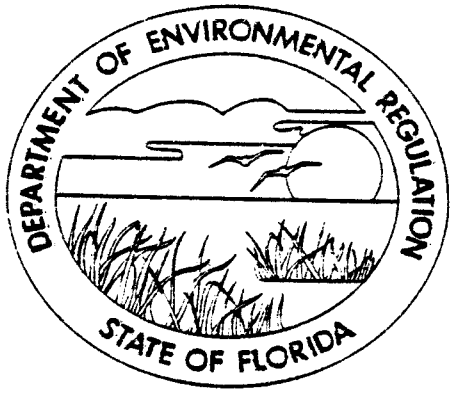


BIOASSAY OF AMERICAN CYANAMID

FIBERS DIVISION

MILTON, SANTA ROSA COUNTY, FLORIDA

NPDES #FL0002593

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Biology Section
Bureau of Water Analysis
July 10, 1984

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EXECUTIVE SUMMARY

American Cyanamid, NPDES #FL0002593 on 8 to 11 May, 1984

The sample of wastewater effluent from American Cyanamid proved to be acutely toxic to Mysidopsis bahia, producing a 48 hour LC_{50} of 99.3% and a 72 hour value of 84.6%. In a similar test, in which the pH of the 100% effluent concentration was lowered to a value similar to that of the original effluent at the time of sampling, slightly less toxic 48 and 72 hour LC_{50}'s of 178.6% and 111.9% were calculated. These latter LC_{50}'s are, however, still considered acutely toxic according to the definition in Chapter 17-3, and will be reported here as probably best characterizing American Cyanamid's effluent.

A portion of the toxicity reported above can most likely be attributed to the significant concentrations of total ammonia (up to 14.8 mg/l) and unionized ammonia (up to 1.97 mg/l) measured in the effluent. Chemical analyses indicated the presence of moderate concentrations of copper (21 µg/l) and zinc (10.9 µg/l), and what may be a significant concentration of silver (< 2.5 µg/l), which may have contributed to the observed toxicity. No organic chemicals were detected in American Cyanamid's effluent.

INTRODUCTION

American Cyanamid, Fibers Division, is located near Milton in Santa Rosa County, Florida (Fig. 1). According to the NPDES file, the facility produces acrylic fibers. Concentrated process water is disposed of in a deep well, while remaining wastewater is discharged through a long diffuser pipe to Escambia Bay.

The Biology Section has previously performed toxicity tests on effluent from this facility, in February, 1980, and again, in October, 1980. The results of the February tests indicated tht the effluent was extremely toxic to Daphnia pulex (48 hour LC_{50}=7.2%) and also toxic to Mysidopsis bahia (48 hour LC_{50}=74.8%) (FDER, 1980). Chlorine was present in the effluent during these tests, so a portion of the toxicity was attributed to that compound. The results of the October tests indicated that the effluent was not acutely toxic to Mysidopsis bahia, although significant mortality of shrimp was noted in one of the tests (FDER, 1981). Chlorine was not present in the effluent during these latter tests. Additionally, analyses for ammonia, a known ingredient of American Cyanamid's effluent (letter from Halladay to Kriegel), were not performed during these 1980 tests.

Since it has been some time since the last toxicity test of American Cyanamid's effluent, the Northwest District requested a bioassay to again evaluate the effluent. Additionally, results of self-monitoring bioassays performed by Cyanamid's consultants have exhibited occasional toxicity in the effluent (Mr. Robert Brazzell and Mr. Charles Logue, personal communication). Therefore, the Biology Section obtained a grab sample of effluent on 8 May, 1984 and performed a 72 hour static acute toxicity bioassay using Mysidopsis bahia as the test organism. The results of this test are presented in this report. Those involved in performing this test and in preparation of this report were: P. D. Brooks, C. W. Dye, R. B. Frydenborg, and M. L. Roll.

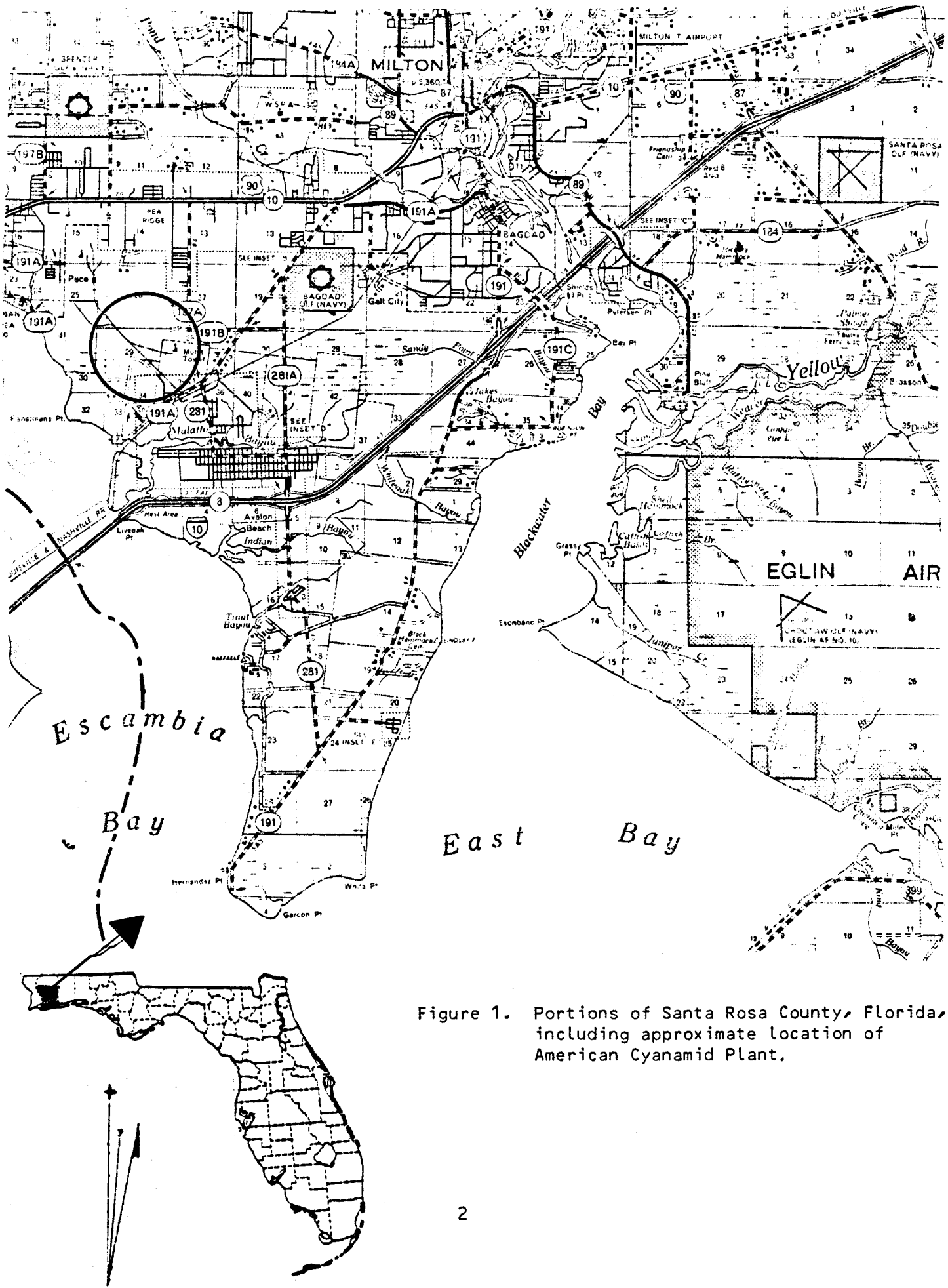


Figure 1. Portions of Santa Rosa County, Florida, including approximate location of American Cyanamid Plant.

METHODS AND MATERIALS

Test methods used to conduct this static bioassay are extensively described by Peltier (1978) and the FDER Biology Section (1983). The organism selected for this study is among those recommended by Peltier (1978), the U.S. EPA (1978), and the FDER Biology Section (1983). 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 and once daily on weekends with freshly hatched Artemia (brine shrimp) and are kept at a constant temperature of 23° plus or minus 3°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

A 72 hour static acute toxicity bioassay was performed on a grab sample of effluent from the American Cyanamid facility. The test was performed from 0920 hours on 8 May to 1020 hours on 11 May, 1984. The sample was collected by Mr. Charles Logue of DER's Northwest District at 1345 hours on 7 May, 1984, 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 bioassay were unpreserved. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 24 hours of collection.

Saltwater for dilution of the effluent sample was obtained from the FSU Marine Laboratory at Turkey Point (salinity = 23.3 ppt). For test concentrations of 56% and below, this diluent was mixed directly with the effluent, achieving a range of final salinities (10.5 ppt to 22.2 ppt). For the 100% effluent test concentrations, freshly autoclaved artificial sea salts (Instant Ocean®) were added until salinities of approximately 7 ppt were achieved (the lower limit of mysid shrimp salinity tolerance). Four control groups (Table 3) 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 (23.3 ppt). This represents the highest concentration of Turkey Point water used in the bioassay, and tests for toxicity in the diluent. This is also the highest salinity used in the bioassay, and therefore tests the tolerance of the organisms to this salinity.

2) Sea Salts Control - Well water, adjusted with artificial sea salts to approximately 7 ppt salinity. This tests for toxicity of the highest concentration of artificial sea salts used in the bioassay, as well as for tolerance of the organisms to the lowest salinity used in the bioassay.

3) pH Adjusted Effluent Control[a] - Effluent (100%) which has been adjusted to a salinity of approximately 7 ppt using artificial sea salts, and which has then been pH adjusted using hydrochloric acid to the original pH of the effluent prior to salinity adjustment. This test ensures that toxicity observed in the bioassay is not merely due to a change in pH caused by the sea salts.

4) Acid Control - Well water, adjusted with artificial sea salts to a salinity of approximately 7 ppt, with an amount of hydrochloric acid added equal to that added in the pH Adjusted Effluent Control. This tests for toxicity in the pH Adjusted Effluent Control due to the hydrochloric acid.

[a] Since this pH adjusted effluent best simulates the effluent at the time of sampling, the mortality observed in this test will be used for the final LC_{50} determination.

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 bioassay, a suitable number of mysids were transferred by net from the 10 ppt, 15 ppt, and 20 ppt salinity aquaria to culture dishes for observation and selection of individuals for the test. 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, 48 hours, and 72 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 diluent control and the 100% effluent test concentrations at the beginning and at hour 48 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 This Static Bioassay

Mysidopsis bahia (mysid shrimp)

Concentration	Dilution Water	Effluent	Total Volume
Controls	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 72 hour, Mysidopsis bahia, static acute toxicity bioassay of American Cyanamid, Milton, Santa Rosa County Florida, NPDES #FL0002593, on 8 to 11 May 1984.

Concentration or %	Number of Live Organisms				Dissolved Oxygen (mg/l)				pH				Temperature (°C)				NH_3/N (mg/l)		Salinity (ppt)		
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72	0	48	0	48	72
5.6%	5	5	5	5	7.9	7.8	6.4	6.3	8.0	7.9	7.6	7.5	21.8	21.0	19.0	18.0					
5.6%	5	5	5	5	7.9	8.0	6.4	6.3	8.0	7.9	7.6	7.5							22.2	22.0	21.8
10.0%	5	5	5	5	7.8	7.9	6.3	6.5	8.1	7.9	7.7	7.6	21.8	20.8	18.8	18.7					
10.0%	5	5	5	5	7.8	7.9	6.3	6.7	8.1	7.9	7.7	7.6							21.4	21.0	21.0
18.0%	5	5	5	5	7.7	7.8	6.3	6.6	8.1	7.8	7.6	7.5	21.5	20.8	18.7	18.5					
18.0%	5	5	5	5	7.7	7.8	6.3	6.6	8.1	7.8	7.6	7.5							19.5	19.2	19.1
32.0%	5	5	5	5	7.5	7.9	6.5	7.2	8.1	7.8	7.5	7.5	21.3	20.8	18.6	18.5					
32.0%	5	5	5	5	7.5	8.0	6.5	7.2	8.1	7.8	7.6	7.5							16.2	16.1	16.0
56.0%	5	4	4	4	7.7	8.3	6.5	7.3	8.0	7.7	7.4	7.3	20.7	20.7	18.6	18.5					
56.0%	5	5	5	5	7.7	8.2	6.5	7.1	8.0	7.7	7.4	7.3							10.5	10.7	9.8
100.0%	5	4	2	1	8.1	7.9	6.5	7.3	8.5	7.7	7.3	7.2	20.4	20.8	18.8	18.7	1.97/14.8	0.1/12.0			
100.0%	5	5	3	2	8.1	7.9	6.5	7.3	8.5	7.7	7.3	7.2							7.0	7.2	7.0

Remarks: Total residual chlorine: None detected (HACH DPD Method).

Table 3. Data recorded during 72 hour, Mysidopsis bahia, static acute toxicity bioassay of American Cyanamid, Milton, Santa Rosa County, Florida, NPDES #FL0002593, on 8 to 11 May, 1984

Controls and pH Adjusted Effluent

Concentration or %	Number of Live Organisms				Dissolved Oxygen (mg/l)				pH				Temperature (°C)				NH_3/N (mg/l)		Salinity (ppt)		
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72	0	48	0	48	72
Diluent Control	5	5	5	5	8.2	7.7	6.7	6.2	8.0	7.8	7.6	7.5	21.8	21.1	19.6	19.2	0/0	0/0			
Diluent Control	5	5	5	5	8.2	7.9	6.7	6.0	8.0	7.8	7.6	7.5							23.3	23.3	23.0
Sea Salts Control	5	5	5	4	8.8	8.6	7.8	7.8	8.3	8.2	8.1	8.0	22.2	21.0	19.3	19.0					
Sea Salts Control	5	5	5	5	8.8	8.6	7.8	7.8	8.3	8.2	8.1	8.0							7.2	7.4	7.5
pH Adjusted Effluent [a]	5	5	5	4	8.4	8.2	6.7	6.6	7.6 [b]	7.3	7.1	7.0	21.1	21.1	19.2	19.0	0.29/14.8	.07/12.8			
pH Adjusted Effluent [a]	5	4	3	2	8.4	8.2	6.7	6.6	7.6	7.3	7.1	7.0							7.0	7.5	7.2
Acid Control	5	5	5	5	8.4	8.3	7.9	6.9	7.6	7.9	7.9	8.0	22.2	21.1	19.3	19.0					
Acid Control	5	5	5	5	8.4	8.4	7.9	6.9	7.6	7.9	7.9	7.8							7.0	7.2	7.0

Remarks: [a] The mortality observed in this pH adjusted 100% effluent will be used with the mortality observed in the 5.6% to 56.0% test concentrations in Table 2 for the final LC_{50} determination.

[b] pH of effluent at time of sampling = 7.7.

Table 4. LC_{50} Data for Bioassay of American Cyanamid, NPDES #FL0002593, on 8 to 11 May, 1984.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals	72 Hour LC_{50} and 95% Confidence Intervals
M. bahia	Binomial	a	100.0% (56 and∞),b	83.4% (56 and∞)
100% effluent	Moving Average	a	100.0% (74 and∞),b	83.4% (67 and 131)
not adjusted	Probit	326.1% (112 and∞),b	99.3% (76 and 327),b,c	84.6% (67 and 119),c
M. bahia	Binomial	a	a	a
100% effluent	Moving Average	a	a	a
pH adjusted	Probit	326.1% (112 and∞),b	178.6% (97 and∞),b	111.9% (82 and 4978),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} is reported in the text since it is the most statistically conservative value of the data set.

Table 5. Results of Chemical Analyses performed on a sample of effluent from American Cyanamid, NPDES #FL0002593, collected 7 May, 1984

Organics

Acid Extractables:	N.D.
Base Neutral Extractables:	N.D.
Pesticide Extractables:	N.D.

Metals

Antimony	N.D.
Arsenic	N.D.
Beryllium	N.D.
Cadmium	N.D.
Chromium	N.D.
Copper	21 µg/l
Lead	<5 µg/l[a]
Mercury	N.D.
Nickel	N.D.
Selenium	N.D.
Silver	<2.5 µg/l[a]
Thallium	N.D.
Zinc	10.9 µg/l

N.D. = None Detected

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

RESULTS

The sample of wastewater effluent from American Cyanamid was lethal to a sufficient number of Mysidopsis bahia to produce a 24 hour LC_{50} of 326.1%, a 48 hour value of 99.3%, and a 72 hour value of 84.6% (Table 4). However, this mortality and these LC_{50}'s must be viewed with some degree of uncertainty since the pH of the 100% effluent test concentration was increased due to the addition of artificial sea salts. This increase in pH resulted in a significant increase in the concentration of toxic unionized ammonia.

A more accurate evaluation of the toxicity of Cyanamid's effluent can be made by observing the mortality in an 100% effluent concentration with its pH lowered to that of the original effluent at sampling (Table 3), and, the mortality in the remaining 5.6% to 56.0% effluent concentrations (Table 2). The mortality observed in this test resulted in the calculation of a 24 hour LC_{50} of 326.1%, a 48 hour value of 178.6% and a 72 hour value of 111.9% (Table 4). As suggested above, these latter 48 and 72 hour LC_{50}'s are less toxic than the LC_{50}'s obtained from the effluent in which no pH adjustment was performed. This decrease in toxicity can directly be attributed to the decrease in the concentration of toxic unionized ammonia, as noted in the initial ammonia measurements in Tables 2 and 3. Nevertheless, the unionized ammonia concentration measured in the pH adjusted effluent (0.29 mg/l, Table 3) may very well have contributed to the observed toxicity, since values similar to this have been reported as being acutely toxic to certain sensitive freshwater fish (Thurston, et al. 1981). The significant concentration of total ammonia (14.8 mg/l) measured in Cyanamid's effluent may also have contributed to the toxicity.

The 48 and 72 hour LC_{50}'s (178.6% and 111.9%) reported above using the pH adjusted 100% effluent, which best characterizes the toxicity of Cyanamid's effluent, are considered acutely toxic based on the definition in Chapter 17-3 of the F.A.C. The 96 hour LC_{50} for this effluent would most likely be even lower than the 72 hour value. Some degree of leniency may have been provided by terminating the test at 72 hours. However, it should be noted that the Biology Section normally performs only 48 hour static tests, which probably provides greater leniency towards a discharger.

In addition to the ammonia component of Cyanamid's effluent, the results of chemical analyses (Table 5) indicated the presence of the metals copper (21 µg/l), zinc (10.9 µg/l), and silver (<2.5 µg/l). These metals, particularly the silver, may have contributed to the toxicity, since a silver concentration as low as 5.8 µg/l was reported as being acutely toxic to oyster larvae (U.S. EPA, 1976). No organic chemicals were detected in American Cyanamid's effluent.

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 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}. 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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