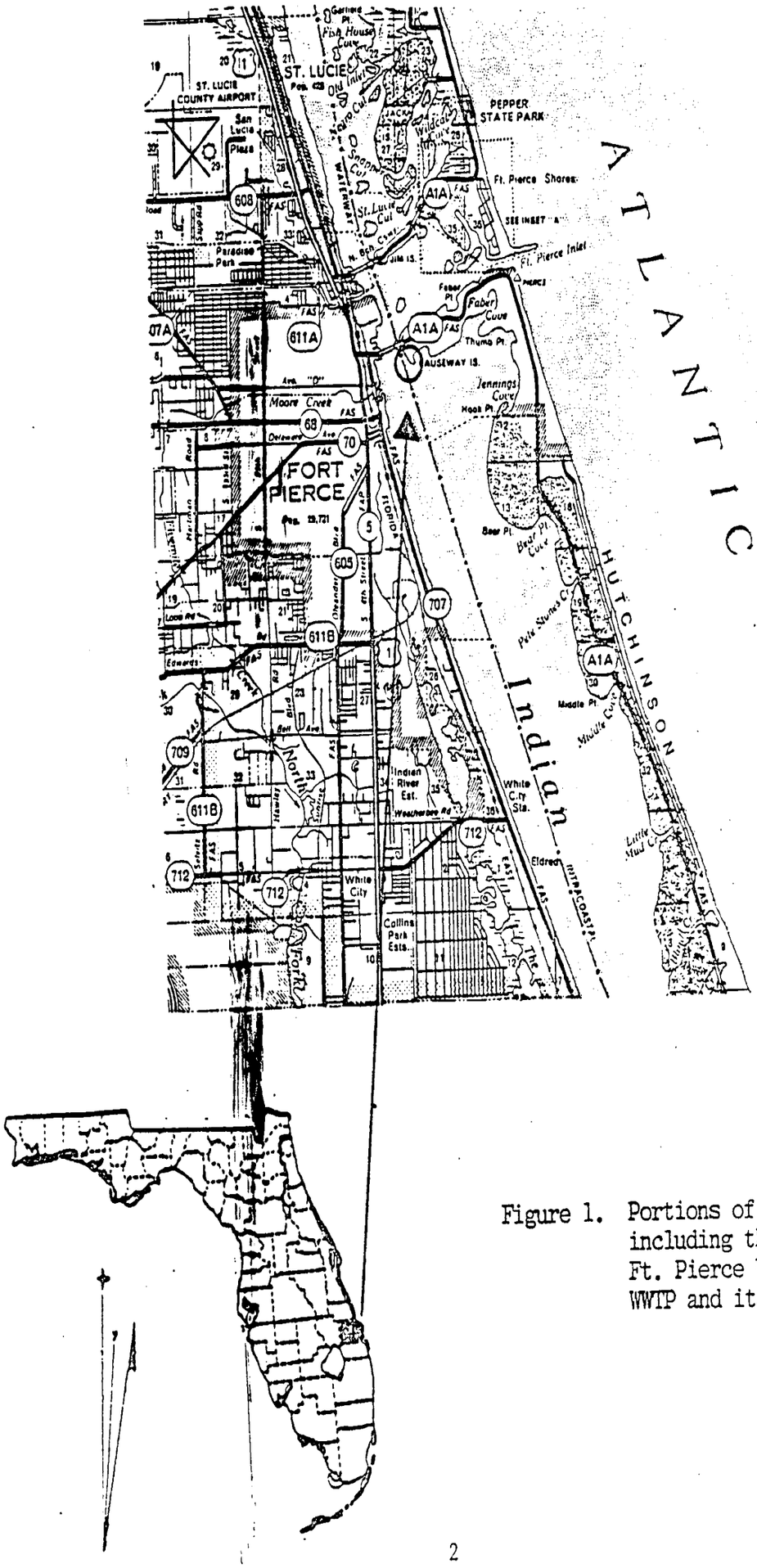


Figure 1. Portions of St. Lucie Co., including the location of Ft. Pierce Utility Authority WWTP and its outfall.

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 20 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

Six separate 48 hour static screening toxicity bioassays were performed on a sample of the discharge from the Ft. Pierce Utilities Authority Wastewater Treatment Plant. Three were performed on a de-chlorinated portion and three on the chlorinated discharge. A Microtox® Assay was performed on both the de-chlorinated and chlorinated sample portions. The tests were performed from 1030 hours on August 4 to 1030 hours on August 6, 1987.

