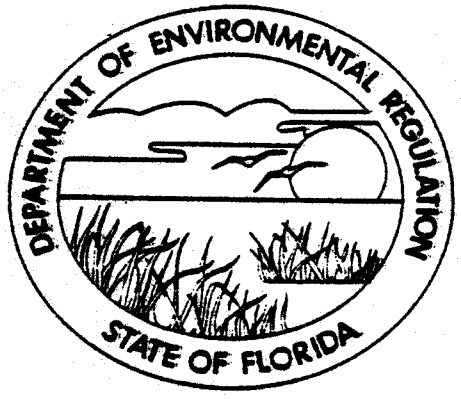


BIOASSAYS OF THE CAPE CANAVERAL AIR FORCE BASE

SEWAGE TREATMENT PLANT

BREVARD COUNTY, FLORIDA

NPDES #FL0022071

SAMPLED 2/22/83

Biology Section
Bureau of Water Analysis
April 12, 1983

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EXECUTIVE SUMMARY

Cape Canaveral A.F.B. STP, NPDES #FL0022071, on 23 to 25 February, 1983.

All samples of pre-chlorinated, chlorinated and de-chlorinated effluents from this U.S. government facility were acutely toxic to Mysidopsis bahia, producing 48 hour LC_{50} values of 18.4%, 24.0% and 69.6%, respectively. Ammonia concentrations measured in these samples were insufficient to have contributed significantly to the observed toxicity. A portion of the toxicity observed in the chlorinated sample can be attributed to the total chlorine residual (1.2 mg/l) measured in that sample. Chemical analysis indicated the presence of a large number of unidentified compounds, eight in the pre-chlorinated effluent and seven in the chlorinated effluent. Also present were measurable concentrations of at least six metals, with mercury (0.6 µg/l) and silver (1.4 µg/l) being the most likely to have acted with the organic compounds and residual chlorine to have caused the observed toxicities of these effluents.

INTRODUCTION

The Cape Canaveral Air Force Base 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.

The Biology Section has previously performed toxicity bioassays on samples of pre-chlorinated, chlorinated and de-chlorinated effluents from this facility, on 29 April to 1 May, 1981. The results of those tests indicated that although the pre-chlorinated and de-chlorinated effluents were not toxic to Mysidopsis bahia, the chlorinated effluent produced a 48 hour LC_{50} of 87.0% (FDER 1981). Toxicity was attributed to the presence of total chlorine residual, although two pesticides, b-BHC and chlordane, were also present in the effluent.

To further evaluate the existence and extent of toxicity of these effluents to the biota of the receiving waters, the Biology Section performed three additional 48 hour static bioassays, on 23 to 25 February, 1983. The results of these latest tests are presented in this report.

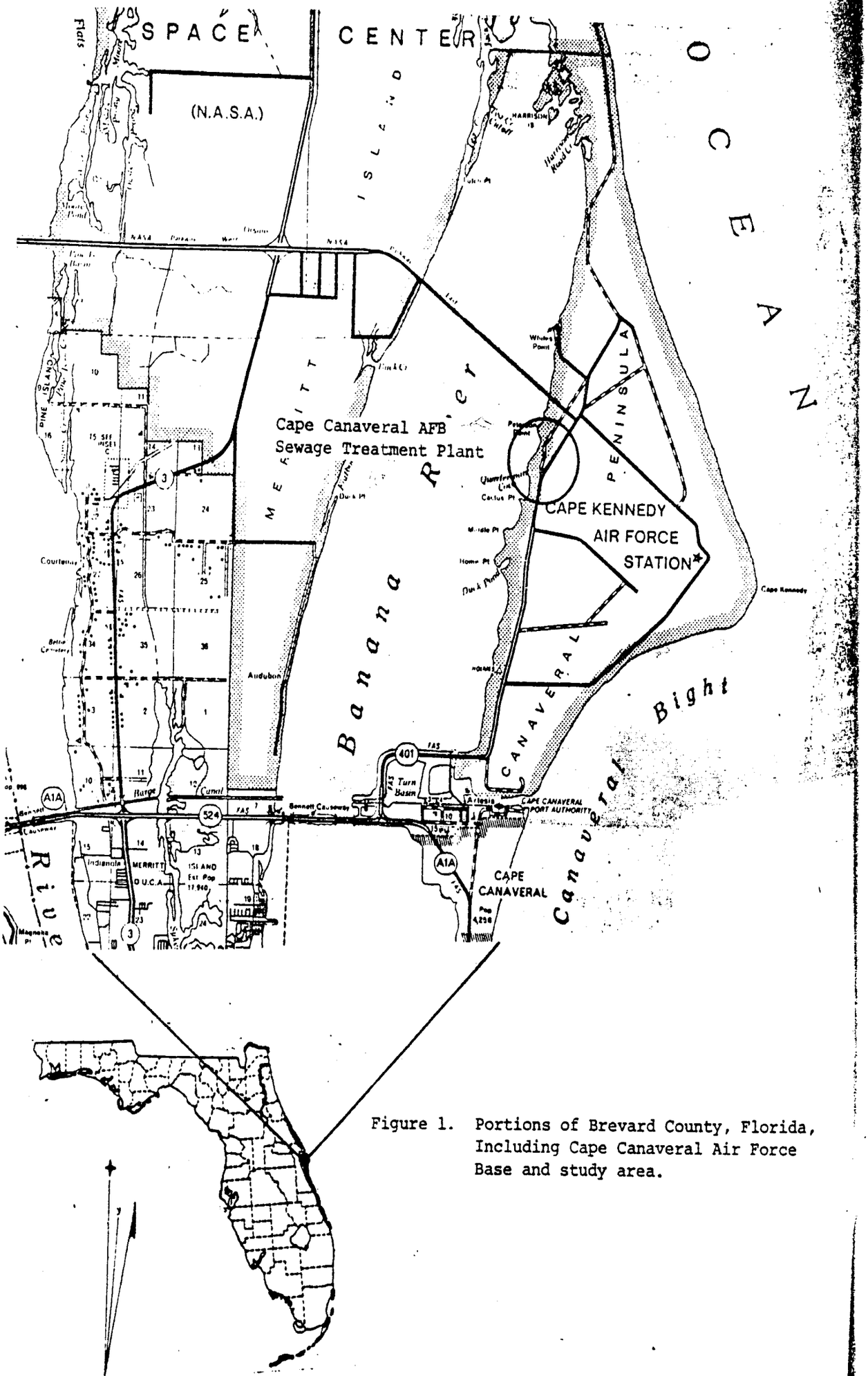


Figure 1. Portions of Brevard County, Florida, Including Cape Canaveral Air Force Base and study area.

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 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 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 Biology 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 on samples of pre-chlorinated, chlorinated and de-chlorinated effluents from the Cape Canaveral A.F.B. STP. The tests were performed from 1120 hours on 23 February to 1130 hours on 25 February, 1983. The samples were collected by Mr. Guy Hadley and Mr. Louis Ley of DER's Orlando office at 1420 hours on 22 February, 1983, 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.

To differentiate between the toxicity resulting from chlorine and the toxicity associated with some other component of the effluent, the Biology 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 = 27.5 ppt). For test concentrations of 56% and below, this diluent was mixed directly with the effluent, achieving a range of final salinities (12.9 ppt to 26.0 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 tolerance). Two 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:

1) Diluent Control - Turkey Point water (27.5 ppt). This represents the highest concentrations of Turkey Point water 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) Sea Salts Control - Well water, adjusted with artificial sea salts (Ocean 50®) to approximately 7 ppt salinity. This tests for toxicity of the highest concentration of artificial sea salts used in the bioassays, as well as 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 the 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 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 the unionized fraction (expressed in mg/l as $NH_3 + NH_4$ and as NH_3, respectively), were recorded in the Sea Salts control 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 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

Mydidopsis bahia (mysid shrimp)

Concentration	Dilution Water	Effluent	Total Volume
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 the Cape Canaveral A.F.B. STP, Brevard County, Florida, NPDES #FL0022071, on 23 to 25 February, 1983.

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	5	8.4	6.5	6.5	8.3	8.1	8.0	20.3	19.0	19.0				
5.6%	5	5	5	8.4	6.5	6.5	8.3	8.1	8.0						26.0	26.0
10.0%	5	5	5	8.3	6.4	6.7	8.3	8.1	8.0	20.3	19.0	19.0				
10.0%	5	5	5	8.3	6.4	6.7	8.3	8.1	8.0						24.9	25.0
18.0%	5	5	1	8.3	6.7	6.6	8.3	8.1	8.0	20.3	19.1	19.0				
18.0%	5	5	2	8.3	6.7	6.6	8.3	8.1	8.0						23.0	23.0
32.0%	5	5	2	8.3	7.0	6.6	8.2	8.1	8.0	20.3	19.2	19.0				
32.0%	5	3	0	8.3	7.0	6.8	8.2	8.1	8.0						19.5	19.8
56.0%	5	3	0	8.0	7.2	6.6	8.1	8.1	8.0	20.3	19.3	19.0				
56.0%	5	4	0	8.0	7.2	6.6	8.1	8.1	8.0						13.0	13.2
100.0%	5	1	0	7.9	7.6	6.9	8.6	8.4	8.3	20.2	19.4	19.2	.08/.62	--		
100.0%	5	1	0	7.9	7.4	6.9	8.6	8.4	8.3						7.1	7.5

Remarks:

Table 3. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of the Cape Canaveral A.F.B. STP, Brevard County, Florida, NPDES #FL0022071, on 23 to 25 February, 1983.

Test #2 Chlorinated Effluent[a]

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	8.3	7.3	6.1	8.3	8.2	8.0	20.2	18.9	18.6				
5.6%	5	5	5	8.3	7.3	6.1	8.3	8.2	8.0						25.8	26.0
10.0%	5	5	5	8.4	7.2	6.7	8.3	8.2	8.1	20.1	18.8	18.5				
10.0%	5	5	5	8.4	7.3	6.7	8.3	8.2	8.1						24.9	24.8
18.0%	5	5	5	8.4	7.3	6.9	8.3	8.2	8.1	20.0	18.8	18.5				
18.0%	5	5	5	8.4	7.4	6.9	8.3	8.2	8.1						23.0	22.8
32.0%	5	1	0	8.2	7.6	7.3	8.1	8.1	8.1	19.9	18.9	18.6				
32.0%	5	1	0	8.2	7.6	7.3	8.2	8.1	8.1						19.5	19.2
56.0%	5	0	0	8.3	7.9	--[b]	7.9	8.1	--[b]	19.8	19.1	--[b]				
56.0%	5	0	0	8.3	7.9	--	7.9	8.1	--						12.9	--[b]
100.0%	5	0	0	8.4	8.2	--	8.5	8.4	--	19.7	19.2	--	.01/.10	--		
100.0%	5	0	0	8.4	8.2	--	8.5	8.5	--						7.0	--

Remarks:

[a] Total Chlorine Residual = 1.2 mg/l (HACH DPD Method), 0.89 mg/l (Iodometric Method I).

[b] Remaining parameters not recorded since all organisms were dead by hour 24 of the test.

Table 4. Data recorded during 48 hour, *Mysidopsis bahia*, static acute toxicity bioassay of the Cape Canaveral A.F.B. STP, Brevard County, Florida, NPDES #FL0022071, on 23 to 25 February, 1983.

Test #3 De-chlorinated Effluent[a]

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	8.3	6.7	6.5	8.3	8.1	8.0	20.4	18.8	18.5				
5.6%	5	5	5	8.3	6.7	6.5	8.3	8.1	8.0						26.0	26.0
10.0%	5	5	5	8.3	6.7	6.5	8.3	8.1	8.0	20.4	18.7	18.3				
10.0%	5	5	5	8.3	6.7	6.5	8.3	8.1	8.0						25.0	24.8
18.0%	5	5	4	8.4	6.5	6.6	8.3	8.1	8.0	20.4	18.5	18.3				
18.0%	5	5	5	8.4	6.5	6.6	8.3	8.1	8.0						23.0	22.8
32.0%	5	5	5	8.4	6.5	6.8	8.2	8.1	8.0	20.5	18.5	18.3				
32.0%	5	5	5	8.4	6.7	6.8	8.2	8.0	8.0						19.5	19.2
56.0%	5	3	2	8.5	7.0	6.4	8.0	8.1	8.0	20.5	18.9	18.4				
56.0%	5	3	3	8.5	7.1	6.4	8.0	8.1	8.0						13.2	13.0
100.0%	5	4	1	8.5	7.4	6.7	8.6	8.4	8.3	21.2	19.1	18.6	.03/.18	--		
100.0%	5	5	3	8.5	7.4	6.7	8.6	8.4	8.3						7.1	7.0

Remarks: [a] Total Chlorine Residual = None Detected (HACH DPD Method).

Table 5. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of the Cape Canaveral A.F.B. STP, Brevard County, Florida, NPDES #FL0022071, on 23 to 25 February, 1983.

Controls

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
Diluent Control	5	5	5	8.5	6.6	6.7	8.3	8.1	8.0	20.1	19.3	19.1				
Diluent Control	5	5	5	8.5	6.6	6.7	8.3	8.1	8.0						27.5	28.0
Sea Salts Control	5	5	5	8.7	7.8	7.4	8.8	8.6	8.3	20.5	19.1	19.0	0/0	--		
Sea Salts Control	5	5	5	8.7	7.8	7.4	8.8	8.6	8.3						7.5	7.7

Remarks:

Table 6 . LC_{50} Data for Bioassays of Cape Canaveral Air Force Base STP, NPDES #FL0022071, on 23 to 25 February, 1983.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Test #1: M. bahia Pre-chlorinated Sample	Binomial	70.3% (18 and ∞)	15.7% (10 and 56)
	Moving Average	67.6% (46 and 155)	19.0% (13 and 26)
	Probit	64.8% (48 and 101), c	18.4% (14 and 24), c
Test #2: M. bahia Chlorinated Sample	Binomial	26.7% (18 and 56), c	24.0% (18 and 32), c
	Moving Average	a	a
	Probit	a	a
Test #3: M. bahia De-chlorinated Sample	Binomial	a	56.0% (32 and ∞), b
	Moving Average	a	69.6% (52 and 118), b, c
	Probit	207.2% (92 and ∞), b, c	74.7% (52 and 161), b

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

Table 7. Results of Chemical Analyses Performed on Effluents from the Cape Canaveral A.F.B. STP, NPDES #FL0022071, collected 22 February, 1983.

Organics

	Pre-chlorinated Effluent	Chlorinated Effluent
Acid Extractables:	N.D.	N.D.
Base Neutral Extractables:	One Unidentified Component	N.D.
Pesticide Extractables:	Seven Unidentified Components - six sensitive to EC detector - one sensitive to Hall detector (halogenated)	Seven Unidentified Components - six sensitive to EC detector - one sensitive to Hall detector (halogenated)

Metals

Arsenic	8.7 µg/l
Cadmium	0.2 µg/l
Chromium	N.D.
Copper	< 25 µg/l[a]
Lead	4.4 µg/l[b]
Mercury	0.6 µg/l
Nickel	N.D.
Selenium	N.D.
Silver	1.4 µg/l

N.D. = None Detected

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

[b] A similar concentration of lead (4.0 µg/l) was found in the blank.

RESULTS AND DISCUSSION

All three effluents from the Cape Canaveral Air Force Base STP were acutely toxic to Mysidopsis bahia. The pre-chlorinated effluent produced 24 and 48 hour LC_{50} values of 64.8% and 18.4%, respectively (Table 6). The chlorinated effluent produced 24 and 48 hour LC_{50}'s of 26.7% and 24.0% (Table 6). The de-chlorinated sample was significantly less toxic, producing a 24 hour LC_{50} of 207.2% and a 48 hour value of 69.6% (Table 6). Ammonia concentrations measured in these effluents were insufficient to have contributed significantly to the observed toxicity. A portion of the toxicity observed in the chlorinated effluent can be attributed to the concentration of total chlorine residual (1.2 mg/l HACH DPD Method, 0.89 mg/l Iodometric Method) measured in that sample.

The DER Chemistry Section has analyzed samples of pre-chlorinated and chlorinated effluents for certain organic compounds, and a sample of chlorinated effluent for certain metals (Table 7). These analyses indicated the presence of one base neutral compound and seven other unidentified compounds (labeled "Pesticide Extractables" by the Chemistry Section), one of which was halogenated, in the pre-chlorinated effluent. The chlorinated effluent also contained seven unidentified "Pesticide Extractable" compounds, one of which was halogenated, which may or may not be the same compounds present in the pre-chlorinated effluent. At least six metals were also present in the final effluent from the Cape Canaveral facility, including arsenic (8.7 µg/l), cadmium (0.2 µg/l), copper (<25 µg/l), lead (4.4 µg/l), mercury (0.6 µg/l) and silver (1.4 µg/l). Of these, the mercury and silver were the most likely to have acted synergistically with the organic compounds and the residual chlorine to have caused the observed toxicities of these effluents.

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 17-3-3.021(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 existance of a mixing zone, whether as a specific part of the permit or based on the existance 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} 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 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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