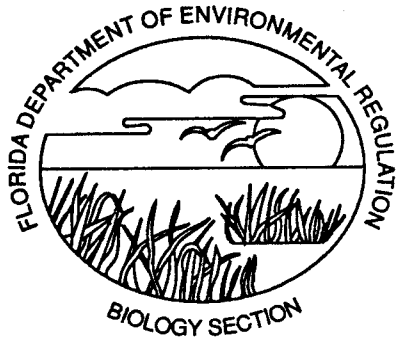


Bioassays of

Avondale Sewage Treatment Plant

Pensacola, Escambia County, Florida

NPDES #FL0031801

Sampled 6/20/88

February 10, 1989

Biology Section
Division of Administrative and Technical Services

Executive Summary

Avondale Sewage Treatment Plant, Pensacola, Escambia County, Florida, NPDES #FL0031801 on 21 to 23 June, 1988.

Six bioassays and two Microtox® assays were performed on a sample of discharge from the Avondale STP outfall #001. The bioassays were performed on chlorinated and dechlorinated portions of the sample. The total residual chlorine concentration was measured at 0.4 mg/l (Hach) and 0.5 mg/l (Iodometric). The bioassay tests indicated that both the chlorinated and dechlorinated samples were toxic to the bioassay test organism, *Ceriodaphnia dubia*, producing LC_{50}'s of 44.4% and 53.1%, respectively. The chlorinated and dechlorinated samples adversely affected the Microtox® test organism, *Photobacterium phosphoreum*, producing EC_{50}'s of 18.5% and 29.3%, respectively.

The sample, both chlorinated and dechlorinated, did not cause deaths in the test organisms, *Daphnia pulex* and *Notropis leedsi*.

Chemical analyses were not performed on the sample. The cause of toxicity was not established, although partial toxicity can be attributed to the total residual chlorine in the sample.

Introduction

The Avondale Sewage Treatment Plant is located west of the intersection of Bellview Road and Muldoon Road in Pensacola, Escambia County, Florida (Figures 1 and 2). The facility is owned and operated by the Escambia County Utilities and treats domestic wastes from the surrounding residential community. The design capacity of the plant is 1.0 MGD and treatment of waste includes screening, grit chamber, contact stabilization, clarification, polishing ponds, and final chlorination. Discharge is to an unnamed tributary, then to Bayou Marcus Creek, which flows a short distance to Perdido Bay. A schematic of the facility is presented in Figure 3 (data from NPDES file). Also noted in the NPDES file of this facility were various orders for the Avondale facility to stop discharging to surface waters.

A bioassay on a sample of discharge from this facility was performed by the Biology Section in June, 1987. The results of these tests indicated that the chlorinated sample was acutely toxic to the test organisms, *Ceriodaphnia dubia* and *Notropis leedsi* (LC_{50}'s < 50%) and the dechlorinated sample was acutely toxic to *C. dubia* (24 hour LC_{50} = 76.3%; 48 hour LC_{50} = 50%). The toxicity was attributed to the total residual chlorine (0.5 mg/l Hach; 0.89 mg/l Iodometric) and to the pesticides, chlorpyrifos (0.38 µg/l), diazinon (0.91 µg/l), and endosulfan sulfate (< .03 µg/l) (FDER 1987).

To again determine the existence and extent of toxicity in the discharge from this facility, the Biology Section, at the request of the FDER Northwest District, performed six acute toxicity bioassays and two Microtox® assays on a sample from the Avondale STP. 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, and L. A. Williams.

