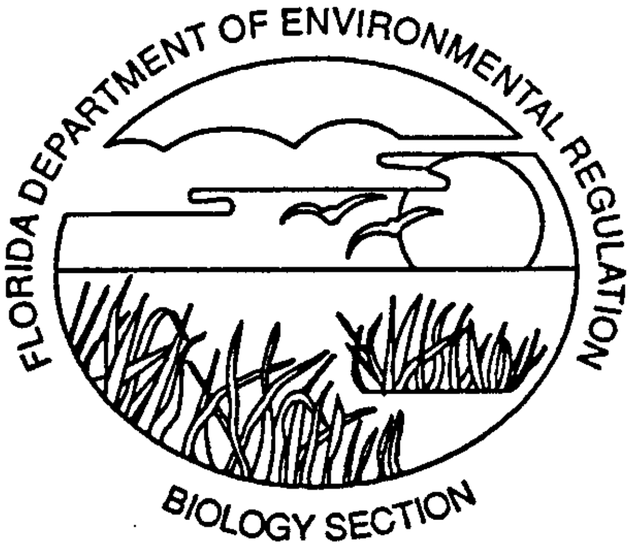


Bioassays of the

City of Carrabelle Sewage Treatment Plant

Carrabelle, Franklin County, Florida

NPDES #FL0026808

Sampled 5/24/88

September 21, 1988

Biology Section
Division of Administrative and Technical Services

Bioassays of The

City of Carrabelle Sewage Treatment Plant

Carrabelle, Franklin County, Florida

NPDES #FL0026808

Biology Section
Bureau of Laboratories
September 21, 1988

Executive Summary

City of Carrabelle Sewage Treatment Plant, Carrabelle, Franklin County, Florida, NPDES #FL0026808, from 24 to 26 May, 1988.

The sample of chlorinated discharge from this facility was acutely toxic to the water fleas, *Ceriodaphnia dubia* and *Daphnia pulex*, and to the fish, *Notropis leedsi*. The dechlorinated sample from this facility was toxic to the test organism, *C. dubia*. The LC_{50}'s were less than 30% in each of the chlorinated tests since all of the test organisms died in the lowest test concentration (30%). Both the chlorinated and dechlorinated samples were toxic to the Microtox® assay organism, *Photobacterium phosphoreum*, producing EC_{50}'s of 0.2% and 2.5%, respectively.

The *C. dubia* dechlorinated test indicated that the sample was toxic, producing an LC_{50} of <30%; however, this test is technically invalid because of the control mortality. The control mortality (35%) exceeded the10% permissible control mortality for static tests. The cause of the control mortality in the one control vial is unknown, but it is consistent with contamination. Though the LC_{50} is probably correct, the test is still invalid and these results are presented for informational purposes only.

The mortality in the chlorinated tests can be attributed primarily to the total residual chlorine concentration (15.9 mg/l Iodometric) in the sample. The mortality in the chlorinated sample is believed to be partially attributable to the pesticide chlorpyrifos (0.18 µg/l); the phthalate, bis(2-ethylhexyl) phthalate (13 µg/l); and possibly the metal, zinc (73 µg/l). Numerous unidentified components probably also contributed to the observed mortality. Although the dechlorinated *C. dubia* test results were invalided due to the control mortality, it is believed that the chemical constituents detected in the sample were contributory to the mortality observed in all the test concentrations.

Introduction

The City of Carrabelle Sewage Treatment Plant is located at 5th Street East and U.S. Highway 98 in Carrabelle, Franklin County, Florida (Figure 1). The facility consists of a 0.15 MGD primary Treatment Plant which discharges to St. George Sound. The facility serves a population of less than 5,000. The City of Carrabelle has been under U.S. EPA administrative orders to cease discharge from this facility to St. George Sound.

To determine the existence and extent of the toxicity of the discharge from this facility, at the request of the FDER Northwest District, the Biology Section performed six acute toxicity bioassays and 2 Microtox® assays on a discharge sample. The results of those tests are presented in this report. Those involved in performing the tests and in preparation of this report were: J. M. Faircloth, R. B. Frydenborg, K. M. Lurding, L. A. Williams, and S. H. Wolfe.

