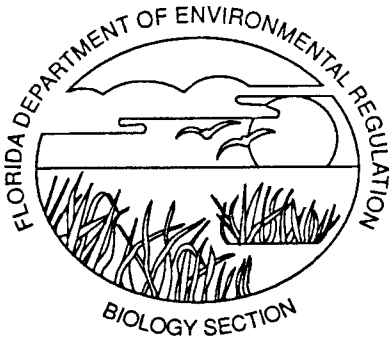


Bioassays of

Key Trailer Court Sewage Treatment Plant

Marathon, Monroe County, Florida

NPDES #FL0023566

Sampled 4/25/88

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DO NOT REMOVE

September 20, 1988

Biology Section
Division of Administrative and Technical Services

Bioassays of

Key Trailer Court Sewage Treatment Plant

Marathon, Monroe County, Florida

NPDES #FL0023566

Biology Section
Bureau of Laboratories
September 20, 1988

Executive Summary

Key Trailer Court Sewage Treatment Plant, Marathon, Monroe County, Florida, NPDES #FL0023566, on 27 to 29 April, 1988.

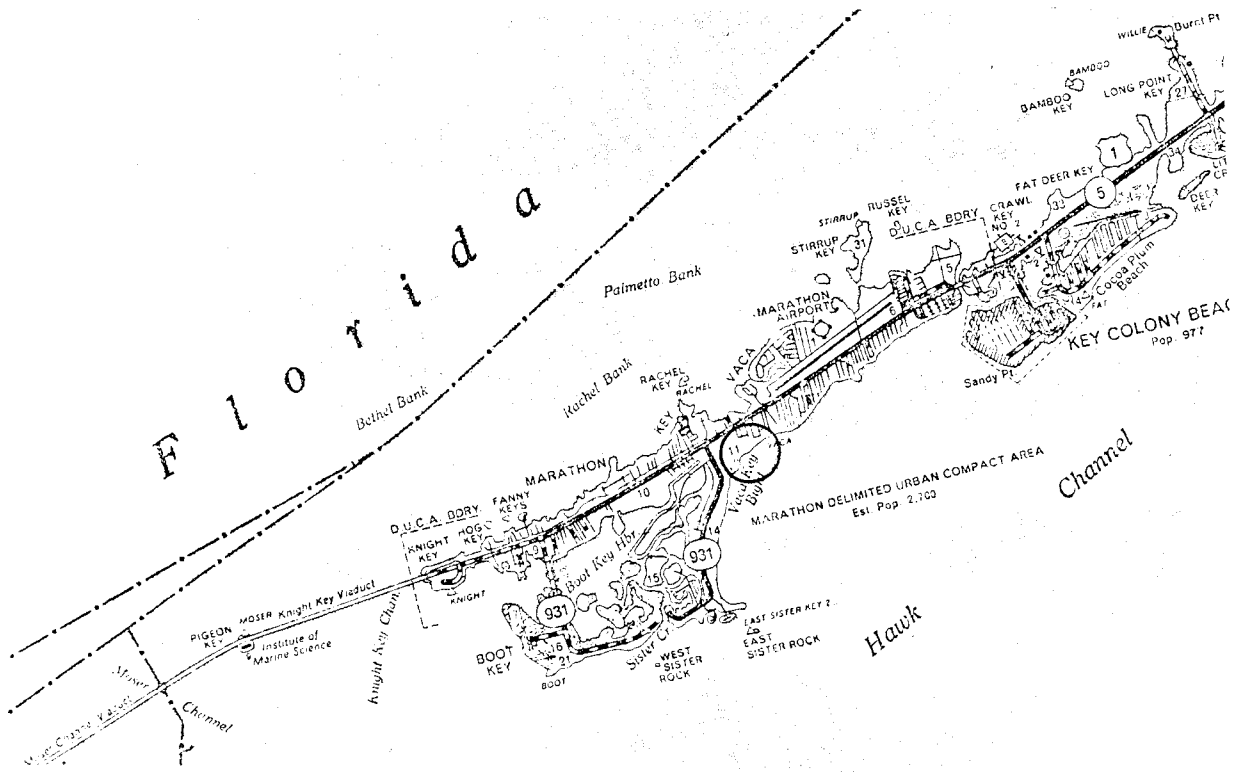
The sample of prechlorinated recovered water from the Key Trailer Court's STP was not toxic to the bioassay test organism, *Mysidopsis bahia*. The sample was stimulatory to the Microtox® assay organism, *Photobacterium phosphoreum*.

