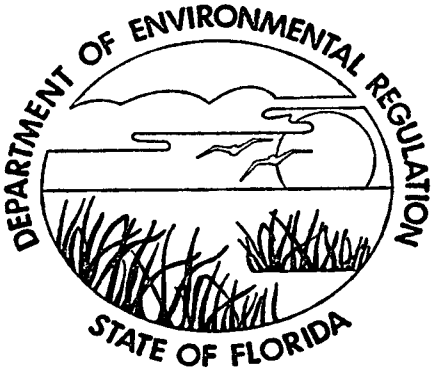


BIOASSAYS OF THE DEPARTMENT OF TRANSPORTATION'S STORMWATER RUN-OFF PROJECT MARATHON, MONROE COUNTY, FLORIDA NO NPDES PERMIT

SAMPLED 5/20/86

JULY 30, 1986

Biology Section
Division of Environmental Programs

BIOASSAYS OF THE DEPARTMENT OF TRANSPORTATION'S STORMWATER RUN-OFF PROJECT MARATHON, MONROE COUNTY, FLORIDA NO NPDES PERMIT

Biology Section
Bureau of Laboratories and
Special Programs
July 30, 1986

EXECUTIVE SUMMARY

DOT Stormwater Runoff, Marathon, on 21 to 23 May, 1986.

The sample of stormwater runoff collected from a drain leading from a DOT holding pond, part of their four lane project at U.S. Highway 1 near Marathon, proved to be toxic to Ceriodaphnia dubia and also produced some Mysidopsis bahia mortality. No mortality, however, occurred in the Notropis leedsi test. A 48 hour LC_{50} of 100% was calculated for the C. dubia test, and approximately 30% of the M. bahia died in the 100% sample. A reason for this toxicity was not readily apparent during the tests. Results of chemical analyses of this stormwater sample indicated the presence of the metals copper (28 ug/l), lead (56 ug/l), and zinc (179 ug/l). These may have contributed to the toxicity noted.

Since the 95% confidence limits for the LC_{50} reported above for the C. dubia test were so large (0 and infinity), we hesitate to call this sample acutely toxic and in violation of state standards. We do recommend, however, that bioassay tests be repeated on a sample of this DOT discharge, and do caution, based on the above results, that toxic effects may be present.

INTRODUCTION

The Florida Department of Transportation is currently widening U.S. Highway 1 in Marathon, Monroe County. As part of this project, several stormwater holding ponds are maintained to catch and settle suspended particulates to prevent their entry into various canals and waterways in the area (Fig. 1). Since roadway construction contaminants may also enter this system, the South Florida District has been concerned of possible toxic effect that the stormwater overflow may have on aquatic organisms. Therefore, the district requested that acute toxicity bioassays be performed on a sample of this DOT project stromwater run-off. A sample was collected from a stromwater drain leading to a nearby waterway, on 20 May, 1986, during or shortly after a rainfall event. Three screening tests (control, 50%, and 100% concentrations) were performed on this sample using both freshwater and marine organisms. The results of these tests are presented in this report. Those involved in performing the tests and in preparation of this report were: R. B. Frydenborg, M. L. Roll, and P. G. Weiland.

