

BIOASSAYS OF CAPE CANAVERAL AIR FORCE BASE (MAIN SEWAGE TREATMENT PLANT)

BREVARD COUNTY, FLORIDA

NPDES #FL0022071
SAMPLED 7/28/81

Biological Section
Bureau of Water Analysis
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INTRODUCTION

The Cape Canaveral Air Force Base Main Sewage Treatment Plant is a U.S. Government facility located on Industry Road, Cape Canaveral Air Force Station, Brevard County, Florida (Figure 1). The plant serves primarily air force base operations and its personnel, but also treats wastewater from 12 industrial sources. Approximately 0.49 MGD of treated effluent is discharged to the Banana River.

Between 29 April and 1 May, 1981, the Biological Section performed static bioassays on samples of effluent from this facility to determine the existence and extent of toxicity to the biota of the receiving waters. The results of these tests are presented in this report.

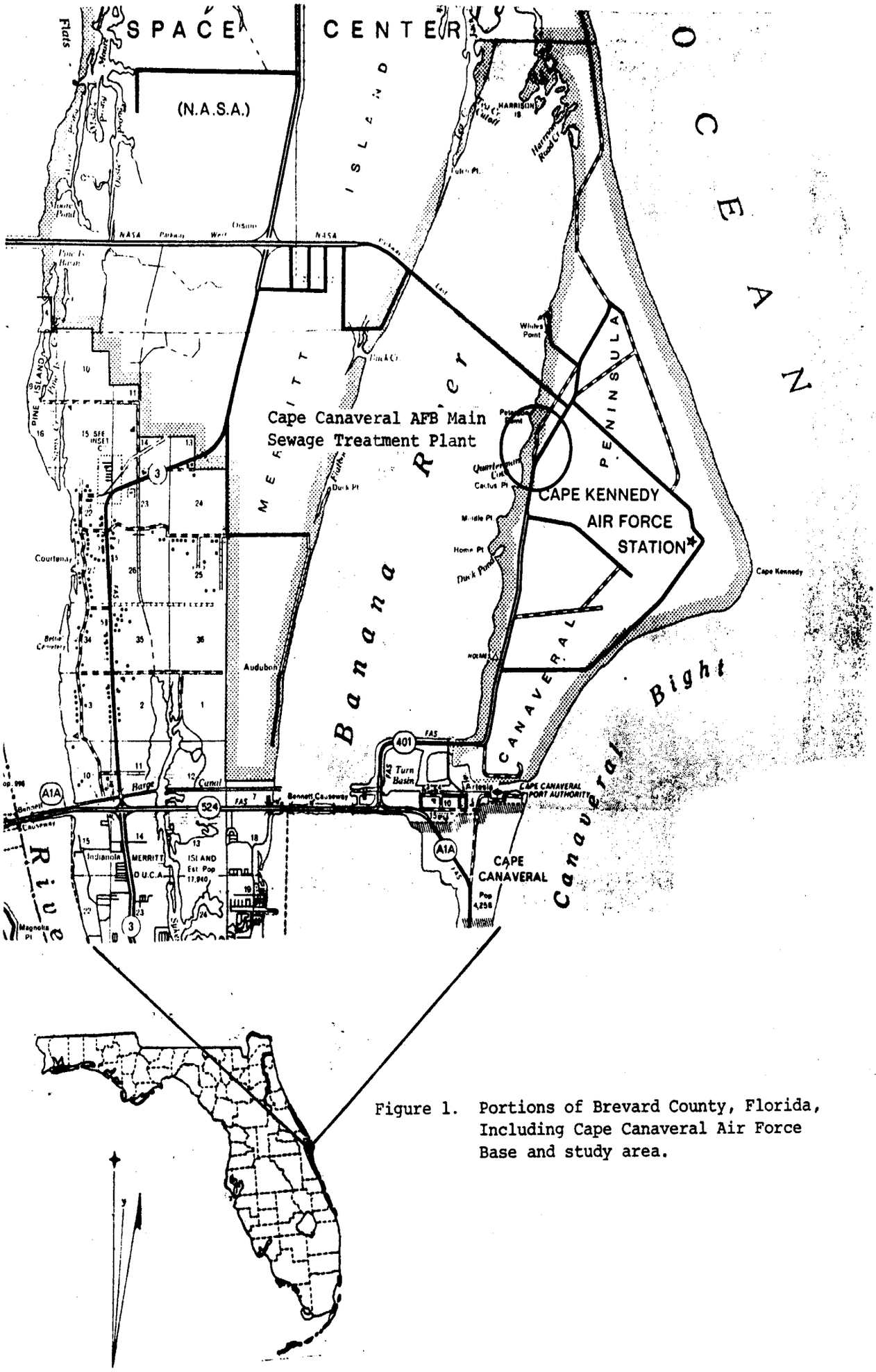


Figure 1. Portions of Brevard County, Florida, Including Cape Canaveral Air Force Base and study area.

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 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 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 $22^{o} \pm 1^{o}C$.

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 1330 hours on 29 April to 1300 hours on 1 May, 1981. Grab samples of chlorinated effluent (Outfall #001) and of effluent collected prior to chlorination were obtained by Mr. Guy Hadley of DER's St. Johns River District at 1545 hours on 28 April, 1981, using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples for metals analysis were preserved with nitric acid. The samples for organic chemical analysis and for the bioassays were unpreserved. The samples were iced and transported to the DER laboratory in Tallahassee 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, it 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[1]. 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 = 31.0 ppt). This saltwater diluent was then adjusted to the salinity of the receiving body of water (Banana River = 24.0 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 (12.0 ppt to 23.0 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 tolerences). A series of three 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 = 24.0 ppt), artificial sea salts (Ocean 50®) adjusted DER well water (salinity = 7.5 ppt), and a salinity control consisting of Turkey Point seawater and DER well water (salinity = 12.0 ppt).

Two replicates of each test concentration and the controls were used in these static tests. 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 test. Individual three-quarter adult size (10 to 15 day old) mysid shrimp were siphoned via 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. The concentration series selected for this study (Table 1) are those recommended by the APHA (1976).

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1. De-Chlorination was accomplished by adding a calculated amount of sodium thiosulfate.

Five organisms were loaded per test chamber, giving ten per concentration. Temperature, pH, dissolved oxygen, and the number of live organisms were recorded at 0, 24, and 48 hours for each test chamber (Tables 2 through 5). Additionally, ammonia concentrations, including ionized and unionized fractions[1], were determined for a control and a 100% test concentration at the beginning and end of the bioassays. 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, the mysids were fed brine shrimp (Artemia) nauplii at the rate of approximately 10 to 20 per mysid shrimp to minimize cannibalism.

[1] Ionized (total) fraction expressed in mg/l as N, unionized fraction expressed in mg/l as NH_3. Measurements were performed with an Orion specific ion probe and a Corning pH/millivolt meter.

Table 1. Concentration Series and Volumes Used for These Static Bioassays.

Mysid shrimp (*Mysidopsis bahia*)

Concentration	Diluent	Effluent	Total Vol.
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 Air Force Base (Main Sewage Treatment Plant) Brevard County, Florida, NPDES #FL0022071, on 29 April to 1 May, 1981.

Test #1. Pre-chlorinated Sample.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ) [b]		Salinity (o/oo)
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0
5.6%	5	5	5	7.3	6.0	5.5	7.7	7.6	7.6	20.4	19.8	20.0			23.5
5.6%	5(4)[a]	4	4	7.3	6.0	5.5	7.7	7.6	7.6						
10%	5	5	5	7.3	5.6	5.4	7.7	7.6	7.6	20.3	19.8	19.9			24.0
10%	5	5	5	7.2	5.6	5.4	7.7	7.6	7.6						
18%	5	5	5	7.2	5.5	5.2	7.7	7.6	7.6	20.3	19.8	19.9			21.5
18%	5	5	5	7.2	5.6	5.6	7.7	7.6	7.6						
32%	5	5	5	7.0	5.8	5.7	7.6	7.6	7.6	20.5	19.8	19.9			18.0
32%	5	5	5	7.1	5.8	5.7	7.6	7.6	7.5						
56%	5	5	5	6.9	6.3	5.8	7.5	7.6	7.5	20.8	19.8	19.8			12.0
56%	5	5	5	6.9	6.3	5.8	7.5	7.6	7.5						
100%	5	5	5	6.9	6.7	6.0	8.6	8.0	7.7	21.3	20.0	20.0	.08/.52	.02/1.15	8.5
100%	5	5	4	6.9	6.6	6.0	8.6	8.0	7.7						

Remarks: a. Number in parentheses used in calculations since one organism jumped out between 0 and 24 hours.
b. Total ammonia is reported as mg/l as N and unionized ammonia is reported as mg/l as NH_3.

Table 3. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Cape Canaveral Air Force Base (Main Sewage Treatment Plant) Brevard County, Florida, NPDES #FL0022071, Outfall #001, on 29 April to 1 May, 1981.

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
5.6%	5	5	5	7.3	5.5	5.5	7.8	7.6	7.6	20.5	20.2	20.2			23.5
5.6%	5	5	5	7.3	5.4	5.5	7.8	7.6	7.6						
10%	5	5	5	7.4	5.6	5.6	7.8	7.6	7.6	20.4	20.1	20.1			23.0
10%	5	5	5	7.4	5.8	5.6	7.8	7.6	7.6						
18%	5	5	5	7.4	6.0	5.3	7.7	7.6	7.5	20.4	20.1	20.1			21.5
18%	5	5	5	7.4	6.1	5.3	7.7	7.6	7.5						
32%	5	5	5	7.5	6.8	5.5	7.7	7.7	7.5	20.5	20.1	20.1			17.5
32%	5	5	5	7.5	6.8	5.3	7.7	7.7	7.5						
56%	5	5	3	7.6	7.2	6.2	7.5	7.7	7.6	20.9	20.2	20.2			11.5
56%	5	5	5	7.6	7.3	6.2	7.5	7.7	7.6						
100%	5	5	4	8.0	7.6	6.5	8.6	8.1	7.7	21.4	20.5	20.4	.12/.84	.04/1.96	7.5
100%	5	0	0	8.0	6.7	6.5	8.6	8.1	7.7						

Remarks: Total chlorine (Residual): 5.6% — N.D (Hach DPD)
10% — .02 (Hach DPD)
18% — .08 (Hach DPD)
32% — .14 (Hach DPD)
56% — .22 (Hach DPD)
100% — .50 (Hach DPDO

a. Total ammonia is reported as mg/l as N and unionized ammonia is reported as mg/l as NH_3.

Table 4. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Cape Canaveral Air Force Base (Main Sewage Treatment Plant) Brevard County, Florida, NPDES #FL0022071, on 29 April to 1 May, 1981.

Test #3. De-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
5.6%	5	5	5	7.4	5.7	5.3	7.8	7.6	7.5	20.7	20.2	20.4			23.0
5.6%	5	5	5	7.4	5.4	5.4	7.8	7.6	7.5						
10%	5	5	5	7.4	5.4	5.3	7.8	7.6	7.5	20.6	20.2	20.4			22.0
10%	5	5	5	7.5	5.4	5.3	7.8	7.6	7.5						
18%	5	5	5	7.5	5.4	5.4	7.7	7.6	7.5	20.5	20.3	20.3			21.5
18%	5	5	5	7.5	5.5	5.4	7.7	7.6	7.5						
32%	5	5	5	7.7	5.4	5.5	7.7	7.5	7.5	20.6	20.3	20.4			17.5
32%	5	5	5	7.7	5.4	5.5	7.7	7.5	7.5						
56%	5	5	5	8.0	5.8	5.3	7.6	7.5	7.4	20.8	20.3	20.5			11.5
56%	5	5	5	8.0	5.7	5.3	7.6	7.5	7.4						
100%	5	5	5	8.3	5.9	5.6	8.8	8.2	7.7	21.3	20.5	20.7	.18/.85	.04/2.03	8.0
100%	5	5	5	8.3	5.9	5.7	8.8	8.2	7.7						

Remarks: a. Total ammonia is reported as mg/l as N and unionized ammonia is reported as mg/l as NH_3.

Table 5. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Cape Canaveral Air Force Base (Main Sewage Treatment Plant) Brevard County, Florida, NPDES FL0022071, Outfall #001, on 29 April to 1 May, 1981.

Test #4. Control Series.

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
Seawater Control	5	5	5	7.2	5.9	5.7	7.7	7.6	7.7	20.7	20.4	20.5	0/.18	.01/.29	24.0
Seawater Control	5	5	5	7.3	5.8	5.8	7.7	7.6	7.7						
Sea Salt Control	5	5	5	8.4	6.9	6.8	8.6	8.3	8.1	21.1	20.4	20.5			7.5
Sea Salt Control	5	5	5	8.4	6.8	6.8	8.6	8.3	8.1						
Salinity Control	5	5	5	8.1	6.2	6.2	7.9	7.7	7.8	21.1	20.6	20.6			11.5
Salinity Control	5	5	5	8.1	6.1	6.2	7.9	7.7	7.8						

Remarks: a. Total ammonia is reported as mg/l as N and unionized ammonia is reported as mg/l as NH_3.

RESULTS AND DISCUSSION

Neither the pre-chlorinated (Table 2) nor the de-chlorinated (Table 4) samples of effluent from the Cape Canaveral Air Force Base Main Sewage Treatment Plant exhibited acute toxicity to Mysidopsis bahia. However, the chlorinated sample (Table 3) was acutely toxic (24 and 48 hour LC_{50}'s = 100% and 87% respectively (Table 6). The 96 hour LC_{50} would, therefore, be $\leq$87%. The toxicity exhibited in the chlorinated sample may be attributed to residual chlorine, since the concentration present in the 100% effluent was 0.5 mg/l (Table 3), which is within the toxic range reported for Mysidopsis bahia (U.S. EPA 1977).

DER's Chemistry Section in Tallahassee has chemically characterized a sample of effluent from this facility with results presented in Table 7. Notable was the identification of two chlorinated hydrocarbon insecticides, BHC (benzene hexachloride) and an isomer of chlordane. Although their concentrations (0.15 µg/l) were below the reported range for acute toxicity, their extreme bioaccumulation in aquatic systems is well documented (U.S. EPA 1976). Also present in the effluent was a significant amount of zinc (40 µg/l). This concentration is approximately one half the amount reported to be acutely toxic to Daphnia, although under slightly different conditions (U.S. EPA, 1976).

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 (95 hour LC_{50}) for a species significant to the indigenous aquatic community..." 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 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.

Table 6. LC_{50} Data for Bioassay of Cape Canaveral Air Force Base (Main Sewage Treatment Plant) NPDES #FL0022071, on 29 April to 1 May 1981.

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. Pre-Chlorinated Sample.	Binomial	No Mortality	A
	Moving Average		A
	Probit		A
Mysidopsis bahia Test #2. Chlorinated Sample.	Binomial	100%(56 and ∞),B	87%(32 and ∞),B
	Moving Average	A	87%(49 and ∞),B
	Probit	A	87%(67 and 160), B
Mysidopsis bahia Test #3. De-Chlorinated Sample.	Binomial	No Mortality	No Mortality
	Moving Average		
	Probit		

A. Due to an insufficient number of dead organisms, or to the irregular distribution of mortality, the 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.

Table 7. Results of Chemical Analyses of Effluent from Cape Canaveral Air Force Base (Main Sewage Treatment Plant) NPDES #FL0022071.

Organics

Acid Extractables	None Detected	
Base Neutral Extractables	None Detected	
Pesticides	b-BHC	0.15 µg/l
	cis isomer of chlordane	0.15 µg/l

Metals

Antimony	<200	µg/l
Arsenic	< 50	µg/l
Beryllium	< 10	µg/l
Cadmium	< 10	µg/l
Chromium	< 50	µg/l
Copper	< 25	µg/l
Lead	< 50	µg/l
Mercury	< 0.2	µg/l
Nickel	< 50	µg/l
Selenium	< 10	µg/l
Silver	< 10	µg/l
Thallium	<100	µg/l
Zinc	40	µg/l[a]

a. Average of two values.

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