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Project Summary

Nonvolatile Organics in Disinfected Wastewater Effluents: Chemical Characterization and Mutagenicity

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The overall objectives of this research program were to examine the effects of three disinfectants on the chemical composition and mutagenic activity of wastewater effluents. The disinfectants studied were chlorine, ozone, and ultraviolet light.

Nine different treatment plant effluents were examined before and after disinfection (Table 1); fractions of concentrates were isolated to determine the identity of mutagenic components.

The investigation used the following methodology: grab sample collection, concentration by lyophilization, high-pressure liquid chromatography (both analytical and preparative scales), and mass spectrometry.

Mutagenic activity was determined largely by the Ames Bacterial Test Method using two strains of organisms (TA 1535 and TA 1538). On one occasion, another test (in vivo unscheduled DNA synthesis in mice), was used to determine whether the sample constituents or metabolites reached the germ cells and damaged DNA in these cells.

No consistent pattern was noted in this study. Interplant comparison revealed considerable variability. Moreover, samples collected at the same plant at different periods varied significantly in chemical composition and in mutagenic activity.

This Project Summary was developed by EPA's Municipal Environmental Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The severity of waterborne disease epidemics has been greatly reduced because of disinfection treatment of wastewater and potable waters, mainly with chlorine. Recently, however, harmful chloro-organics have been established as byproducts of this disinfection process. Ozonation and ultraviolet light irradiation are two disinfectants that are being considered as viable alternatives. But first an evaluation is needed of possible harmful byproducts, like those produced with chlorination.

Within the last decade, rapid progress has been made in the analysis of volatile organic constituents of water and wastewater. The determination and characterization of nonvolatile organics, however, have not advanced as rapidly, even though the greatest portion of

Table 1. Sources of Effluent Samples

Name of Treatment Plant	Location	Type of Treatment	Disinfection Method	Type of Wastewater
Oak Ridge West Wastewater Treatment Plant	Oak Ridge, TN	Primary	Chlorine Ozone[a] Ultraviolet[a]	Primarily residential
Oak Ridge East Wastewater Treatment Plant	Oak Ridge, TN	Secondary[b]	Chlorine Ozone[a] Ultraviolet[a]	Primarily residential
Lyons Bend Fourth Creek Wastewater Treatment Plant	Knoxville, TN	Secondary[b]	Chlorine	Primarily residential
Moccasin Bend Wastewater Treatment Plant	Chattanooga, TN	Secondary[b]	Chlorine	Residential Industrial
Marlborough Easterly Advanced Waste Treatment Plant	Marlborough, MA	Secondary[b]	Ozone Chlorine	Primarily residential
Upper Thompson Sanitation District	Estes Park, CO	Secondary[b]	Ozone	Residential, relatively clean
Taft Center, USEPA Pilot Wastewater Treatment Plant	Cincinnati, OH	Secondary[b]	Ozone	Residential and industrial
Meander Creek Wastewater Treatment Plant	Mineral Ridge, OH	Secondary[b]	Ozone	Primarily residential
Northwest Bergen County Wastewater Treatment Plant	Waldwick, NJ	Secondary[b]	Chlorine Ultraviolet[a]	Primarily residential

[a]Conducted in laboratory tests.
[b]Activated sludge.

soluble organics in wastewater and natural water fall into that category. An understanding of the possible environmental and health-related effects of these pollutants based on definitive data is essential.

Scope

This research program was developed into four major tasks: (1) laboratory-scale disinfection of wastewater effluent to assess the chemical and mutagenic effects of each disinfectant on the effluent; (2) analysis of concentrated samples from eight operational wastewater treatment plants and one pilot plant, representing all three disinfectants and varying levels of domestic and industrial wastewater sources; (3) characterization and identification of constituents separated from the effluent concentrates of operating plants, and (4) screening of concentrated effluents for mutagenic activity. In the later phases of the research program, mutagenic activity was used as the criterion for characterization of the separated chromatographic components.

Results and Conclusion

Disinfection of secondary effluent by chlorination and ozonation was found to destroy some nonvolatile organics and to produce others, as illustrated in Figure 1 showing high-pressure liquid chromatograms of effluent before and after treatment with three disinfectants. This pattern was found to prevail in all samples.

A few more mutagenic fractions were found in chlorinated and ozonated samples than in the corresponding nondisinfected control effluent of most plants sampled. But inconsistent data prevent the conclusion that disinfection caused this effect. Many mutagenic fractions were isolated from a nonmutagenic concentrate and vice versa.

Chlorination of primary effluent produces far more mutagenic components that does chlorination of secondary effluent. This pattern was not observed with ozonation. Ultraviolet irradiation had only a slight effect on the chemical composition of secondary effluent, but it destroyed the mutagenic activity of several fractions.

Samples collected from the same plant on different dates showed varying chemical compositions and mutagenic components.

Table 2 lists the 103 components identified in concentrates of the controls and disinfected effluents. Seventy-seven were in the mutagenic fractions, and 48 were in the nonmutagenic fractions, with 22 of these compounds being common to both sets. None of the identified compounds are known to be mutagenic.

The full report was submitted in fulfillment of Interagency Agreement EPA-IAG-D7-01027, DOE 40-593-76, by Oak Ridge National Laboratory under the sponsorship of the U.S. Environmental Protection Agency.

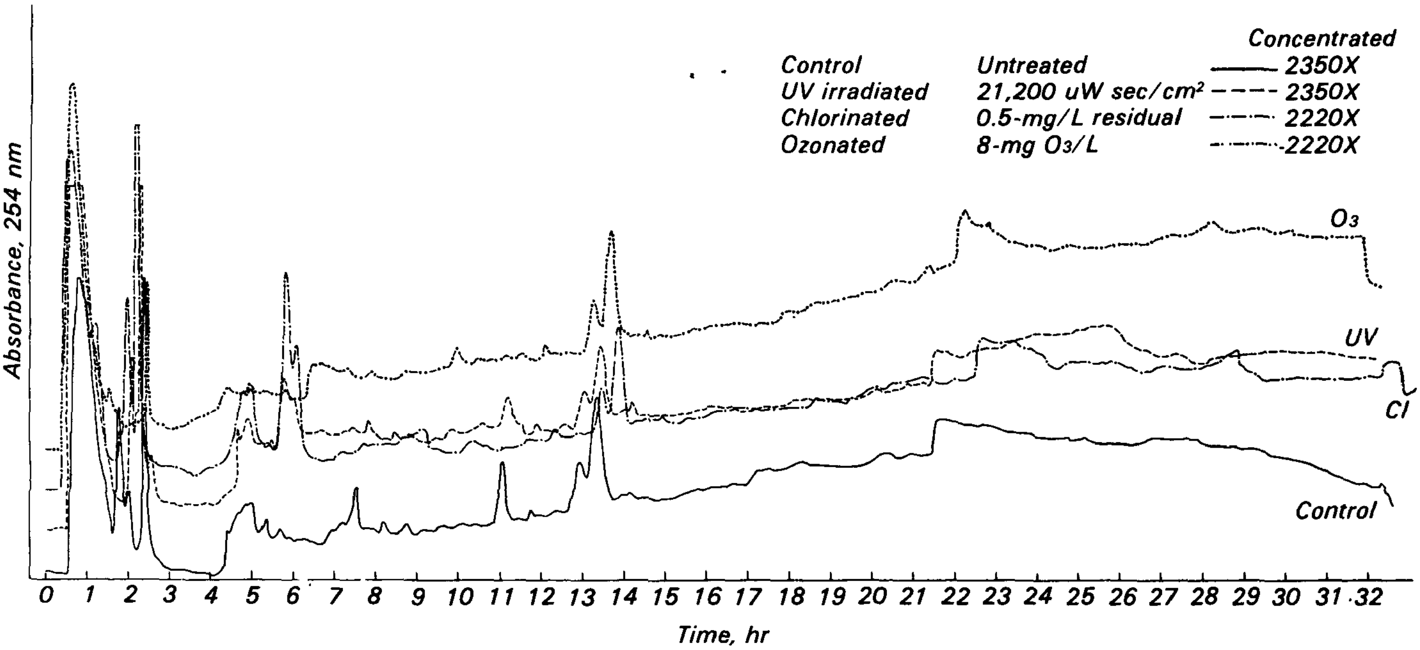

Figure 1. *Chromatograms of ultraviolet- absorbing constituents in secondary effluents disinfected with chlorine, ozone, and ultraviolet light.*

Table 2. *Summary of Identified Compounds in High-Pressure Liquid Chromatography Fractions of Effluent Concentrates*

Alcohols
- 4-Butoxy-1-butanol[a]
- Cyclobutanol[a]
- 2,4-Dimethyl-3-pentanol[a]
- 4-Methyl-2-propyl-1-pentanol[a]

Allphatic Acids
- Diglycolic acid[a,b]
- 2,2-Dimethyl-3-hydroxybutyric acid[b]
- 3,2-Dimethyl-3-hydroxybutyric acid[a]
- Glutamic acid[a]
- 3-Hydroxybutyric acid[b]
- 2-Hydroxyisobutyric acid[a,b]
- 3-Hydroxyisovaleric acid[b]
- Isovaleric acid[a]
- Ketoglutaric acid[a]
- Lactic acid[a,b]
- Malonic acid[b]
- 2-Methyl-2-hydroxybutyric acid[a]
- Muconic acid[a]
- Oxalic acid[a,b]
- Propanoic acid[a]

Amines
- Canavanine[b]
- N,N'-diethyl-N,N'-dimethyl-p-phenylenediamine[a]

Amino Acids
- Alanine[a,b]
- Aminoisobutyric acid[b]
- Aspartic acid[b]
- Cystine[a]
- Glycine[a]
- Histidine[a]
- Homocystine[a]
- Phenylalanine[a]
- Serine[a]
- Tyrosine[a]

Aromatic Acids
- Benzoic acid[a,b]
- 4-Chlorobenzoic acid[a,b]
- Dichlorobenzoic[a]
- O-hydroxyphenylacetic acid[b]
- Phthalic acid[a]
- Phenylpuruvic acid[a]
- 2-Pyridinecarboxylic acid[a]

Carbohydrates
- Erythrose[a,b]
- Erythrono-1,4-lactone[b]
- Galactose[b]
- Glucose[b]
- Gluconic acid[b]
- 1-Methylmannofuranoside[a]
- 2-Methylxylopyranoside[a]
- Rhamnose[b]
- Ribino-1,4-lactone[b]
- Threono-1,4-lactone[a,b]
- Xylono-1,4-lactone[a]
- Xylose[a,b]

Epoxide
- 2-Methyl-3-propyl oxirane[a]

Fatty Acids
- Linoleic acid[a]
- Myristic acid[b]
- Oleic acid[b]
- Palmitic acid[a,b]
- Stearic acid[a,b]
- Tetradecanoic acid[b]

Hydrocarbons
- Decane[b]
- 2,4-Dimethylhexane[a]
- Dodecane[a]
- Ethylindan[a]
- 4-Ethyltetradecane[b]
- Methyleicosane[a]
- 5-Methyl-5-ethyldecane[b]
- 2-Methylhexadecane[b]
- 2-Methyloctane[a]
- 2-Methyl-1,3,6-trioxocane[a]
- Nordodecane[a]
- Pentacosane[a]
- Pentadecane[b]
- 2,2,3,3-Tetramethylhexane[a,b]

Phenols
- Phenol[a,b]

Phthalates
- Di-n-Butylphthalate[b]
- Diethylphthalate[b]
- Diisobutylphthalate[a,b]

Polyols
- Cyclohexane-1,4-diol[a]
- Dihydroxyacetone[b]
- 2,5-Dimethyl-3,4-hexanediol[a]
- Erythritol[a,b]

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Table 2. (continued)

Ethylene glycol[a,b]	*Purines and Pyrimidines*	*Miscellaneous*
Glucitol[a,b]	*Adenosine*[a]	*2-Aminoethanol*[a]
Glycerol[a,b]	*Caffeine*[a]	*Decylhydroxyl amine*[a]
Inositol[a]	*6-Dimethyladenosine*[a]	*2,4-Dimethyl-2,4-disilapentane*[a]
1,3-Propanediol[a,b]	*5-Hydroxyuridine*[a]	*3,7-Dioxa-2,8-disilanonane-2-one-2,2,8,8-tetramethyl*[a]
Propylene glycol[a]	*Thymine*[a]	*L-α-Glycerophosphate*[a]
Xylitol[a]	*Uracil*[a,b]	*Phenylacetamide*[a]
Threitol[a,b]		*Phenylpyruvic oxime*[a]
		1,2,3-Trimethoxypentane[b]
		3,6,9-Trioxa-2-silaundecane-2-3-dimethyl[a]
		Urea[a]

[a]*Identified in mutagenic high-pressure liquid chromatography fraction.*
[b]*Identified in a nonmutagenic high-pressure liquid chromatography fraction.*

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The complete report, entitled "Nonvolatile Organics in Disinfected Wastewater Effluents: Chemical Characterization and Mutagenicity," (Order No. PB 82-254 053; Cost: $18.00, subject to change) will be available only from:
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