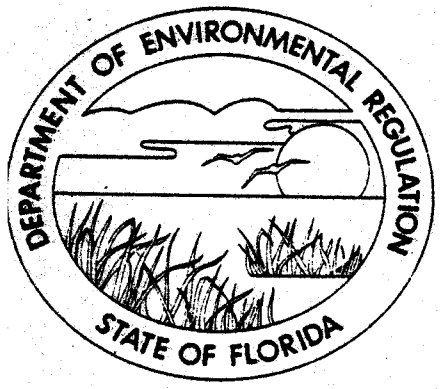


BIOASSAYS OF THE CITY OF CAPE CANAVERAL

WATER POLLUTION CONTROL FACILITY

BREVARD COUNTY, FLORIDA

NPDES #FL0020541

SAMPLED 1/25/82

Biological Section
Bureau of Water Analysis
March 18, 1982

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EXECUTIVE SUMMARY

Cape Canaveral Water Pollution Control Facility, on 26 to 28 January, 1982

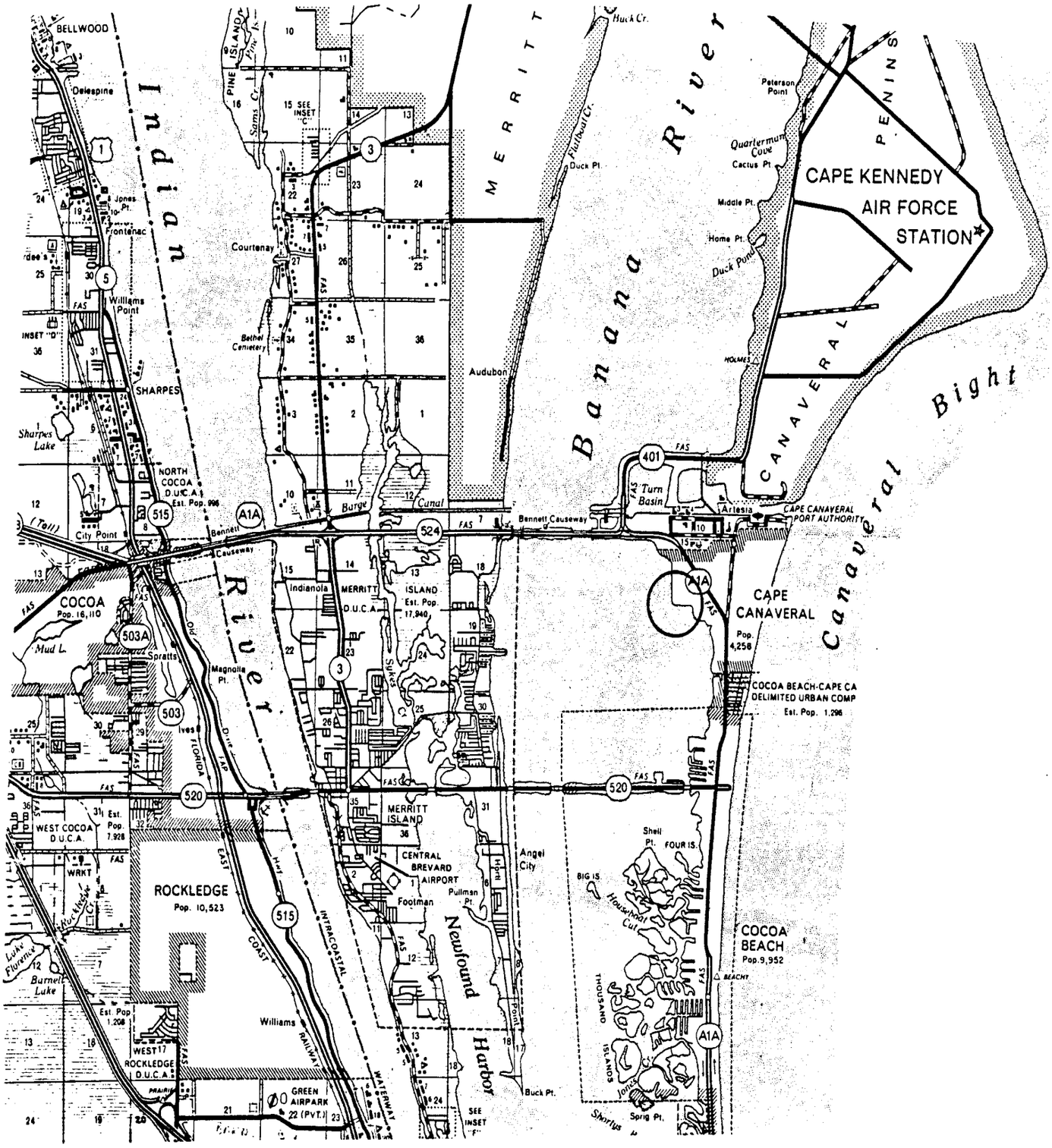
Three 48 hour static acute toxicity bioassays were performed on pre-chlorinated, chlorinated and de-chlorinated samples of effluent from this facility using Mysidopsis bahia as the test organism. Results indicated that all three samples of effluent were acutely toxic to the shrimp, with 48 hour LC_{50} values of 15.4%, 29.8% and 18.7%, respectively. The reason of the toxicity noted in these effluents could not be attributed to a single cause, but to synergistic effects, including ammonia.

INTRODUCTION

The City of Cape Canaveral Water Pollution Control Facility (sewage treatment plant) is located south of Central Boulevard and west of State Road 401 in Cape Canaveral, Brevard County, Florida (Fig. 1). According to the NPDES permit application (dated 24 March 1973), this facility began operations in 1966 and serves a population of approximately 5400. Since there are few private residences in the service area, a majority of the wastewater stems from commercial and industrial sources, including citrus packaging and facilities associated with port activities. The plant has a design capacity of 0.85 MGD but treats an average of only 0.47 MGD. Wastewater treatment at this plant consists of: comminution, contact stabilization, final clarification and chlorination (Fig. 2) prior to discharge to the Banana River through Outfall #001.

The Biological Section had previously performed bioassays on samples of pre-chlorinated, chlorinated and de-chlorinated effluent from this facility, on 10 to 12 December, 1980. The results of those tests indicated that all three samples of effluent were acutely toxic to Mysidopsis bahia, with 48 hour LC_{50}'s of 56%, 42% and 56%, respectively (FDER 1981). The cause of this toxicity was attributed, in part, to ammonia, chlorine and possibly chloramines.

To further evaluate the extent of toxicity of these effluent samples to the biota of the receiving waters, the Biological Section performed additional bioassays on grab samples from this facility on 26 to 28 January, 1982. The results of these latest tests are presented in this report.



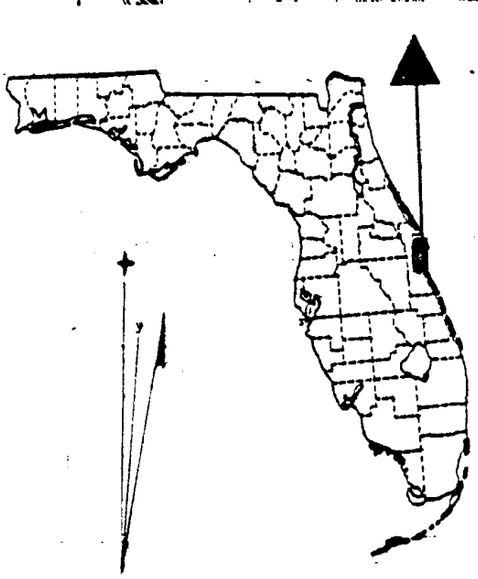
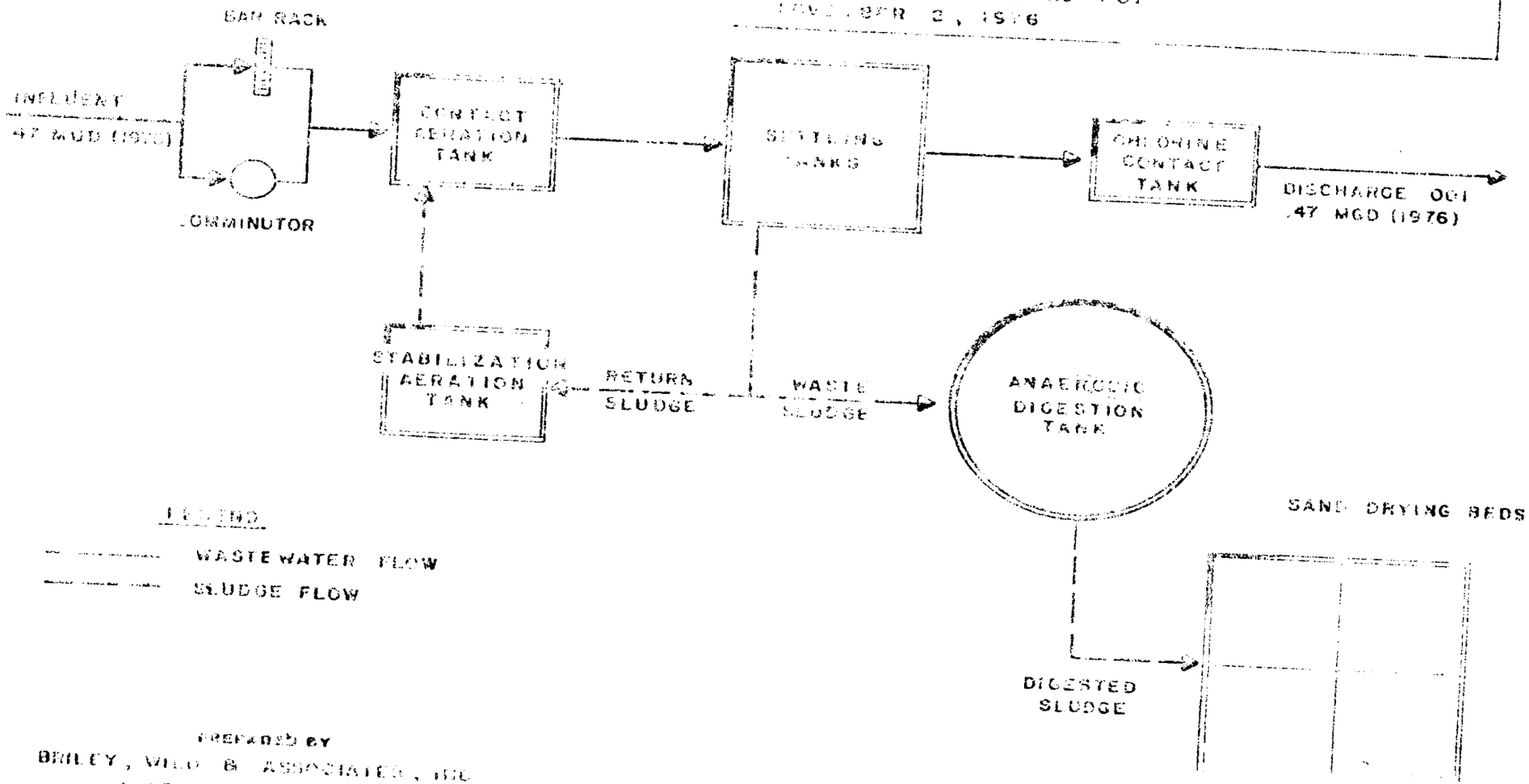


Figure 1. Portions of Brevard County, Florida, including city of Cape Canaveral and study area.

Figure 2. SCHEMATIC OF WASTEWATER FLOW
CAPE CANAVERAL WATER POLLUTION CONTROL PLANT
CAPE CANAVERAL, FLORIDA
DISCHARGE SERIAL NO 001
NOVEMBER 2, 1976



PREPARED BY
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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 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 1345 hours on 26 January, 1982, to 1400 hours on 28 January, 1982. Samples of pre-chlorinated effluent and of chlorinated effluent were collected at 1430 hours on 25 January, 1982. The samples were collected by Mr. Louis Ley of DER's St. Johns River District using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples for metal analysis were preserved with HNO_3. Samples for organic chemical analysis and for the bioassays were unpreserved. 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 = 30 ppt). This saltwater diluent was then adjusted to the salinity of the receiving body of water (Banana River = 28 ppt) 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 (13ppt to 26 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). A series of 3 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 = 27 ppt), artificial sea salt (Ocean 50®) adjusted well water (salinity = 7 ppt), and a salinity control consisting of Turkey Point seawater and well water (salinity 12 ppt). 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, due to a shortage of chlorinated effluent, a 100% concentration of de-chlorinated effluent could not be tested. 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 unionized fractions (expressed in mg/l as N and as NH_3, respectively), were recorded in the seawater controls and the 100% effluent test concentration at the beginning and end of the test. Total ammonia concentrations were determined using a 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 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 Cape Canaveral Water Pollution Control Facility, Brevard County, Florida, NPDES #FL0020541, on 26 to 28 January, 1982.

Test #1. Pre-Chlorinated Effluent

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	4	7.6	7.2	6.7	8.1	7.9	7.9	19.0	19.0	19.5				
5.6%	5	5	5	7.6	7.2	6.7	8.1	7.9	7.9						25.5	27.5
10.0%	5	5	1	7.6	7.3	6.7	8.0	7.9	7.9	19.0	19.0	19.4				
10.0%	5	5	2	7.7	7.3	6.7	8.0	7.9	7.9						25.0	26.0
18.0%	5	5	3	7.6	7.3	6.8	7.8	7.8	7.9	19.0	19.0	19.4				
18.0%	5	5	3	7.6	7.3	6.8	7.8	7.8	7.9						22.5	24.0
32.0%	5	5	2	7.4	7.5	7.0	7.5	7.8	7.9	19.0	19.0	19.4				
32.0%	5	5	2	7.4	7.5	7.1	7.5	7.8	7.9						19.5	21.0
56.0%	5(4)[a]	1	0	6.4	7.9	7.5	7.2	7.8	8.0	19.0	19.0	19.4				
56.0%	5	0	0	6.6	7.9	7.7	7.2	7.7	8.0						13.0	14.0
100.0%	5	0	0	5.1	8.0	7.6	7.2	7.8	8.0	19.0	19.0	19.3	.01/2.24	.43/12.04		
100.0%	5	0	0	5.1	7.9	7.9	7.2	7.8	8.0						7.5	9.5

Remarks: [a] Number in parentheses used in calculations since one organism was cannibalized between 0 and 24 hours.

Table 3. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Cape Canaveral Water Pollution Control Facility, Brevard County, Florida, NPDES #FL0020541, on 26 to 28 January, 1982.

Test #2. Chlorinated Effluent

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	7.5	7.2	6.7	8.0	7.9	8.0	19.0	18.5	19.0				
5.6%	5	5	5	7.7	7.2	6.7	8.0	8.0	8.0						25.0	27.0
10.0%	5	5	5	7.8	7.4	6.8	8.0	7.9	8.0	19.0	18.5	18.8				
10.0%	5	5	5	7.8	7.4	6.9	8.0	7.9	8.0						24.5	26.0
18.0%	5	5	5	7.7	7.6	7.0	7.9	7.9	7.9	19.0	18.5	18.5				
18.0%	5	5	4	7.8	7.6	7.0	7.8	7.9	8.0						22.5	24.0
32.0%	5	4	2	7.6	7.8	7.3	7.5	7.8	8.0	19.0	18.5	18.8				
32.0%	5	5	3	7.6	8.0	7.5	7.5	7.9	8.0						19.0	21.0
56.0%	5	0	0	7.1	8.3	7.7	7.2	7.8	7.9	19.0	19.0	18.8				
56.0%	5	0	0	7.1	8.4	7.7	7.2	7.8	8.0						13.0	14.0
100.0%	5	0	0	6.4	8.7	8.1	7.2	7.8	8.1	19.0	19.0	18.8	.01/2.24	.42/12.04		
100.0%	5	0	0	6.4	8.7	8.1	7.2	7.8	8.1						7.0	8.0

Remarks: Total chlorine residual in the 100% sample: 1.0 mg/l (HACH DPD Method)
0.18 mg/l (Iodometric Method)

Table 4. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Cape Canaveral Water Pollution Control Facility, Brevard County, Florida, NPDES #FL0020541, on 26 to 28 January, 1982.

Test #3. De-Chlorinated Effluent

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	8.0	7.4	6.9	8.1	8.0	8.0	19.7	19.0	19.5				
5.6%	5	5	5	8.0	7.4	6.9	8.1	8.0	8.0						26.0	27.0
10.0%	5	5	4	8.1	7.4	6.8	8.1	8.0	8.0	19.5	19.0	19.3				
10.0%	5	4	3	8.1	7.4	6.8	8.1	8.0	7.9						25.0	26.0
18.0%	5	5	2	8.0	7.5	6.9	8.0	7.9	7.9	19.5	19.0	18.8				
18.0%	5	5	3	8.0	7.5	6.9	8.0	7.9	7.9						23.0	24.0
32.0%	5	5	2	8.1	7.6	7.2	7.8	7.9	7.9	19.5	19.0	18.8				
32.0%	5	5	2	8.1	7.6	7.1	7.8	7.9	7.9						19.0	20.0
56.0%	5	2	0	8.0	7.8	7.5	7.5	7.8	8.0	19.5	19.0	18.8				
56.0%	5	3	0	8.0	7.8	7.8	7.5	7.8	8.0						13.0	14.0

Remarks:

Table 5. Control Series for bioassays of Cape Canaveral Water Pollution Control Facility on 26 to 28 January, 1982.

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	7.8	6.9	7.0	8.2	8.0	7.9	19.7	19.0	19.2	.03/.64	.05/1.96		
Seawater Control	5	5	5	7.9	7.1	6.8	8.2	8.0	7.9						27.0	29.0
Sea Salt Control	5	5	5	7.5	8.3	7.7	8.6	8.3	8.2	20.0	19.0	19.2				
Sea Salt Control	5	5	5	7.5	8.3	7.7	8.6	8.3	8.2						7.0	8.0
Salinity Control	5	5	5	7.5	8.1	7.5	8.1	8.0	8.0	19.5	19.0	19.7				
Salinity Control	5	5	5	7.5	8.1	7.5	8.1	8.0	8.0						12.0	13.0

Table 6. LC_{50} Data for Bioassays of Cape Canaveral Water Pollution Control Facility on 26 to 28 January, 1982.

Organism	Method of Data Analysis	24 LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Test #1: M. bahia	Binomial	45.2% (32 and 56)	13.4% (5.6 and 56)
Pre-chlorinated	Moving Average	a	16.1% (11 and 23)
Sample	Probit	a	15.4% (10 and 22)
Test #2: M. bahia	Binomial	39.9% (32 and 56)	32.0% (18 and 56)
Chlorinated	Moving Average	a	28.8% (21 and 41)
Sample	Probit	a	29.8% (23 and 38)
Test #3: M. bahia	Binomial	56.0% (32 and ∞),b	18.0% (5.6 and 56)
De-chlorinated	Moving Average	a	18.7% (14 and 25)
Sample	Probit	83.6% (46 and 100),b	19.0% (13 and 27)

[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.

RESULTS AND DISCUSSION

The pre-chlorinated effluent from the Cape Canaveral facility proved to be acutely toxic to Mysidopsis bahia, with a 24 hour LC_{50} of 45.2% and 48 hour LC_{50} values of 13.4% to 16.1% (Table 6). The chlorinated effluent was also acutely toxic to the shrimp, with a 24 hour LC_{50} of 39.9% and 48 hour LC_{50} values of 28.8% to 32.0% (Table 6). Likewise, the de-chlorinated effluent exhibited acute toxicity, with 24 hour LC_{50} values of 56.0% and 83.6%, and 48 hour LC_{50} values of 18.0% to 19.0% (Table 6). The 96 hour LC_{50}'s for these effluents would be less than or equal to their 48 hour values.

The DER Chemistry Section in Tallahassee has chemically characterized a sample of effluent from this facility, the results of which are presented in Table 7. Only one unidentified halogenated compound was determined in the effluent, and no metals were present in significant concentrations.

The toxicity noted in these latest bioassays was significantly higher than that noted in the previous 1980 bioassays. The effluents tested here were approximately 2 to 3 times as toxic as those tested in 1980. As in the 1980 bioassays, the reason for the toxicity could not be attributed to a single cause, but rather to the synergistic effects of several components or characteristics of the effluent.

Two curious aspects of these bioassays were the significant increase in ammonia from the beginning to the end of the test, and, the fact that the chlorinated effluent was the least toxic of the three tested. The increase in ammonia may have been due to chemical and biological activity in the effluent, converting the available nitrate to ammonia. This increase in ammonia may account for part of the toxicity noted in these effluents, since the concentration of unionized ammonia determined at 48 hours (0.43 mg/1, Table 2) is above the level reported to be toxic to certain aquatic organisms (Thurston and Russo, 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 (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 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.

Table 7. Results of Chemical Analyses Performed on Effluent from the Cape Canaveral Water Pollution Control Facility, collected on January 25, 1982.

Organics

Parameter	Component/Concentration
Acid Extractables	None detected
Base Neutral Extractables	None detected
Pesticide Extractables	1 unidentified halogenated compound, 0.2 µg/l (estimated)

Metals

Antimony	None detected
Arsenic	None detected
Beryllium	None detected
Cadmium	None detected
Chromium	None detected
Copper	<25 µg/l[a]
Lead	None detected
Mercury	<0.2 µg/l[a]
Nickel	None detected
Selenium	None detected
Silver	None detected
Thallium	None detected
Zinc	<25 µg/l[a]

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

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