UNC-WRRI-94-281

TREATABILITY ASSESSMENT OF JORDAN LAKE

by

Philip C. Singer, Louise M. Ball, Francis A. DiGiano,
Kathryn L. Miller, and Hope C. Taylor

Department of Environmental Sciences and Engineering
University of North Carolina at Chapel Hill
Chapel Hill, NC 27599

The activities on which this report is based were financed in part by the Department of the Interior, U.S. Geological Survey, through the Water Resources Research Institute of The University of North Carolina. The contents of this publication do not necessarily reflect the views and policies of the Department of the Interior, nor does mention of trade names or commercial products constitute their endorsement by the United States government.

Agreement No. 14-08-0001-G1580
USGS Project No. 05 (FY89)
WRRI Project No. 70098

One hundred fifty copies of this report were printed at a cost of $2,173.50 or $14.49 per copy.

ACKNOWLEDGMENT

We thank the operators of local drinking water treatment plants for their cooperation in making their water and analytical results available for this study, and Ms. D.C. Simmons for assistance in preparing this report.

ABSTRACT

At the time this study was initiated, Jordan Lake was being considered as a source of municipal drinking water by several communities in the central Piedmont of North Carolina. Because of the industrialized and urbanized nature of the Jordan Lake watershed, concern had been expressed about the presence of contaminants in the water that may be harmful to public health. Additionally, because of growing concern about disinfection by-products, especially those arising from chlorination of water, advanced water treatment technologies may be appropriate for the treatment of Jordan Lake water.

This research project evaluated three different treatment scenarios for Jordan Lake water. The first consisted of conventional coagulation, sedimentation, and filtration, followed by chlorination. The other two involved advanced treatment schemes, one incorporating adsorption using granular activated carbon to remove disinfection by-product precursors and any synthetic organic chemicals in the raw water, and the other employing ozonation and chloramination as an alternative disinfection program to free chlorine.

Water samples were collected from Segment III of Jordan Lake on a quarterly basis for a full year and subjected to the above three treatment schemes. The treated waters were analyzed for the removal of total organic carbon, and the formation of trihalomethanes and total organic halides. Additionally, in order to test for the presence of potentially carcinogenic contaminants in the raw and treated waters, the respective samples were analyzed by the Ames Salmonella plate incorporation assay. The Ames assay is the most widely-applied test for genotoxicity and is widely accepted as a standard technique for the detection of mutagens. It is often used as a predictor of carcinogenicity.

The results show that the treatment train involving granular activated carbon adsorption produced a water with the lowest total organic carbon concentration and the lowest formation of trihalomethanes and total organic halides. None of the finished waters treated by this scheme exhibited any mutagenic activity in any of the strains of Salmonella tested. In contrast, about half of the raw water samples analyzed exhibited significant mutagenicity in at least one of the strains tested, and more than half of the samples exhibited mutagenic activity following conventional treatment and chlorination. The majority of these were from the tributaries to Jordan Lake. The treatment train involving ozonation and chloramination also produced finished water with low trihalomethane concentrations and also exhibited no mutagenic activity, although some of the samples were Ames-positive following ozonation alone. The findings of this study are expected to be applicable to other drinking water supplies in North Carolina and elsewhere that draw water from urbanized/ industrialized watersheds.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

SUMMARY AND CONCLUSIONS

B. Everett Jordan Lake, in the central Piedmont of North Carolina, was being considered as a source of municipal drinking water for several communities at the time this investigation was initiated. Concern had been expressed about the suitablity of Jordan Lake as a source of drinking water because of the industrialized nature of the watershed that it drains. Advanced water treatment technologies have been suggested to assure that the treated water is free of potentially harmful chemical contaminants.

The objectives of this project were to evaluate three different scenarios for treatment of Jordan Lake water. The three treatment schemes consisted of: Scheme 1, conventional surface water treatment and post-chlorination; Scheme 2, conventional treatment followed by granular activated carbon adsorption and post-chlorination; and Scheme 3, conventional treatment followed by ozonation and post-chloramination in place of disinfection with free chlorine. Finished water quality from each of the process trains was analyzed for removal of total organic carbon (TOC), disinfection by-product (trihalomethanes and total organic halides) formation, and mutagenicity using the Ames bioassay procedure.

Samples of Jordan Lake water were collected during all four seasons and subjected to the three different treatment schemes cited above. The treatments were conducted on a batch basis. The raw water was analyzed for mutagenicity by the Ames assay procedure, and for TOC, trihalomethanes (THMs), and total organic halides (TOX). The Ames mutagenicity assay was run using both strains TA98 and TA100, with and without metabolic activation. The same analyses were performed on water samples following conventional pre-treatment (coagulation with alum, flocculation, sedimentation, and filtration), and pre-treatment followed by chlorination, activated carbon adsorption, ozonation, and chloramination. Chemical doses were selected to match typical treatment practices.

The same treatment scenarios and analytical scheme were applied to four samples of water from nearby Falls Lake, for purposes of comparison; Falls Lake serves as the water supply for the City of Raleigh, NC. Additionally, samples of finished water from two local water treatment plants were analyzed for Ames mutagenicity, THMs, and TOX to serve as comparisons with the laboratory-treated Jordan and Falls Lake water treated by the three different treatment schemes.

The principal findings of this research project are summarized in Table 1 and show that the treatment train consisting of conventional pre-treatment followed by granular activated carbon adsorption and post-chlorination (Scheme 2) produced the highest quality water compared to Scheme 3 consisting of conventional pre-treatment followed by ozonation and chloramination, and Scheme 1 consisting of conventional pre-treatment and post-chlorination. Scheme 2 produced a finished water with an average TOC concentration of 0.3 mg/L for both Jordan and Falls Lakes, amounting to 93% and 91% removal of TOC, respectively, compared to raw water from the two Lakes. By comparison, Scheme 3 produced a finished water with an average TOC concentration of 3.4 mg/L from Jordan Lake and 3.2 mg/L from Falls Lake, and Scheme 1, consisting of conventional treatment, produced a

finished water with an average TOC concentration of 4.0 mg/L from Jordan Lake and 3.6 mg/L from Falls Lake.

Table 1. Summary of Treatability Results

Parameter	Scheme 1 Conventional treatment plus free chlorination	Scheme 2 Conventional treatment plus activated carbon adsorption plus free chlorination	Scheme 3 Conventional treatment plus ozonation plus chloramination
Average TOC Concn, mg/l			
Jordan Lake	4.0	0.3	3.4
Falls Lake	3.6	0.3	3.2
Average THM Formation Potential, µg/L			
Jordan Lake	102	2	6
Falls Lake	97	6	6
Average TOX Formation Potential, µg/L			
Jordan Lake	307	24	129
Falls Lake	320	15	109
Mutagenicity, no. positive responses/ total no. samples analyzed	11/17	0/8	0/7

The average formation of THMs (2 day storage at pH 8.0, in the dark, at room temperature, to simulate distribution system conditions) from Scheme 1 were 102 and 97 ug/L from Jordan and Falls Lake, respectively, compared to only 2 and 6 ug/L for Scheme 2 and 6 and 6 ug/L for Scheme 3. The current maximum contaminant level (MCL) for total THMs is 100 ug/L, and the US Environmental Protection Agency is currently considering adoption of a more stringent standard. The results clearly indicate that the two advanced treatment technologies consisting of ozonation/chloramination and GAC/chlorination can produce a simulated distribution system water with THM concentrations below 10 ug/L.

The corresponding average formation of all organic halides, i.e. TOX, from Scheme 1 were 307 and 320 ug/L for Jordan and Falls Lake, respectively, compared to 129 and 109 ug/L for Scheme 3 and 24 and 15 ug/L for Scheme 2. The treatment train involving GAC (Scheme 2) had the lowest chlorine consumption and, correspondingly, the lowest level of organic halide formation, reflecting the low level of residual TOC in the finished water.

With respect to mutagenicity, 6 out of 13 raw water samples analyzed exhibited significant mutagenicity in at least one of the strains tested. The majority of these were from the tributaries to Jordan Lake. For raw waters that were subjected to Scheme 1 consisting of pre-treatment and chlorination, 11 out of 17 samples exhibited mutagenicity in at least one strain. By comparison, for the 8 samples treated by the GAC-chlorination train (Scheme 2), none exhibited mutagenic activity in any of the strains. The ozonation-chloramination train (Scheme 3) also exhibited no mutagenic activity for the 7 samples processed by this train, although 2 of the 7 samples were Ames-positive in at least one strain following ozonation alone.

The results confirm our general understanding that conventional treatment employing disinfection with chlorine produces disinfection by-products that are mutagenic and potentially carcinogenic, and that advanced treatment approaches using either granular activated carbon adsorption to enhance the removal of DBP precursors or an alternative disinfection program employing ozonation and chloramination in place of free chlorine produces a finished water of higher quality, that is low in disinfection by-product formation, and has no detectable mutagenic activity. The findings that Falls Lake water exhibited similar behavior to Jordan Lake water and that halogenated disinfection by-product concentrations in the finished water from two local water treatment plants were similar to those produced by chlorination of Jordan Lake water following conventional treatment suggest that the results are aplicable to a number of other water supplies as well.

RECOMMENDATIONS

As a result of the findings in this study, and in view of the fact that regulations for halogenated disinfection by-products are going to become more stringent in the near future, it is recommended that utilities using, or contemplating the use of Jordan Lake and Falls Lake as a source of drinking water supply seriously consider the use of granular activated carbon adsorption as part of their treatment train. Pilot-plant studies should be conducted using granular activated carbon either as a filter media in place of the conventional sand and anthracite media, i.e. as a filter/adsorber, or in a separate post-filtration adsorbent bed. In either case, chlorination or chloramination should be practiced downstream of the activated carbon bed.

As part of the pilot-plant studies, ozonation should be considered as a pre-treatment oxidant and as an alternative primary disinfectant in place of free chlorine. The application point of ozone should be after sedimentation, prior to passage of the water through the pilot activated carbon filter, and also prior to passage through the post-filtration activated carbon adsorber.

The studies should be conducted for at least one year to take into account seasonal water quality variations in the two sources, and to allow for the development of biological activity in each of the carbon beds. Ozonation produces biodegradable organic matter which can be removed by microorganisms growing within the activated carbon filter/adsorber or post-filter adsorber. Use of such biologically-active beds of activated carbon has been shown to be an effective treatment method for removing disinfection by-product precursors.

INTRODUCTION

Jordan Lake, located in Chatham County in central North Carolina, was constructed by the Army Corps of Engineers for flood control and recreational use. Prompted by steadily growing development in the area with increasing demands on existing water supplies, Jordan Lake was being considered as a drinking water source for several nearby communities at the time this project was initiated. Tributaries to Jordan Lake drain industrial and urban centers, and samples from these sources have demonstrated toxicity in several bioassays. Elevated concentrations of metals and the presence of synthetic organic chemicals in one of the tributaries have raised concerns over the suitability of the lake as a source of drinking water.

Classified by the N.C. Department of Environmental Management as eutrophic, Jordan Lake water contains relatively high concentrations of total organic carbon (TOC) and humic material. When treated with oxidants and disinfectants such as chlorine, humic substances may react to form compounds generally referred to as disinfection byproducts (DBPs). Trihalomethanes (THMs), such as chloroform, are potentially carcinogenic reaction products resulting from the chlorination of aquatic humic material, and have been regulated in drinking water by the U.S. Environmental Protection Agency since 1979. New maximum contaminant levels (MCLs) in drinking water for these and other DBPs will soon be promulgated. In assessing the suitability of Jordan Lake as a drinking water supply, it is important to determine the possible presence of harmful impurities in the water as well as to evaluate the potential formation of DBPs from various treatment processes.

The primary objective of this research was to test three treatment process trains for Jordan Lake water and to compare the finished water quality from each of the trains. Conventional surface water treatment consisting of coagulation, sedimentation and filtration was applied to samples of the raw water collected during four different seasons. Part of the filtered water was then disinfected with free chlorine. Another portion was filtered through granular activated carbon (GAC) followed by disinfection with free chlorine. A third portion was treated with ozone followed by the addition of monochloramine (combined chlorine). These three processes were applied to four samples of Jordan Lake water during one year and to four samples from Falls Lake near Raleigh, N.C., for purposes of comparison. Falls Lake is currently used as a source of drinking water for the city of Raleigh. Water quality and treatment effectiveness were measured in terms of turbidity reduction, TOC removal, formation of THMs and total organic halides (TOX), and Ames mutagenicity. The last of these is not as familiar to water quality specialists and is discussed further below.

The Ames genotoxicity bioassay is a method of assessing the collective presence of harmful compounds without analyzing for specific chemicals. Results from this assay indicate a relative level of genetic mutation potential in the whole sample rather than from any specific compound. The Ames bioassay has demonstrated a high degree of sensitivity towards mutagenic compounds associated with raw and treated drinking waters. Some chlorinated organic byproducts in drinking water have been found to be mutagenic at nanogram per liter (ng/l) concentrations. Researchers have used the assay to detect the presence of synthetic organic chemicals in waters at levels that are non-detectable by modern analytical methods.

BACKGROUND AND THEORETICAL CONSIDERATIONS

HYDROLOGY OF THE JORDAN LAKE WATERSHED

The Jordan Lake watershed encompasses the Haw River basin, covering 1310 square miles and including large parts of Guilford, Alamance, Rockingham, Caswell, Chatham and Orange counties and the Greensboro and Burlington metropolitan centers. The tributaries New Hope Creek, Morgan Creek and Northeast Creek comprise the New Hope Basin and drain an area of 322 square miles including sections of Orange, Durham and Wake counties. Figure 1 shows the Jordan Lake watershed. The two basins have approximately the same average runoff per area, so essentially 80% of the inflow to Jordan Lake enters from the Haw River and 20% from New Hope Creek (Moreau and Challa, 1985). However, the lake is structured such that about 95% of the storage pool is in the New Hope arm. When the lake surface is at 216.0 feet above mean sea level (MSL), it is at the top of the conservation pool, and volume is estimated by the Army Corps of Engineers to be 215,130 acre-feet.

Figure 2 shows Jordan Lake divided into four geographical segments. In 1985, Moreau and Challa studied the exchange of water between these segments for one year of recorded flow and simulated the number and volume of intersegment flows. Their results estimated that an upstream flow from Segment I to II occurred during 26% of that year, from II to III for 23%, and from III to IV during 19% of the year. Projecting the volume of exchange during the largest backflow period, a maximum of 8% of Segment I water (Haw River inflow) was estimated to occur in Segment III (assuming complete mixing).

Water Quality in Jordan Lake

Jordan Lake was designed by the Army Corps of Engineers as a flood control measure for downstream communities and for recreational use. It began filling in 1981 and was reclassified in October 1983 by the North Carolina Environmental Management Commission (EMC) as Class A-II, raw water supply, in order to protect the water quality while investigations could be undertaken to determine the suitability of the lake as a drinking water source. Because of the intense industrial, urban and agricultural use of the watershed, the water quality in Jordan Lake is susceptible to impact by heavy metals, synthetic organic chemicals (SOCs), and nutrients from both point and nonpoint sources within the basin. With the reclassification, the EMC required an evaluation of toxicant sources in the watershed by the N.C. Department of Environmental Management (DEM).

Point-Source Pollution. The Haw River carries effluents from 18 direct industrial dischargers, 3 small municipal wastewater treatment plants and 7 major municipal dischargers that treat 105 industrial waste streams. The New Hope Creek basin receives treated wastewater from 3 major municipal plants that treat 25 industrial effluents, and from 1 industry directly (Division of Environmental Management, 1985). Direct and indirect industrial dischargers to the Haw River are 50% textile mills, 10% machinery and metals manufacturers, 8% chemical plants, 2% tobacco processors, and 30% miscellaneous. Industries in the New Hope basin are 19% metals and machinery, 12% chemical manufacturers, 7% tobacco and textiles, and 62% miscellaneous. The DEM monitors dischargers who have National Pollution Discharge Elimination System (NPDES) permits for releasing permit-specified concentrations

Figure 1. Jordan Lake Watershed

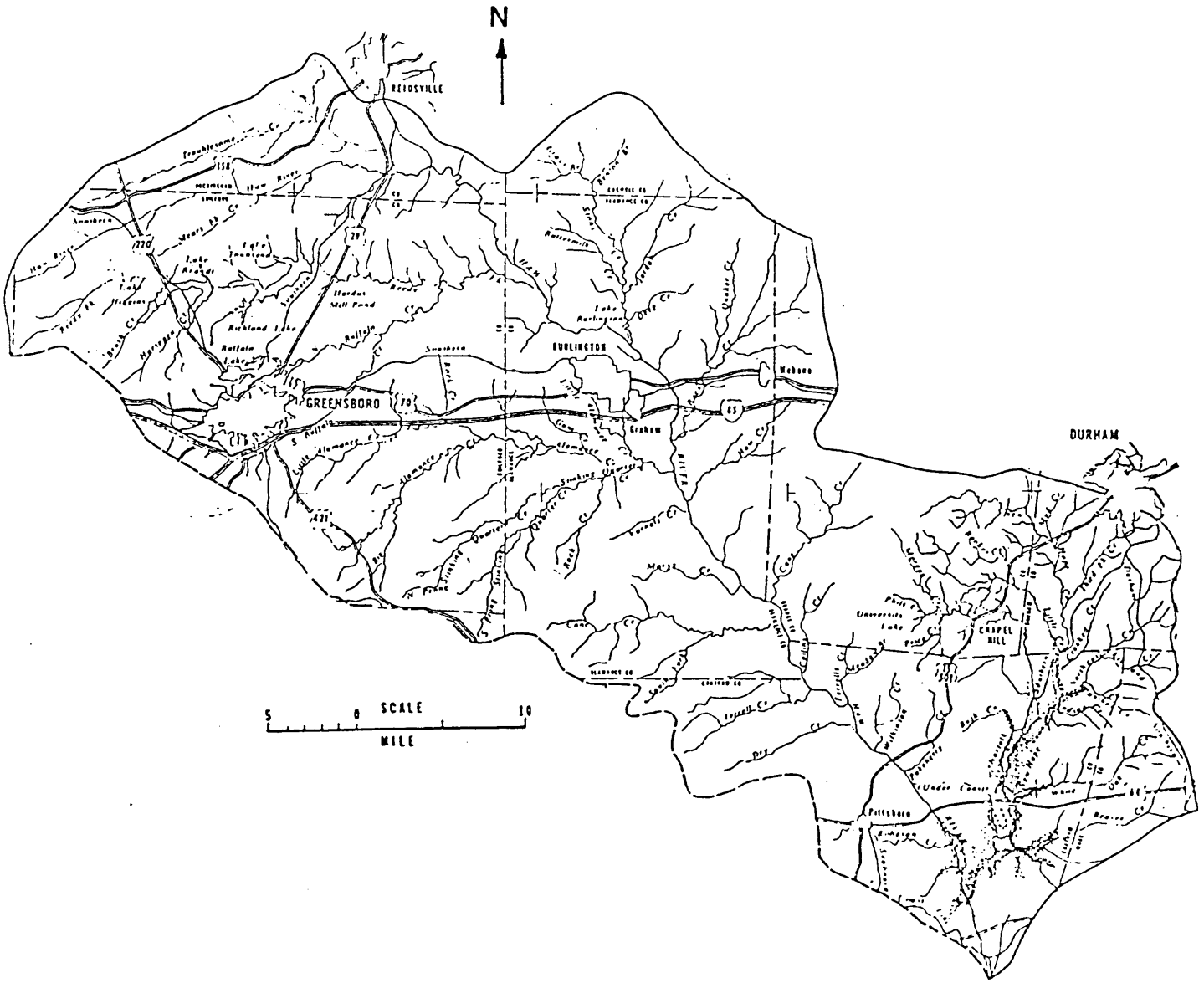
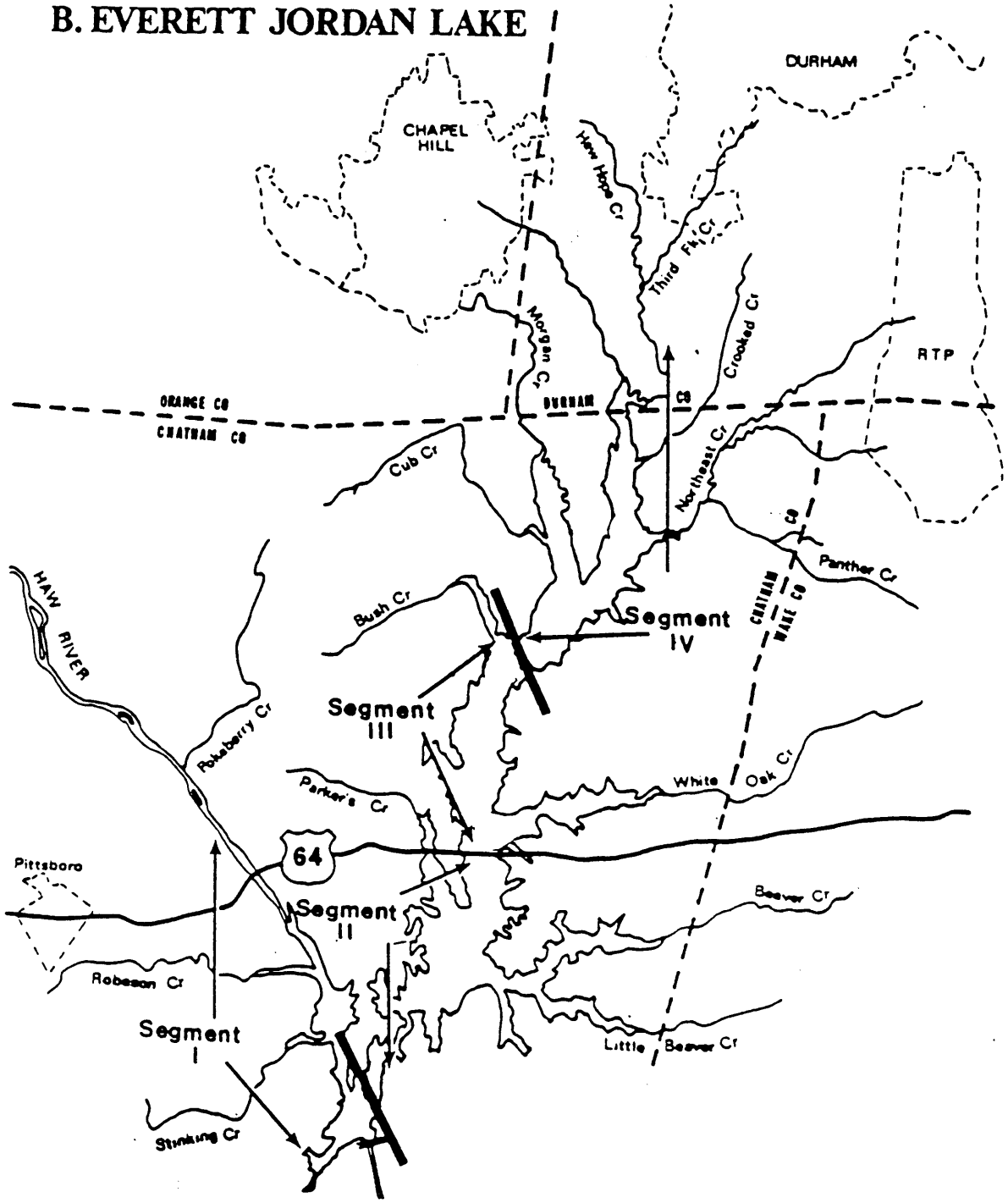


Figure 2. Segments of Jordan Lake



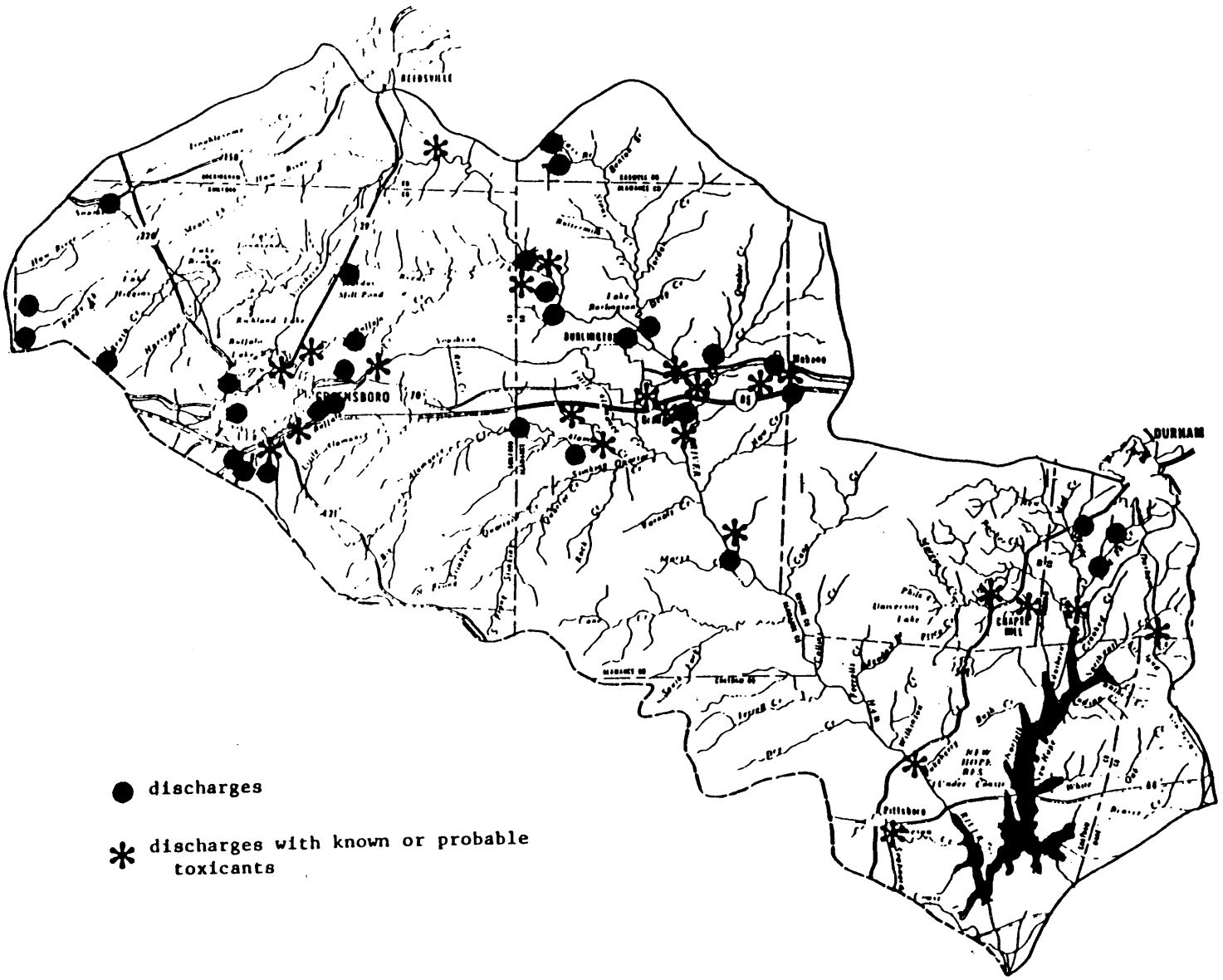
and toxicants in their effluent streams. "Toxicants" refers to substances that cause toxicity in an aquatic bioassay, and thus includes heavy metals and SOCs that may not be listed specifically on the NPDES permit. Of the 169 NPDES permits in the Jordan Lake watershed, 25 permitted dischargers are known sources of toxicants, mostly heavy metals such as chromium, cadmium, copper and zinc (Division of Environmental Management, 1985). Figure 3 shows the locations of the 1985 NPDES permit holders in the watershed. SOC analyses conducted by the Division of Environmental Management in 1984 on the effluents from the East and South Burlington and Durham City wastewater treatment plants (WWTPs) all resulted in no detectable compounds.

Nonpoint-Source Pollution. Metals detected in the Jordan Lake watershed have been attributed primarily to urban runoff (Division of Environmental Management, 1985). A study on land use in three of the major urban areas in the basin accounted for almost all deposited lead, 50-75% of the copper, and 70-95% of the zinc measured in the waters, as well as significant amounts of the detected cadmium, arsenic, chromium, mercury, and nickel. Pesticides from agricultural areas adsorb to soil particles and are deposited in streams and rivers by erosion and stormwater runoff. The U.S. Soil Conservation Service estimated the amount of highly erodible (defined as greater than 12 tons erodible soil per acre) agricultural land in the Jordan Lake watershed to be 8.2% of the Haw River basin and 2.7% of the New Hope Creek basin (Division of Environmental Management, 1985). Growing development in the watershed may cause these percentages to rise with increased forest clearance for agriculture and construction, higher loading rates of stormwater runoff to soils from impervious surfaces, and a greater impact from new highways and traffic. These sources of nonpoint pollution increase the rate of soil released into the tributary streams and directly into the lake, along with the concentration of anthropogenic compounds associated with these soil particles. Thus it can be expected that heavy metals and non-biodegradable pesticides and other anthropogenic matter will continue to be deposited into the sediments of Jordan Lake, and at an increasing rate in the future.

Stream and Lake Measurements. Prior to 1980, heavy metal concentrations in tributary streams often exceeded water quality standards; measurements in the last decade indicate significant reductions in metal concentrations due to wastewater treatment plant improvements and industrial pre-treatment controls (Division of Environmental Management, 1985). The Division of Environmental Management analyzed Haw River water in 1983 for SOCs and identified 52 compounds including the EPA priority pollutants trichlorobenzene, toluene and naphthalene at low ug/l to unquantifiable concentrations. Toxic biocides discovered in the Haw River in 1983 were identified by the Division of Environmental Management as triorganotin compounds common to textile mills. However, three SOC analyses of Jordan Lake in 1983 and 1984 resulted in no detectable compounds, but one analysis by the Division of Environmental Management in 1985 recorded 7 unidentified compounds in water collected from Segment I.

The Triangle Area Water Supply Monitoring Project is a comprehensive water quality study of six water supply reservoirs, including Jordan and Falls Lakes. The project is a joint effort of Chatham, Durham, Johnston, Lee, Orange and Wake counties, through the Triangle J Council of Governments (TJCOG). The U.S. Geological Survey (USGS) and

Figure 3. 1985 NPDES Dischargers in Jordan Lake Watershed



N.C. Department of Natural Resources and Community Development (NRCD) began collecting water and sediment samples at 32 stream and reservoir sites in October 1988, and is continuing to monitor for nutrients, metals, bacteria and SOCs (USGS, 1988).

