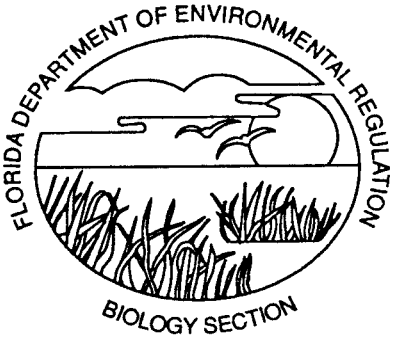


Bioassays of

Lynn Haven Wastewater Treatment Plant

Bay County, Florida

NPDES #FL0027804

Sampled 6/28/88

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February 10, 1989

Biology Section

Division of Administrative and Technical Services

Bioassays of

Lynn Haven Wastewater Treatment Plant

Bay County, Florida

NPDES #FL0027804

Biology Section
Bureau of Laboratories
February 10, 1989

Executive Summary

Lynn Haven Wastewater Treatment Plant, Lynn Haven, Bay County, Florida, NPDES #FL0027804 on 29 June to 1 July, 1988.

The sample of discharge from the Lynn Haven WWTP outfall #001 was not toxic to the bioassay test organisms, *Ceriodaphnia dubia* and *Notropis leedsi*, in either the chlorinated or dechlorinated tests. The tests involving the bioassay test organism, *Daphnia pulex*, were invalidated due to excessive control mortality; however, for informational purposes, neither the chlorinated nor the dechlorinated samples were toxic to this test organism.

The chlorinated and dechlorinated samples adversely affected the Microtox® assay organism, *Photobacterium phosphoreum*, producing EC_{50}'s of 49.5% and 85.6%, respectively. Chemical analyses were not performed on the sample.

Introduction

The Lynn Haven Wastewater Treatment Plant NPDES #FL0027804, is located at 6th Street at Montana Avenue, Lynn Haven, Bay County, Florida. The facility uses an activated sludge process to treat approximately 1 MGD of domestic waste. After treatment, the reclaimed water is spray irrigated. Drainage from the spray field is collected and discharged to a tributary of Beatty Bayou, itself a tributary of North Bay (Figure 1).

In June, 1986, the Biology Section performed four 48-hour static acute bioassays on a sample from outfall #001 of the Lynn Haven WWTP. The chlorinated sample was toxic to the *Daphnia pulex* test organisms, producing an LC_{50} of 48.2%. The chlorinated sample was not toxic to the fish, *Notropis leedsi*. The dechlorinated sample was not toxic to either test organism. Toxicity was attributed to the total residual chlorine (0.25 mg/l Hach) and the ammonia (2.28 mg/l total and 0.17 mg/l unionized) in the sample (FDER 1986).

To again evaluate the existence and extent of toxicity from this facility, at the request of the FDER Northwest District Office, the Biology Section performed six separate static toxicity bioassays and two Microtox® assays on a sample from this facility. 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, L. A. Williams, and S. H. Wolfe.



Figure 1. Portions of Bay County, Florida, including the approximate location of the Lynn Haven 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 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 17 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 from the Lynn Haven WWTP facility. The tests were performed from 1130 hours on 29 June to 1030 hours on 1 July, 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. Mike Clayton of the FDER Northwest District branch office in Panama City, 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.

Four replicates consisting of 30 ml plastic vials containing 15 mls of the control and each test concentration were used in bioassays with the water flea, *Ceriodaphnia dubia*. Two replicates consisting of 250 ml flint glass jars containing 100 mls of the control and each test concentration were used with the water flea, *Daphnia pulex*. Adult *Ceriodaphnia* and *Daphnia* were separated using a nylon mesh sieve and placed into large culture dishes 18 hrs before the beginning of the test. Neonates produced during the intervening period (i.e., <18 hr old) were then captured by glass pipette, examined for injury, and transferred into the test chambers. Five (for *C. dubia*) or

ten individuals (for *D. pulex*) were loaded 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 discharge from the Lynn Haven Wastewater Treatment Plant was not toxic to the bioassay test organisms, *Ceriodaphnia dubia* and *Notropis leedsi*, in either the chlorinated or dechlorinated tests. The bioassays using the test organism, *Daphnia pulex*, were invalidated due to excessive control mortality; however, for informational purposes the chlorinated and dechlorinated *Daphnia pulex* tests indicated that the sample was not toxic (Tables 2 and 3).

The chlorinated and dechlorinated samples adversely affected the Microtox® assay test organism, *Photobacterium phosphoreum*, producing EC_{50}'s of 49.5% sample and 85.6% sample, respectively (Table 4).

Chemical analyses were not performed on the sample

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

Ceriodaphnia dubia (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	15.0 ml	0.0 ml	15 ml
25.0%	11.25 ml	3.75 ml	15 ml
50.0%	7.5 ml	7.5 ml	15 ml
70.0%	4.5 ml	10.5 ml	15 ml
85.0%	2.25 ml	12.75 ml	15 ml
100.0%	0.0 ml	15.0 ml	15 ml

Daphnia pulex (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	100.0 ml	0.0 ml	100 ml
100.0%	0.0 ml	100.0 ml	100 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Sample	Total Volume
Control	500 ml	0 ml	500 ml
25.0%	125 ml	375 ml	500 ml
50.0%	250 ml	250 ml	500 ml
70.0%	150 ml	350 ml	500 ml
85.0%	75 ml	425 ml	500 ml
100.0%	0 ml	500 ml	500 ml

Table 2. Data recorded during 48 hour static acute toxicity bioassays of a dechlorinated sample of discharge from outfall #001 of the Lynn Haven WWTP, Lynn Haven, Bay County, Florida, NPDES #FL0027804, on 29 June to 1 July, 1988.

Facility:	**Lynn Haven WWTP**		**NPDES #FL0027804**	Facility Type:	**Domestic**
Location:	6th St. at Montana Ave., Lynn Haven		Contact/District:	Mike Clayton/NW	
County:	Bay		Test type:	Static Definitive	
Sample Collection Date:	**6/28/88**	Time: 1400	# tests:	6	Receiving Water: Ditch to Beatty Bayou
Test Beginning Date:	6/29/88	Time: 1130	Chlorination Type:	**Dechlorinated**	and N. St. Andrews Bay
Test Ending Date:	7/1/88	Time: 1030		**Page 1 of 2**	

Total Residual Cl2	mg/l	Method
Field:	0.4	Hach
Lab:	0.4	Hach
Lab:	0.4	Iodometric

Ammonia	Total (mg/l)	Unionized (mg/l)
Control Water (fish):	0.00	0.00
Cond. Control Water (daphnids):	0.00	0.00
100% Sample:	0.01	0.00

Alk & Hardness	Alkalinity (mg/l)	Hardness (mg/l)
Control Water (fish):	125	125
Cond. Control Water (daphnids):	130	133
100% Sample:	125	118

Salinity (ppt)	
Control Water:	0
100% Sample:	0

Organism: ***Ceriodaphnia dubia*** Life stage: neonate
LC50: - Binomial: - Moving Average: - Probit: -

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hr	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	4	4	9.1	-	8.9	8.1	-	8.2	19.6	-	20.2	-	-
Control	5	5	4	9.1	-	8.9	8.1	-	8.2	19.6	-	-	-	-
Control	5	5	5	9.0	-	8.9	8.1	-	8.2	19.6	-	-	-	-
Control	5	5	5	9.0	-	8.9	8.1	-	8.2	19.7	-	20.2	-	-
25%	5	5	5	9.0	-	8.9	8.0	-	8.2	19.7	-	20.3	-	-
25%	5	5	5	9.0	-	8.9	8.0	-	8.2	19.7	-	-	-	-
25%	5	5	5	9.0	-	8.9	8.0	-	8.2	19.8	-	-	-	-
25%	5	5	5	9.0	-	8.9	8.0	-	8.2	19.9	-	20.3	-	-
50%	5	5	5	9.0	-	8.9	7.9	-	8.2	19.7	-	20.3	-	-
50%	5	5	5	9.0	-	8.9	7.9	-	8.2	19.7	-	-	-	-
50%	5	5	5	9.0	-	8.9	7.9	-	8.2	19.8	-	-	-	-
50%	5	5	5	9.0	-	8.9	7.9	-	8.2	19.8	-	20.3	-	-
70%	5	5	5	8.9	-	8.9	7.9	-	8.2	19.6	-	20.2	-	-
70%	5	5	5	8.9	-	8.9	7.9	-	8.2	19.6	-	-	-	-
70%	5	5	5	8.9	-	8.9	7.9	-	8.2	19.7	-	-	-	-
70%	5	5	5	8.9	-	8.9	7.9	-	8.2	19.7	-	20.2	-	-
85%	5	5	5	8.9	-	8.9	7.8	-	8.2	19.7	-	20.2	-	-
85%	5	5	5	8.9	-	8.9	7.8	-	8.2	19.7	-	-	-	-
85%	5	5	5	8.9	-	8.9	7.8	-	8.2	19.7	-	-	-	-
85%	5	5	5	8.9	-	8.9	7.8	-	8.2	19.7	-	20.2	-	-
100%	5	5	5	9.0	-	8.9	7.7	-	8.1	19.7	-	20.2	-	-
100%	5	5	5	9.0	-	8.9	7.7	-	8.1	19.7	-	-	-	-
100%	5	5	5	9.1	-	8.9	7.7	-	8.1	19.7	-	-	-	-
100%	5	5	5	9.1	-	8.9	7.7	-	8.1	19.8	-	20.2	-	-

Organism: ***Daphnia pulex*** Life stage: neonate
LC50: - Binomial: - Moving Average: - Probit: -

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hr	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	2	2	9.0	-	9.0	8.2	-	8.1	19.8	-	19.7	-	-
Control	10	3	3	9.1	-	9.0	8.2	-	8.1	-	-	-	-	-
100.0%	10	10	10	9.1	-	9.0	7.6	-	8.0	19.8	-	19.6	-	-
100.0%	10	10	10	9.1	-	9.0	7.6	-	8.0	-	-	-	-	-

Table 2. Continued.

Facility:	**Lynn Haven WWTP**		**NPDES #FL0027804**	Facility Type:	**Domestic**
Location:	6th St. at Montana Ave.	County: Bay	Contact/District:	Mike Clayton/NW	
			Test type:	Static Definitive	
Sample Collection Date:	**6/28/88**	Time: 1400	# tests:	6	Receiving Water: Ditch to Beatty Bayou
Test Beginning Date:	6/29/88	Time: 1130	Chlorination Type:	**Dechlorinated**	and N. St. Andrews Bay
Test Ending Date:	7/1/88	Time: 1030			**Page 2 of 2**

Total Residual Cl2	mg/l	Method
Field:	0.4	Hach
Lab:	0.4	Hach
Lab:	0.4	Iodometric

Ammonia	Total (mg/l)	Unionized (mg/l)
Control Water (fish):	0.00	0.00
Cond. Control Water (daphnids):	0.00	0.00
100% Sample:	0.01	0.00

Alk & Hardness	Alkalinity (mg/l)	Hardness (mg/l)
Control Water (fish):	125	125
Cond. Control Water (daphnids):	130	133
100% Sample:	125	118

Salinity (ppt)	
Control Water:	0
100% Sample:	0

Organism: ***Notropis leedsi*** Age: 17 days
LC50: - Binomial: - Moving Average: - Probit: -

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hr	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	8.6	8.8	8.8	7.6	7.9	7.9	20.0	19.1	20.0	-	-
Control	5	4	4	8.6	8.8	8.8	7.6	7.9	7.9	-	-	-	220	220
25%	5	5	5	8.7	8.9	8.9	7.6	7.8	7.9	20.1	18.8	19.8	-	-
25%	5	5	5	8.8	8.9	9.0	7.6	7.8	7.9	-	-	-	300	300
50%	5	5	4	8.8	8.9	9.0	7.6	7.8	7.9	20.2	18.7	19.6	-	-
50%	5	5	5	8.8	8.9	9.0	7.6	7.8	7.9	-	-	-	380	380
70%	5	5	5	8.6	8.7	9.0	7.6	7.8	7.9	20.2	18.7	19.6	-	-
70%	5	5	5	8.6	8.7	8.9	7.6	7.8	7.9	-	-	-	440	440
85%	5	5	5	8.6	8.6	8.9	7.6	7.8	7.9	20.2	18.6	19.5	-	-
85%	5	5	5	8.6	8.6	8.9	7.6	7.8	7.9	-	-	-	485	500
100%	5	5	5	9.0	8.4	8.9	7.5	7.8	7.9	20.1	18.7	19.6	-	-
100%	5	4	4	9.0	8.4	8.8	7.5	7.8	7.9	-	-	-	580	550

Table 3. Data recorded during 48 hour static acute toxicity bioassay of a sample of chlorinated discharge from outfall #001 of the Lynn Haven WWTP, Lynn Haven, Bay County, Florida, NPDES #FL0027804, on 29 June to 1 July, 1988.

Facility:	**Lynn Haven WWTP**		**NPDES #FL00 27804**	Facility Type:	**Domestic**
Location:	6th St. at Montana Ave., Lynn Haven		Contact/District:	Mike Clayton/NW	
County:	Bay		Test type:	Static Definitive	Receiving Water: Ditch to Beatty Bayou
Sample Collection Date:	**6/28/88**	Time: 1400	# tests:	6	and N. St. Andrews Bay
Test Beginning Date:	6/29/88	Time: 1130	Chlorination Type:	**Chlorinated**	
Test Ending Date:	7/1/88	Time: 1030			

Total Residual Cl2	mg/l	Method
Field:	0.4	Hach
Lab:	0.4	Hach
Lab:	0.4	Iodometric

Ammonia	Total (mg/l)	Unionized (mg/l)
Control Water (fish):	0.00	0.00
Cond. Control Water (daphnids):	0.00	0.00
100% Sample:	0.01	0.00

Alk & Hardness	Alkalinity (mg/l)	Hardness (mg/l)
Control Water (fish):	125	125
Cond. Control Water (daphnids):	130	133
100% Sample:	125	118

Salinity (ppt)	
Control Water:	0
100% Sample:	0

Organism: ***Ceriodaphnia dubia*** Life stage: neonate
LC50: - Binomial: - Moving Average: - Probit: -

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hr	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	8.9	-	8.9	8.2	-	8.2	19.7	-	19.9	-	-
Control	5	5	5	8.9	-	8.9	8.2	-	8.2	-	-	-	-	-
Control	5	5	5	8.9	-	8.9	8.2	-	8.2	-	-	-	-	-
Control	5	5	5	8.9	-	8.9	8.2	-	8.2	-	-	19.9	-	-
100%	5	5	5	9.0	-	9.0	7.7	-	8.2	19.7	-	19.9	-	-
100%	5	5	5	9.0	-	9.0	7.7	-	8.2	-	-	-	-	-
100%	5	5	5	9.0	-	9.0	7.7	-	8.2	-	-	-	-	-
100%	5	5	5	9.0	-	9.0	7.7	-	8.2	-	-	19.9	-	-

Organism: ***Daphnia pulex*** Life stage: neonate
LC50: - Binomial: - Moving Average: - Probit: -

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hr	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	1	1	9.2	-	8.8	8.2	-	8.2	19.7	-	19.6	-	-
Control	10	3	3	9.2	-	8.8	8.2	-	8.2	-	-	-	-	-
100%	10	10	10	9.2	-	8.9	7.6	-	8.1	19.7	-	19.6	-	-
100%	10	9	9	9.2	-	8.8	7.6	-	8.1	-	-	-	-	-

Organism: ***Notropis leedsi*** Age: 17 days
LC50: - Binomial: - Moving Average: - Probit: -

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hr	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	9.0	8.9	8.6	7.6	7.9	8.0	20.3	18.6	19.5	-	-
Control	5	5	5	9.0	8.9	8.7	7.6	7.9	8.0	-	-	-	220	215
100%	5	5	5	9.0	8.7	8.4	7.5	7.7	7.9	20.0	18.6	19.6	-	-
100%	5	5	5	9.1	8.7	8.2	7.5	7.7	7.9	-	-	-	560	550

Table 4. Microtox test results, Lynn HavenWWTP discharge, Bay County, NPDES #FL0027804, on 29 June to 1 July, 1988.

Microtox Test — Culture Lot # M-606

% Sample	Final Reclaimed Water			
	Chlorinated		Dechlorinated	
	5-min	15-min	5-min	15-min
0	85	54	65	48
0	79	50	72	55
0	78	52	79	57
12.38	73	50	69	45
12.38	76	51	69	45
12.38	71	49	70	47
24.75	80	46	63	39
24.75	81	46	68	41
24.75	79	46	69	42
49.5	64	35	59	32
49.5	66	34	61	35
49.5	69	36	58	32
99	51	19	52	25
99	52	19	51	25
99	52	19	53	25

EC_{50} Chlorinated (15 minute, 15 °C) = 49.45% sample
EC_{50} Dechlorinated (15 minute, 15 °C) = 85.6% 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.

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.

_____. 1986. Bioassays of the Lynn Haven Wastewater Treatment Plant Bay County, Florida, NPDES #FL0027804. Biology Section, Tallahassee. 15 p.

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.

Lynn Haven WWTP
6th St. at Montana Ave, Bay Co.
Mike Clayton / NW
FL0027804

L6
Dechlor

6/28/88 1400
6/29/88 1130
7/1/88 1930
No. feeds 1
17 days

																	Conductivity							
Control	113	5	5	5			8.6	8.8	8.8			7.6	7.9	7.9			20.0	19.1	20.0					
Control	2	5	4	4			8.6	8.8	8.8			7.6	7.9	7.9							220		220	
25%	5	5	5	5			8.7	8.9	8.9			7.6	7.8	7.9			20.1	18.8	19.8					
25%	52	5	5	5			8.8	8.9	9.0			7.6	7.8	7.9							300		300	
50%	23	5	5	4			8.8	8.9	9.0			7.6	7.8	7.9			20.2	18.7	19.6					
50%	21	5	5	5			8.8	8.9	9.0			7.6	7.8	7.9							380		380	
70%	49	5	5	5			8.9	8.7	9.0			7.6	7.8	7.9			20.2	18.7	19.6					
70%	38	5	5	5			8.9	8.7	8.9			7.6	7.8	7.9							440		440	
85%	54	5	5	5			8.9	8.6	8.9			7.6	7.8	7.9			20.2	18.6	19.5					
85%	47	5	5	5			8.9	8.6	8.9			7.6	7.8	7.9							485		500	
100%	86	5	5	5			9.0	8.4	8.9			7.5	7.8	7.9			20.1	18.7	19.6					
100%	6	5	4	4			9.0	8.4	8.8			7.5	7.8	7.9							580		550	
Initial of Investigator		RBF	SHW	SHW			RBF	SHW	SHW			RBF	SHW	SHW			RBF	SHW	SHW		RBF		SHW	

Control Water: A 125 / 1 12
100% Sample: 125 / 1 11
Other:
Measured by: MF

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

	in	T
Control Water:		0
100% Sample:		0.011
Other:		

Hour 0

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

Signatures:
Steve H Wolfe
Mark W Faircloth
Russel Frydenborg

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7 at 7, 9 at 9	9.0 at 21°C	1270 @ 20°C @ 1273 micros
24 Hours	7 at 7, 9 at 9	9.2 @ 20°C	
48 Hours	7 at 7, 9 at 9	9.0 @ 21°C	1290 @ 21°C @ 1299 micros
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: 0.4 Method: Hach
Lab: 0.4 Method: Hach
Lab: 0.49 Method: Iodometric

De-chlorinated
Measurements by: RBF

Reviewed by:
Date: 1/20/89

Salinity 0.2 ppt

Remarks:

Industry/Toxicant: Lynn Haven WWTP
Address:
Contact/District:
Effluent Serial Number:
NPDES Permit Number:

BIOASSAY DATA FORM

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

Sample Collection: Date 6/28/88 Time 1400
Test Beginning: Date 6/29/88 Time 1145
Test Ending: Date 7/1/88 Time 1030
Test Organism: D. pulex
Age/Life Stage: neonate

Conc. or %	Test Container Number	Number of Live Orgamisms 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	2	2			9.0		9.0			8.2		8.1			19.8		19.7							
Control	17	10	3	3			9.1		9.0			8.2		8.1			–		–							
100%	119	10	10	10			9.1		9.0			7.6		8.0			19.8		19.6							
100%	28	10	10	10			9.1		9.0			7.6		8.0			–		–							
Initial of Investigator		MF	CH	SHW			RBF		SHW			RBF		SHW			RBF		SHW			RBF				

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

	0	24	48	72	96
Control Water:	130 / 133				
100% Sample:	125 / 118				
Other:					
Measured by:					

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

Hour 0

	u	I
Control Water:	0	0
100% Sample:		0.011
Other:		

Hour 0

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

Signatures:
JH Wolfe
Marshall Fairbett
Asst. Laboratory

Instrument Calibrations:

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

Total Residual Chlorine:

0 Hours:		Method:	
24 Hours:		Method:	
48 Hours:		Method:	

Reviewed by:

Date: 1/20/89

Lynn Haven WWTP

3 6 dechlor

C. dubia neonate

6/28/88 1400
6/29/88 1130
7/1/88 1030

	Beaker	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control	1 / 2	5/5	4/5	4/4			9.1 / 9.1		8.4 / 8.4			8.1 / 8.1		8.2 / 8.2			19.6 / 19.6		20.2							
Control	3 / 4	5/5	5/5	5/5			9.0 / 9.0		8.9 / 8.9			8.1 / 8.1		8.2 / 8.2			19.6 / 19.7		20.2							
25%	5 / 6	5/5	5/5	5/5			9.0 / 9.0		8.9 / 8.9			8.0 / 8.0		8.2 / 8.2			19.7 / 19.7		20.3							
25%	7 / 8	5/5	5/5	5/5			9.0 / 9.0		8.9 / 8.9			8.0 / 8.0		8.2 / 8.2			19.8 / 19.9		20.3							
50%	9 / 10	5/5	5/5	5/5			9.0 / 9.0		8.9 / 8.9			7.9 / 7.9		8.2 / 8.2			19.7 / 19.7		20.3							
50%	11 / 12	5/5	5/5	5/5			9.0 / 9.0		8.9 / 8.9			7.9 / 7.9		8.2 / 8.2			19.5 / 19.8		20.3							
70%	13 / 14	5/5	5/5	5/5			8.9 / 8.9		8.9 / 8.9			7.9 / 7.9		8.2 / 8.2			19.6 / 19.6		20.2							
70%	15 / 16	5/5	5/5	5/5			8.9 / 8.9		8.9 / 8.7			7.9 / 7.9		8.2 / 8.2			19.7 / 19.7		20.2							
85%	17 / 18	5/5	5/5	5/5			8.9 / 8.9		8.9 / 8.9			7.8 / 7.8		8.2 / 8.2			19.7 / 19.7		20.2							
85%	19 / 20	5/5	5/5	5/5			8.9 / 8.9		8.9 / 8.9			7.8 / 7.8		8.2 / 8.2			19.7 / 19.7		20.2							
100%	21 / 22	5/5	5/5	5/5			9.0 / 9.0		8.9 / 8.9			7.7 / 7.7		8.1 / 8.1			19.7 / 19.7		20.2							
100%	23 / 24	5/5	5/5	5/5			9.1 / 9.1		8.9 / 8.9			7.7 / 7.7		8.1 / 8.1			19.7 / 19.8		20.2							
Initial of investigator		SHW	MF	[illegible]			RBF		SHW			RBF		SHW			RBF		SHW							

Control Water: 130/133
100% Sample: 125 118
Other:
Measured by: MF

Ammonia unionized/total (mg/l as NH3/N)
Hour 0

	u	T
Control Water:	0	0
100% Sample:		0.011
Other:		

Hour 0
Control Water:
100% Sample:
Other:
Measured by: MF

Signatures:
SH Wolfe
Marshall Faircloth
Russell [illegible]

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: ______

slope = -60.3

Reviewed by: [signature]
Date: 1/20/89

Industry/Toxicant Lynn Haven WWTP
Address
Contact/District
Effluent Serial Number
NPDES Permit Number

BIOASSAY DATA FORM

Test Number: 4 of 6
Test Type: (Static) Renewal (Screening) Conventional
Other: Cl_2

Sample Collection: Date 6/28/89 Time 1400
Test Beginning: Date 6/29/89 Time 1130
Test Ending: Date 7/1/89 Time 1030
Test Organism N. Ieeds
Age/Life Stage 17 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	99	5	5	5			9.0	8.9	8.6			7.6	7.9	8.0			20.3	18.6	19.5							
Control	131	5	5	5			9.0	8.9	8.7			7.6	7.9	8.0								220		215		
100%	15	5	5	5			9.0	8.7	8.4			7.5	7.7	7.9			20.0	18.6	19.6							
100%	43	5	5	5			9.1	8.7	8.2			7.5	7.7	7.9								560		550		
Initial of Investigator		RBF	SHW	SHW			RBF	SHW	SHW			RBF	SHW				RBF	SHW	SHW			RBF		SHW		

Alkalinity/Hardness (mg/l as $CaCO_3$) A H
Control Water: 125 | 125
100% Sample: 125 | 118
Other:
Measured by: MF

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
U T
Control Water: 0 | 0
100% Sample: | 0.01
Other:

Hour 0
Control Water:
100% Sample:
Other:
Measured by: MF

Signatures:
RBF
Marshall Faircloth
Russell Frydenlund

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:
Reviewed by:
Date: 1/20/89
Data Abstracted:
Abstracted by:

LYNN HAVEN WWTP

5 6 Cl_2

6/28/88 1400
6/29/88 1145
7/1/88 1030
D. pulex
neonate

Control	61	10	1	1			9.2		8.8			8.2		8.2			19.7	-	19.6							
Control	138	10	3	3			9.2		8.8			8.2		8.2												
100%	14	10	10	10			9.2		8.9			7.6		8.1			19.7	-	19.6							
100%	39	10	9	9			9.3		8.5			7.6		8.1												
Initial of Investigator		JHF	JHF	SHW			RBF		SHW			RBF		SHW			RBF		SHW							

	A	I
Control Water:	130	13
100% Sample:	125	11
Other:		
Measured by:	MF	

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

	U	I
Control Water:	0	0
100% Sample:		0.01
Other:		

Hour 0

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

Signatures:
S H Wolfe
Marshall Fairchild
Russell [illegible]

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: [signature]

Date: 1/20/89

Industry/Toxicant Lynn Haven WWTP
Address
Contact/District
Effluent Serial Number
NPDES Permit Number

BIOASSAY DATA FORM

Test Number: 6 of 6
Test Type: Static / Renewal / Screening / Conventional
Other: Cl_2

Sample Collection: Date 6/28/88 Time 1400
Test Beginning: Date 6/29/88 Time 1130
Test Ending: Date 7/4/88 Time 1030
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	0	24	48	72	96
CONTROL	25	5/~~5~~	5	5			8.9	~~8.9~~	8.9			8.2		8.2			19.7		19.9							
CONTROL	26	5/~~5~~	5	5			8.9	~~8.9~~	8.9			8.2		8.2					—							
CONTROL	27	5/~~5~~	5	5			8.9	~~8.9~~	8.9			8.2		8.2												
CONTROL	28	5	5	5			8.9	~~8.9~~	8.9			8.2		8.2					19.9							
100%	29	5	5	5			9.0		9.0			7.7		8.2			19.7		19.9							
100%	30	5	5	5			9.0		9.0			7.7		8.2												
100%	31	5	5	5			9.0		9.0			7.7		8.2												
100%	32	5	5	5			9.0		9.0			7.7		8.2					19.9							
Initial of Investigator		SHW	MF				RBF		SHW			RBF		SHW			RBF		SHW							

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

	A	H
Control Water:	130	133
100% Sample:	125	118
Other:		
Measured by:	MF	

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

	U	T
Control Water:	0	0
100% Sample:		0.011
Other:		
Hour		

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

Signatures:
Marshall Faircloth
Russell Frederking

Instrument Calibration
pH Meter:
D.O. Meter:
Temp. Meter:
0 Hours
24 Hours
48 Hours
72 Hours
96 Hours

Total Residual Chlorine
Probe used: Method:
Lab: Method:
Lab: Method:
Neutralized:
Neutralization by:

Reviewed by:
Date 1/20/89

MICROTOX™ Data Reduction Sheet 104

SOURCE: Lynn Haven STP

GRAPH CALC EC 50 (15 MIN, 15 °C) = 49.5 %SAMPLE mg/L

SAMPLE DATA:

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

DATE ASSAYED: 6-29-88 TIME: 1:45PM

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: -

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

REMARKS:

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	F5	F15	NOTES
A1	0	85	54			
B1		79	50			
C1		78	52			
A2	12.38	73	40 50	(F15)	(F5)	
B2		76	47 51	.040	.010	
C2		71	39 49			
A3	24.75	80	46			
B3		81	46	.130	.008	
C3		79	46			
A4	49.5	64	35			
B4		66	34	.486	.216	
C4		69	36			
A5	99	51	19			
B5		52	19	1.737	.516	
C5		52	19			
EC_{50} (MIN, °C)				49.45	108.2	

REVIEWED BY:

DATE: 1/20/89

MICROTOX™ Data Reduction Sheet 105

SOURCE: Lynn Haven STP

GRAPH CALC EC 50 (15 MIN, 15 °C) = 85.6 (%SAMPLE) mg/L

SAMPLE DATA:

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

DATE ASSAYED: 6-29-88 TIME: 2:30

VISUAL COLOR: none CORRECTION REQUIRED: YES (NO)

VISUAL TURBIDITY: none SEPARATION REQUIRED: YES (NO)

INITIAL pH: pH ADJUSTED TO: WITH:

OSMOTICALLY ADJUSTED WITH: MOAS (DRY NaCl) OTHER

PRIMARY DILUTION OF SAMPLE:

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

REMARKS:

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	Γ5	Γ15	NOTES
A1	0	65	48	X	X	
B1		72	53			
C1		79	57			
A2	12.38	69	45	.038	.168	
B2		69	45			
C2		70	47			
A3	24.75	~~59~~ 63	39	.080	.311	
B3		~~61~~ 69	41			
C3		~~58~~ 69	42			
A4	49.5	~~52~~ 59	32	.213	.616	
B4		~~57~~ 61	35			
C4		~~53~~ 58	32			
A5	99	52	25	.385	1.133	
B5		51	25			
C5		53	25			
EC_{50} (MIN, °C)				211.66	85.63	

REVIEWED BY:

DATE: 1/20/89