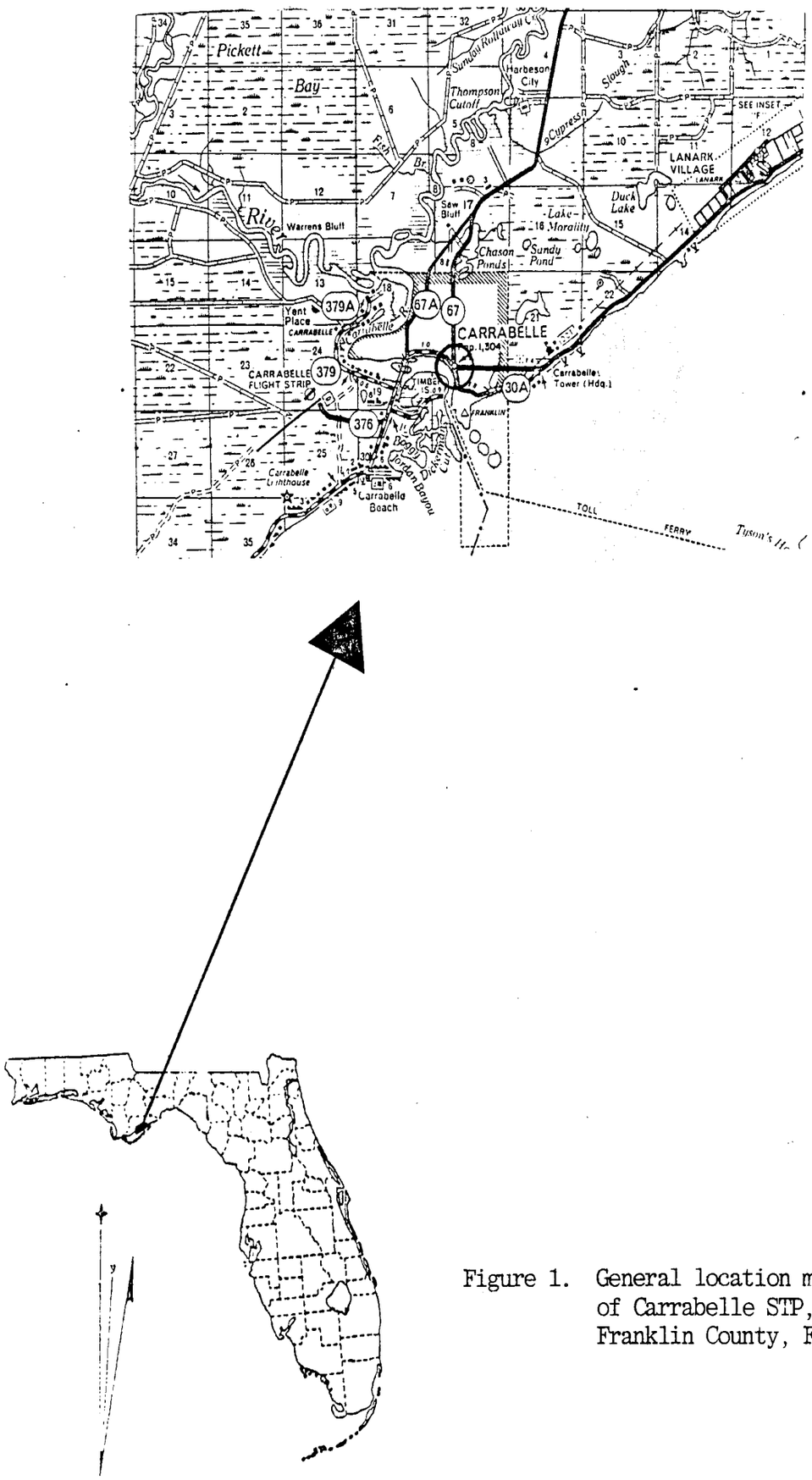


Figure 1. General location map of the City of Carrabelle STP, Carrabelle, Franklin County, Florida.

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).

1. 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 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 Aquarian® flake food 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 Aquarian® flake food. The fish used were 27 days old when the test commenced.

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).

2. 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.

3. 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 acute toxicity bioassays and two Microtox® assays were performed on a sample of chlorinated and dechlorinated discharge from the City of Carrabelle STP. The tests were performed from 1530 hours on 24 May to 1530 hours on 26 May, 1988.

Separate samples were obtained for chemical and biological testing. Samples for metal analyses were preserved with $HN0_3$. Samples for organic chemical analyses and for the bioassays were unpreserved. The samples were collected by Mr. L. V. Olney of the FDER Northwest District Branch office in Panama City, Florida, at 0930 hours on 24 May, 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 ± 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. Dechlorination 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.