Results of the first year's sampling showed that metals in Jordan Lake were safely below water quality standards, and nutrients such as phosphorus were similar in concentrations to previous years (TJCOG, 1989). Trihalomethanes were measured at 5 to 8 ug/l downstream from wastewater treatment plants, indicating the presence of chlorinated organic compounds. One or more of the pesticides diazinon, lindane and dieldrin were detected at levels up to 0.2 ug/l in 34 out of 74 total samples and in 9 of 10 samples collected from the Haw River near Bynum. The North Carolina water quality standard for lindane is 0.01 ug/l and for dieldrin it is 0.002 ug/l; there is presently no standard for diazinon. The same samples collected from the Haw River contained several undifferentiated alkanes and polyaromatic hydrocarbons in the 10 ug/l range.

Comparison to Falls Lake Watershed

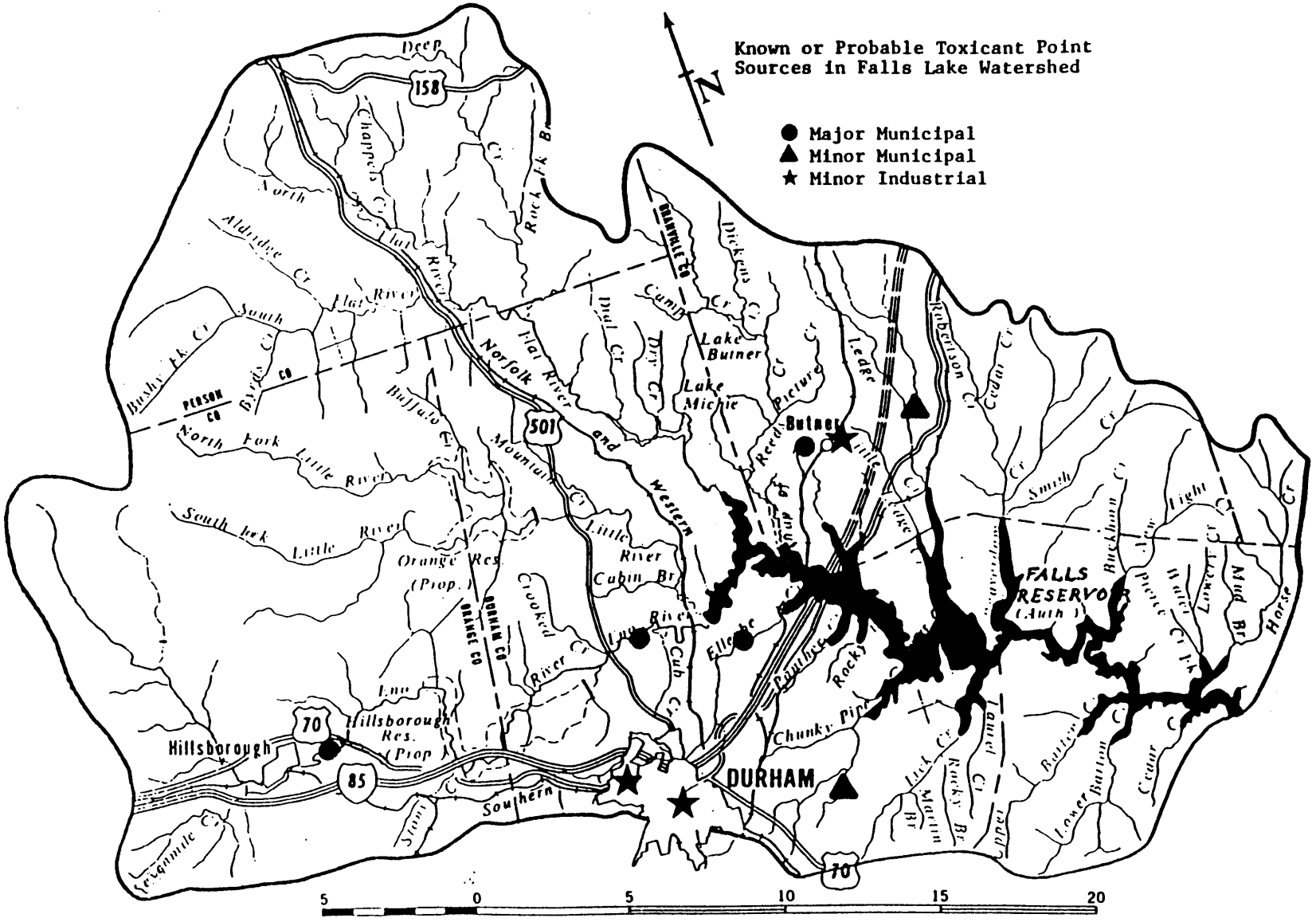
Falls of the Neuse Lake, hereafter referred to as Falls Lake, is located in Durham and Wake Counties, N.C., and is part of the Upper Neuse River system. Tributaries to Falls Lake include the Eno, Flat and Little Rivers, and Ellerbe and Knapp of Reeds Creeks. The Falls Lake watershed covers 770 square miles and 10,703 acres. Figure 4 shows the locations of NPDES dischargers in the watershed in 1985. Point source dischargers to Falls Lake include the Durham Northside wastewater treatment plant via Ellerbe Creek, the City of Hillsborough and the Eno wastewater treatment plants via the Eno River, and the John Umstead Hospital via Knapp of Reeds Creek. Falls Lake currently serves as the primary drinking water source for the City of Raleigh, N.C.

Jordan and Falls Lakes are similar in terms of hydrology, nutrient loading, and non-point source pollution. Classified as hyper-eutrophic by the Division of Environmental Management in 1985 based on total phosphorus, total nitrogen, secchi depth and chlorophyll *A* content, Falls Lake ranked as the most eutrophic lake in N.C. that year. Jordan Lake was similarly classified and ranked the third most eutrophic lake in N.C. by the same survey (Division of Environmental Management, 1986).

CHLORINATION OF DRINKING WATER

Discovery of the formation of organic contaminants in drinking water due to chemical reactions between the applied disinfectant and background organic material in the water has resulted in more than a decade of research aimed at determining the composition and routes of formation of disinfection byproducts (DBPs) and their associated health risks. In 1979, the U.S. Environmental Protection Agency (USEPA) amended the Interim Primary Drinking Water Standards to include a maximum contaminant level (MCL) of 100 ug/l for total trihalomethanes (TTHMs), a group of potential carcinogens that may represent up to 30% of the halogenated organic byproducts in chlorinated drinking water. Amendments to the Safe Drinking Water Act were made in 1986 that require the USEPA to set new MCLs for drinking water DBPs based on health effects research. Although the new

Figure 4. 1985 NPDES Dischargers in Falls Lake Watershed



requirements were scheduled to be published in 1991, the EPA has postponed final regulation until more definitive research can be completed concerning the chemical makeup, toxicological effects and control methods for DBPs created by chlorine and alternative disinfectants, and until a balance between the chemical risks from the application of disinfectants and microbial risks from pathogenic microorganisms can be evaluated.

Chemistry of Free Chlorine

Chlorine is usually applied in water treatment processes as a gas (Cl_2). It is quickly and completely converted in water to hypochlorous acid (HOCl) as follows:

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

Hypochlorous acid is in equilibrium with the unprotonated hypochlorite ion (OCl^-) as shown in Equation 2; the pKa for this relationship at 25°C is 7.5.

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

Reactions with Ammonia. When chlorine is added to water that contains ammonia, or vice versa, the chlorine and ammonia rapidly react to form a class of compounds known as chloramines. The chloramines consist of monochloramine (NH_2Cl), dichloramine ($NHCl_2$), and trichloramine or nitrogen trichloride (NCl_3). The three species, together referred to as combined chlorine, occur simultaneously as described by Equations 3 through 5; their distribution is dependent on the chlorine to ammonia-N ratio (Cl_2:NH_3-N), chlorine dose, temperature, and pH.

$$NH_3(aq) + HOCl = NH_2Cl + H_2O \qquad (3)$$

$$NH_2Cl + HOCl = NHCl_2 + H_2O \qquad (4)$$

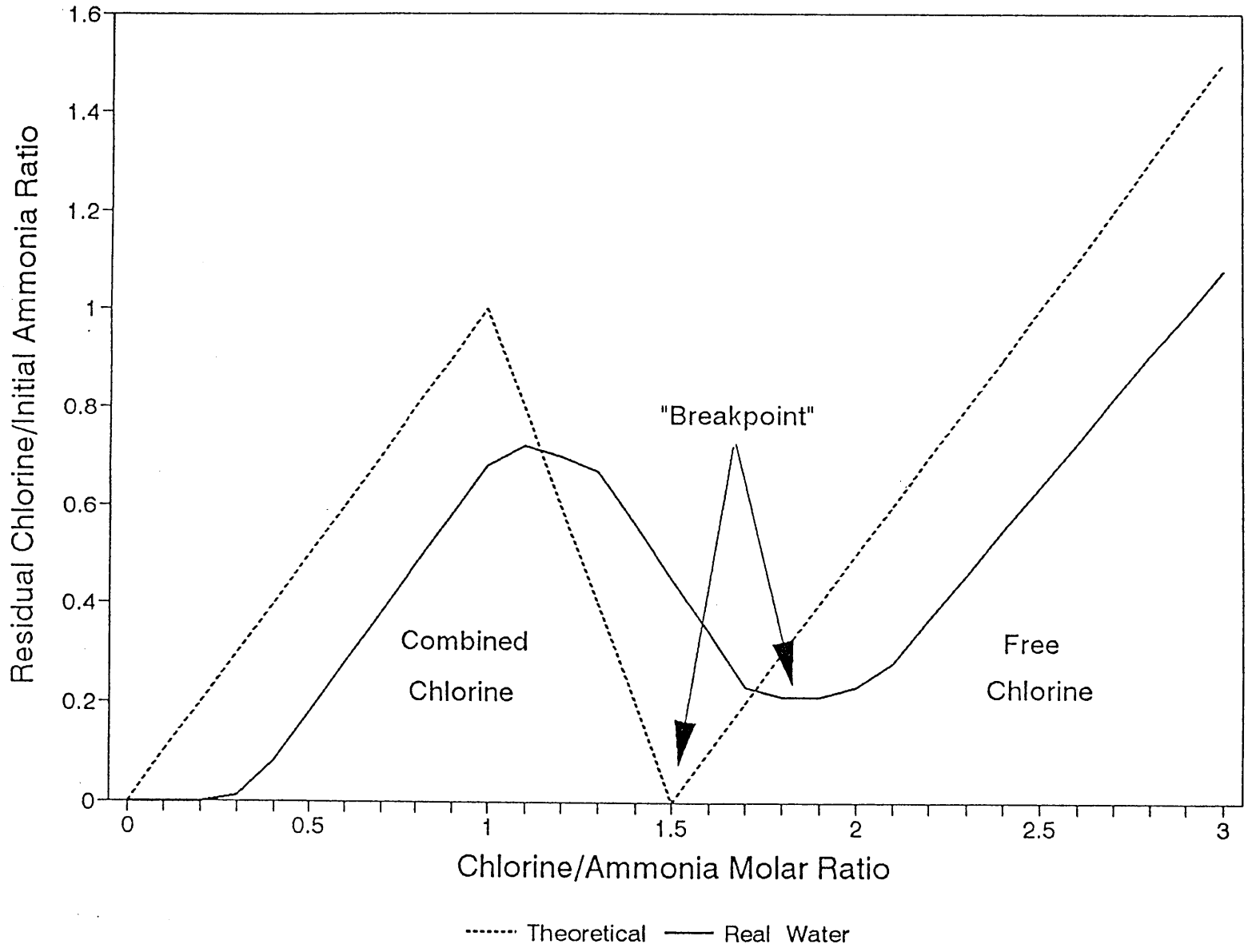
$$NHCl_2 + HOCl = NCl_3 + H_2O \qquad (5)$$

The effect of the chlorine to ammonia ratio on chloramine speciation between pH 6 and 9 is depicted in Figure 5 and is known as breakpoint chlorination. The dashed line represents the theoretical reactions that would occur without competitive reactions with organic compounds in water; the solid line represents speciation in a real water with natural organic material. At chlorine to ammonia-N molar ratios less than one, the chlorine is rapidly converted to monochloramine by Equation 3. The rate constant for this reaction at 25°C and pH 8.0 is 4.6×10^6 L $mol^{-1}s^{-1}$ (Weil and Morris, 1949). This reaction is most rapid at pH 8.5 and is complete in about 1 minute.

Between molar ratios of 1 and 1.5, more chlorine is available to substitute onto the already-formed monochloramine by Equation 4. Dichloramine is formed more slowly than monochloramine, with a rate constant of 2.7×10^2 L $mol^{-1}s^{-1}$ at 20°C (Morris, 1967). Dichloramine is less stable than monochloramine and decomposes by the following simplified reaction:

$$2\ NHCl_2 + H_2O = N_2 + HOCl + 3H^+ + 3Cl^- \qquad (6)$$

Figure 5. Theoretical Breakpoint Chlorination Curves



Some nitrate is also formed during this decomposition (Morris, 1967). The total chlorine residual decreases as more chlorine is added, and the reactions proceed through Equations 3, 4 and 6 until the minimum "breakpoint" is reached. The theoretical breakpoint occurs at a Cl_2:NH_3-N molar ratio equal to 1.5, at which no chlorine residual is found. In real waters, the breakpoint ratio is closer to 2.0 because some of the ammonia is oxidized to nitrate directly.

Furthermore, chlorination reactions with organic compounds (such as amino acids) that are faster than the chloramine formation reactions cause the breakpoint to shift to ratios greater than 2.

As more chlorine is added beyond the breakpoint, the ammonia is entirely converted to N_2 and the total chlorine residual occurs as free chlorine. Nitrogen trichloride is formed by Equation 5 when the Cl_2:NH_3-N molar ratio is greater than 2 and the pH is less than 7.5 (Jolley and Carpenter, 1983). In a real water, the total chlorine residual may also include some organic chloramines that were not oxidized by the free chlorine (Scully et al., 1984).

Reactions with Bromide. Bromide exists in most natural fresh waters at low concentrations (<100 ug/l), but it is found at higher concentrations in brackish water, seawater, and in some industrial effluents. Bromide is quickly oxidized by chlorine to form hypobromous acid with a rate constant at pH 8 and 25°C of 2.95×10^3 L $mol^{-1}s^{-1}$ (Farkas et al., 1949):

$$HOCl + Br^- = HOBr + Cl^- \quad (7)$$

The bromine species Br_2, HOBr and OBr^- are distributed according to the system pH, in a similar manner to chlorine except that Br_2 is present in neutral pH regions and HOBr and OBr^- are equimolar at pH 8.7 and 25°C (Jolley and Carpenter, 1983).

Hypobromous acid reacts with ammonia to produce bromamines at much faster rates than chloramine formation. Contrary to the relative stability of monochloramine, 5 mg/l of monobromamine has a half-life of 19 hours at pH 8 and 25°C, while dibromamine has a half-life under the same conditions of 30 minutes (Jolley and Carpenter, 1983).

Reactions with Natural Organic Material. Hypochlorous acid reacts with organic matter by accepting electrons from the substrate through oxidative reactions, by substituting into a compound at the carbon or nitrogen site, or by addition onto compounds with reactive double bonds (Jolley, 1973). Direct oxidation reactions, i.e. the breaking down of organic matter and particularly carbohydrate-related compounds into nitrogen and carbon dioxide gases and carboxylic acids (Johnson and Jensen, 1986), account for 50- 80% of the chlorine consumption and are responsible for the effectiveness of chlorine as a strong oxidant and disinfectant (Hileman, 1982). The formation of C-chlorinated or N-chlorinated compounds by substitution reactions, especially with humic and aromatic compounds, has been the subject of extensive research since the discovery of trihalomethanes in drinking water by Rook in 1974.

In order to quantify the amount and types of chlorinated organic byproducts in natural waters, researchers have characterized precursor structures that are targets for these reactions. About half of the dissolved organic carbon (DOC) in terrestrial streams is

present as humic substances, hydrophobic macromolecular structures from plant diagenesis (Leenheer, 1981). About 25% of the total organic carbon is arranged in aromatic structures that researchers feel may be most responsible for chlorine consumption (Rook, 1977; Norwood and Christman, 1987; Reckhow, Singer and Malcolm, 1990).

Fulvic and humic acids comprise the majority of humic material in most surface waters. Fulvic acids have molecular weights in the range of 100 to 1000 and are more highly concentrated in most surface waters than the much larger humic acids with molecular weights greater than 10,000 (Trussell and Umphres, 1978; Collins, Amy and Steelink, 1986). Humic acids have been found to produce higher yields of total organic halides (TOX) and THMs than fulvic acids (Babcock and Singer, 1979; Oliver, 1983; Reckhow and Singer, 1984) purportedly due to the greater number of reactive sites on the larger humic acid molecule (Peters, Young and Perry, 1980). However, humic acids are usually a small percentage of the total humic material and may be easier to remove by conventional treatment methods than the smaller fulvic acids (Babcock and Singer, 1979; Collins, Amy and Steelink, 1986). In an investigation of chlorination byproducts from humic and fulvic acids, over 100 reaction products, not all containing chlorine, were identified (Christman et al., 1983). A later study found similar chlorination byproducts from fulvic acid and implicated reactive phenolic structures that undergo ring rupture as primary precursor structures for organohalide formation (Norwood and Christman, 1987).

Chloro-Organic Byproducts and Relationships

While the chemical structure of organic material in the water may help predict chlorinated byproduct formation, relationships between the byproducts and chlorination conditions may also aid in estimating byproduct types and quantities. Specific byproducts that have been isolated in chlorinated drinking waters include the volatile THMs, haloketones (HK) (Suffet, Brenner and Silver, 1976), and haloacetonitriles (HAN) (Oliver, 1983), and the non-volatile compounds trichloroacetic acid (TCAA), dichloroacetic acid (DCAA), dichlorosuccinic acid (DCSA), dichloromalonic acid (DCMA) and chloral hydrate (CH) (Quimby et al., 1980; Christman et al., 1983; Miller and Uden, 1983). Together, these compounds account for up to 50% of the total organic halide (TOX) concentration in drinking waters.

Reckhow and Singer (1990) chlorinated raw drinking waters from several cities at pH 7, a chlorine dose of 20 mg/l and a reaction period of 72 hours. On the average, the waters consumed 2 mg chlorine per mg TOC in the sample under these conditions. An average of 23% of the chlorine consumed became incorporated into the TOX. The major chlorination byproducts consisted of the following average percentages of the TOX: TTHM, 22%; TCAA, 17%; DCAA, 7%; and trichloroacetone and dichloroacetone, 0.7%. Finished waters from the same treatment plants had similar average byproduct percentages of the TOX: TTHM, 23%; TCAA, 11%; DCAA, 9%.

The distribution and quantities of chlorinated compounds produced during drinking water treatment depends on several parameters including the chlorine-to-carbon ratio, free chlorine contact time, system pH and temperature, and background concentrations of ammonia and bromide. Important observations by several researchers about the interrelationships between these parameters are summarized below.

Increasing the chlorine-to-carbon molar ratio beyond 0.1, Reckhow and Singer (1984) observed a sharp increase in TOX, chloroform ($CHCl_3$) and TCAA formation up to a molar ratio of 1.0, then a much smaller increase in the formation of all the species at higher molar ratios. The extent of DCAA production appeared to be independent of the chlorine-to-carbon ratio. Miller and Uden (1983) found that unidentified high molecular weight compounds were formed at chlorine-to-carbon molar ratios up to 2.0, but these byproducts were subsequently broken down at a ratio of 5:1. Other products appeared only at high chlorine-to-carbon ratios, indicating that rigorous oxidation was a prerequisite for their formation.

Reckhow and Singer (1984) observed a similar trend in TOX, THM and TCAA formation by lengthening the free chlorine contact time as by increasing the chlorine-to-carbon ratio: rapid formation of byproducts in the first few hours followed by a more uniform rate of increase. $CHCl_3$ became a larger percentage of the TOX concentration, from 9% after 30 minutes to 27% after 300 hours. The rate of TCAA formation increased at the same rate as $CHCl_3$ formation for 12 hours, then decreased slightly. The DCAA concentration remained at 5-6% of the TOX concentration during the entire 300-hour period of observation. Dichloroacetonitrile (DCAN) formation increased for 30 hours, then decreased to below detection limits at 170 hours. In Miller and Uden's investigation (1983), nearly 90% of the final concentrations of TCAA, DCAA, $CHCl_3$ and chloral hydrate (CH) were attained within 24 hours.

The general effects of pH noted by Reckhow and Singer (1984) were decreasing concentrations of TOX with increasing pH, increasing production of $CHCl_3$ with increasing pH, and higher formation of TCAA and DCAA at decreasing pH. Miller and Uden (1983) reasoned that HOCl is a much stronger oxidant than OCl^-. More highly oxidized chlorination byproducts such as TCAA might be expected at pH values below the pKa for HOCl of 7.5. The formation of $CHCl_3$ is a base-catalyzed reaction that is not as dependent on the oxidation of fulvic material and is thus produced to a greater degree at higher pH values. Chloral hydrate is unstable at high pH values and decomposes to produce $CHCl_3$. Krasner et al. (1989) observed the degradation of haloacetonitriles, haloketones, chloral hydrate, and possibly cyanogen chloride at alkaline pH values. Reckhow, Singer and Malcolm (1990) recently observed that the TOX produced in humic and fulvic acid solutions at pH 12 was only about 50% of the TOX created at pH 7. They also noted that for chlorination at pH 12, the same fraction of TOX as trichloromethyl species was shifted from mostly TCAA at pH 7 to mostly $CHCl_3$. They proposed that the reduced reactivity and the resulting reduced level of TOX formation from humic substances observed at high pH values may be due to a change in reaction mechanism from chlorine substitution to oxidation as the compound's degree of protonation shifts with increasing pH. The reduction in the concentration of TOX at higher pH may also be a result of base-catalyzed hydrolysis of C-Cl bonds.

Fleischacker and Randtke (1983) noted a greater increase in $CHCl_3$ production than TOX production with increasing temperature, and observed that at lower temperatures the TOX was comprised mostly of unhydrolyzed $CHCl_3$ intermediates. Oliver (1980) observed that heating a sample that was chlorinated at pH 7 increased the extent of $CHCl_3$ production to the same amount as that formed at pH 11 at a lower temperature.

Several researchers have attempted to quantify the extent of incorporation of bromide from the raw water into THM production, and to explain the competitive reactions between HOBr and HOCl. Minear and Bird (1980) found a nonlinear increase in THM production with increasing bromide concentrations, and a shift from $CHCl_3$ as the largest fraction of the TTHM concentration at raw water bromide levels less than 0.1 mg/l to bromoform ($CHBr_3$) dominance at bromide concentrations greater than 1.0 mg/l. Because the bromide ion requires oxidation by HOCl, the chlorine demand of the water may be significantly increased in the presence of elevated bromide concentrations. Luong, Peters and Perry (1982) found that chlorine consumption increased by up to 50% in bromide-containing waters.

Models of Disinfection By-Product Formation

Several kinetic models and correlations have been developed by researchers in order to predict and quantify byproduct formation. In 1981, Singer et al. found excellent correlations between the raw water TOC concentration, UV- absorbance (254 nm) and THM formation potential (THMFP). Edzwald, Becker and Wattier (1985) also noted that raw water UV-absorbance correlated well with THMFP and TOC, and that the relationship was unique to each water source and chlorination conditions. For example, different correlations were observed for the THMFP of the same fulvic acid at different pH values. Reckhow and Singer (1984) observed a strong correlation between THMFP and TOXFP and found that about 220 ug TOX were produced per mg TOC at pH 7 when chlorinated with 5 mg chlorine per mg TOC and reacted for 72 hours. Under these same conditions, $CHCl_3$ comprised about 20% of the TOX concentration for a variety of chlorinated fulvic and humic acids. Singer and Chang (1989) analyzed chlorinated byproduct formation at 7 treatment plants and also found an excellent correlation between THMFP and TOXFP. Instantaneous TTHM correlated well with instantaneous TOX, suggesting that for conventional surface water treatment plants that employ coagulation and filtration, TTHM (ug/l) = 0.35 TOX (ug/l).

Oliver and Thurman (1983) correlated the THMFP of several fulvic acids with different variables and found the strongest relationships with molecular size and color. Molecular size and color also correlated well with each other, indicating that color increased with conjugation of the double bond of the humic molecule. A weak correlation between THMFP and phenolic content disputes the theory that THM precursor structures are most likely high in phenolic content and suggests instead that they are highly conjugated aromatic ring systems.

Amy, Chadik and Chowdhury (1987) developed two models, a multiple linear regression model with logarithmic transforms and a nonlinear regression model, to predict TTHM formation based on UV-absorbance, TOC concentration, chlorine dose, reaction time, temperature, pH and bromide level. Over 1000 data points were used to calibrate the models and a sensitivity analysis of parameters and model verification using an external data set were performed. The models predicted THM formation within 20% of measured values except for extremely high or low THM levels. The models are useful to simulate the THMFP of untreated waters and to predict the effect of reaction time on THM formation.

Reckhow, Singer and Malcolm (1990) investigated chlorinated byproduct formation from 10 aquatic humic and fulvic acids and attempted to relate structural characteristics of the humic material to byproduct

yields and chlorine consumption. A model was derived to estimate the probable number of activated aromatic rings that undergo chlorination substitution reactions. The percent chlorine incorporated into TOX was modelled stoichiometrically taking into account chlorine consumed by activated aromatic rings and nitrogenous compounds. TOX production from fulvic acid was predicted very well by these models (r^2=0.96). Good correlations were found between the ratio of TCAA formation potential to TTHMFP and UV-absorbance (254 nm), and between DCAN formation potential and organic N.

REMOVAL OF NATURAL ORGANIC MATERIAL BY COAGULATION

Surface water treatment plants in the United States have traditionally used the conventional process combination of coagulation, sedimentation, filtration and chlorine disinfection to effectively remove particulate matter and to inactivate pathogens. Currently, process design and operation is evolving to achieve more efficient removals of high molecular weight organic material that may react with disinfectants to produce potentially carcinogenic disinfection byproducts (DBPs). Conventional coagulation and filtration can be optimized to achieve average reductions of natural organic material of greater than 50%, substantially lowering the amount of disinfectant required and the concentration of DBPs generated in the treated effluent.

Babcock and Singer (1979) found that aquatic fulvic acid solutions required more alum for coagulation and resulted in lower removals of overall TOC than equivalent concentrations of humic acid solutions. Coagulation of both the humic and fulvic acid solutions resulted in a 70% reduction in chloroform formation, leading the authors to conclude that chloroform precursors were selectively removed by alum coagulation. The color in fulvic acid solutions has been correlated with aromaticity and with THMFP (Oliver, 1983), suggesting that color removal by coagulation of solutions containing fulvic acids may reflect THM precursor removal. Chadik and Amy (1983) also noted greater removals of THMFP than TOC in various natural waters, and high reductions in UV-absorbance indicated that humic acids with greater absorbance at 310 nm and greater THMFP were preferentially removed by coagulation over fulvic acids with low UV-absorbance and lower THMFP. Reckhow and Singer (1984) evaluated the preferential removal of chlorination byproduct precursors by alum coagulation of a fulvic acid and a natural water and ranked precursor removability from greatest to least as follows: UV-absorbing substances, dichloroacetonitrile (DCAN) and trichloroacetic acid (TCAA) precursors, dichloroacetic acid (DCAA) precursors, TOX precursors, THM precursors, TOC and chlorine demand, and 1,1,1-trichloroacetone precursors. Singer and Chang (1989) determined that precursor removals at 7 water treatment plants averaged 51% for TOC, 47% for THMFP and 49% for TOXFP.

Jodellah and Weber (1985) coagulated river water and found that higher doses of alum were necessary to achieve similar TOC reductions for an equivalent amount of humic and fulvic acid. Although TOC removal increased with increasing alum doses, the THMFP reduction decreased, indicating that THM precursor removal does not always parallel TOC removal. The authors concluded that coagulation of the water produced an altered organic speciation that responded differently to chlorination than the untreated water.

Collins, Amy and Steelink (1986) characterized several waters before and after treatment in terms of their molecular weight distribution, humic substance content and functional group carboxylic acidity. Their results agreed with past findings of the removability of humic acids over fulvic acids by showing large removals of organic matter with molecular weight greater than 5000 (humic acids) but ineffective removals of organic substances with molecular weights less than 500 (fulvic acids). Their results also concur with those of others in the preferential removal of UV-absorbing substances (humic material), and a higher percent removal of THMFP than TOC. The authors separated the treated and untreated waters into hydrophobic and hydrophilic fractions, accounting for at least 95% of the organic carbon. The hydrophobic fraction represented humic material and 85-100% of the color in the waters, while hydrophilic substances made up the non-humic organic portion of the aquatic material. The hydrophilic fraction contributed up to 65% of the nonvolatile TOC (NVTOC) and 56% of the THMFP, and was more difficult to remove than the hydrophobic organic material. Finally, carboxylic acidity was determined for the treated and untreated waters because the acidic functional groups have a charge density that affects the electrostatic attraction mechanism of coagulation. The authors found that the low molecular weight fractions corresponded to higher carboxylic acidities and were therefore more difficult to destabilize by coagulation. This concurs with the higher doses of alum required for effective fulvic acid coagulation initially reported by Babcock and Singer (1979).

REMOVAL OF NATURAL ORGANIC MATERIAL BY GRANULAR ACTIVATED CARBON ADSORPTION

Granular activated carbon (GAC) has received wide attention for its effective removal of synthetic organic chemicals from municipal and industrial wastewaters and from drinking water. The adsorption capacity and removal efficiency of a GAC contactor may be optimized by controlling process conditions such as characteristics of the carbon adsorbent, treatment of the water prior to application to the GAC bed, pH, and biological activity.

The tendency of different compounds to adsorb on GAC is dependent on their concentration and on the matrix of other compounds that compete for the same sites. Adsorption isotherms may be developed for individual compounds or surrogate groups of compounds such as TOC; an adsorption isotherm describes the equilibrium concentration of adsorbate on the GAC surface as a function of the bulk equilibrium concentration of adsorbate in solution at a constant temperature. Readily-adsorbed organic compounds include phenols and chlorophenols, pesticides, and high molecular weight hydrocarbons. Poorly adsorbed organics are alcohols, sugars, low molecular weight ketones and aldehydes, acids, and very high molecular weight compounds or colloidal organics (Froelich, 1978).

Humic substances are generally poorly adsorbed because these compounds are usually of such high molecular weight that they may be excluded from smaller GAC pores. McCreary and Snoeyink (1980) found that adsorption of humic material was enhanced at low pH, but was dependent on the specific source of material. Lee et al. (1981) observed that alum coagulation before GAC increased the capacity and rates of adsorption for fulvic and humic acids. They noted that the higher molecular weight fractions of these solutions that were not well adsorbed were significantly reduced by coagulation, resulting in

greater access of the lower molecular weight material to carbon pores that would otherwise be blocked by the higher molecular weight substances. Lengthening the empty bed contact time (EBCT) increases the adsorption of humic materials by allowing more time for removal of compounds that adsorb more slowly. Because humic substances encompass a wide range of organic compounds, competition for active sites on the carbon may result in desorption and displacement of a less sorbable compound by the favored sorption of another. Thus, it is important to monitor the effluent for increased concentrations of specific contaminants, especially those that are not preferentially adsorbed.

GAC provides an excellent medium for microbiological activity because of the rough and irregular texture of the surface and the possibility that sorbed compounds may become available as substrates for microbial growth. In Europe, many water treatment plants have chosen to optimize biological conditions in the GAC bed, resulting in reasonable organic removals and longer service times between carbon reactivation. There is some concern in the United States, however, that microbes may be released into the filter effluent and may not be adequately destroyed by subsequent disinfection, or may result in the production of harmful disinfection byproducts.

OZONE AND MONOCHLORAMINE AS ALTERNATIVE OXIDANTS/DISINFECTANTS TO FREE CHLORINE

Ozone

Ozone has been applied for water treatment in Europe since the early part of the twentieth century, and more recently in the United States, to achieve disinfection without the formation of chlorinated organic byproducts. Ozone can react with compounds in solution by direct molecular activity or by indirect radical reactions (Hoigne and Bader, 1977). Molecular ozone is unstable in water. Decomposition of ozone is first-order with respect to OH^- and ozone concentration in the presence of radical scavengers that prevent secondary reactions.

In natural waters, ozone initially reacts with humic material at sites with carbon double bonds, negatively-charged atoms such as N, P, O and S, complexed metals such as iron, and ortho-activated aromatic rings (Glaze, 1986). Humic substances can initiate free radical reactions that may be impeded by the presence of bicarbonate in the water. The nature of the direct and indirect reactions and their byproducts are dependent on the characteristics of the humic material in the water, the ozone dose and contact time, and the presence of radical initiators and scavengers. Under general treatment conditions, ozonated humic material is not fully oxidized, resulting in macromolecular structures and ring-cleavage products that are more hydroxylated, polar, biodegradable and of lower molecular weight than the parent compounds (Singer, 1990).

Possible ozonation byproducts that may have potential health effects include organic peroxides, hepatotoxic unsaturated aldehydes, and potentially carcinogenic epoxides (Glaze, 1986). Formaldehyde, a low molecular weight carbonyl compound, has been detected in several ozonated surface waters (Jacangelo et al., 1989; Glaze et al., 1989). Ozone oxidizes bromide in raw water to hypobromous acid which then can form bromate and bromo-organic compounds, such as bromoform, similar to the chloro-organic compounds resulting from free chlorine addition. Preozonation followed by chlorination may enhance chloropicrin

formation (Hoigne, 1988), and preozonation followed by chloramination has been shown to increase cyanogen chloride production (Jacangelo et al., 1989).

Ozonation may be placed at various positions in the treatment train to fulfill different objectives. The effectiveness of ozone at any position is dependent on the specific water quality and treatment processes employed, and should thus be investigated prior to permanent installation in a process regime. Preozonation is employed for the oxidation of reduced iron and manganese, color-causing organics, taste and odor compounds, and as a coagulant aid.

Ozone increases the biodegradability of compounds in water and may thus promote biological activity in downstream processes or in the distribution system. When ozone is applied prior to filtration, the filter media provides a stable environment for biodegradation if a sufficient hydraulic residence time is available. This prevents potential downstream regrowth problems. Ozonation followed by activated carbon filtration is termed biologically-enhanced activated carbon (BEAC) and is used by many treatment plants in Europe. This system relies on the partial oxidation of compounds with ozone and further degradation by microorganisms in the filter, as well as by adsorption on the carbon. Effective removals of TOC, chloro-organic precursors and chlorine-demanding impurities have been demonstrated by microbial degradation processes, thereby enhancing the adsorptive capacity of GAC (Rice, 1980) and reducing the frequency and cost of carbon reactivation.

Ozone is toxic to microorganisms due to its oxidative and free-radical reactivity; thus, it is a very effective disinfectant. However, the use of ozone as a disinfectant is limited by its rapid decomposition, resulting in no downstream residual disinfection capacity. Furthermore, ozone generates biodegradable products that may cause regrowth problems in the distribution system. Thus, ozonation must be followed by a biologically-active filter bed to remove the biodegradable organic carbon, or by the addition of a post-disinfectant with a long-term residual. Because ozone does not adequately destroy THM precursors at the doses commonly used in practice (Chang and Singer, 1991), the post-application of chlorine may still produce undesirable quantities of THMs and other chlorinated DBPs in waters with a high THM formation potential. Monochloramine, which produces much lower levels of THMs than chlorine, is a more appropriate disinfectant to follow ozonation in waters with a high THMFP.

Inorganic Chloramines

Since the regulation of THMs (U.S. Environmental Protection Agency, 1979), monochloramine has been used as an alternative drinking water disinfectant to free chlorine in order to reduce THM production. Monochloramine is reasonably stable in water at neutral pH and thus provides good residual disinfection in a distribution system. Chlorine and monochloramine both destroy microorganisms by disrupting the integrity of the cell membrane. A much higher concentration of monochloramine is required to achieve the same pathogenic kill as chlorine for most microorganisms. However, this equivalency may be attained by extending the contact time of the monochloramine.

Byproduct formation from chloramination has not been well researched.

It is commonly understood that while chloramines do not produce the high concentrations of THMs that chlorine produces, substantial amounts of chlorinated compounds, as represented by TOX, are still generated. Fleischacker and Randtke (1983) compared the products of free chlorine and monochloramine in fulvic and humic acid solutions and found 8 to 20% as much nonpurgeable total organic chloride (NPTOCl) produced from monochloramine treatment as that formed by the same doses of free chlorine. Up to 10 mg/l of monochloramine added to a fulvic acid solution of 3 mg/l TOC at pH 7 resulted in <1 ug/l chloroform and up to 86 ug/l TOCl produced after 100 hours. Five real waters were chloraminated and yielded less than 2 ug CHCl3 as Cl^-/mg TOC and 12-52 ug TOCl/mg TOC.

Trichloroacetic acid and dichloroacetic acid were isolated in low concentrations in monochloraminated fulvic acid solutions (Jensen et al., 1985A,B). Jacangelo et al. (1989) found cyanogen chloride and chloropicrin at low levels in water treated with ozone and chloramine. Because chloramines have been shown to produce some halogenated organic byproducts, research still needs to be conducted on this subject.

MUTAGENICITY OF DRINKING WATER

The Ames Assay

The Ames Salmonella plate incorporation assay is the most widely applied, rapid and simply executable test for genotoxicity (Ames et al., 1975; Maron and Ames, 1983). This assay has been extensively validated (Ames et al., 1975; Maron and Ames, 1983) and widely accepted as a standard technique for the detection of mutagens. As genetic damage is an important indicator of both potential carcinogenicity and of germ cell damage which extends its effects to the next generation, the Ames assay continues to grow in importance as screening for a variety of environmental samples and manufactured products. The assay procedure is relatively cheap and much more easily and rapidly performed than most mammalian mutagenicity and cell transformation assays, giving results in three days rather than six or more weeks, and lends itself well to preliminary screening of unknown compounds and to the detection of genotoxic components in complex mixtures. While the Sister Chromatid Exchange and Micronucleus Formation assays may be somewhat more sensitive in detection of genetic damage caused by treated drinking waters in some cases (Douglas et al., 1986), their time requirements and difficulty of execution has made them less useful in the rapid screening of large numbers of samples such as was required in this study.

Specific damage (mutations) to the genome of the modified histidine-dependent Salmonella strains which are used in this assay causes these organisms to regain the ability to synthesize histidine, and therefore grow without exogenous supplementation with this amino acid. Thus exposure of the bacteria to mutagenic chemicals will result in back-mutated colonies (revertants) able to grow on media containing minimal histidine, the number of colonies formed being an indicator of the mutagenic potency of the chemicals. Although carcinogenicity and mutagenicity are not necessarily directly causally linked, both are expressions of damage to the genetic material (genotoxicity). There exists overall a reasonably good concordance between the detection of compounds as mutagens in vitro and as carcinogens in whole animal studies (Claxton et al., 1988; Tennant et

al., 1987). The Ames assay has demonstrated both high sensitivity and high specificity in detecting known carcinogens as mutagens (McCann et al., 1975), and is thus often used as a predictor of carcinogenicity.

Variants of the Salmonella tester strains have been developed which possess different mutational target sites (strains TA98, 1537 and 1538 respond preferentially to frameshift-inducing mutagens whereas strains TA100 and TA1535 are sensitive to agents which cause base-pair substitutions) and with different repair capabilities (strains TA98 and TA100 have plasmid-encoded enhanced error-prone DNA repair mechanisms, and thus increased overall sensitivity). This enables discrimination between different types of chemicals based on the differences in response between Salmonella strains, reflecting differences in the sensitivity of the different target sequences to the initial lesion (Hartman et al., 1986). Conversely, appropriate selection of tester strain can serve to emphasize sensitivity to defined classes or types of mutagen. A further level of discrimination can be introduced by consideration of the mechanism of action of mutagenic agents. Many chemicals, predominantly those which are electrophilic in nature, can interact directly with the cellular genetic material. These compounds, typically alkylating agents and epoxides, are termed direct-acting mutagens. A second class of compounds, indirect-acting mutagens, require metabolic transformation which is typically carried out by the mixed-function oxidase system (cytochrome P-450) to generate the electrophilic species which interacts with DNA. In the Ames assay, this metabolic transformation capability is provided by addition of the 9000 x g supernatant from rat liver, which is rich in cytochrome P-450, and is termed S9. A co-factor generating system is included with the S9 in order to supply the reducing equivalents (NADPH) required for cytochrome P-450 enzyme activity. The indirect-acting mutagens include polycyclic aromatic hydrocarbons and many other environmental contaminants.

The presence of S9 during assay of compounds of the first type can be either neutral (no interaction, therefore no effect) or can result in a decrease in the mutagenic activity detected, either through binding of the compounds to the S9 protein, or through metabolic detoxication. Occasionally an increase in activity is seen when metabolism results in enhancement of reactivity of the electrophile or, more often, when the S9 protein acts to protect the tester bacteria from the toxicity of the compound. Therefore careful selection of the strains and activation conditions used can yield useful information on the nature and mode of action of unknown mutagens, and assay conditions can be selected to maximize detection of previously-characterized mutagens which are specifically sought.

No single bioassay is capable of detecting all possible mutagens, much less carcinogens, and no <u>in vitro</u> system can satisfactorily model all of the intricate interplay of uptake, absorption, distribution and tissue-specific metabolism that will ultimately determine the carcinogenicity or toxicity of a compound in the whole live animal. Therefore the Ames assay screening outlined here will not, and cannot be expected to, detect every potential health hazard which may result from exposure to the samples studied. Notably, volatile agents and certain classes of chemicals (such as halogenated organics) will not be well detected, regardless of strain utilized (Claxton et al., 1988). Conversely, the assay may be over-responsive to other classes of chemicals, such as nitrogen-containing organics (particularly when these are activated by endogenous bacterial enzyme systems).

Nevertheless, this assay does present advantages of being widely accepted and standardized, and therefore is especially useful for comparisons with other studies. When used appropriately, the Ames assay is a valuable tool for detection of genotoxic agents, and to date no other bioassay system has been proved superior, or even complementary, in the detection of carcinogens as mutagens (Tennant et al., 1987).

Mutagenicity of Chlorinated Drinking Water

Mutagenic activity has been detected in chlorinated drinking waters by the Ames assay since the late 1970s (Glatz et al., 1978; Loper, Lang and Smith, 1978), and researchers have attempted to characterize compounds that contribute to the mutagenicity. *Salmonella* strains TA98 and TA100 have been used most frequently to detect the presence of mutagenic compounds due to their sensitivity to mutagenic substances in raw and chlorinated waters. Generally, the addition of rat liver microsome S9 has been found to decrease the mutagenic activity of the water samples. In 1977, Simmon et al. assayed 22 halogenated compounds that might be found in chlorinated waters. Although chloroform did not exhibit mutagenic activity, the three other common THM species dichlorobromomethane, chlorodibromomethane and bromoform showed significant activity in strain TA100 without metabolic activation by S9 (-S9). Kool et al. (1982) found mutagenic activity in 12 out of 15 drinking waters from surface supplies in the Netherlands. They separated the mutagenic fraction and found that the majority of the mutagens were in the slightly polar nonvolatile fraction with molecular weights on the order of 200.

In 1984, Holmbom et al. isolated the strong mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) from chlorinated kraft pulp effluents and reported that at very low concentrations in the effluent (<10 ug/l), MX produced 2800-10,000 net revertants in TA100-S9 per nanomole of MX. Later studies of Finnish chlorinated drinking waters showed that MX in concentrations of 5 to 70 ng/l was responsible for up to 50% of the TA100-S9 mutagenicity exhibited by the samples (Holmbom et al., 1990; Kronberg et al., 1990).

Backlund et al. (1989) chlorinated humic and river water at chlorine to TOC molar ratios of 0.5, 1.0 and 2.0, reaction times of 65 and 240 hours and various pHs. They found that direct mutagenic activity in TA100 was substantially increased with decreasing pH and with increasing chlorine doses at each pH. Although in some cases total mutagenicity increased over the longer reaction period, MX was not detected in the 240-hour samples at pH 7 and 9. Also, the MX contribution to total mutagenic activity was less at pH 7 and 9 than at pH 4 and 2. These findings were substantiated by the work of Holmbom et al. (1990) who demonstrated that MX is stable at low pH but undergoes ring opening and dissociation under alkaline conditions. Although the river water and humic water samples exhibited similar levels of mutagenicity under all conditions, the MX concentration and its contribution to total mutagenic activity was much lower in the river water. Thus, significant mutagenicity was produced by chlorinated compounds other than MX.

Mutagenicity from Alternative Treatment Processes

Many studies have been conducted to assess the formation or removal of

mutagenic activity through various water treatment processes. In general, disinfectants ranked in decreasing order of probable mutagenic activity production are: chlorine, chloramine, chlorine dioxide and ozone (Noot et al., 1989), although ozone has been shown to sometimes produce as much activity as chlorine (Cognet et al., 1986). GAC treatment has most consistently shown high to complete removals of mutagenic activity.

Ozone application in water treatment usually decreases raw water mutagenic activity and subsequent activity produced by chlorination, but occasionally has been shown to create mutagenicity. In an early study of the mutagenic effects from ozonation (Van Hoof, 1983), it was observed that ozonation after prechlorination and filtration of a European river water resulted in effective removal of TA98-S9 frameshift mutagenicity formed by prechlorination. TA100-S9 base-pair substitution mutagens created by chlorination were not as effectively destroyed by ozonation, although subsequent activity in this strain decreased at higher ozone doses. TA100-S9 activity before and after ozonation correlated well with UV-absorbance. Separating the extracts by molecular weight fractions, the authors observed large decreases in the concentration of low molecular weight compounds after ozonation. Kool et al. (1982) found that the majority of mutagenic activity in chlorinated drinking waters was associated with the low molecular weight fraction. However, Van Hoof (1983) noted that oxidation of hydrophobic compounds by ozone was accompanied by the formation of some hydrophilic TA100-S9 mutagens.

In a later study of the same river water, Van Hoof et al. (1985) preozonated the raw water prior to coagulation, filtration and post-chlorination. The ozonated water resulted in up to a ten-fold increase in TA98-S9 mutagenic activity in the treated effluent compared to the same treated water without ozone. Thus, ozone applied before chlorination may increase substances that form TA98 mutagenic activity when chlorinated. However, the previous study (Van Hoof, 1983) showed that ozone following chlorination effectively oxidized TA98 mutagens created by chlorine. Therefore, the mutagenic activity associated with ozone is dependent not only on the raw water quality but also on the location of ozone application relative to chlorine addition in the treatment process.

Cognet et al. (1986) showed that mutagenicity is also related to the ozone dose and contact time. Observing TA98-S9 activity in Seine River water before and after ozonation, they found that a higher ozone dose of 5 mg/l created less mutagenic activity than a lower dose of 1 mg/l. In a batch ozonation study using groundwater, TA98-S9 activity was observed to increase over the influent level after 5 minutes contact time, decrease after 10 minutes, significantly increase again after 30 minutes and decrease to below background after 1 hour. This experiment suggests that different ozonation byproducts are created and destroyed under longer durations of ozone contact, and that no predetermined dose or contact time is optimal for all waters.

Most studies analyzing the effect of GAC adsorption on mutagenic activity have demonstrated high to complete removals. Bourbigot et al. (1986) found that all raw water or ozone-induced activity was removed by GAC. Loper et al. (1985) monitored mutagenic activity in TA98 and TA100 following chlorination, sand filtration and GAC filtration of river water for 32 weeks. They found that during normal operation and even after the GAC contactor was exhausted, mutagenic activity

produced from chlorination was completely removed by GAC. Residues extracted from the GAC contactor following the 32-week period contained all TA98+/-S9 activity in the top (influent) section, while TA100 activity was evident in decreasing concentrations from the top to the bottom of the bed. They concluded that GAC preferentially adsorbs direct and indirect mutagenic compounds over TOC, with a higher preference for TA98-sensitive compounds than for TA100-sensitive compounds. When the effluent from the GAC contactor was disinfected with 2.6 mg/l chlorine and stored for 3 days, the samples produced no mutagenic activity.

Finally, studies by Anderson et al. (1990) and Huck et al. (1990) evaluated pilot scale treatments on river water over a two-year period. Treatment included coagulation and sedimentation, followed by the addition of chlorine, chloramine, chlorine dioxide or ozone. All samples were then filtered through sand and GAC, and final disinfection was applied with one of the above oxidants. During 2 out of 49 sampling periods, raw water mutagenic activity was exhibited in both TA98-S9 and TA100-S9 and was reduced in both strains by metabolic activation. Mutagenic activity was observed in most of the post-filtration chlorinated samples, five chloraminated samples, one chlorine-dioxide-treated sample and one ozonated sample. GAC completely removed all mutagenic activity. Furthermore, mutagenicity was not created by any of the disinfectants added following GAC-treatment. The adsorption of MX and THMs by GAC was evaluated, and resulted in higher removals of MX than THMs. However, the adsorption capacity for MX was reduced by 40% in the presence of high levels of background organic material.

EXPERIMENTAL METHODS

SITE SELECTION AND SAMPLING PROCEDURES

Jordan Lake, located in Chatham County, N.C., was sampled north of the U.S. Highway 64 bridge at the proposed Apex-Cary water supply intake (see Figure 6). This location is in Segment III of the lake, which extends from the northern side of the U.S. Highway 64 bridge to the Farrington Road bridge. The water in this segment has been classified by the Department of Environment, Health and Natural Resources as WS-IV, suitable for drinking water supply.

To compare the finished water quality from Jordan Lake with that of another lake that is already used as a drinking water source, samples were also collected from Falls Lake in Wake County, N.C (see Figure 7). Falls Lake raw water samples were taken at the dam release and are representative of water at a depth of 12 to 16 feet from the lake's surface. This is a similar depth to the intake for the Raleigh water treatment plant. However, the samples collected experienced some degree of turbulence and aeration through the spillway prior to the point of sample collection.

Jordan Lake and Falls Lake samples were collected four times during the year with the intent of comparing seasonal variability, especially during the conditions of summer peak algal productivity, fall turnover (a thermal destratification and mixing process due to the drop in surface water temperature), winter minimum light and temperature, and spring biomass maximum growth.

Tributaries to Jordan Lake were sampled in the summer of 1990 to simulate a "worst case" condition of lake water quality and to analyze the mutagenic effects of the inflows. These samples were processed through the same conventional treatment scheme as the lake water samples (see below). Aliquots from Morgan Creek, New Hope Creek and Northeast Creek, major tributaries flowing into Segment IV of the lake, were collected in July, 1990, at sites currently sampled by the U.S. Geologic Survey (see Figure 6).

The Haw River was sampled at two locations in September, 1990, to compare the relative water quality in the main tributary to the lake. Water was collected from the river near U.S. Highway 64, about one mile upstream from Jordan Lake. The Pittsboro water treatment plant raw water intake is in the Haw River north of U.S. Highway 15-501; a sample was collected at the treatment plant's raw water sample tap to represent Haw River water at this location.

The procedures for collection of lake and stream samples were as follows. Plastic Nalgene 10- and 40-liter bottles were washed with detergent and thoroughly rinsed in the laboratory prior to sample collection. The bottles and a plastic sampling bucket were rinsed with sample water on-site at least five times before collecting the raw water samples. At Jordan Lake, a boat was taken to a location north of the U.S. Highway 64 bridge where Jordan Lake is about 50 feet deep. Samples were pumped by a centrifugal pump from 12 feet below the water surface. At Falls Lake, water was pumped from 3 feet below the surface at the dam release. Tributary samples were collected by immersing a clean plastic bucket beneath the stream surface. Pittsboro water treatment plant influent was collected at the raw water sampling tap

Figure 6. Jordan Lake and Sampling Locations

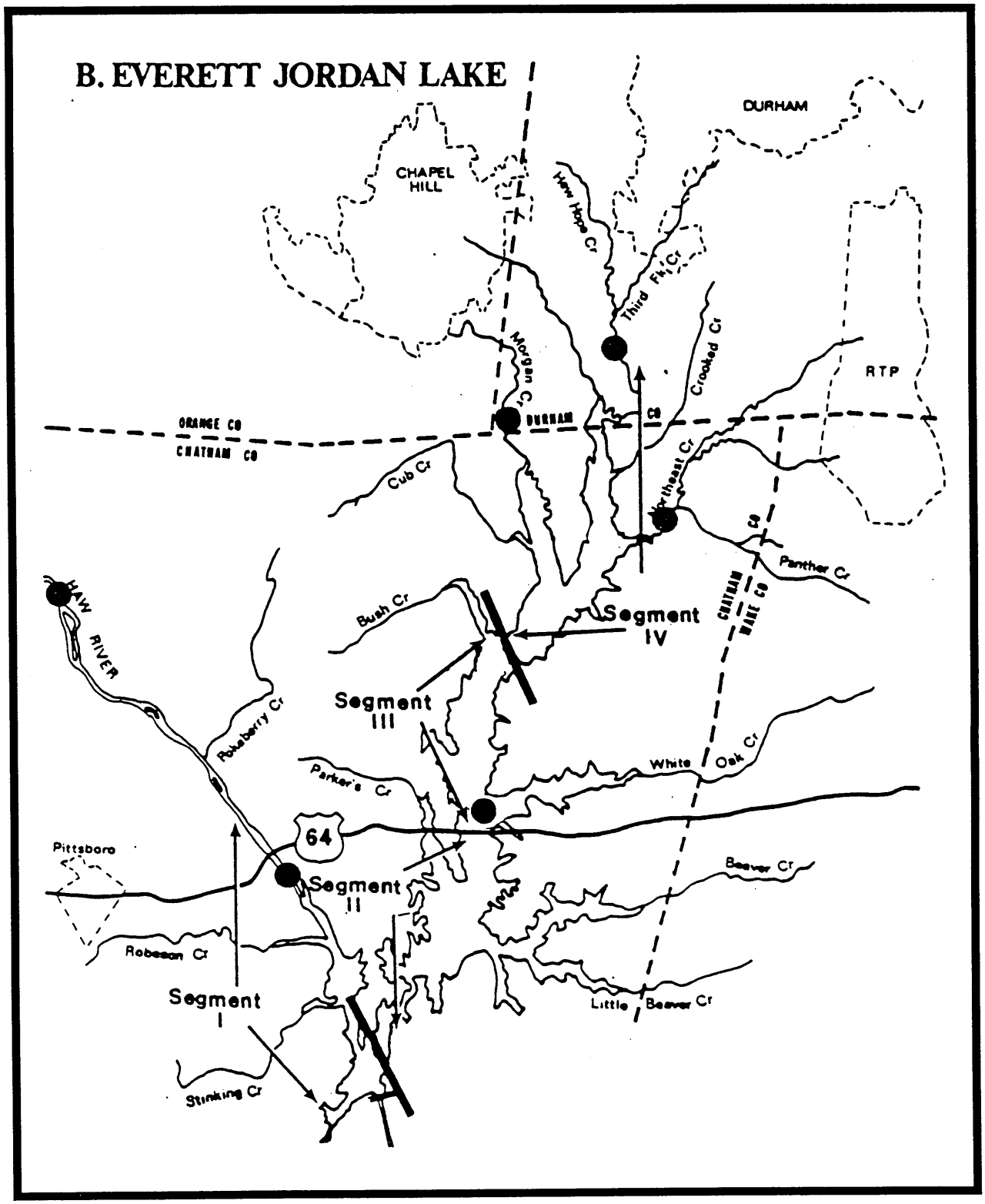
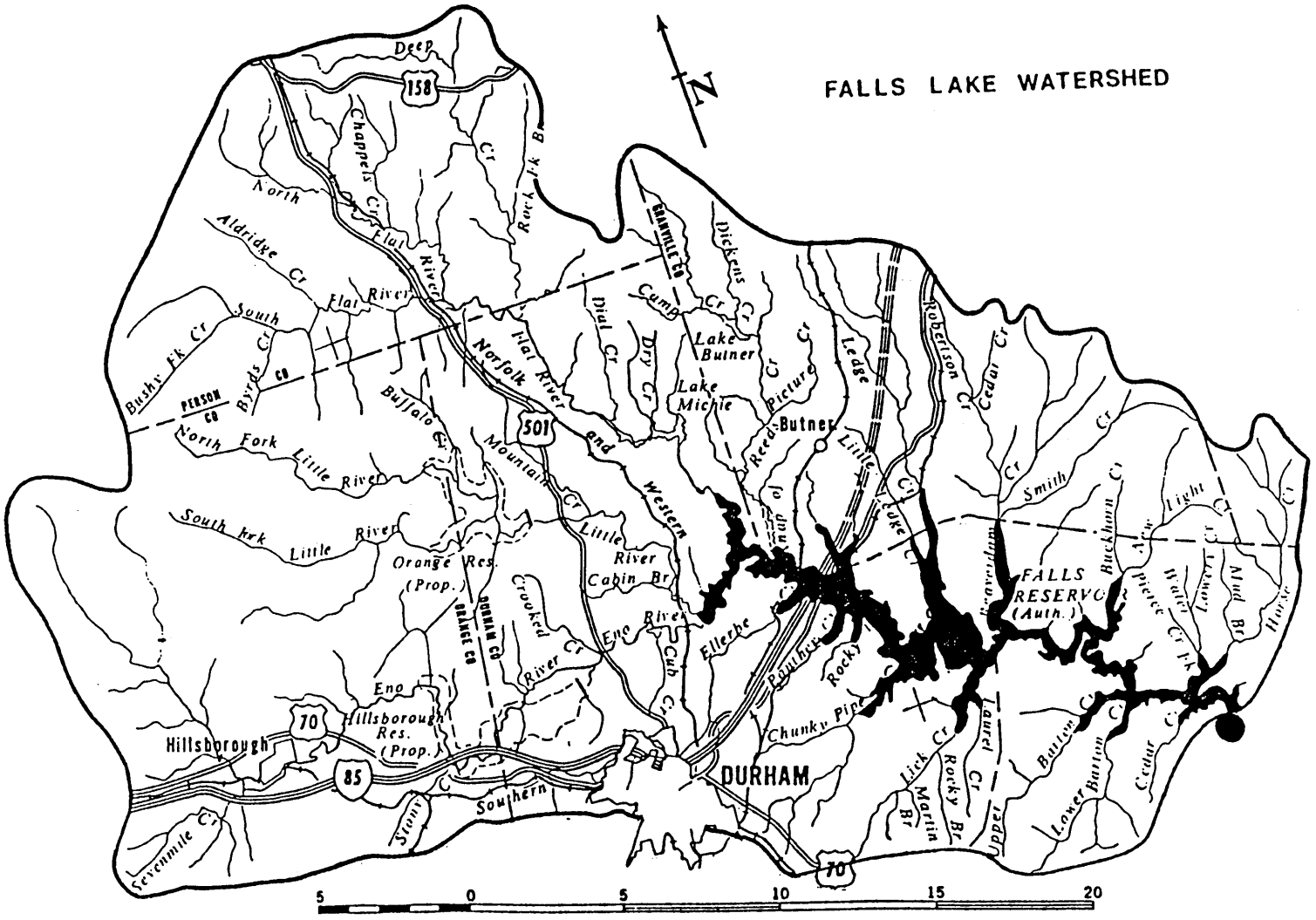


Figure 7. Falls Lake and Sampling Location



● = Sampling Site

in the plant laboratory. Temperatures were recorded on-site. Upon return to the laboratory, pH and turbidity were measured immediately. Raw water samples were taken for analysis of TOC, TOX, THM and Ames mutagenicity.

Finished water samples were collected from two surface water treatment plants that have sources similar to Jordan and Falls Lakes. The water quality of these finished water samples was analyzed for comparison to the finished quality of the laboratory-treated waters. Samples were carefully poured from the finished water sampling tap into clean four-liter glass bottles for the bioassay, and into clean 40-ml glass vials with required preservatives for chemical analysis.

GENERAL TREATMENT SCHEME

The pilot-scale treatment regime depicted in Figure 8 shows the three complete treatment processes applied to Jordan and Falls Lake waters. One of the primary objectives of the project was to determine the mutagenicity of the raw lake waters, if any, and the extent of removal or creation of mutagenic activity provided throughout each treatment process. Reduction in the TOC concentration and in turbidity, and the quantity of TOX and THMs produced during each process were also analyzed to help determine water quality throughout treatment. Conventional coagulation, sedimentation and filtration were applied to all of the raw water samples (Sequence A-B in Figure 8). Then, the conventional practice of post-chlorine disinfection (Sequence A-B-C) was contrasted with the advanced treatment alternatives of GAC adsorption followed by chlorination (A-B-D-E) and ozonation followed by chloramination (A-B-F-G).

