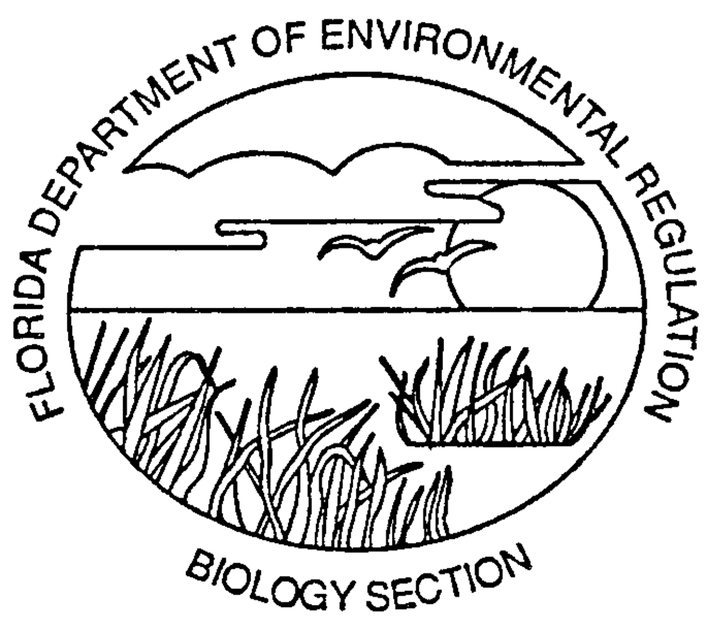


Bioassays of the Dunedin

Sewage Treatment Plant

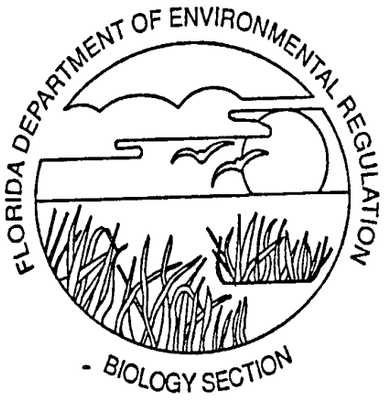
Dunedin, Pinellas County, Florida

NPDES #FL0021326

SAMPLED 3/21/88

August 5, 1988

Biology Section
Division of Administrative and Technical Services



Bioassays of the Dunedin Sewage Treatment Plant

Dunedin, Pinellas County, Florida

NPDES #FL0021326

Biology Section
Bureau of Laboratories
August 5, 1988

Executive Summary

Dunedin Sewage Treatment Plant, Dunedin, Pinellas County, Florida, NPDES #FL0021326, 22 to 24 March, 1988.

The sample of chlorinated discharge from the Dunedin STP was acutely toxic to the water fleas, *Ceriodaphnia dubia* and *Daphnia pulex*, and to the fish *Notropis leedsi*; producing respectively, LC_{50}'s of 42.43%, 39.36%, and 85.15 (Table 6). The Microtox® assay showed that the chlorinated sample was also toxic to the test organism, *Photobacterium phosphoreum*, producing and EC_{50} of 39.9%. Mortality in the chlorinated sample was partly attributable to the total residual chlorine concentration of 0.3 mg/l (Hach) and 0.44 mg/l (Iodometric).

The dechlorinated sample of discharge was non toxic to the water flea, *Daphnia pulex*, the fish, *Notropis leedsi*, and the Microtox® organism, *Photobacterium phosphoreum*. The dechlorinated sample was acutely toxic to the water flea, *Ceriodaphnia dubia*, producing a LC_{50} of ~~42.43~~%. The sample was analyzed for chemical constituents (Table 5). The diazinon fraction (0.3µg/l) of the pesticide extractables most probably contributed to the mortality as concentrations as low as 0.2 µg/l have resulted in mortality.

The Biology Section recommends continued monitoring of the discharge from this facility and, if toxicity continues, the implementation of a Toxicity Reduction Evaluation plan.

Introduction

The Dunedin Sewage Treatment Plant is located at 1140 Highland Avenue, Dunedin, Pinellas County, Florida (Figure 1). The facility has a design capacity of 5.0 MGD and treats an average of 3.78 MGD of waste. Treatment consists of activated sludge and chlorination (Figure 2). The treated water discharges through Outfall #001 to St. Joseph Sound.

To evaluate the existence and extent of the toxicity of the discharge from this facility and possible effects on receiving waters, the Biology Section performed six acute toxicity bioassays and two Microtox® assays on chlorinated and dechlorinated samples. The results of these tests are presented in this report. These involved in performing the tests and in preparation of this report were: J. M. Faircloth and L. A. Williams.

