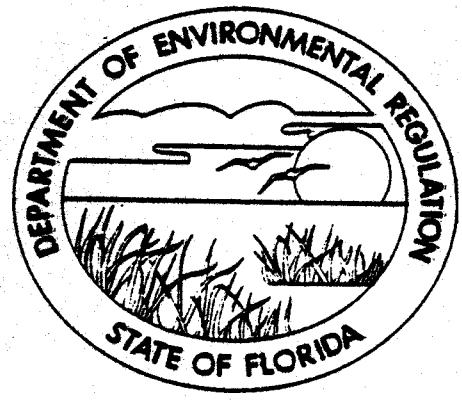


BIOASSAYS OF THE CAPE CANAVERAL AIR FORCE BASE

SEWAGE TREATMENT PLANT

BREVARD COUNTY, FLORIDA

NPDES #FL0022071

SAMPLED 11/1/83

Biology Section
Bureau of Water Analysis
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EXECUTIVE SUMMARY

Cape Canaveral A.F.B. STP, NPDES #FL0022071, on 2 to 4 November, 1983.

Of the pre-chlorinated, chlorinated and de-chlorinated effluents from this U.S. government facility that were tested for acute toxicity, only the chlorinated effluent was toxic to the test organism, *Mysidopsis bahia* (24 and 48 hour LC_{50} = 39.9%). No mortality of shrimp was noted in either the pre-chlorinated or the de-chlorinated effluent tests. The toxicity noted in the chlorinated effluent was attributed to the total chlorine residual detected in the sample (0.9 mg/l field and 0.75 mg/l lab measurements), and therefore, further chemical analyses were not requested.

The results of these latest tests were a marked improvement over the results of previous bioassays (February 1983), in which all three effluents from this facility were acutely toxic to *M. bahia* at that time. Additionally, it was noted that the results of these latest tests were very similar to the results of bioassays first performed on effluents from this facility in 1981.

INTRODUCTION

The Cape Canaveral Air Force Base Sewage Treatment Plant is a U.S. Government facility located on Industry Road, Cape Canaveral Air Force Station, Brevard County, Florida (Figure 1). The plant serves primarily air force base operations and its personnel, but also treats wastewater from 12 industrial sources. Approximately 0.49 MGD of treated effluent is discharged to the Banana River.

The Biology Section has previously performed toxicity bioassays on samples of pre-chlorinated, chlorinated and de-chlorinated effluents from this facility, on 29 April to 1 May, 1981. The results of those tests indicated that although the pre-chlorinated and de-chlorinated effluents were not toxic to *Mysidopsis bahia,* the chlorinated effluent produced a 48 hour LC_{50} of 87.0% (FDER 1981). Toxicity was attributed to the presence of total chlorine residual, although two pesticides, b-BHC and chlordane, were also present in the effluent.

Further bioassay testing was performed on 23 to 25 February, 1983, to continue to evaluate the toxicity of effluents from this facility. Those tests indicated that all three effluents were acutely toxic to *M. bahia,* producing 48 hour LC_{50}'s of 18.4%, 24.0% and 69.6% for pre-chlorinated, chlorinated and de-chlorinated samples, respectively (FDER 1983). Toxicity was attributed to the presence of numerous organic compounds and heavy metals.

To again evaluate the toxicity of these effluents to the biota of the receiving waters, the Biology Section performed three additional 48 hour static bioassays, on 2 to 4 November, 1983. These latest bioassays were requested by DER's St. Johns River District office since it was believed that improvements were made at this facility (Mr. Guy Hadley, personal communication). The results of these latest tests are presented in this report. Those involved in performing the tests and in preparation of this report were: R. B. Frydenborg and M. L. Roll.