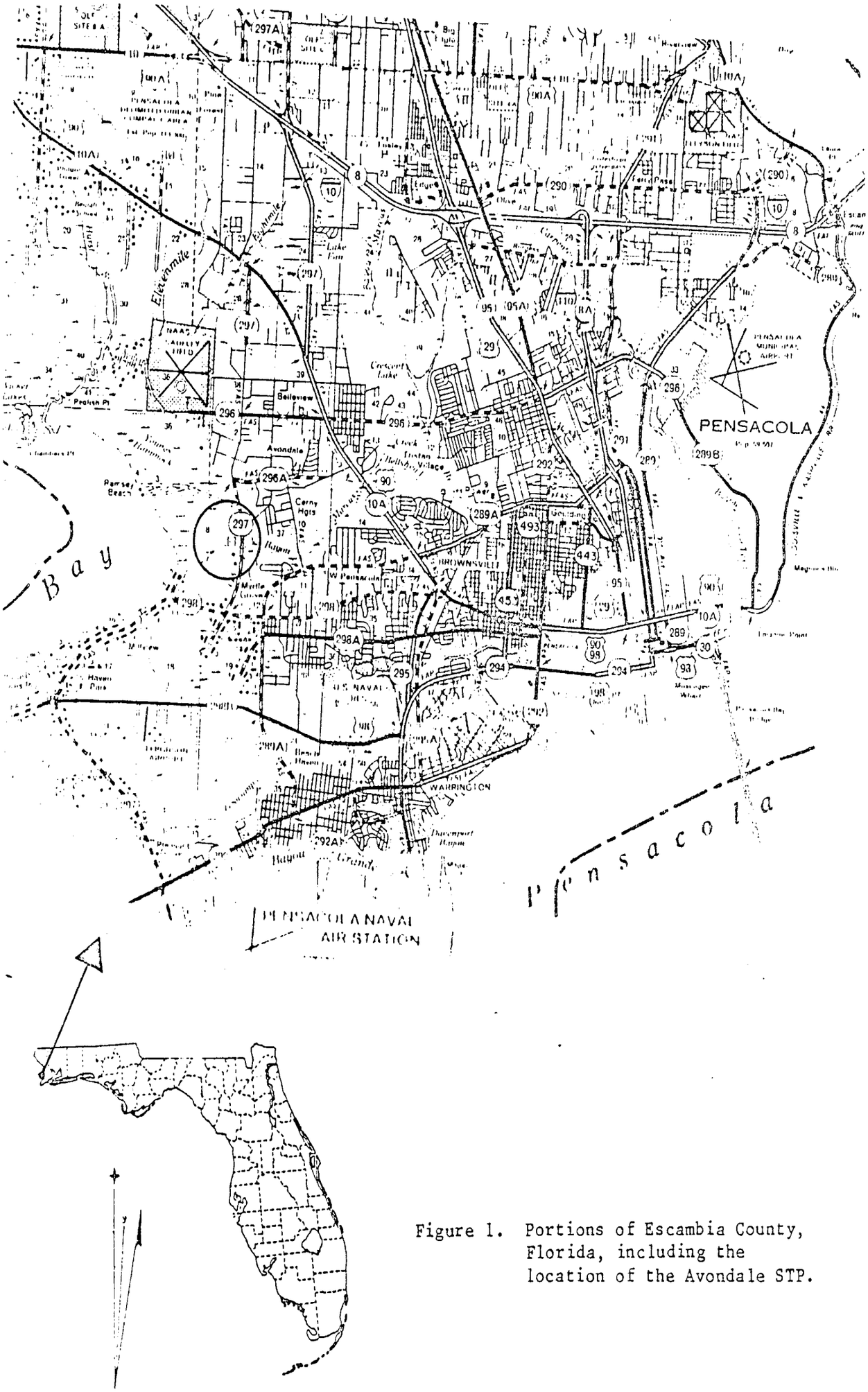


Figure 1. Portions of Escambia County, Florida, including the location of the Avondale STP.

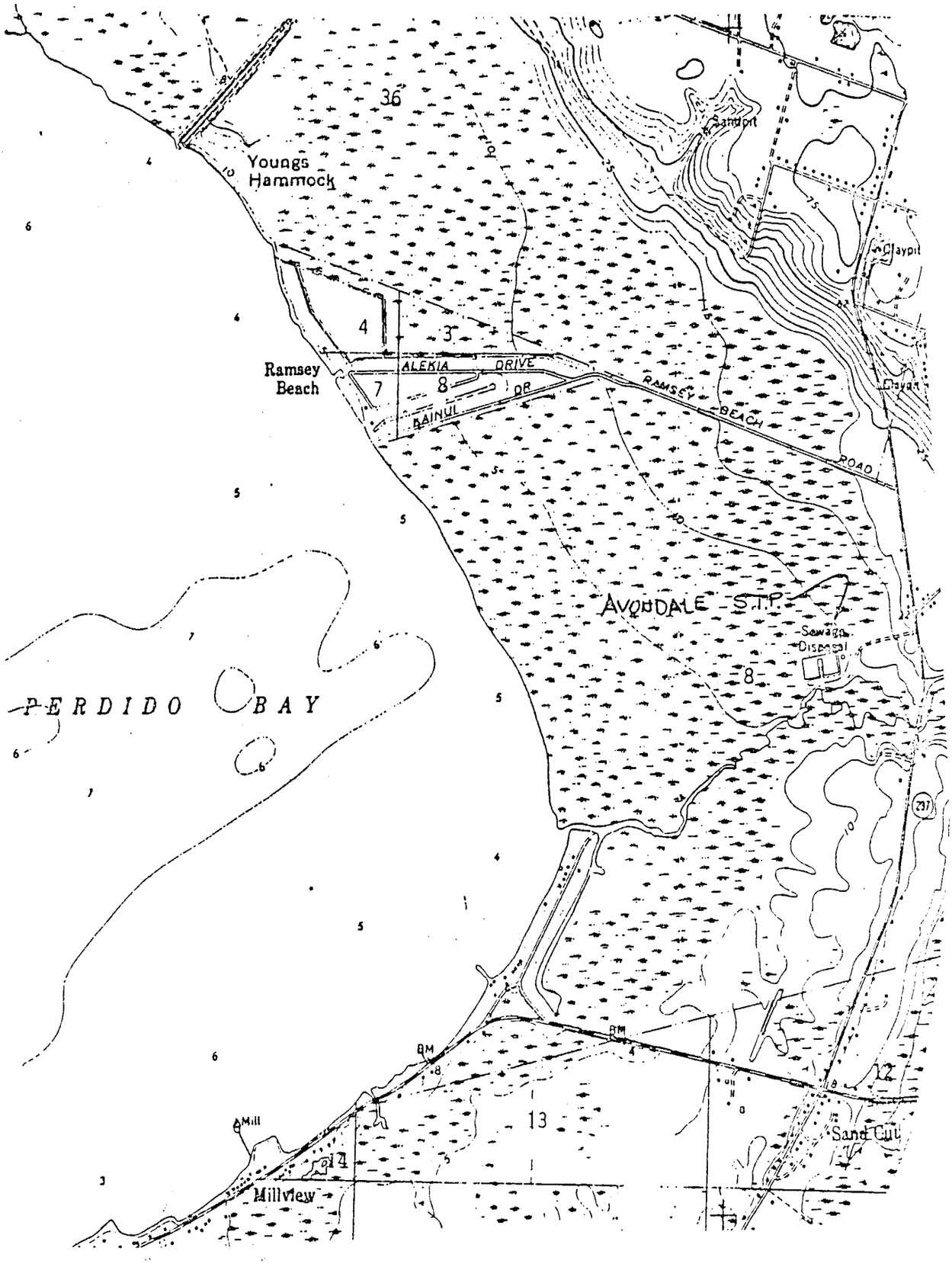


Figure 2. Location of Avondale STP and discharge point to Bayou Marcus Creek and Perdido Bay.