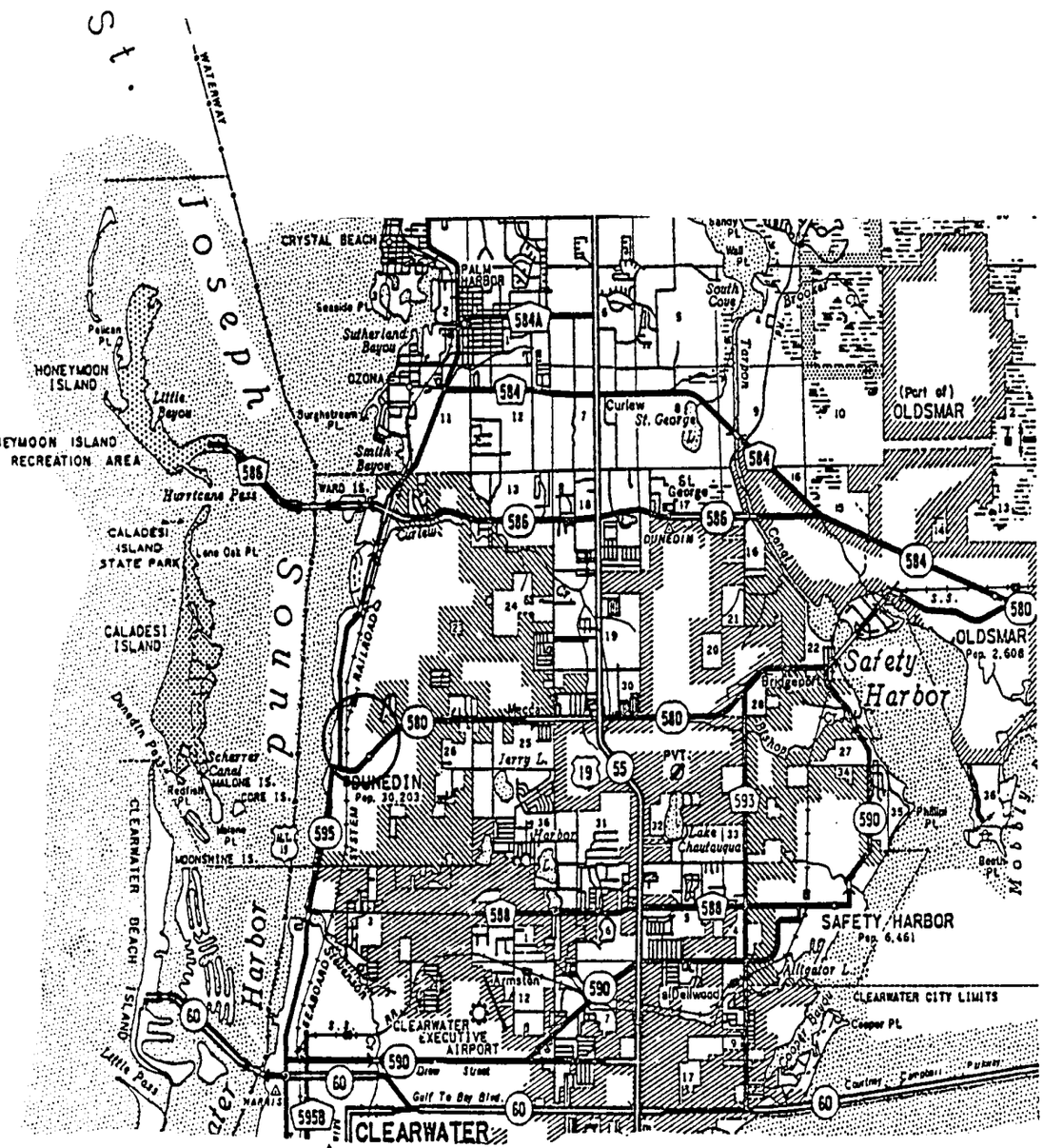


Figure 1. Portion of Pinellas County, Florida, including location of Dunedin Sewage Treatment Plant.

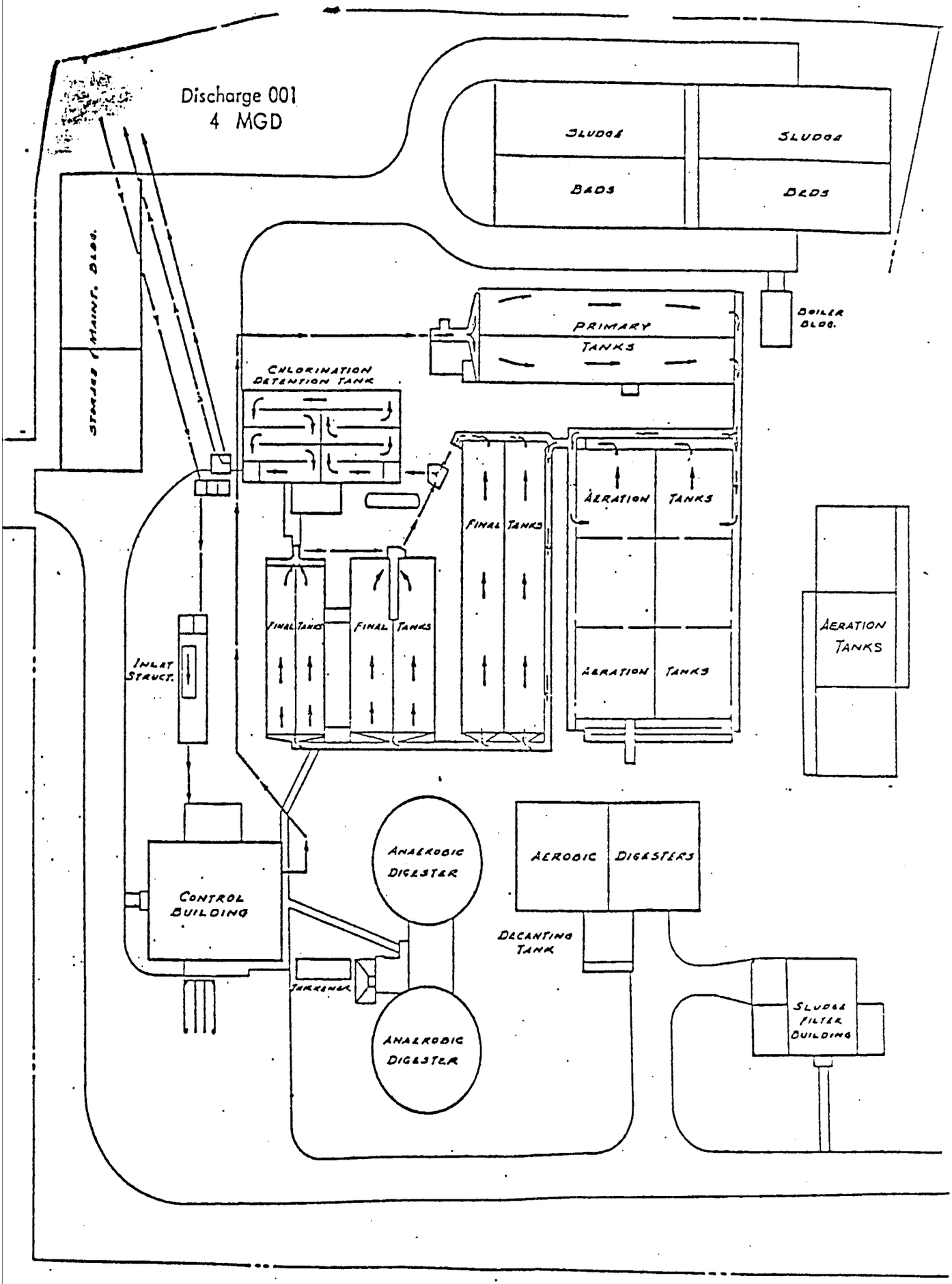


Figure 2

Schematic of Wastewater Flow
Mainland Pollution Control Facility
Dunedin, Florida
Discharge Serial No. 001
May 1976

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), the U.S. EPA (1978), 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 static acute toxicity bioassays and two Microtox® assays were performed on the chlorinated and dechlorinated sample of discharge from 1150 hours on 22 March to 1200 hours on 24 March, 1988.

Separate samples were obtained for chemical and biological testing. Samples for metal analyses were preserved with HNO_3. Samples for organic chemical analyses and for the bioassays were unpreserved. The samples were collected by Mr. David Demmi of the FDER's Southwest District at 1020 hours on 21 March, 1988, using standard wastewater sampling procedures. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 26 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) bioassays. 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 these tests.

Test Parameters

Dissolved oxygen (YSI Model 57 oxygen meter), pH (Corning Model 7 pH meter with Fisher Accuphast® 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. The salinity of the sample was recorded at the beginning 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 chlorinated water sample taken from Outfall #001 of the Dunedin Sewage Treatment Plant was acutely toxic to the test organisms, *Ceriodaphnia dubia*, *Daphnia pulex*, and *Notropis leedsi*. The chlorinated sample was also toxic to the Microtox® organism, *Photobacterium phosphoreum*. The acute toxicity in the chlorinated sample was mainly attributable to the total residual chlorine concentration which was measured 0.3 mg/l (Hach) and 0.44 mg/l (Iodometric).

The dechlorinated sample was non toxic to the bioassay test organisms *D. pulex* and *N. leedsi*. The dechlorinated sample was also non toxic to the Microtox® assay organism, *P. phosphoreum*. The dechlorinated sample was toxic to the bioassay organism, *C. dubia*, producing a LC_{50} of between 50 to 75%. Chemical constituents (Table 5) found in the sample included: total ammonia (7.5 mg/l) and unionized ammonia (0.15 mg/l); the metals zinc (29 µg/l), arsenic (6.0 µg/l), and silver (0.6 µg/l); and the pesticide extractables Diazinon (0.3 µg/l) and chlorpyrifos (0.3 µg/l).

The toxicity to the test organisms of the sample from the Dunedin STP can be only partially attributed to the total residual chlorine in the sample because of the mortality of the *C. dubia* in the dechlorinated sample. Part of the toxicity in the chlorinated sample and the toxicity in the dechlorinated sample can probably be attributed to the pesticide Diazinon which has been shown to be toxic to a species of cladoceran at a concentration of 0.2 µg/l, less than that found in the sample from this facility (Cope 1966).

Biology Section Recommendations

The Biology Section recommends that this facility be required to meet state toxicity standards or undertake a Toxicity Reduction Evaluation with the goal of meeting those standards.

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 48 hour, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi, static acute toxicity bioassay of the Dunedin Sewage Treatment Plant, Chlorinated Discharge, Pinellas County, NPDES #FL0021326, on 22 to 24 March, 1988.

Facility:	Dunedin STP			NPDES # FL0021326		Facility Type:	Municipal
Location:	Pinellas			Contact/District:	D. Demmi		
Sample Collection Date:	3/21/88	Time:	1020	Test type:	Static Screening		
Test Beginning Date:	3/22/88	Time:	1150	# tests:	3	Receiving Water:	St. Joseph Sound
Test Ending Date:	3/24/88	Time:	1130	Chlorination Type:	chlorinated		

Parameter	
Total Residual Cl2:	0.3
Method:	Hach
By:	JMF
Salinity:	1

Ammonia:	Control Water	100% Sample
Unionized NH3:	0.00	0.12
Total NH3:	0.034	7.56

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

LC50: Binomial: 42.43% 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	10	8.8	8.7	8.8	8	7.9	8.3	19.7	19.2	19.3		
Control	10	10	10	8.8	8.7	8.8	8	7.9	8.3					
30%	10	10	10	8.5	8.8	8.8	7.7	8.1	8.3	20.2	19	19.2		
30%	10	10	10	8.5	8.8	8.8	7.7	8.1	8.3					
60%	10	0	0	8.3	9	8.9	7.6	8.2	8.4	20.6	19.2	19.1		
60%	10	0	0	8.3	9	8.9	7.6	8.2	8.4					
100%	10	0	0	7.8	9	8.6	7.5	8.2	8.4	20.8	19.8	19.2		
100%	10	0	0	7.8	9	8.6	7.5	8.2	8.4					

	Control water	100% sample
Alkalinity:	133	225
Hardness:	125	220

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

LC50: Binomial: 39.36% 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	10	8.7	8.7	8.8	8	8	8.3	20.3	19.2	19.1		
Control	10	10	10	8.7	8.7	8.8	8	8	8.3				215	210
30%	10	10	9	8.6	8.9	8.8	7.7	8	8.2	21	18.8	18.9		
30%	10	10	9	8.6	8.9	8.8	7.7	8	8.2				445	420
60%	10	1	0	8.2	9	8.8	7.5	8	8.3	21.8	18.7	18.9		
60%	10	3	0	8.1	9	8.8	7.5	8	8.3				700	680
100%	10	0	0	7.5	9	8.2	7.4	8.1	8.4	22.5	18.8	19.1		
100%	10	0	0	7.5	9	8.2	7.4	8.1	8.4				1000	1000

	Control water	100% sample
Alkalinity:	133	225
Hardness:	125	220

Organism: *Notropis leedsi* — Age/Life stage: 27 days old

LC50: Binomial: 85.15% 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	5	8.3	8.3	8.2	7.5	7.8	8	21.2	19.2	19.3		
Control	5	4	4	8.3	8.3	8.2	7.5	7.8	8				215	208
30%	5	5	5	8.2	8.2	8	7.4	7.8	8.1	21.8	18.6	19.2		
30%	5	5	5	8.2	8.2	8	7.4	7.8	8.1				450	440
60%	5	5	5	7.8	8.2	7.8	7.4	7.9	8.2	22.7	18.6	19		
60%	5	5	5	7.8	8.2	7.8	7.4	7.9	8.2				450	720
100%	5	1	1	7.3	8.2	6.6	7.4	7.9	8.2	23.5	18.8	19.2		
100%	5	1	1	7.3	8.2	5.7	7.4	7.9	8.1				1100	1001

	Control water	100% sample
Alkalinity:	120	225
Hardness:	120	220

Table 3. Data recorded during 48 hour, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi, static acute toxicity bioassay of the Dunedin Sewage Treatment Plant, De-chlorinated Discharge, Pinellas County, NPDES #FL0021326, on 22 to 24 March, 1988.

Facility: **Dunedin STP**
Location: Pinellas
Sample Collection Date: 3/21/88 Time: 1020
Test Beginning Date: 3/22/88 Time: 1210
Test Ending Date: 3/24/88 Time: 1200

NPDES # **FL0021326**
Contact/District: D. Demmi
Test type: Static Screening
tests: 3
Chlorination Type: de-chlorinated

Facility Type: **Municipal**

Receiving Water: St. Joseph Sound

Parameter	
Total Residual Cl2:	0.3
Method:	Hach
By:	JMF
Salinity:	1

Ammonia:	Control Water	100% Sample
Unionized NH3:	0.00	0.15
Total NH3:	0.034	7.56

Organism: ***Ceriodaphnia dubia*** Age/Life stage: Neonates
LC50: Binomial: 42.43% 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	10	8.7	8.8	8.6	8.1	8.1	8.3	20.5	19.1	19.3		
Control	10	10	10	8.7	8.8	8.6	8.1	8.1	8.3					
30%	10	10	10	8.5	8.7	8.3	7.8	8.1	8.3	20.6	19.2	19.3		
30%	10	10	10	8.5	8.7	8.3	7.8	8.1	8.3					
60%	10	6	0	8.1	8.4	8.3	7.7	8.2	8.3	21	19.4	19.6		
60%	10	7	0	8.1	8.4	8.2	7.7	8.2	8.3					
100%	10	0	0	7.7	8.1	8.1	7.6	8.2	8.4	21.1	19.5	19.6		
100%	10	0	0	7.7	8.1	8.1	7.6	8.2	8.4					

	Control water	100% sample
Alkalinity:	133	225
Hardness:	125	220

Organism: ***Daphnia pulex*** Age/Life stage: Neonates
LC50: Binomial: Moving Average: Probit:
non-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	10	8.9	8.7	8.6	8.1	8.1	8.3	20.8	19.2	19.3		
Control	10	10	10	8.9	8.7	8.6	8.1	8.1	8.3				210	215
30%	10	10	10	8.5	8.8	8.4	7.8	8	8.2	21.3	18.8	19.1		
30%	10	10	10	8.5	8.7	8.4	7.8	8	8.2				460	448
60%	10	10	10	8	8.3	8	7.6	8	8.2	22.1	18.8	19.1		
60%	10	10	10	8	8.3	8.1	7.6	8	8.2				780	720
100%	10	10	10	7.4	8.2	8.1	7.5	8	8.3	22.7	19.1	19.3		
100%	10	10	10	7.4	8.2	8.1	7.5	8.1	8.3				1120	1070

	Control water	100% sample
Alkalinity:	133	225
Hardness:	125	220

Organism: ***Notropis leedsi*** Age/Life stage: 27 days old
LC50: Binomial: Moving Average: Probit:
non-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	5	8.3	8	7.9	7.5	7.8	8	21.2	19	19.2		
Control	5	5	5	8.3	8.1	7.9	7.5	7.7	8				210	205
30%	5	5	5	8.1	8	7.6	7.4	7.8	8.1	21.8	18.7	19.1		
30%	5	5	5	8.1	8	7.6	7.4	7.8	8.1				470	450
60%	5	5	5	7.9	7.5	7.7.2	7.4	7.9	8.2	22.5	18.7	19		
60%	5	5	5	7.9	7.5	6.7	7.4	7.9	8.1				800	750
100%	5	5	5	7.5	6.8	6.5	7.4	7.9	8.2	23.2	18.7	19.2		
100%	5	5	5	7.5	6.6	6.5	7.4	7.9	8.2				1180	1050

	Control water	100% sample
Alkalinity:	120	225
Hardness:	120	220

Table 4. Microtox test results, Dunedin WWTP discharge, Pinellas County, NPDES #FL0021326, on 22 March 1988.

Microtox Test Culture Lot # M-606

Final Reclaimed Water

% Sample	Chlorinated		Dechlorinated	
	5-min	15-min	5-min	15-min
0	93	59	78	52
0	94	60	83	54
0	93	60	83	56
12.38	86	51	79	53
12.38	86	53	81	50
12.38	83	50	73	54
24.75	65	39	71	49
24.75	67	40	73	49
24.75	67	41	73	50
49.5	46	25	72	49
49.5	47	25	71	48
49.5	46	25	71	49
99	30	12	67	39
99	31	12	67	40
99	30	12	64	38

EC50 Chlorinated (15 minute, 15 degrees C) = 39.90% sample
EC50 Dechlorinated (15 minute, 15 degree C) = 550%

EC50 (15 minute, 15 degrees C) is calculated using the Data Reduction Computer Program developed by Microbics Corporation for the Sharp Model EL-5100 (Microbics) Systems Operating Manual, 1982.

Table 5. Chemical constituents present in the reclaimed water from the Dunedin Sewage Treatment Plant, NPDES #FL0021326.

Organics

Acid Extractables	None Detected
Base Neutral Extractables	None Detected
Pesticide Extractables	a-BHC - 0.04 µg/l g-BHC - 0.07 µg/l Diazinon - 0.3 µg/l Chlorpyrifos - 0.3 µg/l

Metals

Arsenic	6.0 µg/l
Silver	0.26 µg/l
Zinc	29.0 µg/l

Table 6. LC_{50} Data for Bioassay of Dunedin STP, Pinellas County, NPDES #FL0021326, on 22 to 24 March, 1988.

Organism	Method of Data Analysis	48 Hour LC_{50} (95% Confidence Intervals)
Chlorinated discharge		
Ceriodaphnia dubia	Binomial	42.43 (30-60)
Daphnia pulex	Binomial	39.36 (30-60)
Notropis leedsi	Binomial	85.15 (60-∞)
Dechlorinated discharge		
Ceriodaphnia dubia	Binomial	42.43 (30-60)

Literature Cited

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

Cope, O. B. 1966. Contamination of the freshwater ecosystem by pesticides. J. Appl. Ecol. 3 (suppl.):33-44.

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. 1978. Bioassay procedures for the Ocean Disposal Permit Program. Environmental Research Laboratory. Gulf Breeze, Florida. EPA-600/9-78-010. 121 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(l), 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 concentration of the sampled discharge than allowed above.

MICROTOX™ Data Reduction Sheet

SOURCE: Dunedin STP

GRAPH CALC EC 50 (15 MIN, 15 °C) = 39.9 (%SAMPLE) mg/L

SAMPLE DATA:

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

DATE ASSAYED: 3-21-88 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	93	59	X	X	
B1		94	60			
C1		93	60			
A2	12.38	86	51	.098	.162	
B2		86	53			
C2		83	50			
A3	24.75	65	39	.407	.504	
B3		67	40			
C3		67	41			
A4	49.5	46	25	1.014	1.387	
B4		47	25			
C4		46	25			
A5	99	30	12	2.077	3.972	
B5		31	12			
C5		30	12			
EC_{50} (MIN, °C)				53.04	39.89	

REVIEWED BY:

DATE: 8/4/88

078

MICROTOX™ Data Reduction Sheet

SOURCE: Dunedin STP

GRAPH CALC EC 50 (15 MIN, 15 °C) = >100 (%SAMPLE) mg/L

SAMPLE DATA:

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

DATE ASSAYED: 03-22-88 TIME: 10:35

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	78	52	X	X	
B1		83	54			
C1		83	56			
A2	12.38	79	53	0.047	.032	
B2		81	50			
C2		73	54			
A3	24.75	71	49	.124	.095	
B3		73	49			
C3		73	50			
A4	49.5	72	51 49 LAW	.140	.110	
B4		71	48			
C4		71	49			
A5	99	67	39	.232	.385	
B5		67	40			
C5		64	38			
EC_{50} (MIN, °C)				536.4	~~772~~ 550.85 LAW	

REVIEWED BY:

DATE: 8/4/88

chlorinated

BIOASSAY DATA FORM — 13x1C

Laboratory/Facility: Dunedin STP
Address: 1140 Highland Ave. Dunedin FL
Contact/District: D. Domi SCO
Effluent Serial Number: 001
NPDES Permit Number:

Test Number: 1 of 6
Test Type: (Static) Renewal (Screening) Conventional
Other:

Sample Collection: Date 3-21-85 Time 1020
Test Beginning: Date 3-22-85 Time 1150
Test Ending: Date 3-24-85 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
control	6	10	10	10			8.8	8.7	8.8			8.0	7.9	8.3			19.7	19.2	19.3		
control	5	10	10	10			8.8	8.7	8.8			8.0	7.9	8.3			20.5				
30%	67	10	10	5			8.5	8.8	8.8			7.7	8.1	8.3			20.2	19.0	19.2		
30%	63	10	10	5			8.5	8.8	8.8			7.7	8.1	8.3							
60%	92	10	0	0			8.3	9.0	8.9			7.6	8.2	8.4			20.6	19.2	19.1		
60%	62	10	0	0			8.3	9.0	8.9			7.6	8.2	8.4							
100%	65	10	0	0			7.8	9.0	8.6			7.5	8.2	8.4			20.8	19.8	19.2		
100%	99	10	0	0			7.8	9.0	8.6			7.5	8.2	8.4							
Initial of Investigator	MF	MF	MF	MF			MF	MF	MF			MF	MF	MF			MF	MF	MF		

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

	0	96
Control Water:	133	1
100% Sample:	225	2
Other:		
Measured by:	MF	

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

	Hour 0	Hour ____
Control Water:	.0	
100% Sample:	7.	
Other:		
Measured by:	MF	

Slope = -57

Signatures: Marshall Fairbell

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7@7.9 9	9.0@20°C	9.5@10 27@30
24 Hours	7@7 9.9	9.0@21°C	9.7@10 27@30
48 Hours	7@7 9@9	9.0@21°C	9.5@10 22.2@30
72 Hours			
96 Hours			

Total Residual Chlorine:

Field data: 0.4 Method: Hach
Lab: 0.3 Method: Hach
Lab: 0.44 Method: Colorimetric

De-chlorinated: Measurements by: JMF

Reviewed by:

Date: 5/14/85

Remarks: SCT Ohm cond. 1300@19°C
48h. 1270@20°C

chlorinated

Toxic

BIOASSAY DATA FORM

Industry/Toxicant: Dunedin WWTP
Address: 1140 Highland Ave. Dunedin, FL
Contact/District: B. Demmi / SW
Effluent Serial Number: 001
NPDES Permit Number:

Test Number: 2 of 6
Test Type: (Static) Renewal (Screening) Conventional
Other:

Sample Collection: Date 3-21-88 Time 1020
Test Beginning: Date 3-22-88 Time 1200
Test Ending: Date 3-24-88 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	Conductivity 0	24	48	72	96
control	28	10	10	10			8.7	8.7	8.8			8.0	8.0	8.3			20.3	19.2	19.1							
control	12	10	10	10			8.7	8.7	8.8			8.0	8.0	8.3								215		210		
30%	52	10	10	9			8.6	8.9	8.8			7.7	8.0	8.2			21.0	18.8	18.9							
30%	26	10	10	9			8.6	8.9	8.8			7.7	8.0	8.2								445		420		
60%	60	10	1	0			8.2	9.0	8.8			7.5	8.0	8.3			21.8	18.7	18.9							
60%	2	10	3	0			8.1	9.0	8.8			7.5	8.0	8.3								700		680		
100%	132	10	0	0			7.5	9.0	8.2			7.4	8.1	8.4			22.5	18.8	19.1							
100%	65	10	0	0			7.5	9.0	8.2			7.4	8.1	8.4								1020		1000		
Initial of Investigator	MF	MF	MF	MF			MF	MF	MF			MF	MF	MF			MF	MF	MF			MF		MF		

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

Control Water: 133 / 125
100% Sample: 225 / 220
Other:
Measured by: MF

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

Control Water: 0.03 / 2.5
100% Sample:
Other:

Hour ____

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

Signatures:
Marshall Faircloth

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: 5/4/88

Industry/Toxicant: Dupont WWTP & Kleinfelter
Address: 1410 Highland Ave Deepwater, NJ
Contact/District: D. Dimmick
Effluent Serial Number: 001
NPDES Permit Number:

BIOASSAY DATA FORM - Acute

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

Sample Collection: Date 3-21-88 Time 1020
Test Beginning: Date 3-22-88 Time 1200
Test Ending: Date 3-24-88 Time 1130
Test Organism: M. beryllina
Age/Life Stage: 21 days old

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	47	5	5	5			8.3	8.3	8.2			7.5	7.8	8.0			21.2	19.2	19.3							
Control	16	5	4	4			8.3	8.3	8.2			7.5	7.8	8.0								215		208		
30 %	34	5	5	5			8.2	8.2	8.0			7.4	7.8	8.1			21.8	18.6	19.2							
30 %	52	5	5	5			8.2	8.2	8.0			7.4	7.8	8.1								450		440		
60 %	31	5	5	5			7.8	8.2	7.8			7.4	7.9	8.2			22.7	18.6	19.0							
60 %	99	5	5	5			7.8	8.2	7.8			7.4	7.9	8.2								780		720		
100 %	26	5	5*	1			7.3	8.2	6.6			7.4	7.9	8.2			23.5	18.8	19.2							
100 %	66	5	2	1			7.3	8.2	5.7			7.4	7.9	8.1								1100		1010		
Initial of Investigator		MF	MF	MF			MF	MF	MF			MF	MF	MF			MF	MF	MF			MF		MF		

Salinity 1.0

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 120 / 12
100% Sample: 225 / 22
Other:
Measured by: MF

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0 — U | T
Control Water: 0.
100% Sample: 7.5
Other:
Hour ____
Control Water:
100% Sample:
Other:
Measured by: MF

slope = 57.3

Signatures:
Marshall Faulk
_____
_____
_____
_____

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: _____

De-chlorinated:
Measurements by: _____

Reviewed by: _____
Date: 8/4/88

Remarks:
* erratic swimming pattern in 3 of 5

De-Cl

Toxic

Industry/Toxicant Dunedin WWTP
Address 1140 Highland Ave. Dunedin Fl
Contact/District Dennis/SW
Effluent Serial Number 001
NPDES Permit Number

BIOASSAY DATA FORM

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

Sample Collection: Date 3-21-88 Time 1020
Test Beginning: Date 3-22-88 Time 1210
Test Ending: Date 3-24-88 Time 1120
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	6	10	10	10			8.7	8.8	8.6			8.1	8.1	8.3			20.5	19.1	19.3							
Control	19	10	10	10			8.7	8.8	8.6			8.1	8.1	8.3												
30 %	12	10	10	10			8.5	8.7	8.3			7.8	8.1	8.3			20.6	19.2	19.3							
30%	81	10	10	10			8.5	8.7	8.3			7.8	8.1	8.3												
60 %	85	10	6	0			8.1	8.4	8.3			7.7	8.2	8.3			21.0	19.4	19.6							
60 %	36	10	7	0			8.1	8.4	8.2			7.7	8.2	8.3												
100 %	82	10	0	0			7.7	8.1	8.1			7.6	8.2	8.4			21.1	19.5	19.2							
100 %	87	10	0	0			7.7	8.1	8.1			7.6	8.2	8.4												
Initial of Investigator	mf	mf	mf	mf			mf	mf	mf			mf	mf	mf			mf	mf	mf							

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

Control Water:	133	12
100% Sample:	225	226
Other:		
Measured by:	mf	

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

	Hour 0		
	un	+	
Control Water:			0.034
100% Sample:			7.56
Other:			
Hour ____			
Control Water:			
100% Sample:			
Other:			
Measured by:	mf		

Signatures: Marshall Fandett

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: _____

Reviewed by: [signature]

Dechlorinated:
Measurements by: _____

Date: 5/4/88

Industry/Toxicant: Dinedan WWTP De-Cl
Address: 1140 Beaudoin Ave, Dinedan, MI
Contact/District: D. Ouimet / Swl
Effluent Serial Number: 001
NPDES Permit Number:

BIOMONITORING DATA FORM

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

Sample Collection: Date 3-21-88 Time 1000
Test Beginning: Date 3-22-88 Time 1210
Test Ending: Date 3-24-88 Time 1200
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	8	10	10	10			8.9	8.7	8.6			8.1	8.1	8.3			20.8	19.2	19.3							
Control	119	10	10	10			8.9	8.7	8.6			8.1	8.1	8.3								210		215		
30%	138	10	10	10			8.5	8.8	8.4			7.8	8.0	8.2			21.3	18.8	19.1							
30%	50	10	10	10			8.5	8.7	8.4			7.8	8.0	8.2								460		445		
60%	7	10	10	10			8.0	8.3	8.0			7.6	8.0	8.2			22.1	18.8	19.1							
60%	39	10	10	10			8.0	8.3	8.1			7.6	8.0	8.2								780		720		
100%	2	10	10	10			7.4	8.2	8.1			7.5	8.0	8.3			22.7	19.1	19.3							
100%	55	10	10	10			7.4	8.2	8.1			7.5	8.1	8.3								1120		1070		
Initial of Investigator	MF	MF	MF	MF			MF	MF	MF			MF	MF	MF			MF	MF	MF			MF		MF		

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 133 / 125
100% Sample: 225 / 220
Other:
Measured by: MF

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

Signatures:
Mandi Faircloth

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: 6/14/88

TOXIC

De-Cl

BIOASSAY DATA FORM

Industry/Toxicant Dunedin WWTP
Address 1140 Highland Ave. Dunedin, FL
Contact/District D. Damm; SW
Effluent Serial Number 001
NPDES Permit Number

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

Sample Collection: Date 3-21-88 Time 1020
Test Beginning: Date 3-22-88 Time 1210
Test Ending: Date 3-24-88 Time 1200
Test Organism N. Lohbsi
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	69	5	5	5			8.3	8.0	7.9			7.5	7.8	8.0			21.2	19.0	19.2							
Control	67	5	5	5			8.3	8.1	7.9			7.5	7.7	8.0								210		205		
30 %	68	5	5	5			8.1	8.0	7.6			7.4	7.8	8.1			21.8	18.7	19.1							
30 %	54	5	5	5			8.1	8.0	7.6			7.4	7.8	8.1								470		450		
60 %	15	5	5	5			7.9	7.5	7.2			7.4	7.9	8.2			23.5	18.7	19.0							
60 %	70	5	5	5			7.9	7.5	6.7			7.4	7.9	8.1								800		750		
100 %	6	5	5	5			7.5	6.8	6.5			7.4	7.9	8.2			23.2	18.7	19.2							
100 %	62	5	5	5			7.5	6.6	6.5			7.4	7.9	8.2								1150		1050		
Initial of Investigator	mf	MF	MF	MF			mf	MF	MF			mf	MF	MF			mf	MF	MF			mf		MF		

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

Control Water:	120	120
100% Sample:	225	220
Other:		
Measured by:	mf	

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

Hour 0

	n/t
Control Water:	0.0
100% Sample:	7.5
Other:	

Hour ____

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

Signatures: Marshall Fancett

Instrument Calibrations:

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

Records:

Total Residual Chlorine:

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

Dechlorinated:
Measurements by:

Reviewed by:

Date: 5/4/88