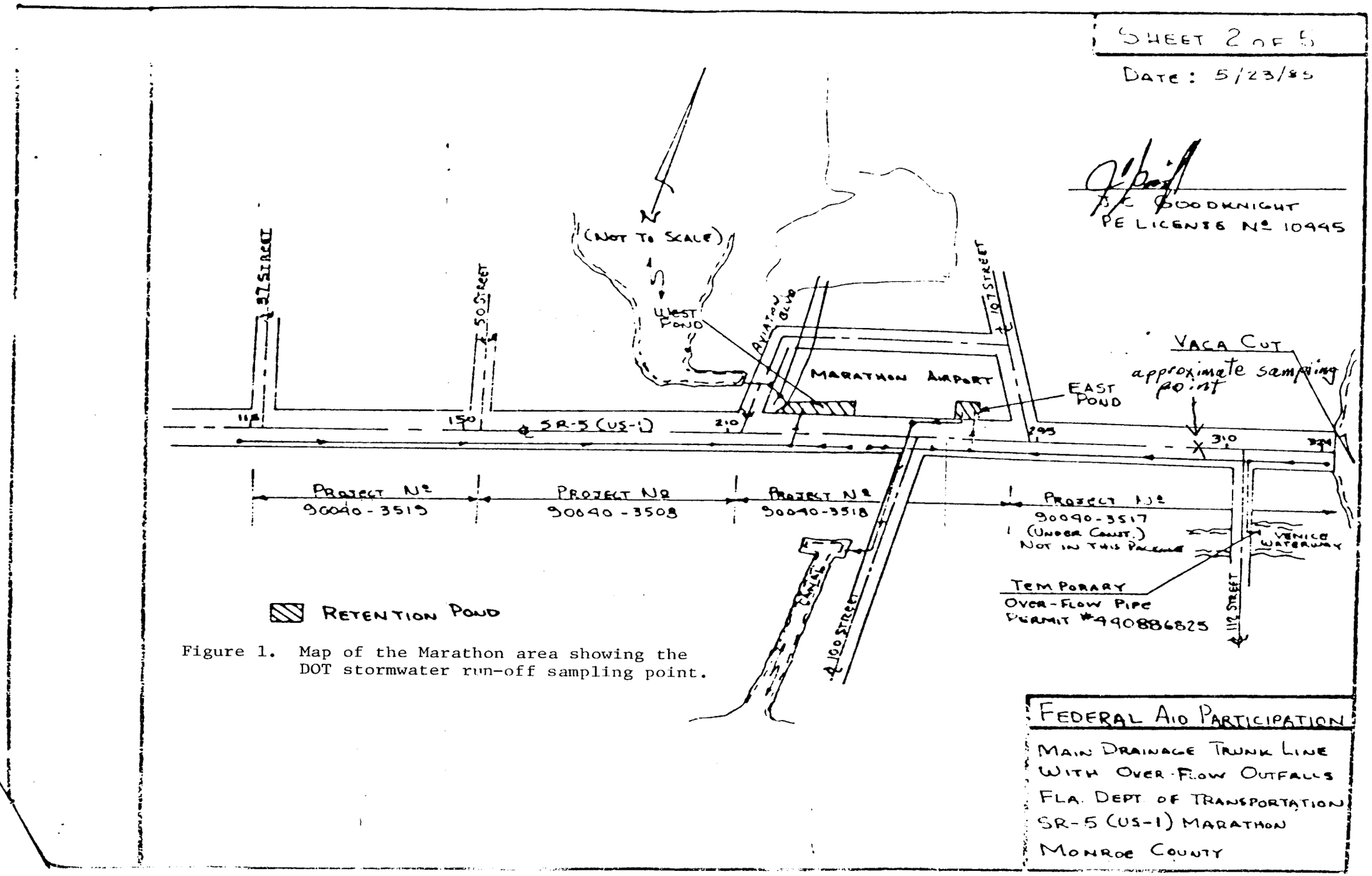


Figure 1. Map of the Marathon area showing the DOT stormwater run-off sampling point.

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), 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.

History and Maintenance of Bioassay Organisms

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 cycle and are fed Tetra-Min® and frozen Artemia (brin 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 approximately three weeks old at the time of the test.

Mysidopsis bahia (mysid shrimp) - In September, 1978, approximately 300 adult mysid shrimp were obtained from the EPA laboratory in Gulf Breeze, Florida. Additional mysid shrimp are obtained from EPA and private labortories as needed. The mysids are cultured under a 16:8 hour light-dark cycle in 10 and 15 gallon all-glass aquaria equipped with undergravel filtration and aeration. The mysid shrimp are cultured in several salinities (20-30 ppt) to minimize acclimation effects to high

salinity effluents. Low salinity effluents are adjusted to approximately 20 ppt. Seawater, used for culturing the test organisms and as diluent in the bioassays, is prepared by mixing artificial sea salts (Forty Fathoms®) and well water to obtain a final salinity of approximately 20 ppt. Freshwater, for dilution of the seawater as necessary, is obtained from a deep well near the DER laboratory. The mysids are fed twice daily and once daily on weekends with freshly hatched Artemia (brine shrimp) and are kept at a constant temperature of 23° ± 2°C. Adult or large juvenile mysids were used in this test.

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 separate 48 hour acute toxicity screening tests were performed on a sample of stormwater run-off from the DOT holding pond in Marathon. The tests were performed from 1012 hours on 21 May to 1000 hours on 23 May, 1986.

The sample was collected by Mr. R. J. Helbling of DER's Marathon office at 1310 hours on 20 May, 1986, using standard wastewater sampling procedures. 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 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.

Salinity Adjustment

Since this particular bioassay sample is discharged to a marine environment, the Biology Section used a saltwater test organism to evaluate toxicity. Therefore, artificial sea salts (Forty Fathoms®) were added to the 100% concentration until a salinity of approximately 23 ppt was obtained. This salinity is well within the salinity tolerance of the test organism. A control was also employed, consisting of artificial seawater at a similar salinity of approximately 23 ppt.

pH Adjustment

Since the toxicity, or lack of toxicity, in a bioassay sample may be dependent on the sample's pH, the Biology Section prefers, and recommends, that the pH of a sample at the start of the bioassay be similar to the pH measured during sampling. In this particular case, the sample used for the saltwater test was adjusted with an acid (0.1N HCl). This was necessary due to the pH having increased between sampling and initiating the test. This pH increase was likely a result of salinity adjusting the sample. The pH of the saltwater sample was adjusted to $\pm$ 0.3 s.u. of the value measured at sampling. This procedure provides the fairest test possible considering the varied chemical substances in complex wastewaters, the toxicities of which are dependent on pH.

Freshwater Tests

Freshwater for dilution of the sample was obtained from the same DER deep well that is used to culture the test organisms. The concentration series selected for this study is presented 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 250 ml capacity were used as test chambers, and 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. 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.

Saltwater Test

The concentration series selected for the M. bahia screening test is presented in Table 1. Two replicates of the 100% concentration and of the control were used to test with Mysidopsis bahia. Immediately prior to the bioassay, a suitable number of mysids were transferred by net from the

appropriate salinity aquarium to a culture dish for observation and selection of individuals for the test. Individual mysid shrimp were siphoned using a glass tube, carefully examined for injury, and transferred to wide mouth quart jars, each containing 500 ml of the appropriate concentration. For each concentration, the mysid shrimp were pre-acclimated to within 4 ppt salinity. Five organisms were loaded per test chamber, giving ten per concentration. Based on the recommendations of EPA (1978), the mysids were fed brine shrimp (Artemia) nauplii at the rate of approximately 10 to 20 nauplii per mysid per day to minimize cannibalism.

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. The specific conductance or salinity (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. These values were recorded on the bioassay data forms and are presented in Tables 2 and 3.

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

Ceriodaphnia dubia (water flea)

Concentration	Dilution Water	Effluent	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)

Mysidopsis bahia (mysid shrimp)

Concentration	Dilution Water	Effluent	Total Volume
Control	500 ml	--------	500 ml
50.0%[a]	250 ml	250 ml	500 ml
100.0%	------	500 ml	500 ml

[a] A 50% concentration was not used in the *M. bahia* test.

Table 2. Data recorded during 48 hour, Ceriodaphnia dubia and Notropis leedsi, static acute toxicity bioassay of the DOT Stormwater Run-off, Marathon, Monroe County, on 21 to 23 May, 1986.

Freshwater Tests

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	7.9	8.8	8.5	8.1	7.9	8.0	21.0	19.7	21.6		
Control	10	9	9	7.8	8.8	8.5	8.1	7.9	8.0				200	225
50.0%	10	10	7	7.6	8.9	8.6	8.1	7.9	7.9	21.1	19.6	21.4		
50.0%	10	9	7	7.6	8.9	8.6	8.1	7.9	7.9				205	230
100.0%	10	10	5	7.6	8.4	8.5	8.2	7.8	7.8	21.2	19.4	21.3		260
-a														
Notropis leedsi														
Control	5	5	5	7.2	8.3	7.9	7.7	7.8	7.8	21.6	19.6	21.1		
Control	5	5	5	7.3	8.3	7.9	7.7	7.	7.				200	210
50.0%	5	5	5	7.4	7.9	8.0	7.7	7.6	7.6	21.7	19.4	21.0		
50.0%	5	5	5	7.4	7.9	7.9	7.7	7.6	7.6				210	225
100.0%	5	5	5	7.4	7.0	6.6	8.1	7.6	7.6	22.0	19.3	20.8		
100.0%	5	5	5	7.4	7.0	6.6	8.1	7.6	7.6				212	255

