BIOASSAYS OF THE NAVAL COASTAL SYSTEMS CENTER

WASTEWATER TREATMENT PLANT

PANAMA CITY BEACH, BAY COUNTY, FLORIDA

NPDES #FL0002551

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FEBRUARY 1, 1988

Biology Section
Division of Environmental Programs

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PANAMA CITY BEACH, BAY COUNTY, FLORIDA

NPDES #FL0002551

Biology Section
Bureau of Laboratories and
Special Programs
February 1, 1988

EXECUTIVE SUMMARY

Naval Coastal Systems Center WWTP, Panama City Beach, Bay County, Florida, NPDES #FL0002551, 1 to 3 September, 1987.

The trickling filter WWTP serving the Naval Coastal Systems Center in Panama City Beach produces a chlorinated (.7 mg/L Hach Method and .9 mg Cl_2/L Iodometric) reclaimed water that was found to be acutely toxic to the two water fleas, Ceriodaphnia dubia and Daphnia pulex, and to the bannerfin shiner, Notropis leedsi, with complete mortality occurring within 24 hours. This discharge was also extremely toxic to the common marine bacterium, Photobacterium phosphoreum used in the Microtox® Assay producing an EC_{50} of 11.25% discharge concentration.

A de-chlorinated portion of the sample was still acutely toxic to the water flea, Ceriodaphnia dubia with a 48 hour LC_{50} between 50% and 100% sample concentration. No mortality occurred with Daphnia pulex or Notropis leedsi. The Microtox® Assay still showed toxicity to Photobacterium phosphoreum with an EC_{50} of 70.11%.

Chemical analyses revealed the presence of the metals copper, zinc, and chromium. The low alkalinity and hardness contributed to the toxicity of the copper. Several organic constituents detected (Table 5) were likely common constituents of sewage. The toxicity may be attributed to the presence of the metals and chlorine.

INTRODUCTION

The Naval Coastal Systems Center Wastewater Treatment Plant is located in Panama City Beach in Bay County (Figure 1). The plant is a high rate trickling filter plant with a design capacity of 200,000 GPD. The plant is operating well below design capacity treating 100,000 GPD. The wastewater is a combination of industrial and municipal wastes from the operation of the center.

To evaluate the existence and extent of toxicity to the receiving water biota the Northwest District requested the Ecotoxicology Laboratory to perform static screening acute toxicity bioassays. Three screening bioassays and one Microtox® Assay were performed on the chlorinated discharge, and on a de-chlorinated portion of the discharge, on 1 to 3 September, 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, and L. A. Williams.

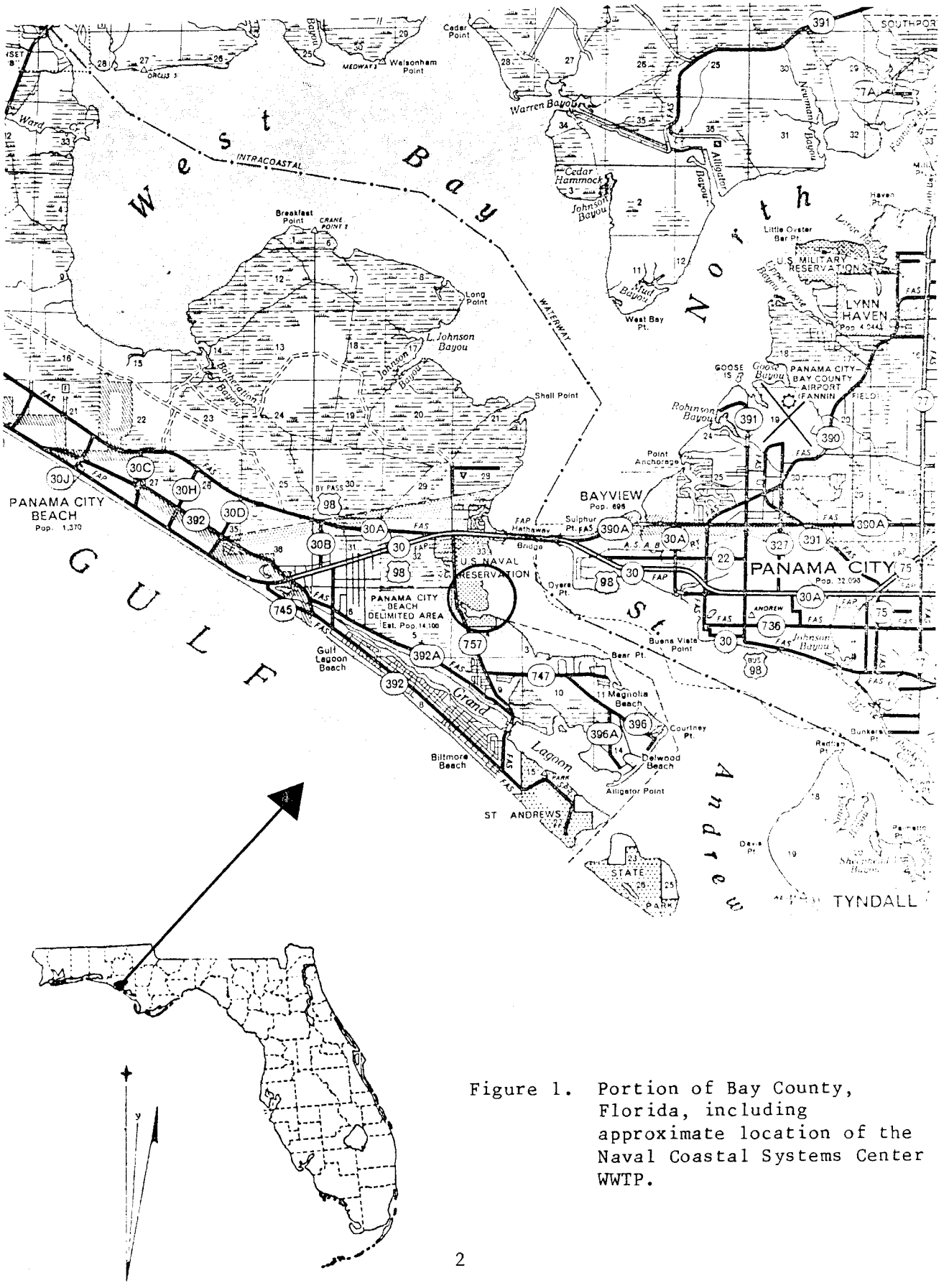


Figure 1. Portion of Bay County, Florida, including approximate location of the Naval Coastal Systems Center WWTP.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier and Weber (1985) and the FDER Biology Section (1985). The organisms selected for this study include species recommended by Peltier and Weber (1985) and the FDER Biology Section (1985). Additionally, the Biology Section maintains cultures of several species of organisms indigenous to the State of Florida. One of these indigenous species was also selected for testing. Life histories of these test organisms and maintenance procedures used to culture them are detailed below.

The Microtox® assay was run according to the manufacturer's protocol described in the Microtox® System Operating Manual (Beckman, 1982).

History and Maintenance of Bioassay Organisms

Daphnia pulex (water flea) - A breeding culture of Daphnia pulex was obtained from the EPA laboratory in Athens, Georgia, in June, 1987. This culture has been maintained since its arrival in 2 1/2 gallon all-glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. Organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, once daily on weekends, and are kept at a constant temperature of 22° ± 2°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms, and then refilling with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances such as: declining populations, ephippia formation, or excessive fungal or bacterial growth.

Ceriodaphnia dubia (water flea) - A breeding culture of Ceriodaphnia dubia was obtained from the EPA laboratory in Athens, Georgia, in June, 1985. The culture is maintained in 2-1/2 gallon all-glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. Organisms are fed a liquified blend of Purina Trout Chow®, Cerophyl® and Fleischmann's® yeast twice daily, once daily on weekends, and are kept at a constant temperature of 26° ± 2°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms, and then refilling with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances such as: declining populations, ephippia formation, or excessive fungal or bacterial growth.

