BIOASSAYS OF FORT LAUDERDALE "B" SEWAGE TREATMENT PLANT

FORT LAUDERDALE, BROWARD COUNTY, FLORIDA

NPDES #FL0020524

SAMPLED 3/15/83

Biology Section
Bureau of Water Analysis
April 22, 1983

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EXECUTIVE SUMMARY

Ft. Lauderdale "B" STP, NPDES #FL0020524, on 16 to 18 March, 1983.

The samples of pre-chlorinated, chlorinated and de-chlorinated effluents from this municipal sewage treatment plant were all acutely toxic to Mysidopsis bahia, producing 48 hour LC_{50} values of 74.8%, 9.0%, and 78.7%, respectively. The concentrations of ammonia detected in these effluents could not have contributed significantly to the observed toxicity. The increased toxicity of the chlorinated sample can be attributed to the large concentration of total residual chlorine measured in that sample (6.0 mg/l field measurement, 2.3 mg/l and 3.01 mg/l lab measurements). Chemical analyses indicated the presence of a variety of organic components in the pre-chlorinated effluent, including the pesticide Lindane (0.05 µg/l), three acid extractable components and numerous other pesticide extractable components, two of which were halogenated. The final chlorinated effluent contained an even greater number of organic components, including eight unidentified acid extractables, one unidentified base neutral, Lindane (0.04 µg/l) and numerous other components, eight of which were halogenated. Also present in the final effluent from the Ft. Lauderdale "B" facility were several heavy metals, including cadmium (0.2 µg/l), lead (10 µg/l), mercury (1.0 µg/l), silver (3.5 µg/l) and zinc (517 µg/l). The toxicities of these effluents, therefore, can be attributed to the synergistic effects of the organic and metal constituents, with particular emphasis on the Lindane, mercury, silver and zinc, although the total residual chlorine level of the chlorinated effluent was sufficient to have caused the toxicity found in that sample.

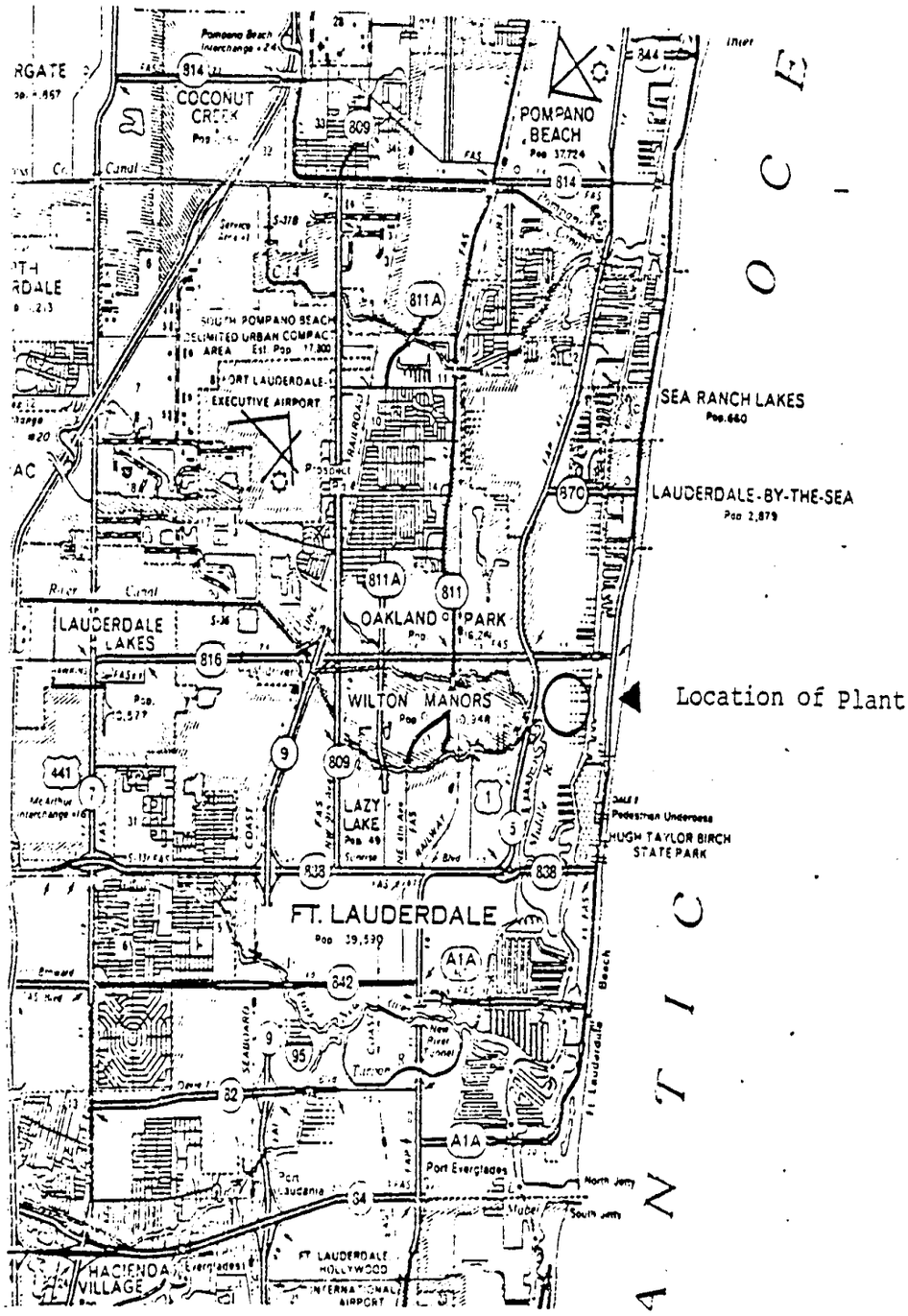
INTRODUCTION

The Fort Lauderdale "B" Sewage Treatment Plant is a municipal facility operated by the City Utilities Department. It is located on N.E. 40th and 25th Avenue, in the town of Coral Ridge, Broward County, Florida (Fig. 1). The plant, operating since 1965, presently has a design capacity of 8.0 MGD and serves a population of approximately 80,000. The plant is primarily an activated sludge treatment plant (7.0 MGD), but also has a 1.0 MGD trickling filter and a 1.0 MGD "Cantex" contact stabilization unit for increased capacity. Domestic wastewater purification consists of comminution, vacuum floatation, degritting, activated sludge treatment, final clarification and chlorination. Discharge of the average 6.77 MGD effluent is to the Intracoastal Waterway through a 36" pipe sixty feet from shore.

This facility is scheduled to cease discharging when the new Central Region sludge facility begins operating. In the past, the plant has received several notices for exceeding its permit limitations for biochemical oxygen demand (BOD) and total suspended solids (TSS).

The Biology Section has previously performed toxicity tests on pre-chlorinated, chlorinated and de-chlorinated samples of effluent from this facility, in July, 1981, and again in June, 1982. The results of 1981 tests indicated that the chlorinated effluent was extremely toxic to Mysidopsis bahia (48 hour LC_{50}=10.5%), while the pre-chlorinated and de-chlorinated samples were only slightly toxic (48 hour LC_{50}'s=>100%, FDER 1981). The results of June, 1982, bioassays demonstrated that all three effluents were acutely toxic to Mysidopsis bahia, producing 48 hour LC_{50} values of 87.1%, 22.6% and 91.9%, respectively. Toxicity at that time was attributed to the wide variety of chemical constituents in the effluent, including halogenated organics, alaphatic acids, alcohols and zinc, and, to total residual chlorine in the chlorinated sample (FDER 1982).

To further evaluate the existence and extent of toxicity of these pre-chlorinated, chlorinated and de-chlorinated effluents to the biota of the receiving waters, the Biology Section performed additional static acute toxicity bioassays on grab samples, on 16 to 18 March, 1983. The results of these latest tests are presented in this report.



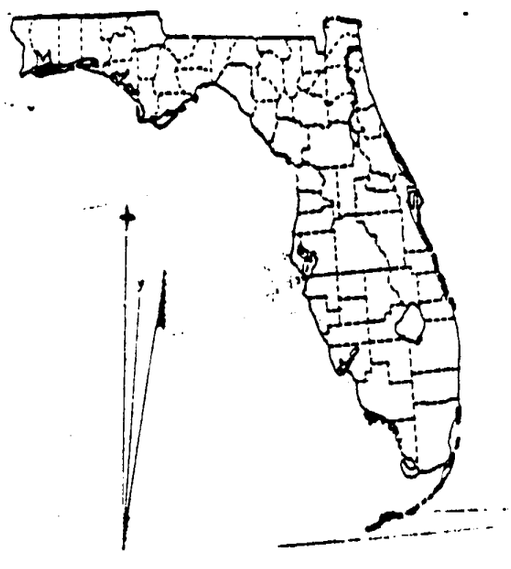


Figure 1. Portions of Broward County, Florida, including approximate location of Fort Lauderdale B Sewage Treatment Plant.

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. Ten to fifteen day old (three-quarter adult size) 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

Three separate 48 hour static acute toxicity bioassays were performed on samples of pre-chlorinated, chlorinated and de-chlorinated effluents from the Ft. Lauderdale "B" STP. The tests were performed from 1000 hours on 16 March to 1015 hours on 18 March, 1983. The samples were collected by Mr. Mike Nichols of DER's Port St. Lucie office at 1020 hours on 15 March, 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 = 28.0 ppt). For test concentrations of 56% and below, this diluent was mixed directly with the effluent, achieving a range of final salinities (13.7 ppt to 26.8 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 5) 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 (28.0 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 the 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 10 ppt, 15 ppt, 20 ppt and 25 ppt salinity aquaria to culture dishes for observation and selection of individuals for the tests. Ten to fifteen day old (three-quarter adult size) mysids were used in these 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, pH, temperature, and the number of live organisms were recorded for each test chamber at 0 hours, 24 hours, and 48 hours. The salinity of each test concentration was recorded at the beginning and end of the test. Ammonia concentrations, including total and the unionized fraction (expressed in mg/l as $NH_3 + NH_4$ and as NH_3, respectively), were recorded in the Sea Salts 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 Corning pH/millivolt meter. The unionized fraction was then determined 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 through 5.

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

Mysidopsis bahia (mysid shrimp)

Concentration	Dilution Water	Effluent	Total Volume
Control	500 ml	-----	500 ml
5.6%	472 ml	28 ml	500 ml
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 the Ft. Lauderdale "B" STP, Broward County, Florida, NPDES #FL0020524, on 16 to 18 March, 1983.

Test #1 Pre-chlorinated Sample[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
5.6%	5	5	5	6.8	6.0	6.2	8.1	7.9	7.8	20.2	18.7	19.0				
5.6%	5	5	5	6.8	6.0	6.2	8.1	7.9	7.8						26.8	26.2
10.0%	5	5	5	6.9	6.2	6.2	8.1	7.9	7.8	20.1	18.5	18.7				
10.0%	5	4	3(4)[b]	6.9	6.2	6.2	8.1	7.9	7.8						25.8	25.2
18.0%	5	5	5	6.8	6.4	6.4	8.0	7.9	7.8	20.0	18.4	18.6				
18.0%	5	5	5	6.8	6.4	6.4	8.0	7.9	7.8						23.8	23.2
32.0%	5	5	5	6.7	6.5	6.5	7.9	7.9	7.8	19.9	18.4	18.5				
32.0%	5	5	5	6.7	6.7	6.5	7.9	7.9	7.8						20.0	19.8
56.0%	5	5	4	6.9	6.8	7.0	7.7	7.8	7.8	19.9	18.5	18.6				
56.0%	5	5	5	6.9	6.8	7.0	7.7	7.8	7.8						14.0	14.0
100.0%	5	5	0	7.3	7.4	7.5	8.6	8.3	8.1	20.0	18.6	18.6	.01/.06	--		
100.0%	5	5	1	7.3	7.5	7.6	8.6	8.3	8.1						7.5	7.5

Remarks: [a] Total Residual Chlorine = None Detected (HACH DPD Method).

[b] Number in parentheses used in calculations since one organism was cannibalized between 24 and 48 hours.

Table 3 . Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of the Ft. Lauderdale "B" STP, Broward County, Florida, NPDES #FL0020524, on 16 to 18 March, 1983.

Test #2 Chlorinated Sample[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
5.6%	5	5	5	6.9	7.2	6.2	8.0	8.0	7.8	20.3	18.7	18.8				
5.6%	5	5	4	6.9	7.2	6.2	8.0	8.0	7.8						26.5	26.0
10.0%	5	2	0	6.8	7.5	6.8	8.0	8.0	7.8	20.3	18.7	18.8				
10.0%	5	4	4	6.8	7.5	7.0	8.0	8.0	7.8						25.2	25.0
18.0%	5	0	0	6.9	7.7	7.3	7.9	8.0	7.9	20.3	18.7	18.8				
18.0%	5	0	0	6.9	7.7	7.3	7.9	8.0	7.9						23.0	23.0
32.0%	5	0	0	7.0	8.0	7.4	7.7	7.9	8.0	20.2	18.7	18.7				
32.0%	5	0	0	7.0	8.0	7.6	7.7	7.9	8.0						20.0	19.5
56.0%	5	0	0	7.2	8.3	8.4	7.5	7.8	8.0	20.2	18.7	18.7				
56.0%	5	0	0	7.2	8.3	8.4	7.4	7.8	8.0						14.0	13.2
100.0%	5	0	0	7.3	8.7	8.5	7.6	8.0	8.1	20.6	18.8	18.8	.01/.55	--		
100.0%	5	0	0	7.3	8.7	8.6	7.7	8.0	8.1						7.3	6.8

Remarks: [a] Total Residual Chlorine = 6.0 mg/l (Field Measurement)
2.3 mg/l (HACH DPD Method - Lab)
3.01 mg/l (Iodometric Method I - Lab)

Table 4 . Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of the Ft. Lauderdale "B" STP, Broward County, Florida, NPDES #FL0020524, on 16 to 18 March, 1983.

Test #3 De-chlorinated Sample[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
5.6%	5	5	5	7.1	6.6	6.2	8.1	7.9	7.8	20.2	18.3	18.3				
5.6%	5	5	5	7.1	6.6	6.2	8.1	7.9	7.8						25.2	25.0
10.0%	5	5	5	7.1	6.5	6.2	8.0	7.9	7.8	20.0	18.2	18.2				
10.0%	5	5	5	7.1	6.5	6.2	8.0	7.9	7.8						24.2	24.0
18.0%	5	5	5	7.0	6.2	5.8	8.0	7.8	7.7	20.1	18.1	18.1				
18.0%	5	5	5	7.0	6.2	5.8	8.0	7.8	7.7						22.8	22.0
32.0%	5	5	5	7.2	6.0	6.1	7.9	7.8	7.7	20.1	18.1	18.1				
32.0%	5	5	5	7.2	6.0	6.1	7.9	7.8	7.7						19.8	19.0
56.0%	5	5	2	7.4	6.2	6.2	7.7	7.7	7.7	20.1	18.1	18.1				
56.0%	5	5	4	7.4	6.2	6.2	7.7	7.7	7.7						13.7	13.2
100.0%	5	5	1	7.7	7.1	6.1	8.3	8.1	7.9	20.2	18.4	18.4	.04/.59	--		
100.0%	5	5	3	7.7	7.1	6.1	8.3	8.1	7.8						7.5	7.2

Remarks: [a] Total Residual Chlorine = <0.02 mg/l (HACH DPD Method)

Table 5 . Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of the Ft. Lauderdale "B" STP, Broward County, Florida, NPDES #FL0020524, on 16 to 18 March, 1983.

Control Series

Concentration or	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/NH_3+NH_4 (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	7.2	6.9	6.9	8.1	8.0	7.9	20.1	18.9	19.2				
Diluent Control	5	5	5	7.2	6.9	6.9	8.1	8.0	7.9						28.0	28.0
Sea Salts Control	5	5	5	8.3	8.3	7.2	8.7	8.5	8.3	21.7	18.9	19.1	.01/.05	--		
Sea Salts Control	5	5	5	8.3	8.3	7.2	8.7	8.5	8.3						7.0	7.5

Remarks:

Table 6. LC_{50} Data for Bioassays of the Ft. Lauderdale "B" STP, NPDES #FL0020524, on 16 to 18 March, 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*	Binomial	a	74.8% (56 and 100)
Pre-chlorinated	Moving Average	a	74.8% (61 and 103), d
Sample	Probit	a	77.1% (0 and ∞), c
Test #2: *M. bahia*	Binomial	10.8% (5.6 and 18), d	9.0% (5.6 and 18)
Chlorinated	Moving Average	a	9.0% (6.9 and 11.1), d
Sample	Probit	a	8.8% (6.9 and 11.3)
Test #3: *M. bahia*	Binomial		74.8% (32 and ∞), b
De-chlorinated	Moving Average	No Mortality	74.8% (56 and 136), b
Sample	Probit		78.7% (59 and 137), b, d

[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] Due to the distribution of mortality, the LC_{50} value calculated by this method of statistical analysis does not meet the criteria established by the Committee on Methods for Toxicity Tests with Aquatic Organisms.

[c] Due to the distribution of mortality, results calculated using the probit method are not statistically sound.