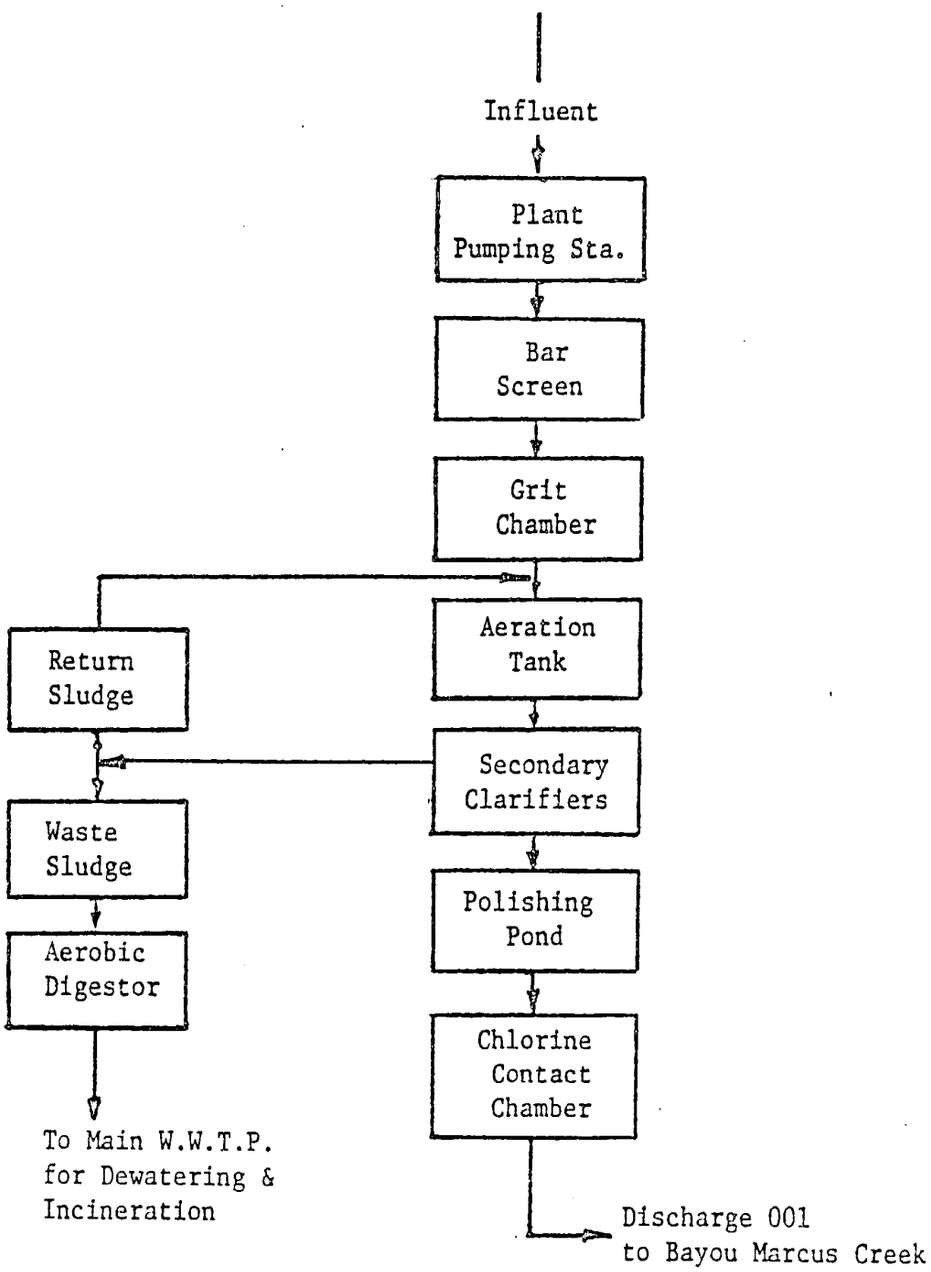


Figure 3. Schematic of Wastewater Flow
Avondale S.T.P.
Pensacola, Florida
Discharge Serial No. 001
1-11-83

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 18 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 theAvondale Sewage Treatment Plant facility outfall #001. The tests were performed from 1130 hrs on 21 June, 1988, to 1130 hrs on 23 June, 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. John Williams of the FDER Northwest district office in Pensacola at 1425 hours on 20 June, 1988, using standard wastewater sampling procedures. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 20 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 of the control and each test concentration were used in bioassays with the water flea *Ceriodaphnia dubia*. Two replicates of the control and each test concentration were used in bioassays with the water fleas, *Daphnia pulex*. Plastic vials of 30 ml capacity (for *C. dubia*) and flint glass jars of 200 ml capacity (for *D. pulex*) were used as test chambers, and either 15 ml or 100 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 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