Method

Freshwater for diluting the water samples being tested 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 the control and each test concentration were used in bioassays 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, respectively, 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. Ten were loaded per chamber, resulting in 20 per test concentration. *Daphnia* were siphoned into a stainless steel screen chamber placed into a large culture dish to isolate the proper size and age organisms (neonates). Then the screen chamber along with any retained adults were removed. The neonates remaining were then captured by glass pipette, examined for injury, and loaded 10 per chamber, resulting in 20 per test concentration. The 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 using a pipette into wide mouth quart jars containing 500 ml each of the appropriate test concentration. Five were loaded per chamber, resulting in 10 per concentration. Fish were not fed during this 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 before use 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 for Microtox® analysis. 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 99%, 49.5%, 24.75%, 12.38%, and 0%. An incubation temperature of 15 °C was maintained throughout the assay. Ten µl 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 sample of chlorinated discharge from the City of Carrabelle Sewage Treatment Plant was toxic to the bioassay test organisms, *Ceriodphnia dubia*, *Daphnia pulex*, and *Notropis leedsi*, producing LC_{50}'s of less than 30% (Table 5). The chlorinated sample was also toxic to the Microtox® organism, *Photobacterium phosphoreum*, producing an EC_{50} of 0.2% sample. The dechlorinated sample was toxic to the Microtox® assay organism, *P. phosphoreum* (EC_{50} = 2.5%). The total residual chlorine (15.9 mg/l Iodometric) was largely responsible for the mortality in the chlorinated tests.

Chemical analyses (Table 6) detected several identifiable components, including Bis(2-ethylhexyl) phthalate (13 µg/l), chlorpyrifos (0.18 µg/l), zinc (73.0 µg/l), lead (6.3 µg/l), numerous unidentified components sensitive to ECD (Electron Capture Detector), and several unidentified components sensitive to NPD (Nitrogen Phosphorus Detector). The total ammonia concentration was 5.69 mg/l and the unionized ammonia concentration was 0.02 mg/l. Phthalate concentrations as low as 0.03 µg/l have affected reproduction in the Cladoceran, *Daphnia magna* (U.S. EPA 1976). Chlorpyrifos concentration as low as 0.11 µg/l have produced acute toxicity in aquatic organisms (U.S. EPA 1986). Unionized ammonia concentrations of 0.01 mg/l have also been toxic to aquatic organisms (U.S. EPA 1973). Zinc toxicity to *Daphnia* species has occurred at levels ranging from 33 µg/l for *Daphnia hyalina* (hardness = 23 mg/ as $CaCO_3$) to 1524 µg/l for *Daphnia magna* (unspecified hardness) . *Daphnia magna* also exhibited zinc LC_{50}'s of 60.9. µg/ to 308 µg/l in the hardness range of about 20 - 45.3 mg/l as $CaCO_3$ (U.S. EPA 1976).

It seems likely that these substances, by themselves or in combination with the numerous unidentified organic compounds in the sample from the City of Carrabelle, were partially responsible for the mortality in the chlorinated and dechlorinated bioassay tests. The dechlorinated *C. dubia* test was invalidated due to control mortality. All of the control mortality occurred in one container. The cause of the control mortality is unknown, but is consistent with contamination by a probe. The components detected in the sample by chemical analyses were of sufficient concentrations to have caused the observed mortality in the remainder of the dechlorinated tests.

Because of the test invalidation (control mortality > 10%), the test results for the *C. dubia* dechlorinated are presented in Table 5 for informational purposes. We feel confident, however, that this test indicates acute toxicity in the dechlorinated sample. In conclusion, the sample of discharge from the City of Carrabelle STP was acutely toxic on 24 May, 1988.

Biology Section Recommendations

The Biology Section recommends that this facility be required to meet state toxicity standards or to undertake a Toxicity Reduction Investigation with the goal of meeting those standards. The Biology Section also recommends that an effort be made to reduce the amount of total residual chlorine in the final discharge from the facility.

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	0.0 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%	0.0 ml	50.0 ml	50 ml

Daphnia pulex (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	200.0 ml	0.0 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%	0.0 ml	200.0 ml	200 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Sample	Total Volume
Control	500 ml	0 ml	500 ml
30.0%	350 ml	150 ml	500 ml
60.0%	200 ml	300 ml	500 ml
100.0%	0 ml	500 ml	500 ml

Table 2. Data recorded during the 48 hour static acute bioassay of the chlorinated sample from the City of Carrabelle STP, Carrabelle, Franklin County, Florida, NPDES #FL0026808 on 24 to 26 May, 1988.

Facility:	City of Carrabelle			NPDES # FL0026808		Facility Type:	Municipal
Location:	Franklin County			Contact/District:	L.V. Olney		
Sample Collection Date:	5/24/88	Time:	0930	Test type: Static Screening			
Test Beginning Date:	5/24/88	Time:	1530	# tests:	6	Receiving Water:	St. George Sound
Test Ending Date:	5/26/88	Time:	1530	Chlorination Type:	chlorinated		

Parameter	
Total Residual Cl2:	15.9
Method:	Iodometric
By:	RBF
Salinity:	0

Ammonia:	Control Water	100% Sample
Unionized NH3:	0.00	0.02
Total NH3:	0	5.69

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	-	8.3	9.0	-	8.0	8.1	-	-	-	-	-	-
Control	10	10	-	8.3	9.0	-	8.0	8.1	-	-	-	-	-	-
30%	10	0	-	8.3	9.2	-	7.5	8.1	-	-	-	-	-	-
30%	10	0	-	8.3	9.2	-	7.5	8.1	-	-	-	-	-	-
60%	10	0	-	8.3	9.2	-	7.1	8.0	-	-	-	-	-	-
60%	10	0	-	8.3	9.2	-	7.0	8.0	-	-	-	-	-	-
100%	10	0	-	7.9	9.2	-	6.8	8.0	-	-	-	-	-	-
100%	10	0	-	7.9	9.2	-	6.8	8.0	-	-	-	-	-	-

	Control water	100% sample
Alkalinity:	117	95
Hardness:	112	103

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	-	8.4	9.0	-	8.0	8.0	-	20.7	20.2	-	-	-
Control	10	10	-	8.4	9.0	-	8.1	8.1	-	-	-	-	230	-
30%	10	0	-	8.5	9.3	-	7.5	7.8	-	20.7	20.1	-	-	-
30%	10	0	-	8.5	9.3	-	7.5	7.8	-	-	-	-	250	-
60%	10	0	-	8.5	9.3	-	7.1	7.8	-	20.7	20.0	-	-	-
60%	10	0	-	8.5	9.3	-	7.0	7.7	-	-	-	-	275	-
100%	10	0	-	8.2	9.3	-	6.7	7.6	-	20.7	20.1	-	-	-
100%	10	0	-	8.2	9.3	-	6.7	7.6	-	-	-	-	300	-

	Control water	100% sample
Alkalinity:	117	95
Hardness:	112	103

Organism: ***Notropis leedsi*** Age/Life stage: 27 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	-	8.4	7.5	-	7.6	7.7	-	21.0	20.2	-	-	-
Control	5	5	-	8.4	7.7	-	7.6	7.7	-	-	-	-	215	-
30%	5	0	-	8.3	8.3	-	7.3	7.6	-	20.8	19.9	-	-	-
30%	5	0	-	8.3	8.4	-	7.3	7.6	-	-	-	-	235	-
60%	5	0	-	8.3	8.9	-	6.9	7.4	-	20.7	19.8	-	-	-
60%	5	0	-	8.3	8.9	-	6.9	7.4	-	-	-	-	270	-
100%	5	0	-	7.9	8.9	-	6.7	7.3	-	20.7	19.9	-	-	-
100%	5	0	-	7.9	8.9	-	6.7	7.3	-	-	-	-	300	-

	Control water	100% sample
Alkalinity:	115	95
Hardness:	105	103

Table 3. Data recorded during the 48 hour static acute bioassay of the dechlorinated sample from the City of Carrabelle STP, Carrabelle, Franklin County, Florida, NPDES #FL0026808 on 24 to 26 May, 1988.

Facility:	City of Carrabelle			NPDES # FL0026808		Facility Type:	Municipal
Location:	Franklin County			Contact/District:	L.V. Olney		
Sample Collection Date:	5/24/88	Time:	0930	Test type:	Static Screening		
Test Beginning Date:	5/24/88	Time:	1530	# tests:	6	Receiving Water:	St. George Sound
Test Ending Date:	5/26/88	Time:	1530	Chlorination Type:	dechlorinated		

Parameter	
Total Residual Cl2:	15.9
Method:	Iodometric
By:	RBF
Salinity:	0

Ammonia:	Control Water	100% Sample
Unionized NH3:	0.00	0.01
Total NH3:	0.0	5.69

Organism: *Ceriodaphnia dubia* Age/Life stage: Neonates
LC50: < 30 toxic 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	8.3	8.9	8.5	8.0	8.0	7.8	-	-	-	-	-
Control	10	3	3	8.3	9.0	8.5	8.0	8.1	7.8	-	-	-	-	-
30%	10	9	6	8.3	8.7	8.3	7.2	8.0	7.7	-	-	-	-	-
30%	10	10	1	8.3	8.7	8.3	7.2	8.0	7.7	-	-	-	-	-
60%	10	0	0	8.5	8.7	8.2	6.9	7.9	7.6	-	-	-	-	-
60%	10	1	0	8.3	8.7	8.2	6.9	7.9	7.6	-	-	-	-	-
100%	10	3	0	7.9	8.9	8.0	6.7	7.9	7.6	-	-	-	-	-
100%	10	0	0	7.9	8.9	8.0	6.7	7.9	7.3	-	-	-	-	-