Separate samples were obtained for chemical and biological testing. Samples for metal analyses were not collected. Samples for organic chemical analyses and for the bioassays were preserved at 0°C on ice. The samples were collected by Mr. Paul Strauss, using standard wastewater sampling procedures on 3 August 1987 at 0950. The samples were transported to the DER laboratory in Tallahassee for analyses within 25 hours of collection.

Test Temperature

Upon arrival of the iced (4°C) bioassay samples in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the samples allowed to rise to 20° $\pm$ 2°C. Although this temperature may be different than the temperature of the sample during collection, it is the temperature range to which the test organisms are acclimatized and

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

Total Residual Chlorine

Since many reclaimed waters are chlorinated, especially those originating from sewage treatment facilities, it is often desirable to differentiate between the toxicity resulting from chlorine and the toxicity associated with other components of the reclaimed water. To accomplish this, the Biology Section performed concurrent bioassays on a sample of chlorinated reclaimed water in which the chlorine had been neutralized. De-chlorination was accomplished by adding a calculated amount of 0.025N sodium thiosulfate. The total residual chlorine concentrations of the tested sample of reclaimed water are given on the bioassay data forms. A comparison of the results of these tests will distinguish between the toxicity due solely to residual chlorine and the toxicity inherently contained in the reclaimed water.

Freshwater Tests

Freshwater for dilution of the reclaimed 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 shown in Table 1.

Two replicates, of each concentration and a control, were used to test with the water fleas, Ceriodaphnia dubia and Daphnia pulex. Flint glass jars of either 60 ml (for C. dubia) or 250 ml (for D. pulex) capacity were used as test chambers, and either 50 ml or 200 ml of the appropriate test concentration was placed in each chamber. Ceriodaphnia were siphoned into a large culture dish and neonates were transferred by glass pipette, and loaded 10 per chamber, resulting in 20 per test concentration. Daphnia were siphoned into a large culture dish to isolate the proper size and age organisms (neonates). To accomplish this, the Daphnia were siphoned through a stainless steel screen. The trapped adults were then discarded. These neonates were then transferred by glass pipette, examined for injury, and were loaded 10 per chamber, resulting in 20 per test concentration. Water fleas were not fed during these tests.

Two replicates, of each concentration and a control, were used for the Notropis leedsi (bannerfin shiner) bioassay. The fish were transferred individually or by twos, into wide mouth quart jars containing 500 ml each of the appropriate test concentration, and were loaded 5 per chamber, resulting in 10 per concentration. Fish were not fed during this 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 from the Ft. Pierce Utilities Authority was acutely toxic (Table 2) to all the test organisms including the two water fleas, Ceriodaphnia dubia and Daphnia pulex and to the bannerfin shiner, Notropis leedsi with LC_{50}'s of < 50% (the lowest concentration used in a static screening test). The discharge had a total chlorine residual of .60 mg/l by the Hach method and .71 mg/l by the Iodometric method. Static screening bioassays of de-chlorinated sample (Table 3) were non- toxic to the representative fish specie (Notropis leedsi) but still exhibited acute toxicities to the water fleas, C. dubia and D. pulex with LC_{50}'s of < 50% and between 50% - 100%, respectively. The Microtox® Assay had an EC_{50} of 15.03% for the chlorinated sample and 81.45% for the de-chlorinated portion of the sample further supporting the conclusion that both fractions are acutely toxic. Part of the toxicity can be attributed to chlorine residual but significant acute toxicity remains in the de-chlorinated sample portion.

Chemical analyses (Table 5) performed on pesticide extractable organics showed a significant concentration of the pesticide lindane (.12 ug/l) in the reclaimed water. This value seems high compared to the Class III water quality standards of .004 ug/l for marine systems. Lindane is a biocidal agent used primarily to control anthropods (i.e., insects-fleas). The water fleas are crustacean anthropods and are seriously affected by the toxicity of the discharge lending support to the suggestion that lindane is the primary toxic factor. Further analysis using a nitrogen-phosphorus detector, indicated the presence of several unidentified nitrogen and phosphorus organic containing compounds. This analysis detects phosphorus based pesticides, nitrogen based pesticides, and other nitrogen containing compounds (i.e., nitrosamines). Chlorine, lindane, and the unidentified organic compounds are the most likely cause of the acute toxicity in the discharge.

The Indian River biota is extremely rich, and the area serves as feeding and nursery ground for many commercial and sport fish and commercial shrimp found in the eastern United States coastal waters as well as the invertebrate animals which serve as their food. Toxic discharges with high concentrations of biocidal compounds (i.e., lindane and other pesticide fractions) may cause serious impact to major strands of the food webs.