Chemical analyses detected numerous unidentified pesticide components sensitive to ECD (Electron Capture Detectors) and NPD (Nitrogen Phosphorus Detectors) in the organics sample. The metal zinc (32.0 µg/l) was detected in the metals sample.

Introduction

The Key Trailer Court's Sewage Treatment Plant is located at 6099 Overseas Highway, Monroe County, Florida (Figure 1). The 0.015 MGD average annual flow of this facility discharges into a dead end canal opening to Class III waters of the Atlantic Ocean. Treatment consists of an activated sludge extended aeration process, including a clarification tank and aerobic sludge tanks. Disinfection is by chlorination in a chlorine contact chamber.

To evaluate the existence and extent of toxicity of the discharge from this facility, the Biology Section performed one acute toxicity bioassay and one Microtox® assay on a prechlorinated sample. The results of those tests are in this report. Those involved in performing the tests and in the preparation of this report were: J. M. Faircloth, R. B. Frydenborg, K. M. Lurding, and L. A. Williams.



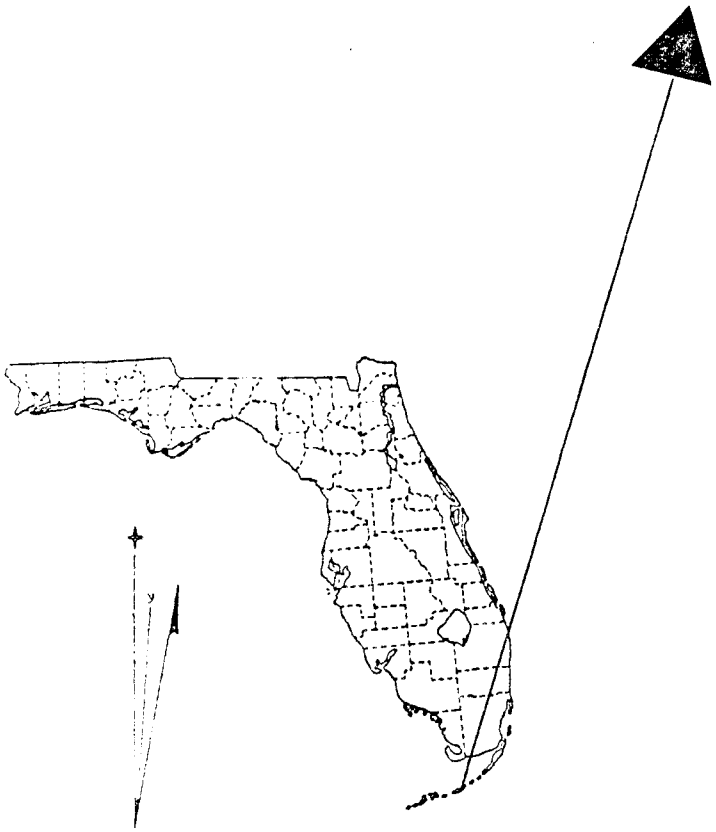


Figure 1. Location map of Key Trailer Court STP Marathon, Monroe County, Florida.

Methods and Materials

Test methods used to conduct these static bioassays are extensively described by Peltier and Weber (1985) and the FDER Biology Section (1985). The organisms selected for this study include a species recommended by Peltier and Weber (1985), the U.S. EPA (1978), and the FDER Biology Section (1985). Additionally, the Biology Section maintains cultures of several species of organisms indigenous to the State of Florida. One of these indigenous species was also selected for testing. Life histories of these test organisms and maintenance procedures used to culture them are detailed below.

The Microtox® assay was run according to the manufacturer's protocol described in the Microtox® System Operating Manual (Beckman 1982).

1. History and Maintenance of Bioassay Organisms

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 laboratories 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 (15–30 ppt) to minimize acclimation effects to high salinity effluents. Seawater, used as diluent in the bioassay, was prepared by mixing artificial sea salts (Forty Fathoms®) and well water to obtain a final salinity of approximately 10 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.

Photobacterium phosphoreum, a luminescent marine bacterium, is maintained as a freeze-dried culture, purchased from Microbics Corporation. Lot number M-606 was used in this assay. Immediately prior to the assay, the culture is rehydrated with 1.0 ml of specially purified water (Microtox Reconstitution Solution).

2. Standard Reference Toxicity Assays

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

3. Test Methods

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

Sampling Methods and Test Dates

One 48 hour acute toxicity bioassay and one Microtox® assay were performed on a prechlorinated sample from the Key Trailer Court facility. The tests were performed from 1015 hours on 27 April to 1015 hours on 29 April, 1988.

Separate samples were obtained for chemical and biological testing. Samples for metal analyses were preserved with $HN0_3$. Samples for organic chemical analyses and for the bioassays were unpreserved. The samples were collected by Mr. Gustavo Rios of the FDER's South Florida District Branch office in Marathon, using standard wastewater

procedures. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 49 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.

Saltwater Tests

Saltwater for dilution of the effluent samples and a control was prepared from artificial sea salts (Forty Fathoms®), and adjusted with DER well water until a salinity of approximately 10 ppt was achieved. For test concentrations of 60% and below, this diluent was mixed directly with the effluent, achieving a range of final salinities (9.4 ppt to 9.8 ppt). One control group (Table 2) was used to ensure that any mortality in the test organisms was not the result of toxic properties associated with the diluent or the salinity range. The control groups included:

1) Diluent Control - Artificial seawater (9.5 ppt). This represents the highest concentration of artificial seawater used in the bioassays, and tests for toxicity in the diluent. This is also the highest salinity used in the bioassays, and therefore tests the tolerance of the organisms to this salinity.

The concentration series selected for this study (Table 1) is that recommended by APHA (1980). Two replicates of each test concentration and of the controls were used to test with *Mysidopsis bahia*. Immediately prior to the bioassays, 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 tests. Individual mysid shrimp were siphoned using a glass tube, 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 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 before use according to manufacturer's recommendations. Ammonia concentrations, including the total and unionized fraction (expressed in mg/l as N and as NH_3, respectively), were recorded in the controls and the 100% reclaimed water test concentration at the beginning of the test. Total ammonia concentrations were determined using an Orion specific ion probe and an Orion Ionalyzer®. The unionized fraction was then calculated based on the total ammonia concentration and the pH, temperature, and salinity of the sample. These values were recorded on the bioassay data forms and are presented in Table 2.

Microtox® Test

Upon receipt at the DER laboratory, a 20 ml subsample was collected for Microtox® analysis. The sample was adjusted to 2% salinity with reagent grade dry sodium chloride to maintain the osmotic balance of the test bacterium during the assay. Four two-fold dilutions were made of the test sample resulting in a test range of 97%, 49.5%, 24.75%, 12.38%, and 0%. An incubation temperature of 15 °C was maintained throughout the assay. Ten µl of bacterial suspension was added to each of the 1.0 ml test dilutions. Light output (luminescence) was read after 5 and 15 minutes incubation, using the Microtox® analyzer and recorded on a Perkin-Elmer strip chart recorder. In the presence of a toxicant, the luminescence of the test organisms will decrease. In the presence of a stimulant, the luminescence will increase. Toxicity is calculated as a function of the light lost in the test sample, normalized to the control, and is reported as the effective concentration at which a 50% reduction, of light output is determined in 15 minutes (EC_{50}). Results are given in Table 3.