	Control water	100% sample
Alkalinity:	117	108
Hardness:	112	100

Organism: *Daphnia pulex* Age/Life stage: Neonates
LC50: 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	8.5	8.8	8.4	8.0	8.0	8.0	20.6	20.3	20.1	-	-
Control	10	9	9	8.5	8.8	8.4	8.0	8.0	8.0	-	-	-	230	238
30%	10	10	10	8.6	8.4	7.2	7.3	7.8	7.7	20.7	20.2	19.9	-	-
30%	10	10	10	8.6	8.4	7.1	7.3	7.8	7.7	-	-	-	305	300
60%	10	10	10	8.4	7.7	5.8	6.9	7.7	7.6	21.0	20.1	19.8	-	-
60%	10	10	10	8.4	7.7	5.8	6.9	7.7	7.6	-	-	-	390	375
100%	10	10	10	7.9	7.7	5.2	6.7	7.5	7.6	21.2	20.1	19.8	-	-
100%	10	10	10	7.8	8.0	5.1	6.7	7.5	7.5	-	-	-	490	480

	Control water	100% sample
Alkalinity:	117	108
Hardness:	112	100

Organism: *Notropis leedsi* Age/Life stage: 27 days old
LC50: 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	7.4	6.2	7.6	7.6	7.6	21.2	20.4	20.1	-	-
Control	5	5	5	8.3	7.5	6.3	7.6	7.6	7.6	-	-	-	220	225
30%	5	5	5	8.3	6.6	5.1	7.1	7.5	7.4	21.2	20.1	19.8	-	-
30%	5	5	5	8.3	6.6	5.1	7.1	7.5	7.5	-	-	-	300	300
60%	5	5	5	8.0	7.0	3.9	6.9	7.4	7.4	21.3	20.0	19.7	-	-
60%	5	5	5	8.0	7.0	4.0	6.9	7.4	7.4	-	-	-	385	380
100%	5	4	4	7.8	7.2	3.6	6.6	7.4	7.4	21.5	20.0	19.7	-	-
100%	5	5	5	7.7	7.2	3.9	6.6	7.4	7.4	-	-	-	500	482

	Control water	100% sample
Alkalinity:	115	108
Hardness:	105	100

Table 4. Microtox test results, City of Carrabelle WWTP discharge, Franklin County, NPDES #FL0026808, on 25 May, 1988.

Microtox Test — Culture Lot # M-606

	Final Reclaimed Water			
% Sample	Chlorinated		Dechlorinated	
	5-min	15-min	5-min	15-min
0	79	65	80	57
0	66	54	78	54
0	73	60	85	59
12.38	69	56	11	6
12.38	73	60	11	6
12.38	72	60	10	6
24.75	66	55	4	3
24.75	66	55	4	3
24.75	70	59	4	3
49.5	11	9	3	1
49.5	10	8	3	1
49.5	12	9	3	1
99	1	0.5	2	0.5
99	1	0.5	2	0.5
99	1	0.5	2	0.5

EC_{50} Chlorinated (15 minute, 15 °C) = 0.2% sample
EC_{50} Dechlorinated (15 minute, 15 °C) =2.5% sample

EC_{50} (15 minute, 15 °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. LC_{50} Data for Bioassay of the City of Carrabelle Sewage Treatment Plant, Carrabelle, Franklin County, Florida, NPDES #FL0026808 on 25 to 27 May, 1988.

Organism	Method of Data Analysis	48 Hour LC_{50} (95% Confidence Intervals)
Dechlorinated discharge		
*Ceriodaphnia dubia**	Binomial	a, < 30.00%[c]
	Moving Average	a
	Probit	a
Daphnia pulex		no toxicity
Notropis leedsi		no toxicity
Chlorinated discharge		
Ceriodaphnia dubia	Binomial	a, < 30.00%[b]
	Moving Average	a
	Probit	a
Daphnia pulex	Binomial	a, < 30.00%[b]
	Moving Average	a
	Probit	a
Notropis leedsi	Binomial	a, < 30.00%[b]
	Moving Average	a
	Probit	a

a Due to an insufficient number of dead organisms, or to the irregular distribution of mortality, an LC_{50} value cannot be calculated using this method of statistical analysis.

b The LC_{50} is < 30.0% since all the organisms died in the lowest test concentration (30.0%).

c The LC_{50} is < 30.0% since 65.0% of the organisms died in the lowest test concentration (30.0%).

* Test invalidated because of death of all animals in 1 of 4 control chambers. Tests are technically invalid if control mortality is > 10%, however, contamination of one control chamber is the probable cause of the observed control mortality, and the results are probably correct.

Table 6. Results of chemical analyses on the sample of discharge from the City of Carrabelle STP, Carrabelle, Franklin County, Florida.

Organics	
Acid Extractables	None Detected
Base Neutral Extractalbes	Bis(2-ethylhexyl) Phthalate (13 µg/l)
	Several unidentified peaks
Pesticide Extractables	Chlorpyrifos (0.18 µg/l)
	Numerous unidentified components sensitive to ECD
	Several unidentified components sensitive to NPD
Metals	
Lead	6.3 µg/l
Zinc	73.0 µg/l

Literature Cited

Beckman Instruments, Inc. 1982. Microtox system operating manual.

