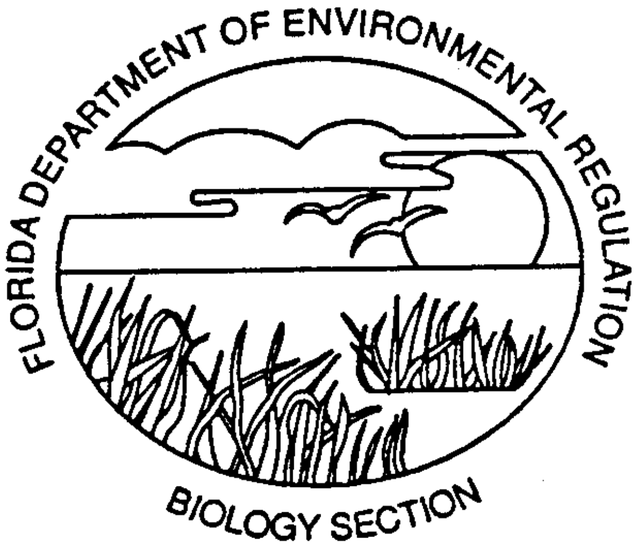


Bioassays of

American Cyanamid

Milton, Santa Rosa County, Florida

NPDES #FL0002593

Sampled 6/14/88

September 22, 1988

Biology Section
Division of Administrative and Technical Services

Bioassays of

American Cyanamid

Milton, Santa Rosa County, Florida

NPDES #FL0002593

Biology Section
Bureau of Laboratories
September 22, 1988

Executive Summary

American Cyanamid, Milton, Santa Rosa County, Florida NPDES #FL0002593, on 15 to 17 June, 1988.

The sample collected from Outfall #001 of the American Cyanamid facility was not toxic to the bioassay test organisms, *Ceriodaphnia dubia, Daphnia pulex*, and *Notropis leedsi*. there were no mortalities in any of the test concentrations. The sample was toxic to the Microtox® assay organism, *Photobacterium phosphoreum*, producing an EC_{50} of 33.32%.

Chemical analyses detected the metal zinc (12 µg/l), cyanide (30 µg/l), several small unidentified base neutral extraction components, and one unidentified pesticide extraction component sensitive to electron capture detection. The total ammonia concentration was 5.85 mg/l and the unionized ammonia concentration was 0.03 mg/l. The toxicity evidenced in the Microtox® assay organism, *P. phosphoreum,* could have been caused by the cyanide concentration (30 µg/l) in the sample. Cyanide has been shown to cause adverse effects on aquatic organisms in concentrations as low as 10 µg/l (U.S. EPA 1976).

Introduction

American Cyanamid, fibers division, is located near Milton in Santa Rosa County, Florida (Figure 1). According to the NPDES file, the facility produces acrylic fiber. Concentrated process water is disposed of in a deep well (constructed in 1976, personal communication J. May), while remaining process water is discharged through a long diffuser pipe to Escambia Bay.

The Biology Section has previously performed toxicity tests on samples from this facility. All tests were conducted on samples from the process water discharged to Escambia Bay. In February, 1980, a sample was acutely toxic to the test organisms, *Daphnia pulex* (LC_{50} = 7.2%) and *Mysidopsis bahia* (LC_{50} = 74.8%). This toxicity was partly attributed to chlorine (FDER 1980). In October, 1980, bioassays indicated that the sample was not toxic, although mortality occurred in the *M. bahia* tests (FDER 1981). In May, 1984, bioassays indicated that the sample was toxic to *M. bahia* (LC_{50} = 111.9%). This toxicity was attributed to ammonia (FDER 1984). In bioassays performed during September, 1986, a sample of chlorinated discharge was shown to be toxic to the bioassay test organisms, *C. dubia* (LC_{50} = 39.0%), *D. pulex* (LC_{50} = 39.9%), and *N. leedsi* (LC_{50} = 90.8%). The dechlorinated portion of this sample was not toxic. The toxicity was attributed to the total residual chlorine (0.25 mg/l), although the presence of other toxic components was not ruled out. Chemical analyses revealed the presence of numerous unidentified organic compounds as well as the metal silver (FDER 1986). In February, 1987, bioassays were performed on a sample from the facility. The results of the tests using the organisms, *C. dubia*, *D. pulex*, and *N. leedsi*, indicated that the sample was not toxic, although some random mortality did occur in the *C. dubia* test (FDER 1987).

To again evaluate the existence and extent of toxicity in this facility's discharged process water, at the request of the FDER Northwest District office, the Biology Section performed three acute toxicity bioassays and one Microtox® assay on a sample of discharge from the facility. The results of these tests are presented in this report. Those involved in performing the tests and in preparation of this report were: J. M. Faircloth, K. M. Lurding, L. A. Williams, and S. H. Wolfe.

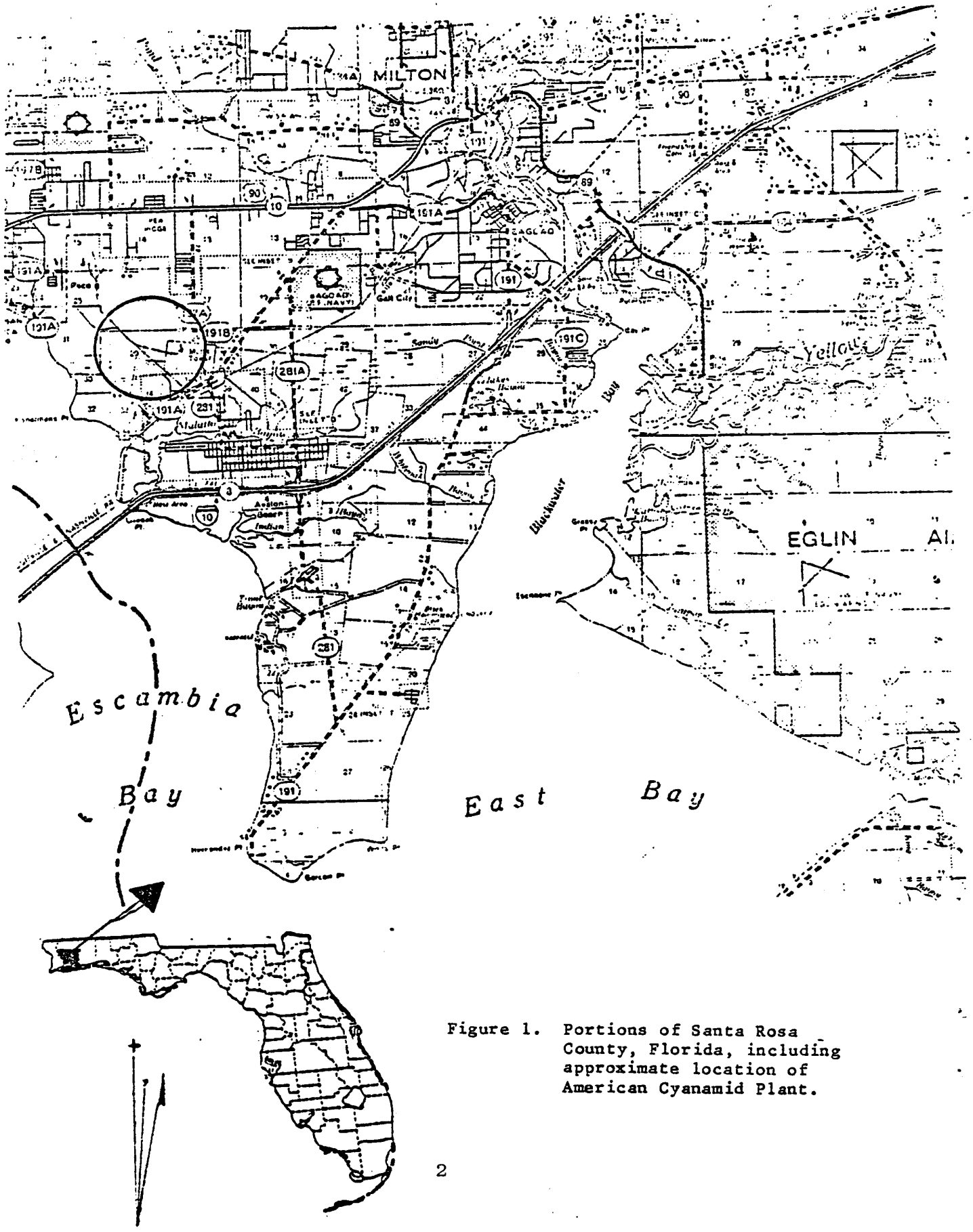


Figure 1. Portions of Santa Rosa County, Florida, including approximate location of American Cyanamid Plant.

Methods and Materials

Test methods used to conduct these static bioassays are extensively described by Peltier and Weber (1985) and the FDER Biology Section (1985). The organisms selected for this study include a species recommended by Peltier and Weber (1985) and the FDER Biology Section (1985). Additionally, the Biology Section maintains cultures of several species of organisms indigenous to the State of Florida. One of these indigenous species was also selected for testing. Life histories of these test organisms and maintenance procedures used to culture them are detailed below.

The Microtox® assay was run according to the manufacturer's protocol described in the Microtox® System Operating Manual (Beckman 1982).

1. History and Maintenance of Bioassay Organisms

Daphnia pulex (water flea)—A breeding culture of *Daphnia pulex* was obtained from the EPA laboratory in Athens, Georgia, in June, 1987. This culture has been maintained since its arrival in 2 1/2 gallon all-glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. Organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, once daily on weekends, and are kept at a constant temperature of 22 ±2 °C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms, and then refilling with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances such as: declining populations, ephippia formation, or excessive fungal or bacterial growth.

Ceriodaphnia dubia (water flea)—A breeding culture of *Ceriodaphnia dubia* was obtained from the EPA laboratory in Athens, Georgia, in June, 1985. The culture is maintained in 2-1/2 gallon all-glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. Organisms are fed a liquified blend of Purina Trout Chow®, Cerophyl® and Fleischmann's® yeast twice daily, once daily on weekends, and are kept at a constant temperature of 26 ±2 °C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms, and then refilling with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances such as: declining populations, ephippia formation, or excessive fungal or bacterial growth.

Notropis leedsi (bannerfin shiner)—Approximately 40 adult bannerfin shiners were obtained from an indigenous population in the upper Suwannee River during October, 1981. The breeding stock is maintained in 125 gallon all-glass aquaria equipped with a flow-through water exchange system. Water for this system is unchlorinated and is pumped from the DER deep well. The bannerfin shiners are reared at 22 ±2 °C under a 16:8 hour light-dark cycle and are fed Aquarian® flake food and frozen *Artemia* (brine shrimp) twice daily, and once daily on weekends. Breeding and egg hatching are encouraged at 25 °C. The eggs are laid between stacked red ceramic tiles separated by a 3mm bead of silicone. Most of the eggs adhere to the tiles and are easily removed to heated, aerated hatching tanks. The narrow space between the tiles eliminates egg predation by adult fish. New hatching tanks are started regularly to segregate fry into even age groups. Newly hatched fry are fed a liquefied blend of Purina Trout Chow® and Cerophyl® twice daily. After one week, feeding is augmented with newly hatched *Artemia* nauplii and finely crushed Aquarian® flake food. The fish used were 19 days old when the test commenced.

Photobacterium phosphoreum, a luminescent marine bacterium, is maintained as a freeze-dried culture, purchased from Microbics Corporation. Lot number M-606 was used in this assay. Immediately prior to the assay, the culture is rehydrated with 1.0 ml of specially purified water (Microtox Reconstitution Solution).

2. Standard Reference Toxicity Assays

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.

3. Test Methods

Methods used to perform acute toxicity bioassays are described by Peltier and Weber (1985) and the FDER Biology Section (1985). Some of the methods described below are more specific than those of Peltier and Weber. These will be noted in more detail.

Sampling Methods and Test Dates

Three separate 48 hour definitive acute toxicity bioassays and one Microtox® assay were performed on a sample from the Outfall #001 of the American Cyanamid facility. The tests were performed from 1000 hours on 15 June to 1000 hours on 17 June, 1988.

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 collected by Mr. Barry Long of the FDER's Northwest District office in Pensacola, using standard wastewater sampling procedures. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 24 hours of collection.

Test Temperature

Upon arrival of the iced (4 °C) bioassay samples in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the samples allowed to rise to 20 ± 2 °C. Although this temperature may be different than the temperature of the sample during collection, it is the temperature range to which the test organisms are acclimatized and is the range recommended in the above referenced bioassay protocols. This avoids stressful effects caused by exposure to unusually cold or warm samples.

Method

Freshwater for diluting the water samples being tested was obtained from the same DER deep well that is used to culture the test organisms. The concentration series selected for this study is given in Table 1.

Four replicates of the control and each test concentration were used in bioassays with the water fleas, *Ceriodaphnia dubia*, and two for *Daphnia pulex*. Test chambers consisting of plastic vials of 30 ml capacity (for *C. dubia*) and flint glass jars of 250 ml capacity (for *D. pulex*) were used and either 15 ml or 100 ml, respectively, of the appropriate test concentration was placed in each chamber. *Ceriodaphnia* were siphoned into a large culture dish and neonates were transferred by glass pipette. Five were loaded per chamber, resulting in 20 per test concentration. *Daphnia* were siphoned into a stainless steel screen chamber placed into a large culture dish to isolate the proper size and age organisms (neonates). Then the screen chamber along with any retained adults were removed. The

neonates remaining were then captured by glass pipette, examined for injury, and loaded 10 per chamber, resulting in 20 per test concentration. The water fleas were not fed during these tests.

Two replicates of each concentration and a control were used for the *Notropis leedsi* (bannerfin shiner) bioassay. The fish were transferred individually or by twos using a pipette into wide mouth quart jars containing 500 ml each of the appropriate test concentration. Five were loaded per chamber, resulting in 10 per concentration. Fish were not fed during this/ test.

Test Parameters

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, and 48 hours. Total alkalinity and total hardness (measured as $CaCO_3$ using Hach Chemical Company reagents) were recorded at the beginning of the test for the controls and the 100% reclaimed water concentration. The specific conductance (YSI Model 33 S-C-T meter) of each test concentration was recorded at the beginning and end of the test. The salinity was measured at the beginning of the test. All instruments were calibrated before use 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 controls and the 100% reclaimed water test concentration 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 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 Table 2.

Microtox® Test

Upon receipt at the DER laboratory, a 20 ml subsample was collected for Microtox® analysis. The sample was adjusted to 2% salinity with reagent grade dry sodium chloride to maintain the osmotic balance of the test bacterium during the assay. Four two-fold dilutions were made of the test sample resulting in a test range of 97%, 49.5%, 24.75%, 12.38%, and 0%. An incubation temperature of 15 °C was maintained throughout the assay. Ten µl of bacterial suspension was added to each of the 1.0 ml test dilutions. Light output (luminescence) was read after 5 and 15 minutes incubation, using the Microtox® analyzer and recorded on a Perkin-Elmer strip chart recorder. In the presence of a toxicant, the luminescence of the test organisms will decrease. In the presence of a stimulant, the luminescence will increase. Toxicity is calculated as a function of the light lost in the test sample, normalized to the control, and is reported as the effective concentration at which a 50% reduction, of light output is determined in 15 minutes (EC_{50}). Results are given in Table 3.

Results and Discussion

Bioassays, performed on the sample of discharge taken at Outfall #001 of the American Cyanamid facility, indicated that the sample was not toxic to the bioassay test organisms, *Ceriodaphnia dubia, Daphnia pulex,* and *Notropis leedsi* (Table 2). The sample was toxic to the Microtox® assay organism, *Photobacterium phosphoreum*, producing an EC_{50} of 33.32% sample (Table 3).

The exact cause of this toxicity was not determined, although the cyanide content of the sample (30 µg/l) may have contributed to the observed toxicity. Cyanide concentrations as low as 10 µg/l have been shown to cause adverse effects in aquatic organisms (U.S. EPA 1976).

The total ammonia concentration and the unionized ammonia concentration were 5.85 mg/l and 0.03 mg/l, respectively. Chemical analyses detected, in addition to cyanide, the metal zinc (12 µg/l), several small unidentified base neutral components, and one unidentified pesticide component sensitive to Electron Capture detection (Table 4).

In conclusion, the sample from the American Cyanamid facility was not toxic to the bioassay test organisms, *C. dubia, D. pulex,* and *N. leedsi*, but was toxic to the Microtox® organism, *P. phosphoreum,* on June 14, 1988.

Biology Section Recommendations

Although standards have not been established for toxicity evidence by the Microtox® assay, the Biology Section recommends that American Cyanamid reduce the cyanide concentration of their discharged process water. The concentration of cyanide in the sample (30 µg/l) of 14 June, 1988, exceeded the concentration at which adverse effects to aquatic organisms have been noted.

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

Ceriodaphnia dubia (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	15.0 ml	0.0 ml	15 ml
5.6%	14.2 ml	0.8 ml	15 ml
10.0%	13.5 ml	1.5 ml	15 ml
18.0%	12.3 ml	2.7 ml	15 ml
32.0%	10.2 ml	4.8 ml	15 ml
56.0%	6.6 ml	8.4 ml	15 ml
100.0%	0.0 ml	15.0 ml	15 ml

Daphnia pulex (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	100.0 ml	0.0 ml	200 ml
5.6%	94.4 ml	5.6 ml	200 ml
10.0%	90.0 ml	10.0 ml	200 ml
18.0%	82.0 ml	18.0 ml	200 ml
32.0%	68.0 ml	32.0 ml	200 ml
56.0%	44.0 ml	56.0 ml	200 ml
100.0%	0.0 ml	100.0 ml	200 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Sample	Total Volume
Control	500 ml	0 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%	0 ml	500 ml	500 ml

Table 2. Data recorded during the 48 hour definitive acute toxicity bioassays of the process water discharge from the American Cyanamid facility, Milton, Santa Rosa County, Florida, NPDES #FL0002593, on 15 to 17 June, 1988.

Facility: American Cyanamid — NPDES # FI#0002593 — Facility Type: Industrial
Location: 1801 Cyanamid Rd., Milton — Contact/District: B. Long/NW
County: Santa Rosa County — Test type: Static Definitive
Sample Collection Date: 6/14/88 Time: 1450 — # tests: 3 — Receiving Water: Escambia Bay
Test Beginning Date: 6/15/88 Time: 1000 — Chlorination Type: nonchlorinated
Test Ending Date: 6/17/88 Time: 1000 — Page 1 of 2

Total Residual Cl2	mg/l	Method
Field:	0.0	-
Lab:	0.0	Hach
Lab:	-	-

Ammonia	Total (mg/l)	Unionized (mg/l)
Control Water (fish):	0.00	0.00
Cond. Control Water (daphnids):	0.00	0.00
100% Sample:	5.85	0.03

Alk & Hardness	Alkalinity (mg/l)	Hardness (mg/l)
Control Water (fish):	125	125
Cond. Control Water (daphnids):	132	140
100% Sample:	15	5

Salinity (ppt)	
Control Water:	0
100% Sample:	0

Organism: *Ceriodaphnia dubia* — Life stage: neonate
LC50: - — Binomial: - — Moving Average: - — Probit: -

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hr	Live at 24 hr	Live at 48 hr	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	48 hour
Control	5	5	5	8.9	-	9.2	8.0	-	8.1	-	-	-	-	-
Control	5	5	5	9.0	-	9.2	8.0	-	8.1	-	-	-	-	-
Control	5	5	5	9.0	-	9.2	8.0	-	8.1	-	-	-	-	-
Control	5	5	5	9.0	-	9.1	8.0	-	8.1	-	-	-	-	-
6%	5	5	5	9.0	-	9.2	8.0	-	8.1	-	-	-	-	-
6%	5	5	5	9.0	-	9.2	8.0	-	8.1	-	-	-	-	-
6%	5	5	5	9.0	-	9.1	8.0	-	8.1	-	-	-	-	-
6%	5	5	5	9.1	-	9.1	8.0	-	8.1	-	-	-	-	-
10%	5	5	5	9.2	-	9.1	8.0	-	8.1	-	-	-	-	-
10%	5	5	5	9.2	-	9.1	8.0	-	8.1	-	-	-	-	-
10%	5	5	5	9.2	-	9.3	8.0	-	8.1	-	-	-	-	-
10%	5	5	5	9.2	-	9.3	8.0	-	8.1	-	-	-	-	-
18%	5	5	5	9.2	-	9.3	8.0	-	8.0	-	-	-	-	-
18%	5	5	5	9.1	-	9.3	8.0	-	8.0	-	-	-	-	-
18%	5	5	5	9.2	-	9.2	8.0	-	8.0	-	-	-	-	-
18%	5	5	5	9.2	-	9.2	8.0	-	8.0	-	-	-	-	-
32%	5	5	5	9.2	-	9.2	7.9	-	8.0	-	-	-	-	-
32%	5	5	5	9.2	-	9.2	7.9	-	8.0	-	-	-	-	-
32%	5	5	5	9.2	-	9.1	7.9	-	8.0	-	-	-	-	-
32%	5	5	5	9.2	-	9.1	7.9	-	8.0	-	-	-	-	-
56%	5	5	5	9.2	-	9.1	7.7	-	7.8	-	-	-	-	-
56%	5	5	5	9.2	-	9.1	7.7	-	7.8	-	-	-	-	-
56%	5	5	5	9.2	-	9.1	7.7	-	7.8	-	-	-	-	-
56%	5	5	5	9.2	-	9.1	7.7	-	7.8	-	-	-	-	-
100%	5	5	5	9.2	-	9.1	7.0	-	7.0	-	-	-	-	-
100%	5	5	5	9.2	-	9.1	7.0	-	7.0	-	-	-	-	-
100%	5	5	5	9.1	-	9.2	7.0	-	7.0	-	-	-	-	-
100%	5	5	5	9.1	-	9.2	7.0	-	7.0	-	-	-	-	-

* Organism: *Daphnia pulex* — Life stage: neonate
LC50: - — Binomial: - — Moving Average: - — Probit: -

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hr	Live at 24 hr	Live at 48 hr	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	48 hour
Control	10	10	10	9.3	-	9.2	8.1	-	8.0	19.5	18.9	18.9	-	-
Control	10	10	10	9.3	-	9.2	8.1	-	8.0	-	-	-	-	-
10.0%	10	10	10	9.4	-	9.3	8.1	-	8.0	19.6	18.5	18.5	-	-
10.0%	10	10	10	9.4	-	9.2	8.1	-	8.0	-	-	-	-	-
32%	10	10	10	9.5	-	9.2	8.1	-	7.9	19.8	18.5	18.5	-	-
32%	10	10	10	9.5	-	9.2	8.1	-	7.9	-	-	-	-	-
100%	10	10	10	9.6	-	9.3	7.0	-	6.8	20.6	18.7	18.7	-	-
100%	10	10	10	9.6	-	9.3	7.0	-	6.8	-	-	-	-	-

* Due to programming constraints, all of the Daphnia pulex test concentrations were not included in this table. Data for these concentrations are available for review in the raw data sheets.

Table 2. Continued.

Facility:	American Cyanamid		NPDES #	FI#0002593	Facility Type: Industrial
Location:	1801 Cyanamid Rd., N	County:	Santa Rosa County/District:	B. Long/NW	
			Test type:	Static Definitive	
Sample Collection Date:	6/14/88	Time: 1450	# tests:	3	Receiving Water: Escambia Bay
Test Beginning Date:	6/15/88	Time: 1000	Chlorination Type:	nonchlorinated	
Test Ending Date:	6/17/88	Time: 1000			Page 2 of 2

Total Residual Cl2	mg/l	Method
Field:	0.0	-
Lab:	0.0	Hach
Lab:	-	-

Ammonia	Total (mg/l)	Unionized (mg/l)
Control Water (fish):	0.00	#VALUE!
Cond. Control Water (daphnids):	0.00	#VALUE!
100% Sample:	5.85	#VALUE!

Alk & Hardness	Alkalinity (mg/l)	Hardness (mg/l)
Control Water (fish):	0.00	0.00
Cond. Control Water (daphnids):	0.00	#VALUE!
100% Sample:	5.85	#VALUE!

Salinity (ppt)	
Control Water:	0
100% Sample:	0

Organism: *Notropis leedsi* Age: 19 days

LC50: - Binomial: - Moving Average: - Probit: -

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hr	Live at 24 hr	Live at 48 hr	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	48 hour
Control	5	5	5	9.1	8.8	8.5	7.6	7.9	7.9	20.0	18.9	18.8	-	-
Control	5	5	5	9.1	8.8	8.5	7.6	7.9	7.9	-	-	-	220	220
6%	5	5	5	9.1	8.8	8.7	7.6	7.9	7.9	19.9	18.5	18.5	-	-
6%	5	5	5	9.1	8.8	8.7	7.6	7.9	7.9	-	-	-	215	215
10%	5	5	5	9.0	8.6	8.7	7.6	7.9	7.9	20.6	18.5	18.4	-	-
10%	5	5	5	9.0	8.7	8.5	7.6	7.9	7.9	-	-	-	220	210
18%	5	5	5	9.2	8.8	8.6	7.6	7.8	7.9	20.6	18.5	18.5	-	-
18%	5	5	5	9.3	8.5	8.6	7.6	7.8	7.9	-	-	-	210	205
32%	5	5	5	9.2	8.7	8.6	7.6	7.7	7.7	20. 8	18.5	18.5	-	-
32%	5	5	5	9.2	8.4	8.6	7.6	7.7	7.7	-	-	-	210	200
56%	5	5	5	9.2	8.5	8.4	7.5	7.6	7.5	21.0	18.5	18.5	-	-
56%	5	5	5	9.2	8.6	8.5	7.5	7.6	7.5	-	-	-	195	185
100%	5	5	5	9.5	8.4	8.4	7.0	6.7	6.5	21.2	18.7	18.6	-	-
100%	5	5	5	9.5	8.5	8.4	7.0	6.7	6.5	-	-	-	180	175

Table 3. Microtox test results, American Cyanamid, Milton, Santa Rosa County, NPDES#FL0002593, on 6/15/88.

Microtox Test Culture lot # M-606

Final Reclaimed Water

% Sample	nonchlorinated	
	5-min	15-min
0	99	79
0	94	75
0	87	70
12.38	84	62
12.38	82	61
12.38	87	64
24.75	73	49
24.75	72	48
24.75	75	51
49.5	51	23
49.5	51	24
49.5	51	25
99	0	0
99	0	0
99	0	0

EC-50 Non-chlorinated (15 minute , 15°C) = 33.32%

EC50 (15 minute, 15°C) is calculated using the Data Reduction Computer Program developed by Microbics Corporation for the Sharp Model EL-5100 (Microbics) Systems Operating Manual, 1982.

Table 4. Chemical components detected in the sample of discharged process water from the American Cyanamid facility, Milton, Santa Rosa County, Florida, NPDES #FL0002593, on 14 June, 1988.

Organics	
Acid Extractables	None Detected
Base Neutral Extractables	Several small unidentified components
Pesticide Extractables	One unidentified compondnet sensitive to ECD
Metals	
Zinc	12 µg/l
Other	
Cyanide	30 µg/l

Literature Cited

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Peltier, W. and C. I. Weber. 1985. Methods for measuring the acute toxicity of effluents to aquatic organisms (Third Edition). Environmental Research Laboratory. Athens, Georgia. EPA-600/4-85-012. 217 p.

U.S. Environmental Protection Agency. 1976. Quality criteria for water. U.S. EPA, Washington, D.C. 256 p.

Facility: American Cyanamid

Address:

Contact/Collector:

Effluent Serial Number:

NPDES Permit Number:

Test Number: 1-3

Test Type: Static Renewal Screening: Definitive

Other:

Test Beginning: Date: June 14 Time: 1450

Test Ending: Date: June 17 Time: 1000

Test Organism: C. dubia

Age/Life Stage: neonate

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96	0	24	48	72	96
Control	33	5	5	5			8.9	–	9.2			8.0	–	8.1												
Control	34	5	5	5			9.0	–	9.2			8.0	–	8.1												
Control	35	5	5	5			9.0	–	9.2			8.0	–	8.1												
Control	36	5	5	5			9.0	–	9.2			8.0	–	8.1												
5.6%	37	5	5	5			9.0	–	9.1			8.0	–	8.1												
"	38	5	5	5			9.0	–	9.1			8.0	–	8.1												
"	39	5	5	5			9.0	–	9.1			8.0	–	8.1												
"	40	5	5	5			9.1	–	9.1			8.0	–	8.1												
10.0%	41	5	5	5			9.2	–	9.1			8.0	–	8.1												
"	42	5	5	5			9.2	–	9.1			8.0	–	8.1												
"	43	5	5	5			9.2	–	9.1			8.0	–	8.1												
"	44	5	5	5			9.2	–	9.1			8.0	–	8.1												
18%	45	5	5	5			9.2	–	9.2			8.0	–	8.0												
"	46	5	5	5			9.1	–	9.2			8.0	–	8.0												
Initial of Investigator		SHW	SHW	SHW			JMF	–	SHW			SHW		SHW												

Alkalinity/hardness (mg/l as $CaCO_3$)

Control Water:	132	140
100% Sample:	15	5
Other:		
Measured by:		

Ammonia unionized/total (mg/l as NH_3/N)

Hour 0

Control Water: 0

100% Sample: 5.85

Other:

Hour 0

Control Water: ~~0~~

100% Sample:

Other:

Measured by: JMF

slope-58.7

Signatures:

[signature]

[signature]

[signature]

[signature]

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7at7.9at9	8.9 at 21.5°	
24 Hours	"		
48 Hours	7at7.9	9.2@20°c	
72 Hours			
96 Hours			

Total Residual Chlorine:

Field data: Method:

Lab: 0 Method: Hach

Lab: Method:

De-chlorinated:

Measurements by:

this sample has an abundance of algae

Reviewed by: [signature]

Date: 9/26/86

Remarks: 0 hours - Cond. 1310 at 1300

48 hour - Cond. 1280 @1273 (20.0°C)

24 hour measurements not taken due to

Industry/Toxicant American Cyanamid
Address
Contact/District
Effluent Serial Number
NPDES Permit Number

BIOASSAY DATA FORM

Test Number: 1 of 3
Test Type: Static Renewal, Screening Conventional
Other: Definitive

Sample Collection: Date 6-14-88 Time 1450
Test Beginning: Date 6-15-88 Time 1000
Test Ending: Date 6-17-88 Time 1000
Test Organism C. dubia
Age/Life Stage neonate

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	43	72	96	0	24	48	72	96
18%	47	5	5	5			9.2	-	9.2			8.0	-	8.0												
"	48	5	5	5			9.2	-	9.2			8.0	-	8.0												
32%	49	5	5	5			9.2	-	9.2			7.9	-	8.0												
"	50	5	5	5			9.2	-	9.1			7.9	-	8.0												
"	51	5	5	5			9.2	-	9.2			7.9	-	8.0												
"	52	5	5	5			9.2	-	9.2			7.9	-	8.0												
56%	53	5	5	5			9.2	-	9.1			7.7	-	7.8												
"	54	5	5	5			9.2	-	9.1			7.7	-	7.8												
"	55	5	5	5			9.2	-	9.1			7.7	-	7.8												
"	56	5	5	5			9.2	-	9.1			7.7	-	7.8												
100%	57	5	5	5			9.2	-	9.3			7.0	-	7.0												
"	58	5	5	5			9.2	-	9.3			7.0	-	7.0												
"	59	5	5	5			9.1	-	9.3			7.0	-	7.0												
"	60	5	5	5			9.1	-	9.3			7.0	-	7.0												
Initial of Investigator		SHW	MF	MF			JMF		SHW			SHW	-	SHW												

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 132 / 140
100% Sample: 15 / 5
Other:
Measured by:

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: 0
100% Sample: 5.85
Other:
Hour 0

Control Water:
100% Sample:
Other:
Measured by: MF

Signatures:
Joseph Tarbell
Steven H. Wolfe
KM Lurding

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours			
24 Hours			
48 Hours			
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: Method:
Lab: 2 Method: 330.4
Lab: Method:
Reviewed by:
Re-Chlorinated:
Measurements by:
Date: 9/26/88

American Cyanamid

Test Number: 2 of 3
Test Type: Static Renewal, Freshwater Chronic
Color: Definitive

June 14 1450
June 15 1000
June 17 1000
Test Organism: D. pulex
Age/Life Stage: neonate

Concentration	Test Chamber Number	Number of Live Organisms: 0	24	48	72	96	Dissolved Oxygen (mg/l): 0	24	48	72	96	pH: 0	24	48	72	96	Temperature °C: 0	24	48	72	96
Control	52	10	10	10			9.3	–	9.2			8.1	–	8.0			19.5		18.9		
Control	119	10	10	10			9.3	–	9.2			8.1	–	8.0							
5.6%	143	10	10	10			9.4	–	9.2			8.1	–	8.1			19.5		18.6		
5.6%	41	10	10	10			9.4	–	9.2			8.1	–	8.1							
10%	64	10	10	10			9.5	–	9.3			8.1	–	8.0			19.6		18.5		
10%	25	10	10	10			9.5	–	9.2			8.1	–	8.0							
18%	27	10	10	10			9.5	–	9.2			8.1	–	8.0			19.7		18.4		
18%	32	10	10	10			9.5	–	9.2			8.1	–	8.0							
32%	43	10	10	10			9.5	–	9.2			8.1	–	7.9			19.8		18.5		
32%	55	10	10	10			9.5	–	9.2			8.1	–	7.9							
56%	23	10	10	10			9.5	–	9.2			7.9	–	7.8			20.3		18.6		
56%	12	10	10	10			9.6	–	9.2			7.9	–	7.8							
100%	14	10	10	10			9.6	–	9.3			7.0	–	6.8			20.6		18.7		
100%	27	10	10	10			9.6	–	9.3			7.0	–	6.8							
Initial of Investigator		JMF KML	NS	MH			JMF	–	SHW			SHW		SHW			SHW		SHW		

Alkalinity (mg/l as CaCO3)

	0	24	48	72	96
Control Water:	132		140		
100% Sample:	15		5		
Other:					
Measured by:	mf				

Ammonia unionized/total (mg/l as NH_3/N)

Hour 0

Control Water:	0	
100% Sample:	5.85	
Other:		
Measured by:	mf	

Hour 0

Control Water:	
100% Sample:	
Other:	
Measured by:	mf

Signatures:

KM Lurding
Lyle Faulstich
Steve Hall

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours			
24 Hours			
48 Hours			
72 Hours			
96 Hours			

Remarks:

Total Residual Chlorine:

Field data: _____ Method: _____
Lab: _____ Method: _____
Lab: _____ Method: _____

De-chlorinated:
Measurements by: _____

Reviewed by: _____
Date: 9/26/85

Industry/Toxicant: American Cyanamid
Address:
Contact/District:
Effluent Serial Number:
NPDES Permit Number:

BIOASSAY DATA FORM

Test Number: 3 of 3
Test Type: Static Renewal, Screening Conventional
Other: Definitive

Sample Collection: Date 6-14-88 Time 1450
Test Beginning: Date 6-15-88 Time 1000
Test Ending: Date 6-17-88 Time 1000
Test Organism: N. Leedsi
Age/Life Stage: 14 days

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96	Conductivity 0	24	48	72	96
Control	34	5	5	5			9.1	8.8	8.5			7.6	7.9	7.9			20.0	18.9	18.8							
Control	76	5	5	5			9.1	8.8	8.5			7.6	7.9	7.9								220	220	215 MF	220	
5.6%	4	5	5	5			9.1	8.8	8.7			7.6	7.9	7.9			19.9	18.5	18.5							
5.6%	2	5	5	5			9.1	8.8	8.7			7.6	7.9	7.9								215	210	215		
10%	44	5	5	5			9.0	8.6	8.7			7.6	7.9	7.9			20.6	18.5	18.4							
10%	21	5	5	5			9.0	8.7	8.5			7.6	7.9	7.9								220	205	210		
18%	38	5	5	5			9.2	8.8	8.6			7.6	7.8	7.8			20.6	18.5	18.5							
18%	16	5	5	5			9.3	8.5	8.6			7.6	7.8	7.8								210	200	205		
32%	113	5	5	5			9.2	8.7	8.6			7.6	7.8 7.7	7.7			20.9	18.5	18.5							
32%	54	5	5	5			9.2	8.4	8.6			7.6	7.8 7.7	7.7								210	190	200		
56%	43	5	5	5			9.2	8.5	8.4			7.5	7.6	7.5			21.0	18.5	18.5							
56%	131	5	5	5			9.2	8.6	8.5			7.5	7.6	7.5								195	195	185		
100%	49	5	5	5			9.5	8.4	8.4			7.0	6.7	6.5			21.2	18.7	18.1							
100%	5	5	5	5			9.5	8.5	8.4			7.0	6.7	6.5								180	175	175		
Initial of Investigator		KMK	MS	MF			JMF	MF	SHW			SHW	SHW	SHW			SHW	SHW	SHW			SHW	SHW	SHW		

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 125 / 125
100% Sample: 15 / 5
Other:
Measured by: MF

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: 0
100% Sample: 5.85
Other:

Hour 0
Control Water:
100% Sample:
Other:
Measured by: MF

Signatures:
H M Lurding
Joseph Faucette
Steven H Cobb

Instrument Calibration:

	pH Meter:	DO Meter:	Specific Meter:
0 Hours			
24 Hours			
48 Hours			
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: Method:
Lab: Method:
Lab: Method:

Reviewed by:
Date: 9/26/88

Recalculated:
Comments by:

MICROTOX™ Data Reduction Sheet 101

SOURCE: American Cyanamid

GRAPH CALC EC 50 (15 MIN, 15 °C) = 33.3 (%SAMPLE) mg/L

SAMPLE DATA:

SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent

DATE ASSAYED: 6-15-88 TIME: 10:00 Am

VISUAL COLOR: none CORRECTION REQUIRED: YES (NO)

VISUAL TURBIDITY: none SEPARATION REQUIRED: YES (NO)

INITIAL pH: pH ADJUSTED TO: WITH:

OSMOTICALLY ADJUSTED WITH: MOAS (DRY NaCl) OTHER

PRIMARY DILUTION OF SAMPLE: none

OPERATOR: Leslie Williams REAGENT VIAL LOT NO. M-606

REMARKS:

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	Γ5	Γ15	NOTES
A1	0	11	79			15° → lockspam
B1		94	75			
C1		21	70			
A2	12.38	84	62	-.107	.198	
B2		82	61			
C2		87	64			
A3	24.75	73	49	.273	.514	
B3		72	48			
C3		75	51			
A4	49.5	51	23	.830	2.11	
B4		51	24			
C4		51	25			
A5	99	0.0	6	—	—	X 10
B5		0.0	0			
C5		0.0	0			
EC_{50} (MIN, °C)				57.17	33.32	

REVIEWED BY:

DATE: 9/26/88