Notropis leedsi (bannerfin shiner) - Approximately 40 adult bannerfin shiners were obtained from an indigenous population in the upper Suwannee River during October, 1981. The breeding stock is maintained in 30 and 125 gallon all-glass aquaria equipped with a flow-through water exchange system. Water for this system is unchlorinated and is pumped from the DER deep well. The bannerfin shiners are reared at 22° ± 2°C under a 16:8 hour light-dark cycle and are fed Tetra-Min® and frozen Artemia (brine shrimp) twice daily, and once daily on weekends. Breeding and egg hatching are encouraged at 25°C. The eggs are laid between stacked red ceramic tiles separated by a 3mm bead of silicone. Most of the eggs

adhere to the tiles and are easily removed to heated, aerated hatching tanks. The narrow space between the tiles eliminates egg predation by adult fish. New hatching tanks are started regularly to segregate fry into even age groups. Newly hatched fry are fed a liquefied blend of Purina Trout Chow® and Cerophyl® twice daily. After one week, feeding is augmented with newly hatched Artemia nauplii and finely crushed Tetra Min®. The fish used in this test were 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-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 acute screening bioassays and one Microtox® Assay were performed on both the reclaimed water and a de-chlorinated portion of the reclaimed water from 1100 hours on 1 September to 1125 hours on 3 September, 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 unpreserved. The samples were collected by Mr. J. E. Villodsen of the Northwest District, 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 sample in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the sample allowed to rise to 20° $\pm$ 2°C. Although this temperature may be different than the temperature of the sample during collection, it is the temperature range to which the test organisms are acclimatized and is the range recommended in the above referenced bioassay protocols. This avoids stressful effects caused by exposure to unusually cold or warm samples.

Total Residual Chlorine

Since many 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 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 Naval Coastal Systems Center in Bay County, Florida was acutely toxic to all test organisms, causing complete mortality in 24 hours (Table 2). These organisms included the two water fleas, (Crustacea:Cladocera) Ceriodaphnia dubia and Daphnia pulex, and the fish, Notropis leedsi. The 24 hour LC_{50} is between 0% sample and the lowest screening concentration of 50%. The Microtox® Assay reveals similar extreme toxicity to the common marine bacterium, Photobacterium phosphoreum, with an EC_{50} of 11.25%. The chlorine residual was .9 mg Cl_2/L by the Iodometric Method.

A de-chlorinated portion of the reclaimed water was still acutely toxic to the water water flea, Ceriodaphnia dubia, producing a 48 hour LC_{50} between 50% and 100% sample concentration (Table 3). The Microtox® Assay had an EC_{50} of 70.1%. No mortality occurred with the other water flea, Daphnia pulex, or the fish, Notropis leedsi.

De-chlorination removed a significant portion of the toxicity to all the organisms. Other toxicants are present in high enough concentration to be acutely toxic to Ceriodaphnia dubia and the bacterium, Photobacterium phosphoreum (EC_{50}). Chemical analysis found a variety of organics (Table 5) which are probably not a significant cause of the toxicity. The metals, copper (28.0 ug/L) and zinc (110 ug/L), were quite high when considered relative to the Class III water quality standards for copper of 15 ug/L in saltwater and 30 ug/L in freshwater and for zinc 30 ug/L in freshwater. Chromium was detectable but at concentrations less than the technique used could precisely quantify. Chromium concentration was less than 100 ug/L.

These metal concentrations, particularly copper and zinc, are well above the suggested criteria by EPA (1976) for freshwater organisms and explain the significant toxicity exhibited by the test organisms. The reclaimed water had a very low total hardness characteristic of the Bay County Region of Florida. As hardness levels decrease, toxicity of both zinc and copper increases significantly. The toxicity of the reclaimed water from this facility may be attributed to the presence of these metals and chlorine.

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 acute screening bioassays on chlorinated reclaimed water from the Naval Coastal Systems Center WWTP, NPDES #FL0002551 on 1 to 3 September, 1987.

Facility:	Naval Coastal Systems Center WWTP			NPDES # FL0002551		Facility Type:	Municipal WWTP
Location:	Bay County			Contact/District:	NW/J.E. Villodsen		
Sample Collection Date:	8/31/87	Time:	1300	Test type:	Static Screening		
Test Beginning Date:	9/1/87	Time:	1100	# tests:	3	Receiving Water:	St. Andrews Bay
Test Ending Date:	9/3/87	Time:	1125	Chlorination Type:	Chlorinated		

Parameter		
Total Residual Cl2:	0.7	0.9
Method:	Hach	Iodometric
By:	RBF	RBF
Salinity:	0	

Ammonia:	Control Water	100% Sample
Unionized NH3:	0.00	0.00
Total NH3:	0	0.505

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	8.2	7.6	-	8.1	8	-	20.5	20.3	-		
Control	10	10	10	8.2	7.6	-	8.1	8	-					
50%	10	0	-	8.4	8	-	7.5	7.7	-	20.2	20.2	-		
50%	10	0	-	8.4	8	-	7.5	7.7	-					
100%	10	0	-	8.5	8.1	-	6.9	7.3	-	19.7	20	-		
100%	10	0	-	8.5	8.1	-	6.9	7.3	-					