Florida Department of Environmental Regulation. 1985. Quality assurance manual for performing acute toxicity tests (Second Edition). Biology Section, Tallahassee.

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. 1973. Nitrogenous Compounds in the Environment. Hazardous Materials Advisory Committee (to the EPA), EPA-ASP-73-001, December, 1973. In Process Design Manual for Nitrogen Control. U.S. EPA. 1975. Section 2.4.2 p. 2-13.

_____. 1976. Quality Criteria for Water. U.S. EPA, Washington, D.C. 256 p.

_____. 1976. Zinc Ambient Water Quality Criteria. Criteria and Standards Division, Office of Water Planning and Standards, U.S. Environmental Protection Agency. Washington, D. C. 144 p.

_____. 1986. Ambient Aquatic Life Water Quality Criteria for Chlorpyrifos. U.S. EPA Office of Research and Development Environmental Research Laboratories. Duluth, Minnesota; Narragansett, Rhode Island. 59 p.

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 $LC_{50} < 2000\%$ at the point of discharge.

   B. With a non-dredge and fill mixing zone the discharge may not exhibit an $LC_{50} < 100\%$ at the point of discharge and may not exhibit an $LC_{50} < 2000\%$ 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 a more dilute concentrations of the sampled discharge than allowed above.

Industry/Toxicant: City of Carrabelle STP
Address: Franklin Co.
Contact/District:
Effluent Serial Number: F
NPDES Permit Number: FL0026808

BIOASSAY DATA FORM

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

Sample Collection: Date 5-24-88 Time 0930
Test Beginning: Date 5-24-88 Time 3:30
Test Ending: Date 5-26-88 Time 1530
Test Organism: C. dubia
Age/Life Stage: neonate

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96
Control	1	10	10	10			8.3	8.9	8.5			8.0	8.0	7.8							
Control	2	10	3	3			8.3	9.0	8.5			8.0	8.1	7.8							
30%	3	10	9	6			8.3	8.7	8.3			7.2	8.0	7.7							
30%	4	10	10	1			8.3	8.7	8.3			7.2	8.0	7.7							
60%	5	10	0	0			8.3	8.7	8.2			6.9	7.9	7.6							
60%	6	10	1	0			8.3	8.7	8.2			6.9	7.9	7.6							
100%	7	10	3	0			7.9	8.8	8.0			6.7	7.9	7.6							
100%	8	10	0	0			7.9	8.9	8.0			6.7	7.9	7.5							
Initial of Investigator		KHL	SHW	KHL			RSF	MF	JMF			SHW	MF	JMF							

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 117/112
100% Sample: 103/100
Other:
Measured by:

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0 — KHL
Control Water: 0
100% Sample: 5.69
Other:
Hour 1
Control Water:
100% Sample:
Other:
Measured by: MF

Instrument Calibrations:
pH Meter:
0 Hours 7@7.9@9
24 Hours 7@7.9@1
48 Hours 7@7.9@9
72 Hours
96 Hours
DO Meter: 9.0@21°C; 8.8@22°C
S-C-T Meter: 10000 @ 20°C; H@10 34@30; Titration, 21 2nd 30 cont 13 1st 21

Total Residual Chlorine:
Field data: Method:
Lab: OFF SCALE Method: HACH
Lab: 1000 Method: 15.9 RSF

Remarks: Salinity of receiving water = 25 ‰
pH = 7.8

Signature: Russell Frazelsburg
KM Lurding
Marshall Faircloth
Henry Wade

Slope = 59.4
sal = 0.04

Reviewed by:
Date: 10/12/88

Carrabelle STP

Test Material: 2-6
Test Type: (Static) Renewal Flow-through (Conventional)
Other: deCl2

5-24-88 : 0930
5-24-88 : 3:30
5-26-88 : 1530
Test Organism: D. pulex
Age/life Stage: neonate

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96	Conductivity 0	24	48	72	96
Control	67	10	10	10			8.5	8.8	8.4			8.0	8.0	8.0			20.6	20.3	20.1							
Control	12	10	9	10			8.5	8.8	8.4			8.0	8.0	8.0								230		238		
30%	37	10	10	10			8.6	8.4	7.2			7.3	7.8	7.7			20.7	20.2	19.9							
30%	28	10	10	10			8.6	8.4	7.1			7.3	7.8	7.7								305		300		
60%	13	10	10	10			8.4	7.7	5.8			6.9	7.7	7.6			21.0	20.1	19.8							
60%	27	10	10	10			8.4	7.7	5.8			6.9	7.7	7.6								390		375		
100%	119	10	10	10			7.9	7.7	5.2			6.7	7.5	7.6			21.2	20.1	19.8							
100%	2	10	10	10			7.8	8.0	5.1			6.7	7.5	7.5								490		480		
Initial of Investigator		RBF	MF	JMF			RBF	MF	JMF			RBF	MF	JMF			RBF	MF	JMF			RBF		JMF		

