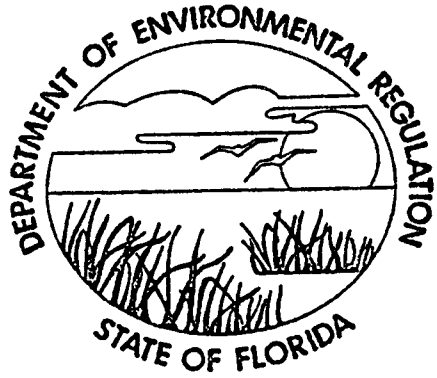


BIOASSAYS OF THE DEPARTMENT OF TRANSPORTATION

STORMWATER RUNOFF PROJECT

MARATHON, MONROE COUNTY, FLORIDA

NO NPDES PERMIT

Sampled 8/5/87

OCTOBER 8, 1987

Biology Section
Division of Environmental Programs

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MARATHON, MONROE COUNTY, FLORIDA

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Biology Section
Bureau of Laboratories and
Special Programs
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EXECUTIVE SUMMARY

DOT Stormwater Runoff, Marathon, Monroe County, Florida, 6 to 8 August, 1987.

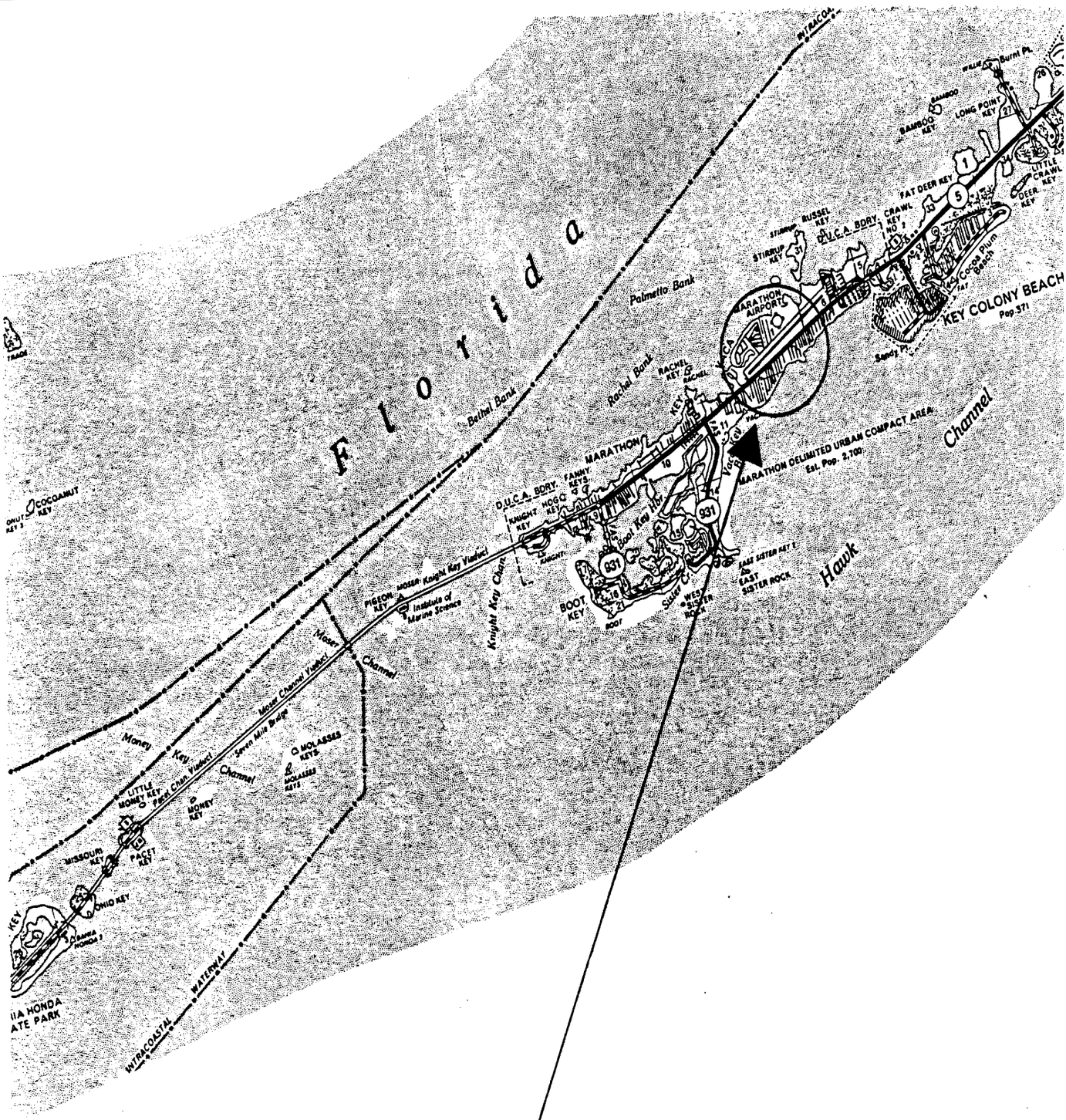
Two static acute toxicity bioassays were performed on a sample of stormwater runoff collected from a drainage pipe leading to the collection system of a Department of Transportation four-lane construction project on U.S. Highway 1 in Marathon, Florida. Results indicated a 48 hour LC_{50} of 216.0% to Ceriodaphnia dubia, which is considered acutely toxic in the absence of a mixing zone. Only one individual died in the Notropis leedsi bioassay; this was not sufficient to calculate an LC_{50}. Chemical analyses were not performed on this stormwater sample.

INTRODUCTION

The Florida Department of Transportation is currently widening U.S. Highway 1 in Marathon, Monroe County (Figure 1). As part of this project, a collection system will be constructed which will route stormwater to two retention ponds to catch and settle suspended particulates (Figure 2). When the project is completed, stormwater overflow will run off into surface waters, but presently is retained by the project collection system and seeps into the groundwater. To evaluate the water quality and potential toxicity of this stormwater, the South Florida District requested that acute toxicity bioassays be performed.

During the first half inch of rainfall on 20 May, 1986, a raw stormwater sample was collected from a drain pipe leading to the collection system. Results indicated that the stormwater was toxic to Ceriodaphnia dubia (LC_{50} = 100%) and caused 30% mortality of Mysidopsis bahia in the 100% sample concentration. No mortality occurred in the Notropis leedsi bioassay. Chemical analyses indicated the presence of the metals copper (28 ug/l), lead (56 ug/l), and zinc (179 ug/l), which may have contributed to the observed toxicity (FDER 1986).

Three followup bioassays were performed on a sample of raw stormwater collected from the same drainage pipe in August, 1987. Results are presented in this report. Those involved in performing the tests and in preparation of this report were: K. M. Lurding and M. L. Roll.



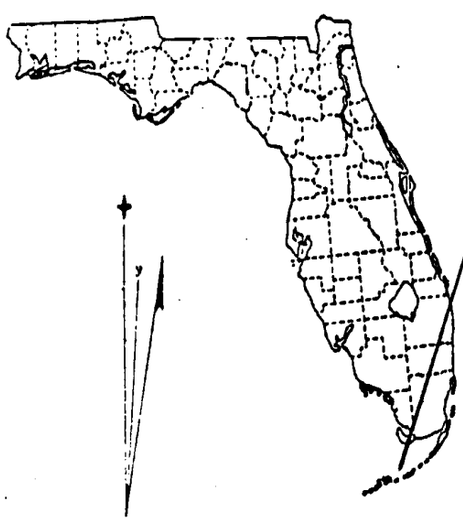


Figure 1. Portions of Monroe County, Florida, including the approximate location of DOT's Stormwater Runoff Project

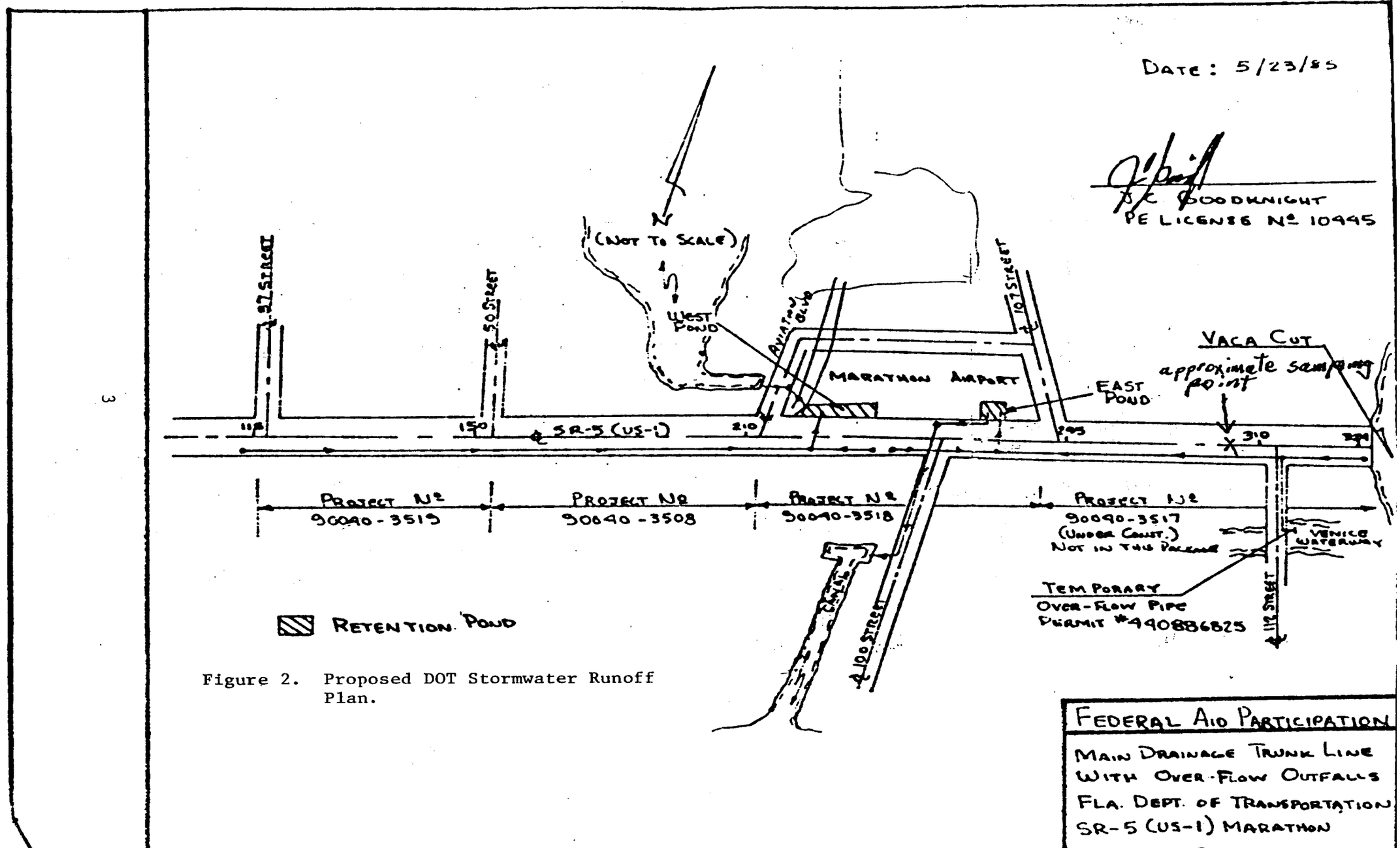


Figure 2. Proposed DOT Stormwater Runoff Plan.

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). The bioassay using one of these organisms, Daphnia pulex, was invalidated due to excessive control mortality (see raw data sheets). 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.

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 22 days old at the time of the test.

Standard Reference Toxicity Assay

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

Two static acute toxicity bioassays were performed on a sample of raw stormwater collected from a drainage pipe leading to the collection system of the DOT Stormwater Runoff Project in Marathon, Florida. The tests were performed from 0920 hours on 6 August to 0920 hours on 8 August, 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 preserved at 4°C on ice. The samples were collected by Mr. R. J. Helbling and Mr. Gus Rios of DER's South Florida District Branch Office in Marathon, Florida at 1040 hours on 5 August, 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 sample in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the sample 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.

Aeration

Since the dissolved oxygen concentrations in certain individual test chambers had decreased and were approaching 40% of saturation (due to BOD), then each of the test chambers and the controls were aerated. This was achieved using a series of separate tubes which were connected to the laboratory compressed air supply. The exact amounts and levels of aeration are noted on the bioassay data forms.

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 flea, Ceriodaphnia dubia. Flint glass jars of 60 ml capacity were used as test chambers, and 50 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. Water fleas were not fed during this test.

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 test.

Test Parameters

Dissolved oxygen (YSI Model 57 oxygen meter), pH (Corning Model 7 pH meter with Orion Ross® combination electrode), temperature (NBS calibrated glass thermometer), and the number of live organisms were recorded for each test concentration at 0 hours, 24 hours, and 48 hours. Total alkalinity and total hardness (measured as $CaCO_3$ using HACH Chemical Company reagents) were recorded at the beginning of the test for the controls and the 100% sample 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% 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.

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

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* and *Notropis leedsi*, static acute toxicity bioassays of Florida Department of Transportation Stormwater Runoff Project, Marathon, Monroe County, Florida, 6 to 8 August, 1987.

Concentration or %	Number of Live Organisms			Dissolved Oxygen[a] (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	8.6	8.1	8.5	8.0	7.9	7.9	20.9	21.1	21.1		
Control	10	10	9	8.6	8.1	8.5	8.0	7.9	8.0					
50%	10	10	10	8.8	6.4	7.5	7.8	7.4	7.7	21.4	21.0	21.1		
50%	10	9	8	8.8	6.4	7.5	7.8	7.4	7.7					
100%	10	9	7	9.0	5.3	7.2	7.6	7.2	7.5	21.8	21.0	21.1		
100%	10	9	8	9.0	5.3	7.2	7.6	7.2	7.5					
Notropis leedsi														
Control	5	5	5	8.2	7.8	8.7	7.5	7.6	8.1	21.6	20.7	20.7		
Control	5	5	5	8.2	7.8	8.7	7.5	7.6	8.1				210	215
50%	5	5	5	8.9	6.6	8.7	7.5	7.2	7.9	21.8	20.5	20.6		
50%	5	5	5	8.9	7.0	8.7	7.5	7.2	7.9				290	281
100%	5	5	4	9.2	6.2	8.3	7.5	7.0	7.8	22.2	20.5	20.7		
100%	5	5	5	9.2	6.2	8.4	7.5	7.0	7.8				360	374

Control Water	
NH_3(as NH_3)(mg/l) 0	Total Alkalinity 108 (mg/l $CaCO_3$)
Total ammonia (as N)(mg/l) 0	Total Hardness 113 (mg/l $CaCO_3$)

100% Sample	
NH_3(as NH_3)(mg/l) 0	Total Alkalinity 96 (mg/l $CaCO_3$)
Total ammonia (as N)(mg/l) 0	Total Hardness 85 (mg/l $CaCO_3$)

[a] All treatments were aerated from 24 to 48 hours.

Table 3. LC_{50} Data for Bioassays of DOT Stormwater Runoff Project, Marthon, Monroe County, Florida, 6 to 8 August, 1987.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Ceriodaphnia dubia	Binomial	a	a
	Moving Average	a	a
	Probit	1153.2% (133.5 and ∞)	216.0% (104.4 and ∞)
Notropis leedsi	Binomial		a
	Moving Average	No Mortality	a
	Probit		a

[a] Due to an insufficient number of dead organisms, or to the irregular distribution of mortality, an LC_{50} value cannot be calculated using this method of statistical analysis.

RESULTS AND DISCUSSION

The bioassay performed on Ceriodaphnia dubia resulted in acute toxicity, with a 48 hour LC_{50} of 216.0% (Table 3). Because this stormwater is not being discharged to surface waters, it does not constitute a violation of water quality standards. Only one individual died in the Notropis leedsi test (Table 2); this was insufficient to calculate an LC_{50}. Chemical analyses were not performed on this stormwater sample.

LITERATURE CITED

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

_____. 1986. Bioassays of the Department of Transportation's Stormwater Runoff Project, Monroe County, Florida. 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.

BIOASSAY DATA FORM

Facility/Toxicant: Ft. Dix Plume Project — Test #: 1, 2, 3
Address: Wrightstown — Type: Screen
NPDES Number: — Organism: C. dubia
Outfall Number: — D. pulex (N. leedsi)

Sample Collected: Date 3/4/87 Time 1040
Test Beginning: Date 3/5/87 Time 9:20
Test Ending: Date 3/8/87 Time 9:20

Test Concentration	Chamber Number	Live Organisms 0	24	48	Dissolved Oxygen (mg/L) 0	24	48	pH 0	24	48	Temperature °C 0	24	48	Conductivity 0	48	Ammonia Total 0	48	Unionized 0	48
Control	15	10	10	10	8.6	8.1	8.5	8.0	7.9	7.9	20.9	21.1	21.1			0		0	
Control	93	10	10	9	8.6	8.1	8.5	8.0	7.9	8.0									
50%	96	10	10	10	8.8	6.4	7.5	7.8	7.4	7.7	21.4	21.6	21.1						
50%	51	10	9	8	8.8	6.4	7.5	7.8	7.4	7.7									
100%	99	10	9	7	9.0	5.3	7.2	7.6	7.2	7.5	21.6	21.0	21.1			0		0	
100%	21	10	9	8	9.0	5.3	7.2	7.6	7.2	7.5									
Control	37	10	10	5	8.7	8.3	8.7	8.0	7.9	8.1*	21.3	20.6	20.9			0		0	
Control	—	10	10	10	8.7	8.5	8.7	8.0	7.9	8.1				185	200				
50%	—	10	10	10	8.8	7.2	8.6	7.8	7.5	7.9	21.4	20.7	20.8						
50%	35	10	8	10	8.8	7.2	8.6	7.8	7.5	7.9				260	270				
100%	55	10	10	10	9.0	5.2	8.4	7.6	7.2	7.8	21.8	20.5	20.8			0		0	
100%	59	10	10	2	9.0	5.2	8.5	7.6	7.2	7.8				340	352				
Control	234	5	5	5	8.2	7.8	8.7	7.5	7.6	8.1	21.6	20.7	20.7			0		0	
Control	—	5	5	5	8.2	7.8	8.7	7.5	7.6	8.1				210	215				
50%	3	5	5	5	8.9	6.6	8.7	7.5	7.2	7.9	21.8	20.5	20.6						
50%	86	5	5	5	8.9	7.0	8.7	7.5	7.2	7.4				290	281				
100%	23	5	5	4	9.2	6.2	8.3	7.5	7.0	7.8	22.1	20.5	20.7						
100%	—	5	5	5	9.2	6.2	8.4	7.5	7.0	7.8				310	374	0		0	
		KML MLR	KML MLR	KML	MLR	MLR	KML	MLR	MLR	KML	MLR	MLR	KML	MLR	KML	MLR		MLR	

NOTES

Control Water

Total Alkalinity (mg/l $CaCO_3$): 108

Total Hardness (mg/l $CaCO_3$): 113

100% Sample

Total Alkalinity (mg/l $CaCO_3$): 96

Total Hardness (mg/l $CaCO_3$): 85

Age of Test Organisms: 22 days-fish

Weight: —

Instrument Calibrations:

	pH Meter: KML MLR	D.O. Meter:	S-C-T Meter:
0 Hours	7 at 7, 9 at 9	9.0 at 21°C	10.7 at 10, 27 at 30
24 Hours	7 at 7; 9 at 9	8.9 at 21.5°C	
48 Hours	7 at 7; 9 at 9	9.0 at 21°C	9.9 at 10; 28 at 30

Chlorine: N.D. (HACH)

Reviewed by: MLR

Date: 10/7/87

Note: D. pulex volumes are only 100 ml due to opacity of sample

* Aeration began 7 Aug - AM - fish and Daphnia and Ceriodaphnia