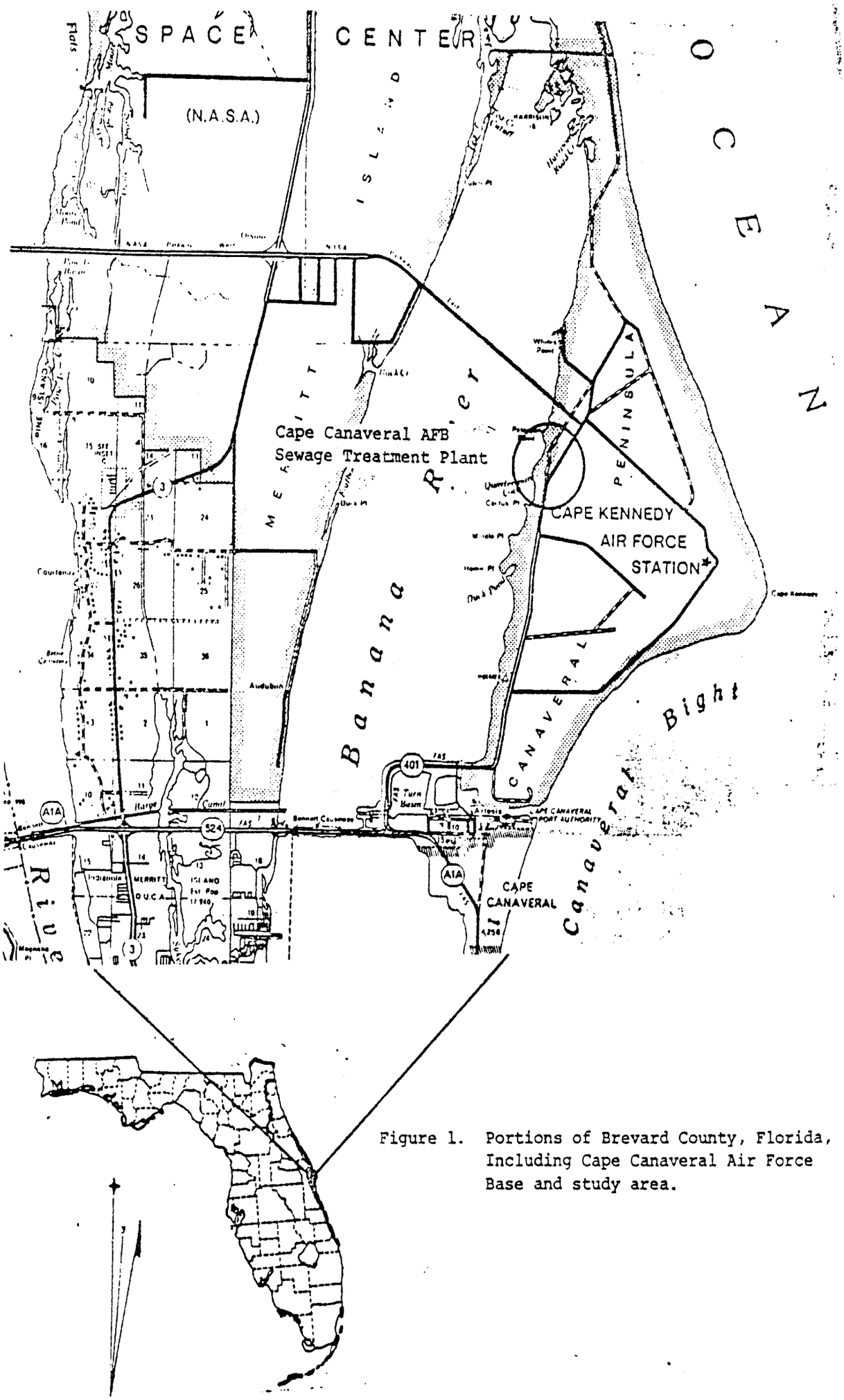


Figure 1. Portions of Brevard County, Florida, Including Cape Canaveral Air Force Base and study area.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978). The organism selected for this study is among those recommended by Peltier (1978) and the U.S. EPA (1978). 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 range of salinities (10-30 ppt) to minimize acclimation periods to low or high salinity effluents. Seawater is obtained from the FSU Marine Laboratory located at Turkey Point, Florida (salinity range 17-31 ppt) and partial water changes are performed weekly. Freshwater, for dilution of the seawater as necessary, is obtained from a deep well near the DER laboratory. The mysids are fed twice daily with freshly hatched Artemia (brine shrimp) and are kept at a constant temperature of 22° plus or minus 1°C. Large juveniles to adult mysids were used in these tests.

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

Three separate 48 hour static acute toxicity bioassays were performed on samples of pre-chlorinated, chlorinated and de-chlorinated effluents from the Cape Canaveral A.F.B. STP. The tests were performed from 1100 hours on 02 November to 1100 hours on 04 November, 1983. The samples were collected by Mr. Louis Ley of DER's Orlando office at 1405 hours on 01 November, 1983 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.

To differentiate between the toxicity resulting from chlorine and the toxicity associated with some other component of the effluent, the Biology Section adopted the following test procedure:

Test #1. Pre-chlorinated Effluent (Table 2). This test was performed on an effluent sample collected at the end of the treatment process but prior to chlorination, and was intended to evaluate the toxicity of the effluent without the added toxic effects of chlorine.