Six 48 hour acute toxicity screening bioassays and two Microtox® assays were performed on a sample of discharge from outfall #001 of the Avondale Sewage Treatment Plant. Both chlorinated and dechlorinated tests were performed. In both the chlorinated and dechlorinated tests, the sample exhibited toxicity to the bioassay test organism, *Ceriodaphnia dubia*, and to the Microtox® assay test organism, *Photobacterium phosphoreum*. The chlorinated and dechlorinated sample produced LC_{50}'s of 44.4% and 53.1%, respectively, in the *C. dubia* test (Table 4) and produced EC_{50}'s of 18.5% and 29.4%, respectively in the *P. phosphoreum* test (Table 5). No deaths were recorded in the chlorinated and dechlorinated sample tests using the bioassay organisms, *Daphnia pulex* and *Notropis leedsi*.

The total residual chlorine (0.4 mg/l Hach, 0.5 mg/l Iodometric) contributed to the observed toxicity. No chemical analyses were performed and the cause of the remaining toxicity was not determined. A prior bioassay of a sample from this facility (FDER 1987) attributed toxicity to total residual chlorine and the pesticides; chlorpyrifos, diazinon, and endosulfan sulfate.

In conclusion, the sample from this facility was toxic on 20 June, 1988, but the cause of toxicity was not determined.

Biology Section Recommendations

The Biology Section recommends that the facility be required to undertake additional toxicity testing of their discharge as the first step in a Toxicity Reduction Evaluation. Should additional toxicity tests show that the discharge from the facility is again toxic, then the facility should be required to continue the Toxicity Reduction Evaluation with the goal of meeting state water quality standards.

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
30.0%	10.5 ml	4.5 ml	15 ml
60.0%	6.0 ml	9.0 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 ml	0 ml	100 ml
30.0%	70 ml	30 ml	100 ml
60.0%	40 ml	60 ml	100 ml
100.0%	0 ml	100 ml	100 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 acute toxicity bioassays of a sample of chlorinated discharge from the Avondale Sewage Treatment Plant, Pensacola, Escambia County, Florida, NPDES #FL0031801, on 21 to 23 June, 1988.

Facility:	**Avondale WWTP**		**NPDES #**	**FI0031801**	Facility Type: **Municipal WWTP**
Location:	Bellview / Mulldoon Rds. Pensacola		Contact/District:	J. Williams	
County:	Escambia		Test type:	Static Screening	
Sample Collection Date:	**6/20/88**	Time: 1425	# tests:	6	Receiving Water: Bayou Marcus Creek
Test Beginning Date:	6/21/88	Time: 1130	Chlorination Type:	**chlorinated**	to Perdido Bay
Test Ending Date:	6/23/88	Time: 1130			**Page 1 of 2**

Total Residual Cl2	mg/l	Method
Field:	0.5	-
Lab:	0.4	Hach
Lab:	0.5	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:	0.00	0.00

Alk & Hardness	Alkalinity (mg/l)	Hardness (mg/l)
Control Water (fish):	120	125
Cond. Control Water (daphnids):	130	135
100% Sample:	108	160

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

Organism: ***Ceriodaphnia dubia*** Life stage: neonate
LC50: - Binomial: 44.4% 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	8.5	-	8.5	8.0	-	7.9	-	-	-	-	-
Control	5	5	5	8.5	-	8.7	8.0	-	7.9	-	-	-	-	-
Control	5	5	5	8.5	-	8.7	8.0	-	7.9	-	-	-	-	-
Control	5	5	5	8.5	-	8.8	8.0	-	7.9	-	-	-	-	-
30%	5	5	5	8.8	-	8.7	7.6	-	7.9	-	-	-	-	-
30%	5	5	5	8.8	-	8.7	7.6	-	7.9	-	-	-	-	-
30%	5	5	5	8.8	-	8.7	7.6	-	7.9	-	-	-	-	-
30%	5	5	5	8.9	-	8.7	7.6	-	7.9	-	-	-	-	-
60%	5	5	0	8.9	-	8.8	7.3	-	7.9	-	-	-	-	-
60%	5	5	0	8.9	-	8.8	7.3	-	7.9	-	-	-	-	-
60%	5	3	0	8.9	-	8.8	7.3	-	7.9	-	-	-	-	-
60%	5	5	1	8.9	-	8.8	7.3	-	7.9	-	-	-	-	-
100%	5	2	0	9.0	-	8.9	7.0	-	7.8	-	-	-	-	-
100%	5	3	0	9.0	-	8.9	7.0	-	7.8	-	-	-	-	-
100%	5	5	0	9.1	-	8.9	7.0	-	7.8	-	-	-	-	-
100%	5	3	0	9.0	-	8.9	7.0	-	7.8	-	-	-	-	-

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	10	10	8.9	-	9.0	8.1	-	7.9	20.2	-	19.5	-	-
Control	10	2*	1*	8.9	-	8.9	8.1	-	7.9	-	-	-	-	-
30%	10	10	10	8.9	-	8.9	7.6	-	7.9	20.2	-	19.5	-	-
30%	10	10	10	8.9	-	8.9	7.6	-	7.9	-	-	-	-	-
60%	10	10	10	9.0	-	9.0	7.2	-	7.9	20.2	-	19.4	-	-
60%	10	10	10	9.0	-	9.0	7.2	-	7.9	-	-	-	-	-
100%	10	10	10	9.1	-	9.0	6.9	-	7.8	20.4	-	19.4	-	-
100%	10	10	10	9.0	-	9.0	6.9	-	7.8	-	-	-	-	-

* lab accident

Table 2. Continued.

Facility:	**Avondale WWTP**		**NPDES #**	**FI0031801**	Facility Type: **Municipal WWTP**
Location:	Bellview / Muldoon Rds. Pensacola		Contact/District:	J. Williams	
County:	Escambia		Test type:	Static Screening	
Sample Collection Date:	**6/20/88**	Time: 1425	# tests:	6	Receiving Water: Bayou Marcus Creek
Test Beginning Date:	6/21/88	Time: 1130	Chlorination Type:	**chlorinated**	to Perdido Bay
Test Ending Date:	6/23/88	Time: 1130			**Page 2 of 2**

Total Residual Cl2	mg/l	Method
Field:	0.5	-
Lab:	0.4	Hach
Lab:	0.5	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:	0.00	0.00

Alk & Hardness	Alkalinity (mg/l)	Hardness (mg/l)
Control Water (fish):	120	125
Cond. Control Water (daphnids):	130	135
100% Sample:	108	160

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	8.7	8.3	8.3	7.5	7.8	7.8	21.0	19.4	19.5	-	-
Control	5	5	5	8.7	8.6	8.3	7.5	7.9	7.8	-	-	-	220	220
30%	5	5	5	8.6	8.4	8.3	7.2	7.7	7.7	21.1	19.3	19.4	-	-
30%	5	5	5	8.6	8.5	8.3	7.2	7.7	7.7	-	-	-	322	320
60%	5	5	5	8.6	8.5	8.5	7.0	7.6	7.6	21.1	19.3	19.3	-	-
60%	5	5	5	8.7	8.5	8.5	7.0	7.6	7.6	-	-	-	420	415
100%	5	5	5	8.7	8.5	8.4	6.9	7.6	7.6	21.0	19.3	19.4	-	-
100%	5	5	5	8.6	8.5	8.5	6.9	7.5	7.6	-	-	-	600	580

Table 3. Data recorded during 48 hour acute toxicity bioassays of the dechlorinated sample of discharge from the Avondale Sewage Treatment Plant, Pensacola, Escambia County, Florida, NPDES #FL0031801, on 21 to 23 June, 1988.

Facility: **Avondale WWTP** NPDES # **Fl0031801** Facility Type: **Municipal WWTP**
Location: Bellview / Mulldoon Rds. Pensacola Contact/District: J. Williams
County: Escambia Test type: Static Screening
Sample Collection Date: **6/20/88** Time: 1425 # tests: 6 Receiving Water: Bayou Marcus Creek
Test Beginning Date: 6/21/88 Time: 1130 Chlorination Type: **dechlorinated** to Perdido Bay
Test Ending Date: 6/23/88 Time: 1130 **Page 1 of 2**

Total Residual Cl2	mg/l	Method
Field:	0.5	-
Lab:	0.4	Hach
Lab:	0.5	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:	0.00	0.00

Alk & Hardness	Alkalinity (mg/l)	Hardness (mg/l)
Control Water (fish):	120	125
Cond. Control Water (daphnids):	130	135
100% Sample:	108	160

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

Organism: ***Ceriodaphnia dubia*** Life stage: neonate
LC50: - Binomial: 53.8% 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	8.1	-	8.4	7.9	-	8.0	-	-	-	-	-
Control	5	5	5	8.2	-	8.5	7.9	-	8.0	-	-	-	-	-
Control	5	5	5	8.3	-	8.6	8.0	-	8.0	-	-	-	-	-
Control	5	5	5	8.3	-	8.6	8.0	-	8.0	-	-	-	-	-
30%	5	5	5	8.9	-	8.7	7.6	-	8.0	-	-	-	-	-
30%	5	5	5	8.9	-	8.7	7.6	-	8.0	-	-	-	-	-
30%	5	5	5	8.9	-	8.7	7.6	-	8.0	-	-	-	-	-
30%	5	5	5	8.9	-	8.7	7.6	-	8.0	-	-	-	-	-
60%	5	5	2	9.0	-	8.7	7.3	-	8.0	-	-	-	-	-
60%	5	5	2	9.0	-	8.7	7.3	-	8.0	-	-	-	-	-
60%	5	5	1	9.0	-	8.7	7.3	-	8.0	-	-	-	-	-
60%	5	5	2	9.0	-	8.8	7.3	-	8.0	-	-	-	-	-
100%	5	2	0	9.0	-	8.8	7.0	-	7.8	-	-	-	-	-
100%	5	1	0	9.0	-	8.8	7.0	-	7.8	-	-	-	-	-
100%	5	3	0	9.0	-	8.8	7.0	-	7.8	-	-	-	-	-
100%	5	5	0	9.0	-	8.8	7.0	-	7.8	-	-	-	-	-

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	10	10	8.8	-	8.7	8.0	-	7.9	19.8	-	19.5	-	-
Control	10	10	10	8.9	-	8.7	8.0	-	7.9	-	-	-	-	-
30%	10	10	10	9.0	-	8.8	7.6	-	7.9	19.9	-	19.5	-	-
30%	10	10	10	9.0	-	8.8	7.6	-	7.9	-	-	-	-	-
60%	10	10	10	9.0	-	8.8	7.2	-	7.8	20.1	-	19.4	-	-
60%	10	10	10	9.0	-	8.8	7.2	-	7.8	-	-	-	-	-
100%	10	10	10	9.1	-	8.9	6.9	-	7.8	20.5	-	19.5	-	-
100%	10	10	10	9.1	-	8.8	6.9	-	7.8	-	-	-	-	-

Table 3. Continued.