[d] This LC_{50} value is reported in the text since it is the most statistically conservative value of the data set.

Table 7. Results of Chemical Analyses Performed on Effluents from the Ft. Lauderdale "B" STP, NPDES #FL0020524, collected 15 March, 1983.

Organics

	Pre-chlorinated Effluent	Chlorinated Effluent
Acid Extractables:	Three unidentified components	Eight unidentified components
Base Neutral Extractables:	N.D.	One unidentified component
Pesticide Extractables:	0.05 µg/l g-BHC (Lindane) Numerous unidentified components, two of which were halogenated	0.04 µg/l g-BHC (Lindane) Numerous unidentified components, eight of which were halogenated

Metals

Arsenic	N.D.
Cadmium	0.2 µg/l
Chromium	N.D.
Copper	< 25 µg/l[a]
Lead	10 µg/l
Mercury	1.0 µg/l
Nickel	N.D.
Selenium	N.D.
Silver	3.5 µg/l
Zinc	517 µg/l

N.D. = None Detected

[a] Actual value is known to be less than value given

RESULTS AND DISCUSSION

The samples of pre-chlorinated, chlorinated and de-chlorinated effluents from the Ft. Lauderdale "B" STP were all acutely toxic to Mysidopsis bahia, producing 48 hour LC_{50} values of 74.8%, 9.0%, and 78.8%, respectively (Table 6). The 96 hour LC_{50}'s for these effluents would be less than or equal to their 48 hour values. The chlorinated effluent also produced a 24 hour LC_{50} of 10.8% (Table 6). The increased toxicity of this chlorinated effluent can be attributed to the large concentration of total residual chlorine measured in that sample (6.0 mg/l field measurement, 2.3 mg/l and 3.01 mg/l lab measurements, Table 3). Minor concentrations of ammonia were detected in these effluents, which could not have contributed significantly to the observed toxicity. The toxicity of the pre-chlorinated and de-chlorinated effluents, therefore, must have been due to factors other than residual chlorine and ammonia.

The DER Chemistry Section has analyzed samples of these effluents for certain organic compounds and metals (Table 7). These analyses indicated the presence of a variety of organic components in the pre-chlorinated effluent, including three unidentified acid extractables, the pesticide Lindane (0.05 µg/l) and numerous other pesticide extractable components, two of which were halogenated. The final chlorinated effluent from the Ft. Lauderdale "B" facility contained an even greater number of components, including eight unidentified acid extractables, one unidentified base neutral, Lindane (0.04 µg/l), and numerous other pesticide extractable components, eight of which were halogenated. Also present in the final effluent were several heavy metals, including cadmium (0.2 µg/l), lead (10 µg/l), mercury (1.0 µg/l), silver (3.5 µg/l) and zinc (517 µg/l).

The toxicities of the Ft. Lauderdale "B" pre-chlorinated and de-chlorinated effluents were very likely due to the presence of the wide variety of organic and metal constituents. The presence of the numerous unidentified acid, base neutral and pesticide extractable organic components probably contributed significantly to the observed toxicities. Of particular importance were the halogenated components (eight in the final effluent). Many halogenated compounds are known to be highly toxic to aquatic organisms. The concentrations of Lindane (.05 and .04 µg/l), although individually sub-lethal to Mysidopsis bahia, probably contributed to the toxicities. Of the metals present, mercury (1.0 µ/gl), silver (3.5 µg/l) and zinc (517 µg/l) were particularly significant. The concentration of mercury present in this effluent (1.0 µg/l) "represents a distinct threat to selected species of marine organisms" (Eisler 1974, in U.S. EPA 1976). The concentration of silver present in this effluent (3.5 µg/l), has been shown to severely affect some organisms. A concentration of 3.2 µg/l silver was acutely toxic to Daphnia magna, 3.0 µg/l was lethal to sticklebacks, 0.5 µg/l affected the development of sea urchins and 5.8 µg/l silver was acutely toxic to oyster larvae in 48 hours (U.S. EPA 1976). The concentration of zinc present in the effluent (517 µg/l), although probably less toxic than either mercury or silver, has been shown to be acutely toxic to certain sensitive freshwater fish at lesser concentrations (U.S. EPA 1976).

The zinc, therefore, may have contributed to the observed toxicities.

From the above discussion of the toxicities of the various components present in the Ft. Lauderdale "B" effluent, it is apparent that a serious problem may exist at this facility.

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 17-3-3.021(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} standard. 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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