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

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

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

Control Water: U 0 / T 0
100% Sample: S
Other:

Hour _____

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

Signature: Richard Anderberg, Kathleen Lundeen, Marshall Faircloth, Steven H. Wolfe

Instrument Calibrations:

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

Remarks:

Total Residual Chlorine:

Field data: _____ Method: _____
Lab: _____ Method: _____
Lab: _____ Method: _____

De-chlorinated:
Measurements by: _____

Reviewed by: _____

Date: 10/12/88

Industry/Toxicant Carrabelle STP
Address
Contact/District
Effluent Serial Number
NPDES Permit Number

BIOASSAY DATA FORM

Test Number: 3 of 6
Test Type: (Static) Renewal, (Screening) Conventional
Other: deCl2

Sample Collection: Date 5-24-88 Time 0930
Test Beginning: Date 5-24-88 Time 1530
Test Ending: Date 5-26-88 Time 1530
Test Organism N. Leptocephalus
Age/Life Stage 27 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	67	5	5	5			8.3	7.4	6.2			7.6	7.6	7.6			21.2	20.4	20.1							
Control	69	5	5	5			8.3	7.5	6.3			7.6	7.6	7.6								220		225		
30%	68	5	5	5			8.3	6.6	5.1			7.1	7.5	7.4			21.2	20.1	19.8							
30%	16	5	5	5			8.3	6.6	5.1			7.1	7.5	7.5								300		300		
60%	12	5	5	5			8.0	7.0	3.9			6.9	7.4	7.4			21.3	20.0	19.7							
60%	47	5	5	5			8.0	7.0	4.0			6.9	7.4	7.4								385		380		
100%	5	5	4	4			7.8	7.2	3.6			6.6	7.4	7.4			21.5	20.0	19.7							
100%	~~170~~ 6 240	5	5	5			7.1	7.2	3.9			6.6	7.4	7.4								500		462		
Initial of Investigator		SW	SHW	JMF			RBF	HF	JMF			SHW	MF	JMF			RBF	MF	JMF			RBF		JMF		

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 115/105
100% Sample: ~~135~~
Other:
Measured by:

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

	u	t
Control Water:	0	0
100% Sample:		5.69
Other:		

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

Signatures:
Russell Frankenberg
K H Lurding
Marshall Fairchild
Steven H Woodfe

Instrument Calibrations:

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

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

Dechlorinated:
Measurements by:

Reviewed by:
Date: 10/12/88

Carrabelle STP

Test Number: 4-6
Test Type: Static / Renewal / Screening / Definitive / Conventional
Other: CP2

5-24-88 0930
5-24-88 1530
5-28-88 1530

Test Organism: C. dubia
Age/Life Stage: neonate

Med Lab used

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	Alkalinity/Hardness (mg/l as $CaCO_3$) 0	24	48	72	96
Control	9	10	10				8.3	9.0				8.0	8.1													
Control	10	10	10				8.3	9.0				8.0	8.1													
10%	11	10	0				8.3	9.2				7.5	8.1													
10%	12	10	0				8.3	9.2				7.5	8.1													
30%	13	10	0				8.3	9.2				7.1	8.0													
30%	14	10	0				8.3	9.2				7.0	8.0													
50%	15	10	0				7.9	9.2				6.8	8.0													
50%	16	10	0				7.9	9.2				6.8	8.0													
Initial of Investigator		KHL	SW				RBF	MF				SHW	MF													

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water:
100% Sample: 99/102
Other:
Measured by:

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0 (U / T)
Control Water: 0 / 0
100% Sample: 5.6
Other:

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

Signatures:
Daniel Fredenburg
Lin Lur Cing
Marshall Faudeth
Steve H Wolf

Instrument Calibrations:

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

Remarks:

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

De-chlorinated:
Measurements by:_____

Reviewed by:
Date: 10/12/88

Industry/Toxicant: Carrabelle STP
Address:
Contact/District:
Effluent Serial Number:
NPDES Permit Number:

BIOASSAY DATA FORM

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

Sample Collection: Date 5-24-88 Time 0930
Test Beginning: Date 5-24-88 Time 1530
Test Ending: Date 5-28-88 Time 1530
Test Organism: D. pulex
Age/Life Stage: neonate

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96	Conductivity 0	24	48	72	96
Control	43	10	10				8.4	9.0				8.0	8.0				20.7	20.2								
Control	59	10	10				8.4	9.0				8.1	8.1									230				
10%	32	10	0				8.5	9.3				7.5	7.8				20.7	20.1								
10%	55	10	0				8.5	9.3				7.5	7.8									29				
30%	7	10	0				8.5	9.3				7.1	7.8				20.7	20.0								
30%	38	10	0				8.5	9.3				7.0	7.7									215				
50%	25	10	0				8.2	9.3				6.7	7.6				20.7	20.1								
50%	41	10	0				8.2	9.3				6.7	7.6									300				
Initial of Investigator		RBF	MF				RBF	HF				SHW	MF				RBF	MF				RBF				

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

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: 0/0
100% Sample: 5.69
Other:
Hour
Control Water:
100% Sample:
Other:
Measured by: MF

Signatures:
Russell Frydenborg
Kathy Lurding
Marshall Faircloth
Steve Wolfe

Instrument Calibrations:

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

Total Residual Chlorine:
Field data: Method:
Lab: Method:
Lab: Method:
Dechlorinated:
Comments by:

Reviewed by:
Date: 10/12/88

Carlsbad STP

Test Initiated: Date 5-24-88 Time 0930
Test Terminated: Date 5-24-88 Time 1530
Test Ended: Date 5-25-88 Time 1530
Test Organism: N. Reticulata
Age/Life Stage: 27 days

Test Chemicals: 6 6
Test Agent: Cl2
Method: Static / Renewal / Recirculating / Conventional

Concentration 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	99	5	5				8.4	7.5				7.6	7.7				21.0	20.2								
Control	62	5	5				8.4	7.7				7.6	7.7									215				
10%	52	5	0				8.3	8.3				7.3	7.6				20.8	19.9								
10%	31	5	0				8.3	8.4				7.3	7.6									235				
30%	11	5	0				8.3	8.9				6.9	7.4				20.7	19.8								
30%	25	5	0				8.3	8.9				6.9	7.4									270				
50%	66	5	0				7.9	8.9				6.7	7.3				20.7	19.9								
50%	26	5	0				7.9	8.9				6.7	7.3									300				
Initial of Investigator		SHW	SHW				RBF/MF					SHW					RBF/MF					RBF				

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

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

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

Control Water: O / C
100% Sample: S
Other:

Hour

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

Signatures:
Ryrel Frydenborg
KHLundring
Marlon Faucett
Steven H Wolfe

Instrument Calibrations:

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

Remarks:

Total Residual Chlorine:

Field data: _____ Method: _____
Lab: _____ Method: _____
Lab: _____ Method: _____

De-chlorinated:
Measurements by: _____

Reviewed by: _____

Date: 10/12/88

.091

MICROTOX™ Data Reduction Sheet

SOURCE: Carrabelle STP

GRAPH CALC EC 50 (15 MIN, 15 °C) = 0.2 (%SAMPLE) mg/L

SAMPLE DATA:

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

DATE ASSAYED: 5-24-88 TIME: 2:00 PM

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: 1:10 · as noted below

OPERATOR: Lisa Williams REAGENT VIAL LOT NO. M-606

REMARKS: 99% test: no response at all dilutions at 5 min. Sample re-run at 1-10 fold dilu

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	Γ5	Γ15	NOTES
A1	0	79	65	X	X	
B1		66	54			
C1		73	60			
A2	~~12.38~~ .099	69	56	.019	.017	span changed to 100% at 15 min.
B2		73	60			
C2		72	60			
A3	~~24.75~~ .099	66	55	.079	.059	
B3		66	55			
C3		70	59			
A4	~~49.5~~ .99	11	9	5.606	5.886	
B4		10	8			
C4		12	9			
A5	~~99~~ 9.9	1	.5	71.667	118.333	
B5		1	.5			
C5		1	.5			
EC_{50} (MIN, °C)				0.345	0.2	

REVIEWED BY:

DATE: 10/12/88

092

MICROTOX™ Data Reduction Sheet

SOURCE: Carrabelle STP

GRAPH CALC EC 50 (15 MIN, 15 °C) = 2.5 (%SAMPLE) mg/L

SAMPLE DATA:

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

DATE ASSAYED: 5-24-88 TIME: 2:45PM

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-606

REMARKS: S. Wolfe noted ~~[illegible]~~ pH of 6.7 in sample

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	Γ5	Γ15	NOTES
A1	0	80	57	X	X	
B1		78	54			
C1		85	59			
A2	12.38	11	6	6.59	8.44	
B2		11	6			
C2		10	6			
A3	24.75	4	3	19.25	17.89	
B3		4	3			
C3		4	3			
A4	49.5	3	1	26.0	55.67	
B4		3	1			
C4		3	1			
A5	99	2	5	39.5	112.3	
B5		2	5			
C5		2	5			
EC_{50} (MIN, °C)				1.30	2.46	

REVIEWED BY:

DATE: 10/12/88