	Control water	100% sample
Alkalinity:	109	30
Hardness:	113	98

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	10	-	8.4	7.9	-	8.2	7.9	-	20.6	20.2	-		
Control	10	10	-	8.4	7.9	-	8.2	7.9	-				210	-
50%	10	0	-	8.6	8.1	-	7.4	7.7	-	20.3	20	-		
50%	10	0	-	8.6	8.1	-	7.4	7.7	-				275	-
100%	10	0	-	8.8	8.2	-	6.8	7.2	-	19.4	19.9	-		
100%	10	0	-	8.8	8.2	-	6.8	7.2	-				340	-

	Control water	100% sample
Alkalinity:	109	30
Hardness:	113	98

Organism: ***Notropis leedsi*** Age/Life stage: 25 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	-	8.3	8	-	7.7	7.7	-	21	20.1	-		
Control	5	5	-	8.3	7.9	-	7.7	7.7	-				205	-
50%	5	0	-	8.4	8	-	7.3	7.4	-	20.3	19.9	-		
50%	5	0	-	8.5	8	-	7.3	7.4	-				280	-
100%	5	0	-	8.8	8.1	-	6.8	7	-	19.3	19.8	-		
100%	5	0	-	8.8	8.2	-	6.8	6.9	-				345	-

	Control water	100% sample
Alkalinity:	109	30
Hardness:	113	98

Table 3. Data recorded during 48 hour, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi static acute screening bioassays on de-chlorinated reclaimed water from the Naval Coastal Systems Center, WWTP, NPDES #FL0002551 on 1 to 3 September, 1987.

Facility:	**Naval Coastal Systems Center WWTP**			NPDES # **FL0002551**		Facility Type:	**Municipal WWTP**
Location:	Bay County			Contact/District:	NW/J.E. Villodsen		
Sample Collection Date:	8/31/87	Time:	1300	Test type:	Static Screening		
Test Beginning Date:	9/1/87	Time:	1100	# tests:	3	Receiving Water:	St. Andrews Bay
Test Ending Date:	9/3/87	Time:	1125	Chlorination Type:	Dechlorinated		

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

Ammonia:	Control Water	100% Sample
Unionized NH3:	0.00	0.00
Total NH3:	0	0.505

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

LC50: Binomial: Moving Average: Probit:

LC50<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	8.4	8	8.7	8.2	7.9	8	20.9	20	19.9		
Control	10	10	10	8.4	8	8.8	8.2	7.9	8					
50%	10	10	10	8.5	8	8.9	7.6	7.5	7.8	20.8	20	19.9		
50%	10	10	10	8.5	8	9	7.6	7.5	7.8					
100%	10	6	0	8.5	7.4	8.7	7	7	7.3	20.2	20	20		
100%	10	5	0	8.5	7.4	8.7	7	7	7.2					

	Control water	100% sample
Alkalinity:	109	30
Hardness:	113	98

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

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	10	10	10	8.5	7.6	8.9	8.2	7.9	8	20.8	20	19.9		
Control	10	10	10	8.5	7.6	9	8.2	7.9	8				215	210
50%	10	10	10	8.5	7.8	7.7	7.5	7.5	7.6	20.6	20	19.8		
50%	10	10	10	8.5	7.8	8.3	7.5	7.5	7.6				272	275
100%	10	10	10	8.7	8	8.4	6.9	7.1	7	20.3	19.9	20		
100%	10	10	10	8.7	8	8.2	6.9	7.1	7				360	380

	Control water	100% sample
Alkalinity:	109	30
Hardness:	113	98

Organism: ***Notropis leedsi*** Age/Life stage: 25 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	7.9	8.2	7.7	7.7	7.7	21.4	19.9	19.8		
Control	5	5	5	8.3	7.9	8	7.7	7.7	7.6				215	210
50%	5	5	5	8.4	7.9	7.8	7.3	7.4	7.4	20.7	19.9	19.7		
50%	5	5	5	8.5	7.9	7.8	7.3	7.4	7.4				290	270
100%	5	5	5	8.7	8	7.7	6.8	6.9	6.9	20.3	19.8	19.9		
100%	5	5	5	8.7	7.9	7.5	6.8	6.9	6.9				360	370

	Control water	100% sample
Alkalinity:	109	30
Hardness:	113	98

Table 4. Microtox test results, Naval Coastal Systems Center WWTP discharge, Bay County, NPDES #FL0002551, on1 September, 1987.