The toxic response exhibited by the crustaceans used in the screening bioassay demonstrates what probably occurs in the receiving waters. An ultimate loss in anthropod biomass and numbers, one of the dominant groups of organisms of the benthos, zooplankton, as well as many commercial shrimps and crabs will send serious impacts cascading through the lagoonal ecosystem.

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 static screening bioassays using Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi on chlorianted discharge from the Fort Pierce Utilities Authority WWTP, St. Lucie County, NPDES #FL0027278, on 4 to 6 August, 1987.

Facility:	Fort Pierce Utilities Authority WWTP			NPDES # FL0027278		Facility Type:	Municipal WWTP
Location:	St. Lucie County			Contact/District:	Paul Strauss/SE		
Sample Collection Date:	8/3/87	Time:	950	Test type: Static Screening			
Test Beginning Date:	8/4/87	Time:	1030	# tests:	3	Receiving Water:	Indian River
Test Ending Date:	8/6/87	Time:	1030	Chlorination Type:	Chlorinated		

Parameter		
Total Residual Cl2:	0.60	0.71
Method:	Hach	Iodometric
By:		

Ammonia:	Control Water	100% Sample
Unionized NH3:	0.00	0.00
Total NH3:	0.00	0.80

Organism: *Ceriodaphnia dubia* — Age/Life stage: Neonates

LC50: <50% — Binomial: — Moving Average: — Probit:

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	9	8.6	8.6	8.3	8	8.2	8	20.8	20.4	21		
Control	10	10	10	8.6	8.6	8.3	8	8.2	8					
50%	10	0	0	8.6	8.9	-	7.3	7.9	-	20.8	20.2	-		
50%	10	0	0	8.6	8.9	-	7.3	7.9	-					
100%	10	0	0	8.5	8.9	-	6.8	7.6	-	20.9	20.2	-		
100%	10	0	0	8.5	8.9	-	6.8	7.6	-					

	Control water	100% sample
Alkalinity:	108	36
Hardness:	113	140

Organism: *Daphnia pulex* — Age/Life stage: Neonates

LC50: <50% — Binomial: — Moving Average: — Probit:

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	8.6	8.5	8.4	8	8.2	8	20.9	20.3	20.5		
Control	10	10	10	8.6	8.6	8.4	8	8.2	8				205	215
50%	10	0	0	8.6	8.8	-	7.3	7.9	-	21.2	20.3	-		
50%	10	0	0	8.6	8.8	-	7.3	7.9	-				465	-
100%	10	0	0	8.5	8.8	-	6.8	7.5	-	21.1	20.2	-		
100%	10	0	0	8.5	8.8	-	6.8	7.5	-				850	-

	Control water	100% sample
Alkalinity:	108	36
Hardness:	113	140

Organism: *Notropis leedsi* — Age/Life stage:

LC50: < 50% — Binomial: — Moving Average: — Probit:

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	8.5	8.4	7.6	8	7.9	21.5	20.9	20.3		
Control	5	5	5	8.3	8.5	8.4	7.6	8	7.9				202	210
50%	5	0	0	8.4	8.7	-	7.1	7.6	-	21.4	20.6	-		
50%	5	0	0	8.4	8.7	-	7.1	7.7	-				480	-
100%	5	0	0	8.5	8.8	-	6.8	7.3	-	21.3	20.5	-		
100%	5	0	0	8.5	8.8	-	6.8	7.3	-				870	-

	Control water	100% sample
Alkalinity:	108	36
Hardness:	113	140

Table 3. Data recorded during 48 hour static screening bioassays using Ceridaphnia dubia, Daphnia pulex, and Notropis leedsi on De-chlorinated discharge from the Fort Pierce Utilities Authority WWTP, St. Lucie County, NPDES #FL0027278, on 4 to 6 August, 1987.

Facility:	Fort Pierce Utilities Authority WWTP			NPDES # FL0027278		Facility Type:	Municipal WWTP
Location:	St. Lucie County			Contact/District:	Paul Strauss/SE		
Sample Collection Date:	8/3/87	Time:	950	Test type: Static Screening			
Test Beginning Date:	8/4/87	Time:	1030	# tests:	3	Receiving Water:	Indian River
Test Ending Date:	8/6/87	Time:	1030	Chlorination Type:	Dechlorinated		

Parameter	
Total Residual Cl2:	0.00
Method:	Hach
By:	

Ammonia:	Control Water	100% Sample
Unionized NH3:	0.00	0.00
Total NH3:	0.00	0.80

Organism: ***Ceriodaphnia dubia*** Age/Life stage: Neonates

LC50: <50% 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	9	9	8.5	8.6	8.5	8	8.1	8.1	21.4	20.8	20.4		
Control	10	10	10	8.5	8.6	8.5	8	8.2	8.1					
50%	10	0	0	8.6	8.3	8.5	7.4	7.9	7.8	21.3	20.5	20.1		
50%	10	1	0	8.6	8.3	8.5	7.4	7.9	7.8					
100%	10	0	0	8.6	8.3	-	6.9	7.6	-	21.1	20.5	-		
100%	10	0	0	8.6	8.3	-	6.9	7.5	-					

	Control water	100% sample
Alkalinity:	108	36
Hardness:	113	140

Organism: ***Daphnia pulex*** Age/Life stage: Neonates

LC50: 50%-100% 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	8.5	8.4	8	8.1	8.1	21.5	20.7	20.1		
Control	10	10	10	8.7	8.5	8.4	8	8.1	8.1				205	220
50%	10	10	10	8.6	8.6	8	7.4	7.8	7.6	21.4	20.4	20		
50%	10	10	9	8.6	8.6	8	7.4	7.8	7.6				490	495
100%	10	6	3	8.5	8.2	7.2	6.8	7.4	7.2	21.3	20.4	20.1		
100%	10	10	4	8.5	8.2	7.2	6.8	7.4	7.2				870	890

	Control water	100% sample
Alkalinity:	108	36
Hardness:	113	140

Organism: ***Notropis leedsi*** Age/Life stage:

LC50: non-toxic Binomial: Moving Average: Probit:

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hour	Live at 24 hr	Live at 48 hr	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	48 hour
Control	5	5	5	8.4	8.5	8	7.6	8	7.9	21.7	20.7	19.9		
Control	5	5	5	8.4	8.5	8	7.6	8	7.9				202	210
50%	5	5	5	8.5	8.3	7.9	7.2	7.6	7.6	21.5	20.5	19.8		
50%	5	5	5	8.5	8.3	7.9	7.2	7.6	7.6				490	490
100%	5	5	5	8.5	8	7.4	6.8	7.2	7.2	21.4	20.4	19.7		
100%	5	5	5	8.5	8	7.4	6.8	7.2	7.2				870	890

	Control water	100% sample
Alkalinity:	108	36
Hardness:	113	140

Table 4. Microtox test results, Fort Pierce Utilities Authority WWTP discharge, St. Lucie County, NPDES #FL0027278, on 4 to 6 August, 1987.

Microtox Test Culture Lot # M-603

Final Reclaimed Water

% Sample	Chlorinated		Dechlorinated	
	5-min	15-min	5-min	15-min
0	57	26	100	52
0	55	34	96	48
0	52	34	99	51
12.38	22	15	95	44
12.38	19	14	85	38
12.38	20	15	92	48
24.75	5.4	4.3	82	34.5
24.75	3.7	4.2	94	44
24.75	2.7	3.6	89	37
49.5	1.8	1.1	78	30
49.5	0.3	0.2	82	32
49.5	0.5	0.3	77	31
99	0.3	0.1	66	23
99	0.3	0.1	84	43
99	LA*	*	80	36

EC50 Chlorinated (15 minute, 15 degrees C) = 15.03% sample
EC50 Dechlorinated (15 minute, 15 degree C) = 81.45%

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.

* LA signifies no data point due to lab accident

TABLE 5. Results of Chemical Analyses Performed on Reclaimed Water Collected from the Ft. Pierce Utilities Authority WWTP, NPDES #FL0027278, on 3 August, 1987.

ORGANICS

Base Neutral Extractables	None Detected
Acid Extractables	None Detected
Pesticide Extractables:	
Lindane	.12 ug/L
N-P Detector	Several unidentified compounds
E-C Detector	Several unidentified compounds

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. 1980. Bioassay of Ft. Pierce Utilities Authority Wastewater Treatment Plant, St. Lucie County, Florida. NPDES #FL0027278. FDER 8 p.

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_____. 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.

