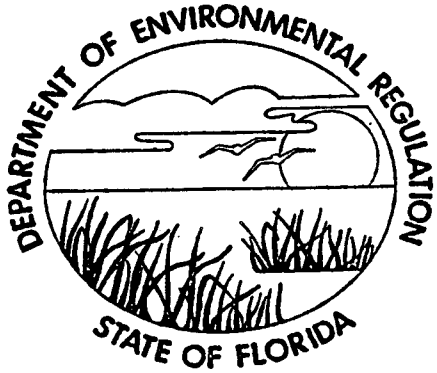


BIOASSAY OF THE KEY WEST

SEWAGE TREATMENT PLANT

MONROE COUNTY, FLORIDA

NPDES #FL0025976

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MAY 15, 1986

Biology Section
Division of Environmental Programs

BIOASSAY OF THE KEY WEST SEWAGE TREATMENT PLANT MONROE COUNTY, FLORIDA NPDES #FL0025976

Biology Section
Bureau of Laboratories and
Special Programs
May 15, 1986

EXECUTIVE SUMMARY

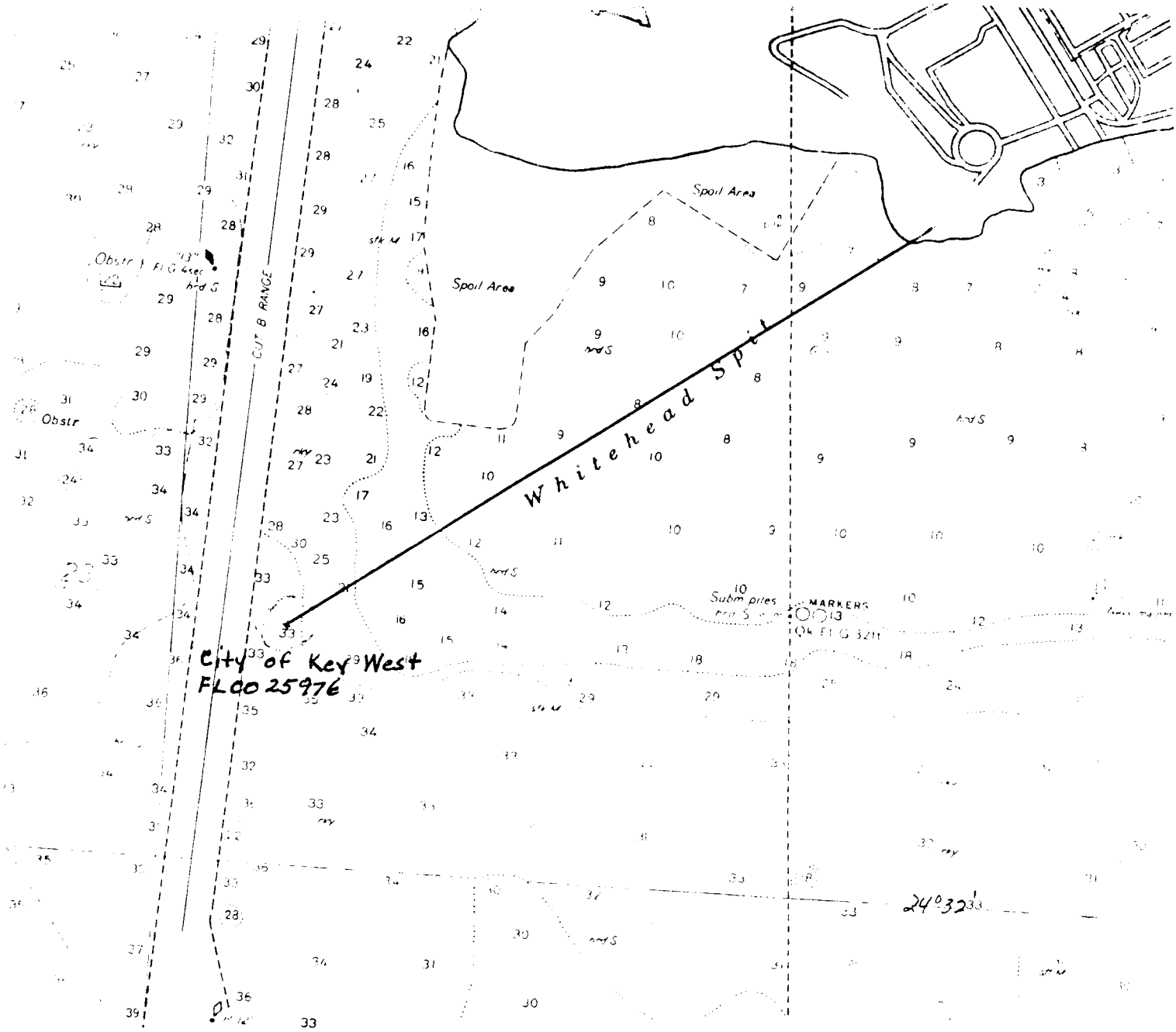
Key West Sewage Treatment Plant, NPDES #FL0025976, on 18 to 20 March, 1986.