Results and Discussion

The water sample (salinity = 10 ppt) taken from the Key Trailer Court STP was not toxic to the bioassay test organism, *Mysidopsis bahia*. The sample was stimulatory to the Microtox® assay organism, *Photobacterium phosphoreum*. The sample was taken prior to chlorination and therefore had no total residual chlorine. No ammonia, either total or unionized, was detected in the sample.

Chemical analyses were performed on the sample. The pesticide extractables analyses detected numerous components sensitive to ECD and NPD. No components were detected in the acid extractables or base neutral extractables analyses. The metal zinc (32.0 µg/l) was detected during the metal analyses. In conclusion, the sample from this facility was not toxic on 25 April, 1988

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

Mysidopsis bahia (mysid shrimp)

Concentration	Dilution Water	Sample	Total Volume
Control	500 ml	0 ml	500 ml
30.0%	350 ml	150 ml	500 ml
60.0%	200 ml	300 ml	500 ml
100.0%	0 ml	500 ml	500 ml

Table 2. Data recorded during th 48 hour acute toxicity bioassay of the pre-chlorinated discharge from the Key Trailer Court STP, Marathon, Monroe County, Florida, NPDES #FL0023566 on 27 to 29 April, 1988.

Facility:	**Key Trailer Court STP**			NPDES # **FL0023566**		Facility Type:	**Private**
Location:	Monroe County			Contact/District:	G. Rios		
Sample Collection Date:	4/25/88	Time:	915	Test type:	Static Screening		
Test Beginning Date:	4/27/88	Time:	1015	# tests:	1	Receiving Water:	Gulf of Mexico via Artificial Canal
Test Ending Date:	4/29/88	Time:	1015	Chlorination Type:	pre-chlorination		

Parameter		
Total Residual Cl2:	0	
Method:	Hach	
By:	RBF	
Salinity:	10.0	
Ammonia:	Control Water	100% Sample
Unionized NH3:	0.00	0.00
Total NH3:	0.008	0.0

Organism: ***Mysidopis bahia*** Age/Life stage: less than 21 days

LC50: Binomial: Moving Average: Probit:

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Salinity	
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	5.0	8.4	6.5	8.5	7.2	7.1	21.5	19.4	19.2	-	-
Control	5	5	5	5.1	8.4	6.3	8.5	7.2	7.1	-	-	-	9.5	9.0
30%	5	3	3	6.2	8.2	6.7	8.0	7.7	7.6	21.4	19.3	19.1	-	-
30%	5	5	5	6.0	8.2	6.8	8.0	7.7	7.6	-	-	-	9.8	9.2
60%	5	5	5	6.7	8.4	6.0	7.8	7.9	7.9	20.4	19.1	19.0	-	-
60%	5	5	5	6.8	8.4	6.0	7.8	7.9	7.9	-	-	-	9.9	9.8
100%	5	5	5	7.6	8.4	6.3	7.7	7.9	8.1	19.5	19.1	19.0	-	-
100%	5	5	4	7.9	8.4	6.5	7.7	7.9	8.1	-	-	-	10.0	10.0

	Control water	100% sample
Alkalinity:	-	-
Hardness:	-	-

Table 3. Microtox test results , Key Trailor Court STP, Marathon, Monroe County, NPDES#FL0023566 , on 4/27/88.

Microtox Test Culture lot # M-606

Final Reclaimed Water

% Sample	Post-chlorination	
	5-min	15-min
0	95	57
0	86	52
0	86	53
12.38	95	60
12.38	94	58
12.38	92	58
24.75	97	61
24.75	96	59
24.75	98	62
49.5	98	63
49.5	99	63
49.5	99	63
99	99	66
99	97	64
99	92	60

EC-50 Non-chlorinated (15 minute , 15°C) = Stimulatory

EC50 (15 minute, 15°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

American Public Health Association. 1980. Standard methods for the examination of water and wastewater. 15th ed. 1,134 p.

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

Florida Department of Environmental Regulation. 1985. Quality assurance manual for performing acute toxicity tests (Second Edition). Biology Section, Tallahassee.

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

U.S. Environmental Protection Agency. 1978. Bioassay procedures for the Ocean Disposal Permit Program. Environmental Research Laboratory. Gulf Breeze, Florida. EPA-600/9-78-010. 121 p.

