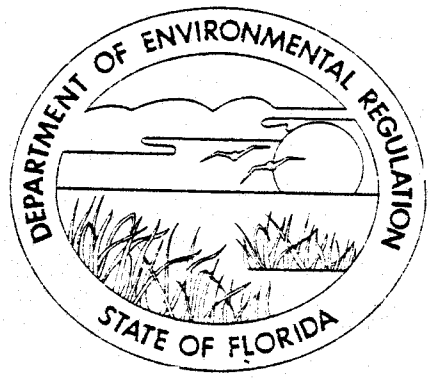


BIOASSAYS OF AMERICAN CYANAMID COMPANY,

FIBERS DIVISION

SANTA ROSA COUNTY, FLORIDA

NPDES #FL0002593

SAMPLED 10/27/80

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Biological Section
Bureau of Water Analysis
January 19, 1981

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INTRODUCTION

American Cyanamid Company, Fibers Division, Santa Rosa Plant is located near Milton, Florida in Santa Rosa County (Fig. 1). During the manufacture of acrylic fibers, this facility produces 2.6 MGD of wastewater which is discharged to Escambia Bay. There is a deep well injection system for the concentrated process water. Information regarding effluent treatment systems and suspected toxic components is included in appended excerpts of the plant's NPDES permit application.

In February, 1980, the Biological Section performed static bioassays on effluent from outfall #001 at this facility. At that time, the effluent was found to be toxic to both Daphnia pulex and Mysidopsis bahia. At the request of DER's Northwest District Office and the American Cyanamid Company we performed another series of bioassays on effluent from this facility. The effluent samples were obtained 28 October 1980 from outfall #001 at the American Cyanamid plant. The following is a report of the results of these tests.

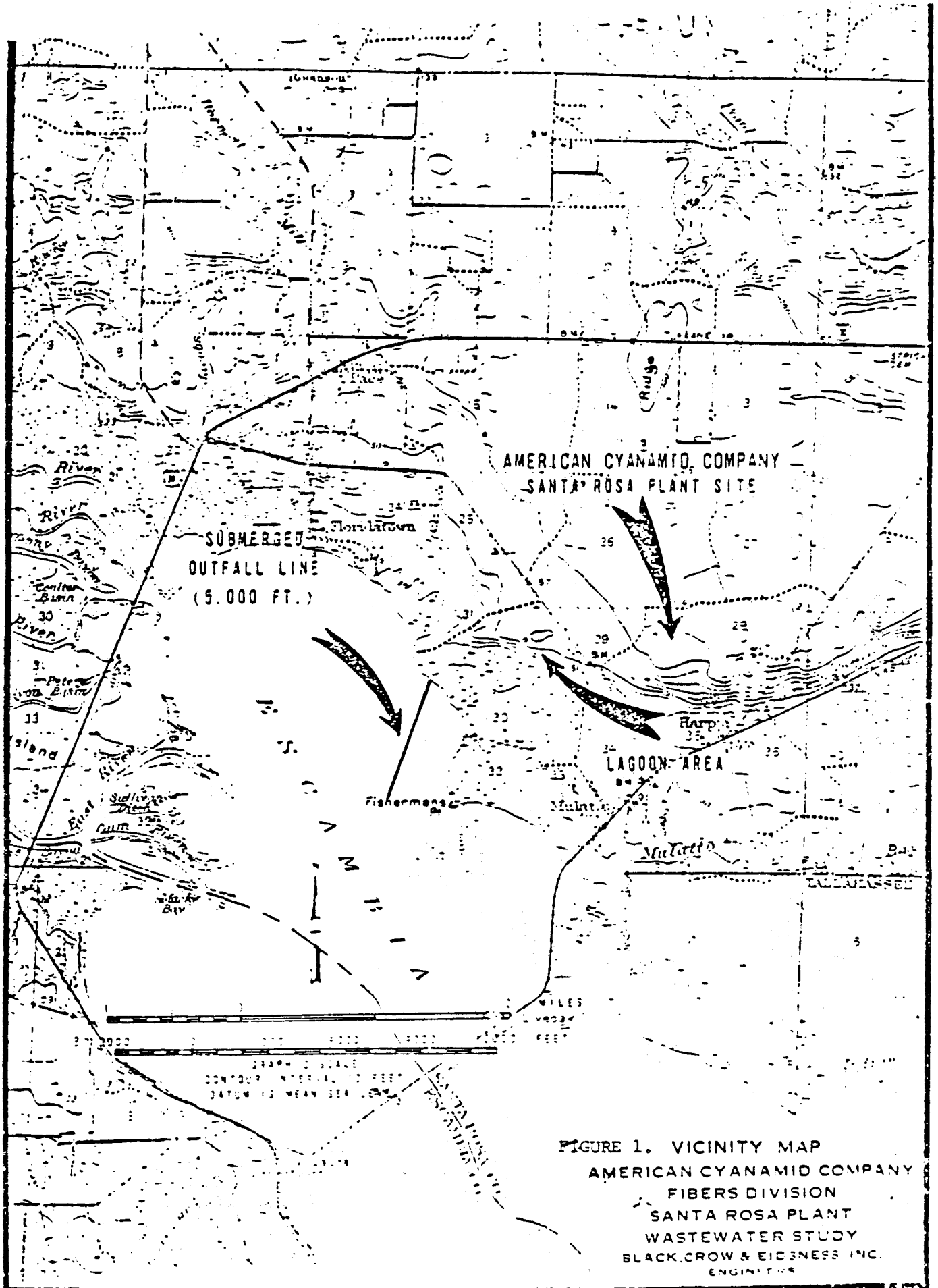


FIGURE 1. VICINITY MAP
AMERICAN CYANAMID COMPANY
FIBERS DIVISION
SANTA ROSA PLANT
WASTEWATER STUDY
BLACK, CROW & EIDSNESS INC.
ENGINEERS

METHODS AND MATERIALS

Test methods used for conducting these static bioassays are extensively described by Peltier (1978). The organisms selected for this study are those recommended by Peltier (1978) and the U.S. EPA (1978). Life histories of these test organisms and maintenance procedures used to culture them are detailed below.

Since the American Cyanamid Company's effluent is composed of freshwater and its discharge is to saline waters, bioassays were conducted with freshwater as well as saltwater organisms. However, due to excessive mortality in the control organisms (i.e. >20%) the freshwater invertebrate (Daphnia pulex and Simocephalus serrulatus) bioassays were invalidated. Therefore, only the methodology and test results of the salt water bioassays will be reported.

History and Maintenance of Bioassay Organisms

Mysidopsis bahia (mysid shrimp) - In September, 1978, approximately 300 adult mysid shrimp were obtained from the Gulf Breeze EPA Laboratory in Pensacola, Florida. The mysids have been cultured under continuous lighting in 10 and 15 gallon all glass aquaria equipped with undergravel filtration and aeration. The mysid shrimp are cultured over a wide range of salinities (10-30 0/00) to minimize acclimation periods to low or high salinity effluents. Sea water is obtained from the FSU Marine Laboratory located at Turkey Point, Florida (salinity range 17-31 0/00) and partial water changes are performed weekly. Fresh water, for dilution of the sea water as necessary, is obtained from a deep well near the DER laboratory. The mysids are fed twice daily with freshly hatched Artemia (brine shrimp) and are kept at a constant temperature of $22^{\circ} \pm 1^{\circ}C$. Immediately prior to the bioassay, a suitable number of mysids were transferred by net to a culture dish for observation and selection of individuals for the test. Fifteen day old (three-quarter adult size) mysids were used in this test. Organisms selected for testing were transferred individually by glass tube and randomly placed in the test chambers.

To ensure the uniform sensitivity of these test organisms to toxic substances, the Biological Section conducts monthly Standard Reference Toxicant Bioassays using sodium lauryl sulfate as the reference toxicant.

Test Methods

Two separate 48 hour static bioassays were conducted from 1100 hours 28 October 1980 to 1100 hours 30 October 1980 on a 24 hour composite sample of effluent from outfall #001. The sample was collected by Mr. Rick Bradburn of the DER Pensacola Office (Northwest District) at 1100 hours on 27 October 1980, using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples for metal analysis were preserved with HNO_3. Samples for organic chemical analysis and the bioassays were unpreserved. The samples were iced and transported to the DER laboratory in Tallahassee within 24 hours of collection.