Facility:	**Avondale WWTP**		**NPDES #**	**FI0031801**	Facility Type: **Municipal WWTP**
Location:	Bellview / Muldoon Rds. Pensacola		Contact/District:	J. Williams	
County:	Escambia		Test type:	Static Screening	
Sample Collection Date:	**6/20/88**	Time: 1425	# tests:	6	Receiving Water: Bayou Marcus Creek to Perdido Bay
Test Beginning Date:	6/21/88	Time: 1130	Chlorination Type:	**dechlorinated**	
Test Ending Date:	6/23/88	Time: 1130			**Page 2 of 2**

Total Residual Cl2	mg/l	Method
Field:	0.5	-
Lab:	0.4	Hach
Lab:	0.5	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:	0.00	0.00

Alk & Hardness	Alkalinity (mg/l)	Hardness (mg/l)
Control Water (fish):	120	125
Cond. Control Water (daphnids):	130	135
100% Sample:	108	160

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	8.7	8.2	8.0	7.5	7.7	7.8	20.9	19.9	19.8	-	-
Control	5	5	5	8.7	8.3	8.0	7.5	7.8	7.8	-	-	-	220	225
30%	5	5	5	8.8	8.2	8.1	7.2	7.7	7.7	21.1	19.6	19.8	-	-
30%	5	5	5	8.8	8.2	8.1	7.2	7.7	7.7	-	-	-	321	320
60%	5	5	5	8.8	8.2	8.1	7.0	7.6	7.6	21.2	19.5	19.6	-	-
60%	5	5	5	8.8	8.2	8.0	7.0	7.6	7.6	-	-	-	425	420
100%	5	5	5	8.8	8.4	8.1	6.9	7.5	7.6	21.2	19.4	19.6	-	-
100%	5	5	5	8.8	8.4	8.0	6.9	7.5	7.6	-	-	-	600	600

Table 4. LC_{50} Data for Bioassay

Organism	Method of Data Analysis	48 Hour LC_{50} (95% Confidence Intervals)
Chlorinated discharge		
Ceriodaphnia dubia	Binomial	44.40% (30.0%-60.0%)
	Moving Average	a
	Probit	a
Daphnia pulex		no toxicity
Notropis leedsi		no toxicity
Dechlorinated discharge		
Ceriodaphnia dubia	Binomial	53.08% (30.0%-100%)
	Moving Average	a
	Probit	a
Daphnia pulex		no toxicity
Notropis leedsi		no toxicity

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.

Table 5. Microtox test results, Avondale WWTP discharge, Escambia County, NPDES #FL0031801, on 21 June, 1988.

Microtox Test

Culture Lot # M-603

% Sample	Final Reclaimed Water			
	Chlorinated		Dechlorinated	
	5-min	15-min	5-min	15-min
0	95	59	102	70
0	78	59	100	67
0	80	51	98	65
12.38	63	32	90	51
12.38	69	35	86	49
12.38	65	33	98	54
24.75	51	22	74	37
24.75	49	21	74	36
24.75	48	21	78	38
49.5	34	12	57	23
49.5	32	11	52	21
49.5	33	12	57	22
99	17	6	36	12
99	17	6	28	10
99	17	6	35	13

EC_{50} Chlorinated (15 minute, 15 °C) = 18.42% sample
EC_{50} Dechlorinated (15 minute, 15 °C) =29.32% 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.

_____. 1987. Bioassays of the Avondale Sewage Treatment Plant, Pensacola, Escambia County, Florida. NPDES #FL0031801. Biology Section. Tallahassee. 17 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.

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.

Arrandale WWTP

Pensacola

001

FL0031801

1 6

de Cl2

6-20-88 1425
6-21-88 1130
6-23-88 1130

C. dubia - neonate

	Test Concentration (%)	Number Dead or Abnormal					Dissolved Oxygen (mg/l)					pH					Conductivity				
		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	5/5	5/5			8.1/8.2	8.8	8.4/8.5			7.9/7.9		8.0/8.0							
Control	3/4	5/5	5/5	5/5			8.3/8.3		8.2/8.6			8.0/9.0		9.0/8.0							
30%	5	5	5	5			8.9		8.7			7.6		8.0							
30%	6	5	5	5			8.9		8.7			7.6		8.0							
30%	7	5	5	5			8.9		8.7			7.6		8.0							
30%	8	5	5	5			8.9		8.7			7.6		8.0							
60%	9	5	5	2			9.0		8.7			7.3		8.0							
60%	10	5	5	2			9.0		8.7			7.3		8.0							
60%	11	5	5	1			9.0		8.7			7.3		8.0							
60%	12	5	5	2			9.0		8.8			7.3		8.0							
100%	13	5	2	0			9.0		8.8			7.0		7.8							
100%	14	5	1	0			9.0		8.8			7.0		7.8							
100%	15	5	3	0			9.0		8.8			7.0		7.8							
100%	16	5	5	0			9.0		8.8			7.0		7.8							
Initial of Investigator		KML	KML	KML			RBF		RBF			RBF		RBF							