Test #2. Chlorinated Effluent (Table 3). This test was performed on an effluent sample collected at the point of discharge.

Test #3. De-chlorinated Effluent (Table 4). This test was performed on a subsample of the chlorinated effluent sample in which the chlorine had been neutralized. De-chlorination was accomplished by adding a calculated amount of 0.025 N sodium thiosulfate. This test was intended to evaluate the combined toxicity of the effluent and the chlorine reaction products.

Saltwater for dilution of the effluent samples was obtained from the FSU Marine Laboratory at Turkey Point (salinity = 26.1 ppt). For test concentrations of 56% and below, this diluent was mixed directly with the effluent, achieving a range of final salinities (12.1 ppt to 23.5 ppt). For the 100% effluent test concentrations, freshly autoclaved artificial sea salts (Ocean 50®) were added until salinities of approximately 7 ppt were achieved (the lower limit of mysid shrimp salinity tolerance). Two control groups (Table 2) were then used to ensure that any mortality in the test organisms was not the result of toxic properties associated with the diluent, the artificial sea salts, or the salinity range. The control groups included:

1) Diluent Control - Turkey Point water (26.1 ppt). This represents the highest concentration of Turkey Point water 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.

2) Sea Salts Control - Well water, adjusted with artificial sea salts (Ocean 50®) to approximately 7 ppt salinity. This tests for toxicity of the highest concentration of artificial sea salts used in the bioassays, as well as for tolerance of the organisms to the lowest salinity used in the bioassays.

The concentration series selected for this study (Table 1) is that recommended by APHA (1980). Two replicates of each randomly arranged 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 10 ppt, 15 ppt, 20 ppt and 25 ppt salinity aquaria to culture dishes for observation and selection of individuals for the tests. 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.

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 chamber 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 concentrations, including the total and unionized fraction (expressed in mg/l as N and as NH_3, respectively), were recorded in the diluent control and the 100% effluent test concentrations 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, 3, and 4.

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

Mysidopsis bahia (mysid shrimp)

Concentration	Dilution Water	Effluent	Total Volume
10.0%	450 ml	50 ml	500 ml
18.0%	410 ml	90 ml	500 ml
32.0%	340 ml	160 ml	500 ml
56.0%	220 ml	280 ml	500 ml
100.0%	------	500 ml	500 ml

Table 2. Data recorded during 48 hour, *Mysidopsis bahia*, static acute toxicity bioassay of Cape Canaveral A.F.B. STP, Brevard County, Florida, NPDES #FL0022071, on 2 to 4 November, 1983.

Control Series and Pre-chlorinated Sample (Test #1)

Concentration or	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/N (mg/ℓ)		Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
Diluent Control	5	5	5	8.1	7.0	6.9	8.5	8.2	8.0	21.0	19.8	19.5	0/0.1	---		
Diluent Control	5	5	5	8.1	7.0	6.9	8.5	8.2	8.0						26.1	26.9
Sea Salts Control	5	5	5	8.5	8.0	7.7	8.3	8.3	8.1	20.9	19.6	19.4				
Sea Salts Control	5	5	5	8.5	8.0	7.7	8.3	8.2	8.1						8.0	8.2
10.0%	5	5	5	8.2	7.1	6.9	8.4	8.1	8.0	21.0	19.7	19.5				
10.0%	5	5	5	8.2	7.1	6.9	8.4	8.1	8.0						23.5	23.8
18.0%	5	5	5	8.2	7.1	7.0	8.4	8.2	8.1	20.6	19.7	19.4				
18.0%	5	5	5	8.2	7.1	7.0	8.4	8.2	8.1						21.5	22.0
32.0%	5	5	5	8.1	7.2	7.2	8.4	8.1	8.1	20.4	19.6	19.4				
32.0%	5	5	5	8.1	7.2	7.2	8.4	8.1	8.1						17.1	18.5
56.0%	5	5	4(5)[a]	7.8	7.4	7.2	8.2	8.1	8.1	19.8	19.6	19.4				
56.0%	5	5	5	7.8	7.4	7.2	8.2	8.1	8.1						12.1	12.5
100.0%	5	5	5	7.3	7.2	6.4	8.1	8.1	8.1	19.5	19.8	19.5	0/0.3	---		
100.0%	5	5	5	7.3	7.2	6.4	8.1	8.1	8.0						8.0	8.2

Remarks: [a] Number in parentheses used since one organism was cannibalized between 24 and 48 hours.

Table 3. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Cape Canaveral A.F.B. STP, Brevard County, Florida, NPDES #FL0022071, on 2 to 4 November, 1983.

Chlorinated Sample[a] (Test #2)

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			NH_3/N (mg/l)		Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
10.0%	5	5	5	8.3	7.3	6.9	8.4	8.1	8.0	20.7	19.6	19.5				
10.0%	5	5	5	8.3	7.3	6.9	8.4	8.1	8.1						22.9	24.0
18.0%	5	5	5	8.4	7.6	7.1	8.4	8.2	8.1	20.8	19.5	19.4				
18.0%	5	5	5	8.4	7.6	7.1	8.4	8.2	8.1						21.5	22.0
32.0%	5	4	4	8.3	7.8	7.5	8.3	8.2	8.2	20.6	19.5	19.4				
32.0%	5	5	5	8.3	7.8	7.5	8.3	8.2	8.2						18.0	18.7
56.0%	5	0	0	8.4	8.1	7.9	8.1	8.1	8.2	20.6	19.4	19.4				
56.0%	5	0	0	8.4	8.1	7.9	8.1	8.1	8.2						12.2	12.5
100.0%	5	0	0	8.2	8.4	8.1	7.8	8.1	8.2	20.3	19.5	19.4	0/0	---		
100.0%	5	0	0	8.2	8.4	8.1	7.8	8.1	8.2						8.0	8.0

Remarks: [a] Total Chlorine Residual: 0.9 mg/l field measurement
0.75 mg/l lab measurement HACH DPD Method
0.48 mg/l lab measurement Iodometric Method I

Table 4. Data recorded during 48 hour, *Mysidopsis bahia*, static acute toxicity bioassay of Cape Canaveral A.F.B. STP, Brevard County, Florida, NPDES #FL0022071, on 2 to 4 November, 1983.

De-chlorinated Sample[a] (Test #3)

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/N (mg/ℓ)		Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
10.0%	5	5	5	8.3	7.0	6.8	8.4	8.2	8.0	20.9	19.7	19.5				
10.0%	5	5	5	8.3	7.0	6.8	8.4	8.2	8.1						23.0	23.9
18.0%	5	5	5	8.4	7.1	7.0	8.4	8.2	8.1	20.8	19.7	19.4				
18.0%	5	5	5	8.4	7.1	7.0	8.4	8.2	8.1						21.2	21.9
32.0%	5	5	5	8.4	7.3	7.2	8.3	8.1	8.1	20.7	19.7	19.4				
32.0%	5	5	5	8.4	7.3	7.2	8.3	8.1	8.1						18.0	18.5
56.0%	5	5	5	8.5	7.4	7.0	8.2	8.1	8.1	20.7	19.7	19.4				
56.0%	5	5	5	8.5	7.4	7.0	8.2	8.1	8.1						12.2	12.5
100.0%	5	5	5	8.4	7.6	7.2	8.0	8.1	8.1	20.8	19.8	19.4	0/0	---		
100.0%	5	5	5	8.4	7.6	7.2	8.0	8.1	8.1						7.8	7.8

Remarks: [a] Total Chlorine Residual: <0.03 mg/l HACH DPD Method.

Table 5. LC_{50} Data for Bioassays of the Cape Canaveral A.F.B. STP, NPDES #FL0022071, on 2 to 4 November, 1983.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Test #1: *M. bahia* Pre-chlorinated Sample	Binomial Moving Average Probit	No mortality	No Mortality
Test #2: *M. bahia* Chlorinated Sample	Binomial Moving Average Probit	39.9% (32 and 56) a a	39.9% (32 and 56) a a
Test #3: *M. bahia* De-chlorinated Sample	Binomial Moving Average Probit	No Mortality	No Mortality

[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 pre-chlorinated and de-chlorinated samples of effluent from the Cape Canaveral A.F.B. STP were not toxic to the test organism, *Mysidopsis bahia*. The chlorinated sample, however, produced a 24 and 48 hour LC_{50} of 39.9% (Table 5). The 96 hour LC_{50} for this effluent would therefore be less than or equal to 39.9%. The toxicity noted in the chlorinated effluent can be attributed to the total chlorine residual (0.75 mg/l, Table 3) detected in that sample, and therefore, additional chemical analyses were not requested.

The results of these latest bioassays were a marked improvement over the results of earlier tests (February 1983), in which all three samples of effluent from this facility were acutely toxic to *M. bahia*. According to district personnel, certain improvements have been made at this facility (Mr. Guy Hadley, personal communication). However, it was interesting to note that the results from these latest tests were very similar to the results of bioassays first performed on effluents from this facility in 1981.

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 existance of a mixing zone, whether as a specific part of the permit or based on the existance 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.

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