BIOASSAYS OF GRANT ST. SEWAGE TREATMENT PLANT

MELBOURNE, BREVARD COUNTY, FLORIDA

NPDES #FL0027693

SAMPLED 12/1/81

Biological Section
Bureau of Water Analysis
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EXECUTIVE SUMMARY

Three 48 hour static acute toxicity bioassays were performed on pre-chlorinated, chlorinated and de-chlorinated samples of effluent from the Grant Street Sewage Treatment Plant, a municipal facility located in Melbourne, on 2-4 December, 1981. Results indicated that all three samples of effluent were acutely toxic to the test organism, Mysidopsis bahia, with 48 hour LC_{50}'s of 24%, 12.6% and 15.7%, respectively. The concentration of total residual chlorine was the probable cause of toxicity in the chlorinated bioassay. Since no chemical analyses were performed on these samples, the exact cause of toxicity in the pre-chlorinated and de-chlorinated samples is uncertain. However, district personnel have indicated that a number of industrial metal plating companies may contribute wastewater to the facility.

INTRODUCTION

The Grant Street Sewage Treatment Plant is located at 200 Grant Street, Melbourne, Brevard County, Florida (Figure 1). This municipal waste treatment facility uses high rate trickling filters and chemical treatment (aluminum sulfate) to treat domestic wastes from a population of approximately 28,000. A treatment plant flow diagram is presented in Figure 2. Discharge of effluent averages 2.1 MGD, and is to Crane Creek, a tributary to the Indian River. However, in wet weather, hydraulic overload may occur at this facility, resulting in a discharge of as much as 4.2 MGD (from the NPDES file).

To evaluate the existence and extent of toxicity of this effluent to the biota of the receiving waters, the Biological Section performed static acute toxicity bioassays on grab samples, on 2-4 December, 1981. The results of these tests are presented in this report.

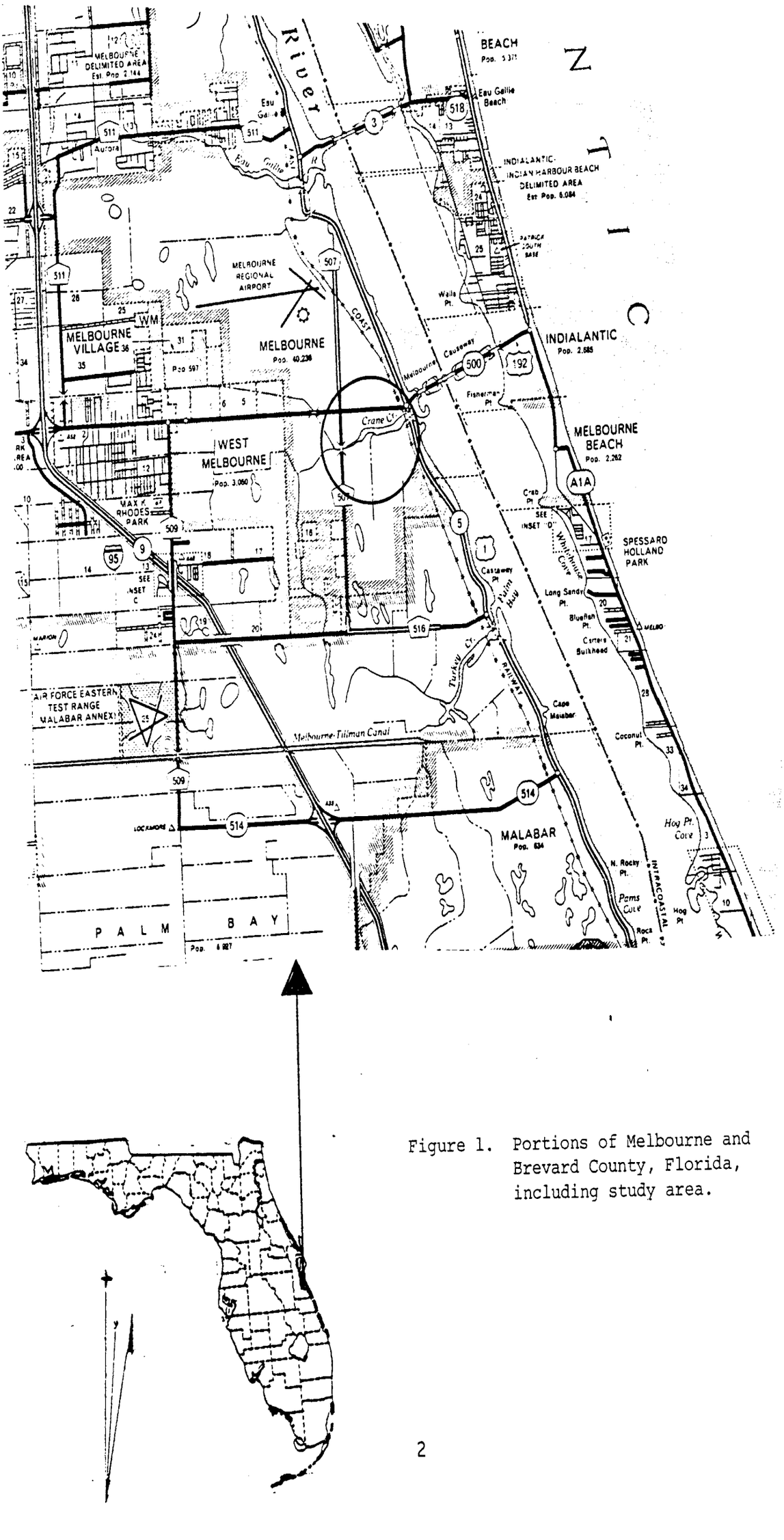


Figure 1. Portions of Melbourne and Brevard County, Florida, including study area.

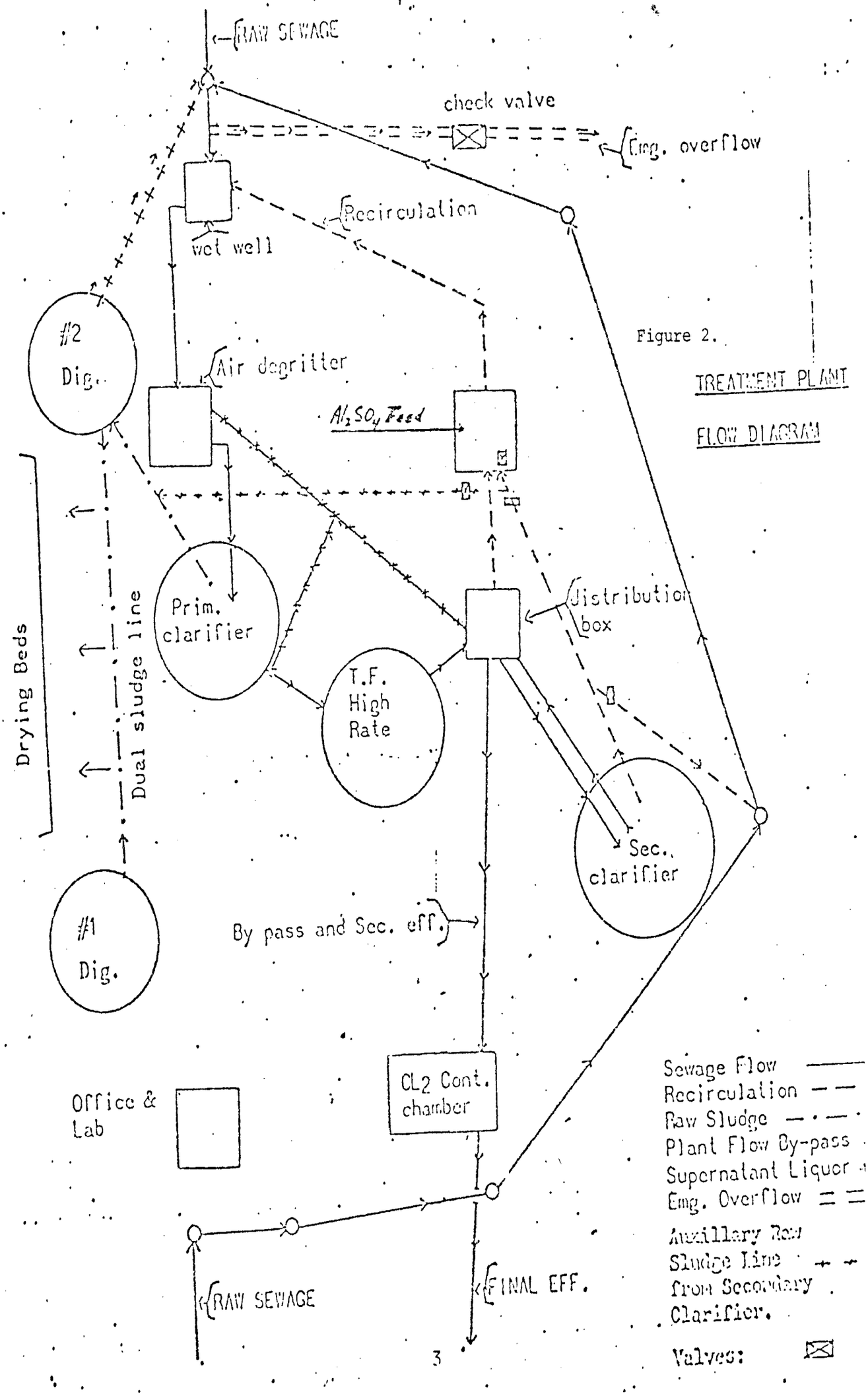


Figure 2.

TREATMENT PLANT

FLOW DIAGRAM

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.

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 continuous lighting 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 Biological 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 from 1200 hours on 2 December, 1981 to 1200 hours on 4 December, 1981. Samples of effluent were collected from the final clarifier before chlorination, and from the point of discharge after chlorination (Outfall #001). The samples were collected by Mr. Guy Hadley of DER's St. Johns River District at 1525 hours on December 1, 1981, using standard wastewater sampling procedures. The samples were iced and transported to the DER laboratory in Tallahassee for analysis 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 Biological 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= 30ppt). This saltwater diluent was then adjusted to the salinity of the receiving body of water (Crane Creek = 18ppt) by adding an appropriate amount of DER well water. For test concentrations of 56% and below, this adjusted diluent was mixed directly with the effluent, achieving a range of final salinities (9ppt to 17.5ppt). 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). A series of 5 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: adjusted Turkey Point seawater (salinity= 18ppt), artificial sea salt (Ocean 50®) adjusted well water (salinity= 8ppt), and a salinity control consisting of Turkey Point seawater and well water (salinity= 11.5ppt). Additionally, two control groups were added consisting of sea salt adjusted 100% sample and sea salt adjusted well water, with their pH's adjusted with hydrochloric acid to the original pH of the sample. Since sea salts increase the pH, thereby increasing the amount of toxic unionized ammonia, these additional controls were necessary to ensure that any mortality was not the result of an artificial rise in pH. 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. However, only single replicates were used in the pH adjusted controls. Immediately prior to the bioassays, a suitable number of mysids were transferred by net from the 10, 15, and 20ppt 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 4ppt salinity. Five organisms were loaded per test chamber, giving ten per concentration. Only one test chamber, containing 5 test organisms, was used in each of the two pH adjusted controls. 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 unionized fractions (expressed in mg/l as N and as NH_3, respectively), were recorded in the controls and the 100% effluent test concentrations at the beginning and end of the test. Total ammonia concentrations were determined using an Orion® specific ion probe and a 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 Grant St. Sewage Treatment Plant, Melbourne, Brevard County, Florida. NPDES #FL0027693, on 2-4, December, 1981.

Test #1 Pre-chlorinated Sample

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			NI_3/NI_3+NI_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	4	7.7	6.8	7.2	7.7	7.7	7.8	21.4	20.9	20.8				
5.6%	5	5	5	7.7	6.8	7.2	7.7	7.7	7.8						17.5	17.5
10.0%	5(4)[a]	4	4	7.5	6.7	6.9	7.7	7.8	7.8	21.4	20.7	20.8				
10.0%	5	5	3	7.5	6.7	6.9	7.7	7.8	7.8						17.0	17.3
18.0%	5	4	4	7.4	6.7	6.9	7.6	7.8	7.8	21.5	20.7	20.7				
18.0%	5	4	3	7.4	6.7	6.9	7.6	7.8	7.8						16.0	16.5
32.0%	5	4	2	6.9	6.5	7.1	7.6	7.7	7.8	21.5	20.6	20.6				
32.0%	5	3	1	6.9	6.5	7.1	7.6	7.7	7.8						13.5	13.5
56.0%	5	0	0	6.2	6.3	6.9	7.5	7.7	7.8	21.5	20.6	20.6				
56.0%	5	0	0	6.1	6.3	6.9	7.5	7.7	7.7						9.0	9.5
100.0%	5	0	0	5.2	5.1	6.2	8.1	7.8	7.8	21.5	20.6	20.6	.42/8.12	.27/10.6		
100.0%	5	0	0	5.2	5.1	6.2	8.1	7.8	7.8						8.0	8.2

Remarks: a. Number in parentheses used in calculations since one organism jumped out between 0 and 24 hours.

Table 3. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Grant St. Sewage Treatment Plant, Melbourne, Brevard County, Florida. NPDES #FL0027693, on 2-4, December, 1981.

Test #2 Chloroinated Sample

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	7.9	7.0	7.3	7.8	7.8	7.8	21.5	20.7	20.6				
5.6%	5	5	5	7.9	7.0	7.3	7.8	7.8	7.8						17.5	17.5
10.0%	5	5	5	7.8	7.1	7.4	7.7	7.8	7.9	21.5	20.5	20.6				
10.0%	5	5	4	7.8	7.2	7.6	7.7	7.8	7.9						17.0	17.2
18.0%	5	0	0	7.9	7.4	—a	7.7	7.8	—a	21.5	20.4	—a				
18.0%	5	0	0	7.9	7.4	—	7.7	7.8	—						16.0	16.1
32.0%	5	0	0	7.9	7.7	—	7.5	7.8	—	21.5	20.4	—				
32.0%	5	0	0	7.9	7.7	—	7.5	7.8	—						13.5	13.8
56.0%	5	0	0	7.8	7.9	—	7.4	7.8	—	21.5	20.3	—				
56.0%	5	0	0	7.8	7.9	—	7.4	7.8	—						9.0	9.3
100.0%	5	0	0	7.8	8.0	—	8.0	7.9	—	21.7	20.3	—	.53/12.6	.42/10.9		
100.0%	5	0	0	7.8	8.0	—	8.0	7.9	—						7.0	7.0

Remarks: a. Since all organisms were dead by hour 24, the 48 hour data are not reported.
Total chlorine residuals: 5.6% - 0.2 mg/l 32.0% - 0.75 mg/l
10.0% - 0.35 mg/l 56.0% - 1.25 mg/l
18.0% - 0.5 mg/l 100.0% - 2.0 mg/l (HACH DPD Method)

Table 4. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Grant St. Sewage Treatment Plant, Melbourne, Brevard County, Florida. NPDES #FL0027693, on 2-4, December, 1981.

Test #3 De-chlorinated Sample

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			NI_3/NI_3+NI_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	7.7	7.2	7.4	7.8	7.8	7.8	21.5	20.5	20.6				
5.6%	5	5	5	7.7	7.2	7.4	7.8	7.8	7.8						17.5	17.5
10.0%	5	5	5	7.7	6.9	7.2	7.8	7.8	7.8	21.5	20.4	20.6				
10.0%	5	5	5	7.7	6.9	7.2	7.8	7.8	7.8						17.0	17.2
18.0%	5	5	0	7.8	6.8	7.2	7.7	7.7	7.8	21.5	20.4	20.6				
18.0%	5	5	3	7.8	6.8	7.2	7.7	7.7	7.8						16.0	16.0
32.0%	5	4	0	7.8	6.9	7.1	7.7	7.7	7.8	21.6	20.3	20.5				
32.0%	5	3	0	7.8	6.9	7.1	7.7	7.7	7.7						13.5	13.9
56.0%	5	1	0	7.9	6.8	6.8	7.6	7.7	7.7	21.8	20.4	20.5				
56.0%	5	0	0	7.9	6.8	6.9	7.6	7.7	7.7						9.0	9.5
100.0%	5	0	0	7.9	6.7	6.3	8.2	7.9	7.7	22.2	20.4	20.5	.47/7.0	.17/8.68		
100.0%	5	0	0	8.0	6.7	6.3	8.2	7.9	7.7						7.0	7.0

Remarks: Chlorine neutralized with 2.25ml of .025 N sodium thiosulfate per liter of sample.
Final residual: <0.01 mg/l (HACH DPD Method)

Table 5. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Grant St. Sewage Treatment Plant, Melbourne, Brevard County, Florida. NPDES #FL0027693, on 2-4 December, 1981.

Control Series

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			NI_3/NI_3+NI_4 (mg/l)		Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
Seawater Control	5	5	5	7.7	6.8	7.2	7.7	7.8	7.8	21.3	20.9	20.8	.0/.22	.02/.81		
Seawater Control	5	5	5	7.7	6.8	7.2	7.7	7.8	7.8						18.0	19.0
Salinity Control	5(4)[a]	4	4	8.0	7.1	7.5	7.7	7.8	7.9	21.3	20.6	20.6				
Salinity Control	5	5	5	8.0	7.2	7.6	7.7	7.8	7.9						11.5	12.0
Sea Salts Control	5	5	5	8.3	7.5	7.5	8.4	8.2	8.1	21.5	20.5	20.6				
Sea Salts Control	5	5	5	8.3	7.6	7.7	8.4	8.2	8.1						8.0	8.0
Sea Salts pH Adjusted	5	5	5	8.5	7.5	7.6	7.3	7.8	7.8	22.4	20.5	20.6			7.0	7.2
100% Effluent pH Adjusted[b]	5	1	0	8.1	6.3	6.1	7.3	7.7	7.6	22.3	20.7	20.7	.06/7.0	.21/13.2	7.0	7.0

Remarks: a. Number in parentheses used in calculations since one organism jumped out between 0 and 24 hours.
b. Pre-chlorinated effluent was used in this control.

RESULTS AND DISCUSSION

The samples of effluent collected from the Grant Street Sewage Treatment Plant proved to be acutely toxic to the test organism, Mysidopsis bahia. Results of pre-chlorinated effluent indicated 24 hour LC_{50}'s of 29.6% to 36.4% and 48 hour LC_{50}'s of 19.2% to 24.0% (Table 6). The chlorinated effluent was slightly more toxic, with a 24 hour LC_{50} of 13.4% and a 48 hour LC_{50} of 12.6% (Table 6). The de-chlorinated effluent resulted in 24 hour LC_{50}'s of 37.9% to 38.4% and a 48 hour LC_{50} of 15.7% (Table 6). The 96 hour LC_{50}'s for these samples would therefore be less than or equal to their 48 hour values.

The cause of the toxicity in the pre-chlorinated sample cannot be established at this time, since no chemical analyses were performed. However, the slight increase in toxicity of the chlorinated sample can be attributed to the added effects of chlorine, since the concentrations found (Table 3) are within the range reported to be toxic to certain aquatic organisms (U.S. EPA, 1976). As with the pre-chlorinated sample, the cause of toxicity in the de-chlorinated sample could not be ascertained since no chemical analyses were performed.

Specific concentrations of total and unionized ammonia were determined in these samples. The concentrations found were slightly below the ranges reported to be toxic to certain aquatic organisms (Thurston and Russo, 1981). District personnel have indicated that a number of industrial metal plating companies may contribute wastewater to the Grant Street Sewage Treatment Plant. The relatively high specific conductance of this effluent (1400 umhos, bioassay field data) may support this, suggesting that substantial concentrations of ionized species are present. If so, it is suggested that additional testing be performed at this facility, particularly a priority pollutant metal scan.

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 tests 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 (3), 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, whether as 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)(o)]; 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, but rather to the average of a number of points within 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 the mixing zone (i.e., at any point within the mixing zone), from exceeding the 96 hour LC_{50}. Therefore, at any point within a mixing zone, including the point of discharge, one-half of the test organisms cannot be killed in 96 hours.

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, the regulations require that the <u>maximum</u> 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.

Table 6. LC_{50} Data for Bioassays of Grant St. Sewage Treatment Plant, Melbourne, on 2-4 December, 1981.

Organism	Method of Data Analysis	24 LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Test #1: *M. bahia* Pre-chlorinated Sample	Binomial	36.4% (10 and 56)	24.0% (5.6 and 56)
	Moving Average	29.6% (22 and 43)	19.5% (14 and 28)
	Probit	31.1% (24 and 40)	19.2% (14 and 27)
Test #2: *M. bahia* Chlorinated Sample	Binomial	13.4% (10 and 18)	12.6% (10 and 18)
	Moving Average	[a]	[a]
	Probit	[a]	[a]
Test #3: *M. bahia* De-chlorinated Sample	Binomial	38.1% (18 and 56)	15.7% (10 and 32)
	Moving Average	38.4% (30 and 53)	[a]
	Probit	37.9% (30 and 48)	[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.

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