BIOASSAY DATA FORM

Facility/Toxicant U. Pacific Waterworks Test # 1-2-3

Address South Carolina, FL 12462 Type Acute

NPDES Number _____ Organism C. dubia / D. pulex / N. laeda

Outfall Number _____

Sample Collected: Date 2/4/87 Time 0930

Test Beginning: Date 2/4/87 Time 1030

Test Ending: Date 3/6/87 Time 1030

Test Concentration	Chamber Number	Live Organisms 0	24	48	Dissolved Oxygen (mg/L) 0	24	48	pH 0	24	48	Temperature °C 0	24	48	Cond. 0	48	Ammonia Total 0	48	Ammonia Unionized 0	48
Control	03	10	10	9	8.6	8.6	8.3	8.0	8.2	8.0	21.8	20.4	21.0			0			
Control	41	10	10	10	8.6	8.6	8.3	8.0	8.2	8.0									
50%	36	10	0	0	8.6	8.9	—	7.3	7.9	—	20.9	20.3	—						
50%	—	10	0	0	8.6	8.9	—	7.3	7.9	—									
100%	75	10	0	0	8.5	8.9	—	6.9	7.6	—	20.6	20.3	—						
100%	85	10	0	0	8.5	8.9	—	6.9	7.6	—						.8			
Control	44	10	10	10	8.6	8.5	8.4	8.0	8.2	8.0	20.9	20.3	20.5			0			
Control	39	10	10	10	8.6	8.6	8.4	8.0	8.2	8.0				215	215				
50%	31	10	0	0	8.6	8.8	—	7.3	7.9	—	21.3	20.3	—						
50%	18	10	0	0	8.6	8.8	—	7.3	7.9	—				465	—				
100%	36	10	0	0	8.5	8.8	—	6.9	7.5	—	21.1	20.3	—						
100%	6	10	0	0	8.5	8.5	—	6.9	7.5	—				950	—	.8			
Control	10	5	5	5	8.3	8.5	8.4	7.1	8.0	7.9	21.5	20.4	20.3			.00			
Control	46	5	5	5	8.3	8.5	8.4	7.6	8.0	7.9				205	210				
50%	70	5	0	0	8.6	8.7	—	7.1	7.6	—	21.6	20.6	—						
50%	50	5	0	0	8.4	8.7	—	7.1	7.7	—				1150	—				
100%	99	5	0	0	8.5	8.8	—	6.8	7.3	—	21.4	20.5	—						
100%	31	5	0	0	8.5	8.5	—	6.9	7.3	—				870	—	.03			
	016	4/11																	
		5																	
		MLR	JNF	MLR	MLR	JNF	MLR	MLR	JNF	MLR	MLR	JNF	MLR	MLR	MLR	JNF			

NOTES

Control Water

Total Alkalinity (mg/l $CaCO_3$) 108 MLR

Total Hardness (mg/l $CaCO_3$) 113

100% Sample

Total Alkalinity (mg/l $CaCO_3$) 36 MLR

Total Hardness (mg/l $CaCO_3$) 140

Age of Test Organisms 20 days - fish

Weight _____

Instrument Calibrations:

	pH Meter: MLR JNF	D.O. Meter:	S-C-T Meter:
0 Hours	7 at 7; 9 at 9	3.4 at 21.5°C	1 at 10; 2 at 30
24 Hours	4 at 11; 7 at 9	8.5 at 22°C	10-7 at 10; 30 at 30
48 Hours	7 at 4; 9 at 9	4.0 at 21°C	

Cl_2: 0.6 HACH DPD
0.71 Iodometric I

Reviewed by: _____

Date: _____

BE-(C)

BIOASSAY DATA FORM

Facility/Toxicant: A.H. Preece (Hg, Cu, Cl)
Address: Smith Creek Way, A.H. Preece
NPDES Number:
Outfall Number:

Test #: 45,6
Type: 20 Cell
Organism: C. dubia
D. pulex
N. acadian

Sample Collected: Date 5/3/87 Time 0950
Test Beginning: Date 5/4/87 Time 1032
Test Ending: Date 5/8/87 Time 1030

Test Concentration	Chamber Number	Live Organisms 0	24	48	Dissolved Oxygen (mg/L) 0	24	48	pH 0	24	48	Temperature °C 0	24	48	Conduc. 0	48	Ammonia Total 0	48	Unionized 0	48
Control	17	10	9	9	8.5	8.5	8.5	8.0	8.1	8.1	21.4	20.8	20.4			0			
Control	—	10	11	10	8.5	8.6	8.5	8.0	8.2	8.1									
50%	15	10	0	0	8.6	8.3	8.5	7.6	7.9	7.8	21.3	20.5	20.1						
50%	42	10	1	0	8.6	8.3	8.5	7.6	7.9	7.8									
100%	55	10	0	0	8.6	8.3	—	6.9	7.6	—	21.1	20.6	—			0.8			
100%	—	10	0	0	8.6	8.3	—	6.9	7.5	—									
Control	15	10	10	10	8.7	8.5	8.4	8.0	8.1	8.1	[illegible]	20.7	20.1			0			
Control	62	10	10	10	8.7	8.5	8.4	8.0	8.1	8.1				205	220				
50%	64	10	10	10	8.1	8.6	8.0	7.4	7.8	7.6	[illegible]	20.4	20.0						
50%	—	10	10	9	8.6	8.6	8.0	7.6	7.8	7.6				165	175				
100%	41	10	6	3	8.5	8.2	7.2	6.8	7.4	7.2	21.3	20.4	20.1	440	[illegible]	0.8			
100%	67	10	10	4	8.5	8.2	7.2	6.8	7.4	7.2				890	890				
Control	56	5	5	5	8.6	8.3	8.0	7.6	8.0	7.9	21.1	20.7	19.9			0			
Control	36	5	5	5	8.4	8.3	8.0	7.6	8.0	7.9				200	210				
50%	17	5	5	5	8.5	8.3	7.9	7.2	7.6	7.6	21.5	20.5	19.8						
50%	38	5	5	5	8.5	8.3	7.9	7.2	7.6	7.6				480	460				
100%	76	5	5	5	8.5	8.0	7.4	6.8	7.2	7.2	21.6	20.4	19.7						
100%	30	5	5	5	8.5	8.1	7.4	6.8	7.3	7.2				870	890	0.8			
		MLR	MLR	MLR	MLR	JMF	MLR	MLR	JMF	MLR	MLR	JMF	MLR	MLR	MLR	JMF			

NOTES

Control Water
Total Alkalinity (mg/l $CaCO_3$): 108 KAL
Total Hardness (mg/l $CaCO_3$): 113

100% Sample
Total Alkalinity (mg/l $CaCO_3$): 36 KAL
Total Hardness (mg/l $CaCO_3$): 140

Age of Test Organisms: 20 days - fish
Weight: —

Instrument Calibrations:

	pH Meter:	D.O. Meter:	S-C-T Meter:
0 Hours	7 at 7.9 at 9	8.9 at 21.5°C	10 at 10, 24 at 30
24 Hours	7 at 7.9 at 9	8.8 at 22°C	[illegible] at 30
48 Hours	7 at 7.9 at 9	9.0 at 21°C	

Cl_2: N.D. HACH

Reviewed by: _____

Date: _____

MICROTOX™ Data Reduction Sheet

SOURCE: Ft. Pierce STP

GRAPH / CALC EC 50 (15 MIN, 15 °C) = 15.03 (%SAMPLE) mg/L

SAMPLE DATA:

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

DATE ASSAYED: 8-4-87 TIME: 11:15 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: Natalie 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	57	26	—	—	
B1		55	34			
C1		52	34			
A2	12.38	22	15	1.69	1.14	
B2		19	14			
C2		20	15			
A3	24.75	5.4	4.3	12.90	6.77	
B3		3.7	4.2			
C3		2.7	3.6			
A4	49.5	1.8	1.1	6.28	57.75	
B4		.3	.2			
C4		.5	.3			
A5	99	.3	.1	272.3	469.0	
B5		.3	.1			
C5		(A)	—			
EC_{50} (MIN, °C)				9.39	15.03	

REVIEWED BY:
DATE:

C04

MICROTOX™ Data Reduction Sheet

SOURCE: Ft. Pierce

GRAPH / CALC EC 50 (15 MIN, 15 °C) = 81.45 (SAMPLE) mg/L

SAMPLE DATA:

SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent - de 3

DATE ASSAYED: 8-4-87 TIME: 10:00AM

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: 0

OPERATOR: Sales Guillard REAGENT VIAL LOT NO. M-603

REMARKS:

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	Γ5	Γ15	NOTES
A1	0	100	52	—	—	
B1		96	48			
C1		99	51			
A2	12.38	95	44	.08	.16	
B2		85	38			
C2		92	48			
A3	24.75	82	34.5	.11	.31	
B3		94	44			
C3		89	37			
A4	49.5	78	30	.24	.62	
B4		82	32			
C4		77	31			
A5	99	66	23	.28	.48	* deleted for CC value.
B5		84	43			
C5		80	36			
EC_{50} (MIN, °C)				502.7	81.45	

REVIEWED BY:
DATE: