

BIOASSAYS OF THE EMBASSY HILLS WASTEWATER TREATMENT PLANT PASCO COUNTY, FLORIDA NO NPDES PERMIT

SAMPLED 11/9/87

MARCH 8, 1988

Biology Section
Division of Environmental Programs

BIOASSAYS OF THE EMBASSY HILLS

WASTEWATER TREATMENT PLANT

PASCO COUNTY, FLORIDA

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Biology Section
Bureau of Laboratories and
Special Programs
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EXECUTIVE SUMMARY

Embassy Hills Wastewater Treatment Plant, Pasco County, Florida, No NPDES Permit, on 10 to 12 November 1987.

The Embassy Hills Wastewater Treatment Plant treats domestic sewage from the Embassy Hills community of Pasco County and discharges to percolation ponds. The discharge, which has a chlorine residual of .53 mg/l by the Iodometric method, was acutely toxic to 4 out of 5 of the test organisms. The two water fleas, (Ceriodaphnia dubia and Daphnia pulex), and the bannerfin shiner (Notropis leedsi) tests produced 48 hour LC_{50}'s between 50% and 100% discharge concentration. The Microtox® assay indicated more extreme toxicity to the bacterium, Photobacterium phosphoreum, producing an EC_{50} of 28.8% discharge concentration. The discharge was not acutely toxic to the fathead minnow Pimephales promelas.

A portion de-chlorinated in the Ecotoxicology Laboratory was non toxic to the water flea, Daphnia pulex and both fish, the bannerfin shiner (Notropis leedsi) and the fathead minnow (Pimephales promelas). The other test, on the water flea, Ceriodaphnia dubia, was invalidated due to excessive control mortality. The Microtox® assay yielded a very toxic EC_{50} of 36.6% on this de-chlorinated sample portion. The cause of the toxicity is unknown.

INTRODUCTION

The Embassy Hills Wastewater Treatment Plant is an extended aeration facility with a design capacity of 2.5 MGD, which discharges to percolation ponds. Due to problems with high nitrates being detected in groundwater monitoring wells, permit limitations were imposed to limit operation to 2.0 MGD. On February 1, 1988, a new permit allowed an increase to 2.8 MGD provided discharge was to five new percolation ponds farther north. The facility is currently constructing these ponds with an EPA funded grant.

To evaluate the extent and existence of toxicity, the Southwest district of DER requested toxicity screening bioassays be performed. The Ecotoxicology Laboratory performed four toxicity screening bioassays and one Microtox® assay on both the discharge sample and a de-chlorinated portion of the discharge. Results are presented in this report. Those involved in performing the tests and in preparation of this report were: D. A. Chasteen, J. H. Epler, J. M. Faircloth, R. B. Frydenborg, K. M. Lurding, and L. A. Williams.

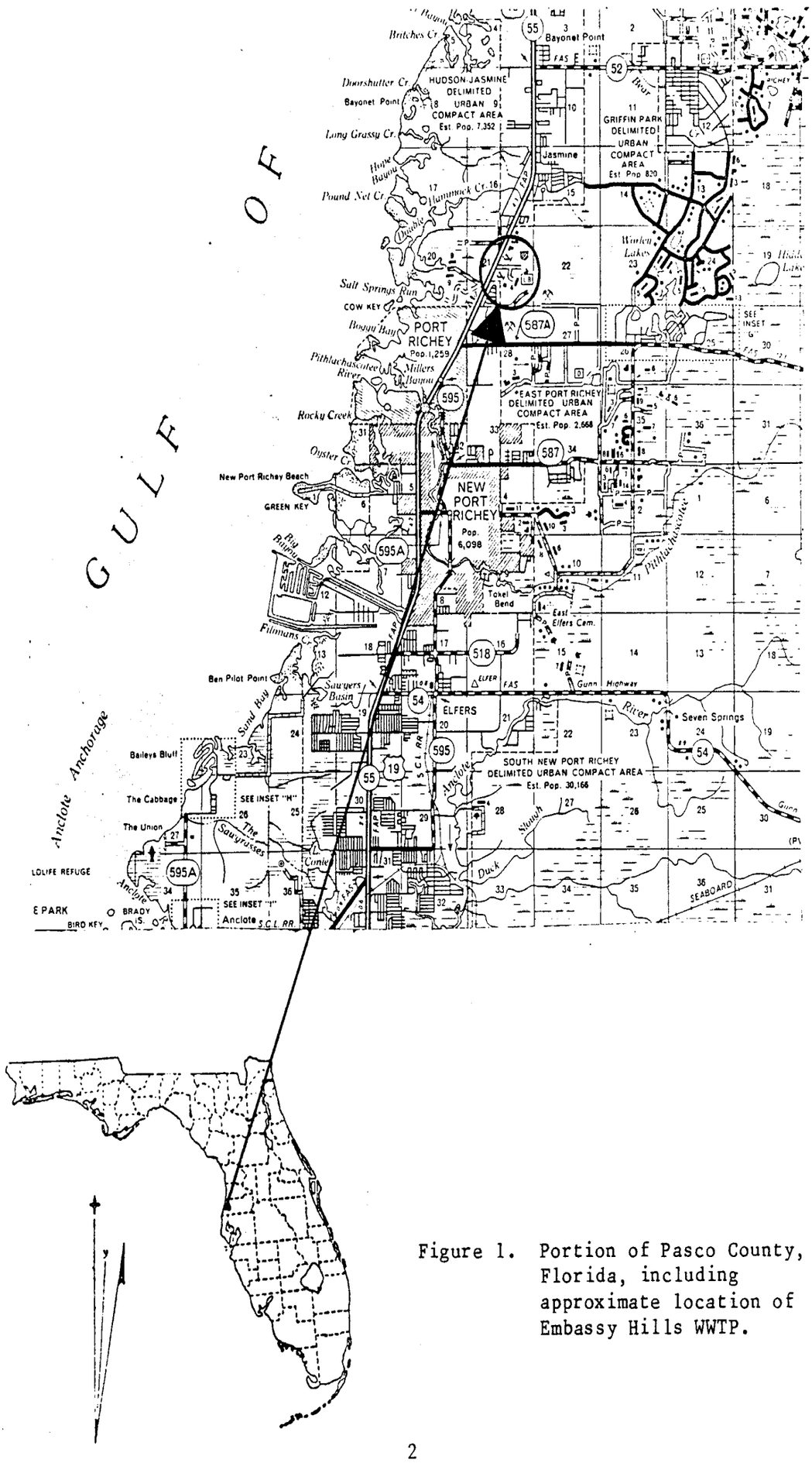


Figure 1. Portion of Pasco County, Florida, including approximate location of Embassy Hills WWTP.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier and Weber (1985) and the FDER Biology Section (1985). The organisms selected for this study include 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).

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.

Pimephales promelas (fathead minnows) - A breeding culture of approximately 100 adult fathead minnows was obtained from the EPA laboratory in Cincinnati, Ohio, in May, 1979. This breeding population is augmented with fry hatched in our laboratory and with additional adult fish from EPA as required. The fish are maintained in all-glass aquaria (15 to 125 gallons), equipped with flow-through water exchange systems, using unchlorinated well water obtained from a deep well near the DER laboratory. The fathead minnows are reared at a temperature of 22° ± 2°C under a 16:8 hour light-dark cycle. These fish are fed Glencoe Mills "#3 size enriched trout fry granules" or Tetra-Min®, and frozen Artemia (brine

shrimp) at a rate of 0.75g/30 fish, twice daily, and once daily on weekends. Breeding has been encouraged by placing several halved clay pots in each tank to serve as substrates for eggs and to provide cover for the fish. Tanks are examined twice daily (at feeding time) for egg production. Clay pots containing egg clusters are immediately transferred to 2-1/2 to 15 gallon aerated aquaria for hatching. A new aquarium is prepared for each week's production of eggs, thereby segregating fish into even age groups to be used for testing. After hatching, fathead minnow fry are fed twice daily with freshly hatched Artemia. The fish used in this test were 16 days old at the time of the test.

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 19 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-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).

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

Four static acute screening toxicity bioassays and one Microtox® assay were performed on both the facility's discharge and a de-chlorinated portion of the discharge from Outfall #001 at 1030 hours on 10 November, to 1030 hours on 12 November, 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 Demmi of the DER Southwest District from the Outfall #001 weir of the facility. They were taken at 1105 hours on 9 November, 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° ± 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. De-chlorination 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.

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 Pimephales promelas (fathead minnow) and 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 these 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% 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 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% 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 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 4.

