EPA-600/2-76-156
October 1976

Environmental Protection Technology Series

DISINFECTION EFFICIENCY AND RESIDUAL TOXICITY OF SEVERAL WASTEWATER DISINFECTANTS

Volume I - Grandville, Michigan

Municipal Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

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Volume I Grandville, Michigan

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FOREWORD

The Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems for the prevention, treatment, and management of wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, for the preservation and treatment of public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research; a most vital communications link between the researcher and the user community.

This study was concerned with comparing the disinfection efficiency of chlorine with and without dechlorination, ozone, and bromine chloride on parallel wastewater streams and evaluating the potential toxicity of those streams to aquatic life. Intimate knowledge of wastewater disinfection principles and the effects of wastewater disinfection practices on man and his environment is vital to the proper control of disease transmission and preservation of wildlife. This project has contributed valuable information in the quest for these goals.

Francis T. Mayo, Director
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ABSTRACT

This study was conducted to determine the comparative effectiveness of chlorine, bromine chloride, and ozone as wastewater disinfectants, and to determine any residual toxicity associated with wastewater disinfection with these agents or with chlorinated wastewater which had been dechlorinated with sulfur dioxide.

Streams of nondisinfected and chlorinated wastewater were pumped from the Grandville, Michigan Wastewater Treatment Plant to the project laboratory. Part of the chlorinated wastewater stream was delivered directly to the toxicity laboratory for bioassay studies while the remainder of the chlorinated stream was dechlorinated with sulfur dioxide prior to its use in bioassay tests. A portion of the nondisinfected wastewater stream was delivered to the toxicity laboratory for use in bioassays while the remaining portion was split to receive bromine chloride and ozone prior to use in the bioassay studies.

Total and fecal coliform densities, suspended solids, volatile solids, COD, ammonia nitrogen, phosphate, turbidity, color, and pH were measured in the wastewater streams. Each of the five wastewater streams was used in acute toxicity tests with several species of fishes and the freshwater macroinvertebrate *Daphnia magna*, and in a life cycle toxicity study with the fathead minnow, *Pimephales promelas*, as the test subject.

Disinfection standards were met most frequently by chlorinated and dechlorinated effluents and less frequently by chlorobrominated effluent. The only time disinfection standards were met consistently by ozonated effluent was when filtration preceded ozone injection.

Chlorine was found to be most toxic to aquatic life while sulfur dioxide dechlorination completely eliminated the toxicological effects of chlorine. Bromine chloride was less toxic than chlorine. Ozone was found to be neither acutely nor chronically toxic to the aquatic animals tested.

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TABLES (continued)

ACKNOWLEDGEMENTS

We are indebted to numerous persons from the cities of Grandville and Wyoming, Michigan, and from The Grand Valley State Colleges, for their assistance on this project. The cooperation and assistance of Mr. Glenn Folk, Superintendent of the Grandville Wastewater Treatment Plant, and all of his staff, was greatly appreciated. Drs. Gary Griffiths and John Quiring, College of Arts and Sciences, The Grand Valley State Colleges, provided assistance in the processing and analysis of data. Dr. Stephen Clark, College of Arts and Sciences, The Grand Valley State Colleges, participated in the early stages of the project. Mr. William Stonebrook, Superintendent of the Wyoming Wastewater Treatment Plant, and Ms. Grace Raven, Secretary, assisted the project in many ways.

Mr. Paul Spelman was instrumental in the design and construction of the water treatment facilities, and served as budget officer and on-site director of the project.

Dr. William Brungs, Mr. Robert Andrew, and Mr. John Arthur, all of the National Water Quality Laboratory in Duluth, Minnesota, and Drs. Quint Pickering and Tim Neiheisel of the Newtown Fish Toxicology Laboratory, provided assistance and advice for the bioassay portion of this project. We also acknowledge the assistance of Dr. Jack Mills and Mr. Sidney Jackson of the Dow Chemical Company, Drs. Allen Filbey and Michael McCuen of Ethyl Corporation, and Dr. Harvey Rosen and other employees of W. R. Grace and Company. The personnel of several Federal and State of Michigan fish hatcheries were generous in providing test animals for acute toxicity studies.

The assistance of our laboratory personnel, Mr. Terry Cruzan, Mr. Dale DeKaaker, Mr. Irwin Jousma, Mr. Richard Lincoln, Ms. Patricia Matthews, Mr. Michael Stifler, and Ms. Bonnie White, is gratefully acknowledged.

The suggestions, assistance and critical evaluations provided by Messrs. Cecil W. Chambers and Albert D. Venosa, EPA Project Officers, were invaluable to the completion of this project.

This report was submitted in fulfillment of Grant Number S802292 by the City of Wyoming, Michigan, under the partial sponsorship of the Environmental Protection Agency.

SECTION I

INTRODUCTION

AN OVERVIEW OF THE PROBLEM

Over the past seventy years chlorination has evolved as the commonly used method of disinfecting water supplies, wastewater, and industrial wastes in the United States. This extensive use of chlorine has been fostered by its powerful disinfection capabilities, availability, ease of application, and relatively low cost.

Even though the toxicity of chlorine has long been recognized, it is only recently that much attention has been given to the possible toxic side effects of chlorination. The principal exceptions to this were the hobbyists and aquaculturists, who quickly recognized the toxicity of chlorinated water supplies to aquatic organisms and tried to solve their toxicity problems as early as 1930.[1,2] However, over the past two decades, and especially since Rachel Carson published her book, Silent Spring,[3] increasing attention has been given to the environmental impact of chlorinated compounds. The various chlorinated hydrocarbon pesticides, such as DDT, elicited much of the early concern, but the chlorine compounds found in chlorinated wastewater also received attention.[4]

An increasing number of laboratory and field investigations have documented the toxic effects potentially associated with the chlorination of wastewater. Principal among these were the studies of Arthur, et al.,[5] Arthur and Eaton,[6] Esvelt, et al.,[7] Tsai,[8,9] and Zillich.[10] The literature on residual chlorine toxicity to aquatic life was reviewed by Brungs.[11]

More recently an interest has arisen in the carcinogenic effects of the chlorinated organics which might be formed in chlorinated water supplies.[12] This concern for public health will undoubtedly lead to a broader understanding of the formation and the effects of chlorinated compounds.

One avenue that some investigators have taken to solve the potential problem of residual toxicity of chlorinated effluent to aquatic life has been dechlorination. Thus, Arthur, et al.,[5] and Collins and Deener,[13] have successfully dechlorinated with SO_2; Esvelt, et al.,[7] with bisulfite; and Zillich[10] with sodium thiosulfate, with no apparent adverse effects on their test animals.

A second approach to the residual toxicity problem has been the substitution of other means of disinfection for chlorination. Ozone has received considerable attention over the past decade as an alternative to chlorine, and during the past several years, a major effort has been initiated to determine the feasibility of bromine chloride as an alternative to chlorine.

Ozone is commonly used in Europe to disinfect water supplies and is a stronger and faster acting oxidizing agent than chlorine.[14,15] Various reports have appeared on the superiority of ozone over chlorine in killing bacteria and viruses,[16-21] and on the ability of ozone to reduce the color, odor, oxygen demand, and turbidity of wastewater.[21-24] While some investigators[14,25] believe that the by-products of the ozonation of wastewater lack the potential toxicity of the by-products of chlorination, some studies[5,26,27] indicate that undesirable biological effects could possibly be associated with the ozonation of wastewater. This point requires additional investigation before final conclusions may be drawn.

In addition to our increased knowledge of the biological characteristics of ozone as a disinfectant of wastewater, technological advances have been made in generating ozone. These advances have decreased the costs of treating wastewater with ozone and, at least in some cases, have apparently made disinfection with ozone economically competitive with disinfection with chlorine followed by dechlorination and reaeration.[28] A general review of the chemical reactivity and characteristics of ozone and its applicability to water and wastewater treatment may be found in Evans' work.[22]

The modern-day interest in the disinfection capabilities of bromine began with the investigations of Wood and Illing[29] and Beckwith and Moser[30] during the 1930's. During the same decade bromine was suggested as an agent for disinfecting water supplies as well as swimming pools.[31] Johnson, et al.[32] concluded that bromine had several advantages over chlorine in the disinfection of water, including the ability to kill both viruses and spores.

Kamlet[33] advocated bromine chloride as a water and wastewater disinfectant on the basis of its greater disinfection effectiveness and economy when compared with either bromine or chlorine. More current estimates of the economic advantages of wastewater disinfection with bromine chloride over disinfection with either ozone or chlorination-dechlorination-reaeration were presented by Wilson.[34] In addition to the potential economic advantages of bromine chloride for wastewater disinfection, Mills[35,36] concluded that, compared to chlorination, chlorobromination produces a better kill of viruses and bacteria and a reduced residual toxicity.

OBJECTIVES OF PROJECT

This project was designed to investigate the problems mentioned above, i.e., the undesirable toxicity problem sometimes associated with the chlorination of wastewater, the bactericidal efficacy of alternative wastewater disinfection processes in parallel on identical wastewater streams, as well as any undesirable toxic effects associated with those alternative processes.

Two contrasting study sites were included in this project, one being the Grandville, Michigan, Wastewater Treatment Plant, the other being the Wyoming, Michigan, Wastewater Treatment Plant. The Grandville plant is an activated sludge facility that treats wastewater derived almost totally from domestic sources. The Wyoming plant has both activated sludge and trickling filter treatment facilities, either of which might treat an influent composed of 35-45 percent industrial wastes. The design of this project called for the study of wastewater treated by the trickling filter process at the Wyoming plant.

This report deals only with the Grandville portion of the project described above. Utilizing a primarily domestic source wastewater receiving secondary treatment by the activated sludge process, the specific objectives of this portion of the project were to determine the disinfection efficiency of chlorine (with and without sulfur dioxide dechlorination), ozone, and bromine chloride in parallel on identical wastewater streams, and any residual toxicity associated with these processes.

THE GRANDVILLE STUDY SITE

The Grandville Wastewater Treatment Plant, which receives primarily domestic wastewater, is an activated sludge plant with chemical removal of phosphates. The plant has a capacity of 12,000 cu m/d (3.2 mgd) and an average flow of 9800 cu m/d (2.6 mgd). The effluent is chlorinated with a manually controlled feed system adjusted with the aid of a continuous residual chlorine analyzer and recorder.

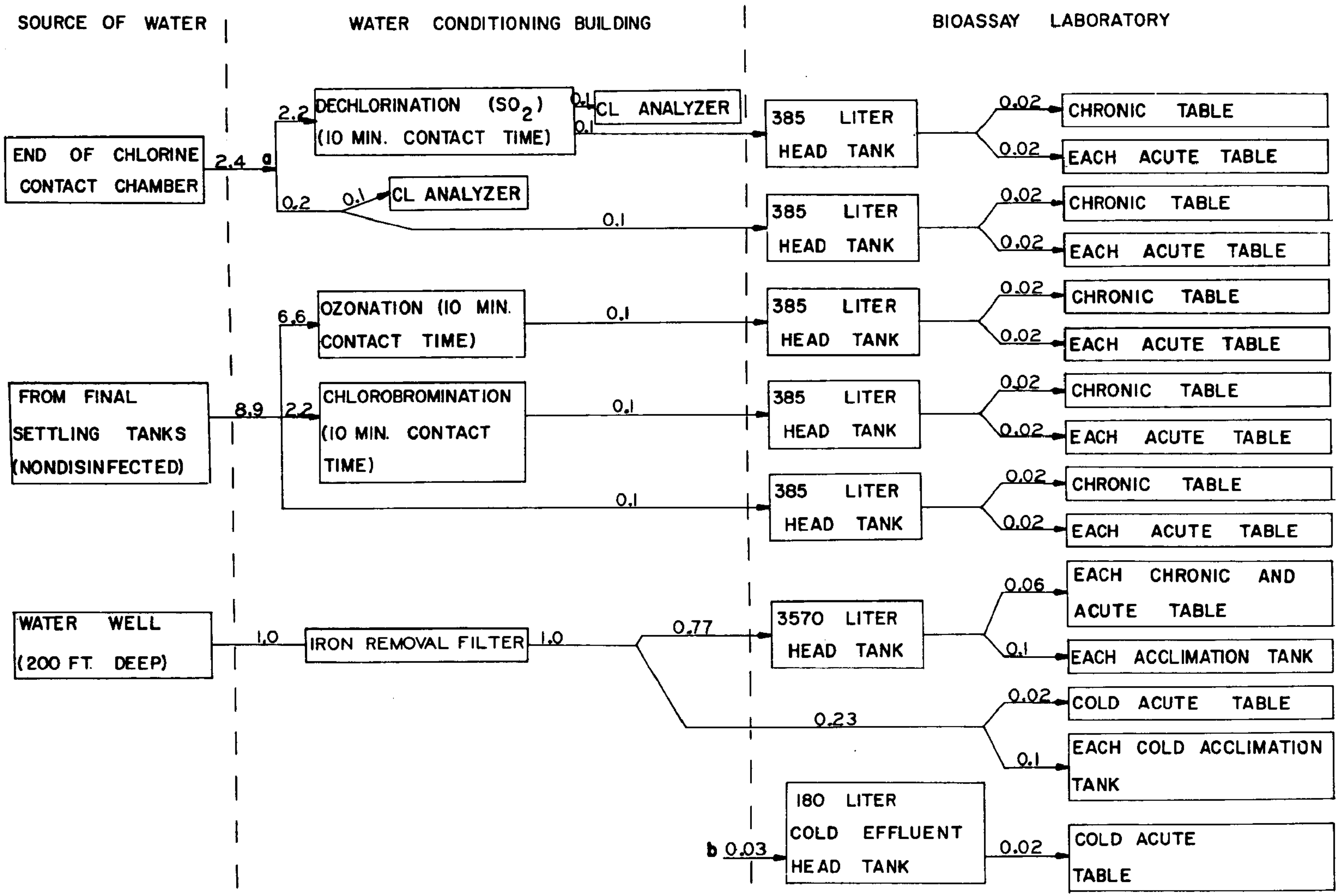
A laboratory building constructed at the plant provided space for the effluent treatment and toxicity studies. Approximately 2.40 liters/sec (37 gpm) of chlorinated final effluent, which normally had a chlorine residual of 1.0 to 2.0 mg/1, was pumped from the chlorine contact chamber of the main plant to the effluent treatment area of the laboratory (Figure 1). Part of this chlorinated stream was pumped directly to the toxicity testing area, while part was dechlorinated with sulfur dioxide and then pumped to the toxicity testing area.

Nondisinfected effluent was pumped from the final settling tanks of the main plant to the effluent treatment area where it was split into three streams, one leading directly to the toxicity testing area, one flowing to the ozone contact system and then to the toxicity testing area, and the third flowing to the bromine chloride contacting system and then to the toxicity testing area. Detailed information on the flow rates, dose rates and resulting residuals, and other characteristics of the wastewater may be found in Section III, while bacteriological data are discussed in Section IV.

After they were warmed to test temperature, the various wastewater streams were each piped to a proportional diluter, which also received a supply of warmed dilution water from a well. Undiluted wastewater and dilution water and six concentrations of wastewater were then delivered to aquaria in which test organisms were maintained. Several species of fishes and invertebrates served as subjects for acute toxicity studies, while life-cycle studies were conducted on fathead minnows (*Pimephales promelas*) in each wastewater stream. A detailed account of the toxicity testing may be found in Sections V and VI.

IMPORTANT DATES

Table 1 shows some of the important dates during the Grandville study and will serve as a useful reference while reading and interpreting the other sections of this report.



a. NUMBERS ON LINE INDICATE THE APPROXIMATE FLOW RATE IN LITERS PER SECOND

b. SOURCE OF EFFLUENT IS ONE OF THE EFFLUENT HEAD TANKS IN THE BIOASSAY LABORATORY.

Figure 1. FLOW OF EFFLUENT AND WELL (DILUTION) WATER

Table 1. DATES[a] OF SOME IMPORTANT EVENTS DURING THE GRANDVILLE STUDY

Start (End) of disinfection studies	January 9	(November 27)
Start (End) of life cycle bioassay studies	January 8	(December 5)
Last day of spring flooding (overloading of treatment plant)		March 24
Alum substituted for ferric chloride in the removal of phosphate from the wastewater		June 21
Average chlorine feed lowered from 2.90 mg/l to 2.73 mg/l (Average chlorine residual reduced from 2.0 to 1.5 mg/l)		July 8
Average chlorine feed lowered from 2.73 mg/l to 2.31 mg/l (Average chlorine residual reduced from 1.5 to 1.0 mg/l)		August 12
Sulfur dioxide feed lowered from 7.0 mg/l to 4.0 mg/l (Measured mean residual sulfite reduced from 5.12 mg/l to 2.88 mg/l)		April 1
Measured mean residual BrCl lowered from 3.6 mg/l to 3.0 mg/l		February 22
Measured mean residual BrCl lowered from 3.0 mg/l to 2.5 mg/l		March 28
Measured mean residual BrCl lowered from 2.5 mg/l to 2.0 mg/l		July 8
Start of filtration of wastewater prior to ozonation		September 26

[a]All dates are during the 1974 calendar year

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SECTION II

CONCLUSIONS

1. With the exception of the residual toxicity imparted to the effluent by some of the disinfection processes, no change in wastewater quality that might create an environmental problem under the more common conditions of effluent release to waterways was observed as the result of disinfection with chlorine, bromine chloride, or ozone, or dechlorination with sulfur dioxide.

2. The frequency of disinfection by chlorine, bromine chloride, and ozone was directly related to wastewater quality, as indicated by suspended solids and biochemical oxygen demand.

3. Aftergrowth of microorganisms was considerably more apparent in the dechlorinated effluent stream than in the chlorinated or chlorobrominated stream. Less prominent aftergrowth was also observed in the ozonated effluent stream.

4. The respective fecal and total coliform densities (MF) in the chlorinated, dechlorinated, and chlorobrominated effluents did not differ significantly during the first four treatment intervals (January through September). Coliform densities in chlorobrominated effluent were significantly higher ($p > 0.99$) than those in chlorinated and dechlorinated effluents during treatment intervals five and six (October 1 - November 27).

5. Fecal and total coliform densities (MF) in the ozonated effluent were significantly higher ($p > 0.99$) than those in the other disinfected effluents (during all intervals) when there was no multimedia pressure filtration. But, when preceded by pressure filtration (October 1 - November 19), ozonated effluent displayed coliform densities which were not significantly higher than those in the other disinfected effluents.

6. The fecal coliform standard ($<$ 200/100 ml) was met more than 80 percent of the time during all treatment intervals by the chlorinated and dechlorinated effluents, during all but one interval (November 19 - November 27) by the chlorobrominated effluent, and during only one interval (October 1 - November 19, during pressure filtration) by the ozonated effluent.

7. The total coliform standard ($<$ 1000/100 ml) was met more than 80 percent of the time during the second through fifth intervals (February 22 - November 19) by the chlorinated and dechlorinated effluents, during the second through fourth intervals (February 22 - September 30) by the chlorobrominated effluent, and during no interval by the ozonated effluent.

8. Examination of the disinfection capability of ozone was often limited by an inadequate dosage resulting from design limitations, mechanical failures, and operator inexperience.

9. Since 100 percent nondisinfected effluent was lethal to fathead minnows (*Pimephales promelas*) less than 60 days old, no conclusions could be drawn on the toxicity of the 100 percent disinfected effluents.

10. Fourteen and twenty percent chlorinated effluent concentrations with mean total chlorine residuals of 0.045 mg/l or more caused growth retardation and mortality of continuously exposed fathead minnows less than 60 days old. The maximum mean residual chlorine concentrations which failed to show such effects varied from 0.01 to 0.03 mg/l, depending upon the quality of the effluent.

11. Dechlorination with sulfur dioxide eliminated the lethal and growth inhibiting effects on fathead minnows reared in 14 and 20 percent chlorinated effluent concentrations. No effects were observed on the growth, reproduction, or survival of fathead minnows continuously exposed to dechlorinated effluent concentrations of 50 percent or less.

12. Continuous exposure to chlorobrominated effluent concentrations of 50 percent or less containing mean residual bromine chloride levels of 0.043 mg/l or less had no effect on the growth, reproduction, or survival of fathead minnows.

13. No effects were observed on the survival or reproduction of fathead minnows continuously exposed to ozonated effluent concentrations of 50 percent or less with mean residual ozone levels of 0.005 mg/l or less. Fathead minnows continuously exposed to 100 percent ozonated effluent with a maximum mean ozone residual of 0.016 mg/l exhibited greater mean lengths at 30, 60, and 330 days of age than their counterparts in 100 percent concentrations of nondisinfected, dechlorinated or chlorobrominated effluent.

14. None of the disinfected effluent streams had any effect on the number of eggs produced by those fathead minnows which survived to maturity or on the probability of those eggs hatching.

15. Acute toxicity tests conducted on several species of fishes and the macroinvertebrate *Daphnia magna* indicated that chlorine was the most toxic disinfectant tested.

16. Fathead minnows exposed to gradually increased residual chlorine and bromine chloride concentrations tolerated higher halogen residuals than fish which lacked prior exposure to the halogens.

SECTION III

THE WASTEWATER TREATMENT SYSTEMS AND THE CHARACTERISTICS OF THE WASTEWATER STREAMS

INTRODUCTION

A variety of interactions and effects have been shown to occur when disinfectants are applied to wastewater. For instance, the organic load and the pH of the wastewater often affect the bactericidal activity of a disinfectant. Conversely, the disinfectant might affect wastewater parameters such as dissolved oxygen, pH, or residual toxicity to aquatic organisms.

This section discusses the characteristics of the Grandville wastewater, and outlines the treatment processes at the Grandville Wastewater Treatment Plant. The design of the test treatment systems and their effects on the characteristics of the wastewater are described.

THE GRANDVILLE WASTEWATER TREATMENT PLANT

The wastewater treatment plant at Grandville, Michigan, is a secondary activated sludge system with chemical removal of phosphate. It has a 20,000 population equivalent[1] of biochemical oxygen demand (BOD) and a 14,000 population equivalent of suspended solids. The wastewater is primarily of domestic origin, with only three industrial inputs which contribute an estimated 20 percent of the plant's BOD load.

Expansion of the plant from 6,000 cu m/day (1.6 mgd) to a design capacity of 12,000 cu m/day (3.2 mgd) was completed just prior to the January 2, 1974 start of this project. Operational and equipment problems at the enlarged plant resulted in highly variable effluent quality during the first months of the project. Heavy precipitation and the subsequent infiltration of Grand River flood waters into the plant's influent lines occurred during the first two weeks of each of the first five months of 1974 and caused numerous treatment problems. The infiltration of flood waters was the main cause of an estimated 568,000 cu m (150 mg) excess annual plant flow over the previous year, and the subsequently lower than average effluent quality.

The plant influent flow for 1974 averaged 9,600 cu m/day (2.54 mgd) with daily flows varying from 6,000 to 19,300 cu m/day (1.60-5.09 mgd). The influent pH ranged from 6.3 to 8.4. Figure 2 shows the average flows for 1974. The high flows occurred in the first five months. The poor removal of solids, BOD, and phosphates in the first months of 1974 were largely the result of the high daily plant flows and the numerous operational problems experienced during this time.

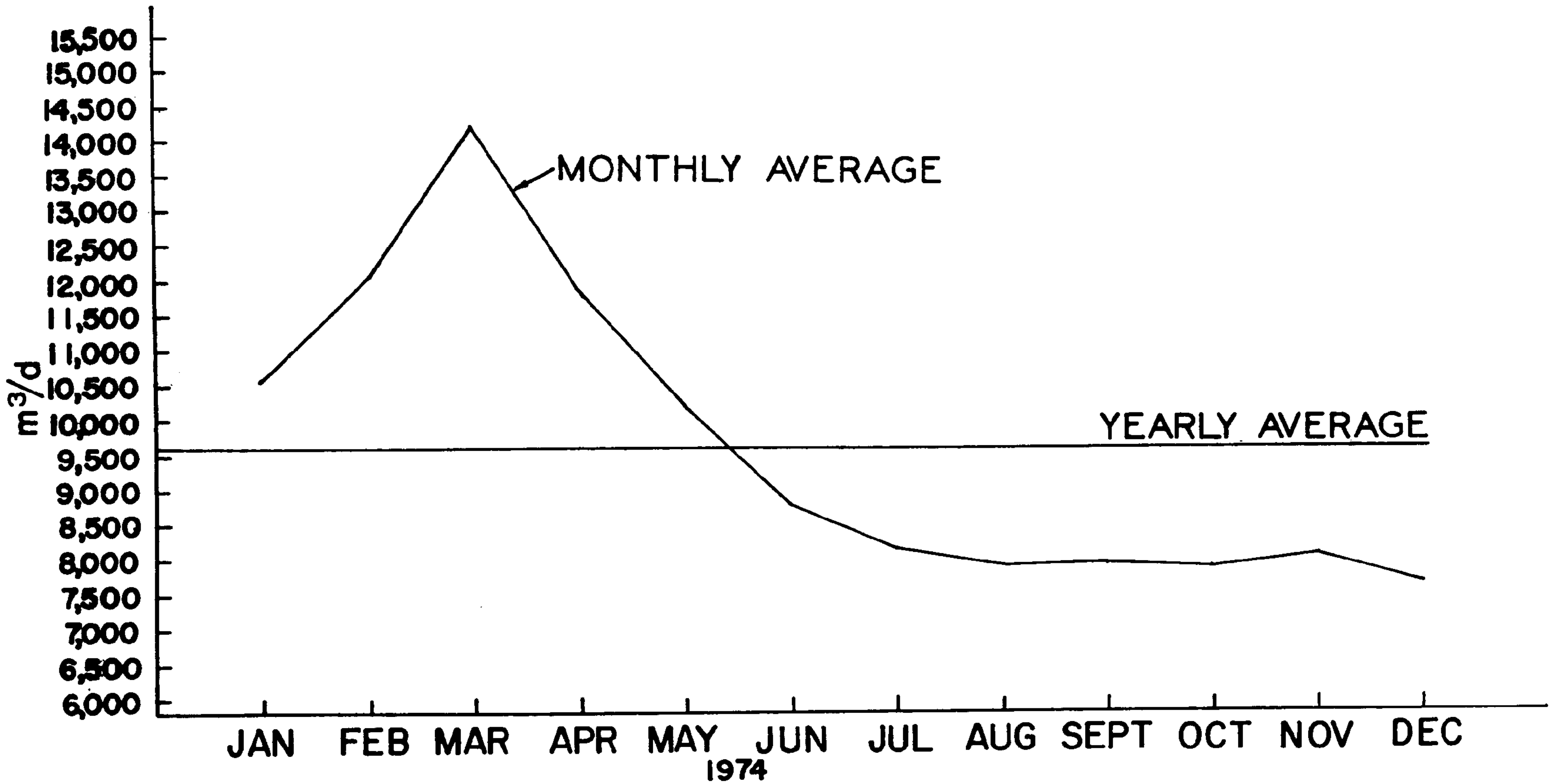

Figure 2. MONTHLY AND YEARLY AVERAGE PLANT FLOWS AT THE GRANDVILLE WASTEWATER TREATMENT PLANT

Sludge removal was also a problem during the first part of the project. This problem was solved by wasting activated sludge to an aerobic digester and primary sludge to an anaerobic digester. These sludges were not mixed together and this type of sludge removal resulted in better effluent quality and fewer problems with bulking.

Figure 3 shows the monthly average total suspended solids content in the influent and final effluent for 1974. The average total suspended solids for the year 1974 was 146 mg/l for the influent and 17 mg/l for the effluent. The daily range of total suspended solids in the influent was 25 to 361 mg/l, while the effluent range was 1 to 179 mg/l. The average removal of total suspended solids was 88.0 percent.

The monthly average BOD concentrations for the influent and effluent streams (as determined by the Grandville Wastewater Treatment Plant laboratory) are given in Figure 4. The daily influent BOD ranged from 23 to 760 mg/l with an average of 158 mg/l, while the daily effluent BOD ranged from 4 to 100 mg/l with an average of 21 mg/l. The average BOD removal over the year was 86.8 percent.

The use of ferric chloride and polymer to remove phosphate from the plant's effluent was begun shortly before this project was started (December, 1973). After extensive experimentation with application sites and feed rates of polymer and ferric chloride, and many problems with the ferric chloride feed system, alum was substituted for ferric chloride on June 21, 1974. In June, alum and polymer were being split-fed 80 percent to the primary and 20 percent to the final settling basins. Feed rates were adjusted to obtain alum concentrations of 150 mg/l and polymer concentrations of 0.5 mg/l. Beginning in October, both alum and polymer were fed at the end of the aeration tanks, giving a retention time of 5 to 7 minutes before entering the final settling tanks. Starting in October, the application of alum and polymer was gradually reduced to achieve concentrations of 80 mg/l and 0.4 mg/l, respectively.

The problems initially encountered with the phosphorus removal system are clearly seen in Figure 5, which illustrates the monthly average total phosphorus in the influent and effluent streams. It was not until July that the plant achieved a monthly average phosphorus removal greater than 75 percent. The influent stream averaged 8.1 mg/l phosphorus with the extremes being 3.6 and 16.8 mg/l. The effluent averaged 3.4 mg/l with a range of 0.6 to 11.9 mg/l. The average total phosphorus removal for the entire year was 57.6 percent.

DESIGN OF TEST WASTEWATER TREATMENT SYSTEMS

Because of the need for chlorine-free makeup water for the dilutions required in the bioassay laboratory, a water well was drilled at the project location. Water from the well was passed through an iron removal filter prior to its delivery to the bioassay laboratory (Figure 1).