Microtox Test Culture Lot # M-603

Final Reclaimed Water

% Sample	Chlorinated		Dechlorinated	
	5-min	15-min	5-min	15-min
0	73	45	63	42
0	70	42	65	43
0	73	43	62	41
12.38	32	19.5	60	37
12.38	28	17	61	37.5
12.38	27	17	57	35
24.75	4	4	60	37
24.75	6.5	5.5	61	38
24.75	6.5	5.5	61	37.5
49.5	0.3	0.2	53	31
49.5	0.6	0.3	50	30
49.5	0.4	0.3	46	27.5
99	0.4	0.1	31	16
99	0.4	0.1	25	12
99	0.4	0.1	31	16

EC50 Chlorinated (15 minute, 15 degrees C) = 11.25% sample
EC50 Dechlorinated (15 minute, 15 degree C) = 70.11%

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 Naval Coastal Systems Center Wastewater Treatment Plant, NPDES #FL0002551 on 31 August, 1987.

Organics

Acid Extractables	None Detected
Base Neutral Extractables	Tentative trace cholestanols
	Tentative trace organic acids
	Bis (2-ethylhexyl) phthalate (10 ug/L**)
Herbicides-HPLC	None Detected
Pesticide Extractables	Numerous unidentified components sensitive to ECD

Metals

Chromium	100.0 ug/L **
Copper	28.0 ug/L
Zinc	110.0 ug/L

* Results for cadmium, lead, and silver were invalidated due to a contaminated field blank.

** Chemical was detected but the analytical technique cannot give a precise value at these levels. Actual value is known to be less than the value given.

Discussion of the Toxicity Criteria

The toxicity standards of Chapters 17-3 and 17-4, F.A.C., are complex. Because of this complexity, they will be briefly reviewed here so that these test results may be more readily compared with the standards. For legal purposes, however, the language of the Florida Administrative Code should be consulted directly. Applicable portions of the F.A.C. are: 17-3.021(1), 17-3.021(4), 17-3.051(1)(d), 17-3.061(3)(p), 17-3.402(1), 17-4.244(4), and 17-4.244(6).

The particular restrictions applied to a given discharger depend upon the type of discharge, the type of water body which is impacted, and the type of mixing zone, if any, which has been permitted. The most stringent toxicity limitations which apply to the various combinations of these factors are listed below, specified in terms of the most dilute concentration of effluent which is allowed to kill 50% of the test organisms in 96 hours. Note that any listed concentration greater than 100% is extrapolated from toxicity curves determined from testing various effluent concentrations of 100% or less. Violations of the toxicity standards occur in the following conditions.

I. Discharges to Surface Water

A. With no mixing zone, or with only a dredge and fill mixing zone, the discharge may not exhibit an $LC_{50} \leq 2000\%$ at the point of discharge.

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

C. With a non-dredge and fill mixing zone, and either discharging to the open ocean through multiport diffusers or meeting the provisions of 17-4.244(4)(b), the LC_{50} at the point of discharge cannot exceed 30% sample concentration.

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

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

LITERATURE CITED

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

Florida Department of Environmental Regulation, Biology Section. 1985. Quality Assurance Manual for Performing Acute Toxicity Tests (Second Edition). FDER.

Peltier, W. and C. I. Weber. 1985. Methods for measuring the acute toxicity of effluents to aquatic organisms (Third Edition). Environmental Research Laboratory. Athens, Georgia. EPA-600/4-85-012. 217 p.

U.S. Environmental Protection Agency. 1976. Quality Criteria for Water. U.S. EPA, Washington, D.C. 256 p.

Industry/Toxicant NCSC W.W.T.P.
Address Panama City
Contact/District
Effluent Serial Number FL0002551
NPDES Permit Number 001

BIOASSAY DATA FORM

Test Number: 1 of 6
Test Type: Static Renewal, Screening Conventional
Other: Cl2

Sample Collection: Date 8/31/87 Time 1300
Test Beginning: Date 9/1/87 Time 1100
Test Ending: Date ____ Time ____
Test Organism C. dubia
Age/Life Stage neonates

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96
Control	92	10	10	-			8.2	7.6	-			8.1	8.0	-			20.5	20.3	-		
Control	85	10	10	-			8.2	7.6	-			8.1	8.0	-							
50%	65	10	0	-			8.4	8.0	-			7.5	7.7	-			20.2	20.2	-		
50%	75	10	0	-			8.4	8.0	-			7.5	7.7	-							
100%	1	10	0	-			8.5	8.1	-			6.9	7.3	-			19.7	20.0	-		
100%	45	10	0	-			8.5	8.1	-			6.9	7.3	-							
Initial of Investigator		RML	RBF				JMF	RBF				JMF	RBF				JMF	RBF			

Conc. or %	Alkalinity/Hardness (mg/l as $CaCO_3$) 0	24	48	72	96
Control Water:	109/113				
100% Sample:	30/98				
Other:					
Measured by:	KML				

Conc. or %	Ammonia unionized/total (mg/l as NH_3/N) Hour 0	Hour ____
Control Water:	0.0	
100% Sample:	.505	
Other:		
Measured by:	RBF	

58.1 slope

Signatures:
Carol Sanderberg
KM Lurding
Jan Faulkner

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7at7;9at9	9.0 at 21C	10at10;25at30
24 Hours	7@7 9@9	9.0 @ 21C	
48 Hours	7@7 9@9	9.2 @ 20°C	10@10 25.36 30
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: Method:
Lab: 0.7 mg/l Method: HACH
Lab: 0.9 mg/l Method: Iodometric

Reviewed by: [signature]
Date: 2/1/88

Dechlorinated:
Measurements by: RBF

BIOASSAY DATA FORM

Industry/Applicant: NCSC W.W.T.P.
Address: Panama City
Contact/District:
Effluent Serial Number: FL0002551
NPDES Permit Number: 001

Test Number: 2 of 2
Test Type: Static Renewal, (Screening) Conventional
Other: Cl2

Sample Collection: Date 8/31/87 Time 1300
Test Beginning: Date 9/1/87 Time 1100
Test Ending: Date Time
Test Organism: M. 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	61	10	10	-			8.4	7.9	—			8.2	7.9	—			20.6	20.4	—			210	—	—		
Control	35	10	10	-			8.4	7.9	—			8.2	7.9	—												
50%	13	10	0	-			8.6	8.1	—			7.4	7.7	—			20.3	20.0	—			275	—	—		
50%	—	10	0	-			8.6	8.1	—			7.4	7.7	—												
100%	151	10	0	-			8.8	8.2	—			6.8	7.2	—			19.4	19.9	—			340	—	—		
100%	55	10	0	-			8.8	8.2	—			6.8	7.2	—												
Initial of Investigator		JMF	JMF				JMF/RBF					JMF/RBF					JMF/RBF					JMF				

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 109/113
100% Sample: 30/98
Other:
Measured by: KML

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: 0.0
100% Sample: .505
Other:
Hour
Control Water:
100% Sample:
Other:
Measured by: RBF

Signature: Russell Frydenborg
KM Lurdiet
JMFairchild

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7@7.9@9	9.0 at 21°C	10 cd 10, 25 at 30
24 Hours	7@7 7@9	9.0 @ 21°C	
48 Hours	7@7 9@9	9.2 @ 20°C	10@10 25.3 @ 30
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: Method:
Lab: 0.7 mg/l Method: HACH
Lab: 0.9 mg/l Method: Iodo

Reviewed by:
Date: 2/1-85

Remarks:

Industry/Toxicant CSC NSES W.W.T.P.
Address
Contact/District
Effluent Serial Number
NPDES Permit Number

BIOASSAY DATA FORM

Test Number: 36
Test Type: Static Renewal, Screening Conventional
Other: Cl2

Sample Collection: Date 8/31/87 Time 1300
Test Beginning: Date 9/1/87 Time 1115
Test Ending: Date Time
Test Organism N. seudi
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	-			8.3	8.0	—			7.7	7.7	—			21.0	20.1	—							
Control	55	5	5	-			6.3	7.9	—			7.7	7.7	—								205	—	-		
50%	47	5	0	-			8.4	8.0	—			7.3	7.4	—			20.3	19.9	—							
50%	285	5	0	-			8.5	8.0	—			7.3	7.4	—								280	—	-		
100%	72	5	0	-			8.8	8.1	—			6.8	7.0	—			19.3	19.9	—							
100%	33	5	0	-			8.8	8.2	—			6.8	6.9	—								345	—	-		
Initial of Investigator		RBF	RBF				JMF	RBF				JMF	RBF				JMF	RBF				JMF				

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 109/113
100% Sample: 30/98
Other:
Measured by: KML

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: 0.0
100% Sample: .505
Other:
Hour
Control Water:
100% Sample:
Other:
Measured by: RBF

Signatures: Russel Frydenborg, KMLurding, J M Fairbrother

Instrument Calibrations:

	pH Meter:	D. O. Meter:	S-C-T Meter:
0 Hours	7@7.9@9	9.0@21°C	10.0@10, 25@30
24 Hours	7@7.9@9	9.0@21°C	
48 Hours	7@7.9@9	9.2@20°C	10@10 25@30
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: Method:
Lab: 0.7 Method: HACH
Lab: 0.4 Method: Iodo

Dechlorinated:
Measurements by:

Reviewed by:
Date: 2/1/88

Industry/Toxicant: CSC NSES W.W.T.P.
Address:
Contact/District:
Effluent Serial Number:
NPDES Permit Number:

BIOASSAY DATA FORM

Test Number: 4 of 6
Test Type: Static Renewal Screening Conventional
Other: de-Cl2

Sample Collection: Date 8/31/87 Time 1300
Test Beginning: Date 9/1/87 Time 1115
Test Ending: Date Time
Test Organism: Ceriodaphnia
Age/Life Stage: neonates

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96	— 0	24	48	72	96
Control	47	10	10	10			8.4	8.0	8.7			8.2	7.9	8.0			20.9	20.0	19.9							
Control	51	10	10	10			8.4	8.0	8.8			8.2	7.9	8.0												
50%	80	10	10	10			8.5	8.0	8.9			7.6	7.5	7.8			20.8	20.0	19.9							
50%	—	10	10	10			8.5	8.0	9.0			7.6	7.5	7.8												
100%	4	10	6	0			8.5	7.4	8.7			7.0	7.0	7.3			20.2	20.0	20.0							
100%	85	10	5	0			8.5	7.4	8.7			7.0	7.0	7.2												
Initial of Investigator		KML	RBF	RBF			JMF	RBF	JE			JMF	RBF	JMF			JMF→	RBF	JE							

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 109/113
100% Sample: 30/98
Other:
Measured by: KML

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: 0.0
100% Sample: .505
Other:
Hour
Control Water:
100% Sample:
Other:
Measured by: RBF

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7a17,9a19	9.0@21°C	10.0@10,2Sat30
24 Hours	7e7 9e9	9.0@21°C	10@10 2S3@30
48 Hours	7e7 9e9	9.2@20°C	
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: Method:
Lab: 0.7 Method: HACH
Lab: 0.9 Method: Iodo

Re-chlorinated:
Concentrations (%):

Remarks:

Signature: Russell Frydenborg
KM Lurding
Jim Faulstich
Jeff Epler

Reviewed by:
Date: 2/1/88

Industry/Toxicant CSC ~~NSES~~ W.W.T.P.
Address
Contact/District
Effluent Serial Number
NPDES Permit Number

BIOASSAY DATA FORM

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

Sample Collection: Date 8/31/87 Time 1300
Test Beginning: Date 9/1/87 Time 1120
Test Ending: Date ____ Time ____
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	25	10	10	10			8.5	7.6	8.9			8.2	7.9	8.0			20.8	20.0	19.9							
Control	132	10	10	10			8.5	7.6	9.0			8.2	7.9	8.0								215	–	210		
50%	70	10	10	10			8.5	7.8	7.7			7.5	7.5	7.6			20.6	20.0	19.8							
50%	5	10	10	10			8.5	7.8	8.3			7.5	7.5	7.6								272	–	275		
100%	2	10	10	10			8.7	8.0	8.4			6.9	7.1	7.0			20.3	19.9	20.0							
100%	72	10	10	10			8.7	8.0	8.2			6.9	7.1	7.0								360 ~~380~~	–	380		
Initial of Investigator		RBF	JMF	JMF			JMF	RBF	JE			JMF	RBF	JMF			JMF	RBF	JE			JMF		JMF		

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 109/113
100% Sample: 97/98
Other:
Measured by: KML

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: 0.0
100% Sample: .505
Other:
Hour ____
Control Water:
100% Sample:
Other:
Measured by: RBF

Signatures:
Russell Frydenborg
KMLurding
JMFandell
JE

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7 at 7; 9 at 9	9.0 at 21°C	10 at 10; 25 at 30
24 Hours	7@7	9@9	9.0 @ 21°
48 Hours	7@7	9@9	9.2 @ 20°C
72 Hours			10@10 25.3@30
96 Hours			

Total Residual Chlorine:
Field data: ____ Method:
Lab: ____ Method:
Lab: ____ Method:

Dechlorination:
Measurement Type:

Reviewed by:
Date: 2/1/88

Industry/Toxicant: ~~NSES~~ CSC W.W.T.P.
Address:
Contact/District:
Effluent Serial Number:
NPDES Permit Number:

BIOASSAY DATA FORM

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

Sample Collection: Date 8/3/87 Time 1300
Test Beginning: Date 9/1/87 Time 1125
Test Ending: Date ____ Time ____
Test Organism: N. Seeds
Age/Life Stage: ~~2 days~~ 25days

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96	Conductivity 0	24	48	72	96
Control	58	5	5	5			8.3	7.9	8.2			7.7	7.7	7.7			21.4	19.9	19.3							
Control	6	5	5	5			8.3	7.9	8.0			7.7	7.7	7.6								215	–	210		
50%	34	5	5	5			8.4	7.9	7.8			7.3	7.4	7.4			20.7	19.9	19.7							
50%	38	5	5	5			8.5	7.9	7.8			7.3	7.4	7.4								290	–	270		
100%	50	5	5	5			8.7	8.0	7.7			6.8	6.9	6.9			20.3	19.8	19.9							
100%	63	5	5	5			8.7	~~8.9~~	7.5			6.8	6.9	6.9								360	–	370		
Initial of Investigator		RBF	RBF	RBF			JMF	RBF	JE			JMF	RBF	JE			JMF	RBF	JE			JMF		JMF		

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

Control Water: 109/113
100% Sample: 30/98
Other:
Measured by: KML

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

Control Water: 0.0
100% Sample: .505
Other:

Hour ____

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

Signatures:
Reed Frydenborg
KMLurding
Jan Faubert
JE Gee

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7@7; 4@9	9.0@21°C	10@10; 35@30
24 Hours	7@7	9.2@21°C	
48 Hours	7@7	9.2@20°C	10@10; 35.3@30
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: ____ Method: ____
Lab: ____ Method: ____
Lab: ____ Method: ____

Reviewed by:

Dechlorinated:
Personnel notified by:

Date: 2/1/88

Remarks:

MICROTOX™ Data Reduction Sheet

SOURCE: ACSC

GRAPH / CALC) EC 50 (15 MIN, 15 °C) = 11.3 (%SAMPLE) mg/L

SAMPLE DATA:

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

DATE ASSAYED: 9-1-87 TIME: 10:45 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	73	45	X	X	
B1		76	42			
C1		73	43			
A2	12.38	32	19.5	1.48	1.49	
B2		28	17			
C2		27	17			
A3	24.75	4	4	11.71	7.81	
B3		6.5	5.5			
C3		6.5	5.5			
A4	49.5	.3	.2	165.2	165.4	
B4		.6	.3			
C4		.4	.3			
A5	99	.4	.1	179.0	442.3	
B5		.4	.1			
C5		.4	.1			
EC_{50} (MIN, ° C)				10.38	11.25	

REVIEWED BY: [signature]

DATE: 2/1/88

014

MICROTOX™ Data Reduction Sheet

SOURCE: NCSC

GRAPH (CALC) EC 50 (15 MIN, 15 °C) = 70.1 (%SAMPLE) mg/L

SAMPLE DATA:

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

DATE ASSAYED: 9-1-87 TIME: 11:10 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: Susan 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	63	42	X	X	
B1		65	43			
C1		62	41			
A2	12.38	60	37	.07	.15	
B2		61	37.5			
C2		57	35			
A3	24.75	60	37	.04	.12	
B3		61	38			
C3		61	37.5			
A4	49.5	53	31	.28	.42	
B4		50	30			
C4		46	27.5			
A5	99	31	16	1.18	1.86	
B5		25	12			
C5		31	16			
EC_{50} (MIN, °C)				87.36	70.11	

REVIEWED BY:

DATE: 2/1/88