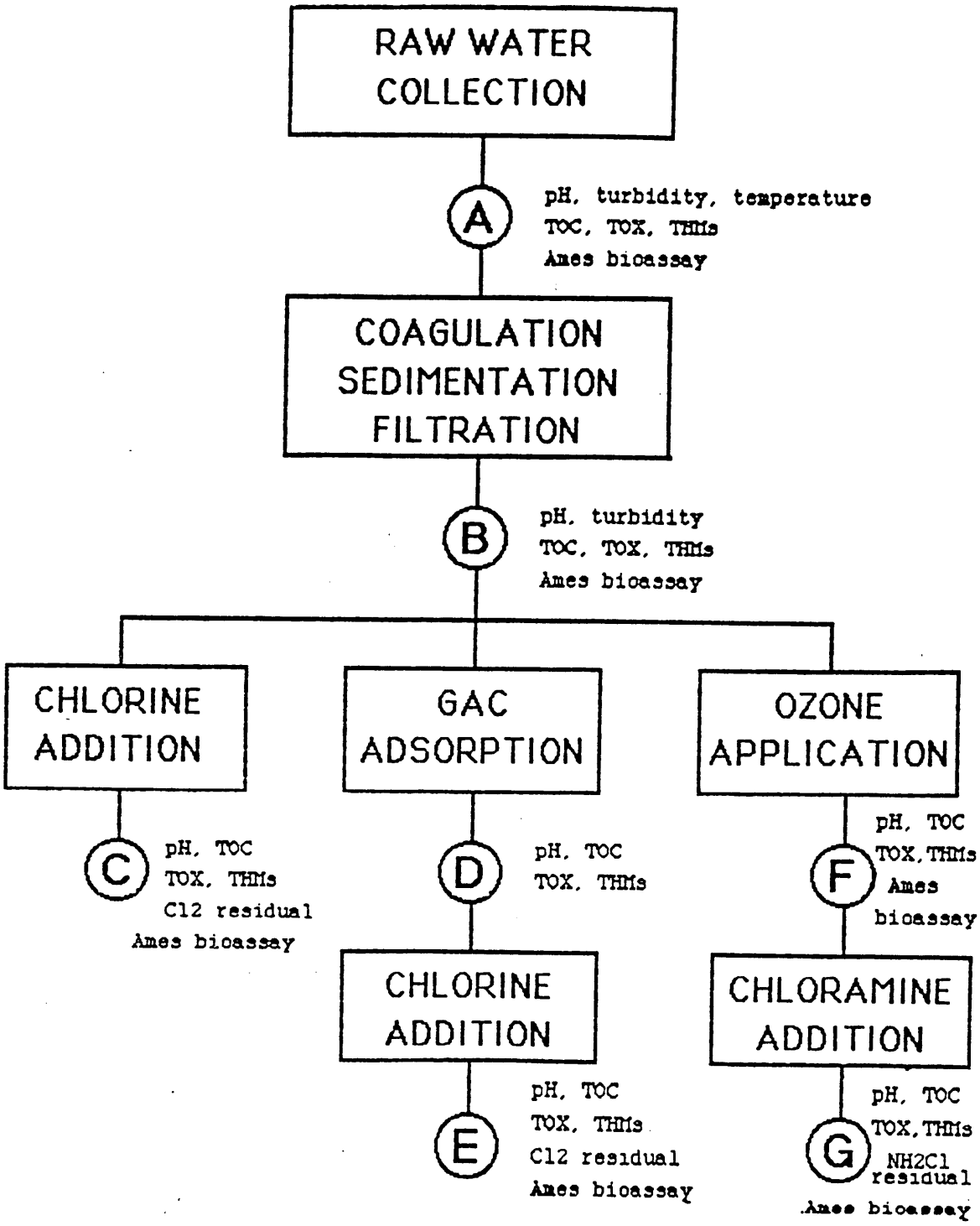
PREPARATION OF GLASSWARE

Deionized distilled water was used for all chemical preparations and final rinsing of glassware. Glassware was soaked in detergent, rinsed with tap water, soaked overnight in 10% sulfuric acid, rinsed with deionized distilled water and baked overnight at 100°C. Glassware used in chlorination experiments was made chlorine-demand-free by soaking overnight in purified chlorinated water (about 30 mg/l) and rinsing with distilled water prior to use. Glassware used in ozonation experiments was made ozone-demand-free immediately prior to sample ozonation by diffusing ozone gas into deionized distilled water in the vessel for 30 minutes followed by rinsing with deionized distilled water.

COAGULATION

Optimal alum doses were determined by conducting standard jar tests in 500-ml sample beakers. The pH was held at 6.0 with 0.01 M sulfuric acid addition while different doses of alum, ranging from 25 to 45 mg/l, were added to the beakers at a rapid mix blade rotational velocity of 80 rpm. After two minutes of rapid mixing during chemical addition, the blade speed was decreased to 45 rpm for seven minutes, 30 rpm for seven minutes and then 15 rpm for seven minutes. The time of the first appearance of floc particles, the water clarity, and the relative floc size after 15 minutes were noted for each beaker. Following flocculation, the samples were settled for 30 minutes. pH and turbidity were measured at the end of this period. The requisite alum dose for bulk flocculation was selected by choosing the lowest

Figure 8. General Treatment Scheme



alum dose that provided a settled water turbidity of less than 1.0 NTU.

Batch coagulation of 100 liters of sample at pH 6.0 followed the jar tests, using the optimal alum and acid dosage determined from the jar tests. The large sample size was dictated by the need to process coagulated water through the three parallel treatment trains and the need for relatively large aliquots in the Ames bioassay. The batch reactor used for coagulation had a center-axle, single rotational blade and four wall baffles. This reactor was designed to achieve a high velocity gradient, G, during the two minute rapid mix at 80 rpm. G was calculated as decreasing from 110 s^{-1} at 45 rpm to 60 s^{-1} at 30 rpm to 21 s^{-1} at 15 rpm during the 21-minute, 3-stage tapered flocculation. Settling was permitted for one hour before 72 liters of the sample was filtered through a 2.0-micron cellular polyester cartridge filter into clean 18-liter glass bottles. The filtered water was analyzed for residual turbidity, TOC, TOX and THMs, and the remainder was stored at 4°C until further experimentation. This water served as the source water for all three final treatment scenarios (see Figure 8).

CHLORINATION

Concentrated chlorine stock solutions were freshly prepared from Fisher purified 4-6% NaOCl. Chlorine concentrations were determined using the DPD Ferrous Titrimetric Method 4500-F described in Standard Methods (1990). The chlorine dose necessary to produce a 48-hour residual free chlorine concentration of 0.5 mg/l for each sample was determined by adding various doses of chlorine to several 350-ml sample aliquots that were first adjusted to pH 8.0 using a 0.1 M sodium carbonate solution. These chlorinated sample aliquots were stored in ground-glass-stoppered bottles headspace-free in the dark at room temperature for 48 hours.

The chlorine dose for large-scale chlorination was selected as the dosage which resulted in a 48-hour free chlorine residual closest to 0.5 mg/l. Twelve liters of each sample was then chlorinated in an 18-liter chlorine-demand-free glass bottle following sample pH adjustment to 8.0. The chlorinated samples were carefully siphoned into headspace-free, 4-liter glass bottles for storage at room temperature in the dark. After 48 hours, the samples were analyzed for turbidity, free and total chlorine residual and pH, and portions were rebottled in 40-ml vials and quenched with sodium sulfite and acid for subsequent TOC, TOX and THM analysis. The remaining sample was stored at 4°C for the subsequent Ames bioassay. Samples chlorinated following GAC filtration were handled similarly.

Samples receiving chloramination were pre-adjusted to pH 8.0 with a 0.1 M sodium carbonate solution. Chloramines were created by adding 0.25 mg ammonium phosphate as N per mg chlorine to the sample prior to chlorine addition. Mixing was achieved using a magnetic stirrer. Optimal chloramine doses were selected by a procedure similar to that used for chlorination with a target 48-hour residual of 1 mg/l total chlorine. During bulk chloramination, ammonia was added to the 12-liter sample and allowed to mix for several minutes before chlorine addition. Sample handling and subsequent analysis were the same as for the chlorinated waters.

GAC TREATMENT

GAC column dimensions were determined using criteria of an empty bed contact time (EBCT) of ten minutes and a loading rate of 5 m/hr to simulate typical applications. Calgon Filtrasorb 400 activated carbon was washed in deionized distilled water, oven-dried and sieved before being packed into a 4.5-cm diameter, 100-cm long Plexiglas column. Samples flowed by gravity into the column and flow rate was controlled at the effluent. The intent of the GAC treatment was to measure removals achievable on fresh GAC, i.e. to determine the maximum removal of TOC, THM and TOX precursors, and mutagenic activity. Grab samples of the effluent from the column were analyzed for TOC every fifteen minutes to determine if column breakthrough had occurred. Testing was limited to the first 24 hours of service time of the GAC bed. Composite effluent samples were stored in glass bottles at 4°C for further analysis.

OZONATION

Three ozonation reactors were used over the course of the project. A 70-liter stainless steel batch reactor was used to ozonate the Falls Lake April and Jordan Lake May 1990 samples. A 27-liter stainless steel reactor was used to ozonate the Falls Lake July and Jordan Lake October samples. The remaining samples were ozonated in an 18-liter glass bottle equipped with a magnetic stirrer. Ozone for all reactions was produced from breathing quality air by a Union Carbide Model No. SG 4060 Ozone Generator.

All of the samples received a target ozone dose such that the mass of ozone absorbed by the sample was equivalent to the mass of TOC in the sample. To achieve this, the ozone concentration in the outlet stream from the generator was measured by trapping the ozone in the off-gas into a 20 g/l potassium iodide (KI) solution. The iodide that was oxidized to iodine by ozone was then titrated back to iodide with a standard 0.1 M sodium thiosulfate solution; the requisite amount to completely convert the iodine back to iodide represents one mole of ozone absorbed per 2 moles of thiosulfate titrated by the following reactions:

Iodide oxidation:

$$\begin{aligned} &3O_3 + I^- = IO_3^- + 3O_2 \\ &\underline{IO_3^- + 5I^- = 3I_2 + 3H_2O} \\ &O_3 + H_2O + 2I^- = O_2 + 2OH^- + I_2 \end{aligned} \qquad (8)$$

Iodine reduction:

$$2S_2O_3^{-2} + I_2 = S_4O_6^{-2} + 2I^- \qquad (9)$$

After determining the ozone concentration produced by the generator, ozone gas was sparged into the water sample while two vertical axis blades mixed the solution vigorously. Off-gas was collected from the top of the tank and diffused directly into KI to detect any ozone that was not absorbed by the sample. After the requisite ozone contact period, purified air was sparged through the sample and gas collection through the KI trap continued for 30 minutes (the estimated time to displace three reactor volumes at that gas flow rate). The KI in the trap was then titrated with sodium thiosulfate. The ozone absorbed by the sample was calculated as the applied ozone dose minus the ozone

trapped in the KI. This procedure was repeated until the ozone absorbed by the sample was equivalent to the TOC of the sample before ozonation. Changes in the concentration of TOC, production of TOX and THMs, and Ames mutagenic activity in the samples were measured following treatment with ozone.

TOC ANALYSIS

Samples collected for TOC analysis were stored headspace-free in 40-ml glass, teflon-sealed vials with 0.5 ml sulfuric acid added for preservation, and held at 4°C in the dark not longer than two weeks before analysis. Samples were analyzed on an Oceanographics International Corporation Model 700 TOC Analyzer with automatic sampler. A one-ml sample was injected per analysis, and each sample was analyzed at least three times to assure precision. The instrument was calibrated using a standard solution prepared from potassium hydrogen phthalate at 5.0 mg/l concentration because the samples analyzed ranged from 0.2 to 10 mg/l TOC. Standards ranging from 0.1 mg/l to 20 mg/l were analyzed under these calibration conditions and consistently resulted in less than one percent error. The calibration was checked every two hours to ensure that precision was maintained throughout the analysis.

TOX ANALYSIS

Samples collected for TOX analysis were stored headspace-free in 40-ml glass, teflon-sealed vials and held at 4°C in the dark not longer than one week before analysis. The samples were preserved with 0.5 ml sulfuric acid, and the residual chlorine was quenched by the addition of approximately 10 mg sodium thiosulfate. Dissolved organic halogen concentration was measured by Method 5320 B described in Standard Methods (1989), using a Dohrmann DX-20 microcoulometric titration system with pyrolysis furnace and AD-2 adsorption module. Carbon columns used for adsorption of halides were packed with fresh, clean 200-mesh activated carbon within 24 hours of sample analysis. Halogens from the samples were adsorbed onto the carbon by acidifying the samples to pH 2.0 with concentrated nitric acid and passing the samples through the carbon columns. The columns were then rinsed with a nitrate solution, causing displacement of the inorganic halides from the carbon, leaving only organic halides on the carbon. The carbon was then pyrolyzed at 800°C, converting the organic halides to HX gas. The gas flowed into a microcoulometric titration cell where silver-ion precipitation by the halide was measured as current produced from silver ions dissolving from a silver electrode in order to maintain a constant silver concentration in the cell. The analyzer then converted this signal into a measurement of micrograms of chloride (ug Cl^-).

All samples were collected and analyzed in duplicate or in triplicate, when necessary, if the two measurements did not agree within 10%. Six to eight blank carbon columns were nitrate-washed and analyzed during each sampling session to determine the average background level of TOX in the columns. This concentration, which did not exceed 0.90 ug Cl^- and averaged 0.66 ug Cl^- per column, was subtracted twice from each sample measurement to account for the background TOX in each of the two columns. The sample TOX was calculated as:

$$\text{TOX (ug/l)} = \frac{(\text{Col 1} - \text{AvgBlnk} + \text{Col 2} - \text{AvgBlnk})\ \text{ug Cl}^-}{(\text{Sample Volume})\ \text{l}} \qquad (10)$$

Of the 75 samples analyzed, duplicates agreed within 10% of each other with the exception of five samples.

The precision of the analysis was tested for low concentrations of organic halides by analyzing ten deionized distilled water samples of volumes varying from 50 to 200 ml. Detected TOX concentrations of 3.8 to 4.5 ug Cl^-/l indicated reasonable precision at low concentrations. Standard Methods (1989) recommends a detection limit of 5 ug Cl-/l. Replicated small volumes of a prepared pentachlorophenol standard exhibited a much wider range of values at high concentrations, indicating that some precision is lost with (1) increased concentrations of TOX, and (2) smaller sample volumes passed through the carbon columns.

THM ANALYSIS

Samples collected for THM analysis were stored headspace- free in 40-ml glass teflon-sealed vials with approximately 10 mg sodium thiosulfate to quench the residual chlorine, and held at 4°C in the dark not longer than three weeks before extraction. Samples were liquid-liquid extracted in THM-grade 99.9% purity pentane, according to the procedure outlined by the U.S. Environmental Protection Agency (1988); 40 to 70 ug/l dibromopropane was injected per sample as an internal standard. Three concentrated stock solutions of the four THM species $CHCl_3$, $CHCl_2Br$, $CHClBr_2$ and $CHBr_3$ were prepared by injecting small volumes of analyte into a measured amount of acetone and verifying the volumes by weighing the entire stock before and after addition. Twelve standards were prepared from these stocks, ranging from 0.1 to 200 ug/l of each analyte, by injecting a pre-determined volume into deionized distilled water covered with a pentane layer for extraction. Because the method measures only the THMs that are extracted into the pentane from water, injecting the standards into water is designed to simulate the extraction efficiency of the samples. Other studies have accomplished at least 95% recovery of the analytes by this extraction method (Reckhow, 1984).

The extracted samples were stored in teflon-sealed glass vials not longer than 7 days prior to analysis. The extracted samples taken from tributaries during the summer were re-tested two months after the initial chromatography and the later results agreed within 10% of the previous results, indicating only minor loss of THM compounds during storage in pentane.

THMs were measured on a Hewlett-Packard 5890A gas chromatograph with electron capture detector. The injector and detector temperatures were 177°C and 297°C, respectively. The carrier gas flowed at 60 ml/minute. The column initial temperature of 35°C was held for 18 minutes after sample injection, then ramped up at a rate of 9° per minute to 110°C, followed by a ramp of 27° per minute to 204°C. The twelve standards were run at the same time as the samples; the peak areas of the standards were used to compute sample concentrations. A linear regression of peak area relative to the area of the internal standard was computed for each analyte and compared to the known concentration of analyte. The sample concentrations were then determined from the slope of the regression line. All standard regressions resulted in an R^2 value greater than 0.95. Samples were collected and analyzed in duplicate and resulted in less than 10% error between samples.

AMES ASSAY

Rationale

The objective of this section of the study was to detect potentially genotoxic chemicals in the waters examined. The study examined genotoxic activity both as it occurred in the raw water and after the water had been treated for drinking purposes in order to evaluate the effects of different treatment trains on genotoxic agents or their chemical precursors.

The major contributors to genotoxicity in the raw water were anticipated to be naturally-occurring substances, industrial wastes, domestic wastes, and in treated waters the disinfection by-products of substances present in natural waters. Therefore a wide variety of different chemicals may be present, each with its own physico-chemical and mutagenic characteristics.

The mutagenic activity detected in treated drinking water by previous studies has been primarily attributed to species produced by chlorination of humic acid, in particular MX (Coleman et al., 1984; Kronberg et al., 1985; Meier et al., 1987). This compound is a potent direct-acting mutagen whose activity is maximal in TA100, and is diminished in the presence of S9. The mutagenicity detected in chlorinated humic waters, and that of chlorinated nutrients such as amino acids (Sussmuth, 1982) also exhibits these characteristics. The strain TA100 without exogenous metabolic activation (S9) is therefore the strain of choice for detection of mutagenic activity due to disinfection by-products of natural water constituents. One of the primary aims of this study was the detection of genotoxins arising from industrial or anthropogenic wastes, which may contribute mutagenicity both in themselves and after undergoing disinfection treatment. While the balance of evidence suggests that chlorination products generally will be detected by TA100 without S9, it is desirable to screen samples with S9, and also with the strain TA98 which is more sensitive to frameshift mutagens in order to maximize chances of detecting a greater diversity of chemical species: industrial wastes may contain aromatic hydrocarbons, which typically require S9 activation, and other chemicals whose mutagenic spectrum has not yet been characterized. Approximately 80% of the pure mutagenic compounds in the National Toxicology Program data base are responsive in the presence of S9 (Zeiger et al., 1985), and a panel of industrial waste samples were predominantly mutagenic towards strain TA98 with S9 (DeMarini et al., 1987).

Thus the initial stages of this study were conducted with both TA98 and TA100 with and without S9. As the type of mutagens present (i.e. the predominant mutagenic species) can be expected to be altered by the disinfection treatment processes, the bacterial strain most responsive to raw water may well not be the same strain which is most sensitive to the treatment products.

Only if a highly potent mutagen is present in high concentrations can mutagenicity be expected to be directly detectable in water, because of the limits of sensitivity and sample size (generally 0.1 mL per plate) in the Ames assay. Therefore water will generally need to be concentrated in order to detect mutagenicity levels on the order of 100 revertants per liter of water reported elsewhere (Loper et al., 1985).

Several techniques have been utilized for concentration of drinking water before bioassay, including adsorption onto solid-phase resins, lyophilization, and liquid-liquid extraction (Wigilius et al., 1985), which each exhibit their own advantages and disadvantages.

The concentration factor used in this study (2.5×10^3) was chosen as sufficient to give statistically significant mutagenic activities for samples from local drinking water sources known to produce 200-300 revertants per liter in TA100 without S9 activation. As we did not need to extract enormous volumes of water to detect mutagenicity at the level of concern and we wished to avoid possible artifacts arising from interaction of unquenched disinfectants with solid phase resins (Cozzie et al., 1993), we chose to use the simpler, more rapid technique of liquid-liquid extraction (LLE) over resin extraction methods. LLE with methylene chloride (DCM) has been shown to extract a wide range of compounds, under neutral and acidic conditions, with fewer artifacts than result from extraction with XAD macroreticular resins (Grabow et al., 1981A), as well as greater recovery of mutagenicity. Monarca et al. (1985) have found that compounds which contribute most to mutagenicity of chlorinated waters are extractable at neutral to acidic pH, as used in this study.

Methylene chloride has been widely used as an extraction solvent for chemical and genotoxic analyses on raw and treated waters (Dietrich et al., 1988). The only disadvantage to use of this solvent in extracting chlorinated samples seems to be the possible production of chlorinated cyclohexenes (Dietrich et al., 1988) resulting from the reaction of residual chlorine with trace amounts of cyclohexene added to prevent formation of phosgene. While such products have been found in highly concentrated samples ($>10^4$), the consequences for genotoxicity testing are not clear. Further, the concentration factor used in this study (2.5×10^3) is lower than that generally used in such work, with the result that any such products would be of less concern.

Extraction conditions were selected to favor concentration of a broad range of chemical classes, whereas the humic acid products are preferentially extracted by acid adsorption onto the more polar resins XAD-4 and XAD-8.

Chlorinated samples for chemical analyses are routinely quenched with sodium sulfite, a practice shown by Cheh et al. (1980) to reduce mutagenicity of samples by 50 to 80%, almost certainly by nucleophilic attack on electrophiles. Consequently, bulk chlorinated samples were not quenched before Ames assay, in order to minimize underestimation of the genotoxicity of such waters.

Procedures

Extraction. Raw and treated waters were stored at 4°C until extraction (less than 2 days for chlorinated and ozonated samples, less than one week for raw water). Chlorinated samples were not quenched, as this has been shown to reduce mutagenicity of treated samples (Cheh et al., 1980).

Five liters of each raw or treated sample were extracted at ambient (or treatment) pH, adjusted to pH 2 with HCl and extracted again. The total volume was divided into 2.5 L aliquots and each aliquot was shaken twice (in a 4 L separatory funnel) at each pH for 30 seconds

with 100 mL methylene chloride (Burdick & Jackson High Purity, Baxter Healthcare, Charlotte, NC). The total 800 mL extract was dried completely by rotary evaporation at about 40°C, stored at 4°C, and resuspended in 2 mL sterile dimethyl sulfoxide (DMSO) (Burdick & Jackson High Purity, Baxter Healthcare, Charlotte, NC) immediately prior to assay, resulting in a concentration factor of 2.5×10^3 (1 uL of extract = 2.5 mL of water).

Ames Salmonella Plate Incorporation Assay. Ames assays were performed according to standard procedures (Ames et al., 1975; Maron and Ames, 1983) in duplicate on three 2-fold dilutions of the extracts dissolved in DMSO. Salmonella strains TA98 (sensitive to frameshifts) and TA100 (sensitive to base-pair substitutions), obtained from Dr. B. N. Ames, University of California-Berkeley and stored and maintained according to standard procedures (Ames et al., 1975; Maron and Ames, 1983), were used to assay all extracts with and without Aroclor 1254-induced rat liver S9 metabolic activation (Mol-Tox Inc., College Park, MD). Molten top agar (approximately 2.5 mL) was mixed with 20 to 100 uL of water extract or control dissolved in DMSO, 100 uL of overnight culture of strain TA98 or TA100 containing $1\text{-}2 \times 10^9$ organisms per ml and, where required, 500 uL of reconstituted S9 preparation, then poured onto 10 x 150 mm Petri dishes containing 25 mL of solidified agar with minimal histidine. Positive controls were included with each experiment: for TA98 without S9, 2-nitrofluorene, 3 ug/plate, 290±116 rev/plate; TA100 without S9, sodium azide, 1,5 ug/plate, 496±112 rev/plate; TA98 and TA100 with S9, 2-anthramine, 0.5 ug/plate, 514±187 rev/plate respectively. Solvent (negative) controls were also performed in duplicate for each strain and S9 condition. TA98 without S9, 26±13; TA98 with S9, 39±16; TA100 without S9, 99±27; TA100 with S9, 108±30. After 48 hours incubation at 37°C, the number of revertant colonies on each plate was counted by hand, or with an automatic colony counter (Model 880 Image Analyzer, Artek Inc., Chantilly, VA) with hand counts carried out at random to check reliability.

Data Analysis. Numbers of revertant colonies were averaged for duplicate plates, except for cases of clear anomalies such as fungal contamination or mispouring. A reversion ratio (highest number of revertant colonies per plate for each sample divided by spontaneous revertants on the solvent control plates) was calculated for each sample. In each case where this ratio exceeded 2.0 for assays with strain TA98, or 1.5 for strain TA100, regression analysis was performed on the dose response data.

The slope of the line fitted by linear regression (Bernstein et al., 1982) on a plot of revertants/plate versus dose (expressed as equivalent water volume extracted) corresponds to the mutagenic activity in revertants/L of the sample under the stated test conditions. In order to evaluate the statistical reliability of the calculated slopes we report them along with r^2 (correlation coefficient, a measure of the scatter of the data) and p-values (the probability of a slope or mutagenic activity being greater than zero). In general, a p-value of less than 0.05 indicates very significant mutagenic activity, whereas a p-value of greater than 0.10 shows that the mutagenicity cannot be estimated with sufficient confidence either due to scatter in the data or to small differences in numbers of revertants with dose or both. In a few cases where reversion ratios did not quite meet our initially stated minimum criteria, we still found statistically significant mutagenicity and so have included

these results as well. The dose range and concentration factor were such that our limits of detectability and statistical significance were around 200 revertants per liter for Salmonella typhimurium strain TA100 and 100 revertants per liter for strain TA98, which is of the order of the activity reported for finished drinking waters derived from surface waters of acceptable quality (Lippincott et al., 1990).

RESULTS AND DISCUSSION

RAW WATER CHARACTERISTICS

The raw waters sampled from Jordan Lake and Falls Lake were similar with respect to their average characteristics and seasonal variations in turbidity, temperature, pH, and total organic carbon (TOC) and total organic halide (TOX) concentrations, as shown in Table 2. Temperatures varied seasonally as did turbidity and TOC. Turbidity in Falls Lake was generally higher than that of Jordan Lake, but this may have been due to the turbulence at the Falls Lake sampling site. Falls Lake samples exhibited slightly higher overall TOC concentrations than Jordan Lake. The average pH of Jordan and Falls Lake samples were 7.0 and 7.1, respectively. TOX concentrations from 11 (limit of detection) to 45 ug/l were detected in the raw water samples, and less than 1 ug/l of THMs were measured.

Jordan Lake tributaries sampled in July and September of 1990 were of poorer quality than the lake waters, as demonstrated by the higher turbidities and TOC and TOX concentrations. Although less than 10 ug/l total THMs were measured in the tributary waters, the TOX concentration was greater than 100 ug/l for Morgan and New Hope Creeks and close to 200 ug/l in Northeast Creek. In a WRRI-sponsored study conducted by Singer, Brown and Wiseman (1988), similar TOX concentrations were found in Morgan and New Hope Creeks near these same sites. The presence of TOX was attributed to the discharge of chlorinated municipal wastewater effluents upstream of the sampling locations. The TOX concentrations of the Haw River samples were less than those of the New Hope tributaries, yet high enough to indicate the presence of chlorinated discharges upstream that may have undergone some dilution and natural decomposition. Because purgeable halogens are significantly volatilized by aeration in the flowing streams, the TOX composition can be assumed to be mostly nonpurgeable organic halogen (NPOX); the volatile THMs are expected to be minimal.

The raw water sources for Treatment Plants A and B have similar characteristics to those of Jordan and Falls Lakes. The raw water turbidities and TOC concentrations were measured by treatment plant personnel and are shown in Table 2.

Results of Coagulation, Settling, and Filtration

Table 3 displays the alum doses selected for coagulation and the resulting filtered water characteristics. Raw and filtered TOC concentrations are depicted for each sample in Figures 9 and 10. The TOC of the lake and tributary samples was reduced an average of 48% (standard deviation = 12.3) by coagulation, sedimentation, and filtration, with removals ranging from 29% to 69%. By comparison, water plants A and B achieved from 29% to 54% removals of TOC on the four sampling days, with an average reduction of 40%.

