BIOASSAYS OF FT. PIERCE UTILITIES AUTHORITY

WASTEWATER TREATMENT PLANT

ST. LUCIE COUNTY, FLORIDA

NPDES #FL0027278

SAMPLED 10/22/84

January 23, 1985

Biology Section
Division of Environmental Programs

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EXECUTIVE SUMMARY

Ft. Pierce Utilities Authority STP, NPDES #FL0027278, St. Lucie Co., on 23 to 25 October, 1984.

The samples of chlorinated and de-chlorinated effluents from this facility were both acutely toxic to Mysidopsis bahia, producing 48 hour LC_{50}'s of 31.5% and 44.8%, respectively. A moderate chlorine residual (0.65 mg/l HACH Method, 0.44 mg/l Iodometric Method) was probably responsible for the slightly more toxic results obtained from the chlorinated sample. However, the presence of chlorine accounted for only a portion of the observed mortality. The total and unionized ammonia detected in these samples were also insufficient to have contributed greatly to the observed mortality. Chemical analyses indicated the presence of several unidentified organic compounds and low concentrations of the metals copper, mercury, and zinc. Therefore, the toxicity in the samples may be attributed to either the unidentified organic compounds or to other constituents or properties not adequately tested for during chemical analyses.

INTRODUCTION

The Ft. Pierce Utilities Authority WWTP is located on Causeway Island, Ft. Pierce, St. Lucie County, Florida (Figure 1). The facility provides secondary treatment to 4.6 MGD (average combined flow) of domestic and industrial wastes from a population of 38,000. Although the design capacity is 6.5 MGD, peak flows of 8.51 MGD have occurred. After comminution, grit removal, aeration (including both activated sludge and contact stabilization), clarification, digestion, sludge removal, and chlorination, the effluent is discharged to the Indian River (data from NPDES file).

A previous bioassay performed on pre-chlorinated effluent from this facility, using Daphnia pulex as the test organism, indicated that this effluent was acutely toxic (48 hour LC_{50} = 73.2%) (FDER 1980). Therefore, Mr. Terry Davis of DER's Southeast District requested further bioassays to determine the current existence and extent of toxicity generated by the Ft. Pierce WWTP. Those involved with conducting the test and preparing this report were: Russel B. Frydenborg, Manitia L. Moultrie, and Martin L. Roll.

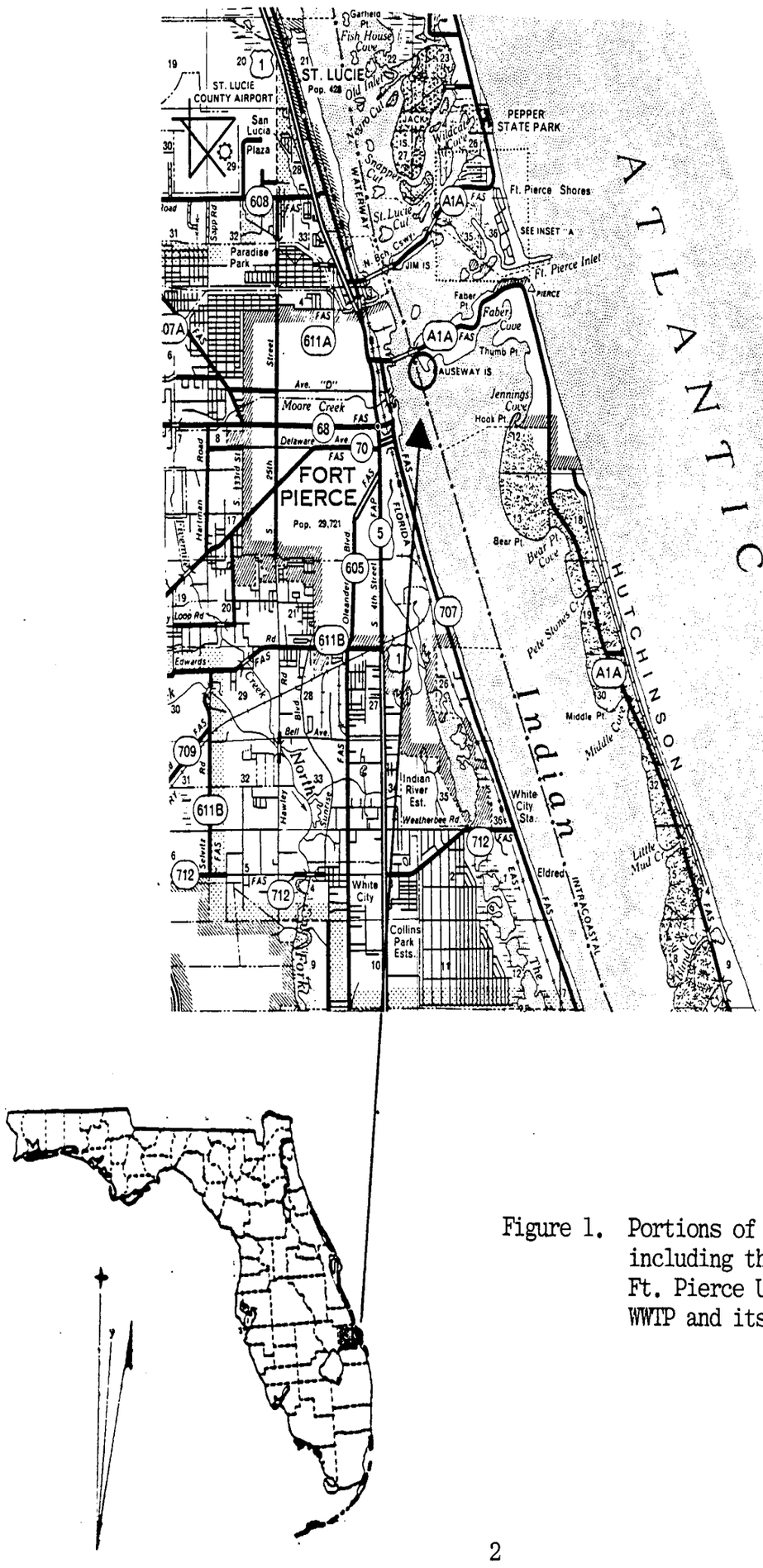


Figure 1. Portions of St. Lucie Co., including the location of Ft. Pierce Utility Authority WWTP and its outfall.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978) and the FDER Biology Section (1983). The organism selected for this study is among those recommended by Peltier (1978), the U.S. EPA (1978), and the FDER Biology Section (1983). The life history and maintenance procedures used to culture the organism are detailed below.

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, 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 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° plus or minus 3°C. Adult or large juvenile mysids were used in this test.

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

Test Methods

Two separate 48 hour static acute toxicity bioassays were conducted from 1000 hours on 23 October to 1013 hours on 25 October, 1984, on samples collected from the Ft. Pierce WWTP.

The samples were collected by Ms. Randi Schwartz of DER's Southeast Florida District Branch Office at 1245 hours on 22 October, 1984, 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. Chlorinated Effluent (Table 2). This test was performed on an effluent sample collected at the point of discharge.

Test #2. De-chlorinated Effluent (Table 3). 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 prepared by mixing artificial sea-salts with well water. For test concentrations of 56% and below, this diluent was mixed directly with the effluent, achieving a range of final salinities of 10.9 ppt to 24.2 ppt. For the 100% effluent test concentrations, freshly autoclaved artificial sea salts 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 - Forty Fathoms® brand sea salt mixed with well water to a salinity of 24.2 ppt. This represents the highest salinity used in the bioassays, and tests for toxicity in the diluent, as well as the tolerance of the organisms to this salinity.

2) Sea Salts Control - Well water, adjusted with artificial sea salts to approximately 7 ppt salinity. This tests for the tolerance of the organisms to the lowest salinity used in the bioassays (the sea salt adjusted 100% effluent).

The concentration series selected for this study (Table 1) is an abbreviated version of 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 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 test 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 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 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% 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 forms and are presented in Tables 2 and 3.

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
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 Ft. Pierce Utilities WWTP, NPDES #FL0027278, St. Lucie Co., on 23 to 25 October, 1984.

Test #1 - Chlorinated Effluent[a]

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
Diluent Control	5	5	4 [b]	7.6	6.6	6.4	8.6	8.3	8.3	22.0	21.0	20.5		
Diluent Control	5	5	5	7.6	6.6	6.4	8.6	8.3	8.3				24.2	23.7
Salinity Control	5	5	5	8.5	7.5	6.8	8.4	8.5	8.2	22.0	21.1	20.4		
Salinity Control	5	5	5	8.4	7.6	6.8	8.4	8.5	8.2				7.1	7.2
10.0%	5	5	5	7.8	7.1	6.8	8.6	8.3	8.4	21.8	21.1	20.4		
10.0%	5	4	4	7.9	7.1	6.8	8.6	8.3	8.4				21.1	21.0
18.0%	5	5	5	8.0	7.3	6.8	8.6	8.4	8.4	21.7	21.0	20.3		
18.0%	5	5	5	8.0	7.4	6.8	8.6	8.4	8.4				19.3	19.1
32.0%	5	3	2	8.1	7.6	7.2	8.5	8.4	8.4	21.6	21.0	20.2		
32.0%	5	5	4	8.1	7.7	7.2	8.5	8.4	8.4				16.3	16.1
56.0%	5	0	0	8.5	8.2	- [c]	8.4	8.4	- [c]	21.5	20.9	- [c]		
56.0%	5	0	0	8.5	8.2	-	8.4	8.4	-				10.9	- [c]
100.0%	5	0	0	8.7	8.5	-	7.6	8.1	-	21.5	20.9	-		
100.0%	5	0	0	8.7	8.5	-	7.6	8.0	-				7.0	-

Control Water

NH_3(as NH_3)(mg/l) 0.02

Total Alkalinity (mg/l $CaOO_3$)

Total ammonia (as N)(mg/l) 0.15

Total Hardness (mg/l $CaOO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) 0.08

Total Alkalinity (mg/l $CaOO_3$)

Total ammonia (as N)(mg/l) 3.85

Total Hardness (mg/l $CaOO_3$)

[a] Total residual chlorine = 0.65 HACH Method and 0.44 Iodometric Method.

[b] One organism jumped and adhered to the side of the test chamber.

[c] Parameters not taken at 48 hours since all organisms were dead at 24 hours.

Table 3. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Ft. Pierce Utilities WWTP, NPDES #FL0027278, St. Lucie Co., on 23 to 25 October, 1984.

Test #2 - De-chlorinated Effluent[a]

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
10.0%	5	5	5	8.2	6.9	6.7	8.6	8.3	8.3	21.7	21.1	20.4			
10.0%	5	4	4	8.2	6.9	6.7	8.6	8.4	8.3				20.9		20.2
18.0%	5	5	5	8.4	6.9	6.6	8.6	8.4	8.3	21.7	21.2	20.3			
18.0%	5	5	5	8.4	6.9	6.6	8.6	8.4	8.3				19.2		19.0
32.0%	5	5	4	8.5	6.7	6.6	8.6	8.4	8.2	21.6	21.2	20.2			
32.0%	5	5	5	8.5	6.7	6.6	8.6	8.4	8.2				16.5		16.1
56.0%	5	5	0	8.6	6.6	6.7	8.4	8.3	8.2	21.9	21.2	20.3			
56.0%	5	4	2	8.6	6.5	6.7	8.4	8.3	8.2				11.0		10.9
100.0%	5	4	0	8.8	6.6	6.9	7.8	8.0	8.0	22.1	21.3	20.4			
100.0%	5	4	0	8.8	6.6	6.9	7.8	7.9	8.0				7.0		7.0

Control Water

NH_3(as NH_3)(mg/l) _____ Total Alkalinity _____ (mg/l $CaOO_3$)

Total ammonia (as N)(mg/l) _____ Total Hardness _____ (mg/l $CaOO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) 0.13 Total Alkalinity _____ (mg/l $CaOO_3$)

Total ammonia (as N)(mg/l) 3.9 Total Hardness _____ (mg/l $CaOO_3$)

[a] Total residual chlorine = None Detected.

Table 4. LC_{50} Data for Bioassays of Ft. Pierce Utilities Authority WWTP, NPDES #FL0027278, St. Lucie Co., on 23 to 25 October, 1984.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Mysidopsis bahia			
Test #1:	Binomial	38.2% (18 and 56)	34.4% (18 and 56)
Chlorinated	Moving Average	35.3% (25.2 and 51.7)	31.5% (22.0 and 44.8),[d]
Sample	Probit	34.1% (0 and ∞),[c]	30.7% (0 and ∞),[c]
Test #2:	Binomial	[a]	44.4% (32 and 100)
De-chlorinated	Moving Average	[a]	44.8% (35.8 and 56.9),[d]
Sample	Probit	2437% (137.8 and ∞),[b]	40.8% (0 and ∞),[c]

[a] Due to an insufficient number of dead organisms, or to the irregular distribution of mortality, and 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.

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

Table 5. Results of Chemical Analyses Performed on Effluent Collected from the Ft. Pierce WWTP, NPDES #FL0027278, on 22 October, 1984.

Organics

Base Neutral Extractables:	N.D.
Pesticide Extractables:	Several unidentified compounds

Metals

Arsenic	-[a]
Cadmium	N.D.
Chromium	N.D.
Copper	< 25 µg/l
Lead	N.D.
Mercury	< 0.1 µg/l
Nickel	N.D.
Selenium	N.D.
Silver	N.D.
Zinc	38 µg/l

N.D. = None Detected

[a] Arsenic analysis invalidated due to blank contamination.

RESULTS AND DISCUSSION

The samples of chlorinated and de-chlorinated effluents collected from the Ft. Pierce Utilities Authority WWTP were both acutely toxic to Mysidopsis bahia, producing 48 hour LC_{50}'s of 31.5% and 44.8%, respectively (Table 4). These latest results indicate a moderate increase in toxicity since the facility was last tested in 1980, when the 48 hour LC_{50} for Daphnia pulex was 73.2% (FDER 1980). Since the de-chlorinated sample was only slightly less toxic than the chlorinated sample, the observed mortality cannot be attributed solely to the presence of residual chlorine. The total and unionized ammonia concentrations in these samples were saml1, and therefore contributed very little to the noted toxicity.

Chemical analyses (Table 5) indicated the presence of several unidentified organic compounds and the metals copper (< 25 ug/1), mercury (< 0.1 ug/1) and zinc (38 ug/1). The toxicity found in these samples, therefore, may be attributed to the unidentified organic compounds, or to other constituents or properties not adequately tested for in chemical analyses.

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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