Alkalinity/Hardness (mg/l as CaCO3)
Cond. with 130/13
Control Water: 130/12
100% Sample: 103/16
Other:
Measured by: JALF

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

Control Water: 0
100% Sample: 0
Other:

Hour 0

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

Signatures:
Russell Frydenborg
KM Lurding
Marshall Faircloth

Instrument Calibrations:

	pH Meter:	DO Meter:	S-T Meter:
0 Hours	7at7; 9at9	9.0at21°C	1300at1300
24 Hours	7at7; 9at9	9.0@21°C	
48 Hours	7at7; 9at9	9.0@21°C	1300at1299.7
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: 0.5 Method:
Lab: .4 Method: HACH
Lab: .53 Method: ICDO
Reviewed by:

De-chlorinated:
Measurements by: RBF

Date: 1/20/89

Remarks:

Industry/Toxicant Avondale 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: dechl2

Sample Collection: Date 6-20-88 Time 1425
Test Beginning: Date 6-21-88 Time 1130
Test Ending: Date 6-23-88 Time 1130
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	0	24	48	72	96
Control	67	10	10	10			8.8		8.7			8.0		7.9			19.8		19.5							
Control	2	10	10	10			8.9		8.7			8.0		7.9												
30%	70	10	10	10			9.0		8.8			7.6		7.9			19.9		19.5							
30%	25	10	10	10			9.0		8.8			7.6		7.9												
60%	60	10	10	10			9.0		8.8			7.2		7.8			20.1		19.4							
60%	119	10	10	10			9.0		8.8			7.2		7.8												
100%	38	10	10	10			9.1		8.9			6.9		7.8			20.5		19.5							
100%	17	10	10	10			9.1		8.8			6.9		7.8												
Initial of Investigator		RBF		RBF			RBF		RBF			RBF JWF		RBF			RBF		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:
100% Sample:
Other:

Hour

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

Signatures: Ronald Applegate
R M Lurding
Monica A Tumlin

Instrument Calibrations:

	pH Meter:	D.O. 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

Remarks:

Arrondale WWTP

Test Number: 36
Test Type: [illegible] Conc. of, Concentration Experiments
Others: de Cl2

6-20-88 1425
6-21-88 1130
6-23-88 1130

Test Organism: N. Seticulis
Age/Life Stage: 18 days

Control	Rep	Number Exposed	Survival					Dissolved Oxygen (mg/l)					Temperature °C					Conductivity				
			0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control	25	5	5	5				8.7	8.2	8.0			7.5	7.7	7.8			20.9	19.9	19.8			
Control	13	5	5	5				8.7	8.3	8.0			7.5	7.8	7.8								220		225		
30%	33	5	5	5				8.8	8.2	8.1			7.2	7.7	7.7			21.1	19.6	19.8			
30%	4	5	5	5				8.8	8.2	8.1			7.2	7.7	7.7								320		320		
60%	120	5	5	5				8.8	8.2	8.1			7.0	7.6	7.6			21.2	19.5	19.6			
60%	44	5	5	5				8.8	8.2	8.0			7.0	7.6	7.6								425		420		
100%	47	5	5	5				8.8	8.4	8.1			6.9	7.5	7.6			21.2	19.4	19.6			
100%	7	5	5	5				8.8	8.4	8.0			6.9	7.5	7.6								600		600		
Initial of Investigator			KMF	MF	RBF			RBF	JMF	RBF			RBF	JMF	RBF			RBF	JMF	RBF			RBF		RBF		

Identify Hardness (mg/l as CaCO3)

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

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

Control Water:
100% Sample:
Other:

Hour

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

Signatures
Richard Frydenborg
KM Lurding
Marshall Fairbell

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours			
24 Hours	7.07, 9.09	8.2 @ 20°C	
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 Avondale 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: Cl2

Sample Collection: Date 6-20-88 Time 1425
Test Beginning: Date 6-21-88 Time 1430
Test Ending: Date 6-23-88 Time 1130
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	17/18	5/5	5/5	5/5			8.5/8.5	/	8.5/8.1			8.0/8.0	/	7.9/7.9			/	/								
Control	19/20	5/5	5/5	5/5			8.5/8.5	/	8.7/8.8			8.0/8.0	/	7.9/7.9			/	/								
30%	21	5	5	5			8.8		8.7			7.6		7.9												
30%	22	5	5	5			8.8		8.7			7.6		7.9												
30%	23	5	5	5			8.8		8.7			7.6		7.9												
30%	24	5	5	5			8.9		8.7			7.6		7.9												
60%	25	5	5	0			8.9		8.8			7.3		7.9												
60%	26	5	5	0			8.9		8.8			7.3		7.9												
60%	27	5	3	0			8.9		8.8			7.3		7.9												
60%	28	5	5	1			8.9		8.8			7.3		7.9												
100%	29	5	2	0			9.0		8.9			7.0		7.8												
100%	30	5	3	0			9.0		8.9			7.0		7.8												
100%	31	5	5	0			9.1		8.9			7.0		7.8												
100%	32	5	3	0			9.0		8.9			7.0		7.8												
Initial of Investigator		KML	MML	KML			RBF		RBF			RBF		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:
100% Sample:
Other:

Hour

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

Signatures: Rachel Shackelford
Mark Lindberg
Marshall Fridden

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:

Personal notified:
Notification made by:

Reviewed by:
Date 1/20/89

Triplicate with

5-6

CO_2

6-20-88 1425
6-21-88 1130
6-23-88 1130

D. pulex neonate

		Initial Conductivity (µmhos/cm)				Dissolved Oxygen (mg/l)				pH				Temperature °C			
		0 hour	24	48	72	0 hour	24	48	72	0 hour	24	48	72	0 hour	24	48	72
Control	42	10	10	10		8.9		9.0		8.1		7.9		20.2		19.5	
Control	11A	10	2	1		8.9		8.9		8.1		7.9					
30%	1	10	10	10		8.9		8.9		7.6		7.9		20.2		19.5	
30%	27	10	10	10		8.9		8.9		7.6		7.9					
60%	32	10	10	10		9.0		9.0		7.2		7.9		20.2		19.4	
60%	—	10	10	10		9.0		9.0		7.2		7.9					
100%	27	10	10	10		9.1		9.0		6.9		7.8		20.4		19.4	
100%	7	10	10	10		9.0		9.0		6.9		7.8					
Initial of Investigator		RBF	MT	RBF		RBF				RBF		RBF		RBF		RBF	

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

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

Control Water:
100% Sample:
Other:

Hour

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

Signatures:
Ronald Frederberg
Kathleen Ding
Marshall Fairchild

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: 1/20/87

Industry/Toxicant Avondale 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: CO2

Sample Collection: Date 6-20-88 Time 1425
Test Beginning: Date 6-21-88 Time 1120
Test Ending: Date 6 23 88 Time 1130
Test Organism N. seudsi
Age/Life Stage 8 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	11	5	5	5			8.7	8.3	8.3			7.5	7.8	7.8			21.0	19.4	19.5							
Control	6	5	5	5			8.7	8.3	8.3			7.5	7.9	7.8								220		220		
30%	54	5	5	5			8.6	8.4	8.3			7.2	7.7	7.7			21.1	19.3	19.4							
30%	46	5	5	5			8.6	8.5	8.3			7.2	7.7	7.7								322		320		
60%	16	5	5	5			8.6	8.5	8.5			7.0	7.6	7.6			21.1	19.3	19.3							
60%	99	5	5	5			8.7	8.5	8.5			7.0	7.6	7.6								420		415		
100%	76	5	5	5			8.7	8.5	8.4			6.9	7.6	7.6			21.0	19.3	19.4							
100%	43	5	5	5			8.6	8.5	8.5			6.9	7.5	7.6								600		580		
Initial of Investigator		KML	MT	RBF			RBF	JMF	RBF			RBF	JMF	RBF			RBF	JMF	RBF			RBF		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:
100% Sample:
Other:

Hour

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

Signatures:
Randall Fagenbaugh
Kathy Harding
Marshall Fairbrother

Instrument Calibrations:

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

Total Residual Chlorine:

Test Data: Method:
Lab: Method:
Lab: Method:

Reviewed by:
Date: 11/20/89

MICROTOX™ Data Reduction Sheet 102

SOURCE: Avondale STP

GRAPH CALC EC 50 (15 MIN, 15 °C) = 18.5 (%SAMPLE) mg/L

SAMPLE DATA:

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

DATE ASSAYED: 9:15 AM 6-21-88 TIME:

VISUAL COLOR: none CORRECTION REQUIRED: YES (NO)

VISUAL TURBIDITY: none SEPARATION REQUIRED: YES (NO)

INITIAL pH: pH ADJUSTED TO: WITH:

OSMOTICALLY ADJUSTED WITH: MOAS (DRY NaCl) OTHER

PRIMARY DILUTION OF SAMPLE:

OPERATOR: Lesie 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	95	59	X	X	
B1		78	49			
C1		80	51			
A2	12.38	63	32	.284	.590	
B2		69	35			
C2		65	33			
A3	24.75	51	22	.709	1.484	
B3		49	21			
C3		48	21			
A4	49.5	34	12	1.556	3.543	
B4		32	11			
C4		33	12			
A5	99	17	6	3.961	7.833	
B5		17	6			
C5		17	6			
EC_{50} (MIN, °C)				33.55	18.47	

REVIEWED BY:

DATE: 1/20/89

MICROTOX™ Data Reduction Sheet 103

SOURCE: Avondale STP

GRAPH CALC EC 50 (15 MIN, 15 °C) = 29.4 (%SAMPLE) mg/L

SAMPLE DATA:

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

DATE ASSAYED: 6-21-88 TIME: 10:00 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: 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	102	70	X	X	
B1		100	67			
C1		98	65			
A2	12.38	90	51	.095	.312	
B2		86	49			
C2		98	54			
A3	24.75	74	37	.327	.820	
B3		74	36			
C3		78	38			
A4	49.5	57	23	.807	2.061	
B4		52	21			
C4		57	22			
A5	99	36	12	2.03	4.771	
B5		28	10			
C5		35	13			
EC_{50} (MIN, ° C)				58.21	29.37	

REVIEWED BY:

DATE: 1/20/89