RESULTS AND DISCUSSION

The discharge from the Embassy Hills WWTP has a chlorine residual of .53 mg/l by the Iodometric method. This residual is high enough to cause acute toxicity to all the organisms tested, except the fathead minnow (Pimephales promelas) with 48 hour LC_{50}'s in the range of 50% and 100% discharge concentration (Table 2). The test organisms included two cladoceran water fleas (Ceriodaphnia dubia and Daphnia pulex) and two fish species, the bannerfin shiner (Notropis leedsi) and the fathead minnow (Pimephelas promelas). The toxicity results are further supported by the Microtox® assay results on the bacterium, Photobacterium phosphoreum, which exhibited an EC_{50} of 28.8% discharge concentration.

When a portion of discharge was de-chlorinated the toxicity was reduced considerably. One water flea (Daphnia pulex) and both fish (Notropis leedsi and Pimephales promelas) exhibited no mortality. The Ceriodaphnia dubia test controls were contaminated causing excessive control mortality and the test had to be invalidated. The Microtox® assay still exhibited extreme toxicity to the bacterium, Photobacterium phosphoreum, with an EC_{50} of 36.6% discharge concentration. Since the chemistry samples exceeded the holding time, analyses were not performed and the nature of the toxicant remains unknown.

Clearly, the de-chlorinated process eliminated must of the toxicity to the test organisms. Since the discharge does not go to surface waters, the acute toxicity standards do not apply. The toxicity however may inhibit some of the organisms providing biological treatment in the percolation ponds since the discharge appears to be quite toxic in the microbial based Microtox® assay. Toxicity reduction through treatment changes or more careful chemical control may optimize the treatment in the percolation ponds.

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

Pimephales promelas (fathead minnow)

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 static screening bioassays using Ceriodaphnia dubia, Daphnia pulex, Notropis leedsi, and Pimephales promelas, of the chlorinated discharge from the Embassy Hills WWTP, Pasco County, Florida, No NPDES permit, on 10 to 12 November, 1987.

Facility:	Embassy Hills STP			NPDES # None		Facility Type:	Municipal WWTP
Location:	Pasco County			Contact/District:	SW/David Demmi		
Sample Collection Date:	11/9/87	Time:	1105	Test type: static screening			
Test Beginning Date:	11/10/87	Time:	1030	# tests:	4	Receiving Water:	Percolation Ponds
Test Ending Date:	11/12/87	Time:	1030	Chlorination Type:	Chlorinated		

Parameter		
Total Residual Cl2:	0.53	0.5
Method:	IODOMETRIC	HACH
By:	JMF	RBF
Salinity:	0	

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

Organism: ***Ceriodaphnia dubia*** Age/Life stage: Neonates
LC50: Binomial: Moving Average: Probit:
<100%

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hour	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	7.9	8.7	8.2	8.1	8.2	20.5	19.3	19.5		
Control	10	10	10	8.9	8	8.7	8.2	8.1	8.2					
50%	10	10	8	8	8.2	8.8	7.6	8.2	8.3	20.6	19.3	19		
50%	10	10	10	8	8.1	8.7	7.6	8.3	8.3					
100%	10	5	0	7.6	8.3	8.9	7.4	8.3	8.5	20.8	19.2	19		
100%	10	6	0	7.6	8.3	8.9	7.4	8.3	8.5					

	Control water	100% sample
Alkalinity:	127	231
Hardness:	130	220

Organism: ***Daphnia pulex*** Age/Life stage: Neonates
LC50: Binomial: Moving Average: Probit:
<100%

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hour	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	8.5	8.1	8.9	8.2	8.1	8.2	20.8	19.3	18.8		
Control	10	10	10	8.5	8.1	8.8	8.2	8.1	8.2				215	250
50%	10	10	10	8.1	8.1	8.8	7.6	8.1	8.3	20.8	19	18.7		
50%	10	10	10	8.2	8.1	8.8	7.6	8.1	8.3				600	600
100%	10	8	2	7.3	8.3	8.9	7.4	8.1	8.4	20.9	19	18.8		
100%	10	6	2	7.3	8.3	8.9	7.4	8.1	8.4				950	900

	Control water	100% sample
Alkalinity:	127	231
Hardness:	130	220

Organism: ***Notropis leedsi*** Age/Life stage: 19 days
LC50: Binomial: Moving Average: Probit:
<100%

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hour	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	8.4	8	8.4	7.7	7.9	8	21.2	19.1	19		
Control	5	5	5	8.5	8	8.4	7.7	7.9	8				210	215
50%	5	5	5	8.2	8.1	8.6	7.4	7.9	8.1	21.3	18.9	18.4		
50%	5	5	5	8.2	8.1	8.7	7.4	7.9	8.1				600	580
100%	5	0	0	7.5	8	-	7.3	7.9	8.2	21.4	18.9	18.4		
100%	5	0	0	7.5	8.1	-	7.3	8	8.2				990	900

	Control water	100% sample
Alkalinity:	112	231
Hardness:	112	220

Organism: ***Pimephales promelas*** Age/Life stage: 16 days
LC50: Binomial: Moving Average: Probit:
non-toxic

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hour	Live at 24 hr	Live at 48 hr	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	48 hour
Control	5	5	5	8.6	7.8	7.9	7.7	7.9	7.9	21.2	19.1	18.5		
Control	5	5	5	8.6	7.8	7.9	7.7	7.9	7.9				205	210
50%	5	5	5	8.1	8	8.2	7.4	7.9	8.1	21.2	19	18.5		
50%	5	5	5	8.1	8	8.2	7.4	7.9	8.1				600	590
100%	5	4	4	7.6	8	7.8	7.3	8	8.2	21.4	19	18.7		
100%	5	5	5	7.6	8	8.2	7.3	8	8.3				980	950

	Control water	100% sample
Alkalinity:	112	231
Hardness:	112	220

Table 3. Data recorded during 48 hour static screening bioassays using Ceriodaphnia dubia, Daphnia pulex, Notropis leedsi, and Pimephales promelas of the de-chlorinated discharge from the Embassy Hills WWTP, Pasco County, Florida, No NPDES Permit, on 10 to 12 November, 1987.

Facility:	Embassy Hills STP			NPDES # None	Facility Type:	Municipal WWTP
Location:	Pasco County			Contact/District:	SW/David Demmi	
Sample Collection Date:	11/9/87	Time:	1105	Test type: static screening		
Test Beginning Date:	11/10/87	Time:	1030	# tests: 4	Receiving Water:	Percolation Ponds
Test Ending Date:	11/12/87	Time:	1030	Chlorination Type:	Dechlorinated	

Parameter	
Total Residual Cl2:	None Detected
Method:	HACH
By:	RBF
Salinity:	0

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

Organism: ***Ceriodaphnia dubia*** Age/Life stage: Neonates
LC50: Binomial: Moving Average: Probit:
Invalidated

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	0	9.2	8.5	8.8	8.1	8	8	20.3	19.4	19.4		
Control	10	10	8	9.2	8.4	8.8	8.1	8.1	8					
50%	10	10	10	8.7	8.5	8.9	7.7	8.1	8.2	20.3	19.3	19.3		
50%	10	10	10	8.5	8.4	8.8	7.7	8.2	8.2					
100%	10	5	3	7.6	8.5	8.7	7.5	8.2	8.4	20.6	19.3	19.4		
100%	10	4	0	7.6	8.4	8.6	7.5	8.3	8.4					

	Control water	100% sample
Alkalinity:	127	231
Hardness:	130	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	8.7	8.3	8.7	8.2	8.1	8.1	20.7	19.4	19		
Control	10	10	10	8.7	8.3	8.7	8.2	8.1	8.1				225	225
50%	10	10	10	8.8	8	8.8	7.6	8.1	8.2	20.8	19.1	18.7		
50%	10	10	10	8.7	8	8.8	7.6	8.1	8.2				680	600
100%	10	10	10	7.5	8.3	8.7	7.4	8.1	8.3	21.1	19.1	18.7		
100%	10	10	10	7.4	8.4	8.7	7.4	8.1	8.3				950	900

	Control water	100% sample
Alkalinity:	127	231
Hardness:	130	220

Organism: ***Notropis leedsi*** Age/Life stage: 19 days
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	[illegible]	5	5	8.4	8.2	7.8	7.7	7.9	7.8	21.3	20	19.4		
Control		5	5	8.4	8.1	7.8	7.7	7.9	7.8				215	208
50%	[illegible]	5	5	8.3	8	7.8	7.4	7.9	8	21.3	19.4	18.8		
50%	5	5	5	8.2	7.9	7.8	7.4	7.9	8				650	590
100%	5	5	5	7.4	7.9	8.1	7.3	8	8.2	21.3	19.1	18.7		
100%	5	5	5	7.5	7.9	8.1	7.3	8	8.2				990	910

	Control water	100% sample
Alkalinity:	112	231
Hardness:	112	220

Organism: ***Pimephales promelas*** Age/Life stage: 16 days
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	8.4	7.8	8.1	7.7	7.9	7.8	21.2	19.3	18.6		
Control	5	5	5	8.4	7.8	8	7.7	7.9	7.9				218	212
50%	5	5	5	8	7.8	7.9	7.4	7.9	8	21.2	19.2	18.6		
50%	5	5	5	8	7.9	7.9	7.4	7.9	8				610	580
100%	5	5	5	7.5	7.9	7.8	7.3	8	8.2	21.3	19.1	18.6		
100%	5	5	5	7.5	7.8	7.8	7.3	8	8.2				960	920

	Control water	100% sample
Alkalinity:	112	231
Hardness:	112	220

Table 4. Microtox test results, Embassy Hills WWTP discharge, Pasco County, no NPDES permit, on 10 to 12 November,1987.

Microtox Test Culture Lot # M-606

Final Reclaimed Water

% Sample	Chlorinated		Dechlorinated	
	5-min	15-min	5-min	15-min
0	99	60	77	48
0	90	54	82	51
0	76	47	75	48
12.38	78	40	71	40
12.38	79	41	69	38
12.38	83	43	74	41
24.75	59.5	32	61	31
24.75	67	31	61	31
24.75	68.5	28	62	32
49.5	40	22	44	20
49.5	35	20	39	17
49.5	34	20	45	21
99	9	4	25	10
99	11	5	23	10
99	11	5	24	10

EC50 Chlorinated (15 minute, 15 degrees C) = 28.8% sample
EC50 Dechlorinated (15 minute, 15 degree C) = 36.6%

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.

LITERATURE CITED

Beckman Instruments, Inc. 1982. Microtox System Operating 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.

* Chemistry samples exceeded holding time in our investigator's office. Be more careful!

Industry/Toxicant: Embassy Hills STP
Address: Pasco Co.
Contact/District: SW
Effluent Serial Number: 001
NPDES Permit Number: FL KML

BIOASSAY DATA FORM

Test Number: 1 of 0
Test Type: (Static) Renewal, (Screening) Conventional
Other: Ce-Cl2

Sample Collection: Date 11-9-87 Time 1105
Test Beginning: Date 11-10-87 Time 1030
Test Ending: Date 11-12-87 Time 1030
Test Organism: C. dubia
Age/Life Stage: neonate

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96	Conductivity 0	24	48	72	96
92	Control	10	10	0			9.2	8.5	8.8			8.1	8.0	8.0			20.3	19.4	19.4							
2T83	Control	10	10	10			9.2	8.4	8.8			8.1	8.1	8.0												
71	50%	10	10	10			8.7	8.5	8.9			7.7	8.1	8.2			20.3	19.3	19.3							
8521	50%	10	10	10			8.5	8.4	8.8			7.7	8.2	8.2												
7525T	100%	10	5	3			7.6	8.5	8.7			7.5	8.2	8.4			20.4	19.3	19.4							
88	100%	10	4	0	*		7.6	8.4	8.6			7.5	8.3	8.4												
Initial of Investigator		JMF	RBF	RBF			RBF	RBF	JMF			RBF	RBF	JMF			JMF	RBF	JMF							

Alkalinity/Hardness (mg/l as $CaCO_3$)
Cond. water: 127/130
Control Water: 112/112
100% Sample: 231/220
Other:
Measured by: KML

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

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

Signatures:
Randi Snyderburg
Joan Faudeth
KM Lindsey

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7@7; 9@9	9.0@21.0°C	10.5@10.0; 29.2@30.0
24 Hours	7@7; 9@9	9.0@21.0°C	
48 Hours	7@7; 9@9	9.2@20.0°C	10.0@10.0 29@30
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: 3.0 Method:
Lab: 0.5 Method: HACH
Lab: 0.53 Method: IODO

Dechlorinated: 0.0 ND
Measurements by: RBF, MF

Reviewed by:
Date: 3/3/88

Remarks:

Discharger/Facility: Embassy Hills STP

Address:

Contact/District:

Effluent Serial Number:

NPDES Permit Number:

Test Number: 2nd D

Test Type: Static Renewal, Screening Conventional

Other: Cu Cl2

Sample Collection: Date 11-9-87 Time 1105

Test Beginning: Date 11-10-87 Time 1030

Test Ending: Date 11-12-87 Time 1030

Test Organism: D. pulex

Age/Life Stage: neonate

KML

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
56	Control	10	10	10			8.2	8.3	8.7			8.2	8.1	8.1			20.7	21.4	17.0							
—	Control	10	10	10			8.7	8.3	8.7			8.2	8.1	8.1								225		225		
17	50%	10	10	10			8.8	8.2	8.8			7.6	8.1	8.2			20.8	21.1	18.7							
61	50%	10	10	10			8.7	8.0	8.8			7.6	8.1	8.2								680		600		
68	100%	10	10	10			7.5	8.3	8.7			7.4	8.1	8.3			21.1	19.1	18.7							
72	100%	10	10	10			7.4	8.4	8.7			7.4	8.1	8.3								950		900		
Initial of Investigator		RBF	RBF	RBF			RBF	RBF	JMF			RBF	RBF	JMF			JMF	RBF	JMF			RBF		JMF		

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

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

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

Control Water:
100% Sample:
Other:

Hour

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

Signatures: Russell Frydenborg, Joseph Faucett, Ann Lurding

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: 3.0 Method:
Lab: 0.5 Method: HACH
Lab: 0.58 Method: ICDO

De-chlorinated: 0.0 M.D.
Measurements by: RBF, MF

Reviewed by:

Date: 3/3/88

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

BIOASSAY DATA FORM

Test Number: 3 of B
Test Type: (Static) Renewal (Screening) Conventional
Other: DECl2

Sample Collection: Date 11-9-87 Time 1105
Test Beginning: Date 11-10-87 Time 1030
Test Ending: Date 11-12-87 Time 1030
Test Organism: N. laticeps
Age/Life Stage: F 19 days

KML

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
30	Control	5	5	5			8.4	8.2	7.8			7.7	7.9	7.8			21.3	20.2	19.4							
34	Control	5	5	5			8.4	8.1	7.8			7.7	7.9	7.8								215		208		
31	50%	5	5	3			8.3	8.0	7.8			7.4	7.9	8.0			21.3	19.9	18.8							
13	50%	5	5	5			8.2	7.9	7.8			7.4	7.9	8.0								650		590		
44	100%	5	5	5			7.4	7.9	8.1			7.3	8.0	8.2			21.3	19.1	18.7							
37	100%	5	5	5			7.5	7.9	8.1			7.3	8.0	8.2								990		910		
Initial of Investigator		KML	RBF	JE			RBF	RBF	JMF			RBF	RBF	JMF			JMF	RBF	JMF			RBF		JMF		

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

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

Hour

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

Signatures: Russel Frydenborg
Joseph Faudeth
KM Lurding

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: 3.0 Method:
Lab: 0.5 Method: HACH
Lab: 0.53 Method: IODO
De-chlorinated: 0.0 (N.D.)
Measurements by: RBF, MF

Reviewed by:
Date: 3/3/88

Facility Name or Discharger: EMVMSD | HLLS 511

Address:

Contact/District:

Effluent Serial Number:

NPDES Permit Number:

Test Number: 4-50

Test Type: Static Renewal, Screening Conventional

Other: DE Cl2

Grab Collection: Date 11-9-87 Time 1105

Test Beginning: Date 11-10-87 Time 1230

Test Ending: Date 11-12-87 Time 1030

Test Organism: P. promelas

Age/Life Stage: 16 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
47	Control	5	5	5			8.4	7.8	8.1			7.7	7.9	7.8			21.2	19.3	18.6			218				
99	Control	5	5	5			8.4	7.8	8.0			7.7	7.9	7.9								218		212		
2	50%	5	5	5			8.0	7.8	7.9			7.4	7.9	8.0			21.2	19.2	18.6			6				
70	50%	5	5	5			8.0	7.9	7.9			7.4	7.9	8.0								610		580		
65	100%	5	5	5			7.5	7.9	7.8			7.3	8.0	8.2			21.3	19.1	18.6							
86	100%	5	5	5			7.5	7.8	7.8			7.3	8.0	8.2								960		920		
Initial of Investigator		RBF	RBF	JE			RBF	RBF	JMF			RBF	RBF	JMF			JMF	RBF	JMF			RBF		JMF		

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

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

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

Control Water:
100% Sample:
Other:

Hour

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

Signatures: Russell Frederking, Joseph Goodall, Hal Wuerzing

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: 3.0 Method:
Lab: 0.5 Method: Hach
Lab: 0.53 Method: 1000

De-chlorinated: 0.0 (N.D.)

Measurements by: RBF, JE

Reviewed by:

Date: 3/3/88

Industry/Toxicant: Embassy Hills STP

Address:

Contact/District:

Effluent Serial Number:

NPDES Permit Number: KML

BIOASSAY DATA FORM

Test Number: 58

Test Type: (Static Renewal, Screening, Conventional)

Other: Cl2

Sample Collection: Date 11-9-87 Time 1105

Test Beginning: Date 11-10-87 Time 1030

Test Ending: Date 11-12-87 Time 1230

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	Conductivity 0	24	48	72	96
67	Control	10	10	16			8.8	7.9	8.7			8.2	8.1	8.2			20.5	19.3	18.5							
—	Control	10	10	16			8.9	8.0	8.7			8.2	8.1	8.2												
47	50%	10	10	8			8.0	8.2	8.8			7.6	8.2	8.3			20.6	19.3	19.0							
95	50%	10	10	10			8.0	8.1	8.7			7.6	8.3	8.3												
80	100%	10	5	0			7.6	8.3	8.9			7.4	8.3	8.5			20.8	19.2	19.0							
13	100%	10	6	0			7.6	8.3	8.9			7.4	8.3	8.5												
Initial of Investigator		JMF	RBF	RBF			RBF	RBF	JMF			RBF	RBF	JMF			JMF	RBF	JMF			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:
Russel Frydenborg
Joseph Faudelt
Tim Lurding

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: 3.0 Method:
Lab: .53 Method: Iodometric
Lab: .50 Method: HACH Reviewed by:

~~De chlorinated~~: no

Measurements by: MF, RBF

Date: 3/3/88

Effluent/Toxicant: Lambsway Hills STP

Address:

Contact/District:

Effluent Serial Number:

NPDES Permit Number:

RML

Test Number: 87-0-19

Test Type: (Static) Renewal, (Screening) Conventional

Other:

Sample Collection: Date 11-9-87 Time 1105

Test Beginning: Date 11-10-87 Time 1030

Test Ending: Date 11-12-87 Time 1030

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
23	Control	10	10	10			8.5	8.1	8.9			8.2	8.1	8.2			20.8	19.3	18.8							
1	Control	10	10	10			8.5	8.1	8.8			8.2	8.1	8.2								215		250		
132	50%	10	10	10			8.1	8.1	8.8			7.6	8.1	8.3			20.3	19.0	18.7							
59	50%	10	10	10			8.2	8.1	8.8			7.6	8.1	8.3								600		600		
49	100%	10	8	2			7.3	8.3	8.9			7.4	8.1	8.4			20.9	19.0	18.8							
66	100%	10	6	2			7.3	8.3	8.9			7.4	8.1	8.4								950		900		
Initial of Investigator		RBF	RBF	RBF			RBF	RBF	JMF			RBF	RBF	JMF			JMF	RBF	JMF			RBF		J4F		

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:

Russel Snyderburg

Joseph Fandolt

RBF Burdick

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: 20 Method:

Lab: 0.5 Method: HACH

Lab: 0.53 Method: IODO

~~De-chlorinated~~:

Measurements by: RBF, MF

Reviewed by:

Date: 3/3/88

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

BIOASSAY DATA SHEET

Test Number: B-8
Test Type: Static Renewal (Screening) Conventional
Other:

Sample Collection: Date 11-9-87 Time 1105
Test Beginning: Date 11-10-87 Time 1030
Test Ending: Date 11-12-87 Time 1030
Test Organism: N. Leeds
Age/Life Stage: 19 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	6	5	5	5			8.4	8.0	8.4			7.7	7.9	8.0			21.2	19.1	19.0							
Control	281	5	5	5			8.5	8.0	8.4			7.7	7.9	8.0								210		215		
50%	52	5	5	5			8.2	8.1	8.6			7.4	7.9	8.1			21.3	19.9	18.4							
50%	23	5	5	5			8.2	8.1	8.7			7.4	7.9	8.1								600		580		
100%	18	5	0	0			7.5	8.0	—			7.3	7.9	8.2			21.4	18.9	18.4							
100%	15	5	0	0			7.5	8.1	—			7.3	8.0	8.2								990		900		
Initial of Investigator		JML	RBF	JE			RBF	RBF	JMF			RBF	RBF	JMF			JMF	RBF	JMF			RBF		JMF		

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

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

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

Signatures:
Russel Frydenborg
Joseph Faiella
V.M. Lurding
J.M. Fe

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: 3.0 Method:
Lab 0.5 Method: HACH
Lab 0.53 Method: DPD
~~Dechlorinated~~:
Measurements by: RBF, MF

Reviewed by:
Date: 3/3/88

Facility/Applicant: Embassy Hills STP
Address:
Contact/District:
Effluent Serial Number:
NPDES Permit Number:

EFFLUENT DATA FORM

Test Number: 87-8
Test Type: Static Renewal, Screening, Conventional
Other:

Sample Collection: Date 11-9-87 Time 1105
Test Beginning: Date 11-10-87 Time 1030
Test Ending: Date 11-17-87 Time 1030
Test Organism: P. promelas
Age/Life Stage: 16 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	25	5	5	5			8.6	7.8	7.9			7.7	7.9	7.9			21.2	19.1	18.5							
Control	42	5	5	5			8.6	7.8	7.9			7.7	7.9	7.9								205		210		
50%	13	5	5	5			8.1	8.0	8.2			7.4	7.9	8.1			21.2	19.0	18.5							
50%	32	5	5	5			8.1	8.0	8.2			7.4	7.9	8.1								600		590		
100%	27	5	4	4			7.6	8.0	7.8			7.3	8.0	8.2			21.4	19.0	18.7							
100%	9	5	5	5			7.6	8.0	8.2			7.3	8.0	8.3								980		950		
Initial of Investigator		JMF	RBF	JE			RBF	RBF	JMF			RBF	RBF	JMF			JMF	RBF	JMF			RBF		JMF		

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

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

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

Signatures: Russell Frydenborg, Joseph Fairchild, Jeff Hurding, Jeff Gibbs

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: 3.0 Method:
Lab: 0.5 Method: HACH
Lab: 0.53 Method: 1000

De-chlorinated:
Measurements by: RBF, MF

Reviewed by:
Date: 3/3/88

C4-1

MICROTOX™ Data Reduction Sheet

SOURCE: Embassy Hills STP

GRAPH (CALC) EC 50 (15 MIN, 15 °C) = 36.6 (%SAMPLE) mg/L

SAMPLE DATA:

SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent - de Cl_2

DATE ASSAYED: 11-10-87 TIME: 9:15

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: Lelee 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	77	48	X	X	
B1		82	51			
C1		75	48			
A2	12.38	71	40	.09	.24	
B2		69	38			
C2		74	41			
A3	24.75	61	31	.27	.56	
B3		61	31			
C3		62	32			
A4	49.5	44	20	.83	1.53	
B4		39	17			
C4		45	21			
A5	99	25	10	2.25	3.90	
B5		23	10			
C5		24	10			
EC_{50} (MIN, °C)				~~22.5~~ 57.4	36.6	

REVIEWED BY:

DATE: 3/3/88

040

MICROTOX™ Data Reduction Sheet

SOURCE: Embassy Hills STP

~~GRAPH~~ CALC EC 50 (15 MIN, 15 °C) = 28.8 %SAMPLE mg/l

SAMPLE DATA:

SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent Cl_2

DATE ASSAYED: 11-10-87 TIME: 8:45 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	97	60	X	X	
B1		90	54			
C1		76	47			
A2	12.38	78	40	.10	.30	
B2		79	41			
C2		83	43			
A3	24.75	59.5	32	.36	.77	
B3		67	31			
C3		68.5	28			
A4	49.5	40	22	1.43	1.60	
B4		35	20			
C4		34	20			
A5	99	9	4	7.55	10.50	
B5		11	5			
C5		11	5			
EC_{50} (MIN, °C)				39.75	28.79	

REVIEWED BY:

DATE: 3/3/88