After final settling, nondisinfected effluent was pumped to a treatment

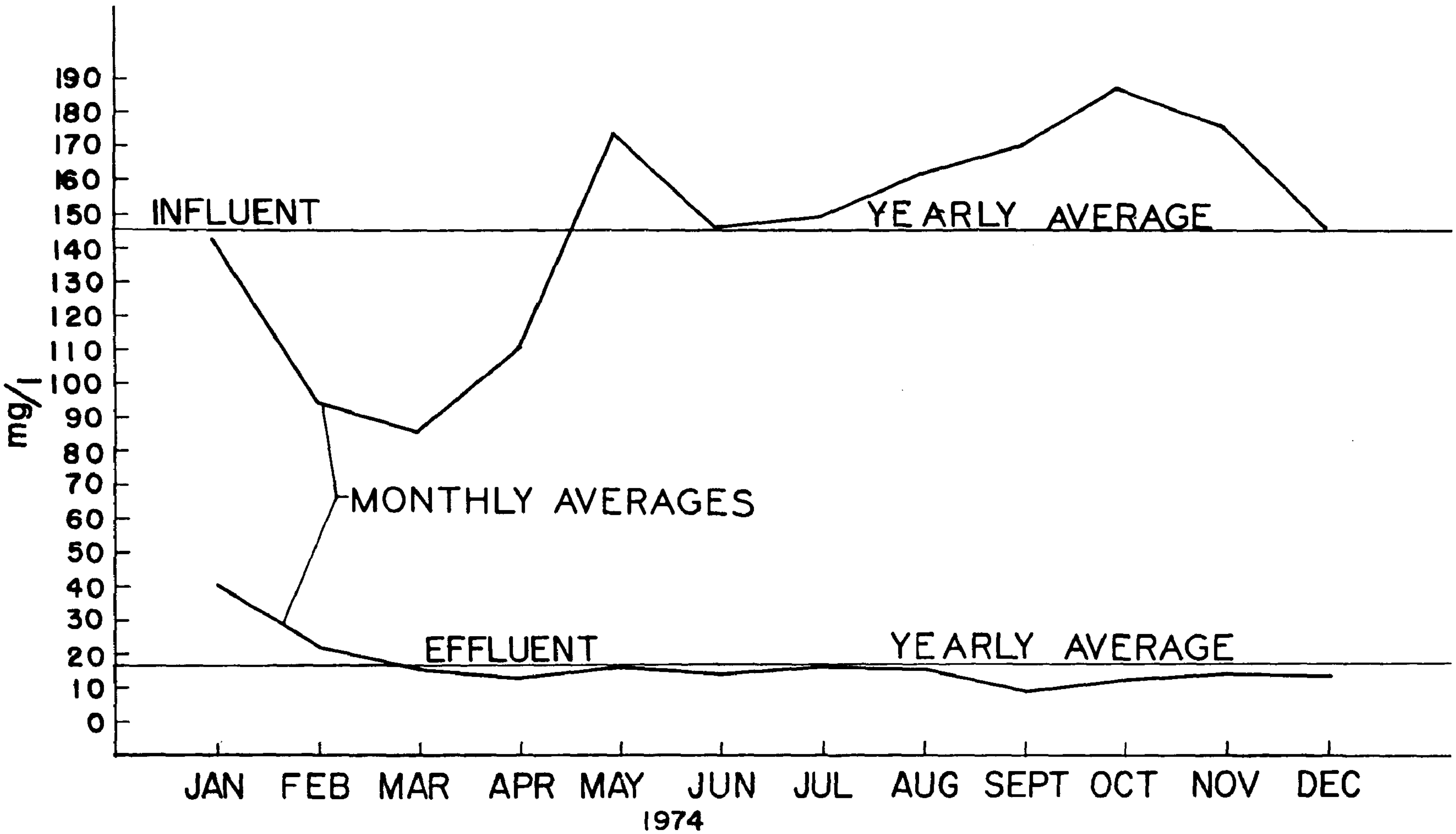

Figure 3. MONTHLY AND YEARLY AVERAGE SUSPENDED SOLIDS AT THE GRANDVILLE WASTEWATER TREATMENT PLANT

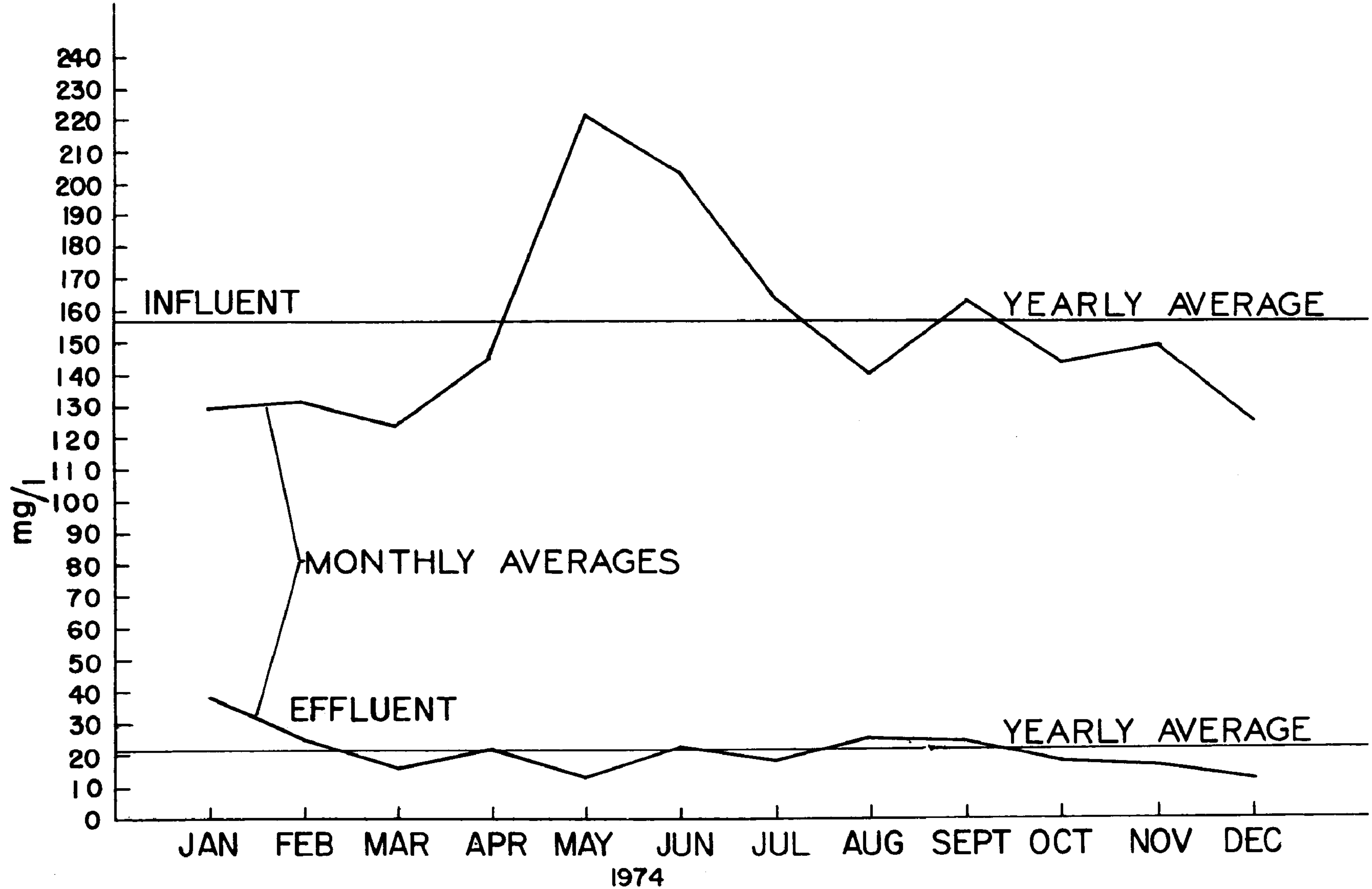

Figure 4. MONTHLY AND YEARLY AVERAGE BIOCHEMICAL OXYGEN DEMAND AT THE GRANDVILLE TREATMENT PLANT

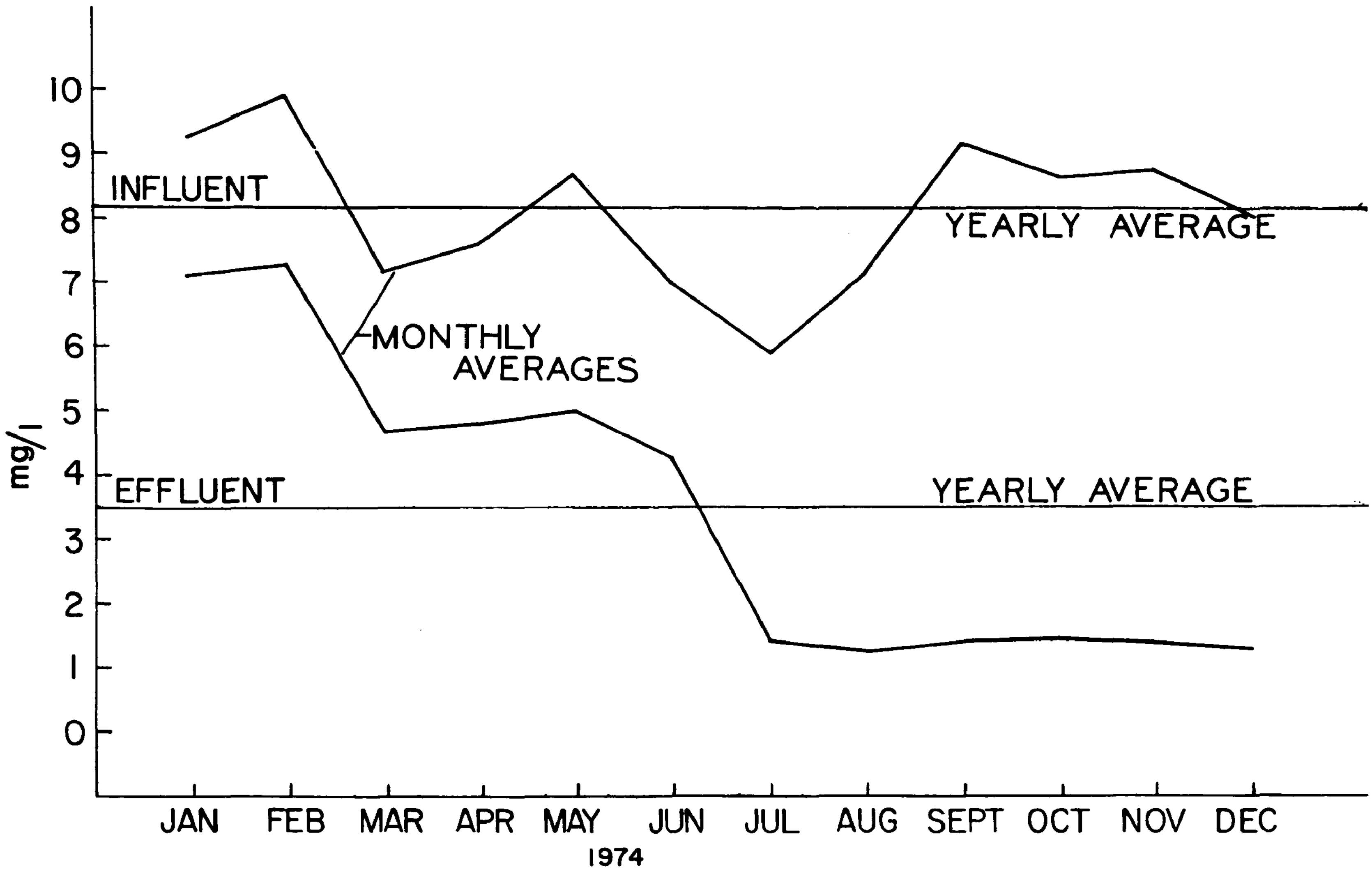

Figure 5. MONTHLY AND YEARLY AVERAGE PHOSPHORUS CONCENTRATIONS AT THE GRANDVILLE TREATMENT PLANT

building and divided into three streams; one was treated with bromine chloride, one with ozone, and one was passed directly to the bioassay laboratory (Figure 1). Chlorinated effluent was pumped from the end of the chlorine contact chamber of the main plant to the treatment building, where a portion of this stream was dechlorinated with sulfur dioxide and the remaining portion was carried directly into the bioassay laboratory.

The plant chlorinating system consisted of two Fisher-Porter chlorinators. A Fisher-Porter Anachlor continuous titrator was installed in the line between the end of the chlorine contact chamber of the main plant and the bioassay laboratory. This unit measured the total chlorine residual and provided information for the manual adjustment of the chlorine feed rate in order to hold a constant chlorine residual. (Normally, adjustments of the feed rate were made at intervals of 1-2 hours and the total chlorine residual was maintained within ±0.3 mg/l.) The main problem with controlling the chlorine residual was that the time between the application of chlorine and the measurement of the residual varied between 20 and 60 minutes, depending upon the plant flow.

The 30 minute residual chlorine concentration was maintained at 2.0 mg/l from the beginning of the study until July 8th. During this period, the chlorine feed averaged 2.90 mg/l (24 lb/mil gal). In order to achieve minimum residual chlorine levels and still obtain adequate disinfection (less than 1000 total coliforms per 100 ml and less than 200 fecal coliforms per 100 ml), the residual concentration was lowered to 1.5 mg/l and was held there until August 12th. The chlorine feed during this period averaged 2.73 mg/l (23 lb/mil gal). Since the 1.5 mg/l residual chlorine concentration appeared to be more than adequate for disinfection, the residual was lowered to 1.0 mg/l on August 12th and was held there throughout the remainder of the project. The feed rate of chlorine while the residual was being held at 1.0 mg/l averaged 2.31 mg/l (19 lb/mil gal).

A portion of the chlorinated stream (2.2 l/sec (35 gpm)) was treated with sulfur dioxide (SO_2). The SO_2 was fed into the chlorinated stream by an aspirator and regulated by a Wallace and Tiernan Model 20-055 chlorinator. The only problem associated with this system was the occasional interruption in liquid flow through the aspirator as a result of high solids levels in the wastewater. The dechlorinated stream flowed into a contact tank having a 30-minute residence time at a flow rate of 2.2 l/sec (35 gpm). The dimensions of the steel contact tank measured 3.66 m long by 1.22 m wide by 0.91 m deep. Steel baffles were welded to the bottom at intervals of 1.22 and 2.44 m from the end, and wooden baffles were inserted from the top at 0.61, 1.83, and 3.0 m from the end. This arrangement of baffles provided an under-over-under flow configuration. The contact tank was constructed with three outlets so that effluent could be pumped to the bioassay laboratory after 10-, 20-, or 30-minute contact times.

Although SO_2 reacts with chlorine in a 1:1 ratio, the initial feed rate of SO_2 was set at 7 mg/l (58 lb/mil gal) to protect the subjects of the bioassay tests from accidental exposure to residual chlorine. This application rate was found to be higher than necessary, and was reduced on April 1, 1974 to 4 mg/l (33 lb/mil gal).

The mean sulfite residual after 30 minutes of contact was 5.12 mg/l when the feed rate was 7.0 mg/l, and 2.88 mg/l when the feed rate was 4.0 mg/l. For the entire project the mean sulfite residual was 3.0 mg/l (105 total analyses).

To insure that no chlorine residual carried over to the bioassay laboratory, another Fisher-Porter anachlor unit continuously monitored the dechlorinated stream. This unit was designed so that the presence of any residual chlorine tripped a switch which caused a signal to be sent to the control panel of the bioassay laboratory. Upon receipt of this signal, the control panel stopped the flow of treated effluent to the fish tanks and simultaneously triggered a bell and light alarm system, thus insuring that fish in the dechlorinated stream were not exposed to residual chlorine.

The components of the bromine chloride (BrCl) dosing system were similar to the dechlorinated system except that the BrCl was vaporized and then injected into the effluent stream. This was accomplished by use of a dip pipe whereby the liquid was removed under its own pressure (2038 newtons/m^2). The liquid BrCl was then vaporized by heat and metered by a Wallace and Tiernan Model 20-055 chlorinator. It was necessary to heat the feeder and the piping to the aspirator in order to keep the BrCl in a gaseous state.

Unlike the chlorination system which was regulated by residual control, the chlorobromination system was regulated by dosage control. Originally the BrCl feed rate was set at 3.6 mg/l. Because the immediately preceding BrCl feed rates were determined to be greater than required for disinfection, the dosage was lowered to 3.0 mg/l on February 22, to 2.5 mg/l on March 28, and to 2.0 mg/l on July 8, where it remained until the completion of the project.

The problems encountered in keeping the BrCl feeder operating included plugging of feeder and feed lines by the condensation of BrCl and by contaminating materials presumably originating in the BrCl tanks. As with the SO_2 system, the aspirator tended to plug when the wastewater contained high levels of solids.

The ozonating system consisted of an Ingersoll-Rand Model ESV-NL compressor, a Pall-Trinity Model 35 HAl dryer, a W. R. Grace Model LG-16 ozonator, a W. R. Grace contactor, and a contact chamber identical to those used for the SO_2 and BrCl systems. The compressor produced approximately 28.4 l/sec (18 scfm) of air, which was dried to a -50°C dew point, and then passed on to the ozone generator. The ozone-air mixture was introduced along with the wastewater at the top of a 3.66 m column through a positive pressure injector. The gas/liquid mixture flowed cocurrently from the injector down a 10.2 cm diameter central pipe. The mixture reversed direction at the base of the central pipe and flowed upwards through a concentric 30.5 cm diameter circular tank, which was open at the top and allowed the water to fall into a steel contact chamber. Detention time was 40 seconds in the vertical contactor and 10 minutes in the steel contact chamber. Most of the ozone contacting appeared to occur in the vertical contactor. A limited amount of analytical data suggested that the ozone contacting system was very inefficient, and that most of the ozone that was introduced was lost in the off-gasses from the contactor.

Because of the limited success in disinfecting with ozone during the early stages of the project, a Baker Model HRC-330D Hi-Rate filter system was installed to filter the wastewater prior to ozonation. The support media consisted of 64 mm pea gravel and 1.4 mm garnet, and the working media of 0.3 mm garnet and 0.6 mm anthracite. This filter was in operation from September 26 until the completion of the project.

Ozone was applied at rates of 2.5 to 8.5 mg/l, calculated on the basis of the amount of ozone in the gas stream and the wastewater flow to the contactor. The variation in application rates was the result of the problems experienced with the ozone system. Some of the problems were largely due to inexperience with the system, while others were of a mechanical nature such as malfunction of the compressor, ozone generator, and drier system.

In general, the performance of the chlorination and dechlorination systems exceeded that of the ozonation and chlorobromination systems because the former were less complex, simpler to operate, and better understood by the operating personnel.

The average detention times in the various systems prior to sampling for chemical analyses are shown in Figure 6. Point A represents the location of the nondisinfected effluent pumps. Identical samples were taken from each stream for the period January 9 to November 26, 1974, at a frequency of once per day, five days per week, usually between the peak flow hours of 8:00 A.M. and 12:00 noon. Thus, the chemical samples did not represent the same effluent due to the different detention times in the various systems. The detention times given for the chlorinated and dechlorinated streams are the average values over the entire project. The contact time for the chlorinated stream varied from 20 minutes to more than 60 minutes, depending on the plant flow. However, even though the different detention times resulted in the sampling of potentially different test streams, this variable was apparently of little significance to the final results, since the characteristics of each treated stream over the entire study period were similar.

REACTIONS OF DISINFECTANTS

The extensive use of chlorine as a disinfectant has resulted in a thorough understanding of the chemistry of chlorine in water.[2] Elemental chlorine hydrolyzes in water to form hypochlorous acid (equation 1). The hypochlorus acid is a weak acid and it dissociates according to equation 2.

$$Cl_2 + H_2O \rightleftharpoons HOCl + H^+ + Cl^- \qquad (1)$$

$$HOCl \rightleftharpoons H^+ + OCl^- \qquad (2)$$

Thus, free available chlorine is present as hypochlorous acid (HOCl), hypochlorite ion (OCl^-), and elemental chlorine (Cl_2). The relative abundance of these three species is temperature and pH dependent. The equilibrium reaction of equation 1 lies far to the right at neutral pH, so that the predominant species at that pH are HOCl and OCl^-. HOCl is a much more effective disinfectant than OCl^-. The ratio of HOCl and OCl^- in aqueous solution is

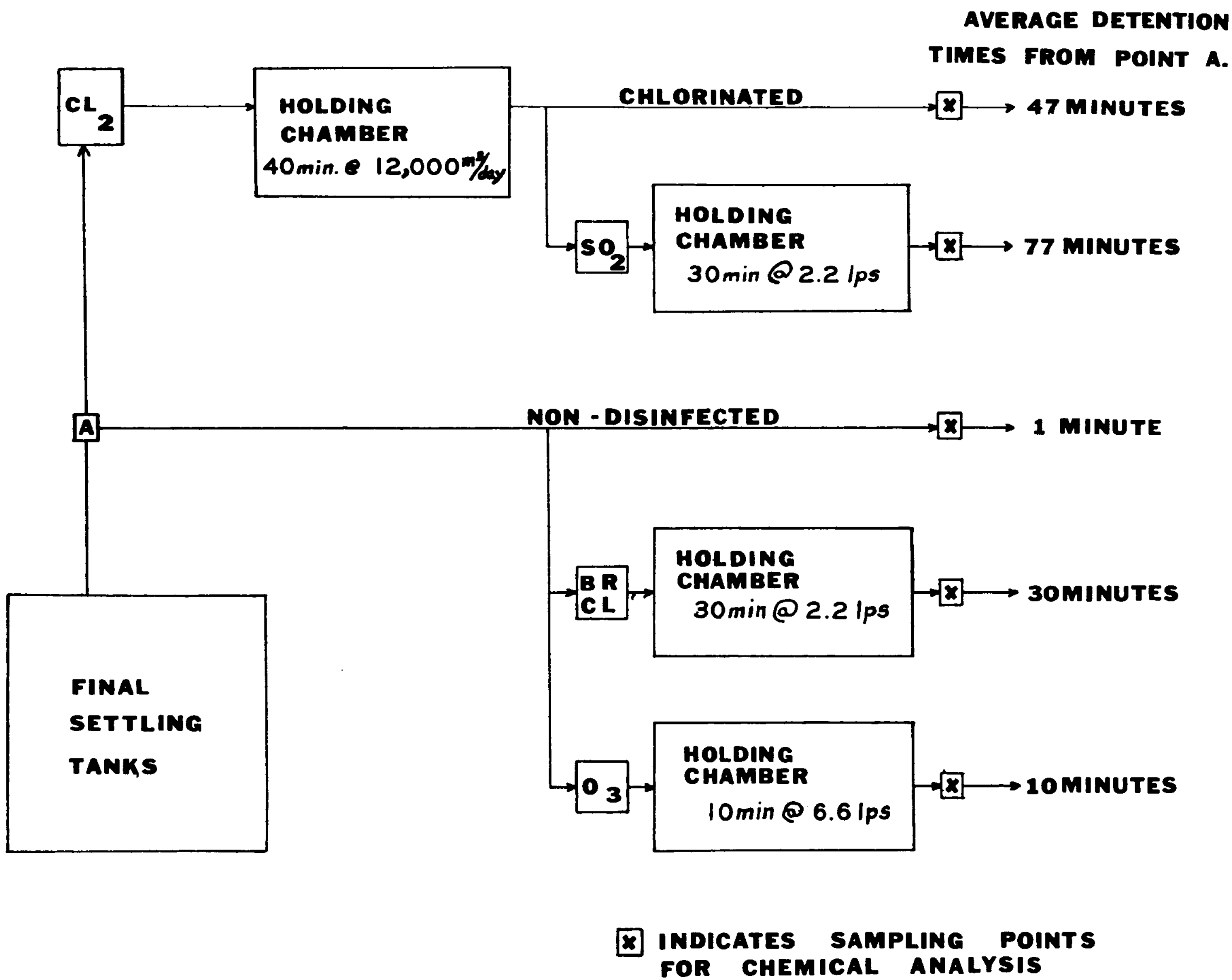

Figure 6. FLOW OF THE VARIOUS WASTEWATER STREAMS IN THE GRANDVILLE DISINFECTION STUDY

inversely proportional to pH. Thus, at 20°C the ratio of HOCl to OCl^- at pH 6, 7, 8, and 9 are approximately 32, 4, 0.39, and 0.04, respectively.[3]

Ammonia is present to a significant degree in most wastewater and is of prime importance in wastewater treatment plants using halogenation for disinfection. The addition of ammonia to water results in the formation of ammonium and hydroxide ions (equation 3).

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^- \quad (3)$$

At pH 4.5-8.5 and 20°C, chlorine reacts with ammonia in wastewater to produce monochloramine (NH_2Cl) and dichloramine ($NHCl_2$) as in equations 4 and 5.

$$HOCl + NH_3 \longrightarrow H_2O + NH_2Cl \quad (4)$$

$$HOCl + NH_2Cl \longrightarrow H_2O + NHCl_2 \quad (5)$$

The ratio of monochloramine to dichloramine increases directly with pH. Only dichloramine exists at pH 4.5, while only monochloramine exists above pH 8.5. When the pH is less than 4.4, trichloramine (nitrogen trichloride or NCl_3) predominates (equation 6).

$$HOCl + NHCl_2 \longrightarrow H_2O + NCl_3 \quad (6)$$

Complex organic chloramines may also be formed upon chlorination of wastewater containing reactive organic amines.

The chloramines are considerably less microbiocidal than free chlorine. Although discussion continues on the relative residual toxicity of "free" and "combined" chlorine to aquatic organisms, the residual toxicity of both forms has been conclusively demonstrated.[4]

Bromine chloride (BrCl) exists in equilibrium with bromine and chlorine in both the gas and liquid phase (equation 7).

$$2BrCl \rightleftharpoons Br_2 + Cl_2 \quad (7)$$

In the vapor phase, BrCl is about 40 percent dissociated over a wide range of temperature.[5] The addition of BrCl vapor to water results in equilibrium solutions represented by the following equations:

$$BrCl + H_2O \rightleftharpoons HOBr + HCl \quad (8)$$

$$Br_2 + H_2O \rightleftharpoons HOBr + HBr \quad (9)$$

$$Cl_2 + H_2O \rightleftharpoons HOCl + HCl \quad (10)$$

BrCl hydrolizes exclusively to hypobromous acid (equation 8). Any HBr formed by dissociation of elemental bromine would be quickly oxidized by HOCl to HOBr.[5]

$$HBr + HOCl \longrightarrow HOBr + HCl \quad (11)$$

Hypobromous acid also dissociates to give hydrogen and hypobromite ions (equation 12).

$$HOBr \rightleftharpoons H^+ + OBr^- \quad (12)$$

Because the hypohalous acids are more active disinfectants than the hypohalite ions, it is of interest to compare the equilibria of chlorine and bromine chloride in wastewater. At pH 8.0, only 19 percent of the chlorine exists as hypochlorous acid, while 90 percent of the dissociated bromine chloride is hypobromous acid.[6] Bromine chloride combines with the ammonia in wastewater to form bromamines (equations 13-15). At the usual pH range of typical wastewater effluent, mono- and di-bromamines are predominant.

$$NH_3 + HOBr \longrightarrow NH_2Br + H_2O \quad (13)$$

$$NH_2Br + HOBr \longrightarrow NHBr_2 + H_2O \quad (14)$$

$$NHBr_2 + HOBr \longrightarrow NBr_3 + H_2O \quad (15)$$

Mills[7] reported that bromamines are unstable in wastewater and exhibit a half life of less than 10 minutes in secondary wastewater effluent. Furthermore, both the bactericidal and virucidal activity[6] of bromamines have been reported to be superior to those of chloramines in situations where the halogen demand is low and the pH is high. Mills[6] also reported that wastewater disinfected with bromine chloride exhibited a lower residual toxicity to aquatic organisms than wastewater disinfected with chlorine.

Sulfur dioxide (SO_2) dissociates in aqueous solution in the following manner:

$$SO_2 + H_2O \rightleftharpoons H_2SO_3 \quad (16)$$

$$H_2SO_3 \rightleftharpoons H^+ + HSO_3^- \quad (17)$$

$$HSO_3^- \rightleftharpoons H^+ + SO_3^{--} \quad (18)$$

Equilibrium of the above reactions favors formation of sulfite (equation 18) at pH $>$ 5, whereas at pH $<$ 5, bisulfite predominates (equation 17).

SO_2 reacts with hypochlorous acid or chloramines as follows:

$$SO_2 + HOCl + H_2O \longrightarrow H_2SO_4 + HCl \quad (19)$$

$$SO_2 + NH_2Cl + 2H_2O \longrightarrow NH_4Cl + H_2SO_4 \quad (20)$$

$$2SO_2 + NHCl_2 + 4H_2O \longrightarrow NH_4Cl + 2H_2SO_4 + HCl \quad (21)$$

$$3SO_2 + NCl_3 + 6H_2O \longrightarrow NH_4Cl + 3H_2SO_4 + 2HCl \quad (22)$$

If the concentration of SO_2 exceeds the stoichiometric amount described in equations 19 to 22, the resulting excess sulfite (equation 18) will react with dissolved oxygen (DO) to form sulfate ion, thereby lowering the DO content of the effluent by the amount of the excess SO_2.

Ozone is one of the most powerful oxidizing agents known. Its high oxidadion-reduction potential is thought to be due to the formation of the highly reactive free radical $O^{\cdot}$ (equation 23).[8]

$$O_3 \longrightarrow O_2 + O^{\cdot} \quad (23)$$

Recent work indicates that reactions of ozone are more dependent on the concentration of decomposition products than on the ozone concentration.[9,10] Hewes and Davison[10] have proposed that the free radicals and ions formed by ozone decomposition are the chief reacting species. Their proposed mechanism for aqueous decomposition of ozone is as follows:

$$O_3 + H_2O \longrightarrow HO_3^+ + OH^- \quad (24)$$

$$HO_3 + OH^- \longrightarrow 2HO_2 \quad (25)$$

$$O_3 + HO_2 \longrightarrow HO + 2O_2 \quad (26)$$

Venosa[11] reports that the same free radicals are produced by irradiation of the water, and that HO and HO_2 radicals contribute significantly to the killing of bacteria by irradiation.

The reaction of ozone with ammonia is first-order with respect to ammonia concentration, and the rate increases with increasing pH over the range 7-9, and with increasing ozone partial pressure. In wastewater, the reaction is only effective if the pH of the wastewater is maintained alkaline.[12] Ozone is a powerful oxidizing agent, and thus its rapid reactions with most compounds in wastewater have limited investigations in this field.

There are many problems involved in identifying the composition of wastewater and the products formed by chemical disinfectants. However, concern about the environmental effects of reaction by-products formed by chemical disinfection is increasing. Studies on the chemical interactions of disinfectants with organic compounds and wastewater components have been carried out by Sawyer and McCarty,[2] Mills,[6] Hewes and Davison,[10] Bailey,[13] and others.

MATERIALS AND METHODS

Chemical tests performed during the project were those routinely used to characterize wastewater. The analyses performed were: total and volatile suspended solids, turbidity, color (apparent and true), pH, ammonia-nitrogen, total phosphorus, dissolved oxygen, and chemical oxygen demand (COD). Biochemical oxygen demand (BOD) was not run due to the uncertain nature of this analysis on disinfected effluents even when neutralizing agents are used, and the error introduced by the seed correction.

Suspended solids were extracted by passing water samples through Gooch crucibles with glass fiber filters.[1] Color, determined by the platinum cobalt method, and turbidity were measured on a Hach-DR colorimeter. Phosphate was measured using the persulfate digestion-stannous chloride method.[1] Ammonia nitrogen was determined by direct Nesslerization after clarification with zinc sulfate and alkali.[1] The colors developed in the phosphate and ammonia tests were read on a Model 300 Turner spectrophotometer. Because of the rather high quality of the activated sludge effluent, the COD test was modified by using 0.0625N dichromate solution instead of the 0.25N solution prescribed in Standard Methods.[1] Dissolved oxygen was measured by use of a membrane probe (Yellow Springs Instrument).

The residual ozone in the ozonated effluent was measured by the iodometric method.[1] The residual chlorine and the residual bromine chloride in the respective chlorinated and chlorobrominated effluent streams were determined with a spectrophotoiodometric method of analysis which has been shown to measure residual chlorine with approximately the same accuracy as the amperometric titration method.[14] Residual sulfite in the dechlorinated effluent stream was measured by an amperometric titration method in which sensitivity was increased through the use of a polarograph and a strip chart recorder for end point determination.[15]

Statistical differences among respective mean test results in the various wastewater streams were determined by subjecting the data to a two-tailed t-test ($P < 0.05$).

RESULTS AND DISCUSSION

Table 2 summarizes the physical-chemical characteristics of the test streams during the test period. The unfiltered values represent samples taken from January through September and two weeks in November. The filtered values represent samples taken after filtration and before ozonation.

In all of the treated streams, the mean suspended solids levels were significantly ($P < 0.05$) lower than the levels in the nondisinfected stream. Sedimentation in the contact chambers accounted for a large part of the observed decrease. Because the systems did not all have the same size contact chambers and flow rates, the settling time for the different effluent streams varied, thus making it difficult to determine if any system removed solids better than another. Suspended solids reduction by an ozone-induced flotation process has been reported by Nebel et al.,[16] and Greening.[17] However, Snider and Porter[9] reported no significant decrease in total or volatile solids when the flotation process was not used, as was the case in this project.

All of the treated wastewater streams exhibited significantly lower turbidity than was observed in the nondisinfected stream. At least part of this reduced turbidity was attributed to the detention times in the respective contact chambers of the treated streams.

No significant difference in turbidity was observed among the four treated test streams. This was of interest, since the settling times resulting from the special treatment systems ranged from 30 minutes in the dechlorinated and chlorobrominated systems to 10 minutes in the ozonated system. No data were collected to determine if the reduced turbidity of the ozonated stream resulted from the additional 10 minutes of settling time or if the ozone per se acted to reduce turbidity.

All but the chlorinated stream showed significantly lower mean apparent and

Table 2. PHYSICAL-CHEMICAL CHARACTERISTICS OF THE TEST STREAMS DURING THE TEST PERIOD - JANUARY 2, 1974 TO NOVEMBER 30, 1974[a]

Parameter	Nondis-infected[b]	Chlor-inated	Dechlor-inated	Chloro-brominated	Unfiltered Effluent		Filtered Effluent	
					Nondis-infected[b]	Ozonated	Nondis-infected[b]	Ozonated
Total Suspended Solids (mg/1)	19.9 (31.0)	13.0 (12.1)	11.0 (7.2)	11.8 (7.1)	20.6 (33.5)	12.2 (8.2)	16.1 (7.9)	5.6 (4.3)
Volatile Suspended Solids (mg/1)	14.2 (19.6)	9.6 (8.8)	8.0 (5.0)	8.8 (5.1)	14.7 (21.1)	8.9 (5.6)	11.1 (6.6)	4.3 (3.2)
Turbidity (J.T.U.)	23.4 (43.3)	15.2 (10.5)	12.6 (7.4)	12.8 (7.9)	25.6 (46.7)	12.3 (8.0)	11.2 (5.5)	4.8 (3.2)
Apparent Color (Platinum Cobalt units)	56.0 (83.6)	37.5 (30.8)	28.9 (23.0)	29.6 (23.1)	58.3 (90.8)	24.8 (22.7)	44.1 (14.4)	17.4 (13.4)
True Color (Platinum Cobalt units)	11.9 (9.5)	10.4 (10.9)	8.1 (8.2)	7.6 (8.4)	11.3 (9.8)	2.5 (4.8)	15.3 (7.1)	6.4 (3.6)
Chemical Oxygen Demand (mg/1)	38.3 (18.7)	28.7 (14.6)	34.1 (17.2)	33.2 (15.5)	38.5 (19.6)	33.4 (19.4)	36.9 (13.4)	17.4 (6.8)
Ammonia Nitrogen (mg/1)	7.58 (2.66)	7.81 (2.60)	8.10 (2.27)	7.74 (2.66)	6.86 (2.74)	6.76 (2.80)	9.36 (1.34)	9.40 (1.38)
Dissolved Oxygen (mg/1)	2.70 (0.99)	5.60 (0.86)	4.88 (0.88)	2.82 (1.01)	2.76 (1.03)	10.16 (0.86)	2.34 (0.62)	9.68 (0.87)
Total Phosphate (mg/1)[c]	0.63 (0.30)	0.64 (0.35)	0.63 (0.38)	0.57 (0.34)	0.56 (0.34)	0.51 (0.42)	0.72 (0.21)	0.33 (0.22)

[a]Values depicted are means, while numbers in parentheses are standard deviations.

[b]The mean values in the "Nondisinfected" columns vary because they represent different time periods. The "Nondisinfected" column on the left was derived from data collected during the entire study period. The second "Nondisinfected" column was derived from samples collected between January 2 and September 26 when no filtration was carried out. The third "Nondisinfected" column was derived from the data gathered between September 26 and November 30 on the test stream before it was passed through pressure filters prior to ozonation.

[c]Measured only from August through November.

true color levels than the nondisinfected stream, and the mean apparent and true color levels were significantly lower in the ozonated stream than in each of the other treated streams. The decolorizing properties of ozone have been widely reported.[9,12,16,17] On several occasions, usually when effluent was of exceptionally good quality or when the filters were in operation, the ozonated effluents exhibited a dilute permanganate color. This was reflected in a higher true color when filtering than when not filtering and was due to some unidentified compound in the effluent.

At the dosages and detention times in this project, chlorination significantly reduced the chemical oxygen demand (COD) of the effluent, while ozonation only reduced the COD of the filtered effluent. Dechlorination and chlorobromination did not result in significantly different COD levels. Several investigators [16-19] have reported COD reductions with ozone, but such a reduction was not demonstrated in this project. The reduction of COD in the filtered and ozonated stream appeared to be due to filtration. The reason that only chlorination showed a significant reduction in COD is presumably due to the longer contact time than the other treatments.

None of the treatments significantly affected the ammonia nitrogen levels. Total phosphorus concentrations were reduced only in the filtered ozonated stream, presumably because of the physical removal of phosphates bound to suspended solids.

The mean DO concentration was significantly greater in the chlorinated, dechlorinated, and ozonated streams than in the nondisinfected stream. The ozonated stream, as expected, exhibited the highest level of dissolved oxygen, because oxygen is the major decomposition product of ozonation. The mean DO concentration in the dechlorinated stream was significantly higher than that in the nondisinfected stream, but lower than that in the chlorinated stream. The reason for the latter observation is that the excess sulfite is oxidized by oxygen to sulfate.

The pH values of the five wastewater streams are summarized in Table 3. Chlorination, dechlorination, and chlorobromination did not significantly affect the pH of the effluent. However, ozonation caused an increase in the recorded pH values. Nebel, et al.[16] and Greening[17] have reported similar increases in pH as a result of ozonation, and have attributed them to the removal of carbon dioxide from the water.

CONCLUSIONS

Since the study of water quality improvement was not a major objective in this project, the experimental design was not optimized for the comparison of the physical and chemical changes of the various wastewater streams induced by disinfection processes. Factors such as flow rates, contact times, contactor designs, and methods of controlling the feed of the disinfectants were not uniform in the various test streams. Nevertheless, some conclusions pertaining to water quality may be drawn from the observations made in this project.

Table 3. A SUMMARY OF THE pH VALUES MEASURED IN THE VARIOUS WASTEWATER STREAMS

	Stream				
	Nondisinfected	Chlorinated	Dechlorinated	Chlorobrominated	Ozonated
No. of samples	212	209	204	202	200
Range in pH	6.7-7.8	6.8-7.8	6.5-7.7	6.8-8.0	6.9-8.1
% of samples:					
pH 7.0	12.2	5.8	7.4	4.0	0.5
pH 7.0-7.4	73.1	80.5	88.7	87.0	53.0
pH 7.5-7.9	14.6	13.9	4.0	8.5	46.0
pH interval with highest percentage of samples	7.1	7.1	7.1	7.1	7.4
Central range of pH in which approx. 90% of samples fell	6.9-7.5 (90.5%)	7.0-7.5 (90.1%)	7.0-7.5 (91.2%)	7.0-7.5 (92.0%)	7.1-7.7 (91.5%)

The application of chlorine, bromine chloride, and ozone for disinfection and sulfur dioxide for dechlorination did not cause any adverse changes in the physical and chemical characteristics of the Grandville effluent. The dechlorination process did significantly lower the mean dissolved oxygen level observed in the chlorinated stream. However, the magnitude of the observed decrease in DO suggests that reaeration may not be necessary if the dechlorination process is adequately controlled. Ozonation caused the pH to rise in the treated effluent, but not enough to be detrimental.

The improvements in physical and chemical quality of the water that were demonstrated in this project were a reduction in COD as the result of chlorination, a reduction in apparent and true color as the result of ozonation, and an increase in DO as the result of both chlorination and ozonation.

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SECTION IV

DISINFECTION STUDIES

INTRODUCTION

The role of disinfection in wastewater treatment is to destroy pathogenic microorganisms in the wastewater and thereby provide a reasonable margin of safety in controlling the spread of disease in natural waters. Total and fecal coliform concentrations have been widely used as fecal pollution indicators, and maximum allowable concentrations of these bacteria have been assigned as public health standards.[1]

The objective of this part of the project was to evaluate the efficiency of wastewater disinfection by chlorination (with and without dechlorination), chlorobromination, and ozonation on parallel wastewater effluent streams. The experimental site was the Grandville, Michigan activated sludge wastewater treatment plant. This plant received primarily domestic wastewater as raw influent and produced a good quality secondary effluent. For a description of the Grandville study site and a detailed discussion of the effectiveness and chemical reactivity of the disinfection processes, refer to Section III.

MATERIALS AND METHODS

The standard membrane filtration (MF) technique[1] was used to enumerate coliform bacteria in the five effluent streams. For isolating total coliforms, samples were filtered through membrane filters (Gelman) having an average pore size of 0.45 micrometers (μm). The filters were placed on pads saturated with M-Endo broth (Difco), and incubated at 35±0.5°C for 24 hours before counting. For isolating fecal coliforms, membranes were placed on absorbent pads saturated with MFC broth (Difco) and incubated for 22 hours at 44.5±0.2°C.

To check the accuracy of the MF technique, the multiple tube fermentation method (MPN)[1] was performed on every fifth sample in addition to membrane filtration. A minimum of three sample dilutions with five tubes per dilution were tested, using Lauryl Tryptose broth (Difco) as the presumptive medium[2] and Brilliant Green Bile broth (Difco) as the confirmatory medium.

Samples from nondisinfected, chlorinated, chlorobrominated, and ozonated streams were placed in sterile bottles containing sodium thiosulfate.[1] During the first 14 weeks, the dechlorinated stream was sampled only once per week. Since coliform densities were found to deviate significantly from those of the chlorinated stream, the dechlorinated stream was subsequently sampled on a daily basis.

A number of tests were conducted to determine the best location and time of day to sample the effluent. It was observed that the highest bacterial density appeared in the late morning, while the lowest density occurred in the early morning hours between 2 and 7 A.M. Bacteriological sampling at specified intervals along each stream was conducted to determine the minimum detention time sufficient to achieve the desired disinfection efficiency with a minimum of bacterial aftergrowth.

Based upon findings from the above study, a routine sampling program was established. Each weekday morning and periodically in the afternoon, samples were collected at the following locations:

(1) Nondisinfected wastewater - immediately prior to entering the treatment systems.

(2) Chlorinated stream - at a site corresponding to a mean detention time of 30 minutes (see Section III) in the chlorine contactor.

(3) Dechlorinated stream - at a site in the dosing unit immediately following the SO_2 injection point, to minimize microbial aftergrowth.

(4) Chlorobrominated stream - at the end of the BrCl contact chamber, corresponding to a mean detention time of 30 minutes.

(5) Ozonated stream - at a site in the holding tank after 10 minutes contact time.

Data were tabulated and statistically analyzed to determine the pertinent relationships among the various wastewater characteristics.

The target coliform densities were arbitrarily established at 200 fecal coliforms per 100 ml and 1000 total coliforms per 100 ml, the former based on the Environmental Protection Agency's 1973 Secondary Effluent Standards[2] and the latter on the onetime State of Michigan standard. Disinfection efficiency was calculated by dividing the number of bacteriological samples which met the above standards by the total number of samples taken from each effluent.

The central tendencies of coliform densities were calculated both as arithmetic and geometric means. The geometric mean was used to minimize the effects of extremely high and low values in a sampling period and because they were the basis of the Federal standard at the time the project was conceived. Standard deviations were calculated as measures of dispersion. Mean differences were analyzed by t-test, analysis of variance, least significant difference (*a priori*) and Tukey's procedure (*a posteriori*). Linear regression analyses were performed to determine the relationship between MF and MPN total coliform densities and between suspended solids and coliform density.

RESULTS

Observations of Accumulated Data

In order to determine and compare the relative bactericidal effects of each disinfectant, coliform data were grouped into time segments during which no changes in experimental design occurred. A new group was formed each time a dose rate, residual, or other controllable system parameter was changed. This resulted in nine groups of data consisting of four different BrCl dosages (changed February 25, March 28, July 8), three different Cl_2 residuals (changed July 8, August 12), four concentration ranges of O_3 dosage (changed April 16, August 12, October 28), and addition of a filtration step prior to ozonation later in the project (i.e., pressure filtration added October 1, removed again November 19). On one occasion, Cl_2 and BrCl were changed simultaneously (July 8) and, on another occasion, O_3 and Cl_2 were changed simultaneously (August 12).

The above data groupings were subjected to analyses of variance followed by Tukey's procedure to determine whether neighboring changes of a disinfectant concentration resulted in significant differences in coliform survival. When no significant difference was found between these neighboring groups, they were combined into one larger group. This resulted in the final formation of six data groups by aggregation of data involving two changes in BrCl dosage (February 25 and March 8), two different concentrations of BrCl dosage and Cl_2 residual (changed July 8), and two changes in ozone application (within the addition of filtration on October 1, a dosage reduction on October 28).

Tables 4 through 9 summarize the disinfectant levels, arithmetic and geometric means of total and fecal coliform concentrations, and standard deviations of the arithmetic means for the six intervals. Table 10 presents the frequency that each disinfectant reduced total and fecal coliform densities to project standards.

Mean total coliform densities (MF) per 100 ml nondisinfected wastewater ranged from a high of 1.4×10^6 (4.0×10^6 arithmetic) in the first interval (Table 4) to a low of 7.2×10^4 (8.5×10^4 arithmetic) in the last interval (Table 9). This corresponds in the same intervals to a high fecal coliform density of 1.6×10^5 (6.6×10^5 arithmetic) and a low of 7.9×10^3 (9.9×10^3 arithmetic) organisms per 100 ml. Overall means for the entire test period were 3.1×10^5 (1.2×10^6 arithmetic) total coliforms per 100 ml and 1.6×10^5 (4.3×10^5 arithmetic) fecal coliforms per 100 ml of nondisinfected wastewater.

Coliform densities in the disinfected streams were highest in the first time segment (Table 4, when wastewater quality was low due to hydraulic overload) and lowest in the third interval (Table 6, when biological treatment was more uniform). Nevertheless, coliform density in the ozonated effluent was lowest in the fifth interval (Table 8) when multimedia filtration preceded ozonation. Variance in coliform data was greatest in the ozonated stream, probably due to frequent breakdowns in the ozone generation equipment.

From the foregoing tables, it is possible to determine the relative ability of each disinfectant to reduce coliform densities to acceptable levels under

Table 4. REDUCTION IN COLIFORM NUMBERS BY CHLORINE, CHLORINE FOLLOWED BY DECHLORINATION, OZONE, AND BROMINE CHLORIDE DURING JANUARY THROUGH FEBRUARY 22, 1974

	Total Coliform Density (number/100 ml)								Fecal Coliform Density (number/100 ml)			
	Membrane Filtration				Multiple Dilution Tube				Membrane Filtration			
Treatment	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	Geom. Mean
Nondisinfected	34	4.0×10^6	5.53×10^6	1.4×10^6	2	2.4×10^5	3.07×10^5	1.1×10^5	33	6.6×10^5	1.33×10^6	1.6×10^5
Chlorinated[a]	35	5,900	9,860	2,000	2	2,500	3,430	500	34	65	86	35
Dechlorinated	13	4,600	5,780	1,700	2	3,600	4,790	1,300	12	33	34	19
Chlorobrominated[b]	34	1,900	3,790	1,100	2	200	42	200	33	210	700	57
Ozonated[c]	36	18,000	24,900	8,900	2	5,200	2,600	4,800	34	1,700	2,360	860

[a] Cl_2 residual 2.0 mg/l after 30 minutes contact

[b] BrCl dosage 3.6 mg/l

[c] O_3 dosage 5 - 8 mg/l

Table 5. REDUCTION IN COLIFORM NUMBERS BY CHLORINE, CHLORINE FOLLOWED BY DECHLORINATION, OZONE, AND BROMINE CHLORIDE DURING FEBRUARY 25 THROUGH APRIL 15, 1974

	Total Coliform Density (number/100 ml)								Fecal Coliform Density (number/100 ml)			
	Membrane Filtration				Multiple Dilution Tube				Membrane Filtration			
Treatment	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	Geom. Mean
Nondisinfected	37	$2.8x10^5$	$2.57x10^5$	$1.8x10^5$	6	$3.9x10^5$	$3.09x10^5$	$1.9x10^5$	32	$8.5x10^4$	$7.3x10^4$	$3.6x10^4$
Chlorinated[a]	33	480	971	71	5	360	312	36	27	64	269	4.5
Dechlorinated	12	1,000	1,510	380	5	540	670	280	9	7	7.8	3.6
Chlorobrominated[b]	35	47	64	22	6	53	62	31	32	8.6	13.1	3.8
Ozonated[c]	30	4,100	4,480	2,500	4	1,800	1,990	1,100	28	1,200	1,750	400

[a] Cl_2 residual 2.0 mg/l after 30 minutes contact

[b] BrCl dosage 3.0 mg/l, then 2.5 mg/l (no significant differences between coliform densities at these two dosages)

[c] O_3 dosage 2.5 - 4 mg/l

Table 6. REDUCTION IN COLIFORM NUMBERS BY CHLORINE, CHLORINE FOLLOWED BY DECHLORINATION, OZONE, AND BROMINE CHLORIDE DURING APRIL 16 THROUGH AUGUST 9, 1974

	Total Coliform Density (number/100 ml)								Fecal Coliform Density (number/100 ml)			
	Membrane Filtration				Multiple Dilution Tube				Membrane Filtration			
Treatment	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	Geom. Mean
Nondisinfected	76	6.7×10^5	1.04×10^6	2.0×10^5	14	5.6×10^5	7.32×10^5	2.0×10^5	77	9.5×10^4	1.98×10^6	2.8×10^4
Chlorinated[a]	75	52	60	27	14	97	141	38	72	3.7	7.0	1.9
Dechlorinated	68	95	115	54	11	160	167	99	66	7.5	206	2.4
Chlorobrominated[b]	68	160	253	56	15	490	1,370	84	66	23	33.0	8.5
Ozonated[c]	75	1,300	1,620	570	13	1,100	2,240	460	74	180	257	80

[a] Cl_2 residual 2.0 mg/l to July 8, then 1.5 mg/l (no significant differences between coliform densities at these two residuals)

[b] BrCl dosage 2.5 mg/l to July 8, then 2.0 mg/l (no significant differences between coliform densities at these two dosages)

[c] O_3 dosage 5-8 mg/l

Table 7. REDUCTION IN COLIFORM NUMBERS BY CHLORINE, CHLORINE FOLLOWED BY DECHLORINATION, OZONE, AND BROMINE CHLORIDE DURING AUGUST 12 THROUGH SEPTEMBER 30, 1974

	Total Coliform Density (number/100 ml)								Fecal Coliform Density (number/100 ml)			
	Membrane Filtration				Multiple Dilution Tube				Membrane Filtration			
Treatment	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	Geom. Mean
Nondisinfected	33	$1.2x10^6$	$1.82x10^6$	$4.2x10^5$	7	$5.0x10^5$	$8.53x10^5$	$1.9x10^5$	31	$2.2x10^5$	$3.61x10^5$	$4.7x10^4$
Chlorinated[a]	31	380	433	210	6	540	328	410	32	17	20.5	8.3
Dechlorinated	33	560	532	380	6	1,000	1,260	600	32	15	16.7	7.5
Chlorobrominated[b]	34	630	965	220	7	270	162	280	31	53	76.7	23
Ozonated[c]	32	2,700	2,350	1,900	7	2,500	3,000	1,700	29	240	250	130

[a] Cl_2 residual 1.0 mg/l

[b] BrCl dosage 2.0 mg/l

[c] O_3 dosage started at 8 mg/l. Due to mechanical breakdown, dropped to 3 mg/l August 28 (no significant differences between coliform densities at these two dosages)

Table 8. REDUCTION IN COLIFORM NUMBERS BY CHLORINE, CHLORINE FOLLOWED BY DECHLORINATION, OZONE, AND BROMINE CHLORIDE DURING OCTOBER 1, THROUGH NOVEMBER 19, 1974

	Total Coliform Density (number/100 ml)								Fecal Coliform Density (number/100 ml)			
	Membrane Filtration				Multiple Dilution Tube				Membrane Filtration			
Treatment	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	Geom. Mean
Nondisinfected	25	7.8×10^5	9.18×10^5	3.4×10^5	5	1.6×10^5	2.16×10^5	8.5×10^4	20	1.3×10^5	1.83×10^5	2.9×10^4
Chlorinated[a]	26	760	638	610	5	570	577	430	22	20	17.2	13
Dechlorinated	25	310	1,430	620	5	1,100	1,340	720	21	26	22.6	16
Chlorobrominated[b]	25	1,700	1,140	620	3	780	724	570	20	120	85.5	82
Ozonated[c]	20	600	561	370	5	280	194	210	14	58	67.2	28

[a] Cl_2 residual 1.0 mg/l

[b] BrCl dosage 2.0 mg/l

[c] O_3 dosage 3 mg/l to October 28, then 6 mg/l (no significant differences between coliform densities at these two dosages) Pressure filtration added October 1

Table 9. REDUCTION IN COLIFORM NUMBERS BY CHLORINE, CHLORINE FOLLOWED BY DECHLORINATION, OZONE, AND BROMINE CHLORIDE DURING NOVEMBER 19 THROUGH NOVEMBER 27, 1974

	Total Coliform Density (number/100 ml)								Fecal Coliform Density (number/100 ml)			
	Membrane Filtration				Multiple Dilution Tube				Membrane Filtration			
Treatment	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	Geom. Mean
Nondisinfected	7	$8.5x10^4$	$5.2x10^4$	$7.2x10^4$	1	$2.2x10^4$	0	$2.2x10^4$	6	$9.9x10^3$	$8.3x10^3$	$7.9x10^3$
Chlorinated[a]	7	760	333	700	0				5	68	48	55
Dechlorinated	7	940	421	880	0				6	71	18	69
Chlorobrominated[b]	7	2,300	1,300	1,800	0				6	170	46	170
Ozonated[c]	5	4,500	1,280	4,400	0				3	290	55	280

[a] Cl_2 residual 1.0 mg/1

[b] BrCl dosage 2.0 mg.1

[c] O_3 dosage 6 mg/1. Pressure filtration bypassed

Table 10. FREQUENCY[a] THAT DAILY SAMPLES OF DISINFECTED EFFLUENTS[b] ACHIEVED PROJECT BACTERIOLOGICAL STANDARDS JANUARY THROUGH NOVEMBER, 1974

Dates	Total Coliforms % of Samples Below 1000 per 100 ml								Fecal Coliforms % of Samples Below 200 per 100 ml			
	Membrane Filtration				Multiple Tube Dilution				Membrane Filtration			
	Cl_2	SO_2	BrCl	O_3	Cl_2	SO_2	BrCl	O_3	Cl_2	SO_2	BrCl	O_3
Jan. - Feb. 22	31.4 (35)[c]	46.2 (13)	26.5 (34)	0 (36)	50 (2)	50 (2)	100 (2)	0 (2)	93.9 (34)	100 (12)	90.9 (33)	0 (34)
Feb. 22 - Apr. 15	84.8 (33)	83.3 (12)	100 (35)	20.7 (30)	83.3 (5)	80 (5)	100 (6)	50 (4)	96.3 (27)	100 (9)	100 (32)	29.6 (28)
Apr. 16 - Aug. 9	100 (75)	100 (68)	100 (68)	64.5 (75)	100 (14)	100 (11)	93.3 (15)	75 (13)	100 (72)	100 (66)	100 (66)	71.1 (74)
Aug. 12 - Sept. 30	93.5 (31)	93.9 (33)	79.4 (34)	21.9 (32)	100 (6)	83.3 (6)	100 (7)	14.3 (7)	100 (32)	100 (32)	93.5 (31)	65.5 (29)
Oct. 1 - Nov. 19	84.6 (26)	84.0 (25)	36.0 (25)	75 (20)	100 (5)	100 (5)	80 (3)	92.9 (5)	100 (22)	100 (21)	80 (20)	92.9 (14)
Nov. 19 - Nov. 27	71.4 (7)	71.4 (7)	14.3 (7)	0 (5)	100 (1)	(0)	(0)	(0)	100 (5)	100 (6)	66.7 (6)	0 (3)

[a]Frequency calculated as number of samples that met coliform standards divided by total number of samples times 100%.

[b]For disinfectant concentrations, refer to Tables 4-9.

[c]Number of samples in parentheses.

a particular set of operating conditions. A number of statistical tests were performed to determine significant differences in disinfection efficiency among the disinfection processes and effective differences among the various disinfectant concentrations.

A two-way analysis of variance was computed on the total coliform and fecal coliform data (MF). The two factors under consideration were treatment effects (Cl_2, SO_2, BrCl, O_3) and time segment effects. A highly significant interaction between treatment and time segment was demonstrated. This was not unexpected and suggested that some of the changes in disinfectant dosage or residual concentration significantly affected relative fecal and total coliform densities in the disinfected streams. Consequently, each of the six time segments was subjected individually to analyses of variance and Least Significant Difference procedures to determine significant differences among treatment means.

In the first time interval (January - February 22), mean coliform densities were higher than in any other interval (Table 4). Poor water quality was most probably responsible (see below) for these increased bacterial levels. Both arithmetic and geometric means for all disinfection processes exceeded the total coliform standard of 1000/100 ml. On the other hand, mean fecal coliform levels from chlorinated, dechlorinated, and chlorobrominated effluents met or closely approached the project standard of 200/100 ml. Mean coliform densities in the ozonated effluent were significantly higher ($p>0.99$) than all other streams. Results from all disinfection processes except ozone were not significantly different from one another.

Table 10 more clearly depicts these findings. All disinfection processes were relatively ineffective in reducing total coliform levels (MF) to below 1000/100 ml more than 50 percent of the time. Nevertheless, fecal coliform levels (MF) were reduced more than 90 percent of the time by all treatments except ozone, which failed to meet either standard at any time during the interval.

The second time interval (Table 5) was initiated by decreasing the BrCl dose from 3.6 to 3.0 mg/l, and later to 2.5 mg/l. The geometric mean coliform densities for chlorinated, dechlorinated, and chlorobrominated effluents were below 1000 total coliforms and 200 fecal coliforms per 100 ml, but mean coliform densities for ozonated effluent were well above the project standards as well as significantly above ($p> 0.99$) mean fecal and total coliform densities for the other disinfected effluents.

Disinfection effectiveness (Table 10) was $>$ 80 percent for total coliforms and $>$95 percent for fecal coliforms in the chlorinated, dechlorinated, and chlorobrominated streams, but only 20 to 30 percent for the ozonated stream.

In the third time segment (Table 6), improvement in ozonation capacity was accomplished by increasing dosage to a range of 5 to 8 mg/l. Concomitantly, BrCl and Cl_2 dose rates were lowered, but coliform densities were not significantly changed. All systems functioned satisfactorily due to a considerable improvement in wastewater quality (see Section III). Thus, during this time segment, mean coliform densities were lowest in all effluents except the ozonated stream. The only other time coliform levels were lower in the ozonated stream was in the fifth interval (October 1 - November 19) when a multimedia pressure filter was installed prior to ozone application. Chlorine with and without dechlorination and bromine chloride achieved project standards 100 percent of the time during this third period, while ozone achieved the standards more than 64 percent of the time (Table 10). Mean coliform densities, however, were significantly higher ($p > 0.99$) in the ozonated stream than in the other disinfected streams.

The fourth interval (Table 7) was initiated by decreasing the chlorine residual from 1.5 to 1.0 mg/l (the BrCl dosage remained the same). Although coliform densities were higher in this interval, chlorine with and without dechlorination and bromine chloride still achieved project standards satisfactorily. Chlorine reduced total and fecal coliform densities to desired levels more than 90 percent of the time. BrCl reduced total coliform densities to desired levels about 80 percent of the time, and fecal coliform densities more than 90 percent of the time (Table 10). Although fecal coliform standards were met by ozone almost as frequently as in the previous interval, total coliform standards were met only about 22 percent of the time. While chlorination, dechlorination, and chlorobromination produced fecal and total coliform means which were not significantly different from each other, ozonation still produced significantly higher ($p > 0.99$) coliform means.

In the fifth time segment, a multimedia filter was installed in front of the ozonation system in an attempt to achieve better disinfection efficiency by reducing the suspended solids level in the effluent. The other streams were not filtered. This treatment was apparently successful, since total and fecal coliform densities in the ozonated stream were lower than in any other interval (Table 8). Total coliform standards were met 75 percent of the time and fecal coliform standards 93 percent of the time (Table 10). Furthermore, mean total coliform density in the ozonated stream was, for the first time, not significantly higher than the levels produced by the other disinfection processes. Disinfection efficiency in the BrCl system fell sharply in this interval, the coliform levels being significantly higher ($p > 0.99$) than the other systems.

The pressure filter was bypassed for the final, short interval (Table 9) to reaffirm its effect on the ozonation process. In this interval, ozone failed to meet project standards all of the time (Table 10). Coliform densities in the ozonated effluent again increased to levels significantly higher ($p > 0.99$) than the other disinfection processes (Table 9). Coliform densities in the chlorobrominated stream were also significantly higher ($p > 0.99$) than the chlorinated and dechlorinated effluents. Table 10 illustrates this marked reduction in disinfection effectiveness of BrCl and also indicates a slight reduction in disinfection effectiveness of Cl_2. These effects were attributed to a substantial decline in effluent quality due to seasonal changes in temperature.

When the analyses of variance for all disinfectants were calculated using the MPN data, it was found that ozone was the only disinfection process which produced data quantitatively higher than the other processes, and this was only significant ($p > 0.90$) in the fourth interval (August 12-September 30).

Because MPN's were performed only on every 5th sample, the above anomaly may be a consequence of an insufficient number of samples. Thus, caution should be exercised in forming conclusions from MPN data alone.

<u>Observations of Monthly Data</u>

Total and fecal coliform levels varied widely over the entire span of the project. In order to analyze the temporal relationships of the various disinfection processes and to present them in a more typical fashion, mean coliform data are here presented in terms of monthly intervals. Additional information is presented in Appendices 1A-1E which show samples sizes, standard deviations, and arithmetic and geometric means.

Figures 7 and 8 show the monthly geometric means of total coliform densities (MF) and fecal coliform densities (MF), respectively, in all test streams.

All process flows exhibited a major peak in total and fecal coliform levels early in the project. This was due to excessive hydraulic flows overloading the final clarifiers, causing poor solids separation and reducing chlorine residence time (see Section III). These high liquid flow rates in January, trailing into February, were caused by heavy seasonal rain and snowfall, flooded river conditions, and heavy infiltration which overloaded the plant's treatment systems. Suspended solids levels and biochemical oxygen demand (BOD) were high in both influent and effluent (see Section III, Figs. 3 and 4). Coliform levels declined in spring and early summer as effluent quality returned to normal.

Fecal and total coliform densities in nondisinfected wastewater remained fairly constant for the remainder of the project period until a decline was seen in the last month. As autumn approached (July-September), coliform concentrations in the chlorinated, dechlorinated, and chlorobrominated streams increased without a concomitant rise in suspended solids or BOD (the ozonated stream was being filtered at this time). The rise in coliform levels was partly due to a lowering of the halogen feed rates to determine the minimal effective concentrations necessary to maintain the desired bacteriological quality. Chlorine residual was decreased from 2.0 to 1.5 mg/l on July 8, and then to 1.0 mg/l on August 12. Bromine chloride dosage was lowered from 2.5 to 2.0 mg/l on July 8.

Figures 9 and 10 indicate the frequency that samples from each treatment met project disinfection criteria. These data closely parallel those discussed above. Disinfection was least effective during the first part of the project. Then, as effluent quality improved, disinfection efficiency rose sharply. Finally, at the end of the project, a decline in bacteriological quality was again observed, partially because minimal concentrations of chlorine and bromine chloride were employed, and partially because effluent quality decreased due to seasonal temperature changes.

Disinfection with ozone varied considerably throughout the project. Although a liquid flow rate of 2.2 lps (35 gpm) was the original project requirement, a 6.3 lps (100 gpm) contactor was provided. The ozone generator was theoretically capable of dosing the wastewater flow to 20 mg/l, but in actuality

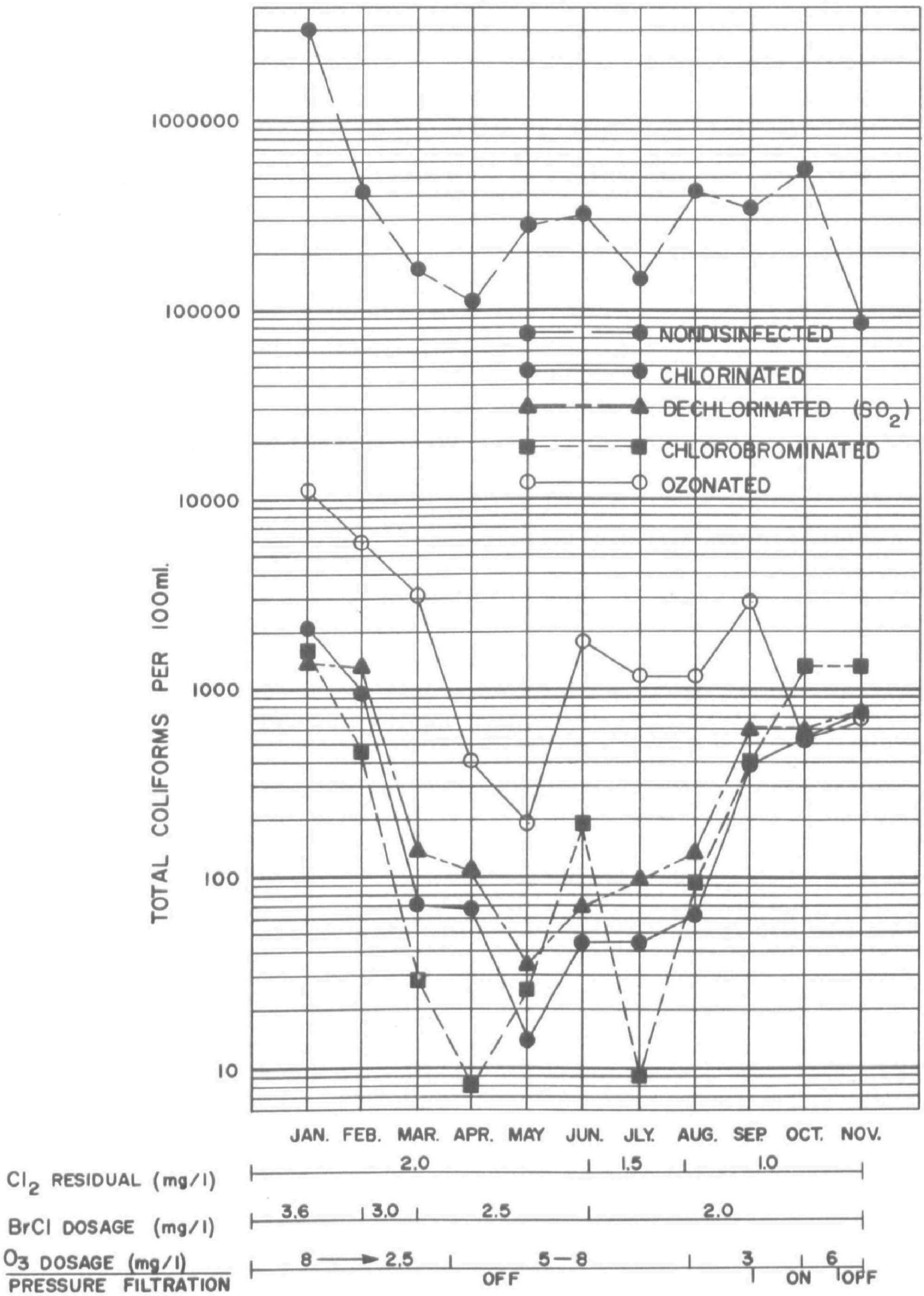

Figure 7. MONTHLY GEOMETRIC MEANS OF TOTAL COLIFORM DENSITIES (MF)

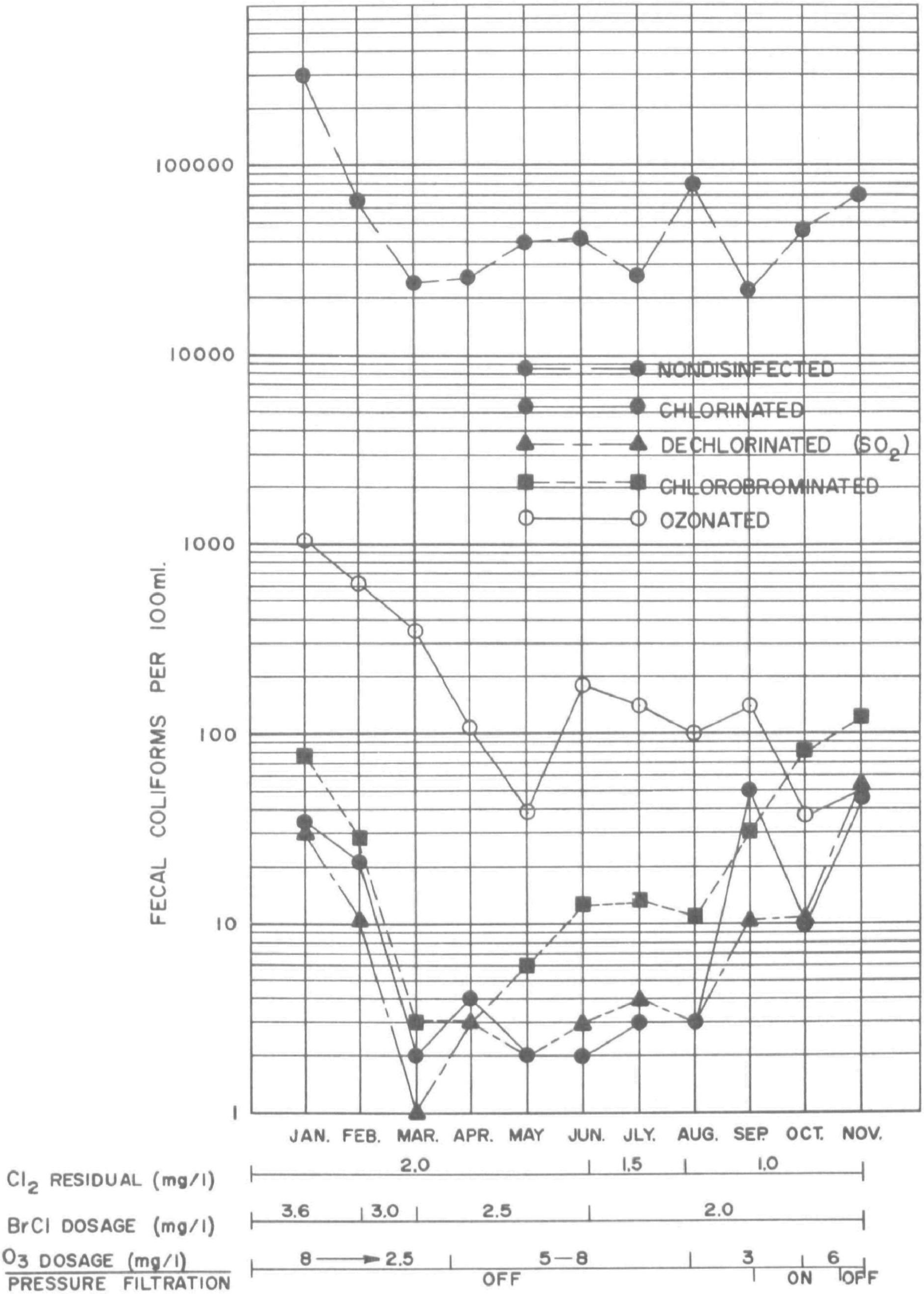

Figure 8. MONTHLY GEOMETRIC MEANS OF FECAL COLIFORM DENSITIES (MF)

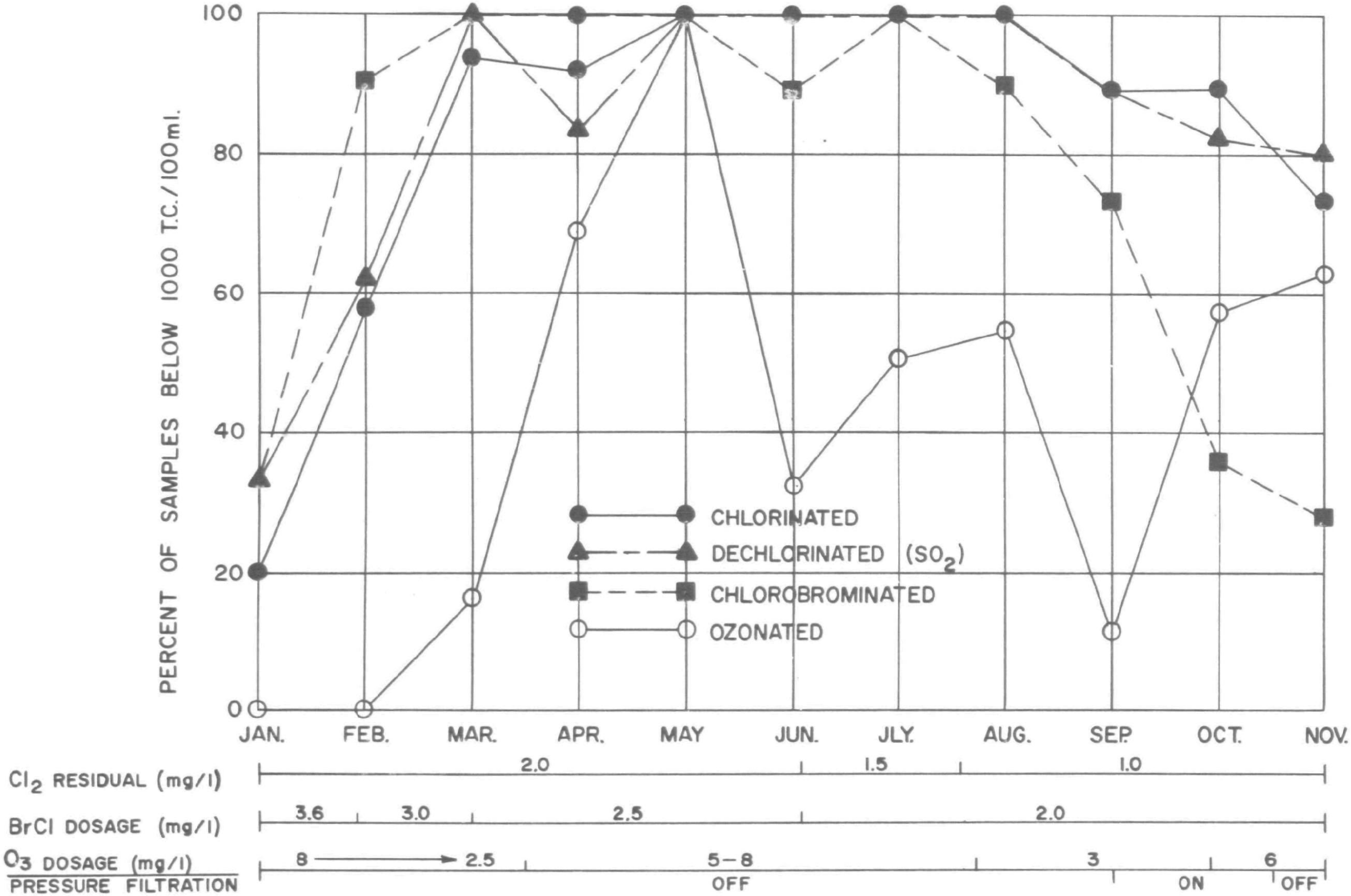

Figure 9. PERCENT OF SAMPLES WITH TOTAL COLIFORM DENSITIES (MF) BELOW 1000 PER 100 ml

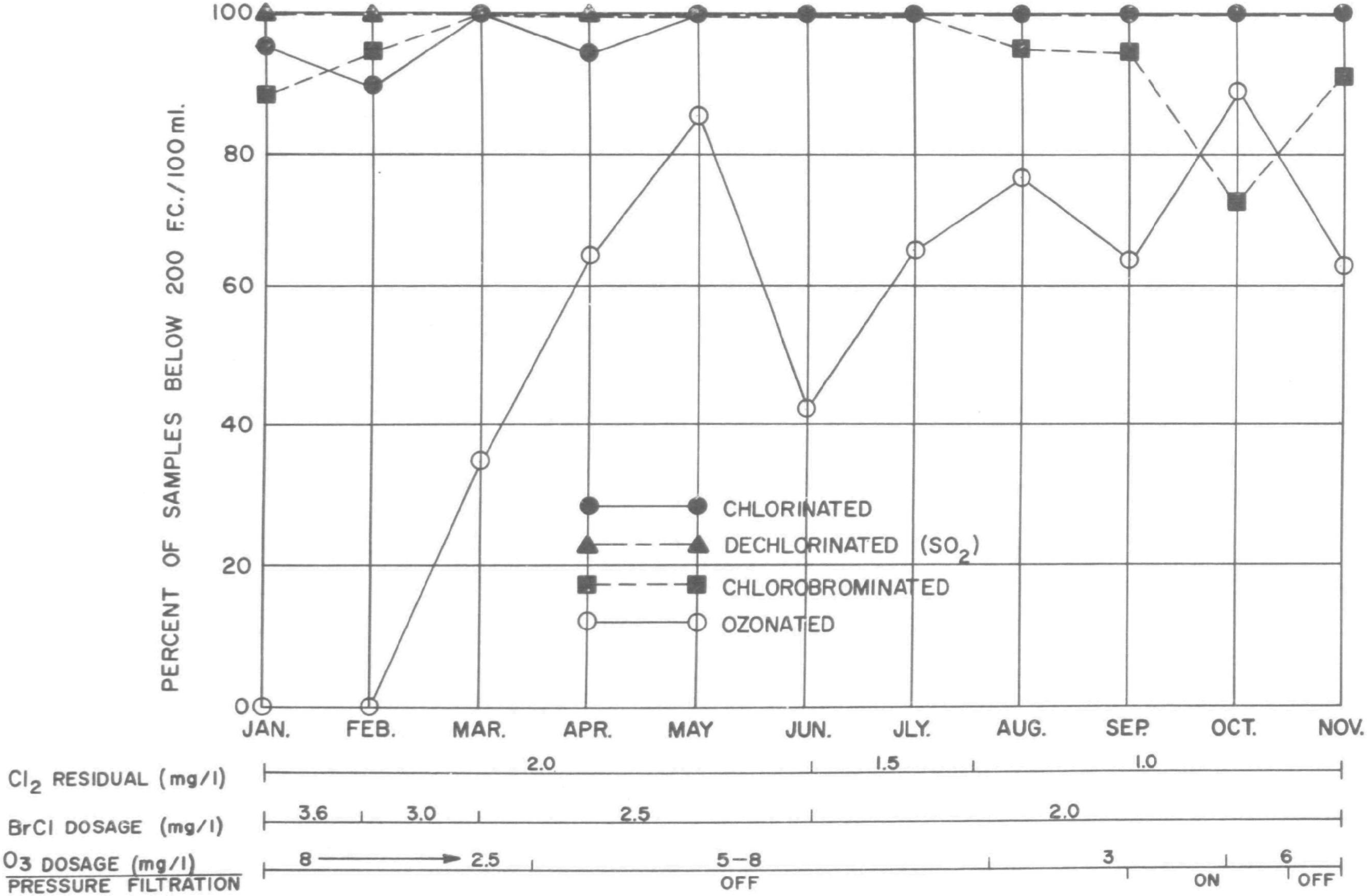

Figure 10. PERCENT OF SAMPLES WITH FECAL COLIFORM DENSITIES (MF) BELOW 200 PER 100 ml

only a maximum of 8 mg/l was ever achieved. This prevented determination of the true minimal dosage required to achieve adequate disinfection without filtration.

During the early weeks of the project, chlorine demand was approximately 1.4 mg/l (see Section III). At this time, chlorine residual was regulated at 2 mg/l after about 30 minutes contact, although the detention time varied with plant flow. In March, chlorine demand declined 50 percent and detention time increased slightly.

Disinfection with bromine chloride was regulated by dosage control rather than residual control, because bromine chloride residual in wastewater declines very rapidly and consequently is difficult to monitor accurately at low concentrations. A dosage of 2.0 mg/l was found to be adequate in meeting project bacteriological standards only occasionally. The mean 30 minute residual at this dosage was calculated to be 0.5 mg/l, but ranged from 0.1 to 1.5 mg/l.

Coliform levels in the ozonated stream fluctuated widely throughout the project because of the difficulty in maintaining a constant dosage. This was a result both of mechanical breakdowns of the ozone generator and inexperience in handling ozonation equipment. At the beginning of the project ozone dosage was 8 mg/l, but the gas-liquid contacting was inefficient. To improve mass transfer, it was necessary to lower the gas flow rate. However, the gas control valve was damaged by abrasives which had been introduced into the ozone generator from the compressor and air dryer. A filter was installed on March 8 to trap the abrasives, but little improvement resulted. It was then found that excessive moisture was present in the air line, thus limiting ozone production. New desiccant was placed in the dryer on April 3, and still no improvement in ozone generation ensued. Upon further examination, it was found that the dielectric cells had been damaged by the abrasives in February. New cells were installed on April 16 and an immediate improvement in ozone dosage and disinfection efficiency occurred.

The above mechanical upsets were not recognized rapidly by the relatively inexperienced on-site personnel. The gas-to-liquid ratio necessary for optimum mass transfer in the positive pressure injector contacting system was about 0.025. But, because at the time there was no good method of quantifying ozone concentration either in the liquid stream or in the exhaust gas, the amount of ozone being lost in the exit gas could only be grossly approximated.

On May 8, 1974, the Air Pollution Control division of the Michigan Department of Natural Resources estimated ozone loss in the contacting unit by a series of measurements with an Ecolyzer (Energetic Sciences, Inc.), a continuous ozone monitor. Ozone concentrations were measured in the inlet and exhaust gas streams, and from these data mass transfer efficiency was calculated. Results indicated that 70 to 90 percent of the applied ozone was lost in the off-gas, based on an assumed flow rate of 850 cfm through the exhausting stack (not a sealed system). Thus, mass transfer efficiency was extremely low.

In September, ozone production declined to 3 mg/l due once again to excessive moisture in the air and remained at this level until late October. New desicant did not arrive until the second week in October.

Effect of Multimedia Filtration on Disinfection Efficiency of Ozone

Table 11 summarizes the effect of ozonation on filtered wastewater effluent compared to a similar unfiltered wastewater effluent. Because the system could not be run in parallel, filtered effluent data (October 1 - November 19) were compared with unfiltered effluent data from the immediately preceeding time interval (August 15 - September 17). The suspended solids levels in the filtered effluent were significantly different ($p > 0.99$) from the suspended solids levels in the unfiltered effluent. Total coliform densities in the filtered wastewater were significantly different ($p > 0.999$) from those in unfiltered wastewater. It was concluded that filtration enhanced the effectiveness of disinfection with ozone.

Correlation and linear regression analyses were performed between suspended solids and total coliform densities (Table 11) before and/or after pressure filtration. The results indicated no significant correlation before filtration but a significant ($p > 0.99$) correlation after filtration. Since the samples were not taken in parallel and did not contain a wide range of suspended solids concentrations, caution should be exercised in forming conclusions. Nevertheless, it appears that "large" particles, which are removed by pressure filtration, may have played an important role in limiting ozone disinfection effectiveness. Further investigations need to be performed to confirm this phenomenon.

Comparison of Membrane Filter and Multiple Tube Fermentation Procedures

MPN total coliform data were compared with MF data statistically by way of t-test and linear regression procedures. Table 12 gives the results of those analyses. The MF means were not significantly different from MPN means ($p < 0.50$). Correlation between the two analytical procedures was linear in all cases at the $p > 0.99$ level. The linear model chosen had an intercept of zero. The results indicated a set of relationships very close to ideal (slope of 1.0). From these tests, it was concluded that the MF data were valid estimates of total coliform densities in all streams studied at Grandville.

DISCUSSION AND CONCLUSIONS

All disinfection systems displayed a capability of achieving adequate disinfection efficiency when effluent quality was good and sufficient disinfectant dosages were maintained. As effluent quality declined, the frequency of failures increased. Chlorine, with and without dechlorination, was least affected by plant upsets.

State-of-the-art disinfection technology with bromine chloride and ozone was not as advanced as chlorination technology, and consequently breakdowns were more pervasive in these two systems. Bromine chloride application was regulated by dosage control rather than residual control, and this was found to be

Table 11. EFFECTIVENESS OF OZONE DISINFECTION ON FILTERED AND UNFILTERED EFFLUENT

		Total Coliform Density[a] (number/100 ml)				Suspended Solids (mg/1)			Total Coliform Density vs Suspended Solids			
	O_3 Feed (mg/1)	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	No. of Samples	Corr. Coeff.(r)	Slope (Coliforms/ Suspended Solids)	Intercept Total Coli-forms/100 ml
Unfiltered Effluent[b]	4.4	30	2700	2400	1800	27	10.5	6.9	27	0.036	13	2700
Filtered Effluent[c]	3.8	20	600	560	370	19	6.3	5.1	19	0.678[d]	75	144

[a]Membrane filter determinations

[b]Samples taken August 15 - September 27

[c]Samples taken October 1 - November 18

[d]Correlation was linear at the $p > 0.99$ level

Table 12. CORRELATION AND REGRESSION OF MPN vs MF TOTAL COLIFORM DENSITIES (NUMBER/100 ml) JANUARY THROUGH NOVEMBER, 1974

Treatment	No. of Samples	Arithmetic Mean Coliform Densities				t-Statistic for Means	Corr. Coeff. (r)	Slope (Intercept = 0)
		MPN[a]		MF[a]				
			S.D.		S.D.			
Nondisinfected	32	3.8×10^5	5.3×10^5	4.3×10^5	6.5×10^5	0.2543[b]	0.7890[c]	1.02
Chlorinated	30	1000	3160	1600	1560	0.5593[b]	0.9352[c]	1.55
Dechlorinated	23	760	1560	1100	3490	0.3886[b]	0.9170[c]	1.93
Chlorobrominated	30	230	327	260	404	0.2090[b]	0.8625[c]	1.08
Ozonated	29	1700	2260	2200	2540	0.5371[b]	0.9429[c]	1.13

[a]MPN data were independent variables, MF were dependent variables

[b]$0.10 \leq p \leq 0.50$

[c]Correlation was linear at the $p > 0.99$ level

the primary cause of system failure. Another frequent repair item in the BrCl dosing system was the evaporator. BrCl must be vaporized prior to injection into the wastewater stream. The evaporator unit tended to accumulate solids with time, thereby blocking flow. If an adequate means of control by residual can be developed and refined, and if improvements in the dosing system can be made, bromine chloride may be an effective wastewater disinfectant.

Mechanical breakdowns in the ozonation system were more frequent and severe and more difficult to diagnose and correct. Experience gained in this project suggest that a fairly extensive shakedown period is needed by plant operating personnel in learning to handle and control ozonation equipment properly. Dosing of ozone appeared to be quite sensitive to shifts in demand and changes in flow.

The duration of this project was 11 months. Data were grouped into six main intervals or time segments, during which experimental conditions were fairly stable in all systems. Within these intervals, fecal and total coliform levels in chlorinated, dechlorinated, and chlorobrominated effluents generally were not significantly different. Only in the last two intervals were coliform densities in chlorobrominated effluent significantly higher than in the chlorinated and dechlorinated streams.

Fecal and total coliform numbers in the ozonated stream were significantly higher than in the other disinfected streams in all intervals but the fifth (when filtration preceded ozonation). It appeared that particles larger than about 10 μm more readily interfered with the disinfection efficiency of ozone than smaller particles.

Fecal coliform standards were met more frequently in each time interval and for each treatment than corresponding total coliform standards. At times the difference in frequency was 60 percent. This suggests that fecal coliforms are more sensitive to disinfectants than those organisms comprising the total coliform population. However, this conclusion may be somewhat questionable, since recovery of fecal coliforms on membrane filters in some cases is inferior to recoveries determined by the MPN technique.

Conclusions regarding the relative disinfection efficiencies of the various disinfectants studied, based on the statistical analyses described in detail, should be viewed from the perspective that dosage control of the various disinfection systems was vastly different. For example, in order to maintain a desired chlorine residual, dosages were changed in accordance with changes in demand. Thus, disinfection efficiency of chlorine was fairly consistent. However, the rate of addition of bromine chloride and ozone could not be correlated with demand fluctuations, but rather was held constant at all times during a specific interval. As a result, sudden changes in demand could easily have caused concurrent changes in disinfection efficiency by BrCl and ozone, and these changes would not have been accounted for in the statistical analysis.

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APPENDIX A-1

COLIFORM DENSITIES OF NONDISINFECTED WASTEWATER
JANUARY THROUGH NOVEMBER, 1974
(number/100 ml)

	Total Coliform Density								Fecal Coliform Density			
	Membrane Filtration				Multiple Dilution Tube				Membrane Filtration			
	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	Geom. Mean
January	18	6.0×10^{6}	6.6×10^{6}	3.0×10^{6}					17	1.8×10^{6}	9.8×10^{5}	3.0×10^{5}
February	20	1.3×10^{6}	2.4×10^{6}	4.4×10^{5}	2	2.4×10^{5}	3.1×10^{5}	1.1×10^{5}	20	2.3×10^{5}	3.0×10^{5}	6.8×10^{4}
March	20	2.7×10^{5}	2.3×10^{5}	1.7×10^{5}	4	3.8×10^{5}	4.3×10^{5}	1.4×10^{5}	18	5.2×10^{4}	7.0×10^{4}	2.4×10^{4}
April	24	2.7×10^{5}	3.8×10^{5}	1.2×10^{5}	4	2.3×10^{5}	1.5×10^{5}	9.2×10^{4}	23	7.7×10^{4}	9.1×10^{4}	2.6×10^{4}
May	20	8.1×10^{5}	1.2×10^{6}	2.8×10^{5}	3	9.8×10^{5}	1.3×10^{6}	2.6×10^{5}	21	1.1×10^{5}	1.6×10^{5}	4.0×10^{4}
June	19	1.2×10^{6}	1.4×10^{6}	3.3×10^{5}	4	6.5×10^{5}	7.8×10^{5}	2.1×10^{5}	19	2.0×10^{5}	2.8×10^{5}	4.1×10^{4}
July	20	3.3×10^{5}	4.9×10^{5}	1.6×10^{5}	4	5.0×10^{5}	4.8×10^{5}	4.4×10^{5}	20	4.7×10^{4}	5.8×10^{4}	2.8×10^{4}
August	20	1.0×10^{5}	1.6×10^{6}	4.3×10^{5}	4	2.6×10^{5}	1.7×10^{5}	2.2×10^{5}	19	2.8×10^{5}	4.0×10^{5}	8.0×10^{4}
September	19	1.0×10^{6}	1.8×10^{6}	3.5×10^{5}	4	6.6×10^{5}	1.2×10^{6}	1.8×10^{5}	18	1.1×10^{5}	2.4×10^{5}	2.1×10^{4}
October	17	1.1×10^{6}	9.8×10^{5}	5.9×10^{5}					15	1.7×10^{5}	2.0×10^{5}	4.8×10^{4}
November	15	1.1×10^{5}	9.5×10^{4}	8.8×10^{4}	6	1.3×10^{5}	2.0×10^{5}	6.8×10^{4}	11	9.5×10^{3}	7.3×10^{3}	7.0×10^{3}

APPENDIX A-2

COLIFORM DENSITIES OF CHLORINATED EFFLUENT
JANUARY THROUGH NOVEMBER, 1974
(number/100 ml)

	Total Coliform Density								Fecal Coliform Density			
	Membrane Filtration				Multiple Dilution Tube				Membrane Filtration			
	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	Geom. Mean
January	20	5000	8900	2100					18	69	59	35
February	19	5700	1000	950	2	2500	3400	490	20	59	96	22
March	16	300	520	71	3	310	419	110	14	5	9.8	2
April	24	460	1200	68	4	230	260	54	20	82	310	4
May	22	27	41	15	3	67	56	53	20	2.2	2.3	2
June	17	72	78	45	3	60	61	42	16	7.1	13	2
July	19	66	48	45	5	160	220	43	19	4.7	4.8	3
August	20	95	69	62	4	350	380	230	21	6.2	9.0	3
September	17	580	500	390	3	660	150	650	17	24	24	14
October	18	700	700	540					17	15	15	10
November	15	830	410	750	5	570	580	430	10	54	36	46

APPENDIX A-3

COLIFORM DENSITIES OF DECHLORINATED EFFLUENT JANUARY THROUGH NOVEMBER, 1974 (number/100 ml)

	Total Coliform Density								Fecal Coliform Density			
	Membrane Filtration				Multiple Dilution Tube				Membrane Filtration			
	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	Geom. Mean
January	6	4100	5500	1500					5	52	46	30
February	8	4400	6200	1400	2	3600	4800	1300	8	17	13	11
March	6	280	370	160	3	220	240	150	5	1.6	0.9	1
April	12	870	1600	120	3	690	890	240	10	8.0	9.2	3
May	21	54	57	35	3	56	12	56	20	4.5	12	2
June	17	140	190	70	3	140	160	85	16	17	38	3
July	17	120	89	97	3	350	190	310	17	6.2	6.0	4
August	20	210	210	140	4	320	320	230	21	7.4	13	3
September	19	770	600	600	3	1700	1600	1200	17	19	18	11
October	17	940	1400	600					16	19	18	12
November	15	850	410	760	5	1100	1300	720	11	60	23	55

APPENDIX A-4

COLIFORM DENSITIES OF CHLOROBROMINATED EFFLUENT
JANUARY THROUGH NOVEMBER, 1974
(number/100 ml)

	Total Coliform Density								Fecal Coliform Density			
	Membrane Filtration				Multiple Dilution Tube				Membrane Filtration			
	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	Geom. Mean
January	18	3100	5000	1700					17	320	950	77
February	20	530	440	460	2	200	42	200	20	85	220	28
March	19	42	50	28	4	70	71	17	17	7.9	12	3
April	23	24	38	8	4	10	10	6	21	8.9	16	3
May	13	110	190	26	3	91	74	68	12	23	33	6
June	18	340	400	190	4	110	76	91	18	34	47	13
July	20	120	91	9	5	1300	2300	350	20	25	25	14
August	21	350	880	94	4	180	71	210	20	36	76	12
September	19	770	910	400	4	340	180	290	17	58	68	31
October	17	1800	1300	1400					15	120	97	80
November	15	1800	1100	1400	3	780	720	570	11	140	62	130

APPENDIX A-5

COLIFORM DENSITIES OF OZONATED EFFLUENT
JANUARY THROUGH NOVEMBER, 1974
(number/100 ml)

	Total Coliform Density								Fecal Coliform Density			
	Membrane Filtration				Multiple Dilution Tube				Membrane Filtration			
	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	Geom. Mean	No. of Samples	Arith. Mean	S.D.	Geom. Mean
January	20	23000	28000	12000					18	2000	2300	1100
February	16	12000	20000	5900	2	5200	2600	4800	16	1300	2400	630
March	19	5000	5200	3100	3	2000	2600	1000	17	2100	1300	350
April	22	1400	2100	420	3	500	700	200	22	510	840	110
May	21	300	260	190	3	300	220	200	21	85	97	38
June	19	2700	2000	1800	3	1900	1500	1400	19	380	410	180
July	20	1600	1500	1200	4	1700	2400	910	20	180	120	150
August	17	1600	1100	1200	4	950	420	870	17	190	240	100
September	19	3600	2600	2800	4	3500	3800	2400	16	260	230	150
October	12	800	630	540					9	74	78	37
November	13	1900	2300	680	5	280	190	210	8	130	140	49

SECTION V. LIFE CYCLE RESIDUAL TOXICITY STUDIES

INTRODUCTION

The current concern for maintaining the quality of our environment requires that chemical agents used in the treatment of wastewater not only function as efficient disinfectants, but that they also show minimal potential to exert a toxic effect on the aquatic life of receiving waters. This project was, therefore, designed to simultaneously investigate the disinfection effectiveness of several microbiocidal agents and their potential residual toxicity to aquatic life.

The toxicity of wastewater effluent disinfected with chlorine (Cl_2) has received attention in several recent studies[1-7] and the potential for undesirable toxic effects by such effluents is generally acknowledged. As a result, research has progressed to the stage where successful elimination of the acute residual toxicity of chlorinated effluents is possible through the use of sulfur dioxide (SO_2)[1], bisulfite[3] and sodium thiosulfate.[6]

On the other hand, both the disinfection effectiveness and the residual toxic properties of bromine chloride (BrCl) and ozone (O_3) have received much less study. Mills[8,9] recently investigated wastewater disinfection with BrCl and performed static tests to determine acute (96-hour) toxic effects, if any, as a result of chlorobromination. No life cycle studies with bromine chloride are reported in the literature. Venosa[10] reviewed the literature on water and wastewater disinfection with ozone and found it to be confusing and contradictory. Investigations of the residual toxicity of ozonated water under different conditions have produced varying results. Arthur, et al.,[1] observed no acute or life-cycle toxic effects on aquatic life exposed to ozonated effluent in which no measurable residual ozone was present. The same investigators found that ozonated effluent containing a measurable ozone residual was lethal to fathead minnows. Likewise, Rosenlund[11] reported that rainbow trout died soon after exposure to ozonated lake water which contained residual ozone. Thus, it is clear that the potential for residual toxicity in both chlorobrominated and ozonated effluents merits additional attention.

This residual toxicity study was designed to simultaneously test in parallel the toxicity of a nondisinfected effluent stream; identical effluent streams disinfected with chlorine, bromine chloride or ozone; and a chlorinated stream dechlorinated with sulfur dioxide. Life cycle studies were run with fathead minnows (*Pimephales promelas*) as test subjects while acute studies were conducted with *P. promelas* and other species of fish, and the freshwater macroinvertebrate *Daphnia magna*.

MATERIALS AND METHODS

Water Supplies

For a detailed description of the treatment site and flow schemes, refer to Section III.

The dilution water used for diluting the treated effluent streams delivered to the fish tanks was well water from which excess iron was removed by passage through an iron removal filter. This water was of high enough quality to enable fathead minnows (*Pimephales promelas*) and *Daphnia magna* to grow and reproduce satisfactorily. The chemical characteristics of the dilution water are shown in Table 13. The pH was 7.6 and the conductivity was 859 micromhos/cm.

Table 13. CHARACTERISTICS OF THE DILUTION WATER

Analysis	Concentration in mg/l
Hardness (as $CaCO_3$)	464
Calcium	160
Magnesium	13
Sulfate	270
Chloride	8
Iron	0.68
NH_3-N	0.16
NO_2^--N	0.0
Alkalinity (as $CaCO_3$)	194
Acidity (as $CaCO_3$)	15

Chemical Analyses

Residual chlorine, bromine chloride, ozone, and sulfite (residual sulfur dioxide was measured as sulfite) were measured daily in the respective effluent storage tanks and in one aquarium containing the highest effluent concentration. These same analyses were performed at least once per week in the aquaria containing each lower effluent concentration. Standard amperometric titration procedures[12] were modified to improve the sensitivity for determining the amperometric end point.[13] The modification included a polarograph (Heath EU-401 Series), a strip chart recorder (Heath model EUW-20A), a synchronous motor electrode rotator, a platinum electrode, a magnetic stirrer, and a microburet. An accuracy test using a volumetric dilution of a known chlorine standard indicated that our procedure was accurate to ±0.002 mg/l for halogen determinations, and to ±0.08 mg/l for sulfite titrations.

A portable dissolved oxygen meter (Yellow Springs Instrument Model 54) was used to measure oxygen concentrations daily in the highest effluent concentration tanks, at least once per week in each of the other test chambers, and three times per week in each of the effluent storage tanks. The temperature of the contents of one aquarium receiving effluent diluted 50 percent was continuously monitored on each bioassay table. Also, the temperature of each effluent storage tank was monitored twice per day.

Acidity, alkalinity, total ammonia, conductivity, hardness, and pH were measured weekly in the effluent storage tanks, in the test aquaria containing the highest effluent concentration, and in the dilution water (control) test tanks. Acidity, alkalinity, and hardness samples were analyzed according to procedures outlined in Standard Methods.[12] Conductivity was measured with a Hach conductivity meter (Model 2510), and pH was measured with an Orion pH meter (Model 701). A modified Seligson-Seligson[14] method was used in running total ammonia samples.

Bioassay Methods

The effluents and well water were heated to 25C for bioassays with warm water species, or chilled to 13C for testing cold water species. Proportional diluters (Mount and Brungs[15]) with some refinements (Figure 11) were used to achieve the desired test concentrations and to mix effluents with dilution water. Only PVC, silicone rubber, stainless steel, neoprene rubber and glass materials were used in the construction of the diluters. Each diluter was operated continuously on a four minute (±10 seconds) cycle time, with each adult test chamber receiving 700 ml per cycle. With the exception of the chlorinated effluent diluter system, all diluters in the life cycle systems were calibrated to deliver 100 percent effluent and 100 percent well water and six intermediate concentrations. The six nominal intermediate concentrations were 50.00, 25.00, 12.50, 6.25, 3.12, and 1.56 percent effluent. The chlorinated life cycle diluter was designed to deliver seven nominal dilutions of chlorinated effluent, 20.00, 14.00, 9.80, 6.86, 4.80, 3.46, and 2.35 percent, and 100 percent dilution water. Lower concentrations of chlorinated effluent were used in the life cycle study because of the previously demonstrated toxicity of chlorinated effluent to aquatic life.[7]

The calibration of all life cycle diluters was checked volumetrically each week and adjusted, if necessary, to maintain the proper effluent concentration and turnover time in each test chamber. Usually only minor adjustments of the diluters were required to maintain proper calibration. Most of the problems encountered with the diluters occurred during the first several months of the project when effluent quality was low.

Test chambers were 60 x 29 x 30 cm (28.4 l) glass aquaria, which received one complete volume change every 2.6 hours, or 9.2 tank volumes per 24-hour period. Duplicate test chambers were randomly located on a 1.2 x 3.0 m table for each type of treated effluent. The life cycle test tables were isolated behind a black curtain to minimize the visual stimulation of the test animals by laboratory traffic. This was particularly important during the reproductive period when the fish were most sensitive to external stimuli. On the life cycle test tables, fry chambers (30 x 30 x 30 cm (14.2 l)) were located on a shelf below the spawning chambers and received the same concentrations of effluent at the same rate as the adult tanks. Those adult and fry tanks receiving the four highest effluent concentrations (100, 50, 25, and 12.5 percent) were continuously aerated with oil-free air to prevent

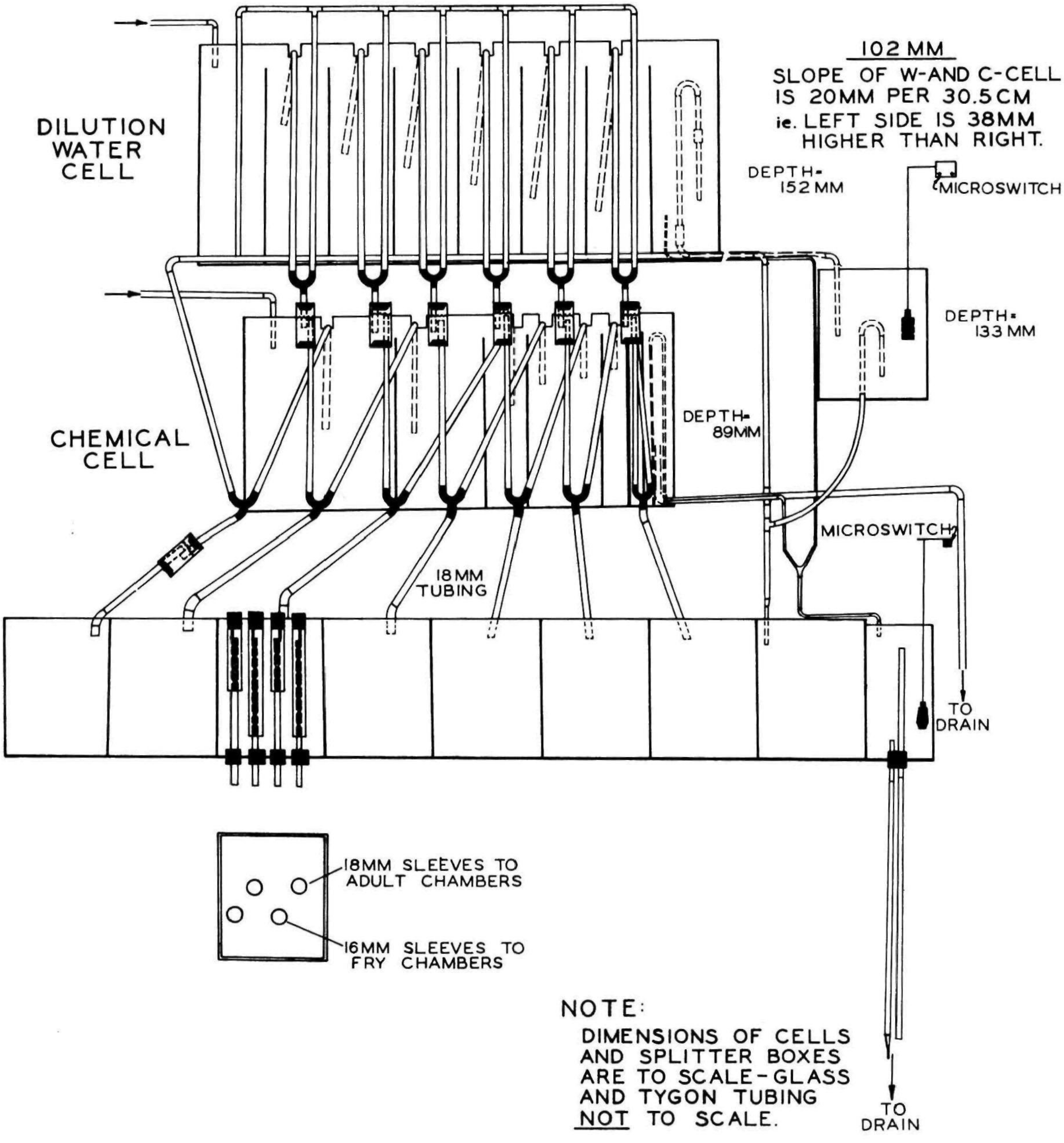

Figure 11. SCHEMATIC DRAWING OF THE MODIFIED MOUNT-BRUNGS PROPORTIONAL DILUTER USED IN THIS STUDY

excessively low dissolved oxygen concentrations. This process was not necessary on the chlorinated effluent life cycle table because of the greater dilutions of effluent in those test chambers.

The bioassay system was equipped with an electrically operated warning system which shut down all diluters, activated a light and bell alarm, and turned on an air supply to each test tank in the event of extreme effluent or dilution water temperature, unacceptably high concentrations of chlorine in the chlorinated effluent, or the presence of chlorine in the dechlorinated effluent stream. The various systems were also equipped with monitors so that any failure in the supply of effluents or dilution water to the head tanks or to the diluters and any malfunction of the diluters would activate the system.

The bioassay laboratory lighting was from artificial sources only and was requlated to approximate seasonal changes in day length. Light intensity was gradually increased in the morning and decreased in the evening to simulate dawn and dusk,[16] respectively. A combination of General Electric F40 daylight fluorescent bulbs, General Electric F40 plant light fluorescent bulbs, and 40 watt incandescent bulbs was used for illumination.

Progeny of fathead minnows (P. promelas), obtained from stock cultures maintained at the U.S. E.P.A. National Water Quality Laboratory in Duluth, Minnesota, were used for the life cycle tests. These tests were started by placing fifty 1 - 2 day old fry in each test aquarium and monitoring their survival. Any test tank containing less than 15 living fry after 15 - 17 days was restocked with 15 - 17 day old fry which had been reared in 100 percent dilution water to bring the number of fish per aquarium back to 50. This process was necessitated because of the high random mortality of the original stock, which we concluded was caused by the scarcity of natural food after the larvae had absorbed their yolk sac and become dependent upon ingested food. The test animals were photographed at 30 and 60 days into the test. The photographs were enlarged to determine the average lengths of the survivors.

At 60 days into the test the fish population in each test tank was thinned to a maximum of 15 apparently healthy fish, and five spawning substrates (7.6 cm (3 in) lengths of 12.7 cm (5 in) asbestos drain tile cut in half) were placed in each tank. These spawning tiles provided refuge for the fathead minnows, sites for males to establish territories, and substrates on which the females could deposit their eggs. When the fatheads were mature enough for us to definitely determine their sex (170-204 days into the test), each tank was thinned to no more than four males (to eliminate territorial conflict), and the number of females was recorded.

During each day of the spawning period, all eggs produced in each tank were removed, counted, and examined microscopically to determine their condition. Those eggs that we felt, based upon past experience, would go on to hatch if incubated under optimum conditions were considered viable eggs. The number of viable eggs in each spawning was recorded within 24 hours of the time those eggs were produced. Some of the eggs produced in each concentration of each effluent type were incubated to determine the hatchability of eggs spawned in that particular environment. For our purposes, hatchability was defined as the ability of eggs to hatch and produce living fry. If fry only partially emerged from the egg, or hatched and died prior to the end of the incubation period, they were not counted as living fry.

Fifty viable eggs from each spawning were incubated in mesh bottom egg cups in the tank in which they were spawned. When the total number of spawnings in any particular tank equaled the number of females in that tank, we incubated only eggs from every third spawning, except that to minimize weekend work, no eggs were incubated from those spawnings which occurred on Mondays and Tuesdays.

Generally, five days of incubation were required for all viable eggs from a spawning to hatch. The eggs were recounted each day of the incubation period and those which had died were removed. After hatching began, the egg cup was left untouched until all eggs had hatched. At that time the numbers of dead eggs and living and dead fry were recorded. Data from one or more incubation in each effluent type and concentration were utilized to calculate the mean percentage hatchability.

In addition, when time and spawnings permitted, hatchability information was obtained on eggs produced in high concentration effluent tanks and incubated in dilution water tanks, and, conversely, on eggs produced in dilution water tanks and incubated in high concentration effluent tanks.

If more than four of the fifty eggs in any one incubation were unaccounted for, that incubation attempt was discarded. Loss of eggs and/or fry was not uncommon due to their small size. The number of successful incubations was also limited by spawnings that were deposited on the mesh bottom of the egg cups, and intermittent periods when effluents were high in suspended solids, which partially plugged the mesh bottoms and thereby precluded adequate water transfer through the mesh screening.

Forty 1-2 day old fry from each reproducing adult tank were placed in fry chambers receiving the same concentration of effluent as the adult tank in which they were produced. Their lengths were also measured photographically at 30 days, and they were directly weighed, measured, examined, and frozen for future analyses at 60 days of age.

The fry were fed three times each day, once with live brine shrimp nauplii, once with frozen trout starter mash, and once with a diatom culture. Juvenile and adult fish were fed twice each day, once with live brine shrimp nauplii, and once with granular frozen trout food. These feedings were occasionally supplemented with feedings of live *Daphnia magna*. Excess food and other debris were siphoned from the test chambers daily. Fry chambers were not cleaned until the fish were thirty days old.

With few exceptions, the duration of acute toxicity tests was 96 hours. Test animals for acute studies were either reared in the laboratory, purchased from private sources, or obtained from State or Federal fish hatcheries. In all cases they were held in the laboratory at test temperatures at least ten days prior to testing, or until they were determined to be disease free.

All data collected during the life cycle study were stored and analyzed on a Xerox Sigma-6 computer. All water chemistry data, disinfectant residuals, egg production and hatchability data, and fish growth and mortality data were keypunched weekly onto cards which were verified and processed. The computer was programmed to provide printouts of means, standard deviations, and ranges during the course of the study.

In addition, a two-way analysis of variance with unbalanced and nested designs was performed on mortality, spawning, hatchability, and growth data for all treatment types and concentrations using the Statistical Analysis System (S.A.S.).[17]

RESULTS AND DISCUSSION

Water Chemistry

The results of residual chemical determinations in the various effluent streams delivered to the adult test chambers are summarized in Table 14. In many cases the standard deviation was high; this was particularly true for dechlorinated samples. The occasional high sulfite residual levels (above 0.100 mg/l only 17 times out of 208 samples) seemed to occur during those times of low total suspended solids (3-11 mg/l), low volatile suspended solids (1-11 mg/l), and low turbidity (6-14 Jackson turbidity units) values.

Ozone and bromine chloride residuals were more uniform than the sulfite residuals, as evidenced by the standard deviations approaching more closely the mean value for most concentrations, while chlorine residuals were the most uniform with standard deviations approximately half the mean values. The relatively narrow distribution of the chlorine residual determinations can be explained by the fact that chlorine was the only disinfectant whose concentration was regulated by residual control rather than by dosage control. Chlorine residuals were adjusted on an hourly basis. This compensated for changes in demand of the effluent, a feature which was lacking from the other disinfection systems (see Section III).

Table 15 summarizes the residual levels measured in the fry tanks. In almost every instance, residual levels were less, in some cases considerably less, than those in the adult tanks (Table 14). This was attributed to differences in the cleaning and feeding procedures between adult and fry chambers. The adult tanks were fed granulated food and newly-hatched brine shrimp daily, and excessive food and other debris were siphoned out. The fry tanks, on the other hand, were not cleaned at all during the first thirty days of their use, while a daily allotment of diatom culture, granulated food, and brine shrimp were added as feed. As a result, an organic layer formed on the bottom of the fry tanks on which the fry were frequently seen feeding. Although this proved to be an effective feeding method, the presence of the excessive material in the tank increased the demand for disinfectant, which in turn resulted in lower residual levels.

Table 14. THE MEAN RESIDUAL CHEMICAL LEVELS (mg/l), SAMPLE SIZES, AND STANDARD DEVIATIONS MEASURED IN HEAD TANKS AND ADULT TEST CHAMBERS DURING THE LIFE-CYCLE TESTS

Effluent Stream	Sampling Site							
	Head Tank	20% Effluent	14% Effluent	9.8% Effluent	6.9% Effluent	4.8% Effluent	3.4% Effluent	2.4% Effluent
Chlorinated								
Chlorine Residual	1.357	0.101	0.067	0.035	0.024	0.022	0.011	0.008
Sample Size	182	200	156	103	105	105	102	101
Standard Deviation	0.435	0.064	0.038	0.020	0.015	0.015	0.008	0.002

	Sampling Site							
	Head Tank	100% Effluent	50% Effluent	25% Effluent	12.5% Effluent	6.25% Effluent	3.12% Effluent	1.56% Effluent
Dechlorinated								
Sulfite Residual	1.572	0.027	0.012	0.005	0.004	0.005	0.001	0.000
Sample Size	175	248	100	97	96	101	99	99
Standard Deviation	1.13	0.119	0.060	0.021	0.011	0.021	0.004	0.000
Ozonated								
Ozone Residual	0.027	0.012	0.005	0.003	0.002	0.001	0.001	0.001
Sample Size	182	250	100	101	97	97	99	98
Standard Deviation	0.025	0.016	0.003	0.003	0.002	0.002	0.002	0.002
Chlorobrominated								
Bromine Chloride Residual	0.478	0.119	0.032	0.017	0.007	0.005	0.004	0.003
Sample Size	185	173	64	103	103	103	104	103
Standard Deviation	0.476	0.128	0.034	0.016	0.006	0.005	0.004	0.003

Table 15. THE MEAN RESIDUAL CHEMICAL LEVELS (mg/l), SAMPLE SIZES, AND STANDARD DEVIATIONS MEASURED IN FRY TEST CHAMBERS DURING THE LIFE-CYCLE TESTS

Effluent Stream	Sampling Site						
	20% Effluent	14% Effluent	9.8% Effluent	6.9% Effluent	4.8% Effluent	3.4% Effluent	2.4% Effluent
Chlorinated							
Chlorine Residual	0.076	0.053	0.033	0.026	0.023	0.014	0.008
Sample Size	84	35	35	34	38	35	42
Standard Deviation	0.049	0.028	0.017	0.016	0.013	0.008	0.007

	Sampling Site						
	100% Effluent	50% Effluent	25% Effluent	12.5% Effluent	6.25% Effluent	3.12% Effluent	1.56% Effluent
Dechlorinated							
Sulfite Residual	0.015	0.000	0.000	0.002	0.004	0.000	0.000
Sample Size	83	34	37	35	43	33	38
Standard Deviation	0.096	0.000	0.001	0.007	0.023	0.000	0.000
Ozonated							
Ozone Residual	0.012	0.005	0.003	0.003	0.002	0.002	0.002
Sample Size	79	38	39	39	38	40	36
Standard Deviation	0.009	0.004	0.002	0.003	0.002	0.002	0.002
Chlorobrominated							
Bromine Chloride Residual	0.045	0.027	0.011	0.008	0.005	0.003	0.003
Sample Size	73	31	38	47	35	40	37
Standard Deviation	0.041	0.018	0.010	0.007	0.004	0.004	0.003

The average dissolved oxygen concentrations in the effluent head tanks ranged from 2.54 mg/l for the nondisinfected effluent to 8.15 mg/l for the ozonated effluent (Table 16). The dilution water averaged 3.80 mg/l dissolved oxygen, and was the only liquid that was aerated to increase the dissolved oxygen levels. The 100 percent through 12.5 percent effluent concentration tanks (adult and fry) on all life cycle test tables except the chlorinated table were aerated to increase the dissolved oxygen levels. This was not necessary for the chlorinated table because of the lower oxygen demand due to higher effluent dilution factors. Thus, the lowest mean dissolved oxygen level for a 100 percent effluent concentration was 3.93 mg/l in the chlorobrominated fry tanks, while the highest was 5.41 mg/l in the 100 percent chlorobrominated adult tanks. These values range between 47 percent and 65 percent of dissolved oxygen saturation at the test temperature of 25C. Since fathead minnows are capable of surviving dissolved oxygen levels as low as 2 mg/l for several days at 25C, the above values were probably within safe limits for P. promelas.

The alkalinity, acidity, hardness, conductivity, and total ammonia nitrogen values measured in the various streams are summarized in Tables 17 and 18. According to McKee and Wolf[18] the results of these tests are within acceptable tolerance limits for fish. The measured pH of the effluents in the head tanks ranged from 6.9 to 8.0 and was usually between 7.4 and 8.0. The pH of the contents of the fish tanks ranged from 7.0 to 8.2 and was usually between 7.4 and 8.0. In every instance, the mean pH values were lower and the mean acidity values higher in the head tanks than in the highest effluent concentration test chambers. These differences may in part be a result of the presence of food, animals and their by-products. Also, aeration of the test tanks, which received high effluent concentrations, to maintain satisfactory dissolved oxygen levels may have contributed to the observed pH and acidity changes.

With the exception of the chlorinated and chlorobrominated test tanks for which lower effluent concentrations were sampled, all water chemistry values and water temperatures (Table 19) were similar for each of the effluent streams. Thus any negative effects that were observed when each treatment type was compared to the nondisinfected stream was attributed to the particular disinfection process applied to that effluent stream, since the values of other chemical parameters measured were within safe limits.

MORTALITY

Mortalities of the first and second generation fish in the various effluent streams are summarized in Tables 20-29. All survivor counts during the first 60 days of the test were made from photographs of the fish in each tank, while the survivor counts after day 60 were determined through direct observations.

The first generation data included the additional variable of restocking some of the tanks in each effluent stream on day 15, as shown in the respective tables. This restocking was necessitated by the nearly total mortality observed in many tanks during the first two weeks of the study. Because this early mortality occurred in a random pattern and because the growth of the test fish was retarded during this interval, it was concluded that the major

Table 16. THE MEAN DISSOLVED OXYGEN CONCENTRATIONS (mg/l) MEASURED IN STORAGE TANKS AND TEST CHAMBERS DURING THE LIFE CYCLE STUDIES

		Storage Tank	Nominal Percent Effluent Concentrations							
			100	50	25	12.50	6.25	3.12	1.56	0.00
Dilution Water		3.80								
Nondisinfected	Adult	2.54	4.87	4.64	4.95	4.79	4.66	4.69	5.45	5.45
	Fry	2.54	4.14	5.14	4.57	4.23	4.68	4.79	5.32	4.50
Dechlorinated	Adult	4.10	5.37	5.02	4.90	4.61	5.08	5.21	5.87	5.49
	Fry	4.10	4.75	5.69	4.55	5.45	4.44	5.31	5.16	5.04
Chlorobrominated	Adult	3.23	5.41	4.82	4.50	4.16	4.62	5.06	5.51	5.38
	Fry	3.23	3.93	4.41	4.50	4.26	4.15	4.72	5.14	4.70
Ozonated	Adult	8.15	4.70	4.29	4.49	4.46	4.77	5.49	5.40	5.89
	Fry	8.15	4.91	5.04	4.56	4.65	4.73	5.28	5.40	5.90
		Storage Tank	20	14	9.8	6.86	4.80	3.36	2.35	0.00
Chlorinated	Adult	5.46	5.46	4.76	4.62	4.56	4.79	5.37	5.46	5.49
	Fry	5.46	4.00	4.43	5.00	5.06	4.85	5.23	5.22	4.81

Table 17. THE MEAN WATER CHEMISTRY VALUES MEASURED IN HEAD TANKS

Head Tanks	Alkalinity as mg/l $CaCO_3$	Acidity as mg/l $CaCO_3$	Hardness as mg/l $CaCO_3$	Conductivity Micromhos/cm	Total Ammonia Nitrogen (mg/l)
Nondisinfected					
Mean	194	27	288	911	9.3
Sample Size	48	46	47	46	46
Standard Deviation	36.7	6.8	36.9	73.9	5.4
Chlorinated					
Mean	193	28	287	908	9.4
Sample Size	46	46	47	45	45
Standard Deviation	35.8	5.0	39.9	85.3	5.2
Dechlorinated					
Mean	185	37	289	918	9.8
Sample Size	47	45	47	44	46
Standard Deviation	35.3	5.0	43.5	64.5	5.4
Chlorobrominated					
Mean	192	31	289	911	9.1
Sample Size	47	45	45	45	46
Standard Deviation	37.3	6.2	38.3	73.9	5.6
Ozonated					
Mean	193	20	289	911	8.7
Sample Size	46	46	46	45	46
Standard Deviation	41.5	3.4	38.1	71.1	5.3
Dilution Water					
Mean	194	15	465	859	0.16
Sample Size	47	46	47	47	36
Standard Deviation	2.3	2.6	9.3	20.4	0.18

Table 18. THE MEAN WATER CHEMISTRY VALUES MEASURED IN THE HIGHEST EFFLUENT CONCENTRATION ADULT TEST TANKS CONTAINING LIVE FISH

Effluent Stream	Alkalinity as mg/l $CaCO_3$	Acidity as mg/l $CaCO_3$	Hardness as mg/l $CaCO_3$	Conductivity Micromhos/cm	Total Ammonia Nitrogen (mg/l)
Nondisinfected					
Mean	189	15	386	905	9.1
Sample Size	48	46	47	47	45
Standard Deviation	36.6	3.7	36.4	67.5	5.1
Chlorinated[a]					
Mean	191	15	437	875	1.3
Sample Size	37	36	37	37	37
Standard Deviation	5.2	2.7	7.2	17.2	0.9
Dechlorinated					
Mean	180	17	293	911	9.2
Sample Size	47	45	47	45	46
Standard Deviation	35.4	4.3	52.4	64.7	4.9
Chlorobrominated[a]					
Mean	184	22	369	874	5.2
Sample Size	29	27	28	29	28
Standard Deviation	20.0	37.0	10.0	27.7	2.8
Ozonated					
Mean	187	16	285	906	8.5
Sample Size	47	46	47	47	46
Standard Deviation	36.4	3.5	35.8	68.5	4.8
Dilution Water					
Mean	193	10	466	856	0.18
Sample Size	48	46	46	46	45
Standard Deviation	2.5	2.5	8.1	26.9	0.35

[a]The chlorinated test tank sampled was 14% effluent, the chlorobrominated test tank sampled was 50% effluent; and the remaining test tanks sampled were 100% effluent, with the exception of dilution water. This accounts for the difference in hardness, conductivity, and ammonia nitrogen levels.

Table 19. MEAN WATER TEMPERATURES ($^{\circ}C$)
MEASURED IN STORAGE TANKS AND ADULT TEST CHAMBERS
DURING THE LIFE CYCLE STUDIES

Effluent Type	Mean Temperature In Storage Tank	Mean Temperature In Aquaria[a]
Nondisinfected	26.5	24.9
Chlorinated	27.3	25.1
Dechlorinated	27.3	24.7
Chlorobrominated	26.4	25.1
Ozonated	26.2	24.9

[a] All temperatures were measured in aquaria containing a 50 percent effluent concentration, except in the chlorinated effluent stream where temperatures were recorded in an aquarium containing a 20 percent effluent concentration.

cause of this mortality was an insufficient supply of food (microscopic organisms) as the fish became dependent upon ingested food rather than their yolk sacs. Those tanks that were restocked showed higher survival rates at days 23 and 53 of the study than did comparable tanks that had not been restocked. However, no difference in survival was detected between restocked and nonrestocked tanks after the tanks were thinned to 15 fish each on day 53.

Nondisinfected Effluent

The only pattern of mortality observed in the first generation fish reared in nondisinfected effluent was that observed during the first two weeks of the study (Table 20). The greatest mortality during that period occurred in the two highest effluent concentrations, 50 and 100 percent, and both duplicates in these concentrations required restocking on Day 15.

The second generation of fish exposed to nondisinfected effluent showed a variable rate of survival which, except for the undiluted effluent, was not clearly dependent upon effluent concentration (Table 21). While only four survivors were observed in the 100 percent effluent concentration, mortality occurred early in life as in the first generation test animals which apparently suffered from an inadequate food supply.

In considering these results, it is necessary to point out that the quality of the effluent to which the two generations were exposed differed substantially. For example, during the first two months of their lives the first generation test animals were exposed to treated wastewater with mean monthly suspended solids levels and total phosphate concentrations of approximately 20-40 mg/l and 8 mg/l, respectively. This contrasts with the exposure of second generation test animals of similar age to mean monthly suspended solids and total phosphate concentrations of 10-15 mg/l and $<$2 mg/l, respectively.

It appeared, then, that both first and second generation P. promelas were subject to significantly higher mortality during the first 15 or 30 days of the test, respectively, when exposed to 100 percent nondisinfected effluent. This mortality probably resulted from a combination of factors, including the supply of microscopic food organisms available to the young fish as well as the generally unfavorable environmental conditions which occurred in the 100 percent effluent concentrations.

Table 20. NUMBER OF FIRST GENERATION P. PROMELAS SURVIVING IN NONDISINFECTED EFFLUENT

	Nominal Percent Nondisinfected Effluent							
	0.00	1.56	3.12	6.25	12.50	25.00	50.00	100.00
No. of fish alive at day 23[a]	87	71[b]	64[b]	76[b]	78[b]	79[b]	96[c]	92[c]
C.I.[d] for prob. of survival thru day 23	0.79-0.92	0.61-0.79	0.54-0.73	0.67-0.83	0.69-0.85	0.70-0.86	0.90-0.98	0.85-0.96
Survival/100 at day 53	84	71	63	74	78	79	89	85
C.I. for prob. of survival thru day 53	0.76-0.90	0.61-0.79	0.53-0.72	0.65-0.82	0.69-0.85	0.70-0.86	0.81-0.94	0.77-0.91
No. of fish alive from day 53 until day 330 or death[e]	26	27	23	20	23	29	25	25
Number of fish alive at: 90 days	26	27	23	20	23	29	25	25
120 days	26	27	23	20	23	29	25	25
150 days	26	26	23	20	23	29	25	25
180 days	26	26	23	20	23	29	25	25
210 days	26	26	23	20	23	29	25	25
240 days	26	26	23	20	23	29	25	25
270 days	26	26	23	20	23	29	25	25
300 days	25	25	22	19	23	28	25	24
330 days	25	25	22	19	23	28	25	24
C.I. for prob. of survival day 53-330	0.81-0.99	0.76-0.99	0.79-0.99	0.76-0.99	0.86-1.00	0.83-0.99	0.87-1.00	0.80-0.99

[a] From the original 100 fish that were stocked in each concentration
[b] One of the duplicate tanks restocked on day 15
[c] Both of the duplicate tanks restocked on day 15
[d] 95 percent confidence interval for the true probability of survival
[e] The number of fish in each effluent concentration was reduced to 30 on day 53 and excess males were subsequently removed prior to the spawning season.

Table 21. NUMBER OF SECOND GENERATION *P. PROMELAS* SURVIVING IN THE NONDISINFECTED EFFLUENT[a]

	Nominal Percent of Nondisinfected Effluent							
	0.00	1.56	3.12	6.25	12.50	25.00	50.00	100.00
30 Days of Age								
No. Surviving	49	35	44	46	37	72	31	4
Conf. Interval[b]	0.50-0.72	0.33-0.55	0.44-0.65	0.47-0.68	0.36-0.57	0.81-0.95	0.62-0.88	0.01-0.12
60 Days of Age								
No. Surviving	49	35	41	46	35	72	31	4
Conf. Interval	0.50-0.72	0.33-0.55	0.40-0.62	0.47-0.68	0.33-0.55	0.81-0.95	0.62-0.88	0.01-0.12

[a]40 fish were started in each of the two duplicate tanks of each effluent concentration, except for the 50 percent concentration for which only one tank with 40 fish was started.

[b]95 percent confidence interval for the true probability of survival.

Chlorinated Effluent

The first generation of fathead minnows reared in chlorinated effluent suffered lethal effects in the two highest effluent concentrations between days 23 and 53 of the study (Table 22). This observation is important not only because the highest concentration of chlorinated effluent to which fish were exposed was only 20 percent, but also because both duplicate tanks of these concentrations were restocked on day 15, an action that normally enhanced survival at days 23 and 53.

Fish reared in both 20 and 14 percent chlorinated effluent concentrations also exhibited a higher mortality after day 53, but the cause of that mortality was apparently not excessively high levels of residual chlorine. On days 72 and 73 of the study, 26 fish died in the 20 percent effluent concentration tanks, and 12 died in the 14 percent effluent concentration tanks. Although the highest total residual chlorine measured on those days was 0.188 mg/l in the 20 percent effluent concentration tanks and 0.163 mg/l in the 14 percent effluent tanks, these temporary residuals were apparently not extreme since no lethal effects were observed at other times when those residuals were exceeded. However, from days 66 to 71 the fish had been exposed to unchlorinated effluent because of mechanical problems. The ensuing mortality brought on by renewed exposure to chlorine suggests that the test animals had lost some of their tolerance to chlorine during that brief period of no exposure. Our data and experience indicate that fathead minnows maintained in sublethal levels of chlorinated effluent develop a tolerance which permits them to survive in concentrations of chlorinated effluent which would normally be lethal to nonacclimated individuals of the same species. Other data which tend to support this apparent acclimation phenomenon are documented and discussed in the section dealing with acute toxicity studies (Section VI).

The second generation test fish exhibited lethal effects in the 20 percent effluent concentration (Table 23). Fish reared in 14 percent effluent showed a tendency toward reduced survival, although the 95 percent confidence intervals for their probability of survival overlapped with those of fish reared in less concentrated effluents.

The principal difference in the mortality patterns of the first and second generation fish in 20 percent chlorinated effluent was that the first generation showed excessive mortality between 23 and 53 days of life, while the second generation showed a lethal effect only during the first 30 days of life. While a lethal effect may have been masked by the restocking of first generation fish on day 15, it is known that the two generations of test animals were exposed to different total residual chlorine levels. A minimum mean total chlorine residual of 0.045 mg/l was apparently necessary to exert a lethal effect during the first 60 days of life. These findings agree well with those of Arthur, _et al._,[1] who found that long-term exposure of _P. promelas_ to a mean chlorine residual of 0.042 mg/l was lethal, but a mean chlorine residual of 0.014 mg/l was not. However, in our study, older fathead minnows survived mean total chlorine residuals as high as 0.074 mg/l for 180 days

Table 22. NUMBER OF FIRST GENERATION *P. PROMELAS* SURVIVING IN CHLORINATED EFFLUENT

	Nominal Percent Chlorinated Effluent							
	0.00	2.35	3.36	4.80	6.86	9.80	14.00	20.00
No. of fish alive at day 23[a]	66[b] (0.000)[d]	71[b] (0.002)	52 (0.003)	67[b] (0.007)	81[b] (0.010)	93[c] (0.013)	95[c] (0.020)	87[c] (0.038)
C.I.[e] for prob. of survival thru day 23	0.56–0.75	0.61–0.79	0.42–0.62	0.57–0.75	0.72–0.87	0.86–0.97	0.89–0.98	0.79–0.92
No. of fish alive at day 53[a]	64 (0.000)	69 (0.004)	52 (0.006)	65 (0.016)	80 (0.016)	85 (0.025)	72 (0.045)	43 (0.076)
C.I. for prob. of survival thru day 53	0.54–0.73	0.59–0.77	0.42–0.62	0.55–0.74	0.71–0.87	0.77–0.91	0.63–0.80	0.34–0.53
No. of fish alive from day 53 until day 330 or death[f]	26	23	24	25	21	24	28	30
Number of fish alive at: 90 days	26 (0.000)	23 (0.004)	24 (0.007)	25 (0.020)	21 (0.020)	23 (0.030)	15 (0.057)	1 (0.088)
120 days	26 (0.000)	23 (0.006)	24 (0.010)	25 (0.023)	21 (0.023)	23 (0.036)	15 (0.063)	1 (0.092)
150 days	26 (0.000)	22 (0.007)	24 (0.011)	25 (0.024)	20 (0.026)	23 (0.038)	15 (0.067)	1 (0.098)
180 days	26 (0.000)	22 (0.009)	24 (0.012)	25 (0.026)	20 (0.028)	23 (0.039)	15 (0.074)	1 (0.103)
210 days	26 (0.000)	22 (0.009)	23 (0.012)	25 (0.025)	20 (0.027)	22 (0.038)	15 (0.074)	1 (0.105)
240 days	25 (0.000)	22 (0.008)	23 (0.012)	25 (0.025)	20 (0.026)	22 (0.038)	15 (0.073)	1 (0.106)
270 days	25 (0.000)	22 (0.008)	23 (0.012)	25 (0.023)	20 (0.026)	22 (0.037)	15 (0.073)	1 (0.105)
300 days	25 (0.000)	22 (0.008)	22 (0.012)	25 (0.023)	20 (0.025)	22 (0.036)	15 (0.071)	1 (0.103)
330 days	25 (0.000)	22 (0.008)	22 (0.011)	25 (0.022)	20 (0.024)	22 (0.035)	15 (0.067)	1 (0.102)
C.I. for prob. of survival day 53–330	0.75–0.99	0.79–0.99	0.74–0.98	0.87–1.00	0.77–0.99	0.74–0.98	0.34–0.72	0.01–0.17

[a] From the original 100 fish that were stocked in each concentration
[b] One of the duplicate tanks restocked on day 15
[c] Both of the duplicate tanks restocked on day 15
[d] Mean total residual chlorine (mg/l)
[e] 95 percent confidence interval for the true probability of survival
[f] The number of fish in each effluent concentration was reduced to 30 on day 53, and excess males were subsequently removed prior to the spawning season.

Table 23. NUMBER OF SECOND GENERATION *P. PROMELAS* SURVIVING IN THE CHLORINATED EFFLUENT[a]

	Nominal Percent Chlorinated Effluent							
	0.00	2.35	3.36	4.80	6.86	9.80	14.00	20.00
30 Days of Age								
No. Surviving	53	52	63	59	67	62	49	26
Conf. Interval[b]	0.55–0.76	0.54–0.75	0.69–0.86	0.63–0.82	0.74–0.90	0.67–0.85	0.50–0.71	0.23–0.43
$\overline{X}$ Residual, mg/l[c]	0.000	0.008	0.004	0.018	0.016	0.028	0.033	0.045
60 Days of Age								
No. Surviving	53	51	64	59	66	62	47	26
Conf. Interval	0.55–0.76	0.53–0.73	0.70–0.87	0.63–0.82	0.73–0.89	0.67–0.85	0.48–0.69	0.23–0.43
$\overline{X}$ Residual, mg/l	0.000	0.008	0.009	0.020	0.016	0.033	0.035	0.033

[a]40 fish were started in each of the two duplicate tanks of each effluent concentration.

[b]95 percent confidence interval for the true probability of survival.

[c]Mean total residual chlorine.

without any lethal effect, indicating that the chlorine tolerance of *P. promelas* increases with age and/or size.

Dechlorinated Effluent

Sixty-one percent of the first generation fish reared in the 100 percent dechlorinated effluent died within the first 23 days of exposure and, by day 53, mortality had increased to 64 percent (Table 24).

The second generation fish exhibited greater mortality in 100 percent dechlorinated effluent (Table 25). However, these test animals were exposed to much lower mean sulfite residuals (0.005-0.010 mg/l) than the first generation fish (0.042-0.080 mg/l).

The significance of the mortality observed in the 100 percent dechlorinated effluent was obscured by the fact that similar mortality occurred in the 100 percent nondisinfected effluent and by the varied restocking histories of different tanks.

Thus it is impossible to ascertain the true cause of the observed mortality in the 100 percent dechlorinated effluent tanks. Since other investigators[1] have found no lethal effect on fathead minnows with long-term exposure to mean residual sulfite concentrations of 0.104 mg/l, it would appear that our sulfite residuals did not account for the observed mortality.

While the lethality of 100 percent dechlorinated effluent is questionable, it is probably not an important consideration since it has no application to normal wastewater disposal practices. The important point to be gained here is that dechlorination eliminated the lethal effect of the 20 percent chlorinated effluent, and appeared to eliminate the toxicity of 50 percent chlorinated effluent.

Chlorobrominated Effluent

First generation fish reared in 100 and possibly 50 percent chlorobrominated effluent appeared to exhibit lethal effects at both 23 and 53 days of age (Table 26), as evidenced by the reduced survival observed even after restocking. Since the fish living in 100 percent chlorobrominated effluent were the only test animals which clearly showed a lethal response to a 100 percent effluent concentration between days 23 and 53, such mortality appeared to be related to the bromine chloride concentration in the effluent. Almost all of the mortality during this interval occurred over a two-day period when high bromine chloride residuals, resulting from lower demand due to better effluent quality, were measured in the test aquaria. The maximum bromine chloride residual measured during this period was 0.651 mg/l in the 100 percent effluent fish tanks. That value was several times higher than the 96 hour TL50 values for fathead minnows discussed in the acute toxicity section (Section VI) of this report.

On days 76 and 77 a mechanical failure of the bromine chloride feed system resulted in a fish kill in the 100 percent effluent tanks, where bromine

Table 24. NUMBER OF FIRST GENERATION *P. PROMELAS* SURVIVING IN DECHLORINATED EFFLUENT

	Nominal Percent Dechlorinated Effluent							
	0.00	1.56	3.12	6.25	12.50	25.00	50.00	100.00
No. of fish alive at day 23[a]	94[c] (0.000)[d]	60[b] (0.000)	56[b] (0.000)	75[c] (0.008)	68[b] (0.002)	83[c] (0.000)	59 (0.000)	39[c] (0.080)
C.I.[e] for prob. of survival thru day 23	0.88-0.97	0.50-0.69	0.46-0.65	0.66-0.82	0.58-0.76	0.74-0.89	0.49-0.68	0.30-0.49
No. of fish alive at day 53[a]	81 (0.000)	49 (0.000)	56 (0.000)	61 (0.010)	64 (0.010)	66 (0.000)	56 (0.000)	36 (0.042)
C.I. for prob. of survival thru day 53	0.72-0.87	0.39-0.59	0.46-0.65	0.51-0.70	0.54-0.73	0.56-0.75	0.46-0.65	0.27-0.46
No. of fish alive from day 53 until day 330 or death[f]	24	24	26	23	24	20	23	21
Number of fish alive at:								
90 days	24 (0.000)	22 (0.000)	26 (0.002)	23 (0.018)	24 (0.012)	20 (0.011)	23 (0.027)	21 (0.060)
120 days	24 (0.000)	22 (0.000)	26 (0.002)	23 (0.013)	24 (0.009)	20 (0.009)	23 (0.021)	21 (0.045)
150 days	24 (0.000)	22 (0.000)	26 (0.002)	23 (0.011)	24 (0.007)	20 (0.008)	23 (0.016)	21 (0.036)
180 days	24 (0.000)	22 (0.000)	26 (0.001)	23 (0.009)	24 (0.006)	20 (0.007)	23 (0.020)	21 (0.034)
210 days	24 (0.000)	22 (0.000)	25 (0.001)	23 (0.008)	24 (0.005)	20 (0.007)	23 (0.018)	21 (0.031)
240 days	24 (0.000)	22 (0.000)	25 (0.001)	23 (0.007)	24 (0.005)	20 (0.006)	23 (0.016)	21 (0.029)
270 days	24 (0.000)	22 (0.000)	25 (0.001)	23 (0.007)	24 (0.005)	19 (0.006)	23 (0.014)	21 (0.027)
300 days	24 (0.000)	22 (0.000)	25 (0.001)	23 (0.006)	24 (0.004)	18 (0.005)	23 (0.014)	21 (0.028)
330 days	24 (0.000)	22 (0.000)	25 (0.001)	23 (0.005)	24 (0.004)	18 (0.005)	23 (0.012)	21 (0.026)
C.I. for prob. of survival day 53-330	0.86-1.00	0.74-0.98	0.81-0.99	0.86-1.00	0.86-1.00	0.69-0.98	0.86-1.00	0.84-1.00

[a] From the original 100 fish that were stocked in each concentration
[b] One of the duplicate tanks restocked on day 15
[c] Both of the duplicate tanks restocked on day 15
[d] Mean residual sulfur dioxide as sulfite (mg/l)
[e] 95 percent confidence interval for the true probability of survival
[f] The number of fish in each effluent concentration was reduced to 30 on day 53, and excess males were subsequently removed prior to the spawning season.

Table 25. NUMBER OF SECOND GENERATION P. PROMELAS SURVIVING IN THE DECHLORINATED EFFLUENT[a]

	Nominal Percent Dechlorinated Effluent							
	0.00	1.56	3.12	6.25	12.50	25.00	50.00	100.00
30 Days of Age								
No. Surviving	45	37	56	45	51	67	39	3
Conf. Interval[b]	0.45-0.66	0.36-0.57	0.59-0.79	0.45-0.67	0.53-0.73	0.74-0.90	0.87-1.00	0.01-0.10
$\overline{X}$ Residual, mg/l[c]	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.010
60 Days of Age								
No. Surviving	43	37	58	44	47	66	39	3
Conf. Interval	0.43-0.64	0.36-0.57	0.62-0.81	0.44-0.65	0.48-0.69	0.73-0.89	0.87-1.00	0.01-0.10
$\overline{X}$ Residual, mg/l	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.005

[a] 40 fish were started in each of the two duplicate tanks of each effluent concentration, except for the 50 percent concentration for which only one tank with 40 fish was started.

[b] 95 percent confidence interval for the true probability of survival

[c] Mean residual sulfite.

Table 26. NUMBER OF FIRST GENERATION *P. PROMELAS* SURVIVING IN CHLOROBROMINATED EFFLUENT

	Nominal Percent Chlorobrominated Effluent							
	0.00	1.56	3.12	6.25	12.50	25.00	50.00	100.00
No. of fish alive at day 23[a]	95[b] (0.000)[d]	62[c] (0.000)	67[c] (0.001)	74[c] (0.000)	96[b] (0.002)	53 (0.008)	65[b] (0.018)	52[b] (0.054)
C.I.[e] for prob. of survival thru day 23	0.89-0.98	0.52-0.71	0.57-0.75	0.65-0.82	0.90-0.98	0.43-0.62	0.55-0.74	0.42-0.62
No. of fish alive at day 53[a]	88 (0.000)	60 (0.000)	67 (0.002)	71 (0.002)	92 (0.006)	51 (0.019)	62 (0.043)	31 (0.129)
C.I. for prob. of survival thru day 53	0.80-0.93	0.50-0.69	0.57-0.75	0.61-0.79	0.85-0.95	0.41-0.61	0.52-0.71	0.23-0.41
No. of fish alive from day 53 until day 330 or death[f]	24	22	23	23	23	20	24	30
Number of fish alive at: 90 days	24 (0.000)	22 (0.001)	23 (0.002)	23 (0.003)	23 (0.007)	20 (0.021)	24 (0.048)	26 (0.144)
120 days	24 (0.000)	22 (0.001)	23 (0.002)	23 (0.004)	23 (0.008)	20 (0.022)	24 (0.052)	26 (0.142)
150 days	24 (0.000)	22 (0.002)	23 (0.003)	23 (0.004)	23 (0.008)	20 (0.020)	24 (0.043)	0 (0.141)
180 days	24 (0.000)	22 (0.003)	23 (0.004)	23 (0.005)	23 (0.008)	20 (0.019)	23 (0.039)	0 (0.141)
210 days	24 (0.000)	22 (0.003)	23 (0.004)	23 (0.005)	23 (0.008)	20 (0.019)	23 (0.038)	0 (0.141)
240 days	24 (0.000)	21 (0.003)	23 (0.004)	23 (0.005)	23 (0.008)	20 (0.018)	23 (0.036)	0 (0.127)
270 days	24 (0.000)	20 (0.003)	21 (0.004)	19 (0.005)	23 (0.008)	20 (0.018)	23 (0.035)	0 (0.122)
300 days	23 (0.000)	20 (0.003)	20 (0.004)	19 (0.005)	23 (0.008)	20 (0.017)	23 (0.033)	0 (0.119)
330 days	23 (0.000)	20 (0.003)	20 (0.004)	19 (0.005)	23 (0.007)	20 (0.017)	23 (0.033)	0 (0.119)
C.I. for prob. of survival day 53-330	0.79-0.99	0.71-0.98	0.67-0.97	0.62-0.97	0.86-1.00	0.84-1.00	0.79-0.99	0.00-0.13

[a] From the original 100 fish that were stocked in each concentration
[b] Both duplicate tanks restocked on day 15
[c] One of the duplicate tanks restocked on day 15
[d] Mean residual bromine chloride (mg/l)
[e] 95 percent confidence interval for the true probability of survival
[f] The number of fish in each effluent concentration was reduced to 30 on day 53, and excess males were subsequently removed prior to the spawning season.

chloride residuals of 0.628 mg/l were measured. The mortality in 100 percent effluent on day 121 occurred after the fish were exposed to measured bromine chloride residuals of 0.020 mg/l following three and one-half days when, because of mechanical failures in the bromine chloride dosing system, the test fish were exposed to nondisinfected effluent. Since other instances of exposure to much higher bromine chloride residuals were not lethal to fathead minnows, it appeared that, just as with chlorinated effluent, fish in chlorobrominated effluent developed a tolerance to levels of residual bromine chloride which might have killed nonacclimated fish. This acclimation was apparently lost during the three and one-half day period when the fish were not exposed to residual bromine chloride.

Mortality of the second generation fish (Table 27) was observed only in the 100 percent and the 3.12 percent effluent concentrations after 30 and 60 days of exposure. Considering that the bromine residual in the 100 percent effluent concentration was almost ten-fold greater than that in the 3.12 percent effluent concentration, the observed mortalities do not appear to be due to residual bromine chloride. Unlike their first generation counterparts, second generation fish in 50 percent effluent did not appear to suffer any lethal effects. However, the latter had the advantage of being subjected to less variation in residual bromine levels because the effluent quality was consistently high during their exposure period. Thus, even though first and second generation fish in 100 and 50 percent effluent were subjected to nearly the same mean residual bromine concentrations during their first month of life, the highest values to which first generation fish were exposed were several times higher than those to which second generation fish were exposed. These data suggest that long-term exposure of first and second generation fathead minnows to chlorobrominated effluent will not be lethal except when fish are temporarily exposed to unnecessarily high levels of residual bromine chloride or are suddenly exposed to chlorobrominated effluent following a period of nonexposure.

Ozonated Effluent

First generation fish exhibited no lethal effects attributable to ozonated effluent (Table 28). Most of the mortality after 53 days occurred between days 219 and 276 of the test. During that interval a total of 14 male and 4 female fathead minnows died for no apparent reason in the 1.56, 3.12, 6.25, and 25.00 percent effluent concentrations. A similar pattern of mortality occurred in the chlorobrominated effluent stream where a total of 8 males died during the same interval in effluent concentrations of 1.56, 3.12, and 6.25 percent. This mortality did not appear to be the result of exhaustion from spawning in either of these effluent streams, since the fish that died had not been the most productive. The exact cause of this mortality was never determined.

Second generation fish reared in the ozonated effluent and exposed to approximately the same residual ozone levels as the first generation test animals also failed to exhibit a definite lethal response pattern (Table 29). These data suggest that long-term exposure to ozonated effluent will not be toxic or lethal to fathead minnows.

Table 27. NUMBER OF SECOND GENERATION _P. PROMELAS_ SURVIVING IN THE CHLOROBROMINATED EFFLUENT[a]

	Nominal Percent Chlorobrominated Effluent							
	0.00	1.56	3.12	6.25	12.50	25.00	50.00	100.00
30 Days of Age								
No. Surviving	71	61	19	64	76	44	36	34
Conf. Interval[b]	0.80-0.94	0.66-0.84	0.16-0.34	0.70-0.87	0.88-0.98	0.44-0.65	0.77-0.96	0.32-0.53
$\overline{X}$ Residual, mg/l[c]	0.000	0.003	0.005	0.006	0.006	0.012	0.020	0.045
60 Days of Age								
No. Surviving	69	60	19	62	73	42	36	33
Conf. Interval	0.77-0.92	0.65-0.83	0.16-0.34	0.67-0.85	0.83-0.96	0.42-0.63	0.77-0.96	0.31-0.52
$\overline{X}$ Residual, mg/l	0.000	0.002	0.004	0.006	0.006	0.014	0.024	0.034

[a]40 fish were started in each of the two duplicate tanks of each effluent concentration, except for the 50 percent concentration for which only one tank with 40 fish was started.

[b]95 percent confidence interval for the true probability of survival.

[c]Mean residual bromine chloride.

Table 28. NUMBER OF FIRST GENERATION P. PROMELAS SURVIVING IN OZONATED EFFLUENT

	Nominal Percent Ozonated Effluent							
	0.00	1.56	3.12	6.25	12.50	25.00	50.00	100.00
No. of fish alive at day 23[a]	97[b] (0.000)[c]	79 (0.000)	56 (0.000)	58 (0.000)	64 (0.000)	71 (0.000)	43 (0.003)	94[b] (0.010)
C.I.[d] for prob. of survival thru day 23	0.92-0.99	0.70-0.86	0.46-0.65	0.48-0.67	0.54-0.73	0.61-0.79	0.34-0.53	0.88-0.97
No. of fish alive at day 53[a]	95 (0.000)	76 (0.000)	56 (0.000)	55 (0.000)	63 (0.000)	69 (0.000)	43 (0.002)	93 (0.016)
C.I. for prob. of survival thru day 53	0.89-0.98	0.67-0.83	0.46-0.65	0.45-0.64	0.53-0.72	0.59-0.77	0.35-0.59	0.86-0.97
No. of fish alive from day 53 until day 330 or death[f]	22	15	26	20	23	29	25	21
Number of fish alive at: 90 days	22 (0.000)	15 (0.000)	26 (0.000)	20 (0.000)	23 (0.000)	29 (0.001)	25 (0.003)	21 (0.014)
120 days	22 (0.000)	15 (0.000)	26 (0.000)	20 (0.001)	23 (0.001)	29 (0.002)	25 (0.004)	20 (0.012)
150 days	21 (0.000)	15 (0.000)	26 (0.001)	20 (0.001)	23 (0.001)	28 (0.002)	25 (0.004)	20 (0.012)
180 days	20 (0.000)	15 (0.001)	26 (0.001)	20 (0.001)	23 (0.002)	28 (0.002)	24 (0.004)	20 (0.012)
210 days	20 (0.000)	15 (0.001)	26 (0.001)	20 (0.001)	23 (0.002)	28 (0.002)	24 (0.004)	20 (0.012)
240 days	20 (0.000)	14 (0.001)	19 (0.001)	18 (0.001)	23 (0.002)	27 (0.003)	24 (0.004)	20 (0.012)
270 days	20 (0.000)	12 (0.001)	19 (0.001)	16 (0.001)	23 (0.002)	26 (0.003)	24 (0.004)	20 (0.011)
300 days	18 (0.000)	12 (0.001)	19 (0.001)	15 (0.001)	23 (0.002)	26 (0.003)	24 (0.004)	20 (0.011)
330 days	18 (0.000)	12 (0.001)	19 (0.001)	15 (0.001)	23 (0.002)	26 (0.003)	24 (0.005)	20 (0.012)
C.I. for prob. of survival day 53-330	0.85-1.00	0.52-0.95	0.53-0.88	0.51-0.91	0.86-1.00	0.73-0.97	0.80-0.99	0.77-0.99

[a] From the original 100 fish that were stocked in each concentration
[b] Both of the duplicate tanks restocked on day 15
[c] Mean residual ozone (mg/l)
[d] 95 percent confidence interval for the true probability of survival
[e] The number of fish in each effluent concentration was reduced to 30 on day 53, and excess males were subsequently removed prior to the spawning season.

Table 29. NUMBER OF SECOND GENERATION *P. PROMELAS* SURVIVING IN THE OZONATED EFFLUENT[a]

	Nominal Percent Ozonated Effluent							
	0.000	1.56	3.12	6.25	12.50	25.00	50.00	100.00
30 Days of Age								
No. Surviving	60	32	60	35	49	46	48	26
Conf. Interval[b]	0.65-0.83	0.30-0.51	0.65-0.83	0.33-0.55	0.50-0.71	0.47-0.68	0.49-0.70	0.23-0.43
$\bar{X}$ Residual, mg/l[c]	0.000	0.002	0.003	0.004	0.004	0.004	0.004	0.013
60 Days of Age								
No. Surviving	60	31	60	35	48	46	46	26
Conf. Interval	0.65-0.83	0.29-0.50	0.65-0.83	0.33-0.55	0.49-0.70	0.47-0.68	0.47-0.68	0.23-0.43
$\bar{X}$ Residual, mg/l	0.000	0.001	0.003	0.003	0.003	0.004	0.005	0.012

[a] 40 fish were started in each of the two duplicate tanks of each effluent concentration.

[b] 95 percent confidence interval for the true probability of surviving.

[c] Mean residual ozone.

A Comparison of Treatments

The comparison of respective effluent concentrations in the various effluent streams was complicated by several major factors. First, the history of all tanks was not uniform since some had been restocked on day 15 of the test. Second, the variability of the mortality data in identical dilution water control tanks of the various treatment streams could not be ignored. While every effort was made to provide similar environments in each dilution water control tank, analysis of the survival data, and other data as well, showed that statistical differences were occasionally observed in the data from these controls. Another factor which caused difficulty in treatment comparisons was that, after 53 days of age, first generation test fish appeared to be more affected by accidents or mechanical failures than by exposure to the various treatment systems per se. Thus, first generation data for the period 53-330 days of age failed to demonstrate a consistent lethal effect by any treatment, even though differences were noted among the various treatments.

Both duplicate 100 percent effluent tanks were restocked with first generation fish in all effluent streams except the chlorinated stream, where the highest effluent concentration was only 20 percent. The data in Tables 20, 24, 26, and 28 showed that fish reared in 100 percent dechlorinated and 100 percent chlorobrominated effluent exhibited lethal effects at 23 and 53 days of age, but fish reared in 100 percent nondisinfected and 100 percent ozonated effluents did not. The data from the second generation test animals neither supported nor refuted this observation due to the exceptionally high mortality in the 100 percent nondisinfected effluent stream and the generally lower chemical residuals to which the test animals were exposed.

Both duplicate tanks of the 50 percent nondisinfected and 50 percent chlorobrominated effluent were restocked with first generation fish, and at 23 and 53 days of age fewer fish survived the chlorobrominated effluent. None of the tanks containing 50 percent concentrations of dechlorinated or ozonated effluent were restocked, and no difference in survival to age 53 days was observed in these two streams. The second generation fish exposed to 50 percent effluent concentrations displayed similar survival patterns in all streams, although survival in ozonated effluent appeared to be lower than in dechlorinated and chlorobrominated effluent.

The survival patterns of first generation fish in 25 percent nondisinfected, dechlorinated, chlorobrominated, and ozonated effluent were similar over the first 53 days of the test. Comparison of the latter survival patterns with those of first generation fish exposed to the 20 percent chlorinated effluent revealed two important points: (1) the chlorinated effluent was toxic, and (2) the toxicity of the chlorinated effluent stream was completely eliminated by dechlorination with SO_2.

Survival of second generation fish in 25 percent nondisinfected effluent exceeded survivals in 25 percent chlorobrominated and ozonated effluent concentrations. However, since this would not correlate with the expected relationship between effluent concentration and mortality, i.e., higher mortality in higher effluent concentrations, we concluded that random mortality accounted for the observed differences. The mortality of second generation fish in 20 percent chlorinated effluent exceeded the mortalities observed in any of the 25 percent effluent streams. However, this lethal effect was eliminated by dechlorination.

No significant trends in mortality were observed in any of the lower effluent concentrations. Thus, only the 100 percent dechlorinated effluent, 100 and 50 percent chlorobrominated effluent, and 20 percent chlorinated effluent were lethal to fathead minnows. The lethality of 20 percent chlorinated effluent was eliminated by dechlorination, and the data in Table 24 suggested that the lethal effects of chlorinated effluent concentrations of up to at least 50 percent were eliminated by SO_2. The excessive mortality in 100 percent dechlorinated effluent may have been the result of products formed by the addition of SO_2, by inadequate neutralization of residual chlorine, by compounds formed in the chlorination process and not destroyed in the dechlorination process, or by the inherent lethality that was observed in the 100 percent concentration of the nondisinfected stream.

The findings on the chronic toxicity of chlorinated effluent agree well with the work of Arthur, et al.,[1] who found that 5 and 10 percent effluent concentrations with mean total residual chlorine levels of 0.042 and 0.110 mg/l, respectively, were lethal to fathead minnows, and that the application of sulfur dioxide eliminated the toxicity of those effluents. Other investigators have reported success in detoxifying chlorinated effluent with sodium thiosulfate[6] and bisulfite.[3]

GROWTH

When reviewing the growth data of all first generation test animals, some consideration of the restocking history of the various tanks must be given. A comparison of growth in restocked and nonrestocked duplicate tanks indicated that restocked fish did not grow as well as their nonrestocked counterparts. Thus, an inherent bias for reduced length was assumed in any test condition where restocking occurred on day 15. Further, one should note the variability in growth of both generations of test animals in the dilution water control tanks, (Tables 35-39) where environmental conditions were, for all practical purposes, identical. One environmental variable which was not measured was the quantity of microorganisms available as a food supply for first generation fish. The importance of this factor was well illustrated by the fact that when special attention was given to insure that an adequate supply of microorganisms was available as food for the second generation fry, they grew approximately twice as long in their first 30 days of life as the first generation test animals grew in their first 23 days of life.

The first generation test fish reared in nondisinfected effluent showed no significant differences in length at the termination (day 330) of the study (Table 30). However, the first generation fish reared in 50 and 100 percent concentrations of nondisinfected effluent were significantly shorter in mean length at 23 and 53 days of age than the fish reared in dilution water control tanks. Similar retardation of growth was not exhibited by the second genera-

Table 30. MEAN LENGTHS (IN mm) OF FIRST AND SECOND GENERATION P. promelas REARED IN NONDISINFECTED EFFLUENT AND IN DILUTION WATER

Age of Fish and Data	Nominal Percent Nondisinfected Effluent Concentration							
	Dilution H_2O (A)	1.56 (B)	3.12 (C)	6.25 (D)	12.50 (E)	25.0 (F)	50.0 (G)	100.0 (H)
First Generation								
23 days								
N	87	71[b]	64[b]	76[b]	77[b]	79[b]	96[c]	92[c]
$\bar{x}$	13.13	11.86	11.86	11.38	11.55	11.65	9.86	9.89
S.D.	1.54	2.22	2.23	1.80	2.26	1.94	1.37	1.24
S >[a]	B-H	G,H	G,H	G,H	G,H	G,H	—	—
S <[a]	—	A	A	A	A	A	A-F	A-F
53 days								
N	84	71	63	74	78	78	89	85
$\bar{x}$	33.29	32.70	32.41	32.66	31.58	31.37	28.92	28.04
S.D.	3.32	5.00	4.47	3.91	3.92	3.71	3.04	2.92
S >	G,H	G,H	G,H	G,H	G,H	G,H	—	—
S <	—	—	—	—	—	—	A-F	A-F
330 days								
N	24	25	21	19	23	28	25	24
$\bar{x}$	68.02	69.50	72.48	71.47	68.07	67.05	68.02	63.48
S.D.	2.02	9.47	9.56	11.15	10.85	7.79	9.35	7.54
S >	—	—	—	—	—	—	—	—
S <	—	—	—	—	—	—	—	—
Second Generation								
30 days								
N	54	35	44	46	37	72	31	4
$\bar{x}$	22.18	22.07	22.78	25.04	21.34	22.58	20.15	19.00
S.D.	3.16	2.35	3.25	1.98	2.94	2.72	1.77	1.15
S >	—	—	G	A-C,E-H	—	—	—	—
S <	D	D	D	—	D	D	C,D	D
60 days								
N	54	35	41	47	35	72	31	4
$\bar{x}$	35.32	38.23	37.43	38.00	35.73	36.28	33.65	33.00
S.D.	3.45	2.78	3.87	3.40	2.84	2.80	1.91	1.41
S >	—	A,G	G	A,G	—	—	—	—
S <	B,D	—	—	—	—	—	B,D	—

[a] Significantly different (P=0.05) by Scheffe's analysis of variance test.
[b] One of the duplicate tanks restocked on day 15 of the test.
[c] Both of the duplicate tanks restocked on day 15 of the test.

tion test animals reared in the two highest effluent concentrations, but this observation should be viewed in light of the fact that only four fish survived in the 100 percent effluent concentration. Thus, it appears that the differences observed in the first generation fathead minnows were probably related to the different restocking histories of the dilution water tanks, which were not restocked, and the 50 and 100 percent effluent tanks, which were both restocked on day 15. Another possible factor, which might have influenced the observed differences, was the poor quality of effluent during the first few months of the project compared to the higher quality effluent during the remainder of the project.

While the data were not always significantly different, fathead minnows reared in the lower concentrations of nondisinfected effluent tended to attain greater mean lengths than those reared in the higher concentrations. A similar tendency toward greater growth at the lower effluent concentrations was also seen in each of the disinfected effluent streams (Tables 31-34). The consistency of this pattern suggests that high concentrations of either nondisinfected or disinfected effluent were detrimental to the growth of *P. promelas*.

First generation fish exposed to various concentrations of chlorinated effluent showed no significant differences in length at day 330 (termination) of the study (Table 31). However, those animals maintained in 20 and 14 percent chlorinated effluent were significantly smaller than the dilution water controls at 53 days and the fish in the lower effluent concentrations at 23 and 53 days. Similarly, second generation test fish were significantly shorter than their dilution water controls at 30 days of age. Thus, mean total residual chlorine levels as low as 0.045 mg/l were adequate to suppress the growth of fathead minnows. This threshold value is less than that found by Arthur, *et al.*[1] (0.079-0.096 mg/l) to retard the growth of *P. promelas*. The maximum total residual chlorine level of 0.01 mg/l recommended by Brungs[7] to protect the more resistant fish species continuously exposed to chlorinated effluent appears to be supported by this study.

The first generation of *P. promelas* reared in various concentrations of dechlorinated effluent showed no significant differences in length at 330 days (termination) of the life-cycle test (Table 32). Although both the first and second generation test animals exposed to 100 percent dechlorinated effluent were smaller than the other fish at 53 and 60 days of age, respectively, the validity of the second generation data is questionable since only three fish survived to the age of 30 days. This pattern is almost identical to that observed in the nondisinfected effluent (Table 30) where only four second generation fish survived in the 100 percent dechlorinated effluent. Thus, it appears that the factor(s) responsible for growth inhibition and mortality in the dechlorinated effluent was (were) also responsible for growth inhibition and mortality in the nondisinfected effluent. This conclusion is further supported by the fact that the maximum mean sulfite residual to which our fish were exposed was 0.025 mg/l (range of 0.000-0.610 mg/l) over the 330 day test period, a concentration considerably below the mean sulfite residual of 0.104 mg/l

Table 31. MEAN LENGTHS (IN mm) OF FIRST AND SECOND GENERATION *P. promelas* REARED IN CHLORINATED EFFLUENT AND IN DILUTION WATER

Generation	Age of Fish and Data	Nominal Percent Chlorinated Effluent Concentration: Dilution H_2O (A)	2.35 (B)	3.36 (C)	4.80 (D)	6.86 (E)	9.80 (F)	14.00 (G)	20.00 (H)
First Generation	23 days								
	N	66[b]	71[b]	52	67[b]	81[b]	93[c]	95[c]	87[c]
	$\bar{X}$	10.53	12.39	12.68	11.10	12.82	10.27	10.37	10.64
	S.D.	1.68	1.95	1.39	1.44	2.22	1.66	1.71	1.29
	S >[a]	—	D,F-H	D,F-H	—	A,D,F-H	—	—	—
	S <[a]	B,C,E	—	—	B,C,E	—	B,C,E	B,C,E	B,C,E
	X Resid.[d]	0.000	0.002	0.003	0.007	0.010	0.013	0.020	0.038
	53 days								
	N	64	69	52	65	80	84	71	43
	$\bar{X}$	30.67	31.92	34.54	30.96	31.83	28.95	27.80	26.84
	S.D.	3.89	4.13	3.29	3.84	3.90	3.90	3.67	2.88
	S >	G,H	F,G,H	A,B,D-H	G,H	F-H	—	—	—
	S <	C	C	—	C	—	B,C,E	A-E	A-E
	X Resid.	—	0.004	0.006	0.016	0.016	0.025	0.045	0.076
	330 days								
	N	24	20	22	24	18	23	13	1
	$\bar{X}$	68.35	69.63	69.34	68.60	70.78	67.24	70.08	52.00
	S.D.	7.84	10.64	10.37	10.20	10.05	9.39	9.79	0.00
	S >	—	—	—	—	—	—	—	—
	S <	—	—	—	—	—	—	—	—
	X Resid.	—	0.008	0.011	0.022	0.024	0.035	0.067	0.102
Second Generation	30 days								
	N	53	52	63	59	67	62	49	26
	$\bar{X}$	19.53	22.78	23.43	21.15	21.42	22.08	17.95	16.35
	S.D.	2.74	2.00	1.96	2.54	2.87	1.94	3.27	2.25
	S >	H	A,G,H	A,D,E,G,H	—	A	A,G,H	—	—
	S <	B,C,E,F	—	—	C	C	—	B-F	A-f
	X Resid.	0.000	0.008	0.004	0.018	0.016	0.028	0.033	0.045
	60 days								
	N	53	51	64	59	66	62	47	26
	$\bar{X}$	34.76	36.18	35.95	35.23	34.92	34.70	34.13	34.77
	S.D.	3.21	2.82	2.97	3.28	3.80	2.27	3.48	3.37
	S >	—	—	—	—	—	—	—	—
	S <	—	—	—	—	—	—	—	—
	X Resid.	0.000	0.008	0.009	0.020	0.016	0.033	0.035	0.033

[a]Significantly different (P=0.05) by Scheffe's analysis of variance test.

[b]One of the duplicate tanks restocked on day 15 of the test.

[c]Both of the duplicate tanks restocked on day 15 of the test.

[d]Residual chlorine in mg/1.

Table 32. MEAN LENGTHS (IN mm) OF FIRST AND SECOND GENERATION *P. promelas* REARED IN DECHLORINATED EFFLUENT AND IN DILUTION WATER

	Age of Fish and Data	Dilution H_2O (A)	Nominal Percent Dechlorinated Effluent Concentration 1.56 (B)	3.12 (C)	6.25 (D)	12.50 (E)	25.0 (F)	50.0 (G)	100.0 (H)
First Generation	23 days								
	N	94[c]	60[b]	56[b]	75[c]	68[b]	83[c]	59	39[c]
	$\bar{X}$	10.15	11.18	11.86	10.37	11.44	10.04	13.72	10.13
	S.D.	1.57	1.85	1.61	1.43	2.69	1.37	1.42	1.19
	S >[a]	—	F	A,D,F,H	—	A,F	—	A-F,H	—
	S <[a]	C,E,G	G	G	C,G,	G	B,C,E,G	—	C,G
	$\bar{X}$ Resid.[d]	0.000	0.000	0.000	0.008	0.002	0.000	0.000	0.080
	53 days								
	N	79	49	56	60	64	65	56	36
	$\bar{X}$	30.79	32.91	34.05	31.53	31.55	29.09	32.51	27.21
	S.D.	3.60	3.09	3.46	3.08	3.91	3.06	1.89	2.72
	S >	H	F,H	A,F,H	H	F,H	—	F,H	—
	S <	C	—	—	—	—	B,C,E,G	—	A-E,G
	$\bar{X}$ Resid.	0.000	0.000	0.000	0.010	0.010	0.000	0.000	0.042
	330 days								
	N	23	21	25	23	24	18	23	21
	$\bar{X}$	69.30	69.50	68.20	71.07	68.71	69.58	69.13	66.67
	S.D.	9.49	11.10	7.66	10.79	8.15	8.52	11.52	9.33
	S >	—	—	—	—	—	—	—	—
	S <	—	—	—	—	—	—	—	—
	$\bar{X}$ Resid.	0.000	0.000	0.001	0.005	0.003	0.005	0.012	0.025
Second Generation	30 days								
	N	45	36	56	45	51	67	39	3
	$\bar{X}$	19.18	23.63	21.92	22.76	24.21	22.58	22.50	16.00
	S.D.	3.03	3.10	3.54	2.52	1.77	2.20	1.94	3.04
	S >	—	A,H	A,H	A,H	A,C,H	A,H	A,H	—
	S <	B-G	—	E	—	—	—	—	B-G
	$\bar{X}$ Resid.	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.010
	60 days								
	N	43	37	58	44	47	66	39	3
	$\bar{X}$	36.15	36.60	34.80	35.51	38.17	33.70	33.64	27.33
	S.D.	3.81	3.46	4.81	3.00	3.16	2.58	2.36	2.25
	S >	F,H	F-H	H	H	C,D,F-H	—	—	—
	S <	—	—	E	E	—	A,B,E	B,E	A-E
	$\bar{X}$ Resid.	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.005

[a] Significantly different (P=0.05) by Scheffe's analysis of variance test.
[b] One of the duplicate tanks restocked on day 15 of the test.
[c] Both of the duplicate tanks restocked on day 15 of the test.
[d] Mg/l residual sulfite.

Table 33. MEAN LENGTHS (IN mm) OF FIRST AND SECOND GENERATION *P. promelas* REARED IN CHLOROBROMINATED EFFLUENT AND IN DILUTION WATER

	Age of Fish and Data	Nominal Percent Chlorobrominated Effluent Concentration							
		Dilution H_2O (A)	1.56 (B)	3.12 (C)	6.25 (D)	12.50 (E)	25.0 (F)	50.0 (G)	100.0 (H)
First Generation	**23 days**								
	N	95[b]	62[c]	67[c]	74[c]	96[b]	53	65[c]	52[b]
	$\bar{x}$	10.51	11.35	11.14	10.93	10.40	12.89	10.10	9.87
	S.D.	1.35	1.54	1.66	1.98	1.39	1.59	1.26	1.42
	S >[a]	—	G,H	H	—	—	A-E,G,H	—	—
	S <[a]	F	F	F	F	F	—	B,F	B,F
	$\bar{X}$ Resid.[d]	0.000	0.000	0.001	0.000	0.002	0.008	0.018	0.054
	53 days								
	N	90	60	67	70	92	50	62	31
	$\bar{x}$	29.57	31.97	30.39	30.71	30.01	34.11	28.35	23.39
	S.D.	3.62	4.05	3.15	3.92	3.15	3.20	3.17	2.14
	S >	H	G,H	H	H	H	A,C,D,E,G,H	H	—
	S <	B,F	—	F	F	F	—	B,F	A-G
	$\bar{X}$ Resid.	—	0.000	0.002	0.002	0.006	0.019	0.043	0.129
	330 days								
	N	24	20	20	17	23	20	21	0
	$\bar{x}$	68.75	67.98	67.45	67.27	70.39	69.80	70.36	—
	S.D.	9.85	9.84	8.88	8.68	11.86	9.48	9.73	—
	S >	—	—	—	—	—	—	—	—
	S <	—	—	—	—	—	—	—	—
	$\bar{X}$ Resid.	—	0.003	0.004	0.005	0.007	0.017	0.033	0.119
Second Generation	**30 days**								
	N	71	61	19	64	76	45	36	34
	$\bar{x}$	21.87	23.53	22.00	23.25	24.18	21.11	20.28	13.25
	S.D.	1.97	2.25	3.33	2.92	2.36	2.58	2.11	2.13
	S >	H	F-H	H	F-H	A	H	H	—
	S <	E	—	—	—	—	B,D,E	B,D,E	A-G
	$\bar{X}$ Resid.	0.000	0.003	0.005	0.006	0.006	0.012	0.020	0.045
	60 days								
	N	69	60	19	62	73	43	36	33
	$\bar{x}$	34.83	35.13	38.45	35.85	36.62	35.62	32.64	22.99
	S.D.	2.13	2.48	2.92	3.13	3.19	3.33	2.41	5.26
	S >	H	H	A,B,G,H	G,H	G,H	G,H	H	—
	S <	C	C	—	—	—	—	C-F	A-G
	$\bar{X}$ Resid.	0.000	0.002	0.004	0.006	0.006	0.014	0.024	0.034

[a] Significantly different (P=0.05) by Scheffe's analysis of variance test.
[b] Both of the duplicate tanks restocked on day 15 of the test.
[c] One of the duplicate tanks restocked on day 15 of the test.
[d] Residual bromine in mg/l.

Table 34. MEAN LENGTHS (IN mm) OF FIRST AND SECOND GENERATION *P. promelas* REARED IN OZONATED EFFLUENT AND IN DILUTION WATER

	Age of Fish and Data	Nominal Percent Ozonated Effluent Concentrations							
		Dilution Water (A)	1.5 (B)	3.1 (C)	6.2 (D)	12.5 (E)	25.0 (F)	50.0 (G)	100.0 (H)
First Generation	23 days								
	N	96[b]	79	55	58	64	71	43	93[b]
	$\bar{X}$	11.18	13.67	13.71	14.25	14.49	14.67	14.01	11.26
	S.D.	1.76	2.52	2.38	2.24	2.64	2.11	1.78	1.29
	S >[a]	—	A,H	A,H	A,H	A,H	A,H	A,H	—
	S <[a]	B-6	—	—	—	—	—	—	B-G
	$\bar{X}$ Resid.[c]	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.010
	53 days								
	N	95	76	56	55	62	69	43	93
	$\bar{X}$	30.03	33.26	33.92	34.24	32.99	33.23	31.86	29.23
	S.D.	3.63	4.10	4.58	6.16	3.21	3.58	4.20	3.24
	S >	—	A,H	A,H	A,H	A,H	A,H	H	—
	S <	B-F	—	—	—	—	—	—	B-G
	$\bar{X}$ Resid.	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.016
	330 days								
	N	17	12	19	14	23	27	24	20
	$\bar{X}$	69.88	75.33	62.79	68.07	68.74	65.22	68.46	68.60
	S.D.	10.64	12.25	5.74	9.47	8.81	7.53	8.92	8.22
	S >	—	—	—	—	—	—	—	—
	S <	—	—	—	—	—	—	—	—
	$\bar{X}$ Resid.	0.000	0.001	0.001	0.001	0.002	0.003	0.005	0.012
Second Generation	30 days								
	N	60	32	60	35	49	46	48	26
	$\bar{X}$	21.04	24.13	22.47	24.16	21.98	23.85	18.37	20.67
	S.D.	3.21	3.85	2.28	2.59	2.35	3.02	3.52	2.03
	S >	G	G,H	G	G,H	G	G,H	—	—
	S <	B,D,F	—	—	—	—	—	A-F	B,D,F
	$\bar{X}$ Resid.	0.000	0.002	0.003	0.004	0.004	0.004	0.004	0.013
	60 days								
	N	61	31	60	35	48	46	46	26
	$\bar{X}$	36.59	36.823	34.44	37.09	36.78	36.99	35.53	36.87
	S.D.	4.04	4.52	2.73	3.15	2.78	3.15	3.88	2.81
	S <	—	—	—	C	—	C	—	—
	S >	—	—	D,F	—	—	—	—	—
	$\bar{X}$ Resid.	0.000	0.001	0.003	0.003	0.003	0.004	0.005	0.012

[a] Significantly different (P=0.05) by Scheffe's analysis of variance test.
[b] Both of the duplicate tanks restocked on day 15 of the test.
[c] Residual ozone in mg/l.

(range of 0.020-0.700 mg/l) found by Arthur, et al.,[1] to have no adverse effects on fathead minnows. Thus, dechlorination with SO_2 appeared to eliminate any inhibition of growth in fathead minnows that may have resulted from the chlorination process.

The fathead minnows that survived in chlorobrominated effluent and dilution water exhibited no significant differences in length at the termination (330 days) of the life cycle test (Table 33). As with the other effluent streams that were tested, maximum growth occurred at some low to intermediate effluent concentration (3.12-25.0 percent). First generation fish reared in 100 percent chlorobrominated effluent were significantly smaller at 53 days than those fish reared in the dilution water control or in any lower dilution of chlorobrominated effluent. The same pattern was observed in second generation fish exposed to 100 percent effluent. Since the fish reared in 100 percent concentrations of some of the other effluent streams, including the non-disinfected stream, exhibited reduced growth, there was no reason to attribute the reduction of the length of fish reared in 100 percent chlorobrominated effluent to the disinfection treatment.

At the termination of the 330 day life cycle study no significant differences were observed between the lengths of fathead minnows reared in dilution water and those reared in ozonated effluent (Table 34). It is important to note that no significant differences in mean length between fish exposed to dilution water and those exposed to 100 percent ozonated effluent were ever recorded. As in all other effluent streams, the maximum growth of fathead minnows always occurred in one of the lower effluent concentrations (1.50 to 25.00 percent) rather than in dilution water or 100 percent effluent.

Tables 35 to 39 summarize all of the foregoing bioassay data by displaying effluent concentrations versus effluent type for each age group of test animals. A comparison of both the statistically significant differences and the trends in the growth of fish in the different effluent streams indicates that both generations of P. promelas reared in 100 percent ozonated effluent grew larger than fish reared in all other 100 percent effluent concentrations. This greater growth in undiluted ozonated effluent may have resulted from a conditioning of the effluent as the result of the relatively high dissolved oxygen concentration in the ozonated stream prior to delivery to the fish tanks.

A second generalization that can be made from a study of Tables 35-39 is that the growth of fathead minnows exposed to mean total chlorine residuals of 0.045 mg/l or higher in 14 or 20 percent effluent concentrations during their first two months of life was inferior to the growth of fish reared in comparable concentrations of the other effluent streams. This growth retardation effect was eliminated by dechlorination as evidenced by the fact that mean lengths of fish in 50 and 100 percent dechlorinated effluent were comparable to those in the respective concentrations of the other effluent streams.

Table 40 summarizes the weights of the first generation test animals measured at the termination of the life cycle study (330 days). No significant differences were observed in either the lengths (Table 37) or weights of the first

Table 35. MEAN LENGTHS (IN mm) OF FIRST GENERATION *P. promelas* AT DAY 23 OF THE LIFE CYCLE TEST

Nominal Effluent Concentration and Data		Effluent Stream Nondis. (A)	Chlor. (B)	Dechlor. (C)	Chlorobr. (D)	Ozon. (E)
Dilution Water	N	87	66[f]	94[g]	95[g]	96[g]
	$\bar{X}$	13.13	10.53	10.15	10.51	11.18
	S.D.	1.54	1.68	1.57	1.35	1.76
	S >[h]	B,C,D,E	—	—	—	C
	S <[h]	—	A	A,E	A	A
1.56%	N	71[f]		60[f]	62[f]	79
	$\bar{X}$	11.86		11.18	11.35	13.67
	S.D.	2.22	a	1.85	1.54	2.52
	S >	—		—	—	A,C,D
	S <	E		E	E	—
	$\bar{X}$ Resid.	—		0.000	0.000	0.000
3.12%	N	64[f]	52[b]	56[f]	67[f]	55
	$\bar{X}$	11.86	12.68	11.86	11.14	13.71
	S.D.	2.23	1.39	1.61	1.66	2.38
	S >	—	D	—	—	A,C,D
	S <	E	—	E	B.E	—
	$\bar{X}$ Resid.	—	0.003	0.000	0.001	0.000
6.25%	N	76[f]	81[c,f]	75[g]	74[f]	58
	$\bar{X}$	11.38	12.82	10.37	10.93	14.25
	S.D.	1.80	2.22	1.43	1.98	2.24
	S >	C	A,C,D	—	—	A,B,C,D
	S <	B,E	E	A,B,E	B,E	—
	$\bar{X}$ Resid.	—	0.010	0.008	0.000	0.000
12.50%	N	77[f]	95[d,g]	68[f]	96[g]	64
	$\bar{X}$	11.55	10.37	11.44	10.40	14.49
	S.D.	2.26	1.71	2.69	1.39	2.64
	S >	B,D	—	B,D	—	A,B,C,D
	S <	E	A,C,E	E	A,C,E	—
	$\bar{X}$ Resid.	—	0.020	0.002	0.002	0.000
25.00%	N	79[f]	87[e,g]	83[g]	53	71
	$\bar{X}$	11.65	10.64	10.04	12.89	14.67
	S.D.	1.94	1.29	1.37	1.59	2.11
	S	B,C	—	—	A,B,C	A,B,C,D
	S	D,E	A,D,E	A,D,E	E	—
	$\bar{X}$ Resid.	—	0.038	0.000	0.008	0.000
50.00%	N	96[g]		59	65[f]	43
	$\bar{X}$	9.86		13.72	10.10	14.01
	S.D.	1.37	a	1.42	1.26	1.78
	S >	—		A,D	—	A,D
	S <	C,E		—	C,E	—
	$\bar{X}$ Resid.	—		0.000	0.018	0.003
100.00%	N	92[g]		39[g]	52[g]	93[g]
	$\bar{X}$	9.89		10.13	9.87	11.26
	S.D.	1.24	a	1.19	1.42	1.29
	S >	—		—	—	A,C,D
	S <	E		E	E	—
	$\bar{X}$ Resid.	—		0.080	0.054	0.010

[a] No comparable chlorinated effluent concentration.
[b] Nominal 3.36 percent chlorinated effluent concentration.
[c] Nominal 6.86 percent chlorinated effluent concentration.
[d] Nominal 14.09 percent chlorinated effluent concentration.
[e] Nominal 20.00 percent chlorinated effluent concentration.
[f] One of the duplicate tanks restocked on day 15.
[g] Both of the duplicate tanks restocked on day 15.
[h] Significantly different $(P < 0.05)$ by Scheffe's analysis of variance test.

Table 36. MEAN LENGTHS (IN mm) OF FIRST GENERATION *P. promelas* AT DAY 53 OF THE LIFE CYCLE TEST

Nominal Effluent Concentration and Data		Effluent Stream Nondis. (A)	Chlor. (B)	Dechlor. (C)	Chlorobr. (D)	Ozon. (E)
Dilution Water	N	84	64[f]	79[g]	90[g]	95[g]
	$\bar{X}$	33.29	30.67	30.79	29.57	30.03
	S.D.	3.32	3.89	3.60	3.62	3.63
	S >[h]	B,C,D,E	—	—	—	—
	S <[h]	—	A	A	A	A
1.56%	N	71[f]		49[f]	60[f]	76
	$\bar{X}$	32.70		32.91	31.97	33.26
	S.D.	5.00	a	3.09	4.05	4.10
	S >	—		—	—	—
	S <	—		—	—	—
	$\bar{X}$ Resid.			0.000	0.000	0.000
3.12%	N	63[f]	52[b]	56[f]	67[f]	56
	$\bar{X}$	32.41	34.54	34.05	30.39	33.92
	S.D.	4.47	3.29	3.46	3.15	4.58
	S >	D	A,D	D	—	D
	S <	B	—	—	A,B,C,E	—
	$\bar{X}$ Resid.		0.006	0.000	0.002	0.000
6.25%	N	74[f]	80[c,f]	60[g]	70[f]	55
	$\bar{X}$	32.66	21.83	31.53	30.71	34.24
	S.D.	3.91	3.90	3.08	3.92	6.16
	S >	D	—	—	—	B,C,D
	S <	—	E	E	A,E	—
	$\bar{X}$ Resid.		0.016	0.010	0.002	0.000
12.50%	N	78[f]	71[d,g,]	64[f]	92[g]	62
	$\bar{X}$	31.58	27.80	31.55	30.01	32.99
	S.D.	3.92	3.67	3.91	3.15	3.21
	S	B	—	B	B	B,D
	S	—	A,D,E	—	E	—
	$\bar{X}$ Resid.		0.045	0.010	0.006	0.000
25.00%	N	78[f]	43[e,g]	65[g]	50	69
	$\bar{X}$	31.37	26.84	29.09	34.11	33.23
	S.D.	3.71	2.88	3.06	3.20	3.58
	S >	B,C	—	B	A,B,C	A,B,D
	S <	D,E	A,D,E	A,D,E	—	—
	$\bar{X}$ Resid.		0.076	0.000	0.019	0.000
50.00%	N	89[g]		56	62[f]	43
	$\bar{X}$	28.92		32.51	28.35	31.86
	S.D.	3.04	a	1.89	3.17	4.20
	S >	—		A,D	—	A,D,
	S <	C,E		—	C,E	—
	$\bar{X}$ Resid.			0.000	0.043	0.002
100.00%	N	85[g]		36[g]	31[g]	93[g]
	$\bar{X}$	28.04		27.21	23.39	29.23
	S.D.	2.92	a	2.72	2.14	3.24
	S >	D		D	—	D
	S <	—		—	A,C,E	—
	$\bar{X}$ Resid.			0.042	0.129	0.016

[a] No comparable chlorinated effluent concentration.
[b] Nominal 3.36 percent chlorinated effluent concentration.
[c] Nominal 6.86 percent chlorinated effluent concentration.
[d] Nominal 14.09 percent chlorinated effluent concentration.
[e] Nominal 20.00 percent chlorinated effluent concentration.
[f] One of the duplicate tanks restocked on day 15.
[g] Both of the duplicate tanks restocked on day 15.
[h] Significantly different ($P < 0.05$) by Scheffe's analysis of variance test.

Table 37. MEAN LENGTHS (IN mm) OF FIRST GENERATION *P. promelas* AT THE TERMINATION (DAY 330) OF THE LIFE CYCLE TEST

Nominal Effluent Concentration and Data		Effluent Stream: Nondis. (A)	Chlor. (B)	Dechlor. (C)	Chlorobr. (D)	Ozon. (E)
Dilution Water	N	24	24[f]	23[g]	24[g]	17[g]
	$\bar{X}$	68.02	68.35	69.30	68.75	69.88
	S.D.	2.02	7.84	9.49	9.85	10.64
	S >[h]	—	—	—	—	—
	S <[h]	—	—	—	—	—
1.56%	N	25[f]		21[f]	20[f]	12
	$\bar{X}$	69.50		69.50	67.98	75.33
	S.D.	9.47	a	11.10	9.84	12.25
	S >	—		—	—	—
	S <	—		—	—	—
	$\bar{X}$ Resid.	—		0.000	0.003	0.001
3.12%	N	21[f]	22[b]	25[f]	20[f]	19
	$\bar{X}$	72.48	69.34	68.20	67.45	62.79
	S.D.	9.56	10.37	7.66	8.88	5.74
	S >	E	—	—	—	—
	S <	—	—	—	—	A
	$\bar{X}$ Resid.	—	0.011	0.001	0.004	0.001
6.25%	N	19[f]	18[c,f]	23[g]	17[f]	14
	$\bar{X}$	71.47	70.78	71.07	67.27	68.07
	S.D.	11.15	10.05	10.79	8.68	9.47
	S >	—	—	—	—	—
	S <	—	—	—	—	—
	$\bar{X}$ Resid.	—	0.024	0.005	0.005	0.001
12.50%	N	23[f]	13[d,g]	24[f]	23[g]	23
	$\bar{X}$	68.07	70.08	68.71	70.39	68.74
	S.D.	10.85	9.79	8.15	11.86	8.81
	S >	—	—	—	—	—
	S <	—	—	—	—	—
	$\bar{X}$ Resid.	—	0.067	0.003	0.007	0.002
25.00%	N	28[f]	1[e,g]	18[g]	20	27
	$\bar{X}$	67.05	52.00	69.58	69.80	65.22
	S.D.	7.79	0.00	8.52	9.48	7.53
	S >	—	—	—	—	—
	S <	—	—	—	—	—
	$\bar{X}$ Resid.		0.102	0.005	0.017	0.003
50.00%	N	25[g]		23	21[f]	24
	$\bar{X}$	68.02		69.13	70.36	68.46
	S.D.	9.35	a	11.52	9.73	8.92
	S >	—		—	—	—
	S <	—		—	—	—
	$\bar{X}$ Resid.			0.012	0.033	0.005
100.00%	N	24[g]		21[g]	0[g]	20[g]
	$\bar{X}$	63.48		66.67	—	68.60
	S.D.	7.54		9.33	—	8.22
	S >	—	a	—	—	—
	S <	—		—	—	—
	$\bar{X}$ Resid.	—		0.025	0.119	0.012

[a] No comparable chlorinated effluent concentration.
[b] Nominal 3.36 percent chlorinated effluent concentration.
[c] Nominal 6.86 percent chlorinated effluent concentration.
[d] Nominal 14.09 percent chlorinated effluent concentration.
[e] Nominal 20.00 percent chlorinated effluent concentration.
[f] One of the duplicate tanks restocked on day 15.
[g] Both of the duplicate tanks restocked on day 15.
[h] Significantly different ($P < 0.05$) by Scheffe's analysis of variance test.

Table 38. MEAN LENGTHS (IN mm) OF 30 DAY OLD SECOND GENERATION *P. promelas* IN THE LIFE CYCLE TEST

Nominal Effluent Concentration and Data		Effluent Stream				
		Nondis. (A)	Chlor. (B)	Dechlor. (C)	Chlorobr. (D)	Ozon. (E)
Dilution Water	N	54	53	45	71	60
	$\bar{X}$	22.18	19.53	19.18	21.87	21.04
	S.D.	3.16	2.74	3.03	1.97	3.21
	S >[f]	B,C	—	—	B,C	C
	S <[f]	—	A,D	A,D,E	—	—
1.56%	N	35		36	61	32
	$\bar{X}$	22.07		23.63	23.53	24.13
	S.D.	2.35	a	3.10	2.25	3.85
	S >	—		—	—	A
	S <	E		—	—	—
	$\bar{X}$ Resid.			0.000	0.003	0.002
3.12%	N	44	63[b]	56	19	60
	$\bar{X}$	22.78	23.43	21.92	22.00	22.47
	S.D.	3.25	1.96	3.54	3.33	2.28
	S >	—	—	—	—	—
	S <	—	—	—	—	—
	$\bar{X}$ Resid.		0.004	0.000	0.005	0.003
6.25%	N	46	67[c]	45	64	35
	$\bar{X}$	25.04	21.42	22.76	23.25	24.16
	S.D.	1.98	2.87	2.52	2.92	2.59
	S >	B,C,D	—	—	B	B
	S <	—	A,D,E	A	A	—
	$\bar{X}$ Resid.		0.016	0.000	0.006	0.004
12.50%	N	37	49[d]	51	76	49
	$\bar{X}$	21.34	17.95	24.21	24.18	21.98
	S.D.	2.94	3.27	1.77	2.36	2.35
	S >	B	—	A,B,E	A,B,E	B
	S <	C,D	A,C,D,E	—	—	C,D
	$\bar{X}$ Resid.		0.033	0.004	0.006	0.004
25.00%	N	72	26[e]	67	45	46
	$\bar{X}$	22.58	16.35	22.58	21.11	23.85
	S.D.	2.72	2.25	2.20	2.58	3.02
	S >	B	—	B	B	B,D
	S <	—	A,C,D,E	—	E	—
	$\bar{X}$ Resid.		0.045	0.000	0.012	0.004
50.00%	N	31		39	36	48
	$\bar{X}$	20.15		22.50	20.28	18.37
	S.D	1.77	a	1.94	2.11	3.52
	S >	—		A,D,E	E	—
	S <	C		—	C	C.D.
	$\bar{X}$ Resid.			0.000	0.020	0.004
100.00%	N	4		3	34	26
	$\bar{X}$	19.00		16.00	13.25	20.67
	S.D.	1.15	a	3.04	2.13	2.03
	S >	D		—	—	D
	S <	—		—	A,E	—
	$\bar{X}$ Resid.			0.010	0.045	0.013

[a]No comparable chlorinated effluent concentration.
[b]Nominal 3.36 percent chlorinated effluent concentration.
[c]Nominal 6.86 percent chlorinated effluent concentration.
[d]Nominal 14.09 percent chlorinated effluent concentration.
[e]Nominal 20.00 percent chlorinated effluent concentration.
[f]Significantly different ($P < 0.05$) by Scheffe's analysis of variance.

Table 39. MEAN LENGTHS (IN mm) OF 60 DAY OLD SECOND GENERATION *P. promelas* IN THE LIFE CYCLE TEST

Nominal Effluent Concentration and Data		Effluent Stream: Nondis. (A)	Chlor. (B)	Dechlor. (C)	Chlorobr. (D)	Ozon. (E)
Dilution Water	N	54	53	43	69	61
	$\bar{X}$	35.32	34.76	36.15	34.83	36.59
	S.D.	3.45	3.21	3.81	2.13	4.04
	s > [f]	—	—	—	—	—
	s < [f]	—	—	—	—	—
1.56%	N	35		37	60	31
	$\bar{X}$	38.23		36.60	35.13	36823
	S.D.	2.78	a	3.46	2.48	4.52
	s >	D		—	—	—
	s <	—		—	A	—
	$\bar{X}$ Resid.			0.000	0.002	0.001
3.12%	N	41	64[b]	58	19	60
	$\bar{X}$	37.43	35.95	34.80	38.45	34.44
	S.D.	3.87	2.97	4.81	2.92	2.73
	s >	C,E	—	—	C,E	—
	s <	—	—	A,D	—	A,D
	$\bar{X}$ Resid.		0.009	0.000	0.004	0.003
6.25%	N	47	66[c]	44	62	35
	$\bar{X}$	38.00	34.92	35.51	35.85	37.09
	S.D.	3.40	3.80	3.00	3.13	3.14
	s >	B,C,D	—	—	—	B
	s <	—	A,E	A	A	—
	$\bar{X}$ Resid.		0.0016	0.000	0.006	0.003
12.50%	N	35	47[d]	47	73	48
	$\bar{X}$	35.73	34.13	38.17	36.62	36.78
	S.D.	2.84	3.48	3.16	3.19	2.78
	s >	—	—	A,B	B	B
	s <	C	C,D,E	—	—	—
	$\bar{X}$ Resid.		0.035	0.002	0.006	0.003
25.00%	N	72	26[e]	66	43	46
	$\bar{X}$	36.28	34.77	33.70	35.62	36.99
	S.D.	2.80	3.37	2.58	3.33	3.15
	s >	C	—	—	—	C
	s <	—	—	A,E	—	—
	$\bar{X}$ Resid.		0.033	0.000	0.014	0.004
50.00%	N	31		39	36	46
	$\bar{X}$	33.65		33.64	32.64	35.53
	S.D.	1.91	a	2.36	2.41	3.88
	s >	—		—	—	D
	s <	—		—	E	—
	$\bar{X}$ Resid.			0.000	0.024	0.005
100.00%	N	4		3	33	26
	$\bar{X}$	33.00		27.33	22.99	36.87
	S.D.	1.41	a	2.25	5.26	2.81
	s >	D		—	—	C,D
	s <	—		E	A,E	—
	$\bar{X}$ Resid.			0.005	0.034	0.012

[a]No comparable chlorinated effluent concentration.
[b]Nominal 3.36 percent chlorinated effluent concentration.
[c]Nominal 6.86 percent chlorinated effluent concentration.
[d]Nominal 14.09 percent chlorinated effluent concentration.
[e]Nominal 20.00 percent chlorinated effluent concentration.
[f]Significantly different ($P < 0.05$) by Scheffe's analysis of variance.

Table 40. MEAN WEIGHTS (IN GRAMS) OF FIRST GENERATION *P. promelas* AT THE TERMINATION (330 DAYS) OF THE LIFE CYCLE TEST

Nominal Effluent Concentration and Data	Effluent Stream				
	Nondisinfected	Chlorinated	Dechlorinated	Chlorobrominated	Ozonated
Dilution Water					
N[a]	24	24	23	24	17
$\bar{X}$ Weight	3.25	3.51	3.39	3.45	3.80
S.D.[b]	1.69	1.71	1.71	1.79	2.10
1.56%					
N	25		21	20	12
$\bar{X}$ Weight	3.56	d	3.63	3.42	5.18
S.D.	1.98		2.16	1.95	2.55
$\bar{X}$ Residual[c]	—		0.000	0.003	0.001
3.12%					
N	21	22[e]	25	20	19
$\bar{X}$ Weight	4.10	3.27	3.20	3.13	2.47
S.D.	2.10	1.92	1.51	1.66	0.97
$\bar{X}$ Residual	—	0.011	0.001	0.004	0.001
6.25%					
N	19	18[f]	23	17	14
$\bar{X}$ Weight	3.86	3.71	3.90	3.01	3.53
S.D.	2.14	1.85	2.16	1.50	1.86
$\bar{X}$ Residual	—	0.024	0.005	0.005	0.001
12.50%					
N	23	13[g]	24	23	23
$\bar{X}$ Weight	3.43	3.67	3.31	3.77	3.54
S.D.	1.98	2.00	1.55	2.39	1.97
$\bar{X}$ Residual	—	0.067	0.003	0.007	0.002
25.0%					
N	28	1[h]	18	20	27
$\bar{X}$ Weight	3.11	1.1	3.89	3.69	2.67
S.D.	1.38	—	1.88	1.90	1.27
$\bar{X}$ Residual	—	0.102	0.005	0.017	0.003
50.0%					
N	25		23	21	24
$\bar{X}$ Weight	3.19	d	3.61	4.03	3.23
S.D.	1.49		1.66	1.89	1.47
$\bar{X}$ Residual	—		0.012	0.033	0.005
100.0%					
N	24		21	0	20
$\bar{X}$ Weight	2.75	d	3.33	—	3.69
S.D.	1.12		1.40	—	1.43
$\bar{X}$ Residual	—		0.025	0.119	0.012

[a] N = Sample size.
[b] S.D. = Standard deviation.
[c] $\bar{X}$ Residual in mg/l.
[d] No comparable chlorinated effluent concentration.
[e] Nominal 3.36 percent chlorinated effluent concentration.
[f] Nominal 6.86 percent chlorinated effluent concentration.
[g] Nominal 14.00 percent chlorinated effluent concentration.
[h] Nominal 20.00 percent chlorinated effluent concentration.

generation test fish. This suggests that time, and perhaps the improved effluent quality, had a normalizing effect on the size of the test animals. This observation lends further support to the conclusion that young P. promelas are more sensitive to adverse environmental conditions than older, more mature members of the species.

REPRODUCTION

The effects of the various effluent streams on the reproductive functions of the fathead minnow were analyzed from the standpoint of egg production and egg hatchability. Egg production is defined as the numbers of viable eggs produced per female or per spawning in each concentration of each effluent stream. Hatchability refers to the percentage of eggs that hatched in incubation attempts in the various concentrations of the different effluent streams. Significant differences (P=0.05) between concentrations within the same effluent stream were determined by Tukey's two-tailed analysis of variance test.[18]

Table 41 summarizes the total number of eggs produced in all effluent concentrations and the mean residual disinfectant levels per concentration. Egg production in nondisinfected effluent was maximum at an intermediate effluent concentration (25 percent). The mean number of eggs produced per female in the 25 percent nondisinfected effluent concentration was significantly greater (P=0.05) than the mean number produced in the 100, 6.25 and 0.00 percent effluent concentrations. Thus, it appeared that the 100 percent concentration of nondisinfected effluent exerted a negative effect on the production of eggs by our test fish. The reductions in egg production observed in the low effluent concentrations were probably not the result of one or more factors present in the nondisinfected effluent, but may have been related to lower levels of nutrients and/or planktonic food in the low effluent concentrations and dilution water tanks.

A similar pattern of reduced egg production per female in the higher and lower effluent concentrations was observed in the dechlorinated stream. Since this reduction in egg production exhibited the same pattern as those in the nondisinfected effluent stream, there was no reason to believe it was related to the chlorination-dechlorination processes.

Comparison of egg production on the chlorinated effluent stream with egg production in similar concentrations of the nondisinfected effluent stream reveals that only the 20 percent chlorinated effluent adversely affected viable egg production. Fish in those tanks were exposed to a mean chlorine residual of 0.103 mg/l, which exceeded the TL50 values of 0.082 to 0.095 mg/l found in the acute tests with chlorinated effluent. This supports the findings of Arthur, et al.,[1] who observed that spawning was completely inhibited by a mean chlorine residual of 0.110 mg/l. The lower average residual chlorine levels to which our fish were exposed (0.069-0.008 mg/l) did not appear to have an adverse effect on egg production. Except for the 20 percent effluent concentration, there were no significant differences in viable egg production observed among the lower concentrations of chlorinated effluent.

Table 41. MEAN NUMBER OF VIABLE EGGS PRODUCED PER FEMALE AND THE MEAN DISINFECTANT RESIDUAL (mg/l) IN EACH CONCENTRATION OF EACH EFFLUENT STREAM

Effluent Stream	Nominal percent effluent concentrations[a]										Mean for All Concentrations
	100.00	50.00	25.00	12.50	9.80	6.25	4.80	3.12	1.56	0.00	
Nondisinfected											
$\bar{x}$ eggs/female	382	1240	2750	1584	b	450	b	1803	1215	715	1315
Chlorinated[a]											
$\bar{x}$ eggs/female	b	b	0[c]	981	1236	1245	1940	778	1606	1929	
$\bar{x}$ residual			0.103	0.069	0.036	0.025	0.023	0.012	0.088	---	1458
Dechlorinated											
$\bar{x}$ eggs/female	90	380	2482	2299	b	1649	b	1385	1454	1359	
$\bar{x}$ residual	0.027	0.013	0.005	0.004		0.006		0.001	0.000	---	1538
Chlorobrominated											
$\bar{x}$ eggs/female	0[d]	649	2788	0	b	1281	b	2126	917	955	
$\bar{x}$ residual	0.119	0.033	0.017	0.008		0.005		0.004	0.003	---	1232
Ozonated											
$\bar{x}$ eggs/female	2054	1182	1988	1063	b	1749	b	1007	1098	806	
$\bar{x}$ residual	0.011	0.004	0.003	0.002		0.001		0.001	0.001	---	1420

[a]The nominal effluent concentrations in the chlorinated stream were 20.00, 14.00, 9.80, 6.86, 4.80, 3.36, 2.35 and 0.00 percent.

[b]No equivalent concentration

[c]Only one fish survived to maturity

[d]No fish survived to maturity

Viable egg production in the dechlorinated stream was maximum in the 25 and 12.5 percent effluent concentrations. This pattern closely parallels that seen in the nondisinfected stream. The considerable variability in egg production observed in the dechlorinated stream (90-2482 viable eggs per female) is similar to the variability in the nondisinfected stream (382-2750 viable eggs per female) and in the dilution water control tanks (715-1929 viable eggs per female). The similarity in the pattern of mean egg production per female in the dechlorinated effluent to productivity in the nondisinfected stream suggests that, at least in effluent concentrations of less than 25 percent, the dechlorination process effectively eliminated the adverse effects of chlorination reported by Arthur, et al.[1]

Fathead minnows reared in chlorobrominated effluent produced from zero to 2780 viable eggs per female. Egg production in the 25 percent effluent level (0.017 mg/l residual bromine chloride) was significantly higher than the other effluent concentrations. No eggs were produced in 100 percent effluent (0.119 mg/l residual bromine chloride) because none of the test fish survived to reproductive age. Fish exposed to 50 percent effluent concentration (0.033 mg/l residual bromine chloride) produced about half as many viable eggs per female as fish in the same concentration in the nondisinfected stream. Although some fish survived exposure to 12.5 percent effluent (0.008 mg/l residual bromine chloride), no eggs were produced. It appears unlikely that this lack of egg production was caused by excessive levels of residual bromine chloride, since egg production was greater in two higher effluent concentrations (25 and 50 percent).

Fish reared in the ozonated effluent stream exhibited the least variation in egg production (1007-2065 viable eggs per female) with no statistically significant differences occurring between any two concentrations. Even in 100 percent effluent egg production and viability were normal. In fact, maximum egg production per female occurred in the latter effluent concentration, in contrast to the pattern established in all other effluents. The ozonation process apparently eliminated or significantly reduced the inherent toxicity of the nondisinfected effluent.

As previously mentioned, the total number of eggs in each spawning was determined within 24 hours after the eggs were deposited. The mean percentage of viable eggs was then calculated for all concentrations of each effluent type. The mean percent viability in all effluent streams was 92.6 percent, while the range was 78.9 to 97.6 percent. Neither the concentration nor the type of effluent markedly affected percent viability, as the variance among test streams was negligible.

The greatest mean number of eggs per spawning was produced by fish in nondisinfected effluent and in ozonated effluent (respective means of 234 and 235 eggs per spawning) (Table 42). There were no significant differences among the mean number of eggs per spawning in the various concentrations of the nondisinfected effluent stream. In the chlorinated stream no spawnings occurred in the 20 percent effluent concentration because only one fish survived to reproductive age. Likewise no fish survived to reproduce in the 100 percent chlorobrominated effluent concentration and,

Table 42. MEAN NUMBER OF EGGS PER SPAWNING IN THE VARIOUS CONCENTRATIONS OF EACH EFFLUENT STREAM

Effluent Stream	Nominal percent effluent concentrations[a]										Mean for all Concentrations
	100.00	50.00	25.00	12.50	9.80	6.25	4.80	3.12	1.56	0.00	
Nondisinfected											
$\bar{x}$ eggs/spawning	181	314	266	292	b	160	b	197	161	237	234
No. of spawnings	31	66	230	83		31		122	150	53	96
Chlorinated[a]											
$\bar{x}$ eggs/spawning	b	b	0[c]	166	179	176	261	186	169	210	199
No. of spawnings			0	51	112	78	130	60	133	171	92
Dechlorinated											
$\bar{x}$ eggs/spawning	146	93	198	260	b	171	b	163	213	217	210
No. of spawnings	6	48	159	148		136		158	144	105	113
Chlorobrominated											
$\bar{x}$ eggs/spawning	0[d]	170	259	0	b	184	b	240	172	170	211
No. of spawnings	0	48	136	0		100		142	83	94	75
Ozonated											
$\bar{x}$ eggs/spawning	249	218	333	210	b	218	b	202	144	132	235
No. of spawnings	83	96	146	82		99		97	57	75	92

[a] The nominal effluent concentrations in the chlorinated stream were 20.00, 14.00, 9.80, 6.86, 4.80, 3.36, 2.35, and 0.00 percent.

[b] No equivalent concentration

[c] Only one fish survived to maturity

[d] No fish survived to maturity

while both sexes were present in the 12.5 percent chlorobrominated effluent concentration, no spawnings occurred. The patterns of egg production per spawning in the dechlorinated and ozonated effluent streams were generally similar to the pattern observed in the nondisinfected effluent. These results indicate that the mean number of eggs produced per spawning by fathead minnows is not adversely affected by the disinfection processes studied.

Some of the viable eggs produced were incubated to determine their hatchability (i.e., the percent of eggs per spawning that hatched and produced fry that were living at the end of the five-day incubation period). Fry that had hatched, or partially hatched, but that were not alive at the end of the incubation period were not considered living fry.

The hatchability of eggs spawned and incubated in nondisinfected effluent improved with decreasing effluent concentration, with optimum hatchability (90 percent) occurring in 6.25 percent nondisinfected effluent (Table 43). Hatchability in the 100 percent effluent concentration was significantly less than in other concentrations of nondisinfected effluent. The hatchability of eggs produced in dilution water and incubated in 100 percent nondisinfected effluent was only 3 percent, while the hatchability of eggs produced in 100 percent nondisinfected effluent and incubated in dilution water was 39 percent (Table 44). The hatchability of eggs spawned in dilution water and incubated in 50 percent effluent, or eggs spawned in 50 percent effluent and incubated in well water, exceeded the hatchability of eggs spawned and incubated in 50 percent nondisinfected effluent. Thus, the 100 percent concentration of nondisinfected effluent appeared to have an adverse effect on the hatching of fathead minnow eggs.

There were no data available on the hatchability of eggs produced and incubated in the highest concentration of chlorinated effluent (20 percent) due to adult mortality in those tanks. Hatchability in the lower effluent concentrations (77-86 percent) was similar to hatchability in comparable nondisinfected effluent concentrations. This supports the findings of Arthur, *et al.*[1] Eggs spawned in 14 and 9.8 percent chlorinated effluent and incubated in well water had mean hatchability values of 70 and 90 percent respectively. The mean hatchabilities of eggs spawned in well water and incubated in 20, 14, and 9.8 percent chlorinated effluent were 63, 66, and 59 percent respectively, which were lower than values for eggs spawned and incubated in similar concentrations of nondisinfected effluent (78-85 percent). The values for eggs transferred from dilution water to 14 and 9.8 percent chlorinated effluent were lower than eggs spawned and incubated in the chlorinated effluent, which suggests that the eggs spawned and incubated in the presence of chlorinated effluent may have developed a higher tolerance to chlorine than eggs produced in well water and then exposed to chlorinated effluent during incubation.

With the exception of the 100 percent concentration where only one incubation was attempted, hatchability in all dechlorinated effluent concentrations compared favorably with the hatchability recorded in respective concentrations of nondisinfected effluent (Table 43). When eggs spawned in dilution water were incubated in 100 percent dechlorinated effluent (Table 44) mean hatch-

Table 43. PERCENT HATCHABILITY, MEAN DISINFECTANT RESIDUAL (mg/l), AND INCUBATION ATTEMPTS IN THE VARIOUS EFFLUENT STREAMS

Effluent Type	Nominal Percent Effluent Concentration[a]									
	100.00	50.00	25.00	12.50	9.80	6.25	4.80	3.12	1.56	0.00
Nondisinfected										
% Hatchability	29	61	78	85		90		88	83	76
No. of incubations	4	11	35	13		2		23	21	24
Chlorinated[a]										
% Hatchability			b	86	82	77	84	81	86	83
No. of incubations			0	9	22	15	26	11	27	42
Mean residual			0.114	0.072	0.034	0.023	0.019	0.011	0.009	0.000
Dechlorinated										
% Hatchability	0	50	82	77		88		87	79	91
No. of incubations	1	8	29	32		25		19	18	22
Mean residual	0.012	0.014	0.001	0.001		0.001		0.000	0.000	0.000
Chlorobrominated										
% Hatchability	b	73	80	b		77		89	82	87
No. of incubations	0	10	25	0		15		20	15	18
Mean residual	0.069	0.028	0.014	0.006		0.006		0.007	0.005	0.000
Ozonated										
% Hatchability	64	59	80	85		84		88	79	84
No. of incubations	10	13	28	16		14		16	15	12
Mean residual	0.010	0.006	0.004	0.003		0.002		0.003	0.002	0.000

[a]The nominal chlorinated effluent concentrations were 20.0, 14.0, 9.8, 6.9, 4.8, 3.4, and 2.4 percent.

[b]No spawnings occurred in this concentration.

Table 44. PERCENT HATCHABILITY OF EGGS INCUBATED IN WATER DIFFERENT FROM THAT IN WHICH THEY WERE SPAWNED

Effluent Type	Test Group 1	Test Group 2	Test Group 3	Test Group 4	Test Group 5
Nondisinfected					
Spawned in:	dilution water	dilution water	50% effluent	100% effluent	
Incubated in:	100% effluent	50% effluent	dilution water	dilution water	
% Hatchability:	3	87	87	39	
No. of attempts:	2	3	7	4	
Chlorinated					
Spawned in:	dilution water	dilution water	dilution water	14% effluent	9.8% effluent
Incubated in:	20% effluent	14% effluent	9.8% effluent	dilution water	dilution water
% Hatchability:	63	66	59	70	90
No. of attempts:	18	8	4	5	6
Dechlorinated					
Spawned in:	dilution water	dilution water			
Incubated in:	100% effluent	50% effluent			
% Hatchability:	56	82			
No. of attempts:	4	5			
Chlorobrominated					
Spawned in:	dilution water	25% effluent			
Incubated in:	100% effluent	dilution water			
% Hatchability:	67	71			
No. of attempts:	13	5			
Ozonated					
Spawned in:	12.5% effluent				
Incubated in:	dilution water				
% Hatchability:	68				
No. of attempts:	6				

ability (56 percent) was lower than the mean of eggs that were both spawned and incubated in dilution water (84 percent) (Table 43), but higher than eggs both produced and incubated in 100 percent dechlorinated effluent (0 percent, based on one incubation attempt). Similarly, eggs spawned in dilution water and incubated in 50 percent dechlorinated effluent showed improved hatchability (82 percent) over those produced and incubated in 50 percent dechlorinated effluent (50 percent). Thus, egg hatchability in dechlorinated effluent was similar to egg hatchability in nondisinfected effluent.

Eggs produced and incubated in chlorobrominated effluent exhibited a hatchability pattern similar to that of the nondisinfected stream, except for the 100 and 12.5 percent effluent concentrations in which no spawnings occurred (Table 43). Since no adults survived exposure to 100 percent chlorobrominated effluent, no eggs were produced. There was no apparent reason for the lack of egg production in the 12.5 percent effluent concentration. The hatchability of eggs spawned in dilution water and incubated in 100 percent chlorobrominated effluent was 67 percent. This result was similar to that which occurred with eggs that were spawned in dilution water and incubated in 100 percent dechlorinated effluent. The hatchability of eggs spawned in 25 percent chlorobrominated effluent and incubated in dilution water was similar to the hatchability of eggs spawned and incubated in the 25 percent chlorobrominated effluent. These findings indicate that chlorobrominated effluent has no adverse effect on the hatchability of fathead minnow eggs.

Eggs spawned and incubated in various concentrations of ozonated effluent had hatchability values similar to the respective concentrations of nondisinfected effluent. While the mean hatchability in ozonated effluent was higher than the mean hatchability in any other 100 percent effluent concentration, limited sample sizes precluded an objective statistical analysis of the data. However, ozonated effluent did not appear to have any adverse effect on the hatchability of fathead minnow eggs.

The mean hatchability in each effluent treatment was calculated by dividing the sum of the percent hatchability values in each concentration of a treatment by the number of concentrations of the respective treatment for which hatchability values were determined (Table 43). A statistical comparison of the mean hatchability values for each effluent treatment showed that no significant differences ($p = 0.05$) existed between the mean hatchability values of any two treatment types.

In summary then, the reproduction studies show that egg production and egg hatchability were reduced in the highest effluent concentrations of all but the ozonated effluent stream. Intermediate effluent concentrations generally tended to be optimum for egg productivity except in the ozonated effluent where the greatest egg production occurred in the 100 percent concentration. This difference may have been due to the fact that during part of the spawning period the ozonated effluent was filtered, while the other effluent streams were not, or to the higher dissolved oxygen levels observed in the ozonated stream prior to delivery to the fish tanks. Except for the 20 percent chlorinated effluent concentration where no fish survived to reproduce, no adverse effects on reproduction occurred as the result of any disinfection process.

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SECTION VI. ACUTE TOXICITY TESTS

INTRODUCTION

Acute toxicity tests are valuable for determining an organism's tolerance to some lethal agent during a relatively short exposure time (usually one week or less). Although acute toxicity tests are not nearly as comprehensive as life cycle tests, they do permit the rapid collection of toxicity data for many species. Thus, concurrently with our life cycle toxicity tests, we conducted acute toxicity tests on each effluent stream as time and the availability of test animals permitted.

METHODS

Acute tests of 96 hours in duration were run using a variety of cold and warm water fishes. Acute tests of 48 hours in duration were also run with the freshwater macroinvertebrate Daphnia magna. In most cases, each species was exposed to all five types of effluent available to us, i.e., chlorinated, dechlorinated, ozonated, chlorobrominated, and nondisinfected. In general, procedures were followed as outlined by the Committee on Methods for toxicity tests with Aquatic Organisms.[1] Tests were conducted at 25C (±1C) for warm water species, and at 14C (±1C) for cold water forms. Diluters similar to those described for the life cycle tests were used to dilute the effluent and deliver the proper concentrations to the test chambers. Dilution water was identical to that used in the life cycle studies. During the tests, the animals were exposed to 753-1346 lumen/sq m of light, with light intensity increasing and decreasing gradually over 30-minute morning and evening periods, respectively. Since the acute tests were conducted in the same area as the life cycle studies, the photoperiod was varied from 10-14 hours of light per day, depending upon the stage of the life cycle tests during which each acute test was run.

Alkalinity, pH, acidity, hardness, conductivity, and ammonia analyses were made once during each acute test on the dilution water, the effluent storage tanks, the control (dilution water) test chambers, and the 100 percent effluent test chambers. Dissolved oxygen concentrations were measured in each test tank at 0, 48, and 96 hours after the start of each test period. The results of these analyses are similar to the results obtained from the identical analyses conducted during the life cycle bioassays (Table 16).

Total residual chlorine, bromine chloride, ozone, and sulfite were measured in the test chambers with a polarograph using methods identical to those described for the life cycle tests. Residuals from one duplicate test tank were measured for each effluent concentration prior to the introduction of the test animals. Immediately after the animals were introduced, the residual analyses were run on the other set of duplicate test chambers. Subsequently, residual disinfectant concentrations were measured at three and six hours after starting time in the highest effluent concentration tanks in which subjects remained alive. After 24 hours, residuals were determined in one duplicate tank of each effluent concentration containing living fish. For the duration of the test, residuals were determined at least two times each day in the highest effluent concentration tanks with living test subjects. In the event of partial mortality of test animals in an effluent concentration, the residual in the next lower effluent concentration tank was also measured.

The TL50 for each test species was calculated using the graphical interpolation method described in Standard Methods[2]. All results of residual determinations for each pair of duplicate test tanks were averaged together to approximate a mean residual level for that concentration for the 96-hour period.

The first two acute bioassays with chlorinated effluent and the first ten with chlorobrominated effluent were conducted using diluters calibrated to deliver to the duplicate test tanks 100 percent effluent, 100 percent dilution water, and six intermediate concentrations, each having 50 percent less effluent than the immediately preceding higher concentration. For the remaining tests the diluters were recalibrated to deliver 100 percent effluent, 100 percent dilution water, and six intermediate test concentrations, each having 40 percent less effluent than the immediately preceding higher concentration. This change was made to minimize the difference in effluent concentration between those tanks having 100 percent mortality and those having 100 percent survival of test animals. Ideally under these conditions, concentrations which would kill 50 percent of the test animals would be maintained. However, under actual conditions, the Grandville effluent was so variable that maintaining a constant disinfectant residual concentration was virtually impossible.

Test animals were either reared in the laboratory, purchased from private sources, or obtained from State of Michigan or National fish hatcheries. In all cases they were held in the laboratory at test temperatures and lighting conditions for at least ten days prior to testing. During the acclimation period, the test fish were observed for signs of disease or parasites. Fish exhibiting symptoms of bacterial infections were treated with Neomycin, Tetracycline, Furox 50 or Furanace; fish having ectoparasites were treated with formalin; and fish having fungal infections were treated with Dexon.

Acute tests were started between 8 A.M. and 12 noon. Small beakers were used to capture and transport the test animals to the test tanks where they were randomly distributed. Ten animals were added to each test tank except when numbers of animals were limited, in which case as few as five were used per tank. Dead individuals were removed every half hour for the first 3 hours, again at 6 hours, and daily thereafter.

Observations on the behavior and general condition of the test subjects were also made at these times. With the exception of the first three tests with bromine chloride, standard lengths (±0.5 mm) and weights (±0.1 g) were recorded for each animal that died. Test subjects were not fed for 96 hours prior to the beginning of the test, nor for the duration of the test.

Most acute tests were performed during the chronic testing period with the same effluent used in the life cycle bioassays. However, some acute bioassays were conducted either before or after the chronic tests with effluent receiving exceptionally high doses of disinfectants or sulfur dioxide in an attempt to produce a toxic response in the test animals. Thus, the reader is cautioned against drawing any conclusions on the efficacy of the various disinfection treatments from the residuals reported in the acute toxicity studies.

As previously mentioned, the quality of the effluent varied throughout the study period and thus was not identical for all acute tests. Also, the feed systems for bromine chloride and ozone occasionally malfunctioned or failed completely. We attempted to conduct our acute toxicity tests on chlorobrominated and ozonated effluents during those periods of time when we had the most confidence in the latter dosing systems. Any tests that we felt contained an unreasonable amount of variation due to mechanical failure were discontinued and not included in this report. Although the residual disinfectant levels in our test tanks varied moderately, such variation probably approximated a natural situation that exists at the discharge point of most wastewater treatment plants.

RESULTS AND DISCUSSION

Acute Toxicity Tests with Nondisinfected Effluent

Nondisinfected effluent was examined for its acute toxic effects on all test species studied in the project. Specific results are not listed, because nondisinfected effluent produced no acute toxicity response in those species of fish tested. However, the freshwater macroinvertebrate, Daphnia magna, was unable to tolerate 100 percent nondisinfected effluent.

Acute Toxicity Tests with Chlorinated Effluent

The acute toxicity of chlorinated effluents on fishes is well documented. Zillich[3] reported 100 percent mortality of fathead minnows exposed to five percent chlorinated effluent after 96 hours. In a literature review, Brungs[4] reported 96 hour TL50 values ranging from 0.014 mg/l total residual chlorine for rainbow trout to 0.19 mg/l total residual chlorine for golden shiners. McKee and Wolf[5] reported various species of fish killed at total residual chlorine levels ranging from 0.03 to 2.0 mg/l. They also discussed the effects of pH, temperature, and dissolved oxygen on the toxicity of chlorinated effluents.

Chlorinated effluent was acutely toxic to all species of fish tested (Table 45), with TL50 values for fish ranging from 0.045 mg/l to 0.278 mg/l total residual chlorine, and greater than 50 percent mortality occurring in effluent concentrations of 3.12 percent to 60 percent. These results generally

Table 45. RESULTS OF ACUTE TOXICITY TESTS WITH CHLORINATED EFFLUENT

Species	Test Temp (C)	96 Hour TL50[a] (mg/1)	Comments
Fathead Minnow Test #1 *Pimephales promelas*	25	0.095	100% mortality at 0.145 mg/1[a] (12.5% effluent) $\bar{x}$ length 32 mm, $\bar{x}$ weight 0.6 g
Fathead Minnow Test #2 *Pimephales promelas*	25	0.082	70% mortality at 0.099 mg/1 (3.12% effluent) $\bar{x}$ length 26 mm, $\bar{x}$ weight 0.4 g
Pugnose Shiner *Notropis anogensus*	25	0.045	75% mortality at 0.057 mg/1 (21.6% effluent) $\bar{x}$ length 43 mm, $\bar{x}$ weight 0.5 g
Northern Common Shiner *Notropis cornutus*	25	0.051	60% mortality at 0.057 mg/1 (21.6% effluent) $\bar{x}$ length 49 mm, $\bar{x}$ weight 0.7 g
Western Golden Shiner *Notemigonus crysoleucas*	25	0.040	100% mortality at 0.047 mg/1 (21.6% effluent) $\bar{x}$ length 98 mm, $\bar{x}$ weight 10.4 g
Goldfish Test #1 *Carassius auratus*	25	0.153	90% mortality at 0.264 mg/1 (21.6% effluent) $\bar{x}$ length 40 mm, $\bar{x}$ weight 2.3 g
Goldfish Test #2 *Carassius auratus*	25	0.210	100% mortality at 0.270 mg/1 (60% effluent) $\bar{x}$ length 55 mm, $\bar{x}$ weight 5.6 g
Lepomis sp. Test #1	25	0.278	100% mortality at 0.370 mg/1 (36% effluent) $\bar{x}$ length 50 mm, $\bar{x}$ weight 3.7 g
Lepomis sp. Test #2	25	0.195	100% mortality at 0.276 mg/1 (21.6% effluent) $\bar{x}$ length 50 mm, $\bar{x}$ weight 3.2 g

[a]Total residual chlorine

(continued)

Table 45. RESULTS OF ACUTE TOXICITY TESTS WITH CHLORINATED EFFLUENT
Page 2.

Species	Test Temp. (C)	96 Hour TL50[a] (mg/1)	Comments
Pomoxis sp.	25	0.127	100% mortality at 0.183 mg/1 (7.8% effluent) $\bar{x}$ length 67 mm, $\bar{x}$ weight 5.8 g
Lake Trout *Salvelinus namaycush*	14	0.060	90% mortality at 0.078 mg/1 (7.8% effluent) $\bar{x}$ length 38 mm, $\bar{x}$ weight 0.5 g
Rainbow Trout *Salmo gairdnerii*	14	0.069	85% mortality at 0.087 mg/1 (7.8% effluent) $\bar{x}$ length 55 mm, $\bar{x}$ weight 2.6 g
Coho Salmon *Oncorhynchus kisutch*	14	0.059	100% mortality at 0.087 mg/1 (7.8% effluent) $\bar{x}$ length 44 mm, $\bar{x}$ weight 1.3 g
Largemouth Bass *Micropterus salmoides*	25	0.241	95% mortality at 0.320 mg/1 (60% effluent) $\bar{x}$ length 71 mm, $\bar{x}$ weight 7.6 g
Yellow Walleye *Stizostedion vitreum*	25	0.108	100% mortality at 0.181 mg/1 (60% effluent) $\bar{x}$ length 70 mm, $\bar{x}$ weight 3.4 g
Daphnia magna Test #1 (3 days old)			100% mortality in 5.5 hours at 0.220 mg/1 (13% effluent), and total mortality in 10.5 hours at 0.070 mg/1 (4% effluent)
Daphnia magna Test #2 (Less than 1 day old)	25	0.017	30% mortality at 0.011 mg/1 (23% effluent)

agree with those of Arthur, et al., who reported TL50 values ranging from 0.08 to 0.154 mg/l total residual chlorine.[6,7] Salmonids and shiners generally exhibited the lowest tolerance of residual chlorine, while members of the sunfish family exhibited the greatest tolerance. The typical signs of stress in fish exposed to chlorinated effluent were gasping at the surface, rapid gill movements, loss of equilibrium, hemorrhaging at the gills and base of fins, loss of slime coat, rapid erratic movements, and a passive floating or lying on the bottom prior to death. These are similar to the symptoms described by Zillich.[3] In most cases, any mortality which occurred during a 96-hour test was complete by the end of the first 48 hours. The final two days of most acute tests were generally uneventful.

D. magna was more sensitive to residual chlorine toxicity than any species tested. Total residual chlorine concentrations of 0.220 mg/l and 0.070 mg/l were lethal to 3-day old D. magna in 5.5 and 10.5 hours, respectively. In a 48-hour acute test with D. magna less than 1 day old, a TL50 of 0.017 mg/l total residual chlorine was observed. This was the lowest TL50 value of any acute test. Thus, extremely low levels of chlorinated effluent may adversely affect the survival of some invertebrates which are potential food supplies for many species of fish.

Acute Toxicity Tests with Dechlorinated Effluent

The same species that were tested with chlorinated effluent were also tested with chlorinated effluent that had been dechlorinated with sulfur dioxide (Table 46). The results show that the dechlorination process effectively detoxified the chlorinated effluent. Arthur, et al.,[6] Coventry,[8] Allen,[9] and Zillich[3] all reported that addition of sufficient quantities of sodium thiosulfate or sulfur dioxide to chlorinated water or wastewater effectively removed the toxic properties.

Acute tests conducted with dechlorinated effluent having sulfite residuals in the range that we normally maintained (0.00-1.937 mg/l) caused little mortality, and hence a TL50 value could not be calculated. The only TL50 values obtained for fish were for western golden shiners (4.82 mg/l) and pugnose minnows (5.68 mg/l), which, in an attempt to determine lethal sulfite concentrations, were exposed to elevated sulfite residual levels (9.52 to 10.44 mg/l). The mortality observed in these tests was at least partially attributable to the depressed dissolved oxygen concentrations which occurred in the highest effluent concentrations (0.95-1.6 mg/l in 100 percent effluent).

In the remainder of the acute tests, fish survived in 100 percent dechlorinated effluent as well as they did in 100 percent nondisinfected effluent. It is interesting to note that the salmonids and shiners, which were relatively sensitive to chlorinated effluent, were able to survive in 100 percent dechlorinated effluent with little mortality. D. magna was the only species tested which exhibited mortality in 100 percent dechlorinated effluent at normal sulfite residual levels. This is not surpising considering the sensitivity of this species and the fact that two acute tests using this same species in full-strength nondisinfected effluent resulted in 50 percent and 100 percent mortality, respectively.

Table 46. RESULTS OF ACUTE TOXICITY TESTS WITH DECHLORINATED EFFLUENT

Species	Test Temp. (C)	96 Hour TL50[a] (mg/1)	Comments
Fathead Minnow Test #1 *Pimephales promelas*	25		No mortality at 0.041 mg/1[a] (100% effluent)
Fathead Minnow Test #2 *Pimephales promelas*	25		No mortality at 8.417 mg/1 (100% effluent)
Fathead Minnow (Fry) *Pimephales promelas*	25		30% mortality at 2.10 mg/1 (100% effluent)
Pugnose Minnow *Opsopoeodus emiliae*	25	5.68	30% mortality at 4.36 mg/1 (60% effluent)
Pugnose Shiner *Notropis anogenus*	25		25% mortality at 0.364 mg/1 (100% effluent)
Northern Common Shiner *Notropis cornutus*	25		No mortality at 0.364 mg/1 (100% effluent)
Western Golden Shiner *Notemigonus crysoleucas*	25	4.820	40% mortality at 4.15 mg/1 (60% effluent) $\bar{x}$ length 98 mm, $\bar{x}$ weight 16.5 g
Goldfish Test #1 *Carassius auratus*	25		No mortality at 0.033 mg/1 (100% effluent)
Goldfish Test #2 *Carassius auratus*	24		No mortality at 0.00 mg/1 (100% effluent)
Lepomis sp.	25		No mortality at 0.671 mg/1 (100% effluent)

[a]Residual sulfite

(continued)

Table 46. RESULTS OF ACUTE TOXICITY TESTS WITH DECHLORINATED EFFLUENT
Page 2.

Species	Test Temp. (C)	96 Hour TL50[a] (mg/l)	Comments
Lake Trout Test #1 *Salvelinus namaycush*	14		No mortality at 1.973 mg/l (100% effluent)
Lake Trout Test #2 *Salvelinus namaycush*			Nine juveniles ($\bar{x}$ length 43 mm, $\bar{x}$ wt. 0.80 g) were exposed to dechlorinated effluent shortly after introduction of the sulfur dioxide. In 8½ hours one had died, and in 10½ hours all were dead. Residuals were not monitored during this time period.
Rainbow Trout *Salmo gairdnerii*	14		5% mortality at 0.287 mg/l (100% effluent)
Brown Trout *Salmo trutta*	15		No mortality at 0.002 mg/l (100% effluent)
Coho Salmon *Oncorhynchus kisutch*	14		No mortality at 0.287 mg/l (100% effluent)
Chinook Salmon *Oncorhynchus tshawytscha*	15		15% mortality at 0.002 mg/l (100% effluent)
Large Mouth Bass *Micropterus salmoides*	25		No mortality at 0.000 mg/l (100% effluent)
Yellow Walleys *Stizostedion vitreum*	24		60% mortality in 60% effluent, 30% mortality in 36% effluent. Residual levels were too inconsistent to calculate a TL50.
Daphnia magna Less than 24 hours old	25	0.018	50% mortality at 0.018 mg/l (100% effluent)

With the exceptions of the two instances noted above where sulfite residuals were intentionally elevated to unusually high levels (9.52-10.44 mg/l), low dissolved oxygen concentrations were not a cause for concern in our acute tests. The mean dissolved oxygen levels in the 100 percent effluent concentrations for all the dechlorinated, nondisinfected, and chlorinated acute tests were 4.8, 5.1, and 6.4 mg/l respectively.

Acute Toxicity Tests with Chlorobrominated Effluent

Table 47 summarizes the results of acute toxicity tests performed with chlorobrominated effluent. The chlorobrominated effluent was less toxic than chlorinated effluent, as evidenced by the higher TL50 values. This supports the findings of Mills,[10] who concluded that chlorobromination produced a less toxic effluent because bromamines are less stable than chloramines and thus do not persist as long. However, chlorobrominated effluent was more toxic than the other effluents tested, which contrasts with Zillich's[11] observation that there is no appreciable difference in the toxicity of chlorobrominated and nondisinfected wastewater. These apparently conflicting results are probably due to differences in methodology. Zillich conducted his tests under static conditions, while we utilized flow-through techniques.

Ten of the acute tests listed on Table 47 were conducted with effluent dosed with 10.3 mg/l of bromine chloride (the amount generally required to achieve disinfection was 2.0-3.0 mg/l). This high feed rate was used to generate sufficient toxicity data to compute TL50 values for bromine chloride, which is a relatively new and untested wastewater disinfectant. The signs of stress associated with bromine chloride toxicity were identical to those produced by chlorinated effluent.

Bromine chloride was found to be approximately half as toxic to fathead minnows and shiners as chlorine (Table 45). Of the species tested in chlorobrominated effluent at normal dosage levels (2-3 mg/l), fathead minnows, lake trout, chinook salmon, and D. magna were sufficiently sensitive to permit the calculation of TL50 values. The lowest chlorobrominated effluent concentration producing significant mortality was 36 percent (TL50 of 0.102 mg/l total residual bromine chloride for lake trout), while effluent disinfected with chlorine produced mortality in the same species at 3.12 percent effluent (TL50 of 0.060 mg/l total residual chlorine).

Acute Toxicity Tests with Ozonated Effluent

No mortality was observed in those acute toxicity tests conducted during periods when ozone was effectively disinfecting the effluent. Thus, TL50 values could not be calculated for any test.

In experiments where fish were placed in aquaria receiving 100 percent ozonated effluent within 10 minutes after the injection of ozone, goldfish and fathead minnows survived residual ozone concentrations of 0.047-0.185 mg/l for seven to fifteen days without any mortality (Table 48). However, under similar conditions, lake trout fingerlings died within 5 hours when the residual ozone concentration was 0.322 mg/l. Similarly, Arthur et. al[6]

Table 47. RESULTS OF ACUTE TOXICITY TESTS WITH CHLOROBROMINATED EFFLUENT

Species	Test Temp. (C)	96 Hour TL50[a] (mg/l)	Comments
Fathead Minnow Test #1 *Pimephales promelas*	25	0.185[b]	100% mortality at 0.286 mg/l[a] (25% effluent)
Fathead Minnow Test #2 *Pimephales promelas*	25	0.173[b]	100% mortality at 0.246 mg/l (50% effluent)
Fathead Minnow Test #3 *Pimephales promelas*	25	0.193[b]	100% mortality at 0.328 mg/l (25% effluent) $\bar{x}$ length 31 mm, $\bar{x}$ weight 0.6 g
Fathead Minnow Test #4 *Pimephales promelas*	25		5% mortality at 0.082 mg/l (100% effluent) This test was 14 days in duration.
Fathead Minnow Test #5 *Pimephales promelas*	25	0.148	100% mortality at 0.321 mg/l (50% effluent) $\bar{x}$ length 23 mm, $\bar{x}$ weight 0.3 g
Fathead Minnow Test #6 *Pimephales promelas*	25	0.133	85% mortality at 0.175 mg/l (50% effluent) $\bar{x}$ length 28 mm, $\bar{x}$ weight 0.5 g
Northern Common Shiner Test #1 *Notropis cornutus*	25	0.120[b]	100% mortality at 0.161 mg/l (60% effluent) $\bar{x}$ length 45 mm, $\bar{x}$ weight 0.8 g This test was only 24 hours in duration due to brominator failure.
Northern Common Shiner Test #2 *Notropis cornutus*	25	0.140[b]	100% mortality at 0.211 mg/l (100% effluent) $\bar{x}$ length 50 mm, $\bar{x}$ weight 1.0 g

(continued)

[a]Total residual bromine chloride

[b]Tests in which high dosages of bromine chloride were intentionally applied to produce a toxic response in the test animals.

Table 47. RESULTS OF ACUTE TOXICITY TESTS WITH CHLOROBROMINATED EFFLUENT
Page 2.

Species	Test Temp. (C)	96 Hour TL50[a] (mg/1)	Comments
Pugnose Shiner Test #1 *Notropis anogenus*	25	0.109[b]	100% mortality at 0.161 mg/1 (60% effluent) $\bar{x}$ length 44 mm, $\bar{x}$ weight 0.6 g This test was only 24 hours in duration due to brominator failure.
Pugnose Shiner Test #2 *Notropis anogenus*	25	0.136[b]	100% mortality at 0.211 mg/1 (100% effluent) $\bar{x}$ length 47 mm, $\bar{x}$ weight 0.8 g
Western Golden Shiner *Notemigonus crysoleucas*	25	0.090[b]	55% mortality at 0.095 mg/1 (50% effluent) $\bar{x}$ length 96 mm, $\bar{x}$ weight 8.9 g
Goldfish *Carassius auratus*	25		35% mortality at 0.127 mg/1 (100% effluent)
Lake Trout *Salvelinus namaycush*	14	0.102	100% mortality at 0.154 mg/1 (36% effluent) $\bar{x}$ length 39 mm, $\bar{x}$ weight 0.7 g
Rainbow Trout *Salmo gairdnerii*	16		43% mortality at 0.153 mg/1 (100% effluent) $\bar{x}$ length 73 mm, $\bar{x}$ weight 6.4 g
Brown Trout *Salmo trutta*	16		20% mortality at 0.066 mg/1 (100% effluent) $\bar{x}$ length 53 mm, $\bar{x}$ weight 2.9 g
Coho Salmon *Oncorhynchus kisutch*	16		21% mortality at 0.153 mg/1 (100% effluent) $\bar{x}$ length 65 mm, $\bar{x}$ weight 4.5 g
Chinook Salmon *Oncorhynchus tschawytscha*	16	0.059	60% mortality at 0.066 mg/1 (100% effluent) $\bar{x}$ length 63 mm, $\bar{x}$ weight 3.9 g
Largemouth Bass *Micropterus salmoides*	25		No mortality at 0.095 mg/1 (100% effluent)

(continued)

Table 47. RESULTS OF ACUTE TOXICITY TESTS WITH CHLOROBROMINATED EFFLUENT
Page 3.

Species	Test Temp. (C)	96 Hour TL50[a] (mg/l)	Comments
Lepomis sp.	25		No mortality at 0.063 mg/l (100% effluent)
Northern Yellow Bullhead *Ictalurus natalis*	25	0.177[b]	100% mortality at 0.285 mg/l (25% effluent) $\bar{x}$ length 95 mm, $\bar{x}$ weight 19.0 g
Northern Black Bullhead *Ictalurus melas*	25	0.283[b]	50% mortality at 0.283 mg/l (25% effluent) $\bar{x}$ length 99 mm, $\bar{x}$ weight 21.4 g
Crayfish *Orconectes propinqus*	25		No mortality at 0.071 mg/l (100% effluent)
Daphnia magna Test #1 Less than 24 hours old	25	0.047	90% mortality at 0.068 mg/l (60% effluent)
Daphnia magna Test #2 Less than 24 hours old	25	0.055	70% mortality at 0.072 mg/l (37% effluent)

Table 48. RESULTS OF ACUTE TOXICITY TESTS WITH OZONATED EFFLUENT

Species	Test Temp. (C)	96 Hour TL50 (mg/1)	Comments
Fathead Minnow Test #1 Pimephales promelas	18		Ten juvenile fatheads were exposed for 11 days to 100% ozonated effluent shortly after contact. The mean residual during that period was 0.058 mg/1.[a] There were no mortalities.
Fathead Minnow Test #2 Pimephales promelas	18		Ten males in spawning condition and five juveniles were exposed for 15 days to 100% ozonated effluent shortly after contact. The mean ozone residual during that period was 0.047 mg/1, and there were no mortalities.
Pugnose Shiner Notropis anogenus	25		No mortality at 0.016 mg/1 (100% effluent)
Northern Common Shiner Notropis cornutus	25		No mortality at 0.016 mg/1 (100% effluent)
Goldfish Test #1 Carassius auratus	25		No mortality at 0.007 mg/1 (100% effluent)
Goldfish Test #2 Carassius auratus	25	b	No mortality at 0.038 mg/1 (100% effluent)
Goldfish Test #3 Carassius auratus	16		Ten adult goldfish were exposed for 7 days to 100% ozonated effluent shortly after contact. The mean residual during that period was 0.185 mg/1. No mortalities were attributed to ozone toxicity.
Lepomis sp.	25		No mortality at 0.002 mg/1 (100% effluent)

(continued)

[a]Residual ozone

[b]Tests conducted during periods when ozonation disinfected the effluent to project standards.

Table 48. RESULTS OF ACUTE TOXICITY TESTS WITH OZONATED EFFLUENT
Page 2.

Species	Test Temp. (C)	96 Hour TL50 (mg/1)	Comments
Large Mouth Bass *Micropterus salmoides*	25	b	No mortality at 0.012 mg/1 (100% effluent) This test was terminated after 72 hours when the ozone generator failed.
Lake Trout Test #1 *Salvelinus namaycush*	14	b	No mortality at 0.016 mg/1 (100% effluent)
Lake Trout Test #2 *Salvelinus namaycush*	14	b	Nine fingerlings ($\bar{x}$ length 43 mm and $\bar{x}$ weight 0.83 g) were exposed to ozonated effluent shortly after contact. The mean ozone residual was 0.322 mg/1. All test animals died within 5 hours.
Rainbow Trout *Salmo gairdnerii*	15		No mortality at 0.010 mg/1 (100% effluent) This test was terminated after 48 hours when the ozone generator failed.
Brown Trout *Salmo trutta*	17	b	No mortality at 0.018 mg/1 (100% effluent)
Coho Salmon *Oncorhynchus kisutch*	15		No mortality at 0.010 mg/1 (100% effluent) This test was terminated after 48 hours when the ozone generator failed.
Chinook Salmon *Oncorhynchus tschawytscha*	17	b	No mortality at 0.018 mg/1 (100% effluent)
Daphnia magna (Less than 24 hours old)	25		30% mortality at 0.030 mg/1 (100% effluent)

found that residual ozone concentrations of 0.2-0.3 mg/l were lethal to fathead minnows. While this indicates that high ozone concentrations in effluent are lethal to fish, such high ozone residuals are unlikely to occur in receiving waters. Our lake trout were exposed to undiluted effluent approximately 6 to 7 minutes after ozone injection. Under actual operating conditions, the time between ozone injection and effluent discharge will be greater and the effluent will be diluted by the receiving waters.

Acclimation Tests

In several instances we observed that if residual chlorine or bromine chloride levels were relatively low at the start of an acute test, and then gradually increased to a higher level, test animals were able to tolerate higher residual levels for the duration of the test than they would have been able to tolerate had they not had previous exposure. In other words, they were achieving a certain degree of acclimation to chlorine or bromine chloride in effluent. To test this hypothesis, we conducted two experiments, one with fathead minnows exposed to chlorinated effluent, the other with lake trout exposed to chlorobrominated effluent.

The first experiment consisted of eight individual tests utilizing five fathead minnows each. These tests were conducted over a 7-week period. Each test group was exposed to one sub-lethal concentration of residual chlorine for 1 week, then transferred to a slightly higher concentration for another week. This procedure was continued until each group of fish was exposed to chlorine concentrations which were higher than our TL50 values for fathead minnows. At that time a control group of non-exposed fish was isolated in the same test tank and the mortality of both groups was monitored. If that group survived for 1 week, it was transferred to the next higher concentration.

The second acclimation experiment consisted of 4 tests, each utilizing 18 fingerling lake trout. The fish were exposed to sub-lethal concentrations of chlorobrominated effluent for 4 to 9 days, then placed in effluent having bromine chloride residual levels well above their TL50 values. Mortality was monitored to ascertain the degree of acclimation achieved by the test animals.

Table 49 indicates that fathead minnows previously exposed to sub-lethal levels of residual chlorine were able to tolerate residual chlorine levels greater than our observed TL50 values (0.082-0.095 mg/l). Fathead minnows survived for 1 week at residual chlorine levels of 0.113, 0.116, 0.110, 0.134, and 0.138 mg/l, while all control fish died in less than 68 hours. Also, at higher residual levels (0.215 to 0.512 mg/l) previously exposed fathead minnows survived 11 to 44 times longer (20-142 hours) than control groups.

Our data also suggest that there is a direct relationship between increased resistance of fathead minnows to high residual concentrations and length of exposure time to sub-lethal chlorine levels. For example, in tests numbers

Table 49. FATHEAD MINNOW ACCLIMATION TEST IN CHLORINATED EFFLUENT

Test No.		Week Number 1	2	3	4	5	6	7
1	A	0.056	0.063	0.113	0.504			
	B	6.3%	12.5%	25%	50%			
	C	NM	NM	NM	20 hrs.			
	D			20 hrs.	1.5 hrs.			
2	A	0.052	0.064	0.116	0.512			
	B	6.3%	12.5%	25%	50%			
	C	NM	NM	NM	20 hrs.			
	D			68 hrs.	1 hr.			
3	A	0.021	0.036	0.064	0.233			
	B	3.1%	6.3%	12.5%	25%			
	C	NM	NM	NM	20 hrs.			
	D							
4	A	0.018	0.047	0.069	0.215			
	B	3.1%	6.3%	12.5%	25%			
	C	NM	NM	NM	28 hrs.			
	D							
5	A	0.007	0.016	0.033	0.110	0.306		
	B	1.6%	3.1%	6.3%	12.5%	25%		
	C	NM	NM	NM	NM	142 hrs.		
	D					4 hrs.		
6	A	0.007	0.029	0.043	0.113	0.318		
	B	1.6%	3.1%	6.3%	12.5%	25%		
	C	NM	NM	NM	NM	45 hrs.		
	D					4 hrs.		
7	A		0.008	0.021	0.042	0.134	0.241	
	B		1.6%	3.1%	6.3%	12.5%	25%	
	C		NM	NM	NM	NM	142 hrs.	
	D					4 hrs.		
8	A		0.012	0.022	0.070	0.138	0.224	0.359
	B		1.6%	3.1%	6.3%	12.5%	25%	50%
	C		NM	NM	NM	NM	(20%)	44 hrs.
	D						9 hrs.	1 hr.

A = Average total residual chlorine (mg/l) to which fish were exposed.
B = Percent effluent to which fish were exposed.
C = Time required for total mortality of all previously exposed test animals (or percent of test animals dying during the one week exposure period). NM indicates no mortality.
D = Time required for total mortality of test animals not previously exposed. (Blanks indicate no fish tested.)

three and four (Table 49), after 3 previous weeks of exposure to sub-lethal chlorine residuals, fathead minnows survived for 20 hours and 28 hours when subjected to 0.233 and 0.215 mg/l residual chlorine, respectively. Tests five and six indicate that fathead minnows exposed previously for 4 weeks survived exposure to chlorine residual levels of 0.318 and 0.306 mg/l for 45 and 142 hours, respectively. Tests numbers seven and eight show similar trends.

Four separate acclimation tests were conducted with lake trout in chlorobrominated effluent (Table 50). Test one involved 18 trout which were initially exposed to an average of 0.068 mg/l total residual bromine chloride for 4 days, and then were exposed to a residual bromine chloride concentration of 1.066 mg/l. After 3 hours of exposure, 72 percent were still alive. (Our TL50 value for lake trout in chlorobrominated effluent was 0.102 mg/l.) In the second test lake trout were subjected to mean bromine chloride residuals of 0.029 mg/l for 9 days and then were placed in a tank having 0.664 mg/l residual bromine chloride. The first mortality occurred at 9 hours, and all of the test animals were dead 4 hours later. Unexposed lake trout (test three), which were subjected to effluent having similar residual levels (0.647 mg/l), were all dead in 4-1/2 hours. The fish in test four were exposed to 0.011 mg/l bromine chloride for 9 days and were then placed in effluent having 0.635 mg/l residual bromine chloride. They began dying in 6 hours and were all dead after 8 hours. This suggests that group two was able to tolerate similar residual levels for a longer period of time, because they were exposed to a slightly higher bromine chloride concentration (0.029 mg/l) than group three (0.011 mg/l).

The above data indicate that at least two species of fish, if previously exposed to sublethal residual concentrations of chlorine or bromine chloride, are capable of tolerating levels of chlorine and bromine chloride higher than their 96-hour TL50 values for longer periods of time than fish which were not previously exposed to either disinfectant.

SUMMARY

In summary, the results of our acute tests indicate that nondisinfected, ozonated, and dechlorinated effluents were nontoxic to all fish species tested, and toxic only in high effluent concentrations to D. magna. Chlorobrominated effluent, however, was toxic to all fish exposed to elevated doses, but toxic to some fish species only in high effluent concentrations with normal residuals. D. magna, an invertebrate which serves as food for fish, was more sensitive than any fish species tested. Effluent disinfected with chlorine was the most toxic of those we tested. Sufficient mortality to calculate a TL50 value was recorded for each species exposed to chlorine. This was not true for any other effluent type. Also, residual levels and effluent concentrations that produced mortality in fish and D. magna were lower for chlorinated effluent than any other effluent tested.

Table 50. LAKE TROUT FINGERLING ACCLIMATION TESTS IN CHLOROBROMINATED EFFLUENT

Test Number		Eighteen fish were used in each test ($\bar{x}$ length 39 mm, $\bar{x}$ weight 0.7 g)	
1	A	0.068 mg/l	(12.5% effluent)
	B	4 days	
	C	1.066 mg/l	(100% effluent)
	D	72% survival after 3 hours, 22% survival after 5 hours. Time to total mortality unknown.	
2	A	0.029 mg/l	(6.3% effluent)
	B	9 days	
	C	0.664 mg/l	(100% effluent)
	D	Total mortality in 13 hours.	
3	A	0.000 mg/l	(0% effluent)
	B		
	C	0.647 mg/l	(100% effluent)
	D	Total mortality in 4.5 hours.	
4	A	0.011 mg/l	(1.6% effluent)
	B	9 days	
	C	0.635 mg/l	(100% effluent)
	D	Total mortality in 8 hours.	

A = Total residual bromine chloride to which the lake trout were initially exposed.
B = Length of time the fish were exposed to the initial residual.
C = Total residual bromine chloride to which lake trout were subsequently exposed.
D = Elapsed time prior to the death of all test animals in the subsequent exposure.

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TECHNICAL REPORT DATA
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1. REPORT NO. EPA-600/2-76-156	2.	3. RECIPIENT'S ACCESSION NO.
4. TITLE AND SUBTITLE DISINFECTION EFFICIENCY AND RESIDUAL TOXICITY OF SEVERAL WASTEWATER DISINFECTANTS Volume I. Grandville, Michigan		5. REPORT DATE October 1976 (Issuing Date) 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Ronald W. Ward,* Randall D. Giffin,** G. Michael DeGraeve,* and Richard A. Stone**		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS **City of Wyoming, Michigan 1155 - 28th Street, S.W. Wyoming, Michigan 49509		10. PROGRAM ELEMENT NO. 1BC611 11. CONTRACT/GRANT NO. S802292
12. SPONSORING AGENCY NAME AND ADDRESS Municipal Environmental Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Cincinnati, Ohio 45268		13. TYPE OF REPORT AND PERIOD COVERED Final Report, Jan.-Nov. 1974 14. SPONSORING AGENCY CODE EPA-ORD

15. SUPPLEMENTARY NOTES
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16. ABSTRACT
This study was conducted to determine the comparative effectiveness of chlorine, bromine chloride, and ozone as wastewater disinfectants, and to determine any residual toxicity associated with wastewater disinfection with these agents or with chlorinated wastewater which had been dechlorinated with sulfur dioxide.

Streams of nondisinfected and chlorinated wastewater were pumped from the Grandville, Michigan, Wastewater Treatment Plant to the project laboratory. Part of the chlorinated wastewater stream was delivered directly to the toxicity laboratory for bioassay studies while the remainder of the chlorinated stream was dechlorinated with sulfur dioxide prior to its use in bioassay tests. A portion of the nondisinfected wastewater stream was delivered to the toxicity laboratory for use in bioassays while the remaining portion was split to receive bromine chloride and ozone prior to use in the bioassay studies.

Total and fecal coliform densities, suspended solids, volatile solids, COD, ammonia nitrogen, phosphate, turbidity, color, and pH were measured in the wastewater streams. Each of the five wastewater streams was used in acute toxicity tests with several species of fishes and the freshwater macroinvertebrate Daphnia magna, and in a life cycle toxicity study with the fathead minnow, Pimephales promelas, as the test subject.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Waste water, Disinfection, Disinfectants, Bactericides, Effectiveness, Efficiency, Chlorination, Chlorine, Dechlorination, Sulfur dioxide, Bromine halides, Ozone, Ozonization, Coliform bacteria, Toxicity, Bioassay, Aquatic animals, Daphnia, Fishes	Wyoming (Michigan) parallel streams, Acute bioassays, Chronic bioassays, Residual toxicity	13B 6F

18. DISTRIBUTION STATEMENT Release unlimited	19. SECURITY CLASS *(This Report)* UNCLASSIFIED	21. NO. OF PAGES 144
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE