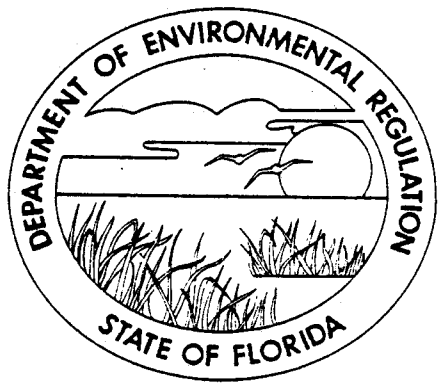


BIOASSAYS OF THE CITY OF

CAPE CANAVERAL WATER POLLUTION CONTROL FACILITY

BREVARD COUNTY, FLORIDA

NPDES PERMIT # FL0020541

SAMPLED 12/9/80

Biological Section
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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, and facilities associated with port ativities. The plant has a design capacity of 0.85 MGD but treats an average of only 0.47 MGD. Most of this flow (0.40 MGD) is from a shellfish harvesting operation which processes approximately 20 tons of scallops per day. 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.

On 10 December 1980, the Biological Section obtained prechlorinated and chlorinated effluent samples from the City of Cape Canaveral sewage treatment plant and conducted static bioassays to determine the existence and extent of toxicity to the biota of the receiving waters. The results of these tests are reported here.

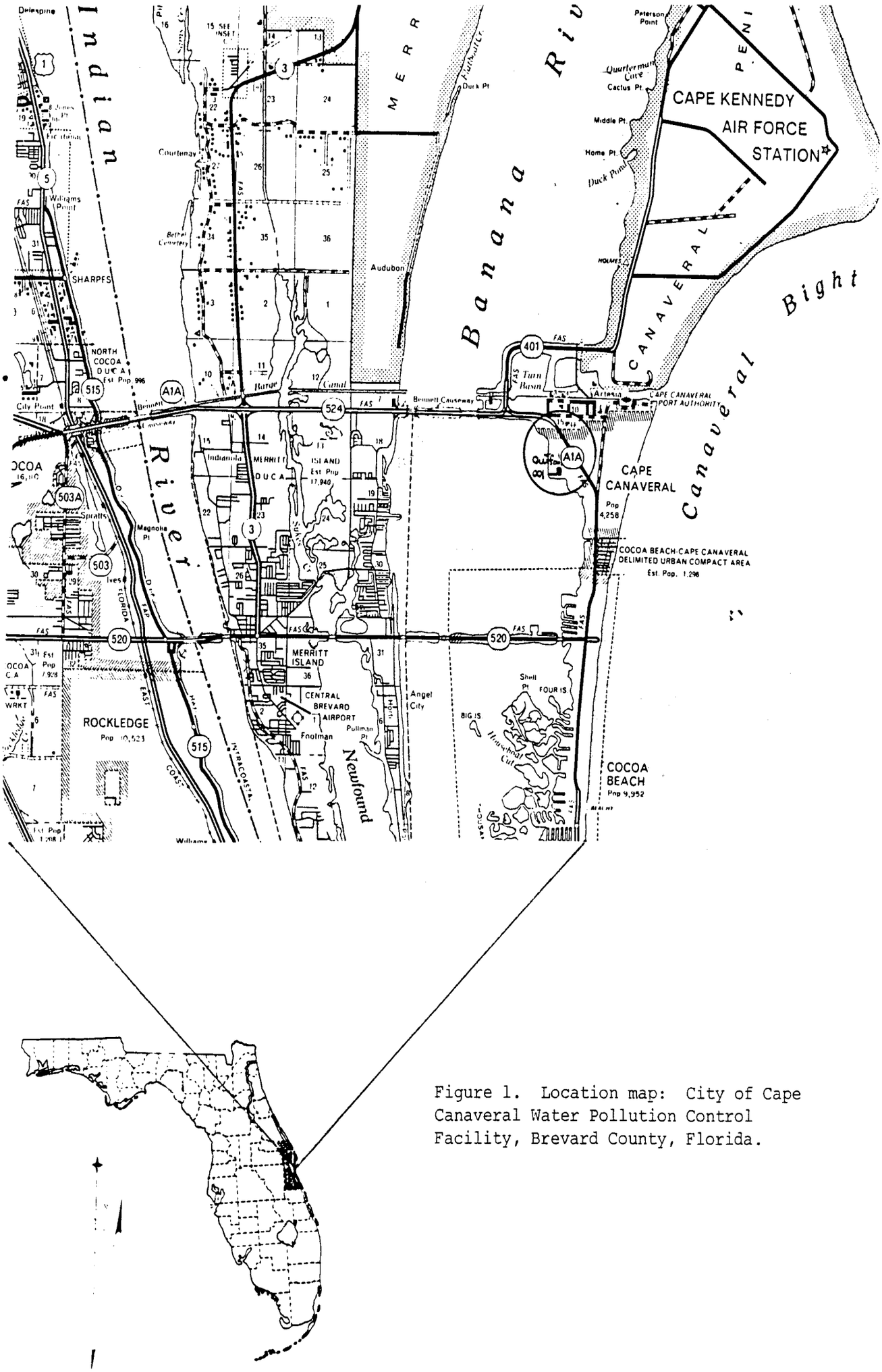


Figure 1. Location map: City of Cape Canaveral Water Pollution Control Facility, Brevard County, Florida.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978). The organisms selected for this study are among those recommended by Peltier (1978) and the U.S. EPA (1978). Life histories of these test organisms and maintenance procedures used to culture them 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 Gulf Breeze EPA Laboratory in Pensacola, Florida. The mysids have been 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 0/00) to minimize acclimation periods to low or high salinity effluents. Sea water is obtained from the FSU Marine Laboratory located at Turkey Point, Florida (salinity range 17-31 0/00) and partial water changes are performed weekly. Fresh water, for dilution of the sea water 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 $22^{\circ} \pm 1^{\circ}C$. Immediately prior to the bioassay, a suitable number of mysids were transferred by net to a culture dish for observation and selection of individuals for the test. Fifteen day old (three-quarter adult size) mysids were used in this test. Organisms selected for testing were transferred individually by glass tube and randomly placed in the test chambers.

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 bioassays were conducted from 1100 hours on 10 December 1980 to 1100 hours on 12 December 1980 on two grab samples of effluent from the City of Cape Canaveral Pollution Control Facility. One grab sample was obtained before chlorination and one from Outfall #001, after chlorination. The samples were collected at 1715 hours by Mr. Louis Ley of the DER St. Johns River District Office on 9 December 1980, using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples for metal analysis were preserved by acidification with nitric acid. Samples for organic chemical analysis and bioassay tests were unpreserved. The samples were iced and transported to DER's Tallahassee Laboratory within 24 hours of collection. Field parameters for the pre-chlorinated and chlorinated effluent samples are presented in Table 1.

These bioassays were performed using Mysidopsis bahia as the test organism. To evaluate the effects of chlorination on the toxicity of this effluent, tests were performed on prechlorinated (Test #1), chlorinated (Test #2), and dechlorinated (Test #3) samples. Dechlorination was accomplished by: obtaining a subsample of the chlorinated sample, determining its total chlorine content /Hach (DPD) and Iodometric Method I (APHA 1976)/, then neutralization of this chlorine by adding the proper amount of 0.025N sodium thiosulfate. To ensure that all the chlorine had been neutralized, the procedure was repeated until the total residual chlorine content of the subsample reached the limit of detection.

Saltwater for dilution of the effluent sample was obtained from the FSU Marine Laboratory at Turkey Point (Salinity = 29.0 ppt). To minimize salinity differences between the various concentrations, freshly autoclaved artificial sea salt (Ocean 50 ®) was added to the prechlorinated, chlorinated and dechlorinated effluent samples and mixed with a magnetic stirrer until dissolved and a salinity of 30.0 ppt achieved in each.

For each of these static tests, two replicates, each of six concentrations of effluent and a sea water control were used. Additionally, a second set of controls consisting of salinity adjusted DER well water (salinity = 27.5 ppt) was instituted to ensure that the sea salts used were not toxic to mysid shrimp. Immediately prior to the bioassays, a suitable number of mysids were transferred by net to a culture dish for observation and selection of individuals for the test. Individual mysid shrimp were siphoned or pipetted via glass tube, carefully examined for injury and transferred to wide mouth quart jars, each containing 500 ml of the appropriate concentration. The concentration series selected for this study (Table 2) are those recommended by APHA (1976).

Five organisms were loaded per test chamber, giving 10 per concentration. Temperature, pH, dissolved oxygen, and the number of live organisms were recorded at 0, 24, and 48 hours (Tables 3, 4, and 5). According to EPA (1978), "Although it is generally undesirable to feed most fish and macroinvertebrates during static toxicity tests, an exception is necessary for M. bahia". Therefore, mysids were fed brine shrimp nauplii (Artemia) daily at a rate of approximately 10-20 per mysid shrimp to minimize cannibalism. These tests were conducted at a temperature of 22.0± 1°C.

Table 1. Field Parameters of the Prechlorinated and Chlorinated Effluent Samples from the City of Cape Canaveral Water Pollution Control Facility.

Parameter	Prechlorinated Sample	Chlorinated Sample
Date of Collection[a]	9 December 1980	9 December 1980
Time of Collection[a]	1715 hours	1715 hours
Temperature[a]	25°C	25°C
Conductivity[a]	1900 µmhos	1900 µmhos
pH[a]	7.7	7.7
Dissolved Oxygen[a]	<1 mg/l	<1 mg/l
Residual Chlorine, Free[b]	None Detected	0.08 mg/l (Hach DPD)
Residual Chlorine, Total[b]	None Detected	1.00 mg/l (Hach DPD)
Un-ionized Ammonia[b]	0.12 mg/l as NH_3	0.06 mg/l as NH_3 ~~9.5 mg/l as NH_3~~
Total Ammonia[b]	10.5 mg/l as NH_3	9.5 mg/l as NH_3 ~~0.65 mg/l (Hach DPD)~~
Total Chlorine Residual After Salinity Adjustment[b]	---	0.65 mg/l (Hach DPD) 0.66 mg/l (Iodometric Method I)

a. Field measurements by Mr. Guy Hadley.

b. Lab measurements by Mr. Steve Wolfe. The sample pH at the time of the ammonia measurements was 7.7 and 7.9 for the prechlorinated and the chlorinated sample respectively and the temperature was 22.5°C. Ammonia and ammonium concentrations were determined using an ion specific Orion Ion Analyzer ® Model 95-10 electrode.

Table 2. Concentration Series and Volumes Used for these Static Bioassays.

Concentration	Seawater diluent	Effluent
Control	500 ml	---
5.6%	472 ml	28 ml
10.0%	450 ml	50 ml
18.0%	410 ml	90 ml
32.0%	340 ml	160 ml
56.0%	220 ml	280 ml
100%	---	500 ml

Table 3. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of the City of Cape Canaveral Water Pollution Control Facility, Cape Canaveral, Brevard County, Florida, NPDES #FL0020541, Outfall #001, on 10 to 12 December 1980.
Test #1 Prechlorinated Sample

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Salinity (o/oo)
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0
Control A	5	5	5	7.5	5.7	4.8	7.8	7.6	7.4	22.7	21.4	20.9	≤.02		29.0
Control B	5	4(5[b])	4(5[b])	7.7	5.9	4.7	7.8	7.6	7.4						
Control C	5	5	5	7.4	5.8	4.9	8.8	8.5	8.2	22.7	21.2	20.6			27.5
Control D	5	5	5	7.4	5.8	4.9	8.8	8.5	8.2						
5.6% A	5	5	4	7.3	5.8	4.2	7.9	7.7	7.4	22.5	21.1	20.5			29.0
5.6% B	5	4(5[b])	4(5[b])	7.3	5.8	4.2	7.9	7.7	7.7						
10.0% A	5	5	4	7.2	5.8	4.1	7.9	7.6	7.4	22.5	21.1	20.5			29.0
10.0% B	5	5	4(5[c])	7.3	5.7	4.1	7.9	7.7	7.4						
18.0% A	5	5	5	7.0	5.7	4.0	8.0	7.7	7.4	22.5	21.1	20.4			28.5
18.0% B	5	5	5	7.1	5.7	4.1	8.0	7.7	7.4						
32.0% A	5	5	5	6.6	5.4	3.9	8.0	7.7	7.5	22.4	21.2	20.4			28.0
32.0% B	5	5	5	6.7	5.4	4.0	8.0	7.7	7.5						
56.0% A	5	5	2[d]	6.2	5.5	4.1	8.1	7.8	7.6	22.5	21.2	20.5			28.0
56.0% B	5	4	3[d]	6.2	5.5	4.2	8.1	7.8	7.6						
100% A	5	2[d]	0	5.3[a]	5.1	4.2	8.1	7.9	7.7	22.5	21.6	20.9	See Table 1.		27.5
100% B	5	2[d]	0	5.4[a]	5.2	4.3	8.1	7.9	7.7						

Control A and B consist of Turkey Point seawater. Control C and D consist of salinity adjusted DER well water.

Remarks:
a. Gently aerated to 6.5 mg/l.
b. Number in parentheses used in calculations since one death was due to the organism jumping out of the test container.
c. Number in parentheses used in calculations since one death was due to cannibalism.
d. Test organisms stressed - at or near surface.

Table 4. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of the City of Cape Canaveral Water Pollution Control Facility, Cape Canaveral, Brevard County, Florida, NPDES # FL0020541, Outfall #001, on 10 to 12 December 1980. Test #2 Chlorinated Sample.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Salinity (o/oo)
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0
Control A	5	5	5	7.1	5.0	3.5	7.9	7.5	7.3	22.7	21.4	20.7	≤.02		29.0
Control B	5	5	5	7.2	5.1	3.5	7.9	7.5	7.3						
Control C	5	5	5	7.1	5.2	4.1	8.8	8.5	8.2	22.7	21.2	20.6			27.5
Control D	5	5	4(5[b])	7.1	5.2	4.4	8.8	8.5	8.2						
5.6% A	5	5	5	7.0	4.9	3.8	7.9	7.6	7.4	22.6	21.1	20.5			29.0
5.6% B	5	5	5	7.0	4.9	3.9	7.9	7.6	7.4						
10.0% A	5	4	4	7.0	5.0	4.1	7.9	7.6	7.4	22.5	21.1	20.5			29.5
10.0% B	5	5	5	7.0	5.1	4.2	7.9	7.6	7.4						
18.0% A	5	5	4(5[b])	6.9	5.2	4.3	8.0	7.7	7.5	22.5	21.1	20.4			29.5
18.0% B	5	4(5[c])	4(5[c])	6.8	5.1	4.3	8.0	7.7	7.5						
32.0% A	5	5	4	6.8	5.2	4.3	8.0	7.8	7.5	22.5	21.1	20.5			30.0
32.0% B	5	4 (5[b])	3(4[b])	6.7	5.1	4.4	8.0	7.8	7.5						
56.0% A	5	3[d]	1[d]	6.4	5.3	4.4	8.0	7.8	7.6	22.4	21.1	20.5			30.0
56.0% B	5	2[d]	1[d]	6.3	5.5	4.4	8.0	7.9	7.6						
100% A	5	0	0	5.5[a]	5.5	---	8.1	7.9	---	22.3	21.7	20.9	See Table 1		30.0
100% B	5	0	0	5.6[a]	5.5	---	8.1	7.9	---						

Control A and B consist of Turkey Point seawater. Control C and D consist of salinity adjusted DER well water.

Remarks:

a. Gently aerated to 6.5 mg/l.
b. Number in parentheses used in calculations since one death was due to the organism jumping out of the test container.
c. Number in parentheses used in calculations since one death was due to cannibalism.
d. Test organisms stressed - at or near surface.

Table 5. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of the City of Cape Canaveral Water Pollution Control Facility, Cape Canaveral, Brevard County, Florida, NPDES #FL0020541, Outfall #001, on 10 to 12 December 1980.

Test #3 Dechlorinated Sample

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Salinity (o/oo)
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0
Control A	5	5	5	7.3	5.6	4.2	7.8	7.6	7.4	22.4	21.5	20.7			29.0
Control B	5	4	4	7.3	5.5	4.3	7.8	7.6	7.4						
Control C	5	4(5[c])	4(5[c])	7.2	5.6	4.5	8.8	8.5	8.2	22.4	21.4	20.6			27.5
Control D	5	4(5[c])	4(5[c])	7.2	5.5	4.6	8.8	8.5	8.2						
5.6% A	5	3(5[b])	3(5[b])	7.1	5.7	4.5	7.9	7.7	7.4	22.4	21.1	20.6			29.0
5.6% B	5	5	5	7.1	5.6	3.7	7.9	7.6	7.4						
10.0% A	5	5	5	7.1	5.5	4.2	7.9	7.6	7.4	22.3	21.1	20.5			29.0
10.0% B	5	4(5[c])	4(5[c])	7.1	5.3	3.9	7.9	7.6	7.4						
18.0% A	5	5	5	7.0	5.3	4.3	8.0	7.7	7.4	22.1	21.0	20.4			29.0
18.0% B	5	5	5	7.0	5.4	4.4	8.0	7.7	7.5						
32.0% A	5	5	5	6.8	5.4	4.4	8.0	7.7	7.5	22.0	20.9	20.2			29.0
32.0% B	5	5	5	6.8	5.2	4.4	8.0	7.7	7.5						
56.0% A	5	5	2[d]	6.4	5.2	4.5	8.0	7.7	7.6	21.6	20.9	20.3			28.5
56.0% B	5	4(5[b])	3[d](4[b])	6.3	5.2	4.5	8.0	7.7	7.6						
100% A	5	1[d]	0	5.5[a]	5.1	4.6	8.1	7.8	7.7	21.4	21.2	20.4			28.5
100% B	5	0	0	5.6[a]	4.9	---	8.1	7.9	---						

Remarks: Control A and B consist of Turkey Point seawater. Control C and D consist of salinity adjusted DER well water.

a. Gently aerated to 6.5 mg/l.
b. Number in parentheses used in calculations since the deaths were due to the organisms jumping out of the test container.
c. Number in parentheses used in calculations since the death was due to cannibalism.
d. Test organisms stressed - at or near surface.

RESULTS AND DISCUSSION

All three bioassays, performed on the prechlorinated, chlorinated, and dechlorinated effluent grab samples from the City of Cape Canaveral Water Pollution Control Facility were toxic to Mysidopsis bahia. The chlorinated sample (Test #2) was the most toxic, having a 24 hour LC_{50} of 52 to 56% and a 48 hour LC_{50} of 39 to 42% (Table 6). The total residual chlorine content after salinity adjustment (0.65 mg/l, Table 1) in combination with the ammonia content of this sample (0.6 mg/l, Table 1) at least in part, were the cause of this toxicity. The prechlorinated sample and dechlorinated sample (Tests #1 and #3, respectively) exhibited 24 hour LC_{50}'s of 90 to >100% and 80%, respectively, while at 48 hours both LC_{50}'s were 56% (Table 6). The probable cause of toxicity for these two samples was the un-ionized ammonia content (Table 1). Additionally, ammonia reacts with chlorine to form chloramines (U.S. EPA 1977) which may have caused the increased lethality observed after 24 hours of exposure in the dechlorinated sample. The 96 hour LC_{50} for samples from the Cape Canaveral Water Pollution Control Facility is less than or equal to the 48 hour values reported in Table 6.

The chlorinated effluent sample was chemically characterized by DER's Chemistry Section in Tallahassee. These results are presented in Table 7. None of the parameters tested for was found in toxic concentrations.

Table 6. LC_{50} Data for Bioassays of The City of Cape Canaveral Water Pollution Control Facility on 10 to 12 December 1980.

	Method of Data Analysis	24 hour LC_{50} and 95% confidence intervals	48 hour LC_{50} and 95% confidence intervals
Test #1. Prechlorinated Sample. (From Table 3) *Mysidopsis bahia*	Binomial	89.9% (56-∞), B	56.0% (32-100)
	Moving Average	89.9% (70-300), B	A
	Probit	≥100% (0-∞), B, C	52.0% (0-∞), C
Test #2.Chlorinated Sample (From Table 4) *Mysidopsis bahia*	Binomial	56.0% (32-100)	42.3% (18-100)
	Moving Average	A	42.4% (34-53)
	Probit	52.0% (0-∞), C	38.7% (0-∞), C
Test #3. Dechlorinated sample (From Table 5) *Mysidopsis bahia*	Binomial	79.6% (56-100)	56.0% (32-100)
	Moving Average	A	A
	Probit	A	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.

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 using the probit method are not statistically sound.

Table 7. Summary of Chemical Data for Samples from the City of Cape Canaveral Water Pollution Control Facility collected at the point of discharge on 10 December 1980.

Parameter	Value
Organics	
Pesticides	Not Detectable
Base neutral extractables	Not Detectable
Acid extractables	Not Detectable
Metals (ug/l)	
Antimony	50 k
Arsenic	50 k
Beryllium	10 k
Cadmium	10 k
Chromium	50 k
Copper	15 k
Lead	50 k
Mercury	0.2 k
Nickel	50 k
Selenium	10 k
Silver	5 k
Thallium	100 k
Zinc	30

k - Actual value is known to be less than value given.

Chapter 17-3.051, Florida Administrative Code, indicates that "all waters of the State shall at all places and at all times be free from...components of discharges which...are acutely toxic." "Acute toxicity" is defined in Chapter 17-3.021(1) to mean "the presence of one or more substances...in amounts which are greater than one-third (1/3) of the amount lethal to 50% of the test organisms in 96 hours (96 hour LC_{50})...". In the absence of a mixing zone, this 1/3 of the 96 hour LC_{50} applies at the point of discharge. Chapter 17-4.244(4) states that "in no event shall the maximum concentration of wastes in the mixing zone exceed the amount lethal to 50% of the test organisms in 96 hours (96 hour LC_{50}) for a species significant to the indigenous aquatic ommunity..." This criterion applies at the point of discharge if there is a mixing zone. The distinction between presence or absence of a mixing zone is not relevant here, however, since the testing procedure used does not involve effluent concentration. The 1/3 of the 96 hour LC_{50} criterion is three times as stringent as the 96 hour LC_{50} criterion, and therefore, in the absence of a mixing zone, the procedure used is very lenient. The procedure followed is also lenient in that testing is only done for 48 hours. The 96 hour LC_{50} derived from this this testing is therefore reported as "less than or equal to" the 48 hour measurement. The value found after 48 hours can never be more restrictive than the value found after 96 hours. It must be noted that all organisms 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.

LITERATURE CITED

American Public Health Association. 1976. Standard Methods for the Examination of Water and Wastewater. 14th ed. pp. 1193.

Peltier, William. 1978. Methods for measuring the acute toxicity of effluents to aquatic organisms. Environmental Research Laboratory. Athens, Georgia. EPA-600/4-78-012 (Revised July 1978) 51p.

U. S. Environmental Protection Agency. 1977. Bioassay for toxic and hazardous material. EPA-430/1-74-014.

U. S. Environmental Protection Agency. 1978. Bioassay procedures for the Ocean Disposal Permit Program. Environmental Research Laboratory. Gulf Breeze, Florida. EPA-600/9-78-010.