TOXICITY ASSESSMENT OF THE NORTHFIELD, MINNESOTA WASTEWATER TREATMENT PLANT EFFLUENT

June and July 1979

Minnesota Pollution Control Agency
Division of Water Quality
Surface and Ground Water Section
Bioassay Program

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SUMMARY

A 96-hour acute toxicity test on bluegills was conducted with effluent from the Northfield Wastewater Treatment Plant. The effluent was found to be acutely toxic. Effluent LC50 values of 74.5% and 67.6% were calculated from the bluegill toxicity test using the moving average and probit methods, respectively. A comparison of the concentrations of compounds found in the effluent with LC50 values reported in the literature, suggests that cyanide, un-ionized ammonia and silver are toxic constituents of the effluent. However, wastewater treatment plant effluents are complex mixtures, and other compounds may also be involved in the effluent's toxicity.

RECOMMENDATION

1. The City of Northfield should remove or treat to non-acutely toxic levels those effluent components causing a violation of Section (C) (6) of WPC 14

2. The Enforcement Section of the Division of Water Quality should closely monitor the City of Northfield's progress in complying with Section (C) (6 of WPC 14.

MINNESOTA POLLUTION CONTROL AGENCY

Division of Water Quality

Toxicity Assessment of the Northfield, Minnesota

Wastewater Treatment Plant Effluent

INTRODUCTION

The purpose of the toxicity test is to determine compliance with the water quality and effluent standards for the Cannon River. In particular, Section (c)(6) of WPC 14, which states that no effluent may be acutely toxic to plant or animal life.

The scope of the toxicity test involves only the determination of acute toxicity of the effluent. No attempt is made to completely characterize the chemical nature of the effluent. If the effluent is toxic the test will provide a starting point from which the discharger can establish compliance with Section (c)(6) of WPC 14.

METHODS

General

A flow-through toxicity test of the Northfield Wastewater Treatment Plant (NWWTP) effluent was conducted on site in a mobile laboratory. A description of the mobile laboratory is provided in Appendix A. The test was conducted by Harold Wiegner and Gary Fandrei, Pollution Control Specialist Intermediates.

Unchlorinated effluent was provided to the mobile laboratory by continuous pumping from the NWWTP final clarifier. Dilution water was collected twice a day, in the morning and in the late evening, from the Cannon River approximately 100 meters (109 yards) upstream of the NWWTP discharge, and stored outside the mobile laboratory in a covered, polyethylene lined pool. Water was continuously pumped from the pool into the mobile laboratory.

The procedures used in conducting the flow-through toxicity test follow the requirements for effluent testing recommended by the USEPA (Peltier 1978) and the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). The procedures used in conducting the toxicity test are described in Appendix B and in "Methods for Measuring the Acute Toxicity of Effluents to Aquatic Organisms" (Peltier 1978).

The flow-through diluters used in the toxicity test are described in Appendix C.

It was necessary to aerate all test chambers during the toxicity test due to low dissolved oxygen levels in the NWWTP effluent.

Fish

Bluegills (*Lepomis macrochirus* Rafinesque) were obtained on July 23, 1979, from the U. S. Fish and Wildlife Service, National Fishery Research Laboratory in LaCrosse, Wisconsin. The average length of the fish was 56mm (2.2 in.), the average weight 3.2g (0.11 oz.) (Table 1). The fish had been held by the National Fishery Research Laboratory for the previous week. Mortalities during that period were minimal. The bluegills were transported to a holding tank in the mobile laboratory and acclimated to the dilution water and a test temperature of 20°C (68°F). No mortalities were observed during the acclimation period. Ten fish were transferred to each test chamber and the test begun on July 26, 1979.

Chemistry

A system of Martek instruments was used to automatically monitor pH, dissolved oxygen, specific conductance and temperature of the effluent and the dilution water during the bluegill toxicity test. The effluent was monitored in the final clarifier and the dilution water was monitored in the holding pool.

Instrumentation Specialties Company (ISCO) water samples were set up to take 24-hour composite samples of the effluent during the bluegill toxicity test. The samples were collected from the final clarifier once every two hours. An ISCO water sampler was also set up to take 24-hour composite samples of the NWWTP influent during the bluegill toxicity test. The samples were collected once every two hours.

Daily grab samples were taken of the effluent and dilution water during the bluegill toxicity test. The samples were collected after the effluent and dilution water were pumped into the mobile laboratory. All samples were analyzed by the Minnesota Department of Health.

RESULTS

General

Diluter calibration before and after the bluegill toxicity test did not reveal excessive fluctuations in the concentration of effluent or the volume of test solution delivered to each test chamber (Table 2).

Fish

In the toxicity test with bluegills, mortalities from the 100% effluent concentration of Diluter No. 2 was 65% (Figure 1). An LC50 value was not calculated from these data because the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) recommend greater than 65% mortality in the highest effluent concentration for the calculation of an LC50 value. Mortality from the 100% effluent concentration of Diluter No. 1 did exceed 65% and LC50 values were calculated. The 96-hour LC50 values were 74.5% and 67.6% effluent as calculated by the moving average and probit methods, respectively (Table 3). No mortality occurred below an effluent concentration of 10%.

Chemistry

The Martek monitoring data of the effluent reveals little variation of the pH, conductivity and temperature during the bluegill toxicity test (Figure 2 and Table 4). The dissolved oxygen variation observed in the effluent was moderated by continuous aeration of the test chambers.

The Martek monitoring data of the dilution water reveals little variation of the pH, dissolved oxygen and conductivity during the bluegill toxicity test (Figure 3 and Table 4). The slight temperature variation observed in the dilution water was moderated by the water bath in the mobile laboratory.

A slight difference in the pH and the conductivity between the effluent and dilution water was observed. Average values of effluent pH and conductivity were 7.66 and 1.2 mmhos (Table 4). Average values of dilution water pH and conductivity were 8.12 and 0.6 mmhos (Table 4).

Chemical data of the effluent and dilution water grab and composite samples from the bluegill toxicity test reveals higher concentrations of ammonia, cadmium, chromium, copper, lead, zinc and cyanide in the effluent than in the dilution water (Tables 5, 6, 7 and 8). The chemical data also reveals the presence of detectable levels of PCB in the influent and the effluent of the NWWTP (Tables 8 and 9).

To determine the effect of aeration and the presence of fish on the ammonia nitrogen concentration in the 100% effluent test chamber, several grab samples were analyzed (Table 10). No significant change in the ammonia nitrogen concentration was detected.

DISCUSSION

General

The concentration of effluent delivered to most of the test chambers varied little during the toxicity test on bluegills. The 7.7% effluent concentration in the bluegill toxicity test did show considerable variation. The variation occurred in diluter No.2. Mortality data from diluter No. 2 was not used to calculate LC50 values for the bluegill toxicity test.

Fish

Ninety-six hour LC50 values were calculated from one of the replicates of the bluegill toxicity test. Effluent LC50 values of 74.5% and 67.6% were calculated by the moving average and probit methods, respectively. It is interesting to note that these LC50 values are similar to the LC50 values of 76.1% and 74.7% calculated from a preliminary shiner toxicity test. Effluent LC50 values could not be calculated for the other replicate of the bluegill toxicity test because the mortality of the highest concentration, 100% effluent, was not greater than 65% (Figure 1).

Chemistry

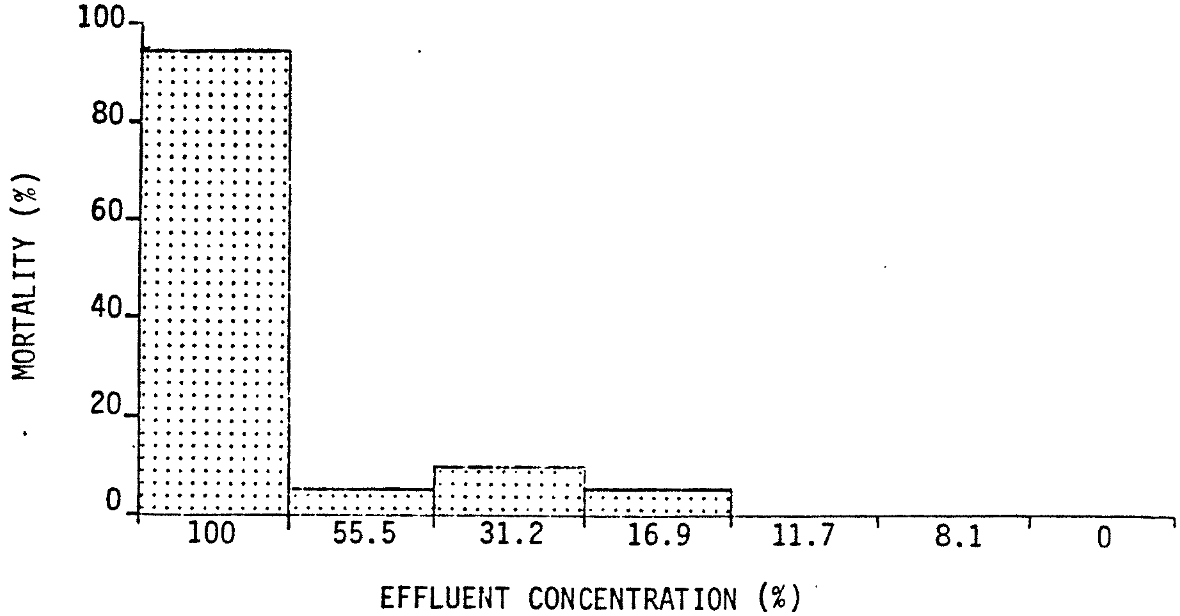
The USEPA, in an extensive literature review, tabulated LC50 data for many chemical compounds (USEPA 1979. Ambient Water Quality Criteria. Draft Document). A comparison of the LC50 data reported in the literature with the chemical data from the NWWTP effluent (Tables 7 and 8), reveals a compound that may be a toxic constituent of the effluent. Bluegill LC50 values for free cyanide tabulated by the USEPA ranged from 7.4 to 364 ug/l. Mean total cyanide concentrations reported from grab samples and composite samples taken during the bluegill toxicity test, were 626 and 500 ug/l, respectively. Although, as Smith et al. (1979) reported, total cyanide measurements would not be representative of the actual cyanide toxicity, they can be used as a liberal estimate for it. Because the total cyanide concentrations of the NWWTP effluent are much greater than the reported LC50 values for free cyanide it seems likely that cyanide was a toxic constituent of the effluent. Smith et al. (1979) and Doudoroff (1976) present thorough reviews of the toxicity of cyanide to fish.

The Minnesota Pollution Control Agency (MPCA), in an extensive literature review, tabulated LC50 data for un-ionized ammonia (MPCA 1979. Ammonia. Standard Justification). Un-ionized ammonia concentrations can be calculated from the ammonia nitrogen concentrations reported in Tables 7 and 8 by the method of Thurston et al. (1974). Bluegill LC50 values for un-ionized ammonia tabulated by the MPCA ranged from 0.40 to 1.65 mg/l. Mean un-ionized ammonia concentrations calculated from effluent grab and composite samples taken during the bluegill toxicity test were 0.38 and 0.29 mg/l, respectively. These concentrations approximate the lower LC50 values reported in the literature, and suggest a possible toxic effect.

In June of 1979, the MPCA conducted a Compliance Sampling Inspection for toxics in the NWWTP effluent. In this study a high level of silver (48 μg/l) was detected. This level approximates a bluegill LC50 value tabulated by the USEPA (1979) and may also contribute to the toxicity of the effluent.

Municipal wastewater treatment plant effluents are generally complex mixtures of many potentially harmful compounds. It is often difficult to predict how these compounds interact, and how they may affect aquatic organisms. It is also difficult and impractical to monitor every potentially harmful compound in municipal wastewater treatment plant effluents. Because of these difficulties, sources of toxicity in municipal wastewater treatment plant effluents can be very elusive. The evidence presented here only suggests that cyanide, un-ionized ammonia and silver are toxic constituents of the Northfield wastewater treatment plant effluent. Other compounds may also be involved.

DILUTER NO. 1



DILUTER NO. 2

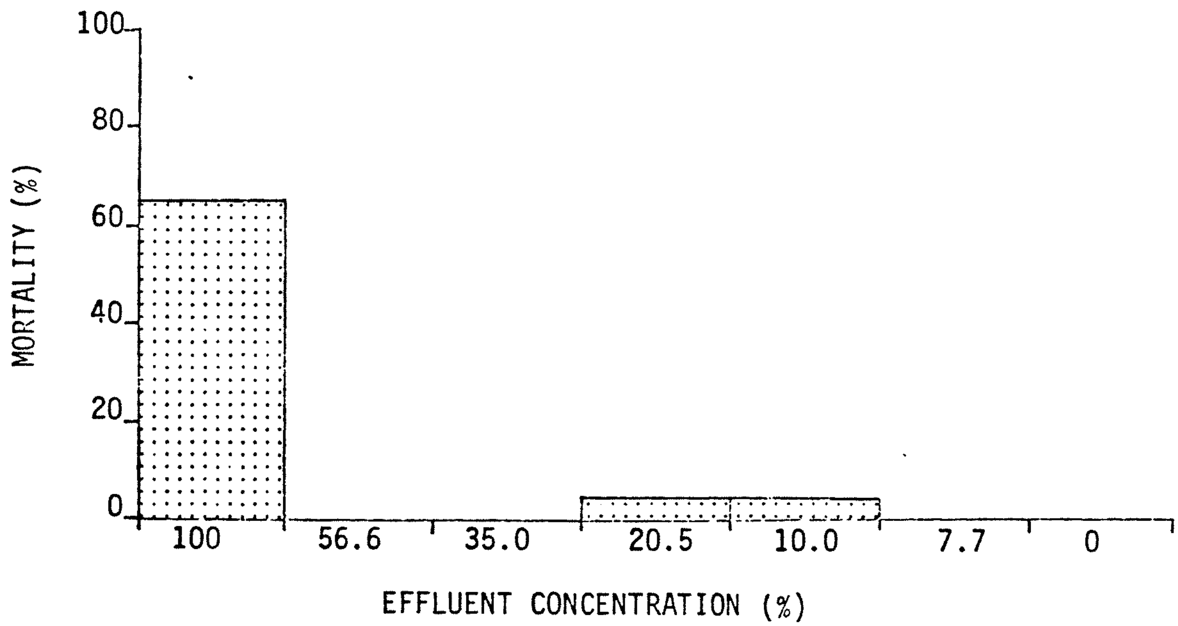


Figure 1. Mortality (%) vs. effluent concentration (%) for the bluegill (Lepomis macrochirus Rafinesque) toxicity test. July 1979.

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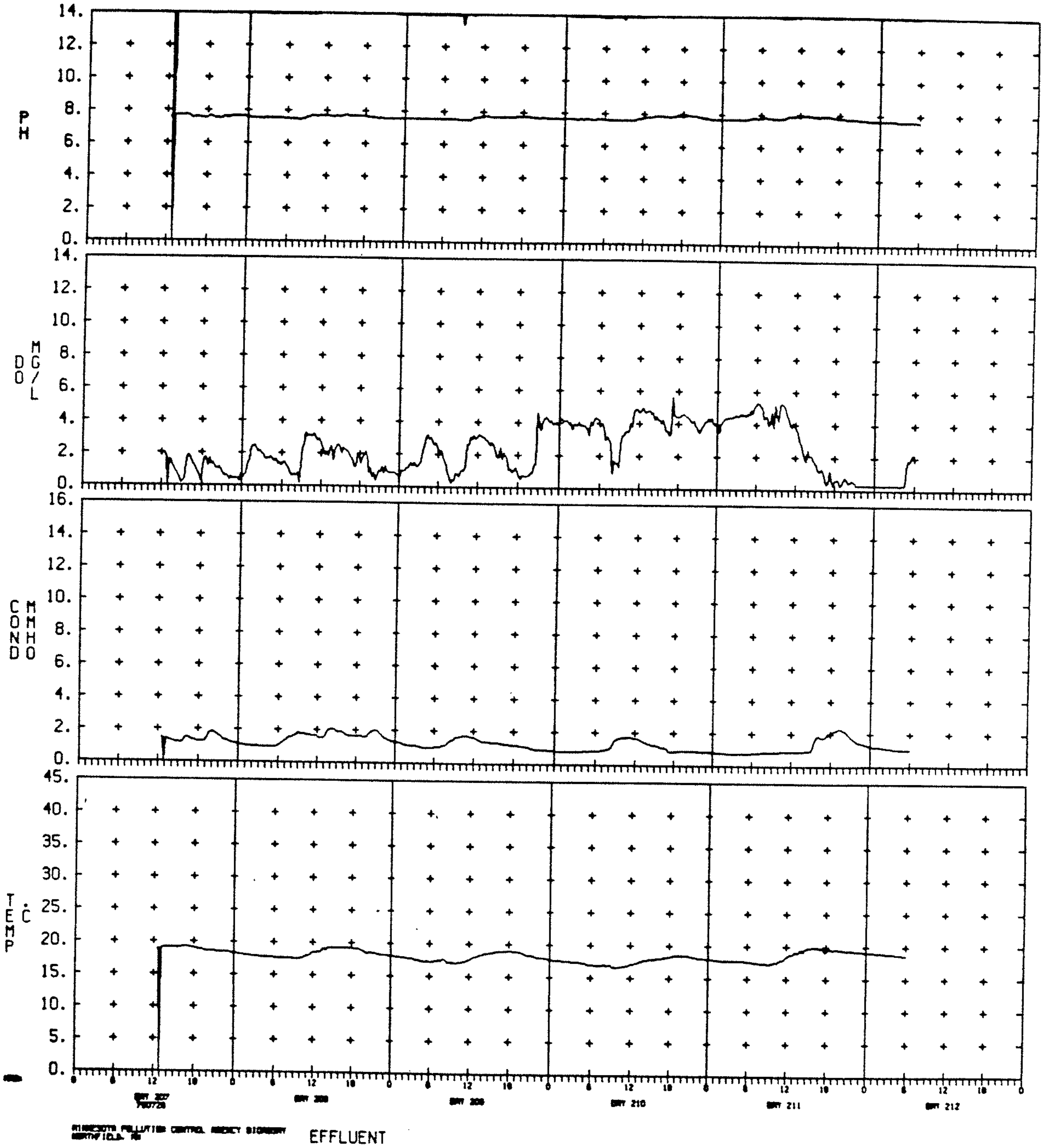

Figure 2. Effluent characteristics as determined by a system of Martek instruments during the bluegill (Lepomis macrochirus Rafinesque) toxicity test. July 1979.

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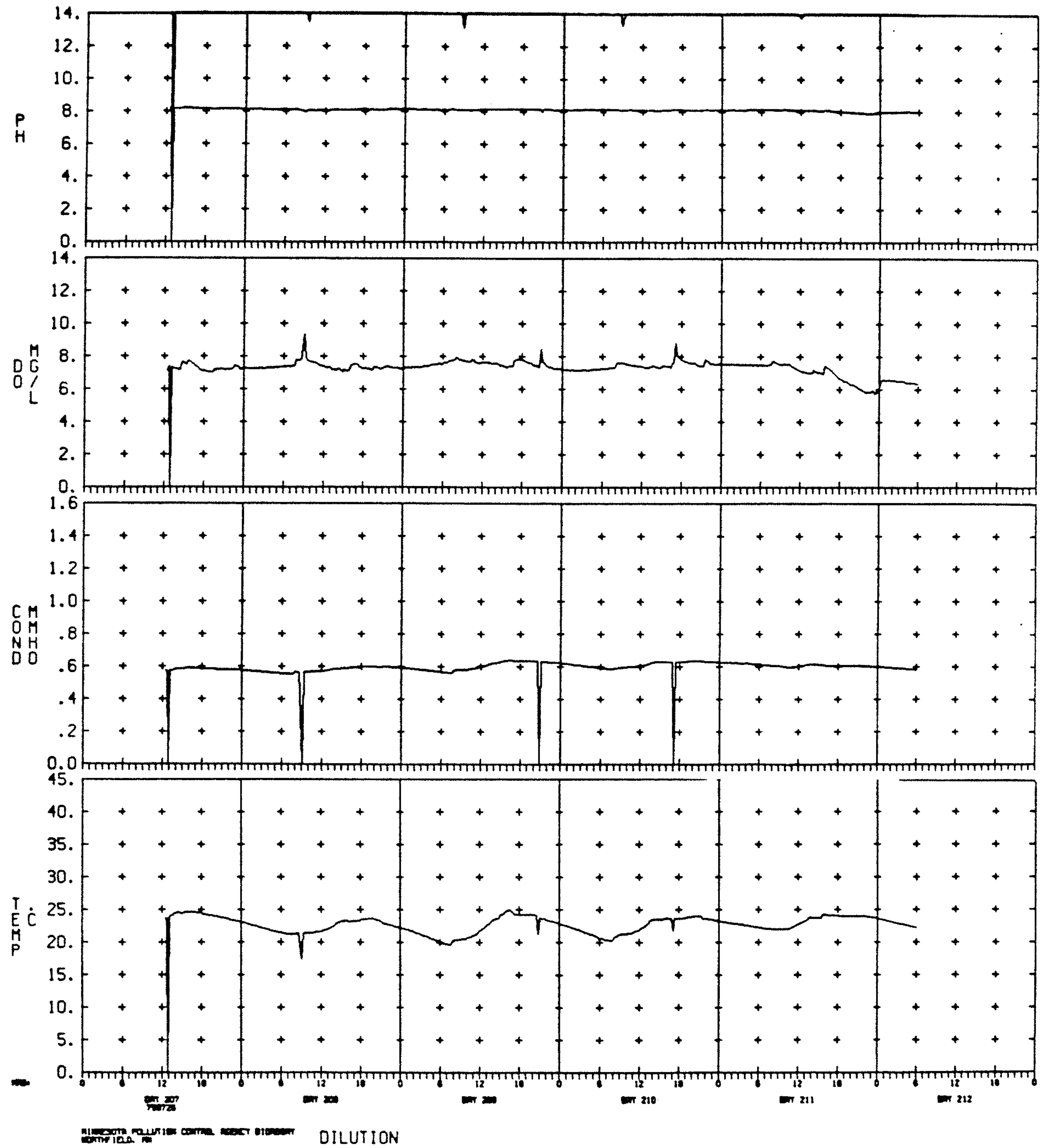


Figure 3. Dilution water characteristics as determined by a system of Martek instruments during the bluegill (Lepomis macrochirus Rafinesque) toxicity test. July 1979.

TABLE 1. Physical characteristics of the fish used in the Northfield, Minnesota wastewater treatment plant toxicity tests. June and July 1979.

Fish		Number Measured	$\bar{x}$ wt. (g)	Range (g)	$\bar{x}$ Total Length (mm)	Range (mm)	Size Difference
Bluegills	Mortalities	38	3.2	2.0 - 5.2	56	49 - 64	1.3
Bluegills	Survivors	51	3.1	2.1 - 5.1	56	51 - 64	1.2
Bluegills	Combined	89	3.2	2.0 - 5.2	56	49 - 64	1.3

TABLE 2. Calibration of the diluters used in the bluegill (Lepomis macrochirus Rafinesque) toxicity test. July 1979.

Diluter No. 1				Diluter No. 2			
Calibration before test		Calibration after test		Calibration before test		Calibration after test	
Effluent Concentration (%)	Volume (mls)	Effluent Concentration (%)	Volume (mls)	Effluent Concentration (%)	Volume (mls)	Effluent Concentration (%)	Volume (mls)
0.0	1070	0.0	1080	0.0	1020	0.0	988
8.1	1042	8.4	1125	7.7	1030	8.9	1043
11.7	1065	12.4	1050	10.0	1070	10.7	1077
16.9	1060	17.9	1005	20.5	1032	21.1	1042
31.2	1145	31.4	1115	35.0	1005	35.5	1015
55.5	1115	53.3	1145	56.6	1045	56.5	1080
100.0	1120	100.0	1069	100.0	1037	100.0	995

TABLE 3. The LC50 analysis of the 96-hour Northfield, Minnesota wastewater treatment plant toxicity tests. June and July 1979.

		Moving Average Method		Probit Method			Approx. Method
Fish	Diluter	LC50 Value (% effluent)	95% Confidence Limits (%)	LC50 Value (% effluent)	95% Confidence Limits (%)	Goodness of fit	LC50 Value (% effluent)
*Shiners	No. 1	76.1	60.8 - 97.5	74.7	62.4 - 96.3	0.96	76.1
Blue-gills	No. 1	74.5	63.1 - 94.1	67.6	0→ + infinity	>0.001	74.5
Blue-gills	No. 2	NC	NC	NC	NC	NC	90.4

NC = Not calculated

*Preliminary Test

TABLE 4. A statistical synopsis of the Martek monitoring data for the effluent and dilution water from the bluegill (*Lepomis macrochirus* Rafinesque) toxicity test. July 1979. Aberrant data generated through equipment maintenance or malfunctions are not included in the statistical synopsis.

Variable Name	Mean	Standard Deviation	Standard Error of Mean	Coefficient of Variation	Range	Total Frequency
Effluent temperature (°C)	18.082	0.669	0.0344	0.03710	16.650 - 19.260	377
Effluent conductivity (mmho)	1.205	0.352	0.0181	0.29181	0.740 - 2.060	377
Effluent D.O. (mg/l)	2.612	1.476	0.0760	0.56516	0.150 - 5.320	377
Effluent pH	7.665	0.114	0.0059	0.01486	7.460 - 7.970	377
Dilution water temperature (°C)	22.580	1.232	0.0635	0.05458	19.620 - 25.010	377
Dilution water conductivity (mmho)	0.599	0.023	0.0012	0.03826	0.552 - 0.641	377
Dilution water D.O. (mg/l)	7.421	0.195	0.0100	0.02624	6.980 - 8.030	377
Dilution water pH	8.124	0.024	0.0012	0.00298	8.050 - 8.200	377

TABLE 5. Results of the laboratory analysis of grab samples collected from the Cannon River on 21 June 1979 at 1615 hours.

Parameter	Concentration
Arsenic as As (μg/l)	5.1
Cadmium as Cd (μg/l)	0.08
Total Chromium as Cr (μg/l)	4.9
Copper as Cu (μg/l)	3.6
Iron as Fe (μg/l)	1400.0
Lead as Pb (μg/l)	5.6
Manganese as Mn (μg/l)	260.0
Nickel as Ni (μg/l)	5.1
Zinc as Zn (μg/l)	31.0
Cyanide (μg/l)	21.0

TABLE 6. Results of the laboratory analysis of grab samples collected from the dilution water during the bluegill (Lepomis macrochirus Rafinesque) toxicity test. July 1979.

Parameter	July 26 1600 hrs	July 27 0930 hrs	July 28 1130 hrs	July 29 2130 hrs	Average for test
Suspended Solids (mg/l)	10.0	9.6	6.8	8.0	8.6
Calcium as $CaCO_3$ (mg/l)	230.0	240.0	250.0	260.0	245.0
Magnesium as $CaCO_3$ (mg/l)	110.0	110.0	120.0	120.0	115.0
Total Hardness as $CaCO_3$ (mg/l)	340.0	350.0	370.0	380.0	360.0
Ammonia nitrogen as N (mg/l)	0.42	0.22	0.376	0.114	0.282
Arsenic as As (µg/l)	9.7	9.2	3.5	3.8	6.6
Cadmium as Cd (µg/l)	0.12	0.14	0.11	0.07	0.11
Total Chromium as Cr (µg/l)	2.2	3.4	3.4	2.8	3.0
Copper as Cu (µg/l)	2.4	2.3	2.0	2.1	2.2
Iron as Fe (µg/l)	320.0	340.0	320.0	340.0	330.0
Lead as Pb (µg/l)	1.2	1.6	1.6	1.7	1.5
Manganese as Mn (µg/l)	71.0	65.0	60.0	69.0	66.0
Mercury as Hg (µg/l)	0.54	0.37	0.30	0.23	0.36
Nickel as Ni (µg/l)	3.1	2.8	2.5	2.7	2.8
Zinc as Zn (µg/l)	5.6	5.9	6.0	5.2	5.7
Cyanide (µg/l)	23.0	28.0	24.0	15.0	22.0
PCB 1016 (µg/l)	<0.1	<0.1	---	<0.1	
PCB 1254 (µg/l)	<0.1	<0.1	---	<0.1	
PCB 1260 (µg/l)	<0.1	<0.1	---	<0.1	

TABLE 7. Results of laboratory analysis of grab samples collected from the effluent during the bluegill (<u>Lepomis</u> <u>macrochirus</u> Rafinesque) toxicity test. July 1979.

Parameter	July 26 1600 hrs	July 27 0930 hrs	July 28 1130 hrs	July 28 1715 hrs	July 29 1400 hrs	July 29 2130 hrs	Average for test
Suspended Solids (mg/l)	27.0	---	35.0	---	---	30.0	30.6
Calcium as $CaCO_3$ (mg/l)	240.0	280.0	260.0	---	---	200.0	245.0
Magnesium as $CaCO_3$ (mg/l)	130.0	150.0	140.0	---	---	110.0	132.0
Total Hardness as $CaCO_3$ (mg/l)	370.0	430.0	400.0	---	---	310.0	378.0
Ammonia nitrogen as N (mg/l)	16.6	17.0	34.0	26.0	29.0	23.0	24.3
Arsenic as As (µg/l)	6.3	8.0	12.0	---	---	3.3	7.4
Cadmium as Cd (µg/l)	1.4	0.78	0.72	---	---	0.65	0.89
Total Chromium as Cr (µg/l)	19.0	3.3	16.0	---	---	9.8	12.0
Copper as Cu (µg/l)	230.0	380.0	210.0	---	---	170.0	248.0
Iron as Fe (µg/l)	1600.0	1200.0	620.0	---	---	500.0	980.0
Lead as Pb (µg/l)	64.0	59.0	50.0	---	---	33.0	52.0
Manganese as Mn (µg/l)	200.0	110.0	92.0	---	---	81.0	121.0
Mercury as Hg (µg/l)	0.68	0.87	0.82	---	---	0.96	0.83
Nickel as Ni (µg/l)	9.2	13.0	9.4	---	---	6.9	9.6
Zinc as Zn (µg/l)	54.0	84.0	85.0	---	---	76.0	75.0
Cyanide (µg/l)	1000.0	1300.0	160.0	---	---	45.0	626.0

TABLE 8 . Results of the analysis of composite samples collected from the effluent during the bluegill (*Lepomis macrochirus* Rafinesque) toxicity test. July 1979.

Parameter	July 26	July 27	July 28	July 29	Average for test
Ammonia nitrogen as N (mg/l)	16.7	19.6	19.3	20.0	18.9
Arsenic as As (μg/l)	14.0	23.0	6.8	7.3	12.8
Cadmium as Cd (μg/l)	8.1	3.8	2.8	1.6	4.1
Total chromium as Cr (μg/l)	15.0	13.0	9.4	12.0	12.4
Copper as Cu (μg/l)	270.0	95.0	210.0	160.0	184.0
Iron as Fe (μg/l)	2100.0	1000.0	740.0	780.0	1155.0
Lead as Pb (μg/l)	54.0	47.0	42.0	26.0	42.0
Manganese as Mn (μg/l)	150.0	110.0	8.4	93.0	109.0
Nickel as Ni (μg/l)	10.0	7.8	4.3	4.7	6.7
Zinc as Zn (μg/l)	140.0	160.0	75.0	110.0	121.0
Cyanide (μg/l)	910.0	910.0	57.0	130.0	500.0
PCB 1016 (μg/l)	<0.1	<0.1	<0.1	<0.1	
PCB 1254 (μg/l)	0.86	<0.1	<0.1	<0.1	
PCB 1260 (μg/l)	<0.1	<0.1	<0.1	<0.1	

TABLE 9. Results of the analysis of composite samples collected from the influent during the bluegill (<u>Lepomis macrochirus</u> Rafinesque) toxicity test. July 1979.

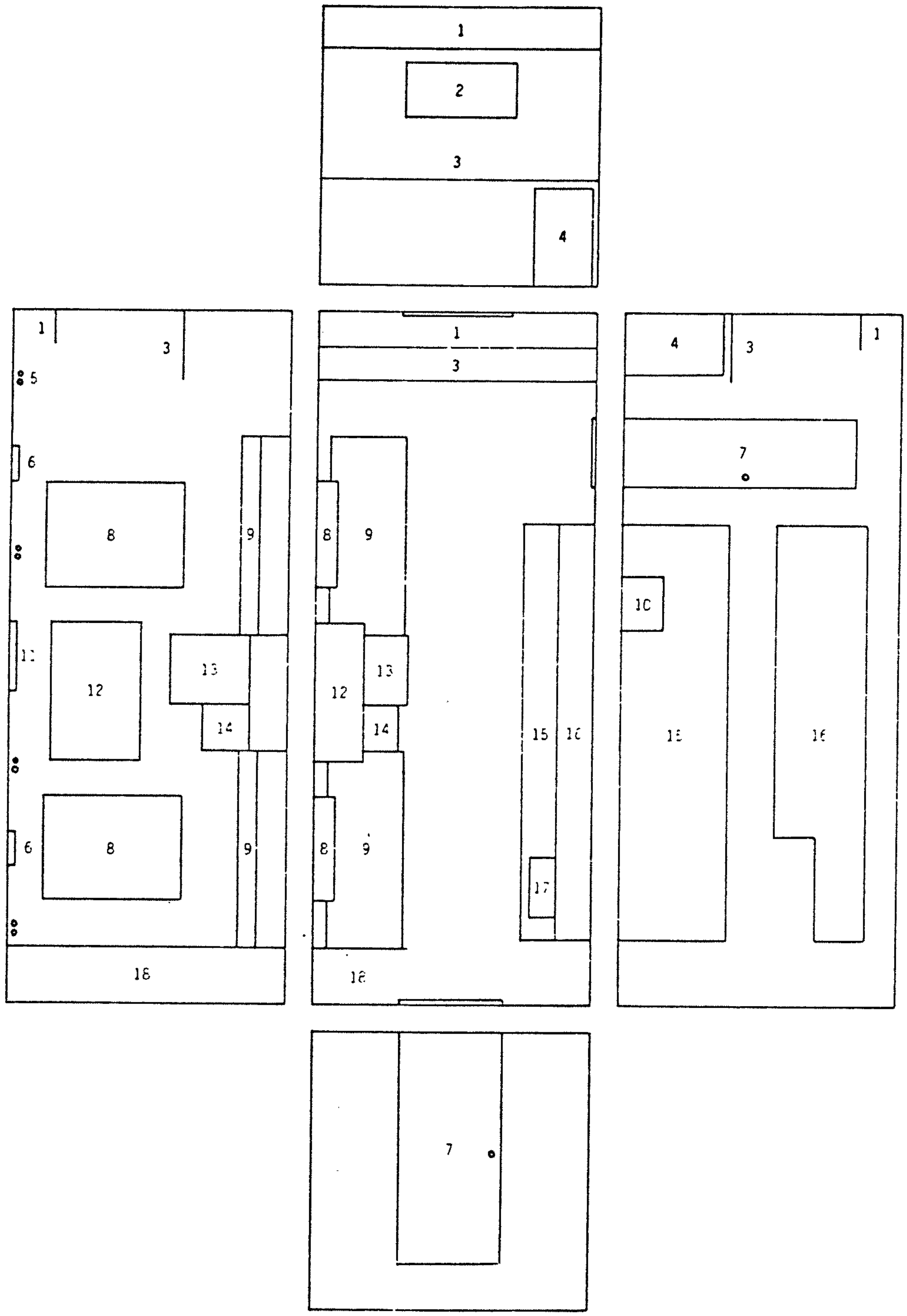
Parameter	July 26	July 27	July 28	July 29
PCB 1016 (μg/l)	<0.1	<0.1	<0.1	<0.1
PCB 1254 (μg/l)	<0.1	0.43	<0.1	<0.1
PCB 1260 (μg/l)	<0.1	<0.1	<0.1	<0.1

TABLE 10. Results of the analysis of grab samples collected from the 100% effluent test chambers during the bluegill (*Lepomis macrochirus* Rafinesque) toxicity test. July 1979.

Date	Ammonia Nitrogen as N (mg/l)	Comments
July 27	26.0	Aerated, with fish in the test chamber
July 29	27.2	Aerated, with fish in the test chamber
July 29	28.0	Aerated, without fish in the test chamber
July 29	26.0	Nonaerated, without fish in the test chamber

APPENDIX A

MOBILE LABORATORY DESCRIPTION



APPENDIX A CONTINUED

1. Storage Shelf
2. Window
3. Workbench
4. Refrigerator
5. Fluorescent Lights
6. Power Vent
7. Door
8. Diluter
9. Aquaria Trays
10. Furnace
11. Air Conditioner
12. Martek Water Quality Analyzers and Data Logger
13. Fish Holding/Acclimation Tank
14. Water Bath
15. Base Cabinets
16. Wall Cabinets
17. Sink
18. Storage Area

APPENDIX B

Toxicity Test Procedures

The first step in conducting a toxicity test is to evaluate the test site. At this time the location of the mobile bioassay trailer is chosen, and an approximate test temperature selected. An assessment of the dissolved oxygen characteristics of the effluent is also made, to determine if aeration of the test chambers is necessary. Other known chemical and physical characteristics of the effluent and dilution water are also reviewed.

The mobile bioassay trailer is then brought to the test site. All plumbing systems are connected. Unchlorinated effluent is pumped to the bioassay trailer in Tufflex® hose by a Flotec® or well pump. Once in the trailer the effluent is run through stainless steel coils in a water bath for temperature regulation, and then to the diluters.

Dilution water is obtained from the water body receiving the discharge at a point unaffected by the discharge. The water is trucked to the trailer in a 300 gallon fiberglass tank and stored in a 1000 gallon polyethylene lined pool, from which it is pumped into the bioassay trailer through Tufflex® hose by a Flotec® pump. Or, if conditions permit, the dilution water is pumped directly from the water body receiving the discharge to the trailer. Once in the trailer the dilution water is run through stainless steel coils in a water bath for temperature regulation. The dilution water is then delivered to the diluters and the fish holding tank.

The fish are then transported to the bioassay trailer. An insulated fiberglass tank with aeration is used to transport the fish. The fish and the transport water are placed in the holding tank in the bioassay trailer. Dilution water is delivered to the holding tank at a rate to provide one tank volume during the first 24 hours. The fish are acclimated to the dilution water for an additional 48 hours.

While the fish are acclimating to the dilution water, the diluters and water analysis equipment are calibrated and the automatic water samplers are set-up. Diluter calibration involves measuring the volume of effluent and dilution water delivered from each cell of each diluter during one diluter cycle. Adjustments to achieve the six desired concentrations in a 56% dilution series are made at this time. After calibration is completed, the diluters are operated for at least four hours to monitor for diluter malfunctions. All the test chambers (two per concentration) are filled at this time. Water analysis equipment (Martek monitoring system, and YSI dissolved oxygen meter) are calibrated according to the manufacture's recommendations. Instrumentation Specialties Company automated sampling equipment (ISCO samplers) are set-up to take samples of the effluent.

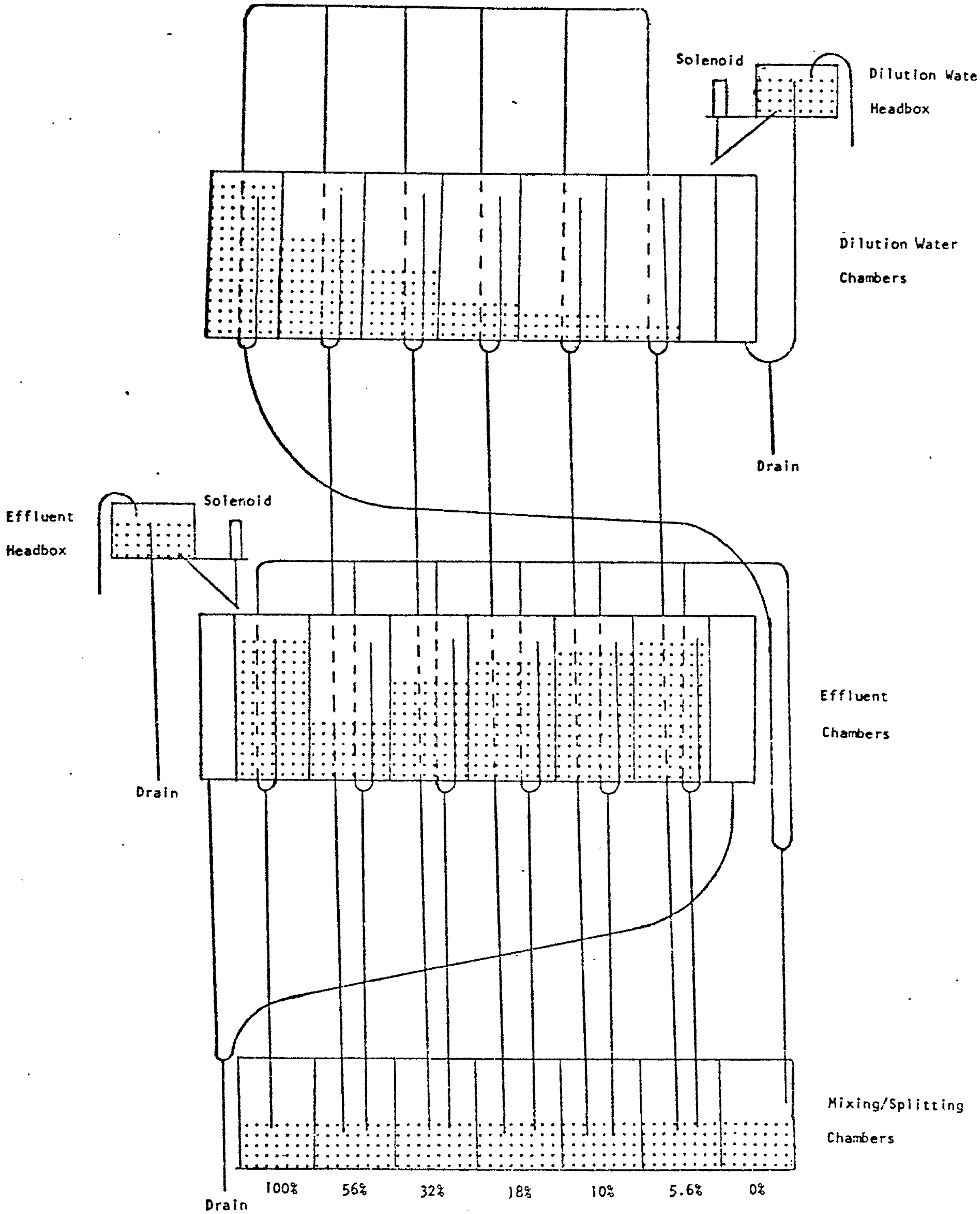
At this point ten fish are placed in each test chamber, the sampling program initiated, and the test begun. The test involves two diluters with six effluent concentrations and a control. Through a flow splitter, test water is delivered to two test chambers per concentration, making a total of 28 test chambers per test. Temperature is monitored daily in each tank. Mortalities are counted, weighed and measured daily. The toxicity test is run for 96 hours. Upon completion of the test all surviving fish are counted, a subsample weighed and measured and the diluters recalibrated.

After the toxicity test all test equipment is washed in detergent, hydrochloric acid, acetone and sodium hypochlorite. The equipment is rinsed in tap water after each washing step. All tygon tubing, neoprene stoppers and hose in contact with the effluent are discarded.

If the mortality data of a toxicity test meet the minimum requirements established by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) for calculating an LC50 value, the results of a toxicity test are reported as an LC50 value. An LC50 value is defined as the concentration of a test material in a suitable dilutant at which 50% of the exposed organisms die. An LC50 value can be calculated by several methods. The moving average method and the probit method are both used in this study. Stephan (1977) provides a good discussion of these and other methods used to calculate LC50 values. A computer program was obtained from Charles Stephan of the U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, Minnesota, and used for LC50 calculations.

If the mortality data of a toxicity test do not meet the minimum requirements established by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) for calculating an LC50 value, the results of a toxicity test are reported as the percent mortality in the highest effluent concentration.

APPENDIX C Proportional Diluter



Constant volumes of dilution water and effluent siphon from their respective chambers and mix in the mixing/splitting chambers at 5 minute intervals. The resulting effluent/dilution water mixtures then flow to the test chambers.

Literature Cited

The Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Ecological Research Series. EPA-660/3-75-009. U.S. Environmental Protection Agency. Corvallis, Or. 61 pages.

Doudoroff, P. 1976. Toxicity to fish of cyanides and related compounds. A review. Ecological Research Series EPA-600/3-76-038. U.S. Environmental Protection Agency. Duluth, MN. 155 pages.

Minnesota Pollution Control Agency. 1979. Ammonia Standard Justification. Draft report prepared in support of proposed water qualty standards 32 pages

Peltier, W. 1978. Methods for measuring the acute toxicity of effluents to aquatic organisms. Ecological Research Series. EPA-600/4-78-012. U.S. Environmental Protection Agency. Cincinnati, OH. 52 pages.

Smith, L.L. Jr., S.J. Broderius, D.M. Oseid, G.L. Kimball, W.M. Koenst and D.L. Lind. 1979. Acute and chronic toxicity of HCN to fish and invertebrates. Ecological Research Series. EPA 600/3-79-009. U.S. Environmental Protection Agency. Duluth, MN. 115 pages.

Stephan, C.E. 1977. Methods for calculating an LC50. Aquatic Toxicology and Hazard Evaluation, ASTM STP 634. F.L. Mayer and J.L. Hamelink, Eds. American Society for Testing and Materials. Pages 65-84.

Thurston, R.V., R.C. Russo and K. Emerson. 1974. Aqueous ammonia equilibrium calculations. Technical Report No. 74-1. Fisheries Bioassay Laboratory Bozeman, MT. 18 pages.

U.S. Environmental Protection Agency. 1979. Ambient Water Quality Criteria. Draft document. Criteria and Standards Division. Office of Water Planning and Standards. U.S. Environmental Protection Agency. Washington, D.C. 144 pages.