Through optimization of coagulation, TOC removals of 50 to 75 percent are reasonably achievable and, in most cases, will effectively reduce subsequent disinfection byproduct formation (Kavanaugh, 1978; Babcock and Singer, 1979). Reduction in TOC concentrations reflects the removal of TOX and THM precursors in most waters (Reckhow and Singer, 1984). Singer et al. (1981) found average TOC removals at 13 water treatment plants in North Carolina of 38% after coagulation and

Table 2. Raw Water Characteristics

Location	Sampling Date	pH	Temp. (Deg.C)	Turbidity (ntu)	TOC (mg/l)	TOX (ug Cl/l)	TTHM (ug/l)
Jordan Lake	05/23/90	7.0	22	7.6	7.3	NA	NA
Jordan Lake	10/10/90	7.0	26	8.8	6.6	45	<1
Jordan Lake	12/23/90	6.9	15	15.1	6.9	39	<1
Jordan Lake	03/07/91	7.1	8	8.7	7.8	21	ND
Falls Lake	04/11/90	6.3	22	10.0	7.7	NA	ND
Falls Lake	07/27/90	6.9	24	6.8	7.3	22	ND
Falls Lake	11/19/90	6.8	26	16.3	10.7	11	ND
Falls Lake	02/26/91	8.4	16	14.0	7.2	20	ND
Falls Lake	04/19/91	6.9	19	8.1	7.1	18	<1
Morgan Creek	07/02/90	7.3	29	16.5	8.7	128	6
New Hope Creek	07/02/90	7.2	30	11.9	9.5	155	5
Northeast Creek	07/02/90	7.1	29	16.6	8.6	194	4
Haw River	09/12/90	7.4	27	5.0	6.9	74	<1
Pittsboro Water Plant Raw Water	09/12/90	7.4	27	6.1	6.7	60	ND
Water Plant A	06/23/90	7.4	26	6.3	6.8	NA	NA
Water Plant A	04/25/91	7.3	20	3.8	4.8	NA	NA
Water Plant B	06/23/90	6.6	29	5.0	6.8	NA	NA
Water Plant B	04/26/90	6.8	18	5.0	7.7	NA	NA

ND: Not detected in analysis

NA: Parameter not analyzed

Table 3. Results of Coagulation, Settling and Filtration

Location	Sampling Date	Alum Dose (mg/l)	Filtered Turbidity (ntu)	Raw TOC (mg/l)	Filtered TOC (mg/l)	TOC Reduction %	Raw TOX (ug/l)	Filtered TOX (ug/l)	TOX Reduction %
Jordan Lake	05/23/90	35.0	0.06	7.3	3.6	50.7	NA	NA	NA
Jordan Lake	10/10/90	30.0	0.04	6.6	4.5	31.8	45	14	69
Jordan Lake	12/23/90	35.0	0.06	6.9	4.1	40.6	39	15	62
Jordan Lake	03/07/91	33.3	0.05	7.8	3.8	51.3	21	8	62
Falls Lake	04/11/90	36.7	0.05	7.7	3.1	59.7	NA	23	NA
Falls Lake	07/27/90	35.0	0.12	7.3	3.2	56.2	22	17	23
Falls Lake	11/19/90	35.0	0.08	10.7	3.3	69.2	15	19	-27
Falls Lake	02/26/91	30.0	0.09	7.2	5.1	29.2	20	12	40
Morgan Creek	07/02/90	45.0	0.12	8.7	4.7	46.0	128	90	30
New Hope Creek	07/02/90	35.0	0.07	9.5	4.3	54.7	155	107	31
Northeast Creek	07/02/90	42.0	0.08	8.6	4.3	50.0	194	156	20
Haw River	09/12/90	35.0	0.05	6.9	4.5	34.8	74	58	22
Pittsboro Water Plant Raw Water	09/12/90	35.0	0.06	6.7	4.2	37.3	60	70	-17
Plant A	06/23/90	37.7	0.15	7.4	4.8	35.1	NA	NA	NA
Plant A	04/25/91	35.0	0.11	7.3	2.1	71.2	NA	NA	NA
Plant B	06/23/90	35.0	0.30	6.6	4.0	39.4	NA	NA	NA
Plant B	04/26/91	35.0	0.18	6.8	3.1	54.4	NA	NA	NA
Mean		35.6	0.10	7.6	3.9	47.7	59	45	29

NA: Parameter not analyzed

Figure 9. TOC Removal by Coagulation and Filtration: Jordan and Falls Lake Samples

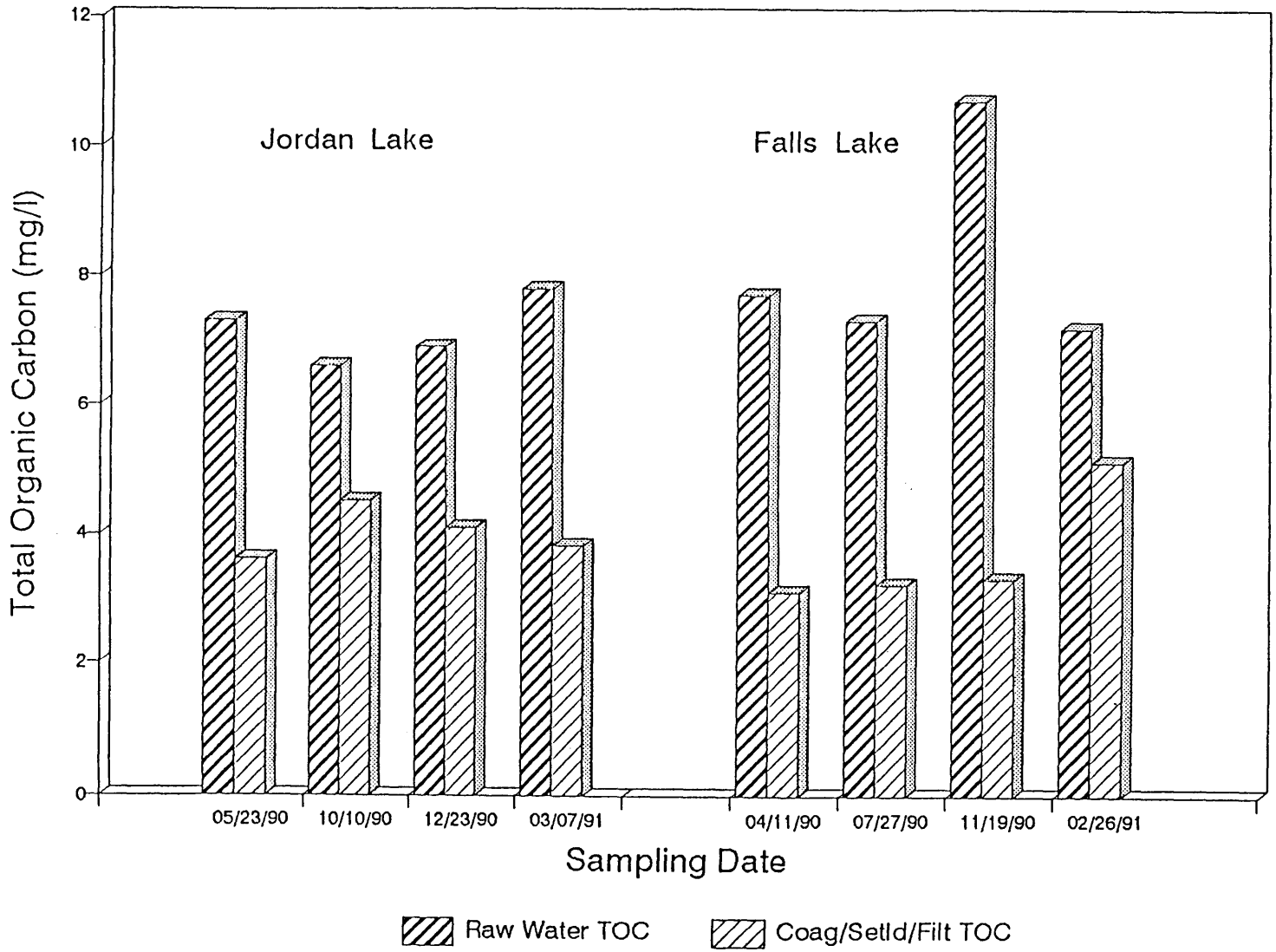
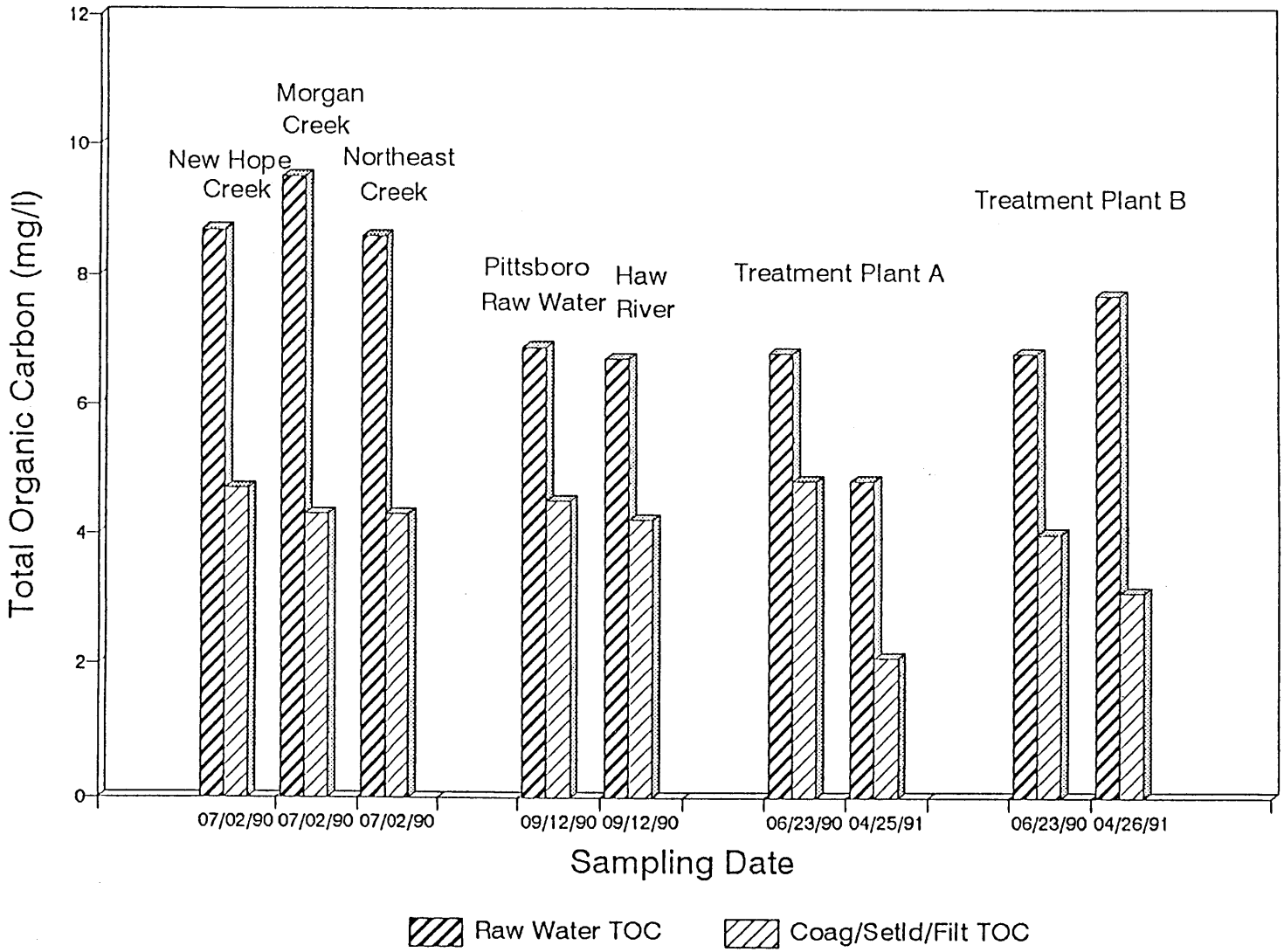


Figure 10. TOC Removal by Coagulation and Filtration: Tributary Samples and Treatment Plant Finished Water



settling and 47% following filtration. THMFP was reduced an average of 42% by coagulation and settling, and 54% after filtration. Because the source waters in this project were similar to those sampled by Singer et al. (1981), it was expected that a similar degree of removal of THM precursors and TOC would have been achieved by coagulation, sedimentation, and filtration in this study.

Removals of TOX from the raw waters varied (see Table 3); TOX was more effectively removed in the lake water samples than in the tributaries, which carried larger concentrations of TOX. Chlorinated organic substances that are macromolecular and hydrophobic in nature are readily coagulated by alum. In a study by Singer (1989), raw water TOX concentrations at a surface water treatment plant in South Carolina were found to range from 150 to 550 ug/l. Coagulation and settling removed an average of 62% of the raw water TOX at this plant.

CHLORINATION RESULTS

General Results - All Samples

Table 4 presents the chlorination results for each sample treated. The concentration of chlorine consumed by each sample after 48 hours correlates well with the TOC concentration of the sample prior to chlorine addition (see Figure 11). This correlation for all chlorinated samples, including treated tributaries and treatment plant finished waters, has a Pearson correlation coefficient of 0.85 (r^2=0.72).

The amounts of TOX and total THMs produced by chlorination are directly related to the organic content of the water and the chlorination conditions of dose, pH, temperature and contact time. Figures 12 and 13 illustrate the formation of TOX and THMs as a function of chlorine consumption. The correlation coefficient for both of these relationships is 0.58 (r^2=0.34), reflecting a significant correlation.

Figures 14 and 15 relate TOX and THM production in the chlorinated samples with the concentration of TOC in the filtered water prior to the addition of chlorine. These regressions have correlation coefficients of 0.62 and 0.59 (r^2=0.39 and 0.35, respectively), again demonstrating a significant correlation. The correlations suggest that about 79 ug TOX and 30 ug THMs are created per mg of TOC in the water.

Several researchers have quantified relationships among the TOC concentration, chlorine consumption, and TOX and THM production in order to better understand the byproduct formation potential of a chlorinated water. Table 5 displays the results from several studies comparing chlorine consumption and TOX and THMs produced to the initial TOC concentration of the water. The results of the present study are consistent with those found by previous researchers. It should be noted that the studies involving fulvic and humic acids generally consumed more chlorine and generated higher concentrations of TOX and THMs than the studies with natural waters. This leads to two important conclusions. First, some of the precursors of THM and TOX formation may have been preferentially removed by alum coagulation and filtration prior to chlorination. Second, the TOC of these natural waters is comprised of a variety of organic compounds, including but not limited to humic and fulvic acids, that may not be as susceptible

Table 4. Characteristics of Chlorinated Water

Location	Sampling Date	TOC (mg/l)	Chlorine Consumed (mg/l)	TOX (ug/l)	TTHM (ug/l)	THM as Chloride (ug Cl/l)	THM as Bromide (ug Br/l)	% TOX as THM (ug Cl)
Jordan Lake	05/23/90	3.6	4.2	374	122	98.1	11.3	27.6
Jordan Lake	10/10/90	4.3	4.5	332	115	84.8	18.8	28.1
Jordan Lake	12/23/90	4.1	4.2	242	103	74.6	18.3	34.2
Jordan Lake	03/07/91	3.8	3.0	278	68	53.1	7.9	20.4
Falls Lake	04/11/90	3.0	3.5	220	50	36.9	8.3	18.4
Falls Lake	07/27/90	3.1	3.6	256	70	55.9	6.9	23.0
Falls Lake	11/19/90	3.3	3.8	304	90	67.9	13.1	24.2
Falls Lake	02/26/91	5.1	4.8	499	179	154.6	5.2	31.4
Morgan Creek	07/02/90	4.7	4.7	342	150	85.8	43.4	24.3
New Hope Creek	07/02/90	4.3	4.6	246	72	47.9	17.3	15.7
Northeast Creek	07/02/90	4.3	4.3	239	93	57.2	27.3	17.6
Haw River	09/12/90	4.4	4.8	307	129	85.7	31.1	27.3
Pittsboro Water Plant Raw Water	09/12/90	4.2	4.7	512	167	99.5	52.5	21.1
Plant A Finished Water	06/23/90	4.8	5.1	294	57	47.1	4.2	16.6
Plant A Finished Water	04/25/91	2.1	2.7	139	48	38.4	4.2	29.0
Plant B Finished Water	06/23/90	4.0	4.5	362	86	71.5	5.6	20.4
Plant B Finished Water	04/26/91	3.1	4.1	200	99	79.9	8.3	41.8
Mean		3.9	4.2	303	100	72.9	16.7	24.8

Conditions for all samples except Water Plants A and B:

Chlorine dose = Chlorine consumed + 0.5 mg/l; pH = 8; 25 C; 48 hour holding time

Figure 11. Correlation Between TOC of Filtered Water and Chlorine Consumed

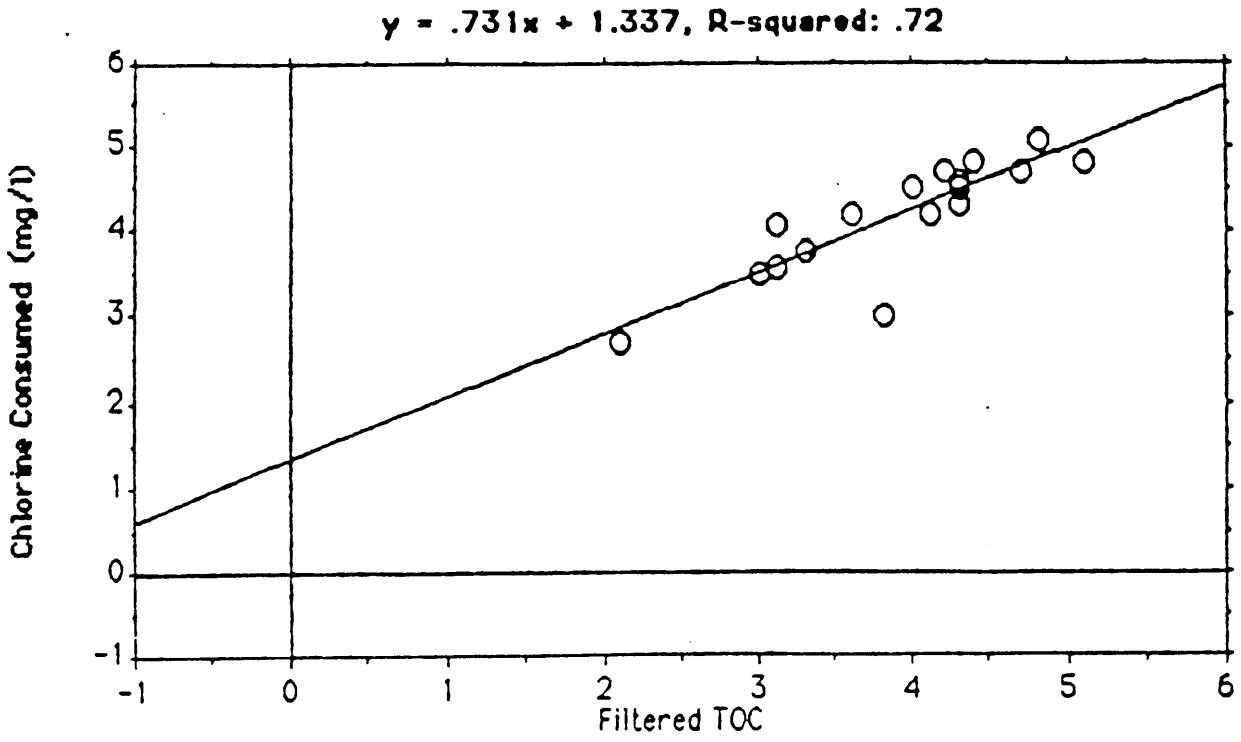


Figure 12. Correlation Between Chlorine Consumed and TOX Formation

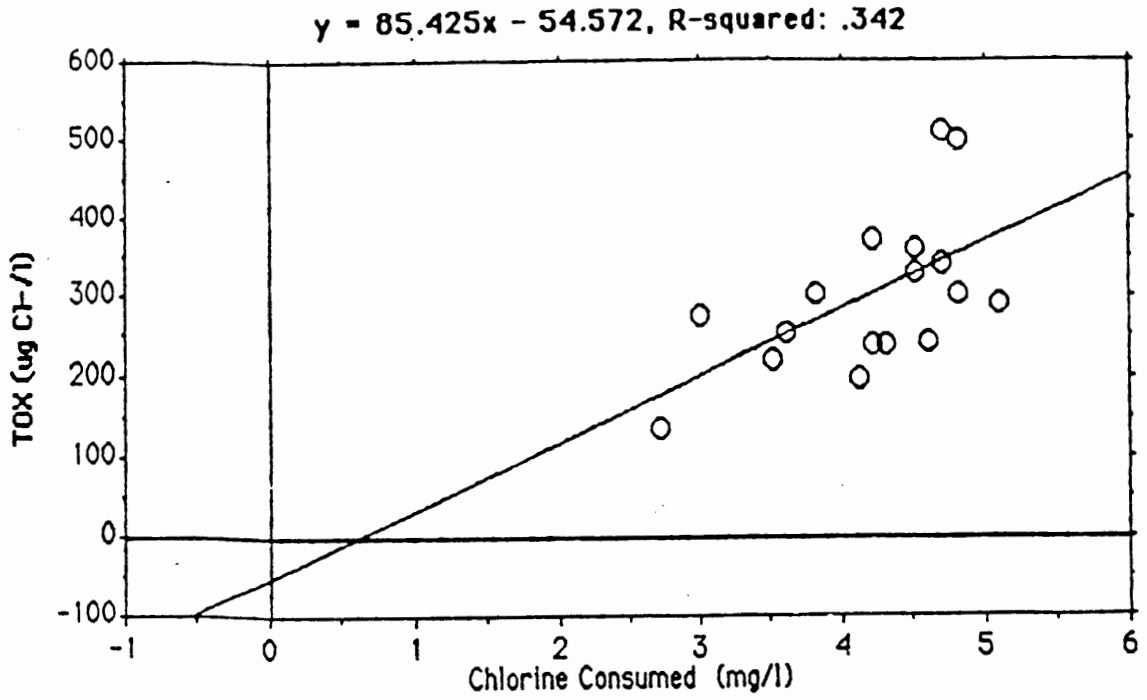


Figure 13. Correlation Between Chlorine Consumed and TTHM Formation

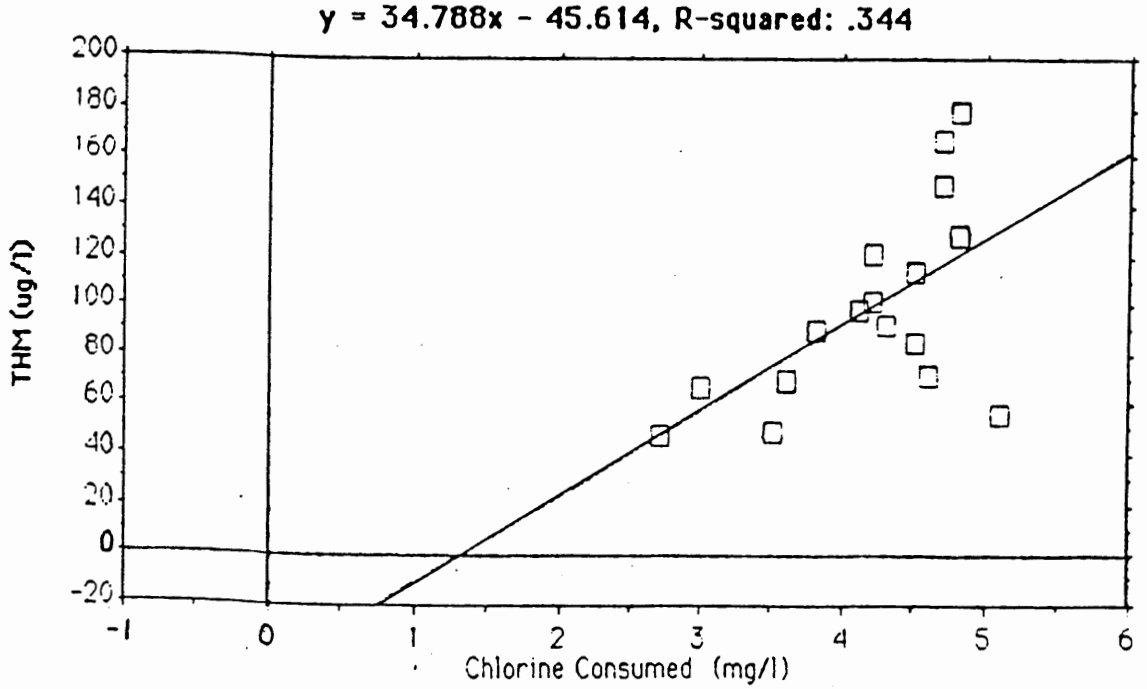


Figure 14. Correlation Between TOC of Filtered Water and TOX Formation

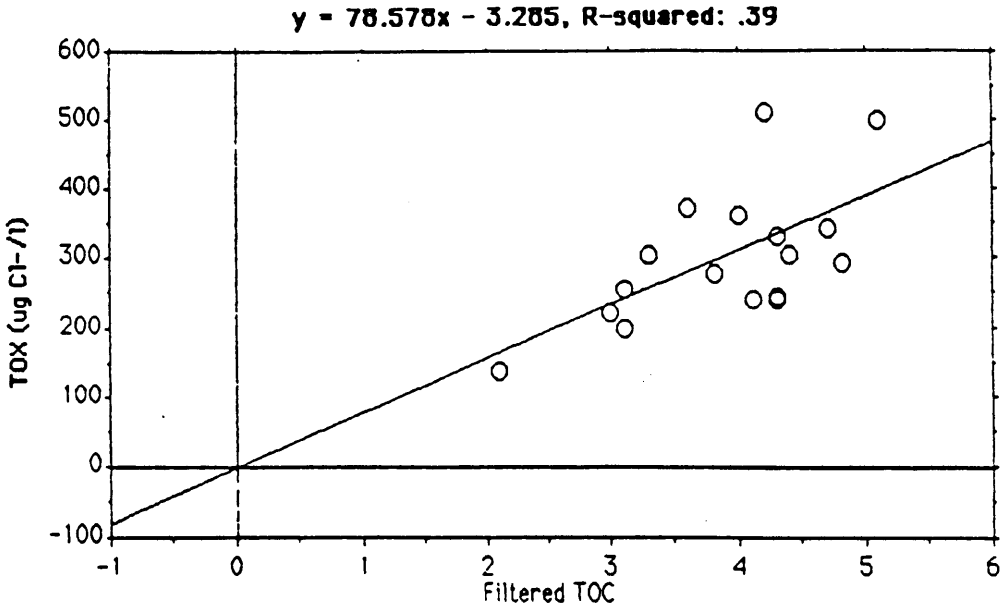


Figure 15. Correlation Between TOC of Filtered Water and TTHM Formation

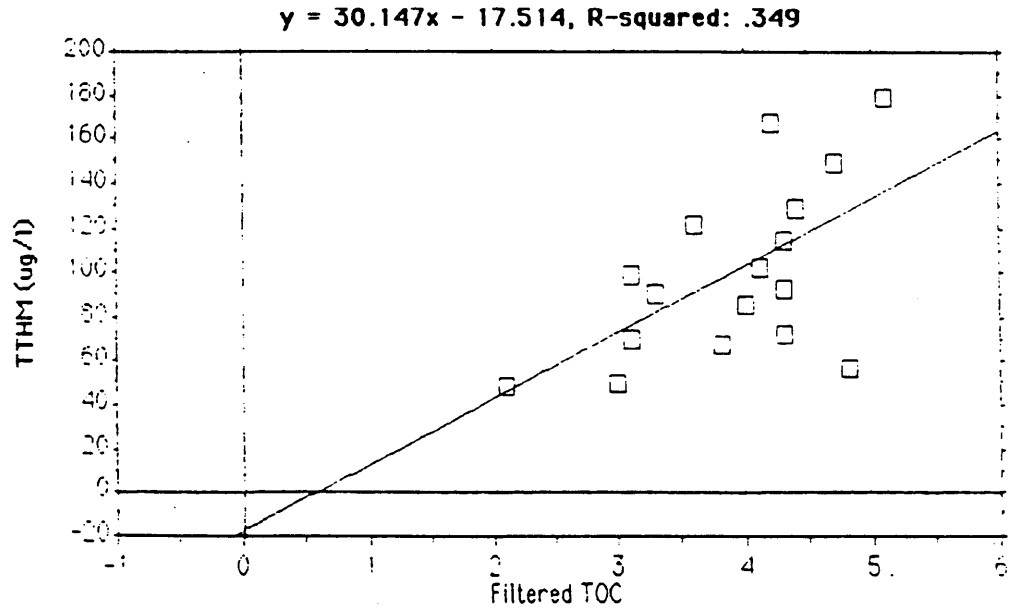


Table 5. Summary of Chlorine Consumption and Disinfection By-Product Relationships

Author and Water Source	pH	Contact Time (hours)	mg Cl_2 Consumed per mg TOC	ug TOX as Cl^- per mg TOC	ug TTHM per mg TOC	% TOX as THM (ug Cl^-)
Babcock & Singer, 1981						
Humic Acid	6.5	48	3.5	NA	120	NA
Fulvic Acid	6.5	48	NA	NA	50	NA
Fleischacker and Randtke, 1983						
Fulvic Acid	7.0	100	1.7	246	63	22.7
Reckhow & Singer, 1984						
Fulvic Acid	7.0	48	NA	195	49	20.0
Fulvic Acid	7.0	72	NA	138-210	29-50	18.7-23.4
Humic Acid	7.0	72	NA	246-333	61-77	18.5-21.2
Fulvic Acid	8.0	72	NA	195	75	31.0
Jensen et al., 1985A,B						
Fulvic Acid	8.9	24	1.6	NA	NA	NA
Reckhow, Singer and Malcolm, 1990						
Fulvic Acid	7.0	72	1.4	191	43	19.9
Humic Acid	7.0	72	2.1	256	57	19.9
Reckhow & Singer, 1990						
Raw Drinking Waters	7.0	72	1.9	215	52	21.5
Amy et al., 1990						
Conventional Pretreatment						
Groundwater	8.9-9.5	1	0.3-1.6	35-66	19-39	39-48
Groundwater	8.9-9.5	96	0.9-1.5	58-117	29-64	33-44
Singer & Chang, 1989 Treated Waters	Varies	Varies	NA	NA	NA	31.0
Present Study						
N.C. Treated Waters	8.0	48	1.1	81	30	26.4

All samples were chlorinated in excess of amount consumed

NA: Parameter not analyzed

to chlorinated byproduct formation as the humic and fulvic acids themselves.

Because THMs comprise most of the purgeable organic halide (POX) included as part of the TOX concentration, a correlation between THMs and TOX should exist for samples treated under the same conditions. Figure 16 displays this relationship; the correlation coefficient of 0.83 (r^2=0.69) indicates a strong correlation. The linear regression suggests that 26 ug of THMs as Cl- (33 ug/l of total THMs) are created per 100 ug TOX. This relationship is in good agreement with that found by Singer and Chang (1989) in 59 samples from 13 conventional water treatment plants, and with Reckhow and Singer's (1984) fulvic acid chlorination results at pH 8.0. Researchers have generally found that the ratio of THM-to-TOX under the same conditions of pH, temperature, chlorine-to-carbon ratio and holding time remains fairly constant regardless of the source of aquatic carbon.

Results by Source

Table 4 displays TOX and THM concentrations created by chlorination following conventional treatment in all of the samples examined. It should be noted that, in all cases, the values represent TOX and THM concentrations produced by chlorination and do not include pre-existing concentrations in the raw water. The average TOX and THM concentrations of the four Jordan Lake samples were 307 ug/l and 102 ug/l, respectively. The Falls Lake samples produced similar quantities of byproducts, averaging 320 ug/l for TOX and 97 ug/l for THMs. The five tributary samples collected in the summer months produced an average of 329 ug/l TOX and 122 ug/l THMs; these concentrations were similar to those exhibited by the chlorinated lake samples collected during the summer. Byproduct formation measured in the finished water samples from the local treatment plants was slightly lower, averaging 249 ug/l for TOX and 73 ug/l for THMs. It should be noted that the residence times for byproduct formation in the treatment plants was shorter (on the order of several hours) compared to the 2-day residence time of the laboratory-chlorinated lake water samples. Hence, it is not surprising that the samples from the treatment plants had slightly lower TOX and THM concentrations.

Figure 17 shows the TOX and THM concentrations produced in the Jordan and Falls Lake samples. From this figure, it can be seen that the current EPA regulatory limit of 100 ug/L for the total THM concentration was slightly exceeded in three out of four of the Jordan Lake samples. One Falls Lake sample exceeded the THM limit at 179 ug/l in February 1991. It is important in evaluating these results to compare the conditions of treatment employed in the laboratory with those that might be encountered under actual treatment conditions. First, all waters regardless of lake temperature were processed and held for 48 hours at 25°C. This temperature is higher than would be encountered by a treatment facility during the winter months; thus the production of THMs and TOX in these laboratory-treated samples may be somewhat elevated compared to the levels produced at an on-site treatment plant. Second, a holding time of 48 hours was chosen to represent a typical residence time for a distribution system. An actual distribution network may have shorter or longer residence times depending on the distances between the plant and consumer taps, system demands, and storage time within the distribution system. Because chlorinated organic compounds continue to form in the presence of

Figure 16. Correlation Between TOX and TTHM Formation

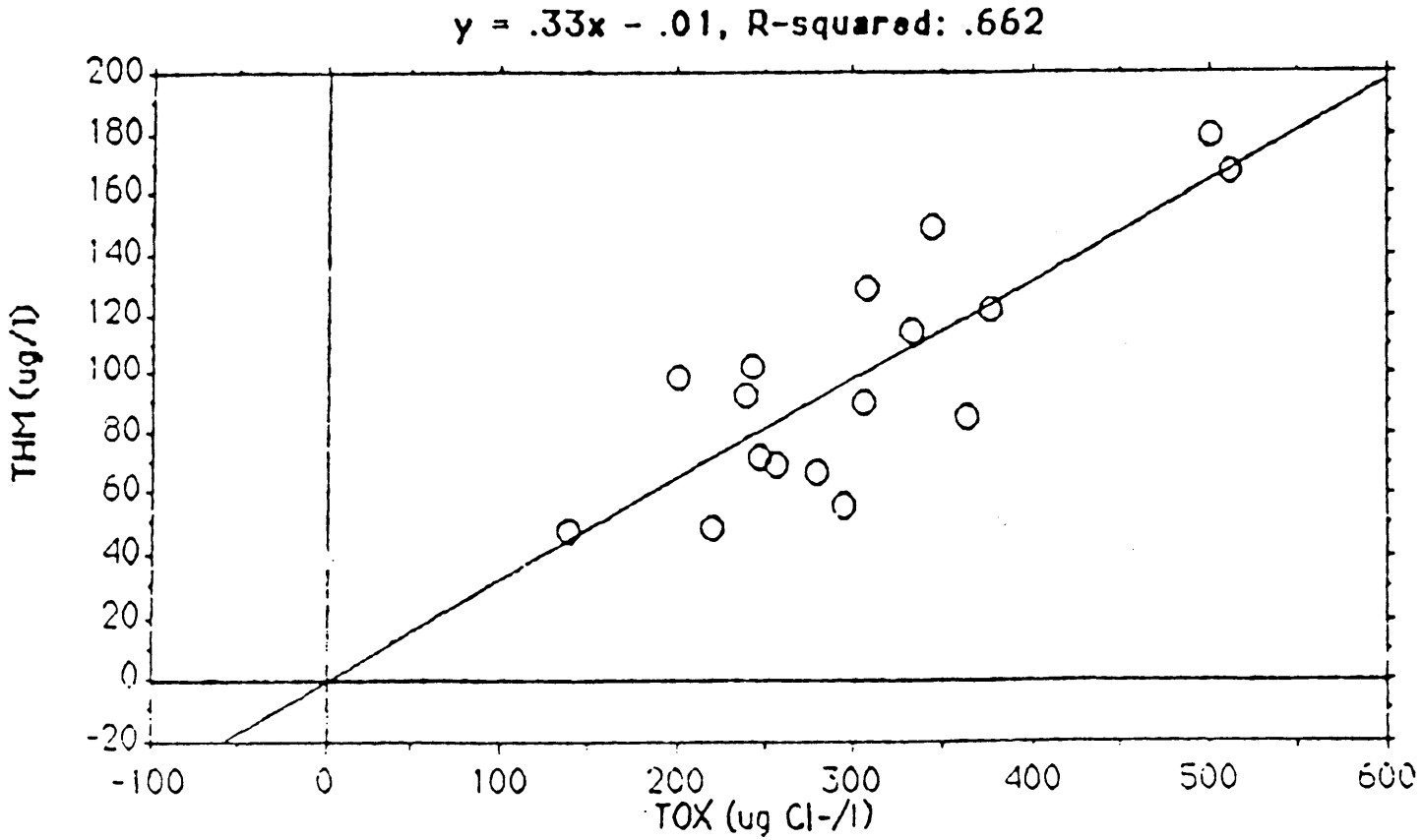
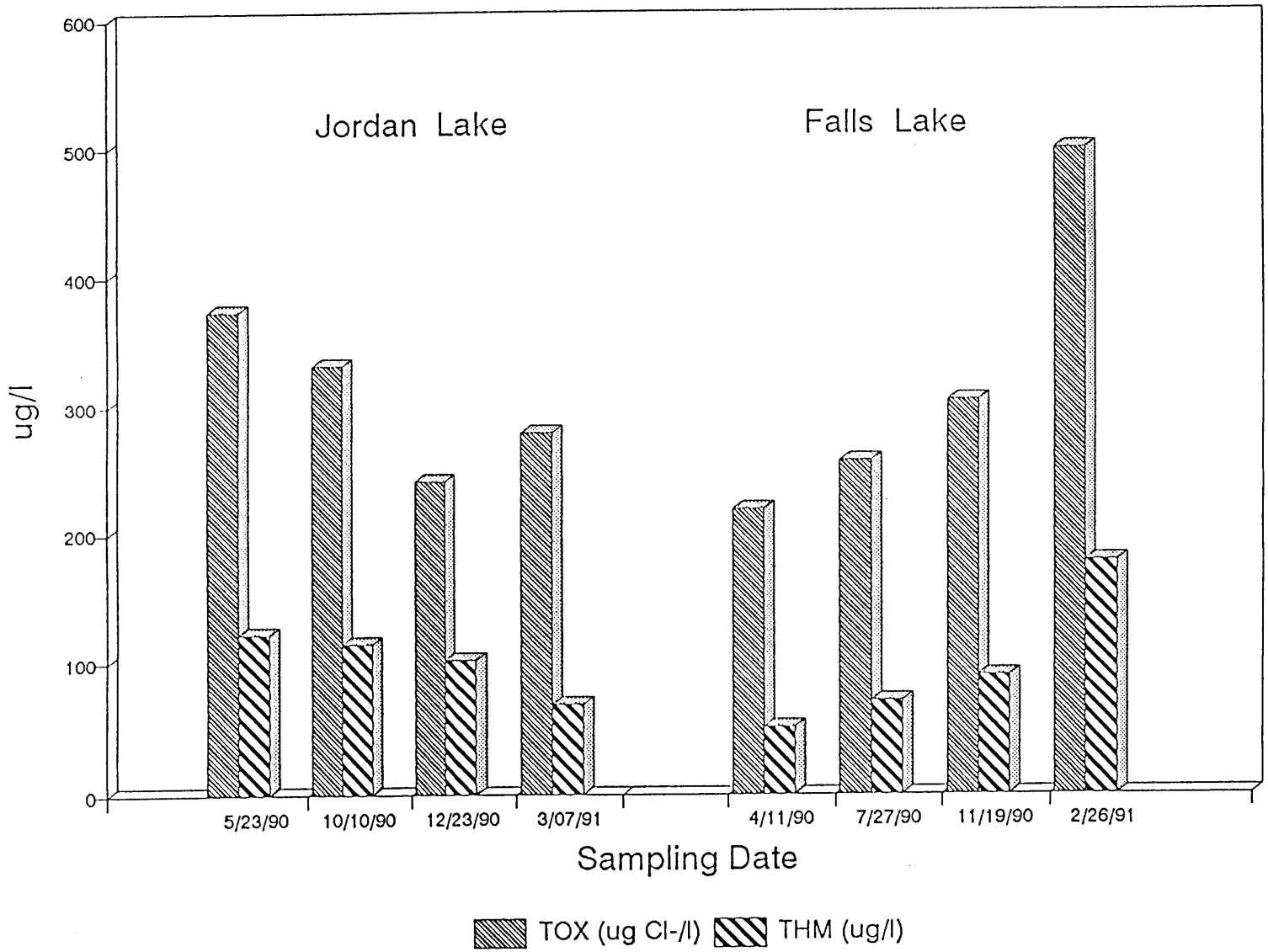


Figure 17. Jordan and Falls Lake Samples: TOX and TTHM Formation



chlorine, even in the distribution system, longer residence times will result in higher concentrations of TOX and THMs.

The five tributary samples collected in the summer of 1990 all contained appreciable quantities of TOX; after coagulation, settling, filtration and post-chlorination, these samples exhibited an increase in TOX concentrations along with the appearance of THMs. Figure 18 shows the TOX and THM concentrations created by chlorination. Although low levels of brominated THMs were measured in the chlorinated lake samples, the tributaries displayed higher concentrations of the brominated THM species. The concentration of bromide incorporated into the THM species by each sample is reported in Table 4. While the lake samples incorporated between 5 and 19 ug/l bromide into THM species, the THMs produced by the tributaries contained from 17 to 53 ug/l bromide.

When waters containing bromide are chlorinated, the bromide is oxidized to hypobromous acid which competes with hypochlorous acid in organic substitution reactions. The distribution of THMs shifts to include brominated species; the extent of speciation, while known to be dependent on the concentration of bromide, the dose of chlorine, and the amount of precursors, has not been quantified (Rook, 1974; Cooper et al., 1985). Figure 19 demonstrates the speciation of THMs in the tributaries, indicating that approximately 40% of the total THMs consist of bromide-containing species. It should be noted that chloroform constitutes only about 60% of the total THMs in these tributary samples, compared to the lake samples in which 90% of the total THM concentration consists of chloroform.

The source of bromide in these streams is probably anthropogenic; concentrations are likely elevated by the low flow conditions encountered during summer and early fall when treatment plant effluents are a large percentage of the stream flow. It may be important to consider the impact of bromide and brominated compounds in the tributary streams on bromide concentrations in the lake, especially during drought conditions. Bromide is not easily removed by conventional water treatment processes and, due to its high reactivity when oxidized by chlorine, low concentrations may significantly increase THM production (Symons et al., 1987; Cooper et al., 1985). The health effects of brominated byproducts are not clearly understood at this time, but future regulations may limit individual THM species, in which case potential bromide interactions should be considered in the design and operation of water treatment facilities.

Figure 20 displays the TOX and THM concentrations in the finished waters from Treatment Plants A and B. THM levels reported in Table 4 are lower than the regulatory mandate of 100 ug/l. However, Plant B produced concentrations that were near this limit, and most likely exceeded the MCL in the distribution system. Chloroform was the principal THM species in these waters, accounting for over 90% of the total THM concentration by mass.

RESULTS OF GAC ADSORPTION AND CHLORINATION

Part of the coagulated, settled and filtered lake samples was passed through granular activated carbon (GAC) with an empty bed contact time (EBCT) of ten minutes. The TOC concentration in the effluent was monitored to detect breakthrough. Breakthrough did not occur during any of the experiments. It should be noted, however, that

Figure 18. Jordan Lake Tributaries: TOX and TTHM Formation

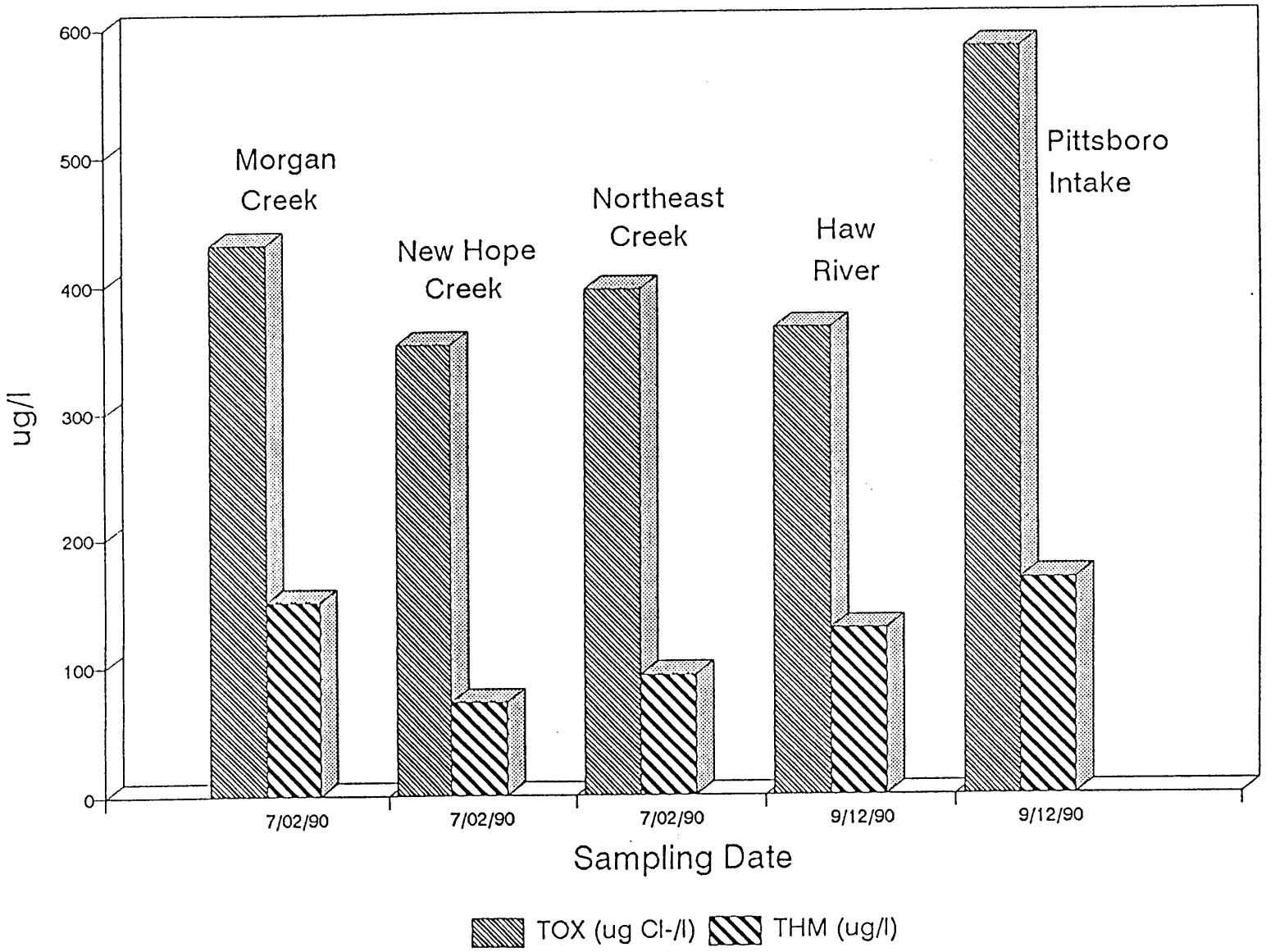


Figure 19. Jordan Lake Tributaries: Distribution of THM Species

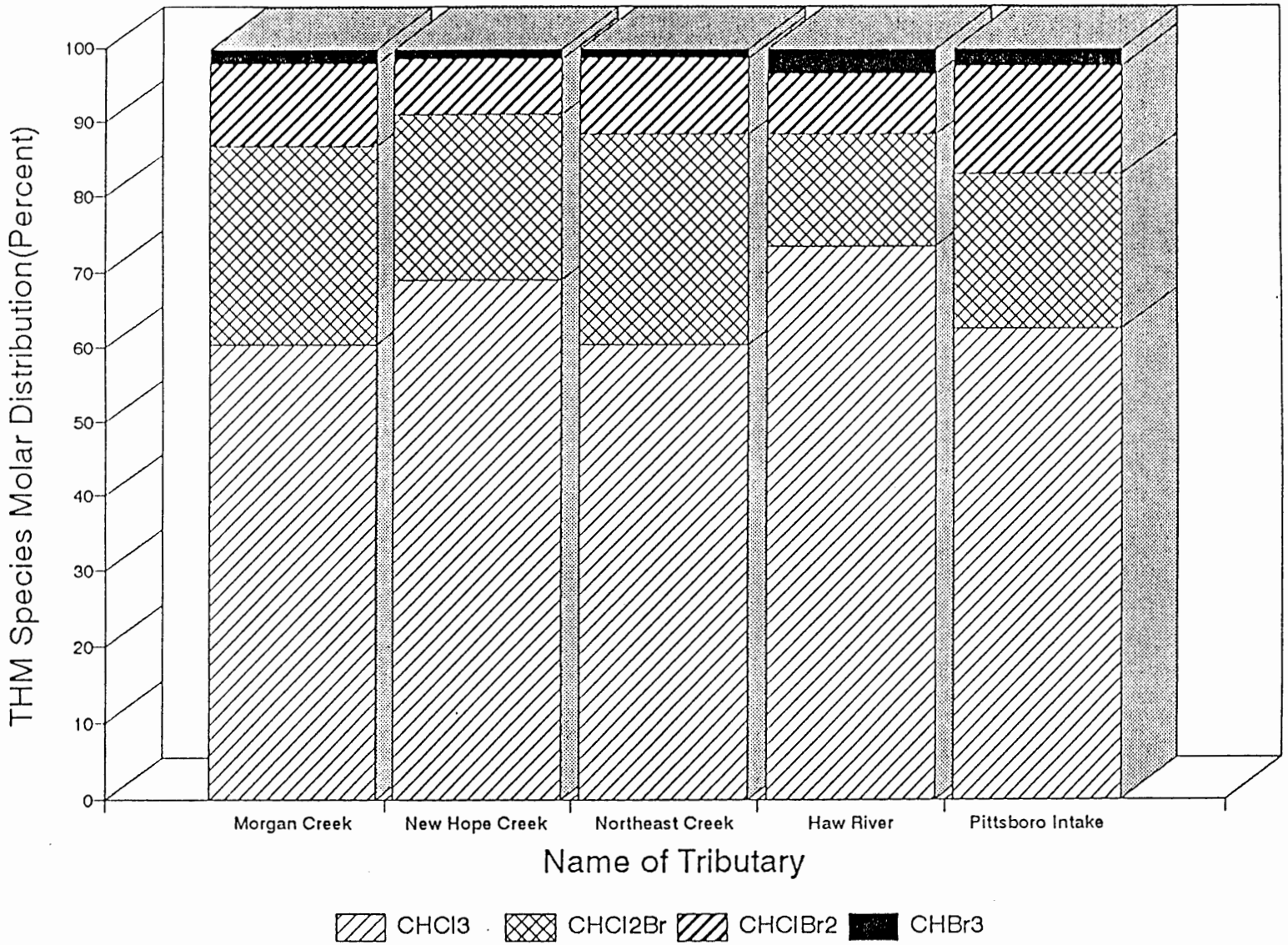
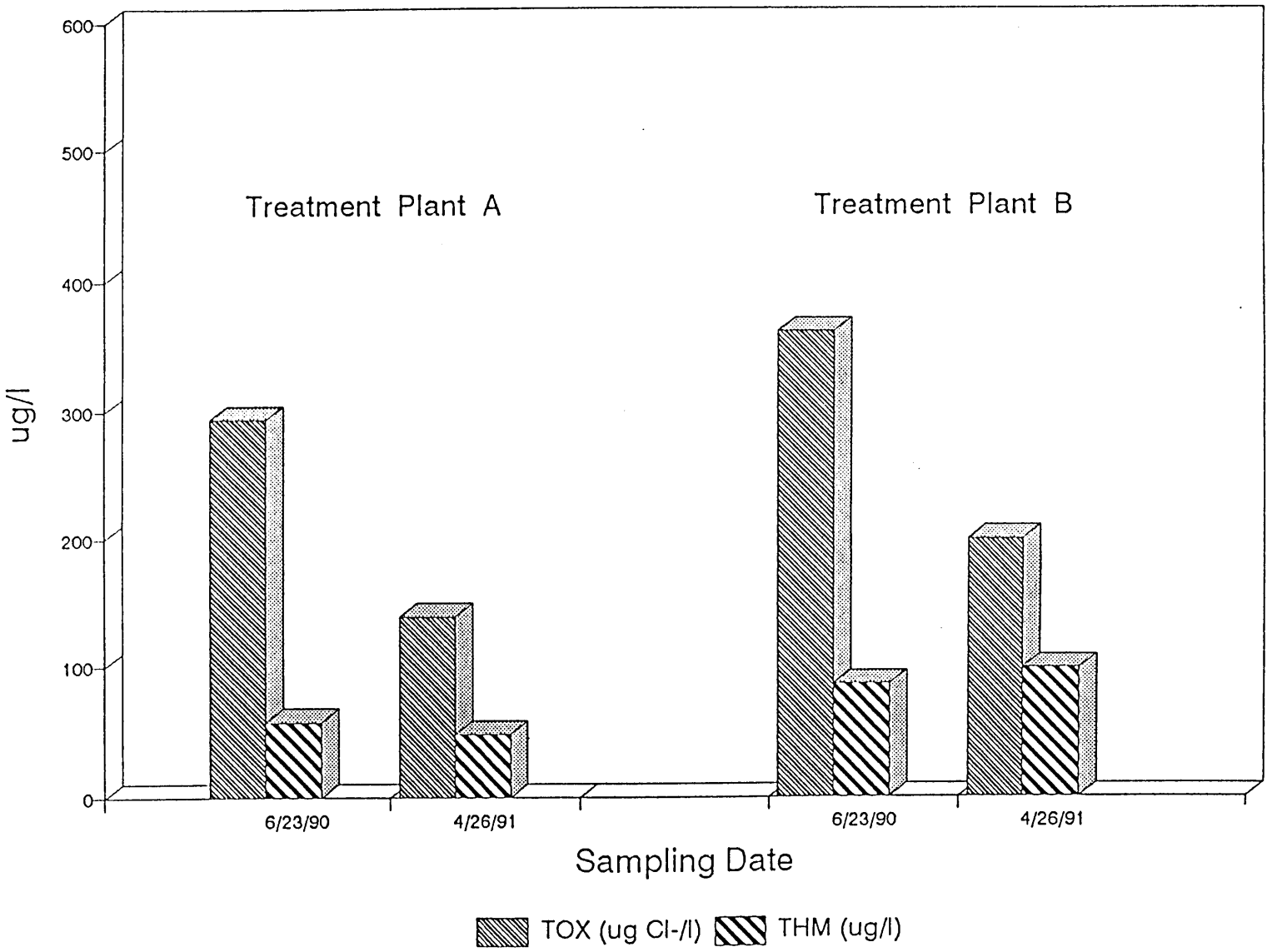


Figure 20. Treatment Plants A and B: TOX and TTHM Formation



measurements were made only during the first 24 hours of service time. Thus, the results show that the EBCT was long enough to contain the mass transfer zone for TOC adsorption, but projection of service time before significant breakthrough is not possible.

Table 6 displays the TOC removals achieved by the GAC. GAC adsorption removed 92% of the residual TOC in the coagulated and filtered samples; the non-adsorbable TOC concentration was 0.2-0.4 mg/L. It should be pointed out that removals of natural organic material and THM precursors are highest when GAC is freshly activated. A decline in the extent of TOC removal occurs as the adsorptive capacity of the GAC is depleted. GAC treatment in these experiments was conducted over a relatively short period of time so that maximum removals of TOC were achieved. Such removals may not accurately reflect the long-term TOC performance of GAC in a practical water treatment situation.

The chlorine demand of the GAC-treated water ranged from 0.3 to 1.1 mg/l for the eight lake samples, averaging 2.3 mg chlorine consumed per mg residual TOC. Because of the excellent removal of organic material by the GAC, the source of this residual chlorine demand is not clear. It is likely that part of the chlorine may have been consumed by ammonia or by reduced nitrogenous compounds remaining in the filtered water. Concentrations of TOX following chlorination ranged from 10 to 39 ug/l, which is approaching the analytical limits of detection for TOX of 10 ug/l. Minimal concentrations of THMs (less than 5 ug/l) were measured in most samples.

Because of the high removals of TOC and THM precursors achieved by GAC-adsorption, a low dose of free chlorine can be applied for final disinfection, resulting in low production of DBPs while assuring adequate disinfection. The TOC removals achieved in these experiments may be higher than the steady-state reductions expected by a water treatment plant operating over longer periods of time. Reactivation of the carbon may be required at a frequency of once every 3-6 months; such reactivation is not inexpensive. Nevertheless, the GAC/chlorine disinfection strategy most effectively and consistently reduced the TOC concentration to very low levels, and the production of TOX and THMs was minimal in these water samples.

RESULTS OF OZONE AND CHLORAMINE TREATMENT

Table 7 displays the results of the ozone and mono-chloramine treatment. Ozonation lowered the TOC concentration of the filtered samples by an additional 15%. Chloramine consumption was low, ranging from 0.4 to 1.8 mg as Cl_2/l. The concentration of TOX produced by chloramination varied between 69 and 202 ug/l, but the THM concentration ranged from only 2 to 13 ug/l and comprised an average of only 5 percent of the TOX concentration.

The low chloramine consumption and the reduced formation of TOX and THMs compared to the formation of DBPs produced by chlorination alone demonstrates the effectiveness of the ozone/chloramine treatment. There are several considerations associated with this strategy. First, ozonation oxidizes TOX and THM precursors to some degree. Other researchers have found that oxidation of THM precursors by ozone resulted in a 10 to 15% destruction in the concentration of THM precursors (Werdehoff and Singer, 1987; Singer and Chang, 1989). Second, ozone is a strong disinfectant, reducing the amount of chloramine needed for final disinfection and, correspondingly,

Table 6. Characteristics of GAC-Treated/Chlorinated Water

Location	Sampling Date	Filtered TOC (mg/l)	Post-GAC TOC (mg/l)	TOC Reduction %	Chlorine Consumed (mg/l)	mg Cl_2 Consumed per mg TOC	TOX [a] (ug Cl/l)	TTHM (ug/l)
Jordan Lake	05/23/90	3.6	0.2	94	0.7	3.5	39	3
Jordan Lake	10/10/90	4.5	0.4	91	1.0	2.5	34	<2
Jordan Lake	12/23/90	4.1	0.2	95	0.3	1.5	13	<2
Jordan Lake	03/07/91	3.8	0.4	89	0.3	0.7	10	4
Falls Lake	04/11/90	3.1	0.5	84	1.1	2.2	21	10
Falls Lake	07/27/90	3.2	0.2	94	0.7	3.5	14	5
Falls Lake	11/19/90	3.3	0.2	94	0.9	4.5	13	3
Falls Lake	02/26/91	5.1	0.4	92	0.5	1.3	10	5
Mean		3.8	0.3	92	0.7	2.5	19	4

Samples were alum coagulated, settled, filtered, GAC filtered and chlorinated at pH 8.0 at a dose resulting in 0.5 mg/l free chlorine residual after 48 hours.

(a): TOX Detection Limit = 10 ug/l

Table 7. Characteristics of Ozonated/Chloraminated Water

Location	Sampling Date	Filtered TOC (mg/l)	Post-Ozone TOC (mg/l)	TOC Reduction %	Ozone Consumed (mg/l)	Chloramine Consumed (mg/l)	mg NH_2Cl Consumed per mg TOC	TOX (ug Cl/l)	TTHM (ug/l)
Jordan Lake	05/23/90	3.6	3.1	14	4.1	0.6	0.19	128	4
Jordan Lake	10/10/90	4.5	3.1	31	3.5	1.0	0.32	NA	NA
Jordan Lake	12/23/90	4.1	3.4	17	3.5	1.8	0.53	116	3
Jordan Lake	03/07/91	3.8	3.7	3	3.6	0.7	0.19	142	10
Falls Lake	04/11/90	3.1	2.7	13	2.5	0.4	0.15	69	6
Falls Lake	07/27/90	3.2	2.7	16	3.4	1.8	0.67	77	3
Falls Lake	11/19/90	3.3	2.5	24	4.3	1.0	0.40	87	2
Falls Lake	02/26/91	5.1	4.8	6	4.8	0.9	0.19	202	13
Mean		3.8	3.3	15	3.7	1.0	0.33	103	6

Samples were alum coagulated, settled, filtered, ozonated and chloraminated at pH 8.0 and a mass dose ratio of 0.25 parts ammonia as N to 1 part chlorine, resulting in a total chlorine residual of 1.0 mg/l after 48 hours.

lowering the subsequent production of TOX. Third, the oxidant demand per unit of TOC is significantly less for monochloramine than for other oxidants (Jensen et al., 1985A,B). Fourth, monochloramine is a weak oxidant that creates some substitution byproducts but produces less overall TOX and THMs than free chlorine (Johnson and Jensen, 1986). Last, monochloramine reacts slowly; thus, when preceded by a strong oxidant and primary disinfectant such as ozone, monochloramine can provide long-term residual disinfection in a distribution system.

Table 8 compares observations by other researchers with those of the present study concerning the relationships between chloramine consumption, TOX and THM production, and the organic carbon content of the source water. Jordan and Falls Lake samples consumed an average of 0.33 mg monochloramine as $Cl_2$2/mg TOC. Chloramine consumption was less than 1 mg as Cl_2/mg TOC in each of the other studies, except for the study by Fleischacker and Randtke (1983) which involved a very high chloramine dose and long contact times. Average TOX production by Jordan and Falls Lake ozone and chloramine-treated samples was 27 ug TOX/mg TOC. Other researchers found that TOX production ranged from 11 to 36 ug/mg TOC. Chloramination of the Jordan and Falls Lake samples generated 1.3 ug THMs/mg TOC, while other studies reported THM formation from 0.6 to 13 ug/mg TOC. The THM content of the TOX averaged only 4.2% in this study; other waters produced from 3 to 30% of TOX as THMs. Overall, Jordan and Falls Lake samples consumed less monochloramine and produced less TOX and THMs than observed for chloramination of other treated waters. This may be attributed to the following considerations. First, the content of monochloramine byproduct precursors of the lake waters is probably not as high as that of model humic and fulvic acid solutions. Second, precursors may have been substantially removed by pretreatment through coagulation and filtration. Third, ozonation may have oxidized byproduct precursors to some degree; except for the study by Jacangelo et al. (1989), the reported results are for chloramination alone, not chloramination preceded by ozonation.

Because this study did not compare the formation of byproducts from monochloramine alone to ozone and monochloramine, the study by Jacangelo et al. (1989) using similar source waters and treatment processes may provide insight to the beneficial effects of preozonation. Table 8 shows some of the results of their research. Jacangelo and colleagues found reductions of 60% for TOX and 85% for THMs in one utility by switching from chloramination only to pre-ozonation and chloramination, but reductions of only 10% for TOX and 20% for THMs were found for the same process change at another treatment facility. In the same two utilities, THM formation was reduced from 29% to 11% and from 7% to 3% of the TOX by the application of ozone before chloramine addition. Haloacetic acid and haloacetonitrile concentrations were also decreased with the addition of ozone in both plants, but small increases in chloropicrin, haloketones, aldehydes and cyanogen chloride were observed. These increases might be due to ozonation byproducts such as nitrophenols that become targets for chloramine substitution reactions, producing organic halides such as chloropicrin (Duguet et al., 1985). The ozonated and chloraminated Jordan and Falls Lake samples resulted in similar TOX and THM productions as found by Jacangelo et al.; thus it is possible that the Jordan and Falls Lake samples may also have contained these other compounds in the finished waters.

Table 8. Summary of Chloramine Consumption and Disinfection By-Product Relationships

Author and Water Source	pH	Contact Time (hours)	mg Cl_2 Consumed per mg TOC	ug TOX as Cl^- per mg TOC	ug TTHM per mg TOC	% TOX as THM (ug Cl^-)
Fleischacker & Randtke, 1983						
Groundwater Fulvic Acid	7.0	100	2.8	34	2.0	5.0
Peat Fulvic Acid	7.0	100	1.5	27-31	NA	NA
Jensen et al., 1985						
Fulvic Acid	9.0	24	0.36	NA	NA	NA
Amy et al., 1990						
Conventional Pretreatment						
Groundwater 1	10.0	1	0.30	32	11	31
Groundwater 1	10.0	96	NA	36	13	32
Groundwater 2	9.6	1	0.18	15	3.5	21
Groundwater 2	9.6	96	NA	29	3.8	12
Groundwater 3	9.5	1	0.47	23	5.4	21
Groundwater 3	9.5	96	NA	36	5.8	14
Jacangelo et al., 1989						
Conventional Pretreatment						
Lake 1 - O_3, NH_2Cl	8.2	0	0.08	15	1.3	7.9
Lake 2 - NH_2Cl	9.0	20	0.94	31	10	29
Lake 2 - O_3, NH_2Cl	9.3	20	0.71	11	1.5	11
Lake 3 - NH_2Cl	7.6	24	0.29	20	1.4	6.6
Lake 3 - O_3, NH_2Cl	7.8	24	0.33	18	0.6	2.8
Present Study						
Conventional Pretreatment						
N.C. Lakes - O_3, NH_2Cl	8.0	48	0.33	27	1.3	4.2

All samples were chloraminated in excess of amount consumed

NA: Parameter not analyzed

Chloramines have been shown to produce 20 to 49 percent of the TOX produced by equimolar doses of chlorine in humic acid solutions and humic waters (Fleischacker and Randtke, 1983; Chow and Roberts, 1981). Comparing the ratios of chlorine or chloramine consumption and TOX and THM formation in Tables 5 and 8 indicates that, in the Jordan and Falls Lake samples, 0.8 mg less Cl_2 per mg TOC was required for disinfection with chloramines compared to free chlorine, a 70% reduction. An average of 81 ug TOX/mg TOC was created by chlorine compared to 27 ug TOX/mg TOC by chloramine, resulting in a 67% reduction in TOX by the ozone/chloramine strategy compared to free chlorine. Chlorination produced 30 ug THMs/mg TOC while chloramination formed only 1.3; this corresponds to a 96% reduction in THMs produced by the combination of ozone/chloramine than by chlorine alone.

Hence, it is clear that ozone and chloramine disinfection effectively reduced the formation of THMs and TOX in Jordan Lake waters compared to that of chlorine alone. While ozone is capable of effectively oxidizing many harmful SOCs and chlorinated byproduct precursors and minimizing the quantity of residual disinfectant required, the health effects and concentrations of ozonation byproducts are not well understood. Further assessment of the formation of other byproducts associated with ozone and monochloramine should be carefully addressed. The relative cost of ozone treatment should also be considered, taking into account the other attributes of ozone, as well as the possible savings that might be achieved by a lowered final disinfectant requirement.

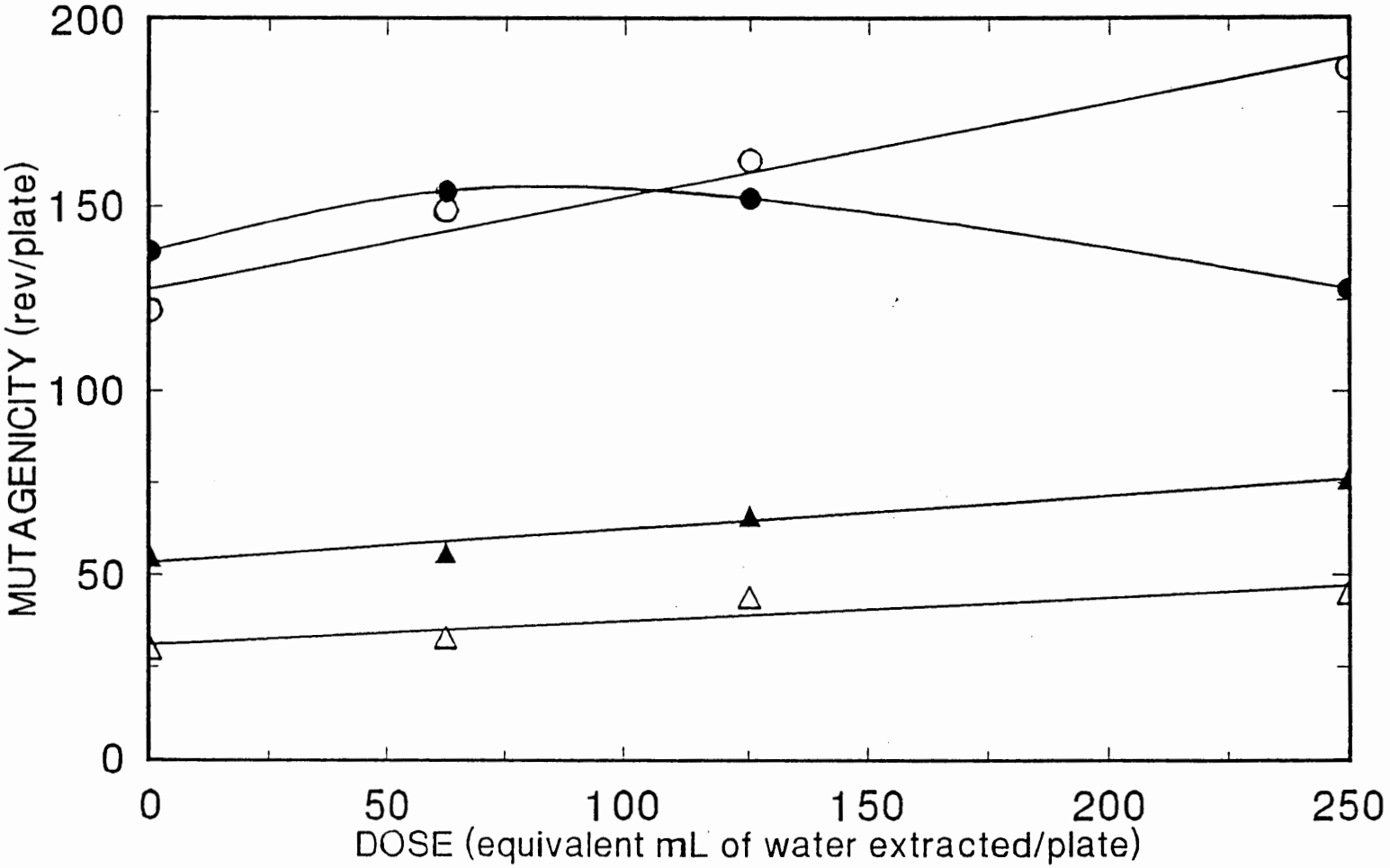
AMES BIOASSAY RESULTS

Figure 21 illustrates typical results obtained in the Ames assays, for a sample which was judged to be positive on TA98 and TA100 without S9, but inactive in both strains in the presence of S9.

Treated Jordan Lake water samples (Table 9) produced genotoxicity, as detected in the Ames assay, on isolated occasions only. In only one conventionally chlorinated sample (collected 5/23/90) was any mutagenic activity found, at the very low end of what has been found for local drinking waters (Kronberg et al., 1991), and of marginal statistical significance ($p=0.11$). Ozonation of the 10/10/90 sample resulted in a substantial activity in TA100, both with and without S9 activation. This suggests the creation of oxidized species during ozonation that are directly mutagenic and are not deactivated by further oxidation by S9. The reason for the lower TA100 activity upon chloramination of this sample is not clear, but may be due to further oxidation and detoxication of a reactive species. Late fall and winter samples, when raw water quality is generally best, did not yield any mutagenic activity with any of the treatment trains, other than with chlorinated raw water itself.

In contrast (Table 10), Falls Lake spring (4/11/90) and summer (7/27/90) raw water samples showed low but significant activity in TA98 without S9, typical of anthropogenic sources. The very high mutagenicity seen in the chlorinated 4/11/90 sample is not consistent with the pattern usually found for chlorinated waters, i.e., lowest activity in TA100 without S9. That the highest activity is seen in TA98 without S9 is strongly suggestive of an anthropogenic source. Since this is the only sample which exhibited such high activity, the possibility of laboratory contamination cannot be excluded, nor could it be conclusively demonstrated. Ozonation of this sample resulted in

Figure 21. Mutagenicity of A Representative Water Sample



Circles, assay performed with Salmonella typhimurium strain TA 100, triangles, assay performed with Salmonella typhimurium strain TA 98; open symbols, without S9; closed symbols, with S9.

Table 9. Mutagenicity of Jordan Lake Water in the Ames Plate Incorporation Assay

Jordan Lake Water Sampling Date	Treatment Train	Rev/L in TA98 Without S9	Rev/L in TA98 With S9	Rev/L in TA100 Without S9	Rev/L in TA100 With S9
05/23/90	Raw	0	0	0	0
	Coag, setl, filt, Cl_2	0	0	200	0
	Coag, setl, filt, GAC, Cl_2	0	0	0	0
	Coag, setl, filt, ozone	0	0	0	0
	Coag, setl, filt, ozone, chlorine/NH_3	0	0	0	0
10/10/90	Raw	0	0	0	0
	Raw, chlorinated	0	0	248	0
	Coag, setl, filt, Cl_2	0	0	0	0
	Coag, setl, filt, GAC, chlorine	0	0	0	0
	Coag, setl, filt, ozone	0	137	1,189	1,085
	Coag, setl, filt, ozone, chlorine/NH_3	0	128	252	304
12/23/90	Raw	0	0	0	0
	Coag, setl, filt, Cl_2	0	0	0	0
	Coag, setl, filt, GAC, chlorine	0	0	0	0
	Coag, setl, filt, ozone	0	0	0	0
	Coag, setl, filt, ozone, chlorine/NH_3	0	0	0	0
03/07/91	Raw	0	0	0	0
	Coag, setl, filt, Cl_2	0	0	0	0
	Coag, setl, filt, GAC, chlorine	0	0	0	0
	Coag, setl, filt, ozone	0	0	0	0
	Coag, setl, filt, ozone, chlorine/NH_3	0	0	0	0

Table 10. Mutagenicity of Falls Lake Water in the Ames Plate Incorporation Assay

Falls Lake Water Sampling Date	Treatment Train	Rev/L in TA98 Without S9	Rev/L in TA98 With S9	Rev/L in TA100 Without S9	Rev/L in TA100 With S9
04/11/90	Raw	223	0	0	0
	Coag, setl, filt, Cl_2	9,416	3,319	3,370	4,724
	Coag, setl, filt, GAC, Cl_2	0	0	0	0
	Coag, setl, filt, ozone	107	87	154	0
	Coag, setl, filt, ozone, chlorine/NH_3	0	0	0	0
07/27/90	Raw	80	0	0	0
	Coag, setl, filt, Cl_2	0	0	0	0
	Coag, setl, filt, GAC, chlorine	0	0	0	0
	Coag, setl, filt, ozone	184	0	0	0
	Coag, setl, filt, ozone, chlorine/NH_3	0	0	0	0
11/19/90	Raw	0	0	0	0
	Coag, setl, filt, Cl_2	0	0	0	0
	Coag, setl, filt, GAC, chlorine	0	0	0	0
	Coag, setl, filt, ozone	0	0	0	0
	Coag, setl, filt, ozone, chlorine/NH_3	0	0	0	0
02/26/91	Raw	0	0	0	0
	Coag, setl, filt, Cl_2	0	0	0	0
	Coag, setl, filt, GAC, chlorine	0	0	0	0
	Coag, setl, filt, ozone	0	0	0	0
	Coag, setl, filt, ozone, chlorine/NH_3	0	0	0	0
04/26/91	Raw, chlorinated	99	0	510	0

low but significant activity in both TA98 and TA100, without S9. No mutagenicity was observed in any of the samples from either lake which had undergone GAC filtration prior to chlorination.

All tributaries of Jordan Lake (Table 11) tested manifested some raw water mutagenicity in TA98 without S9, except for Northeast Creek (the extract of which was toxic to the bacteria in the concentrations used) and the Haw River sample taken from just above Jordan Lake. When all tributary samples were conventionally treated with chlorination, the extracts were mutagenic in TA100 without S9, ranging to above 600 revertants per liter in laboratory-treated Pittsboro water.

Table 11. Mutagenicity of the Major Tributaries of Jordan Lake in the Ames Plate Incorporation Assay

Jordan Tributaries		Rev/L in TA98		Rev/L in TA100	
Sampling Date	Treatment Train	Without S9	With S9	Without S9	With S9
7/02/90 Northeast Creek	Raw	-133 (tox)	0	0	0
	Coag, setl, filt, Cl_2	54	0	101	0
07/02/90 New Hope Creek	Raw	83	0	90	0
	Coag, setl, filt, Cl_2	0	0	0	228
07/02/90 Morgan Creek	Raw	39	0	0	0
	Coag, setl, filt, Cl_2	0	0	247	0
09/12/90 Haw River	Raw	0	0	0	0
	Coag, setl, filt, Cl_2	59	0	347	0
09/12/90 Pittsboro	Raw	131	143	143	234
	Coag, setl, filt, Cl_2	53	0	639	190

It is notable that the mutagenic waters sampled from tributaries all exhibited high pre-treatment TOX values and produced much higher levels of brominated THMs after chlorination, compared to other waters seen in this study (see Table 11).

Finished waters from the two local water treatment plants (Table 12) produced from 0 to 357 revertants per liter in TA100 without S9.

Table 12. Mutagenicity of Some Local Finished Drinking Waters in the Ames Plate Incorporation Assay

Finished Water		Rev/L in TA98		Rev/L in TA100	
Sampling Date	Treatment Train	Without S9	With S9	Without S9	With S9
07/02/90	Coag, setl, filt, Cl_2	27	104	0	0
09/20/90	Coag, setl, filt, Cl_2	0	0	357	0
04/26/91	Coag, setl, filt, Cl_2	99	0	239	0
06/23/90	Coag, setl, filt, Cl_2	0	0	0	0
04/25/90	Coag, setl, filt, Cl_2	105	0	274	0

Raw waters from both lakes (Tables 9 and 10) were also chlorinated without prior coagulation, settling or filtration, to examine a "worst-case" scenario for mutagen production. TOC levels were far higher than for finished waters, and therefore would be expected to yield significantly higher mutagenicity upon chlorination. These raw chlorinated samples gave TA100 (without S9) activities that were generally higher than the conventionally chlorinated samples for both lakes.

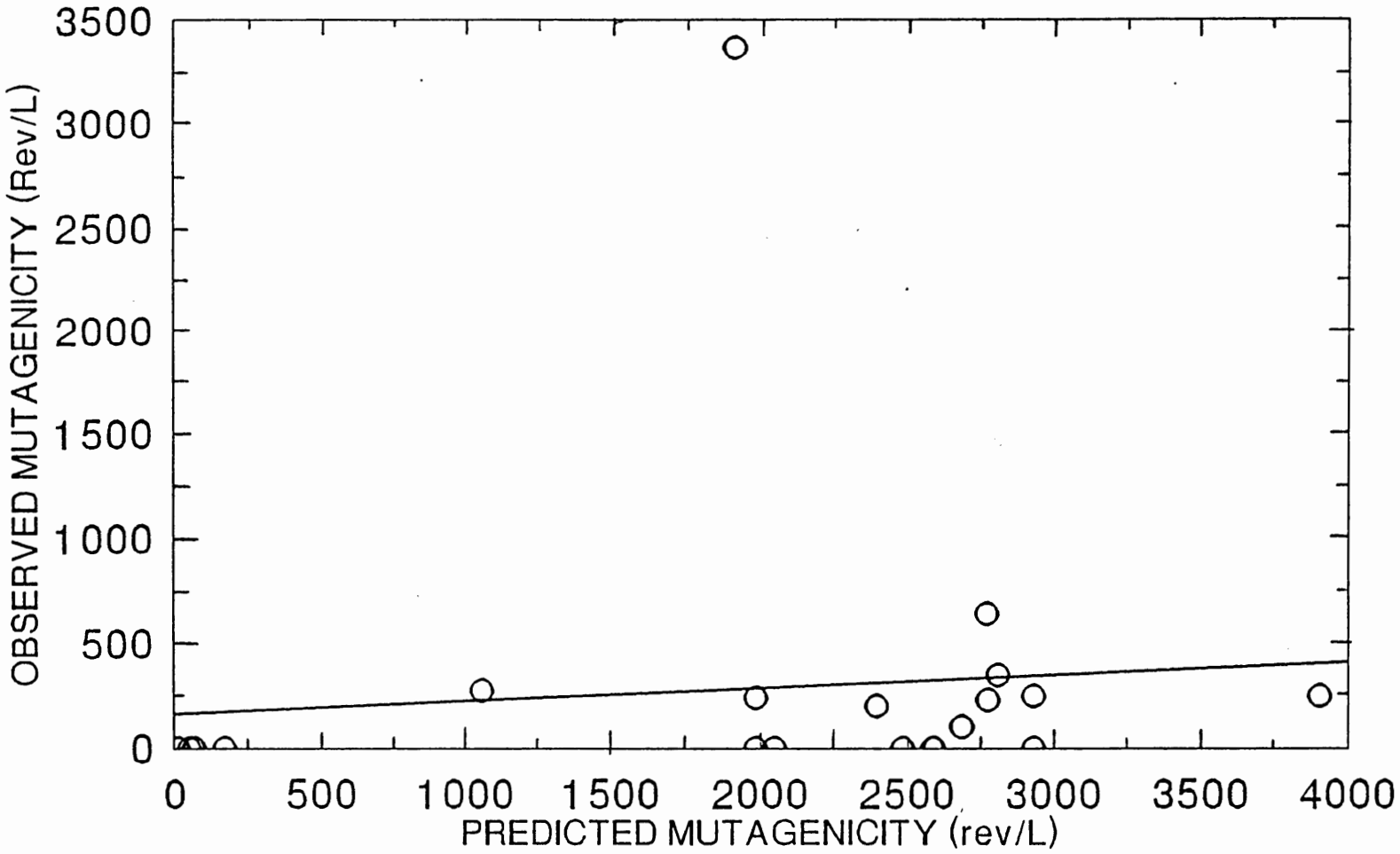
No close correlation was observed beween the mutagenicity of the extracts and either pre- or post-treatement water quality parameters (turbidity, TOC, TOX, THM) except as noted above for the tributary waters.

A model proposed by Vartiainen et al. (1988), based on initial TOC and chlorine oxidant dose and optimized for the highly humic surface waters of Finland, would predict activity about one order of magnitude higher than that which was observed in this study (Figure 22). However the calculations performed with this model did not take into account variations in temperature, pH, contact times and other possible differences in treatment practices which may influence final mutagenic potency. The values reported here for finished waters are in fact consistent with those reported for other North American finished surface waters (Loper et al., 1985; Lippincott et al., 1990).

DISCUSSION OF BIOASSAY RESULTS

It is a reasonable anticipation that direct-acting activity towards TA100 detected in chlorinated samples is due to chlorination products of natural organic substances. Higher activity in the presence than in the absence of S9, or higher activity in strain TA98 than in TA100, suggests the presence of anthropogenic mutagens, or at least, mutagenicity due to substances other than the previously-characterized chlorinated humic acid products, as does also substantial activity before chlorination. The effects of chlorination on mutagenic industrial wastes will depend on the nature of the wastes, and could either increase or decrease overall activity. Halogenated materials may be expected to occur in agricultural runoffs (from pesticides and herbicides) and thus be detectable as chlorinated mutagenic material in raw waters before the chlorination step.

Figure 22. Regression of Observed Mutagenicity and Predicted Mutagenicity for Chlorinated and Chloraminated Water Samples



Therefore the overall presumption is that direct activity towards TA100 which appears only on chlorination is due to chlorinated natural products, primarily MX or similar by-products.

Review of Ames Testing in Previous Water Treatment Studies

Wide variations in sampling, treatment and extraction methodologies continue to be a serious limitation in comparisons of studies of genotoxicity of treated waters. An extensive review of the subject (Noot et al., 1989) was only able to point out major trends in Ames assay mutagenicity for a given type of treatment, with no results truly consistent for studies related to the treatment trains explored in this work. The following brief summary will attempt to provide a context for mutagenicity results obtained in this study for raw water chlorination, ozonation and ozonation/chloramination.

Raw Water. Investigators have found considerable variation in Ames mutagenicity of raw waters depending on the source, season, flow conditions and Salmonella strain used. Surface waters are mutagenic more commonly than ground waters (Kool et al., 1982; Monarca et al., 1985) and more frequently in TA98(-S9) than in other strains. In fact, mutagenicity found under these test conditions has commonly been considered indicative of anthropogenic sources, more often encountered in surface than ground waters.

In most studies, raw water genotoxicity, where it occurs, is lowest in the winter months (Huck et al., 1990; Kool et al., 1985), but can increase markedly under either very low or very high flow conditions (Huck et al., 1990; Grabow et al., 1981B).

Chlorination. Conventionally treated chlorinated drinking waters nearly always exhibit at least moderate Ames mutagenicity, whether treatment occurred in a laboratory or in a treatment plant setting. Strain TA100 without S9 addition is generally most sensitive to the products of chlorination (Noot et al., 1989), but direct-acting mutagenicity was highest towards S. typhimurium strain TA98 in studies of chlorinated surface waters in both moderately and highly industrialized regions (Grabow et al., 1981B; Kool et al., 1984).

Despite the fact that the potent mutagen MX, a common chlorination product of humic waters, is acid extractable (Kronberg et al., 1985), highest activity is most frequently observed in neutral extracts. Direct mutagenicity of raw waters is always increased by chlorination, following which activity is often decreased or eliminated by metabolic activation (Backlund et al., 1985; Noot et al., 1989). One study (Xie et al., 1990) suggests that mutagenicity produced during chlorination is enhanced by the presence of ferric ions.

GAC Treatment and Post Chlorination. GAC filtration is an extremely effective means of removing mutagenicity from treated drinking waters under a variety of conditions and for extended time periods (Bourbigot et al., 1986; Cognet et al., 1986; Huck et al., 1990) even after measurable breakthrough of TOC (Loper et al., 1985; Monarca et al., 1985). No quantitative capacity of GAC for retention of mutagenicity has been determined as yet, but it is likely that background TOC will be a significant modifying factor (Noot et al., 1989).

Post GAC chlorination, however, results in mutagenicity ranging from negligible to pre-GAC treatment levels. Several groups (Loper et al., 1985; Bourbigot et al., 1986; Huck et al., 1990) report no mutagenicity in waters pre-treated with a variety of disinfectants, GAC filtered and post-chlorinated. Other investigators, including Kool (1984) and Cognet et al. (1986) found intermediate to pre-GAC mutagenicity levels, with some dependence on the pre-disinfection method used (ozone being the lowest).

Ozonation and Chloramination. Perhaps no treatment method has produced more widely varying mutagenicity than ozonation. Backlund et al. (1985), Bourbigot et al. (1986), Glaze et al. (1989) and Huck et al. (1990) all reported negligible activity resulting from ozone disinfection. By contrast, significant mutagenicity - up to levels seen in chlorinated waters - has been reported by Nakamuro et al. (1989) and Cognet et al. (1986), especially for strain TA100 without metabolic activation. These studies differ widely, however in ozone dose used, contact time, extraction method and genotoxicity test system, all of which have been shown to affect mutagenicity results. For instance, while low doses of ozone are more likely to create mutagenicity, higher doses can actually contribute to detoxification of organically contaminated waters and lowering of mutagenicity (Bourbigot et al., 1986; Glaze et al, 1989). Most of the above studies used macroreticular resins XAD-2, -4, -7 or -8 to extract ozonation products, which tend to be more polar and more difficult to extract by such resins.

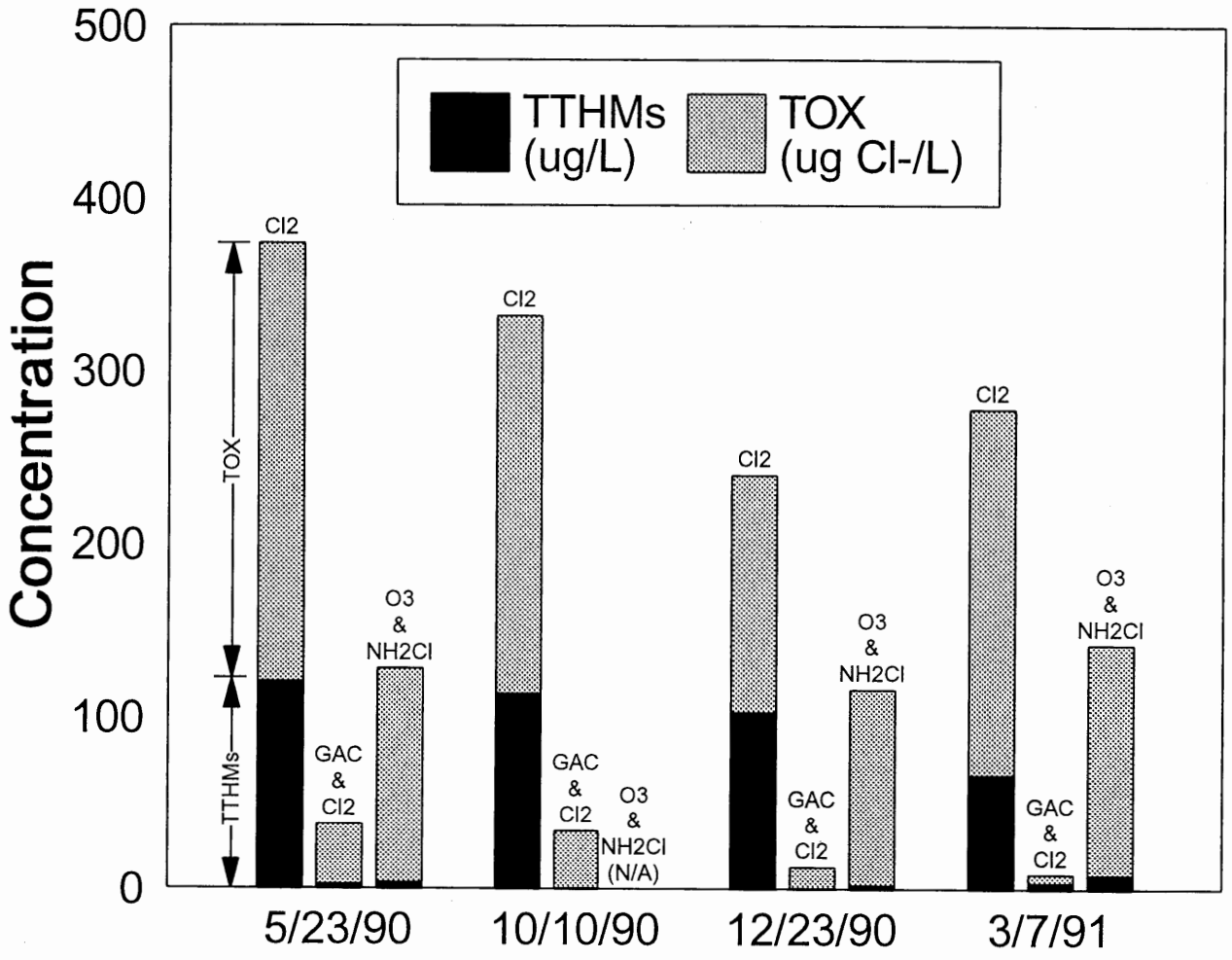
Chloramination usually produces less direct mutagenicity in the same surface waters than chlorination (Noot et al., 1989). However, in one study (Clark and Johnson, 1982), chloramination produced higher levels of promutagenicity than did chlorination. Post-GAC chloramination produces lower activity than post chlorination in general (Noot et al., 1989); studies of genotoxicity of ozonated and chloraminated waters, as a complete treatment train, have been reported by Vartiainen et al., 1988, and resulted in substantial reductions in mutagenicity compared to conventional chlorination.

Since the goal of this project was evaluation of potential health hazards arising from the use of Jordan Lake as a source of drinking water, the origins of any mutagenicity observed are ultimately secondary in importance to their actual presence and to the potential for removing them. The GAC treatment was particularly effective in reducing activity, as was chloramination in the rare instance where ozonation considerably enhanced the activity of a Falls Lake sample.

SUMMARY OF RESULTS

Figure 23 compares the concentrations of TOX and THMs produced in Jordan Lake water by each of the three treatment sequences. The significant differences in TOX formation among the three process options is well illustrated. Ozone and chloramines produced less than half of the TOX created by free chlorine, and the TOX produced from GAC and free chlorine was less than 10% of that produced by free chlorine alone. While THM formation from chlorination alone exceeded 100 ug/l in three of the samples, the maximum THM concentration observed in both GAC-chlorine and ozone-chloramine-treated samples was 10 ug/l. Thus, either of the two advanced treatment process seems capable of meeting a stringent THM regulation while still providing effective disinfection of the water. The GAC, operating with a maximum

Figure 23. Summary of TTHM and TOX Production in Jordan Lake Water by Three Treatment Schemes



adsorptive capacity during these experiments, more effectively removed TOX precursors than the ozone-chloramine treatment. As emphasized earlier, however, the effectiveness of the GAC treatment was only tested during the first 24 hours of service time; long-term performance cannot be extrapolated from these results.

The Ames bioassay results suggested that the limited mutagenicity observed in Jordan Lake water is associated with anthropogenic contaminants, as well as chlorination of the water. GAC, under the optimal adsorption conditions examined in this study, removed all mutagenic activity observed.

Potential adverse health effects from Jordan Lake, as judged by mutagenicity in the Ames assay, are more likely in the summer months than in winter. Overall, the quality of Jordan Lake water at present is no lower than that of alternative water sources (e.g. Fall Lake). The major sources of concern appear to be the tributaries to Jordan Lake, which uniformly exhibited mutagenicity even prior to chlorination. Therefore, with respect to the use of Jordan Lake as a source of drinking water, particular attention should be paid to: (1) controlling point and non-point source discharges into Jordan Lake and its tributaries and thereby arresting deterioration of these bodies of water, and (2) providing water treatment trains designed to remove influent anthropogenic SOCs, and NOM that is responsible for the formation of halogenated disinfection byproducts. With regard to the latter point, water treatment trains involving the use of granular activated carbon and/or ozonation and chloramination should be strongly considered.

REFERENCES

Ames, B.N., J. McCann, and E. Yamasaki. 1975. Methods for detecting carcinogens. *Mutat. Res.* 31:347.

Amy, G.L., J.H. Greenfield, and W.J. Cooper. 1990. Correlations between measurements of organic halide and specific halogenated volatile organic compounds in contaminated groundwater, p.691 in Water Chlorination: Chemistry, Environmental Impact and Health Effects, R.L. Jolley, Ed., Lewis Publishers, Chelsea, MI.

Amy, G.L., P.A. Chadik, and Z.K. Chowdhury. 1987. Developing models for predicting trihalomethane formation potential and kinetics. *J. Am. Water Works Assoc.* 79:7:89.

Anderson, W.B., P.M. Huck, S.A. Daignault, G.A. Irvine, D.W. Rector, E. Savage, R.C. von Borstel, and D.T. Williams. 1990. Comparison of Drinking Water Disinfectants Using Mutagenicity Testing, p. 201 in Water Chlorination: Chemistry, Environmental Impact and Health Effects, Vol. 6, R.L. Jolley et al., Eds., Lewis Publishers, Inc., Chelsea, MI.

Arguello, M.D., C.D. Chriswell, J.S. Fritz, L.D. Kissinger, K.W. Lee, J.J. Richard, and H.J. Svec. 1979. Trihalomethanes in water: A Report on the Occurrence, Seasonal Variation in Concentrations, and Precursors of Trihalomethanes. *J. Am. Water Works Assoc.* 71:504-508.

Babcock, D.B. and P.C. Singer. 1979. Chlorination and coagulation of humic and fulvic acids. *J. Am. Water Works Assoc.* 71:3:149.

Backlund, P. L. Dronbert, G. Pensar, and L. Tikkanen. 1985.Mutagenic activity in humic water and alum flocculated humic water treated with alternative disinfectants. *Sci. Total Environ.* 47:257-264.

Backlund, P., E. Wondergem, K. Voogd, and A. de Jong. 1989. Influence of Chlorination pH and Chlorine Dose on the Formation of Mutagenic Activity and the Strong Bacterial Mutagen 3-Chloro-4-(Dichloromethyl)-5-Hydroxy-5(2H)-Furanone (MX) in Water. *Chemosphere.* 18:9/10, 1903-1911.

Ball, L.M., D.A. Cozzie, R. Kanniganti, J.D. Johnson, and M.J. Charles. 1991. Identification of Mutagenic Byproducts from Chloramination of Aquatic Humic Substances. CR #814900 Report to the Health Effects Laboratory, Office of Research and Development, U.S. Environmental Protection Agency.

Bernstein, L., J. Kaldor, J. McCann, and M.C. Pike. 1982. An empirical approach to the statistical analysis of mutagenesis data from the Salmonella test. *Mutat. Res.* 97:267-281.

Bourbigot, M.M., M.C. Hascoet, Y. Levi, F. Erb, and N. Pomerry. 1986. Role of ozone and granular activated carbon in the removal of mutagenic compounds. *Environ. Health Perspectives.* 96:159-163.

Bull, R.J. 1982. Health Effects of Drinking Water Disinfectants and Disinfectant By-Products. *Environ. Sci. & Technol.* 16:554A-559A.

Chadik, P.A., and G.L. Amy. 1983. Removing trihalomethane precursors from various natural waters by metal coagulants. *J. Am. Water Works Assoc.* 75:10:532.

Chang, S.D., and P.C. Singer. 1991. The impact of ozonation on particle stability and the removal of TOC and THM precursors. *J. Am. Water Works Assoc.* 83:3:71.

Cheh, A.M., J. Skochdopole, P. Koski, and L. Cole. 1980. Nonvolatile mutagens in drinking water: Production by chlorination and destruction by sulfite. *Science.* 207:90-92.

Chow, B.M., and P.V. Roberts. 1981. Halogenated byproduct formation by ClO_2 and Cl_2. *J. Envr. Engr. Div. ASCE.* 107:EE4:609.

Christman, R.F., D.L. Norwood, D.S. Millington, J.D. Johnson, and A.A. Stevens. 1983. Identity and Yields of Major Halogenated Products of Aquatic Fulvic Acid Chlorination. *Environ. Sci. & Technol.* 17:625-628.

Clark, R.R., and J.B. Johnson. 1982. Influence of Chlorination and the Distribution System on Mutagens in the Potable Water Supply. U.I.L.U.-WRC-80-0168, Research Report. 168.

Claxton, L.D., A.G. Stead, and D. Walsh. 1988. An analysis by chemical class of Salmonella mutgenicity tests as predictors of animal carcinogenicity. *Mutat. Res.* 205:197-225.

Cognet, L., Y. Courtois, and J. Mallevialle. 1986. Mutagenic activity of disinfection byproducts. *Environ. Health Perspectives.* 69:165-175.

Coleman, W.E., J.W. Munch, W.H. Kaylor, R.P. Streicher, H.P. Ringhand, and J.R. Meier. 1984. Gas chromatography/mass spectroscopy analysis of mutagenic extracts of aqueous chlorinated humic acid. A comparison of the by-products to drinking water contaminants. *Environ. Sci. & Technol.* 18:674-681.

Collins, M.R., G.L. Amy, and C. Steelink. 1986. Molecular weight distribution, carboxylic acidity, and humic substances content of aquatic organic matter: Implications for removal during water treatment. *Environ. Sci. & Technol.* 20:1028.

Cooper, W.J., R.G. Zika, and M.S. Steinhauer. 1985. Bromide-oxidant interactions and THM formation: A literature review. *J. Am. Water Works Assoc.* 77:4:116.

Cozzie, D.A., R. Kanniganti, M.J. Charles, J.D. Johnson, and L.M. Ball. 1993. Formation of bacterial mutagens from reaction of the alternative disinfectant monochloramine with aqueous solutions of fulvic acid. *Environ & Molecular Mutagenesis.* 21:237.

DeMarini, D.M., J.P. Inmon, J.E. Simmons, E. Berman, T.C. Pasley, S.H. Warren, and R.W. Williams. 1987. Mutagenicity in *Salmonella* of hazardous wastes and urine from rats fed these wastes. *Mutat. Res.* 189:205-216.

Dietrich, A.M., D.S. Millington, and Y.-H. Seo. 1988. Specific identification of organic chemicals in river water. *J. Chromatography.* 436:229-241.

Division of Environmental Management, Water Quality Progress in North Carolina 1984-1985. 1986. Report No.305b, N.C. Department of Natural Resources and Community Development, Raleigh, N.C.

Division of Environmental Management. 1985. Toxic Substances in Surface Waters of the B. Everett Jordan Lake Watershed. Report No. 85, N.C. Department of Natural Resources and Community Development, Raleigh, N.C.

Douglas, G.R., E.R. Nestmann, and G. LeBel. 1986. Contribution of chlorination to the mutagenic activity of drinking water extracts in *Salmonella* and Chinese hamster ovary cells. *Environ. Health Perspectives.* 69:81-87.

Duguet, J.P., Y. Tstutsumi, A. Bruchet, and J. Mallevialle. 1985. Chloropicrin in Potable Water: Conditions of Formation and Production During Treatment Processes, p. 1201 in *Water Chlorination: Environmental Impact and Health Effects, Vol.5.* Lewis Publishers, Inc., Chelsea, MI.

Edzwald, J.K., W.C. Becker, and K.L. Wattier. 1985. Surrogate parameters for monitoring organic matter and trihalomethane precursors. *J. Am. Water Works Assoc.* 77:4:122.

Farkas, L., M. Lewin, and R. Bloch. 1949. The Reactions of Hypochlorite and Bromides. *J. Am. Chem. Soc.* 71:1988-1991.

Fleischacker, S.J., and S.J. Randtke. 1983. Formation of Organic Chlorine in Public Water Supplies. *J. Am. Water Works Assoc.* 75:132-138.

Froelich, E.M. 1978. Control of Synthetic Organic Chemicals by Granular Activated Carbon: Theory, Application and Reactivation Alternatives. Presented at the Seminar on Control of Organic Chemical Contaminants in Drinking Water, Cincinnati, October 10-11.

Fukui, S., Y. Yoshimura, S. Ogawa, and Y. Hanazaki. 1990. Formation of non-volatile potent mutagens in domestic sewage by chlorination. *Chemosphere.* 21:705-716.

Glatz, B.A., C.D. Chriswell, M.D. Arquello, H.J. Svec, J.S. Fritz, S.M. Grimm, and M.A. Thomson. 1978. Examination of drinking water for mutagenic activity. *J. Am. Water Works Assoc.* 70:465.

Glaze, W.H. 1986. Reaction products of ozone: A review. *Environ. Health Perspectives.* 69:151.

Glaze, W.H., M. Koga, D. Cancilla, K. Wang, M.J. McGuire, S. Liang, M.K. Davis, C.H. Tate, and E.M. Aieta. 1989. Evaluation of ozonation by-products from two California surface waters. *J. Am. Water Works Assoc.* 81:8:66.

Grabow, W.O.K., J.S. Burger, and C.A. Hilner. 1981A. Comparison of liquid-liquid extraction and resin adsorption for concentrating mutagens in Ames Salmonella/microsome assays on water. *Bull. Environ. Contam. Toxicol.* 27:442-449.

Grabow, W.O.K., P.G. van Rossum, N.A. Grabow, and R. Denkhaus. 1981B. Relationship of the raw water quality to mutagens detectable by the Ames salmonella/microsome assay via drinking water supply. *Water Research.* 15:1037.

Hartman, P.E., B.N. Ames, J.R. Roth, W.M. Barnes, and D.E. Levin. 1986. Target sequences for mutagenesis in Salmonella histidine-requiring mutants. *Environ. Mutagenesis.* 8:631-641.

Hileman, B. 1982. The chlorination question. *Environ. Sci.and Technol.* 16:1.

Hoigne, J., and H. Bader. 1977. Ozonation of water: Selectivity and rate of oxidation of solutes. Proc. 3rd Intl. Ozone Assoc. Congress. Paris, France.

Hoigne, J. The formation of trichloronitromethane (chloropicrin) and chloroform in a combined ozonation/chlorination treatment of drinking water. *Water Research.* 22:3:313.

Holmbom, B., L. Kronberg, P. Backlund, V.A. Langvik, J. Hemming, M. Reunanen, A. Smeds, and L. Tikkanen. 1990. Formation and properties of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone, A potent mutagen in chlorinated waters, p. 125 in Water Chlorination: Chemistry, Environmental Impact and Health Effects, Vol.6, R.L.Jolley et al., Eds., Lewis Publishers Inc., Chelsea, MI.

Holmbom, B., R. Voss, R. Mortimer, A., and A. Wong. 1984. Fractionation, isolation, and characterization of Ames mutagenic compounds in Kraft chlorination effluent. *Environ. Sci. & Technol.* 18:333-337.

Holmbom, B., L. Kronberg, P. Backlund, V.A. Langvik, J. Hemming, M. Reunanen, A. Smeds, and L. Tikkanen. 1990. Formation and Properties of 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone, A Potent Mutagen in Chlorinated Water, p. 125 in Water Chlorination: Chemistry, Environmental Impact and Health Effects, Vol. 6, Lewis Publishers, Inc., Chelsea, MI.

Huck, P.M., W.B. Anderson, S.A. Daignault, G.A. Irvine, R.C.von Borstel, and D.T.Williams. 1990. Evaluation of Alternative Drinking Water Treatment Processes at Pilot Scale by Means of Mutagenicity Testing. Report to American Water Works Association Research Foundation, Denver, CO.

Ishizaki, C. 1973. Surface Chemistry of Activated Carbon: Its Influence on Adsorption from Aqueous Solution. Ph.D. Thesis, University of Maryland.

Jacangelo, J.G., N.L. Patania, K.M. Reagan, E.M. Aieta, S.W. Krasner, and M.J. McGuire. 1989. Ozonation: Assessing its role in the formation and control of disinfection by-products. *J. Am. Water Works Assoc.* 81:74.

Jensen, J.N., J.J. St. Aubin, R.F. Christman, and J.D. Johnson. 1985A. Characterization of the reaction between monochloramine and isolated aquatic fulvic acids, p. 939 in Water Chlorination: Environmental Impact and Health Effects, R.J. Jolley, Ed., Lewis Publishers, Chelsea, MI.

Jensen, J.N., J.J. St. Aubin, R.F. Christman, and J.D. Johnson. 1985B. Effect of monochloramine on isolated fulvic acid. *Organic Geochem.* 8:1:71.

Jodellah, A.M., and W.J. Weber Jr. 1985. Controlling trihalomethane formation potential by chemical treatment and adsorption. *J. Am. Water Works Assoc.* 77:10:95.

Johnson, J.D., and J.N. Jensen. 1986. THM and TOX formation: Routes, rates, and precursors. *J. Am. Water Works Assoc.* 78:4:156.

Jolley, R.L., and J.H. Carpenter. 1983. A review of the chemistry and environmental fate of reactive oxidant species in chlorinated water, p. 3 in Water Chlorination: Environmental Impacts and Health Effects, Vol. 4, R.L. Jolley et al., Eds., Ann Arbor Science Publishers, Inc., Ann Arbor, MI.

Kavanaugh, M.C. 1978. Modified coagulation for improved removal of trihalomethane precursors. *J. Am. Water Works Assoc.* 70:11:613.

Kool, H.J., J. Hrubec, C.F. van Kreijl, and G.J. Piet. 1985. Evaluation of different treatment processes with respect to mutagenic activity in drinking water. *Sci. Total Environ.* 47:229.

Kool, H.J., C.F. van Kreijl, E. de Greef, and H.J. van Kranen. 1982. Presence, introduction and removal of mutagenic activity during the preparation of drinking water in the Netherlands. *Environ. Health Perspectives.* 46:207.

Kool, H.J., C.F. van Kiejl, and H. Van Oeurs. 1984. Mutagenic activity in drinking water in the Netherlands: A survey and a correlation study. *Toxic Environ. Chem.* 7:111.

Krasner, S.W., M.J. McGuire, J.G. Jacangelo, N.L. Patania, K.M. Reagan, and E.M. Aieta. 1989. The occurrence of disinfection by-products in US drinking water. *J. Am. Water Works Assoc.* 81:8:41.

Kronberg, L., B. Holmbom, and L. Tikkanen. 1985. Fractionation of mutagenic compounds. *Sci. Total Environ.* 47:343-347.

Kronberg, L., B. Holmbom, and L. Tikkanen. 1985. Mutagenic acid in drinking and humic water after chlorine treatment. Vatten (Lund). 41:106-109.

Kronberg, L., R.F. Christman, R. Singh, and L.M. Ball. 1991. Identification of oxidized and reduced forms of the strong bacterial mutagen (Z)-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid (MX) in extracts of chlorine-treated water. *Environ. Sci. & Technol.* 25:99.

Kronberg, L., B. Holmbom, and L. Tikkanen. 1990. Identification of the Strong Mutagen 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone and of its Geometric Isomer, E-2-Chloro-3-(dichloromethyl)-4-oxobutenoic Acid in Mutagenic Fractions of Chlorine-Treated Humic Water and in Drinking Waters, p. 137 in Water Chlorination: Chemistry, Environmental Impact and Health Effects, Vol. 6, Lewis Publishers, Inc., Chelsea, MI.

Lee, M.C., V.L. Snoeyink, and J.C. Crittenden. 1981. Activated carbon adsorption of humic substances. *J. Am. Water Works Assoc.* 73:440.

Leenheer, J.A. 1981. Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural waters and wastewaters. *Environ. Sci. & Technol.* 15:578.

Lippincott, R.L., E.A. Ibrahim, J.B. Louis, T.B. Atherholt, and I. H. Suffet. 1990. Continuous liquid-liquid extraction for the preparation of chlorinated water samples for the Ames bioassay. *Water Resources.* 24:709-716.

Loper, J.C., M.W. Tabor, L. Rosenblum, and J. DeMarco. 1985. Continuous removal of both mutagens and mutagen-forming potential by an experimental full-scale granular activated carbon treatment system. *Environ. Sci. & Toxicol.* 19:333-339.

Loper, J.C., D.R. Lang, and C.C. Smith. 1978. Mutagenicity of complex mixtures from drinking water, p. 433 in Water Chlorination: Environmental Impact and Health Effects, Vol. 2., R.J. Jolley, H. Gorchev, and D.H. Hamilton Jr., Eds., Ann Arbor Science Publishers, Inc., Ann Arbor, MI.

Luong, T.V., C.J. Peters, and R. Perry. 1982. Influence of bromide and ammonia upon the formation of trihalomethanes under water-treatment conditions. *Environ. Sci. & Technol.* 16:8:473.

Lykins, B., and W. Koffskey. Products identified at an alternative disinfection pilot plant. *Environ. Health Perspectives.* 69:119-125.

Maron, D., and B.N. Ames. 1983. Revised methods for *Salmonella* mutagenicity test. *Mutat. Res.* 113:173-212.

McCann, J., E. Choi, E. Yamasaki, and B.N. Ames. 1975. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 compounds. Proc. Natl. Acad. Sci., U.S.A. 72:5135-5139.

McCreary, J.J, and V.L. Snoeyink. 1980. Characterization and Activated Carbon Adsorption of Several Humic Substances. *Water Research.* 14:151.

Meier, J.R., H.P. Ringhand, W.E. Coleman, K.M. Schenck, J.W. Munch, R.P. Streicher, W.H. Kaylor, and F.C. Kopfler. 1986. Mutagenic byproducts from chlorination of humic acid. *Environ. Health Perspectives.* 69:101-107.

Meier, J.R., W.F. Blazak, and R.B. Knohl. 1987. Mutagenic and clastogenic properties of 3-chloro-4-dichloromethy-5-hydroxy-2-(5H)-furanone: A potent bacterial mutagen in drinking water. *Environ. Molec. Mutagenesis.* 10:411-424.

Miller, J.W., and P.C. Uden. 1983. Characterization of nonvolatile aqueous chlorination products of humic substances. *Environ. Sci. & Technol.* 17:150-157.

Minear, R.A., and J.C. Bird. 1980. Trihalomethanes: Impact of bromide ion concentration on yield, species distribution, rate of formation and influence of other variables, p.151 in Water Chlorination: Environmental Impact and Health Effects, Vol.3, R.L. Jolley et al., Eds., Ann Arbor Science Publishers, Inc., Ann Arbor, MI.

Monarca, S., R. Pasquini, and G.S. Sforzolini. 1985. Mutagenicity assessment of different drinking water supplies before and after treatments. *Bull. Environ. Contam. Toxicol.* 34:815-823.

Moreau, D.H., and R. Challa. 1985. An Analysis of the Water Balance of B.Everett Jordan Lake Including Estimates of Flows Between Segments. Report No. 221, The Water Resources Research Institute of the University of North Carolina, Raleigh, N.C.

Morris, J.C. 1967. Kinetics of reaction between aqueous chlorine and nitrogen compounds, in Principles and Applications of Water Chemistry, S.D. Faust and J.V. Hunter, Eds., Wiley, New York.

Nakamuro, K., H. Ueno, and Y. Sayato. 1989. Mutagenic activity of organic concentrates from municipal river water and sewage effluent after chlorination or ozonation. *Water Sci. Technol.* 21:1895-1898.

Nestmann, E.R., G.L. LeBel, D.T. Williams, and D.J. Kowbel. 1979. Mutagenicity of organic extracts from Canadian drinking water in the *Salmonelle*/Mammalian-microsome assay. *Environ. Mutagen.* 1:337-345.

Noot, D.K., W.B. Anderson, S.A. Daignault, D.T. Williams, and P. M. Huck. 1989. Evaluating treatment processes with the Ames mutagenicity assay. *J. Am. Water Works Assoc.* 81:9:87.

Norwood, D.L., and R.F. Christman. 1987. Structural characterization of aquatic humic material. 2. Phenolic content and its relationship to chlorination mechanism in an isolated aquatic fulvic acid. *Environ. Sci. & Technol.* 21:791-798.

Oliver, B.G. 1983. Dihaloacetonitriles in drinking water: Algae and fulvic acid as precursors. *Environ. Sci. & Technol.* 17:80-83.

Oliver, B.G. 1980. Effect of temperature, pH, and bromide concentration on the trihalomethane reaction of chlorine with aquatic humic material, p.141 in Water Chlorination: Environmental Impacts and Health Effects, Vol. 3, R.L. Jolley, Ed., Ann Arbor Science Publishers, Inc., MI.

Peters, C.J., R.J. Young, and R. Perry. 1980. Factors influencing the formation of haloforms in the chlorination of humic materials. *Environ. Sci. & Technol.* 14:1391.

Quimby, B.D., M.F. Delaney, P.C. Uden, and R.M. Barnes. 1980. Determination of aqueous chlorination products of humic substances by gas chromatography with microwave emission detection. *Anal. Chem.* 52:259.

Reckhow, D.A., P.C. Singer, and R.L. Malcolm. 1990. Chlorination of humic materials: Byproduct formation and chemical interpretations. *Environ. Sci. & Technol.* 24:11:1655.

Reckhow, D.A., and P.C. Singer. 1990. Chlorination by-products in drinking waters: From formation potentials to finished water concentrations. *J. Am. Water Works Assoc.* 82:4:173.

Reckhow, D.A. 1984. Organic Halide Formation and the Use of Pre-ozonation and Alum Coagulation to Control Organic Halide Precursors. Ph.D. Thesis, University of North Carolina at Chapel Hill.

Reckhow, D.A., and P.C. Singer. 1984. The removal of organic halide precursors by preozonation and alum coagulation. *J. Am. Water Works Assoc.* 76:4:151.

Rice, R.G. 1980. The use of ozone to control trihalomethanes in drinking water treatment. *Ozone Sci. Engr.* 2:1:75.

Rook, J.J. 1977. Chlorination reactions of fulvic acids in natural waters. *Environ. Sci. & Technol.* 11:478-482.

Scully, F.E., J.P. Yang, K. Mazina, and F.B. Daniel. 1984. Derivatization of organic and inorganic N-chloramines for high-performance liquid chromatographic analysis of chlorinated water. *Environ. Sci. & Technol.* 18:787.

Simmon, V.F., and R.G. Tardiff. 1978. The mutagenic activity of halogenated compounds found in chlorinated drinking water, p. 417 in Water Chlorination: Environmental Impact and Health Effects, Vol.2, R.J. Jolley, H. Gorchev, and D.H. Hamilton, Jr., Eds., Ann Arbor Science, Ann Arbor, MI.

Singer, P.C. 1990. Assessing ozonation research needs in water treatment. *J. Am. Water Works Assoc.* 82:10:78.

Singer, P.C. 1989. *Complying with Trihalomethane Reduction Requirements in Water Treatment Facilities*, Pollution Technology Review No.167, Noyes Data Corporation, Park Ridge, New Jersey.

Singer, P.C., and S.D. Chang. 1989. Correlations between trihalomethanes and total organic halides formed during water treatment. *J. Am. Water Works Assoc.* 81:8:61.

Singer, P.C., R.A. Brown, and J.F. Wiseman Jr. 1988. Formation of Halogenated Organics During Wastewater Disinfection. Report No.239, Water Resources Research Institute of the University of North Carolina.

Singer, P.C., J.J. Barry III, G.M. Palen, and A.E. Scrivner. 1981. Trihalomethane formation in North Carolina drinking waters. *J. Am. Water Works Assoc.* 73:8:392.

Standard Methods for the Examination of Water and Wastewater. 1989. 17th edition. American Public Health Association.

Suffet, I.H., L. Brenner, and B. Silver. 1976. Identification of 1,1,1-trichloroacetone (1,1,1-trichloropropanone) in two drinking waters: A known precursor in haloform reaction. *Environ. Sci. & Technol.* 10:1273.

Sussmuth, R. 1982. Genetic effects of amino acids after chlorination. Mutat.Res. 105:23.

Symons, J.M., P.L.K. Fu, R.C. Dressman, and A.A. Stevens. 1987. The influence of bromide ion on organic chlorine and organic bromine formation during free chlorination. *J. Am. Water Works Assoc.* 79:9:114.

Tedder, S.W. 1984. Statewide Toxics Program in North Carolina. Testimony presented on the behalf of the N.C.Department of Natural Resources and Community Development, Division of Environmental Management, to the Haw River Legislative Study Commission (October 26).

Tennant, R.W., B.H. Margolin, M.D. Shelby, E. Zeiger, J.K. Haseman, J. Spalding, W. Caspary, M. Resnick, S. Stasiewicz, B. Anderson, and R. Minor. 1987. Prediction of chemical carcinogenicity in rodents from in vitro genetic toxicity assays. *Science.* 236:933-941.

Trussell, R.R., and M.D. Umphres 1978. The formation of trihalomethanes. *J. Am. Water Works Assoc.* 70:604-612

Turner, A.G., F.A. DiGiano, and P.M. DeRosa. 1984. A Survey of Potential Population Exposures to Chemical Contaminants Present in Unprotected Surface Water Supplies in North Carolina. Report No.213, The Water Resources Research Institute of the University of North Carolina, Raleigh, N.C.

U.S. Environmental Protection Agency. 1979. National interim primary drinking water regulations for the control of trihalomethanes in drinking water. *Federal Register.* 22:231:68624.

U.S. Environmental Protection Agency. 1988. Methods for the determination of organic compounds in drinking water. Cincinnati, OH.

U.S. Geological Survey. 1988. Surface water quality assessment for region J, North Carolina. Project proposal in cooperation with the Local Governments in the Triangle Area.

van Hoof, F., J. Janssens, and H. van Dyck. 1985. Formation of oxidation by products in surface water preozonation and their behavior in water treatment. *Chemosphere*. 14:501-510.

van Hoff, F. 1983. Influence of ozonation on direct-acting mutagens formed during drinking water chlorination, p.1211 in Water Chlorination: Environmental Impact and Health Effects, Vol. 4., R.L. Jolley et al., Eds., Ann Arbor Science Publishers, Ann Arbor, MI.

Vartiainen, T., A. Liimatainen, P. Keranen, T. Ala-Peijari, and P. Kalliokokoski. 1987. Effect of permanganate oxidation and chlorination on the mutagenic activity and other quality parameters of artificially recharged ground water processed from humus-rich surface water. *Chemosphere*. 16:1489-1499.

Vartiainen, T., A. Liimatainen, P. Kauranen, and L. Hiisirta. 1988. Relations between drinking water mutagenicity and water quality parameters. *Chemosphere*. 17:189-202.

Wang, Y.Y., P. Fiessel, K.-I. Chang, D. Hollander, P. Marsden, and L. Williams. 1990. Evaluation of a protocol for preparing drinking water samples for Ames mutagenicity testing. Water Chlorination. 6:261-277.

Weil, I., and J.C. Morris. 1949. Kinetic studies on the chloramines. I. The rates of formation of monochloramine, N-chloromethylamine and N-chlorodimethylamine. *J. Am. Chem. Soc.* 71:1664.

Werdehof, K.S., and P.C. Singer. 1987. Chlorine dioxide effects on THMFP, and the formation of inorganic by-products. *J. Am. Water Works Assoc.* 79:9:107.

Wetzel, R.G. 1983. Limnology, Second Ed., Saunders College Publishing, Philadelphia.

Wigilius, B., H. Boren, G.E. Carlberg, A. Grimvall, and M. Moller. 1985. A comparison of methods for concentrating mutagens in drinking water - recovery aspects and their implications for the chemical character of major unidentified mutagens. *Science Tot. Environ.* 47:265-272.

Xie, Y., and Z. Wang. 1990. Effect of water treatment on mutagenic activity and concentration MX in water. Abstract for AWWA meeting.

Zeiger, E., K.J. Risko, and B.H. Margolin. 1985. Strategies to reduce the cost of mutagenicity screening with the *Salmonella* assay. *Environ. Mutag.* 7:901-911.

LIST OF PATENTS AND PUBLICATIONS

None to date.

GLOSSARY OF TERMS, ABBREVIATIONS AND SYMBOLS

°C	degrees of centigrade
DCM	dichloromethane, methylene chloride
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
g	gram
GAC	granular activated charcoal
L	liter
LLE	liquid-liquid extraction
mg/L	milligrams per liter
mL	milliliter
MX	3-chloro-4-(dichlorodimethyl)-5-hydroxy-2(5H)-furanone
pKa	negative logarithm of acidity constant
rev/plate	revertants per plate
S9	9,000 g supernatant fraction of tissue homogenate (used here to describe 9,000 g supernatant fraction of livers of Aroclor 1254-treated male rats)
SOC	synthetic organic chemicals
THM	trihalomethane
TOC	total organic carbon (in mg/L)
TOX	total organic halogen (in µg/L)
TTHM	total trihalomethanes (in µg/mL)
µL	microliter