Saltwater for dilution of the effluent in Mysidopsis bahia tests was obtained from the FSU marine laboratory at Turkey Point (salinity = 31.5 0/00). To account for differences in salinity that might be encountered within a mixing zone when a freshwater effluent is discharged to saltwater we adopted the following test scheme:

Test #1 (Table 2) - Effluent was adjusted to approximately 7.0 0/00 salinity with autoclaved artificial sea salts (Ocean 50 ®). Turkey Point seawater was then used as the diluent in this bioassay. The salinity chosen for the effluent was the approximate lower salinity tolerance limit for Mysidopsis (Peltier, 1978).

Test #2 (Table 3) - Effluent was adjusted to approximately the same salinity as the diluent, Turkey Point seawater (i.e. 31.5 0/00) using artificial sea salts (Ocean 50 ®). This test would presumably eliminate salinity as a variable if any toxicity was noted. This test also employed two salinity controls consisting of a DER well water control adjusted to the diluent salinity (i.e. 31.5 0/00) using artificial sea salts (Ocean 50 ®). A set of standard controls (i.e. Turkey Point seawater) was also used.

Test #3 (Table 4) - A salinity control series was prepared using salinity adjusted DER well water (approximately 7.0 0/00) as the highest concentration. Turkey Point seawater was then used as diluent. This control series was used to account for any unknown effects that might be produced by the artificial sea salt salinity adjustments in Test #1 and #2.

Two replicates, each of six concentrations of effluent and a control were used in these static tests (Table 1). Individual mysid shrimp were siphoned or pipetted via glass tube, carefully examined for injury and randomly transferred to wide mouth quart jars, each containing 500 ml of the appropriate concentration. The concentration series selected for this study (Table 1) are those recommended by APHA (1976). Five organisms were loaded per test chamber giving 10 per concentration. Salinity was measured in all chambers at the beginning of the saltwater test. The pH, dissolved oxygen and the number of live organisms were recorded at 0, 24 and 48 hours

(Tables 2, 3 and 4). "Although it is generally undesirable to feed most fish and macroinvertebrates during static toxicity tests, an exception in necessary for M. bahia" (U.S. EPA, 1978). Therefore, mysids were fed brine shrimp (Artemia) nauplii daily at a rate of approximately 10-20 per mysid to minimize cannibalism among the mysids.

Table 1. Concentration Series and Volumes Used for This Static Bioassay

Mysid shrimp (Mysidopsis bahia)

Concentration	Seawater 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 the American Cyanamid Company, Milton, Santa Rosa County, Florida. NPDES #FL0002593. 1100 hours 28 October to 1100 hours 30 October 1980. Test #1.

Conc. or %	Test Container Number	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)			Total Hardness (mg/l as $CaCO_3$)			Salinity (0/00)		
		0	24	48	0	24	48 (b.)	0	24	48	0	24	48	0	24	48	0	24	48
Control	A	5	5	5	6.5	5.0	5.1	7.4	7.3	7.4									
Control	B	5	5	5	6.5	5.2	5.2	7.4	7.3	7.5							31.5		
5.6%	A	5	5	5	6.4	4.6	46.	7.5	7.3	7.4									
5.6%	B	5	5	5	6.4	4.8	4.9	7.5	7.3	7.4							30.5		
10.0%	A	5	5	5	6.8	4.4	4.6	7.5	7.3	7.4									
10.0%	B	5	5	5	6.9	5.0	4.5	7.6	7.3	7.4							29.0		
18.0%	A	5	4[c]	4	6.9	4.8	4.6	7.6	7.3	7.4									
18.0%	B	5	5	5	7.0	5.0	4.6	7.7	7.3	7.4							27.5		
32.0%	A	5	5	5	7.2	4.5	4.6	7.8	7.2	7.4									
32.0%	B	5	5	5	7.2	3.8	4.3	7.8	7.2	7.4							24.0		
56.0%	A	5	3	3	7.7	5.0	4.3	8.0	7.3	7.3									
56.0%	B	5	5	5	7.8	5.4	4.8	8.1	7.4	7.3							18.0		
100%	A	5	3	3	9.2	5.6	5.1	8.6	7.4	7.3									
100%	B	5	5	3	9.2	5.1	4.7	8.7	7.5	7.3							7.0		

REMARKS: a. Test temperature range 20.0° to 20.9 °C
b. All test containers aerated to 7.0+ mg/l D.O.
c. Cannibalized

Table 3 . Data recorded during 48 hour, *Mysidopsis bahia*, static acute toxicity bioassay of the American Cyanamid Company, Milton, Santa Rosa County, Florida. NPDES #FL0002593. 1100 hours 28 October to 1100 hours 30 October 1980. Test #2.

Conc. or %	Test Container Number	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)			Total Hardness (mg/l as $CaCO_3$)			Salinity (0/00)		
		0	24	48	0	24	48 (b)	0	24	48	0	24	48	0	24	48	0	24	48
Control	A	5	4	4	6.7	4.7	4.1	7.6	7.3	7.4									
Control	B	5	5	5	6.8	3.9	3.9	7.6	7.3	7.3							31.0		
Control	C[e]	5	5	5	7.0	3.8	3.2	8.7	8.4	8.2									
Control	D[e]	5	5	5	7.2	4.4	3.7	8.7	8.4	8.2							29.5		
5.6%	A	5	5	5	6.7	3.8	2.9	7.9	7.3	7.4									
5.6%	B	5	5	5	6.8	3.6	3.6	7.8	7.3	7.4							31.5		
10.0%	A	5	5	5	6.7	3.8	3.5	7.9	7.3	7.4									
10.0%	B	5	5	5	6.8	3.8	3.6	7.9	7.3	7.4							31.5		
18.0%	A	5	5	5	6.8	3.8	3.2	8.1	7.4	7.4									
18.0%	B	0[c]	0	0	--	--	--	--	--	--							31.5		
32.0%	A	5	5	5	6.9	3.2	2.9	8.3	7.7	7.4									
32.0%	B	5	5	5	7.0	3.4	2.9	8.3	7.7	7.4							31.5		
56.0%	A	5	5	5	7.1	3.4	3.1	8.7[d]	7.8	7.3									
56.0%	B	5	5	5	7.2	3.5	3.7	8.7[d]	7.9	7.4							31.5		
100%	A	5	5	5	7.7	2.7	3.2	9.4[d]	7.7	7.1									
100%	B	5	5	5	7.8	2.7	3.1	9.5[d]	7.3	7.1							30.5		

REMARKS:
a. Test temperature range 19.8° to 22.5 °C
b. All test containers aerated to 7.0+ mg/l D.O.
c. Container spilled.
d. pH adjusted to 8.5
e. Salinity Adjusted Well Water Control

Table 4. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay. Salinity control. (well water adjusted to 6.5 0/00 salinity diluted with Turkey Point seawater 31.5 0/00). 1100 hours 28 October to 1100 hours 30 October 1980. Test #3

Conc. or %	Test Container Number	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)			Total Hardness (mg/l as $CaCO_3$)			Salinity (0/00)		
		0	24	48	0	24 (b.)	48	0	24	48	0	24	48	0	24	48	0	24	48
Control	A	5	5	5	6.4	4.2	3.7	7.6	7.1	7.3									
Control	B	5	5	5	6.5	4.2	3.5	7.6	7.2	7.3							31.5		
5.6%	A	5	5	5	6.6	3.6	3.5	7.6	7.2	7.3									
5.6%	B	5	5	5	6.7	3.9	3.7	7.6	7.2	7.3							29.0		
10.0%	A	5	5	5	6.7	4.0	3.6	7.6	7.2	7.3									
10.0%	B	5	5	5	6.8	3.5	3.2	7.6	7.2	7.3							29.0		
18.0%	A	5	5	5	7.0	4.8	3.5	7.7	7.3	7.3									
18.0%	B	5	5	5	7.0	4.2	3.7	7.7	7.3	7.4							27.0		
32.0%	A	5	5	5	7.1	3.9	3.4	7.7	7.3	7.4									
32.0%	B	5	5	4[c]	7.2	4.2	3.8	7.8	7.3	7.4							23.0		
56.0%	A	5	5	5	7.7	4.6	3.7	7.9	7.4	7.5									
56.0%	B	5	5	5	7.7	4.6	3.9	7.9	7.5	7.5							17.5		
100%	A	5	5	5	8.3	5.9	3.9	8.3	7.9	7.6									
100%	B	5	5	5	8.3	6.5	4.1	8.3	7.9	7.6							6.5		

Remarks:

a. Test temperature range 19.7° to 21.7 °C.
b. All test containers aerated to 7.0+ mg/l D.O.
c. One mysid jumped out and stuck to jar.

Table 5. Summary of Chemical Data for Sample of American Cyanamid Co. Collected 27 October 1980

Parameter	Value
Pesticides	Not Detected
Base neutral extractables	Not Detected
Acid extractables	Not Detected

Metals	ug/l
Sb	50k
As	50k
Be	11k
Cd	1k
Cr	50k
Cu	15k
Pb	30k
Hg	0.2k
Ni	20k
Se	50k
Ag	1k
Tl	100k
Zn	29

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

RESULTS AND DISCUSSION

This particular sample of effluent from the American Cyanamid Company was essentially non-toxic. Although some mortality was noted in the highest concentration in the variable salinity test (Table 2), the distribution and amount of mortality observed was such that a statistically valid LC_{50} could not be calculated.

This composite effluent sample was chemically characterized by DER's Chemistry Section in Tallahassee. These results are presented in Table 5.

LITERATURE CITED

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

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

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

APPENDIX

DISCHARGE SERIAL NUMBER

001

FOR AGENCY USE

ctices
batement
is discharge
ve. (see

219a Narrative: Weak wastewaters are collected separately through screen boxes and transported to aerated lagoon treatment system. Wastewaters are neutralized and nutrient added (phosphorus). Approximately 5 days retention under aeration are provided and 4 days under oxidation polishing pond conditions. Treated wastewater is chlorinated and discharged either to Escambia Bay or ≈25% to a spray irrigation disposal site and the remainder to Escambia Bay. Discharge into the Bay is through a submerged outfall (continue below)

Waste Abatement Codes
Using the codes listed in Table II of the Instruction Booklet, describe the waste abatement processes for this discharge in the order in which they occur if possible.

219b

(1) ESEPAR, (2) ESEGRE, (3) ESURFA,
(4) ECOUNT, (5) EPUMPS, (6) DCHANG,
(7) DCHEMI, (8) DSTOPD, (9) RCOVE,
(10) RECYCL, (11) REVAPO, (12) LEVAPO,
(13) LOCALS, (14) OMONIT, (15) CNEUTR,
(16) CNUTRI, (17) BAERAT, (18) BPOLIS,
(19) EMERGE, (20) PSKIMC, (21) CCLDIS,
(22) MOTHER, (23) _____, (24) _____,
(25) _____.

FOR AGENCY USE

16. Wastewater Characteristics

Check the box beside each constituent which is present in the effluent (discharge water). This determination is to be based on actual analysis or best estimate.(see instructions)

Parameter 216.	Present	Parameter 216	Present
Color 00080	X	Copper 01042	X
Ammonia 00610	X	Iron 01045	X
Organic nitrogen 00605	X	Lead 01051	A
Nitrate 00620	X	Magnesium 00927	X
Nitrite 00615	X	Manganese 01055	X
Phosphorus 00665	X	Mercury 71900	X
Sulfate 00945	X	Molybdenum 01062	X
Sulfide 00745	A	Nickel 01067	X
Sulfite 00740	A	Selenium 01147	X
Bromide 71870	A	Silver 01077	A
Chloride 00940	X	Potassium 00937	X
Cyanide 00720	X	Sodium 00929	X
Fluoride 00951	X	Thallium 01059	A
Aluminum 01105	A	Titanium 01152	X
Antimony 01097	X	Tin 01102	A
Arsenic 01002	A	Zinc 01092	X
Beryllium 01012	A	Algicides* 74051	X
Barium 01007	X	Chlorinated organic compounds* 74052	X
Boron 01022	A	Pesticides* 74053	A
Cadmium 01027	A	Oil and grease 00550	X
Calcium 00916	X	Phenols 32730	A
Cobalt 01037	A	Surfactants 38260	X
Chromium 01034	X	Chlorine 50060 Total Residual	X
Fecal coliform bacteria 74055	X	Radioactivity* 74050	A

*Specify substances, compounds and or elements in Item 26.

Pesticides (insecticides, fungicides, and rodenticides) must be reported in terms of the acceptable common names specified in *Acceptable Common Names and Chemical Names for the Ingredient Statement on Pesticide Labels*, 2nd Edition, Environmental Protection Agency, Washington, D.C. 20250, June 1972, as required by Subsection [illegible] of the Regulations for the Enforcement of the Federal Insecticide, Fungicide, and Rodenticide Act.