

BIOASSAYS OF SHERWOOD PARK

SEWAGE TREATMENT PLANT

MELBOURNE, BREVARD COUNTY, FLORIDA

NPDES #FL0024503

June 11, 1985

Biology Section
Division of Environmental Programs

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EXECUTIVE SUMMARY

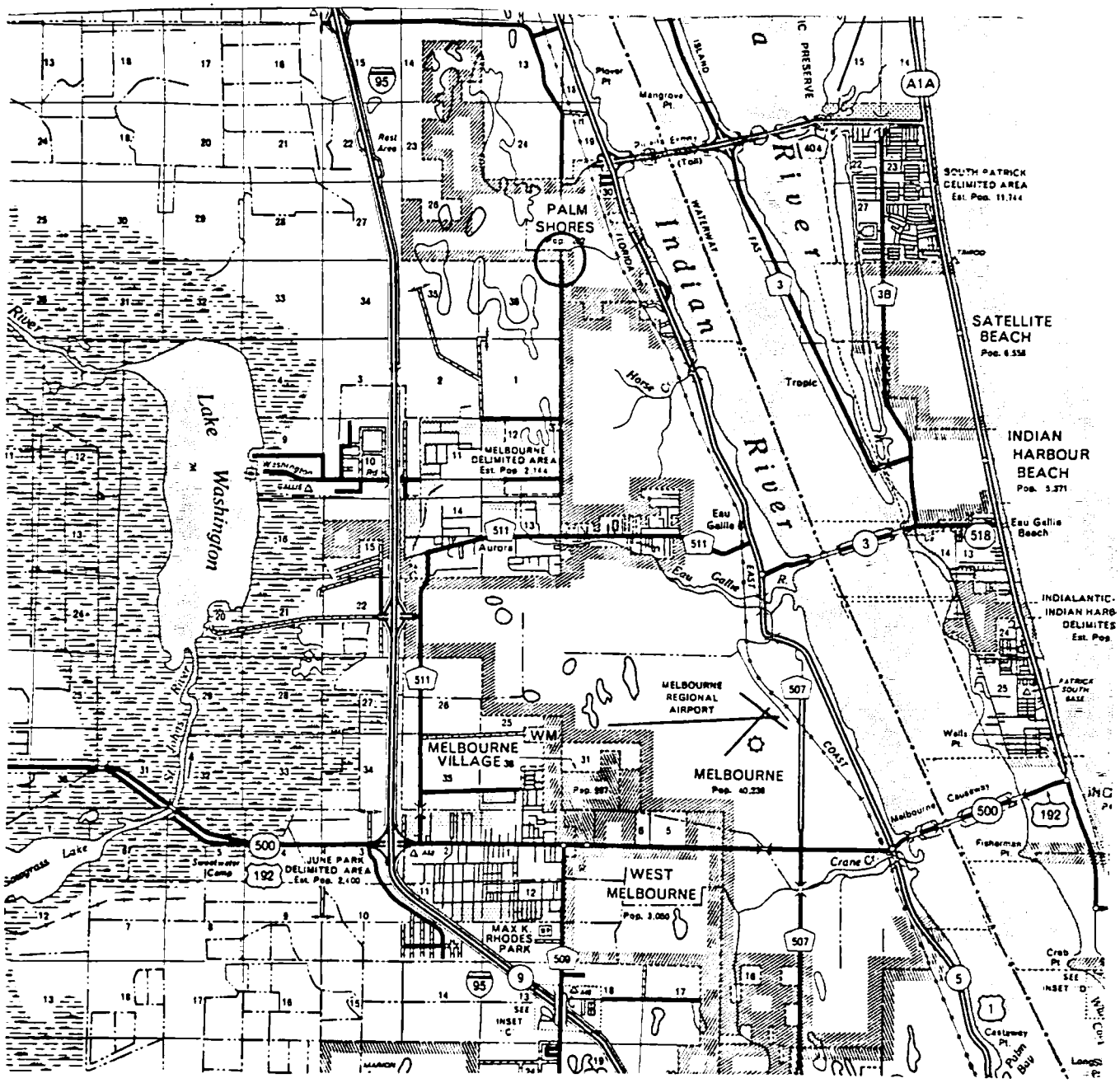
Sherwood Park STP, Melbourne, NPDES #FL0024503, on 2 to 4 April, 1985.

The sample of chlorinated effluent collected from the Sherwood Park Sewage Treatment Plant was acutely toxic to the saltwater organism, Mysidopsis bahia, producing both 24 and 48 hour LC_{50}'s of <10.0%. A chlorine residual of 4.4 mg/l (Iodometric method) was measured in the sample, which may be responsible for the extreme toxicity observed. The de-chlorinated effluent sample was also toxic to M. bahia, producing a 48 hour LC_{50} of 99.0%. The total and unionized ammonia detected in these samples (maximum measured = 15.5 mg/l and .226 mg/l, respectively) may have contributed to the observed toxicity. Chemical analyses indicated the presence of small concentrations of nickel (50 ug/l) and silver (0.2 ug/l). Organic analyses were not performed.

INTRODUCTION

The Sherwood Park Sewage Treatment Plant is located on Robinhood Drive in North Melbourne, Brevard County, Florida (Figure 1). The facility is owned and operated by the City of Melbourne. It has a design flow capacity of 0.69 MGD and discharges secondarily treated wastewater to Otter Creek, a tributary of the Indian River. The wastewater treatment system consists of preliminary treatment (comminutors and grit chambers), clarifiers, trickling filters, secondary clarifiers, chlorination and activated sludge aeration. A schematic diagram of wastewater treatment is outline in Figure 2. According to NPDES files, this facility will discontinue discharging wastewater to Otter Creek by August, 1985.

At the request of the St. Johns River District, the Biology Section performed two static acute toxicity bioassays on April 2, 1985, to evaluate the toxicity of wastewater from the Sherwood Park STP. The results of these tests are presented in this report. Those involved in performing these tests and in preparation of this report were: R. B. Frydenborg, M. L. Moultrie, and M. L. Roll.



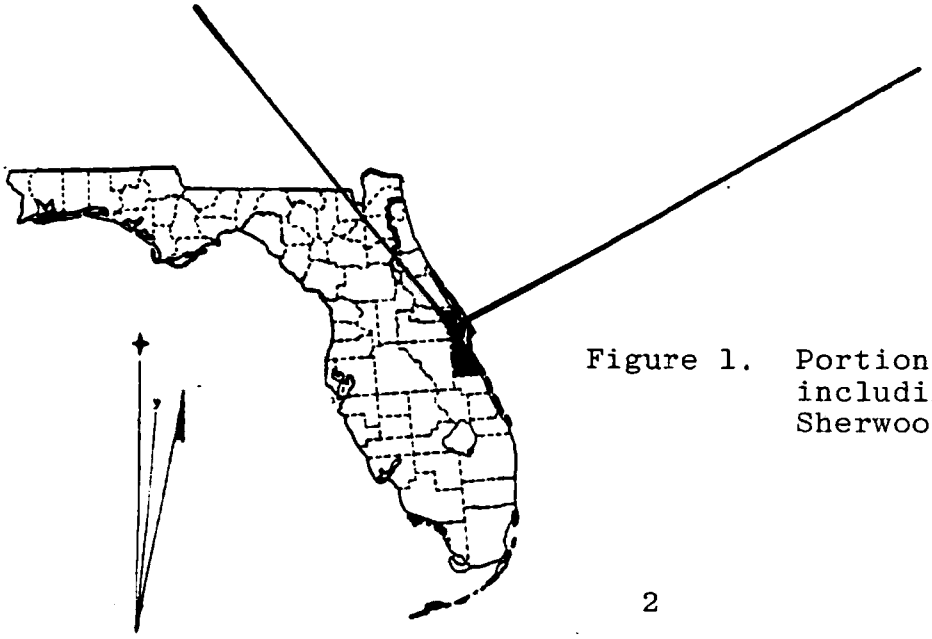


Figure 1. Portions of Brevard County, including the location of Sherwood Park STP.

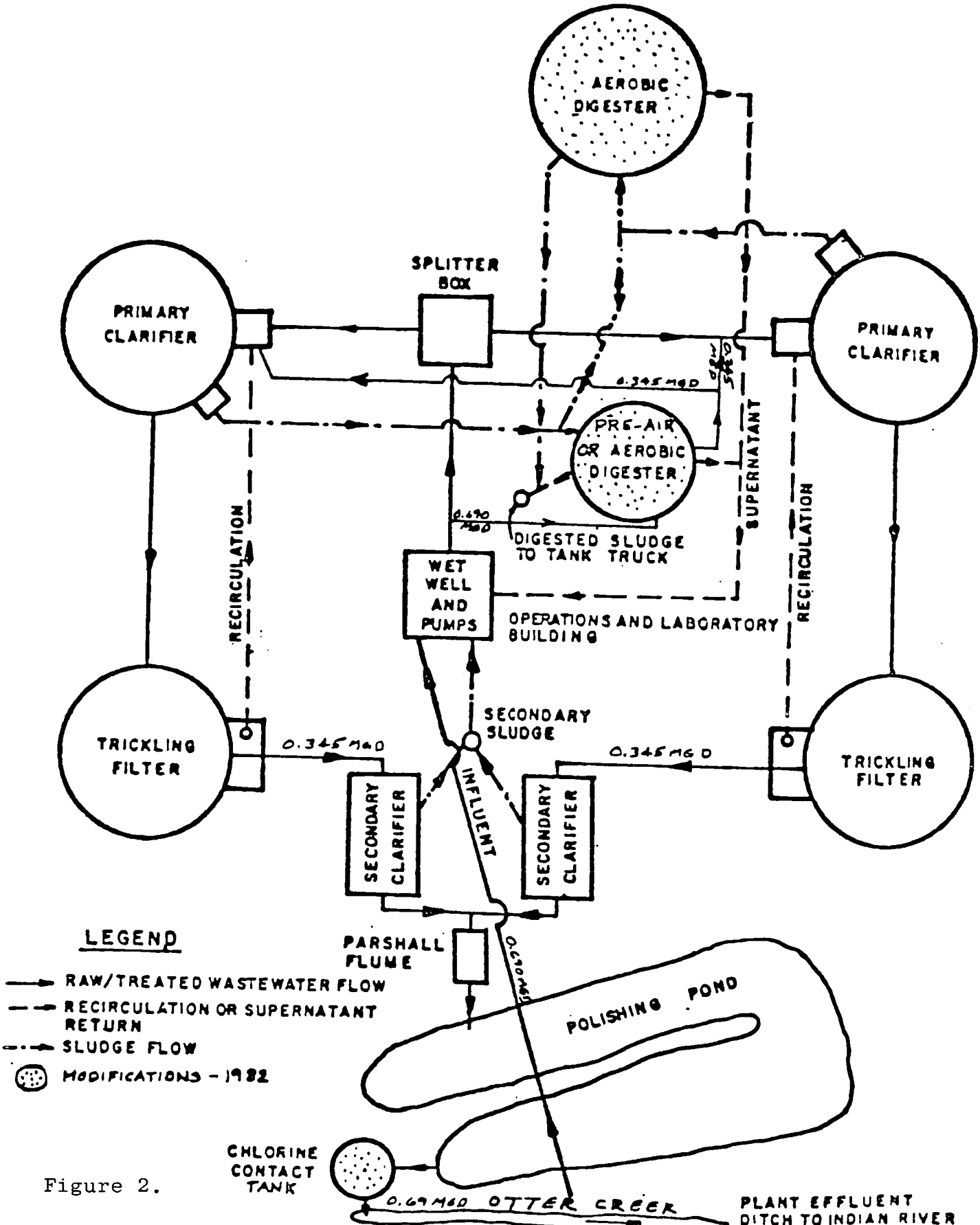


Figure 2. FLOW SCHEMATIC - SHERWOOD PARK WASTEWATER TREATMENT PLANT
MELBOURNE, FLORIDA

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier and Weber (1985) and the FDER Biology Section (1985). The organism 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 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° $\pm$ 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

Two 48 hour static acute toxicity bioassays were performed on samples of chlorinated and de-chlorinated wastewater collected from the Sherwood Park Sewage Treatment Plant. The tests were performed from 1045 hours on 2 April to 1100 hours on 4 April, 1985.

The samples were collected by Mr. Louis Ley of DER's St. Johns River District office at 1420 hours on 1 April, 1985, 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 20° $\pm$ 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 the range recommended in the above referenced bioassay protocols. This avoids stressful effects caused by exposure to unusually cold or warm samples.

Salinity Adjustment

Since this particular bioassay sample eventually enters an estuarine 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 8 ppt was obtained. This salinity is well within the salinity tolerance of the test organism. Nevertheless, a Salinity Control was also employed, consisting of artificial seawater diluted with well water until a similar salinity of approximately 8 ppt was obtained. Additional information concerning salinity adjustment and saltwater tests is given below.

Total Residual Chlorine

Since many wastewaters are chlorinated, especially those originating from sewage treatment facilities, it is often desirable to differentiate between the toxicity resulting from chlorine and the toxicity associated with other components of the wastewater. To accomplish this, the Biology

Section performed a concurrent bioassay on a sample of chlorinated wastewater in which the chlorine had been neutralized. De-chlorination was accomplished by adding a calculated amount of 0.025N sodium thiosulfate. The total residual chlorine concentrations of the tested effluents are given on the bioassay data forms. A comparison of the results of these tests will distinguish between the toxicity due solely to residual chlorine and the toxicity inherently contained in the wastewater.

Saltwater Tests

Saltwater for dilution of the effluent samples was prepared from artificial sea salts (Forty Fathoms®), and adjusted with DER well water until a salinity of approximately 20 ppt was achieved. For test concentrations of 56% and below, this diluent was mixed directly with the effluent, achieving a range of final salinities (8.1 ppt to 20.1 ppt). For the 100% effluent test concentration, freshly autoclaved artificial sea salts were added until a salinity of approximately 8 ppt was achieved (well within 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 - Artificial seawater (20.1 ppt). This represents the highest concentration of artificial seawater 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) Salinity Control - Artificial seawater (20.1 ppt) adjusted with well water to approximately 8 ppt salinity. This tests 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 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 appropriate 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 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 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 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%[a]	------	500 ml	500 ml

[a] The 100.0% concentration was not used in the chlorinated test due to the large concentration of residual chlorine present.

Table 2. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Sherwood Park STP, Melbourne, Brevard County, Florida, NPDES #FL0024503 on 2 to 4 April, 1985.

Chlorinated Sample[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	5	6.2	7.1	7.9	8.4	8.4	8.3	21.8	21.4	19.2			Control Water
Diluent Control	5	5	5	6.2	6.5	8.0	8.4	8.4	8.4				20.1	19.2	NH_3(as NH_3)(mg/l) 0 / Total Alkalinity (mg/l $CaCO_3$)
Salinity Control	5	5	5	7.4	6.2	6.6	7.3	8.0	8.1	22.0	21.2	19.5			
Salinity Control	5[b]	4	4	7.4	6.2	6.6	7.3	8.0	8.0				8.1	8.1	
10.0%	5	0	-[c]	6.0	7.0	-[c]	8.4	8.4	-[c]	21.7	21.4	-[c]			Total ammonia (as N)(mg/l) / Total Hardness (mg/l $CaCO_3$)
10.0%	5	0	-	6.0	7.1	-	8.4	8.4	-				18.5	-[c]	0
18.0%	5	0	-	6.0	7.2	-	8.3	8.4	-	21.6	21.3	-			
18.0%	5	0	-	6.0	7.3	-	8.3	8.4	-				16.8	-	
32.0%	5	0	-	6.0	7.4	-	8.2	8.4	-	21.2	21.0	-			
32.0%	5	0	-	6.0	7.5	-	8.2	8.3	-				14.1	-	56% Effluent
56.0%	5	0	-	6.0	7.5	-	7.8	8.3	-	20.8	21.0	-			NH_3(as NH_3)(mg/l) / Total Alkalinity (mg/l $CaCO_3$)
56.0%	5	0	-	6.0	7.5	-	7.8	8.3	-				9.5	-	.220
															Total ammonia (as N)(mg/l) / Total Hardness (mg/l $CaCO_3$)
															7.5

[a] Total residual chlorine detected: 4.4 mg/l (Iodometric) and >3.0 mg/l (HACH).

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

[c] All organisms were dead at 24 hours. Parameters not measured.

Table 3. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Sherwood Park STP, Melbourne, Brevard County, Florida, NPDES #FL0024503 on 2 to 4 April, 1985.

De-chlorinated Sample[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	5.9	5.8	6.1	8.4	8.4	8.2	21.1	21.6	19.7			Control Water
10.0%	5	5	5	6.1	5.8	6.2	8.4	8.4	8.2				18.5	18.0	NH_3(as NH_3)(mg/l) 0 / Total Alkalinity (mg/l $CaCO_3$)
18.0%	5	5	5	6.1	5.6	6.8	8.3	8.4	8.3	21.6	21.5	19.4			
18.0%	5	5	5	6.1	5.6	6.8	8.3	8.4	8.1				17.0	16.5	
32.0%	5	5	5	6.0	5.5	6.3	8.2	8.3	8.2	21.4	21.4	19.4			Total ammonia (as N)(mg/l) 0 / Total Hardness (mg/l $CaCO_3$)
32.0%	5	5	5	6.0	5.4	6.4	8.2	8.3	8.1				14.0	14.0	
56.0%	5	5	4	6.1	5.8	6.5	8.0	8.2	8.1	21.1	21.4	19.4			
56.0%	5	5	5	6.1	5.8	6.5	8.0	8.1	8.1				9.8	9.5	
100.0%	5	5	3	6.7	5.8	4.1	7.5	8.1	7.7	20.4	21.2	19.5			
100.0%	5(3)[b]	2	1	6.7	5.8	5.5	7.5	8.1	8.0				8.2	8.5	100% Effluent
															NH_3(as NH_3)(mg/l) .226 / Total Alkalinity (mg/l $CaCO_3$)
															Total ammonia (as N)(mg/l) 15.5 / Total Hardness (mg/l $CaCO_3$)

[a] Total residual chlorine detected: <.05 mg/l (HACH).

[b] Two organisms jumped from container, number in parentheses used for LC_{50} calculation.

Table 4. LC_{50} Data for Bioassay of Sherwood Park STP, Melbourne, Brevard County, Florida, NPDES #FL0024503 on 2 to 4 April, 1985.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Chlorinated Sample *M. bahia*	Binomial Moving Average Probit	<10.0%,[a]	<10.0%,[a]
De-chlorinated Sample *M. bahia*	Binomial	b	100.0% (56.0 and ∞),[c]
	Moving Average	b	100.0% (72.8 and ∞),[c]
	Probit	b	99.0% (74.6 and 511.2),[c],[d]

[a] Since the lowest test concentration (10.0%) killed all the test organisms, the LC_{50} is <10.0%.

[b] 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.

[c] 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.

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

Table 5. Results of Metal Analyses[a] performed on Sherwood Park STP's Wastewater, Brevard County, NPDES #FL0024503, collected on 1 April, 1985.

Metals	
Arsenic	N.D.
Cadmium	N.D.
Chromium	<25 ug/l
Copper	N.D.
Lead	N.D.
Mercury	N.D.
Nickel	50 ug/l
Selenium	N.D.
Silver	0.2 ug/l
Zinc	N.D.

N.D. = None Detected.

[a] Samples for organic analyses were invalidated.

RESULTS AND DISCUSSION

The sample of final chlorinated effluent collected from the Sherwood Park Sewage Treatment Plant was acutely toxic to the test organism, Mysidopsis bahia, producing both 24 and 48 hour LC_{50}'s of <10.0% (Table 4). A chlorine residual of 4.4 mg/l (Iodometric Method) was measured in the sample, which may be responsible for the extreme toxicity observed. The de-chlorinated sample was also toxic to M. bahia, producing a 48 hour LC_{50} of 99.0% (Table 4). The total and unionized ammonia detected in these samples (maximum measured = 15.5 mg/l and .226 mg/l, respectively) may have contributed to the observed toxicity. Chemical analyses detected a small concentration of nickel (50 ug/l), and silver (0.2 ug/l). Organic analyses were not performed.

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.

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