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AWWA RESEARCH FOUNDATION
6666 West Quincy Avenue
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RESEARCH REPORT

SUBJECT AREA: Water Treatment and Operations

Mechanism of Inactivation of Microorganisms by Combined Chlorine

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FOREWORD

This report is part of the on-going research program of the AWWA Research Foundation. The research described in the following pages was funded by the Foundation in behalf of its members and subscribers in particular and the water supply industry in general. Selected for funding by AWWARF's Board of Trustees, the project was identified as a practical, priority need of the industry. It is hoped that this publication will receive wide and serious attention and that its findings, conclusions, and recommendations will be applied in communities throughout the United States and Canada.

The Research Foundation was created by the water supply industry as its center for cooperative research and development. The Foundation itself does not conduct research; it functions as a planning and management agency, awarding contracts to other institutions, such as water utilities, universities, engineering firms, and other organizations. The scientific and technical expertise of the staff is further enhanced by industry volunteers who serve on Project Advisory Committees and on other standing committees and councils. An extensive planning process involves many hundreds of water professionals in the important task of keeping the Foundation's program responsive to the practical, operational needs of local utilities and to the general research and development needs of a progressive industry.

All aspects of water supply are served by AWWARF's research agenda: resources, treatment and operations, distribution and storage, water quality and analysis, economics and management. The ultimate purpose of this effort is to assist local water suppliers to provide the highest possible quality of water, economically and reliably. The Foundation's Trustees are pleased to offer this publication as contribution toward that end.

The purpose of this project was directed at determining the mechanisms of inactivation of combined chlorine on bacteria. With many utilities using combined chlorine rather then free chlorine, it is important to know and understand the mechanisms of bacterial disinfection so that treatment practices can be improved. Chloramines and chlorine were found to react differently with the nucleic components of bacteria and that some chloramine reactions with cellular components may be reversible.

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Chairman, Board of Trustees
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ABSTRACT

A study was undertaken to provide a more perspicuous understanding of the mechanism of bacterial inactivation by monochloramine. The chemical reactivity of the disinfectant with various biochemical substrates was studied at 25°C and pH 7.0. The disinfectant readily reacts with 4 compounds: cysteine, cystine, methionine and tryptophan. Rapid reactions with only these compounds provided a partial explanation for its lesser biocidal efficiency when compared to free chlorine, which reacts with a wide variety of substrates. The extremely slow chemical and biological reactions with nucleic acids ruled out these bacterial components as primary lethal targets. Monochloramine did not severely damage the cell envelope of _Escherichia coli_ B as indicated through cell lysis and cell leakage experiments. Since monochloramine-reactive compounds were amino acids, it appeared that the mode of action of the disinfectant involved proteins or protein-mediated reactions. Inhibition of typical protein-associated biological activities such as bacterial transport, respiration and substrate dehydrogenation were observed at concentrations normally employed under disinfection conditions. However, in all cases, the kinetics of bacterial inactivation were not consistent with the kinetics of biological inhibition. The inactivation of _E. coli_ B was generally more rapid than the inhibition of biological activity.

Inactivation curves for _Escherichia coli_ B were characterized by initial shoulders followed by exponential kill. Bacterial clumping and time required for the disinfectant to penetrate the cell and promote leakage were ruled out as possible causes for the initial lag in the curves. The observed differences in rates of kill and inhibition of various biological activities suggested that the shoulder formation was due to reactions at many sensitive sites in the bacterium which preceded inactivation. The mode of action of monochloramine appeared to involve multiple hits by the disinfectant on the bacterial cell before death occurred.