The sample of untreated sewage from the Key West facility, primarily a collection and pumping station, was acutely toxic to Mysidopsis bahia. The sample, under constant aeration, produced a 24 hour LC_{50} of 54.6%, and a 48 hour LC_{50} of < 50%. Chlorine was not responsible for this toxicity since this effluent is not chlorinated. Additionally, the small concentration of unionized ammonia (0.04 mg/l) measured in the sample was insufficient to have caused any toxicity. The results of chemical analyses indicated the presence of butyl benzyl phthalate (1.7 ug/l), and the metals cadmium, copper, and silver in this effluent. These constituents probably contributed to the toxicity noted.

INTRODUCTION

The Key West Sewage Treatment Plant, actually a collection and pumping station, is located at 1200 Thomas Street, Key West, Monroe County, Florida. Municipal sewage, averaging 4.3 MGD, is simply collected and pumped to the Atlantic Ocean (Hawk Channel) through a pipe 4200' from shore and 33' deep (Fig. 1). This discharge is neither chlorinated nor treated in any manner (Mr. R. J. Helbling, personal communication).

To evaluate the toxicity of this discharge, the South Florida District requested that an acute toxicity bioassay be performed. A sample of this untreated sewage was collected from the pump station and a bioassay was performed from 18 to 20 March, 1986. The results of this test are presented in this report. Those involved in performing the test and in preparation of this report were: C. W. Dye, M. L. Roll, and P. G. Weiland.



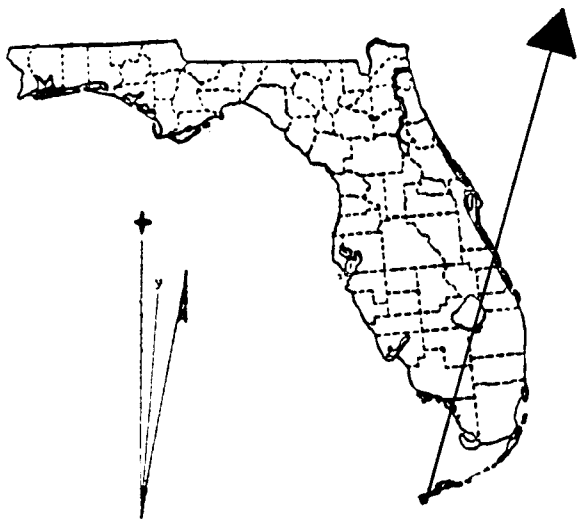


Figure 1. Location of the Key West Sewage Outfall

METHODS AND MATERIALS

Test methods used to conduct this static bioassay are extensively described by Peltier and Weber (1985), the U.S. EPA (1978), and the FDER Biology Section (1985). The organisms selected for this study is among those recommended by Peltier and Weber (1985), the U.S. EPA (1978), and the FDER Biology Section (1985). The life history and maintenance procedures used to culture the organism are detailed below.

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. 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 over a wide rnge of salinities (10-30 ppt) to minimize acclimation periods to low or high salinity effluents. Seawater, used for culturing the test organisms and as diluent in the bioassays, is prepared by mixing artificial sea salts (Forty Fathom®) and well water to obtain a final salinity of 20-30 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° $\pm$ 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

A 48 hour static acute toxicity bioassay was performed on a sample of untreated sewage collected from the Key West pumping station. The test was performed from 1130 hours on 18 March to 1130 hours on 20 Mrch, 1986.

The sample was collected by Mr. R. J. Helbling of DER's Marathon Office during the morning of 17 March, 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 22° ± 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 near the range recommended in the above referenced bioassay protocols. This avoids stressful effects caused by exposure to unusually cold or warm samples. The temperature range in this particular test was slightly higher than usual due to the test being performed beneath an exhaust hood in a warmer laboratory instead of in the temperature-controlled laboratory.

Aeration

Since the sample to be used for bioassay analysis contained a dissolved oxygen concentration of less than 40% of saturation, the entire sample was aerated to approximately 6.0 mg/l. This was achieved using a single airstone connected to the laboratory compressed air supply. Additionally, since the dissolved oxygen concentrations were expected to decrease (due to BOD), each test chamber was aerated throughout the test. The exact amounts and levels of aeration are noted on the bioassay data form.

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 16 ppt was obtained. This salinity is well within the salinity tolerance of the test organism. Additional information concerning salinity adjustment and saltwater tests is given below.

Saltwater Tests

Saltwater for dilution of the effluent sample was prepared from artificial sea salts (Forty Fathoms®), and adjusted with DER well water until a salinity of approximately 20 ppt was achieved. For the 50% test concentration, this diluent was mixed directly with the effluent, achieving a final salinity of approximately 15 ppt. The control used for this test was the same artificial seawater used to dilute the effluent.

The concentration series selected for this study is presented in Table 1. Two replicates of each test 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 tests. Individual mysid shrimp were siphoned using glass tube, carefully examined for injury, and tranferred

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 oaded 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 daily according to manufacturer's recommendations. Ammonia concentations, including the total and unionized franction (expressed in mg/l as N and as NH_3, respectively), were recorded in the control and the 100% effluent 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 from and are presented in Table 2.

Table 1. Concentration Series and Volumes Used for This Static Bioassay.

Mysidopsis bahia (mysid shrimp)

Concentration	Dilution Water	Effluent	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, Mysidopsis bahia, static acute toxicity bioassay of the Key West Sewage Treatment Plant, Monroe County, NPDES #FL0025976, on 18 to 20 March, 1986.

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	8.7	6.2	7.2	8.1	8.2	8.4	23.7	25.1	23.9			
Control	5	5	5	8.7	6.2	7.2	8.1	8.2	8.4				19.7		20.5
50.0%	5	4	1	6.1	6.9	7.6	7.8	8.2	8.5	24.0	25.2	24.3			
50.0%	5	2	0	6.2	6.9	7.6	7.8	8.2	8.5				15.9		17.2
100.0%	5	0	0	6.5	7.0	-b	7.3	8.1	-b	24.7	25.2	-b			
100.0%	5	0	0	6.0	7.0	-	7.3	8.1	-				16.0		-b

Control Water

NH_3(as NH_3)(mg/l) 0

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

Total Alkalinity (mg/l $CaCO_3$)

Total Hardness (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) 0.04

Total ammonia (as N)(mg/l) 3.2

Total Alkalinity (mg/l $CaCO_3$)

Total Hardness (mg/l $CaCO_3$)

[a] All effluent test chambers were continuously aerated throughout the test.

[b] Remaining test parameters were not recorded since all organisms were dead by 24 hours.

Table 3. LC_{50} Data for Bioassay of Key West Sewage Treatment Plant, NPDES #FL0025976, on 18 to 20 March, 1986.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Mysidopsis bahia	Binomial	54.6% (0 and 100)	
	Moving Average	a	< 50.0%, b
	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.

[b] The LC_{50} is < 50.0% since more than half the test organisms died in this test concentration.

Table 4. Results of Chemical Analyses Performed on wastewater collected from the Key West Sewage Treatment Plant, NPDES #FL0025976, on 17 March, 1986.

Organics

Acid Extractables:	—a
Base Neutral Extractables:	—a
Pesticide Extractables:	butyl benzyl phthalate (1.7 ug/l)

Metals

Antimony	N.D.
Arsenic	N.D.
Beryllium	N.D.
Cadmium	3.8 ug/l
Chromium	N.D.
Copper	4.9 ug/l
Lead	< 10 ug/l
Mercury	-b
Nickel	N.D.
Selenium	< 6 ug/l
Silver	0.3 ug/l
Thallium	N.D.
Zinc	-b

[a] Analysis incomplete at the time of the report
[b] Lead analysis was invalidated
N.D. = None Detected

RESULTS AND DISCUSSION

The sample of untreated municipal sewage collected from the Key West Sewage Treatment Plant proved to be acutely toxic to Mysidopsis bahia. All test organisms were dead in the 100% effluent at 24 hours, and most were dead in the 50% effluent concentration by 48 hours (Table 2). The LC_{50}'s calculated for this test were 54.6% at 24 hours and < 50.0% at 48 hours (Table 3). All test chambers were aerated during this test. Dissolved oxygen, therefore, was plentiful. Chlorine was not responsible for this toxicity since this wastewater is not chlorinated. Additionally, the ammonia present in the sample (0.04 mg/l unionized) was insufficient to have caused the toxicity.

The results of chemical analyses (Table 4) indicated the presence of butyl benzyl phthalate (1.7 ug/l), and the metals cadmium (3.8 ug/l), copper (49 ug/l), and silver (0.3 ug/l) in this effluent. These constituents probably contributed to the toxicity noted in the sample.

Biology Section's Recommendation

It is recommended that Key West construct a treatment facility to properly treat its wastes and to eliminate the toxicity.

Discussion of the Toxicity Criteria

The toxicity standards of Chapters 17-3 and 17-4, F.A.C., are complex. Because of this complexity, they will be briefly reviewed here so that these test results may be more readily compared with the standards. For legal purposes, however, the language of the Florida Administrative Code should be consulted directly.

The two basic concepts utilized in the standards are acute and chronic toxicity (defined in 17-3.021(1) and (4), respectively). Simplifying the actual F.A.C. language somewhat, acute toxicity is defined as the presence of a substance in a concentration one-third as great as that concentration which kills one-half of the test organisms in 96 hours, i.e., one-third of the 96 hour LC_{50}. Chronic toxicity is similarly defined as one-twentieth of the 96 hour LC_{50}. Therefore, if a test solution kills one-half or more of a group of test organisms in 96 hours, it must contain at least three times the defined "acutely toxic" concentration and at least twenty times the "chronically toxic" concentration of some substance or substances. If a test solution causes that same mortality in less than 96 hours, (for example, in 24 or 48 hours), it is at least as toxic as the example given above, and actually is probably considerably more toxic.

The particular restrictions applied to a given discharger depend upon the existence of a mixing zone, whetheras a specific part of the permit or based on the existence of a currently valid permit predating the language in Chapter 17-4 requiring mixing zone designation. In the absence of a mixing zone, the general criteria for surface waters [17-3.061] are applicable at all points, beginning at the point of discharge. This section prohibits the discharge of substances in concentrations which are chronically toxic [17-3.061(2)(p)]; that is, the concentrations cannot exceed one-twentieth of the 96 hour LC_{50} as discussed above. If a mixing zone does exist, then this chronic toxicity standard is only applicable from the edge of the mixing zone and beyond. Within the mixing zone, the less stringent acute toxicity standard of the minimum criteria for all waters [17-3.051(1)(d)] applies: one-third of the 96 hour LC_{50}. The acute toxicity standard does not apply, however, to each point within a mixing zone not designated for dredge and fill permitting, but rather to the average of a number of points within such a mixing zone, based on the provisions of the surface water mixing zones section of Chapter 17-4 [17-4.244(4)]. At the same time, though, this same rule prohibits waste concentrations within such a mixing zone (i.e., at any point within the mixing zone), from exceeding the 96 hour LC_{50}. Therefore, at any point within such a mixing zone, including the point of discharge, one-half of the test organisms cannot be killed in 96 hours. Within a mixing zone designated for dredge and fill permitting, the acute toxicity standard (one-third of the 96 hour LC_{50}) applies at all points, including the point of discharge.

From the above discussion, it should be clear that if the least stringent toxicity standard, the 96 hour LC_{50}, is exceeded at any point, from the point of discharge outwards, by any discharger, then a violation of the standards has occurred. This is an important consideration since the test procedure here reported involves the measurement of the 50% lethal concentration, and not some fraction of that concentration. Any discharger required to meet the one-twentieth of the 96 hour LC_{50} standard is therefore being tested very leniently. Any reduction in the test period from 96 hours provides an added degree of leniency to a discharger.

A number of different dilutions were examined in order to determine by how much, if any, the discharge exceeds the 96 hour LC_{50}. For example, if the sample, after two-fold dilution with diluent (a 33% concentration), kills one-half of the test organisms after 96 hours, then the sample is three times as toxic as is allowed by that standard. Similarly, if a 5% solution of the sample kills 50% of the organisms, the sample is twenty times as toxic as is allowed. Toxicities are reported here in that manner, i.e., in terms of the percentage dilution of the test sample. The smaller the percentage which is reported means a correspondingly greater toxicity.

Note that even if a discharger receives a very large mixing zone not designated for dredge and fill permitting, the regulations require that the maximum toxicity at any point within the mixing zone be restricted to the 96 hour LC_{50}. The results here reported are based on actual effluent samples, representative of the effluent concentration which enters waters of the state at the point of discharge. If it is demonstrated that, under the conditions existing at the point of discharge, effluent concentrations will be less than at the actual sampling point (if the two points are different), the reported bioassay results will reflect the reduced discharge concentration.

With respect to the species of organisms used in these tests, the language of the F.A.C. indicates that they should be "significant to the indigenous aquatic community". All species used in these tests are significant to the indigenous aquatic community since they are sensitive to toxic substances which may be expected to impact the indigenous community.

The toxicity reported here is in excess of allowable limits.

LITERATURE CITED

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