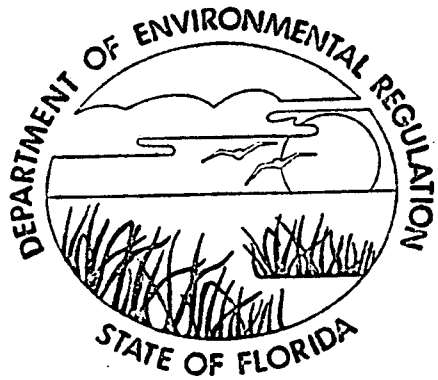


BIOASSAYS OF THE DEER PARK SEWAGE TREATMENT PLANT

PASCO COUNTY, FLORIDA

NO NPDES PERMIT

Sampled 9/15/87

OCTOBER 27, 1987

Biology Section
Division of Environmental Programs

IOASSAYS OF THE DEER PARK SEWAGE TREATMENT PLANT

PASCO COUNTY, FLORIDA

NO NPDES PERMIT

Biology Section
Bureau of Laboratories and
Special Programs
October 27, 1987

EXECUTIVE SUMMARY

Deer Park Sewage Treatment Plant, Pasco County, Florida, on 16 to 18 September, 1987.

The discharge from the Deer Park STP percolation pond #2 was not found to be acutely toxic to the water fleas, Ceriodaphnia dubia and Daphnia pulex, or the bannerfin shiner, Notropis leedsi. No mortality occurred during the 48 hour static bioassays. Chlorine residual was not found during field measurement.

The Microtox® assay, using the bacterium Photobacterium phosphoreum, was also found to be non-toxic. Due to the non-toxic nature of the discharge, chemical analyses were not performed.

INTRODUCTION

The Deer Park Sewage Treatment Plant, an extended aeration facility, serves the residents of Deer Park with an approximate flow of .0025 MGD. The plant discharges to percolation ponds which in overflow conditions would discharge into unnamed wetlants.

The facility was applying the recovered water as golf course irrigation and is currently approved for experimental wetlands discharge once the permits (NPDES and DER) are finalized. The wetlands will drain to the Pithlachascotee River. The plant has not discharged into the wetlands for about one year.

The Southwest District of DER requested the Biology Section in Tallahassee to perform bioassays on this facility to evaluate the toxicity of it's discharge. Bioassays using two invertebrates, both water fleas (Ceriodaphnia dubia and Daphnia pulex) and one native vertebrate, the bannerfin shiner (Notropis leedsi) were performed from 16 to 18 September, 1987. The Microtox® assay, using the bacterium Photobacterium phophoreum was performed on 16 September, 1987. Using organisms from widely divergent animal phyla provides more valid approximations of the toxicity to receiving water ecosystems. Those involved in performing the tests and report preparation were: D. A. Chasteen, J. H. Epler, J. M. Faircloth, R. B. Frydenborg, K. M. Lurding, and L. A. Williams.

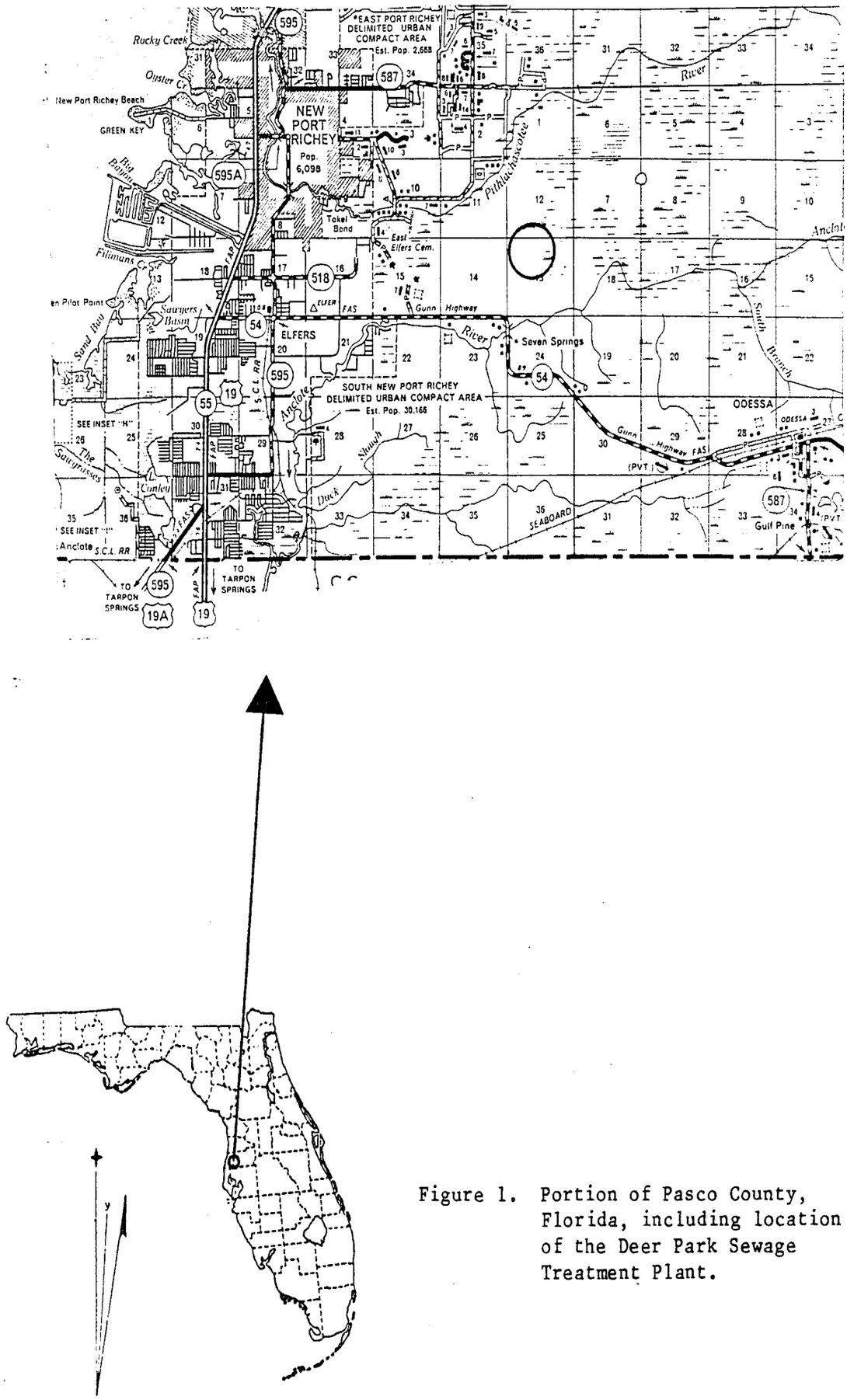


Figure 1. Portion of Pasco County, Florida, including location of the Deer Park Sewage Treatment Plant.

METHODS AND MATERIALS

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

The Microtox® assay was run according to the manufacturer's protocol described in the Microtox® System Operating Manual.

History and Maintenance of Bioassay Organisms

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

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

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

adhere to the tiles and are easily removed to heated, aerated hatching tanks. The narrow space between the tiles eliminates egg predation by adult fish. New hatching tanks are started regularly to segregate fry into even age groups. Newly hatched fry are fed a liquefied blend of Purina Trout Chow® and Cerophyl® twice daily. After one week, feeding is augmented with newly hatched Artemia nauplii and finely crushed Tetra Min®. The fish used in this test were 27 days old at the time of the test.

Photobacterium phosphoreum, a luminescent marine bacterium, is maintained as a freeze-dried culture, purchased from Microbics Corporation. Lot number M-603 was used in this assay. Immediately prior to the assay, the culture is rehydrated with 1.0 ml of specially purified water (Microtox® Reconstitution Solution).

Standard Reference Toxicity Assays

To ensure the uniform sensitivity of these test organisms to toxic substances, the Biology Section conducts monthly standard reference toxicant bioassays using sodium lauryl sulfate as the reference toxicant.

Test Methods

Methods used to perform acute toxicity bioassays are described by Peltier and Weber (1985) and the FDER Biology Section (1985). Some of the methods described below are more specific than those of Peltier and Weber. These will be noted in more detail.

Sampling Methods and Test Dates

Three static acute toxicity bioassays were performed on a sample of discharge from the Deer Park STP percolation pond #2. The tests were performed from 1115 hours on 16 September to 1115 on 18 September, 1987.

Separate samples were obtained for chemical and biological testing. Samples for metal analyses were preserved with HNO_3. Samples for organic chemical analyses and for the bioassays were unpreserved. The samples were collected by Mr. David Killingsworth of DER's Southwest District at 1045 hours on 15 September, 1987, 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° $\pm$ 2°C. Although this temperature may be different than the temperature of the sample during collection, it is the temperature range to which the test organisms are acclimatized and is the range recommended in the above referenced bioassay protocols. This avoids stressful effects caused by exposure to unusually cold or warm samples.

Freshwater Tests

Freshwater for dilution of the recovered water samples was obtained from the same DER deep well that is used to culture the test organisms. The concentration series selected for this study is given in Table 1.

Two replicates, of each concentration and a control, were used to test with the water fleas, Ceriodaphnia dubia and Daphnia pulex. Flint glass jars of either 60 ml (for C. dubia) or 250 ml (for D. pulex) capacity were used as test chambers, and either 50 ml or 200 ml of the appropriate test concentration was placed in each chamber. Ceriodaphnia were siphoned into a large culture dish and neonates were transferred by glass pipette, and loaded 10 per chamber, resulting in 20 per test concentration. Daphnia were siphoned into a large culture dish to isolate the proper size and age organisms (neonates). To accomplish this, the Daphnia were siphoned through a stainless steel screen. The trapped adults were then discarded. These neonates were then transferred by glass pipette, examined for injury, and were loaded 10 per chamber, resulting in 20 per test concentration. Water fleas were not fed during these tests.

Two replicates, of each concentration and a control, were used for the Notropis leedsi (bannerfin shiner) bioassay. The fish were transferred individually or by twos, into wide mouth quart jars containing 500 ml each of the appropriate test concentration, and were loaded 5 per chamber, resulting in 10 per concentration. Fish were not fed during this tests.

Test Parameters

Dissolved oxygen (YSI Model 57 oxygen meter), pH (Corning Model 7 pH meter with Orion Ross® combination electrode), temperature (NBS calibrated glass thermometer), and the number of live organisms were recorded for each test concentration at 0 hours, 24 hours, and 48 hours. Total alkalinity and total hardness (measured as $CaCO_3$ using HACH Chemical Company reagents) were recorded at the beginning of the test for the controls and the 100% recovered water concentration. The specific conductance (YSI Model 33 S-C-T meter) of each test concentration was recorded at the beginning and end of the test. All instruments were calibrated daily according to manufacturer's recommendations. Ammonia concentrations, including the total and unionized fraction (expressed in mg/l as N and as NH_3, respectively), were recorded in the controls and the 100% recovered 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 Table 2.

Microtox® Test

Upon receipt at the DER laboratory, a 20 ml subsample was collected in a small beaker. The sample was adjusted to 2% salinity with reagent grade dry sodium chloride to maintain the osmotic balance of the test

bacterium during the assay. Four two fold dilutions were made of the test sample resulting in a test range of 97%, 49.5%, 24.75%, 12.38%, and 0%. An incubation temperature of 15°C was maintained throughout the assay. Ten microliters of bacterial suspension was added to each of the 1.0 ml test dilutions. Light output (luminescence) was read after 5 and 15 minutes incubation, using the Microtox® analyzer and recorded on a Perkin-Elmer strip chart recorder. In the presence of a toxicant, the luminescence of the test organisms will decrease. In the presence of a stimulant, the luminescence will increase. Toxicity is calculated as a function of the light lost in the test sample, normalized to the control, and is reported as the effective concentration at which a 50% reduction, of light output is determined in 15 minutes (EC_{50}). Results are given in Table 3.

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

Ceriodaphnia dubia (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	50.0 ml	--------	50 ml
50.0%	25.0 ml	25.0 ml	50 ml
100.0%	-------	50.0 ml	50 ml

Daphnia pulex (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	200.0 ml	--------	200 ml
50.0%	100.0 ml	100.0 ml	200 ml
100.0%	--------	200.0 ml	200 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Sample	Total Volume
Control	500 ml	--------	500 ml
50.0%	250 ml	250 ml	500 ml
100.0%	------	500 ml	500 ml

Table 2. Data recorded during 48 hour, *Ceriodaphnia dubia*, *Daphnia pulex*, and *Notropis leedsi*, static acute toxicity bioassays of the Deer Park Sewage Treatment Plant, Pasco County, No NPDES permit, on 16 to 18 September, 1987.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Ceriodaphnia dubia														
Control	10	10	10	8.7	9.0	9.2	7.8	8.0	7.9	21.6	19.2	19.7		
Control	10	10	10	8.7	9.1	9.2	7.9	8.0	7.9					
50%	10	10	10	8.4	9.2	9.3	8.3	8.0	7.9	21.4	19.4	19.5		
50%	10	10	10	8.4	9.2	8.3	8.3	8.0	7.9					
100%	10	10	10	7.9	9.2	9.3	8.6	8.0	8.0	21.6	19.8	19.6		
100%	10	10	10	8.0	9.1	9.3	8.6	8.1	8.0					
Daphnia pulex														
Control	10	10	10	8.8	9.4	9.4	8.0	7.9	7.9	21.5	19.8	19.5		
Control	10	10	10	8.8	9.4	9.4	8.0	7.9	7.9				190	220
50%	10	10	10	8.5	9.4	8.9	8.4	7.9	7.9	22.2	18.9	19.3		
50%	10	10	10	8.5	9.4	8.9	8.4	7.9	7.9				440	430
100%	10	10	10	8.0	9.3	8.9	8.7	8.0	8.0	23.1	19.0	19.4		
100%	10	10	10	8.0	9.3	8.9	8.7	8.0	8.0				800	760
Notropis leedsi														
Control	5	5	5	8.5	9.2	8.9	7.6	7.7	7.8	21.4	18.5	19.4		
Control	5	5	5	8.6	8.3	8.9	7.6	7.6	7.8				205	200
50%	5	5	5	8.0	9.2	8.9	8.2	7.7	7.7	23.4	19.7	19.3		
50%	5	5	5	8.0	9.2	8.8	8.2	7.7	7.8				490	440
100%	5	5	5	8.0	9.1	8.4	8.7	7.9	7.8	24.3	18.9	19.3		
100%	5	5	5	8.0	8.8	8.4	8.7	7.9	7.9				900	785

Control Water

NH_3(as NH_3)(mg/l) 0

Total ammonia (as N)(mg/l) 0

Total Alkalinity 120 (mg/l $CaOO_3$)

Total Hardness 115 (mg/l $CaOO_3$)

100% Sample

NH_3(as NH_3)(mg/l) 0

Total ammonia (as N)(mg/l) 0

Total Alkalinity 135 (mg/l $CaOO_3$)

Total Hardness 120 (mg/l $CaOO_3$)

Table 3. Microtox test results, for Deer Park Sewage Treatment Plant, Pasco County, Florida, on 16 September, 1987.

Microtox Test Culture Lot #M-603

	Final Readings	
% Sample	5-min (I_5)	15-min (I_{15})
0	71	46
0	78	40
0	97	51
12.38	84	45
12.38	90	47
12.38	91	49
24.75	87	48
24.75	92	51
24.75	89	48
49.5	77	45
49.5	86	49
49.5	86	50
99	64	40
99	64	40
99	67	42

EC_{50} (15 min., 15°C) > 100% sample
EC_{50} (15 min, 15°C) calculated using the Data Reduction Computer Program developed by Microbics Corporation for the Sharp Model EL-5100 (Microtox®) Systems Operating Manual, 1982.

RESULTS AND DISCUSSION

The water sample taken from percolation pond #2 of the Deer Park Sewage Treatment Plant was not acutely toxic to the test organisms, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi. Microtox® assay results (EC_{50} > 100%) further support the conclusion that the recovered water from this facility was non-toxic on the sample date, September 15, 1987.

Measurements made in the field indicate no sign of residual chlorine. Ammonia was below measurable concentrations in the laboratory.

Since the sample was not toxic, chemical analyses were not performed.

LITERATURE CITED

Beckman Instruments, Inc. 1982. Microtox® System Oprating Manual.

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

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

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

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

BIOASSAY DATA FORM

Test Number: 1 of 3
Test Type: (Static) Renewal (Screening) Conventional
Other:

Sample Collection: Date 9/15/87 Time 1045
Test Beginning: Date 9/16/87 Time 1115
Test Ending: Date 9-18-87 Time 1115
Test Organism C. dubia
Age/Life Stage Neonates

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96
Control	85	10	10	10			8.7	9.0	9.2			7.8	8.0	7.9			~~20.0~~ 21.6	19.2	19.7		
Control	36	10	10	10			8.7	9.1	9.2			7.9	8.0	7.9							
50%	20	10	10	10			8.4	9.2	9.3			8.3	8.0	7.9			~~20.3~~ 21.4	19.4	19.5		
50%	21	10	10	10			8.4	9.2	9.3			8.3	8.0	7.9							
100%	2	10	10	10			7.9	9.2	9.3			8.6	8.0	8.0			~~20.1~~ 21.6	19.8	19.6		
100%	87	10	10	10			8.0	9.1	9.3			8.6	8.1	8.0							
Initial of Investigator		KML	~~JMF~~ KML	KML			RBF	JE	KML			RBF	RBF	KML			RBF KML	RBF	KML		

Conc. or %	Test Container Number	0	24	48	72	96

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 120/115
100% Sample: 135/120
Other:
Measured by: JMF

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

Hour: ~~72~~
Control Water:
100% Sample:
Other:
Measured by: JMF

Signatures:
KM Lundin
Joseph McCulloch
Russell Frydenborg
[illegible]

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7 at 7; 9 at 9	9.0 at 21°C	11.5 at 10; 29.5 at 30
24 Hours	7 at 7; 9 at 9	9.2 at 20°C	10 at 10; 25 at 30
48 Hours	7 at 7; 9 at 9	8.9 at 21.5	10.1 at 10; 25 at 30
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: 0.0 Method:
Lab: N.D Method: HACH
Lab: Method:
De-chlorinated:
Measurements by:

Reviewed by:
Date: 10/27/87

Remarks:

Industry/Toxicant Deer Park STP
Address Pasco County
Contact/District SW
Effluent Serial Number
NPDES Permit Number

BIOASSAY DATA FORM

Test Number: 2 of 3
Test Type: Static Renewal, Screening Conventional
Other:

Sample Collection: Date 9/15/87 Time 1045
Test Beginning: Date 9/16/87 Time 1115
Test Ending: Date 9/18/87 Time 1115
Test Organism D. pulex
Age/Life Stage neonates

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96	Conductivity 0	24	48	72	96
Control	72	10	10	10			8.8	9.4	9.4			8.0	7.9	7.9			21.0	18.9	19.5							
Control	71	10	10	10			8.8	9.4	9.4			8.0	7.9	7.9			21.5					190	195	220		
50%	35	10	10	10			8.5	9.4	8.9			8.4	7.9	7.9			20.5	18.9	19.3							
50%	18	10	10	10			8.5	9.4	8.9			8.4	7.9	7.9			20.3					440	420	430		
100%	31	10	10	10			8.0	9.3	8.9			8.7	8.0	8.0			21.1	19.0	19.4							
100%	40	10	10	10			8.0	9.3	8.9			8.7	8.0	8.0			20.1					800	720	760		
Initial of Investigator		JW	RBF	KML			RBF	JE	KML			RBF	JE	KML			RBF KML	RBF	KML			RBF	RBF	KML		

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:
KMLurding
Joseph Fairbrother
Reed Frydenborg

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:

Dechlorinated:
Measurements by:

Reviewed by
Date: 10/27/87

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

BIOASSAY DATA FORM

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

Sample Collection: Date 9/15/87 Time 1045
Test Beginning: Date 9/16/87 Time 1115
Test Ending: Date 9/18/87 Time 1115
Test Organism: N. incerta
Age/Life Stage: 07 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	22	5	5	5			8.5	9.2	8.9			7.6	7.7	7.8			22.0	18.5	19.4							
Control	239	5	5	5			8.6	9.3	8.9			7.6	7.6	7.8			21.4					205	200	200		
50%	76	5	5	5			8.0	9.2	8.9			8.2	7.7	7.7			24.5	18.7	19.3							
50%	71	5	5	5			8.0	9.2	8.8			8.2	7.7	7.8			23.4					440	445	440		
100%	—	5	5	5			8.0	9.1	8.4			8.1	7.9	7.8			20.6	18.9	19.3							
100%	3	5	5	5			8.0	8.8	8.4			8.7	7.9	7.9			24.3					900	710	785		
Initial of Investigator		KML	RBF	KML			RBF	JE	KML			RBF	JE				RBF KML	RBF	KML			RBF	RBF	KML		

@

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: K M Linding, Joseph Faiella, Russel Frydenborg, Jeff Gyr

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: 10/27/87

Remarks: @ allowed to cool before loading organisms.

MICROBICS MICROTOX™ DATA REDUCTION SHEET

SAMPLE ID NO. _____ COLLECTED BY: _____

SOURCE: Deer Park STP DATE _____

TIME _____

REPORTED RESULTS: TOXIC ☐ NON-TOXIC ☒ MAX I⁻ = _____ FOR CONCENTRATION = _____

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

CONFIDENCE FACTOR = _____ ☐ OTHER _____

95% CONFIDENCE INTERVAL _____ TO _____

REMARKS: _____

SAMPLE DATA: SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent - no dilution

DATE ASSAYED: 9 - 16 - 87; TIME: 9:15

VISUAL COLOR: none; CORRECTION REQUIRED: YES ☐ NO ☒

VISUAL TURBIDITY: none; SEPARATION REQUIRED: YES ☐ NO ☒

INITIAL pH: 8.6; pH ADJUSTED TO: _____ WITH _____

OSMOTICALLY ADJUSTED WITH: MOAS ☐ DRY NaCl ☒ OTHER ☐ _____

PRIMARY DILUTION OF SAMPLE: none

OPERATOR: Leslee; REAGENT VIAL LOT NO. M - 603

REMARKS: _____

MICROTOX 99% TEST

Sample Cuvette	% Sample	Final Readings 5 min	Final Readings 15 min	Γ5	Γ15	Notes
A1	0	91	46			
B1		78	40			
C1		97	51			
A2	12.28	84	45	0.00	-.03	
B2		90	47			
C2		91	49			
A3	24.75	87	48	-.01	-.07	
B3		92	51			
C3		89	48			
A4	49.5	77	45	.07	-.05	
B4		86	49			
C4		86	50			
A5	99	64	40	.36	.12	
B5		64	40			
C5		67	42			

EC50 (15 min, 15 °C)			
	By Graph		
	By Calculator		7100

Reviewed by _____

Date 10/25/87