Industry/Toxicant Key Trailer Court STP
Address 6097 Overseas Hwy, Marathon FL, Monroe Co.
Contact/District G. Rios / South FL Basin, Marathon
Effluent Serial Number 001 Type Chlorination
NPDES Permit Number FL0023566

BIOASSAY DATA FORM

Test Number: 1 of 1
Test Type: Static / Renewal / Screening / Definitive
Other:

Sample Collection: Date 4-25-88 Time 0915
Test Beginning: Date 4-27-88 Time 1015
Test Ending: Date 4-29-88 Time 1015
Test Organism Mysidopsis Bahia
Age/Life Stage less than 24 hrs, 48 hrs
NOTE: 96 hr = 4-29, 45 min

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	SALINITY 0	24	48	72	96
Control	43	5	5	5			5.0	8.4	6.5			8.5	7.2	7.1			21.5	19.4	19.2							
Control	5	5	5	5			5.1	8.4	6.3			8.5	7.2	7.1								9.5		9.0		
30%	52	5	3*	3			6.2	8.2	6.7			8.0	7.7	7.6			21.4	19.3	19.1							
30%	33	5	5	5			6.0	8.2	6.8			8.0	7.7	7.6								9.8		9.2		
60%	25	5	5	5			6.7	8.4	6.0			7.8	7.9	7.9			20.4	19.1	19.0							
60%	66	5	5	5			6.8	8.4	6.0			7.8	7.9	7.9								9.9		9.8		
100%	131	5	5	5			7.6	8.4	6.3			7.7	7.9	8.1			19.5	19.1	19.0							
100%	11	5	5	4			7.9	8.4	6.5			7.7	7.9	8.1								10.0		10.0		
Initial of Investigator		RBF	MF	RBF			RBF	MF	RBF			RBF	MF	RBF			RBF	MF	RBF			RBF		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	U	T
Control Water:		0.003
100% Sample:		0.0
Other:		

Hour 0

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

alga - 56.4

Signatures:
Marshall Faulstich
Ruth Frydenborg
KM Ludwig

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7@7.96@9	9.0@21°C	10.5@15°C, 47.5@30
24 Hours	7@7.96@9	9.2@20°C	
48 Hours	7@7.96@9	9.2@20°C	10.1@15, 35@30
72 Hours			
96 Hours			

Total Residual Chlorine: sample taken prior to chlorination

Recalibrated: 0 Method: —
Lab: C Method: Hach
Lab: Method:

Reviewed by:

Date: 9/16/88

Recalibrated Measurements by:

Other:
* 1 dead body, 1 missing

Obs Cond = 1350 umhos @ 1250 umhos, 4Kh

C87

MICROTOX™ Data Reduction Sheet

SOURCE: Fla. Keys MHP

Stimulatory

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

SAMPLE DATA:

SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent - non C22

DATE ASSAYED: 4.27.88 TIME: 9:00 AM

VISUAL COLOR: none CORRECTION REQUIRED: YES (NO)

VISUAL TURBIDITY: none SEPARATION REQUIRED: YES (NO)

INITIAL pH: pH ADJUSTED TO: WITH:

OSMOTICALLY ADJUSTED WITH: MOAS (DRY NaCl) OTHER .1 gm

PRIMARY DILUTION OF SAMPLE:

OPERATOR: Leslie Williams REAGENT VIAL LOT NO. M- 606

REMARKS: 5‰ = 10

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	Γ5	Γ15	NOTES
A1	0	95	57	X	X	
B1		86	52			
C1		86	53			
A2	12.38	95	60	-.050	-.080	
B2		94	58			
C2		92	58			
A3	24.75	97	61	-.082	-.110	
B3		96	59			
C3		98	62			
A4	49.5	98	63	-.098	-.143	
B4		99	63			
C4		99	63			
A5	99	99	66	-.073	-.147	
B5		97	64			
C5		92	60			
EC_{50} (MIN, ° C)						

REVIEWED BY:

DATE: 9/16/88