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OXIDANT EFFECTS ON COMPLEX MIXTURES OF NONVOLATILE ORGANICS IN POLLUTED WATERS: EXAMINATION BY HPLC AND BIOSCREENING*

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DISCLAIMER

OXIDANT EFFECTS ON COMPLEX MIXTURES OF NONVOLATILE ORGANICS IN POLLUTED WATERS: EXAMINATION BY HPLC AND BIOSCREENING*

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ABSTRACT

Chemical oxidants such as ozone and chlorine are used for antifoulant treatment of cooling waters and disinfection of polluted waters. The effects of these oxidants on the nonvolatile organic constituents in such waters are being examined using the complementary techniques of high-pressure liquid chromatography (HPLC) and bioscreening. HPLC is used to separate the nonvolatile organic constituents present in complex mixtures in the waters of environmental concern, and the separated organics are detected by UV absorbance or cerate oxidimetry. Bioscreening tests are used to facilitate the examination of only those separated constituents with biological and possible health significance. The bioscreening method principally used is determination of bacterial mutagenic activity. Both ozone and chlorine destroy some nonvolatile organic constituents and produce others. To date, no statistically significant mutagenic activity has been determined for constituents separated from ozonated effluents from wastewater treatment plants.

INTRODUCTION

The use of chemical oxidants for antifoulant treatment of cooling water systems for electric power production and for the disinfection of effluents from wastewater treatment plants has been accepted technology for several decades. Commercial availability, relatively low cost, ease of application and control, and effectiveness have made chlorine an obvious choice for disinfection and antifoulant treatment.[1] Only within this decade have the environmental and

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potential health effects of water chlorination been questioned.[2-7] Although stastically conclusive risk-benefit analyses of water chlorination have not been published, alternate biocides such as ozone are being evaluated with respect to potential environmental and health effects as well as biocidal efficacy.[7-10]

Since nonvolatile organic constituents comprise the bulk of dissolved organic material in natural (cooling waters) and polluted (wastewater effluents) waters, a major question in the evaluation of any biocide is, "What are the chemical effects on the nonvolatile organic constituents?" The corollary question is, "Are toxic or hazardous products formed from the reaction of the biocide with nonvolatile organics in the treated water?" It has been demonstrated that the chemical effects of powerful oxidants such as chlorine and ozone may be numerous and varied.[2-10] Therefore, complete and exhaustive answers to these questions could be obtained only with much effort and expense. The fact that nonvolatile organic material in natural and polluted waters consists of a vast array or mixture of organic constituents, many of which lack precise chemical definition per se, only intensifies the problem. Thus we propose that selected significant biological tests be used to facilitate the study of only those constituents which may have potential environmental or health effects. With judicious choice of chemical separations methods and bioscreening of separated constituents, characterization and identification of the compounds with biological significance become more economically feasible.

In this paper we report the initial results from the use of bacterial mutagenic activity as a bioscreening test to select constituents separated from wastewater effluents by high-pressure liquid chromatography (HPLC) for further characterization by gas chromatography-mass spectrometry (GC/MS).

Mutagenicity was selected for this application because it is one biological end point which is relevant to the evaluation of the safety of environmental chemicals. A significant increase in mutation rate could represent a direct, chronic health hazard to humans or could adversely affect animal or plant populations and thus profoundly damage the environment. Furthermore, mutagenicity has been correlated with mammalian carcinogenicity[11] and, consequently, may give a preliminary indication of danger of an increase in human cancer.[12] HPLC is used because it is capable of separating a wide variety of compounds, particularly those of the nonvolatile category, and GC/MS is the most powerful instrument available for identification of complex organic constituents.

METHODS

High-pressure liquid chromatography

Liquid chromatography has proved to be a useful technique for separating numerous constituents in polluted waters. In a previous HPLC study of primary and secondary effluents from municipal wastewater treatment plants, approximately 70 organic compounds that had been separated from the effluents were subsequently identified by GC/MS.[13] In that study, HPLC using anion exchange resin was demonstrated to have sensitivity in the microgram range.

Both a preparative-scale and an analytical-scale chromatograph are used to separate and detect uv-absorbing compounds and/or cerate-oxidizable compounds. Each chromatograph consists primarily of a heated, high-pressure ion exchange column; a sample injection valve; a two-wavelength dual-beam uv photometer; a cerate oxidative monitor; and a strip-chart recorder.[14]

Generally, samples of polluted waters are concentrated 1000- to 3000-fold by lyophilization before chromatography. A 0.05- to 5.0-ml aliquot of the concentrate is then applied to the top of the resin column (typically strongly basic anion

exchange resin), and the chromatograms are developed by eluting the constituents with an ammonium acetate--acetic acid buffer (pH 4.4) whose acetate concentration gradually increases from 0.015 M to 6.0 M during elution. The uv absorbances at 254 and 280 nm of the column effluent and the Ce(III) fluorescence measured in the cerate oxidative monitor are printed on a strip-chart for a permanent chromatographic record.[14] Eluate fractions are collected for bioscreening tests and possible constituent identification. The fractions are frozen and then lyophilized to remove the ammonium acetate salts from the chromatographic nonvolatile constituents. The constituents are stored in the form of dry powder at -70°C or as a methanol solution at 0°C.

Bioscreening

Reversion mutational assays in several strains of Escherichia coli and Salmonella typhimurium are used for bioscreening nonvolatile chromatographic constituents. The tests involve standard techniques.[11,15,16] The E. coli WP2 strain measures reversion to tryptophan prototrophy by a base-pair substitution mechanism and is used in a suspension test in which the bacteria are exposed to the presumptive mutagen for different periods of time and then plated for survival and mutation frequency.[15] Four strains of S. typhimurium which test for reversion to histidine prototrophy are used.[11] These strains are TA-100 and TA-1535, which are sensitive to base-pair substitution mutagens, and TA-98 and TA-1538, which detect mutagens that operate by a frame-shift mechanism. Each strain is used in a plate test in which the bacteria are included with the presumptive mutagen in a top agar overlay, and the effect is scored as mutant colonies per plate.[16]

Gas chromatography--mass spectrometry

GC/MS analyses are usually performed on aliquots of trimethylsilyl (TMS)-derivatized samples. Conversion of the nonvolatile organics to volatile compounds

is a necessary prerequisite for this technique. The principal method for forming volatile derivatives from nonvolatile constituents is silylation with bis(trimethylsilyl)-trifluoroacetamide.[17]

After bioscreening, significant nonvolatile constituents are derivatized and run routinely on a Finnigan model-3000 gas chromatograph--mass spectrometer. The results provide data concerning the molecular weights of the constituents and the number of active hydrogen atoms per molecule. Comparison of the fragmentation pattern with that of reference standards is necessary for absolute identification.[18] Several other mass spectrometers are available, including two high-resolution instruments. All of the mass spectrometers have computerized data-handling and library search systems.

RESULTS AND DISCUSSION

The primary objectives of this study were: (1) to determine whether treatment of polluted waters with the oxidants ozone and chlorine had chemical effects on nonvolatile organic constituents that were detectable by HPLC and (2) to determine whether the bacterial mutagenic activity tests were suitable for testing concentrates of the polluted waters. After we had established the utility of HPLC for examining chemical effects of ozonation and chlorination and the capabilities of the mutagenesis test procedure, testing of nonvolatile organics separated by HPLC was initiated.

HPLC examination of ozonation and chlorination effects

Samples of both chlorinated and unchlorinated primary effluent were collected in 23-liter carboys at the Oak Ridge West Wastewater Treatment Plant and filtered through a 8-μ Millipore filter. Both the control effluent and the chlorinated effluent (0.9 mg/liter chlorine residual) were concentrated by lyophilization and chromatographed (see Figure 1). In this figure, the chromatogram of the

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uv-absorbing constituents in the control (nonchlorinated) sample is offset above the chromatogram of the chlorinated sample to facilitate comparison. This comparison suggests that treatment of the primary effluent with 10 mg/liter chlorine to a chlorine residual of 0.9 mg/liter destroys many nonvolatile uv-absorbing constituents while producing others. As previously reported,[2] some of the constituents formed during chlorination are probably chloro-organics.

A 20-liter aliquot of the control (untreated) effluent was ozonated in the laboratory at 25°C to a dosage of 6.9 mg ozone per liter of effluent at an ozone utilization efficiency of approximately 70%. The ozonated primary effluent was concentrated and chromatographed. Figure 2 compares the chromatogram of the ozonated sample with that of the control. The chromatograms of the ozonated and control samples are offset to facilitate comparison. The high oxidation potential of ozone is clearly evident in Figure 2 because most of the chromatographic peaks in the control sample have been destroyed.

In another experiment, samples of undisinfected secondary effluent were collected at the Oak Ridge East Wastewater Treatment Plant and filtered through 8-μ Millipore filters. Two 6-liter aliquots were ozonated in the laboratory at 25°C to dosages of 3.5 and 20 mg ozone per liter of effluent at an ozone utilization efficiency of approximately 70%. Chromatograms of the uv-absorbing and cerate-oxidizable constituents in the concentrates of two ozonated effluents are compared in Figure 3 with those of control or untreated samples. It is deduced that most of the destruction of uv-absorbing constituents occurs rapidly and within the first 3.5-mg/liter effluent dosage of ozone. With respect to the cerate-oxidizable constituents, apparently there is initial destruction of some compounds followed by an increase in formation and concentration of others with increasing ozone dosage (e.g., note the greater numbers and sizes of peaks during the 5- to 15-hr elution period).

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It was concluded from this series of experiments that both chlorination and ozonation destroy some nonvolatile organic constituents in polluted waters and produce others.

Bioscreening tests

Initially, concentrates of both control and treated effluents were tested to determine the utility of the bacterial mutagenic activity procedure for bioscreening. Most of the concentrates examined gave negative results. We obtained no evidence for mutagenicity for either chlorinated or unchlorinated secondary effluents from the Oak Ridge East Wastewater Treatment Plant using a variety of tester strains. In contrast, we obtained clear, positive results for chlorinated primary effluent from the Oak Ridge West Wastewater Treatment Plant in S. typhimurium strain TA-1535. However, there was no positive effect for these samples in E. coli WP2 and only a slight effect in S. typhimurium TA-100. The positive effect of these samples is substantial, and the results were reproducible for several samples collected at different periods of the year. (The wastewater treatment plants at Oak Ridge treat principally residential waste.)

No positive results have yet been obtained for ozonated effluents, although ozonated primary and secondary effluents from the Oak Ridge treatment plants, as well as ozonated secondary effluents from treatment plants at Estes Park, Colorado, Mahoning County, Ohio, and Cincinnati, Ohio, have been tested.

A sample of the strongly mutagenic concentrate of the chlorinated primary effluent discussed above was subjected to HPLC. Eluates were collected and pooled to give subsamples of the original concentrate. These subsamples were frozen and then lyophilized to remove the ammonium acetate buffer salts. The residual nonvolatile organics were dissolved in distilled water and tested for mutagenic activity. Of the 29 subsamples obtained from this HPLC separation,

8 were weakly positive in a mutational assay with S. typhimurium strain TA-1535. The HPLC chromatogram and fractions with mutagenic activity are shown in Figure 4. The total mutagenic activity of the fractions is far less than the mutagenicity of the original crude concentrate. This suggests that some of the mutagenic agents present in the concentrate were either lost or inactivated in passage through the anion exchange resin column. Further studies are planned to investigate this phenomenon. The nonvolatile organics possessing mutagenic activity are currently being examined by GC/MS.

Based on the results of this study, we conclude that bacterial mutagenic activity tests may be useful for bioscreening polluted waters and biocides for possible harmful chemical effects. Although preliminary in nature, the data presented here indicate that chlorine may produce potentially harmful nonvolatile organics during the disinfection process from precursor materials in wastewater effluents. Therefore, it is important to generate the information necessary for a complete evaluation of the problem and to determine whether similar difficulties would be encountered with the use of other strong oxidants such as ozone.

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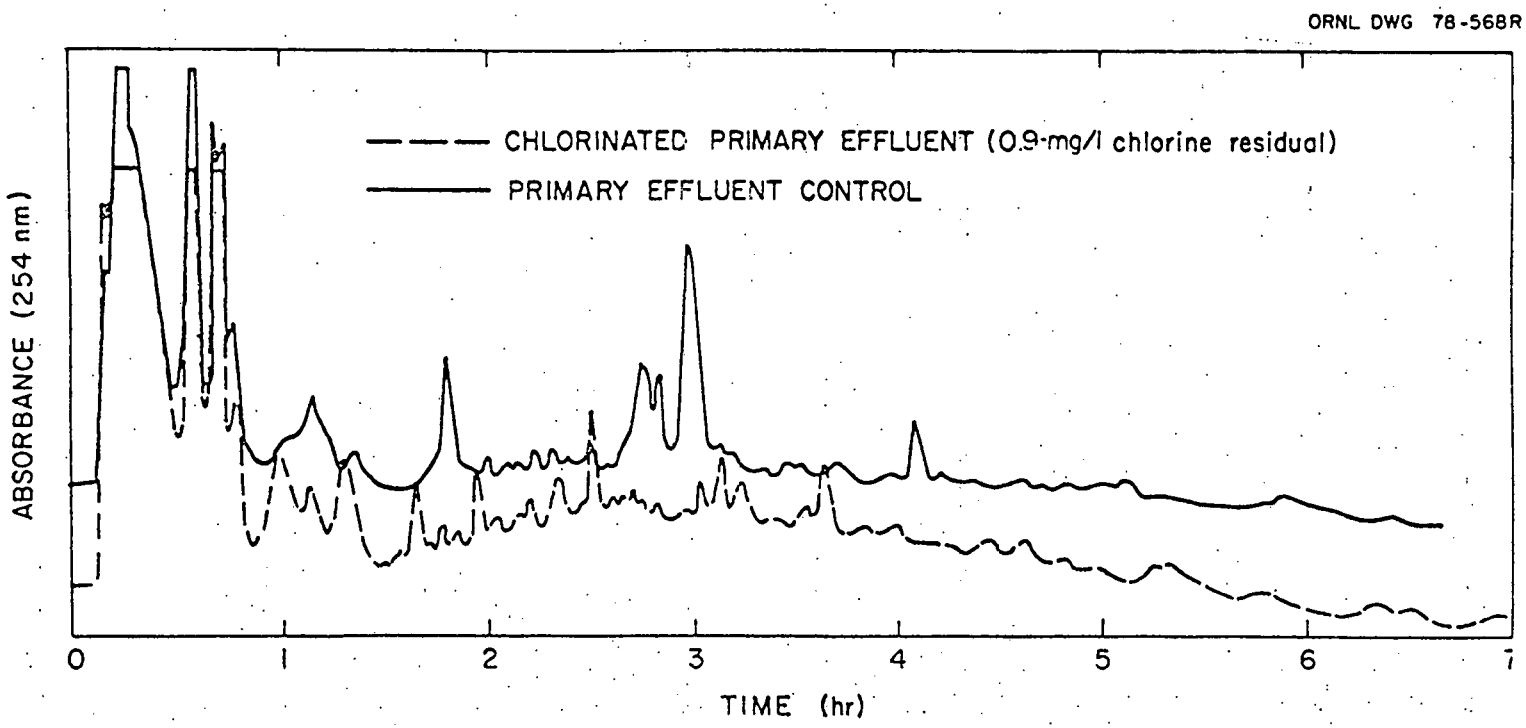
FIGURE LIST

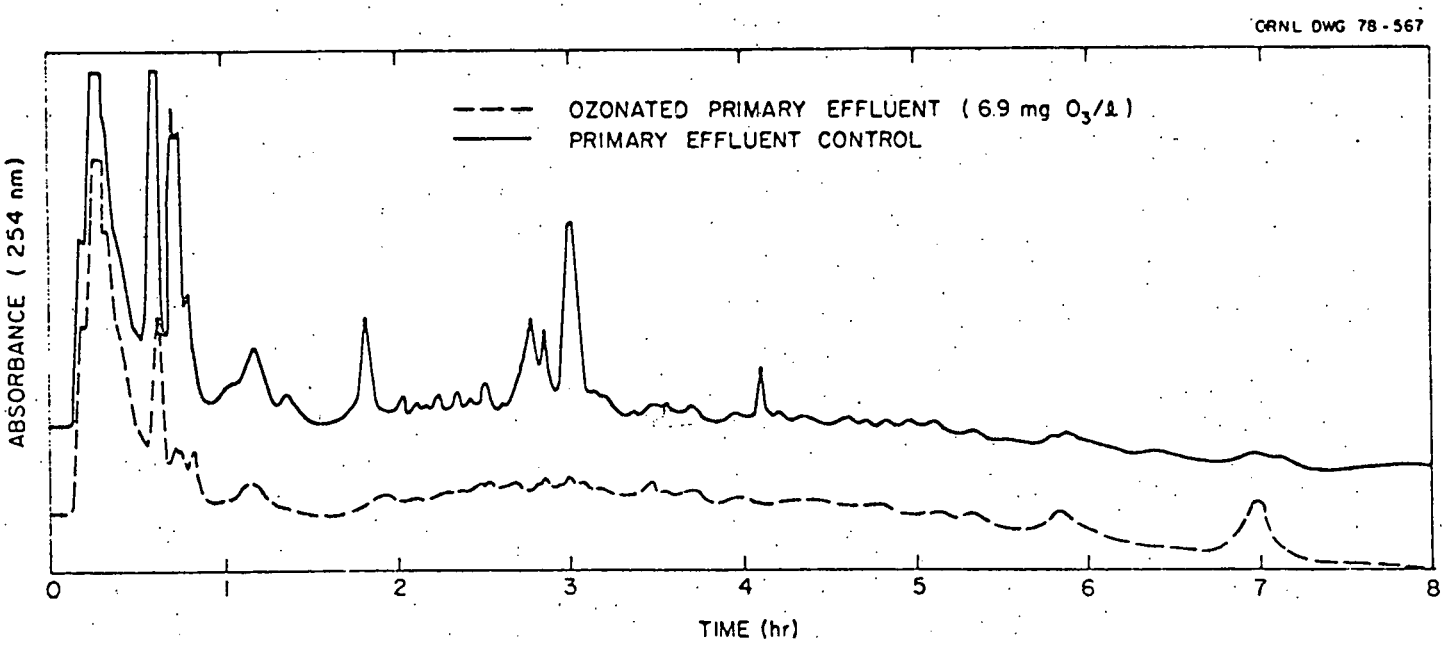
Fig. 1. Chromatograms of uv-absorbing constituents in chlorinated primary effluent (chlorine residual, 0.9 ppm) compared with undisinfected control primary effluent. Samples were collected from the Oak Ridge West Wastewater Treatment Plant. Chlorinated sample, 0.77 ml of 800X concentrate. Unchlorinated sample, 0.77 ml of 700X concentrate.

Fig. 2. Chromatograms of uv-absorbing constituents in ozonated primary effluent (ozone dosage, 6.9 mg/ℓ) compared with undisinfected control primary effluent. Samples were collected from the Oak Ridge West Wastewater Treatment Plant, and the ozonated sample was ozonated in the laboratory. Ozonated sample, 0.77 ml of 790X concentrate. Unozonated sample, 0.77 ml of 700X concentrate.

Fig. 3. Chromatograms of uv-absorbing constituents and cerate-oxidizable constituents in secondary effluent ozonated with 0, 3.5, and 20 mg O_3 per liter of effluent.

Fig. 4. HPLC chromatogram of the uv-absorbing nonvolatile organic constituents in a 2000-fold concentrate of a typical chlorinated (1 ppm chlorine residual) primary effluent from the Oak Ridge West Wastewater Treatment Plant.[12] The notation $\pm$ indicates weak mutagenicity.


ORNL DWG 78-568R
CHLORINATED PRIMARY EFFLUENT (0.9-mg/l chlorine residual)
PRIMARY EFFLUENT CONTROL
ABSORBANCE (254 nm)
0
1
2
3
4
5
6
7
TIME (hr)


ORNL DWG 78-567
OZONATED PRIMARY EFFLUENT (6.9 mg O_3/L)
PRIMARY EFFLUENT CONTROL
ABSORBANCE (254 nm)
0
1
2
3
4
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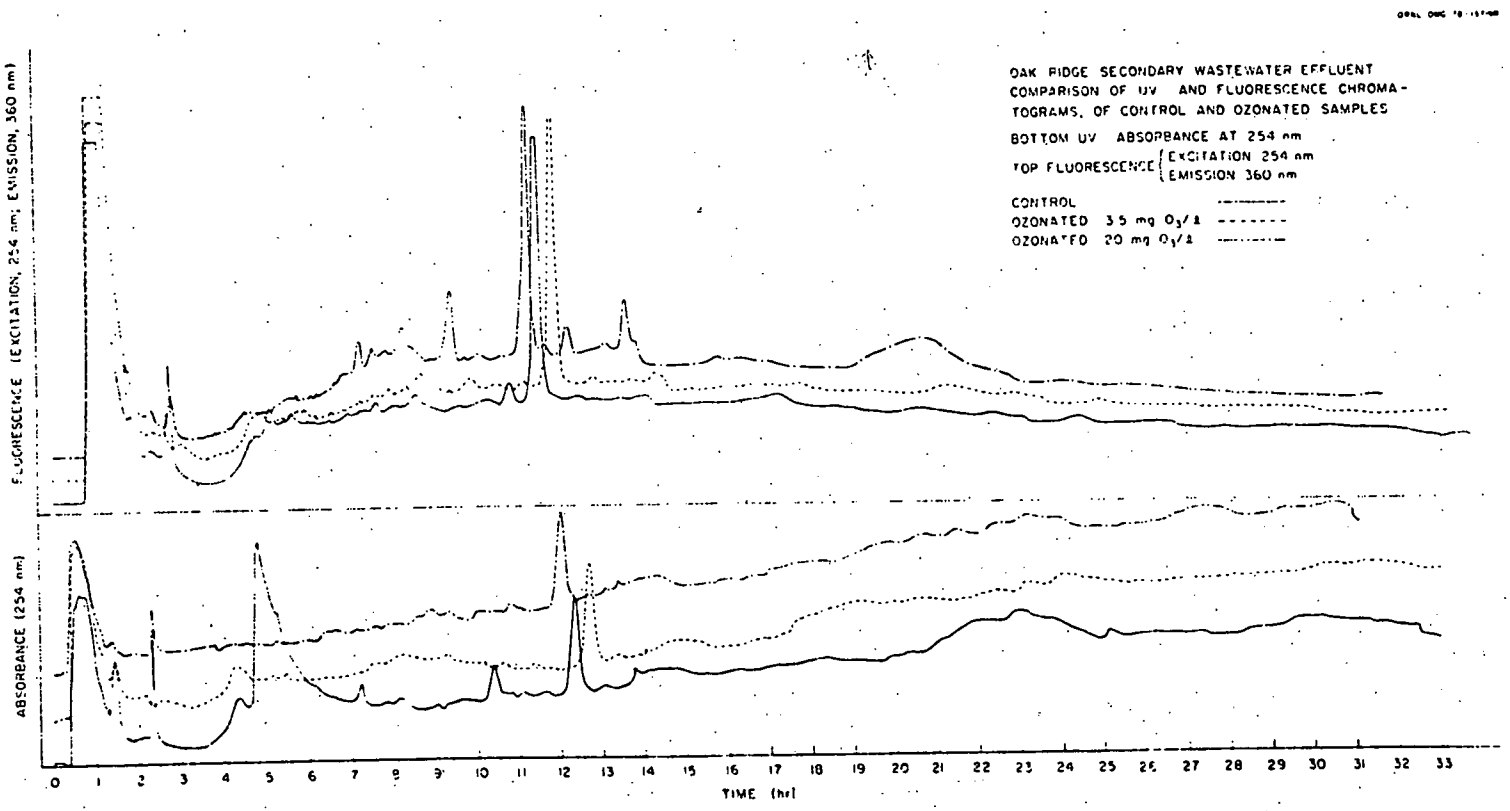
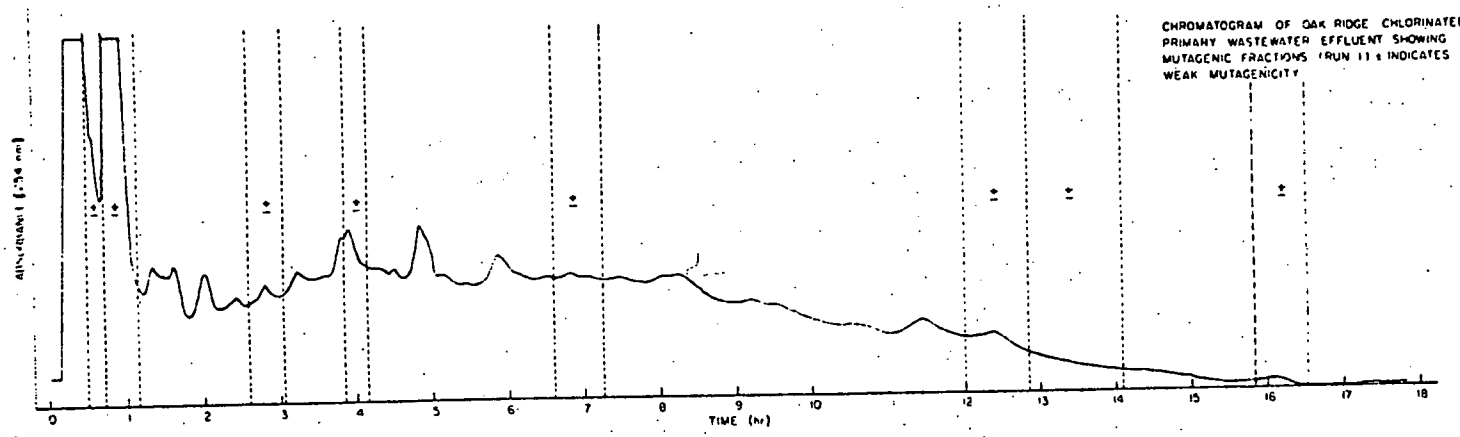
6
7
8
TIME (hr)


OAK RIDGE SECONDARY WASTEWATER EFFLUENT
COMPARISON OF UV AND FLUORESCENCE CHROMATOGRAMS, OF CONTROL AND OZONATED SAMPLES
BOTTOM UV ABSORBANCE AT 254 nm
TOP FLUORESCENCE { EXCITATION 254 nm / EMISSION 360 nm
CONTROL
OZONATED 3.5 mg O3/l
OZONATED 20 mg O3/l
FLUORESCENCE (EXCITATION, 254 nm; EMISSION, 360 nm)
ABSORBANCE (254 nm)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33
TIME (hr)

ORNL DWG 78-13943RA



CHROMATOGRAM OF OAK RIDGE CHLORINATED PRIMARY WASTEWATER EFFLUENT SHOWING MUTAGENIC FRACTIONS (RUN 11 ± INDICATES WEAK MUTAGENICITY