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

100% Effluent	
NH_3(as NH_3)(mg/l) _____	Total Alkalinity _____ (mg/l $CaCO_3$)
Total ammonia (as N)(mg/l) _____	Total Hardness _____ (mg/l $CaCO_3$)

[a] Due to an oversight, a second 100% concentration was not used.

Table 3. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of the DOT Stormwater Run-off,,Marathon, Monroe County, on 21 to 23 May, 1986.

Saltwater Test

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Control	5	5	5	8.2	8.1	7.5	8.6	8.5	8.2	22.0	20.1	21.2		
Control	5	5	5	8.2	8.1	7.5	8.6	8.5	8.2				22.5	23.0
100.0%	6[a]	6	6	7.1	7.4	7.0	7.8[b]	7.7	7.6	22.0	19.8	21.2		
100.0%	5	2	2	7.1	7.4	7.0	7.8[b]	7.7	7.6				22.5	23.0

Control Water

NH_3(as NH_3)(mg/l) _____ Total Alkalinity _____ (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) _____ Total Hardness _____ (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) _____ Total Alkalinity _____ (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) _____ Total Hardness _____ (mg/l $CaCO_3$)

[a] Extra organism loaded by mistake, allowed to remain.

[b] The pH of these test chambers was adjusted to the values shown due to the significant increase in pH caused by the salinity adjustment.

Table 4. LC_{50} Data for Bioassays of the DOT Stormwater Run-off, Marathon, on 21 to 23 May, 1986.

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	100.0% (0 and ∞)
	Moving Average	a	100.0% (0 and ∞)
	Probit	a	100.0% (0 and ∞)
Notropis leedsi	Binomial	No Mortality	No Mortality
	Moving Average		
	Probit		
Mysidopsis bahia	Binomial	a	a
	Moving Average	a	a
	Probit	a	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.

Table 5. Results of Chemical Analyses Performed on the Sample of DOT Stormwater Run-off, Marathon, collected 20 May, 1986.

Organics	
Acid Extractbles:	N.D.
Base Neutral Extractables:	N.D.
Pesticide Extractables:	N.D.
Metals	
Arsenic	< 3 ug/l
Cadmium	< 2 ug/l
Chromium	N.D.
Copper	28 ug/l
Lead	56 ug/l
Mercury	N.D.
Selenium	N.D.
Silver	N.D.
Zinc	179 ug/l

N.D. = None Detected

RESULTS AND DISCUSSION

The sample of stromwater runoff collected from a drain leading from a DOT holding pond, part of their four lane project of U.S. Highway 1 near Marathon, proved to be toxic to Ceriodaphnia dubia and also produced some Mysidopsis bahia mortality. No mortality, however, occurred in the Notropis leedsi test. A 48 hour LC_{50} of 100% was calculated for the C. dubia test, and approximately 30% of the M. bahia died in the 100% sample. A reason for this toxicity was not readily apparent during the tests. Results of chemical analyses of this stormwater sample indicated the presence of the metals copper (28 ug/l), lead (56 ug/l), and zinc (179 ug/l). These may have contributed to the toxicity noted. No organic compounds were detected in the stormwater.

Biology Section's Recommendation

Since the 95% confidence limits for the LC_{50} reported above for the C. dubia test were so large (0 and infinity), we hesitate to call this sample acutely toxic and in violation of state standards. We do recommend, however, that bioassay tests be repeated on a sample of this DOT discharge, and do caution, based on the above results, that toxic effects may be present.

LITERATURE CITED

Florida Department of Environmental Regulation, Biology Section. 1985. Quality Assurrance 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 pp.

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.